UNIVERZITET U BANJOJ LUCI

MEDICINSKI FAKULTET

Žana Maksimović

ANTIDOTSKI POTENCIJAL NOVOSINTETISANOG OKSIMA K870 U POREĐENJU SA OBIDOKSIMOM KOD TROVANJA PARAOKSONOM U PACOVA

DOKTORSKA DISERTACIJA

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ANTIDOTAL POTENTIAL OF THE NEWLY SYNTHESISED OXIME K870 IN COMPARISON WITH OBIDOXIME IN PARAOXON POISONING IN RATS

DOCTORAL DISSERTATION

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Mentor: Prof. dr Miloš P. Stojiljković, redovni profesor, Medicinski fakultet, Univerzitet u Banjoj Luci

Naslov doktorske disertacije: Antidotski potencijal novosintetisanog oksima K870 u poređenju sa obidoksimom kod trovanja paraoksonom u pacova

Rezime: Usljed široke primjene organofosfatnih pesticida, trovanja ovim pesticidima su česta, kako zadesna, tako i namjerna. Djeluju tako što nepovratno inhibišu enzim acetilholinesterazu (AChE), čija je reaktivacija moguća samo pomoću oksima. Inhibicija AChE dovodi do nakupljanja acetilholina u neuromuskularnoj sinapsi i do akutnih holinergičkih efekata. Do sada je pokazano da je obidoksim najefikasniji oksim kod trovanja paraoksonom. Cilj istraživanja je bio da se utvrdi antidotski potencijal novog oksima K870 kod trovanja paraoksonom i da se uporedi sa obidoksimom. Studija je sprovedena kroz nekoliko povezanih cjelina. Nakon što je utvrđena srednja smrtna doza (LD_{50}) oksima, 20% od LD_{50} je uzeto kao doza za dalji tretman. Utvrđena je LD_{50} paraoksona (odnosno zaštitni indeksi) kod pacova tretiranih atropinom, obidoksimom, oksimom K870, kao i atropinom sa obidoksimom odnosno oksimom K870. Praćeni su znakovi trovanja (vrijeme pojave i intenzitet), tokom četiri časa za: konvulzije, tremor, ataksiju, fascikulacije, stereotipne pokrete, egzoftalmus, lakrimaciju, salivaciju, dispneju i piloerekciju. Preživjelim pacovima su analizirani hematološki i biohemijski parametri. Nakon toga je analizirana inhibicija AChE tokom 24 časa, kao i stepen reaktivacije pomoću obidoksima i oksima K870 u mozgu (posebno u velikom mozgu, malom mozgu i moždanom stablu), eritrocitima i dijafragmi. Inhibicija i reaktivacija karboksilesteraze (CarbE) je analizirana u jetri i plazmi. Promjene kontrakcija na neuromuskularnoj sinapsi kod trovanja paraoksonom u zavisnosti od tretmana analizirane su metodom *n. phrenicus* – dijafragma *in situ*, po Ćetkoviću i Boškoviću (1988). Potom su praćeni kardiovaskularni i plućni parametri: broj i dubina respiracija, srčana frekvenca, pojava i tip poremećaja srčanog ritma, vrijednost arterijskog pritiska, gasna analiza arterijske krvi kod pacova trovanih paraoksonom i tretiranih fiziološkim rastvorom, atropinom, N-butilskopolaminom, obidoksimom ili oksimom K870. Pokazano je da oksim K870 bolje od obidoksima smanjuje smrtnost kod trovanja paraoksonom kod pacova. Oksim K870 je imao značajan zaštitni indeks i kao monoterapija, ali je zaštitni indeks bio izraženo potenciran

istovremenom primjenom atropina i bio je statistički značajno bolji od tretmana kombinacijom obidoksima i atropina. Primjena oksima K870 reaktivirala je i AChE i CarbE, a što je posebno značajno reaktivirala je AChE u mozgu. Prilikom aplikacije apsolutne smrtne doze paraoksona tretman oksimom K870 je popravljao neuromuskularnu transmisiju. Oksim K870 je smanjivao acidozu, poboljšavao vrijednosti gasnih analiza u arterijskoj krvi, disanje, smanjivao učestalost aritmija kao i oscilacije arterijskog krvnog pritiska. Oksim K870 je pokazao veći antidotski potencijal od obidoksima.

Ključne riječi: Oksim K870, obidoksim, paraokson, acetilholinesteraza, ireverzibilni inhibitori acetilholinesteraze, organofosfatna jedinjena.

Naučna oblast: Medicinske i zdravstvene nauke

Naučno polje: Medicinske nauke

Uža naučna oblast: Farmakologija i toksikologija

Klasifikaciona oznaka za naučnu oblast prema CERIF šifrarniku: B740

Mentor: Full Professor, Miloš P. Stojiljković, MD, PhD, Faculty of Medicine, University of Banja Luka

Doctoral thesis: Antidotal potential of newly synthesised oxime K870 in comparison to obidoxime in paraoxon poisoning in rats

Summary: Organophosphate pesticides are widely used pesticides, and poisonings are frequent, both intentional and accidental. They act by irreversibly inhibiting the enzyme acetylcholinesterase (AChE), the reactivation of which is possible only with oxime. AChE inhibition leads to the accumulation of acetylcholine at the synaptic cleft and to acute cholinergic effects. Obidoxime is the most effective oxime for paraoxon poisoning known up to date. The aim of the research was to determine the antidotal potential of oxime K870 in paraoxon poisoning and to compare it with obidoxime. The study was conducted through several connected phases. After the median lethal dose (LD_{50}) of the oxime was determined, 20% of the LD_{50} was taken as the dose for further treatment. The LD_{50} of paraoxon (ie protective ratio) of rats treated with atropine, obidoxime, oxime K870, as well as atropine with obidoxime or oxime K870 was determined. Signs of poisoning, their onset and intensity were monitored during four hours for: convulsions, tremors, ataxia, fasciculations, stereotypic movements, exophthalmos, lacrimation, salivation, dyspnoea and piloerection. The surviving rats were analysed for haematological and biochemical parametres. The inhibition of AChE was analysed during 24 hours, as well as the degree of reactivation by obidoxime and oxime K870 in the brain (separately in cerebrum, cerebellum and brainstem), erythrocytes and diaphragm. Inhibition and reactivation of carboxylesterase (CarbE) was analysed in liver and plasma. Changes in contractions at the neuromuscular synapse in paraoxon poisoning, depending on the treatment, were analysed using method *n. phrenicus* – diaphragm *in situ*, according to Ćetković and Bošković (1988). Cardiovascular and pulmonary parameters were monitored: number and depth of respiration, heart rate, occurrence and type of heart rhythm disorders, arterial pressure value, arterial blood gas analysis in rats poisoned with paraoxon and treated with saline, atropine, N-butylscopolamine, obodoxime or oxime K870. Oxime K870 reduced mortality in paraoxon poisoning in rats better than obidoxime. Oxime K870 had a

significant protective ratio even as monotherapy, but the protective ratio was significantly potentiated by the simultaneous application of atropine, significantly better than the treatment with obidoxime and atropine. Administration of oxime K870 significantly reactivated both AChE and CarbE, and especially significantly reactivated AChE in the brain. When applying an absolute lethal dose of paraoxon, oxime K870 treatment improved neuromuscular transmission. Oxime K870 reduced acidosis, improved gas analysis values in arterial blood, improved breathing, reduced the frequency of arrhythmias, reduced arterial blood pressure oscillations. Oxime K870 has a higher antidote potential than obidoxime.

Key words: Oxime K870, obidoxime, paraoxon, acetylcholinesterase, irreversible acetylcholinesterase inhibitors, organophosphorus compounds.

Scientific area: Medical and health sciences

Scientific field: Medical sciences

Narrow scientific area: Pharmacology and toxicology

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SADRŽAJ

1. UVOD

1.1. Acetilholin

Acetilholin (ACh) je neurotransmiter centralnog i perifernog nervnog sistema, estar acetilne kiseline i holina. ACh se sintetiše u nervnim završecima iz acetil koenzima A (acetil CoA, koji nastaje iz glukoze) i aminoalkohola holina, u reakciji koju katalizuje holin acetiltransferaza [1]. Zbog naelektrisane amonijum grupe, ACh je izrazito hidrofoban i ne prodire kroz lipidne membrane [2]. Zbog toga, kada se molekul unese spolja, on ostaje u ekstracelularnom prostoru. Holin je prisutan u plazmi u koncentraciji od oko 10 mM i preuzima se u holinergičke neurone transporterom Na^+/holin visokog afiniteta [3]. Oko 10,000 molekula ACh je upakovano u svaku vezikulu pomoću vezikularnog ACh transportera [4].

Receptori na koje djeluje ACh se nazivaju holinergički, lijekovi koji potenciraju dejstva ACh su holinergički lijekovi, a koji smanjuju su antiholinergički lijekovi [5,6]. ACh se oslobađa na svim parasimpatikusnim i simpatikusnim preganglijskim nervnim vlaknima, na svim postgangliskim parasimpatikusnim vlaknima kao i na holinergičkim simpatikusnim nervnim vlakanima koja inervišu znojne žlijezde, mišiće dlake i krvne sudove kože [7]. U perifernom nervnom sistemu, oslobađa se i depolariše nervno-mišićnu ploču [8]. Sekrecijom ACh u različitim dijelovima mozga, kao što su prugasto tijelo (*Corpus striatim*), Majnertovo bazalno jedro (*Nucleus basalis Meynerti*), velike piramidne ćelije motorne kore i moždano stablo regulišu se brojni procesi kao npr. spavanje, pamćenje, moždana aktivnost [9].

Istorijat otkrića ACh počeo je početkom 20. vijeka, prvenstveno kroz pionirske radove Henry Hallett Dalea i Otta Loewija, koji su za svoje doprinose zajedno dobili Nobelovu nagradu za medicinu i fiziologiju 1936. godine [10]. Daleova istraživanja su osvijetlila prisustvo ovog jedinjena u životinjskim tkivima, dok su Loewijevi eksperimenti u srcima žaba pokazali njegovu ulogu kao hemijskog posrednika [11,12]. Ovo otkriće ne samo da je potvrdilo postojanje neurohemijske transmisije, već je i postavilo temelj za dalja istraživanja neurotransmitera. ACh je bio prvootkriveni neurotransmiter, kada su za njega otkrivene i dvije grupe receptora: muskarinski i nikotinski [13]. Imena su dobili po supstancama koje ih aktivirajaju: po alkaloidu gljive iz roda Musca (*Amanita muscaria*): muskarinu i po alkaloidu nikotinu iz duhana. Za razliku od većine malih molekula, dejstvo ACh na postsinaptičkom holinergičkom receptoru ne prestaje ponovnim

preuzimanjem (eng. *reuptake*) ACh, već pomoću enzima acetilholinesteraze (AChE) koji se nalazi u sinaptičkoj pukotini [14–16].

1.2. Holinergički receptori

1.2.1. Muskarinski receptori

Muskarinski receptori pripadaju tipu receptora vezanih za G protein koji se vezuje za ACh kao svoj primarni endogeni ligand. Imaju karakterističnu strukturu sastavljenu od sedam transmembranskih spirala (TM1-TM7) koje obuhvataju ćelijsku membranu, sa ekstracelularnim N-terminusom i intracelularnim C-terminusom [17]. Ekstracelularni N-terminus sadrži mjesto glikozilacije koje pomaže u savijanju i stabilnosti receptora. Takođe uključuje domen koji vezuje ligand odgovoran za vezivanje ACh i drugih liganada. TM spirale prelaze ćelijsku membranu i označene su od TM1 do TM7 [18]. Ove spirale su povezane intra- i ekstracelularnim petljama, formirajući snop koji obuhvata lipidni dvosloj. Džep za vezivanje liganda nalazi se unutar transmembranskog regiona i formiran je od ostataka iz nekoliko TM spirala [19]. Ovaj džep je mjesto gde se ACh ili drugi ligandi vezuju i aktiviraju receptor. Intracelularni C-terminus igra ulogu u povezivanju receptora sa intracelularnim signalnim putevima kroz interakcije sa G proteinima i drugim signalnim molekulima. Muskarinski receptori mogu usvojiti različite konformacije nakon vezivanja liganda, što dovodi do aktivacije nizvodnih signalnih puteva. Ova aktivacija uključuje vezivanje G proteina za intracelularni dio receptora, inicirajući kaskadu događaja koji moduliraju ćelijske odgovore [20]. Iako je ukupna struktura muskarinskih receptora očuvana među različitim podtipovima (M1-M5), postoje varijacije u specifičnim ostacima aminokiselina unutar transmembranskog regiona i drugih regiona receptora, koji doprinose selektivnosti i funkcionalnim razlikama među podtipovima [21]. Postoji pet podtipova muskarinskih receptora, označenih od M1 do M5, koji su raspoređeni po cijelom tijelu i igraju različite uloge u fiziološkim procesima.

M1 receptori se primarno nalaze u centralnom nervnom sistemu (CNS), posebno u moždanoj kori i hipokampusu. Aktivacija M1 receptora poboljšava kognitivne funkcije kao što su učenje i pamćenje. Takođe su uključeni u regulisanje lučenja želudačne kiseline i modulaciju kontrakcije glatkih mišića u gastrointestinalnom traktu [22].

M2 receptori se nalaze u srcu, glatkim mišićima i presinaptičkim nervnim završecima. U srcu, M2 receptori posreduju u negativnim hronotropnim (usporavanje srčanog ritma) i negativnim inotropnim (smanjenje kontraktilnosti) efektima parasimpatikusnog nervnog sistema. Oni takođe regulišu oslobađanje neurotransmitera tako što inhibišu oslobađanje drugih neurotransmitera, kao što je noradrenalin [23]. M2 receptori se nalaze u presinaptičkim završecima i regulišu oslobađanje neurotransmitera tako što inhibišu aktivnost adenilat ciklaze i moduliraju kalijumove kanale.

M3 receptori su široko rasprostranjeni u glatkim mišićima i tkivima žlijezda, uključujući pluća, gastrointestinalni trakt, mokraćnu bešiku i pljuvačne žlijezde. Aktivacija M3 receptora dovodi do kontrakcije glatkih mišića, povećanog lučenja žlijezda i proizvodnje sluzi. U kardiovaskularnom sistemu, M3 receptori doprinose vazodilataciji koja zavisi od endotela [24].

M4 receptori se prvenstveno nalaze u CNS-u, posebno u oblastima uključenim u kontrolu motorne i kognitivne funkcije. Oni regulišu oslobađanje nekoliko neurotransmitera, uključujući dopamin, glutamat i gama-aminobuternu kiselinu (GABA). M4 receptori mogu igrati ulogu u patofiziologiji shizofrenije i drugih neuropsihijatrijskih poremećaja [25].

M5 receptori se takođe pretežno nalaze u CNS-u, posebno u limbičkom sistemu i bazalnim ganglijima. Oni su uključeni u modulaciju dopaminergičke neurotransmisije i mogu igrati ulogu u nagradi, motivaciji i zavisnosti. M5 receptori se takođe nalaze u nekim perifernim tkivima, kao što su srce i ćelije Langerhansovih ostrvaca pankreasa [26].

1.2.2. Nikotinski receptori

Nikotinski receptori su tip jonotropnog receptora koji su široko rasprostranjeni po cijelom tijelu i igraju bitnu ulogu u sinaptikusnoj transmisiji i neuronskoj signalizaciji [27]. Nikotinski receptori su pentamerni proteini sastavljeni od pet podjedinica raspoređenih oko centralne pore. Svaka podjedinica se sastoji od četiri transmembranske spirale (TM1-TM4), pri čemu je N-terminus lociran ekstracelularno, a C-terminus intracelularno. Postoji nekoliko podtipova podjedinica nikotinskih receptora (a1-a10 i b1-b4 kod ljudi), a različite kombinacije podjedinica mogu formirati različite podtipove receptora sa različitim farmakološkim svojstvima i funkcionalnim karakteristikama. Nikotinski receptori su klasifikovani u dva glavna tipa na osnovu njihovog sastava podjedinica i farmakoloških svojstava: mišićni i neuronski tip.

Nikotinski receptori mišićnog tipa se prvenstveno nalaze na neuromuskularnoj sinapsi i posreduju u kontrakciji skeletnih mišica. Sastoje se od dvije a podjedinice i jedne b, g ili e podjedinice, zajedno sa jednom d ili e podjedinicom u fetalnom obliku [28]. Prototip nikotinskog receptora mišićnog tipa sastoji se od a1, b1, g i d podjedinica (a1b1gd). Na neuromuskularnoj sinapsi, nikotinski receptori mišićnog tipa prenose signale od motornih neurona do vlakana skeletnih mišića, pokrećući kontrakciju mišića.

Nikotinski receptori neuronskog tipa se uglavnom nalaze u CNS-u, autonomnim ganglijama i nekim perifernim neuronima. Oni igraju ključnu ulogu u sinaptičkom prenosu i modulaciji neuronske aktivnosti. Neuronski nikotinski receptori imaju različite sastave podjedinica, kao što su a2-a10 i b2-b4, koji se kombinuju u različite podtipove receptora sa različitim funkcionalnim svojstvima [29].

Kada se ACh ili drugi agonisti vežu za nikotinske receptore, on indukuje konformacijske promjene koje otvaraju jonski kanal u kompleksu receptora. Ovo omogućava protok katjona, prvenstveno natrijuma (Na^+) i kalijuma (K^+), kroz ćelijsku membranu, što dovodi do depolarizacije i ekscitacije postsinaptičke ćelije. Priliv jona kalcijuma (Ca^{2+}) kroz određene podtipove nikotinskih receptora takođe igra ulogu u različitim ćelijskim procesima [30].

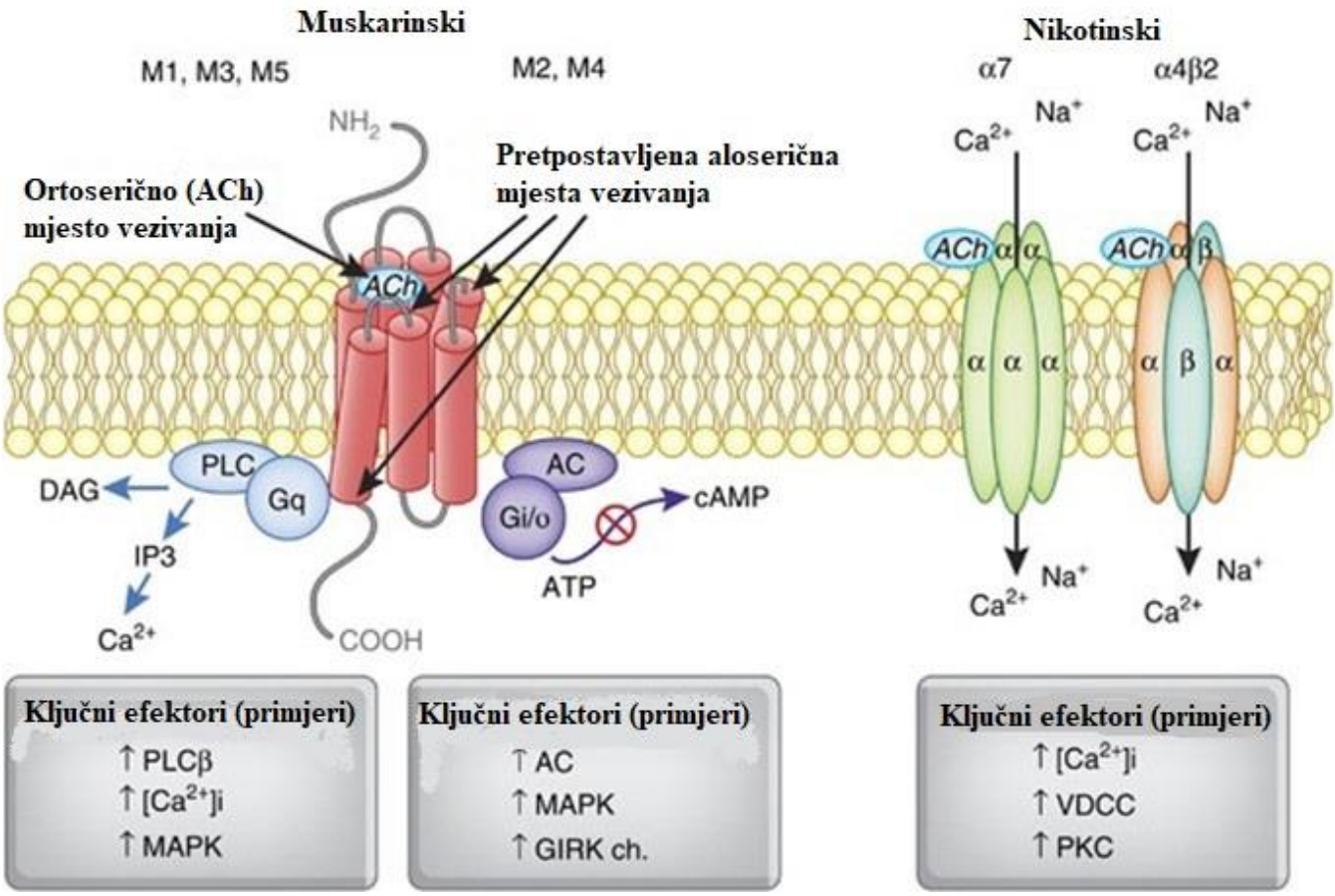
U CNS-u, neuronski nikotinski receptori moduliraju oslobađanje neurotransmitera, sinaptičku plastičnost i kognitivne funkcije kao što su učenje i pamćenje. Oni su uključeni u razvoj zavisnost od nikotina i ciljno su mjesto za terapijske intervencije kod neuroloških poremećaja kao što su Alzheimerova bolest, Parkinsonova bolest i shizofrenija. U autonomnim ganglijama, nikotinski receptori posreduju u brzoj ekscitatornoj sinaptičkoj transmisiji između preganglijskih i postganglijskih neurona simpatikusnog i parasimpatikusnog autonomnog nervnog sistema [31].

1.2.3. Holinergički receptori u mozgu

ACh olakšava formiranje memorije, konsolidaciju i pronalaženje. Holinergičke projekcije od bazalnog prednjeg mozga do hipokampusa i neokorteksa su posebno važne za procese pamćenja. ACh podstiče sinaptičku plastičnost, pospješuje jačanje sinaptičkih veza i formiranje novih, doprinoseći kodiranju i skladištenju sjećanja. Holinergičke projekcije od bazalnog prednjeg mozga do talamusa i korteksa moduliraju kortikalnu aktivnost i senzornu obradu [34]. Promjene u

nivou ACh mogu uticati na procese pažnje i sposobnost filtriranja i odabira relevantnih informacija. ACh je uključen u regulaciju ciklusa spavanja i buđenja [35]. Tokom spavanja, oslobađanje ACh je smanjeno, što omogućava konsolidaciju sna i stvaranje sporotalasnog sna [36]. ACh igra ulogu u kontroli motorne funkcije i koordinaciji. U bazalnim ganglijima, holinergički interneuroni moduliraju aktivnost dopaminergičkih neurona, utičući na motornu snagu i pokretanje pokreta.

ACh djeluje u ravnoteži sa drugim neurotransmiterskim sistemima, kao što su dopamin, serotonin i noradrenalin. U interakciji sa ovim sistemima reguliše raspoloženje, motivaciju i emocionalnu obradu. ACh ima neuroprotektivne efekte promovišući preživljavanje neurona, smanjujući zapaljenje i modulirajući oksidativni stres. Može igrati ulogu u zaštiti od neurodegenerativnih bolesti 33]. Degeneracija holinergičkih neurona u bazalnom prednjem mozgu je obilježje Alzheimerove bolesti. Smanjeni nivoi ACh doprinose kognitivnom padu i oštećenju pamćenja uočenim u ovom stanju. Holinergička disfunkcija kod Parkinsonove bolesti utiče na ravnotežu između dopamina i ACh u bazalnim ganglijama, što dovodi do motoričkih simptoma i kognitivnih oštećenja. Promjene u holinergičkoj signalizaciji primjećene su kod osoba sa shizofrenijom, što doprinosi kognitivnim deficitima i oštećenoj senzornoj obradi [34]. Kompleksnost uloga ACh u CNS-u dovodi do toga da se one često opisuju kao poseban entitet [35,36]. Holinergički receptori su prikazani na Slici 1.



Slika 1. Holinergički receptori (muskarinski i nikotinski)

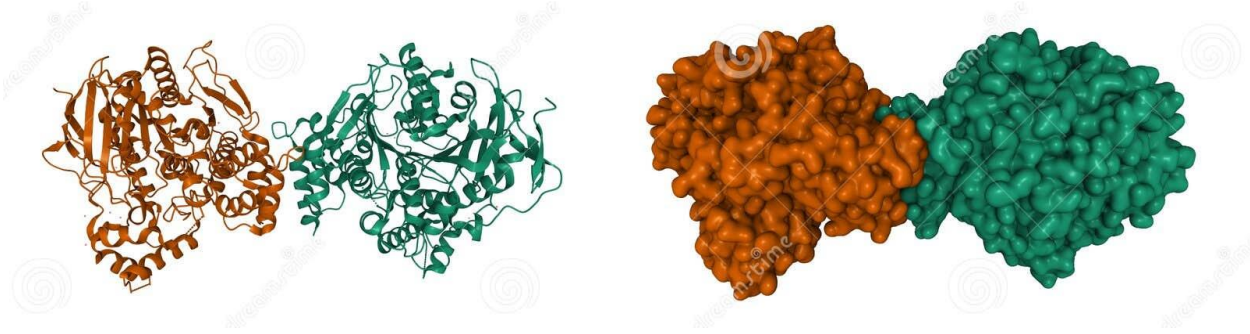
IP3: inozitol-3-fosfat, DAG: diacilglicerol; PLC: fosfolipaza; MAPK: mitogen-aktivirana protein kinaza; GIRK: kanal u unutrašnjosti povezan sa G-proteinom; PKC: protein-kinaza C; VDCC: voltažno-zavisni kalcijumski kanali; Slika je preuzeta i prilagođena od Jones i saradnici (2012) [37].

1.3. Esteraze

Holinestreraze su ubikvitarne u životinjskom svijetu. Od 1943. godine poznato je da postoje dva enzima sposobna za hidrolizu ACh: acetilholinesteraza (AChE) i butirilholinesteraza (BuChE).

1.3.1. Acetilholinesteraza

AChE je ključni enzim u holinergičkom sistemu, odgovoran za brzu hidrolizu ACh na neuromuskularnim sinapsama u CNS i perifernog nervnog sistema (PNS). Ova enzimska aktivnost je neophodna za prekid holinergičke signalizacije, čime se obezbjeđuje precizna neuronska komunikacija i sprečava kontinuirana stimulacija mišića i neurona [38]. AChE, odnosno acetilholin-acetilhidrolaza (EC 3.1.1.7) ima i trivijalni, sada već napušteni naziv prava-holinesteraza (Slika 2).



Slika 2. Struktura humane acetilholinesteraze

Slika je preuzeta sa www.dreamstime.com [39]

Otkriće AChE datira s početkom 20. vijeka kada je identifikovana kao enzim odgovoran za razlaganje ACh, čime je regulisano njegovo djelovanje u sinapsama. Ključni eksperimenti Sir Henry Dalea i njegovih kolega istakli su brzu deaktivaciju ACh, sugerišući postojanje specifične esteraze. Do 1930-ih, enzim je izolovan i okarakterisan, što je dovelo do dubljeg razumijevanja njegovog mehanizma i uloge u sinaptičkom prenosu [40].

AChE je enzim serinska-hidrolaza koji karakteriše visoko očuvana trijada aktivnog mjesta koja se sastoji od ostataka serina, histidina i glutamata. Ovo aktivno mjesto je strateški locirano u dubokoj klisuri na bazi enzima, gde se hidrolizuju molekuli ACh. AChE postoji u više molekularnih oblika, u rasponu od monomera do tetramera i može biti vezana za membranu ili rastvorljiva, odražavajući njene raznovrsne funkcionalne kapacitete u različitim tkivima.

Trodimenzionalna struktura enzima, razjašnjena kristalografijom, otkriva složen aranžman koji olakšava visoku katalitičku efikasnost enzima i specifičnost za ACh [41].

Primarna funkcija AChE je da katalizuje hidrolizu ACh u sirćetnu kiselinu i holin. Ova reakcija se odvija izuzetnom brzinom. Mehanizam uključuje proces u dva koraka gde se acetil grupa ACh prenosi na serinsku hidroksilnu grupu AChE, formirajući intermedijer acetil-enzim. Ovaj intermedijer se zatim hidrolizuje, oslobađajući holin i regenerišući aktivni enzim. Ovaj brzi obrt je od suštinskog značaja za sprečavanje produžene aktivacije ACh receptora, što bi inače moglo dovesti do kontinuirane stimulacije i rezultirajućeg zamora ili desenzibilizacije neurona i mišića [42].

AChE igra vitalnu ulogu u nervnom sistemu tako što obezbjeđuje preciznu regulaciju holinergičke signalizacije. Na neuromuskularnoj sinapsi, aktivnost AChE prekida mišićne kontrakcije, omogućavajući opuštanje mišića, sprečava hiperaktivnost ili grčeve. U CNS-u, enzim reguliše sinaptičku plastičnost, utičući na procese učenja i pamćenja kontrolišući holinergični ton. Štaviše, AChE je uključena u neklasične uloge, kao što je modulacija ćelijske athezije i neuronskog razvoja, što sugeriše uključenost enzima u šire fiziološke kontekste [43].

Disregulacija aktivnosti AChE povezana je sa različitim neurološkim poremećajima, prije svega Alchajmerovom bolešću. Kod Alchajmerove bolesti, smanjeni nivoi ACh u mozgu su usko povezani sa kognitivnim deficitima, što dovodi do upotrebe AChE inhibitora kao terapijske strategije za poboljšanje holinergičkog prijenosa. Lijekovi kao što su donepezil, rivastigmin i galantamin, koji inhibišu aktivnost AChE, široko se koriste za liječenje simptoma demencije [44]. Nasuprot tome, prekomjerna inhibicija AChE, kao što se vidi kod trovanja organofosfatnim jedinjenjima (OP), dovodi do toksične akumulacije ACh, izazivajući holinergičku krizu.

1.3.2. Butirilholinesteraza

Butirilholinesteraza (BuChE), ranije poznata kao pseudoholinesteraza, zvaničnog naziva acetilhlin acil-hidrolaza (EC 3.1.1.8), dijeli funkcionalne sličnosti sa AChE, ali pokazuje različite biološke uloge i specifičnosti supstrata. Iako je istorijski zasjenčen AChE, BuChE je zaslužila naučni interes zbog svog učešća u metabolizmu lijekova, potencijalne terapijske primjene i sve veće uloge u neurobiologiji [45].

Enzim BuChE je prvi put identifikovan kao različit od AChE sredinom 20. vijeka tokom studija koje su imale za cilj razumijevanje holinergičkog prenosa i metabolizma u tijelu. U početku se smatralo da je od manjeg fiziološkog značaja. Dok su istraživači izolovali i karakterisali BuChE u ljudskim i životinjskim tkivima, njegovi izraziti obrasci ekspresije i aktivnosti istakli su složenu interakciju unutar holinergičkog sistema [46,47].

BuChE je član porodice serinskih-hidrolaza i sastoji se od jednog polipeptidnog lanca koji se savija u trodimenzionalnu strukturu karakterističnu za holinesteraze. Enzim dijeli katalitički mehanizam koji uključuje trijadu aminokiselina - serin, histidin i glutamat - koji olakšavaju hidrolizu estarskih veza. Za razliku od AChE, BuChE pokazuje manju specifičnost za supstrate, pripisanu većoj i fleksibilnijoj klisuri aktivnog mjesta, omogućavajući mu interakciju sa širim spektrom jedinjenja koja sadrže estre.

BuChE je široko rasprostranjen enzim u ljudskim tkivima, uključujući plazmu, jetru i CNS, gde obavlja niz bioloških funkcija. Iako je njegova precizna fiziološka uloga i dalje nedovoljno shvaćena, poznato je da djeluje kao biološki čistač, hidrolizujući toksine i jedinjenja lijekova, čime doprinosi procesima detoksikacije [48,49]. U nervnom sistemu, BuChE modulira holinergičku signalizaciju dopunjujući aktivnost AChE u razgradnji ACh, iako njegova specifičnost za butirilholin i druge estre sugeriše dodatne uloge u metabolizmu lipida i regulaciji neurotransmitera [48].

BuChE metaboliše nekoliko lijekova koji sadrže estar, uključujući sukcinilholin, mišićni relaksant koji se koristi u anesteziji i koji zahtijeva precizno doziranje kako bi se izbegle komplikacije koje nastaju usljed produžene neuromuskularne blokade kod osoba sa nedostatkom BuChE [50]. Genetske varijacije u BuChE genu mogu dovesti do atipične aktivnosti enzima, utičući na efikasnost lijeka i profile toksičnosti, što zahtijeva personalizovane terapijske pristupe za optimizaciju ishoda liječenja [51].

BuChE ima terapijski potencijal kao biološki čistač za detoksikaciju nervnih agenasa, obezbeđujući pufer protiv izlaganja OP jedinjenjima razlaganjem toksičnih supstanci prije nego što one inhibišu AChE. Pored toga, inhibitori BuChE su istraženi kao terapijski agensi za neurološke poremećaje, kao što je Alzeimerova bolest, gde poboljšanje holinergičke funkcije može pomoći u ublažavanju kognitivnog pada. Istraživanje o učešću BuChE-a u metaboličkim

sindromima i njegovoj sposobnosti da modulira profile lipida sugeriše šire implikacije na kardiovaskularno i metaboličko zdravlje.

Aktivnost i ekspresija BuChE podliježu genetskoj varijabilnosti, sa nekoliko polimorfizama identifikovanih u BuChE genu koji utiču na funkciju i stabilnost enzima. Ove genetske razlike mogu značajno uticati na osjetljivost pojedinca na neželjene efekte lijekova, posebno na one koji se odnose na anestetičke agense i puteve detoksikacije. Etničke i populacione studije su pokazale varijacije u BuChE aktivnosti u različitim grupama, informišući o potrebi za prilagođenim medicinskim intervencijama i farmakogenetskim uvidima radi optimizacije kliničke njege [52].

1.3.3. Karboksilesteraze

Karboksilesteraze (CarbE) (EC 3.1.1.1) su raznolika grupa enzima unutar porodice esteraza, koji igraju ključnu ulogu u hidrolizi estarskih i amidnih veza u različitim supstratima. Ovi enzimi su ključni u metabolizmu lijekova, procesima detoksikacije i modulaciji puteva transdukcije signala. CarbE se sveprisutno eksprimiraju u živim organizmima, pretežno locirani u jetri, tankom crijevu i drugim tkivima uključenim u detoksikaciju [53].

Istorija istraživanja CarbE seže do ranih studija enzimski posredovanih reakcija hidrolize. Prvobitno identifikovani kroz njihovu sposobnost da katalizuju hidrolizu jednostavnih estara, CarbE su izolovani iz ekstrakata jetre, otkrivajući njihovo obilje i varijacije među vrstama. Tokom decenija, napredak u tehnikama prečišćavanja i molekularnoj biologiji omogućio je izolaciju i karakterizaciju više izoformi CarbE, što je dovelo do otkrića njihove široke distribucije i vitalne biološke uloge [53].

CarbE su tipično globularni proteini koje karakteriše očuvani a/b-hidrolazni nabor, koji uključuje katalitičku trijadu sastavljenu od serina, histidina i glutamata ili aspartata. Ova trijada olakšava nukleofilni napad na estarske veze, što dovodi do njihove hidrolize. Strukturne nijanse CarbE igraju ključnu ulogu u specifičnosti za supstrate i katalitičkoj efikasnosti, sa različitim izoformama koje pokazuju različite aktivnosti prema endogenim i ksenobiotičkim jedinjenjima [53]. Strukturne studije su otkrile da suptilne razlike u arhitekturi aktivnog mjesta među

izoformama CarbE određuju njihove jedinstvene funkcionalne atribute, pružajući uvid u interakcije enzima i supstrata.

CarbE služi višestrukim fiziološkim funkcijama, posebno u metabolizmu lipida i detoksikaciji ksenobiotičkih supstanci [54]. Hidrolizom estarskih i amidnih veza, CarbE modulira bioraspoloživost i aktivnost signalnih molekula izvedenih iz lipida, utičući na procese kao što su zapaljenje i ćelijska proliferacija. U gastrointestinalnom traktu i jetri, CarbE detoksikuje unesene otrove i lijekove, igrajući suštinsku ulogu u zaštiti organizma od štetnih jedinjenja. Štaviše, CarbE učestvuje u metabolizmu estara holesterola i triglicerida, naglašavajući njihov značaj u održavanju homeostaze lipida i energetskog balansa.

Farmakološki značaj CarbE se uglavnom pripisuje njihovoj ulozi u metabolizmu lijekova. Mnogi terapijski agensi, uključujući prolijekove i lijekove na bazi estra, aktiviraju se ili deaktiviraju hidrolizom posredovanom CarbE [54]. Ova enzimska aktivnost utiče na farmakokinetiku, efikasnost i toksičnost različitih lijekova, naglašavajući značaj CarbE u razvoju lijekova i personalizovanoj medicini. Na primjer, CarbE pretvara irinotekan u njegov aktivni metabolit SN-38, što objašnjava njegove citotoksične efekte [55]. Razumijevanje aktivnosti CarbE je ključno u predviđanju interakcija lijekova i optimizaciji terapijskih režima.

Disfunkcija ili abnormalna ekspresija CarbE može doprinijeti patofiziologiji određenih bolesti. Izmijenjena aktivnost CarbE je umiješana u metaboličke poremećaje, kao što su gojaznost i masna bolest jetre, gdje poremećaj metabolizma lipida dovodi do progresije bolesti. Pored toga, genetski polimorfizmi u CarbE genima mogu uticati na individualnu osjetljivost na toksičnost lijekova ili terapijski neuspjeh, posebno u tretmanima koji uključuju lijekove na bazi estra. Istraživanja CarbE kao biomarkera za bolest ili mete za terapijsku intervenciju su u toku, sa potencijalom da poboljšaju dijagnozu i strategije liječenja za različita stanja [56].

Količina CarbE varira među različitim vrstama sisara, pod uticajem genetskih, fizioloških faktora i faktora sredine. Različita toksičnost OP jedinjenja za različite životinjske vrste je u značajnoj mjeri uslovljena različitom koncentracijom CarbE u njihovim tkivima [57].

1.4. Inhibitori AChE

1.4.1. Reverzibilni AChE inhibitori: karbamatna jedinjenja

Inhibitori AChE su najvažnija klasa jedinjenja koja se koriste za pojačanje holinergičke transmisije. Među ovim inhibitorima, karbamati su posebno značajni zbog njihovog reverzibilnog i relativno selektivnog mehanizma djelovanja. Karbamati imaju široku primjenu, u rasponu od terapijske upotrebe kod kognitivnih poremećaja do upotrebe kao pesticida u poljoprivredi [58].

Razvoj karbamata počinje sredinom 20. vijeka kada je postala očigledna potreba za boljom kontrolom poljoprivredni štetočina i poboljšanjem holinergičke funkcije u različitim medicinskim stanjima [59]. Otkriće fizostigmina, alkaloida dobijenog iz kalabarskog pasulja, pružilo je početni uvid u potencijalnu terapijsku primjenu karbamata [60]. Kasniji sintetički napori doveli su do stvaranja raznolikog niza derivata karbamata, od kojih svaki pokazuje jedinstven inhibitorni potencijal i specifičnost prema AChE. Kao rezultat toga, karbamati su postali sastavni dio istraživanja i u medicini i u poljoprivredi [61].

Karbamate karakteriše prisustvo karbamatne funkcionalne grupe (–NHCOO–) povezane sa različitim supstituentima koji utiču na njihova fizičko-hemijska svojstva i biološku aktivnost [62]. Ova strukturna karakteristika olakšava formiranje reverzibilne kovalentne veze između karbamata i serinskog ostatka u aktivnom mjestu AChE [63]. Varijabilnost strukture karbamata omogućava modulaciju njihovih farmakokinetičkih svojstava, optimizujući ravnotežu između efikasnosti i sigurnosti. Fino podešavanje ovih strukturnih elemenata igra vitalnu ulogu u selektivnoj inhibiciji AChE, čime se minimiziraju neželjeni efekti.

Karbamati funkcionišu tako što reverzibilno karbamiliraju serin aktivnog mjesta AChE, stvarajući privremeno stabilan kompleks enzim-inhibitor [64]. Ovaj proces sprečava razgradnju ACh unutar sinaptičke pukotine, što dovodi do produžene aktivacije holinergičkih receptora. Za razliku od ireverzibilnih inhibitora, karbamati omogućavaju regeneraciju aktivne AChE pošto karbamilovani enzim prolazi kroz spontanu hidrolizu, obnavljajući aktivnost enzima tokom vremena. Ova reverzibilna inhibicija je korisna u kliničkim uslovima gdje je poželjno privremeno poboljšanje holinergičke funkcije bez trajne inaktivacije enzima [65].

Karbamati imaju značajnu primjenu u liječenju neuroloških poremećaja kao što su Alzeimerova bolest, mijastenija gravis i glaukom [66]. Na primjer, rivastigmin i neostigmin se

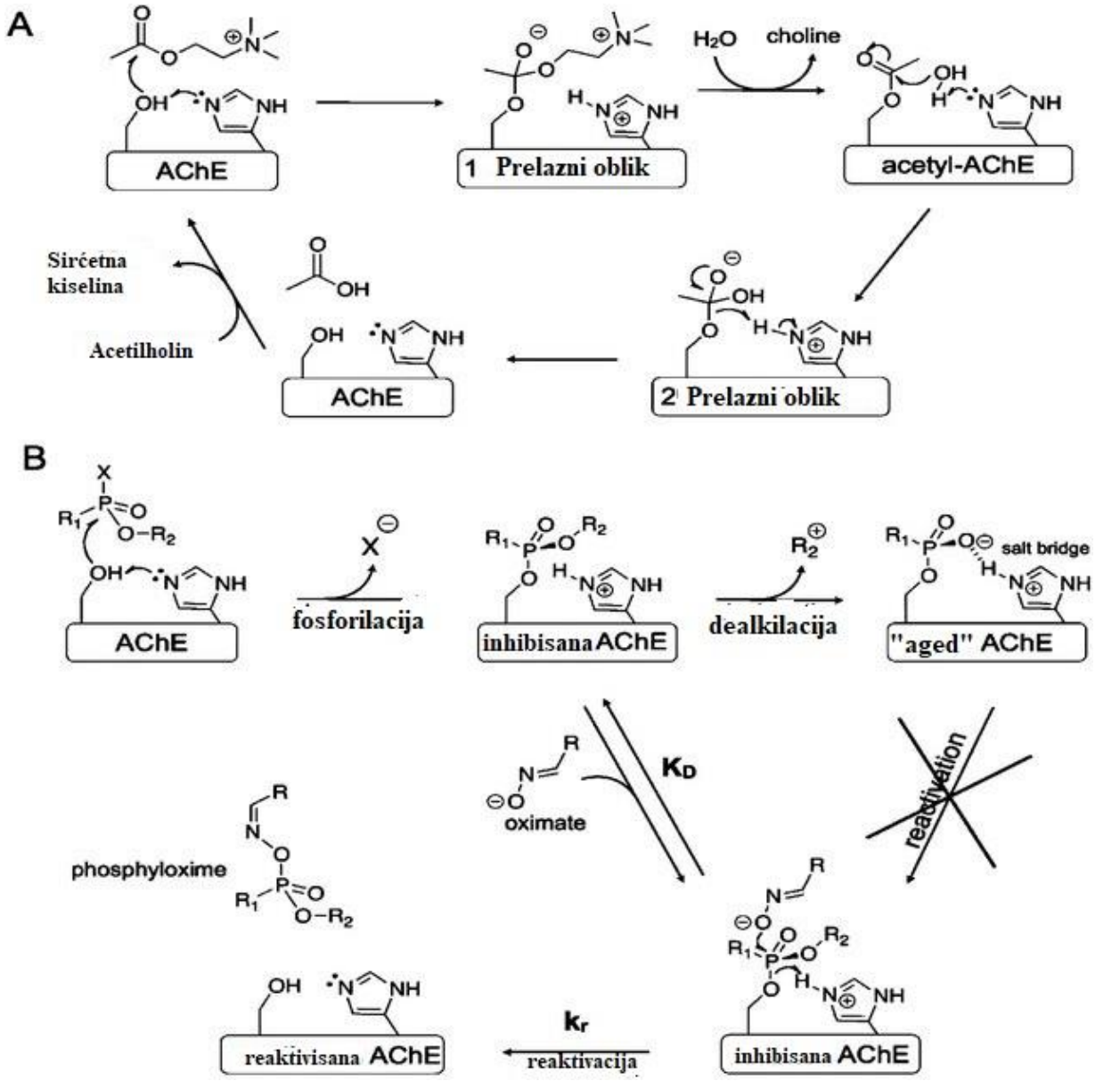
klinički koriste za liječenje ovih stanja povećanjem holinergičkog prenosa i ublažavanjem simptoma povezanih sa holinergičkim deficitom [67].

Pored medicinske upotrebe, karbamati se u velikoj mjeri koriste kao pesticidi u poljoprivredi. Jedinjenja kao što su karbaril i aldikarb se koriste za kontrolu populacija insekata tako što remete funkciju njihovog nervnog sistema putem AChE inhibicije. Ovi pesticidi, zbog svoje reverzibilne prirode, predstavljaju manje dugoročnu opasnost po životnu sredinu u poređenju sa sličnim OP jedinjenjima. Bez obzira na to, upotreba karbamata zahtijeva pažljivo upravljanje kako bi se spriječila potencijalna toksičnost za neciljne vrste, uključujući ljude i korisne insekte [68]. Dok reverzibilna priroda inhibitora karbamata generalno smanjuje rizik od produžene inhibicije AChE, akutna izloženost ovim jedinjenjima može dovesti do simptoma akutne holinergičke krize kao i kod OP jedinjenja.

1.4.2. Ireverzibilni inhibitori AChE: organofosfatna jedinjenja

OP su klasa hemijskih jedinjenja koja su široko poznata po svojoj ulozi ireverzibilnih inhibitora AChE. Porijeklo OP datira još od ranog 20. vijeka, sa pionirskim istraživanjima jedinjenja koja sadrže fosfor [69]. Sinteza i primjena ovih supstanci dobija na zamahu kada su naučnici otkrili njihove moćne biološke efekte, posebno inhibiciju enzima AChE. Tokom Drugog svjetskog rata, OP su se proizvodili u vojne svrhe kao nervni bojni otrovi. Poslije rata, fokus je prebačen na njihov razvoj kao pesticida, značajno povećavajući poljoprivrednu produktivnost, ali i izazivajući zabrinutost u pogledu izloženosti ljudi i životne sredine [70].

OP jedinjenje se karakteriše prisustvom atoma fosfora vezanog za kiseonik i druge alkil ili aril grupe, formirajući OP estre. Ova molekularna struktura olakšava formiranje kovalentne veze sa aktivnim mjestom serina AChE, što dovodi do nepovratne inaktivacije enzima. Proces "starenja" (eng. *ageing*), u kome fosforilisani enzim prolazi kroz dalje hemijske promjene dealkilacije te postaje otporan na reaktivaciju, komplikuje liječenje nakon izlaganja OP (Slika 3).



Slika 3. Inhibicija acetilholinesteraze (AChE) organofosfatnim (OP) jedinjenjem i put kompleksa AChE-OP

Pravovremeno otkrivanje izloženosti OP je od ključne važnosti za ublažavanje njegovih efekata. Dijagnostički pristupi obično uključuju mjerenje aktivnosti AChE u uzorcima krvi, gdje značajno smanjena aktivnost enzima ukazuje na ekspoziciju [71]. Napredak u analitičkoj tehnologiji, kao što su hromatografija i masena spektrometrija, poboljšao je osjetljivost i

specifičnost detekcije OP u biološkim uzorcima i uzorcima životne sredine, pomažući u procjeni izloženosti i epidemiološkim studijama.

Visoko reaktivna priroda fosfora omogućava modifikacije koje mogu prilagoditi OP za specifične namjene, u rasponu od insekticida do hemijskog oružja. Ove strukturne adaptacije utiču na isparljivost, stabilnost i hidrofobnost jedinjenja, svojstva kritična za njihovu funkciju i postojanost u različitim sredinama. S obzirom na široku upotrebu i potencijalne opasnosti OP-a, uspostavljeni su regulatorni okviri za kontrolu upotrebe OP. Agencije kao što su Agencija za zaštitu životne sredine (eng. *Environment Protection Agency*, EPA) i Svjetska zdravstvena organizacija (SZO) daju smjernice za bezbjednu upotrebu, rukovanje i odlaganje OP u cilju zaštite zdravlja ljudi i životne sredine [72,73]. Regulatorne mjere, uključujući ograničenja dozvoljenih nivoa izloženosti i ograničenja za određene OP, imaju za cilj da uravnoteže njihove poljoprivredne koristi sa potrebom da se minimiziraju zdravstveni rizici.

1.4.2.1. Organofosfatni nervni bojni otrovi

OP nervni agensi predstavljaju mračni vrhunac u tehnologiji hemijskog ratovanja, koji se odlikuju snažnom inhibicijom AChE i njihovim posljedičnim, katastrofalnim efektima na biološke sisteme. Ova jedinjenja, razvijena i naoružana prvenstveno tokom 20. vijeka, spadaju među najsmrtonosnije hemijske agense poznate čovječanstvu [74].

Razvoj OP nervnih agenasa započeo je ranih 1930-ih, predvođen radom dr Gerharda Schradera, hemičara koji je radio za njemačku kompaniju *IG Farbenindustrie*. Njegovo istraživanje koje je prvobitno imalo za cilj stvaranje efikasnijih pesticida nenamjerno je dovelo do sinteze tabuna (GA), prvog nervnog agensa, 1936 (G – od eng. *German*) [75]. Ovo otkriće je ubrzo praćeno razvojem drugih agenasa, uključujući sarin (GB) 1938 i soman (GD) 1944, svaki smrtonosniji od prethodnog [76]. Od trenutka kada je utvrđena toksičnost ovih jedinjenja, nervni bojni otrovi su prijetili da postanu najpodlija sredstva za masovno uništenje. Tokom Drugog svjetskog rata, ovi agensi su bili nagomilavani, iako nisu bili korišteni u borbi. Isto važi i za drugu potklasu nervnih bojnih otrova (V-agensi; V za „venomous“, otrovan) koje su Britanci sintetizovali 1950-ih. Nakon rata i SAD i Sovjetski Savez istraživali su vojni potencijal ovih agenasa tokom Hladnog rata, dodatno pojačavajući njihovu ulogu u globalnoj vojnoj strategiji i međunarodnoj diplomatiji. Najpoznatiji među njima je VX koji je postao dio američkog naoružanja. Vodeće vojne

sile nagomilale su 1950-ih i 1960-ih znatan arsenal raznih nervnih bojnih otrova. Kasnije, 1993. ih je zabranila Konvencija o hemijskom oružju (eng. *Chemical Weapons Convention,* CWC), koja je stupila na snagu 1997.

Prva dokumentovana upotreba nervnih bojnih otrova bila je u Iračko-iranskom ratu (1980-1988), kada je dokazana upotreba tabuna i sarina od strane Iračana [77]. Upotreba sarina se spominje u sirijskom sukobu 2013. i 2017. godine [78]. Iako svaka upotreba nervnih bojnih otrova predstavlja čin kršenja CWC i automatski predstavlja krivično djelo protiv čovječnosti, ono što je problemu dalo novu dimenziju jeste njihova zloupotrebu od strane nevladinih subjekata u terorističke svrhe.

Japanski religiozni kult AUM Shinrikyo je koristio sarin dva puta za masovnu intoksikaciju, u Macumotu juna 1994. i u tokijskom metrou u martu 1995, sa 1050 otrovanih i 14 letalnih intoksikacija [79,80]. VX su koristili u decembru 1994. i januaru 1995. za ubistvo pojedinaca – bivših pripadnika AUM i anti-AUM aktivista, sa troje otrovanih i jednim smrtnim ishodom [81]. U februaru 2017. godine u Maleziji, na međunarodnom aerodromu u Kuala Lumpuru, korišten je perkutano VX za atentat Kim-Jong Nama, polubrata predsjednika Sjeverne Koreje [82].

Član posebne, najotrovnije, a najmanje javno poznate podklase ruskih nervnih agensasa koji se zovu A-agensi ili Novichok („pridošlica“ na ruskom) [83], navodno je korišten u trovanju u Salisburyju u Velikoj Britaniji u martu 2018, kada su ruski dvostruki agent Sergey Skripal i njegova ćerka Yulia Skripal bili otrovani, zajedno sa policajcem koji istraživao slučaj [84]. Novichok agent A234 je bio pronađen u rezidenciji Skripal u bočici parfema. Nekoliko mjeseci kasnije, dvije osobe su se otrovale, od kojih je jedna preminula, sa identičnom kliničkom slikom nakon što su se poprskali iz bočice pronađene bačene u parku u Amesburyju, nekih 13 km od Salisburyja [85].

Potentnost i postojanost OP nervnih agenasa usko su vezani za njihovu hemijsku strukturu. Ova jedinjenja dijele centralni atom fosfora vezan za različite supstituente koji daju njihovu reaktivnost i biološku aktivnost. Iako se često nazivaju „nervni gasovi“ oni nisu gasovi već tečnosti. Na primjer, sarin je lako isparljiva tečnost koju karakteriše visok pritisak pare što olakšava izlaganje udisanjem. Suprotno tome, VX posjeduje viskozniji i uljniji sastav, čineći ga manje isparljivim, ali postojanijim u okolini, što omogućava produženo lokalno djelovanje [74].

Čak i niska izloženost nervnim bojnim otrovima može imati ozbiljne i trenutne zdravstvene posljedice zbog njihove brze repsorpcije kroz kožu, respiratorne i gastrointestinalne puteve [86]. Akutni simptomi uključuju konvulzije, paralizu i gušenje, koji mogu biti fatalni ako se ne liječe. Preživjeli od akutnog trovanja mogu doživjeti dugoročne neurološke posljedice, kao što su uporna kognitivna disfunkcija i psihijatrijski poremećaji, što naglašava potrebu za sveobuhvatnom njegom nakon izlaganja. Ovi efekti na zdravlje naglašavaju kritičnu važnost lične zaštitne opreme (PPE), brze dekontaminacije i medicinskih protivmjera kao odgovora na incidente sa nervnim agensima. Lična zaštitna oprema, uključujući gas maske i specijalizovana odjela, čini prvu liniju odbrane od izlaganja nervnim agensima, omogućavajući bezbjedne operacije u kontaminiranim područjima. Ove zaštitne mjere se stalno usavršavaju kako bi se suočile sa prijetnjama koje se razvijaju i poboljšale operativnu spremnost [87].

1.4.2.2. Organofosfatni pesticidi

OP pesticidi su kritična grupa sintetičkih hemikalija koje se u velikoj mjeri koriste u poljoprivredi za zaštitu usjeva od štetočina, čime se povećava poljoprivredna produktivnost i globalno snabdijevanje hranom. Neki od najranijih i najčešće korištenih OP pesticida uključuju paration, malation i hlorpirifos [88], koji su postali sastavni dio posljeratne poljoprivredne prakse. Tokom vremena, OP pesticidi su u velikoj mjeri zamijenili organohlorne pesticide, kao što je dihloro-difenil-trihloroetan (DDT), koji su se suočili sa negativnim posljedicama životne sredine zbog njihove postojanosti i bioakumulacije [89].

OP pesticidi su hemijski raznovrsni, sa centralnim atomom fosfora vezanima za kiseonik, sumpor i organske supstituente, koji definišu njihovo hemijsko ponašanje i toksičnost. Ova jedinjenja su tipično estri, amidi ili tiolati, što im omogućava efikasnu interakciju sa biološkim sistemima. Njihova volatilnost, rastvorljivost i stabilnost u različitim sredinama životne sredine utiču na njihovu distribuciju i postojanost, utičući na način na koji komuniciraju sa neciljanim organizmima, uključujući ljude [90]. Specifične hemijske modifikacije pojedinačnih OP pesticida su dizajnirane da ciljaju specifične štetočine, dok istovremeno imaju za cilj da minimiziraju neželjene efekte (npr. malation).

Inhibicija AChE u ciljanim štetočinama ometa normalno širenje nervnih impulsa uzrokujući paralizu i na kraju smrt. Visoka specifičnost i moć ovog mehanizma čine OP pesticide

visoko efikasnim, ali takođe izaziva značajnu zabrinutost u pogledu bezbijednosti zbog njihovog potencijalnog uticaja na neciljne vrste [91].

OP pesticidi igraju vitalnu ulogu u modernoj poljoprivredi, koriste se za kontrolu širokog spektra insekata koji ugrožavaju usjeve, uključujući voće, povrće i žitarice [92]. Osiguravajući zaštitu usjeva, OP pesticidi pomažu u maksimiziranju prinosa i obezbeđivanju snabdijevanja hranom neophodnom za održavanje rastuće globalne populacije. Poljoprivrednici favorizuju ova jedinjenja zbog njihove efikasnosti, ekonomičnosti i širokog spektra štetočina na koje ciljaju, a sa kojima se tradicionalne biološke metode ne mogu lako izboriti [93]. Međutim, njihova upotreba zahtijeva pažljivo upravljanje kako bi se spriječili negativni efekti na korisne insekte, kao što su oprašivači, koji doprinose stabilnosti ekosistema. EPA i SZO definisale su granice izloženosti, bezbjednosne protokole i sisteme za praćenje kako bi regulisali upotrebu OP. Nedavni trendovi naglašavaju smanjenje oslanjanja na hemijske pesticide, promovišući alternative kao što su biopesticidi i uticaj na genetsku otpornost štetočina [72].

1.4.2.2.1. Paraokson

Paraokson je OP jedinjenje poznato kao aktivni metabolit parationa, pesticida široko korištenog u poljoprivredi [94,95]. Paraokson spada među najotrovnija jedinjenja koja utiču na nervni sistem, sa implikacijama koje se protežu na toksikologiju, farmakologiju i nauku o životnoj sredini [95].

Paraokson se sastoji od atoma fosfora vezanog za atom kiseonika, tiol grupe iz njegovog matičnog jedinjenja parationa i raznih bočnih organskih lanaca [96]. Ova struktura daje visoku reaktivnost, posebno prema hidroksilnim grupama serina u enzimima kao što je AChE. Specifičan raspored i supstituenti u paraoksonu doprinose njegovom snažnom inhibitornom dejstvu. Biotransformacija parationa u paraokson se dešava kroz oksidativnu desulfuraciju, uglavnom katalizovanu enzimima citohroma P450 u jetri. Ova metabolička konverzija pogoršava toksikološki profil parationa, pošto paraokson pokazuje jaču inhibiciju AChE u poređenju sa njegovim matičnim jedinjenjem [97].

Uticaj paraokona na životnu sredinu je značajan zbog njegovog potencijala da kontaminira zemljište, vodu i neciljane organizme. Na postojanost jedinjenja u ekosistemima utiču faktori kao

što su temperatura, pH i mikrobna aktivnost, koji utiču na njegovu degradaciju i mobilnost. Vodena sredina je posebno ranjiva, jer paraokson može akumulirati i ispoljiti toksične efekte na vodene organizme, narušavajući ekosisteme i lance ishrane.

1.5. Klinička slika trovanja organofosfatima

Izlaganje OP jedinjenjma može da dovede do četiri različita sindroma koji se javljaju nakon različitog latentnog perioda od izlaganja organizma OP jedinjenju: akutna holinergička kriza; intermedijarni sindrom; odložena polineuropatija i hronično psihijatrijsko oboljenje.

1.5.1. Akutna holinergička kriza

Akutna holinergična kriza je jedna od najkritičnijih i najopasnijih posljedica trovanja OP, koja nastaje usljed inhibicije AChE od strane OP. Ona je rezultat masivne akumulacije ACh u sinaptičkim pukotinama, što dovodi do kontinuirane stimulacije holinergičkih receptora [98]. Akutnu holinergičku krizu karakteriše prekomjerna stimulacija i muskarinskih i nikotinskih ACh receptora usljed porasta nivoa ACh. Klinički, ova kriza se može razumjeti kroz tri glavne komponente: muskarinske, nikotinske i CNS manifestacije [99].

Aktivacija muskarinskih receptora dovodi do sljedećih simptoma: bronhokonstrikcija, bronhoreja, bradikardija, hipotenzija, mučnina, povraćanje, povećana pokretljivost crijeva i bešike, mioza, hipersalivacija, lakrimacija. Prekomjerna parasimpatikusna stimulacija izazvana toksičnošću OP može ozbiljno da ugrozi respiratornu funkciju zbog bronhoreje i bronhospazma, što zahtijeva hitnu medicinsku intervenciju [100].

Nikotinske manifestacije prvenstveno uključuju neuromuskularnu sinapsu, što dovodi do fascikulacija mišića, slabosti i paralize. Prekomjerna stimulacija nikotinskih receptora na neuromuskularnim sinapsama može dovesti do mišićnog umora i paralize respiratornih mišića, naglašavajući potrebu za hitnom intervencijom kako bi se spriječila respiratorna insuficijencija. Ovi efekti takođe mogu da pogoršaju kardiovaskularnu nestabilnost, dovodeći do tahikardije i hipertenzije, doprinoseći složenosti liječenja tokom holinergične krize [101].

Uključenost CNS-a može dovesti do neurokognitivnih efekata uključujući glavobolju, agitaciju, konfuziju, tremor, nedostatak koordinacije, ataksiju, konvulzije, centralnu respiratornu i kardiovaskularnu depresiju, komu i smrt [102].

1.5.2. Intermedijarni sindrom

Intermedijarni sindrom (IMS) je posebno kliničko stanje koje se javlja kod pacijenata koji su bili izloženi OP jedinjenjima, a javlja se nakon razrješenja akutne holinergičke krize, ali prije početka odložene neuropatije. Prvi put opisan 1970-ih, IMS predstavlja kritičnu fazu u napredovanju trovanja OP-om, koju karakteriše specifična neuromuskularna paraliza koja može dovesti do teškog respiratornog distresa i drugih komplikacija [103].

Koncept IMS-a su prvi uveli Wadia i saradnici 1974. godine, koji su primijetili odloženi početak mišićne slabosti kod pacijenata koji su se oporavljali od početne akutne faze trovanja OP [102]. Za razliku od akutne holinergičke krize, koja se manifestuje u roku od nekoliko sati nakon izlaganja, IMS obično postaje očigledan 24 do 96 h nakon izlaganja i traje nekoliko dana ako se ne liječi. Identifikacija IMS-a je bila ključna u oblikovanju kliničkih protokola i smjernica za liječenje trovanja OP [104].

Patofiziološki mehanizmi koji stoje iza IMS ostaju nepotpuno shvaćeni, ali trenutni dokazi sugerišu da je IMS rezultat produžene disfunkcije neuromuskularne sinapse. Primarna hipoteza je da je IMS uzrokovan produženom inhibicijom AChE, zajedno sa mogućim direktnim efektima OP jedinjenja na nikotinske receptore na neuromuskularnoj sinapsi. Ova produžena neuromuskularna blokada dovodi do trajne depolarizacije i eventualne desenzibilizacije neuromuskularnih receptora, što rezultira slabošću mišića i paralizom [105].

IMS karakteriše specifičan obrazac mišićne slabosti, koja posebno zahvata proksimalne mišiće ekstremiteta, pregibače vrata i respiratorne mišiće. Pacijenti mogu da ispolje ptozu, oftalmoplegiju i slabost mišića lica, uz značajan respiratorni kompromis usljed paralize dijafragme. Ove manifestacije zahtijevaju pažljivu kliničku procjenu, jer se lako mogu pogrešno protumačiti kao tekući efekti akutnog trovanja ili klinički znaci drugih komplikacija [106].

Dijagnostikovanje IMS-a uključuje pažljivo kliničko posmatranje i isključivanje drugih mogućih uzroka neuromuskularne slabosti nakon izlaganja OP. Liječenje IMS-a se fokusira na

suportivnu njegu i simptomatsko liječenje. Za razliku od akutne faze, upotreba standardnih antidota kao što su atropin i oksimi neće biti efikasna u IMS-u, jer ne rješavaju osnovnu disfunkciju neuromuskularnih receptora. Umjesto toga, intervencije uključuju respiratornu podršku, kao što je mehanička ventilacija, na osnovu težine zahvaćenosti respiratornih mišića. Fizioterapija i praćenje sekundarnih komplikacija kao što je aspiraciona pneumonija takođe su bitne komponente njege. Istraživanje novih opcija liječenja usmjerenih na neuromuskularnu transmisiju može poboljšati strategije tretmana IMS [107].

1.5.3. Odložena polineuropatija indukovana organofosfatima (OPIDN)

OPIDN je ozbiljno neurološko stanje koje nastaje usljed izlaganja određenim OP jedinjenjima, posebno onima sa jakim afinitetom za neuropatske efekte. Ova odložena manifestacija neurotoksičnosti, koja se može javiti nekoliko dana do nedjelja nakon akutnog trovanja OP, uključuje ozbiljnu degeneraciju perifernog i CNS-a [108].

Prepoznavanje OPIDN-a datira još od sredine 20. vijeka kada je upotreba OP pesticida postala široko rasprostranjena [109]. Klinički slučajevi su se pojavili i u poljoprivrednim sredinama i među vojnim osobljem koje je bilo izloženo nervnim bojnim otrovima, što je stvorilo potrebu da se obrati pažnja na hronične posljedice nakon akutnog izlaganja. Tokom godina, istraživači su razjasnili odnos između OP jedinjenja i odložene neuropatije, što je dovelo do sistematičnijih istraživanja osnovnih mehanizama i kliničkih karakteristika OPIDN-a [110].

Mehanizmi iza OPIDN-a su zamršeni i uključuju i holinergičke i neholinergičke puteve. Klinička slika OPIDN-a se obično manifestuje 7 do 21 dan nakon izlaganja OP jedinjenjima. Simptomi mogu početi nejasnim tegobama, uključujući slabost mišića, umor i senzorne poremećaje, koji mogu napredovati do izraženijih motoričkih deficita i senzornog gubitka. Uobičajeni klinički znaci uključuju ataksiju, drhtanje i poteškoće sa finim motoričkim vještinama koje se mogu pripisati zahvaćenosti perifernih nerava [111]. Posebno je karakteristična slabost distalnih mišića, pri čemu pacijenti često imaju poteškoća pri hodanju i obavljanju svakodnevnih zadataka koji zahtijevaju koordinaciju ruku. U teškim slučajevima, pacijenti mogu razviti sindrome hroničnog bola i invalidnost, što značajno utiče na njihov kvalitet života [112].

Pošto ne postoji specifičan antidot za ovo stanje, liječenje OPIDN-a se prvenstveno fokusira na suportivnu njegu i rehabilitaciju, fokusirajući se na poboljšanje mišićne snage, koordinacije i funkcionalne nezavisnosti [113]. Dugoročni ishodi OPIDN-a su promjenljivi, neki pojedinci doživljavaju uporne neurološke deficite i smanjen kvalitet života, dok se drugi mogu potpuno oporaviti. Faktori kao što su veličina i trajanje izloženosti, individualna osjetljivost i blagovremena medicinska intervencija utiču na ishode oporavka. Epidemiološke studije sugerišu povezanost između izloženosti OP-u i odloženih neurobihejvioralnih komplikacija, što izaziva širu zabrinutost u vezi sa dugoročnim efektima na kognitivne funkcije i mentalno zdravlje.

1.5.4. Hronično psihijatrijsko oboljenje indukovano ogranofosfatima

Hronične psihijatrijske bolesti izazvane OP privukle su značajnu pažnju posljednjih godina jer naučnici i zdravstveni radnici nastoje da razumiju dugoročne neuropsihijatrijske posljedice izlaganja OP. Profesionalne studije poljoprivrednih radnika otkrile su zabrinjavajuću korelaciju između hronične izloženosti OP pesticidima i neuropsihijatrijskih simptoma, što je navelo istraživače da istraže potencijalne dugoročne efekte na mentalno zdravlje. Ova zapažanja su rezultirala sve većom količinom literature koja naglašava potrebu za daljim istraživanjem neurotoksičnih efekata ovih sveprisutnih hemikalija [114].

Hronična izloženost OP može doprinijeti neuropsihijatrijskim poremećajima kroz različite mehanizme. Primarni način djelovanja uključuje inhibiciju AChE, što dovodi do produženog povećanja nivoa ACh u sinaptičkoj pukotini. Ova prekomjerna stimulacija holinergičkih receptora može izazvati disregulaciju neurotransmisije i u CNS i u PNS [115].

Dugoročne promjene u holinergičkoj signalizaciji su umiješane u kognitivne deficite i poremećaje raspoloženja, što dovodi do stanja kao što su anksioznost, depresija i druga kognitivna oštećenja. Pored toga, OP mogu izazvati oksidativni stres, što dovodi do oštećenja neurona kroz mehanizme kao što su lipidna peroksidacija i mitohondrijalna disfunkcija. Složena interakcija između holinergičke disregulacije, oštećenja neurona i neuroinflamacije doprinosi hroničnim psihijatrijskim poremećajima uočenim kod osoba izloženih OP jedinjenjima [116].

Kliničke karakteristike hronične psihijatrijske bolesti izazvane OP mogu uveliko varirati, što komplikuje dijagnozu i liječenje [117]. Često prijavljeni simptomi uključuju kognitivna

oštećenja, promjene raspoloženja, poremećaje anksioznosti i deficite pamćenja. Mnogi pacijenti imaju simptome koji podsjećaju na posttraumatski stresni poremećaj (PTSP), uz anksioznost, razdražljivost i emocionalnu nestabilnost. Takve manifestacije mogu poremetiti svakodnevno funkcionisanje i negativno uticati na kvalitet života.

Hronična izloženost OP je takođe povezana sa različitim neurokognitivnim deficitima, uključujući smanjen raspon pažnje, oštećeno pamćenje i poteškoće u učenju. Istraživanja su pokazala da poljoprivredni radnici sa istorijom izloženosti OP pokazuju lošiji učinak na kognitivnim testovima u poređenju sa neeksponiranim kontrolama, što ukazuje na opipljiv uticaj na kognitivne funkcije [118].

Liječenje hronične psihijatrijske bolesti izazvane OP često zahtijeva višestrani pristup koji se bavi neurološkim i psihijatrijskim komponentama. Antidepresivi i anksiolitici se obično propisuju da pomognu u ublažavanju poremećaja raspoloženja i anksioznosti kod pogođenih osoba. Kognitivno-bihejvioralna terapija (CBT) može pružiti vrijedne alate za upravljanje psihijatrijskim simptomima i poboljšanje strategija suočavanja [119].

1.6. Liječenje akutnog trovanja organofosfatima

Liječenje akutnog trovanja OP sastoji se od nekoliko ključnih koraka koji imaju za cilj obnavljanje normalne signalizacije ACh i podržavanje vitalnih funkcija [120]. Prvi korak u liječenju OP trovanja je dekontaminacija, koja uključuje i uklanjanje pacijenta sa izvora ekspozicije. U slučajevima dermalne izloženosti, dekontaminaciju treba odmah izvršiti temeljnim pranjem kože sapunom i vodom i skidanjem kontaminirane odjeće. Dekontaminacija je ključna za sprječavanje dalje apsorpcije toksina i smanjenje morbiditeta. U slučaju oralnog unošenja OP, preporučuje se gastrična lavaža ukoliko se pacijent javio 1 h nakon ingestije OP, ali ni tad tretman atropinom i oksimima ne smije biti odložen [121]. Aktivni ugalj se *in vitro* veže za OP, te se njegova primjena preporučuje, iako nema studija gdje je pokazana jasna klinička efikasnost [122]. Lavažu ne treba izvoditi ako je pacijent bez svijesti i ako nema obezbeđen disajni put, zbog rizika od aspiracije [123].

Farmakološki tretman trovanja OP prvenstveno uključuje davanje atropina, oksima i benzodiazepina. Suportivna terapija je sastavna komponenta liječenja trovanja OP. Pacijentima

može biti potrebna respiratorna podrška, posebno ako dođe do respiratorne insuficijencije i neuromuskularne paralize. Praćenje vitalnih znakova, uključujući srčanu frekvenciju i krvni pritisak, je od suštinskog značaja za adekvatnu nadoknadu tečnosti i dodatni tretman [124].

1.6.1. Antiholinergici

1.6.1.1. Muskarinski antagonisti

Muskarinski antagonisti, u prvom redu atropin, su najznačajniji lijekovi kod trovanja OP [125]. Iako atropin ostaje glavni oslonac terapije i drugi antagonisti muskarinskih receptora su proučavani na životinjama. Važna razlika između tih lijekova je u intenzitetu njihovog prodora u CNS [126]. Glikopironijum bromid i skopolamin metobromid ne ulaze u CNS, dok skopolamin ima odličan prodor, bolji i od atropina [127]. Glavni neželjeni efekat atropina je antiholinergički delirijum kod pacijenata koji su primali previsoku dozu. Neki ljekari stoga preferiraju glikopironijum za liječenje perifernih efektata OP trovanja. Međutim, njegov slab prodor u CNS sugeriše da je neefikasan u borbi protiv kome i depresije disanja. Skopolamin je uspješno korišten za liječenje pacijenata sa teškim ekstrapiramidalnim sindromom [127]. Studije na životinjama sugerišu da je efikasniji od atropina za kontrolu konvulzija izazvanih udisanjem OP nervnih bojnih otrova. Ekstrapiramidalni efekti i konvulzije nisu uobičajeni kod trovanja OP pesticidima, za razliku od trovanja nervnim bojnim otrovima.

Atropin će vjerovatno ostati antimuskarinski lijek izbora dok neka visokokvalitetna randomizovana ispitivanja ne pokažu da neki drugi muskarinski antagonist ima bolji odnos koristi i rizika [128]. On je široko dostupan, pristupačan i umjerene sposobnosti da prodre u CNS. Atropin blokira djelovanje ACh na muskarinskim receptorima, ali je neefikasan u tretiranju nikotinskih simptoma i znakova trovanja. Antiholinergici imaju kritičnu ulogu u rješavanju akutnih efekata trovanja OP [129]. Tokom godina, istraživanja su pružila uvid u optimizaciju primjene atropina i njegovih kombinacija sa drugim terapijski agensima, učvršćujući njegovu ulogu u liječenju akutne holinergičke krize.

Klinička primjena atropina kod trovanja OP uključuje agresivan, titrirani pristup zasnovan na težini simptoma [99]. Početni tretman obično počinje visokim dozama atropina, primijenjenim intramuskularno (im) ili intravenski (iv), u zavisnosti od kliničkog okruženja. Doza se može

ponavljati svakih 5 do 15 min dok se ne postigne dovoljan klinički odgovor, koji se karakteriše povlačenjem muskarinskih simptoma, poboljšanjem respiratorne funkcije i povećanjem srčane frekvencije.

Optimalna doza atropina nije određena. Razlikuje se kod otrovanih ljudi zbog varijacije u dozi i vrsti uzetog OP jedinjenja i vjerovatno zbog istovremene primjene oksima (smatra se da oksimi imaju antiholinergičko dejstvo pri visokim dozama). Prve doze se daju kao bolusi da bi se poništili muskarinski znaci (0,6–3,0 mg iv u zavisnosti od težine; u situaciji primarne njege, može se koristiti autoinjektor koji daje 2 mg im) [130]. Nedavne studije u Šri Lanki su imale za cilj da brzo daju dovoljno atropina za poboljšanje kardiovaskularne (sistolni krvni pritisak > 80 mm Hg, puls > 80 bpm) i respiratorne funkcije (liječenje bronhoreje i bronhospazma) u cilju spriječavanja insuficijencije. Zaključak navedene studije je da kada se kod osobe postigne saturacija atropinom, preporuke su da se dalje ordinira infuzija atropina u dozi koja ima za cilj da održi kardiorespiratornu funkciju i istovremeno spriječi toksičnost samog atropina (normalni zvukovi crijeva, bez uznemirenosti ili konfuzije) [131]. Prospektivna opservaciona kohortna studija koja je ispitivala *ad hoc* režim doziranja u odnosu na titrirani protokol doziranja otkrila je da je toksičnost atropina bila više povezana sa *ad hoc* dozom u poređenju sa dozom titriranom prema kliničkom efektu.

1.6.1.2. Nikotinski antagonisti

Nikotinski antagonisti se ne koriste rutinski u liječenju OP trovanja zbog ozbiljnih neželjenih dejstava, u prvom redu paralize respiratornih mišića i posljedične respiratorne insuficijencije [132]. Iako su u eksperimentalnim studijama pokazali značajan zaštitni indeks, u kliničkim istraživanjima se pokazalo da ne smanjuju smrtnost, pa čak i da ne skraćuju vrijeme koje pacijenti provedu na mehaničkoj ventilaciji kod onih pacijenata kod kojih je ista neophodna [133–137]. Značaj nikotinskih antagonista bi mogao biti u prevenciji intermedijarnog sindroma, čiji je predloženi mehanizam nastanka prekomjerna stimulacija post- i/ili presinaptičkih nikotinskih receptora na neuromišićnoj sinapsi. Istovremena nikotinska blokada, sa lijekom kao što je rokuronijum, može spriječiti pretjeranu stimulaciju i oštećenje skeletnih mišića [132]. Studije faze II su potrebne da bi se istražio najbolji način davanja nikotinskog antagoniste pacijentima otrovanim OP.

1.6.2. Reaktivatori acetilholinesteraze

Reaktivatori AChE (oksimi) reaktiviraju OP-inhibisanu AChE [138]. Pralidoksim (PAM-2) je otkriven sredinom 1950-ih od strane Wilsona i njegovih kolega, a ubrzo je uspješno uveden u kliničku praksu za pacijente sa trovanjem parationom. Drugi oksimi, kao npr. obidoksim (ili LüH-6, po Arthuru Lüttringhausu i Ilse Hagedornu koji su prvi sintetisali obidoksim 1964) i trimedoksim, su razvijeni za kliničku upotrebu, ali u praksi PAM-2 ostaje najčešće korišten [139]. Ima četiri soli: hlorid, jodid, metilsulfat i mesilat. Hloridne i jodidne soli se široko koriste, ali metilsulfat i mesilat se najviše koriste u Francuskoj, Belgiji i UK. Hloridna so ima prednosti u odnosu na jodid - manja molekulska težina obezbjeđuje 1,5 puta više aktivnog jedinjenja/g soli nego jodid. Visoke doze PAM-2 jodida dovode pacijente u rizik od toksičnosti za štitnu žlijezdu, posebno ako se primjenjuje tokom dužeg perioda [140]. Obidoksim se često koristi u određenim zemljama Evrope. PAM-2 i obidoksim su veoma slične strukture, ali obidoksim ima dva priridinijumska prstena povezana molekulom kiseonika [141].

Klinička upotreba oksima zahtijeva pažljivo razmatranje vremena, doze i načina primjene [142]. Standardni protokol za liječenje trovanja OP podrazumijeva hitnu medicinsku intervenciju oksimima čim se posumnja na trovanje [143]. U slučajevima akutnog trovanja, oksimi se obično primjenjuje u dozama koje mogu da variraju u zavisnosti od težine manifestacije i specifičnog uključenog OP. Preporučuje se primjena PAM-2 hlorida 2 g (ili obidoksima 250 mg) iv tokom 20-30 min, potom infuzija PAM-2 0,5–1 g/h (ili obidoksima 30 mg/h) u fiziološkom rastvoru [144]. Vrijeme primjene ostaje kritičan faktor efikasnosti liječenja [145,146].

Na stepen efikasnosti oksima značajno utiče njihov afinitet vezivanja za fosforilisani enzim. Strukturne modifikacije i hemijske grupe oksima mogu uticati na njihovu sposobnost da funkcionišu kao nukleofil i isprave OP-indukovanu inhibiciju. Kao takva, istraživanja nastavljaju da se fokusiraju na razvoj efikasnijih jedinjenja koja bi mogla da obezbijede bržu i pouzdaniju reaktivaciju AChE. Obnavljanje aktivnosti AChE ima kritičnu važnost jer pomaže povratku holinergičke ravnoteže i ublažava komplikacije opasne po život povezane sa trovanjem OP. Brzim smanjenjem nivoa neregulisanog ACh, oksimi imaju suštinsku ulogu u ublažavanju simptoma i obnavljanju normalne fiziološke funkcije.

1.6.2.1. Oksim K870

Oksimi su jedini kauzalni antidoti kod trovanja OP. Međutim, oni imaju i značajna ograničenja. Dva najveća problema do sada sintetizovanih oksima su nedovoljna efikasnost protiv specifičnih OP i nedovoljna univerzalnost protiv različitih OP. Upravo zbog toga se stalno traga za novim i efikasnijim oksimima. Takozvani K-oksimi su dio tih napora. Do danas objavljena literatura nudi informacije o K-seriji mono- i bis-piridinijumskih oksima, među kojima su oksimi K027, K048 i K203 pokazali najperspektivniju sposobnost reaktivacije protiv raznih OP i *in vitro* i *in vivo* [147,148]. Važno je napomenuti da oksim K203 štiti od trovanja tabunom bolje od trimedoksima. Hlorisani i dvostruko naelektrisani monooksim K870 je izveden iz strukture oksima K203. Ova strukturna modifikacija K203 treba da donese povećanu lipofilnost i poboljšano prodiranje oksima u mozak i smanjen pKa u poređenju sa nehlorisanim analozima. Hlorisanje K203 smanjuje pKa sa 8,33 na 7,55, povećavajući količinu jonizovanog oblika pri fiziološkom pH od ~10% oksima K203 na ~40% oksima K870, što bi moglo povećati antidotski potencijal oksima K870 [149].

1.6.3. Antikonvulzivi

Dok se akutno liječenje trovanja OP prvenstveno fokusira na upotrebu antiholinergičkih agenasa i oksima, pojava konvulzija koja se češće javlja kod nervnih bojnih otrova nego pesticida zahtijeva primjenu antikonvulziva. Među različitim efektima, konvulzije su posebno zabrinjavajuće jer ne samo da povećavaju morbiditet, već mogu iskomplikovati ili destabilizovati stanje pacijenta. Ovi napadi se obično pripisuju prekomjernoj holinergičkoj aktivnosti i naknadnim ekscitatornim efektima na CNS. Kod pacijenata koji pate od akutnog trovanja, konvulzije se mogu razviti kako se klinička slika razvija, naglašavajući važnost predviđanja i rješavanja potencijalne aktivnosti konvulzija. Uloga antikonvulziva je da kontrolišu aktivnost konvulzija i smanje rizik od pratećih neuroloških oštećenja [150].

Benzodiazepini su često tretman prve linije za konvulzije kod trovanja OP zbog njihovog brzog početka djelovanja i efikasnosti u kontroli konvulzija. Lijekovi kao što su lorazepam i diazepam djeluju tako što pojačavaju djelovanje inhibitornog neurotransmitera GABA na GABA-A receptorima, čime promovišu sedaciju i opuštanje mišića. Studije na životinjama sugerišu da

diazepam smanjuje oštećenje CNS i sprečava respiratornu insuficijenciju i smrt, ali je malo takvih studija na ljudima. Kod trovanja OP kod pacijenata se može javiti agitacija i delirijum. Uzrok je složen, od efekata samog pesticida, toksičnosti atropina, do hipoksije CNS. Akutno uznemireni pacijenti će imati koristi od liječenja diazepamom [151].

Iako su benzodiazepini neophodni za liječenje akutnih napada, možda neće obezbijediti dovoljnu dugoročnu kontrolu kod nekih pacijenata. U takvim slučajevima, dodatni antiepileptički lijekovi kao što su fenitoin ili levetiracetam mogu se koristiti kao dodatna terapija. Izbor antikonvulzivne terapije može zavisiti od faktora specifičnih za pacijenta, uključujući težinu trovanja, odgovor na početni tretman i prisustvo komorbidnih stanja. Doze benzodiazepina mogu da variraju u zavisnosti od kliničkog konteksta, sa dozama koje se obično kreću od 4 do 8 mg lorazepama datih iv kod odraslih. Zbog mogućnosti produžene aktivnosti i kumulativnih efekata u slučajevima ponovljenih doza, neophodna je pažljiva titracija i praćenje [152].

Pored početne doze, treba pažljivo pratiti učestalost primjene antikonvulziva. Pacijenti sa rizikom za kontinuiranu aktivnost konvulzija mogu zahtijevati kontinuiranu infuziju ili ponovljene boluse, posebno u slučajevima teškog trovanja. Ovo naglašava važnost individualizovanih planova liječenja u postizanju efektivne kontrole konvulzija uz minimiziranje neželjenih efekata.

Kod OP trovanja konvulzije su u početku holinergičke, a zatim pod uticajem GABA-A, potom glutamatergične. Stoga, na početku, atropin je efikasan antikonvulziv, zatim benzodiazepini ili barbiturati (npr. tiopental-natrijum) i konačno antagonisti receptora N-metil-D-aspartata (NMDA) (npr. memantin) [153,154].

1.6.4. Simptomatska i suprotivna terapija

Pored antiholinergika, oksima i antikonvulziva, razni drugi lijekovi mogu pomoći u liječenju trovanja OP. Ovi agensi pomažu u stabilizaciji kardiovaskularnog sistema, poboljšanju respiratorne funkcije i pružanju metaboličke podrške.

Kardiovaskularna nestabilnost je karakteristična kod trovanja OP, pri čemu pacijenti često imaju bradikardiju, hipotenziju i druge poremećaje ritma. U ovim slučajevima mogu se primijeniti adrenergički lijekovi kao što su adrenalin ili noradrenalin. Pacijenti sa OP trovanjima često imaju respiratorni distres, bronhospazam i povećano lučenje sekreta. Primjena bronhodilatatora, kao što

je salbutamol, može biti opravdana za ublažavanje bronhokonstrikcije i poboljšanje protoka vazduha, posebno kod pacijenata sa već postojećim respiratornim stanjima kao što je astma [155]. Štaviše, dodatni kiseonik i respiratorna podrška, uključujući mehaničku ventilaciju kada je to neophodno, su kritične komponente sveobuhvatne njege, omogućavajući adekvatnu oksigenaciju i ventilaciju u slučajevima paralize respiratornih mišića.

Hronična izloženost OP jedinjenjima može dovesti do oksidativnog stresa i neurotoksičnih oštećenja, naglašavajući važnost metaboličke podrške u strategiji liječenja. Antioksidansi, kao što je N-acetilcistein, su istraživani zbog njihovog kapaciteta da se suprotstave oksidativnom stresu povezanim sa izlaganjem OP [156]. Dopunjavanjem intracelularnih nivoa glutationa, esencijalnog antioksidansa, N-acetilcistein može ublažiti ćelijsko oštećenje i podržati procese detoksikacije. Nekoliko studija je pokazalo da primjena N-acetilcisteina može poboljšati testove funkcije jetre i smanjiti stepen oksidativnog oštećenja nakon akutnog izlaganja OP [157]. Iako su potrebna dodatna istraživanja da bi se usvojili definitivni klinički protokoli, upotreba antioksidanata u kombinaciji sa standardnim tretmanom mogla bi predstavljati obećavajući put za poboljšanje ishoda OP trovanja kod pacijenata.

Magnezijum-sulfat blokira kalcijum vezan za ligand kanala, što dovodi do smanjenog oslobađanje ACh iz presinaptičkih završetaka, čime se poboljšava funkcija na neuromuskularnoj ploči i smanjuje prekomjerna stimulacija CNS-a posredovana aktivacijom NMDA receptora [158]. Natrijum-bikarbonat se ponekad koristi za liječenje trovanja OP. Povećanje pH krvi (do 7,45–7,55) se pokazalo da poboljšava ishod kod pasa nepoznatim mehanizmom. U eksperimentalnim uslovima se pokazao korisnim, ali pregledni članak zasnovan na podacima iz Cochraine baze je zaključio da ne postoji dovoljno dokaza da bi se utvrdilo da li natrijum bikarbonat treba koristiti kod ljudi otrovanih OP [159–162].

OP insekticidi su rastvorljivi u lipidima, pa je moguće je da iv lipidne emulzije mogu redistribuirati otrov, ali takođe mogu i povećati resorpciju iz crijeva, čime se povećava toksičnost [163]. Studija na pacovima je sugerisala potencijalnu korist od ovog tretmana [163]. Međutim, nedavna *in vitro* studija je sugerisala da lipidna emulzija zapravo može zaptititi OP od degradacije i produžiti njihovo poluvrijeme eliminacije [164].

1.6.5. Predtretman trovanja organofosfatima

Profilaksa ima za cilj da spriječi ili ublaži efekte trovanja OP prije izlaganja ili neposredno poslije njega. Kao zaštitni pristup, strategije profilaktičkog liječenja su dizajnirane da minimiziraju vezujuće i toksične efekte OP-a na AChE, čime se održava normalna holinergička transmisija uprkos izloženosti.

Nekoliko farmakoloških agenasa se koristi za profilaktički tretman trovanja OP [165,166]. Ovi agensi se mogu klasifikovati u dvije osnovne kategorije: zaštitni agensi i strategije rane intervencije. Istraživanja su u toku o dodatnim profilaktičkim opcijama liječenja koje mogu ublažiti toksičnost OP. Suština istraživanja profilaktične primjene se prvenstveno odnosi na zaštitu od nervnih bojnih otrova [167,168]. Karbamati fizostigmin i piridostigmin su korišteni kao predtretman protiv trovanja nervnim agensima kako bi se reverzibilno inhibisao i tako zaštitio od ireverzibilne inhibicije dio AChE u mozgu i respiratornim mišićima koji je ključan za preživljavanje [169]. Naime, kada se profilaktički primijene reverzibilni inhibitori AChE, djelimično inhibisana ACE je zaštićena od naknadne inaktivacije bojnim otrovom [170,171]. U tom slučaju, inhibicija preostale AChE ne predstavlja životnu opasnost, jer se tokom ireverzibilne inhibicije oslobađa dio enzima privremeno inhibisan karbamatima u količini dovoljnoj za regulaciju sinaptičke funkcije [172]. Najuspješnije profilaktičke sheme uključuju karbamatne AChE inhibitore fizostigmin, neostigmin, piridostigmin i neke novije sintetičke karbamate poput ferocenskih karbamata.

Memantin-hidrohlorid i atropin samostalno pokazuju skromne, ali značajne zaštitne indekse kod trovanja somanom [173,174]. Poseban značaj memantina je u potenciranju zaštitnih indeksa u kombinovanoj profilaktičkoj primjeni zajedno sa oksimima i atropinom [175]. Istraživanje upotrebe antioksidativnih agenasa, kao što je N-acetilcistein, takođe je dobilo na snazi, s obzirom na njihov potencijal da smanje oksidativni stres izazvan izlaganjem OP. Štaviše, istraživanje novih jedinjenja koja pokazuju neuroprotektivna svojstva protiv OP-indukovane disregulacije neurotransmisije nastavlja da se razvija, naglašavajući potrebu za inovativnim pristupima u profilaksi. Iako memantin obezbjeđuje bolju i dugotrajniju zaštitu od trovanja somanom kod pacova od karbamata, njegov mali terapijski indeks i uska terapijska širina ozbiljno ograničavaju njegov potencijal kao profilaktičkog agensa [154].

2. CILJEVI ISTRAŽIVANJA

Glavni cilj istraživanja jeste da se utvrdi u kojoj je mjeri novosintetisani oksim K870 efikasan kao antidot prilikom trovanja paraoksonom, u poređenju sa klasičnim oksimom obidoksimom.

Ciljevi istraživanja:

- Utvrditi toksičnost oksima K870 i obidoksima.

- Utvrditi zaštitni indeks oksima K870 i obidoksima prilikom trovanja paraoksonom, kao monoterapije i u kombinaciji sa atropinom.

- Utvrditi intenzitet, učestalost i vrijeme pojave znakova trovanja prilikom trovanja paraoksonom i uticaj terapije (monoterapija oksimom K870, obidoksimom i atropinom ili kombinacija oksima i atropina) na znakove trovanja. Uporediti intenzitet učestalost i vrijeme pojave znakova trovanja u odnosu na dozu paraoksona, smrtni ishod i terapiju.

- Uporediti zaštitni indeks oksima K870 i obidoksima prilikom trovanja paraoksonom (kao monoterapije i u kombinaciji sa atropinom).

- Uporediti uticaj oksima K870 i obidoksima na vremenski profil aktivnosti acetilholinesteraze prilikom trovanja visokom subletalnom dozom paraoksona.

- Utvrditi efekat terapije oksima K870 i obidoksima na neuromuskularnu transmisiju u preparaciji n. *phrenicus* – dijafragma pacova *in situ* nakon primjene visoke subletalne doze paraoksona.

- Utvrditi efekat terapije oksima K870 i obidoksima na kardiorespiratorne parametre (arterijski pritisak (AP), srčanu frekvencu, disanje – broj i dubinu respiracija, acido-bazni status) pacova nakon primjene visoke subletalne doze paraoksona.

3. RADNE HIPOTEZE

- Oksim K870 bolje od obidoksima smanjuje smrtnost kod trovanja paraoksonom kod pacova.

- Oksim K870 ima značajan zaštitni indeks kod trovanja paraoksonom, koji je potenciran istovremenom primjenom atropina.

- Oksim K870 značajno reaktiviše acetilholinesteraze (AChE) i karboksilesteraze (CarbE) poslije trovanja visokom subletalnom dozom paraoksona kod pacova.

- Oksim K870 značajno popravlja neuromuskularnu transmisiju poslije trovanja visokom subletalnom dozom paraoksona kod pacova.

- Oksim K870 značajno pobošljava kardiorespiratorne parametre poslije trovanja visokom subletalnom dozom paraoksona kod pacova.

- Oksim K870 ima veći antidotski potencijal od obidoksima

4. METODOLOGIJA

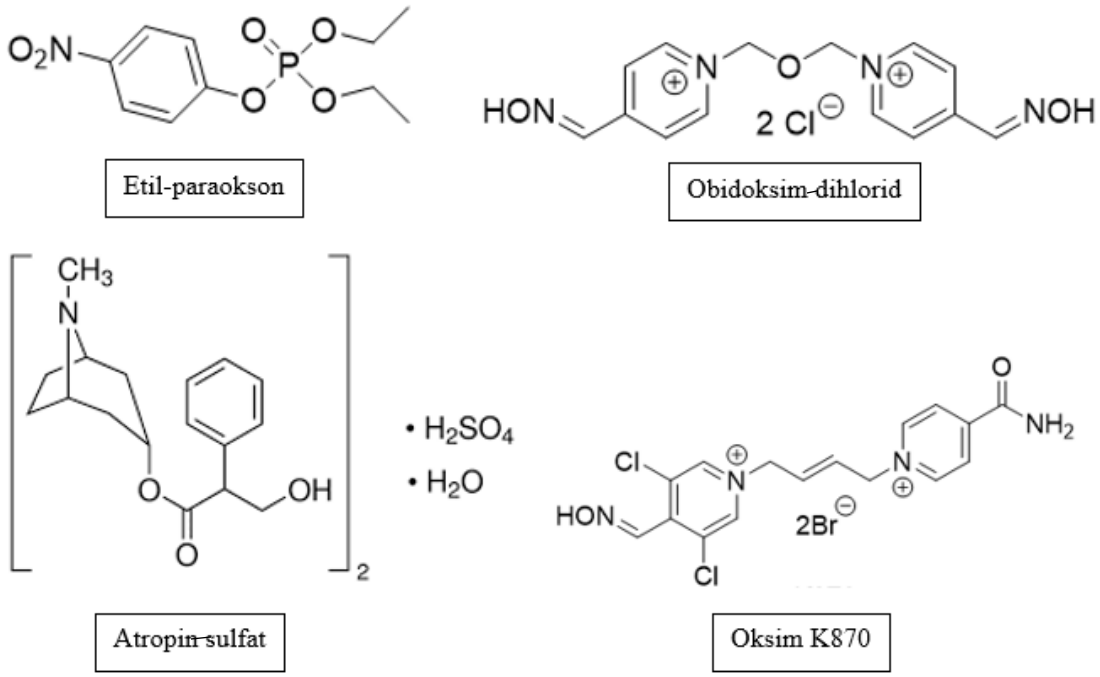
4.1. Životinje

Studija je sprovedena na odraslim albino *Wistar* pacovima, težine 200-340 g, nabavljenih od Prirodno-matematičkog fakulteta Univerziteta u Banjoj Luci, Republika Srpska, Veterinarskog fakulteta Univerziteta u Sarajevu i iz vlastitog uzgoja. Životinje su dobijale vodu i hranu *ad libitum*, držane su na temperaturi od 20-22 °C, uz ciklus svjetlosti i tame od 12 h. Studiju je odobrio Etički odbor za zaštitu i dobrobit eksperimentalnih životinja u biomedicinskim istraživanjima, Medicinskog fakulteta Univerziteta u Banjoj Luci (Odluka br. 18/1/20). Životinje su tretirane u skladu sa *The Guide for the Care and Use of Laboratory Animals* [176]. Studija je sprovedena u Centru za biomedicinska istraživanja Medicinskog fakulteta Univerziteta u Banjoj Luci.

4.2. Hemikalije

Paraokson (dietil-(4-nitrofenil)-fosfat), atropin (atropin-sulfat) i N-butilskopolamin su nabavljeni od *Merck*, St Louis, MO, SAD. Obidoksim [1,3-bis(4-hidroksiiminometilpiridinijum)-2-oksapropan-hlorid] i oksim K870 (4-karbamoil-1-[(2E)-4-{3,5-dihloro-4-[(hidroksiimino)metil]-piridinijum-1-il}but-2-en-1-il]-piridinijum dibromid) sintetizovani su na Odsjeku za hemiju Prirodno-matematičkog fakulteta Univerziteta u Hradec Kralove, Češka Republika i donirani su Medicinskom fakultetu.

Paraokson je rastvaran u izopropil-alkoholu do koncentracije od 50 mg/mL, dok su dalja razblaženja osnovnog rastvora pravljena u fiziološkom rastvoru (0,9% rastvor NaCl) (FR). Atropin i oksimi su rastvarani u FR. Konačna razblaženja su pravljena neposredno prije tretmana. Sve supstance su davane u zapremini od 1 mL/kg, osim oksima K870 u dozi od 200 mg/kg, kada je on, zbog loše rastvorljivosti u FR, davan u zapremini od 2 mL/kg (1 mL/kg u svaki butni mišić). Paraokson je primjenjivan subkutano (sc), a FR, atropin i oksimi im. Hemijske strukture paraoksona, atropin-sulfata, obidoksima i oksima K870 prikazane su na Slici 4.



Slika 4. Hemijske strukture: paraoksona, atropina, obidoksima i oksima K870

4.3. Dizajn studije

4.3.1. Srednja smrtna doza (LD_{50}) i zaštitni indeksi

U ovim eksperimentima smrtnost paraoksona, obidoksima i oksima K870, kao i zaštitni indeksi antidota konstatovani su na osnovu 24-h preživljavanja. Doze su određivane metodom „up and down“, a LD_{50}, kao i zaštitni indeksi su određivani prema Litchfieldu i Wilcoxonu (1949) [177].

LD_{50} paraoksona. Primijenjene su rastuće doze paraoksona sc. Svaka grupa se sastojala od 6 pacova, a doze su određivane metodom „up and down“ (doze su bile: 0,2, 0,3, 0,35, 0,4 mg/kg sc). Da bi se eliminisao uticaj rastvarača, kao i same traume prilikom im aplikacije antidota, jedan min nakon injekcije paraoksona, pacovima je ubrizgavan FR od 1 mL/kg im.

LD_{50} obidoksima i oksima K870. Primjenjene su rastuće doze oksima im. Doze obidoksima su bile: 50, 100, 150 i 200 mg im, a oksima K870 100, 150 i 200 mg im. Svaka grupa se sastojala od 5 pacova.

Paraokson i atropin. Primijenjene su rastuće doze paraoksona sc (doze su bile: 0,6, 0,9, 1,2 mg/kg) i fiksne doze atropina. Svaka grupa se sastojala od 6 pacova. Jedan min nakon injekcije paraoksona, pacovima je ubrizgano 10 mg/kg atropina im.

Paraokson i obidoksim. Primijenjene su rastuće doze paraoksona sc (doze su bile: 0,35, 0,4, 0,5 mg/kg) i fiksna doza obidoksima: 22 mg/kg im (20% prethodno utvrđenog LD_{50} obidoksima). Jedan min nakon injekcije paraoksona, pacovima je ubrizgan obidoksim im.

Paraokson i oksim K870. Primijenjene su rastuće doze paraoksona sc (doze su bile: 0,35, 0,4, 0,5 mg/kg) i fiksna doza oksima K870: 22 mg/kg im (20% prethodno utvrđenog LD_{50} oksima K870). Jedan min nakon injekcije paraoksona, pacovima je ubrizgan oksim K870 im.

Paraokson i atropin i obidoksim. Primijenjene su rastuće doze paraoksona sc (doze su bile: 1, 3, 6, 12, 24, 36, 42 i 48 mg/kg) i fiksna doza atropina (10 mg/kg im) i obidoksima (22 mg/kg im). Atropin i obidoksim su rastvarani zajedno u FR, tako da je ukupni volumen aplikovanih antidota bio 1 mL/kg. Jedan min nakon injekcije paraoksona, pacovima je ubrizgan atropin i obidoksim im.

Paraokson i atropin i oksim K870. Primijenjene su rastuće doze paraoksona sc (doze su bile: 0,35, 0,4, 0,5 mg/kg) i fiksna doza atropina (10 mg/kg) i oksima K870 (35 mg/kg) im. Atropin i oksim K870 su rastvarani zajedno u FR, tako da je ukupni volumen aplikovanih antidota bio 1 mL/kg. Jedan min nakon injekcije paraoksona, pacovima je ubrizgan atropin i oksim K870 im.

4.3.2. Inhibicija acetilholinesteraze i karboksilesteraze

Paraokson je aplikovan 0,25 mg/kg sc (0,75% LD_{50}), a zatim 1 min kasnije oksim K870 (35 mg/kg im) ili obidoksim (22 mg/kg im). Životinje su žrtvovane: 5, 15, 30, 45, 60, 120, 240, 480, 960 i 1440 min (24 h) nakon primjene paraoksona. Aktivnosti AChE su određene spektrofotometrijski u velikom mozgu, malom mozgu, moždanom stablu, dijafragmi i eritrocitima [178]. Aktivnosti CarbE određivane su titrimetrijski u plazmi i jetri [179].

Za svaki vremenski okvir žrtvovane su 4 životinje. Životinje su anestezirane ketaminom 90 mg/kg i ksilazinom 10 mg/kg intraperitonealno (ip). Krv je uzimana iz donje šuplje vene. Nakon vađenja krvi, uklonjeni su organi za analizu: dijafragma, jetra, bubrezi. Nakon toga, mozak je ispran od prisustva krvi, prethodno uspostavljenom MM metodom [180].

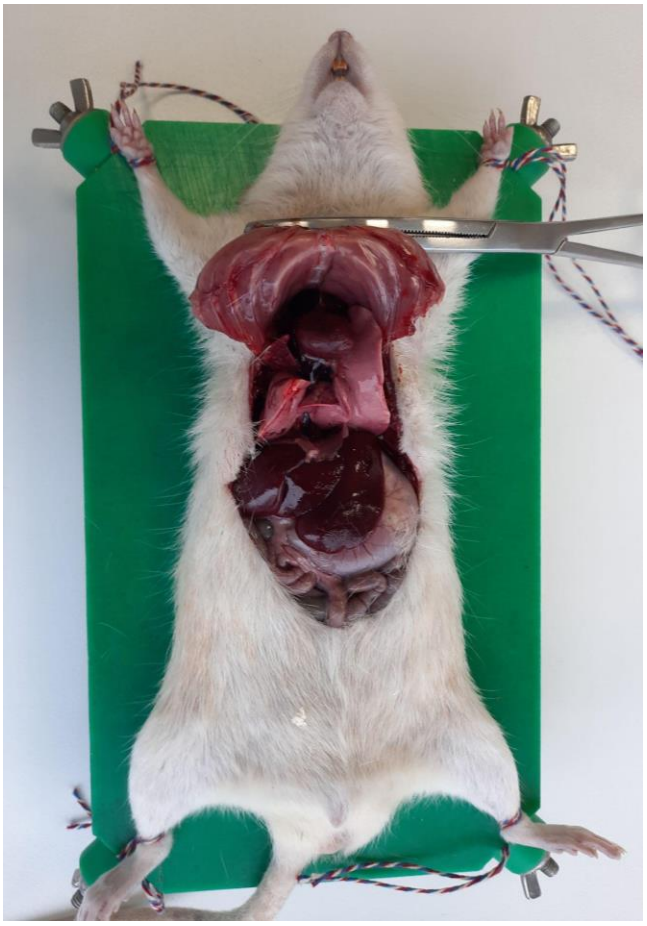
Aktivnost AChE iz eritrocita je određena odmah nakon uzorkovanja, a mozak, dijafragma i jetra su zamrznuti na -20 °C. Homogenizacija tkiva velikog mozga, malog mozga i moždanog stabla je izvršena korištenjem fosfatnog pufera (0,1 M, pH 8,0) u odnosu 1:20. Uzorci su homogenizovani na 10.000 rpm tokom 1 min. Homogenizovano tkivo je centrifugirano na 4 °C, 15 min na 15.000 rpm, a supernatant je korišten kao uzorak za određivanje aktivnosti AChE. Aktivnost AChE mjerena je kolorimetrijskom metodom po Ellmanu, pomoću spektrofotometra Shimadzu UV-1800 (Kjoto, Japan) i softvera UV Probe 2.17 (Kjoto, Japan) [178]. Aktivnost AChE u lizatu eritrocita izražena je kao mmol/min/mL, dok je aktivnost u mozgu i dijafragmi izražena kao mmol/min/g. Nakon toga, AChE je izražen kao procenat osnovnih vrijednosti dobijenih od kontrolnih životinja tretiranih FR. Vrijednosti CarbE su određene u plazmi i jetri. Homogenizacija jetre je izvršena FR u odnosu 1:20.

4.3.3. Metoda Marinković-Maksimović („MM“ metoda)

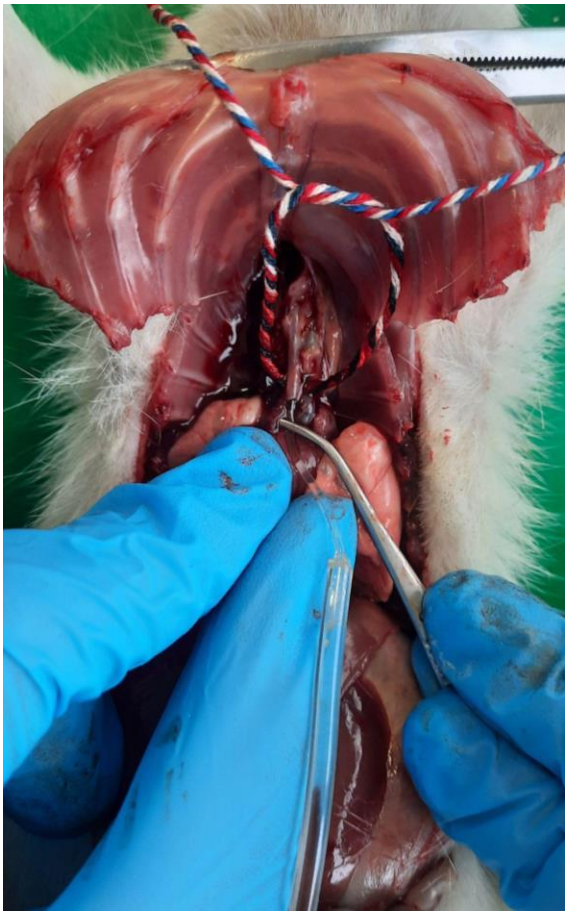
Za pristup srcu pacova i glavnim krvnim sudovima pacovi su bili vezani za operativne daske veličine 15 x 25 cm. Velikim hirurškim makazama napravljen je rez kože i mišića neposredno ispod *processus xyphoideus*, zatim su napravljeni bilateralni rezovi, prateći medijalnu aksilarnu liniju. Nakon odvajanja dijafragme od prednjeg torakalnog zida, djelimično torakotomisani prednji torakalni zid je stegnut i odložen kako bi se ostavilo čisto operativno polje (Slika 5). Da bi se postiglo iskrvarenje, donja šuplja vena je presečena neposredno iznad jetre.

Prije kanulacije aorte, ispod ascendentne aorte je uvučen konac dužine 10 cm. Uz pomoć mikromakaza napravljen je rez malog prečnika (oko 1-2 mm) na prednjem zidu ascendentne aorte, neposredno iznad korijena aorte. Vrh cijevi za kanulaciju je zatim umetnut u lumen aorte, pazeći da ne prelazi tačku prve grane luka aorte (Slika 6). Korišćenjem ranije pripremljenog konca, cijev je vezana na svoje mjesto. Trbušna aorta je klemovana, kako bi se osiguralo da FR koji se koristi za ispiranje ide samo u gornju polovinu tijela. Takođe, da bi se postigao slobodan odliv FR, gornja

šuplja vena je presječena neposredno iznad srca. Nakon toga se uradila perfuzija krvnih sudova gornjeg dijela tela sa ukupno 100 mL FR kroz sistem kanile, koristeći špriceve od 10 mL (Slika 7). Efikasnost perfuzije je posmatrana tokom same procedure posmatranjem očnih jabučica pacova – bljedilo je ukazivalo na eliminaciju krvi iz krvnih sudova oka (Slika 8).



Slika 5. Pristup torakalnoj šupljini pacova



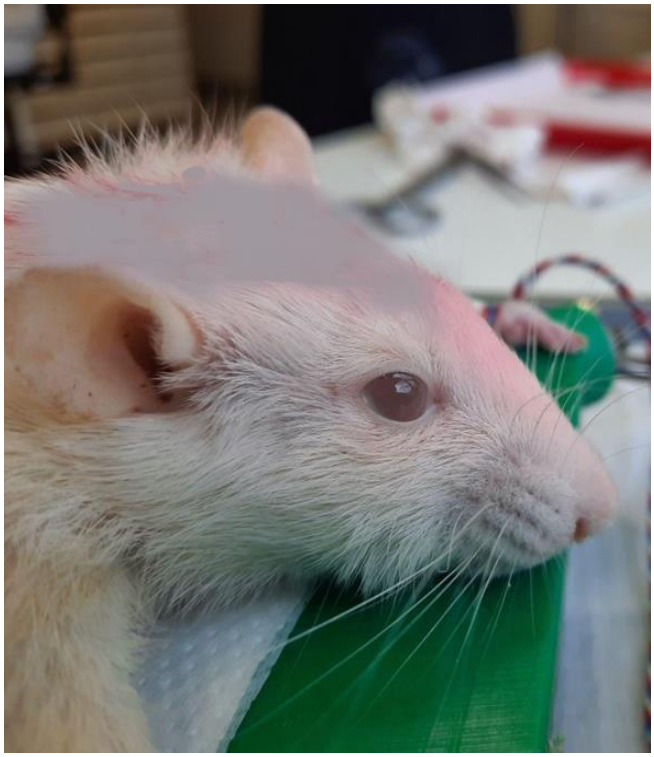
Slika 6. Kanulacija aorte pacova

Da bi se utvrdila efikasnost metode cirkulatorne perfuzije, pacovi su podijeljeni u 4 grupe. Grupe: 1. grupa: kontrola; 2. grupa: paraokson; 3. grupa: kontrola sa ispiranjem; 4. grupa: paraokson sa ispiranjem. Druga i četvrta grupa su tretirane sa 0,25 mg/kg (0,25 mg/mL rastvora) paraoksona sc, dok su prva i treća grupa tretirane sa 1 mL/kg sc FR. Pola sata kasnije, pacovi su anestezirani, torakalna šupljina pacova je otvorena i uzorci krvi su sakupljani iz donje šuplje vene u epruvete sa litijum-heparinom, nakon čega su životinje žrtvovane iskrvarenjem. Metoda „MM“ je sprovedena kod pacova iz grupe 3 i 4. Uzeti su mozgovi iz obe grupe, uključujući veliki mozak,

mali mozak i moždano stablo i čuvani na – 20 °C do homogenizacije. Određene su vrijednosti AChE u sve četiri grupe u eritrocitima i mozgu i analizirana statistička značajnost između grupa. Analizirana je i razlika u moždanoj masi između grupa.

Makroskopski pregled mozga pokazao je da je ispiranjem postignuta dobra eliminacija krvi iz moždanog tkiva (Slika 9).

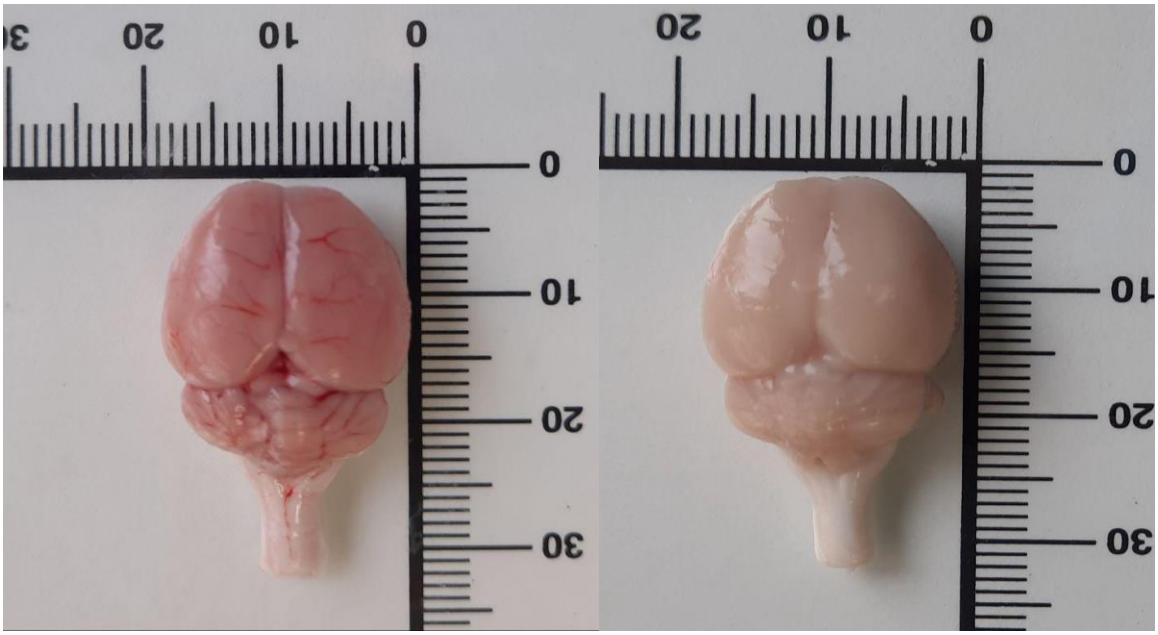
Slika 7. Postupak ispiranja



Slika 8. Bljedilo oka uočeno nakon primjene „MM“ metode

Nije bilo značajne razlike u srednjim vrijednostima moždane mase pacova između grupa kojima jeste i kojima nije sprovedena „MM“ metoda (t = 2,29, p = 0,262). Nije bilo značajne razlike između srednjih vrijednosti aktivnosti AChE u eritrocitima između grupe 1 i 3 (kontrola i kontrola sa ispiranjem) (Studentov t-test: t = 0,88, p = 0,472), kao ni između grupa 2 i 4 (grupa koja je tretirana sa paraoksonom i grupa koja je tretirana sa paraoksonom sa ispiranjem) (t = 0,45, p = 0,708). Nije bilo značajne razlike između srednje vrijednosti aktivnosti AChE u mozgu pacova

između grupa 1 i 3 (t = 0,52, p = 0,655), ali je postojala značajna razlika između srednje vrijednosti aktivnosti AChE u mozgu pacova 2 i 4 grupe (t = 5,00, p = 0,044). Aktivnost AChE je bila viša kod pacova trovanih paraoksonom kod kojih je sprovedena MM metoda u odnosu na kontrolu.



Slika 9. Makroskopski rezultati „MM“ metode. Makroskopska razlika u izgledu mozga pacova iz bez ispiranja (A) i sa ispiranjem (B)

4.3.4. Praćenje znakova trovanja

Prisustvo i intenzitet znakova trovanja paraoksonom kod životinja posmatrani su i bilježeni tokom 4 h. Posmatrani su sledeći znaci: dispneja, salivacija, lakrimacija, egzoftalmus, fascikulacije, tremor, ataksija, konvulzije, stereotipni pokreti. Njihovo prisustvo i intenzitet zabilježeni su: 5, 10, 15, 30, 60, 90, 120, 150, 180, 210 i 240 min nakon primjene paraoksona. Zabilježeno je i vrijeme od ubrizgavanja paraoksona do prve pojave znaka trovanja. Intenzitet je ocjenjivan kao: 0 - odsutan, 1 - blag / umjeren, 2 - izražen.

Praćeni su svi pacovi kojima je dat paraokson, a min kasnije tretman (FR ili antidot). Analizirani su znaci trovanja u odnosu na dozu paraoksona, kao i na ishod trovanja (preživljavanje

ili smrt). Poređen je intenzitet, prisustvo i vrijeme pojave znakova trovanja u odnosu na primijenjene antidote.

Za analizu intenziteta simptoma tokom posmatranog perioda (4 h) uveden je *ToxScore* kao parametar koji sumira intenzitet svih znakova trovanja u svim vremenskim intervalima.

„ToxScore“ je bila mjera koja je predstavljala sumu svih znakova trovanja za jednu životinju. Prilikom računanja sabirani su svi skorovi za pojedinačne znakove osim za stereotipne pokrete koji su oduzimani od ukupnog skora s obzirom da su rezultati ukazivali da je prisustvo stereotipnih pokreta prediktor preživljavanja i pozitivnog ishoda. Zbog ambivalentnih rezultata, intenzitet i pojava ataksije nisu ulazili u skor. U obzir se uzimalo: brzina nastanka znaka (0-5 bodova), intenzitet za svaki sat posmatranog vremena (do 2 boda/h). Ukoliko je pacov uginuo u toku posmatranog vremena, preostali posmatrani period se računao kao u momentu kada je uginuo.

4.3.4. Određivanje hematoloških i biohemijskih parametara kod preživelih pacova

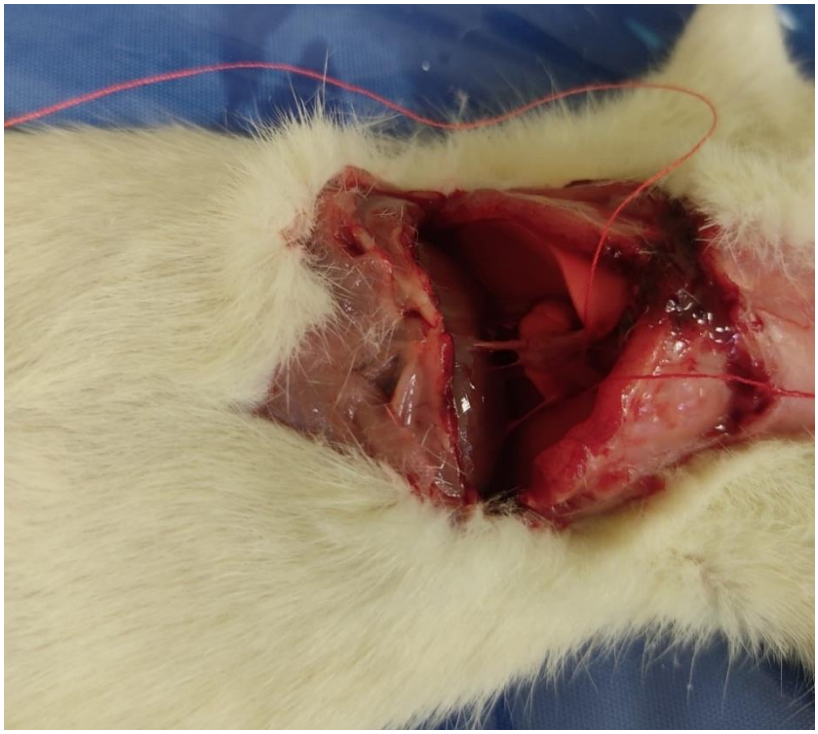
Analiza LD_{50} paraoksona, obidoksima i oksima K870 i zaštitnih indeksa rađena je na osnovu 24-h preživljavanja. Preživjeli pacovi su nakon 24 h anestezirani i uzimana im je krv za analize iz donje šuplje vene. Uzeta je jetra za određivanje aktivnosti CarbE, dijafragma za određivanje AChE, nakon čega je sprovedena „MM“ metoda, te mozak za određivanje aktivnosti AChE. Organi su čuvani na – 20 °C do homogenizacije.

Krv za hematološke analize uzimana je u epruvete sa K2-etilendiamintetrasirćetnom kiselinom (K2-EDTA), za analizu AChE iz eritrocita u epruvete sa litijum-heparinom, a za ostale biohemijske parametre iz seruma u epruvetu sa crvenim čepom bez aditiva. Iz pune krvi je analizirana kompletna krvna slika koja podrazumijeva: broj eritrocita, leukocita i trombocita, eritrocitne konstante (prosječni volumen eritrocita – *mean corpuscular volume* (MCV), prosječna količina hemoglobina u eritrocitu - *mean corpuscular haemoglobin* (MCH), prosječna koncentraciju hemoglobina na litar eritrocita - *mean cell haemoglobin concentration* (MCHC)), prosječni volumen trombocita - *mean platelet volume* (MPV), leukocitarna formula (broj neutrofila, eozinofila, bazofila, monocita i limfocita i njihov procenat u odnosu na ukupni broj leukocita), hemoglobin, hematokrit.

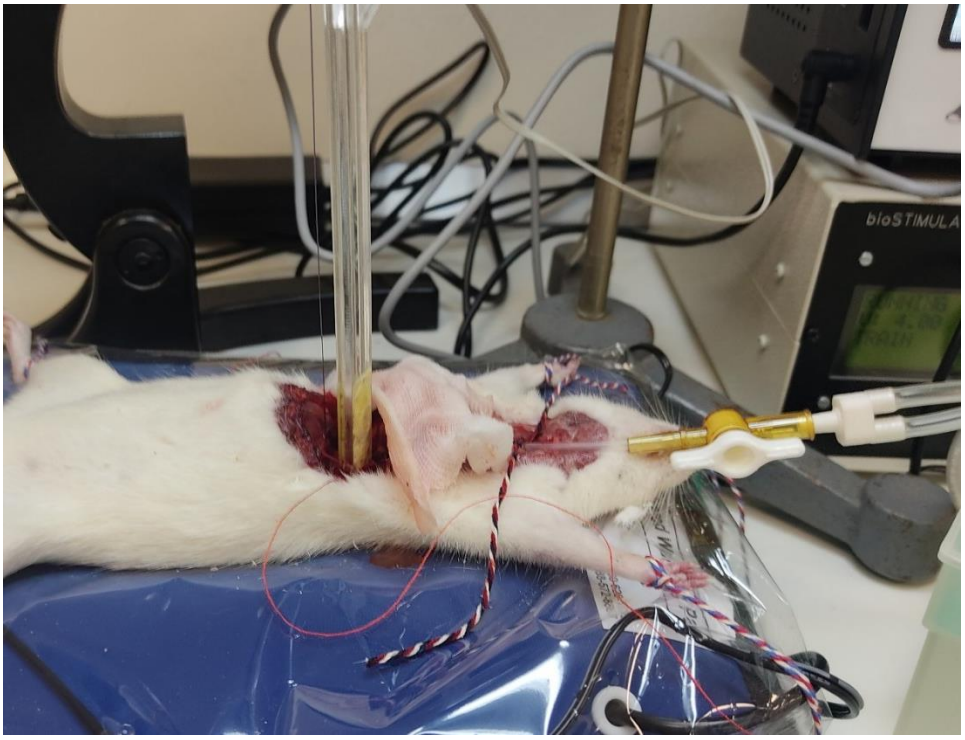
Uzorci krvi za serum su ostavljeni da stoje na sobnoj temperaturi 20 min dok se ne formira ugrušak, a zatim centrifugirani 5 min na 3.000 rpm. Svi uzorci su čuvani na -80 °C do mjerenja. Iz seruma su analizirani: visoko senzitivni troponin I (hsTnI), kreatin kinaza (CK), kreatin kinaza – izoenzim MB (CK-MB), aspartat aminotransferaza (AST), alanin aminotransferaza (ALT), γ-glutamil transferaza (GGT), amilaza, ukupni i direktni bilirubin, laktat dehidrogenaza (LDH), glukoza, ukupni holesterol (*total cholesterol* - TC), holesterol velike gustine (*high density lipoprotein* - HDL), holesterol male gustine (*low density lipoprotein* – LDL), trigliceridi (*triglycerides* - TG), urea, kreatinin, albumini i ukupni proteini, jonogram (natrijum, kalijum, kalcijum, fosfati).

4.3.5. *Nervus phrenicus*-dijafragma *in situ*

Aktivnosti AChE na neuromuskularnoj sinapsi je ispitivana na modelu *n. phrenicus* - dijafragma *in situ* po Ćetkoviću i Boškoviću (1988) [181]. Ukratko, urađena je torakotomija i stimulacija torakalnog dijela freničnog nerva uz direktnu registraciju mišićnih kontrakcija (Slika 10, 11).



Slika 10. Torakotomija i podvezivanje *n. phrenicus*-a



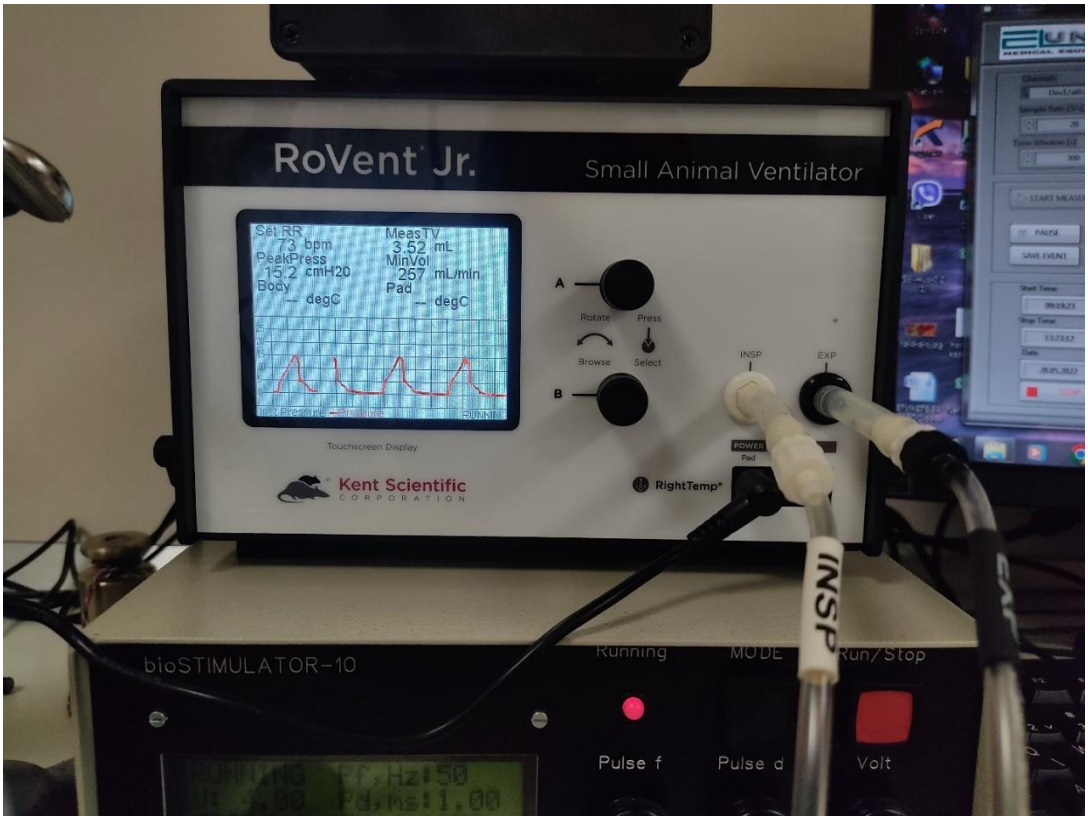
Slika 11. *Nervus phrenicus*-dijafragma *in situ* model

Životinje su anestezirane sa 25% uretanom (7 mL/kg sc), a eksperimenti su izvedeni 30 min kasnije. Traheja je intubirana i povezana sa mehaničkim ventilatorom za male životinje (RoVent Jr, Kent Scientific, Torrington, CT, USA) prije torakotomije (Slika 12).

Oba frenična nerva su bila presječena, a lijevi je bio pričvršćen za elektrodu. Tetivni dio dijafragme je bio povezan sa sistemom za snimanje pomoću transdjusera. Frenični nerv je stimulisan nizom električnih impulsa generisanih svakih 12,5 sekundi sa 50 Hz u trajanju od 0,25 sekundi. Trajanje pojedinačnog impulsa je bilo 1 ms, a napon 4 V. Korišten je bioSTIMULATOR-10 (ElUnit, Beograd, Srbija) sa pripadajućim elektrodama, transjuserom i softverom. Tetaničke kontrakcije dijafragme su bile kontinuirano snimane, a analizirana je promjena visine amplitude kontrakcije.

Prvih 15-30 min je služilo za uspostavljanje ravnoteže modela. Nakon toga, aplikovan je paraokson 0,25 mg/kg sc, a min kasnije FR 1 mL/kg im. S obzirom da na navedenu dozu nije dolazilo do promjene u intenzitetu kontrakcija, aplikovana je doza od 0,4 mg/kg paraoksona, a zatim min kasnije FR (1 mL/kg im), oksim K870 (35 mg/kg im) ili obidoksim (22 mg/kg im).

Pacov je ležao na grijanoj podlozi, a temperatura tijela je provjeravana digitalnim bezkontaktnim toplomjerom, provjerom na korijenu repa.

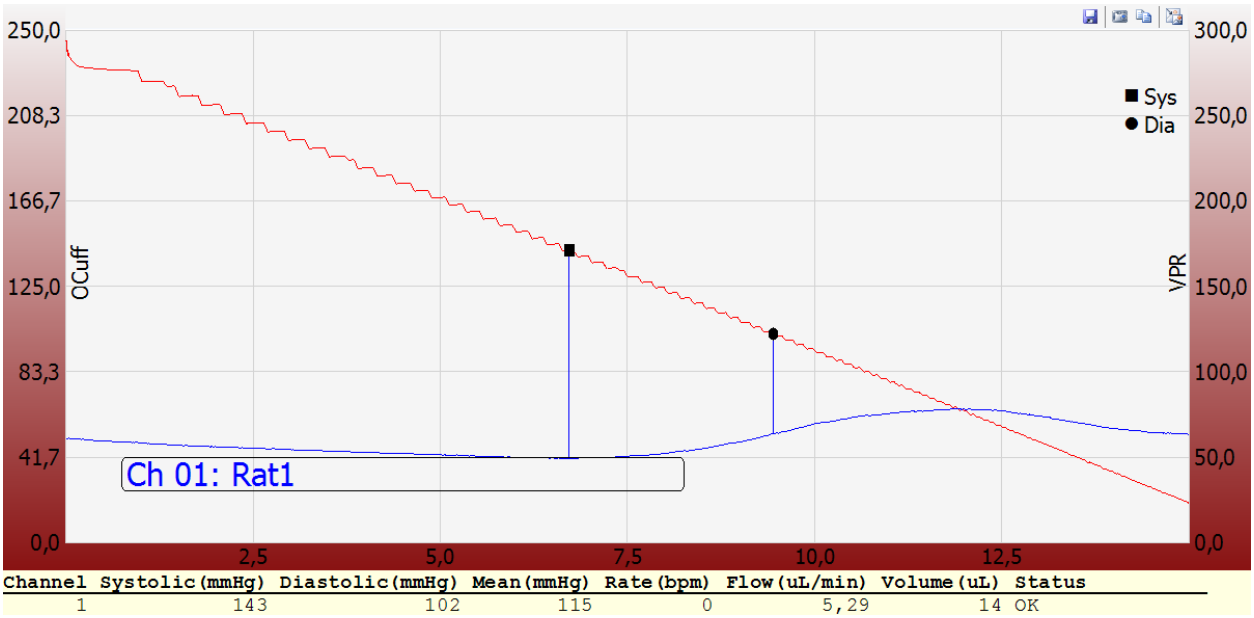


Slika 12. Ventilator za male životinje i bioSTIMULATOR-10

4.3.6. Praćenje kardiovaskularnih i plućnih parametara

Analiziran je efekat paraoksona, kao i uticaj antidota na kardiovaskularne i respiratorne parametre pacova. Učinjen je minimalni rez ispod *processus xyphoideusa*, a *ligamentum falciformis* je bio povezivan za transdjuser povezan sa odgovarajućim softverom (eLABS, ElUnit, Beograd, Srbija). Oscilacije su prikazivale intenzitet, učestalost i trajanje kontrakcija dijafragme, te su služile za analizu respiracija pacova.

Krv za gasne analize je uzorkovana iz arterijske krvi punkcijom *a. femoralis* neposredno prije, 5, 15, 30, 60, 90, 120, 150 i 180 min nakon aplikacije paraoksona. U istim vremenima, rađen je i elektrokardiogram (EKG), kao i mjerenje krvnog pritiska. Analizirani su pH krvi, parcijalni pritisak kiseonika i ugljen-dioksida, vrijednosti laktata, bikarbonata, bazni višak (*base excess* – BE).



Slika 13. Softverski prikaz neinvazivno izmjerenog krvnog pritiska

Sistolni, dijastolni, kao i srednji AP mjeren je na repu pomoću CODA Non-Invasive Blood Pressure System (Kent Scientific, Torrington, CT, USA) (Slika 13). Korišten je EKG aparat CardioTouch 3000 za humanu upotrebu, ali sa prilagođenim elektrodama koje su se stavljale na sva četiri ekstremiteta pacova. Rađen je 6-kanalni EKG. Za analizu je korišten II odvod, u kom su praćeni srčana frekvencija (otkucaj/min), QT interval (s) i amplituda QRS mjerena od vrha do vrha (mV). Osim toga, praćena je i bilježena pojava specifičnih poremećaja srčanog ritma.

Aplikovan je paraokson 0,25 mg/kg sc, a zatim min kasnije FR (1 mL/kg im), atropin (10 mg/kg im), N-butilskopolamin (63,36 mg/kg im – doza ekvimolarna dozi atropina), oksim K870 (35 mg/kg im) ili obidoksim (22 mg/kg im).

4.4. **Statistika**

LD_{50} i zaštitni indeksi su analizirani metodom prema Litchfieldu i Wilcoxonu (1949) na *Pharm/PCS* statističkom softveru [177]. Ostale analize su obavljene na *IBM SPSS for Windows*, verzija 18.0. Za provjeru ravnomjernosti distribucije podataka rađen je Kolmogorov-Smirnov test, a nakon toga odgovarajuća parametrijska ili neparametrijska statistika. Kategorijski podaci su poređeni pomoću Fisher egzaktnog testa, a kontinualni pomoću Student t-testa (ili Man Whitney U-testa) ako se radilo o dvije grupe i jednosmjerna analiza varijanse (ANOVA) (ili neparametrijski Kruskal-Wallis test) ako se radilo o više grupa. Dvije varijable sa kontinualnim podacima su poređene pomoću Spirmanove korelacije. Nivo statističke značajnosti je postavljen na $p < 0,05$.

5. REZULTATI

5.1. Srednja smrtna doza (LD_{50}) paraoksona i oksima

Primijenjene doze paraoksona, obidoksima i oksima K870, broj uginulih pacova u grupi i LD_{50} paraoksona i oksima su prikazani u Tabeli 1. LD_{50} paraoksona iznosio je 0,33 mg/kg sc. Svi pacovi su uginuli unutar prvog sata od primjene otrova. LD_{50} obidoksima iznosio je 108,33 mg/kg im, a oksima K870 172,32 mg/kg im. To odgovara LD_{50} = 301,59 μmol/L za obidoksim i 326,96 μmol/L za oksim K870.

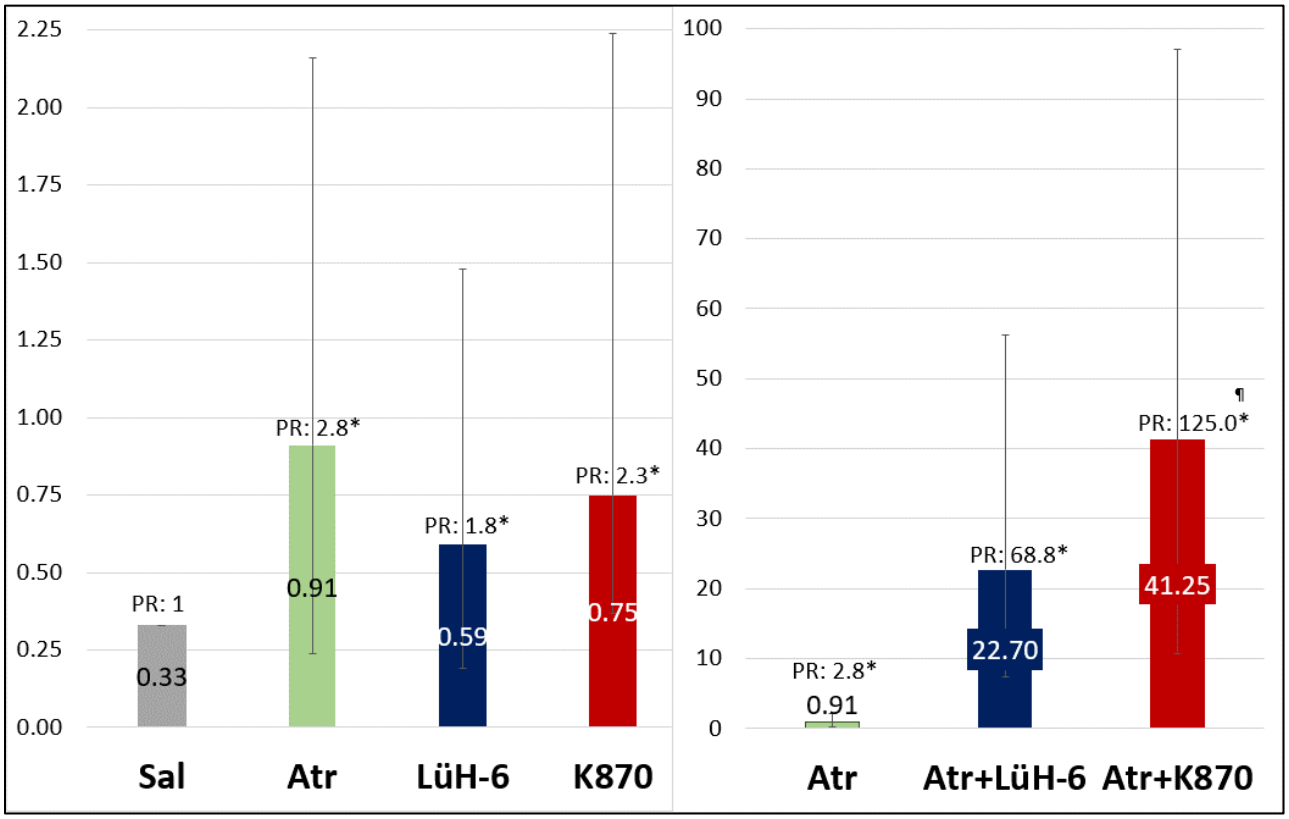
Tabela 1: Srednja smrtna doza (LD_{50}) paraoksona i oksima

Doza	**Broj pacova**	**Litchfield i Wilcoxon (1949)**		
(mg/kg)	**(D/T)**	**LD_{50}**	**F**	**95% CI**
Paraokson (sc)				
0,25	0/6			
0,30	3/12	0,33	1,14	0,31-0,36
0,35	7/12			
0,40	11/12			
Obidoksim (im)				
50	0/5			
100	2/5	108,33	1,46	73,99-158,61
150	4/5			
200	5/5			
Oksim K870 (im)				
100	0/5			
150	2/5	172,32	1,18	145,49-204,09
200	4/5			

D/T: *dead/total* – uginuli/ukupni broj pacova u grupi; LD_{50}: *median lethal dose*; CI: *confidence interval* – interval povjerenja; sc: subkutano; im: intramuskularno; Primijenjene zapremine su bile 1 mL/kg, osim za oksim K870 u dozi 200 mg/kg, gde je dato 2 mL/kg im, po 1 mL/kg u svaki butni mišić.

5.2. Zaštitni indeksi

LD_{50} paraoksona kada su primijenjeni atropin i/ili oksimi je prikazan na Slici 14. Zaštitni indeks atropina je bio 2,76 (LD_{50} = 0,91), obidoksima 1,79 (LD_{50} = 0,59), a oksima K870 2,27 (LD_{50} = 0,75). Zaštitni indeks sva tri ispitivana antidota bio je statistički značajano više u odnosu na FR. Kombinacija atropina i oksima dala je veoma visok zaštitni indeks: 125,00 (LD_{50} = 41,25) kod kombinacije atropina i oksima K870 i 68,79 (LD_{50} = 22,70), kod atropina i obidoksima. Kombinacija atropin-oksim K870 je značajno boje štitila od trovanja paraoksonom od kombinacije atropin-obidoksim, sa relativnim zaštitnim indeksom od 1,82 (Slika 14).



Slika 14. Srednja smrtna doza (LD_{50}) paraoksona i zaštitni indeksi (PRs) u zavisnosti od tretmana

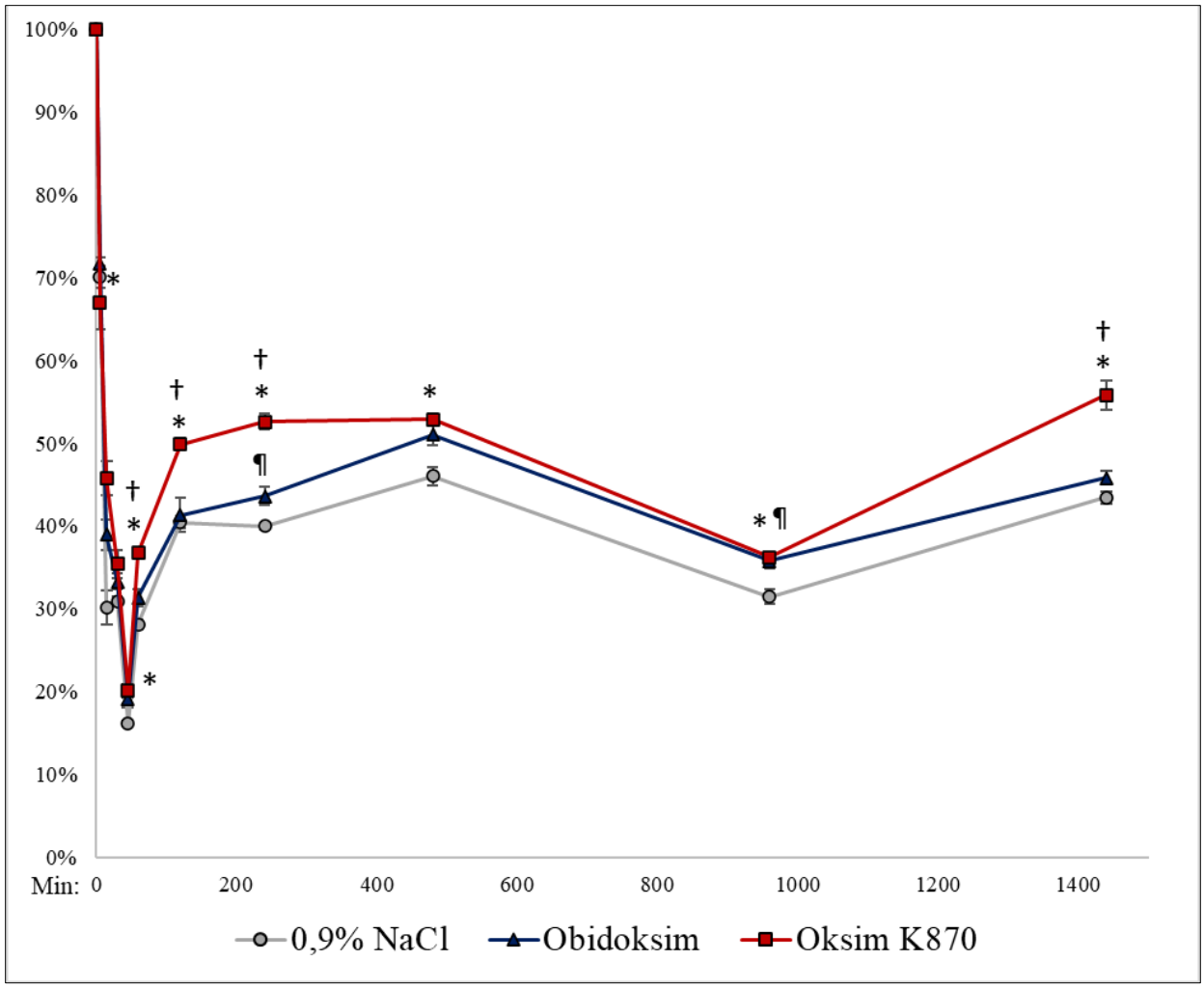
Sal: *saline* – fiziološki rastvor (1 mL/kg –im); Atr: atropin (10 mg/kg im); LüH-6 (obidoksim): (22 mg/kg im), K870: oksim K870 (35 mg/kg im); Y osa pokazuje dozu paraoksona (mg/kg sc). Bar linije pokazuju 95%-interval povjerenja. *: statistički značajno u odnosu na fiziološki rastvor; ¶: statistički značajno u odnosu na atropin + obidoksim (relativni zaštitni indeks: 1,82);

Antidoti su značajno uticali i na vremenski period od aplikacije paraoksona do pojave smrtnog ishoda. Pri dozi 0,30 mg/kg paraoksona, prosječno vrijeme smrti nakon aplikacije paraoksona je iznosilo 22,00 ± 3,61 min, pri dozi 0,35 16,67 ± 7,53 min kada je 1 min kasnije ordiniran FR, a 188,00 min kada je dat obidoksim. Pri dozi 0,40 mg/kg sc paraoksona, prosječno vrijeme smrti je iznosilo 18,09 ± 6,99 min, a nakon aplikacije oksima, svi pacovi su živjeli duže od posmatranog perioda (4 h). I pri visokim dozama paraoksona samo je pri dozi 42,0 mg/kg sc, kada je 1 min kasnije dat atropin i obidoksim jedan pacov uginuo u 12. min, a pri dozi 48,0 mg/kg sc i tretmanom atropinom i oksimom K870 jedan pacov uginuo u 115. min. Svi ostali pacovi su živjeli duže od opserviranog perioda (4 h).

5.3. Inhibicija acetilholinesteraze i karboksilesteraze

5.3.1. Aktivnost acetilholinesteraze

Aktivnost AChE u mozgu su prikazane na Slikama 15-17. Aktivnost AChE u velikom mozgu naglo je opala na minimum od 16,1% kontrolnih vrijednosti 45 min nakon primjene paraoksona. Spontana reaktivacija je primijećena nakon toga, sa najvišom vrijednošću od 46,0% registrovanom 480 min nakon primjene paraoksona. Konačna registrovana aktivnost enzima (poslije 24 h) u velikom mozgu iznosila je 43,4%. Aktivnosti AChE u velikom mozgu kod pacova štićenih oksimima takođe su bile najniže 45 min nakon paraoksona, 20,1% nakon oksima K870 i 19,1% nakon obidoksima. Najveća aktivnost AChE kod pacova štićenih oksimom K870 registrovana je 24 h poslije paraoksona (55,8%), dok je najveća aktivnost AChE kod pacova štićenih obidoksimom registrovana 480 min nakon paraoksona (51,0%). Značajna razlika u inhibiciji AChE kod pacova tretiranih oksimom K870 u poređenju sa FR nađena je u svim vremenima, a kod pacova tretiranih obidoksimom u poređenju sa FR značajna razlika je nađena samo 240 i 960 min nakon primjene paraoksona. Aktivnost AChE u velikom mozgu kod pacova tretiranih oksimom K870 u poređenju sa obidoksimom bila je značajno veća 60, 120, 240 i 1440 min nakon administracije paraoksona (Slika 15).

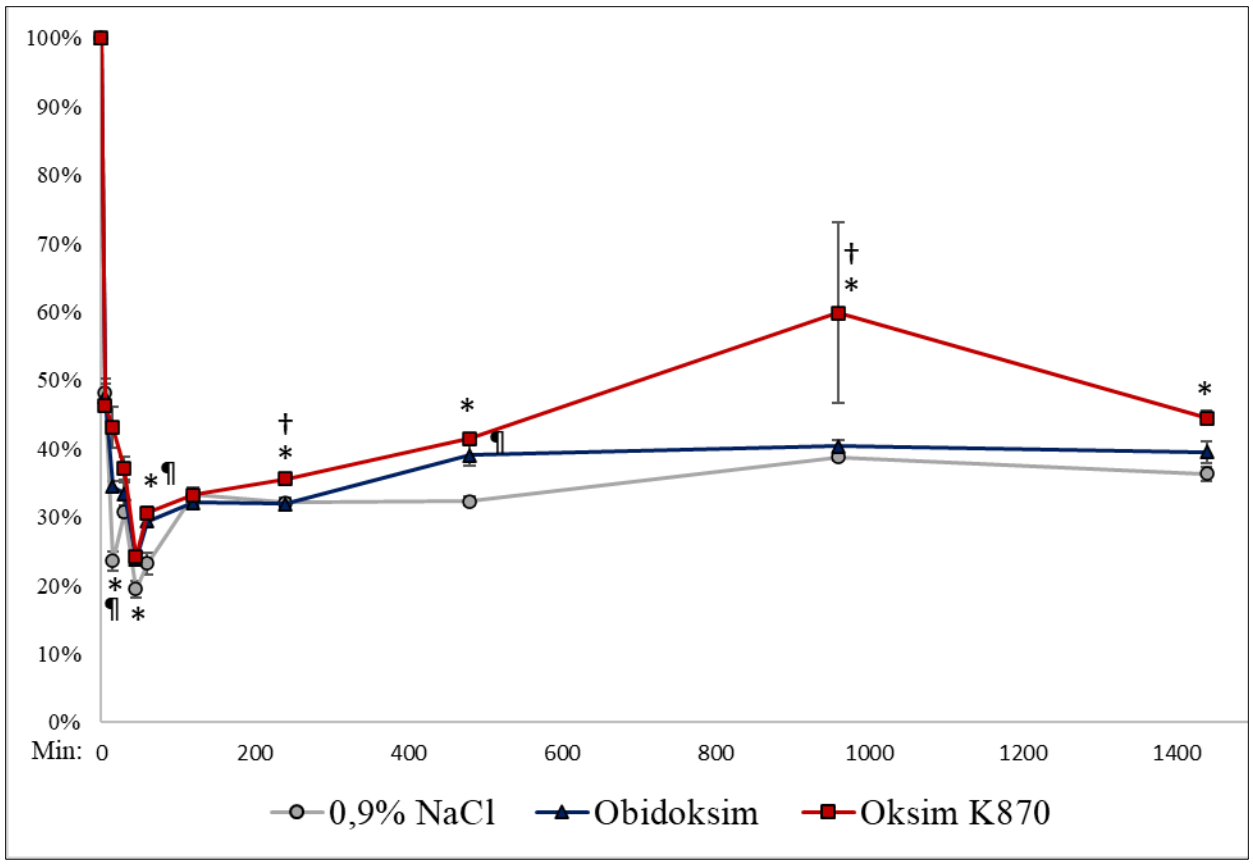


Slika 15. Inhibicija acetilholinesteraze (AChE) tokom 24 časa u velikom mozgu pacova trovanih paraoksonom i tretiranih fiziološkim rastvorom (0,9% NaCl), obidoksimom ili oksimom K870

Kruskal-Wallisov test, statistička značajnost: *Oksim K870 vs fiziološki rastvor; ¶Obidoksim vs fiziološki rastvor; †Oksim K870 vs obidoksim.

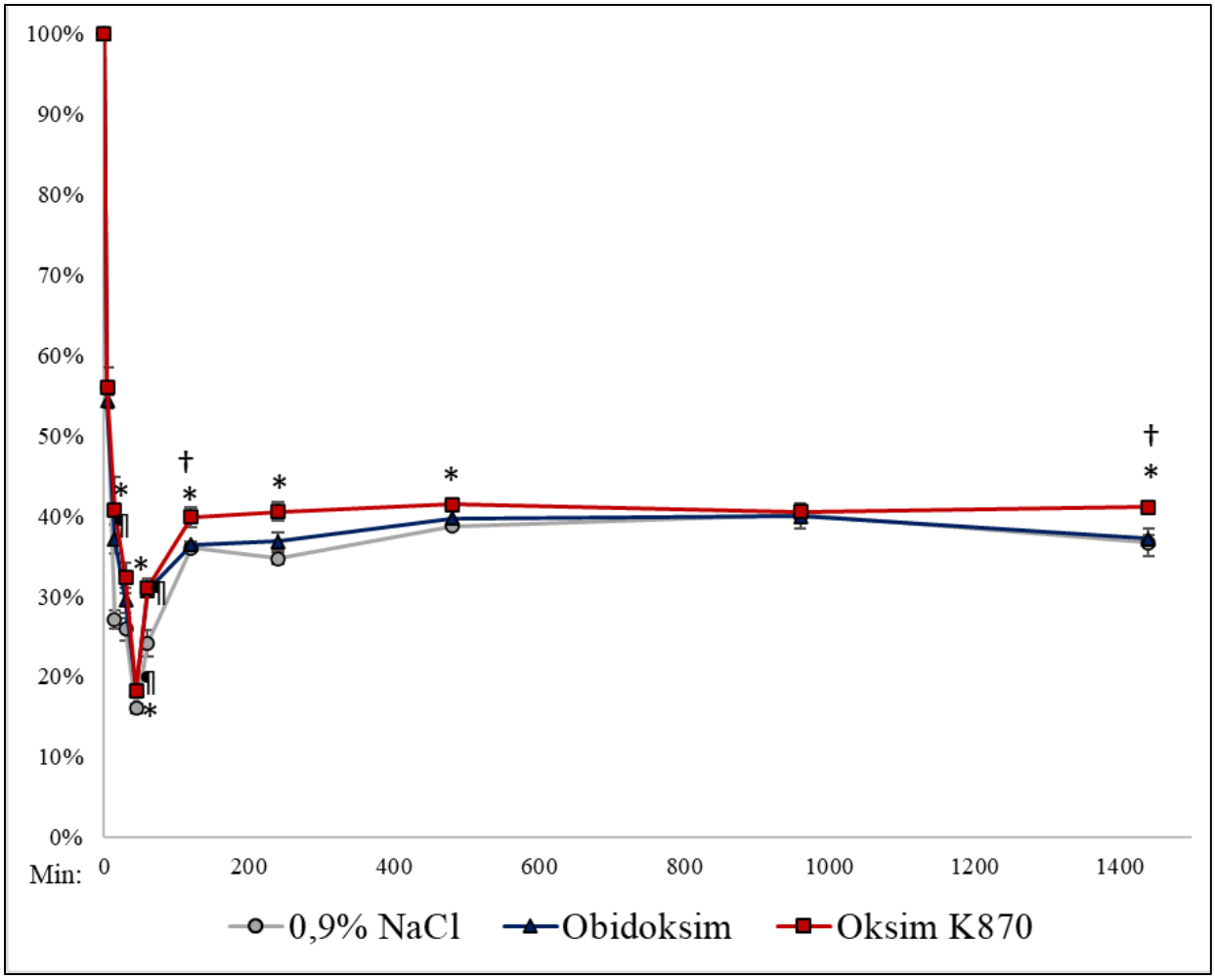
Nakon primjene paraoksona aktivnost AChE u malom mozgu je naglo opala na minimum od 19,4% kontrolnih vrijednosti 45 min nakon primjene paraoksona. Spontana reaktivacija je primijećena nakon toga, sa najvišom vrijednošću od 38,8% 960 min nakon primjene paraoksona. Konačna registrovana aktivnost enzima (poslije 24 h) iznosila je 36,3%. Aktivnosti AChE u malom mozgu kod pacova štićenih oksimima takođe su bile najniže 45 min nakon paraoksona, 24,2% za oksim K870 i 23,8% za obidoksim. Najveća vrijednost AChE aktivnosti kod pacova štićenih

oksimima registrovana je 960 min poslije paraoksona, 59,9% za oksim K870 i 40,4% za obidoksim. Značajna razlika u inhibiciji AChE kod pacova tretiranih oksimom K870 u poređenju sa FR nađena je u svim vremenima, a kod pacova tretiranih obidoksimom u poređenju sa FR značajna razlika je nađena samo 15, 60 i 480 min nakon paraoksona. Vrijednosti AChE u malom mozgu kod pacova tretiranih oksimom K870 u poređenju sa obidoksimom bile su značajno veće 240 i 960 min nakon paraoksona (Slika 16).



Slika 16. Inhibicija acetilholinesteraze (AChE) tokom 24 časa u malom mozgu pacova trovanih paraoksonom i tretiranih fiziološkim rastvorom (0,9% NaCl), obidoksimom ili oksimom K870

Kruskal-Wallisov test, statistička značajnost: *Oksim K870 vs fiziološki rastvor; ¶Obidoksim vs fiziološki rastvor; †Oksim K870 vs obidoksim.



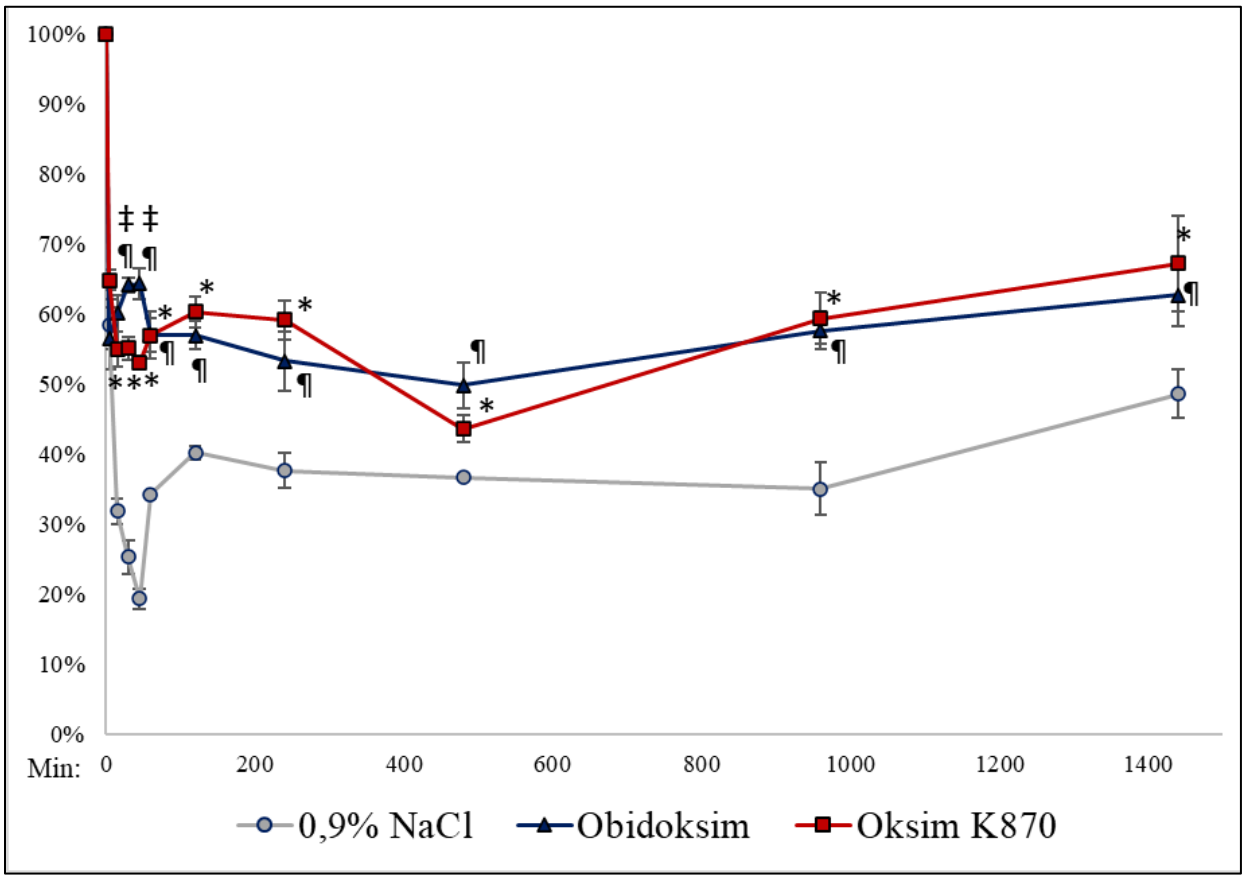
Slika 17. Inhibicija acetilholinesteraze (AChE) tokom 24 časa u moždanom stablu pacova trovanih paraoksonom i tretiranih fiziološkim rastvorom (0,9% NaCl), obidoksimom ili oksimom K870

Kruskal-Wallisov test, statistička značajnost: *Oksim K870 vs fiziološki rastvor; ¶Obidoksim vs fiziološki rastvor; †Oksim K870 vs obidoksim.

Aktivnost AChE u moždanom stablu se takođe naglo smanjila nakon primjene paraoksona na minimum od 16,1% kontrolnih vrijednosti 45 min nakon primjene paraoksona. Spontana reaktivacija je primijećena nakon toga, sa najvišom vrijednošću od 40,1% 960 min nakon primjene paraoksona. Konačna registrovana aktivnost enzima (poslije 24 h) bila je 36,7%. Aktivnosti AChE kod pacova štićenih oksimima takođe su bile najniže 45 min nakon paraoksona, 18,3% kod pacova tretiranih oksimom K870 i 18,4% obidoksimom. Najveća vrijednost aktivnosti AChE kod pacova

štićenih oksimom K870 registrovana je 480 min nakon paraoksona (41,4%), dok je najveća aktivnost AChE kod pacova štićenih obidoksimom registrovana 960 min (39,9%) nakon paraoksona. Reaktivacija AChE oksimima bila je niža nego u drugim analiziranim regionima mozga. Ipak, aktivnost AChE bila je značajno veća kod pacova tretiranih oksimom K870 oksimom u poređenju sa FR u svim vremenskim tačkama, a pacova tretiranih obidoksimom u poređenju sa FR samo u 15, 45. i 60. min. Aktivnosti AChE u moždanom stablu bile su statistički značajno veće kod pacova tretiranih oksimom K870 u poređenju sa obidoksimom u 120. i 1440. min (Slika 17).

Inhibicija aktivnosti AChE u dijafragmi prikazana je na Slici 18.

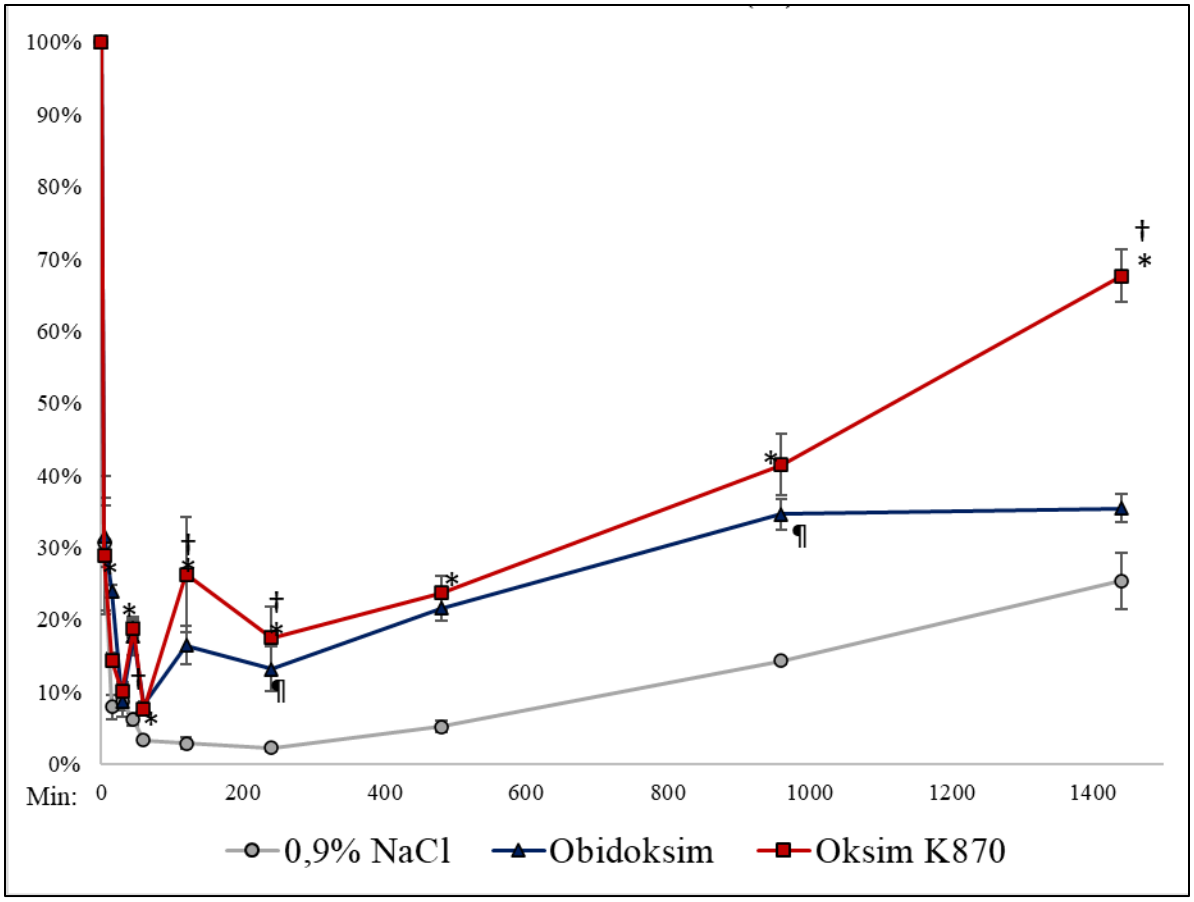


Slika 18. Inhibicija acetilholinesteraze (AChE) tokom 24 časa u dijafragmi pacova trovanih paraoksonom i tretiranih fiziološkim rastvorom (0,9% NaCl), obidoksimom ili oksimom K870

Kruskal-Wallisov test, statistička značajnost: *Oksim K870 vs fiziološki rastvor; ¶Obidoksim vs fiziološki rastvor; †Oksim K870 vs obidoksim.

Najmanja inhibicija aktivnosti AChE nakon primjene paraoksona od svih analiziranih tkiva zabilježena je u dijafragmi. Najniža aktivnost je zabilježena nakon 45 min (19,3%), a nakon toga je primijećena spontana reaktivacija, sa 48,7% od kontrolnih vrijednosti zabilježenih 24 h nakon primjene paraoksona. Inhibicija AChE bila je značajno niža kod pacova tretiranih sa oba oksima u svim vremenskim tačkama. Najniže aktivnosti AChE za oksim K870 i obidoksim su zabilježene 480 min nakon primjene paraoksona, 43,6%, odnosno 49,9%. Najveća vrijedost AChE aktivnosti kod pacova štićenih oksimom K870 registrovana je 24 h nakon paraoksona (67,2%), dok je najveća aktivnost AChE kod pacova štićenih obidoksimom registrovana 45 min nakon paraoksona (64,4%). Razlika između stepena inhibicije AChE kod pacova tretiranih obidoksimom u odnosu na pacove tretiranih oksimom K870 bila je značajna samo u 15. i 30. min - vrijednosti AChE su bile više kod pacova tretiranih obidoksimom (Slika 18).

Inhibicija AChE u eritrocitima prikazana je na Slici 19. Najizraženija inhibicija AChE nakon trovanja paraoksonom u analiziranim tkivima zabilježena je u eritrocitima. Aktivnost AChE je naglo opala, nakon 15 min iznosila je samo 7,9% kontrolnih vrijednosti, dok je minimum od 2,2% zabilježen 240 min nakon primjene paraoksona. Spontana reaktivacija je polako počinjala nakon toga, sa najvišom vrijednošću od 25,4% zabilježenom 24 h nakon primjene paraoksona. Aktivnosti AChE kod pacova štićenih oksimima bile su najniže 60 min nakon primjene paraoksona, 7,6% i 8,2% nakon oksima K870 i obidoksima. Najveće vrijednosti aktivnosti AChE kod pacova štićenih oksimom K870 i obidoksimom registrovane su 24 h poslije paraoksona, 67,6% i 35,5%. Uočena je značajna razlika u inhibiciji AChE kod pacova tretiranih i oksimom K870 i obidoksimom u poređenju sa FR u svim vremenskim tačkama. Vrijednosti AChE u eritrocitima kod pacova tretiranih oksimom K870 u poređenju sa obidoksimom bile su značajno niže 15 min, a više 960 i 1440 min nakon paraoksona.



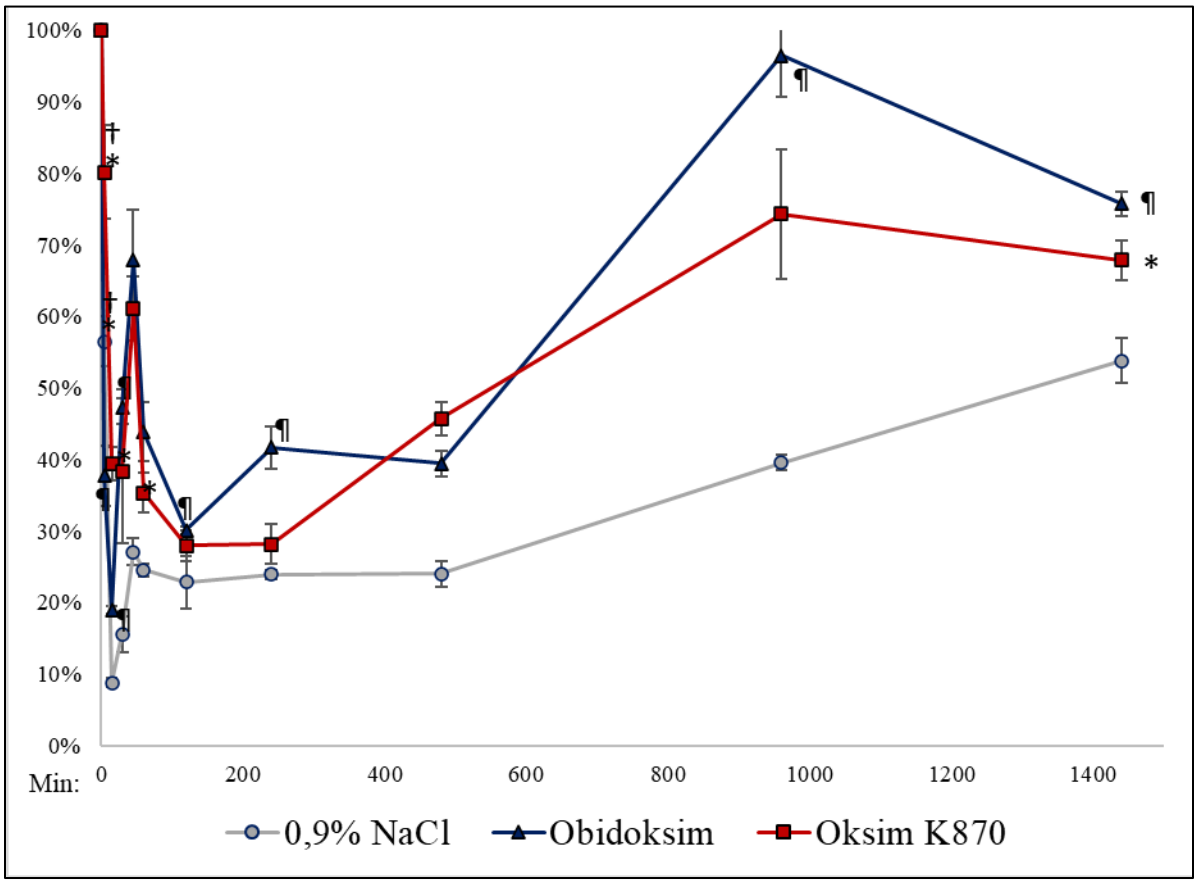
Slika 19. Inhibicija acetilholinesteraze (AChE) tokom 24 časa u eritrocitima pacova trovanih paraoksonom i tretiranih fiziološkim rastvorom (0,9% NaCl), obidoksimom ili oksimom K870

Kruskal-Wallisov test, statistička značajnost: *Oksim K870 vs fiziološki rastvor; ¶Obidoksim vs fiziološki rastvor; †Oksim K870 vs obidoksim.

5.3.2. Aktivnost karboksilesteraze

Procenat inhibicije CarbE u plazmi pacova otrovanih paraoksonom i tretiranih FR, obidoksimom ili oksimom K870 prikazan je na Slici 20. U plazmi je nakon primjene paraoksona aktivnost CarbE naglo opala - nakon 15 min zabilježen je minimum - 8,8% kontrolnih vrijednosti. Nakon toga, primijećena je spontana reaktivacija, sa najvišom vrijednošću od 53,8% registrovanom 24 h nakon primjene paraoksona. Aktivnosti CarbE kod pacova štićenih obidoksimom su takođe bile najniže 15 min nakon paraoksona (19,0%), dok su kod oksima K870 najniže vrijednosti zabilježene 120 min nakon primjene paraoksona (27,9%). Najviše vrijednosti

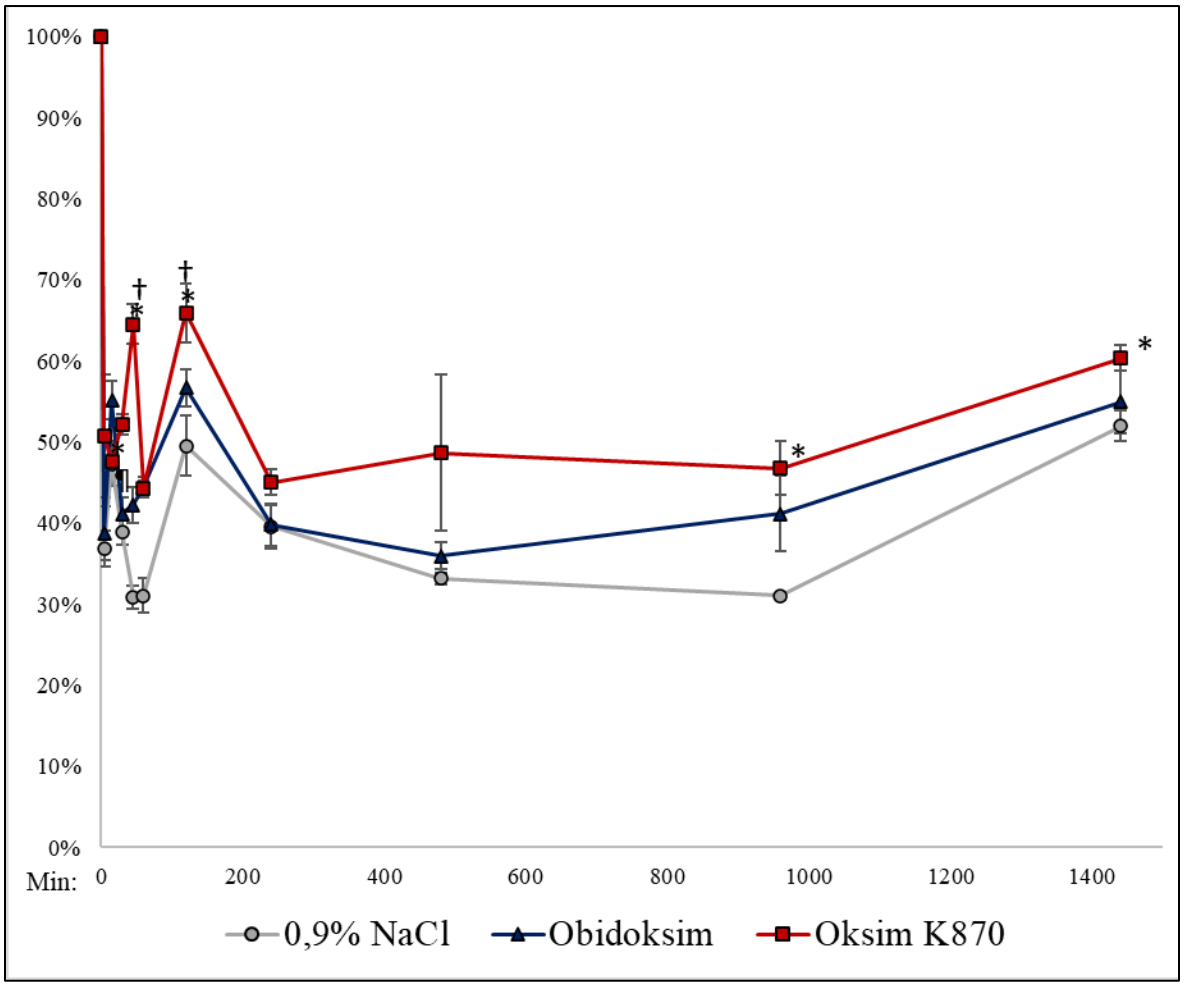
aktivnosti CarbE kod pacova štićenih oksimom K870 i obidoksimom registrovane su 960 min nakon primjene paraoksona (74,3% i 96,5%). Post-hoc analiza je pokazala značajnu razliku u inhibiciji CarbE kod pacova tretiranih obidoksimom u poređenju sa FR u 5, 15, 30, 45, 240. i 1440. min, a kod pacova tretiranih oksimom K870 u poređenju sa FR u 5, 15, 30, 45. i 1440. min. Vrijednosti CarbE u plazmi kod pacova tretiranih oksimom K870 u poređenju sa obidoksimom bile su značajno više 5 i 15, a niže 240 i 1440 min nakon primjene paraoksona.



Slika 20. Inhibicija karboksilesteraze (CarbE) tokom 24 časa u plazmi pacova trovanih paraoksonom i tretiranih fiziološkim rastvorom (0,9% NaCl), obidoksimom ili oksimom K870

Kruskal-Wallisov test, statistička značajnost: *Oksim K870 vs fiziološki rastvor; ¶Obidoksim vs fiziološki rastvor; †Oksim K870 vs obidoksim.

Inhibicija CarbE u jetri prikazana je na Slici 21.



Slika 21. Inhibicija karboksilesteraze (CarbE) tokom 24 časa u jetri pacova trovanih paraoksonom i tretiranih fiziološkim rastvorom (0,9% NaCl), obidoksimom ili oksimom K870

Kruskal-Wallisov test, statistička značajnost: *Oksim K870 vs fiziološki rastvor; ¶Obidoksim vs fiziološki rastvor; †Oksim K870 vs obidoksim.

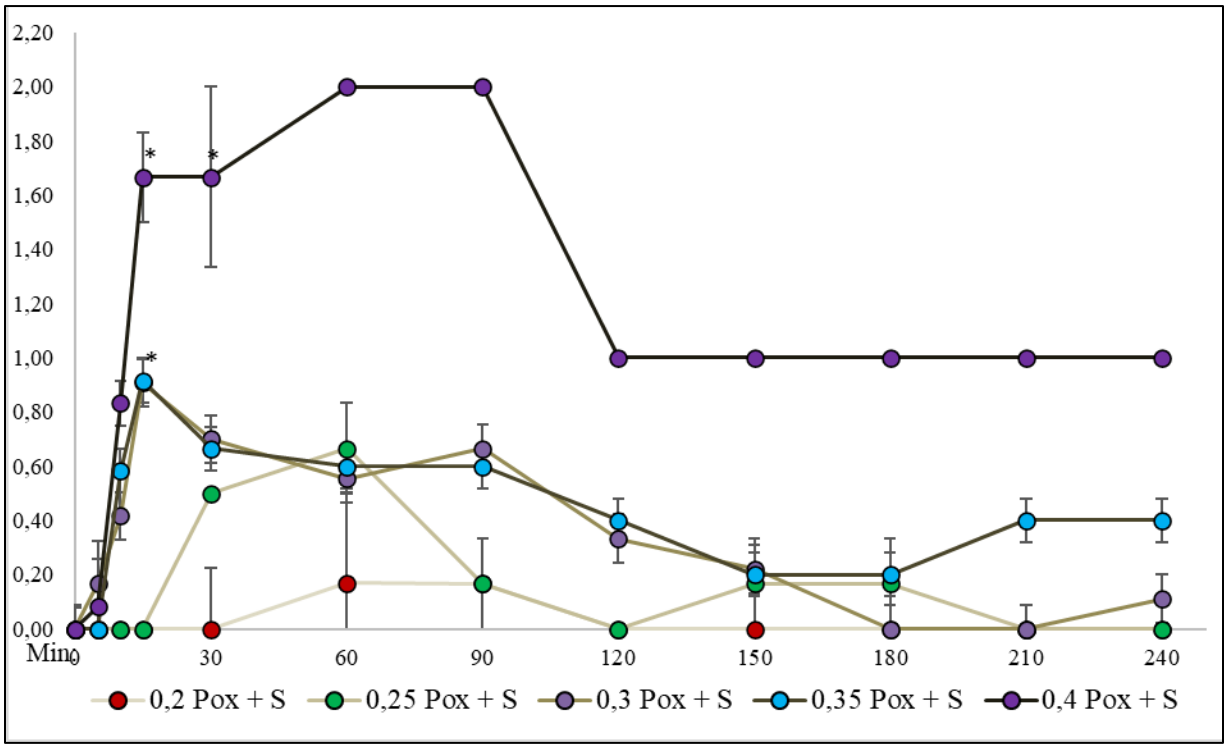
U jetri se aktivnost CarbE naglo smanjila na 36,8% početnih vrijednosti 5 min nakon primjene paraoksona, a minimum aktivnosti CarbE zabilježen je nakon 45 min (30,9%). Nakon početnog smanjenja, zabilježene su blage oscilacije vrijednosti CarbE, sa najvišom vrijednošću od 52,0%, 24 h nakon paraoksona. Sličan trend je zabilježen i kod pacova tretiranih oksimima. Kod

pacova tretiranih oksimom K870 najniža aktivnost CarbE je zabilježena 60 min nakon paraoksona (44,3%), a najviša 120 min nakon paraoksona (66,0%). Kod pacova tretiranih obidoksimom najniža aktivnost CarbE je zabilježena 480 min nakon paraoksona (36,0%), a najviša 120 min nakon paraoksona (56,7%). Primijećena je značajna razlika u inhibiciji CarbE kod pacova tretiranih obidoksimom u poređenju sa FR nakon 45 min i pacovima tretiranim K870 oksimom u poređenju sa FR nakon 30, 45, 120, 960 i 1440 min. Vrijednosti CarbE u jetri bile su značajno više kod pacova tretiranih oksimom K870 u poređenju sa obidoksimom nakon 30 i 45 min.

5.4. Praćenje znakova trovanja

5.4.1. Konvulzije

Intenzitet i vrijeme pojave konvulzija kod pacova tretiranih FR prikazani su na Slici 22 i u Tabeli 2.



Slika 22. Intenzitet konvulzija pri različitim dozama paraoksona kod neštićenih pacova

Pox: paraokson, sc; S: *saline* – fiziološki rastvor, 1 mL/kg, im; *: statistički značajno;

Značajna razlika u intenzitetu konvulzija nađena je u 10. (Kruskal-Wallis test, $\chi^2 = 10{,}168$, $p = 0{,}038$), 15. ($\chi^2 = 20{,}301$, $p < 0{,}001$) i 30. min ($\chi^2 = 9{,}704$, $p = 0{,}046$). U 15. min intenzitet konvulzija pri dozi paraoksona 0,4 je bio veći nego pri dozi 0,20 i 0,25 ($p < 0{,}001$ i $p < 0{,}001$), a u 30. min veći nego pri dozi 0,20 ($p = 0{,}013$). Nakon sat vremena kod većine preživjelih pacova nisu bilježene dalje konvulzije, s tim da je prosječan intenzitet grupe i dalje bio viši kod većih doza, ali bez značajne razlike.

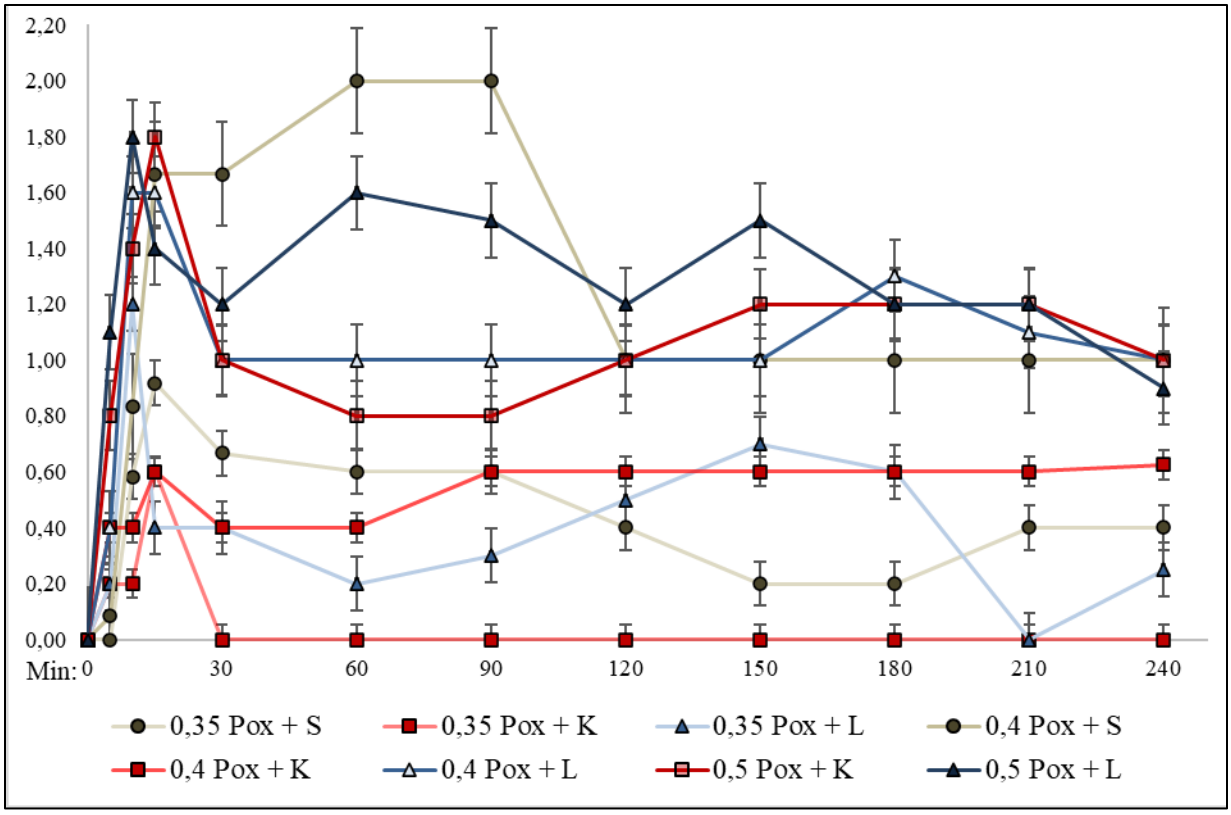
Tabela 2. Vrijeme pojave i učestalost konvulzija pri različitim dozama paraoksona kod neštićenih pacova

POX doza	**Vrijeme pojave konvulzija (min)**				**Učestalost pojave konvulzija (%)**			
	Svi	P	U	p (P)	Svi	P	U	p (P)
0,2	57,00 ± 0,00	57,00 ± 0,00	-	-	16,67	16,67	-	-
0,25	35,25 ± 20,81	35,25 ± 20,81	-	-	66,67	66,67	-	-
0,3	24,78 ± 25,67	33,67 ± 27,69	7,00 ± 3,00	**0,020**	75,00	60,00	100,00	0,509
0,35	18,10 ± 18,73	36,00 ± 29,51	10,43 ± 2,64	**0,015**	83,33	66,69	100,00	0,152
0,4	10,42 ± 3,23	13,00 ± 0,00	10,18 ± 3,28	0,459	100,00	100,00	100,00	-
p (D)	**p = 0,024**	11,53 ± 5,33	8,70 ± 4,33	0,110	70,27	59,26	100,00	**< 0,001**

POX: paraokson, doza subkutano; P: preživjeli 24 h nakon aplikacije paraoksona; U: uginuli; p(P): statistička značajnost u odnosu na to da li je pacov preživio; p(D): statistička značajnost u odnosu na dozu paraoksona;

Konvulzije su se javljale češće ($U = 93{,}00$, $p = 0{,}003$) i ranije ($\chi^2 = 11{,}207$, $p = 0{,}024$) pri većim dozama, kao i kod pacova koji nisu preživjeli. Nađena je značajna negativna korelacija između doze i vremena pojave konvulzija (Spearman, $r = -0{,}521$, $p = 0{,}001$). Bez obzira na dozu, svi pacovi koji su uginuli imali su konvulzije, a preživelih samo 60% pri dozi 0,3, 67% pri dozi 0,35% i 100% pri dozi 0,4 mg/kg sc paraoksona (Fisher test, $\chi^2 = 12{,}444$, $p < 0{,}001$).

Intenzitet i vrijeme pojave konvulzija kod pacova tretiranih oksimima (kao i uporedne doze sa FR) prikazani su na Slici 23 i u Tabeli 3.



Slika 23. Vrijeme pojave i učestalost konvulzija pri različitim dozama paraoksona kod pacova tretiranih oksimima

Pox: paraoxon sc; Sal: *saline* – fiziološki rastvor (1 mL/kg, im); L: (LüH-6 - obidoksim): (22 mg/kg im), K: oksim K870 (35 mg/kg im);

Pri istim dozama paraoksona, pacovi tretirani oksimima su imali niži intenzitet konvulzija nego oni koji su tretirani sa FR, međutim, usljed velike devijacije intenziteta unutar grupe, razlike nisu bile statistički značajne. Sat vremena nakon aplikacije paraoksona, intenzitet i učestalost konvulzija su se znatno smanjili.

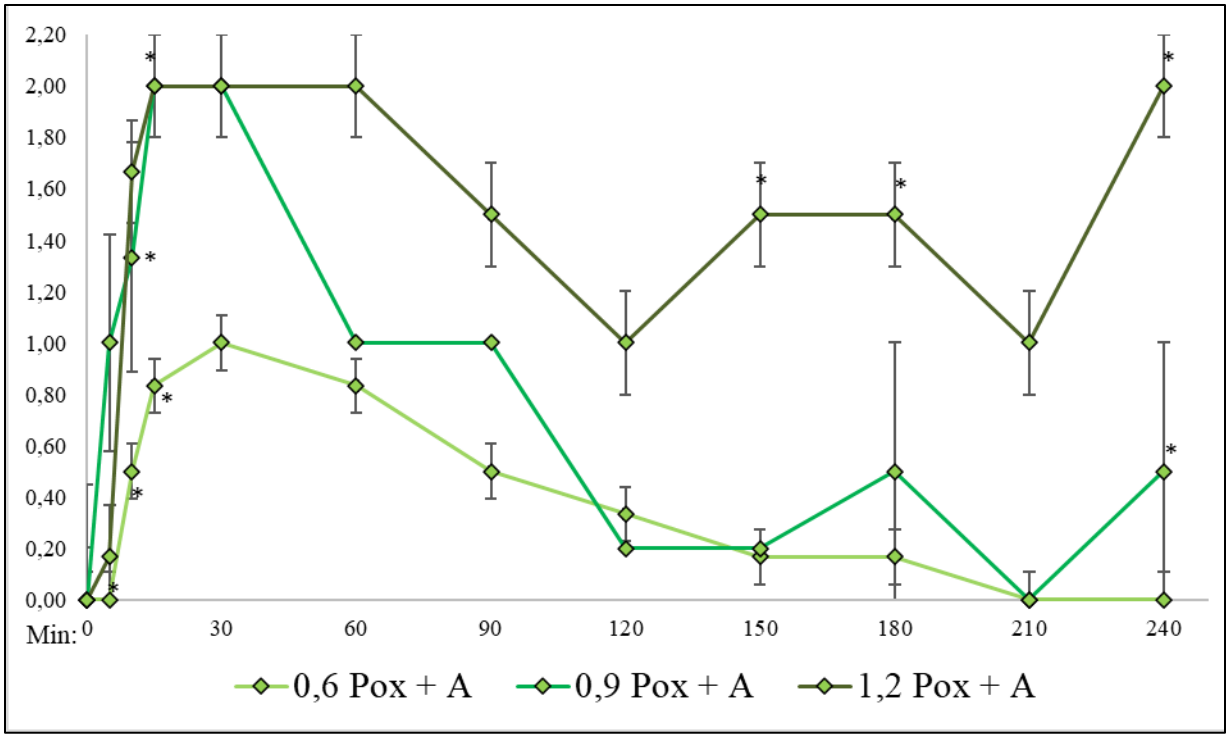
Doza paraoksona je bila u korelaciji sa učestalosti konvulzija ($\chi^2 = 8,025$, $p = 0,020$), kao i sa brzinom pojave konvulzija – konvulzije su se pri višim dozama paraoksona javljale ranije bez obzira na tretman ($r = -0,525$, $p < 0,001$). Konvulzije su se javljale kod svih pacova koji su uginuli i kod 90% pacova koji su preživjeli, što je bilo blizu statističke značajnosti ($p = 0,059$). Pri dozi od 0,35 mg/kg paraoksona, kod tretmana oksimom K870, samo kod 60% pacova su se javile konvulzije (83% kod tretmana FR i 80% kod tretmana obidoksimom), ($\chi^2 = 3,241$, $p = 0,071$), pri čemu su svi pacovi preživjeli.

Tabela 3. Vrijeme pojave i učestalost konvulzija pri različitim dozama paraoksona kod pacova tretiranih oksimima

Tretman	Vrijeme pojave konvulzija (min)				Učestalost pojave konvulzija (%)			
	Svi	P	U	p (P)	Svi	P	U	p (P)
0,35 POX								
S	18,10 ± 18,73	36,00 ± 29,51	10,43 ± 2,64	0,015	83,33	66,69	100,00	0,152
L	12,25 ± 0,01	8,33 ± 2,08	24,00 ± 0.00	1,000	80,00	75,00	100,00	1,000
K	15,65 ± 3,61	12,00 ± 3,61	-	-	60,00	60,00	-	-
p (T)	p = 0,628	18,78 ± 19,78	12,12 ± 5,38	0,734	63,60	77,33	100,00	0,071
0,4 POX								
S	10,42 ± 3,23	13,00 ± 0,00	10,18 ± 3,28	0,459	100,00	100,00	100,00	-
L	9,60 ± 5,07	10,75 ± 5,06	5,00 ± 0,00	0,277	100,00	100,00	100,00	-
K	13,40 ± 4,78	15,50 ± 1,00	5,00 ± 0,00	0,114	100,00	100,00	100,00	-
p (T)	p = 0,265	9,28 ± 3,57	8,67 ± 4,42	0,455	100,00	100,00	100,00	-
0,5 POX								
L	5,40 ± 3,21	8,50 ± 2,12	3,33 ± 1,53	0,083	100,00	100,00	100,00	-
K	6,33 ± 2,31	7,00 ± 2,83	5,00 ± 0,00	0,480	100,00	100,00	100,00	-
p (T)	p = 0,647	4,80 ± 2,68	7,33 ± 2,51	0,169	100,00	100,00	100,00	-
p (T)	p = 0,113	13,78 ± 13,39	10,04 ± 4,33	0,310	83,33	90,74	100,00	0,059

POX: paraokson; P: preživjeli 24 h nakon aplikacije paraoksona; U: uginuli; p(P): statistička značajnost u odnosu na to da li je pacov preživio; p(T): statistička značajnost u odnosu na tretman; S: Saline – fiziološki rastvor; L: LüH-6 – obidoksim; K: oksim K870;

Intenzitet i vrijeme pojave konvulzija kod pacova tretiranih atropinom prikazani su na Slici 24 i u Tabeli 4. Intenzitet konvulzija se značajno razlikovao pri različitim dozama paraoksona u 10. min (χ^2 = 6,129, p = 0,047), 15. min (χ^2 = 8,205, p = 0,017), 150. min (χ^2 = 6,000, p = 0,050), 210. min (χ^2 = 9,000, p = 0,011) i 240. min (χ^2 = 7,667, p = 0,022). Jaka pozitivna korelacija između doze paraoksona i intenziteta trovanja nađena je u 15. min (r = 0,659, p = 0,006), 180. min (r = 0,693, p = 0,026), 210. min (r = 0,791, p = 0,006) i 240. min (r = 0,885, p = 0,001). Kod svih pacova su se javile konvulzije, nešto kasnije pri dozi 0,6 mg/kg paraoksona, ali bez značajne razlike i korelacije između doze i brzine pojave konvulzija (r = -0,150, p = 0,136).



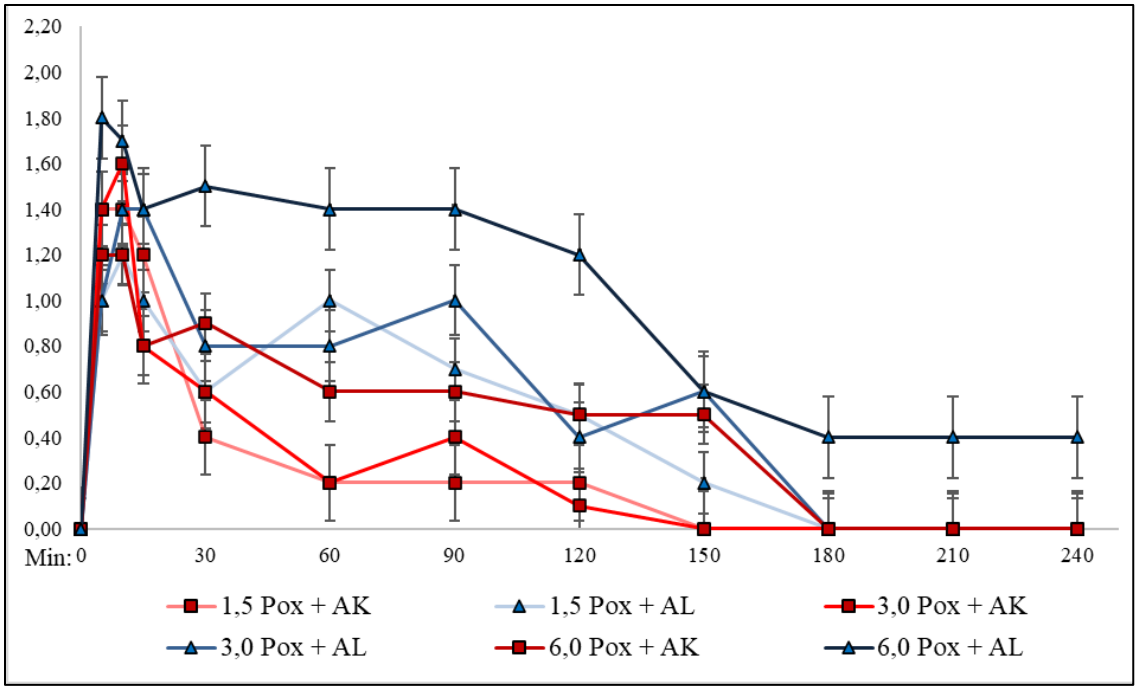
Slika 24. Vrijeme pojave i učestalost konvulzija pri različitim dozama paraoksona kod pacova tretiranih atropinom

Pox: paraoxon sc; A: atropin (10 mg/kg im); *: statistički značajno;

Tabela 4. Vrijeme pojave konvulzija pri različitim dozama paraoksona kod pacova tretiranih atropinom

POX doza (mg/kg sc)	**Vrijeme pojave konvulzija (min)**			
	Svi	P	U	p (P)
0,6	18,20 ± 15,21	18,20 ± 15,21	-	-
0,9	8,50 ± 4,64	10,00 ± 5,66	7,75 ± 5,78	0,475
1,2	9,50 ± 1,64	10,50 ± 0,71	9,00 ± 1,83	0,340
Svi	11,71 ± 9,18	14,67 ± 11,72	8,37 ± 3,42	0,147
p (D)	p = 0,399			

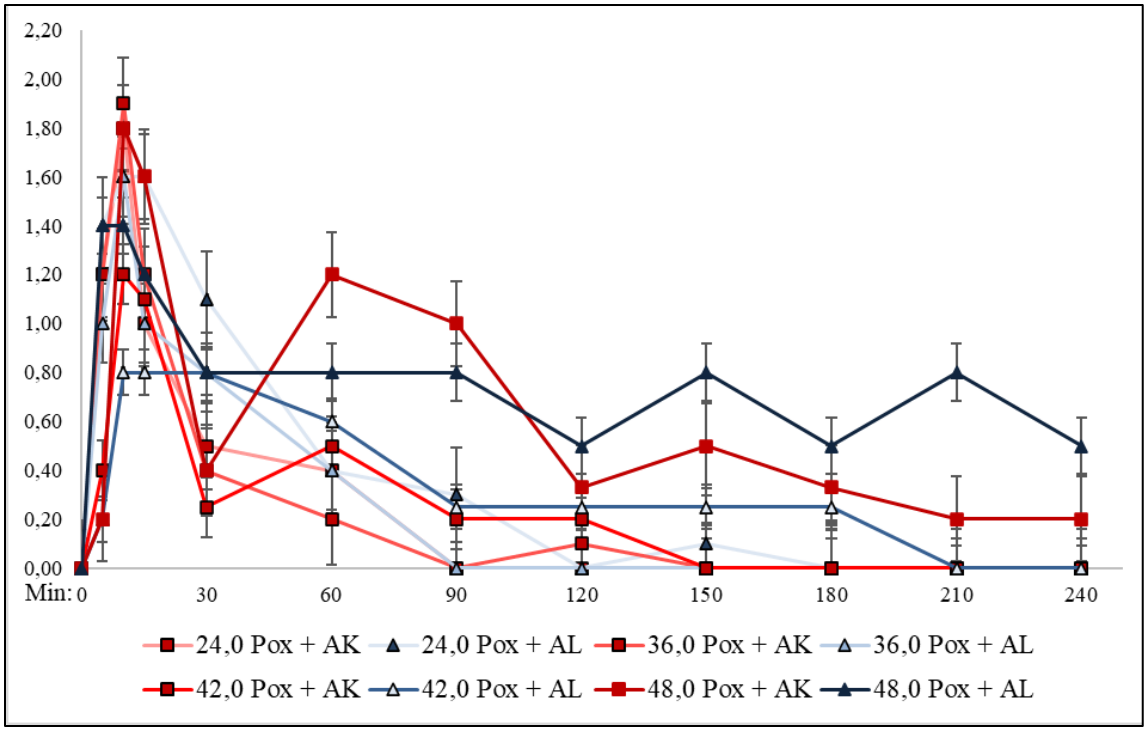
POX: paraokson; P: preživjeli 24 h nakon aplikacije paraoksona; U: uginuli; p(P): statistička značajnost u odnosu na to da li je pacov preživio; p(T): statistička značajnost u odnosu na dozu paraoksona; Svi pacovi 1 min nakon paraoksona tretirani sa 10 mg/ks im atropina;



Slika 25. Intenzitet konvulzija pri različitim dozama paraoksona kod pacova tretiranih atropinom i oksimom K870 ili obidoksimom (A)

Pox: paraoxon, doza sc; A: atropin (10 mg/kg im); L: (LüH-6 - obidoksim): (22 mg/kg im), K: oksim K870 (35 mg/kg im);

Intenzitet i vrijeme pojave konvulzija kod pacova tretiranih obidoksimom ili oksimom K870 u kombinaciji sa atropinom prikazani su na Slikama 25 i 26 i u Tabeli 5. Značajna razlika u intenzitetu konvulzija nađena je u 5. min ($\chi^2 = 28{,}706$, $p = 0{,}002$), 60. min ($\chi^2 = 33{,}053$, $p = 0{,}002$), 90. min ($\chi^2 = 36{,}369$, $p = 0{,}001$), 120. min ($\chi^2 = 28{,}924$, $p = 0{,}007$) i 150. min ($\chi^2 = 25.276$, $p = 0{,}021$). Intenzitet je najčešće bio viši kod većih doza, ali bez statistički značajnih razlika. Nije nađena značajna razlika između tretmana obidoksimom i atropinom i oksimom K870 i obidoksimom. Konvulzije su se javile kod svih pacova, poslije 60 min je dolazilo do smirivanja i/ili povlačenja konvulzija, sa povremenim kratkotrajnim epileptičnim konvulzijama i 4 h nakon aplikacije paraoksona.



Slika 26. Intenzitet konvulzija pri različitim dozama paraoksona kod pacova tretiranih atropinom i oksimom K870 ili obidoksimom (B)

Pox: paraoxon, doza sc; A: atropin (10 mg/kg im); L: (LüH-6 - obidoksim): (22 mg/kg im), K: oksim K870 (35 mg/kg im);

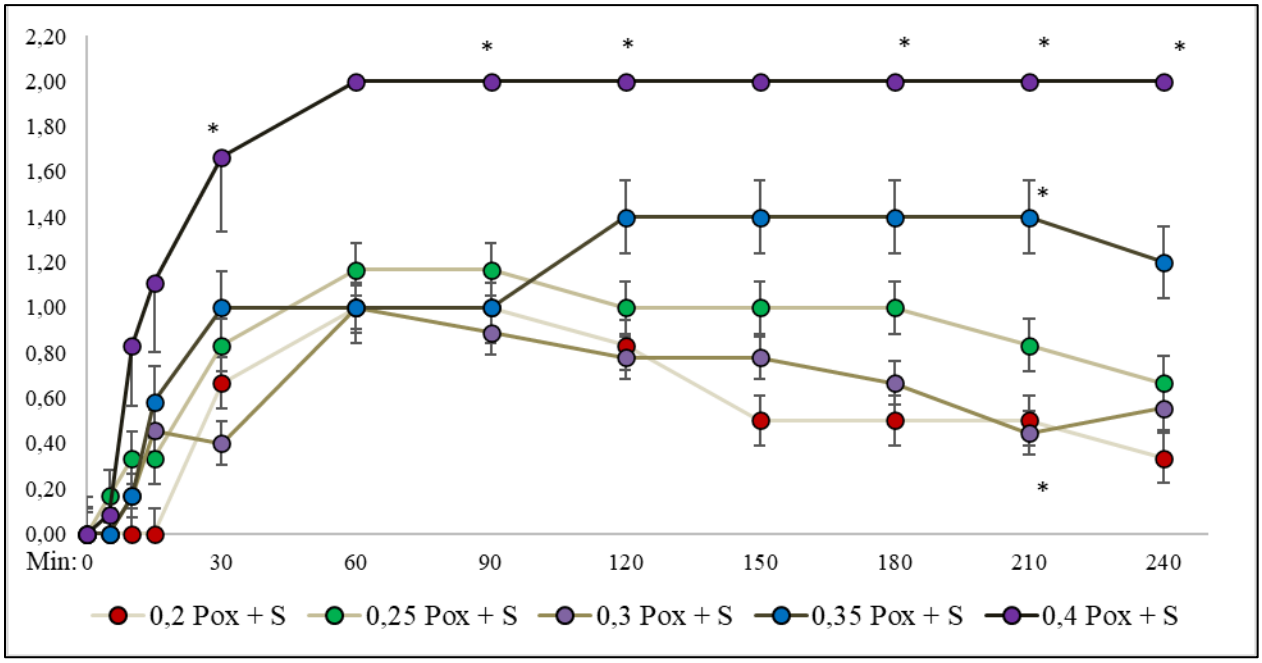
Tabela 5. Vrijeme pojave konvulzija pri različitim dozama paraoksona kod pacova tretiranih atropinom i oksimom K870 ili obidoksimom

Tretman		**Vrijeme pojave konvulzija (min)** Prosjek ± SD	p (T)
1,5 POX			
	L	3,80 ± 2,68	U = 11,000;
	K	3,20 ± 3,19	p = 0,735
3,0 POX			
	L	2,80 ± 1,09	U = 5,000;
	K	4,00 ± 0,71	p = 0,090
6,0 POX			
	L	2,80 ± 1,31	U = 11,500;
	K	3,00 ± 1,41	p = 0,827
24,0 POX			
	L	3,80 ± 0,83	U = 11,500;
	K	3,80 ± 1,64	p = 0,828
36,0 POX			
	L	6,20 ± 4,15	U = 12,000;
	K	5,20 ± 0,84	p = 0,915
42,0 POX			
	L	7,00 ± 2,45	U = 2,000;
	K	5,00 ± 2,00	p = 0,097
48,0 POX			
	L	4,00 ± 1,00	U = 0,000;
	K	7,00 ± 0,71	p = 0,080

POX: paraoxon, doza sc; L: (LüH-6 - obidoksim): (22 mg/kg im), K: oksim K870 (35 mg/kg im); p(T): statistička značajnost u odnosu na tretman; L: (LüH-6 - obidoksim): (22 mg/kg im), K: oksim K870 (35 mg/kg im);

5.4.2. Fascikulacije

Intenzitet i vrijeme pojave fascikulacija kod pacova tretiranih FR prikazani su na Slici 27 i u Tabeli 6.



Slika 27. Intenzitet fascikulacija pri različitim dozama paraoksona kod neštićenih pacova

Pox: paraoxon, doza sc; Sal: *saline* – fiziološki rastvor (1 mL/kg, im); *: statistički značajno;

Intenzitet fascikulacija je bio u pozitivnoj korelaciji sa dozom paraoksona u svim posmatranim vremenima, sa značajnom razlikom u 10. min (Spearman ρ, r = 0,316, p = 0,029), 15. min (r = 0,427, p = 0,004), 120. min (r = 0,379, p = 0,047), 150. min (r = 0,484, p = 0,009), 180. min (r = 0,483, p = 0,009), 210. min (r = 0,459, p = 0,014) i 240. min (r = 0,506, p = 0,006). Doza od 0,4 mg/kg paraoksona je kod preživjelog pacova pokazivala maksimalni intenzitet fascikulacija do kraja opserviranog perioda, značajno jači u odnosu na ostale doze u 30, 90, 120, 180, 210. i 240. min. U 210. min doza od 0,3 mg paraoksona je pokazivala značajno niži intenzitet u odnosu na više doze (0,35 i 0,40 mg).

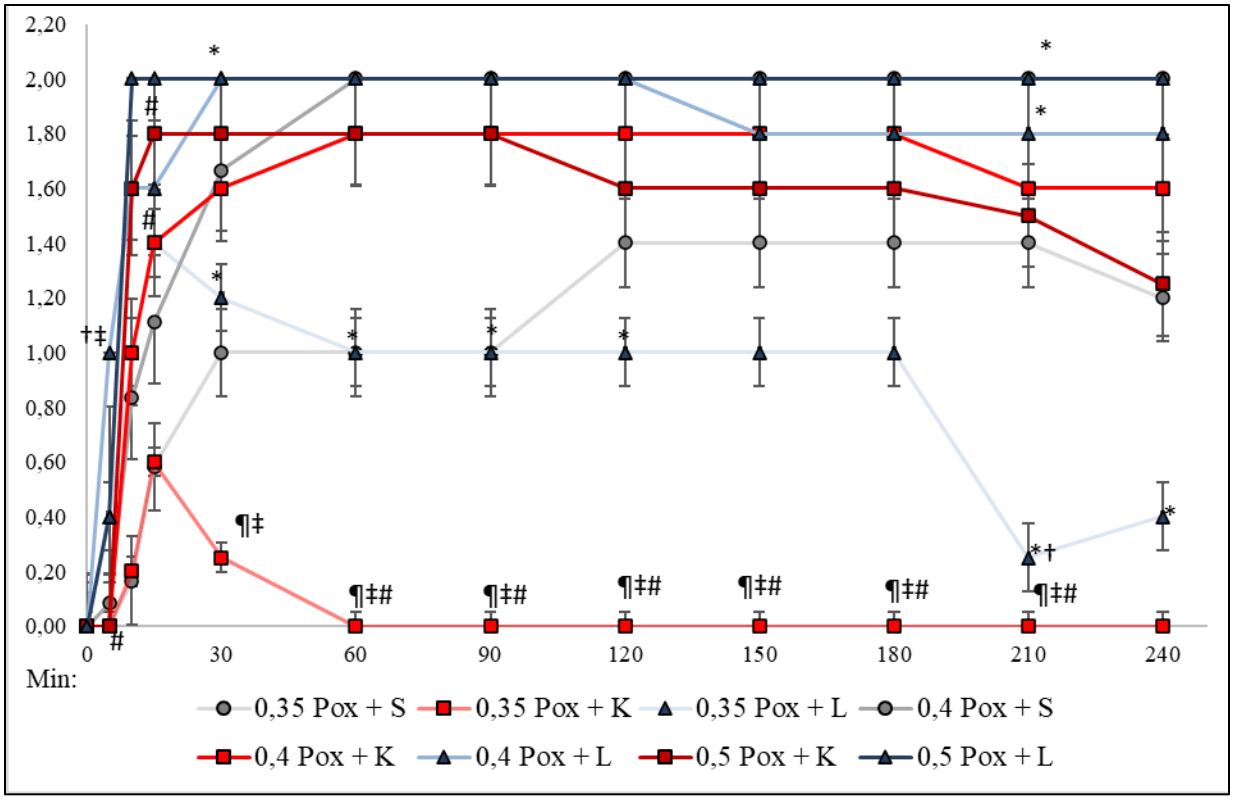
Tabela 6. Vrijeme pojave i učestalost fascikulacija pri različitim dozama paraoksona kod neštićenih pacova

POX doza	Vrijeme pojave fascikulacija (min)				Učestalost pojave fascikulacija (%)			
	Svi	P	U	p (P)	Svi	P	U	p (P)
0,2	35,67 ± 17,95	35,67 ± 17,95	-	-	100,00	100,00	-	-
0,25	30,17 ± 21,98	30,17 ± 21,98	-	-	100,00	100,00	-	-
0,3	34,25 ± 22,17	42,00 ± 1,98	11,00 ± 1,41	0,143	83,33	83,33	83,33	1,000
0,35	20,83 ± 11,84	30,20 ± 12,94	14,14 ± 4,49	0,012	100,00	100,00	-	-
0,4	12,70 ± 7,23	21,00 ± 0,00	11,78 ± 4,41	-	83,33	100,00	90,90	-
p (D)	p = 0,022	p < 0,001			p = 1,000	p = 1,000		

POX: paraokson, doza sc; P: preživjeli 24 h nakon aplikacije paraoksona; U: uginuli; p(P): statistička značajnost u odnosu na to da li je pacov preživio; p(D): statistička značajnost u odnosu na dozu paraoksona;

Fascikulacije su u toku opserviranog perioda primijećene kod skoro svih pacova. Četiri pacova kod kojih nisu primijećene fascikulacije su bili pacovi kod kojih je smrtni ishod nastupio veoma brzo, a konvulzije bile jakog intenziteta. Brzina pojave fascikulacija bila je u negativnoj korelaciji sa dozom paraoksona (ranije se javljali simptomi kod veće doze, Spearman ρ, r = -0,435, p = 0,003). Fascikulacije su se javljale značajno ranije pri dozi od 0,4 mg/kg sc paraoksona u odnosu na dozu 0,3 (U = 0,20, p = 0,020) i 0,2 (U = 20,50, p = 0,052). Takođe, fascikulacije su se javljale ranije kod pacova koji su uginuli (Man-Whitney U-test, U = 61,50, p < 0,001).

Intenzitet i vrijeme pojave fascikulacija kod pacova tretiranih oksimima (kao i uopredne doze sa FR) prikazani su na Slici 28 i u Tabeli 7.



Slika 28. Intenzitet fascikulacija pri različitim dozama paraoksona kod pacova tretiranih oksimima

Pox: paraoxon, doza sc; Sal: *saline* – fiziološki rastvor (1 mL/kg, im); L: (LüH-6 - obidoksim): (22 mg/kg im); K: oksim K870 (35 mg/kg im); ¶: S vs K; †: S vs L; ‡: K vs L; #: K vs K; *: L vs L (statistički značajne razlike)

Oksimi su ublažili intenzitet fascikulacija, naročito pri nižim dozama paraoksona. Pri dozi 0,35 mg/kg paraoksona, pacovi tretirani oksimom K870 su nakon 60 min bili bez znakova trovanja, što je bilo značajno i u poređenju sa obidoksimom i FR, tako i u poređenju sa višim dozama paraoksona. I kod pacova tretiranih obidoksimom, pri dozi paraoksona od 0,35 mg/kg sc, intenzitet fascikulacija je bio značajno niži u odnosu na ostale doze.

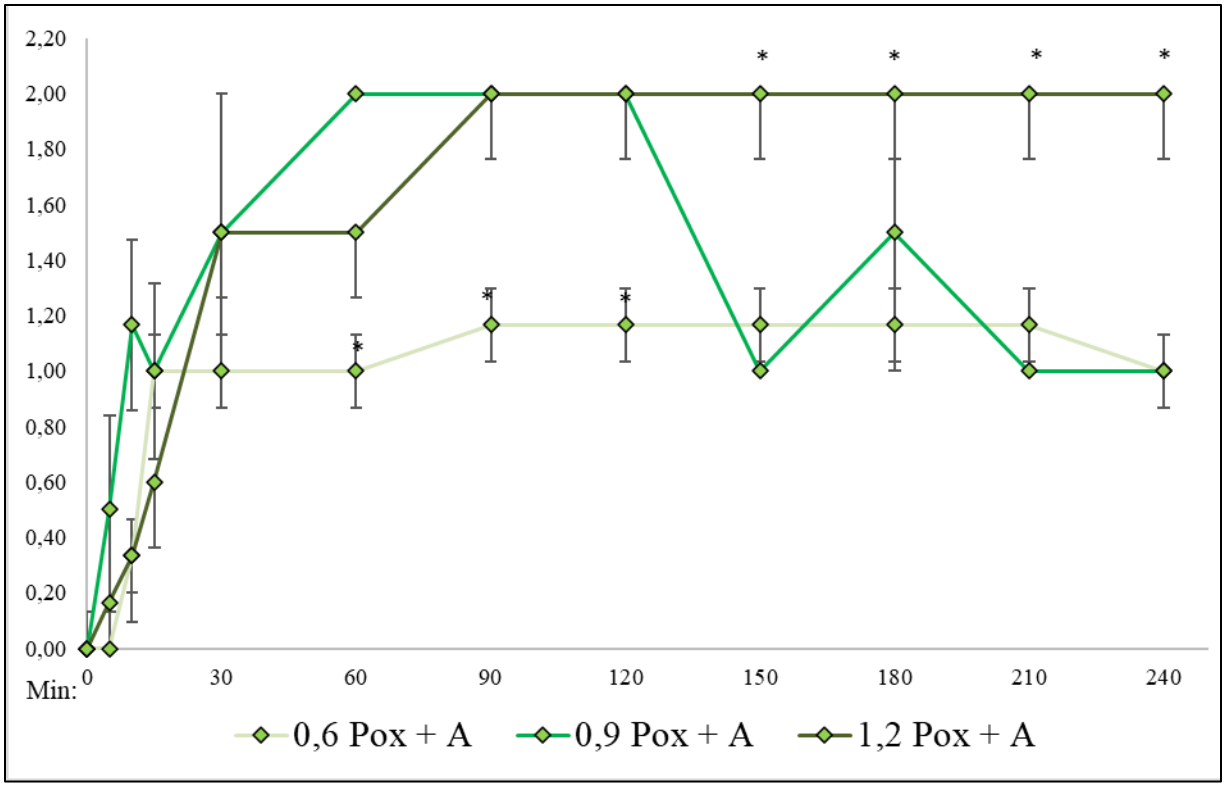
Tabela 7. Vrijeme pojave i učestalost fascikulacija pri različitim dozama paraoksona kod pacova tretiranih oksimima

Tretman	Vrijeme pojave fascikulacija (min)				Učestalost pojave fascikulacija (%)			
	Svi	P	U	p (P)	Svi	P	U	p (P)
0,35 POX								
S	20,83 ± 11,84	30,20 ± 12,94	14,14 ± 4,49	0,012	100,00	100,00	-	-
L	12,40 ± 7,23	9,50 ± 3,70	24,00 ± 0,00	0,039	100,00	100,00	100,00	-
K	18,00 ± 7,21	18,00 ± 7,21	-	-	60,00	60,00	-	-
p (T)	p = 0,340	p = 0,870			p = 1,000	p = 1,000		
0,4 POX								
S	12,70 ± 7,23	21,00 ± 0,00	11,78 ± 4,41	0,083	83,33	100,00	90,90	-
L	6,20 ± 2,49	6,75 ± 2,50	4,00 ± 0,00	0,387	100,00	100,00	100,00	-
K	12,70 ± 5,08	9,25 ± 3,77	15,00 ± 0,00	0,266	100,00	100,00	100,00	-
p (T)	p = 0,047	p = 0,870			p = 1,000	p = 1,000		
0,5 POX								
L	5,20 ± 2,49	4,00 ± 1,73	7,00 ± 2,83	0,346	100,00	100,00	100,00	-
K	9,80 ± 1,30	9,75 ± 1,50	10,00 ± 0,00	0,891	100,00	100,00	100,00	-
p (T)	p = 0,016	p = 0,634			p = 1,000	p = 1,000		

POX: paraokson; P: preživjeli 24 h nakon aplikacije paraoksona; U: uginuli; p(P): statistička značajnost u odnosu na to da li je pacov preživio; p(T): statistička značajnost u odnosu na tretman; Sal: *saline* – fiziološki rastvor (1 mL/kg, im); L: (LüH-6 - obidoksim): (22 mg/kg im); K: oksim K870 (35 mg/kg im);

Pri dozi 0,35 mg/kg sc paraoksona i tretmana oksimom K870, kod 2/5 (40,00%) pacova nije došlo do pojave fascikulacija, dok je kod pacova tretiranih FR i obidoksimom došlo do pojave fascikulacija kod svih pacova (Fisher exact test $\chi^2 = 7,480$, p = 0,087). Kod ostalih doza i tretmana kod svih pacova su primijećene fascikulacije. Vrijeme pojave fascikulacija nije bilo povezano sa preživljavanjem, ali jeste sa dozom paraoksona i tretmanom. Prilikom tretmana oksimom K870 (r = -0,565, p = 0,044) i obidoksimom (r = -0,609, p = 0,016) fascikulacije su se javljale ranije pri višim dozama paraoksona. Pri istim dozama paraoksona, fascikulacije su se javljale ranije kod pacova tretiranih obidoksimom nego oksimom K870.

Intenzitet i vrijeme pojave fascikulacija kod pacova tretiranih atropinom prikazani su na Slici 29 i u Tabeli 8. Intenzitet fascikulacija je bio u korelaciji sa dozom paraoksona u 60. min (r = 0,690, p = 0,027), 90. min (r = 0,791, p = 0,006), 120. min (r = 0,791, p = 0,006), 180. (r = 0,645, p = 0,044) i u 240. min (r = 0,791, p = 0,006).



Slika 29. Intenzitet fascikulacija pri različitim dozama paraoksona kod pacova tretiranih atropinom

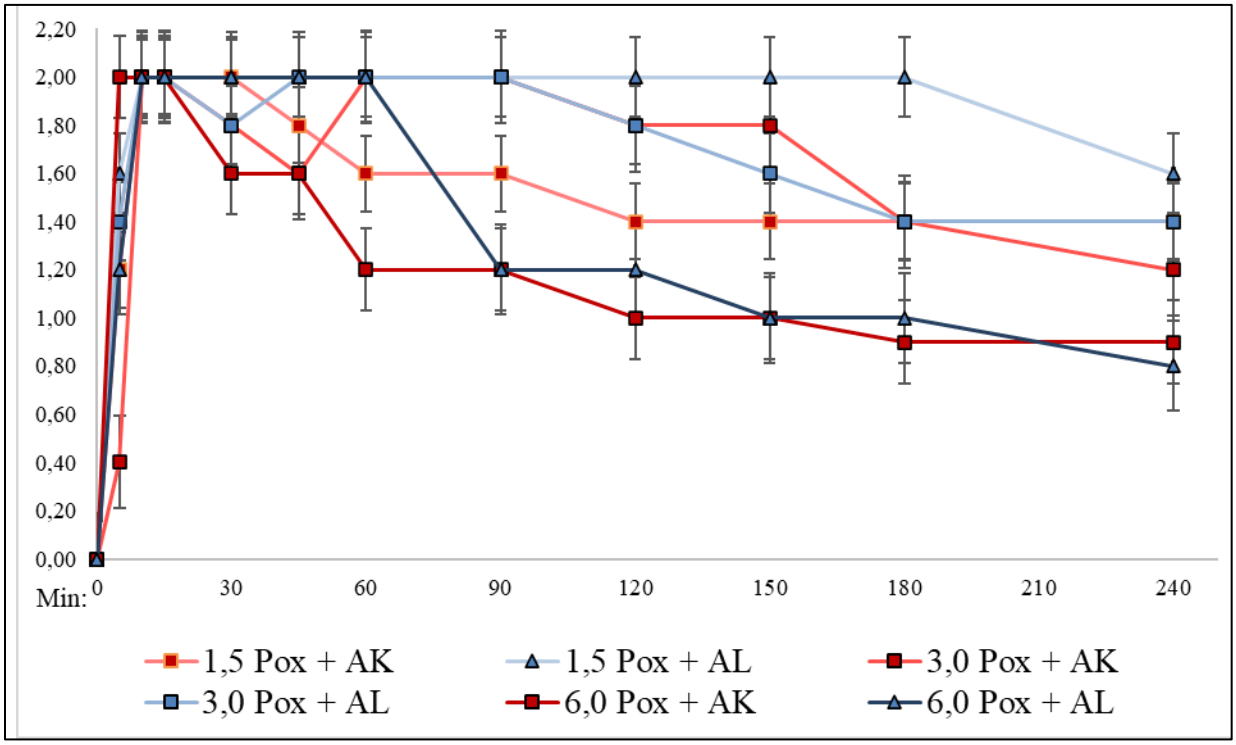
Pox: paraoxon, doza sc; A: atropin (10 mg/kg im); *: statistički značajno;

Kod pacova tretiranih atropinom, vrijeme pojave fascikulacija bilo je u negativnoj korelaciji sa dozom paraoksona – pri višim dozama, fascikulacije su se javljale ranije ($r = -0{,}520$, $p = 0{,}050$). Fascikulacije se nisu javile kod jednog pacova pri dozi 0,9 i dva pacova pri dozi 1,2 mg/kg paraoksona, ali su ti pacovi uginuli već nakon 10 min, i već od 3. min imali veoma intenzivne konvulzije, što je vjerovatno onemogućilo primjećivanje fascikulacija. Fascikulacije su se javile znatno kasnije pri dozi 0,6 mg/kg paraoksona u odnosu na druge doze (Kruskal-Wallis test, $\chi^2 = 6{,}519$, $p = 0{,}038$).

Tabela 8. Vrijeme pojave fascikulacija pri različitim dozama paraoksona kod pacova tretiranih atropinom

POX doza (mg/kg sc)	Vrijeme pojave fascikulacija (min)			
	Svi	P	U	p (P)
0,6	28,83 ± 30,75	18,20 ± 15,21	-	-
0,9	7,60 ± 1,95	6,67 ± 0,58	9,00 ± 2,83	0,197
1,2	10,33 ± 4,73	13,00 ± 1,41	5,00 ± 0,00	0,221
Svi	17,29 ± 21,84	14,67 ± 11,72	8,37 ± 3,42	0,007
p (D)	p = 0,038			

POX: paraokson; P: preživjeli 24 h nakon aplikacije paraoksona; U: uginuli; p(P): statistička značajnost u odnosu na to da li je pacov preživio; p(T): statistička značajnost u odnosu na dozu paraoksona; Svi pacovi 1 min nakon paraoksona tretirani sa 10 mg/ks im atropina;

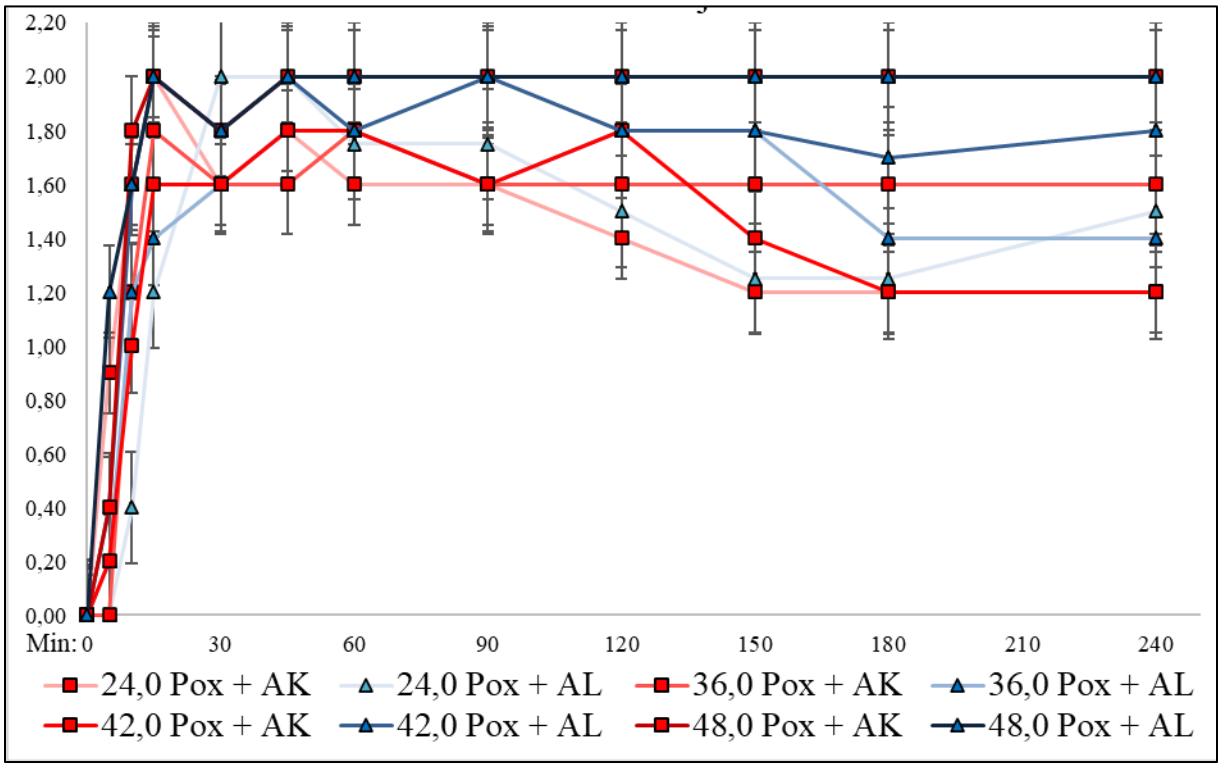


Slika 30. Intenzitet fascikulacija pri različitim dozama paraoksona kod pacova tretiranih atropinom i oksimom K870 ili obidoksimom (A)

Pox: paraoxon, doza s; A: atropin (10 mg/kg im); L: (LüH-6 - obidoksim): (22 mg/kg im), K: oksim K870 (35 mg/kg im);

Intenzitet fascikulacija kod pacova tretiranih atropinom i oksimom K870 ili obidoksimom prikazani su na Slici 30 i 31. Facikulacije su bile izražene pri svim dozama tokom cijelog opserviranog perioda, bez statistički značajne razlike između komparabilnih grupa. Pozitivna korelacija između doze paraoksona i intenziteta fascikulacija nađena je u 5. (r = 0,415, p = 0,001), 10. (r = 0,531, p = 0,001), 15. (r = 0,272, p = 0,023) i 240. min (r = 0,314, p = 0,009).

Fascikulacije su primijećene kod svih pacova. Javljale su se u prosjeku 6,50 ± 4,48 min nakon primjene paraoksona, bez značajne razlike, kako ni u odnosu na dozu, tako ni u odnosu na dati oksim.

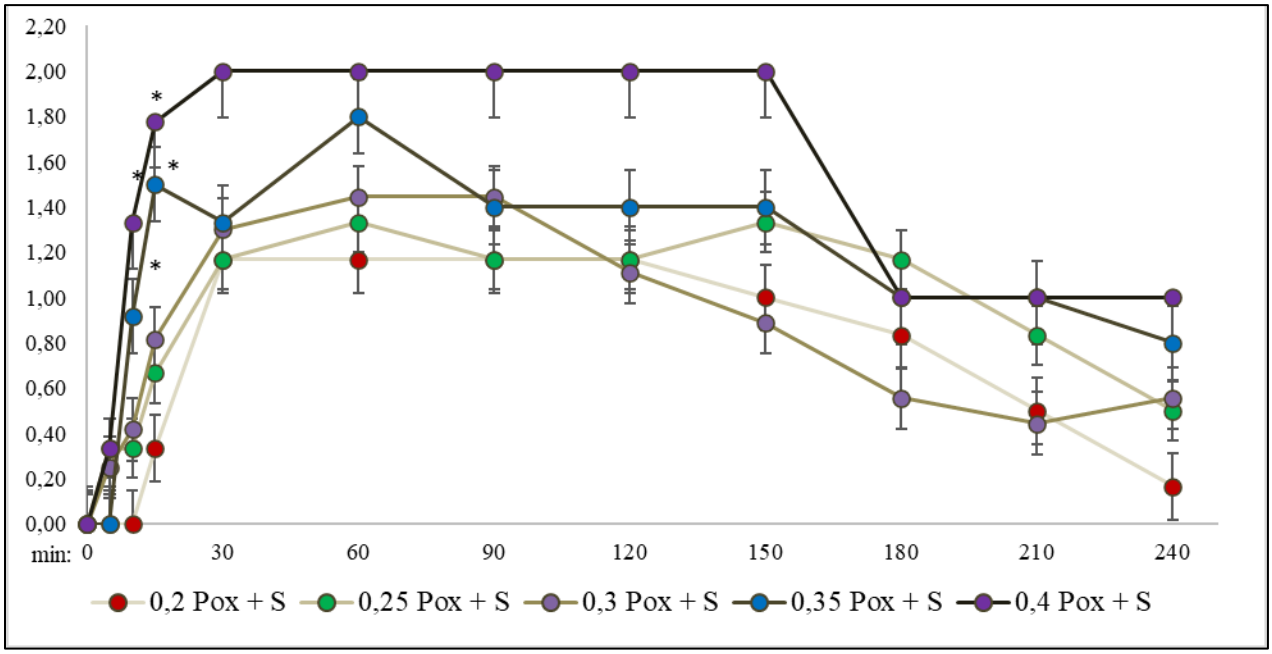


Slika 31. Intenzitet fascikulacija pri različitim dozama paraoksona kod pacova tretiranih atropinom i oksimom K870 ili obidoksimom (B)

Pox: paraoxon, doza sc; A: atropin (10 mg/kg im); L: (LüH-6 - obidoksim): (22 mg/kg im), K: oksim K870 (35 mg/kg im);

5.4.3. Tremor

Intenzitet i vrijeme pojave tremora kod pacova tretiranih FR prikazani su na Slici 32 i u Tabeli 9.



Slika 32. Intenzitet tremora pri različitim dozama paraoksona kod neštićenih pacova

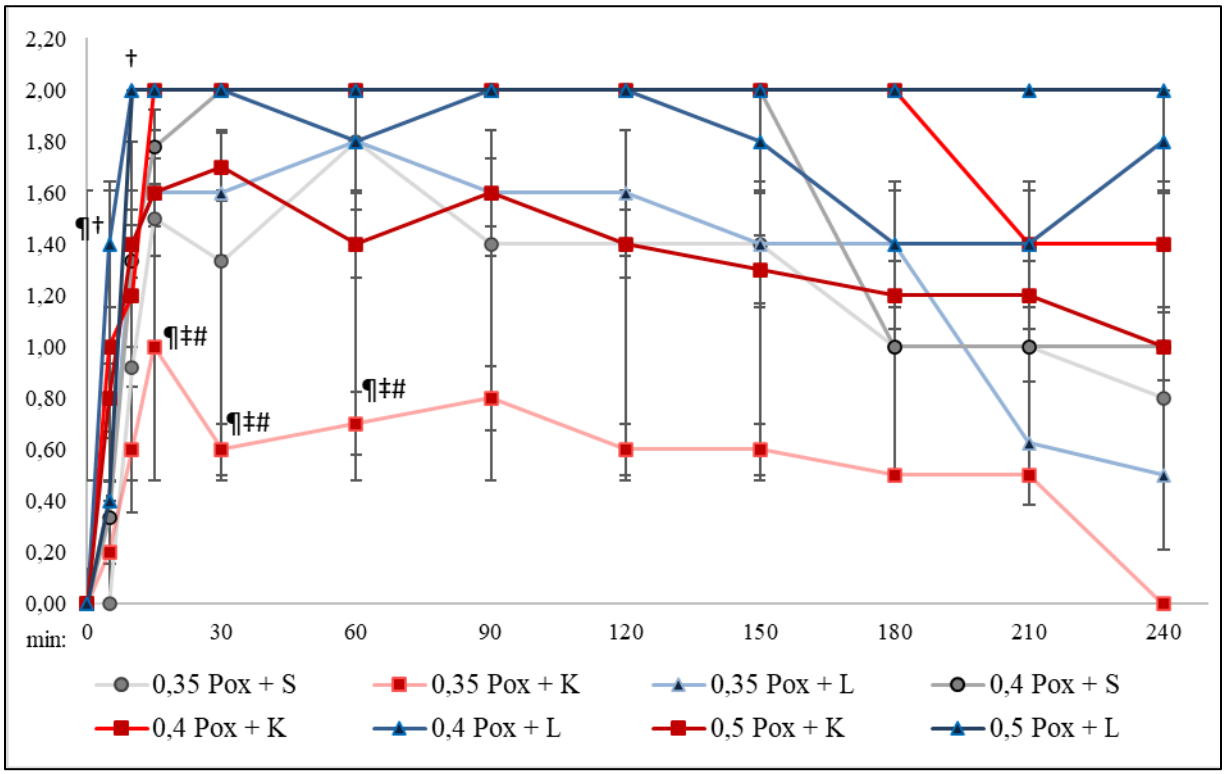
Pox: paraoxon; Sal: *saline* – fiziološki rastvor (1 mL/kg, im); *: statistički značajno;

Tremor je bio jačeg intenziteta pri višim dozama. Značajna razlika u intenzitetu tremora nađena je u 10. (χ^2 = 18,414, p = 0,001) i 15. min (χ^2 = 16,293, p = 0,003). Pri dozi 0,4 i 0,35 mg/kg sc paraoksona, tremor je bio intenzivniji u odnosu na niže doze. Tremor se javljao ranije pri višim dozama (Kruskal-Wallis test, χ^2 = 12,719, p = 0,013) i kod pacova koji su uginuli (Man-Whitney U test, U = 43,500, p < 0,001) (p = 0,009 pri dozi 0,3 i p = 0,004 pri dozi 0,35 mg/kg sc paraoksona).

Tabela 9. Vrijeme pojave i učestalost tremora pri različitim dozama paraoksona kod neštićenih pacova

POX doza	Vrijeme pojave tremora (min)				Učestalost pojave tremora (%)			
	Svi	P	U	p (P)	Svi	P	U	p (P)
0,2	42,83 ± 54,34	42,83 ± 54,34	-	-	83,33	83,33	-	-
0,25	24,60 ± 7,92	24,60 ± 7,92	-	-	83,33	83,33	-	-
0,3	19,33 ± 14,18	23,78 ± 13,63	6,00 ± 2,65	**0,009**	100,00	100,00	100,00	1,000
0,35	19,67 ± 31,29	36,20 ± 45,83	7,86 ± 1,86	**0,004**	100,00	100,00	100,00	1,000
0,4	7,67 ± 1,83	10,00 ± 0,00	7,45 ± 1,75	-	83,33	100,00	100,00	1,000
p (D)	**p = 0,013**	**p < 0,001**			p = 1,000	p = 1,000		

POX: paraokson; P: preživjeli 24 h nakon aplikacije paraoksona; U: uginuli; p(P): statistička značajnost u odnosu na to da li je pacov preživio; p(D): statistička značajnost u odnosu na dozu paraoksona;



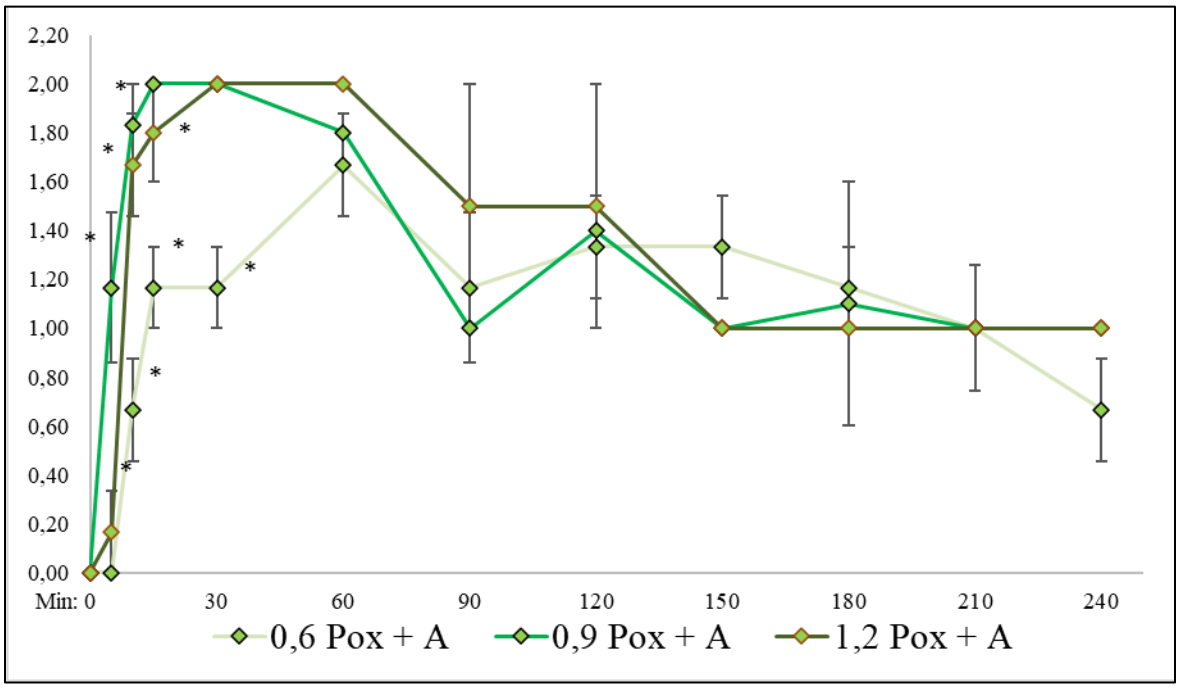
Slika 33. Intenzitet tremora pri različitim dozama paraoksona kod pacova tretiranih oksimima

Pox: paraoxon, doza sc; Sal: *saline* – fiziološki rastvor (1 mL/kg, im); L: (LüH-6 - obidoksim): (22 mg/kg im), K: oksim K870 (35 mg/kg im); ¶: S vs K; †: S vs L; ‡: K vs L; #: K vs K; *: L vs L (statistički značajne razlike);

Intenzitet i vrijeme pojave tremora kod pacova tretiranih oksimima (kao i uopredne doze sa FR) prikazani su na Slici 33. Intenzitet trovanja se razlikovao u odnosu na tretman u 10. (χ^2 =

11,684, p = 0,004), 60. (χ^2 = 9,831, p = 0,007), 90. (χ^2 = 6,156, p = 0,046) i 120. min (χ^2 = 8,006, p = 0,018). Pri dozi 0,35 mg/kg paraoksona, kod pacova štićenih oksimom K870 intenzitet tremora je u prvih sat vremena bio značajno niži. Pri dozi 0,4 mg/kg paraoksona, kod pacova tretiranih obidoksimom intenzitet tremora u 10. i 15. min je bio viši nego u ostalim grupama. Nije postojala razlika u odnosu na dozu i tretman i brzinu pojave tremora.

Intenzitet i vrijeme pojave tremora kod pacova tretiranih atropinom prikazani su na Slici 34 i u Tabeli 10. Značajna razlika u intenzitetu tremora nađena je 5 (χ^2 = 10,181, p = 0,006), 10 (χ^2 = 9,888, p = 0,007), 15 (χ^2 = 8,468, p = 0,015) i 30 min (χ^2 = 6,000, p = 0,050) nakon aplikacije paraoksona.



Slika 34. Intenzitet tremora pri različitim dozama paraoksona kod pacova tretiranih atropinom

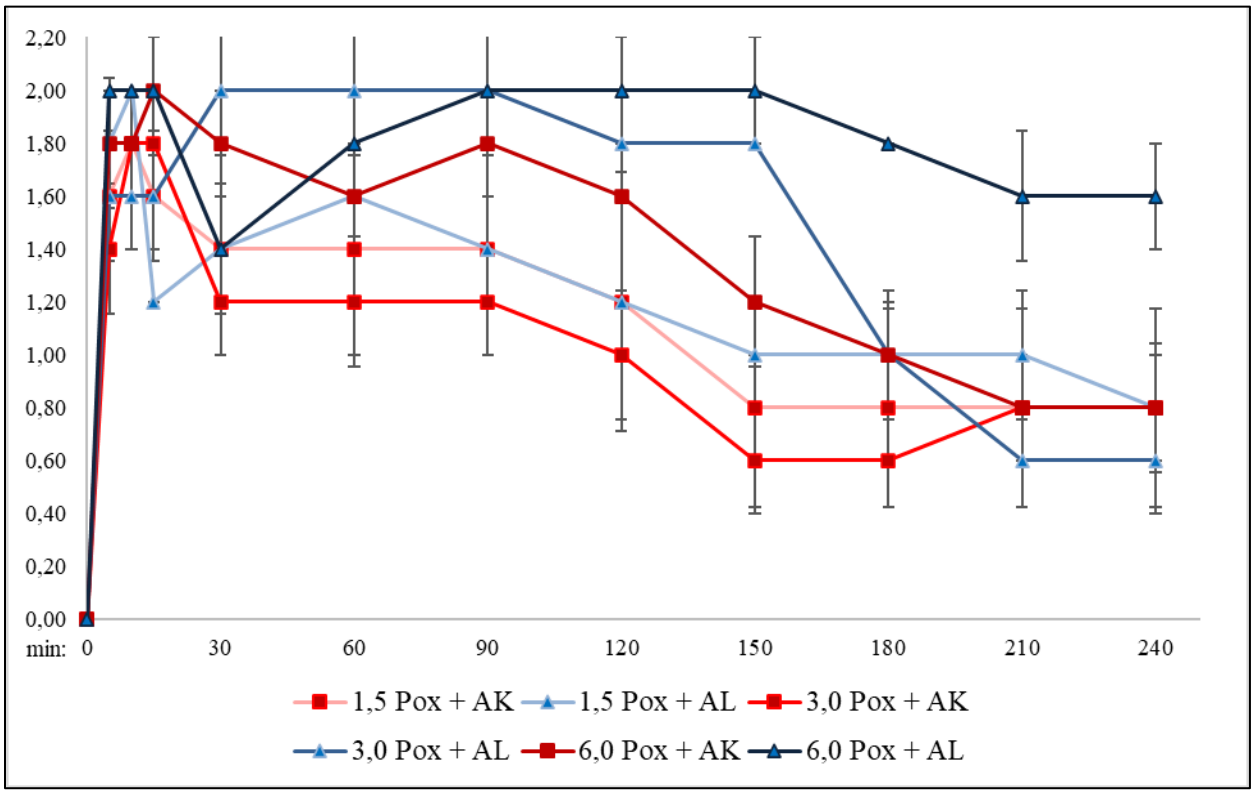
Pox: paraoxon; A: atropin (10 mg/kg im); *: statistički značajno;

Kod pacova tretiranih atropinom pri nižim dozama paraoksona tremor se javljao značajno kasnije (χ^2 = 9,580, p = 0,008). Kod pacova koji su uginuli tremor se javljao ranije, ali bez značajne razlike (U = 20,000, p = 0,073). Tremor se javio kod svih pacova.

Tabela 10. Vrijeme pojave tremora pri različitim dozama paraoksona kod pacova tretiranih atropinom

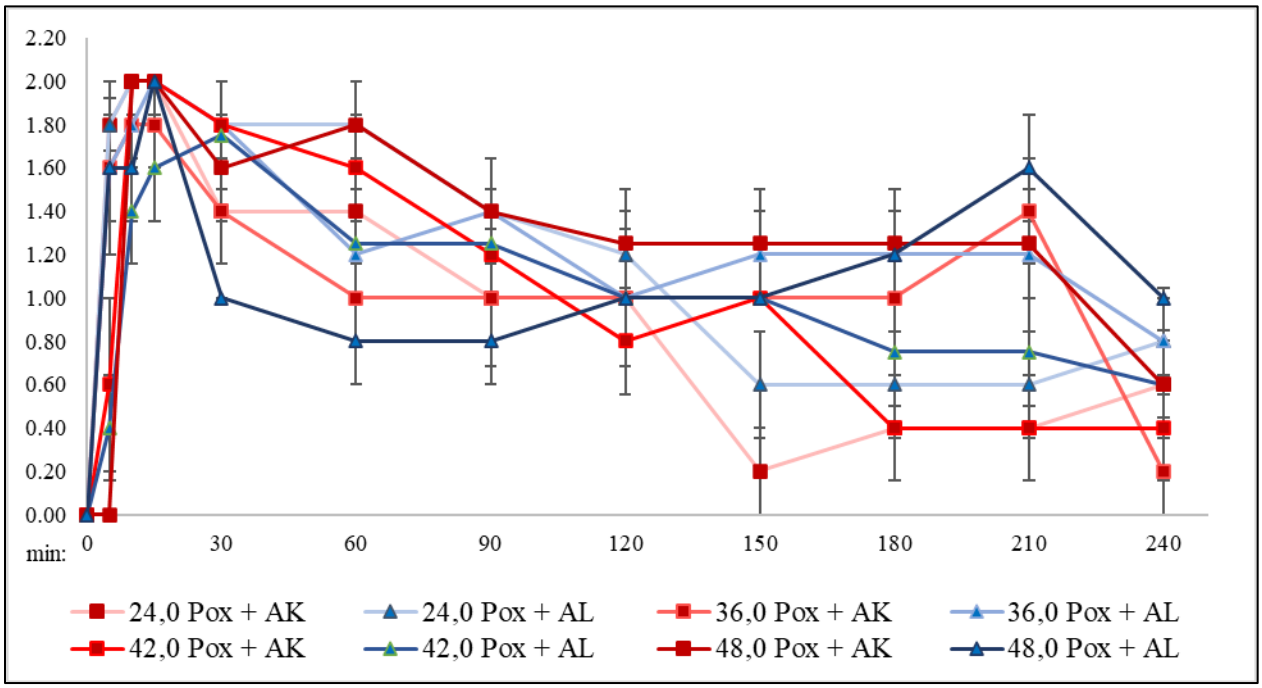
POX doza	**Vrijeme pojave tremora (min)**			
	Svi	P	U	p (P)
0,6	12,83 ± 5,85	12,83 ± 5,85	-	-
0,9	8,33 ± 1,03	9,00 ± 1,41	8,00 ± 0,82	0,325
1,2	5,00 ± 2,19	5,00 ± 2,83	5,00 ±0,00	0,615
Svi	8,72 ± 4,76	10,50 ± 5,48	6,50 ± 2,51	0,073
p (D)	**p = 0,08**			

POX: paraokson, doza sc; P: preživjeli 24 h nakon aplikacije paraoksona; U: uginuli; p(P): statistička značajnost u odnosu na to da li je pacov preživio; p(T): statistička značajnost u odnosu na dozu paraoksona; Svi pacovi 1 min nakon paraoksona tretirani sa 10 mg/ks im atropina;



Slika 35. Intenzitet tremora pri različitim dozama paraoksona kod pacova tretiranih atropinom i oksimom K870 ili obidoksimom (A)

Pox: paraoxon, doza sc; A: atropin (10 mg/kg im); L: (LüH-6 - obidoksim): (22 mg/kg im), K: oksim K870 (35 mg/kg im);



Slika 36. Intenzitet tremora pri različitim dozama paraoksona kod pacova tretiranih atropinom i oksimom K870 ili obidoksimom (B)

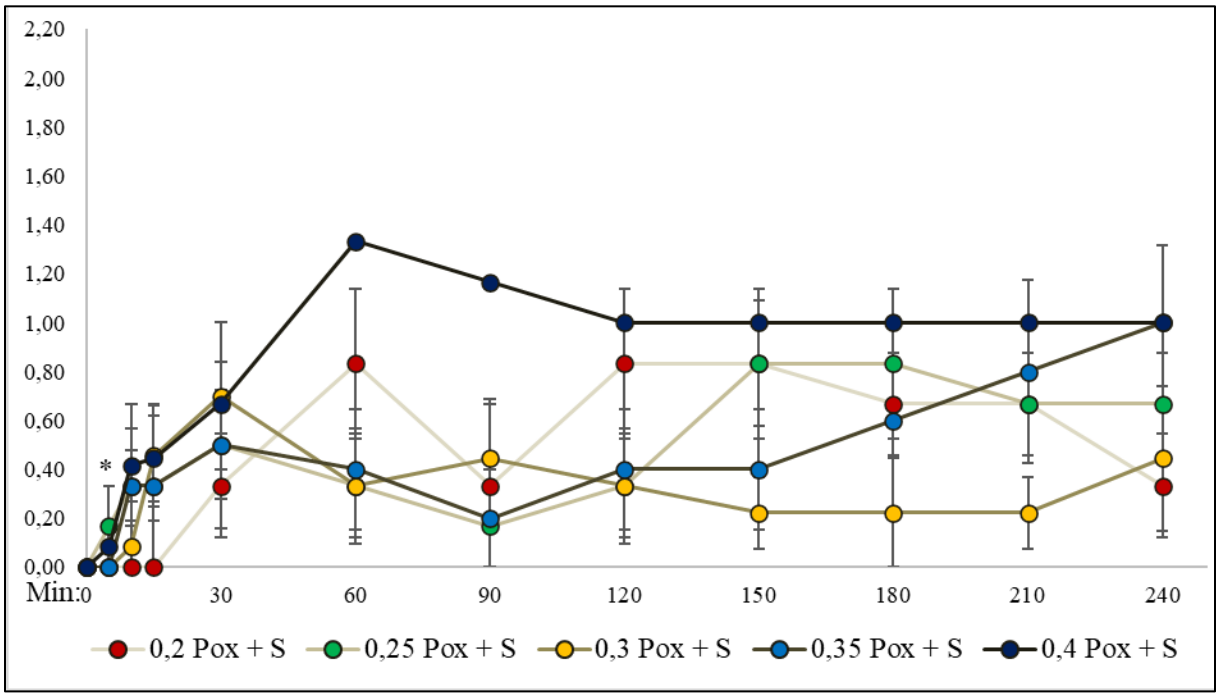
Pox: paraoxon, doza sc; A: atropin (10 mg/kg im); L: (LüH-6 - obidoksim): (22 mg/kg im), K: oksim K870 (35 mg/kg im);

Intenzitet tremora kod pacova tretiranih atropinom i oksimom K870 ili obidoksimom prikazani su na Slici 35 i 36. Intenzitet tremora je značajno varirao unutar posmatranog vremena, kako između grupa, tako i unutar grupe. Iako se uočava da je u prosjeku tremor bio intenzivniji pri višim dozama, razlika nije bila statistički značajna. Tremor se javio kod svih pacova veoma brzo, od 1-9 min (prosjek 4,01 ± 1,95), bez razlike u odnosu na dozu i tretman. Kod pacova kojima je dat i atropin i obidoksim prosječno vrijeme do pojave tremora je bilo 3,85 ± 2,03 min, a kod kojih je aplikovan atropin i oksim K870 4,17 ± 1,89 min (U = 527,500, p = 0,311). Tremor se javljao kasnije kod pacova koji nisu preživjeli - kod pacova koji su preživeli prosječno vrijeme do pojave tremora je bilo 3,59 ± 1,58 min, a koji su uginuli 6,27 ± 2,28 min (U = 113,500, p = 0,001).

Ostali znakovi trovanja su pokazivali manju povezanost, češća odstupanja unutar grupe, te su prikazani samo najznačajniji rezutati.

5.4.4. Ataksija

Kod neštićenih pacova pozitivna korelacija između doze i intenziteta ataksije nađena je 10 min nakon trovanja paraoksonom (r = 0,321, p = 0,026), dok je negativna korelacija nađena u 90. (r = -0,426, p = 0,027), 120. (r = -0,379, p = 0,050) i u 150. min (r = -0,461, p = 0,015) (Slika 37). Nađena je negativna korelacija između doze i brzine javljanja ataksije (r = -0,583, p < 0,001).

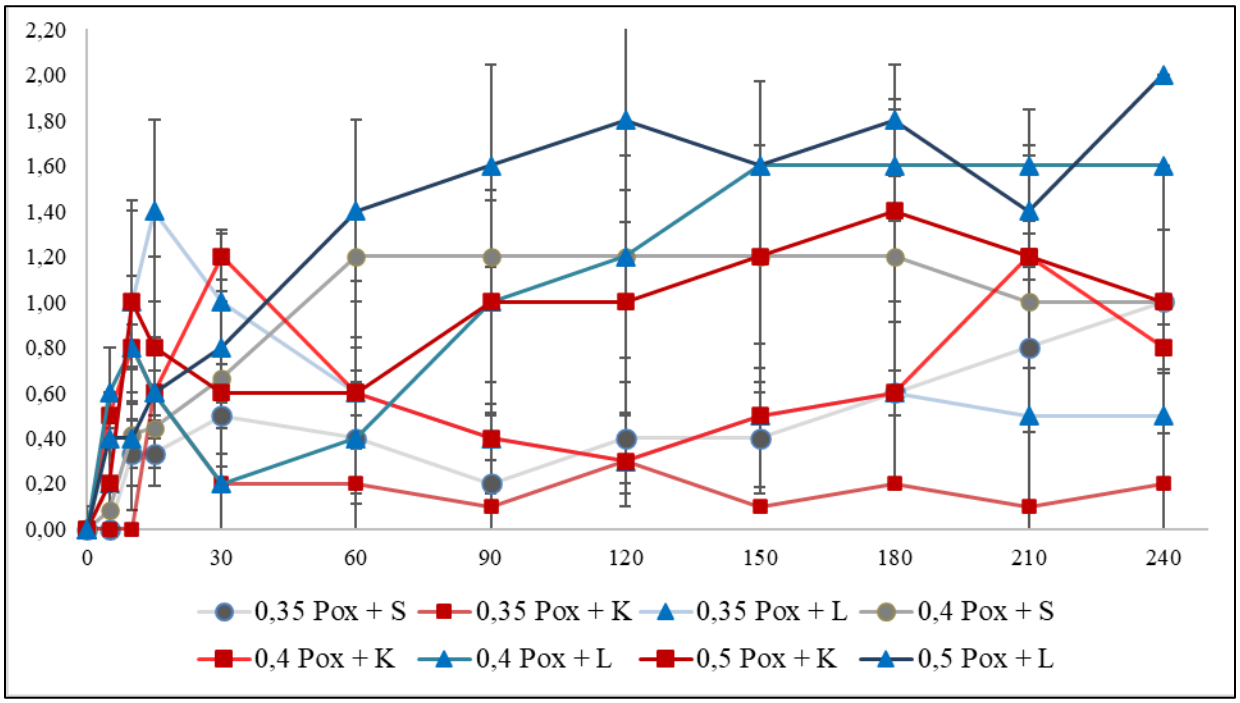


Slika 37. Intenzitet ataksije pri različitim dozama paraoksona kod pacova tretiranih fiziološkim rastvorom

Pox: paraoxon, doza sc; l: *saline* – fiziološki rastvor (1 mL/kg, im); *statistički značajno;

Kod pacova tretiranih oksimima, iako je uočen veći intenzitet pri višim dozama, kao i nešto veći intenzitet kod pacova tretiranih obidoksimom u odnosu na oksim K870, zbog velike devijacije unutar grupe, nije nađena statistička značajnost ni u jednom vremenskom periodu (Slika 38). Nije nađena statistički značaja razlika ni u brzini pojave ataksije, kako u odnosu na tretman i dozu

paraoksona, tako ni u odnosu na ishod trovanja. Kod pacova tretiranih atropinom, kao i atropinom i oksimima, pojava i intenzitet ataksije su bili bez značajne razlike između grupa.

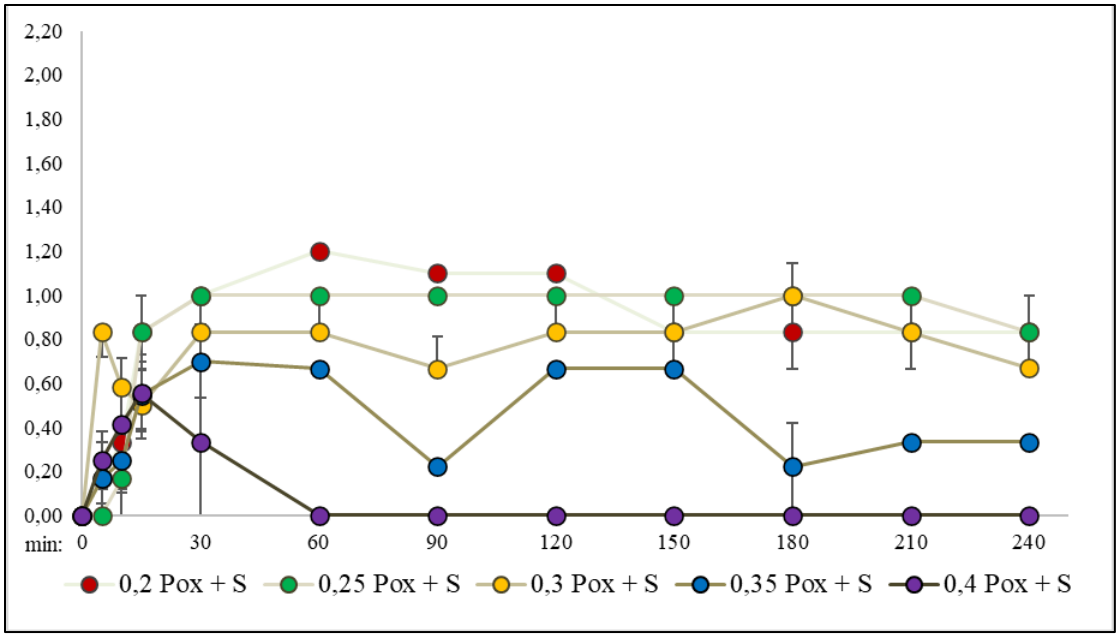


Slika 38. Intenzitet ataksije pri različitim dozama paraoksona kod pacova tretiranih oksimima

Pox: paraoxon, doza sc; S: saline – fiziološki rastvor (1mL/kg); L: (LüH-6 - obidoksim): (22 mg/kg im), K: oksim K870 (35 mg/kg im);

5.4.5. Stereotipni pokreti

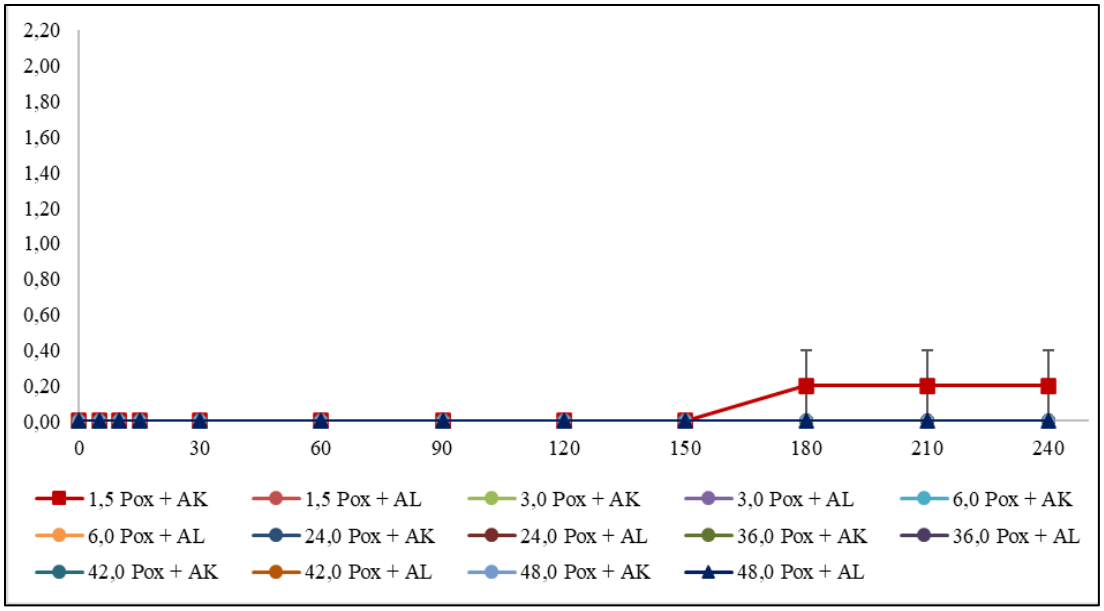
Kod neštićenih pacova, stereotipni pokreti su bili manje izraženi pri višim dozama (pri dozi 0,2 mg/kg paraoksona bili su prisutni u 100% pacova, a pri dozi 0,4 samo kod 41,26%; p = 0,44). Stereotipni pokreti su bili povezani i sa preživljavanjem – stereotipne pokrete je imalo 85,71% preživjelih pacova, nasuprot 52,23% uginulih pacova, p = 0,043). Razlika u intenzitetu u pojedinačnim posmatranim vremenima nije bila značajna (Slika 39).



Slika 39. Intenzitet stereotipnih pokreta pri različitim dozama paraoksona kod pacova tretiranih fiziološkim rastvorom

Pox: paraoxon, doza sc; l: *saline* – fiziološki rastvor (1 mL/kg, im);

Stereotipni pokreti su pokazivali dobar prognostički znak – ranije su se javljali (r = -528, p = 0,001) i bili su izraženiji kod pacova kojima je ordinirana niža doza paraoksona, kao i kod pacova koji su preživjeli (r = -0,394). Kod viših doza paraoksona, kada su ordinirni i oksimi i atropin, stereotipni pokreti su skoro u potpunosti izostajali u toku cijelog posmatranog perioda (Slika 40).



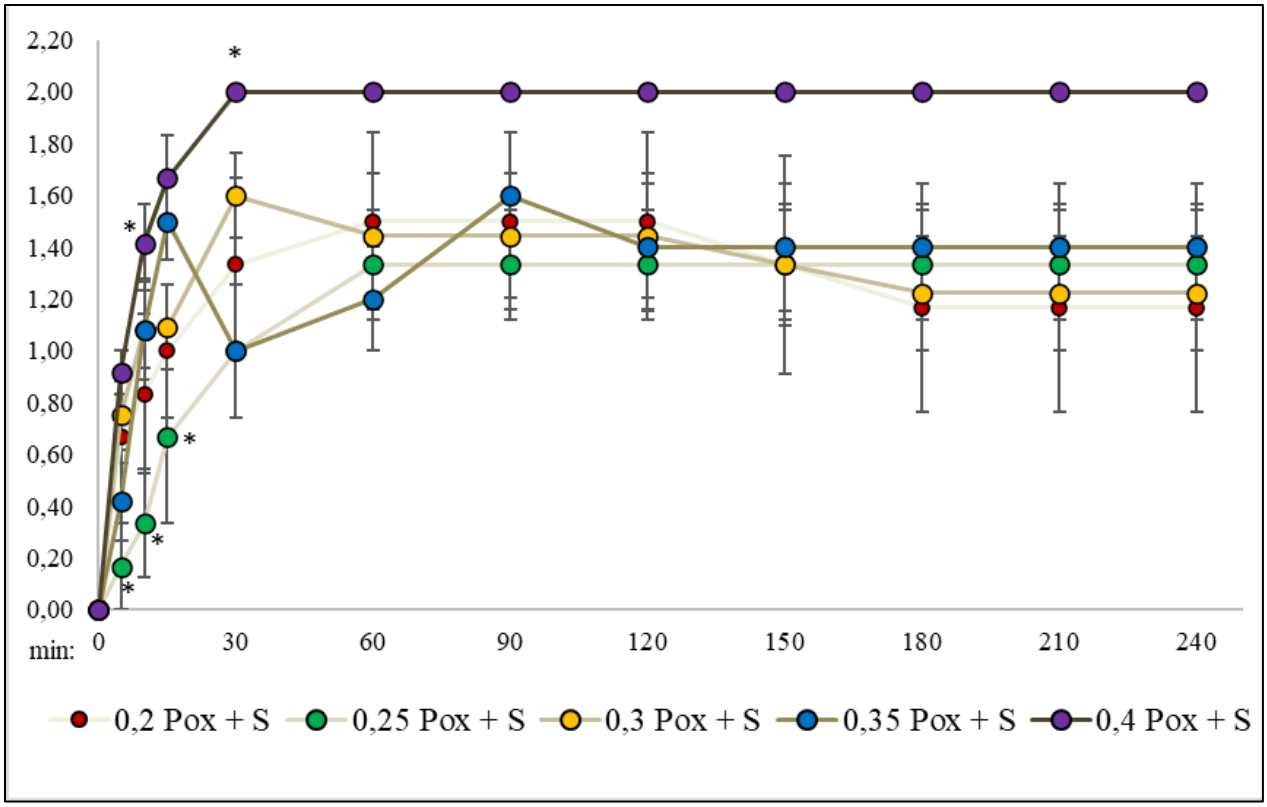
Slika 40. Intenzitet stereotipnih pri različitim dozama paraoksona kod pacova tretiranih atropinom i oksimima

Pox: paraoxon, doza sc; A: atropin (10 mg/kg im); L: (LüH-6 - obidoksim): (22 mg/kg im), K: oksim K870 (35 mg/kg im);

5.4.6. Egzoftalmus

Kod neštićenih pacova, postojala je razlika u intenzitetu egzoftalmusa u 5. (χ^2 = 11,386, p = 0,023), 10. (χ^2 = 11,605, p = 0,021), 15. (χ^2 = 11,076, p = 0,026) i 30. min (χ^2 = 10,013, p = 0,040) u odnosu na različitu dozu paraoksona (Slika 41).

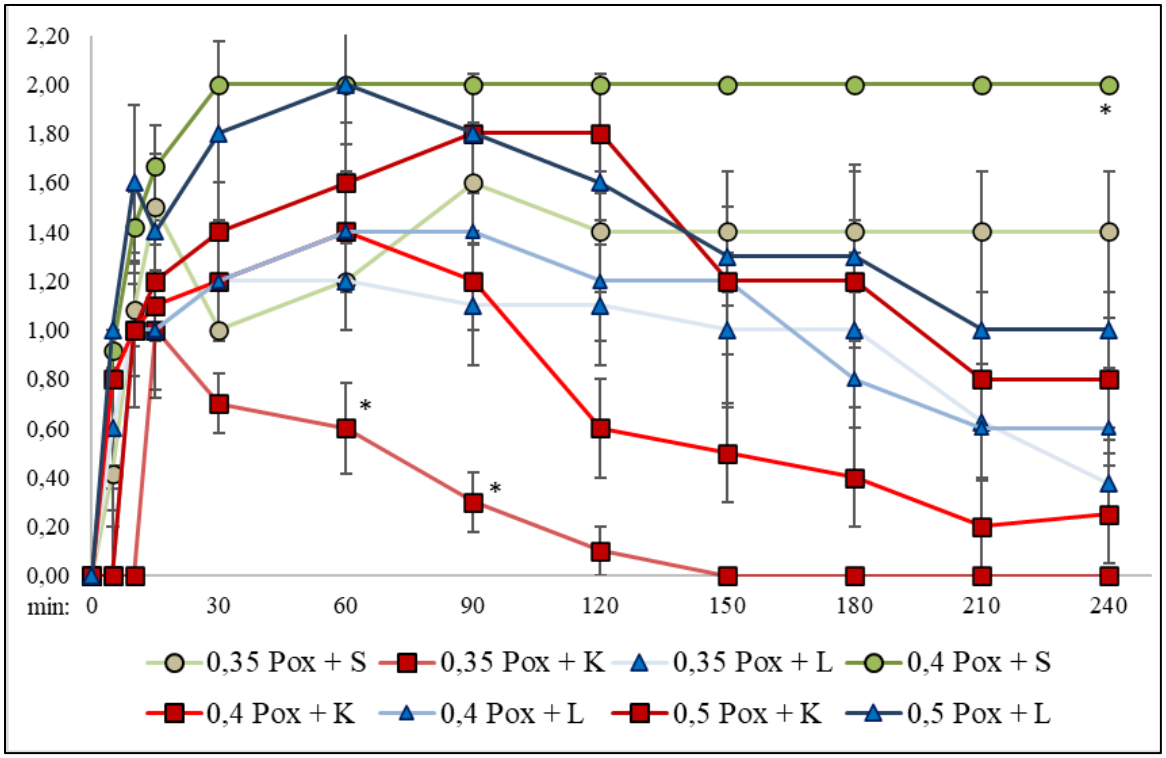
Postojala je statistički značajna razlika u intenzitetu egzoftalmusa u odnosu na različitu dozu paraoksona (χ^2 = 14,199, p = 0,007). Egzoftalmus se javljao ranije pri višim dozama (r = -0.423, p = 0,003). Egzoftalmus se javljao kasnije kod pacova koji su preživjeli (U = 128,000, p = 0,003).



Slika 41. Intenzitet egzoftalmusa pri različitim dozama paraoksona kod pacova tretiranih fiziološkim rastvorom

Pox: paraoxon, doza sc; l: *saline* – fiziološki rastvor (1 mL/kg, im); *statistički značajno;

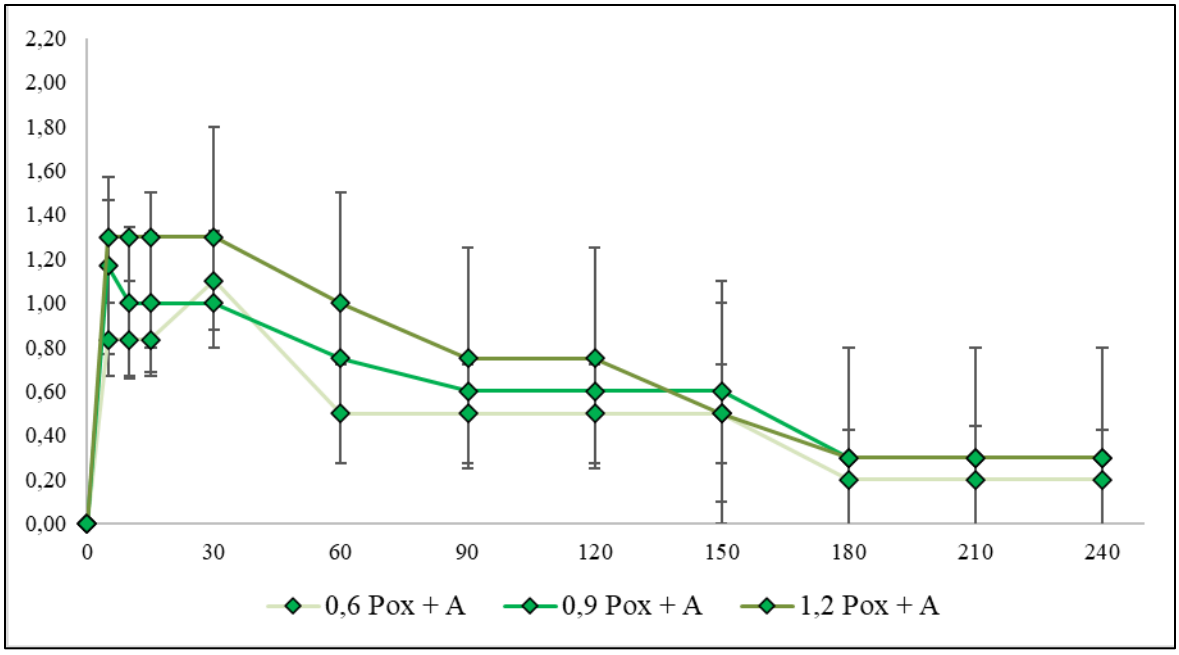
Kod pacova tretiranih oksimima, primijećen je niži intenzitet egzoftalmusa u odnosu na tretman. Kod pacova tretiranih oksimom K870 niži intenzitet egzoftalmusa je nađen u 60. (χ^2 = 10,360, p = 0,043) i 90 min. (χ^2 = 9,603, p = 0,031) (Slika 42).



Slika 42. Intenzitet egzoftalmusa pri različitim dozama paraoksona kod pacova tretiranih oksimima

Pox: paraoxon, doza sc; L: (LüH-6 - obidoksim): (22 mg/kg im), K: oksim K870 (35 mg/kg im); l: *saline* – fiziološki rastvor (1 mL/kg, im); *statistički značajno;

Kod pacova tretiranih atropinom, intenzitet je bio neznatno veći kod viših doza paraoksona, ali su primjećena velika odstupanja unutar grupe. Nije bilo statistički značajne razlike ni u odnosu na intenzitet, brzinu nastajanja, kao ni učestalost egzoftalmusa i kako doze paraoksona tako ni u odnosu na ishod trovanja (Slika 43).

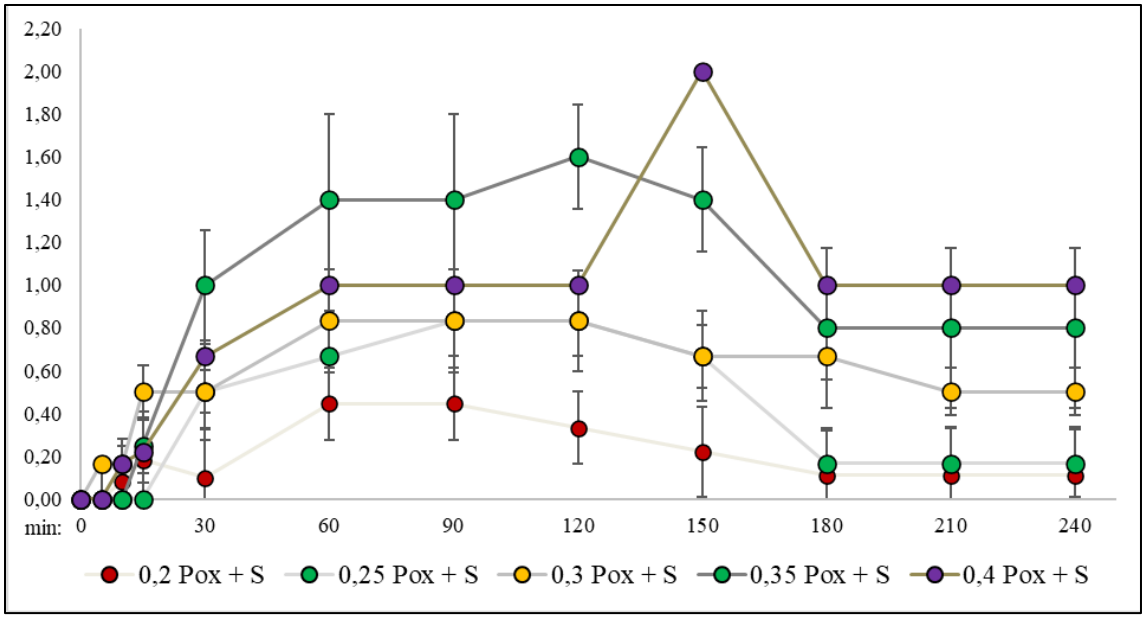


Slika 43. Intenzitet ataksije pri različitim dozama paraoksona kod pacova tretiranih atropinom

Pox: paraoxon, doza sc; A: atropin (10 mg/kg im);

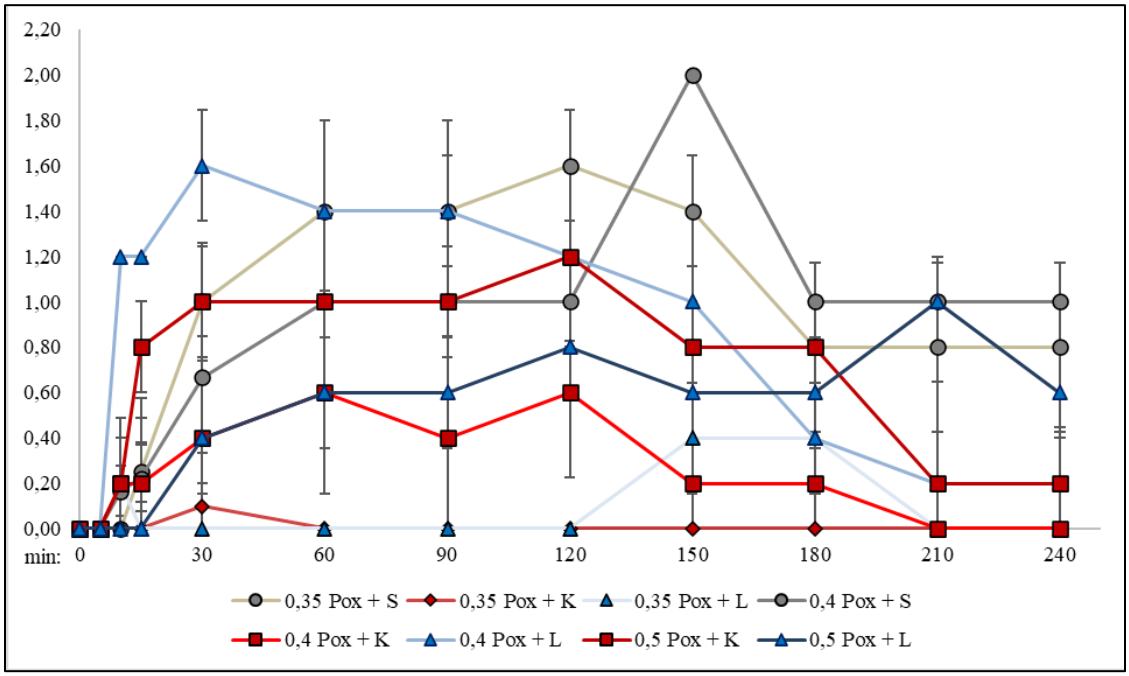
5.4.7. Lakrimacija

Kod neštićenih pacova ni intenzitet ni učestalost lakrimacije nisu bili u značajnoj korelaciji kako sa dozom paraoksona, tako ni sa smrtnim ishodom. Postojale su velike razlike unutar grupe. Kod monoterapije oksimima, lakrimacija se činila skoro nasumična. Međutim, ono što je uočeno kada je atropin primijenjen poslije paraoksona, bilo kao monoterapija ili u kombinaciji sa oksimima, jeste da je lakrimacija bila skoro u potpunosti odsutna. Čak i kod doze paraoksona koja je bila više od 100 puta veća od LD_{50}, lakrimacija se javljala samo kratkotrajno i bila blagog intenziteta (Slike 44-46).



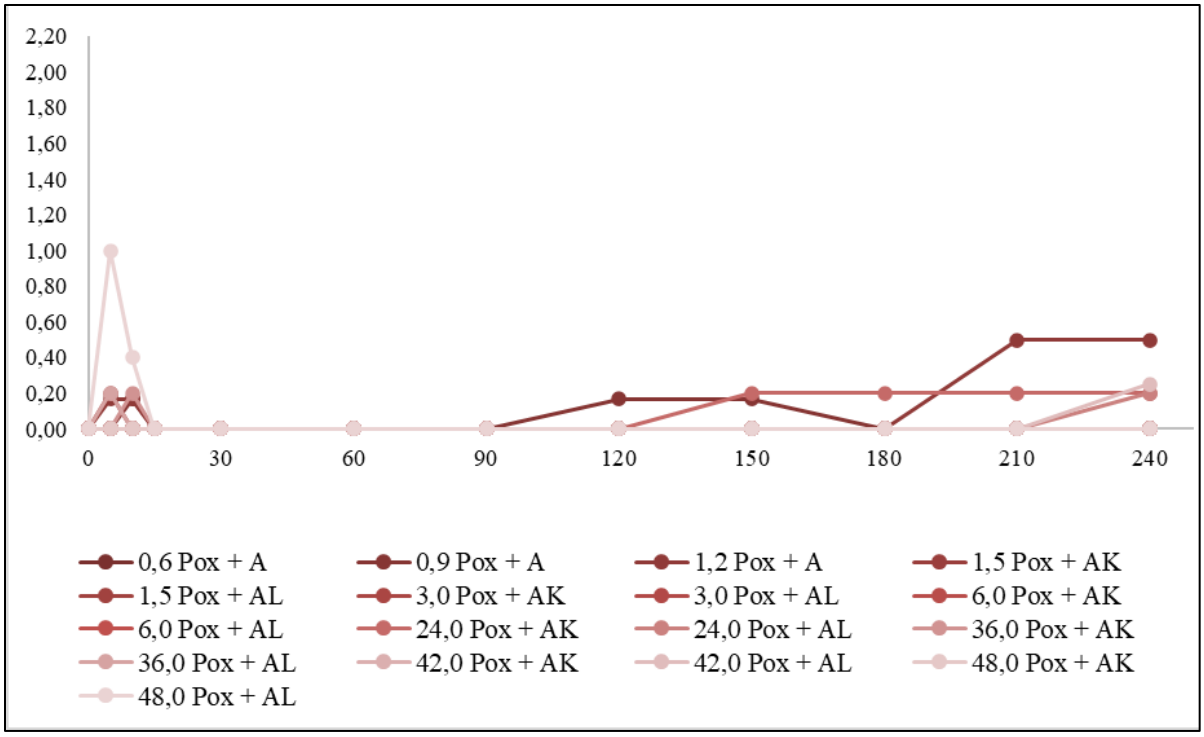
Slika 44. Intenzitet lakrimacije pri različitim dozama paraoksona kod pacova tretiranih fiziološkim rastvorom

Pox: paraoxon, doza sc; l: *saline* – fiziološki rastvor (1 mL/kg, im); *statistički značajno;



Slika 45. Intenzitet lakrimacije pri različitim dozama paraoksona kod pacova tretiranih oksimima

Pox: paraoxon, doza sc; L: (LüH-6 - obidoksim): (22 mg/kg im), K: oksim K870 (35 mg/kg im); S: *saline* – fiziološki rastvor;

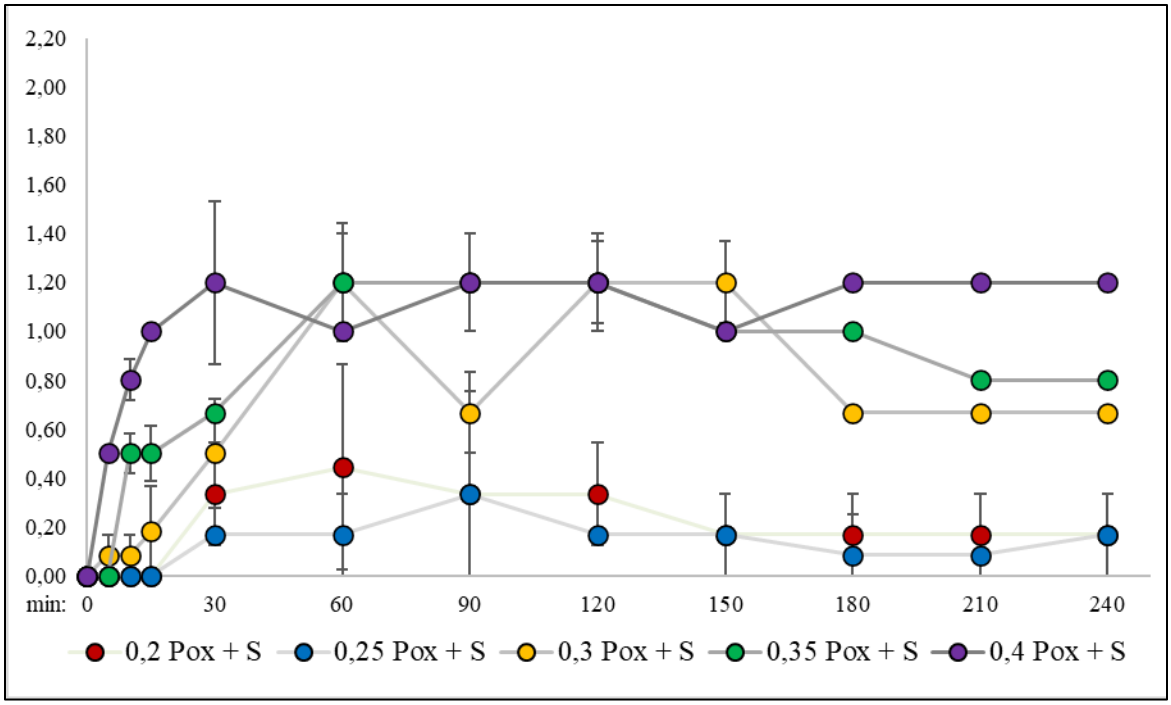


Slika 46. Intenzitet lakrimacij pri različitim dozama paraoksona kod pacova tretiranih atropinom (i) oksimom

Pox: paraoxon, doza sc; A: atropin (10 mg/kg im); L: (LüH-6 - obidoksim): (22 mg/kg im), K: oksim K870 (35 mg/kg im);

5.4.8. Dispneja

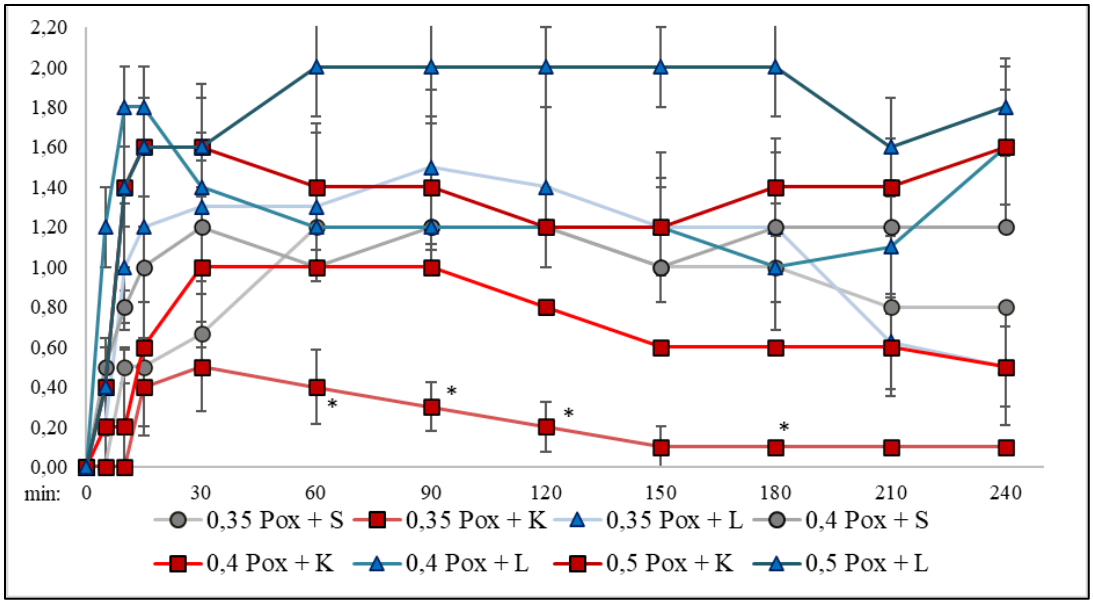
Kod netretiranih pacova, dispneja je bila u slaboj korelaciji sa dozom paraoksona ($r = 0,430$, $p = 0,048$). Nije bilo statistički značajne razlike u pojedinim vremenima. Nije bilo statistički značajne razlike u odnosu na ishod trovanja (Slika 47).



Slika 47. Intenzitet dispneje pri različitim dozama paraoksona kod pacova tretiranih fiziološkim rastvorom

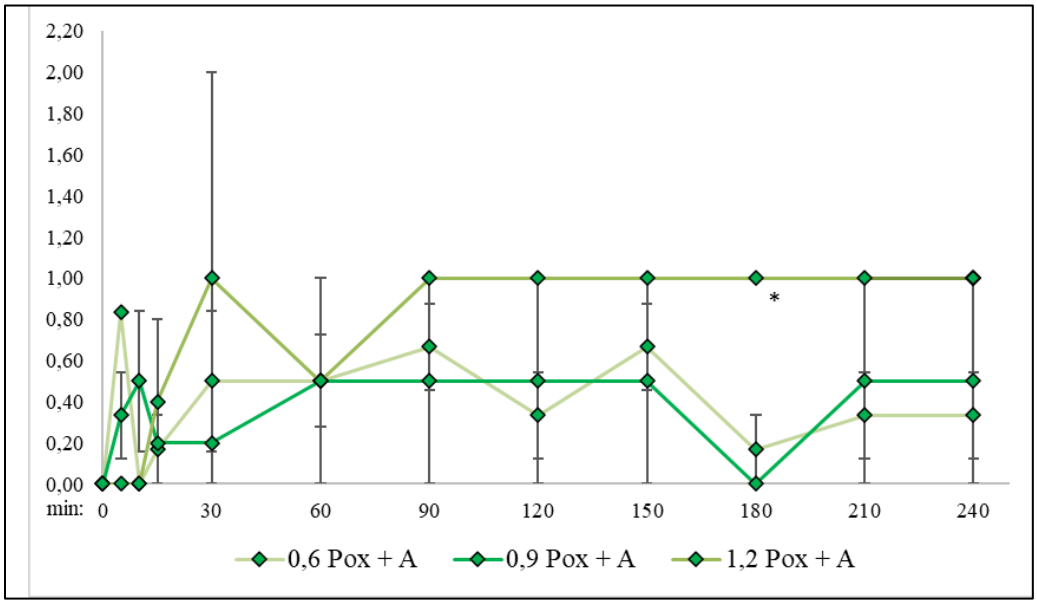
Pox: paraoxon, doza sc; l: *saline* – fiziološki rastvor (1 mL/kg, im); *statistički značajno;

Kod pacova tretiranih oksimima, nađeno je da je dispneja bila statistički značajno nižeg intenziteta kod pacova koji su dobili oksim K870 u poređenju sa obidoksimom, pri dozi paraoksona od 0,35 mg/kg sc (U = 23,304, p = 0,03) (Slika 48).



Slika 48. Intenzitet dispneje pri različitim dozama paraoksona kod pacova tretiranih fiziološkim rastvorom

Pox: paraoxon, doza sc; L: (LüH-6 - obidoksim): (22 mg/kg im), K: oksim K870 (35 mg/kg im); S: *saline* – fiziološki rastvor (1 mL/kg, im); *statistički značajno;



Slika 49. Intenzitet dispneje pri različitim dozama paraoksona kod pacova tretiranih atropinom

Pox: paraoxon, doza sc; A: atropin (10 mg/kg im); *statistički značajno;

Pri tretmanu oksimima i atropinom, dispneja je bila izražena tokom posmatranog perioda, bez značajnih razlika između grupa. Značajne varijacije su se javljale unutar same grupe (Slika 49).

5.4.9. Salivacija

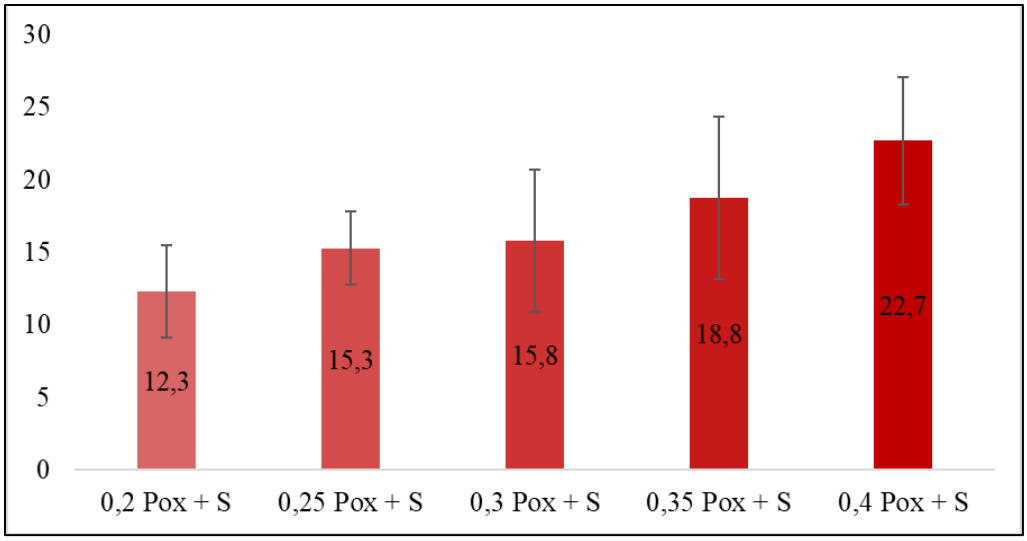
Nije bilo korelacije između intenziteta salivacije i doze paraoksona, kao ni sa primijenjenom terapijom. Pri dozama 0,6-1,2 mg/kg paraoksona, kada je min kasnije ordiniran i atropin, salivacija se nije javila ni kod jednog pacova. Pri višim dozama paraoksona, kada je uz atropin ordiniran i jedan od oksima, povremeno je dolazilo do pojave salivacije različitog intenziteta, nekad uz dispneju, nekad uz konvulzije, ali bez vidljive, korelacije između doze, tretmana, preživljavanja i pojave i intenziteta salivacija.

5.4.10. Piloerekcija

Kod neštićenih pacova piloerekcija se javljala u prvih pola sata nakon trovanja paraoksonom i bila je kratkotrajna. Piloerekcija se češće javljala (33,33% pri dozi 0,3, a 75,00% pri dozi 0,4 paraoksona, $p = 0,09$) i bila je jačeg intenziteta pri višim dozama (razlika značajna u 10. i 15. min: $p = 0.052$; $p = 0.012$). Kod pacova koji nisu preživjeli, piloerekcija se javljala češće (kod 76,19 uginulih pacova, nasuprot 23,81% preživjelih, $p = 0,02$). Piloerekcija se javljala u prvih sat vremena trovanja. Pri višim dozama, naročito u prvih sat vremena, intenzitet konvulzija, tremora i fascikulacija otežao je objektivno posmatranje pojave piloerekcije, te su podaci isključeni iz studije.

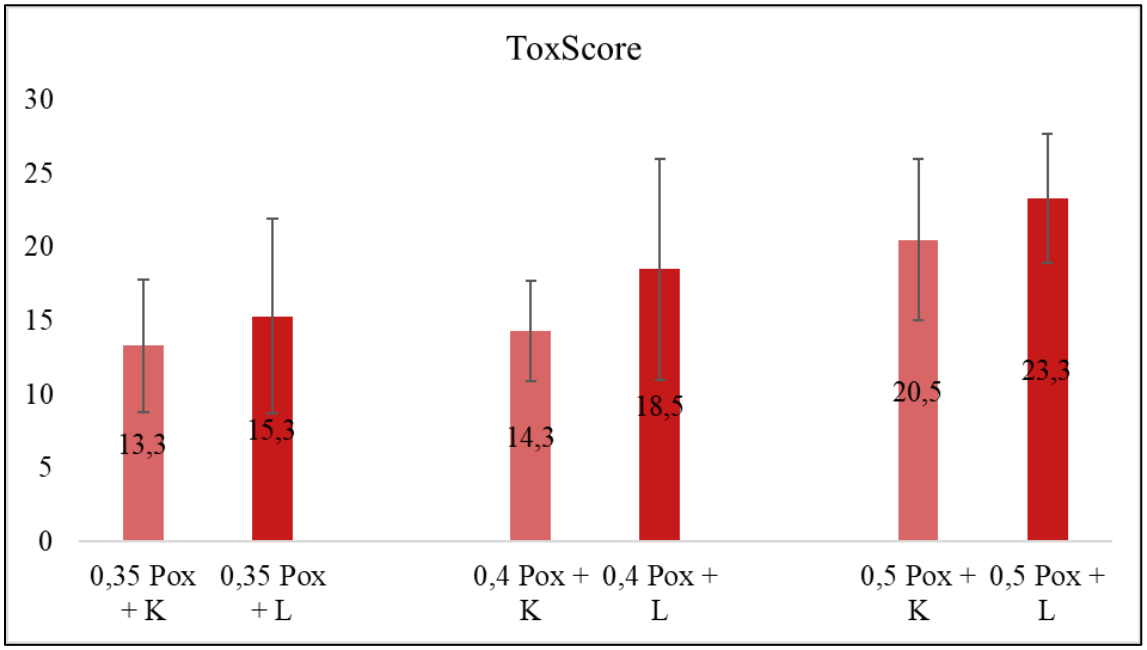
5.4.11. *ToxScore*

Kod netretiranih pacova postojala je jasna korelacija između doze paraoksona i *ToxScore* ($r = 0,657$ $p = 0,02$) (Slika 50). *ToxScore* je bio viši kod pacova koji su uginuli ($\chi^2 = 13,241$, $p = 0,03$).



Slika 50. Prosječni *ToxScore* pri različitim dozama paraoksona kod pacova tretiranih fiziološkim rastvorom

Pox: paraoxon; S: *saline* – fiziološki rastvor (1 mL/kg, im);



Slika 51. Prosječni *ToxScore* pri različitim dozama paraoksona kod pacova tretiranih fiziološkim rastvorom

Pox: paraoxon, doza sc; L: (LüH-6 - obidoksim): (22 mg/kg im), K: oksim K870 (35 mg/kg im);

Kod pacova tretiranih oksimima, pri istoj dozi paraoksona, *ToxScore* je bio niži kod oksima K870 u poređenju sa obidoksimom, ali razlika nije bila statistički značajna (p = 0,09) (Slika 51).

5.5. Hematološki i biohemijski parametri kod preživjelih pacova

5.5.1. Uticaj visokih doza oksima na hematološke i biohemijske parametre

Hematološki parametri kontrolne grupe i pacova tretiranih oksimima su prikazani u Tabelama 11. i 12.

Tabela 11. Hematološki parametri pacova tretiranih visokim dozama oksima

Grupa	Er		Hgb		HCT		MCV		Tr	
	M	SD	M	SD	M	SD	M	SD	M	SD
S + S	8,06	0,70	135,80	4,97	0,46	0,02	56,80	2,27	969,00	48,38
S + L50	8,25	1,23	142,00	17,71	0,49	0,07	59,62	1,29	1042,00	142,95
S + L100	8,49	0,44	143,00	11,27	0,49	0,04	57,43	1,91	966,67	279,90
S + L150	8,76	0,00	153,00	0,00	0,51	0,00	58,10	0,00	1215,00	0,00
S + K100	**10,33**	**0,99**	**178,75**	**16,15**	**0,55**	**0,05**	**53,18**	**0,41**	**1342,75**	**234,02**
S + K150	9,57	0,00	160,00	0,00	0,50	0,00	52,50	0,00	1097,00	0,00
S + K200	9,49	0,00	168,00	0,00	0,51	0,00	54,10	0,00	868,00	0,00
p*	0,061		**0,041**		0,231		**0,027**		0,168	

P*: Kruskal-Wallis test; S + S: 1 mL/kg sc fiziološkog rastvora + 1 min kasnije 1 mL/kg im; L: obidoksim: 50, 100 i 150 mg/kg im; K: oksim K870: 100, 150 i 200 mg/kg im; M: prosjek; SD: standardna devijacija; Er: eritrociti – referentne vrijednosti (RV): 6;71-10,9 x 10^6/µL; Hgb: hemoglobin – RV: 126-160 g/L; HCT: hematokrit – RV: 0,4-0,5, MCV: prosječni volumen eritrocita – RV: 49-61 fL; Tr: trombociti – RV: 380-930 x 10^3/µL;

Tabela 12. Leukociti i leukocitarna formula pacova tretiranih visokim dozama oksima

Grupa	Le		Ne (%)		Li (%)	
	M	SD	M	SD	M	SD
S + S	3,83	1,24	17,54	5,83	77,20	4,47
S + L50	5,37	0,84	11,68	2,43	81,16	7,33
S + L100	4,51	0,62	15,60	3,37	72,00	8,34
S + L150	5,39	0,00	11,60	0,00	75,70	0,00
S + K100	8,01	4,08	59,10	8,14	27,08	7,89
S + K150	4,90	0,00	64,60	0,00	22,00	0,00
S + K200	8,62	0,00	79,00	0,00	18,00	0,00
p*	0,097		**0,016**		**0,035**	

P*: Kruskal-Wallis test; S + S: 1 mL/kg sc fiziološkog rastvora + 1 min kasnije 1 mL/kg im; L: obidoksim: 50, 100 i 150 mg/kg im; K: oksim K870: 100, 150 i 200 mg/kg im; M: prosjek; SD: standardna devijacija; Le: leukociti (N × 10^9/L); Ne: neutrofili; Li: limfociti;

Više vrijednosti hemoglobina, kao i skretanje leukocitarne formule ulijevo primijećeni su kod pacova tretiranih oksimom K870 u odnosu na iste doze obidoksima.

Tabela 13. Vrijednosti parametara oštećenja jetre u serumu pacova tretiranih visokim dozama oksima

Grupa	AST		ALT		LDH	
	M	SD	M	SD	M	SD
S + S	236,00	140,33	65,00	25,05	1048,40	972,29
S + L50	557,00	92,76	86,00	13,29	1781,80	603,97
S + L100	726,33	171,22	92,50	25,36	1855,00	1095,15
S + L150	895,00	0,00	114,00	0,00	1838,00	0,00
S + K100	207,60	80,82	52,00	60,68	1090,00	0,00
S + K150	360,00	0,00	71,00	79,20	1900,00	0,00
S + K200	392,00	0,00	73,20	0,00	1435,00	0,00
p*	**0,049**		**0,043**		0,258	

P*: Kruskal-Wallis test; S + S: 1 mL/kg sc fiziološkog rastvora + 1 min kasnije 1 mL/kg im; L: obidoksim: 50, 100 i 150 mg/kg im; K: oksim K870: 100, 150 i 200 mg/kg im; M: prosjek; SD: standardna devijacija; AST: aspartat aminotransferaza (RV: 67-215 U/L); ALT: alanin aminotransferaza (RV: 17-54 U/L); LDH: laktat dehidrogenaza (RV: 600-1450 U/L);

Uticaj oksima na parametre oštećenja jetre i pankreasa prikazani su u Tabelama 13 i 14. Obidoksim je pokazao hepatotoksične karakteristike i to dozno-zavisne – pri većim dozama obidoksima vrijednosti transaminaza (AST i ALT) su bile više ($r = 0,423$, $p = 0,022$). Oksim K870 nije značajno uticao na vrijednosti transaminaza. Ni obidoksim ni oksim K870 nisu doveli do značajnog povećanja pankreasnih enzima.

Tabela 14. Aktivnost pankreasnih enzima u serumu pacova tretiranih visokim dozama oksima

Grupa	p-amilaza (U/L)		lipaza (U/L)	
	M	SD	M	SD
S + S	1900,40	161,84	8,00	2,92
S + L50	2003,80	272,87	6,80	0,84
S + L100	2065,67	512,25	8,67	2,08
S + L150	1907,00	0,00	6,00	0,00
S + K100	2230,00	0,00	7,50	0,71
S + K150	1718,00	0,00	8,00	0,00
S + K200	2305,00	0,00	8,00	0,00
p*	0,260		0,323	

P*: Kruskal-Wallis test; S + S: 1 mL/kg sc fiziološkog rastvora + 1 min kasnije 1 mL/kg im; L: obidoksim: 50, 100 i 150 mg/kg im; K: oksim K870: 100, 150 i 200 mg/kg im; M: prosjek; SD: standardna devijacija;

Tabela 15. Koncentracija parametara bubrežne funkcije u serumu pacova tretiranih visokim dozama oksima

Grupa	Urea		Cr		UP		Alb	
	M	SD	M	SD	M	SD	M	SD
S + S	5,22	0,66	35,20	5,63	62,20	3,96	40,40	2,51
S + L50	5,38	0,79	36,00	2,12	63,40	1,82	41,00	1,58
S + L100	4,67	0,38	40,33	1,53	63,67	2,31	39,67	1,53
S + L150	4,30	0,00	47,00	0,00	66,00	0,00	44,00	0,00
S + K100	7,40	0,71	82,00	16,97	51,50	0,71	38,00	1,41
S + K150	8,20	10,61	97,00	0,00	54,00	0,00	34,00	1,41
p*	**0,045**		**0,042**		0,109		0,090	

P*: Kruskal-Wallis test; S + S: 1 mL/kg sc fiziološkog rastvora + 1 min kasnije 1 mL/kg im; L: obidoksim: 50, 100 i 150 mg/kg im; K: oksim K870: 100, 150 i 200 mg/kg im; M: prosjek; SD: standardna devijacija; Urea (RV: 2,5-6,6 mmol/L); Cr: kreatinin (RV: 32-59,6 mmol/L); UP: ukupni proteini (RV: 6,1-7,8 g/L); Alb: albumini (RV: 34,3-48,5 g/L);

Uticaj oksima na parametre bubrežne funkcije prikazani su u Tabeli 15. Oksim K870 je pokazao nefrotoksične efekte pri višim dozama (r = 0,234, p = 0,047). Obidoksim nije značajno uticao na bubrežne parametre (vrijednosti uree i kreatinina) ni pri višim dozama. Nijedan od oksima nije dovelo do značajne promjene u vrijednostima proteina i elektrolita u serumu pacova (Tabela 16).

Tabela 16. Koncentracije elektrolita u serumu pacova tretiranih visokim dozama oksima

Grupa	Na^+		K^+		Cl^-		Ca^{++}		PO_4-	
	M	SD	M	SD	M	SD	M	SD	M	SD
S + S	141,40	0,89	6,02	3,42	102,60	2,07	2,64	0,10	3,11	0,76
S + L50	139,80	2,49	6,64	0,44	101,00	1,22	2,81	0,17	2,88	0,36
S + L100	139,00	1,00	7,47	0,40	98,67	1,15	2,67	0,20	3,35	0,18
S + L150	137,00	0,00	6,00	0,00	95,00	0,00	2,81	0,00	2,43	0,00
S + K100	127,25	4,43	6,43	1,75	89,25	1,26	2,39	0,05	4,26	0,21
S + K150	123,50	7,78	5,95	5,47	85,25	2,33	2,41	4,57	4,24	0,00
p*	0,114		0,078		0,154		0,213		0,442	

P*: Kruskal-Wallis test; S + S: 1 mL/kg sc fiziološkog rastvora + 1 min kasnije 1 mL/kg im; L: obidoksim: 50, 100 i 150 mg/kg im; K: oksim K870: 100, 150 i 200 mg/kg im; M: prosjek; SD: standardna devijacija; Na^+: natrijum (RV: 136-149 mEq/L); K^+: kalijum (3,9-6,4 mmol/L); Cl^-: hloridi (94-116 mEq/L); Ca^{2+}: kalcijum (2,2-2,4 mmol/L); PO_4^-: fosfati (4-6 mg/dL):

Oksimi nisu značajno uticali na vrijednosti kardio-specifičnih enzima (CK, CK-MB, hsTnI), ni pri višim dozama (Tabela 17). Pri višim vrijednostima obidoksima nađene su više vrijednosti CK, ali s obzirom na mali broj preživelih pacova, kao i velike varijacije vrijednosti u grupi kontrolnih pacova razlika nije bila statistički značajna.

Tabela 17. Parametri srčanog oštećenja u serumu pacova tretiranih visokim dozama oksima

Grupa	CK		CK - MB		hsTnI	
	M	SD	M	SD	M	SD
S + S	10851,20	10091,36	814,60	581,00	49,65	64,05
S + L50	8125,80	7039,17	623,40	380,54	41,54	38,23
S + L100	19273,00	14673,63	1355,67	600,05	28,75	7,28
S + L150	20541,00	0,00	1077,00	0,00	157,67	0,00
S + K100	6433,00	0,00	884,00	810,55	46,67	0,00
S + K150	8444,00	0,00	862,00	0,00	105,54	0,00
S + K200	11280,00	0,00	1262,00	0,00	123,32	0,00
p*	0,505		0,271		0,921	

P*: Kruskal-Wallis test; S + S: 1 mL/kg sc fiziološkog rastvora + 1 min kasnije 1 mL/kg im; L: obidoksim: 50, 100 i 150 mg/kg im; K: oksim K870: 100, 150 i 200 mg/kg im; M: prosjek; SD: standardna devijacija; CK: kreatin-kinaza (RV: 100-900 IU/L); CK-MB: kreatin-kinaza izoenzim MB (< 500 U/L); hsTnI: visokosenzitivni troponin I (< 15 pg/mL);

Oksimi nisu uticali na vrijednosti glukoze i lipida ni pri visokim dozama (Tabela 18).

Tabela 18. Metabolički parametri u serumu pacova tretiranih visokim dozama oksima

Grupa	GUK		Chol		HDL		LDL		Tgl	
	M	SD	M	SD	M	SD	M	SD	M	SD
S + S	13,60	6,42	0,76	0,05	0,54	0,05	0,12	0,04	0,46	0,15
S + L50	17,08	2,27	0,82	0,13	0,56	0,09	0,12	0,04	0,40	0,12
S + L100	16,20	3,18	0,93	0,12	0,67	0,15	0,10	0,00	0,43	0,06
S + L150	18,50	0,00	0,60	0,00	0,40	0,00	0,10	0,00	0,50	0,00
S + K100	19,40	0,99	0,85	0,07	0,55	0,07	0,15	0,07	0,60	0,14
S + K150	14,25	0,92	1,40	0,71	0,25	0,21	0,20	0,00	0,60	0,00
S + K200	14,00	0,00	0,80	0,00	0,50	0,00	0,20	0,00	0,80	0,00
p*	0,113		0,099		0,127		0,215		0,134	

P*: Kruskal-Wallis test; S + S: 1 mL/kg sc fiziološkog rastvora + 1 min kasnije 1 mL/kg im; L: obidoksim: 50, 100 i 150 mg/kg im; K: oksim K870: 100, 150 i 200 mg/kg im; M: prosjek; SD: standardna devijacija; GUK: glukoza (3,9-,9 mmol/L); Chol: ukupni holesterol (1,2-1-3 mmol/L); HDL: holesterol velike gustine (0,33-1,02 mmol/L); LDL: holesterol male gustine (0,3-1,5 mmol/L); Tgl: trigliceridi (0,8-1,5 mmol/L);

5.5.2. Uticaj paraoksona i tretmana na hematološke i biohemijske parametre

Hematološki parametri pri različitim dozama paraoksona, nakon kojih je ordiniran tretman sa FR, obidoksimom ili oksimom K870 su prikazani u tabelama 19 i 20. Nije bilo statistički značajne razlike između grupa ni u vrijednostima parametara krvne slike (broj eritrocita, leukocita, trombocita, vrijednosti hemoglobina i hematokrita), tako ni na leukocitarnu formulu i broj i odnos specifičnih leukocita.

Tabela 19. Hematološki parametri pacova tretiranih različitim dozama paraoksona i fiziološkim rastvorom, obidoksimom ili oksimom K870

Grupa	Er		Hgb		HCT		MCV		Tr	
	M	SD	M	SD	M	SD	M	SD	M	SD
0,25 P + S	8,42	0,32	142,60	7,27	0,48	0,03	57,28	1,65	923,00	154,69
0,3 P + S	10,02	0,00	150,00	0,00	0,52	0,00	52,30	0,00	1246,00	0,00
0,35 P + S	8,13	0,00	141,00	0,00	0,45	0,00	55,80	0,00	1215,00	0,00
0,35 P + K	8,59	0,92	146,60	13,72	0,50	0,05	57,72	2,14	1022,60	309,32
0,35 P + L	9,62	1,21	162,25	17,63	0,53	0,06	55,38	2,02	814,50	303,97
0,4 P + K	10,55	0,66	179,25	9,14	0,60	0,02	56,45	2,04	1319,00	243,22
0,4 P + L	9,59	0,86	163,25	12,50	0,55	0,04	56,98	1,68	1086,75	177,96
0,5 P + K	9,65	1,81	164,50	35,05	0,54	0,10	56,00	1,09	1214,50	212,21
0,5 P + L	9,09	0,26	155,00	4,00	0,52	0,02	57,03	1,36	1069,00	78,81
p*	0,107		0,105		0,094		0,543		0,124	

P*: Kruskal-Wallis test; S: 1 mL/kg im fiziološkog rastvora; L: obidoksim 22 mg/kg im; K: oksim K870 35 mg/kg m; 0,25-0,5 P: različite doze paraoksona sc; M: prosjek; SD: standardna devijacija; Er: eritrociti – referentne vrijednosti (RV): 6;71-10,9 x 10^6/μL; Hgb: hemoglobin – RV: 126-160 g/L; HCT: hematokrit – RV: 0,4-0,5, MCV: prosječni volumen eritrocita – RV: 49-61 fL; Tr: trombociti – RV: 380-930 x 10^3/μL;

Tabela 20. Leukociti i leukocitarna formula pacova tretiranih različitim dozama paraoksona i fiziološkim rastvorom, obidoksimom ili oksimom K870

Grupa	Le		Ne (%)		Li (%)	
	M	SD	M	SD	M	SD
0,25 P + S	5,17	1,83	29,78	9,34	63,28	11,52
0,3 P + S	4,02	0,00	28,90	0,00	64,20	0,00
0,35 P + S	4,30	0,00	11,20	0,00	86,00	0,00
0,35 P + K	7,15	2,42	26,46	10,02	65,70	11,67
0,35 P + L	6,40	1,02	30,35	17,24	56,10	25,30
0,4 P + K	6,53	0,55	58,28	11,47	27,68	12,56
0,4 P + L	6,37	1,80	49,30	22,87	38,30	28,02
0,5 P + K	5,27	1,21	46,35	20,13	45,85	21,79
0,5 P + L	4,45	2,16	47,90	17,37	45,30	19,08
p*	0,364		0,090		0,134	

P*: Kruskal-Wallis test; S: 1 mL/kg im fiziološkog rastvora; L: obidoksim 22 mg/kg im; K: oksim K870 35 mg/kg m; 0,25-0,5 P: različite doze paraoksona sc; M: prosjek, SD: standardna devijacija; Le: leukociti ($N \times 10^9$/L); Ne: neutrofili; Li: limfociti;

Uticaj paraoksona na parametre oštećenja jetre i gušterače prikazani su u Tabelama 21 i 22. Transaminaze su rasle porastom doze paraoksona i bile više u grupi kojoj je ordiniran obidoksim u odnosu na oksim K870 pri istoj dozi paraoksona, ali razlika nije bila statistički značajna. Porast pankreasne amilaze primjećen je pri višim dozama paraoksona, ali bez statističke značajnosti.

Tabela 21. Vrijednosti parametara oštećenja jetre u serumu pacova pacova tretiranih različitim dozama paraoksona i fiziološkim rastvorom, obidoksimom ili oksimom K870

Grupa	AST		ALT		LDH	
	M	SD	M	SD	M	SD
0,25 P + S	367,50	123,47	100,50	39,20	1376,50	122,39
0,3 P + S	350,00	0,00	87,00	0,00	2201,00	0,00
0,35 P + S	840,00	0,00	421,00	0,00	1077,00	0,00
0,35 P + K	313,25	117,76	70,75	12,84	1237,40	197,33
0,35 P + L	376,60	75,22	82,00	12,59	822,00	471,83
0,4 P + K	260,33	115,08	66,67	4,04	1331,67	345,89
0,4 P + L	483,33	41,04	55,67	5,03	1724,00	2,83
0,5 P + K	518,75	247,96	82,75	45,32	651,33	481,73
0,5 P + L	592,25	166,59	89,50	30,80	1130,67	430,00
p*	0,070		0,247		0,083	

P*: Kruskal-Wallis test; S: 1 mL/kg im fiziološkog rastvora; L: obidoksim 22 mg/kg im; K: oksim K870 35 mg/kg m; 0,25-0,5 P: različite doze paraoksona sc; M: prosjek, SD: standardna devijacija; AST: aspartat aminotransferaza (RV: 67-215 U/L); ALT: alanin aminotransferaza (RV: 17-54 U/L); LDH: laktat dehidrogenaza (RV: 600-1450 U/L);

Tabela 22. Aktivnost pankreasnih enzima u serumu pacova tretiranih različitim dozama paraoksona i fiziološkim rastvorom, obidoksimom ili oksimom K870

Grupa	p-amilaza (U/L)		lipaza (U7L)	
	M	SD	M	SD
0,25 P + S	2063,25	747,68	8,25	1,26
0,3 P + S	2346,00	0,00	8,00	0,00
0,35 P + S	2815,00	0,00	6,00	0,00
0,35 P + K	2770,60	865,75	8,20	2,17
0,35 P + L	2349,75	438,13	6,25	0,50
0,4 P + K	2168,00	202,08	6,67	0,58
0,4 P + L	1958,00	282,28	7,50	0,71
0,5 P + K	2035,00	533,16	7,67	1,15
0,5 P + L	3410,00	292,74	7,00	0,00
p*	0,113		0,131	

P*: Kruskal-Wallis test; S: 1 mL/kg im fiziološkog rastvora; L: obidoksim 22 mg/kg im; K: oksim K870 35 mg/kg m; 0,25-0,5 P: različite doze paraoksona sc; M: prosjek, SD: standardna devijacija;

Tabela 23. Koncentracija parametara bubrežne funkcije u serumu pacova tretiranih različitim dozama paraoksona i fiziološkim rastvorom, obidoksimom ili oksimom K870

Grupa	Urea		Cr		UP		Alb	
	M	SD	M	SD	M	SD	M	SD
0,25 P + S	8,13	2,53	45,75	6,85	68,25	1,71	42,75	1,26
0,3 P + S	9,90	0,00	48,00	0,00	66,00	0,00	37,00	0,00
0,35 P + S	10,50	0,00	69,00	0,00	70,00	0,00	44,00	0,00
0,35 P + K	10,81	8,52	70,00	4,50	66,00	4,47	44,40	4,45
0,35 P + L	9,53	3,46	62,50	3,51	70,25	5,85	40,00	6,38
0,4 P + K	10,15	5,73	67,25	11,62	69,67	4,73	37,25	0,96
0,4 P + L	10,80	2,55	62,00	1,41	73,00	1,41	45,67	3,06
0,5 P + K	10,50	8,55	65,33	5,01	62,00	8,72	38,00	6,08
0,5 P + L	11,37	1,64	61,67	7,77	72,67	2,89	41,00	3,61
p*	0,294		0,375		0,157		0,077	

P*: Kruskal-Wallis test; S: 1 mL/kg im fiziološkog rastvora; L: obidoksim 22 mg/kg im; K: oksim K870 35 mg/kg m; 0,25-0,5 P: različite doze paraoksona sc; M: prosjek, SD: standardna devijacija; Urea (RV: 2,5-6,6 mmol/L); Cr: kreatinin (RV: 32-59,6 mmol/L); UP: ukupni proteini (RV: 6,1-7,8 g/L); Alb: albumini (RV: 34,3-48,5 g/L);

Uticaj paraoksona na parametre oštećenja bubrežne funkcije prikazani su u Tabeli 23. Više doze paraoksona su blago uticale na bubrežne parametre (vrijednosti uree i kreatinina), bez razlike između grupa. Oksim K870 nije pokazivao nefrotoksičnost pri terapijskim dozama. Diskretno niže vrijednosti jona natrijuma i hlora nađene su kod tretmana oksimom K870 u odnosu na obidoksim pri istim dozama paraoksona, ali bez statističke značajnosti (Tabela 24).

Tabela 24. Koncentracije elektrolita u serumu pacova tretiranih različitim dozama paraoksona i fiziološkim rastvorom, obidoksimom ili oksimom K870

Grupa	Na^+		K^+		Cl^-		Ca^{++}		PO_4-	
	M	SD	M	SD	M	SD	M	SD	M	SD
0,25 P + S	147,50	2,89	8,48	0,51	106,50	2,52	2,75	0,14	3,47	0,46
0,3 P + S	145,00	0,00	9,90	0,00	101,00	0,00	2,98	0,00	3,60	0,00
0,35 P + S	143,00	0,00	6,10	0,00	101,00	0,00	2,83	0,00	2,59	0,00
0,35 P + K	141,24	5,67	6,68	4,12	99,75	4,09	2,64	0,19	2,71	0,06
0,35 P + L	144,50	1,91	5,93	1,20	101,75	3,77	2,74	0,30	2,80	0,86
0,4 P + K	138,33	1,53	7,57	1,07	96,33	1,53	2,63	0,09	3,67	0,60
0,4 P + L	146,00	2,71	6,63	0,63	106,50	3,11	2,79	0,15	2,97	0,30
0,5 P + K	138,67	3,21	8,47	1,42	98,67	0,58	2,71	0,15	3,34	1,11
0,5 P + L	149,00	1,00	7,17	1,36	106,33	1,15	2,80	0,14	3,47	0,54
p*	0,342		0,267		0,198		0,585		0,519	

P*: Kruskal-Wallis test; S: 1 mL/kg im fiziološkog rastvora; L: obidoksim 22 mg/kg im; K: oksim K870 35 mg/kg m; 0,25-0,5 P: različite doze paraoksona sc; M: prosjek, SD: standardna devijacija; Na^+: natrijum (RV: 136-149 mEq/L); K^+: kalijum (3,9-6,4 mmol/L); Cl^-: hloridi (94-116 mEq/L); Ca^{2+}: kalcijum (2,2-2,4 mmol/L); PO_4^-: fosfati (4-6 mg/dL):

Parametri srčanog oštećenja pacova tretiranih različitim dozama paraoksona i FR, obidoksimom ili oksimom K870 prikazani su u Tabeli 25. CK i hsTnI su rasli porastom doze paraoksona, ali bez značajne razlike. Nađene vrijednosti su imale značajne devijacije unutar grupe i u prosjeku, iako statistički bez značajne razlike, bile više u grupama koje su tretirane obidoksimom u odnosu na oksim K870. Usljed malog broja pacova unutar grupe i velikih SD, iako postoji trend porasta razlika nije statistički značajna.

Tabela 25. Parametri srčanog oštećenja u serumu pacova tretiranih različitim dozama paraoksona i fiziološkim rastvorom, obidoksimom ili oksimom K870

Grupa	CK		CK-MB		hsTnI	
	M	SD	M	SD	M	SD
0,25 P + S	6616,75	3183,85	1102,00	158,28	100,43	41,30
0,3 P + S	4386,00	0,00	655,00	0,00	864,50	0,00
0,35 P + S	9576,00	0,00	921,00	0,00	1193,20	0,00
0,35 P + K	3101,00	3607,39	585,50	365,94	170,88	252,84
0,35 P + L	7975,20	3866,50	712,66	422,89	2913,90	1198,93
0,4 P + K	3466,00	4662,66	670,33	506,59	194,50	0,00
0,4 P + L	5683,00	3844,69	846,67	327,88	1194,50	0,00
0,5 P + K	8423,00	1243,09	1361,25	271,18	1066,60	67,74
0,5 P + L	12875,00	4741,86	1097,00	186,68	4147,10	0,00
p*	0,284		0,100		0,188	

P*: Kruskal-Wallis test; S: 1 mL/kg im fiziološkog rastvora; L: obidoksim 22 mg/kg im; K: oksim K870 35 mg/kg m; 0,25-0,5 P: različite doze paraoksona sc; M: prosjek, SD: standardna devijacija; CK: kreatin-kinaza (RV: 100-900 IU/L); CK-MB: kreatin-kinaza izoenzim MB (< 500 U/L); hsTnI: visokosenzitivni troponin I (< 15 pg/mL);

Tabela 26. Metabolički parametri u serumu pacova tretiranih različitim dozama paraoksona i fiziološkim rastvorom FR, obidoksimom ili oksimom K870

Grupa	GUK		Chol		HDL		LDL		Tgl	
	M	SD	M	SD	M	SD	M	SD	M	SD
0,25 P + S	9,93	10,07	1,30	0,34	0,83	0,22	0,18	0,05	0,68	0,17
0,3 P + S	16,30	0,00	1,20	0,00	0,80	0,00	0,10	0,00	0,60	0,00
0,35 P + S	14,60	0,00	0,80	0,00	0,60	0,00	0,10	0,00	1,20	0,00
0,35 P + K	19,34	1,89	1,10	0,24	0,70	0,16	0,18	0,04	1,16	0,64
0,35 P + L	18,78	4,03	0,80	0,18	0,55	0,13	0,08	0,05	0,78	0,26
0,4 P + K	13,97	1,37	1,73	1,11	0,97	0,25	0,57	0,64	1,27	0,31
0,4 P + L	16,05	4,31	1,15	0,35	0,80	0,28	0,15	0,07	0,95	0,35
0,5 P + K	17,37	8,46	1,20	0,26	0,80	0,20	0,20	0,10	0,93	0,35
0,5 P + L	16,60	2,69	1,17	0,32	0,83	0,21	0,13	0,06	0,93	0,15
p*	0,347		0,419		0,367		0,236		0,244	

P*: Kruskal-Wallis test; S: 1 mL/kg im fiziološkog rastvora; L: obidoksim 22 mg/kg im; K: oksim K870 35 mg/kg m; 0,25-0,5 P: različite doze paraoksona sc; M: prosjek; SD: standardna devijacija; GUK: glukoza (mmol/L); GUK: glukoza (3,9-,9 mmol/L); Chol: ukupni holesterol (1,2-1-3 mmol/L); HDL: holesterol velike gustine (0,33-1,02 mmol/L); LDL: holesterol male gustine (0,3-1,5 mmol/L); Tgl: trigliceridi (0,8-1,5 mmol/L);

Paraokson nije uticao na vrijednosti glukoze i lipida ni pri visokim dozama (Tabela 26).

Pomenuti hematološki parametri su posmatrani i kada je pri visokim dozama paraoksona (1,5, 3,0, 6,0, 24,0, 36,0, 42,0 i 48,0 mg/kg sc paraoksona) aplikovan tretman atropinom i obidoksimom ili atropinom i oksimom K870. Osim umjerenog skretanja leukocitarne formule ulijevo ($p = 0,007$), nije bilo značajne razlike u vrijednostima hematoloških parametara.

Više vrijednosti paraoksona su dovodile do porasta jetrenih enzima, pri dozi od 42 mg/kg paraoksona vrijednosti AST je iznosila 1000,00 ± 0,00 kod tretmana atropinom i oksimom K870 i 1110,50 ± 507,00 kod tretmana atropinom i obidoksimom, a ALT 170,50 ± 82,73 za tretman atropinom i oksimom K870 i 310,00 ± 0,00 kod tretmana atropinom i obidoksimom. Pri svim dozama nije bilo statistički značajne razlike u odnosu na tretman ($p = 0,121$ za AST i $p = 0,310$ za ALT).

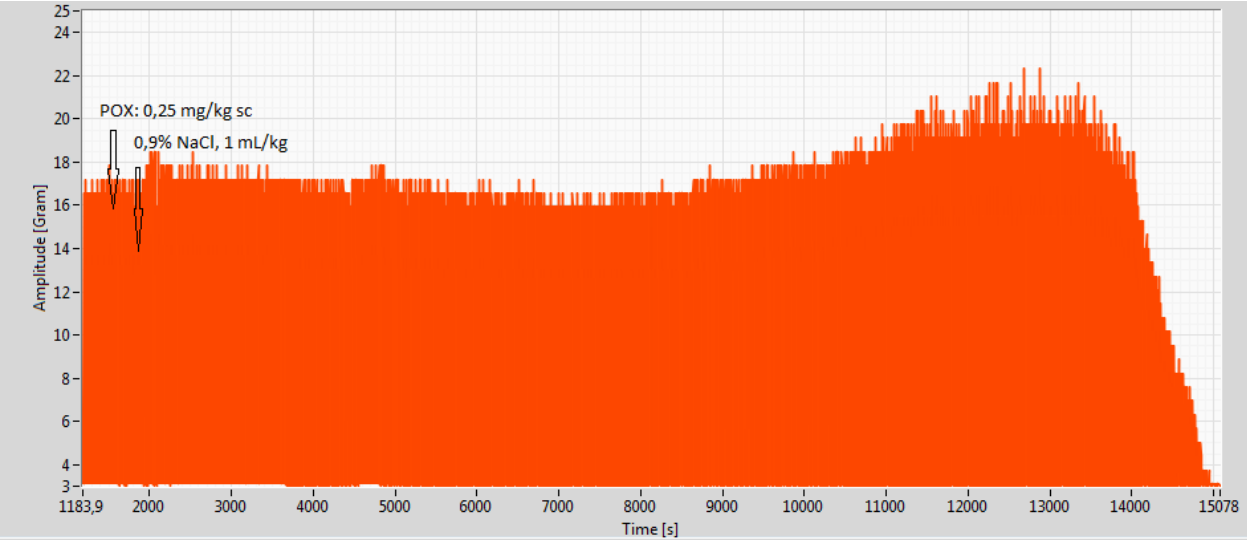
Kod pojedinih pacova vrijednosti amilaze i lipaze bile su mnogostruko više od kontrolnih vrijednosti. Pojedinačni pacovi kojima su bili povišeni pankreasni enzmi nisu bili u vezi ni sa dozom paraoksona ni sa tretmanom, dovodeći do velikih devijacija parametara unutar grupe.

Diskretno povećanje uree i kreatinina nađeno je pri visokim dozama paraoksona, ali bez razlike u odnosu na dozu paraoksona. Nešto više vrijednosti uree i kreatinina bile su u grupama koje su uz atropin tretirane i sa oksimom K870 u poređenju sa onima tretiranih obidoksimom ($p = 0,058$). Varijacije u koncentraciji elektrolita u serumu nisu bile u korelaciji ni sa dozom paraoksona ni sa tretmanom.

Paraokson je pri visokim dozama bio kardiotoksičan bez obzira na tretman, pokazujući višestruko povećanje CK, CK-MB i hsTnI u odnosu na kontrolne vrijednosti ($p < 0,001$), međutim, nije bilo jasne korelacije između stepena kardijalnog oštećenja i doze paraoksona, kao ni između tretmana ($p = 0,077$ za CK, $p = 0,109$ za CK-MB i $p = 0,518$ za hsTnI).

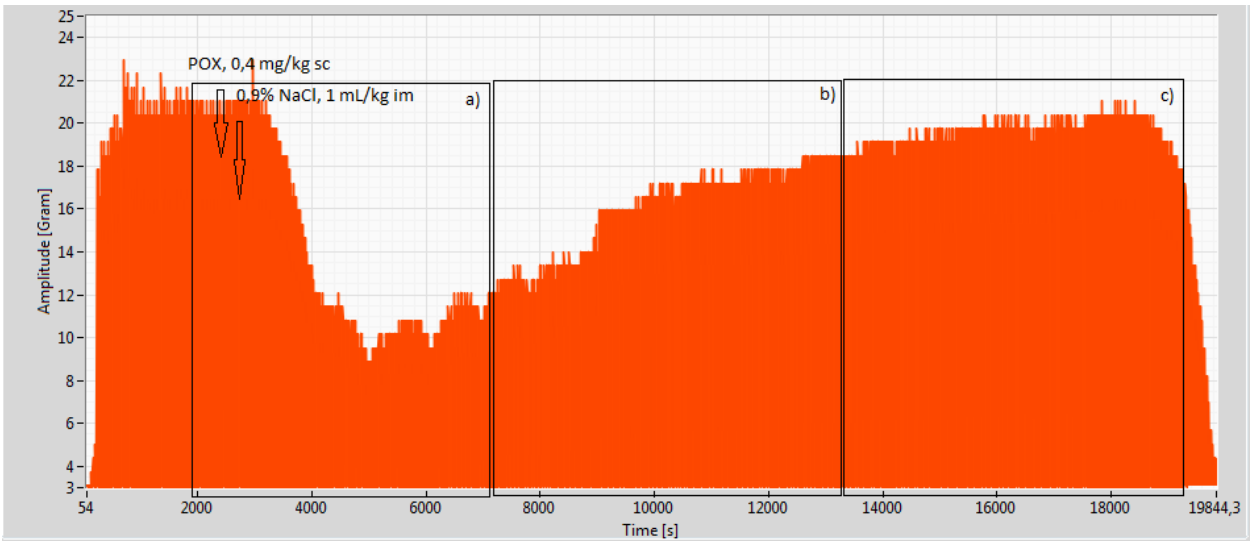
5.6. *Nervus phrenicus*-dijafragma *in situ*

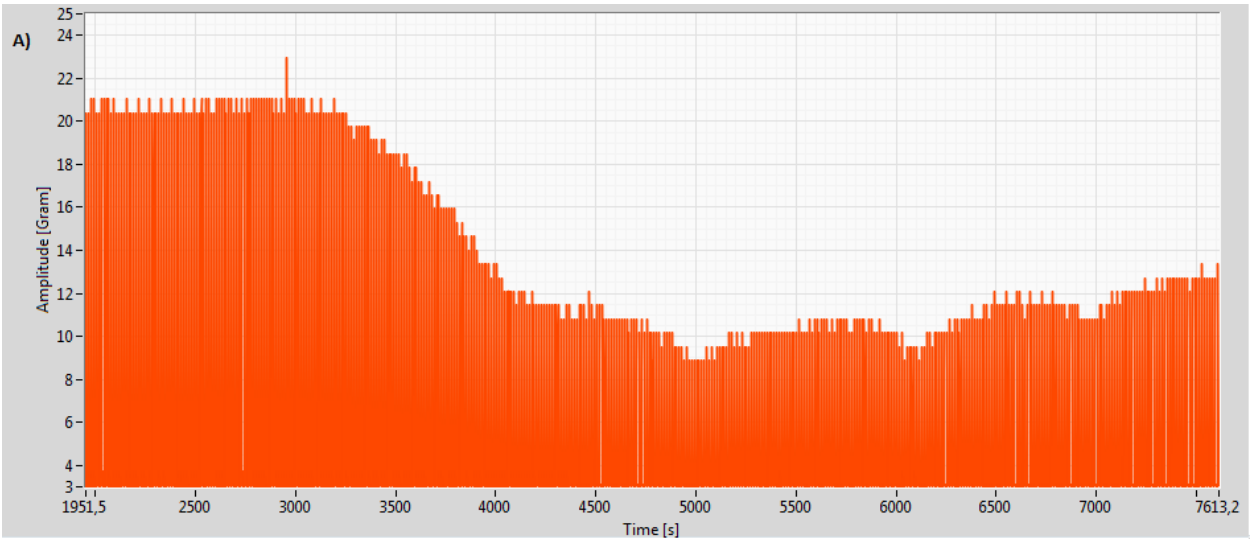
Kod pacova kojima je aplikovana visoka subletalna doza paraoksona (0,25 mg/kg sc) nije došlo do promjene u kontrakciji dijafragme (Slika 52).

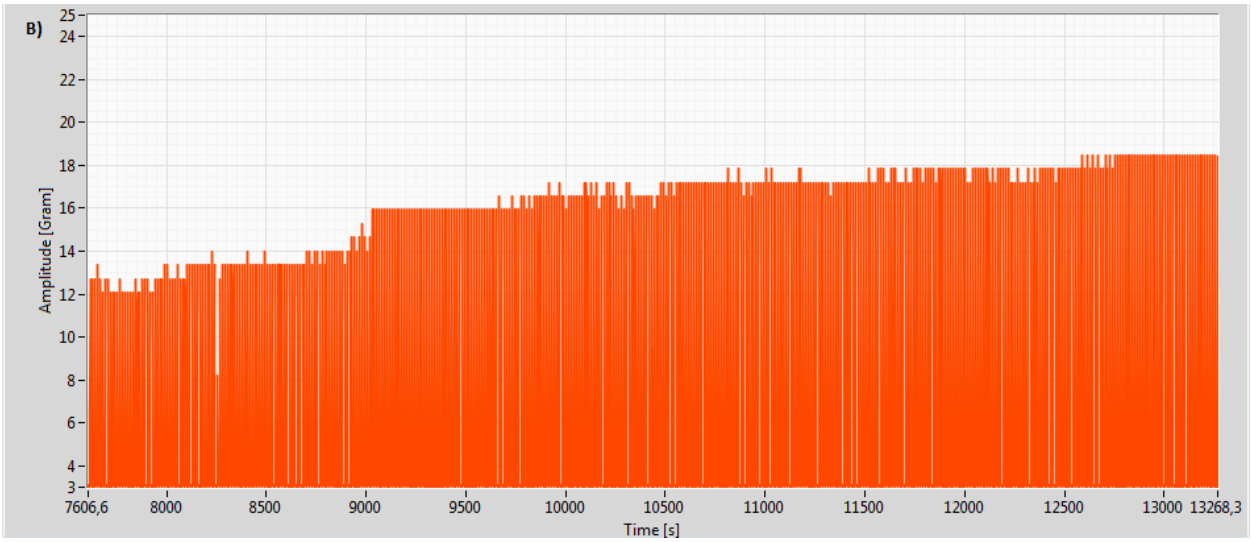


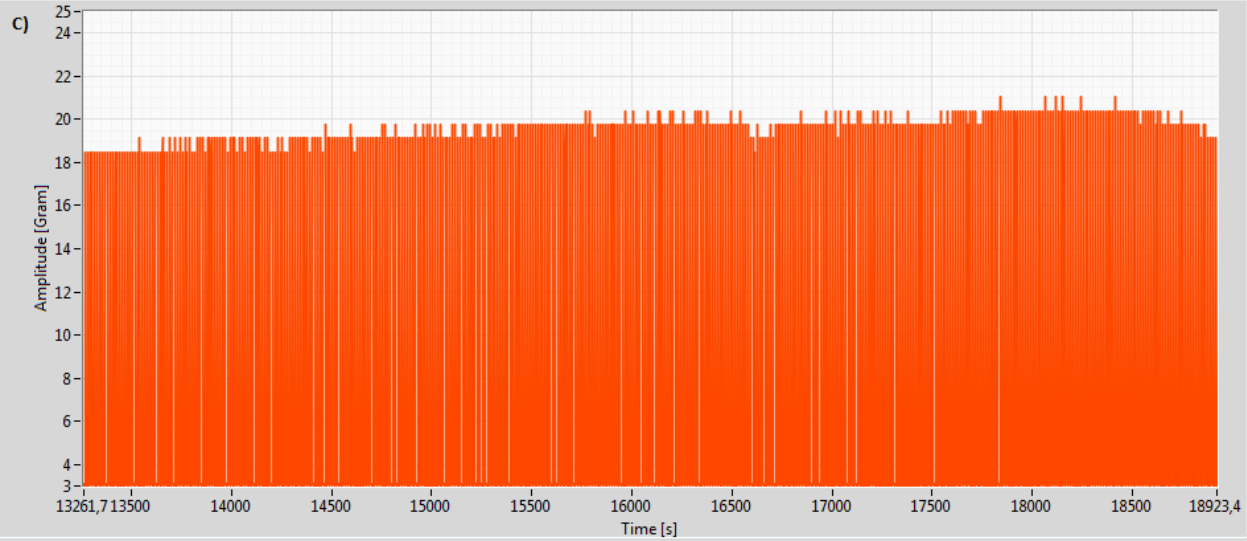
Slika 52. Kontrakcije dijafragme pri dozi paraoksona 0,25 mg/kg (visoka subletalna doza) subkutano (sc)

S obzirom da visoka subletalna doza nije dovela do smanjenja intenziteta kontrakcija na neuromišičnoj sinapski, primjenjena je apsolutna letalna doza paraoksona. Prilikom aplikacije 0,4 mg/kg sc paraoksona, već nakon 10 min dolazilo je do smanjenja intenziteta kontrakcija na < 50% od osnovnih vrijednosti. Sat vremena nakon aplikacije paraoksona, počinjalo je oporavljanje snage kontrakcije dijafragme, da bi 3 sata nakon aplikacije paraoksona, amplituda dolazila na približno početne vrijednosti (Slika 53, Slika 53: A-C).


POX, 0,4 mg/kg sc
0,9% NaCl, 1 mL/kg im
a)
b)
c)
Amplitude [Gram]
Time [s]
54
19844,3


A)
Amplitude [Gram]
Time [s]
1951,5
7613,2

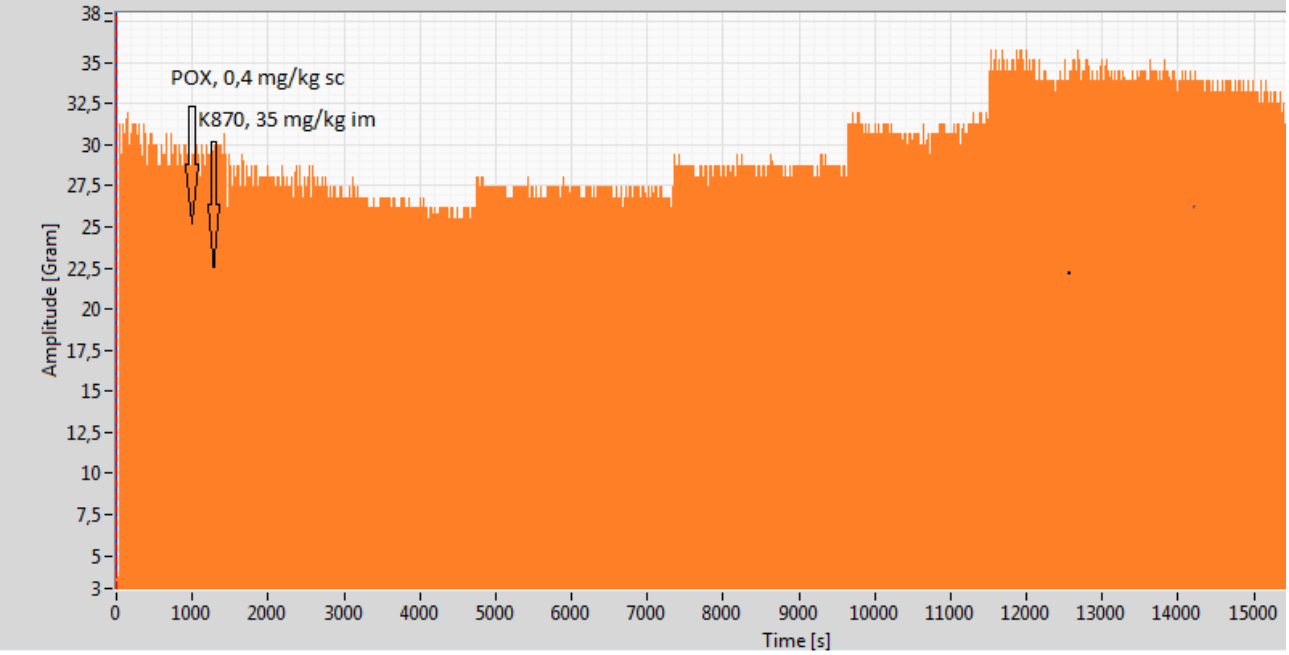

B)
Amplitude [Gram]
Time [s]
7606,6
13268,3



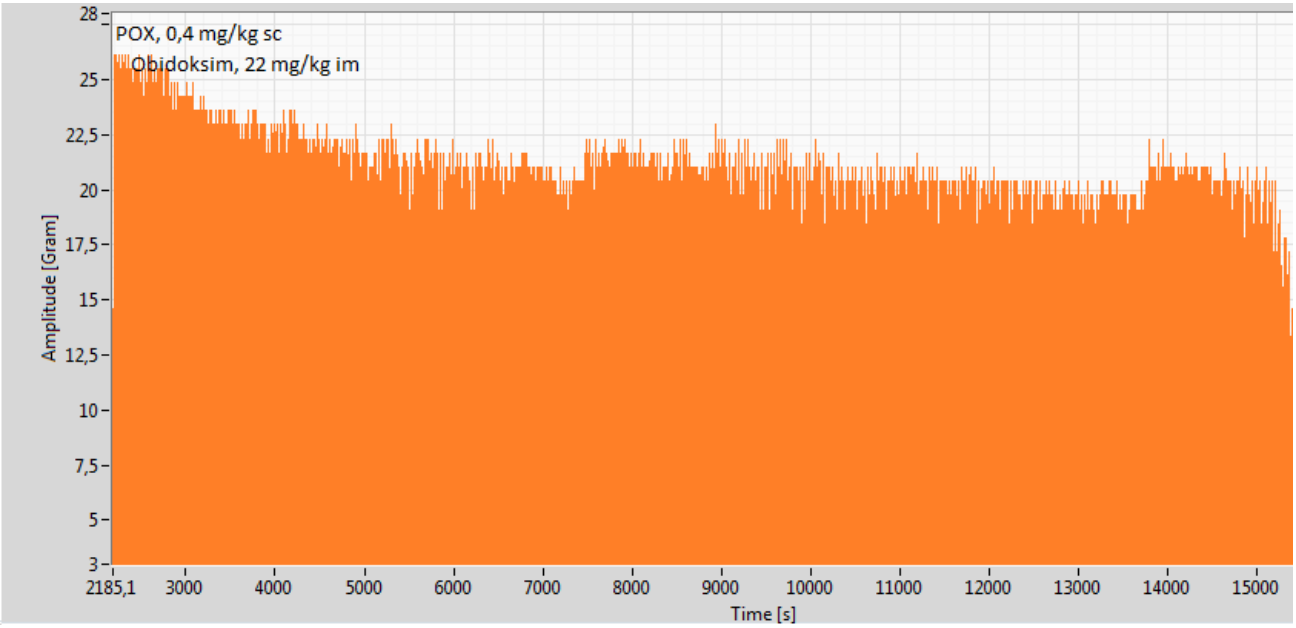
Slika 53. Kontrakcije dijafragme pri dozi paraoksona 0,4 mg/kg sc (apsolutna letalna doza). Markirani su dijelovi zapisa (A-C), u nastavku prikazani uvećano

A-C: obilježeni uvećani prikazi kontrakcija dijafragme; A: par minuta nakon aplikacije paraoksona dolazi do značajne redukcije intenziteta kontrakcija; B: 1 h nakon aplikacije paraoksona počinje spontani oporavak intenziteta kontrakcija; C: 3 h nakon aplikacije paraoksona intenzitet kontrakcija približan početnim vrijednostima;

Softverski prikazi kontrakcija dijafragme kada je 1 min nakon aplikacije 0,4 mg/kg sc paraoksona dat obidoksim, odnosno oksim K870 prikazani su na Slici 54 i 55. Kada je nakon paraoksona 1 min kasnije aplikovan obidoksim, odnosno oksim K870, nije dolazilo do smanjenja intenziteta kontrakcija, već su cijeli posmatrani period kontrakcije bile približne početnim vrijednostima.

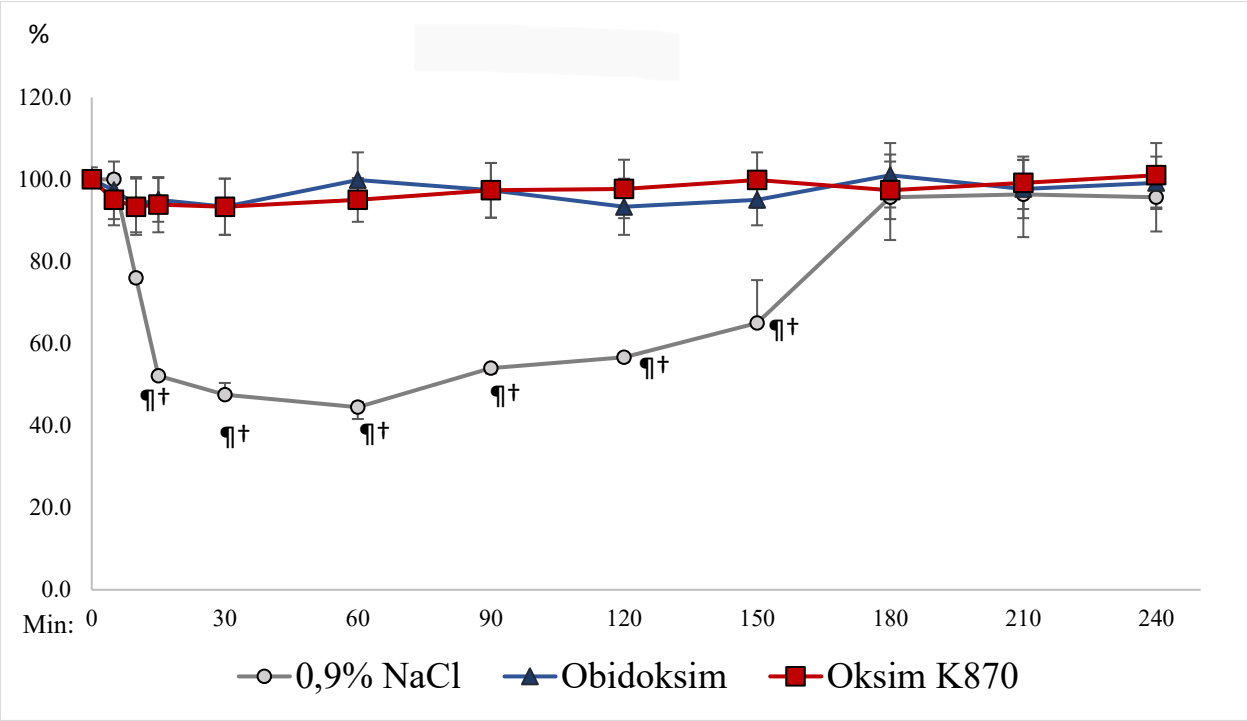


Slika 54. Kontrakcije dijafragme pri dozi paraoksona 0,4 mg/kg sc (apsolutna letalna doza), kada je 1 min kasnije dat oksim K870



Slika 55. Kontrakcije dijafragme pri dozi paraoksona 0,4 mg/kg sc (apsolutna letalna doza), kada je 1 min kasnije dat obidoksim

Razlike u intenzitetu kontrakcija dijafragme između grupa tretiranih FR, obidoksimom i oksimom K870 nađena je u 15. (χ^2 = 11,368, p = 0,003), 30. (χ^2 = 11,439, p = 0,003), 60. (χ^2 = 11,556, p = 0,003), 90. (χ^2 = 11,439, p = 0,003), 120. (χ^2 = 11,497, p = 0,003) i u 150. min (χ^2 = 11,673, p = 0,003). Nakon tretmana oksimima, u navedenim vremenima nije došlo do redukcije kontraktilnosti dijafragme u poređenju sa FR. Nije bilo značajne razlike između grupa tretiranih obidoksimom odnosno oksimom K870 (Slika 56).



Slika 56. Intenzitet kontrakcija dijafragme pri dozi paraoksona 0,4 mg/kg sc (apsolutna letalna doza), kada je 1 min kasnije dat fiziološki rastvor, obidoksim ili oksim K870

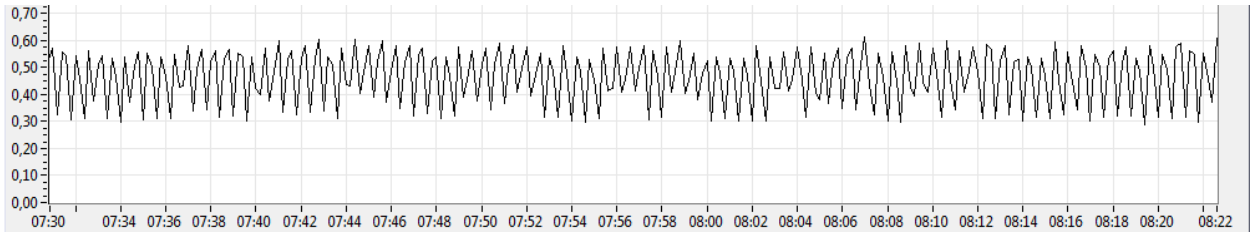
Kruskal-Wallis test, p < 0,05: ¶: fiziološki rastvor vs oksim K870; †: fiziološki rastvor vs obidoksim; %: procenat u odnosu na početne vrijednosti, prije aplikovanja paraoksona;

5.7. Praćenje kardiovaskularnih i plućnih parametara

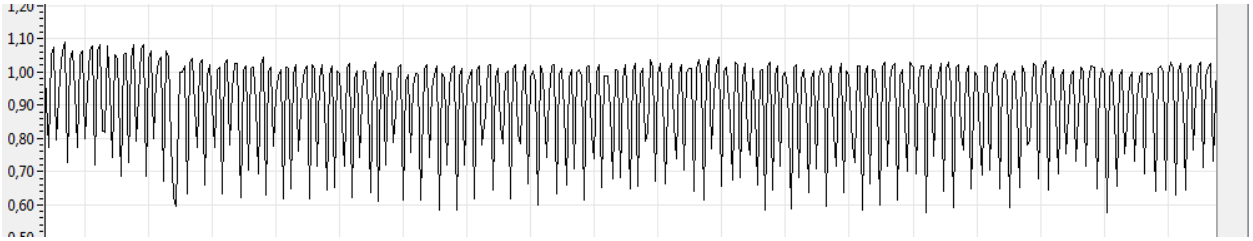
Nakon uspostavljanja stabilizacije modela, za šta je trebalo približno 10 min, ordinirana je visoka subletalna doza paraoksona (0,25 mg/kg sc), a min kasnije FR, atropin, N-butilskopolamin, obidoksim ili oksim K870.

5.7.1. Respiracije

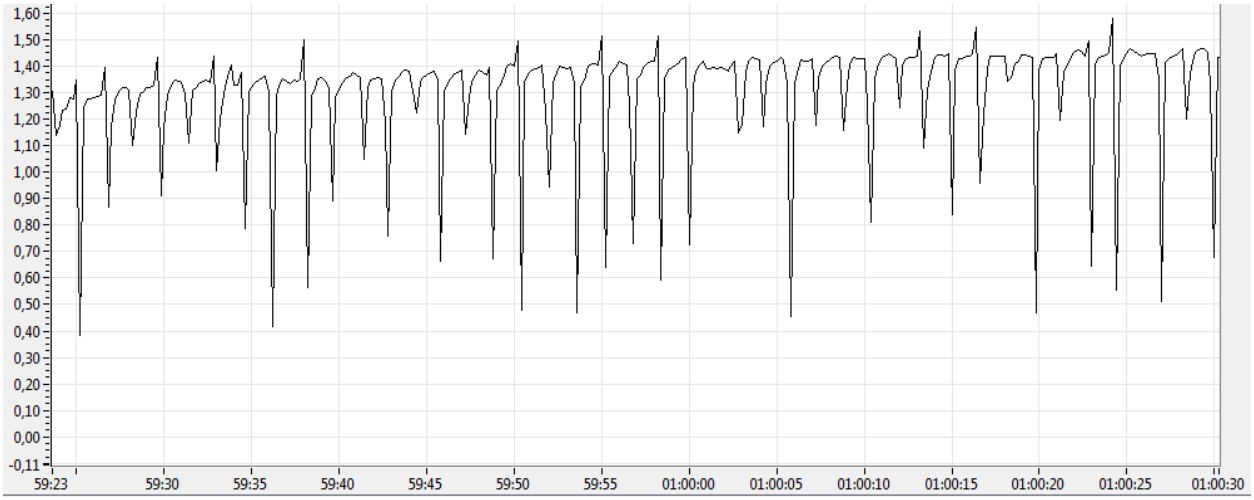
Analizirana je dubina i broj respiracija. Kada je amplituda i broj respiracija proporcionalno rastao, tada se radilo o tahipneji (Slika 57), a kada je padao radilo se o bradipneji (Slika 58). Međutim, kad je amplituda rasla, a frekvencija disanja padala, tada se radilo o dispneji (Slika 59).



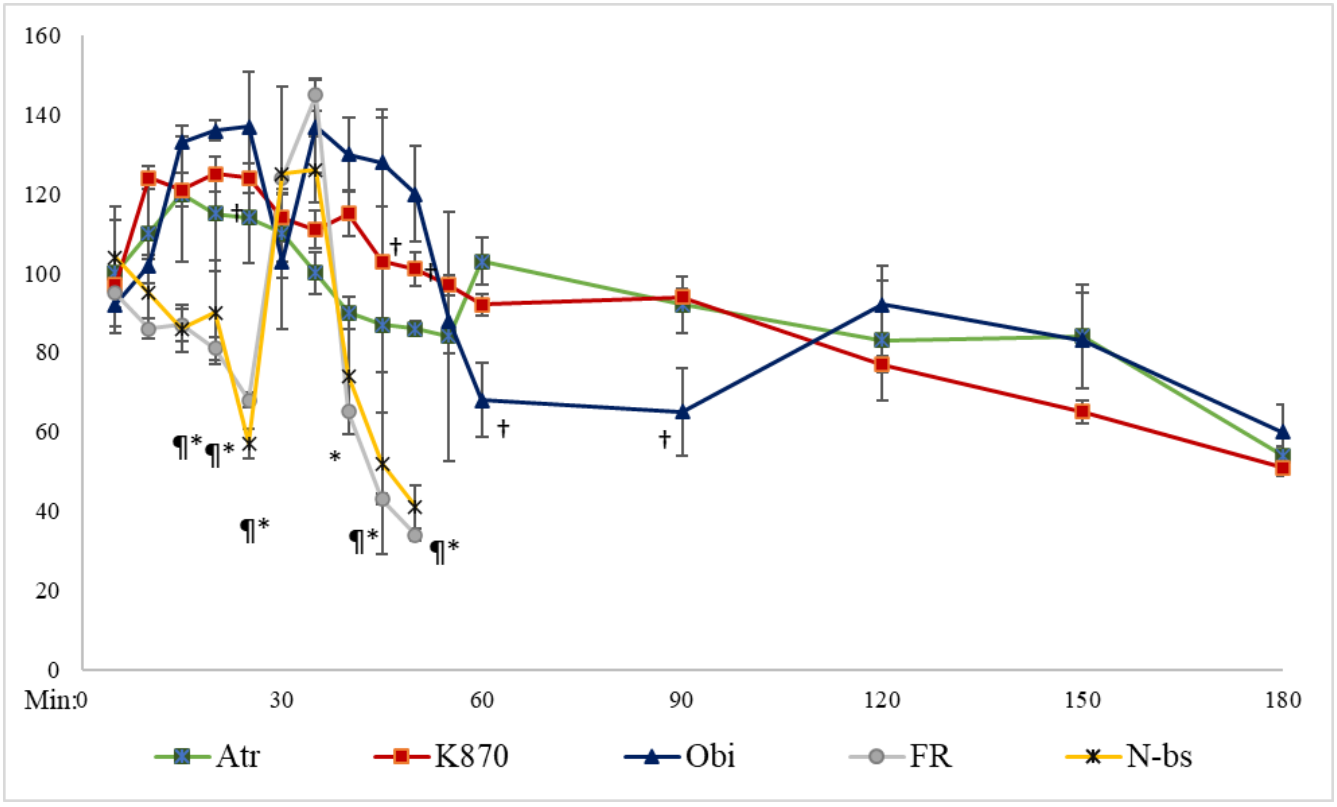
Slika 57. Grafički prikaz normalnih respiracija



Slika 58. Grafički prikaz tahipneje

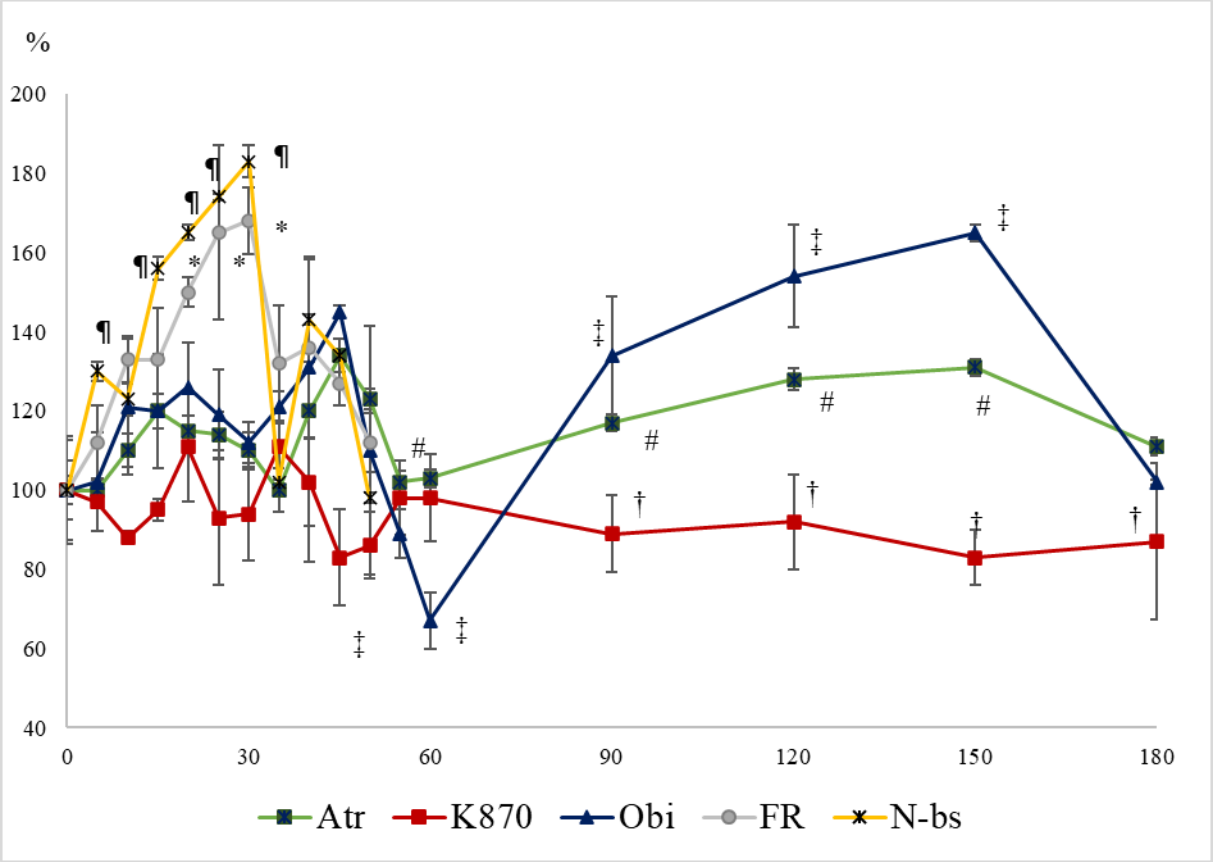


Slika 59. Grafički prikaz dispneje



Slika 60. Broj respiracija pacova trovanog visokom subletalnom dozom paraoksona (0,25 mg/kg sc) u odnosu na orinirani antidot

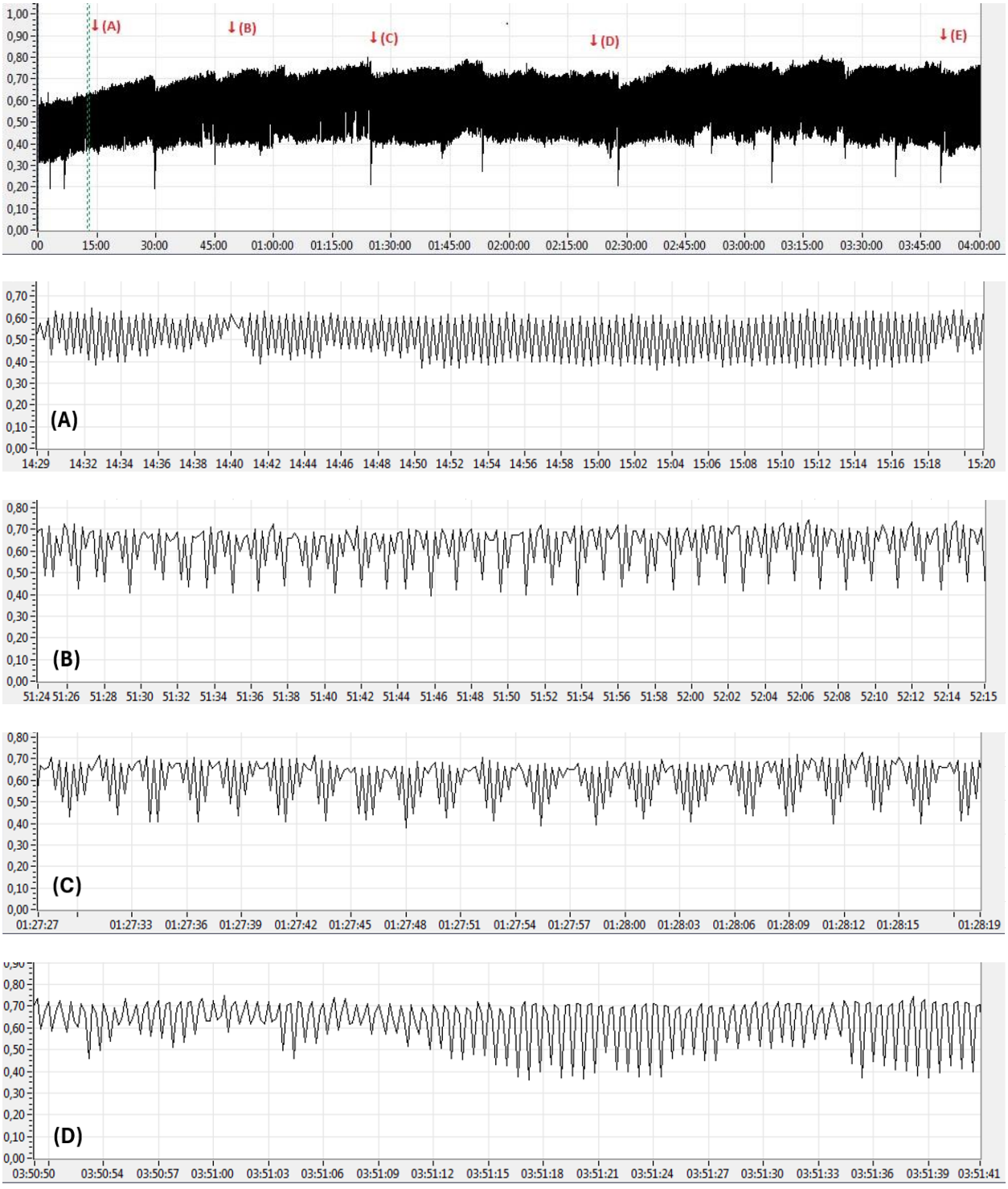
1 min kasnije: FR: 0,9% NaCl (1 mL/kg im), K870: oksim K870 (35 mg/kg im), Obi: obidoksim (22 mg/kg im), Atr: atropin (10 mg/kg im), N-bs: N-butilskopolamin (63,36 mg/kg im);



Slika 61. Broj respiracija pacova tretiranog visokom subletalnom dozom paraoksona (0,25 mg/kg sc) u odnosu na orinirani antidot

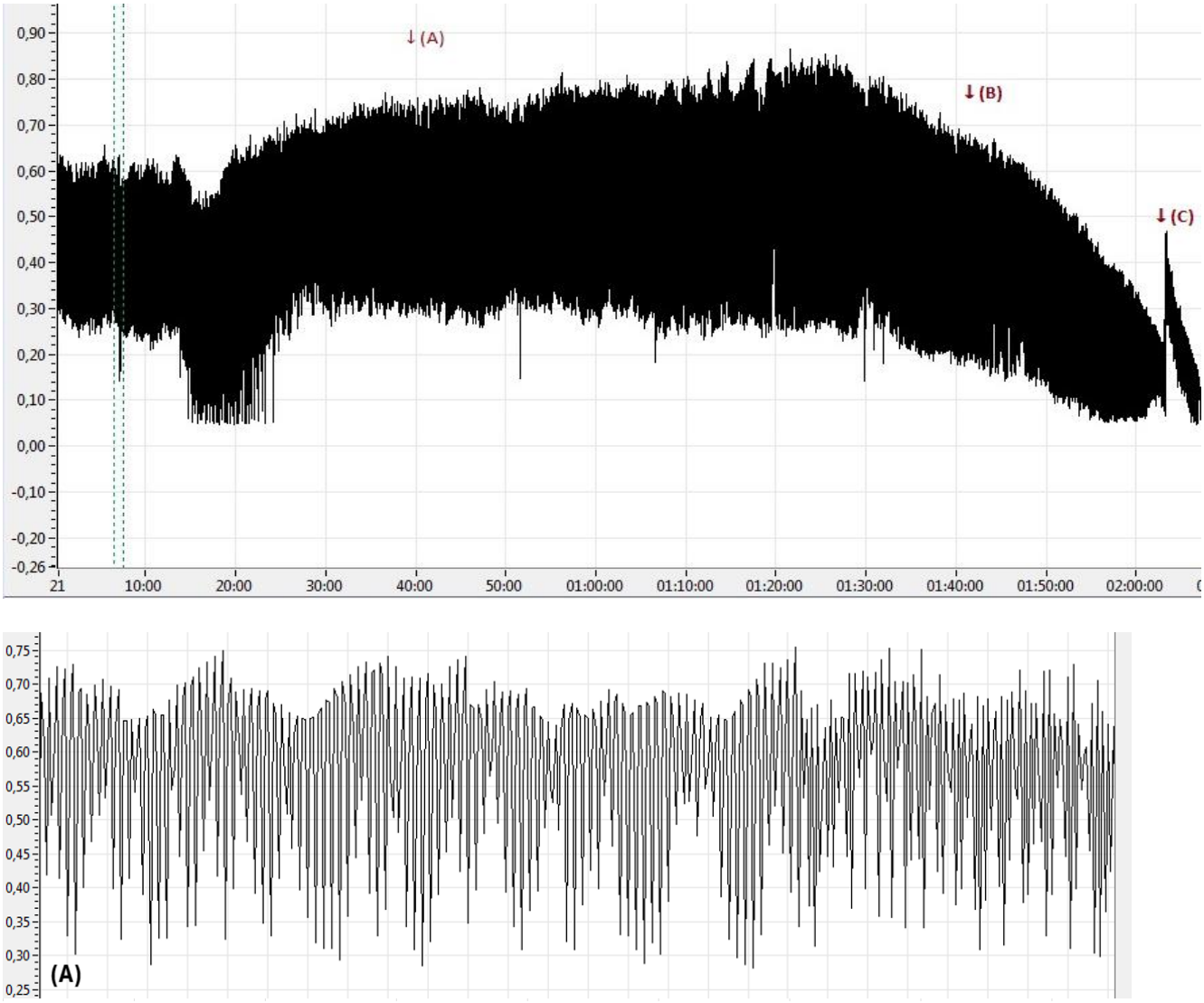
1 min kasnije: FR: 0,9% NaCl (1 mL/kg im), K870: oksim K870 (35 mg/kg im), Obi: obidoksim (22 mg/kg im), Atr: atropin (10 mg/kg im), N-bs: N-butilskopolamin (63,36 mg/kg im);

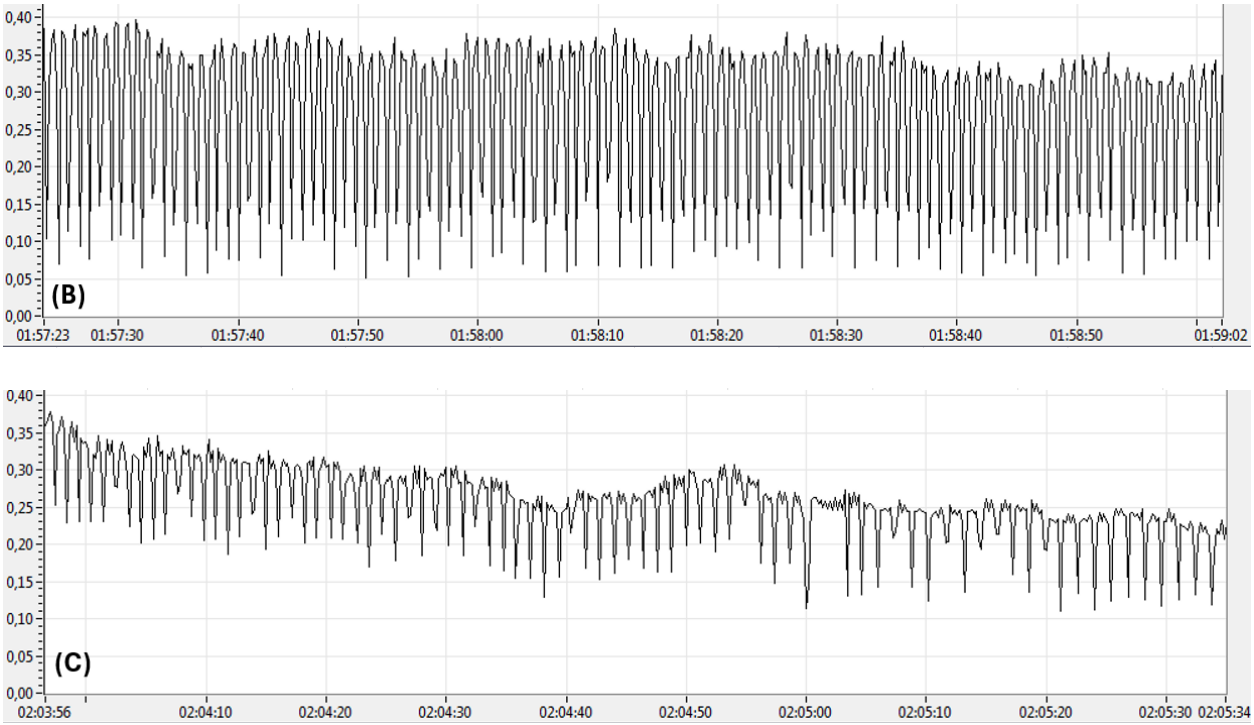
Broj respiracija i dubina disanja po grupama prikazani su na Slikama 60 i 61. Pacovi tretirani atropinom su preživljavali posmatrani period od 3 sata. Tokom opserviranog perioda pacovi su imali periode tahipneje, kratkotrajne povremene dispneje i normalnog disanja. Deset min nakon aplikacije paraoksona došlo je do porasta broja respiracija sa početnih 80 na 110 respiracija u min, a sat vremena kasnije broj respiracija je počeo lagano da pada, i kretao se približnim vrijednostima prije trovanja paraoksonom, da bi nakon tri sata bile 54 respiracije u min. Dubina disanja je blago porasla i bila iznad bazičnih vrijednosti tokom cijelog opserviranog perioda. Slika 62 pokazuje softverski prikaz respiracija kod pacova tretiranog atropinom.



Slika 62. Softverski prikaz respiracija pacova kome je 1 min nakon paraoksona (0,25 mg/kg sc) ordiniran atropin (10 mg/kg im); A-D: uvećani prikazi strelicama obilježenih regija

Kod pacova tretiranih obidoksimom, 10 min nakon aplikacije paraoksona broj respiracija je počeo da raste, zajedno sa dubinom disanja, ukazujući na tahipneju. Tokom prvog sata pacovi su bili tahipnoični (110-130 respiracija u mini), sa povremenim kratkotrajnim epizodama dispneje. Sat nakon aplikacije paraoksona broj respiracija je počeo da pada ispod bazičnih vrijednosti, što je bilo praćeno i povećanjem amplitude disanja (dispneja). Bradipneja je bila prisutna tokom preostalog posmatranog perioda, sa povremenim epizodama tahipneje i duboke dispneje (Slika 63).

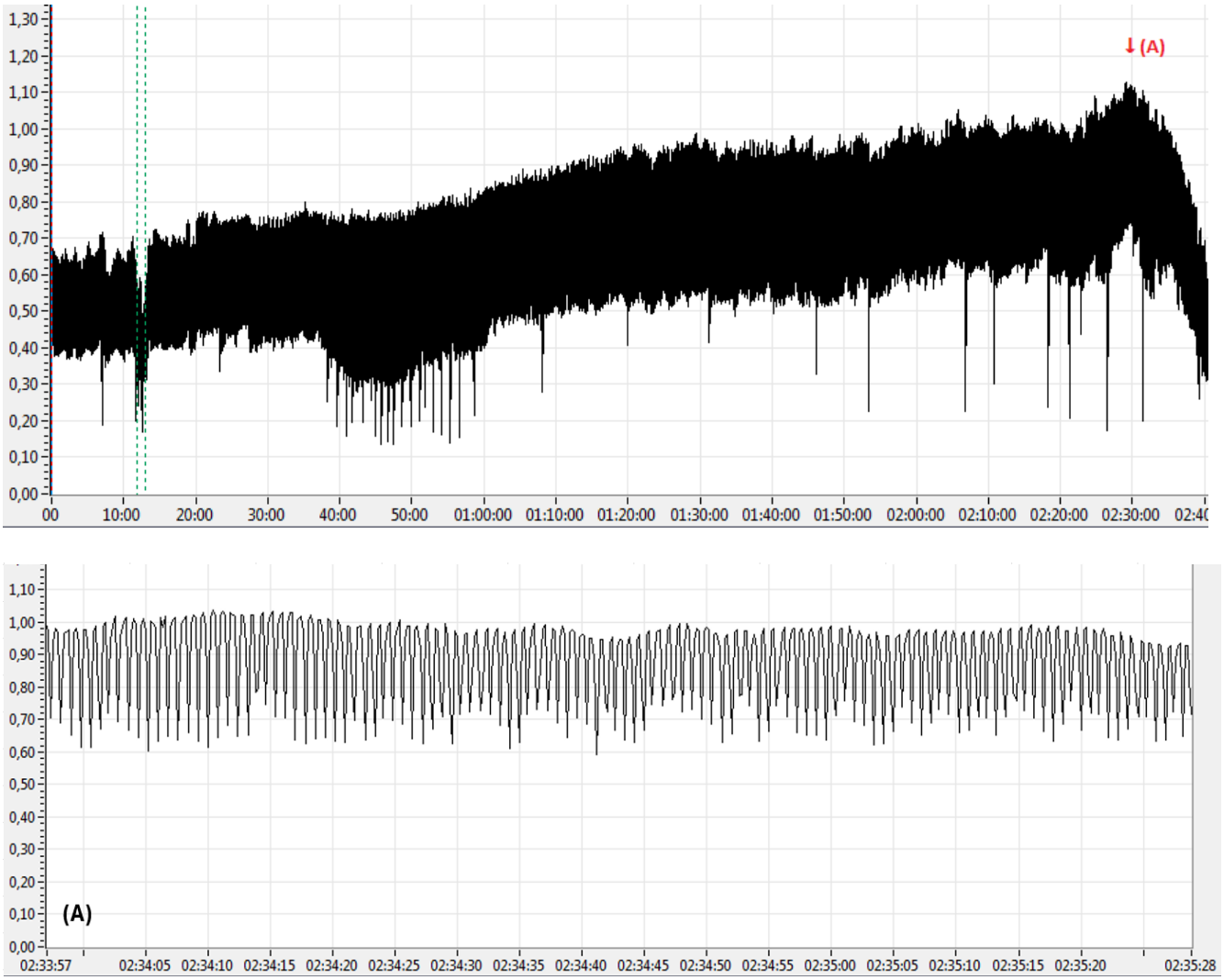




Slika 63. Softverski prikaz respiracija, pacova kome je 1 min nakon paraoksona (0,25 mg/kg sc) ordiniran obidoksim (22 mg/kg im); A-C: uvećani prikazi strelicama obilježenih regija

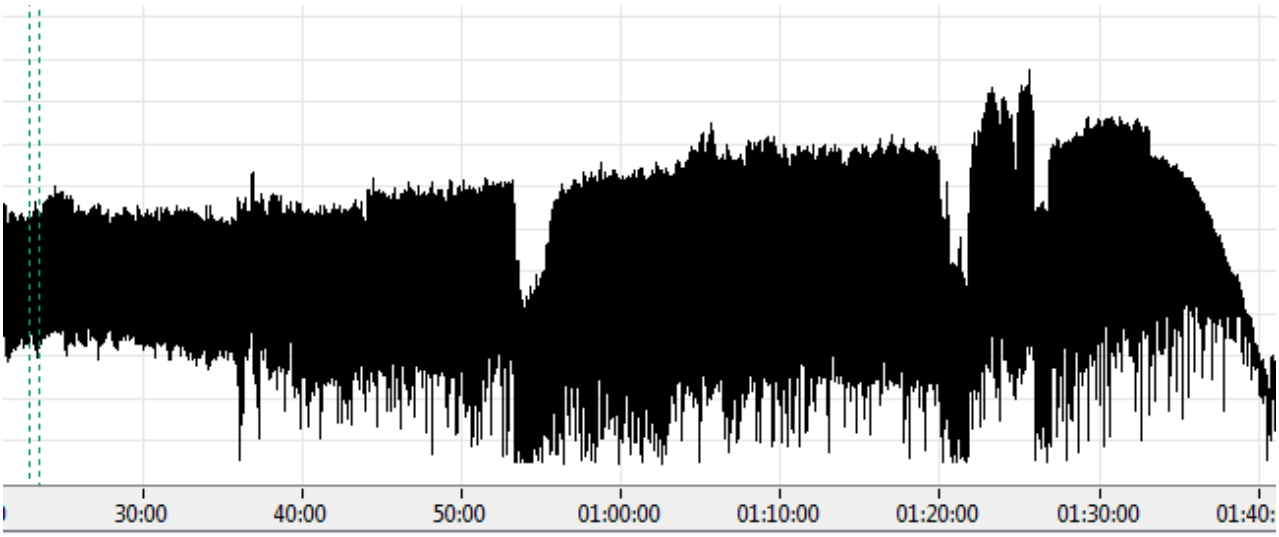
Kod pacova tretiranih oksimom K870, 10 min nakon aplikacije paraoksona broj respiracija je blago porastao (do 115/min), zajedno sa dubinom disanja. Broj respiracija tokom posmatranog perioda je bio blizu bazičnih vrijednosti (95-110/min). Amplituda disanja je bila blago ispod bazičnih vrijednosti, sa umjerenim oscilacijama tokom posmatranog vremena. Bradipneja je bila prisutna tokom preostalog posmatranog perioda, sa povremenim epizodama tahipneje i dispneje (Slika 64).

Pacovi tretirani oksimom K870 su u prosjeku živjeli duže (217,62 ± 55,49 min) u odnosu na pacove tretirane obidoksimom (144,2 ± 103,13 min) ($U = 213{,}500$, $p = 0{,}039$) i imali duže periode normalnog disanja.

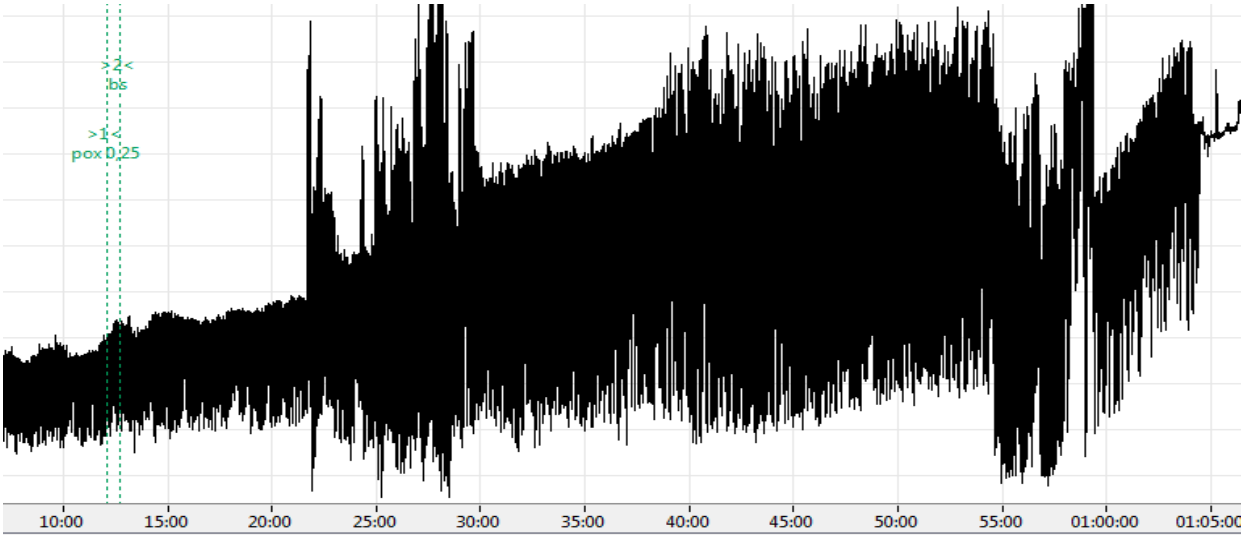


Slika 64. Softverski prikaz respiracija kome je 1 min nakon paraoksona (0,25 mg/kg sc) ordiniran oksim K870 (35 mg/kg im); A: uvećani prikazi obilježene regije – normalno disanje i 2 h nakon paraoksona

Pacovi kojima je ordiniran FR ili N-butilskopolamin imali su veoma slične respiracije i preživljavanje, bez značajne razlike u posmatranom periodu (Slike 65 i 66). Prosječno preživljavanje je iznosilo 45,13 ± 10,33 min nakon aplikacije paraoksona za FR i 47,54 ± 8,83 min za N-butilskopolamin ($p = 0,672$). Vrlo brzo nakon aplikacije paraoksona pacovi su pokazivali znakove značajne dispneje.



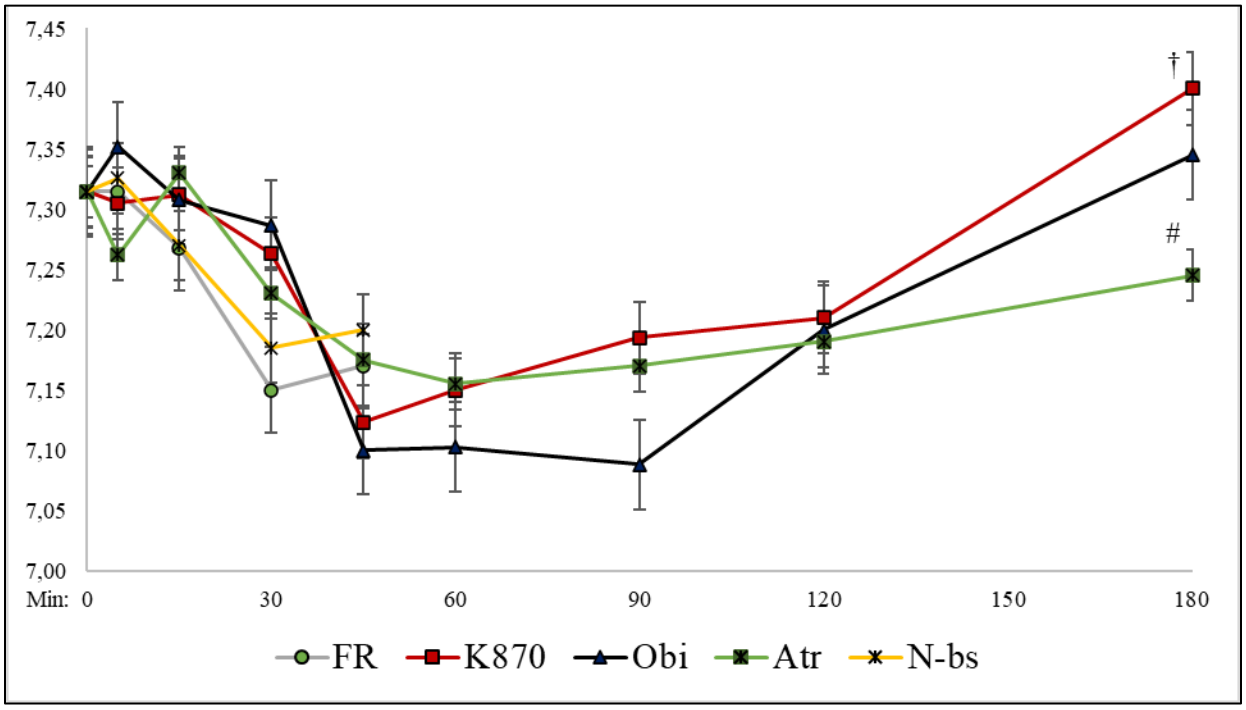
Slika 65. Softverski prikaz respiracija, pacova kome je 1 min nakon paraoksona (0,25 mg/kg sc) ordiniran N-butilskopolamin (63,36 mg/kg im)



Slika 66. Softverski prikaz respiracija, pacova kome je 1 min nakon paraoksona (0,25 mg/kg sc) ordiniran fiziološki rastvor (1 mL/kg im)

5.7.2. Gasne analize

Pet min nakon trovanja paraoksonom, pH arterijske krvi je bio niži kod pacova tretiranih obidoksimom u poređenju sa oksimom K870. Tri sata nakon aplikacije paraoksona pH (χ^2 = 5,400, p = 0,05), pO_2 (χ^2 = 8,100, p = 0,017), laktat (χ^2 = 6,750, p = 0,034), bikarbonati (χ^2 = 6,750, p = 0,034), BE (χ^2 = 8,100, p = 0,017) i sO_2 (χ^2 = 8,100, p = 0,017) su se značajno razlikovali između grupa. Vrijednosti pH, bikarbonata, laktata, pCO_2, pO_2, sO_2, BE su prikazani na Slikama 67-73.

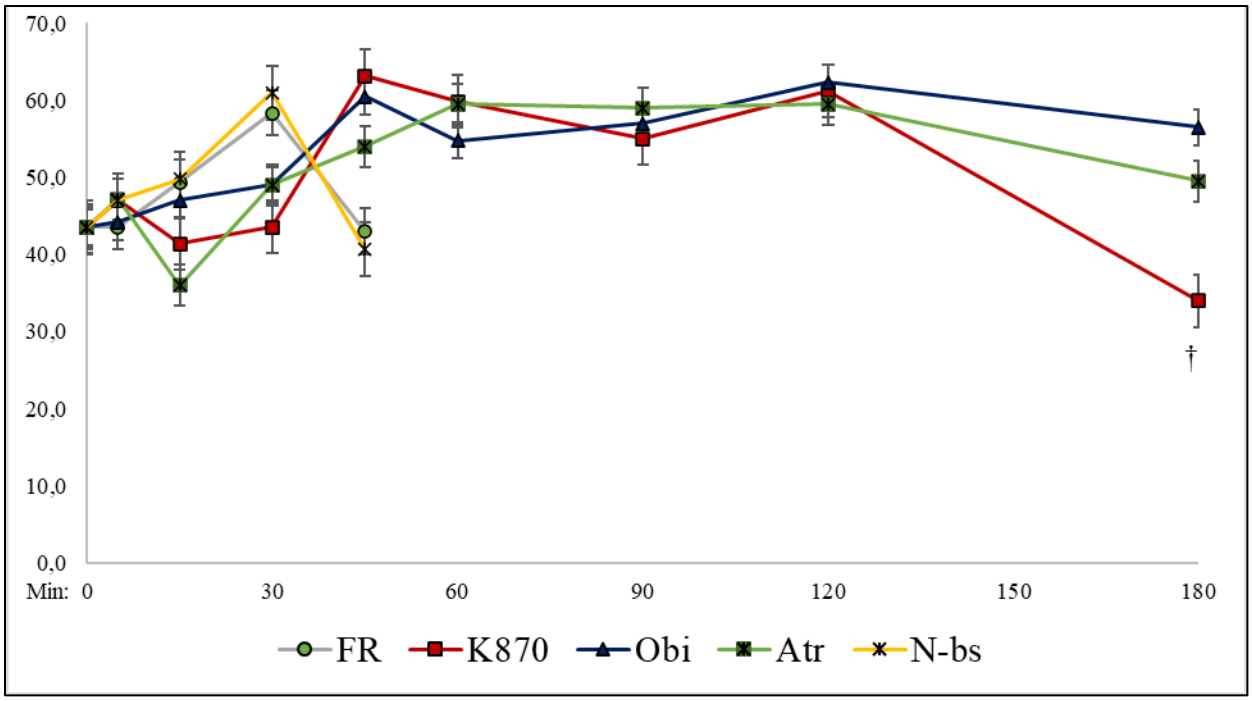


Slika 67. pH arterijske krvi pacova tretiranog visokom subletalnom dozom paraoksona (0,25 mg/kg sc) u odnosu na orinirani antidot

1 min kasnije: FR: 0,9% NaCl (1 mL/kg im), K870: oksim K870 (35 mg/kg im), Obi: obidoksim (22 mg/kg im), Atr: atropin (10 mg/kg im), N-bs: N-butilskopolamin (63,36 mg/kg im); #: K870 vs FR; †: K870 vs Obi;

PH arterijske krvi je nakon aplikacije paraoksona veoma brzo počeo da pada, u 30. min je kod pacova tretiranih sa FR iznosio samo 7,15 (Slika 67). Pacovi tretirani atropinom su imali blaži pad pH (7,25), koji je uz minimalne oscilacije bio prisutan tokom cijelog posmatranog perioda. U 45. min pacovi tretirani oksimima imali su izraženu acidozu (pH 7,11 za obioksim i 7,16 za oksim

K870). Pacovi tretirani obidoksimom imali su izraženu acidozu, do 90. min (pH 7,10), kada je pH krvi počeo da raste i dostigao početne vrijednosti nakon 3 h. Kod pacova tretiranih oksimom K870, acidoza je nastupila kratkotrajno, pH krvi je počeo da pada nakon 30 min, dostigao najniže vrijednosti 45 min nakon trovanja paraoksonom (pH 7,14), da bi nakon toga pH počeo da raste i bio viših vrijednosti u odnosu na grupe tretirane obidoksimom i atropinom tokom posmatranog perioda.

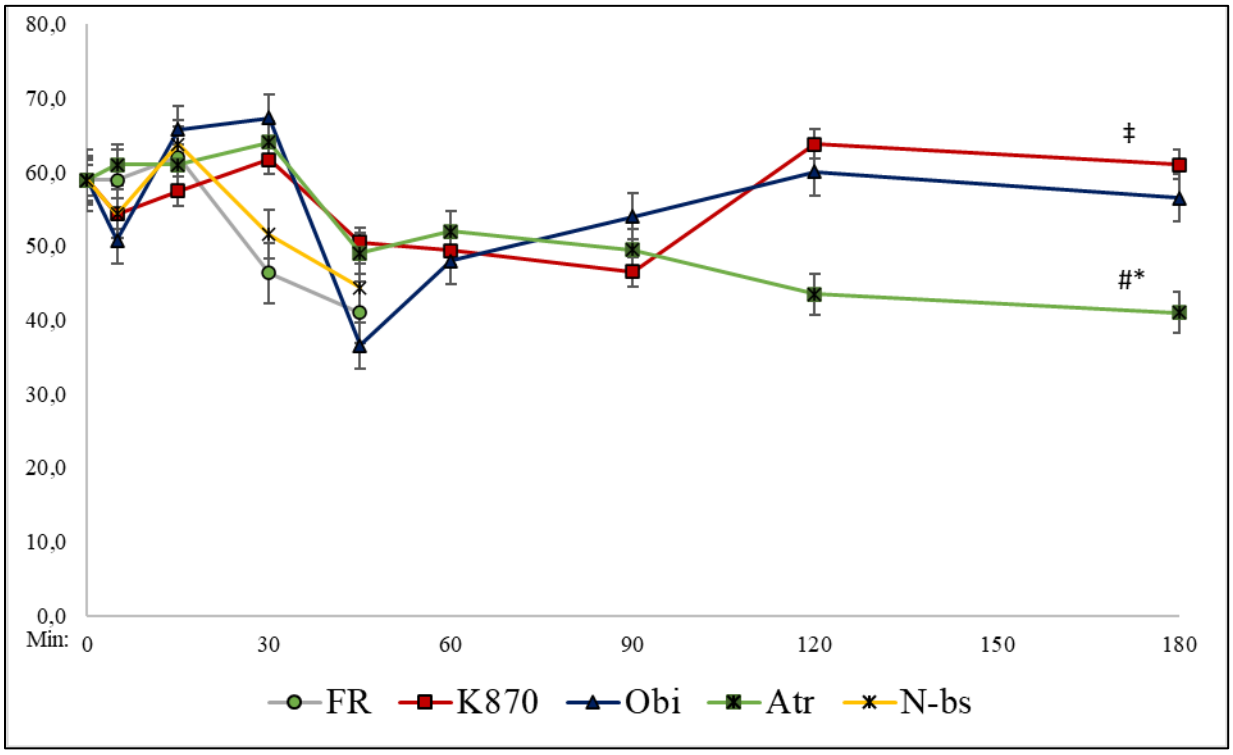


Slika 68. Parcijalni pritisak ugljen-dioksida - pCO_2 (mm Hg) arterijske krvi pacova tretiranog visokom subletalnom dozom paraoksona (0,25 mg/kg sc) u odnosu na orinirani antidot

1 min kasnije: FR: 0,9% NaCl (1 mL/kg im), K870: oksim K870 (35 mg/kg im), Obi: obidoksim (22 mg/kg im), Atr: atropin (10 mg/kg im), N-bs: N-butilskopolamin (63,36 mg/kg im); †: K870 vs Obi i FR;

Parcijalni pritisak ugljen-dioksida (Slika 68) je nakon tretmana paraoksonom u arterijskoj krvi počeo da raste kod pacova tretiranih FR i N-butilskopolaminom već nakon 5 min, dostižući najviše vrijednosti u 30. min. Kod pacova tretiranih atropinom u prvih 15 min pCO_2 je pao na 33 mmHg, da bi nakon 30 min ponovo bio približan bazalnim vrijednostima. Nakon toga dolazi do

blagog porasta pCO_2, do maksimalnih 59,50 mm Hg u 60. min. Kod pacova tretiranih obidoksimom porast pCO_2 je započeo od 15. min, do maksimalnih 62,3 mm Hg dva sata nakon tretmana paraoksonom. Kod pacova tretiranih oksimom K870 porast pCO_2 je počeo u 30. min, dostigao maksimum u 45. min (63,1 mm Hg), nakon toga počeo lagano da pada, da bi 3 sata nakon trovanja paraoksonom bio značajno niži u odnosu na grupe tretirane obidoksimom i atropinom.

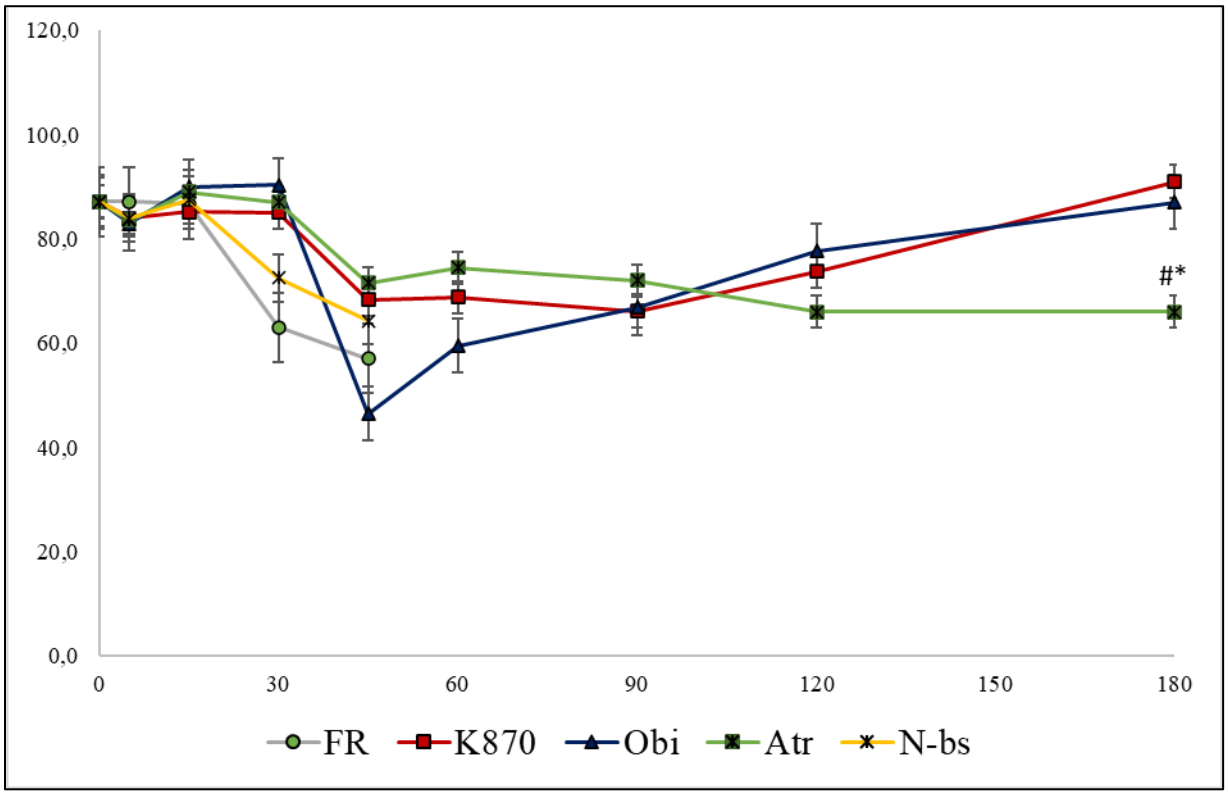


Slika 69. Parcijalni pritisak kiseonika - pO_2 (mm Hg) arterijske krvi pacova tretiranog visokom subletalnom dozom paraoksona (0,25 mg/kg sc) u odnosu na orinirani antidot

1 min kasnije: FR: 0,9% NaCl (1 mL/kg im), K870: oksim K870 (35 mg/kg im), Obi: obidoksim (22 mg/kg im), Atr: atropin (10 mg/kg im), N-bs: N-butilskopolamin (63,36 mg/kg im); #: K870 vs Atr; *: Obi vs Atr; ‡: K870 vs Obi;

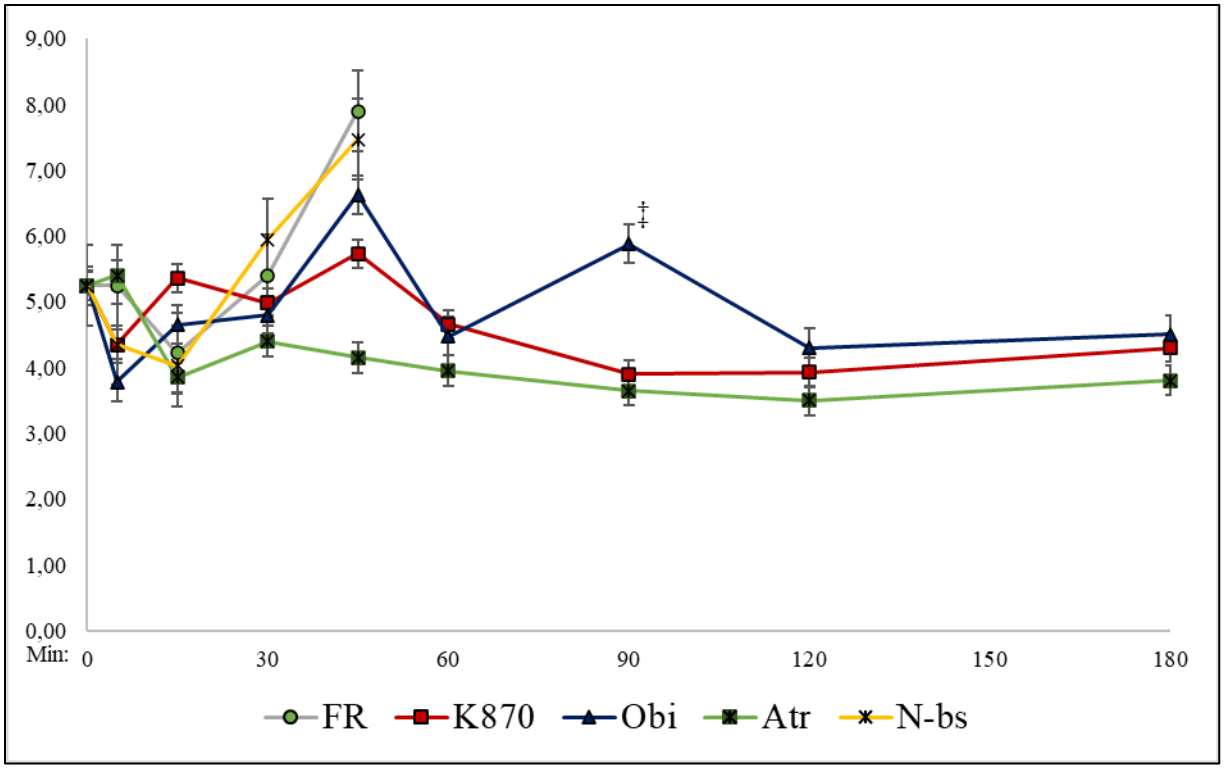
Bazalne vrijednosti parcijalnog pritiska kiseonika (PaO_2) u arterijskoj krvi pacova su bile niže od očekivanih (59 mm Hg). Oscilacije u PaO_2 primijećene su kod svih grupa. Tri sata nakon trovanja paraoksonom, PaO_2 pacova tretiranih oksimom K870 bio je značajno viši u odnosu na pacove tretirane obidoksimom i aropinom (Slika 69).

Najniže vrijednosti saturacije kiseonikom (sO_2) zabilježene su kod pacova tretiranih obidoksimom u 45. min, samo 46,5%. Pacovi tretirani obidoksimom su nakon 30. min od trovanja paraoksonom imali značajan pad sO_2, najizraženiji u 45. min. Nakon toga sO_2 je počeo da raste, da bi na kraju posmatranog perioda iznosio 87%. Pacovi tretirani oksimom K870 imali su manje izraženu hipoksiju tokom posmatranog perioda, najniža saturacija je bila 68%, a tri sata nakon trovanja paraoksonom sO_2 je iznosila 91%. Pacovi tretirani atropinom su imali niže vrijednosti sO_2 u odnosu na pacove tretirane oksimima na kraju posmatranog vremena (Slika 70).



Slika 70. Saturacija kiseonikom - sO_2 (%) arterijske krvi pacova tretiranog visokom subletalnom dozom paraoksona (0,25 mg/kg sc) u odnosu na orinirani antidot

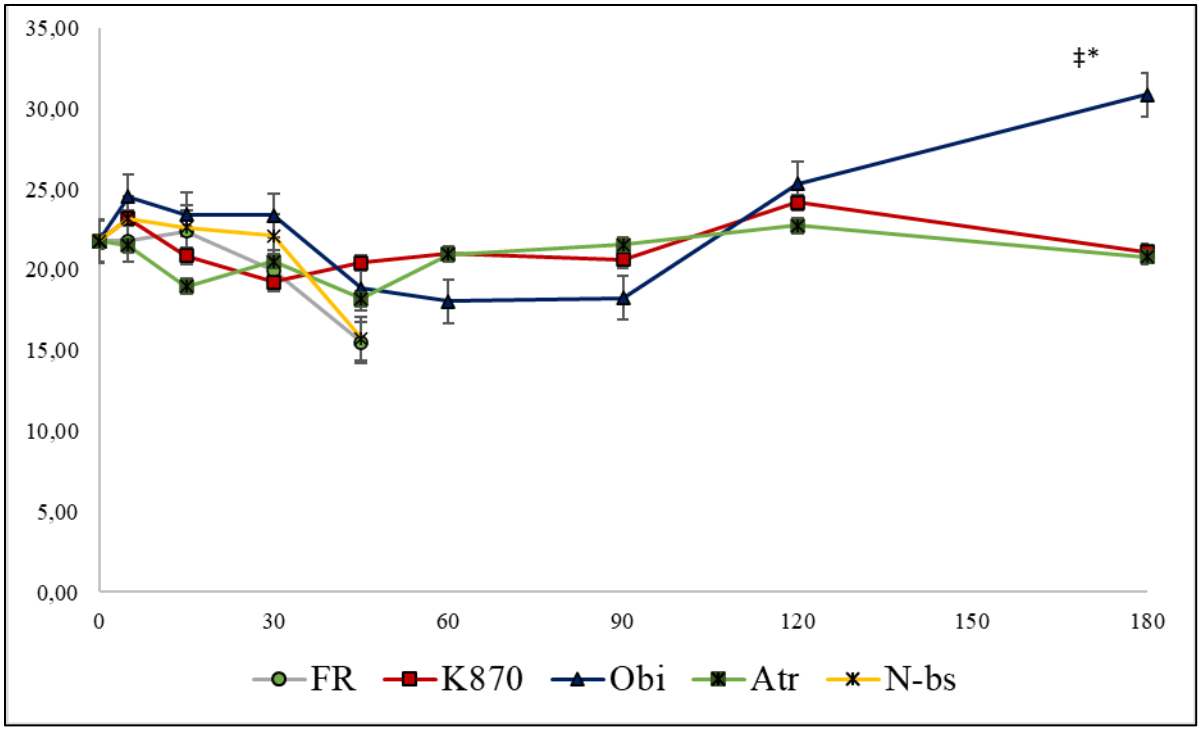
1 min kasnije: FR: 0,9% NaCl (1 mL/kg im), K870: oksim K870 (35 mg/kg im), Obi: obidoksim (22 mg/kg im), Atr: atropin (10 mg/kg im), N-bs: N-butilskopolamin (63,36 mg/kg im); #: K870 vs Atr; *: Obi vs Atr;



Slika 71. Laktat (mmol/L) arterijske krvi pacova tretiranog visokom subletalnom dozom paraoksona (0,25 mg/kg sc) u odnosu na orinirani antidot

1 min kasnije: FR: 0,9% NaCl (1 mL/kg im), K870: oksim K870 (35 mg/kg im), Obi: obidoksim (22 mg/kg im), Atr: atropin (10 mg/kg im), N-bs: N-butilskopolamin (63,36 mg/kg im); ‡: K870 vs Obi;

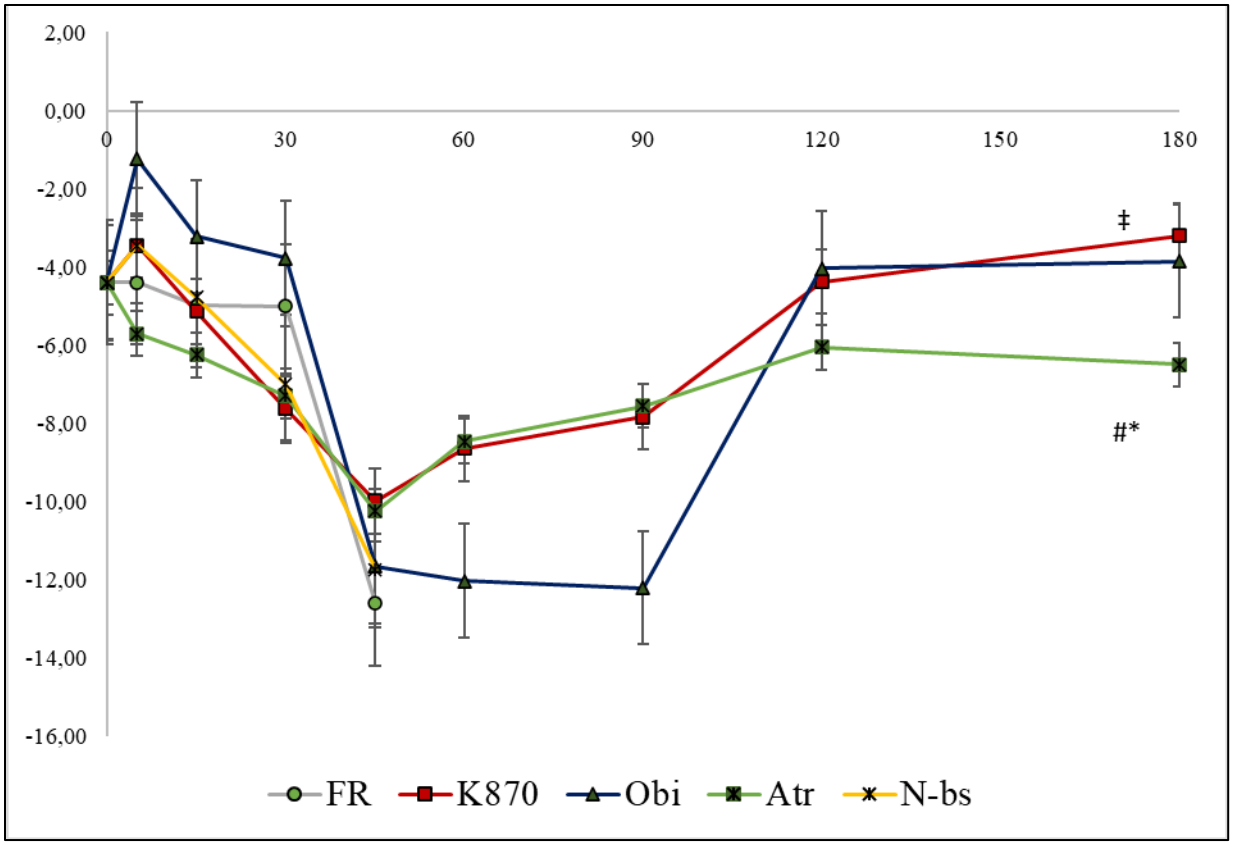
Laktat je bio značajno viši u odnosu na normalne vrijednosti tokom cijelog ispitivanog perioda. U 45. min (približno vrijeme umiranja) kod pacova tretiranih sa FR i N-butilskopolaminom su iznosili čak 7,9 i 7,5 mmol/L. Laktat je kod pacova tretiranih atropinom bio najniži tokom cijelog posmatranog perioda. Kod pacova tretiranih obidoksimom, vrijednosti laktata su oscilirale i bile značajno više u 90. min, a kod pacova tretiranih oksimom K870 bile su blago povišene i stabilne tokom posmatranog perioda (Slika 71).



Slika 72. Bikaronati (mmol/L) arterijske krvi pacova tretiranog visokom subletalnom dozom paraoksona (0,25 mg/kg sc) u odnosu na orinirani antidot

1 min kasnije: FR: 0,9% NaCl (1 mL/kg im), K870: oksim K870 (35 mg/kg im), Obi: obidoksim (22 mg/kg im), Atr: atropin (10 mg/kg im), N-bs: N-butilskopolamin (63,36 mg/kg im); *: Obi vs Atr; ‡: K870 vs Obi;

Bikarbonati su u svim grupama diskretno varirali tokom posmatranog perioda, s tim da je zabilježen pad kod pacova tretiranih sa FR i N-butilskopolaminom neposredno prije uginjavanja. Osim toga primjećen je skok bikarbonata u trećem satu nakon trovanja paraoksonom kod pacova tretiranih obidoksimom, vrijednosti su bile značajno više u odnosu na pacove tretirane atropinom i oksimom K870 (Slika 72).



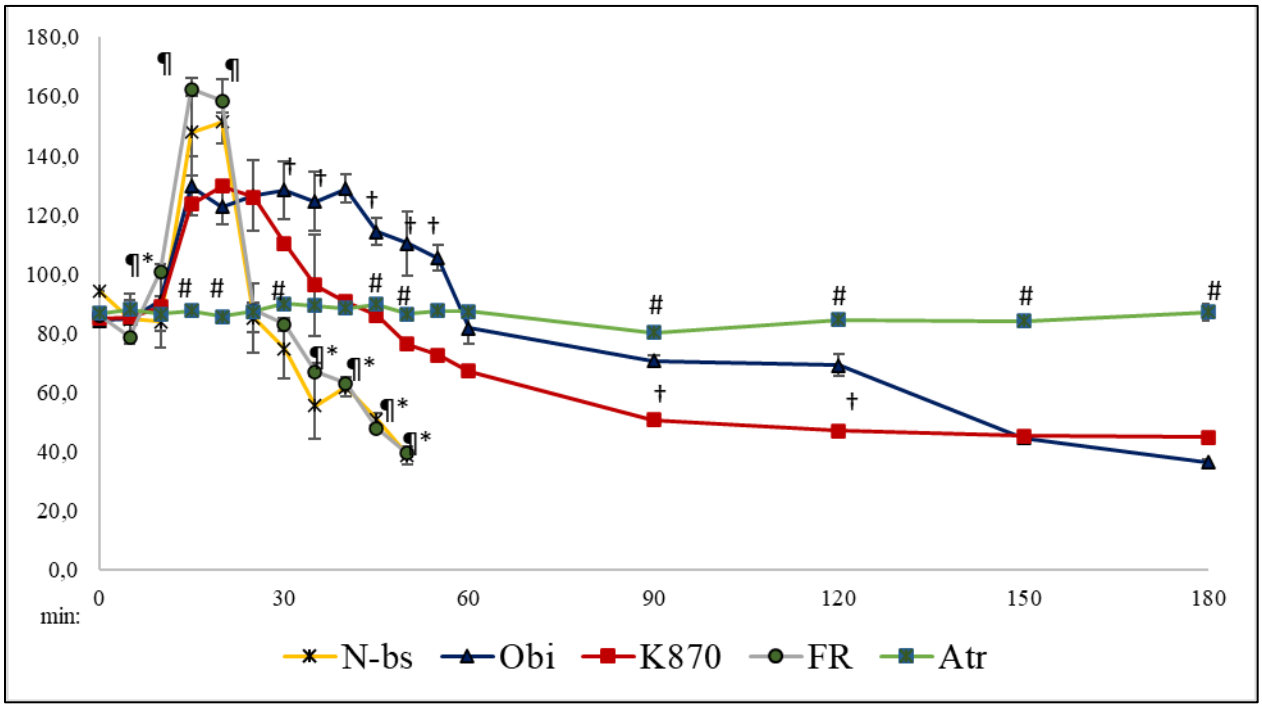
Slika 73. Bazni višak - BE arterijske krvi pacova tretiranog visokom subletalnom dozom paraoksona (0,25 mg/kg sc) u odnosu na orinirani antidot

1 min kasnije: FR: 0,9% NaCl (1 mL/kg im), K870: oksim K870 (35 mg/kg im), Obi: obidoksim (22 mg/kg im), Atr: atropin (10 mg/kg im), N-bs: N-butilskopolamin (63,36 mg/kg im); #: K870 vs Atr; *: Obi vs Atr; ‡: K870 vs Obi;

BE je tokom cijelog posmatranog perioda za sve grupe imao negativne vrijednosti, ukazujući na acidozu (Slika 73). Vrijednosti BE su naglo počele da padaju nakon 30 min, kod neštićenih pacova u 45. min iznosio je -12,00, a kod pacova tretiranih N-butilskopolaminom -11,00. Pacovi tretirani obidoksimom imali su statistički značajno niže vrijednosti BE u odnosu na pacove tretirane oksimom K870 i atropinom u 60. i 90. min ($p = 0,040$ i $p = 0,033$, a 3 h nakon trovanja paraoksonom, pacovi tretirani oksimom K870 su imali najviše vrijednosti (-2,00; $p = 0,089$).

5.7.3. Arterijski pritisak

5.7.3.1.Srednji arterijski pritisak (MAP)



Slika 74. Vrijednosti srednjeg arterijskog pritiska (mm Hg) pacova tretiranog visokom subletalnom dozom paraoksona (0,25 mg/kg sc) u odnosu na orinirani antidot

1 min kasnije: FR: 0,9% NaCl (1 mL/kg im), K870: oksim K870 (35 mg/kg im), Obi: obidoksim (22 mg/kg im), Atr: atropin (10 mg/kg im), N-bs: N-butilskopolamin (63,36 mg/kg im); #: K870 vs Atr; †: K870 vs Obi; ¶: FR vs K870 i Obi; *N-bs vs K870 i Obi;

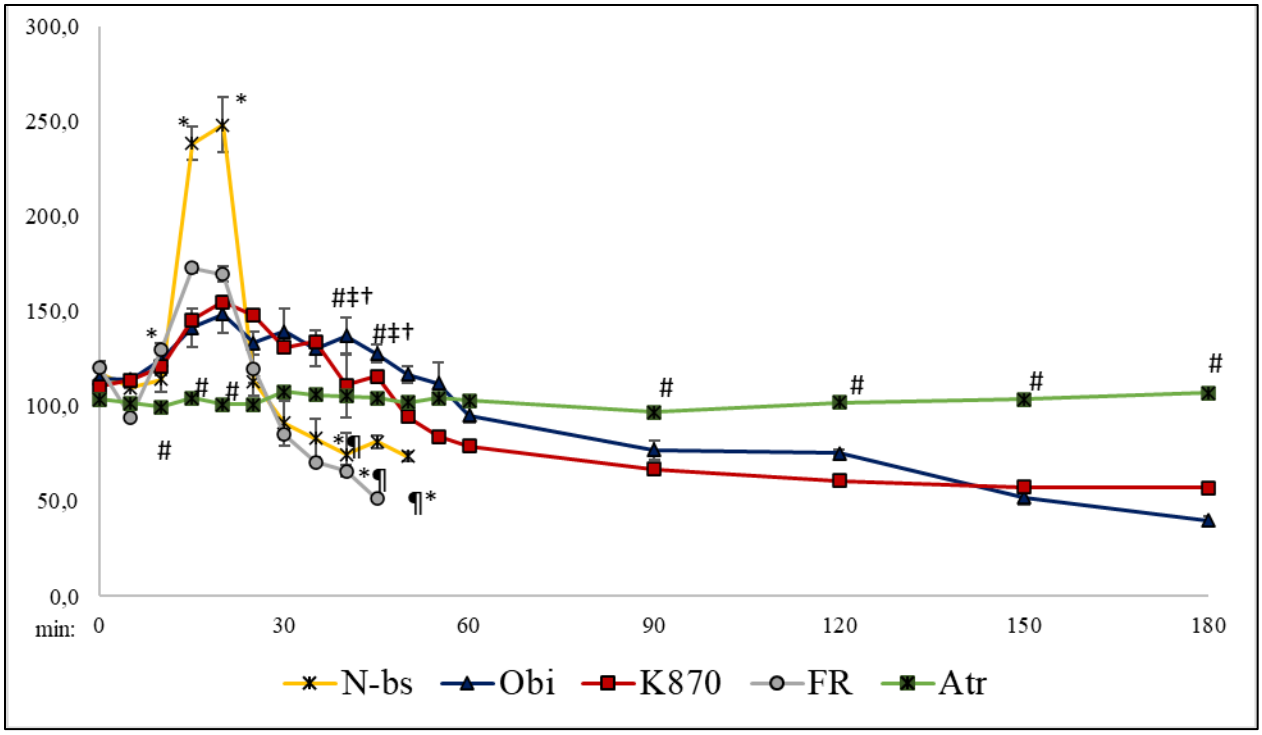
Na početku vrijednost srednjeg arterijskog pritiska (eng. *mean arterial pressure* - MAP) kod neštićenih pacova je počela da opada, da bi već u 10. min ponovo počela da raste i dostigla maksimalne vrijednosti od 162,50 ± 9,19 mm Hg u 15. min (Slika 74). Vrijednost MAP je naglo opala u 25. min, a u 45. min izmjeren je MAP od 39,26 ± 3,20 mm Hg. Kod pacova tretiranih N-butilskopolaminom, MAP je na početku počeo da pada - u 10. min iznosio je 83,85 mm Hg, poslije toga je počeo da raste do maksimalnih 151,42 mm Hg u 20. min. Poslije toga počinje pad MAP uz blage oscilacije, da bi u poslijednjem mjerenju u 50. min iznosio 38,61 mm Hg. Kod pacova tretiranih atropinom osciliranje MAP je bilo minimalno od 80,17 mm Hg registovanom u 90. min

do 90,00 mm Hg registrovanom u 30. min nakon tretmana paraoksonom. Kod pacova tretiranih obidoksimom u 15. min dolazi do porasta vrijednosti MAP na maksimalnih 129,68 mm Hg.

Osim u prvih 10 min, nađena je značajna razlika u vrijednosti MAP između grupa u svim posmatranim vremenima: 15. ($\chi^2 = 19{,}411$, $p = 0{,}001$), 20. ($\chi^2 = 21{,}217$, $p < 0{,}001$), 25. ($\chi^2 = 14{,}697$, $p = 0{,}005$), 30. ($\chi^2 = 23{,}063$, $p < 0{,}001$), 35. ($\chi^2 = 18{,}387$, $p < 0{,}001$), 40. ($\chi^2 = 24{,}482$, $p < 0{,}001$), 45. ($\chi^2 = 25{,}775$, $p < 0{,}001$), 50. ($\chi^2 = 25{,}368$, $p < 0{,}001$), 55. ($\chi^2 = 15{,}158$, $p = 0{,}001$), 60. ($\chi^2 = 11{,}591$, $p = 0{,}003$), 90. ($\chi^2 = 14{,}749$, $p = 0{,}001$), 120. ($\chi^2 = 15{,}158$, $p = 0{,}001$), 150. ($\chi^2 = 11{,}474$, $p = 0{,}003$) i u 180. min ($\chi^2 = 15{,}158$, $p = 0{,}001$). Od 45. min AP počinje lagano da pada. Kod pacova tretiranih oksimom K870 najviše vrijednosti MAP su mjerene u 20. min (129,66 mm Hg), Pola sata nakon administracije paraoksona, vrijednosti MAP su počele lagano na padaju. Na kraju posmatranog perioda vrijednosti MAP su bile 44,68 mm Hg. Vrijednosti MAP u 45. min (posljednje vrijeme kada su mjerene vrijednosti za sve grupe) su bile značajni niže kod pacova tretiranih sa FR i N-butilskopolaminom u odnosu na ostale grupe ($p < 0{,}001$). Vrijednosti MAP pacova tretiranih obidoksimom su bile značajno više u odnosu na oksim K870 i atropin ($p < 0{,}001$).

5.7.3.2.Sistolni arterijski pritisak (SAP)

Na početku vrijednost SAP kod neštićenih pacova je krenula da pada, da bi već u 10. min ponovo počela da raste i dostigla maksimalne vrijednosti od 172,83 ± 19,30 mm Hg u 20. min. Vrijednosti SAP su počele da padaju od 30. min, u 45. min mjeren je SAP od 51,33 ± 14,20 mm Hg. Kod pacova tretiranih N-butilskopolaminom, pritisak je na početku bio stabilan, da bi u 15. i u 20. min naglo porastao na 238,00, odnosno 248 mm Hg. Poslije toga SAP naglo pada na početne vrijednosti, da bi u posljednjem mjerenju u 50. min iznosio 73,50 mm Hg. Kod pacova tretiranih atropinom osciliranje SAP je bilo minimalno od 96,83 mm Hg registovanom u 90. min do 107,65 mm Hg registrovanom u 30. min nakon tretmana paraoksonom. Kod pacova tretiranih obidoksimom od 10. min počinje postepeni porast vrijednosti SAP do maksimalnih 148,33 mm Hg registrovanih u 20. min, od 45. min pritisak počinje lagano da pada. Kod pacova tretiranih oksimom K870 najviše vrijednosti SAP su mjerene u 25. min (147,83 mm Hg), uz diskretno osciliranje SAP u toku posmatranog perioda. Jedan sat nakon paraoksona, vrijednosti SAP su počele lagano na padaju (Slika 75).



Slika 75. Vrijednosti sistolnog arterijskog pritiska (mm Hg) pacova tretiranog visokom subletalnom dozom paraoksona (0,25 mg/kg sc) u odnosu na orinirani antidot

1 min kasnije: FR: 0,9% NaCl (1 mL/kg im), K870: oksim K870 (35 mg/kg im), Obi: obidoksim (22 mg/kg im), Atr: atropin (10 mg/kg im), N-bs: N-butilskopolamin (63,36 mg/kg im); #: K870 vs Atr; †: K870 vs Obi; ‡: Obi vs Atr; ¶: FR vs K870 i Obi; *N-bs vs K870 i Obi;

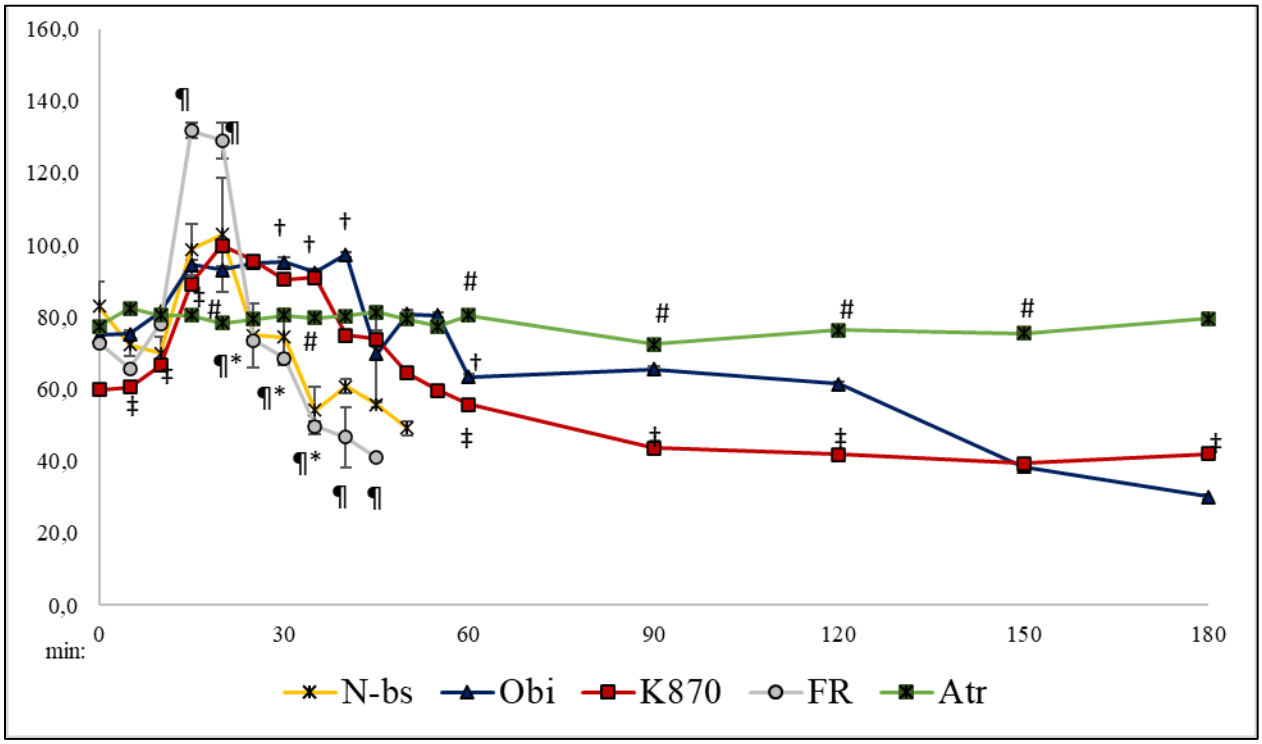
Nađena je značajna razlika u vrijednosti SAP između grupa u svim posmatranim vremenima: 5. ($\chi^2 = 13{,}284$, $p = 0{,}010$), 10. ($\chi^2 = 15{,}367$, $p = 0{,}004$), 15. ($\chi^2 = 26{,}115$, $p < 0{,}001$), 20. ($\chi^2 = 23{,}089$, $p < 0{,}001$), 25. ($\chi^2 = 16{,}340$, $p = 0{,}003$), 30. ($\chi^2 = 25{,}599$, $p < 0{,}001$), 35. ($\chi^2 = 19{,}200$, $p = 0{,}001$), 40. ($\chi^2 = 26{,}334$, $p < 0{,}001$), 45. ($\chi^2 = 26{,}451$, $p < 0{,}001$), 50. ($\chi^2 = 20{,}467$, $p < 0{,}001$), 55. ($\chi^2 = 14{,}981$, $p = 0{,}001$), 60. ($\chi^2 = 14{,}966$, $p = 0{,}001$), 90. ($\chi^2 = 15{,}158$, $p = 0{,}001$), 120. ($\chi^2 = 15{,}158$, $p = 0{,}001$), 150. ($\chi^2 = 14{,}966$, $p = 0{,}001$) i u 180. min ($\chi^2 = 15{,}205$, $p < 0{,}001$).

Vrijednosti SAP u 45. min (poslijednje vrijeme kada su mjerene vrijednosti za sve grupe) su bile značajno niže kod pacova tretiranih sa FR u odnosu na sve ostale grupe ($p < 0{,}001$), kod pacova tretiranih sa N-butilskopolaminom niže u odnosu na sve druge grupe osim FR ($p < 0{,}001$).

Vrijednosti AP kod pacova tretiranih obidoksimom bile su značajno više u odnosu na one tretirane oksimom K870 ($p = 0,020$) i atropinom ($p < 0,001$), a AP kod pacova tretiranih oksimom K870 značajno viši nego kod pacova tretiranih atropinom ($p = 0,021$).

5.7.3.3.Dijastolni arterijski pritisak (DAP)

Na početku vrijednost DAP kod neštićenih pacova je krenula da pada, da bi već u 10. min ponovo počela da raste i dostigla maksimalne vrijednosti od 131,50 ± 13,09 mm Hg u 20. min. Poslije toga, vrijednosti DAP su počele da padaju, u 45. min mjeren je DAP od 41,00 ± 4,20 mm Hg.



Slika 76. Vrijednosti dijastolnog arterijskog pritiska (mmHg) pacova tretiranog visokom subletalnom dozom paraoksona (0,25 mg/kg sc) u odnosu na orinirani antidot

1 min kasnije: FR: 0,9% NaCl (1 mL/kg im), K870: oksim K870 (35 mg/kg im), Obi: obidoksim (22 mg/kg im), Atr: atropin (10 mg/kg im), N-bs: N-butilskopolamin (63,36 mg/kg im);); #: K870 vs Atr; †: K870 vs Obi; ‡: Obi vs Atr; ¶: FR vs K870 i Obi; *N-bs vs K870 i Obi;

Kod pacova tretiranih N-butilskopolaminom, pritisak je na početku počeo da pada, u 10. min iznosio je 70 mm Hg, poslije toga je počeo da raste do maksimalnih 103 mm Hg u 20. min. Poslije toga počinje pad DAP uz blago osciliranje, da bi u posljednjem mjerenju u 50. min iznosio 49 mm Hg. Kod pacova tretiranih atropinom osciliranje DAP je bilo minimalno od 75,50 mm Hg registrovanom u 150. min do 82,50 mm Hg registrovanom u 5. min nakon tretmana paraoksonom (Slika 76).

Na početku vrijednost DAP kod neštićenih pacova je krenula da pada, da bi već u 10. min ponovo počela da raste i dostigla maksimalne vrijednosti od 131,50 ± 13,09 mm Hg u 20. min. Poslije toga, vrijednosti DAP su počele da padaju, u 45. min mjeren je DAP od 41,00 ± 4,20 mm Hg. Kod pacova tretiranih N-butilskopolaminom, pritisak je na početku počeo da pada, u 10. min iznosio je 70 mm Hg, poslije toga je počeo da raste do maksimalnih 103 mm Hg u 20. min. Poslije toga počinje pad DAP uz blago osciliranje, da bi u posljednjem mjerenju u 50. min iznosio 49 mm Hg. Kod pacova tretiranih atropinom osciliranje DAP je bilo minimalno od 75,50 mm Hg registrovanom u 150. min do 82,50 mm Hg registrovanom u 5. min nakon tretmana paraoksonom (Slika 76).

Kod pacova tretiranih obidoksimom od 10. min kreće postepeni porast vrijednosti DAP do maksimalnih 97,33 mm Hg registrovanih u 40. min, nakon toga pritisak počinje lagano da pada. Kod pacova tretiranih oksimom K870 najviše vrijednosti DAP su mjerene u 20. min (99,83 mm Hg), uz diskretno osciliranje AP u toku posmatranog perioda. Jedan sat nakon paraoksona, vrijednosti TA su počele lagano na padaju.

Nađena je značajna razlika u vrijednosti DAP između grupa u svim posmatranim vremenima: 5. ($\chi^2 = 23{,}119$, $p < 0{,}001$), 10. ($\chi^2 = 15{,}708$, $p = 0{,}003$), 15. ($\chi^2 = 21{,}681$, $p < 0{,}001$), 20. ($\chi^2 = 18{,}711$, $p = 0{,}001$), 25. ($\chi^2 = 18{,}711$, $p = 0{,}001$), 30. ($\chi^2 = 20{,}795$, $p < 0{,}001$), 35. ($\chi^2 = 26{,}146$, $p < 0{,}001$), 40. ($\chi^2 = 26{,}862$, $p < 0{,}001$), 45. ($\chi^2 = 19{,}589$, $p = 0{,}001$), 50. ($\chi^2 = 19{,}693$, $p < 0{,}001$), 55. ($\chi^2 = 13{,}542$, $p = 0{,}001$), 60. ($\chi^2 = 15{,}174$, $p = 0{,}001$), 90. ($\chi^2 = 15{,}221$, $p < 0{,}001$), 120. ($\chi^2 = 15{,}158$, $p = 0{,}001$), 150. ($\chi^2 = 11{,}758$, $p = 0{,}003$) i u 180. min ($\chi^2 = 15{,}205$, $p < 0{,}001$).

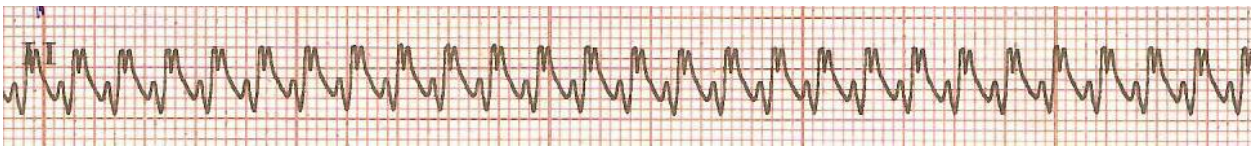
Vrijednosti DAP u 45. min (poslijednje vrijeme kada su mjerene vrijednosti za sve grupe) su bile značajni niže kod pacova tretiranih FR u odnosu na atropin ($p < 0{,}001$), oksim K870 ($p =$

0,006) i obidoksim (p = 0,020). Vrijednosti dijastolnog pritiska u 45. min su bile značajni niže kod pacova tretiranih sa N-butilskopolaminom odnosu na atropin (p = 0,048).

5.7.4. Srčani ritam

Srčani ritam je posmatran na EKG-u. Posmatrana je srčana frekvenca, kao i pojava aritmija u navedenom periodu. Primijećeno je da je frekvenca bila veoma slična kod pacova kojima je dat FR i N-butilskopolamin. Smjenjivali su se periodi tahikardije i bradikardije. Ono što je značajno jeste da u toku posmatranog perioda, skoro u svim vremenima je dolazilo do specifičnih poremećaja ritma – ubrzan rad srca je prvenstveno bio posljedica ventrikularnih tahikardija, a usporen različitih blokova u provođenju. S druge strane, iako je tokom posmatranog perioda kod pacova tretiranih atropinom i oksimima postojala tahikardija, radilo se o sinusnoj tahikardiji i pravilnom srčanom ritmu.

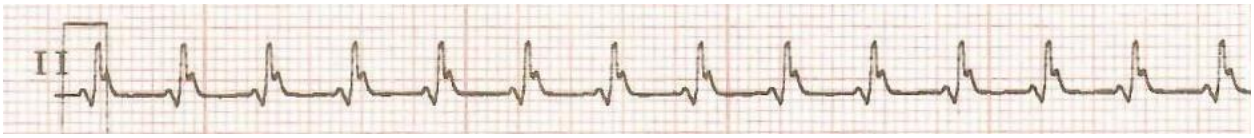
Primjer normalnog srčanog ritma prikazan je na Slici 77.



Slika 77. Normalan srčani ritam, frekvencija 320 otkucaja/min

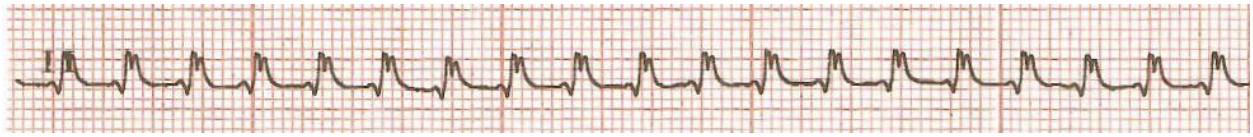
Pacov tretiran paraoksonom (0,25 mg/kg sc) i minut kasnije atropinom (10 mg/kg im). Snimak zabilježen 10 min nakon aplikacije paraoksona.

Primjeri usporenog rada srca su prikazani na Slikama 78-85. Usporen rad srca je kod pojedinih pacova bio jednostavna sinusna bradikardija kao što je prikazano na Slici 78. i 79.



Slika 78. Sinusna bradikardija, frekvencija 170 otkucaja/min

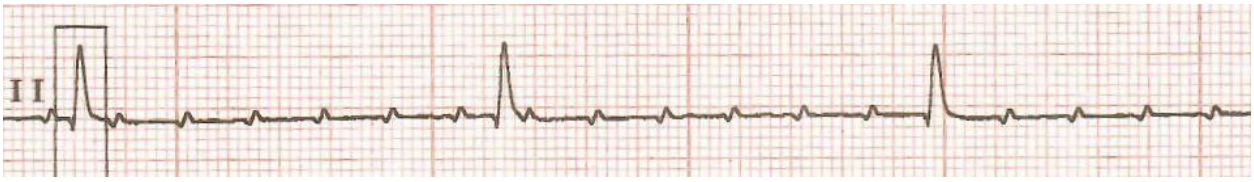
Pacov tretiran paraoksonom (0,25 mg/kg sc) i minut kasnije obidoksimom (22 mg/kg im). Snimak zabilježen 90 min nakon aplikacije paraoksona.



Slika 79. Sinusna bradikardija, frekvencija 210 otkucaja/min

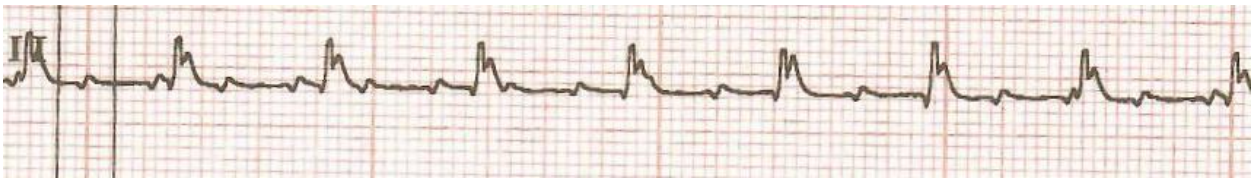
Pacov tretiran paraoksonom (0,25 mg/kg sc) i minut kasnije oksimom K870 (35 mg/kg im). Snimak zabilježen 90 min nakon aplikacije paraoksona.

Nizak broj otkucaja je bio posljedica i različitih poremećaja srčanog ritma, kao što su AV blok različitog stepena, prikazani na Slici 80-85.



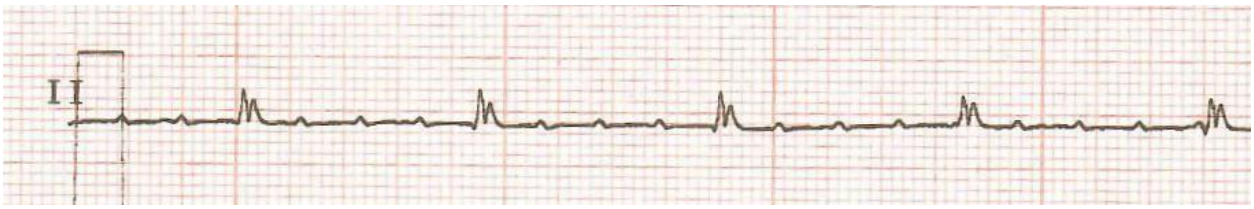
Slika 80. Teška bradikardija i potpuni atrioventikularni (AV) blok, frekvencija komora 70/min

Pacov tretiran paraoksonom (0,25 mg/kg sc) i minut kasnije N-butilskopolaminom (63,36 mg/kg im). Snimak zabilježen 30 min nakon aplikacije paraoksona.



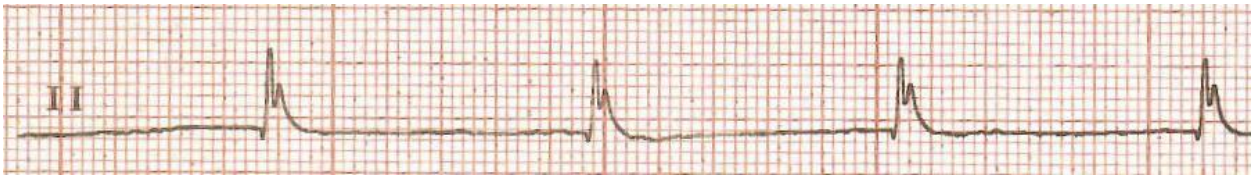
Slika 81. Atrioventrikularni (AV) blok 1:2, frekvencija komora 120/min

Pacov tretiran paraoksonom (0,25 mg/kg sc) i minut kasnije fiziološkim rastvorom (1 mL/kg im). Snimak zabilježen 45 min nakon aplikacije paraoksona.



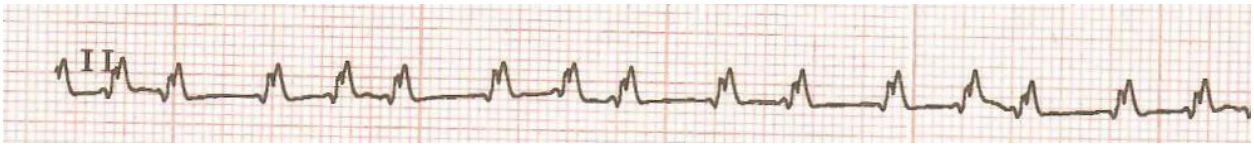
Slika 82. Atrioventrikularni (AV) blok 1:4, frekvencija komora 60/min

Pacov tretiran paraoksonom (0,25 mg/kg sc) i minut kasnije N-butilskopolaminom (63,36 mg/kg im). Snimak zabilježen 60 min nakon aplikacije paraoksona.



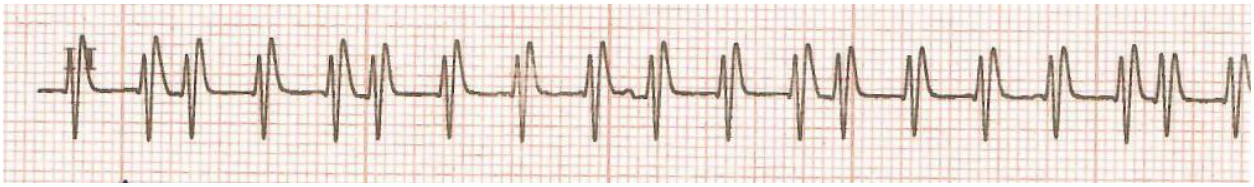
Slika 83. Izražena bradikardija, neposredno pred srčani zastoj, frekvencija 50 otkucaja /min

Pacov tretiran paraoksonom (0,25 mg/kg sc) i minut kasnije fiziološkim rastvorom (1 mL/kg im). Snimak zabilježen 40 min nakon aplikacije paraoksona.



Slika 84. Bradiaritmija (fibrilacija pretkomora uz spor odgovor komora), frekvencija komora 230 otkucaja /min

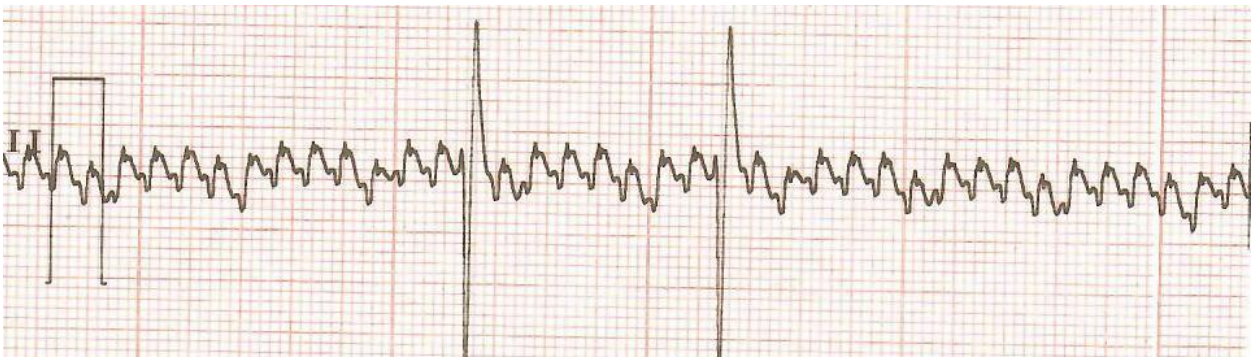
Pacov tretiran paraoksonom (0,25 mg/kg sc) i minut kasnije atropinom (10 mg/kg im). Snimak zabilježen 150 min nakon aplikacije paraoksona.



Slika 85. Bradiaritmija (fibrilacija pretkomora uz aberantno provođenje i blok grane), frekvencija komora 230 otkucaja /min

Pacov tretiran paraoksonom (0,25 mg/kg sc) i minut kasnije obidoksimom (22 mg/kg im). Snimak zabilježen 40 min nakon aplikacije paraoksona.

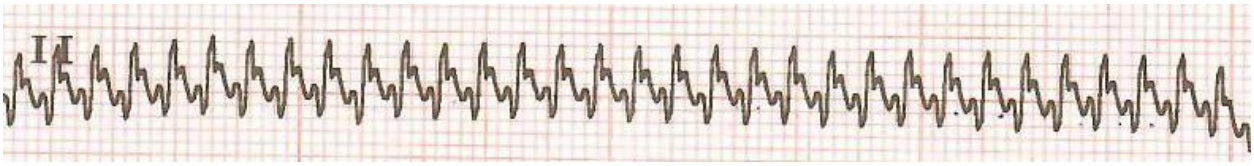
Pojedinačne ekstrasistole nisu bile maligne kada su bile jedini poremećaj ritma (Slika 86).



Slika 86. Pojedinačne ventrikularne ekstrasistole (VES), sa osnovnim ritmom flater pretkomora, frekvencija 480 otkucaja /min

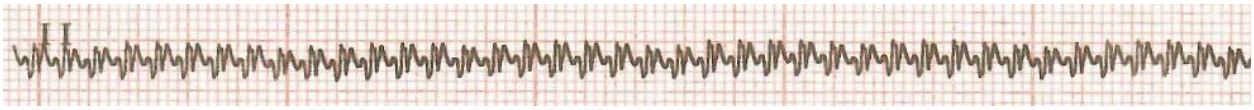
Pacov tretiran paraoksonom (0,25 mg/kg sc) i minut kasnije fiziološkim rastvorom (1 mL/kg im). Snimak zabilježen 50. min nakon aplikacije paraoksona.

Ubrzan rad srca je bio pravilnog ritma kao što je prikazano na Slikama 87-89. Ovo je bio najčešći ritam kod pacova trovanih i atropinom i oksimima. Kada su kod pacova postojale i smetnje provođenja, poput parcijalnog bloka grane, veoma ubrzana srčana frekvenca je prethodila nastanku aritmija (Slika 90. i 91).



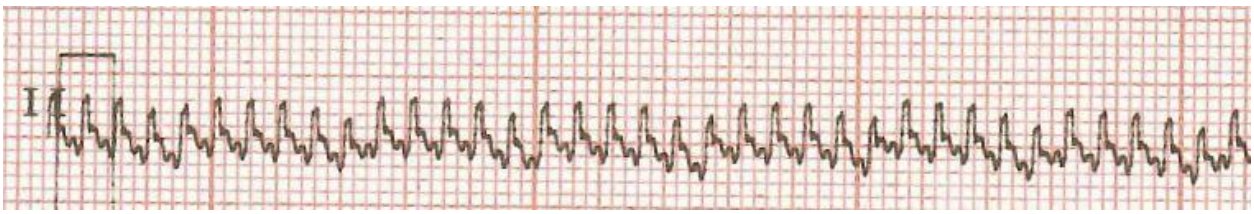
Slika 87. Sinusna tahikardija, frekvencija 470 otkucaja /min

Pacov tretiran paraoksonom (0,25 mg/kg sc) i minut kasnije atropinom (10 mg/kg im). Snimak zabilježen 180 min nakon aplikacije paraoksona.



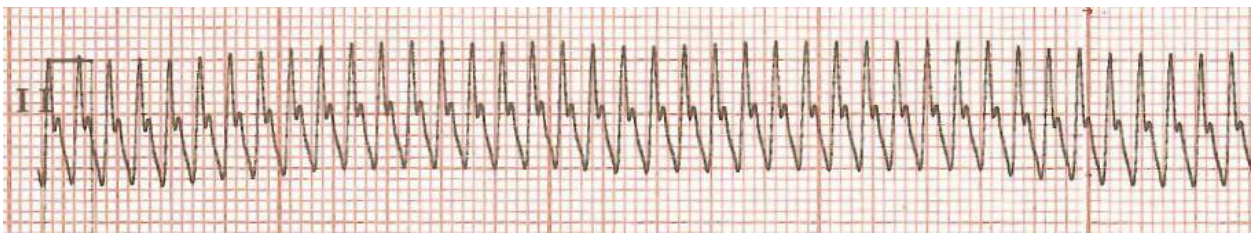
Slika 88. Sinusna tahikardija, frekvencija 480 otkucaja /min

Pacov tretiran paraoksonom (0,25 mg/kg sc) i minut kasnije oksim K870 (35 mg/kg im). Snimak zabilježen 150 min nakon aplikacije paraoksona.



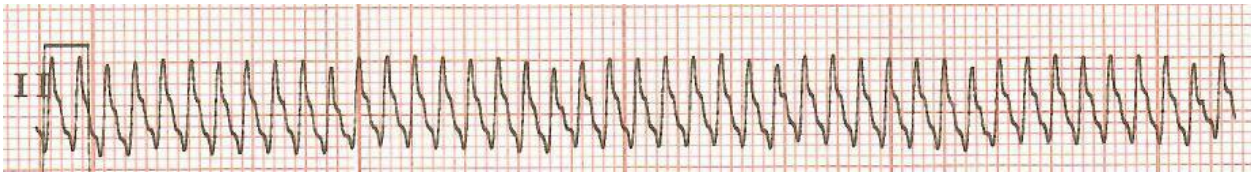
Slika 89. Sinusna tahikardija, frekvencija 600 otkucaja /min

Pacov tretiran paraoksonom (0,25 mg/kg sc) i minut kasnije obidoksim (22 mg/kg im). Snimak zabilježen 180 min nakon aplikacije paraoksona.



Slika 90. Sinusna tahikardija uz aberantno provođenje frekvencija 540 otkucaja/min

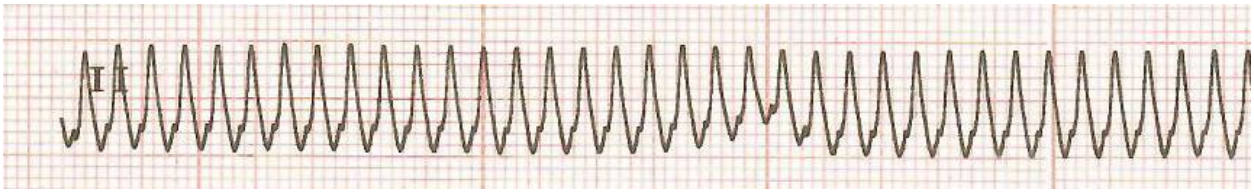
Pacov tretiran paraoksonom (0,25 mg/kg sc) i minut kasnije obidoksim (22 mg/kg im). Snimak zabilježen 120 min nakon aplikacije paraoksona.



Slika 91. Sinusna tahikardija uz aberantno provođenje frekvencija 570 otkucaja/min

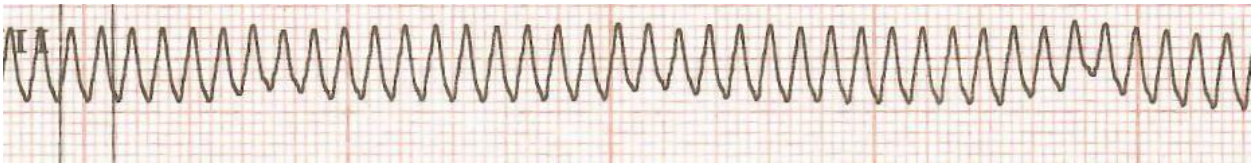
Pacov tretiran paraoksonom (0,25 mg/kg sc) i minut kasnije oksim K870 (35 mg/kg im). Snimak zabilježen 90 min nakon aplikacije paraoksona.

Ventrikularna tahikardija je imala veliki potencijal ka malignim aritmijama – ventrikularnoj fibrlaciji i *Torsades de Pointes* (Slike 92-96).



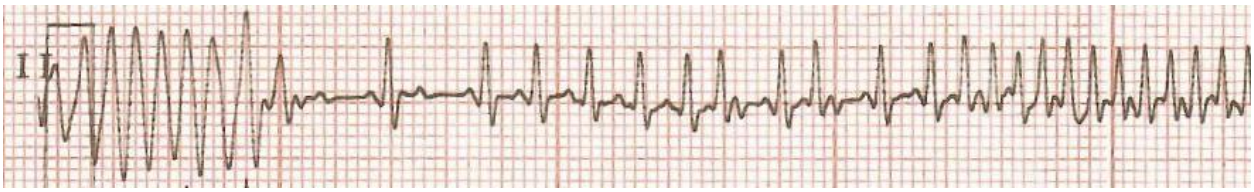
Slika 92. Ventrikularna tahikardija, frekvencija 520 otkucaja/min

Pacov tretiran paraoksonom (0,25 mg/kg sc) i minut kasnije N-butilskopolaminom (63,36 mg/kg im). Snimak zabilježen 40 min nakon aplikacije paraoksona.



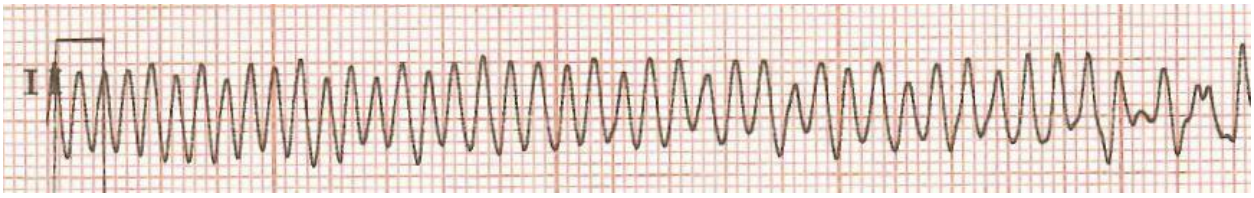
Slika 93. Ventrikularna tahikardija, frekvencija 540 otkucaja/min

Pacov tretiran paraoksonom (0,25 mg/kg sc) i minut kasnije fiziološkim rastvorom (1 mL/kg im). Snimak zabilježen 40 min nakon aplikacije paraoksona.



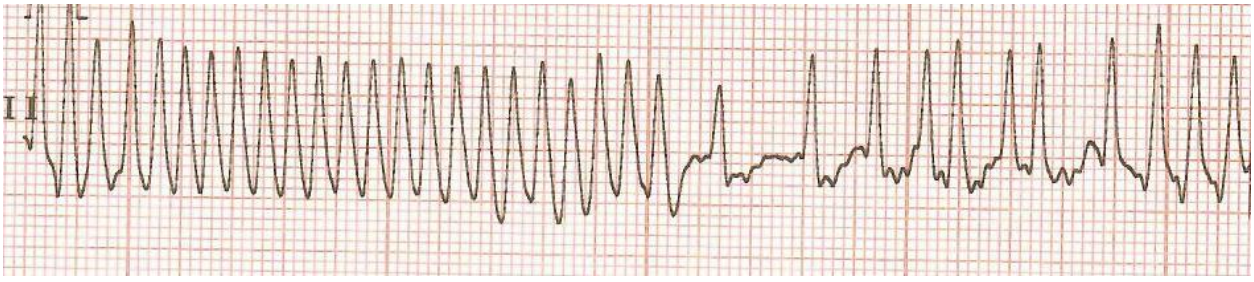
Slika 94. Ventrikularna tahikardija, koja prelazi u apsolutnu aritmiju 310 otkucaja/min

Pacov tretiran paraoksonom (0,25 mg/kg sc) i minut kasnije fiziološkim rastvorom (1 mL/kg im). Snimak zabilježen 45 min nakon aplikacije paraoksona.



Slika 95. Ventrikularna tahikardija koja prelazi u ventrikularnu fibrilaciju, frekvencija 500-540 otkucaja/min

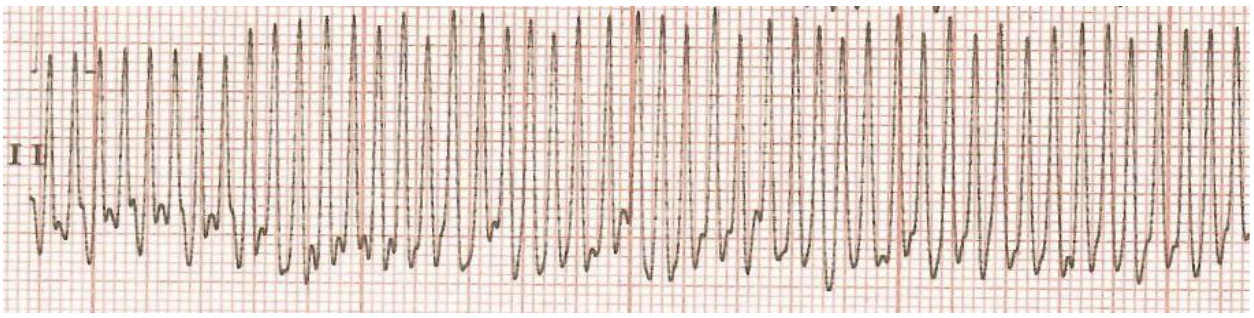
Pacov tretiran paraoksonom (0,25 mg/kg sc) i minut kasnije oksim K870 (35 mg/kg im). Snimak zabilježen 180 min nakon aplikacije paraoksona.



Slika 96. Ventrikularna tahikardija koja prelazi u ventrikularnu fibrilaciju, frekvencija 600 otkucaja/min

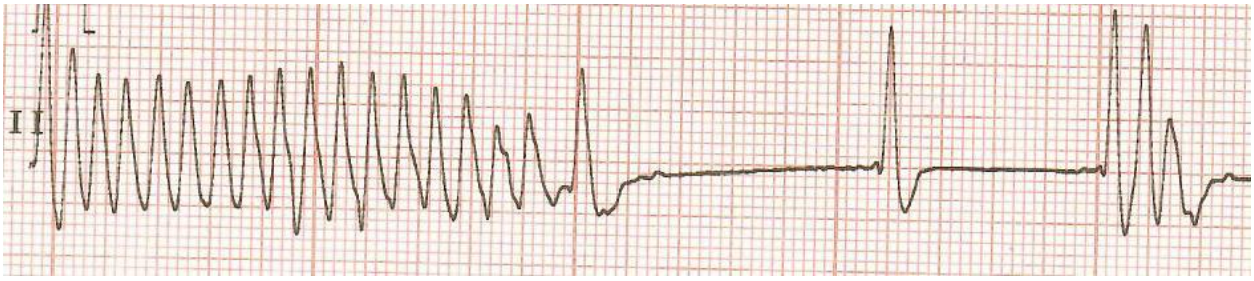
Pacov tretiran paraoksonom (0,25 mg/kg sc) i minut kasnije obidoksim (22 mg/kg im). Snimak zabilježen 180 min nakon aplikacije paraoksona.

Poseban problem predstavljale su maligne aritmije, kod kojih je kontrakcija samog srčanog mišića ograničena. Nakon kratkotrajnih ventrikularnih fibrilacija uglavnom je nastupala asistolija i smrt (Slike 97-100).



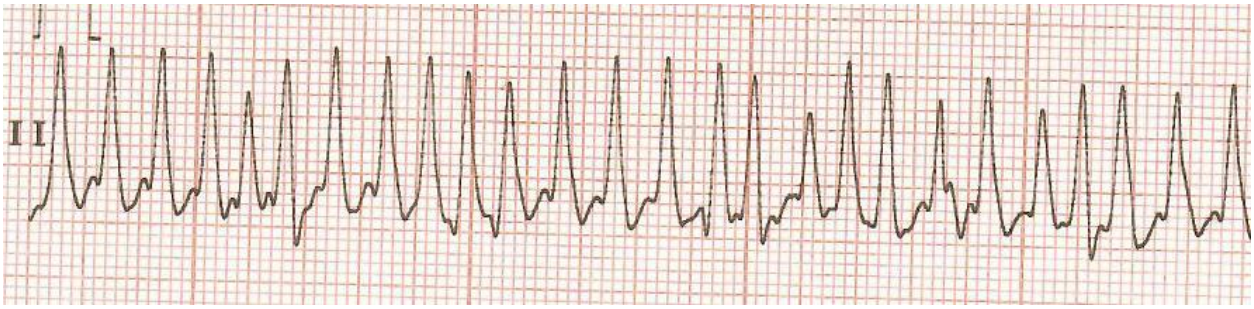
Slika 97. *Torsades de Pointes*, frekvencija 600 otkucaja/min

Pacov tretiran paraoksonom (0,25 mg/kg sc) i minut kasnije obidoksim (22 mg/kg im). Snimak zabilježen neposredno pred smrt



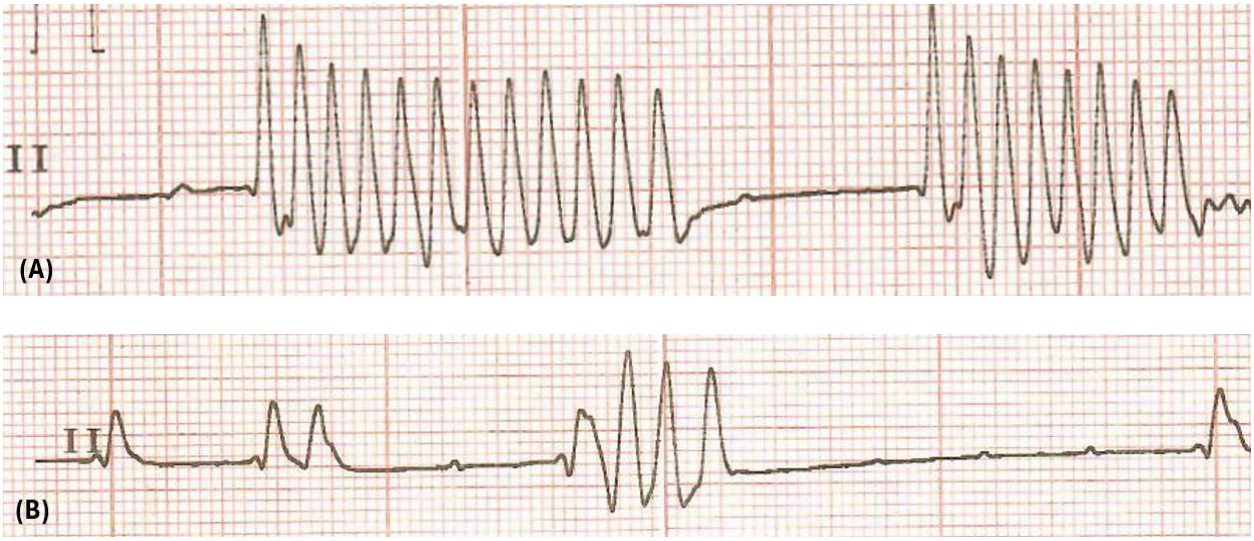
Slika 98. Ventrikularna tahikardija, *Torsades de Pointes*, asistolija

Pacov tretiran paraoksonom (0,25 mg/kg sc) i minut kasnije fiziološki rastvor (1 mL/kg im). Snimak zabilježen neposredno pred smrt



Slika 99. *Torsades de Pointes*, frekvencija 360 otkucaja /min, prethodila epizoda ventrikularne tahikardije

Pacov tretiran paraoksonom (0,25 mg/kg) i minut kasnije oksim K870 (35 mg/kg im). Snimak zabilježen 210 min nakon aplikacije paraoksona

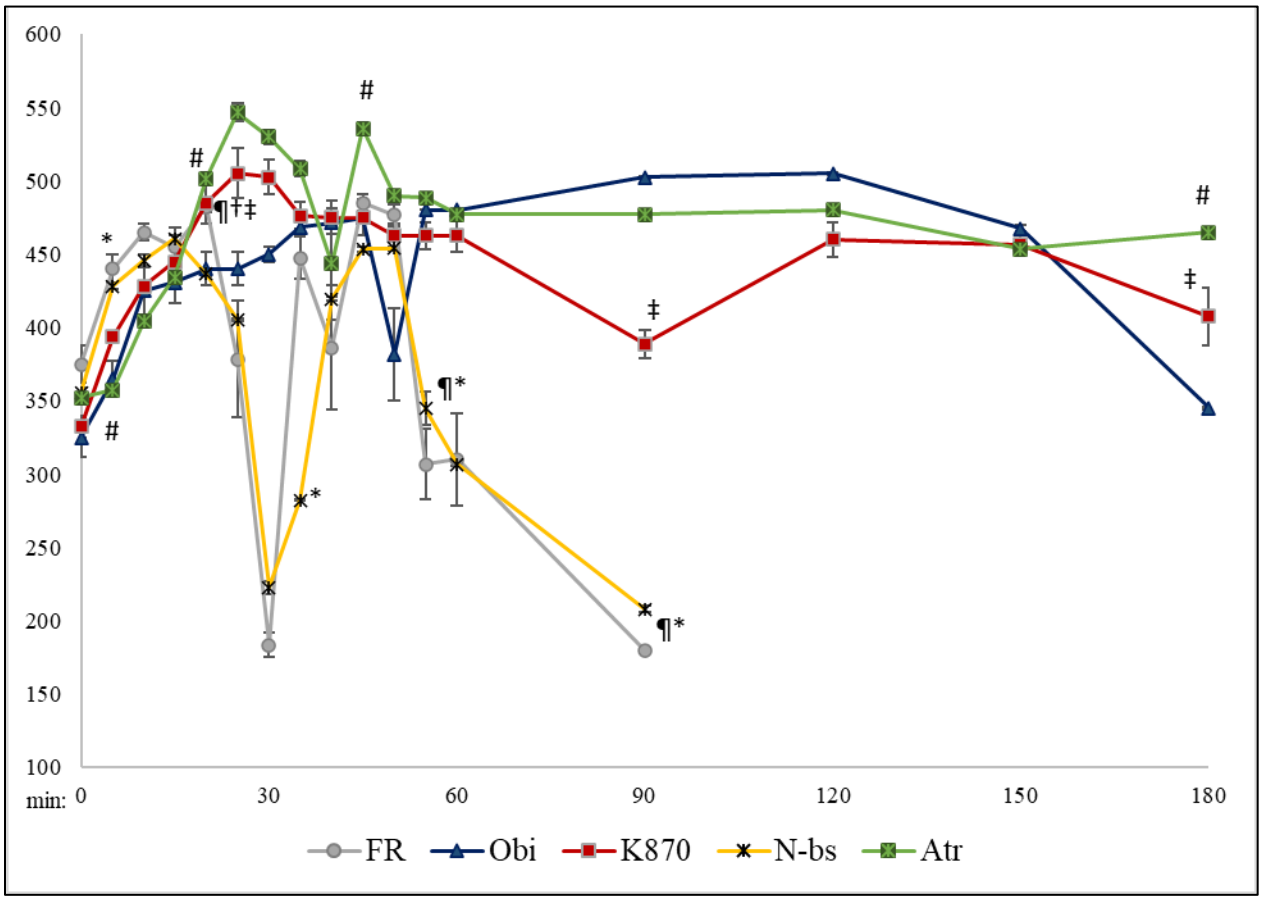


Slika 100. Agonalni poremećaji ritma: A i B: promjene ventriklarna tahikardija, *Torsades de Pointes*, atrioventrikularni blok, asistolija

Pacov tretiran paraoksonom (0,25 mg/kg sc) i minut kasnije obidoksim (22 mg/kg im). Snimak zabilježen 180 min nakon aplikacije paraoksona

Nađena je značajna razlika u vrijednosti srčane frekvence u 5. (χ^2 = 13,710, p = 0,008), 20. (χ^2 = 12,144, p = 0,016), 25. (χ^2 = 13,124, p = 0,011), 30. (χ^2 = 14,406, p = 0,006), 35. (χ^2 = 14,883, p = 0,005), 45. (χ^2 = 12,102, p = 0,017), 50. (χ^2 = 9,976, p = 0,041), 55. (χ^2 = 13,612, p = 0,009), 60.

(χ^2 = 13,468, p = 0,009), 90. (χ^2 = 11,657, p = 0,009) i u 180. min (χ^2 = 5,600, p = 0,061). U 90. min pacovi tretirani sa FR i sa N-butilskopolaminom imali su značajno nižu frekvencu u odnosu na ostale grupe. Pacovi tretirani atropinom, kao i obidoksimom imali su značajno višu frekvencu u odnosu na ostale grupe. Pacovi tretirani oksimom K870 imali su znatno nižu frekvencu u odnosu na one tretirane obidoksimom u atropinom, a više u odnosu na one tretirane N-butilskopolaminom i FR (Slika 101).



Slika 101. Srčana frekvenca pacova tretiranog visokom subletalnom dozom paraoksona (0,25 mg/kg sc) u odnosu na orinirani antidot

1 min kasnije: FR: 0,9% NaCl (1 mL/kg im), K870: oksim K870 (35 mg/kg im), Obi: obidoksim (22 mg/kg im), Atr: atropin (10 mg/kg im), N-bs: N-butilskopolamin (63,36 mg/kg im);); #: K870 vs Atr; †: K870 vs Obi; ‡: Obi vs Atr; ¶: FR vs K870 i Obi; *N-bs vs K870 i Obi;

6. DISKUSIJA

Trovanja OP pesticidima, kako zadesna tako i namjerna, i dalje su česta, naročito u zemljama u razvoju [182,183]. Samoubilačko trovanje insekticidima predstavlja ozbiljan medicinski problem, posebno u nerazvijenim zemljama, gde je uobičajen metod pokušaja samoubistva i metod sa najčešćim smrtnim ishodom [184]. U razvijenim zemljama trovanja pesticidima su tri puta češća kod muškaraca nego kod žena, dok je u manje razvijenim zemljama ovaj odnos blizu 50:50 [185,186]. Žene češće pokušavaju, a muškarci češće realizuju samoubistvo zbog smrtonosnijih metoda koje biraju. Međutim, kada se radi o samotrovanju pesticidima, prvenstveno OP, trovanja sa smrtnim ishodom su jednako česta kod oba pola [187]. Letalni ishod je češći kod starijih osoba [124,184,186,188,189].

Među pokušajima samoubistva sa smrtnim ishodom, 30% njih u Indiji, 62% u Kini i 71% u Šri Lanki bilo je zbog trovanja insekticidima [190]. Studija Centra za kontrolu trovanja Vojnomedicinske akademije, Beograd, pokazala je da je od 60 pacijenata hospitalizovanih u petogodišnjem periodu zbog trovanja inhibitorima AChE, čak kod 85% bilo u pitanju namjerno samotrovanje [124]. Zbog njegove ekstremne toksičnosti, SZO je klasifikovala paration, matično jedinjenje paraoksona, u klasu Ia (izuzetno opasan) pesticid [191]. Zbog svoje toksičnosti i postojanosti u životnoj sredini, paraokson se ne koristi kao pesticid, a i paration je zabranjen u većini razvijenih zemalja. Uvođenje zakona koji zabranjuje upotrebu najotrovnijih insekticida u poljoprivredi, je mjera koja se pokazala efikasnom i u smanjenu smrtnosti od samotrovanja insekticidima [188,189]. Danas, paraokson se načešće koristi u istraživanjima o neurotoksičnosti, liječenju trovanja OP i razvoju antidota.

LD_{50} paraoksona u ovom istraživanju iznosio je 0,33 mg/kg sc. Slične rezultate dobili su i drugi istraživači [96,192]. Kod neštićenih pacova, uginuće skoro svih životinja javlja se u prva dva sata trovanja OP, što je u skladu sa podacima dobijenim u ovoj studiji [192]. LD_{50} paraoksona varira među različitim životinjskim vrstama i zavisi od puta primjene. Za pacove, oralna LD_{50} iznosi približno oko približno 1,8 mg/kg (1,5–2,1 mg/kg), dok je kod miševa nešto niža (oko 0,9 mg/kg). Kod pasa, letalna doza se procjenjuje na 5-10 mg/kg. Iv LD_{50} kod pacova iznosi približno 0,15 mg/kg, dermalna LD_{50} kod kunića iznosi oko 3,5 mg/kg.

Kod ljudi, iako konkretni LD_{50} nije široko dogovoren, procjenjuje se da je izloženost nekoliko mg dovoljna za izazivanje ozbiljnih simptoma trovanja, ako ne i smrtnog ishoda, posebno kroz inhalaciju ili apsorpciju kroz kožu, zbog visoke liposolubilnosti paraoksona. Procijenjena smrtna doza za ljude smatra se da je u rasponu 0,1–0,5 mg/kg na osnovu studija na životinjama i pojedinačnih prikaza slučajeva. Put primjene paraoksona ima značajan uticaj na njegovu toksičnost. Kod zadesnog trovanja inhalacija je najopasnija, jer omogućava direktan i brz ulazak u krvotok, dok dermalna izloženost zahtjeva sporiju apsorpciju, ali je i dalje veoma opasna s obzirom na dugotrajnu izloženost. Oralni put, iako manje efikasan zbog metaboličkih puteva koji mogu djelimično da detoksifikuju agens, i dalje predstavlja visok rizik, posebno kroz kontaminiranu hranu ili vodu.

6.1. Antidotski potencijal atropina

LD_{50} paraoksona, kada je nakon 1 min dato 10 mg/kg im atropina, iznosio je 0,91, tako da je zaštitni indeks atropina 2,73, slično podacima u literaturi [193,194]. Atropin je efikasan u blokiranju efekata ACh na muskarinske receptore, ali je neefikasan protiv nikotinskih znakova trovanja OP [195]. Atropin je umjerene liposolubilnosti i prolazi krvno-moždanu barijeru [196]. Stoga, on u izvjesnoj mjeri antagonizuje toksične efekte prekomjerne holinergičke stimulacije u mozgu [197]. Čini se da bi lipofilniji antimuskarinski lijekovi kao što su aprofen i skopolamin, bili efikasniji od atropina [198,199]. Naime, atropin je 10-20 puta manje efikasan od ostalih antiholiergičkih lijekova (benaktizin, skopolamin, aprofen, triheksifenidil), i, iako prolazi krvno-moždanu barijeru i ostvaruje i centralne i periferene efekte, centralni efekti su samo djelimično ispoljeni. Atropin je mnogo efikasniji kod trovanja karbamatima nego OP [200]. Logično je da je zaštitni indeks atropina znatno veći kod karbamata nego kod trovanja OP, s obzirom da su karbamati reverzibilni AChE inhibitori, pa je zadatak atropina samim tim manje zahtjevan [196,197].

Krutak-Krol i Domino [24] su ispitivali različite doze atropina: 10, 33 i 100 mg/kg i došli do podatka da je doza od 10 mg/kg im optimalna u eksperimentalnim studijama, a to je doza koja je korištena i u ovoj studiji [201]. Veće doze nisu povećavale preživljavanje – štaviše, doza od 100 mg/kg je bila značajno manje efikasna od one od 10 mg/kg. Ponavljane, titrirane doze atropina u eksperimentalnim studijama bolje bi odgovarale kliničkim uslovima kod trovanja OP i

potencijalno dale bolje rezultate, ali i otežale uporedivost rezultata različitih studija. Minimalna apsolutna smrtna doza OP je 1,3 LD_{50} [150]. Primjena atropina omogućila je pacovima da prežive apsolutnu smrtnu dozu paraoksona. Preporuke kada i koliko atropina treba davati razlikuju se od smjernica do smjernica [202].

Preporuke su da se inicijalno obezbijedi venski put i da 1-3 mg atropina, te 5 min nakon inicijalnog davanja atropina, provjeri puls, krvni pritisak, veličina zjenica, prisustvo znoja, te uradi auskultacija [99]. Ako nije došlo do poboljšanja, preporučuje se davanje dvostruke prvobitne doze atropina, nastavi provjera svakih 5 min i davanje udvostručenih doza atropina ako je odgovor i dalje odsutan. Kada parametri počnu da se poboljšavaju, preporučuje se prestanak udvostručavanja doze. Boluse atropina je potrebno davati sve dok broj otkucaja srca ne bude veći od 80 otkucaja u min, sistolni krvni pritisak veći od 80 mm Hg, a grudni koš auskultatorno čist [203]. U većini slučajeva znojenje prestaje. Tahikardija nije kontraindikacija za atropin jer je mogu izazvati mnogi faktori. Zjenice se obično šire, međutim, ovaj znak nije koristan za početni tretman atropinom jer se maksimalni efekti javljaju odloženo. Međutim, veoma proširene zjenice su pokazatelj toksičnosti atropina. Potrebna je klinička procjena o dodatnim dozama atropina ako su broj otkucaja srca i krvni pritisak malo ispod ciljnih vrijednosti, a grudni koš auskultatorno čist [204]. Teška hipotenzija bi mogla imati koristi od primjene vazopresora. Prednost vazopresora u odnosu na više doze atropina još uvijek nije jasna. Kada je pacijent stabilan, preporučuje se infuzija atropina svakih sat vremena, 10-20% ukupne doze potrebne za stabilizaciju pacijenta. Potreban je čest nadzor pacijenta da se procijeni da li se daje previše ili premalo atropina. Ako se daje premalo, holinergične karakteristike će se ponovo pojaviti nakon nekog vremena. Ako se daje previše, pacijenti će postati uznemireni i povišene tjelesne temperature, odsutne crijevne peristaltike uz zadržavanje urina. Prijavljeni su slučajevi trovanja OP insekticidima gdje su davane izuzetno visoke doze atropina - LeBlanc et al dali su čak 30 g atropina pacijentu otrovanom dimetoatom, u periodu od 5 nedjelja [205].

Ova studija je analizirala i akutne znakove trovanja paraoksonom, među kojima je posmatrana i lakrimacija koja se preporučuje u opservaciji pacijenata. Lakrimacija se javlja usljed prekomjerne stimulacije muskarinskih receptora. Iako nije opasna po život, može poslužiti kao dobar pokazatelj muskarinske ekscitacije, kao i mjera adekvatne atropinizacije tokom liječenja [206]. Kod neštićenih pacova ni intenzitet ni učestalost lakrimacije nisu bili u značajnoj korelaciji

kako sa dozom paraoksona, tako ni sa smrtnim ishodom. Međutim, ono što je uočeno kada je atropin primijenjen poslije paraoksona, bilo kao monoterapija ili u kombinaciji sa oksimima, lakrimacija je bila skoro u potpunosti odsutna. Čak i kod doze paraoksona koja je bila više od 100 puta veća od LD_{50}, lakrimacija se javljala samo kratkotrajno i bila je blagog intenziteta. Sami oksimi su blago smanjili intenzitet lakrimacije, ali ne i značajno. Postojanje lakrimacije može direktno ukazivati na to da postoje i drugi efekti muskarinske ekscitacije (npr. hipotenzija, bradikardija).

6.2. Antidotski potencijal oksima

U ovom istraživanju, zaštitni indeks obidoksima je iznosio 1,79, a oksima K870 2,27. Kod trovanja nervnim bojnim otrovima zaštitni indeks samih oksima je još niži [207]. Antidotski značaj oksima se ogleda u njihovom sinergističkom djelovanju sa atropinom, što je pokazano i u ovom istraživanju. Relativno mali pojedinačni zaštitni indeks atropina i oksima su mnogostruko potencirani - zaštitni indeks obidoksima i atropina zajedno je iznosio ~69, a oksima K870 i atropina čak 125. Ovi nalazi su u skladu sa rezultatima Žunec i saradnici koji su našli da zaštitni indeks kod mužjaka miševa otrovanih paraoksonom i tretiranih atropinom i različitim oksimima varira od 60,0 do 100,0 [208].

Iako je u principu PAM-2 efikasniji sa dietil-, a obidoksim sa dimetil-OP-ima, pokazalo se da je obidoksim efikasniji kod trovanja paraoksonom [209]. Obidoksim je do sada smatran najefikasnijim reaktivatorom AChE inhibisane paraoksonom [196]. Jun i saradnici su našli da *in vitro*, samo obidoksim i trimedoksim efikasno reaktivišu AChE inhibisanu paraoksonom [210]. Treba istaći da su prikazani rezultati pokazali da oksim K870 značajno bolje poboljšava preživljavanje kod trovanja paraoksonom od obidoksima. Oksimi su jedini kauzalni antidoti kod trovanja OP. Međutim, oksimi pokazuju značajna ograničenja. Iako su oksimi nesumnjivo dokazali efikasnost u poboljšanju stope preživljavanja u *in vivo* studijama na životinjama, ova ideja nije dovoljno potvrđena u kliničkim uslovima [211]. Liječenje oksimima je komplikovano činjenicom da u slučaju namjerne ingestije insekticida, pacijent često uzima dozu 100 puta veću od smrtonosne, pošto visoke koncentracije otrova prisutne u krvi stalno ponovo inhibišu prethodno reaktivirane esteraze [161]. Pored vrste pesticida, koncentracija pesticida u tjelesnim tečnostima je glavni faktor koji utiče na težinu i ishod trovanja.

Eyer i saradnici su sugerisali da bi terapiju oksimom trebalo započeti što je ranije moguće, da se primjenjuje dovoljno dugo i da bi koncentracija obidoksima u plazmi trebala da bude 10-20 mmol/L. Smatra se da ova koncentracija nema većih nuspojava, ali je neefikasna pri veoma velikim koncentracijama otrova, jer se ponovna inhibicija dešava brže od reaktivacije [212]. Kao što je ranije pomenuto, primjena obidoksima je ograničena njegovom hepatotoksičnošću, koju Zdarova Karasova i saradnici nisu pronašli čak ni poslije veće doze oksima K870, što je potvrđeno i u ovoj studiji [149,213]. Preporučuje se da svaki pacijent otrovan OPI kome je potreban atropin dobije i oksim, dok kod trovanja CI ne postoje univerzalne preporuke [59]. Primjena oksima trebalo bi da traje 12-24 h nakon što je pacijentu potreban atropin i nakon što je ekstubiran [145]. Oksime bi trebalo davati sve dok postoji inhibicija AChE i dok se metaboliti AChE inhibitora više ne nalaze u urinu. Preporuke su da bi bilo korisno razviti protokole za specifičan tretman trovanja izazvanog pojedinačnim OP insekticidom. Liječenje trovanja OP insekticida dodatno je komplikovano činjenicom da toksičnost OP značajno varira među životinjskim vrstama, uključujući ljude [150]. Toksičnosti OP takođe varira i od samih karakteristika OP u pogledu lipofilnosti pojedinih OP, selektivnosti za AChE, te ostavljajuće grupe koje su vezane za fosfat.

Tri glavna faktora koja ograničavaju uspjeh terapije oksimom su kontinuirano prisustvo visokih koncentracija inhibitora u plazmi, starenje („ageing“) i formiranje fosforil oksima [214]. Sa tipičnom di-metoksi i dietoksi strukturom pesticida, stope starenja su takve da ovaj fenomen postaje problem samo kada se tretman odloži i/ili produži izlaganje. Uobičajeno, ali pogrešno, vjeruje se da će 1 dan nakon intoksikacije OP insekticidom skoro sav fosforilisani enzim biti u ostarjelom obliku, tako da će terapija oksimom biti neefikasna nakon ovog vremena. Međutim, ovo tumačenje proizilazi iz *in vitro* studija u kojima se AChE brzo inhibiše i nakon toga održava potpuno inhibisan prisustvom viška inhibitora i u odsustvu oksima tokom eksperimenta. Takvi eksperimenti ne odražavaju situaciju *in vivo* i ne bi trebalo da se koriste kao razlog za odustajanje od upotrebe oksimske terapije nakon 24 sata. *Ageing* je prvenstveno problem kod trovanja OP nervnim bojnim otrovima, gdje se kod somana starenje kompleksa OP-AChE mjeri u minutama. Kod trovanja nervnim bojnim otrovima, najefikasnijim se pokazao asoksim (HI-6) (osim kod trovanja tabunom), potom obidoksim, dok je PAM-2 ograničene efikasnosti. Naročito zahtjevan nervni bojni otrov za reaktivaciju jeste tabun, gdje se obidoksim pokazao nešto efikasniji u odnosu na HI-6 [212].

Postoje dokazi iz kliničke prakse da kod trovanja dimetoksi pesticidima, efikasnost oksima može biti smanjena usljed starenja kompleksa AChE-OP. Manje se zna o drugim strukturama osim tipičnih dimetil i metilfos-foril enzima i efektima oksima. U slučaju profenofosa [215], starenje ovog kompleksa se dešava brzo i stoga se očekuje da oksimi budu neefikasni. Kod fosforamidotioata, kao što je metamidofos, dolazi do spontane i oksimom indukovane reaktivacije i izgleda da starenje ne predstavlja problem: međutim, ove podatke ne bi trebalo ekstrapolirati na sve fosforamidotioate. AChE inhibisana propetamfosom i krofomatom (prvi je fosforamidotioat, a drugi fosforamidat) izgleda da se ne podvrgava spontanoj reaktivaciji [216]. Treći faktor koji može da ometa sposobnost reaktivacije oksima je formiranje fosforil oksima u reakciji reaktivacije AChE jer fosfilovani oksimi mogu djelovati kao inhibitori AChE [217].

U studiji na zamorcima koju su sproveli Inns i Leadbeater koja je uključivala predtretman piridostgminom u oba režima liječenja, primjena bis-piridinijumskih jedinjenja koja nisu oksimi sa atropinom i diazepamom bila je efikasnija od samog atropina i diazepama kod trovanja određenim OP nervnim agensima [218]. Ovo sugeriše da antidotski efekti bis-piridinijumskih jedinjenja ne zavise u potpunosti od oksimske grupe.

Postoje dokazi da oksimi mogu direktno reagovati sa OP [219]. Oksimi mogu imati direktne aktivirajuće i inhibitorne efekte na AChE [134,220]. Pokazano je da i PAM-2 i HI-6 reaguju sa kompleksom nikotinskih receptor-jonskih kanala [134]. Nejasno je koliko ove akcije doprinose efikasnosti oksima. Van Helden et al tretirali su atropinizovane pacove iv sa trostrukim LD_{50} krotilsarina, O-(2-butenil) metilfosfonofluoridata. Ovo jedinjenje proizvodi inhibisani AChE koji trenutno stari, čime se isključuju bilo kakve korisni reaktivirajući efekti oksima. Poslije 5 min, otrovani pacovi su tretirani fiziološkim rastvorom ili oksimom (obidoksim, HI-6, HL6-7, HGG-12 ili HGG-42) [221]. Oksimi su značajno produžili vrijeme preživljavanja u poređenju sa pacovima tretiranim fiziološkim rastvorom i autori su sugerisali da ovaj efekat mora biti drugačiji od reaktivacije holinesteraze.

U mnogim relevantnim farmakodinamskim studijama terapija oksimom obično se daje nekoliko min nakon OP. Ovo nije posebno reprezentativno za kliničku situaciju, u kojoj pacijent stiže u bolnicu nekoliko sati nakon trovanja: u takvim okolnostima, ove studije mogu dati nerealnu ideju o vjerovatnoj efikasnosti oksimske terapije, posebno kod dimetoksi-OP, gde može doći do starenja ako se terapija oksimom odloži.

Jokanović i Maksimović su sproveli studiju na pacovima u kojoj je testirana efikasnost PAM-2, obidoksima, HI-6 i trimedoksima, datih sa atropinom i diazepamom 1 min nakon trovanja, u liječenju trovanja sa dva LD_{50} 25 različitih OP. Pokazalo se da su oksimi moćni antidoti kod trovanja fosfatnim insekticidima. Obidoksim, PAM-2 i HI-6 su imali nisku efikasnost u liječenju trovanja fosfonatnim trihlorfonom. Međutim, nijedan od ovih oksima nije bio efikasan antidot kod trovanja dimetoatom i piridafentionom. Razlozi za neke od ovih razlika su nejasni [222].

Najznačajnije ograničenje u efikasnosti oksima je njihova loša penetracija kroz krvno-moždanu barijeru [223,224]. Piridinijum aldoksimi kao što je PAM-2 imaju veoma nisku stopu penetracije kroz krvno-moždanu barijeru, što je uglavnom povezano sa njihovom visokom hidrofilnošću i trajnim katjonskim nabojem [225,226]. Postoje studije koje tvrde da nakon trovanja OP sa konvulzijama krvno-moždana barijera postaje propusnija za oksime i da oksimi tada bolje prodiru u mozak, ali postoje studije koje pokazuju da čak i nakon trovanja OP, oksimi ne prodiru ili prodiru u mozak vrlo malo [227]. Musilek i saradnici su analizirali idealne karakteristike koje bi oksim trebalo da ima kod trovanja OP pesticidima [228]. Utvrđeno je da: oksimi sa funkcionalnom grupom na poziciji 4 najbolje reaktiviraju AChE inhibisanu OP pesticidima; oksimi sa biskvaternarnom strukturom su efikasniji od monokvaternarnih; idealno, linker (spoj između dva prstena) treba da ima 3-5 C-C veza; dvostruka veza u linkeru povećava i efikasnost i toksičnost. Osim toga, ustanovljeno je da dodavanje molekula hlora blago povećava lipofilnost oksima i prodiranje oksima kroz krvno-moždanu barijeru [229,230]. Oksim K870 je dihlorisani analog oksima K203. Hlorisanje K203 snižava pKa i povećava količinu jonizovanog oblika oksima K870 [229].

Kassa i saradnici su našli da je neuroprotektivna efikasnost oksima K870 kod trovanja tabunom nešto veća ili slična u poređenju sa PAM-2 i HI-6, što je značajno imajući u vidu da je AChE inhibisanu tabunom posebno teško reaktivirati [231]. U eksperimentu sa intoksikacijom sarinom, HI-6 se pokazao kao najbolji reaktivator, zatim oksim K870 i PAM-2, u svim ispitivanim tkivima [149]. Za intoksikaciju tabunom, oksim K870 je bio antidot koji najviše obećava u odnosu na HI-6 i PAM-2 u krvi i bio je nešto slabiji od HI-6 u dijafragmi, ali je pokazao minimalnu reaktivaciju AChE i u mozgu. U slučaju VX intoksikacije, svi oksimi su pokazali sličnu reaktivaciju u krvi, ali su HI-6 i PAM-2 bili bolji od oksima K870 za reaktivaciju AChE u dijafragmi i mozgu. Pri trovanju paraoksonom, svi oksimi su pokazali visoku i uporedivu

reaktivaciju AChE u krvi i generalno nisku reaktivaciju u dijafragmi (PAM-2 > HI-6 > oksim K870), ali je oksim K870 bio jedini u stanju da reaktivira AChE u mozgu, što je u skladu i sa našim rezultatima [149].

In vitro se oksim K870 se vezuje i za AChE i za BuChE sa ukupnom stopom reaktivacije humane AChE inhibisane VX-om koja je uporediva sa najefikasnijim oksimom otkrivenim do sada, HI-6 i sa većim afinitetom od PAM-2 ili oksima K203 [147]. Zandona i saradnici su otkrili efekte imidazolijuma i hlorisanih bispiridinijum oksima, uključujući oksim K870, koji su povezani sa citotoksičnošću, u ćelijama SH-SI5I [232]. Citotoksični efekti su se povećavali sa brojem atoma hlora i dodatno su pojačani dvostrukom vezom u linkeru oksima K870. Produkti razgradnje izoksazol-piridinijum hlorisanih oksima nisu bili citotoksični za ćelijsku liniju HK-2 [233], što bi se moglo smatrati pozitivnom osobinom jer je glavni organ za izlučivanje oksima K870 bubreg [149].

In vivo, Zdarova Karasova i saradnici su utvrdili da je maksimalna podnošljiva doza oksima K870 130 mg/kg i 150 mg/kg za mužjake i ženke miševa [149]. Kada je ista doza data pacovima, primijećena je nekroza, otok i izliv na mjestu injekcije. Kada je doza smanjena na 20% MTD, obdukcija je pokazala blagi otok i hematome na mjestu injekcije. Otkrili su krvarenje i edem na mjestu injekcije nakon 240 min, praćeno inflamatornom infiltracijom i degenerativnim promjenama u miofibrilima u naredna dva dana. Poslije 7 dana, akutna upala je zamijenjena okruglim ćelijskim infiltratom, koji je bio povezan sa regeneracijom miocita i proizvodnjom kolagenih vlakana. Slične doze (35 mg/kg) su korištene u ovom eksperimentu i uočene su slične lokalne promjene. Kalasz i saradnici su pokazali da supstitucija hlorom smanjuje rastvorljivost i apsorpciju oksima K870 nakon njegove im primjena [234].

Prema saznanjima autora, ovo je prva studija koja je odredila LD_{50} oksima K870. Rezultati ove studije pokazuju da je LD_{50} oksima K870 175 mg/kg, u poređenju sa 108 mg/kg obidoksima. Biohemijske analize iz seruma su pokazale da ni obidoksim ni oksim K870 ne utiču na vrijednosti pankreasnih enzima, albumina, elektrolita, kao ni parametara srčanog oštećenja u serumu. Obidoksim je povećavao vrijednosti jetrenih enzima, proporcionalno datoj dozi. Oksim K870 je pri visokim dozama dovodio do porasta uree i kreatinina u serumu, ali ne i pri terapijskim dozama.

6.3. Toksičnost različitih organofosfatnih i karbamatnih insekticida

Podaci regionalnog Centra za kontrolu trovanja pokazuju da su među organofosfatnim i karbamatnim pesticidima, najčešća trovanja malationom i hlorpirifosom. Na sreću, malation (S-[1,2-dikarbetoksietil] O,O-dimetil fosforoditioat) je jedan od najmanje toksičnih OP insekticida. S obzirom na to da je malation manje toksičan u odnosu na druge OP insekticida, njegova upotreba je rasprostranjena u poljoprivredi, pa su pacijenti otrovani malationom češći od onih koji su intoksicirani drugim OP [235–237]. Iako je registrovan kao pesticid, u nekim zemljama malation se koristi i za liječenje pedikuloze [238]. Obidoksim reaktiviše AChE inhibisanu malationom, dok je PAM-2 pokazao ograničenu efikasnost [239].

Hlorpirifos (0,0-dietil 0-[3,5,6-trihloro-2-piridinil]-fosforotioat) je najčešće prodavan OP insekticid na svijetu [240]. Liu et al je utvrdila da je smrtnost kod pacijenata otrovanih hlorpirifosom 15% [241]. Fatalni ishod je konstatovan kod pacijenata sa razvijenom respiratornom insuficijencijom, produženim QT intervalom i zatajenjem bubrega. AChE inhibisana hlorpirifosom se reaktiviše oksimom PAM-2 [124,150].

Diazinon (O,O-dietil O-[4-metil-6-(propan-2-il)pirimidin-2-il] fosforotioat) je visoko lipofilan i umjereno toksičan OP insekticid. Hidrolizovani diazinon je toksičniji i manje liposolubilan. Iz tog razloga, proizvođači često koriste proizvode poput sojinog ulja kao stabilizatore koji sprečavaju hidrolizu diazinona [242]. PAM-2 uspješno reaktiviše AChE inhibisanu diazinonom [124,243].

Dimetoat ima mnogo veći afinitet za AChE nego za BuChE, pa se stoga javlja mnogo veći stepen inhibicije ovog enzima i ispoljavanje pune kliničke slike trovanja OP. Dimetoat ima nisku rastvorljivost u mastima, što rezultira njegovom visokom koncentracijom u krvi [150]. Iako dimetoat pokazuje sličan profil akutne toksičnosti kao hlorpirifos kod životinja, smrtnost od dimetoata je tri puta veća kod ljudi. Dimetoat izaziva tešku hipotenziju koja je otporna na inotropne lijekove [244]. AChE inhibisana dimetoatom ne može se reaktivisati oksimima [124].

Kliničke studije pokazuju da je stopa mortaliteta povezana sa namjernim trovanjem karbofuranom oko 2,2% i da je niža u poređenju sa drugim karbamatnim i OP insekticidima [245]. Većina razvijenih zemalja je zabranila upotrebu metomila, karbamatnog insekticda, zbog njegove visoke toksičnosti, ali se slučajevi trovanja metomilom i dalje bilježe čak i u zemljama u kojima

je njegova upotreba zabranjena [189]. Šri Lanka je zabranila upotrebu karbofurana, karbarila, dimetoata i hlorpirifosa 2014. godine, smanjujući stopu morbiditeta i mortaliteta od trovanja pesticidima za 50% u roku od 3 godine [188]. AChE inhibisana karbamatskim insekticidima će se spontano reaktivirati i primjena oksima se ne preporučuje. Kod trovanja karbarilom, oksimi čak potenciraju njegovu toksičnost [246,247].

Toksičnost OPI i CI zavisi od formulacije datog preparata, uzimajući u obzir da se pesticidi često rastvaraju u organskim rastvaračima koji su i sami toksični. Organski rastvarači dovode do depresije CNS-a, što se manifestuje različitim nivoima poremećaja svijesti [150,248].

Lipofilniji AChE inhibitori se redistribuišu u masnom tkivu i postepeno ponovo ulaze u krv, inhibišući esteraze i produžavajući njihovu inhibiciju [249]. Fosforotioati (P = S) (npr. diazinon, paration) su lipofilniji od fosfata (P = O) (npr. dihlorvos, paraokson). Spontana reaktivacija dimetil-fosforilisanog AChE se često dešava brzo čak i bez terapije oksimom.

Iako se pokazalo da je obidoksim najefikasniji oksim protiv OP insekticida kod eksperimentalnih životinja, PAM-2 i dalje ostaje najčešće korišten i dostupan oksim u kliničkim uslovima [250]. Jedan od razloga je hepatotoksičnost obidoksima koja ograničava upotrebu njegovih viših i vjerovatno efikasnijih doza kod ljudi [251].

Nedostatak efikasnosti oksima je i njihova nejednaka efikasnost kod akutnih trovanja izazvahih različitim OP. Fosforilacija se dešava gubitkom „odlazeće grupe“ OP i formiranjem kovalentne veze sa AChE preko serinske hidroksilne grupe. Specifičnost „odlazeće grupe“ doprinosi složenosti toksičnog odgovora na OP [252]. AChE inhibisana sa nekoliko OP insekticida (dimetoat, triamifos, profenofos, fenamifos) opire se svakom pokušaju reaktivacije bilo kojim oksimom, vjerovatno zbog varijacija u fosforilnom dijelu i distribuciji elektronskog naelektrisanja [206].

6.4. Acetilholinesteraza u mozgu

Kao što je već pomenuto, osnovno ograničenje efikasnosti oksima je njihov slab prodor u mozak. U ovoj studiji je analizirana inhibicija AChE u mozgu i to posebno u velikom mozgu, malom mozgu i moždanom stablu, zbog specifičnih funkcija koje ACh obavlja u pojedinim regijama. Takođe je analizirano da li i u kom procentu oksimi reaktivišu AChE tokom 24 h.

Primijećeno je značajno smanjenje aktivnosti AChE u mozgu u prvom satu, kao i ponovni pad aktivnosti AChE 8 odnosno 16 h nakon trovanja paraoksonom. U navedenom periodu vjerovatno je došlo do dodatnog oslobađanja paraoksona iz depoa i reinhibicije enzima. U studiji Eyer et al, *postmortem* analiza masnog tkiva pacijenta pokazala je 66 puta veću koncentraciju parationa od one u plazmi 16 dana nakon trovanja, dok je koncentracija u mozgu bila slična onoj u plazmi [253]. I obidoksim i oksim K870 su statistički značajno reaktivirali AChE u velikom i malom mozgu, pri čemu je oksim K870 bio bolji reaktivator od obidoksima. Oksim K870 je u svim vremenima pokazao značajnu reaktivaciju AChE u moždanom stablu.

Iz prikazanih rezultata, očigledno je da je do spontane reaktivacije AChE došlo čak i kod pacova koji nisu tretirani oksimima. Brojne *in vitro* studije su pokazale da se tokom formiranja paraokson-AChE veze istovremeno javljaju i reakcije starenja i spontane reaktivacije [216,254–256]. Masson i saradnici su otkrili da je poluvrijeme starenja za AChE 46,3, odnosno 12,6 h za BuChE inhibisane paraoksonom [216]. Poluživot spontane reaktivacije AChE bio je 49 h, sa stopom od 1,4%/h. Worek i saradnici su otkrili da starenje i spontana reaktivacija AChE u slučaju inhibicije paraoksona prate kinetiku prvog reda [254]. Poluvrijeme starenja i spontane reaktivacije kretalo se od 30 do 43 h, odnosno od 28 do 46 h. Konstanta reaktivacije u značajnoj mjeri zavisi od koncentracije inhibitora u krvi, odnosno da li dolazi do ponovne inhibicije ili spontane reaktivacije AChE u većoj mjeri [255,256]. Eyer et al su otkrili da se proces starenja dietil fosforilisane AChE javlja kod pacijenata sličnom brzinom kao u *in vitro* studijama [253].

Ždarova Karasova i kolege su pokazale da oksim K870 u početku bolje prodire u mozak i efikasno reaktiviše AChE u krvi inhibisanoj paraoksonom [149]. Ovi rezultati pokazuju da oksim K870 može ući u mozak, ali da je ukupna izloženost mozga oksimu K870 niska. Čini se da je ovo ujedno i glavno ograničenje oksima K870.

Osim mjerenja aktivnosti AChE u mozgu, praćena je brzina nastajanja, intenzitet i dužina trajanja konvulzija do 4 h nakon trovanja paraoksonom. Brzina i intenzitet konvulzija bili su jasno povezani sa dozom paraoksona, a posebno sa smrtnim ishodom. Konvulzije se javljaju značajno rjeđe kod OP insekticida nego kod nervnih bojnih otrova [257]. U ovoj studiji, konvulzije su se javile kod 83% pacova, dok su svi uginuli pacovi imali konvulzije, što dalje govori o karakteristikama paraoksona da se ponaša kao nervni bojni otrov. Primjena antidota je usporila

pojavu, smanjila intenzitet i dužinu trajanja konvulzija. I uz atenuaciju konvulzija, postojala je jasna veza između ovih parametara i doze paraksona, kao i fatalnog ishoda.

Od posmatranih znakova trovanja, pojava konvulzija je, uz fascikulacije, bila u najboljoj korelaciji sa težinom trovanja, pri čemu je intenzitet konvulzija je bio direktno povezan i sa dozom paraoksona i smrtnim ishodom trovanja. Konvulzije se javljaju na početku trovanja OP usljed prekomjerne holinergičke stimulacije CNS-a. Postoje tri perioda liječenja nakon pojave OP-indukovanih konvulzija: muskarinska, GABA-A/ benzodiazepinska i glutamatergična [207,258]. Tokom prvog, antimuskarinski lijekovi (atropin i, poželjno, još lipofilniji antimuskarinski lijek, kao što je skopolamin) mogu se efikasno koristiti za zaustavljanje konvulzija, pod uslovom da se odabere prava doza [258]. Međutim, posle ovog perioda antimuskarinski lijekovi postaju neefikasni u suzbijanju konvulzija, bez obzira na primijenjenu dozu. U drugoj fazi ovo bi se moglo nadoknaditi primjenom agonista GABAA/benzodiazepinskih receptora, kao što su barbiturati (npr. pentobarbital, tiopental natrijum) i benzodiazepini (npr. diazepam ili midazolam) [136,259]. U trećoj fazi, ovi napadi se mogu zaustaviti samo davanjem antagonista receptora N-metil-D-aspartata (NMDA), kao što su memantin, dizocilpin (MK-801) ili ketamin [153,154,260,261]. Razlog za to je činjenica da su u međuvremenu konvulzije postale glutamatergične po svom nastanku [257]. Za sada se konvulzije liječe kombinacijama antiholinergika i benzodiazepina, ali, s obzirom na ulogu glutamata, buduće terapije bi mogle da uključuju i antagoniste NMDA receptora [262].

Tremor je posredovan raznim neurotransmiterima – dopaminom, glutamatom, serotoninom, adenozinom i acetilholinom [263]. M2 muskarinski receptori su visoko eksprimirani u *nucleus basalis* i okcipitalnom korteksu, zatim u hipokampusu i drugim kortikalnim regionima. Prekomerna stimulacija M2 receptora dovodi do tremora [264]. Postoje kontradiktorni dokazi o ulozi M3 i M4 receptora u etiologiji tremora [265]. U ovoj studiji utvrđena je jasna veza između intenziteta tremora, s jedne strane, i doze paraoksona i ishoda trovanja, s druge strane kod neštićenih pacova. Tremor se često nalazi kao dio ekstrapiramidnog sindroma koji nastaje kao posljedica trajnog oštećenja CNS kod onih koji su preživeli trovanja OP [196,266,267].

6.5. Respiratorni simptomi i znaci trovanja inhibitorima acetilholinesteraze

Rezultati pokazuju da je od analiziranih regija mozga, reaktivacija AChE bila najniža u moždanom stablu. Važno je napomenuti da je reaktivacija AChE oksimom K870 bila značajna u svim posmatranim vremenima i bolja nego obidoksimom. Ovo je posebno značajno kada se zna da je centralna respiratorna depresija glavni uzrok smrti kod trovanja OP i da se respiratorni centar nalazi upravo u moždanom stablu [202,256,268]. Čak i mala reaktivacija AChE u mozgu, posebno u moždanom stablu, može značajno poboljšati preživljavanje [269]. Bajgar i saradnici su našli da je za preživljavanje pacova trovanih sarinom ili somanom neophodno da preostala aktivnost AChE u pontomedularnom uglu bude 10-20 %, čime se može objasniti i značajno bolje preživljavanje pacova koji su tretirani atropinom i oksimom K870 u odnosu na one tretirane atropinom i obidoksimom [270].

Većina studija navodi respiratornu insuficijenciju kao vodeći uzrok smrti [150,271,272]. Ova studija je ispitivala različite parametre respiratorne funkcije kao i promjene pri tretmanu različitim antidotima. Pri nižim dozama paraoksona nađena je veza između učestalosti dispneje i smrtnog ishoda. Dispneja je sa jedne strane uzrokovana perifernom bronhostrikcijom, bronhorejom i zamorom respiratornih mišića, a sa druge strane centralnom depresijom disanja [273].

Houze i saradnici su analizirali da li je respiratorna insuficijencija kod trovanja OP posljedica centralnih ili perifernih muskarinskih efekata [274]. Pokazali su da je respiratorna insuficijencija skoro potpuno korigovana atropinom, a da uopšte nije korigovana isključivo periferno djelujućim N-metil-atropinom datom u 100 puta većoj od ekvimolarne doze atropina, te je zaključeno da je respiratorna insuficijencija posljedica centralnih muskarinskih efekata. Kada su ispitivali efekte dimetil- nasuprot dietil-paraoksona, našli su da iako i jedan i drugi uzrokuju depresiju disanja i kod trovanja dimetil-paraoksonom, nakon 2 h dolazi do spontanog oporavka respiratorne funkcije, dok je kod dietil-paraoksona respiratorna insuficijencija prolongirana [273]. Villa i saradnici su otkrili da 50% i 75% LD_{50} paraoksona izaziva promjene u ventilaciji, ali ne izaziva respiratornu insuficijenciju [275]. U ovom eksperimentu kod životinja tretiranih atropinom nakon primjene paraoksona, atropin je odlagao i smanjivao intenzitet dispneje, iako su to bile

značajno veće doze paraoksona. S druge strane, oksimi su samo blago ublažili dispneju, što dodatno potvrđuje centralno porijeklo respiratorne insuficijencije.

Kada je objektivno analizirana dubina i frekvencija disanja, uočene su značajne razlike između grupa pacova tretiranih različitim antidotima. Životinje kojima je ordiniran FR su u prosjeku živjeli samo ~45 min nakon aplikacije paraoksona. Nekoliko min nakon aplikacije paraoksona pacovi su pokazivali znakove značajne dispneje, bez perioda normalnog disanja.

Pacovi tretirani atropinom su preživljavali posmatrani period od 3 sata. Kada je pacovima pri istoj dozi paraoksona ordinirana ekvimolarna doza antimuskarinskog lijeka koji ne prolazi krvno-moždanu barijeru, N-butilskopolamina, pacovi su imali veoma slične respiracije i preživljavanje kao oni tretirani FR. Pacovi koji su tretirani oksimima su živjeli značajno duže u odnosu na one tretirane sa FR ili N-butilskopolaminom, ali kraće u odnosu na pacove tretirane atropinom. Pri poređenju efikasnosti dva oksima, pacovi tretirani oksimom K870 su živjeli duže i imali duže periode normalnog disanja u poređenju sa obidoksimom. Ovi rezultati potvrđuju sa jedne strane centralnu prirodu respiratorne insuficijencije i same smrti nastale depresijom disanja, a sa druge strane značaja i minimalne reaktivacije AChE u moždanom stablu, koja je bila značajno izraženija kod oksima K870.

Acido-bazni status pacova pokazao je da trovanje paraoksonom vrlo brzo dovodi do teške acidoze. Još uvijek nema jasne preporuke u vezi sa alkalizacijom plazme kao dijelom protokola liječenja trovanja OP, uprkos tome što postoje studije koje su pokazale potencijalnu korist od takvog protokola [276,277]. U kliničkoj praksi, bikarbonati se najčešće ne koriste rutinski, već za liječenje acidobaznog poremećaja [124].

Inhibicija AChE u dijafragmi je bila manjeg stepena, najniže vrijednosti od 19,3% početnih vrijednosti zabilježene su 45 min nakon trovanja paraoksonom kod neštićenih pacova. Kod pacova koji su tretirani oksimima, vrijednosti AChE su se održavale na više od 50% početnih vrijednosti, što ukazuje da se mnogo veći stepen inhibicije javlja u CNS-u nego na neuromuskularnoj sinapsi, što dalje podržava činjenicu da je respiratorna depresija centralnog, a ne perifernog porijekla [274]. To je dodatno podržano dijelom eksperimenta gdje se ispitivala neuromuskularna sinapsa metodom *n. phrenicus in situ* po Ćetkoviću i Boškoviću. Kod pacova kojima je aplikovana visoka subletalna doza paraoksona (0,25 mg/kg sc) nije došlo do promjene u kontrakcijama dijafragme.

Tek prilikom aplikacije 0,4 mg/kg sc paraoksona, već nakon 10 min dolazilo je do smanjenja intenziteta kontrakcija na < 50% od početnih vrijednosti.

S obzirom da je respiratorna insuficijencija glavni uzrok smrti kod trovanja OP, obezbjeđivanje disajnih puteva je izuzetno važno [202,268,278]. Uz antidote, osnovne mjere podrške i simptomatska terapija su ključne za preživljavanje pacijenata kao i adekvatna edukacija zdravstvenih radnika, posebno u ruralnim sredinama gde se najčešće javljaju trovanja insekticidima [88]. Pojava intermedijarnog sindroma dodatno komplikuje kliničku sliku i vrlo često dovodi do respiratorne insuficijencije koja zahtijeva reintubaciju i mehaničku ventilaciju [279,280]. Postoje eksperimentalne studije koje ukazuju da bi upotreba naloksona mogla biti korisna kod centralne respiratorne insuficijencije [281].

6.6. Kardiovaskularni simptomi i znaci trovanja inhibitorima acetilholinesteraze

Kao i respiratorni znaci trovanja OP, tako i kardiovaskularni znaci mogu biti i centralnog i perifernog tipa [282]. Dok će stimulacija muskarinskih receptora dovesti do bradikardije i hipotenzije, suprotno, stimulacija nikotinskih receptora u simpatikusnim ganglijama dovodi do tahikardije i hipertenzije. U kliničkoj praksi se rijetko očekuju nikotinski znakovi trovanja kod pacijenata sa intoksikacijom OP i često dovode ljekare u zabludu. Saadeh et al i Maksimović et al su pronašli tahikardiju kod čak 35-60% pacijenata otrovanih OP [124,283]. To znači da se tahikardija češće javlja nego bradikardija, što ukazuje da je predrasuda ne očekivati nikotinske efekte kod trovanja OP [284]. Kod trovanja AChEI mogu se javiti i hipertenzija i tahikardija kao posljedica prekomjerne stimulacije *locusa coreuleusa*. Stimulacija ovih holinergično inervisanih simpatikusnih jezgara dovodi do centralno nastale hipertenzije [285,286]. Prva istraživanja AChE inhnibitora ukazivala su na tešku bradikardiju i hipotenziju [193,287], dok je 1955. zabilježeno da sarin i fizostigmin mogu izazvati tahikardiju i hipertenziju [285,286]. Već tad je primijećeno da su tahikardija i hipertenzija centralnog porijekla s obzirom da se nisu javljale kod spinalnih pacova. Činjenica da je regulacija krvnog pritiska kod trovanja OP prvenstveno centralnog porijekla, potvrđena je i u eksperimentima Varagića i saradnika, kada je atropin koji prolazi krvno-moždanu barijeru modifikovao kardiovaskularne parametre, a metil-atropin, periferni antimuskarinski lijek, nije imao nikakvog efekta [288]. Dalji eksperimenti sa brojnim drugim AChE - i OP i karbamatima

- reprodukovali su isti fenomen. Uključenost simpatikusnog nervnog sistema je prekinuta heksametonijumom, antagonistom ganglionskih nikotinskih receptora. U ovim ranim eksperimentima je takođe pokazano da se ovaj kardiovaskularni efekat ne može pokazati kod pacova kojima je uklonjena koža, što implicira da je konstrikcija kožnih arteriola odgovorna za povećanje krvnog pritiska [285].

U ovom istraživanju ispitivani su i kardiovaskularni parametri, odnosno vrijednosti krvnog pritiska (sistolnog, dijastolnog i srednjeg arterijskog pritiska), srčane frekvence, kao i pojava specifičnih poremećaja srčanog ritma. Paraokson je nakon početne tahikardije i hipertenzije dovodio do bradikardije i hipotenzije i konačno smrtnog ishoda. Pacovi tretirani antidotima, bilo atropinom, bilo oksimima, imali su znatno manje oscilacije AP i održavali vrijednosti AP blizu bazalnih.

Srčani ritam je posmatran na EKG-u. Posmatrana je srčana frekvenca, kao i pojava aritmija u navedenom periodu. Primijećeno je da je frekvenca bila veoma slična kod pacova kojima je dat FR i N-butilskopolamin. Smjenjivali su se periodi tahikardije i bradikardije. Ono što je značajno jeste da u toku posmatranog perioda, skoro u svim vremenima je dolazilo do specifičnih poremećaja ritma – ubrzan rad srca je prvenstveno bio posljedica ventrikulskih tahikardija, a usporen rad srca različitih blokova u provođenju. S druge strane, iako je tokom posmatranog perioda kod pacova tretiranih atropinom i oksimima postojala tahikardija, radilo se o sinusnoj tahikardiji i pravilnom srčanom ritmu. Srčanom zastoju su prethodile bilo ventrikulske tahikardije ili smetnje u AV provođenju koje su dovodile do ventikulske fibrilacije, te konačno, srčanog zastoja.

U studiji sa neostigminom na izolovanom srcu, odnosno pretkomori zamorca, gdje su direktno stimulisani parasimpatikusni nervi, neostigmin je pri nižim koncentracijama potencirao stimulaciju dovodeći do bradikardije, a pri višim je blokirao stimulaciju [289]. U istom eksperimentu, reverzibilni inhibitor AChE, edrofonijum, je djelovao samo kao antimuskarinski lijek. Skor težine trovanja (eng. *Poisoning severity score, PSS*), kojim se procjenjuje težina trovanja kod pacijenata, pojavu nikotinskih znakova trovanja svrstava u minimalno srednje teško trovanje [290], što je u korelaciji sa nalazom da pri nižim dozama neostigmin dovodi do bradikardije, a pri višim do tahikardije. Fizostigmin, edrofonijum, takrin i soman su na desnu

pretkomoru atropinizovanog zamorca imali pozitivno hronotropno i inotropno dejstvo, kao posljedica direktnog nekompetitivnog dejstva na nikotinske receptore srca [291].

Kod ljudi, fizostigmin, ali ne i neostigmin, takođe izaziva hipertenzivni i tahikardni odgovor centralnog porijekla [292]. Postoje tri regiona mozga za koje se vjeruje da su odgovorni za održavanje tonusa simpatikusa i, posledično, periferne vazokonstrikcije odgovorne za kardiovaskularnu homeostazu. Ovi regioni su *locus coeruleus, Nucleus tracti solitarii* i rostralna ventrolateralna medula [288]. Noradrenergički neuroni *locus coeruleusa* dobijaju muskarinske projekcije i fizostigmin na njih djeluje ekscitatorno [293]. Uloga *Nucleus tractus solitarii* u kontroli krvnog pritiska je suprotna, fizostigmin preko njega izaziva hipotenziju i bradikardiju [294]. Čini se da se primarna holinergička aktivacija dešava u retikulospinalnim simpatoekscitatornim neuronima rostralne ventrolateralne medule [295–297]. Receptori preko kojih AChE inhibitori ispoljavaju ovaj presorski efekat su muskarinski, jer je jasno pokazano da se može spriječiti predtretmanom atropinom [298]. Ovi holinergički efekti acetilholina i AChE inhibitora nisu posredovani centralnim nikotinskim receptorima, pošto na njih ne utiče primjena mekamilamina, antagoniste nikotinskih receptora centralnog djelovanja [299].

Hipertenzivni ili hipotenzivni odgovor na primjenu AChE inhibitora zavisi od životinjske vrste, izbora AChE inhibitora, doze i načina njegove primjene i prisustva ili odsustva anestezije. Kod pacova, iv primena fizostigmina izaziva hipertenziju i tahikardiju, ali su ovi fenomeni naglašeni kod životinja anesteziranih uretanom [300]. Kod nekih životinjskih vrsta i nakon primjene većih doza AChE, umesto hipertenzije i tahikardije dolazi do progresivne hipotenzije i/ili bradikardije. Na primer, iako donepezil ispoljava blago povećanje krvnog pritiska kod svjesnih miševa, on takođe izaziva izraženu bradikardiju koja se može ukinuti i antimuskarinskim atropinom centralnog djelovanja i N-metil-atropinom, antimuskarinskim lijekom sa perifernim dejstvom [301].

6.7. Simptomi i znaci perifernog nervnog sistema kod trovanja inhibitorima acetilholinesteraze

Kao što je već pomenuto, ACh je takođe neurotransmiter PNS. Prekomjerna stimulacija nikotinskih receptora na neuromuskularnoj sinapsi dovodi do fascikulacija, toksičnog fenomena

koji je primijećen u ovoj studiji. Fascikulacije su bile najdosledniji znak težine trovanja pacova. Oni su bili intenzivniji pri većim dozama paraoksona i kod uginulih pacova tokom posmatranog perioda. To ide u prilog činjenici da se kod teških trovanja javljaju nikotinski znaci trovanja [290]. Kada je sarin upotrijebljen u terorističkom napadu u prepunom metrou u Tokiju, otrovano je preko 5.000 ljudi, a poginulo je 12 osoba [80,302,303]. Objavljeni izvještaji navode učestale nikotinske znakove trovanja kod teško otrovanih pacijenata [304,305].

Dodatni problem je nedostatak efikasnog antidota koji bi funkcionisao kao antagonist nikotinskih receptora, a bez ozbiljnih neželjenih dejstava [132,284]. Eksperimentalne studije sa antinikotinskim lijekovima su pokazale njihovu značajnu antidotsku efikasnost protiv karbamata i OP [196,306,307]. Međutim, blokatori nikotinskih receptora se rijetko koriste u kliničkoj praksi u liječenju OP trovanja, zbog ozbiljnih neželjenih efekata pri terapijskim dozama ovih lijekova, prije svega depresije respiratornih mišića [132]. Očekivano, primjena atropina nije uticala na brzinu i intenzitet fascikulacija (bile su većeg intenziteta i javljale su se ranije, jer je doza paraoksona bila veća).

Kod neštićenih pacova, intenzitet fascikulacija bio je u pozitivnoj korelaciji sa dozom paraoksona u svim posmatranim vremenima, primijećene kod skoro svih pacova. Fascikulacije su se javljale ranije kod pacova koji su uginuli i pri većim dozama paraoksona. Oksimi su ublažili intenzitet fascikulacija pri nižim dozama paraoksona, pri čemu je oksim K870 bio efikasniji od obidoksima. Atropin nije ublažio intenzitet i pojavu fascikulacija.

ACh se takođe nalazi u preganglijskim nervnim završecima simpatikusnog nervnog sistema [284,308]. Stimulacija alfa-1-adrenergičkih receptora takođe dovodi do piloerekcije [262]. Stoga, piloerekcija može poslužiti kao indirektni indikator simpatikusne stimulacije. Piloerekcija se češće javljala i bila je jačeg intenziteta kod smrtnog ishoda i pri višim dozama paraoksona kod neštićenih pacova. Alfa-1-adrenergički receptor pored piloerekcije [262], kod pacova takođe izaziva povlačenje očnih kapaka, što dovodi do egzoftalmusa. Za razliku od piloerekcije, egzoftalmus je ostao najuporniji tokom posmatranog perioda i nije bilo statističke značajnosti u intenzitetu i stopi egzoftalmusa u odnosu na dozu paraoksona i mortalitet pacova. Isti rezultati su dobijeni kada su primijenjeni antidoti.

U ovoj studiji najveća inhibicija AChE od strane paraoksona, kao i najizraženija reaktivacija paraoksonom inhibisane AChE od strane oksima pronađena je u eritrocitima. Oba

oksima su bila efikasni reaktivatori AChE inhibisane paraoksonom, a oksim K870 je bio efikasniji u kasnijim posmatranim vremenima. Očekivano je da najveća reaktivacija AChE bude u krvi, s obzirom da sami oksimi dostižu najveću koncentraciju u krvi. To pokazuje sposobnost oksima da reaktivišu AChE, te da bi se, ukoliko bi se pronašao oksim koji odlično prodire u mozak, neuporedivo poboljšala antidotska efikasnost oksima. Stopa preživljavanja zavisi od prisustva oksima u krvotoku što je prije moguće nakon intoksikacije. Oksimi su efikasniji u reaktivaciji AChE nego BuChE, pa se posljedično više OP vezuje za BuChE i druge prirodne biološke čistače OP [230]. Sa ove tačke gledišta, čini se da je farmakokinetički profil oksima K870 savršen: brza apsorpcija iz mišića sa koncentracijom u plazmi do 20 mg/mL u roku od 5 min, dostizanje vršne koncentracije od preko 34 mg/mL u roku od 20 min i poluvrijeme eliminacije od 40 min. Visok AUCtotal u plazmi (1485,78 ± 322,19 min × mg/mL) ukazuje na visok potencijal za obnavljanje aktivnosti AChE/BuChE u krvi u slučaju trovanja OP.

6.8. Značaj karboksilesteraze

U ovoj studiji CarbE u plazmi je bolje reaktivirana obidoksimom, ali u jetri oksimom K870. CarbE su esteraze koje su prisutne u različitom stepenu kod različitih vrsta. Li i saradnici su otkrili da ljudska plazma sadrži 4 esteraze: BuChE, paraoksonazu (PON1), albumin i samo tragove AChE [309]. Ljudska plazma ne sadrži CarbE, za razliku od miša, pacova, zeca, konja, mačke i tigra. Miševi takođe imaju, za razliku od ljudi, značajnu količinu rastvorljive AChE u svojoj plazmi [309]. Najveću aktivnost enzima ima miš, zatim pacov, zatim zamorac [310].

Fu et al i Williams et al su takođe pokazali da plazma pacova ima visoku ekspresiju CarbE dok kod ljudi i pasa nema CarbE. Pored razlika u ekspresiji, postoje razlike u vrstama u pogledu CarbE aktivnosti. Generalno, pokazalo se da CarbE 2 ima mnogo veći afinitet za lipofilne supstrate u poređenju sa CarbE 1; u međuvremenu, CarbE 1 preferira jedinjenje koja sadrže male, a CarbE 2 jedinjenje koja sadrže veće alkoholne grupe. Te karakteristike CarbE 1 mogle bi imati važne implikacije za prolijekove lipofilnih estra, kao što je C2E5 [311,312]. Prisustvo CarbE utiče na toksičnost OP [179]. Naime, kada je CarbE inhibisana, toksičnost OP se povećava. Tri-orto-krezil-fosfat (TOCP) i njegov metabolit 2-(orto-krezil)-4H-1,2,3-benzodioksafosforan-2-on (CBDP) mogu da inhibišu CarbE [313]. Kada je CarbE inhibisana, toksičnost OP se višestruko povećava

(Jokanović, 1989). Stoga, toksičnost OP kod pacova ne treba posmatrati samo preko inhibicije AChE, već i CarbE.

Tatekani i saradnici su pokazali da je CarbE najzastupljenija hidrolaza u jetri i tankom crijevu ljudi, majmuna, pasa, zečeva i pacova. Jetra sadrži i CarbE 1 i CarbE 2 enzime kod svih ovih vrsta. Tanko crijevo sadrži samo enzime iz porodice CarbE 2 kod ljudi i pacova, dok su kod zečeva i majmuna prisutna oba enzima. Zanimljivo je da u tankom crijevu psa uopšte nije pronađena aktivnost CarbE. Ljudska jetra je pokazale R-preferencijalnu hidrolizu, a jetre pacova S-preferencijalnu hidrolizu, uprkos ne-enantioselektivnoj hidrolizi u tankim crijevima kako ljudi, tako i pacova [314].

U studiji o uticaju ishrane na intestinalnu CarbE, aktivnost se povećala kod pacova koji su unosili hranu sa visokim sadržajem masti, a smanjila kod onih koji su postili ili su imali bezmasnu ishranu, dok aktivnost CarbE kod pacova tretiranih fenobarbitonom nije pokazala značajnu promjenu. CarbE iz ljudskog crijeva je okarakterisana i upoređena sa esterazom pacova, miševa, zečeva, zamoraca i pasa. Postojala je razlika u specifičnosti supstrata esteraze i bilo je značajnih vrsta razlika u elektroforetskom ponašanju enzima među ispitivanim vrstama. Studija je pokazala da se CarbE u crijevima razlikuje od onih u jetri, kao i CarbE kod eksperimentalnih životinja (pacova, miševa, zečeva, zamoraca i pasa) [315].

Studija koja je analizirala zdravo stanovništvo Srbije utvrdila je da su normalne vrijednosti AChE i BuChE u krvi bile 8.090,6 ± 1.976,7 IU/L i 14.556,6 ± 4.078,1 U/L [316]. U većini studija, postoji jasna korelacija između stepena inhibicije AChE i BuChE i težine trovanja [268]. Iako su vrijednosti aktivnosti holinesteraze u snažnoj korelaciji sa kliničkom slikom, težinom i ishodom trovanja, očigledno postoje i drugi mehanizmi kojima OP i karbamatna jedinjenja ispoljavaju svoju toksičnost. Sa kliničkog aspekta, stepen i trajanje inhibicije AChE i BuChE mnogo je važnije za dužinu liječenja i nadzora pacijenta, a ne za pouzdano predviđanje ishoda trovanja [317]. Inhibicija BuChE značajno varira među različitim OPI. Vrijednosti BuChE su značajne samo kada je poznat OP kojim je pacijent otrovan. Takođe, s obzirom na velike varijacije u bazalnim vrijednostima AChE i BuChE, jasno je da je značaj vrijednosti istih u kliničkoj praksi umanjen ako se ne znaju njihove bazalne vrijednosti kod pacijenta.

6.9. Toksičnost organofosfatnih jedinjenja nezavisna od inhibicije acetilholinesteraze

Iako je glavni fokus i drugih i ovog istraživanja toksičnost OP koja zavise od inhibicije AChE, važno je napomenuti da sam višak ACh (bez konvulzija) ili drugi mehanizmi koji su nezavisni od inhibicije AChE mogu doprinijeti toksičnosti i neurotoksičnosti OP. U zavisnosti od brojnih faktora, kao što su tip OP, stepen inhibicije AChE ili čak individualna varijabilnost u stopi nove sinteze AChE, vraćanje normalnih nivoa aktivnosti AChE nakon akutne izloženosti OP može potrajati nedjeljama ili mjesecima kod ljudi (potrebne su 1 do 2 nedjelje da se ekspozicija AChE kod pacova trovanih somanom oporavi, što odgovara skoro jednoj ljudskoj godini) [318]. Zamislivo je, dakle, da bi stalno povišeni ACh iznad normalnih nivoa mogao direktno da naškodi neuronima povećanjem intracelularnog Ca^{2+} kroz aktivaciju nikotinskih i muskarinskih receptora, narušavajući ravnotežu drugih neurotransmiterskih sistema i druge moguće mehanizme. Produžena stimulacija nikotinskih receptora povišenim koncentracijama ACh, dovodi do nishodne regulacije receptora, što je potencijalni mehanizam nastanka mišićne slabosti, prvenstveno respiratornih mišića koja se javlja u intermedijarnom sindromu [103]. Međutim, povišeni ACh takođe mogu imati korisne efekte, kao što su antiinflamatorni efekti koje ispoljava preko aktivacije nikotinskih receptora koji sadrže a7 podjedinicu na makrofagima [319–321].

Što se tiče neurotoksičnih efekata OP koji su nezavisni od inhibicije AChE, uvid se može steći iz studija niske doze, dugotrajne izloženosti, koje ne uključuju konvulzije. Kao i kod akutnog izlaganja OP, oksidativni stres i inflamacija igraju važnu ulogu u oštećenju neurona uzrokovanim hroničnom izloženošću OP [322–324]; oksidativni stres u hroničnoj izloženosti može bar djelimično da zavisi od inhibicije AChE, pošto je kod radnika koji formulišu OP pesticide, povezan sa smanjenom aktivnošću AChE eritrocita [325]. Međutim, drugi autori nisu pronašli značajnu povezanost između neurobihejvioralnih deficita uzrokovanih profesionalnom izloženošću OP i smanjene aktivnosti holinesteraze u krvi [326]. Istraživanja hronične izloženosti malim dozama OP na životinjskim modelima takođe pokazuju dugoročne toksične efekte [321,327]. Mehanizmi koji proizvode ove efekte nisu potpuno jasni. Na primer, Phillips i saradnici su otkrili da je ponovljeno izlaganje niskim dozama pacova diizopropil fluorofosfatu (DFP) izazvalo trajno povišenje Ca^{2+} u neuronima hipokampusa, koje su bile praćene simptomima depresije i kognitivnih deficita [328]. Povećanje intracelularnog Ca^{2+} djelimično je posljedica priliva kroz NMDA

receptore, a u većoj mjeri oslobađanja Ca^{2+} iz intracelularnih skladišta [328]. Nije poznato da li je sam povišen ACh i/ili povećana bazalna glutamatergična ekscitacija doprinose ovim efektima. U ćelijskim kulturama primarnih fetalnih humanih astrocita, OP insekticidi su povećali ekspresiju inflamatornih proteina, što vjerovatno nije posljedica holinergičkih mehanizama [329]. Takođe treba napomenuti da iako svi OP dijele zajednički mehanizam toksičnosti inhibicije AChE, postoje neke razlike između njih u tome na koje makromolekule ciljaju i u kojoj mjeri; ove razlike bi mogle donekle da modifikuju kaskadu događaja nakon inhibicije AChE ili različito angažuju neholinergičke mehanizme [330].

Smrtonosne doze dimetoata izazvale su srčanu insuficijenciju kod zamoraca koja nije bila u korelaciji sa nivoom inhibicije AChE, već sa koncentracijom dimetoata u miokardu. To sugeriše da dimetoat ispoljava kardiotoksično dejstvo putem direktnog dejstva na srčani mišić [331]. Koristeći tehniku *patch-clamp* za cijelu ćeliju, utvrđeno je da N-metilkarbamatni insekticid BPMC ne samo da inhibiše AChE, već i blokira kalcijumske kanale L-tipa [332]. U modelu izolovanog srca pacova, OP insekticid fosforamidon je produžio QT-interval preko neholinergičkog mehanizma [333]. Pokazalo se da ovaj efekat napreduje do polimorfne ventrikularne tahikardije tipa *Torsades de Pointes*.

Kod zečeva trovanih višekratnim niskim dozama OP insekticida tokom tri mjeseca ultrazvukom je uočeno blago kardiotoksično dejstvo, praćeno teškim histološkim promjenama u miokardu, posebno u slučaju diazinona, čija je koncentracija u miokardiocitima bila najveća. VX je izazivao ventrikularne aritmije slične digitalisu koje se ne mogu povezati sa njegovim holinergičkim djelovanjem. Ispostavilo se da je osnovni mehanizam ovog fenomena inhibicija alfa1 izoforme enzima Na^+, K^+-ATPaze srca pacova [334].

Trideset pet dana nakon izlaganja jednoj injekciji sarina ili somana, 50% pacova koji su imali posljedice u mozgu takođe je imalo značajno oštećenje miokarda, od akutne miolize i nekroze. Čini se da su konvulzivne doze sarina ili somana praćene kardiovaskularnim oštećenjima [335].

Diazinon izaziva izraženu hipotenziju kod pacova koji ne reaguje na primjenu atropina. *In vitro,* diazinon smanjuje kontraktilne odgovore na KCl i fenilefrin i slabi relaksantni odgovor na ACh. Pošto su krocin i vitamin E ublažavali vaskularne efekte diazinona, ali nisu uspjeli da utiču na inhibiciju AChE, pretpostavljena je hipoteza da je vaskularna toksičnost diazinona u aorti

pacova posredovana oksidativnim stresom [336]. U posebnom eksperimentu pokazano je da su aorte, dobijene od pacova tretiranih 90 dana dihlorvosom ili hlorpirifosom, smanjenog odnosa stres-naprezanje, u poređenju sa aortom kontrolnih životinja. Zaključeno je da ova dva OP insekticida smanjuju snagu aorte [337].

7. ZAKLJUČCI

Na osnovu rezultata dobijenih u ovom istraživanju mogu se izvesti sledeći zaključci:

- Oksim K870 bolje od obidoksima smanjuje smrtnost kod trovanja paraoksonom kod pacova. U kombinaciji sa atropinom štiti od 125 LD_{50}, statistički začajno više u poređenju sa atropinom i obidoksimom.

- Oksim K870 značajno reaktiviše i AChE i CarbE poslije trovanja visokom subletalnom dozom paraoksona kod pacova. Oksim K870 reaktviše značajno i AChE u mozgu, bolje od obidoksima.

- Oksim K870 značajno popravlja neuromuskularnu transmisiju poslije trovanja apsolutnom letalnom dozom paraoksona kod pacova.

- Oksim K870 značajno pobošljava kardiorespiratorne parametre poslije trovanja visokom subletalnom dozom paraoksona kod pacova.

Uzimajući sve u obzir može se zaključiti da oksim K870 ima veći antidotski potencijal od obidoksima.

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LISTA SKRAĆENICA

ACh: acetiholin

AChE: acetlholinesteraza

ALT: alanin aminotransferaza

AST: aspartat aminotransferaza,

BE: bazni višak (eng. *base excess*)

CarbE: karboksilesteraza

CBDP: 2-(orto-krezil)-4H-1,2,3-benzodioksafosforan-2-on

CBT: Kognitivno-bihejvioralna terapija (eng. *cognitive behavioural therapy*)

CK: kreatin.kinaza

CK-MB: kreatin-kinaza izoenzim MB

CNS: centralni nervni sistem

DAP: dijastolni arterijski pritisak;

EKG: elektrokardiogram;

EPA: Agencija za zaštitu životne sredine (eng. *Environment Protection Agency*)

FR: fiziološki rastvor, 0,9% rastvor NaCL (eng. *saline*)

GABA: gama-aminobuterna kiselina

GGT: γ-glutamil transferaza

HDL: holesterol velike gustine (eng. *high density lipoprotein*)

HI-6: asoksim hlorid

hsTnI: visoko senzitivni troponin I;

Im: intramuskularno

Iv: ntravenski

Ip: intraperitonealno

K2-EDTA: K2-etilendiamintetrasirćetna

LDH: laktat dehidrogenaza

LDL: holesterol male gustine (eng. *low density lipoprotein*)

LD_{50}: srednja smrtna doza (eng. *median lethal dose*)

MAP: srednji arterijski pritisak (eng. *mean arterial pressure*)

MCV: prosječni volumen eritrocita (eng. *mean corpuscular volume*)

MCH: prosječna količina hemoglobina u eritrocitu (eng. *mean corpuscular haemoglobin*)

MCHC: prosječna koncentraciju hemoglobina na litar eritrocita (eng. *mean cell haemoglobin concentration*

MPV: prosječni volumen trombocita (eng. *mean platelet volume*),

NMDA: N-metil-D-aspartat

OP: organofosfatna jedinjenja

OPIDN: organofosfatima indukovana odložena neuropatija (eng. *organophosphate-induced delayed neuropathy*)

pCO_2: parcijalni pritisak ugljen-dioksida

pO_2: parcijalni pritisak kiseonika

PAM-2: pralidoksim

PNS: periferni nervni sistem

PTSP: posttraumatski stresni poremećaj

SAP: sistolni arterijski pritisak

SZO: Svjetska zdravstvena organizacija

TOCP: Tri-orto-krezil-fosfat

BIOGRAFIJA

Žana M. Maksimović rođena je 14.6.1985. u Sarajevu. Osnovnu školu je završila u Brčkom kao učenik generacije. Srednju medicinsku školu – fizioterapeutski tehničar završila je u Biljeljini sa odličnim uspjehom. Medicinski fakultet, Univerziteta u Banjoj Luci završila je 2011. godine sa prosječnom ocjenom 8,13.

Specijalizaciju iz urgentne medicine završila je u decembru 2021. godine. Studijski program III ciklusa Biomedicinskih nauka na Medicinskom fakultetu Univerziteta u Banjoj Luci upisala je 2016. godine. te je tokom studija imala prosječnu ocjenu 9,60.

Od 2011. godine zaposlena u Domu zdravlja Modriča. Od 2021. stručni saradnik na katedri za Farmakologiju, toksikologiju i kliničku farmakologiju, Medicinskog fakulteta Univerziteta u Banjoj Luci. Od 2024. zaposlena u Centru za biomedicinska istraživanja, Medicinskog fakulteta Univerziteta u Banjoj Luci. Mlađi je urednik časopisa *Scripta Medica*.

Član Srpskog lekarskog društva, Udruženja doktora urgentne medicine Republike Srpske i Udruženja toksikologa Republike Srpske. Autor je i koautor velikog broja naučnih i stručnih radova objavljenih u međunarodnim i domaćim časopisima te aktivni učesnik međunarodnih i domaćih naučnih i stručnih skupova.

Slobodna.

ADDENDUM

Изјава 1

Прилог 3.

ИЗЈАВА О АУТОРСТВУ

Изјављујем
да је докторска дисертација

Наслов рада Антидотски потенцијал новосинтетисаног оксима К870 у поређењу са обидоскимом код тровања параоксоном у пацова

Наслов рада на енглеском језику Antidotal potential of newly sintesised oxime K870 in comparison with obidoxime in paraoxon poisoning in rats

- ☒ резултат сопственог истраживачког рада,
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У Бањој Луци, дана 22.04.2025. године

Потпис докторанта
Жана Максимовић
Жана Максимовић

Изјава 2

Изјава којом се овлашћује Универзитет у Бањој Луци
да докторску дисертацију учини јавно доступном

Овлашћујем Универзитет у Бањој Луци да моју докторску дисертацију под насловом Антидотски потенцијал новосинтетисаног оксима К870 у поређењу са обидоскимом код тровања параоксоном у пацова

која је моје ауторско дјело, учини јавно доступном.

Докторску дисертацију са свим прилозима предао/ла сам у електронском формату погодном за трајно архивирање.

Моју докторску дисертацију похрањену у дигитални репозиторијум Универзитета у Бањој Луци могу да користе сви који поштују одредбе садржане у одабраном типу лиценце Креативне заједнице (*Creative Commons*) за коју сам се одлучио/ла.

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(Молимо да заокружите само једну од шест понуђених лиценци, кратак опис лиценци дат је на полеђини листа).

У Бањој Луци, дана 22.04.2025. године

Потпис докторанта
Жана Максимовић
Жана Максимовић

Изјава 3

Изјава о идентичности штампане и електронске верзије докторске дисертације

Име и презиме аутора	Жана Максимовић
Наслов рада	Антидотски потенцијал новосинтетисаног оксима К870 у поређењу са обидоскимом код тровања параоксоном у пацова
Ментор	Проф. др Милош П. Стојиљковић

Изјављујем да је штампана верзија моје докторске дисертације идентична електронској верзији коју сам предао/ла за дигитални репозиторијум Универзитета у Бањој Луци.

У Бањој Луци, дана 22.4.2025. године

Потпис докторанта
Жана Максимовић

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ORIGINAL ARTICLE

Preparing a Rat Brain Tissue Samples for Acetylcholinesterase Activity Measurement - the MM method

Sonja Marinković,[1] Đorđe Đukanović,[1] Nebojša Mandić-Kovačević,[2] Tanja Cvjetković,[3] Snežana Uletilović,[3] Žana M Maksimović[1]

(1) Centre for Biomedical Research, Faculty of Medicine, University of Banja Luka, Banja Luka, the Republic of Srpska, Bosnia and Herzegovina.
(2) Department of Pharmacy, Faculty of Medicine, University of Banja Luka, Banja Luka, the Republic of Srpska, Bosnia and Herzegovina.
(3) Faculty of Medicine, University of Banja Luka, Banja Luka, the Republic of Srpska, Bosnia and Herzegovina.

Abstract

Background/Aim: Organophosphorus compounds (OP) bind to acetylcholinesterase (AChE) causing an irreversible inhibition of the enzyme. When doing *in vivo* studies of OP intoxication, to precisely measure AChE activity in the brain tissue it is necessary to remove as much blood from the brain as possible. By doing so, interference of the OPs present in the blood is avoided. Usually this demands expensive equipment, therefore, the aim of this study was to find a simple and economical method to eliminate the blood from brain blood vessels.
Methods: Wistar albino rats were divided into four groups named Control (C), Control washout (CW), Paraoxon (Pox) and Paraoxon washout (PoxW) group. Rats in Pox and PoxW were treated with 0.25 mg/kg paraoxon subcutaneously (sc), while C and CW received 1 mL/kg sc saline instead. The "Marinković-Maksimović" ("MM") method was performed in rats from PoxW and CW groups. Activity of AChE was measured both in erythrocyte lysate and in brain tissue using spectrophotometry.
Results: Macroscopic examination revealed that the elimination of blood was achieved in CW and PoxW groups. Activity of AChE in homogenised brain tissue was expectedly lower in the Pox and PoxW group, when compared to C and CW group, respectively. The CW group had a lower value of AChE activity in the brain tissue compared to C group, while activity of AChE in the PoxW group was statistically higher than in the Pox group ($p = 0.044$).
Conclusion: The MM method provides good elimination of blood from the brain. Together with blood, present confounding factors that interfere with analysis in homogenised brain tissue, were also eliminated.

Key words: Organophosphorus compounds; Oximes; Acetylcholinesterase; Enzyme activity; Rat brain.

Correspondence:
SONJA MARINKOVIĆ
E: sonja.trbojevic@med.unibl.org

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Introduction

Organophosphorus compounds (OP) are synthetic compounds first synthesised in the 1930s, that are vastly used as pesticides, but also developed as warfare nerve agents.[1] OP poisoning causes about 3,000,000 acute intoxications annually, 300,000 of which lead to fatalities.[2] They bind to the serine group of acetylcholinesterase (AChE), form stable covalent bonds, making the enzyme inactive.[3] The OP-AChE bond does not spontaneously dissociate, which is why OPs are called irreversible AChE inhibitors. Inactivated AChE cannot perform its function - the breakdown of acetylcholine (ACh) in the synaptic cleft. Accumulated ACh stimulates muscarinic and nicotinic

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postsynaptic receptors causing acute cholinergic effects.[4] OPs are mostly lipophilic substances that penetrate the blood-brain barrier (BBB) easily.[5] More lipophilic OPs are vastly distributed in fat tissue, from which they are gradually released to re-inhibit AChE. This leads to a prolonged inhibition of AChE and slower elimination of OPs.[6]

OP-AChE bonds can be reactivated by oximes. As such, they are an important therapeutic agent in the therapy of OP intoxication.[5] Unlike the OPs, oximes generally have a low degree of penetration through the BBB, because of which they have little to no ability to reactivate brain AChE.[7] Knowing that central respiratory depression is a main cause of death in OP intoxication,[8, 9] when researching a new oxime, it is very important to determine the degree of its penetration through the BBB. Finding an oxime that is more penetrable, would mean a greater therapeutic success.

In animal models of OP intoxication, to determine the level of AChE inhibition in the central nervous system, a brain tissue is homogenised and the homogenate is analysed for AChE activity. To get accurate test results, it is necessary to remove as much blood from the brain tissue sample as possible. Otherwise, test results would be altered because of the OPs present in blood.[10] When searching the literature on this matter, a method of circulatory perfusion using an isotonic buffer solution and a Masterflex pump (Cole-Parmer, Chicago, IL, USA) was found.[11] As such equipment was not available to us at short notice, the aim of this study was to find a simple and affordable method for circulatory perfusion of Wistar albino rat brain.

Methods

Experimental animals

Wistar albino rats, male and female, weighing between 260 - 350 g were used. The animals were housed 4 per cage, in air-conditioned rooms with controlled temperature (21 ± 1 °C) and a 12 h light/dark cycle. They had access to food and water *ad libitum*. For this study ethical approval was given by the Ethics Committee for the Protection and Welfare of Experimental Animals in Biomedical Research, Faculty of Medicine, University of Banja Luka (Decision No 18/1/20). During the entire experiment, the "Guiding principles in the care of and use of laboratory animals" have been observed.

Experimental protocol

To determine the efficacy of the circulatory perfusion method, rats were divided into 4 groups, 2 animals each. Groups were named Control (C), Paraoxon (Pox), Control with washout (CW) and Paraoxon with washout (PoxW). Rats in the Pox and PoxW group were treated with 0.25 mg/kg (0.25 mg/mL solution) paraoxon (Sigma Aldrich, St Louis, MO, USA) subcutaneously (sc), while C and CW groups received 1 mL/kg sc of saline instead. Half an hour later, rats were anaesthetised using 90 mg/kg ketamine and 10 mg/kg xylazine administered intraperitoneally (ip). The rat thoracic cavity was opened and blood samples were collected from the *vena cava inferior* into Lithium Heparin plasma tubes after which the animals were sacrificed via exsanguination. The "MM" method was performed in the rats from both PoxW and CW groups. Brains from both groups were excised, including *cerebrum, cerebellum* and the *truncus encephali* and stored at – 20 °C until homogenisation.

The Marinković-Maksimović method (MM method)

To access the rat heart and major blood vessels the rats were tied to operating boards 15 x 25 cm in size. A skin and muscle incision was made just below the *processus xiphoideus* using large surgical scissors. Next, bilateral incisions were made, following the medial axillary line. After separating the diaphragm from the anterior thoracic wall, the semi-attached anterior thoracic wall was clamped and put away to leave a clear operating field (Figure 1). To achieve exsanguination, *inferior vena cava* was cut just above the liver.

Prior to the cannulation of the aorta, a 10 cm long thread was pulled under the ascending aorta. Using microscissors a small diameter incision (approx. 1-2 mm) was made in the anterior wall of the ascending aorta, just above the aortic root. The tip of the cannulation tube was then inserted into the lumen of the aorta, making sure that it does not surpass the point of the first aortic arch branch (Figure 2). Using the earlier prepared tread, the tube was tied into place. The abdominal aorta was clamped, to ensure that the saline used for washout goes only to the upper half of the body. Also, to achieve a free outflow of saline

Figure 1: Access to the thoracic cavity of the rat

solution, *superior vena cava* was cut just above the heart. The upper body blood vessels were then perfused with a total of 100 mL of saline through the cannulation system, using 10 mL syringes (Figure 3). The efficiency of perfusion is observed during the procedure itself by observing the eyeballs of rats - pallor indicates the elimination of blood from the blood vessels of the eye (Figure 4).

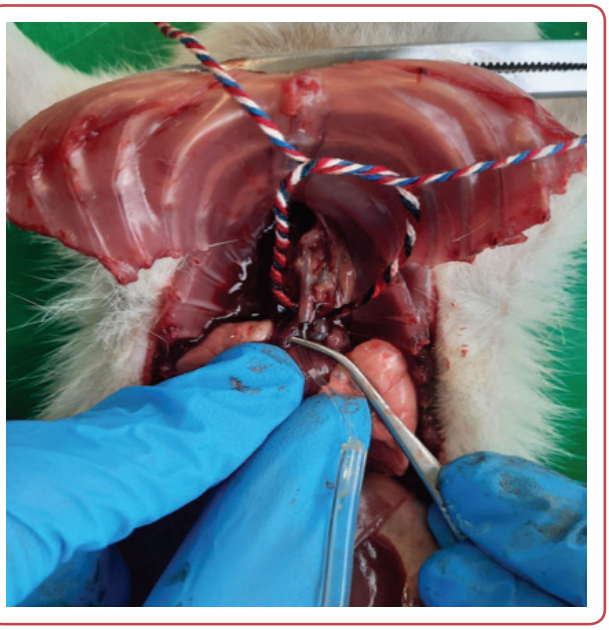

Figure 2: Cannulation of the rat aorta

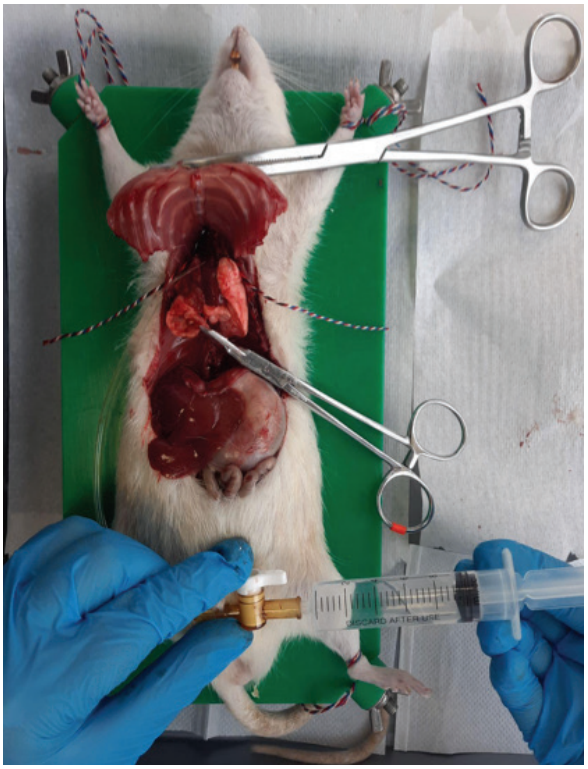

Figure 3: Washout procedure

Tissue homogenisation and measuring of the AChE activity

The right side *cerebrum, cerebellum* and *truncus encephali*, priorly frozen at - 20 °C were cut into smaller tissue fragments and weighted. Fragments were then mixed with phosphate buffer (0.1 M, pH 8.0) in a 1:20 ratio and homogenised at 10,000 rpm for 1 minute. Cold chain was main-

Figure 4: Iris pallor observed after implementation of the "MM" method

tained during the whole procedure. Homogenised tissue was centrifuged at 4 °C, for 15 minutes at 15,000 rpm and the supernatant was used as a sample for determining AChE activity in the brain tissue.

The AChE activity was measured by the Ellman colorimetric method using a Shimadzu UV-1800 spectrophotometer (Kyoto, Japan) and UV Probe 2.17 software (Kyoto, Japan).[12] Activity was determined from the brain tissue samples and from blood samples (erythrocyte lysate). Activity of AChE in erythrocyte lysate was expressed as µmol/min/mL, while its activity in the brain tissue was expressed as µmol/min/g.

Statistical analysis

Normality of data was analysed and confirmed by Kolmogorov-Smirnov test. Appropriate parametric test was performed (Student t-test). Data were shown as mean values with their standard deviations (SD). Statistical significance was set at $p < 0.05$. IBM SPSS version 23.0 for Windows software was used for data analysis.

Results

Macroscopic examination of the brain indicated that good elimination of blood from brain tissue was achieved by washout (Figure 5).

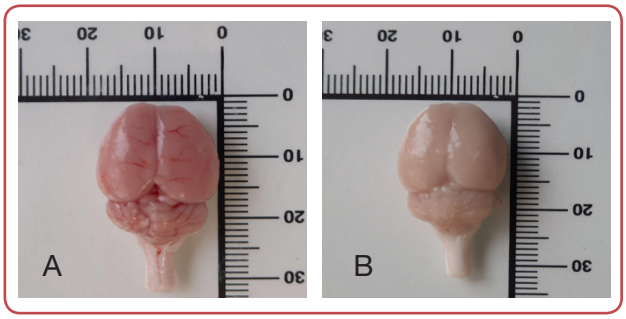

Figure 5: Macroscopic results of the "MM" method. The macroscopic difference in appearance of the rat brain from the control group (A) and from the control with washout group (B)

Detailed values of weight, brain weight and AChE activity for each experimental animal are given in Table 1.

No significant difference in mean values of rat brain mass between groups with and without washout was found (t = 2.29, p = 0.262). There was no significant difference between mean values of AChE activity in erythrocytes between C and CW group (Student t-test: t = 0.88, p = 0.472), as well as between Pox and PoxW group (t = 0.45, p = 0.708) (Figure 6).

Table 1: Acetylcholinesterase (AChE) values in rat brain and erythrocytes related to treatment

Group	Rat No	Rat mass (g)	Rat brain mass (g)	AChE activity in erythrocyte (µmol/min/mL)	AChE activity in brain tissue (µmol/min/g)
C	1	263	1.92	3.36	1.63
	2	271	1.84	2.99	1.26
CW	3	342	2.16	2.76	1.50
	4	354	2.30	3.13	1.11
Pox	5	271	2.30	2.24	0.92
	6	350	2.13	2.61	0.89
PoxW	7	307	2.02	2.24	1.05
	8	315	2.02	2.99	1.01

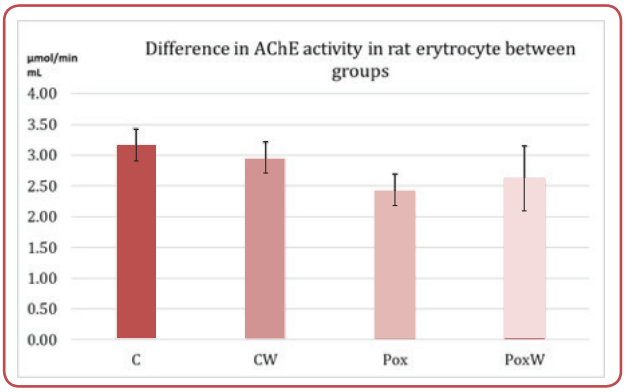

Figure 6: Acetylcholinesterase (AChE) activity in the rat erythrocyte lysate samples
C - control group; CW - control with washout group; Pox - paraoxon group; PoxW - paraoxon with washout group.
Values are presented as mean values ± standard deviations;

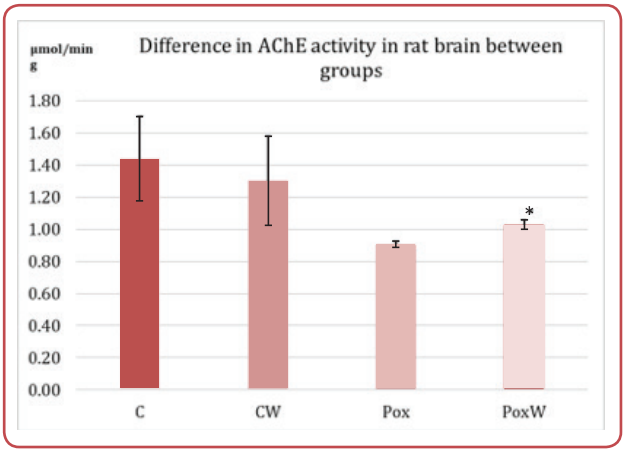

Figure 7: Acetylcholinesterase (AChE) activity in the rat brain tissue samples
C - control group; CW - control with washout group; Pox - paraoxon group; PoxW - paraoxon with washout group;
Values are presented as mean values ± standard deviations;
**There was significant difference between AChE activity in rat brain between Pox and PoxW group (Student t-test: t = 5.00, p = 0.044).*

There was no significant difference between mean values of AChE activity in rat brain between C and CW group (Student t-test: t = 0.52, p = 0.655), but there was significant difference between Pox and PoxW group (t = 5.00, p = 0.044) (Figure 7).

Discussion

When analysing the AChE activity in brains of rats poisoned with OP and treated with oximes, the presence of blood in the brain can distort the results for several reasons, primarily due to lipophilicity of OPs and poor penetration of oximes through the BBB. Following absorption, OPs accumulate rapidly in fat, liver, kidneys and salivary glands. Lipophilic OPs are released more slowly from adipose tissue and have a milder acute but prolonged effect. When the applied oxime reactivates AChE, the leakage of lipophilic OPs from the depot re-inhibits AChE. Paraoxon (diethyl (4-nitrophenyl) phosphate) is the active metabolite of the OP insecticide parathion.[13] The phosphorothioates (P=S) (eg, diazinon, parathion) are more lipophilic than phosphates (P=O) (eg, dichlorvos, paraoxon). Spontaneous reactivation of dimethyl-phosphorylated AChE often occurs rapidly even without oxime therapy. There is no such expectation for AChE inhibited with diethyl phosphoryl insecticides.[5]

How to increase therapeutic potential of oxime, by improving their BBB penetration remains a challenge in modern toxicology. As a very selectively permeable membrane, the BBB imposes several limitations for oxime penetration to the brain tissue, such as hydrophilicity, polarity and molecule size. Consequently, pyridinium aldoximes (PyAls) such as pralidoxime (2-PAM) have a very low rate of BBB penetration,[14, 15] which is mostly associated with their high hydrophilicity[10] and permanent cationic charge. 2-PAM, which is most commonly used oxime in the treatment of OP poisonings, has a relatively low level of transport through the BBB.[15-17]

Respiratory failure has been identified in numerous studies as the leading cause of death in OP poisoning.[18-20] Houze et al[21] have shown that respiratory failure is most likely the result of central respiratory depression, rather than peripheral bronchoconstriction, bronchorrhoea and respiratory muscle fatigue.[8, 9] Knowing that, a crucial point in therapy would be reactivation of AChE in brain tissue.[15] Because of that, non-quaternary organic compounds are introduced as novel antidotes in OP poisoning.[22, 23] Not having a quaternary group gives them a better penetrability through the BBB. Data from the literature indicate that the chlorine atoms in the oxime increase its lipophilicity.[24] In OP insecticides, it has been shown that oximes with a functional group at the position 4 reactivate OP insecticides most effectively. Oximes with a bis-quaternary structure are more efficient than those ones with mono-quaternary structure. It is best that linker has 3-5 C-C connections. The double bond in the linker increases both their efficiency and toxicity.[25, 26]

There are several ways to determine the efficiency of brain AChE reactivation by oximes, but most widely used is an indirect method. With this method the level of BBB of an oxime penetration is measured by the level of AChE reactivation in the brain tissue. To achieve precise results, it is necessary to remove blood from the brain. This way OPs present in the blood will not interfere with the results. Washing out blood from the brain by using the "MM" procedure, showed reduced detected AChE activity in homogenised brain tissue, which resulted in 0.15 µmol/min/g difference between control groups (C and CW). This decrease was due to the removing of the AChE present in red blood cells, whose activity could be measured in the brain homogenate in the absence of washing out. In both groups treated with paraoxon AChE activity was expectedly lower when compared with controls (37.24 % drop when comparing C and Pox group and 21.37 % difference between CW and PoxW). Comparing the results between Pox and PoxW group, AChE activity has remained higher in the PoxW group that was subjected to the "MM" procedure. Evidently the washout prevented remaining paraoxon from blood to bind with AChE and thus to distort the level of AChE activity in the brain after homogenisation.

By introducing the "MM" method, few things were tried to be avoided; one of them is that OP re-arrives from the blood into the brain to re-inhibit the AChE, thus obtaining lower values of AChE activity than the real ones. On the other hand, a new oxime that would penetrate BBB better could reactivate AChE in the brain. The presence of blood in homogenates would call into question

the origin of reactivated AChE - by eliminating the blood, the obtained values of AChE activity are those from the brain and not from the blood and brain.

Conclusion

The "MM" method provides good elimination of blood from the rat brain and thus eliminates confounding factors in the analysis of parameters from brain homogenates. The introduction of the procedure in the Centre for Biomedical Research, Faculty of Medicine, University of Banja Luka enables the universality of the procedure and the reproducibility of the results.

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Conflict of interest

The authors declare that there are no conflicts of interest. This study is partially funded by the Ministry of Scientific and Technological Development, Higher Education and Informational Society of the Government of the Republic of Srpska (Grant No 125 7030).

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ORIGINAL ARTICLE

Onset Rate and Intensity of Signs of Organophosphate Poisoning Related to Paraoxon Dose and Survival in Rats

Žana M Maksimović,[1, 2] Dajana Duka,[3] Nataša Bednarčuk,[3] Ranko Škrbić,[2, 4] Miloš P Stojiljković[2, 4]

(1) Primary Healthcare Centre, Modriča, the Republic of Srspka, Bosnia and Herzegovina.
(2) Centre for Biomedical Research, Faculty of Medicine, University of Banja Luka, Banja Luka, the Republic of Srpska, Bosnia and Herzegovina.
(3) Medical student, Faculty of Medicine, University of Banja Luka, Banja Luka, the Republic of Srspka, Bosnia and Herzegovina.
(4) Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine, University of Banja Luka, Banja Luka, the Republic of Srspka, Bosnia and Herzegovina.

Abstract

Introduction: Oganophosphorus compounds (OP) bind to acetylcholinesterase (AChE) and inactivate it. In the synaptic cleft, undestroyed and accumulated acetylcholine produce the acute cholinergic effects. The aim of this study was to determine the frequency, speed of onset and intensity of certain signs of paraoxon poisoning depending on dose and outcome of poisoning.
Methods: The study was conducted in adult Wistar rats. The median lethal dose (LD_{50}) of paraoxon as well as protective ratio (PR) of atropine (10 mg/kg intramuscularly) was determined. Clinical signs of poisoning were observed: fasciculations, tremor, seizures, ataxia, piloerection, lacrimation, exophthalmos, bizzare/stereotypic behaviour and dyspnoea. The time from paraoxon injection to the first appearance of the sign of poisoning was recorded as well as the intensity of poisoning with evaluation at 10 time intervals throughout the 4 h observational period.
Results: The LD_{50} of paraoxon was 0.33 mg/kg (subcutaneously) and PR of atropine was 2.73. Dose-dependent, piloerection occurred more often ($p = 0.009$) and at higher intensity ($p = 0.016$) at higher doses. Fasciculations, tremor, seizures and ataxia occurred significantly earlier at higher doses of paraoxon ($p = 0.015$, 0.002, 0.021 and 0.016, respectively), as well as the intensity of seizure, tremor and fasciculation. Piloerection ($p = 0.002$) and seizures occurred more frequently ($p = 0.009$) in non-survivors. Fasciculations, tremor, seizures and ataxia occurred significantly earlier and at higher intensity in non-survivors ($p < 0.001$, for all parameters), as well as dyspnoea ($p = 0.009$ and $p = 0.048$). In atropine-protected rats, nicotinic effects persevered, so they were the prognostic parameter of the severity of the poisoning.
Conclusion: Seizures and fasciculations followed by tremor were strong prognostic parameters of the probability of lethal outcome of paraoxon poisoning. Also, the mentioned poisoning signs were with their intensity and speed of occurrence in a clear positive correlation with the administered dose of paraoxon. Even at high doses of paraoxon, atropine blocked the muscarinic (but not nicotinic) effects and somewhat mitigated the CNS toxic effects.

Keywords: Organophosphate; Acetylcholinesterase inhibitor; Paraoxon; Insecticide; Poisoning; Atropine.

Correspondence:
ŽANA MAKSIMOVIĆ
E: maksimoviczana85@gmail.com
M: +387 65 970 593

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Introduction

In the body, organophosphosphorus compound (OPC) binds to the serine group of the enzyme acetylcholinesterase (AChE, EC 3.1.1.7), phosphorylates it and creates a stable covalent bond

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with it.[1] By binding to it, AChE becomes inactive and cannot perform its function, which is the breakdown of acetylcholine in the synaptic cleft.[2] Accumulated acetylcholine (ACh) stimulates various postsynaptic cholinergic receptors and exhibits its acute cholinergic effects: muscarinic (bronchorrhoea, bronchoconstriction, bradycardia, hypotension, miosis, hypersalivation, lacrimation, nausea, vomiting, increased motility of the bowels and of the bladder), nicotinic (mydriasis, tachycardia, hypertension, fibrillation, fasciculation and necrosis of skeletal muscles) and central (tremor, convulsions, motor incoordination, respiratory depression and coma).[3] Besides acute cholinergic crisis, OP poisoning can manifest itself as intermediate syndrome, organophosphate-induced delayed neuropathy (OPIDN) and chronic organophosphate-induced neuropsychiatric disorder due to long-term low-level exposure.[4]

The OP-AChE bond does not spontaneously dissociate (hydrolyses) in the body, which is why OPs are called irreversible AChE inhibitors. The bond can be separated using AChE reactivators, so-called oximes, which are causal antidotes in OP poisonings.[5] Unfortunately, the diversity of OPs makes oximes insufficiently universal and effective antidotes.[6] An additional limitation of its efficacy is the process of dealkylation (so-called "ageing") through which the OP-AChE bond passes, after which it is not possible to separate OP and AChE.[7] Ageing in some OPCs like the nerve agent soman is measured in minutes, while in organophosphorus insecticide (OPI) it is mostly measured in days.[8]

Although oximes are the only causal antidotes, their insufficient efficacy, especially in clinical practice, makes anticholinergic drugs still the main antidotes in OP poisoning. The most commonly used and most available anticholinergic drug is atropine, which blocks the muscarinic effects of OP poisoning, but not the nicotinic ones.[9] The contribution to the protective effect of atropine is its lipophilicity, ie the ability to penetrate the CNS. There is an assumption whether more lipophilic anticholinergics would have better protection against OP poisoning than atropine. However, there are no clinical studies to corroborate this notion.

In addition to atropine and oximes (pralidoxime and obidoxime), anticonvulsants, primarily diazepam and midazolam, are also used in the treatment of OP poisoning. Anticonvulsants are important, especially when the fact that the fatal outcome in OP poisoning most often occurs due to seizures and respiratory failure is taken into account.[10, 11]

Although most developed countries have banned the use of the most toxic pesticides, OPI poisonings, especially the intentional ones, are still common, especially in undeveloped and developing countries.[12, 13] Unfortunately, due to the high toxicity of OPCs, they continue to be abused for terrorist purposes and as nerve agents (tabun, sarin, soman and VX).[14] Paraoxon (diethyl (4-nitrophenyl) phosphate) is the active metabolite of the OPI parathion.[15] Due to its toxicity and chemical characteristics, it serves as a good experimental tool in the field of research of toxicity of nerve agents.

The aim of the study was to determine whether increasing doses of paraoxon affect the rate and intensity of signs of poisoning with an AChE inhibitor. The aim was also to determine whether the speed of onset and intensity of the appearance of certain signs of poisoning can be a prognostic sign of the fatal outcome of poisoning. In addition, the goal was to determine how clinical signs are manifested at high (> LD_{100}) doses of paraoxon in the presence of an antidote (atropine).

Methods

Animals

The study was conducted in adult male and female Wistar rats, weighing 200-240 g, purchased from Faculty of Natural Sciences and Mathematics, University of Banja Luka, the Republic of Srpska. The animals had access to food and water *ad libitum*. The room temperature was maintained at 20-22 ^{0}C, with a 12 h light and dark cycle. The study was approved by Ethics Committee for the Protection and Welfare of Experimental Animals in Biomedical Research, Faculty of Medicine, University of Banja Luka (Decision No 18/1/20). During the entire experiment, the "Guiding principles in the care of and use of laboratory animals" have been observed.

Chemicals

Paraoxon was purchased from Sigma Aldrich, St Louis, MO, USA. Application volume of the chemical was 1 mL/kg. Paraoxon was injected subcutaneously (sc) into the abdominal region, while atro-

pine was administered intramuscularly (im) into the right thigh. Stock solution of paraoxon (100 mg/mL) was dissolved in isopropyl alcohol. Final dilution for sc administration was made from saline (0.9 % NaCl) before injection. Atropine sulphate monohydrate was dissolved in saline up to the concentration of 10 mg/mL

Study design

In these experiments, lethality of paraoxon and an antidote potential of atropine was based on the 24 h survival.

a) Paraoxon and saline

Increasing doses of paraoxon were administered sc. Each group consisted of 6 rats and doses were determined by the "up and down" method (doses were: 0.2, 0.3, 0.35, 0.4 mg/kg). One minute after paraoxon injection, rats were injected with 1 mL/kg saline im. Based on the 24 h lethality, median lethal dose (LD_{50}) was calculated.

b) Paraoxon and atropine

Increasing doses of paraoxon were administered sc (doses were: 0.6, 0.9, 1.2 mg/kg). Each group consisted of 6 rats and doses were determined by the "up and down" method. One minute after the injection of paraoxon, the rats were injected with 10 mg/kg of atropine im. The outcome was protective ratio (PR) ie, ratio of LD_{50} in protected and in unprotected rats.

c) Clinical signs of poisoning

The following clinical signs of poisoning were observed: fasciculations, tremor, seizures, ataxia, piloerection, lacrimation, exophthalmos, bizzare/stereotypic behaviour, dyspnoea. The time from paraoxon injection to the appearance of the sign of poisoning was recorded. The intensity of poisoning was recorded as: 0 - absent, 1 - mild / moderate, 2 - severe. Evaluation was performed in the 5th, 10th, 15th, 30th, 60th, 90th, 120th, 150th, 180th, 210th, and 240th minute. To analyse the intensity of symptoms throughout the observed period (4 h), ToxScore was introduced, as a parameter that summed the intensity of each sign in all time intervals. TotToxScore was a measure that represented the sum of all ToxScore for one animal.

Statistics

The LD_{50} values were computed by means of the Pharm/PCS statistical software, according to the Litchfield and Wilcoxon (1949).[16] IBM SPSS 21.0 software was used for other statistical procedures. After the normality of data distribution was analysed (Kolmogorov–Smirnov test), the appropriate parametric / nonparametric test was used (Student t-test / Man-Whitney U test, One Way ANOVA / Kruskal-Wallis test) as well as Chi-Squared test for categorical data.

Results

Paraoxon

a) Dose

The LD_{50} of paraoxon was 0.33 mg/kg sc (95 % CI: 0.31 - 0.36). In all rats, death occurred in the first hour of poisoning (mean ± SD: 19.19 ± 6.19, 95 % CI: 16.37 - 22.01).

Fasciculations occurred in 85.71 % of all rats, piloerection in 50.00 %, exophthalmos in 95.23 %, lacrimation in 54.76 %, tremor in 97.62 %, seizures in 83.33 %, ataxia in 66.67 %, stereotypic behaviour in 69.05 % and dyspnoea in 26.19 % rats.

The frequency of symptoms at different doses of paraoxon was not significant, except in the case of piloerection, where it occurred after doses of paraoxon 0.2, 0.3, 0.35 and 0.4 mg/kg in 0 %, 33.33 %, 66.67 % and 75.00 %, respectively (χ^2 = 11,667, p = 0.009). The onset rate of poisoning clinical signs at different doses of paraoxon is shown in Table 1. Bizzare-stereotypic behaviour, although frequent (69.05 % of all animals), was not significantly different in either the intensity or the rate of sign on any parameter (data not shown).

Fasciculation, tremor, seizures, and ataxia occurred significantly earlier at higher doses of paraoxon. The intensity of signs measured 15 min and 4 h after paraoxon application relative to the dose applied is shown in Table 2. The time-point of 15th minute was taken as the time period when most of the signs of poisoning were manifested, yet most of the rats were still alive, and the time period of 4th hour was taken as the end of the observation period.

In the presented time intervals (15 min and 4 h after paraoxon), the intensity of seizures, tremor and fasciculations increased significantly with increasing doses. Piloerection was significantly

Table 1: Onset rate of clinical signs of poisoning at different doses of paraoxon (minutes after application of paraoxon)

Sign (ToO)	Paraoxon dose (mg/kg sc) 0.2	0.3	0.35	0.4	p value
Fasciculations	35.67 ± 17.95	34.25 ± 22.17	20.83 ± 1.84	12.70 ± 5.08	0.015*
Tremor	24.60 ± 7.92	19.33 ± 14.18	19.67 ± 31.29	7.67 ± 1.83	0.002*
Seizures	35.25 ± 20.81	24.78 ± 25.67	18.10 ± 18.73	10.42 ± 3.23	0.021*
Ataxia	47.60 ± 28.02	41.43 ± 51.82	33.89 ± 63.30	9.43 ± 3.10	0.016*
Piloerection	-	6.50 ± 3.32	7.75 ± 6.23	7.00 ± 2.83	0.972*
Exophthalmos	7.00 ± 3.46	5.82 ± 3.63	6.67 ± 3.75	3.92 ± 2.07	0.120*
Lacrimation	28.60 ± 24.34	39.50 ± 27.5	19.67 ± 9.58	17.00 ± 9.74	0.412*
Dyspnoea	29.50 ± 4.95	22.75 ± 12.53	27.33 ± 24.85	15.00 ± 7.07	0.795**

ToO – time of occurrence (minutes after application of paraoxon)
** Kruskal-Wallis test, **One-Way ANOVA, Red colour – statistically significant*
Values: mean ± standard deviation

Table 2: Intensity of clinical signs of poisoning 15 min and 4 h after administration of increasing doses of paraoxon

Sign (Intensity)	Paraoxon dose (mg/kg sc) 0.2	0.3	0.35	0.4	p value*
Fasciculations					
15th min	0	0.45 ± 0.82	0.58 ± 0.67	1.11 ± 0.93	0.052
240th min	0.33 ± 0.52	0.56 ± 0.52	0.56 ± 0.73	2.00 ± 0.00	0.061
Tremor					
15th min	0.33 ± 0.52	0.82 ± 0.87	1.50 ± 0.67	1.78 ± 0.44	0.002
240th min	0.17 ± 0.41	0.56 ± 0.53	1.00 ± 0.00	1.00 ± 0.00	0.044
Seizures					
15th min	0	0.91 ± 0.94	0.92 ± 0.79	1.67 ± 0.50	0.004
240th min	0	0.11 ± 0.33	0.40 ± 0.55	1.00 ± 0.00	0.069
Ataxia					
15th min	0	0.45 ± 0.69	0.33 ± 0.49	0.44 ± 0.52	0.329
240th min	0.33 ± 0.52	0.44 ± 0.88	1.00 ± 0.71	1.00 ± 0.00	0.271
Piloerection					
15th min	0	0.27 ± 0.47	0.83 ± 0.72	0.67 ± 0.50	0.016
240th min	0	0	0	0	-
Exophthalmos					
15th min	1.00 ± 0.63	1.09 ± 0.53	1.50 ± 0.52	1.67 ± 0.50	0.055
240th min	1.17 ± 0.98	1.22 ± 0.67	1.40 ± 0.55	2.00 ± 0.00	0.721

** Kruskal-Wallis test, Red colour – statistically significant. Values: mean ± standard deviation (Intensity: 0 - absent, 1 - mild/moderate, 2 - severe)*

higher at higher doses at the 15th min, however, it disappeared in all rats 45 min after poisoning.

To analyse the intensity of symptoms throughout the observed period (4 h), ToxScore was introduced, as a sum of the intensity of each sign in all time intervals. A significant difference was found in ToxScore values relative to dose regarding fasciculations and seizures (Figure 1). Other parameters were not significant.

TotToxScore is a measure that represents the sum of all ToxScores for one animal. TotToxScore values are shown in Figure 2. Although the difference was not statistically significant, an increase in the value of TotToxScore is noticeable, as a joint parameter of the intensity and duration of signs of

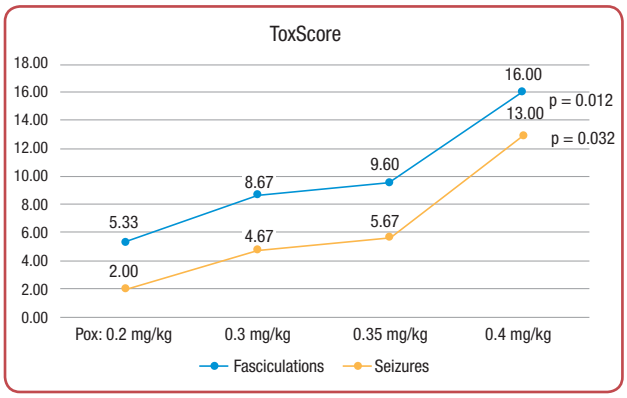

Figure 1: ToxScore for fasciculations and seizures relative to dose of paraoxon (Pox)
** Kruskal-Wallis test; ToxScore: the sum of the values of each sign in each point of time*

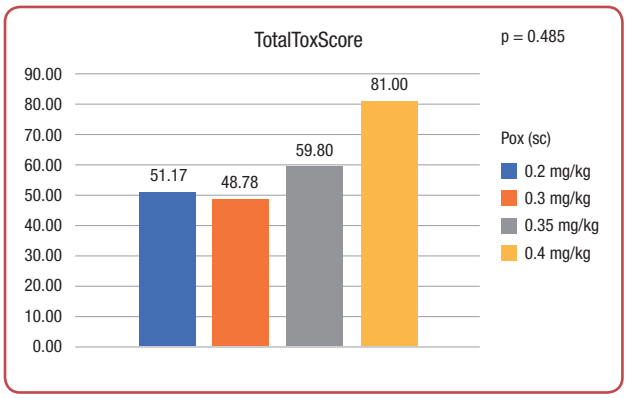

Figure 2: Mean TotToxScore relative to dose of paraoxon (Pox)
**TotToxScore: the sum of the values of all sign in each point of time; Kruskal-Wallis test*

poisoning, especially at high doses.

b) Survival

Analysing the frequency of clinical signs in relation to whether the rat survived or not, a significant difference was found regarding the piloerection: 23.81 % of survivors and 76.19 % of non-survivors had piloerection (χ^2 = 11,524, p = 0.002). The same goes for seizure frequency (66.67 % in survivors vs 100.00 % in non-survivors have had seizures, χ^2 = 8,400, p = 0.009). The speed of onset of clinical signs of poisoning relative to whether or not the animals survived is shown in Table 3. All signs of poisoning occurred earlier in non-survivors compared to survivors.

The intensity of the signs at 15 min after paraoxon application in relation to whether the rat survived

Table 3: Onset rate of clinical signs of paraoxon poisoning in relation to whether rats survived or not

Sign (ToO)	Survived		
	Yes	No	p-value
Fasciculations	35.44 ± 16.94	12.61 ± 4.25	<0.001*
Tremor	26.40 ± 24.02	7.38 ± 1.91	<0.001*
Seizures	33.14 ± 23.74	9.81 ± 3.12	<0.001*
Ataxia	52.33 ± 55.77	8.77 ± 2.05	<0.001*
Piloerection	8.40 ± 5.64	6.81 ± 3.97	0.646*
Exophthalmos	6.60 ± 3.35	4.70 ± 3.16	0.025*
Lacrimation	32.33 ± 22.28	14.37 ± 6.59	0.086*
Dyspnoea	33.67 ± 11.18	12.00 ± 5.83	0.004**

ToO – time of occurrence (minutes after application of paraoxon)
**Man-Whitney U-test, ** Student t-test, Red colour – statistically significant*
Values: mean ± standard deviation

Table 4: Signs intensity at 15 min after paraoxon administration in relation to whether the rat survived or not

Sign (ToO)	Survived		
	Yes	No	p-value*
Fasciculations	0.09 ± 0.44	1.17 ± 0.73	<0.001
Tremor	0.71 ± 0.78	1.76 ± 0.44	<0.001
Seizures	0.43 ± 0.75	1.59 ± 0.51	<0.001
Ataxia	0.19 ± 0.40	0.53 ± 0.62	0.059
Piloerection	0.19 ± 0.40	0.88 ± 0.60	0.646
Exophthalmos	1.14 ± 0.48	1.59 ± 0.62	0.011
Lacrimation	0.33 ± 0.48	0.18 ± 0.39	0.416
Dyspnoea	0	0.24 ± 0.56	0.048

** Man-Whitney U-test, Red colour – statistically significant. Values: mean ± standard deviation (Intensity: 0- absent, 1 - mild/moderate, 2-severe)*

or not is shown in Table 4.
Apart from lacrimation and stereotypy, all other signs were of higher intensity in non-survivors. The most significant difference in intensity was recorded in fasciculations, tremor and seizures, where high significance was found ($p < 0.001$).

Paraoxon and atropine

a) Dose

The PR of atropine was 2.73 (LD_{50} of paraoxon when atropine was administered 1 min after was 0.91 mg/kg sc (95 % CI: 0.67 - 1.25)). In all rats, death occurred during the first hour of poisoning (mean ± SD: 14.00 ± 3.74, 95% CI: 10.87 - 17.13). Fasciculations occurred in 77.78 % of all rats, piloerection in 33.33 %, exophthalmos in 94.44 %, lacrimation in 22.22 %, tremor in 100.00 %, seizures in 94.44 %, ataxia in 44.44 %, stereotypic behaviour in 38.89 % and dyspnoea in 55.56 % of rats.

In atropine-protected rats, the dose had a significantly smaller effect on the speed and frequency of symptoms. The frequency of any poisoning signs was not affected by the dose. As for the onset of clinical signs manifestation, a significant difference was found only in fasciculations ($p = 0.038$) and tremor ($p = 0.007$) (Table 5).

The intensity of the signs persisted throughout the observation period. The intensities of certain symptoms at 15th min and after 4 h relative to the dose are shown in Table 6. The cross-section at the 15th minute was taken as the time period when most of the signs of poisoning were present, yet most of the rats were still alive, while the 4th h was taken as the end of the observation period.

Table 5: Onset rate of clinical signs of poisoning at different doses of paraoxon with atropine protection (10 mg/kg im) (one minute after application of paraoxon)

Sign (ToO)	Paraoxon dose (mg/kg sc)			
	0.6	0.9	1.2	p-value
Fasciculations	28.83 ± 30.75	7.60 ± 1.95	10.33 ± 4.73	0.038*
Tremor	12.83 ± 5.85	8.33 ± 1.03	5.00 ± 2.19	0.007*
Seizures	18.20 ± 15.21	9.50 ± 1.64	8.50 ± 4.64	0.170**
Ataxia	51.50 ± 79.20	8.00 ± 2.83	70.50 ± 85.56	0.444*
Piloerection	4.33 ± 1.15	6.00 ± 3.60	5.17 ± 2.56	0.507*
Exophthalmos	3.20 ± 1.64	4.00 ± 2.89	6.00 ± 0.89	0.134*
Lacrimation	111.00 ± 0.00	105.00 ± 140.00	6.00 ± 0.00	0.632*
Dyspnoea	63.40 ± 46.47	25.33 ± 31.77	52.50 ± 44.55	0.504**

ToO – time of occurrence (minutes after application of paraoxon)
** Kruskal-Wallis test, **One-Way ANOVA. Red colour – statistically significant*
Values: mean ± standard deviation

Table 6: Intensity of clinical signs of poisoning 15 min and 4 h after the application of paraoxon and atropine (10 mg/kg, im, one minute after) depending on the dose of paraoxon

Sign (Intensity)	Paraoxon dose (mg/kg sc)			
	0.6	0.9	1.2	p-value*
Fasciculations				
15th min	0.89 ± 0.37	0.71 ± 0.32	0.55 ± 0.24	0.613
240th min	1.00 ± 0.00	1.00 ± 0.00	2.00 ± 0.00	0.011
Tremor				
15th min	1.17 ± 0.41	2.00 ± 0.00	1.8 ± 0.45	0.015
240th min	0.67 ± 0.52	1.00 ± 0.00	1.00 ± 0.00	0.472
Seizures				
15th min	0.83 ± 0.98	2.00 ± 0.00	2.00 ± 0.00	0.017
240th min	0	0.50 ± 0.71	2.00 ± 0.00	0.022
Ataxia				
15th min	0.33 ± 0.52	0.40 ± 0.55	0.20 ± 0.45	0.797
240th min	0.33 ± 0.52	0.50 ± 0.71	2.00 ± 0.00	0.073
Dyspnoea				
15th min	0.17 ± 0.41	0.20 ± 0.45	0.40 ± 0.89	0.961
240th min	0.33 ± 0.52	0.50 ± 0.71	1.00 ± 0.00	0.301

** Kruskal-Wallis test, Red colour – statistically significant. Values: mean ± standard deviation (Intensity: 0 - absent, 1 - mild/moderate, 2 - severe)*

ToxScore, although without statistical significance, showed a clear increase in fasciculations, tremor, seizures, ataxia, and dyspnoea with increasing doses of paraoxon (Figure 3).

TotToxScore values for paraoxon poisoning followed by atropine administration are shown in Figure 4. Total Tox Score, although without statistical significance, showed a clear increase with higher dose.

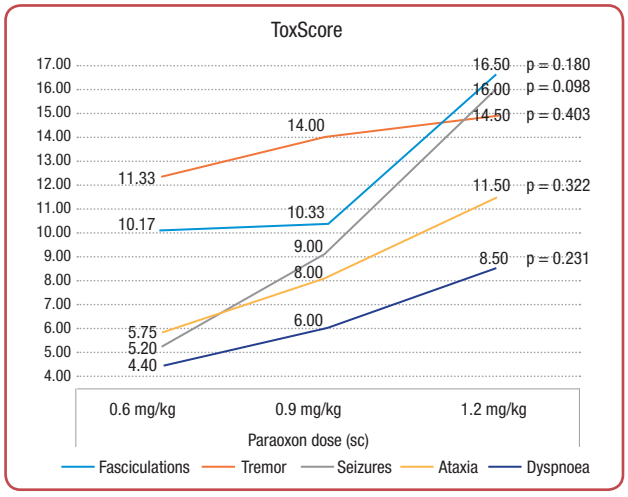

Figure 3: Dose-dependent ToxScore (paraoxon with atropine protection)

** Kruskal-Wallis test; ToxScore: the sum of the values of each sign in each point of time*

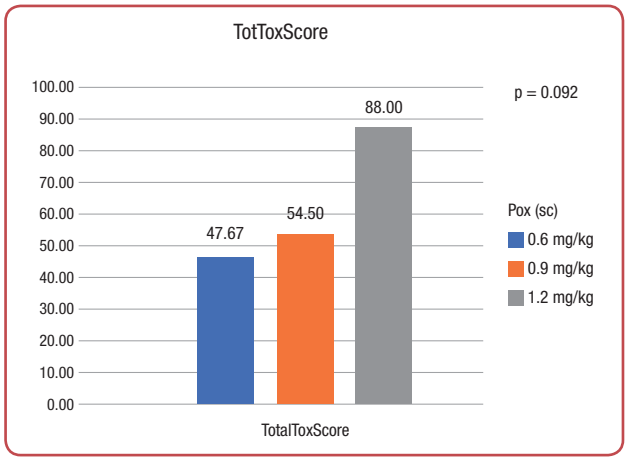

Figure 4: Mean TotToxScore relative to dose of paraoxon with atropine protection

**TotToxScore: the sum of the values of all sign in each point of time; Kruskal-Wallis test*

b) Survival

There was no significant difference in the frequency of symptoms in relation to whether the rat survived or not. It was only observed that ataxia was more common in survivors than in non-survivors (70.00 % vs 12.50 %, respectively; χ^2 = 5,591, p = 0.025) as well as fasciculations (100.00 % vs 50.00 %, respectively; χ^2 = 6,429, p = 0.023).

Table 7: Onset rate of clinical signs of paraoxon poisoning with atropine administration in relation to whether rats survived or not

Sign (ToO)	Survived		p-value
	Yes	No	
Fasciculations	21.70 ± 24.76	6.25 ± 0.96	0.004*
Tremor	10.50 ± 5.58	6.50 ± 2.50	0.083*
Seizures	14.67 ± 11.72	8.38 ± 3.42	0.117**
Ataxia	50.43 ± 69.43	10.00 ± 0.00	-
Piloerection	5.00 ± 1.63	5.50 ± 4.95	-
Exophthalmos	4.22 ± 1.86	4.75 ± 2.71	0.673*
Lacrimation	157.50 ± 65.76	6.00 ± 0.00	0.333*
Dyspnoea	60.50 ± 39.27	7.00 ± 1.41	0.098**

*ToO – time of occurrence (minutes after application of paraoxon). Red colour – statistically significant; *Man-Whitney U-test, ** Student t-test; Values: mean ± standard deviation*

Table 8: Signs intensity at 15 min after paraoxon administration with atropine protection in relation to whether the rat survived or not

Sign (ToO)	Survived		p-value*
	Yes	No	
Fasciculations	1.10 ± 0.74	0.50 ± 0.55	0.147
Tremor	1.50 ± 0.53	1.83 ± 0.41	0.313
Seizures	1.30 ± 0.95	2.00 ± 0.00	0.220
Ataxia	0.40 ± 0.52	0.17 ± 0.41	0.492
Piloerection	0.10 ± 0.32	0.17 ± 0.41	0.875
Exophthalmos	0.80 ± 0.42	1.00 ± 0.63	0.635
Dyspnoea	0.30 ± 0.67	0.17 ± 0.41	0.875

** Man-Whitney U-test; Values: mean ± standard deviation (Intensity: 0 - absent, 1 - mild/moderate, 2 - severe*

A significant difference in the onset of symptoms relative to whether or not a rat survived was observed only regarding fasciculations. The onset rate of poisoning clinical signs relative to whether or not the animals survived is shown in Table 7. Most signs of poisoning occurred earlier in non-survivors, but statistical significance was found only regarding fasciculations (p = 0.004). The intensity of any signs of poisoning did not differ significantly in survivors and non-survivors (Table 8).

Discussion

Paraoxon is one of the most toxic OPIs and is therefore banned from use as an insecticide in most countries. The obtained LD_{50} of paraoxon was 0.33 mg/kg sc, which roughly corresponds to the data from the literature.[17] Death in almost all animals occurs in the first two hours of OP

poisoning, which is consistent with the data obtained in this study.[18]

The LD_{50} of paraoxon, when 10 mg/kg of atropine is administered after 1 minute, was 0.91, so the PR of atropine was 2.73, similar to the data in the literature.[19] Atropine is used as an antidote for poisoning with AChE inhibitors. It is much more effective in carbamate poisoning than in those caused by OPCs.[19] It is logical that PR will be significantly higher in carbamates than OP poisoning given that carbamates are reversible AChE inhibitors, so the spontaneous reactivation of AChE makes the task of atropine as an antidote less demanding.[21-23] Considering the dose of atropine, 10 mg/kg im was selected because it is the most frequently used dose; therefore the results obtained in this experiments are comparable to the results of other researchers.[24] Krutak-Kol and Domino tried different doses of atropine against paraoxon poisoning: 10, 33 and 100 mg/kg and found that higher doses did not increase survival. Moreover, a dose of 100 mg/kg was significantly less effective than the dose of 10 mg/kg.[25] Repeated lower doses of atropine over time might potentially give better results.[26]

The presence, time of occurrence and intensity of paraoxon poisoning signs were examined. The same parameters of poisoning were also observed at high lethal doses of paraoxon, in animals given atropine. Those signs that can be noticed only by observation without manipulation of the animal were analysed.

Lacrimation occurs by excessive stimulation of muscarinic receptors. Although not life-threatening, it can serve as a good indicator of muscarinic excitation, as well as sign of adequate atropinisation during treatment.[3] Neither the intensity nor the rate of lacrimation occurred statistically significantly at different doses of paraoxon and in relation to the survival rate. However, when atropine was administered after paraoxon, lacrimation was significantly less common and occurred later, although significantly higher doses of paraoxon were administered (only 22 % vs 55 % when atropine was not administered). Regardless of the dose, the results obtained were expected since it is well known that atropine is an anti-muscarinic drug. The existence of lacrimation can indirectly indicate that there are other effects of muscarinic excitation (hypotension, bradycardia).

Muscarinic effects that are directly life-threatening are bronchoconstriction and bronchorrhoea. Dyspnoea was monitored as a parameter indicating respiratory failure, which in studies by other researchers proved to be the main cause of lethality in OPI poisoning.[27-29] The disadvantage of this study is that dyspnoea was only subjectively observed, without objective measuring of respiratory failure degree. Sometimes, in severe seizures dyspnoea could be noticed only when the intensity of seizures subsided. Nevertheless, a clear link was found in the intensity and speed of occurrence of dyspnoea in relation to whether the animal survived or not. In animals treated with atropine after paraoxon administration, atropine delayed and reduced the intensity of dyspnoea, although these were significantly higher doses of paraoxon. Houze et al analysed whether respiratory failure in OP poisoning was due to central or peripheral muscarinic effects.[30] He showed that respiratory failure was almost completely corrected by atropine and not corrected at all by 100 times the equimolar dose of N-methylatropine and concluded that respiratory failure is due to central muscarinic effects. On the other hand, Villa et al found that 50 % and 75 % of LD_{50} paraoxon caused alterations in ventilation, but did not cause respiratory failure.[31]

Fasciculations were a sign that, along with seizures, proved to be the most consistent parameter of the poisoning severity. Fasciculations occurred faster and were of higher intensity when the dose of paraoxon as well as survival of rats was observed. This can be explained by the fact that among the examined signs, fasciculations are those that follow overstimulation of nicotinic receptors on the neuromuscular junction. If it is assumed that according to the Poison Severity Score (PSS) in humans, any occurrence of nicotine sings of poisoning is considered at minimum as moderate poisoning, then it can be concluded that fasciculations, as a parameter of nicotine effects of poisoning, directly indicates the severity of poisoning.[32] An additional problem is lack of effective antidote that would function as a nicotine antagonist, without serious side effects.[33, 34] As expected, atropine administration did not affect the rate and intensity of fasciculations - they were of higher intensity and occurred earlier, because the dose of paraoxon was higher.

Muscle tremor was also a sign of inappropriate stimulation of nicotinic receptors on the neuro-

muscular joint, ie weakness of skeletal muscles. Tremor has been more often described in the literature as part of the delayed onset of extrapyramidal syndrome, rather than acute OP poisoning sign.[35] Since the monitoring of poisoning signs was based on observation, tremor served as a good indicator of muscle weakness. A strong relationship was found between both the speed of onset and the intensity of tremor, and the dose of paraoxon or the lethal outcome in rats. As expected, the results did not change when atropine was given after paraoxon, but the tremor occurred earlier and was of stronger intensity at higher doses and in non-survivors.

ACh is also found in the preganglionic fibres of the sympathetic nervous system. Piloerection is a sign that occurs as a consequence of excessive stimulation of preganglionic nicotinic receptors of the sympathetic nervous system.[36] Therefore, piloerection can be considered as potential predictor of sympathetic stimulation. The results of this study showed a strong relationship between the dose of paraoxon and the frequency of piloerection. Studies from other researchers show that the incidence of tachycardia in OP poisoning is 35-60 %, making it more common than bradycardia.[37] The alpha-1-adrenergic receptor, in addition to piloerection, in rats is also involved in retraction of the eyelids, leading to exophthalmos.[38] Unlike piloerection, exophthalmos remained most persistent throughout the observed period and had no statistical significance in the intensity and rate of exophthalmos in relation to paraoxon dose and rat mortality. The same results were obtained when atropine was administered after high doses of paraoxon.

Seizures initially occur due to stimulation of cholinergic receptors in the CNS and at this stage can be reversed by atropine. Prolonged seizure activity is due to excessive release of glutamate, when atropine is not effective.[39] For now, seizures are treated with combinations of anticholinergics and benzodiazepines but, given the role of glutamate in seizures, future therapies should include N-methyl-D-aspartate receptor (NMDA) antagonists.[40] The rate and intensity of seizures were clearly correlated to dose and especially to death in paraoxon poisoning. Seizures occur significantly less frequently in OPI than in nerve agents.[41] In this study, seizures occurred in 83 % of rats and 100 % of non-survivors, which additionally contribute to the characteristics of paraoxon as a nerve agents. Administration of atropine somewhat postponed and decreased the intensity of seizures. There was still a clear relationship between these parameters and paraoxon dose as well as fatal outcome, but seizures occurred later and were less intense than would be expected, given the paraoxon doses administered.

Ataxia is caused by stimulation of muscarinic receptors in the CNS. In addition, in acute OP poisoning, ataxia occurs as a consequence of muscle fatigue, but ataxia is more intense than would be expected from muscle fatigue alone.[42] The results of this study show that ataxia occurred earlier at higher doses of paraoxon and in non-surviving rats.

ToxScore and TotToxScore are parameters that were introduced in order to see the persistence of symptoms related to the dose. Thus, the values of each sign in each point of time were summed, which made up the ToxScore, and the values of all ToxScores were added the calculate the TotToxScore for each animal. The pre-condition is that the animal survived the observation period. These parameters can also be used in future studies, when the protective effect of antidotes (either individually or in combination) can be compared, not only as a measure of survival, but also their ability to alleviate the signs of poisoning. ToxScore showed that the same parameters (seizure, fasciculation, tremor) that were significant indicators of the severity of poisoning lasted longer at higher doses. The TotToxScore had higher value at higher dose and, although the difference was not significant, showed a clear trend.

Conclusion

Seizures and fasciculations followed by tremor were strong prognostic parameters of the probability of lethal outcome of paraoxon poisoning. Also, the mentioned signs of poisoning were with their intensity and speed of occurrence in a clear correlation with the dose of paraoxon. Even at high doses of paraoxon, atropine blocked the muscarinic (but not nicotinic) effects, and somewhat mitigated the CNS effects.

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Conflict of interest

None.

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Dose-Dependency of Toxic Signs and Outcomes of Paraoxon Poisoning in Rats

Žana M. Maksimović[1,*], Ranko Škrbić[1,2], Miloš P. Stojiljković[1,2]

ABSTRACT

Organophosphorus compounds induce irreversible inhibition of acetylcholinesterase, which then produces clinically manifested muscarinic, nicotinic and central effects. The aim of the study was to analyse the clinical signs of acute paraoxon poisoning in rats and to determine the relationship between the intensity of signs of poisoning and the dose of paraoxon and/or the outcome of poisoning in rats. Animals were treated with either saline or atropine (10 mg/kg intramuscularly). The median subcutaneous lethal dose (LD_{50}) of paraoxon was 0.33 mg/kg and protective ratio of atropine was 2.73. The presence and intensity of signs of poisoning in rats (dyspnoea, lacrimation, exophthalmos, fasciculations, tremor, ataxia, seizures, piloerection, stereotypic movements) were observed and recorded for 4 h after the injection of paraoxon. Intensity of these toxic phenomena was evaluated as: 0 – absent, 1 – mild/moderate, 2 – severe. Fasciculations, seizures and tremor were more intense at higher doses of paraoxon and in non-survivors. In unprotected rats piloerection occurred more often and was more intense at higher doses of paraoxon as well as in non-survivors. In atropine-protected rats, piloerection did not correlate with paraoxon dose or outcome of poisoning. The intensity of fasciculations and seizures were very strong prognostic parameters of the poisoning severity.

KEYWORDS

organophosphate; insecticide; paraoxon; poisoning; acetylcholinesterase inhibitor; atropine

AUTHOR AFFILIATIONS

[1] Centre for Biomedical Research, Faculty of Medicine, University of Banja Luka, Banja Luka, the Republic of Srpska, Bosnia and Herzegovina
[2] Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine, University of Banja Luka, Banja Luka, the Republic of Srpska, Bosnia and Herzegovina
* Corresponding author: Centre for Biomedical Research, Faculty of Medicine, University of Banja Luka, Banja Luka, the Republic of Srpska, Bosnia and Herzegovina; e-mail: zana.maksimovic@med.unibl.org

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INTRODUCTION

Acetylcholinesterase (AChE, EC 3.1.1.7) is a very potent enzyme whose role is to break down acetylcholine (ACh) in the synaptic cleft (1, 2). Inhibition of AChE results in the accumulation of ACh and excessive stimulation of cholinergic receptors. AChE inhibitors (AChEI) can be reversible (carbamate compounds) and irreversible (organophosphorus compounds – OPCs) (3–5). OPCs form a stable covalent bond with AChE, which is not spontaneously hydrolysed (6). They are divided into two major groups, nerve agents (tabun, sarin, soman, VX) (7) and organophosphorus insecticides (OPI). Paraoxon (diethyl (4-nitrophenyl) phosphate) is an active metabolite of the highly toxic OPI parathion (8). Among the OPIs, paraoxon is very similar to nerve agents, in terms of its median lethal dose (LD_{50}), profile of inhibition of cholinesterases and general toxicity (9).

Acute OPC poisoning manifests itself with muscarinic effects (bronchoconstriction, bronchorroea, bradycardia, hypotension, nausea, vomiting, increased motility of bowels and bladder, miosis, hypersalivation, lacrimation), nicotinic effects (tachycardia, hypertension, fibrillation, fasciculations, skeletal muscle necrosis, mydriasis) and CNS effects (tremor, convulsions, coma, respiratory depression) (10). Intermediate syndrome can occur after 1–4 days and, 1–2 weeks later, organophosphate-induced delayed neuropathy (OPIDN) can be seen.

Treatment of OPC poisoning is based on a triple regimen: symptomatic anticholinergic therapy (atropine), AChE reactivators (oximes) and anticonvulsants (mainly diazepam) (11). Atropine as an antimuscarinic drug, alleviates the muscarinic effects of OPC poisoning, but has no impact on the nicotinic ones. Oximes bind to OPC already bound to AChE, which leads to the reactivation of AChE, with variable affinity for different OPCs between oximes. Diazepam inhibits the excitability of the neurons in the CNS; by increasing the effect of GABA, it increases cAMP, decreases the level of cGMP, leading to the cessation of convulsions (11).

The aim of the study was to analyse the clinical signs of acute paraoxon poisoning in rats and to determine whether there is a relationship between the intensity of toxicity signs and the dose of paraoxon and/or outcome of poisoning.

MATERIAL AND METHODS

ANIMALS

The study was conducted in adult Wistar rats weighing 200–240 g, purchased from the Faculty of Natural Sciences and Mathematics, University of Banja Luka, the Republic of Srpska. The animals were given water and food *ad libitum*, kept at a temperature of 20–22 °C, with a 12 h cycle of light and darkness. The study was approved by the Ethics Committee for the Protection and Welfare of Experimental Animals in Biomedical Research, Faculty of Medicine, University of Banja Luka (Decision No 18/1/20). The animals were treated in accordance with the Guide for the Care and Use of Laboratory Animals (12). The study was conducted at the Centre for Biomedical Research, Faculty of Medicine, University of Banja Luka.

CHEMICALS

Paraoxon was purchased from Sigma Aldrich, St Louis, MO, USA. Paraoxon was dissolved in isopropyl alcohol up to a concentration of 100 mg/mL and final dilution to the desired concentration was made with saline (0.9% NaCl). Atropine sulphate was dissolved in saline to a concentration of 10 mg/mL. The volumes of administered paraoxon and atropine were 1 mL/kg. Paraoxon and atropine were administered subcutaneously (sc) and intramuscularly (im), respectively. Final dilutions were made immediately before application.

STUDY DESIGN

The LD_{50} of paraoxon was determined by the "up and down" method according to Litchfield and Wilcoxon (1949) (13). In the first part of the experiment, then following doses of paraoxon were administered: 0.2, 0.3, 0.35, 0.4 mg/kg sc. One minute after paraoxon the saline (1 mL/kg, im) was administered. In the second part of the experiment, the following doses of paraoxon were administered: 0.6, 0.9 and 1.2 mg/kg sc. Atropine 10 mg/kg im was injected 1 minute after paraoxon application.

The presence and intensity of signs of paraoxon poisoning in animals were observed and recorded for 4 h. The following signs have been observed: dyspnoea, lacrimation, exophthalmos, fasciculations, tremor, ataxia, seizures, piloerection, stereotypic movements. Their presence and intensity were noted at the minutes: 5, 10, 15, 30, 60, 90, 120, 150, 180, 210 and 240 after paraoxon application. Intensity was evaluated as: 0 – absent, 1 – mild/moderate, 2 – severe. Signs of poisoning were observed in relation to the dose of paraoxon, as well as the outcome of the poisoning (survival or death).

Tab. 1 Time of death from paraoxon administration depending on paraoxon dose.

POX dose (mg/kg sc)		Time of death (minute)	
		Mean ± SD	95% CI
With saline	0.2	–	–
	0.3	16.67 ± 7.51	-1.98–35.31
	0.35	22.00 ± 3.61	18.67–25.33
	0.4	18.09 ± 6.99	13.39–22.78
	All	19.19 ± 6.19	16.37–22.01
With atropine	0.6	–	–
	0.9	14.00 ± 3.83	7.91–20.09
	1.2	14.00 ± 4.24	7.25–20.75
	All	14.00 ± 3.74	10.87–17.13
Total		**17.76 ± 6.04**	**15.46–20.06**

SD: standard deviation; CI: confidence interval; POX: paraoxon; Administered volumes of paraoxon, atropine and saline were 1 mL/kg; Atropine at a dose of 10 mg/kg im was given 1 minute after paraoxon application.

STATISTICS

The LD_{50} and the PR were analysed by the method of Litchfield and Wilcoxon (1949) on Pharm/PCS statistical software. Other analyses were performed on IBM SPPS for Windows, Version 18.0. After the Kolmogorov-Smirnov test showed an unequal distribution of data, appropriate nonparametric tests were applied: Chi-square test (or Fisher exact test) and Kruskal-Wallis test. Statistical significance level was set at $p < 0.05$.

RESULTS

The LD_{50} of paraoxon was 0.33 mg/kg sc (95% CI: 0.31–0.36). The LD_{50} of paraoxon when atropine was administered was 0.91 mg/kg sc (95% CI: 0.67–1.25). Therefore, the PR of atropine was 2.73. All deaths occurred during the first hour of poisoning (Table 1).

CLINICAL SIGNS OF POISONING

1. Fasciculations

Frequency of fasciculations was not correlated with the dose of paraoxon (Table 2).

In atropine-protected rats, fasciculations occurred more often in non-survivors ($p = 0.023$) (Table 3).

Fasciculations occurred earlier and were more intense at higher doses of paraoxon (Figure 1). Although the intensity of fasciculations were related to the dose of paraoxon throughout the observed period, the difference was significant in the minutes 10, 15, 30, 210 and 240 (Kruskal-Wallis test, $p = 0.035$, $p = 0.045$, $p = 0.038$, $p = 0.014$ and $p = 0.034$, respectively).

Due to atropine protection, higher doses of paraoxon could be administered. The intensity of fasciculations depending on paraoxon dose when atropine was administered is shown in Figure 2. Atropine did not influence the

Tab. 2 Frequency of signs of poisoning related to paraoxon dose.

Sign	Paraoxon + Saline						Paraoxon + Atropine				
	Paraoxon (mg/kg, sc)				Total	p*	Paraoxon (mg/kg, sc)			Total	p*
	0.2	0.3	0.35	0.4			0.6	0.9	1.2		
Fasciculations	100.00	66.67	100.00	83.33	85.71	0.080	100.00	83.33	50.00	77.78	0.250
Seizures	66.67	75.00	83.33	100.00	83.33	0.225	83.33	100.00	100.00	94.44	1.000
Tremor	83.33	100.00	100.00	100.00	97.61	0.143	100.00	100.00	100.00	100.00	1.000
Piloerection	0.00	33.33	66.67	75.00	50.00	**0.009**	50.00	50.00	0.00	33.33	0.149
Exophthalmos	83.33	91.67	100.00	100.00	95.23	0.498	83.33	100.00	100.00	94.44	1.000
Lacrimation	83.33	50.00	50.00	50.00	54.76	0.501	16.67	33.33	16.67	22.22	1.000
Ataxia	83.33	58.33	75.00	58.33	66.67	0.691	66.67	33.33	33.33	44.44	0.589
Stereotypy	100.00	66.67	83.33	41.67	69.05	**0.044**	66.67	0.00	50.00	38.89	0.095
Dyspnoea	33.33	33.33	25.00	16.67	26.19	0.862	50.00	50.00	33.33	44.44	1.000

* Chi-squared test (Fisher exact), **bold**: statistical significance. Values in the table are in percentages; sc: subcutaneously; im: intramuscularly; Administered volumes of paraoxon, atropine and saline were 1 mL/kg; Atropine at a dose of 10 mg/kg im was given 1 minute after paraoxon application.

Tab. 3 Frequency of signs of poisoning related to poisoning outcome.

Sign	Paraoxon + Saline				Paraoxon + Atropine			
	Rat survived		Total	p*	Rat survived		Total	p*
	Yes	No			Yes	No		
Fasciculations	85.71	85.71	85.71	1.000	50.00	100.00	77.78	**0.023**
Seizures	66.67	100.00	83.33	**0.009**	90.00	100.00	94.44	1.000
Tremor	95.23	100.00	97.61	1.000	100.00	100.00	100.00	1.000
Piloerection	23.81	76.19	50.00	**0.002**	40.00	25.00	33.33	0.638
Exophthalmos	95.23	95.23	95.23	1.000	90.00	100.00	94.44	1.000
Lacrimation	71.43	38.10	54.76	0.062	20.00	25.00	22.22	1.000
Ataxia	71.43	61.90	66.67	0.744	70.00	12.50	44.44	**0.025**
Stereotypy	85.71	52.23	69.05	**0.043**	60.00	12.50	38.89	0.066
Dyspnoea	28.57	23.81	26.19	1.000	60.00	25.00	44.44	0.239

* Chi-squared test (Fisher exact), **bold**: statistical significance; Values in the table are in percentages; im: intramuscularly; Administered volumes of paraoxon, atropine and saline were 1 mL/kg; Atropine at a dose of 10 mg/kg im was given 1 minute after paraoxon application.

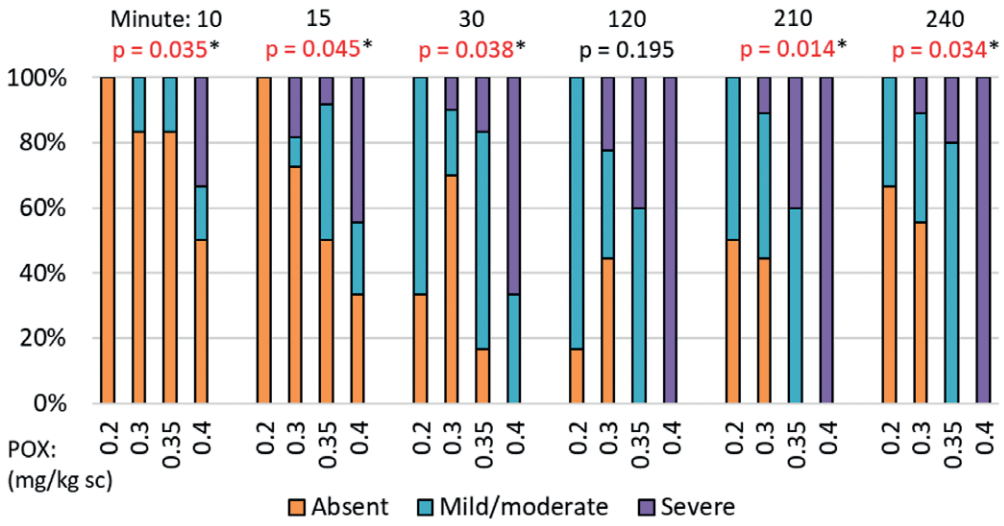

Fig. 1 Intensity of fasciculations in rats depending on paraoxon dose.
* Kruskal-Wallis test, red colour: statistical significance; POX: paraoxon; sc: subcutaneously; Minute: minutes 10, 15, 30, 120, 210 and 240 after paraoxon application.

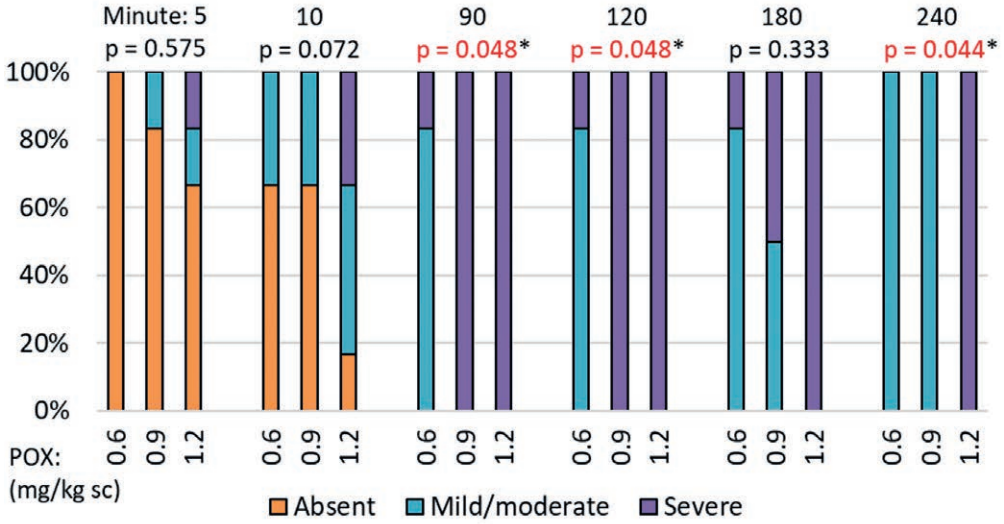

Fig. 2 Intensity of fasciculations depending on paraoxon dose in rats protected with atropine.
* Kruskal-Wallis test, red colour: statistical significance; POX: paraoxon; sc: subcutaneously; Minute: minutes 5, 10, 90, 120, 180 and 240 after paraoxon application; Atropine at a dose of 10 mg/kg im was given 1 minute after paraoxon application.

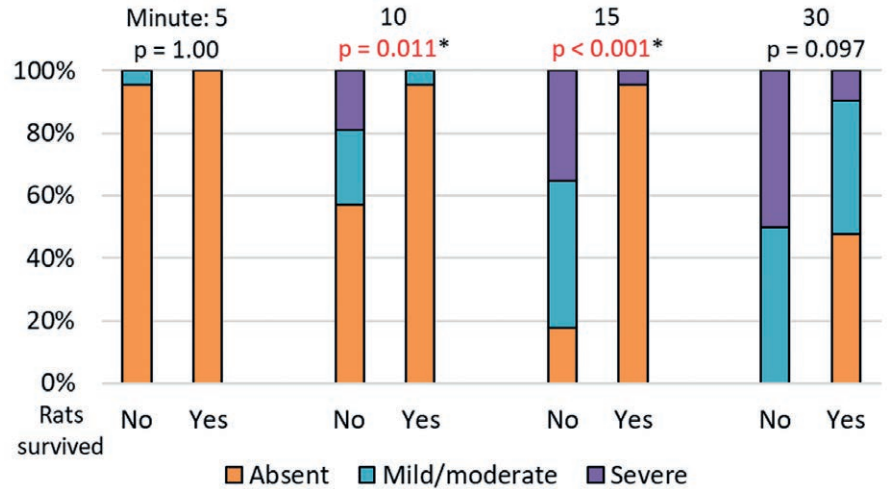

Fig. 3 Intensity of fasciculations in relation to whether rat treated with paraoxon survived or not.
* Fisher exact test, red colour: statistical significance; Minute: minutes 5, 10, 15 and 30 after paraoxon application.

intensity of fasciculations. Fasciculations occurred earlier and were more intense at higher doses of paraoxon. Although the intensity of fasciculations was related to the dose of paraoxon throughout the observed period, the difference was significant in the minute 60 (data not shown), 90, 120 and 240 (Kruskal-Wallis test, p = 0.033, p = 0.048, p = 0.048 and p = 0.044, respectively).

In unprotected rats intensity of fasciculations was in correlation with the outcome of poisoning (higher intensity was in non-survivors) (Figure 3). In atropine-protected rats, fasciculation intensity did not correlate with the outcome of poisoning.

2. Seizures

Frequency of seizures was not correlated with the dose of paraoxon (Table 2), but seizures were more often in non-survivors compared to survivors (Table 3). Seizures occurred earlier and were more intense at higher doses of paraoxon (Figure 4). Although the intensities of seizures were related to the dose of paraoxon throughout the observed period, the difference was significant only at minutes 15, 180 and 210 (Kruskal-Wallis test, p = 0.002, p = 0.024 and p = 0.015, respectively).

A clear relation between paraoxon dose and seizure intensity can be seen in atropine-protected rats (Figure 5). Although the intensity of seizures was related to the dose of paraoxon throughout the observed period, the difference was significant only at minutes 10, 15, 210 and 240 (Kruskal-Wallis test, p = 0.031, p = 0.014, p = 0.044 and p = 0.011, respectively).

In unprotected rats the intensity of seizures was in correlation with the outcome of poisoning (higher intensity was in non-survivors) (Figure 6). In atropine-protected rats, the intensity of seizures did not correlate with the outcome of poisoning.

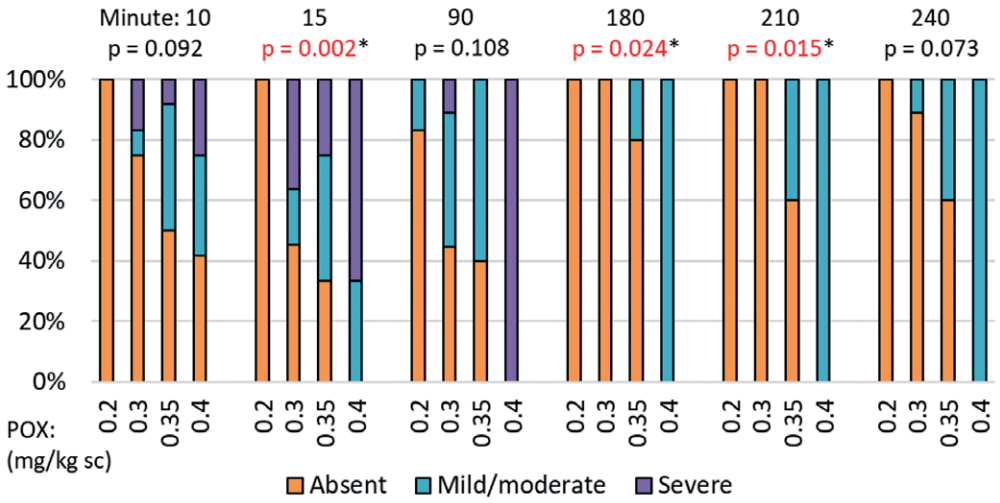

Fig. 4 Intensity of seizures in rats depending on paraoxon dose.
* Kruskal-Wallis test, red colour: statistical significance; POX: paraoxon; sc: subcutaneously; Minute: minutes 10, 15, 90, 180, 210 and 240 after paraoxon application.

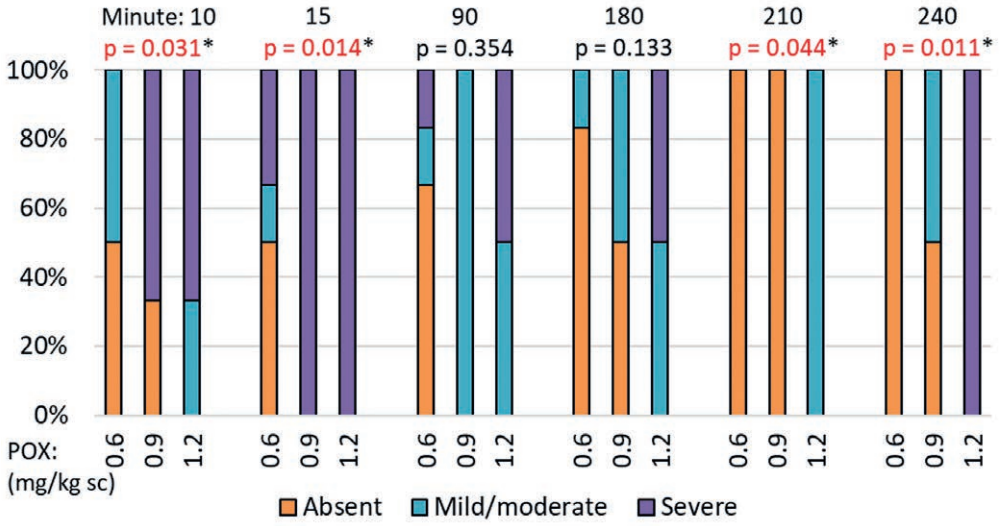

Fig. 5 Intensity of seizures depending on paraoxon dose in rats protected by atropine.
* Kruskal-Wallis test, red colour: statistical significance; POX: paraoxon; sc: subcutaneously; Minute: minutes 10, 15, 90, 180, 210 and 240 after paraoxon application; Atropine at a dose of 10 mg/kg im was given 1 minute after paraoxon application.

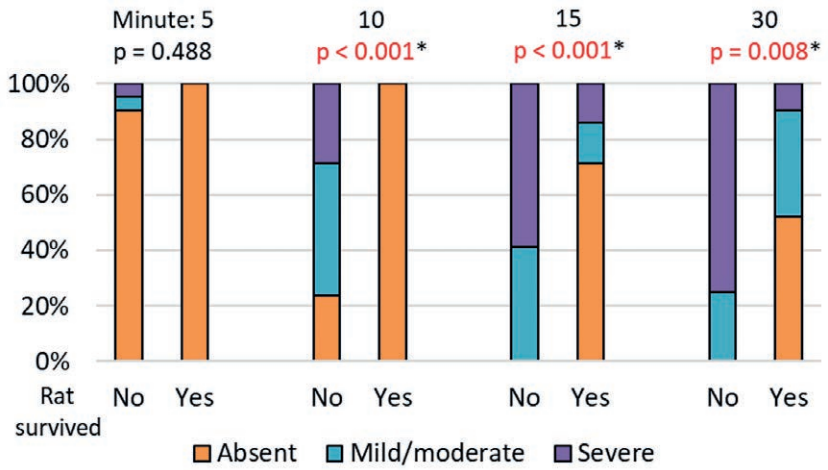

Fig. 6 Intensity of seizures in relation to whether rat treated with paraoxon survived or not.
* Fisher exact test, red colour: statistical significance; Minute: minutes 5, 10, 15 and 30 after paraoxon application.

3. Tremor

Frequency of tremor was not correlated with the paraoxon dose (Table 2) or the outcome of poisoning (Table 3). Tremor occurred earlier and was more intense at higher doses of paraoxon (Figure 7). Although the intensity of tremor was related to the dose of paraoxon throughout the observed period, the difference was significant at minutes 10, 15 and 240 (Kruskal-Wallis test, $p = 0.001$, $p = 0.002$ and $p = 0.044$, respectively).

In the atropine-protected rats, although a higher intensity of tremor was observed at higher doses of paraoxon, the difference was not significant, except at minute 10 ($\chi^2 = 9.88$, $p = 0.007$) and 30 ($\chi^2 = 6.00$, $p = 0.050$).

In unprotected rats intensity of tremor was in correlation with the outcome of poisoning (higher intensity was in non-survivors) (Figure 8). In atropine-protected rats, the intensity of tremor did not correlate with the outcome of poisoning.

4. Piloerection

Piloerection as a clinical sign of poisoning occurred early (within the first half hour of poisoning) and lasted for a short time (Figure 9). Piloerection occurred more often (Table 2) and was more intense at higher doses of paraoxon administered to unprotected rats. The difference was significant at minutes 10 and 15 (Kruskal-Wallis test, $p = 0.052$ and $p = 0.012$, respectively).

In atropine-protected rats, piloerection did not correlate with paraoxon dose.

In unprotected rats intensity of piloerection was in correlation with the outcome of poisoning. Piloerection was

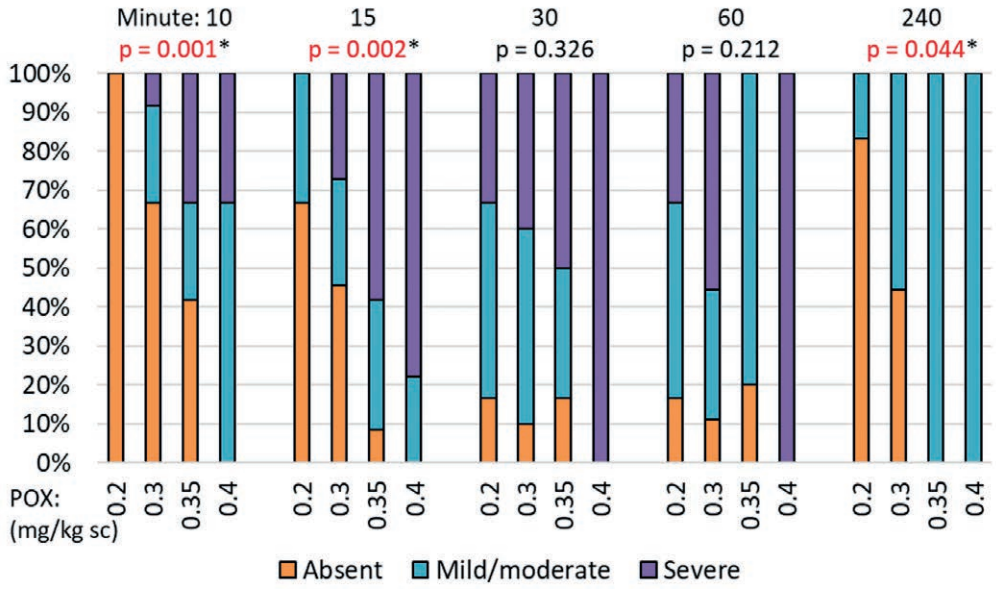

Fig. 7 Intensity of tremor in rats depending on paraoxon dose.
* Kruskal-Wallis test, red colour: statistical significance; POX: paraoxon; sc: subcutaneously; Minute: minutes 10, 15, 30, 60 and 240 after paraoxon application.

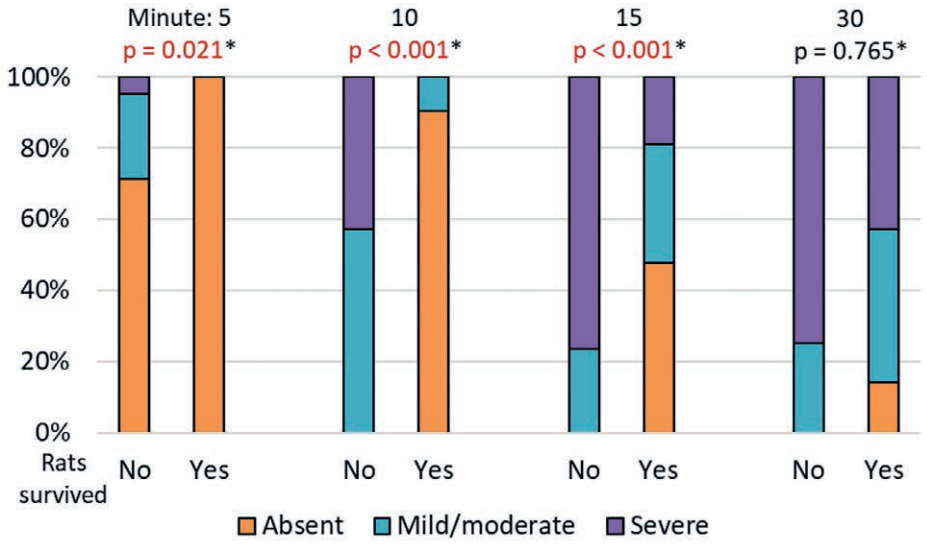

Fig. 8 Intensity of tremor in relation to whether rat treated with paraoxon survived or not.
* Fisher exact test, red colour: statistical significance; Minute: minutes 5, 10, 15 and 30 after paraoxon application.

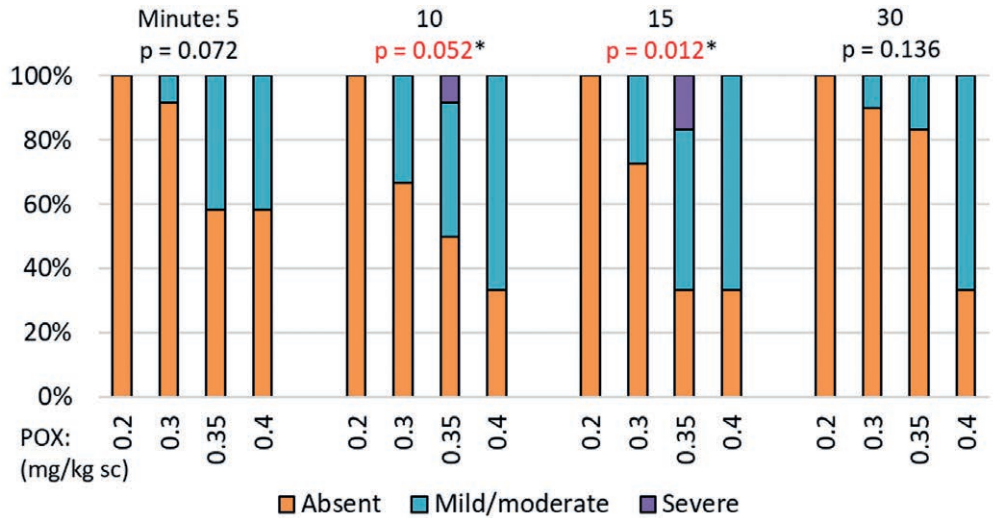

Fig. 9 Intensity of piloerection in rats depending on paraoxon dose.
* Kruskal-Wallis test, red colour: statistical significance; POX: paraoxon; sc: subcutaneously; Minute: minutes 5, 10, 15 and 30 after paraoxon application.

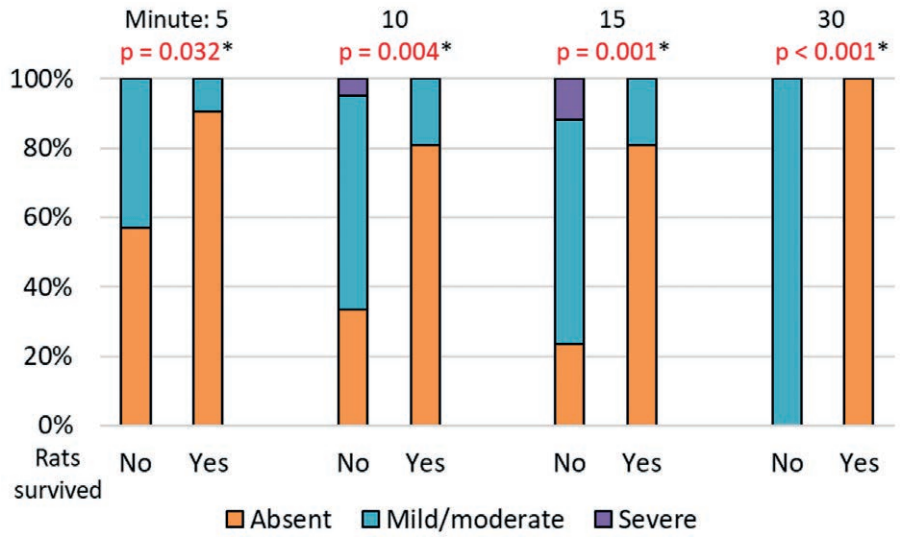

Fig. 10 Intensity of piloerection in relation to whether rat treated with paraoxon survived or not.
* Fisher exact test, red colour: statistical significance; Minute: minutes 5, 10, 15 and 30 after paraoxon application.

more often (Table 3) and of higher intensity in non-survivors (Figure 10). In atropine-protected rats, piloerection did not correlate with the outcome of poisoning.

The intensity of stereotypical movements, exophthalmos, lacrimation, ataxia and dyspnoea was not related to the dose of paraoxon or to the outcome of poisoning. The above results are not shown. Of the listed signs, stereotypical movements were less often with higher doses of paraoxon ($p = 0.044$) (Table 2). Related to poisoning outcome, ataxia ($p = 0.025$) was observed more often in survivors from the group of atropine-protected rats, while stereotypical movements ($p = 0.043$) were observed more often in survivors from the group of unprotected rats (Table 3).

DISCUSSION

Due to its extreme toxicity, the World Health Organization (WHO) has classified parathion, parent compound of paraoxon, as a class Ia (extremely hazardous) pesticide (14). Due to its toxicity, it is banned in most developed countries. Tabun, sarin, soman and VX represent OP compounds similar to parathion and paraoxon, with the same mechanism of action (inhibition of acetylcholinesterase) and their extreme toxicity classifies them as nerve agents. Their production, stockpiling, weaponosing and use is strictly prohibited by the 1993 Chemical Weapons Convention (CWC) (7). In undeveloped and developing countries, OPI poisonings, both accidental and intentional, are common (15, 16). In Sri Lanka and China, pesticide poisoning is the most common method of fatal self-harm (17).

The LD_{50} of paraoxon obtained in this study was 0.33 mg/kg sc, which corresponds with the results of other researchers (18). The PR of atropine was 2.73, which is in accordance with the known publications (19). Atropine is effective in blocking the effects of muscarinic but is ineffective against the nicotinic signs of OPC poisoning (20). This antimuscarinic drug is liposoluble and passes the blood-brain barrier (21). Therefore, it to some extent, antagonises the toxic effects of excessive cholinergic stimulation in the brain (22). It seems that a more lipophilic antimuscarinic drug would be more effective than atropine (23). Krutak-Krol and Domino (24) found that the atropine dose of 10 mg/kg im is optimal in experimental studies. The minimum absolute lethal dose of OPCs is 1.3 LD_{50} (25). The administration of atropine made it possible for rats to survive the absolute lethal dose of paraoxon. That enabled monitoring of signs of poisoning at high doses of paraoxon. As expected, atropine blocked to some extent the muscarinic effects, but not the nicotinic ones. Since different doses of paraoxon were administered in rats treated with saline or atropine, the results are not comparable. However, this makes it possible to compare the signs of poisoning in future studies with other antidotes.

In clinical settings, mainly muscarinic signs of OPC poisoning are expected. Bronchoconstriction and bronchorrhea are life-threatening muscarinic effects. Most studies have cited respiratory failure as the leading cause of death (26–28). Dyspnoea was observed as a sign of poisoning in the present experiment. No clear relationship was found between the intensity of dyspnoea and the dose of paraoxon. However, in the recent study, a clear relationship was found between the onset rate of dyspnoea, as well as the overall intensity of dyspnoea and lethal outcome of poisoning (29). Respiratory failure is a consequence of both peripheral and central cholinergic effects (30). Therefore, it is very important to administer an antidote that can cross the blood-brain barrier and prevent central respiratory depression (31).

Another muscarinic sign of poisoning that was observed is lacrimation. It is a sign that is easily noticeable. It is not a sign that directly implies whether the animal is endangered or not, but it is a good indicator of excessive muscarinic stimulation. In the treatment of OPC poisoning, the lack of lacrimation is one of the signs of achieving the so-called patient atropinisation (5). The results of this study also support this assumption. Although significantly higher doses of paraoxon were administered, the lacrimation occurred significantly less frequently in rats treated with atropine (22% vs 55%).

ACh is also found in the preganglionic nerve endings of the sympathetic nervous system (32). Stimulation of alpha-1-adrenergic receptors also leads to piloerection (33). Therefore, piloerection can serve as an indirect indicator of sympathetic stimulation. The results of this study showed a clear relationship between both the frequency and intensity of piloerection and the dose of paraoxon. Besides, piloerection occurred more often and was of stronger intensity in non-survivors.

Tachycardia and hypertension are rarely expected in patients with OPI intoxications and they often mislead physicians in practice. Saadeh et al found tachycardia in as many as 35–60% of patients poisoned by OPCs (34). It means that tachycardia occurs more often than bradycardia, which indicates that it is a prejudice not to expect nicotinic effects in OPC poisonings. Nicotinic signs of poisoning occur as a consequence of excessive stimulation of ganglionic nicotinic receptors (hypertension, tachycardia, diaphoresis) as well as receptors at the neuromuscular junction (fibrillation and fasciculation) (35). In AChEI poisoning, hypertension and tachycardia can also occur as a consequence of excessive stimulation of the *locus coeruleus*. Stimulation of this cholinergically innervated sympathetic nuclei leads to the centrally-originated hypertension (36, 37).

As already mentioned, ACh is a neurotransmitter of the peripheral nervous system, as well. Excessive stimulation of nicotinic receptors at the neuromuscular junction leads to fasciculations, a toxic phenomenon observed in this study. Fasciculations were the most consistent sign of the severity of rat poisoning. They were more intense at higher doses of paraoxon and in non-survivors throughout the observed period. This is in favour of the fact that nicotinic signs of poisoning appear in severe poisonings (38). When sarin was used in a terrorist attack in the crowded subway in Tokyo, over 5,000 people were injured and 12 people died (7, 39). Published reports cited nicotinic signs of poisoning in severely poisoned patients (40, 41). In rats treated with high doses of paraoxon and atropine, fasciculations were more common in survivors. This can be explained by the rapid lethal outcome of poisoning, which

left non-survivors without this toxic sign. Fasciculations often did not occur in the first 10 minutes of poisoning, but were present even after 4 hours in all survivors. In other words, it means that the non-survivors died too quickly to develop fasciculations. Experimental studies with antinicotinic drugs showed their significant antidotal efficacy against carbamates and OPCs (21). However, nicotinic receptor blockers are rarely used in clinical practice in the treatment of OPC poisonings, due to serious side effects at therapeutic doses of these drugs, primarily the respiratory muscle depression (42).

Tremor is mediated by a variety of neurotransmitters – dopamine, glutamate, serotonin, adenosine and acetylcholine (43). The M_2 muscarinic receptors are highly expressed in the nucleus basalis and occipital cortex, then in hippocampus and other cortical regions. Overstimulation of M_2 receptors leads to tremor (44). There is a conflicting evidence regarding the role of M_3 and M_4 receptors in tremor aetiology (45). In this study, a clear relationship was found between the intensity of poisoning, on the one hand and the dose of paraoxon and the outcome of the poisoning, on the other hand. In atropine-protected rats, tremor occurred in all animals. Tremor is often found as a part of the extrapyramidal syndrome that occurs as a consequence of permanent CNS damage in OPC poisoning survivors (21, 46, 47).

Stereotypical movements were registered more often in survivors and at lower dose of paraoxon. The heavily poisoned animals had significantly decreased spontaneous motor activity. Thus, the appearance of stereotypical movements could be a good prognostic sign of a positive outcome of poisoning. At the highest doses of paraoxon (0.9 and 1.2 mg/kg), ataxia was more common in survivors. Atropine prevented the death of rats, but not the skeletal muscle fatigue. As a consequence, only surviving rats could attempt to move in the cage and these movements were ataxic.

Seizures intensity was directly related to the dose of paraoxon and the lethal outcome of the poisoning. A total of 66.67% of survivors vs 100% of non-survivors had seizures. Seizures occur at the beginning of OPC poisoning due to the excessive cholinergic stimulation of the CNS. There are three treatment periods after the onset of OPC-induced convulsions: muscarinic, gamma-aminobutyric acid A ($GABA_A$)/benzodiazepine and glutamatergic ones (48). During the first one, antimuscarinic drugs (atropine and, preferably, more lipophilic drugs, such as scopolamine) can be efficiently used to stop the seizures, provided the right dose is chosen (49). However, beyond this period antimuscarinic drugs become ineffective in counteracting the seizures, irrespective of the dose applied. In the second phase this could be compensated by the administration of the $GABA_A$/benzodiazepine receptor antagonists, such as barbiturates (e.g., pentobarbital, thiopental sodium) and benzodiazepines (e.g., diazepam or midazolam) (50, 51). In the third phase, these seizures can be stopped by the administration of N-methyl-D-aspartate (NMDA) receptor antagonists, such as memantine, dizocilpine (MK-801) or ketamine (52–55). The reason for this is the fact that in the meanwhile the seizures became glutamatergic in its origin (56). Along with fasciculations, seizures were the most constant sign of the severity of the poisoning.

CONCLUSION

Among all the studied signs of paraoxon toxicity, the intensity of fasciculations and seizures were strong prognostic parameters of the severity of poisoning. They are easily observed and are directly related to both the dose of paraoxon and the lethal outcome of the poisoning. Based on the relationship between the frequency and intensity of muscarinic or nicotinic signs and the doses of paraoxon or outcomes of the poisoning, there are two strong prognostic parameters of the severity of poisoning (fasciculations and seizures) and a good prognostic sign of a positive outcome of poisoning (stereotypical movements). These signs of poisoning may be useful to researchers in monitoring the expected treatment outcome. Also, the appearance of nicotinic and central signs of poisoning in patients indicates the severity of poisoning and provides guidance to clinicians on which potential therapy to use.

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ABBREVIATIONS

ACh: acetylcholine; AChE: acetylcholinesterase; AChEI: acetylcholinesterase inhibitor; OPC: organophosphorus compounds; OPI: organophosphate insecticide

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RESEARCH ARTICLE

Novel chlorinated oxime K870 protects rats against paraoxon poisoning better than obidoxime

Žana M. Maksimović[a,b], Sonja T. Marinković[c], Đorđe Đukanović[a,d], Nebojša Mandić-Kovačević[d], Snežana Uletilović[e], Mladen Duran[a], Kamil Kuča[f,g], Kamil Musilek[f,g], Dragana Lončar-Stojiljković[h], Ranko Škrbić[a,b] and Miloš P. Stojiljković[a,b]

[a]Centre for Biomedical Research, Faculty of Medicine, University of Banja Luka, Banja Luka, Bosnia and Herzegovina; [b]Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine, University of Banja Luka, Banja Luka, Bosnia and Herzegovina; [c]Paediatric Clinic, University Clinical Centre of the Republic of Srpska, Banja Luka, Bosnia and Herzegovina; [d]Department of Pharmacy, Faculty of Medicine, University of Banja Luka, Banja Luka, Bosnia and Herzegovina; [e]Department of Medical Biochemistry and Chemistry, Faculty of Medicine, University of Banja Luka, Banja Luka, Bosnia and Herzegovina; [f]Department of Chemistry, Faculty of Science, University of Hradec Kralove, Hradec Kralove, Czech Republic; [g]Biomedical Research Centre, University Hospital Hradec Kralove, Hradec Kralove, Czech Republic; [h]Department of Anaesthesiology and Reanimatology, Faculty of Medicine, University of Banja Luka, Banja Luka, Bosnia and Herzegovina

ABSTRACT

The aim of this study was to determine the antidotal potential of the chlorinated oxime K870 compared to obidoxime, as a monotherapy and in combination with atropine, in paraoxon (POX)-poisoned rats. The treatment doses of oximes were chosen as 20% of their LD_{50} values. The protective ratio (PR) of oxime K870 with atropine was significantly higher than that of obidoxime with atropine (68.8 and 125.0, respectively). In the biochemical part of the experiment POX subcutaneously (s.c.) (0.75% LD_{50}) was administered and followed by oxime K870 or obidoxime i.m. 1 min later. Acetylcholinesterase (AChE) activity was determined spectrophotometrically in cerebrum, cerebellum, brainstem, diaphragm, and erythrocytes. Carboxylesterase activity was determined in plasma and liver. Both oximes successfully reactivated AChE in brain (cerebrum, cerebellum, and brainstem), diaphragm and erythrocytes, but the oxime K870 performed better than obidoxime. Both oximes reactivated carboxylesterase, obidoxime better in plasma and oxime K870 better in liver. In conclusion, the oxime K870, when co-administered with atropine, is a more effective antidote than the obidoxime-atropine combination in POX-poisoned rats.

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1. Introduction

Organophosphorus compounds (OPCs) are highly toxic chemicals. Although nerve agents (tabun, sarin, soman, VX, and the Novichoks) (Stojiljković, 2019) production, stockpiling, and usage are strictly prohibited by the 1993 Chemical Weapons Convention (CWC), their sporadic use still occurs. In the last three decades, mass poisonings of OPC nerve agents have been recorded. Sarin was used in the Iraq–Iran war (1980–1988), as well as in the Syrian conflict in 2013 and 2017 (Marshall, 1984, John et al., 2018). The AUM Shinrikyo, a Japanese religious cult, used sarin for massive intoxications in Matsumoto in 1994 and in Tokyo subway in 1995 and VX for assassination of individuals in 1994 and 1995 (Morita et al., 1995, Okumura et al., 1996, Nozaki et al., 1995). Nerve agents were used for the purpose of assassination of Kim Jong Nam with VX in Kuala Lumpur (2017), attempted assassination of Sergey Skripal and his daughter Yulia Skripal with Novichok in Salisbury in 2018 and attempted assassination of Alexei Navalny in Omsk in 2020 (Chemist: Kim Had 1.4 Times Lethal Dosage of VX on Face, 2017; Franca et al., 2019, Steindl et al., 2021). On the other hand, the most toxic OPC pesticides are prohibited and those that are used are categorized by the World Health Organization (WHO) as extremely hazardous pesticides (class Ia for parathion) (WHO, 2019). About 100,000 fatal OPC pesticide poisonings occur annually, mostly in the developing countries (Maksimović et al., 2021).

Paraoxon (POX) is the active metabolite of the OPC pesticide parathion (CAMEO Chemicals, 2024). It causes well-documented and described convulsions, which are rare in other OPC pesticide poisoning (Zare et al., 2020, 2022). In the body, POX binds to the serine group of the enzyme acetylcholinesterase (EC 3.1.1.7) (AChE), leading to the enzyme inactivation (Henretig et al., 2019). The POX–AChE bond is a stable covalent bond that does not spontaneously hydrolyze, which is why POX is an irreversible AChE inhibitor (AChEI) (Antonijević et al., 2005). OPCs inhibit not only AChE but also other esterases, including butyrylcholinesterase (EC 3.1.1.8, BuChE) and carboxylesterase (EC 3.1.1.1, CarbE) (Maxwell, 1992).

CONTACT Žana M. Maksimović zana.maksimovic@med.unibl.org Centre for Biomedical Research, Faculty of Medicine, University of Banja Luka, Save Mrkalja 16, 78000 Banja Luka, The Republic of Srpska, Bosnia and Herzegovina

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Inactivated AChE does not degrade acetylcholine (ACh) in the synaptic cleft (Pope and Brimijoin, 2018). Thus, ACh accumulates and produces the symptoms and signs of an acute cholinergic crisis (Eddleston, 2019). Hyperstimulation of muscarinic receptors can cause bronchoconstriction, bronchorrhoea, bradycardia, hypotension, increased motility of the gastrointestinal tract, abdominal cramps, miosis, and hypersalivation. Overstimulation of nicotinic receptors causes hypertension, tachycardia, fibrillations, fasciculations, skeletal muscle necrosis, diaphragm, and intercostal muscle weakness progressing to peripheral respiratory failure. Tremor, incoordination, convulsions, central respiratory and cardiovascular depression, coma and death can occur due to cholinergic stimulation in central nervous system (Reddy et al., 2020).

Besides supportive therapy, treatment includes the use of antidotes-antimuscarinic drugs, AChE reactivators and anticonvulsants (Vanova et al., 2018, Parkes & Sacra, 1954, Eddleston et al., 2005). Antimuscarinic drugs, primarily atropine, are the main symptomatic antidotes for OPC intoxications (Jokanović et al., 2020). The causal therapy is mediated by AChE reactivators (so called oximes) that bind to the OPC in the active site of AChE, which leads to its reactivation (Eddleston et al., 2005, Worek et al., 2020). Convulsions are initially cholinergic, then influenced by gamma-aminobutyric acid A ($GABA_A$) receptors and finally glutamatergic (Shih et al., 1999, Bokonjić & Rosić, 1991). Therefore, at the beginning, atropine is an effective anticonvulsant, then benzodiazepines (e.g. diazepam) or barbiturates (e.g. thiopental sodium) and finally *N*-methyl-D-aspartate (NMDA) receptor antagonists (e.g., memantine) (Spampanato et al., 2020, Stojiljković et al., 2019, Weissman & Raveh, 2008, Stojiljković et al., 2021, Škrbić et al., 2017).

Oximes are the only causal antidotes in OPC poisoning. However, oximes exhibit significant limitations. The two biggest problems of the oximes synthesized so far are insufficient efficiency against specific OPC and the other is insufficient universality against different OPC (Antonijevic & Stojiljkovic, 2007). This is precisely why, there has been a constant search for new and more efficient oximes. The so-called K-oximes are part of those efforts, among which the chlorinated oxime K870 was prepared.

Out of the established oximes, obidoxime is the most effective in POX poisoning (Kuca et al., 2009). The newly synthesized oxime K870 is a dichlorinated analogue of the oxime K203, has a functional group at the position 4 and a double bond in the linker (Kalász et al., 2020). Along with oxime K027 and K048, K203 is one of the K-oximes that showed better efficiency in experimental studies in OPC poisoning compared to some other known oximes (Kovarik et al., 2009, Antonijevic et al., 2016, Antonijevic, Kotur-Stevuljevic, et al., 2018, Antonijevic, Musilek, et al., 2018, Antonijevic et al., 2019). Chlorination of K203 lowers the pKa from 8.33 to 7.55, increasing the amount of the ionized form at physiological pH from ~10% in K203 to ~40% in oxime K870 (Zorbaz et al., 2018). The addition of halogen atoms should ensure higher nucleophilicity of the oxime moiety (i.e., formation of oximate anion as an effective nucleophile) and thus higher reactivation ability of K870 compared to non-halogenated oximes (Zorbaz et al., 2018, 2019, Zdarova Karasova et al., 2022).

The aim of this study was to determine the antidotal potential of oxime K870 compared to obidoxime *in vivo* as monotherapy and in combination with atropine.

2. Material and methods

2.1. Animals

The study was conducted in Wistar rats of both sexes, weighing 200–240 g. They were kept in cages in groups of 5, receiving water and food *ad libitum*, with a 12-h light-dark cycle. The animals were treated in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals (National Research Council Committee, 2011) and associated guidelines, EU Directive 2010/63 for the protection of animals used for scientific purposes. The study was approved by the Ethics Committee for the Protection and Welfare of Experimental Animals in Biomedical Research, Faculty of Medicine, University of Banja Luka (Decision No 18/1/20). The study was conducted at the Center for Biomedical Research, Faculty of Medicine, University of Banja Luka.

2.2. Chemicals

POX was purchased from Merck, St Louis, MO. Obidoxime [1,3-bis(4-hydroxyiminomethylpyridinium)-2-oxapropane chloride] and the oxime K870 (4-carbamoyl-1-[(2E)-4-{3,5-dichloro-4-[(hydroxyimino)methyl]pyridinium-1-yl}but-2-en-1-yl] pyridinium dibromide) were synthesized at the Department of Chemistry of the Faculty of Science, University of Hradec Kralove, Czech Republic and were donated to the Medical Faculty of the University of Banja Luka. POX was dissolved in isopropyl alcohol to a concentration of 50 mg/mL and further dilutions of the stock solution were made in saline (0.9% NaCl solution). Atropine (atropine sulphate) and oximes were dissolved in saline. The final solutions were made immediately before the treatment. All substances were administered in a volume of 1 mL/kg, except for oxime K870 in a dose of 200 mg/kg, due to the poor water solubility of the oxime, it was administered in a volume of 2 mL/kg (1 mL/kg in each thigh muscle). POX was administered subcutaneously (s.c.) and atropine and oximes intramuscularly (i.m.). Chemical structures of POX, obidoxime, and oxime K870 are presented in Figure 1.

2.3. Median lethal dose (LD_{50}) and protective ratio (PR)

In a previous study, the LD_{50} of POX was determined, as well as the protective ratio (PR) of atropine (10 mg/kg i.m.) (Maksimović et al., 2022). The LD_{50} of obidoxime and oxime K870 was determined by the 'up and down' method and calculated according to Litchfield and Wilcoxon (1949). After that, 20% of LD_{50} of oximes was taken as the treatment doses for further experiments.

Further, PRs of: obidoxime, oxime K870, obidoxime with atropine and oxime K870 with atropine were determined. One minute after POX was injected s.c., antidotes were given

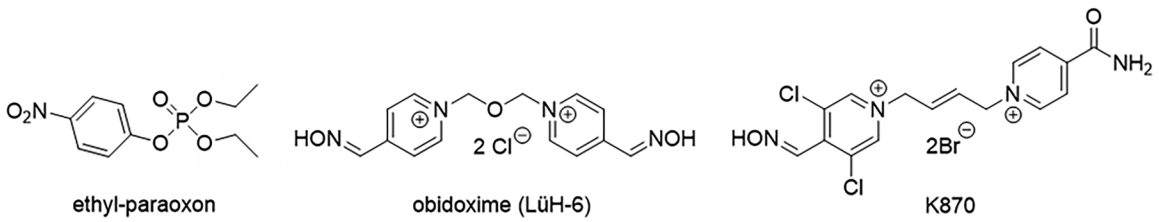

Figure 1. Chemical structures of tested organophosphate and active antidotes.

Table 1. Median lethal dose of tested oximes (LD_{50}).

Dose (mg/kg i.m.)	Animals (D/T)	Litchfield and Wilcoxon (1949)		
		LD_{50}	F	95% CI
Obidoxime				
50	0/5	108.33	1.46	73.99-158.61
100	2/5			
150	4/5			
200	5/5			
Oxime K870				
100	0/5	172.32	1.18	145.49-204.09
150	1/5			
200	4/5			

D/T: dead/total number of animals in group; LD_{50}: median lethal dose; CI: confidence interval; Administered volumes were 1 mL/kg, except for oxime K870 200 mg/kg, where 2 mL/kg were administered i.m.

in fixed doses i.m. The oximes and atropine were dissolved together, so that all rats were administered a volume of 1 mL/kg i.m. For determining the LD_{50} of POX only, rats were administered 1 mL/kg saline 1 min after POX.

2.4. Inhibition of acetylcholinesterase and carboxylesterase

POX 0.25 mg/kg s.c. (0.75% LD_{50}) was administered followed one minute later by the oxime K870 (35 mg/kg i.m. or obidoxime (22 mg/kg i.m.) (20% LD_{50}). Animals were sacrificed: 5, 15, 30, 45, 60, 120, 240, 480, 960, and 1440 min after POX administration. AChE activities were determined spectrophotometrically in cerebrum, cerebellum, brainstem, diaphragm, and erythrocytes (Ellman et al., 1961). CarbE activities were determined titrimetrically in plasma and liver (Jokanović et al., 1996).

Animals were anesthetized with ketamine 90 mg/kg and xylazine 10 mg/kg intraperitoneally (i.p.). Blood was drawn from the inferior vena cava. After blood extraction, organs were removed for analysis: diaphragm, liver, kidneys. After that, the brain was washed out of the presence of blood, using the already established MM method (Marinković et al., 2021). Shortly, the ascending part of the aorta was cannulated, just above the aortic root, the superior vena cava was cut, the abdominal part of the aorta was clamped and 100 mL of saline was administered through the cannula. After that, the heart and brain were removed.

AChE activity from erythrocytes was determined immediately after sampling and the brain, diaphragm and liver were frozen at −20 °C. Homogenization of cerebrum, cerebellum, and brainstem was performed using phosphate buffer (0.1 M, pH 8.0) in a 1:20 ratio. The samples were homogenized at 10,000 rpm for 1 min. Homogenized tissue was centrifuged at 4 °C, for 15 min at 15,000 rpm and the supernatant was used as a sample for determination of the AChE activity. The AChE activity was measured by the Ellman colorimetric method using a Shimadzu UV-1800 spectrophotometer (Kyoto, Japan) and UV Probe 2.17 software (Kyoto, Japan). Activity of AChE in erythrocyte lysate was expressed as µmol/min/mL, while its activity in the brain and diaphragm was expressed as µmol/min/g. Thereafter, AChE was expressed as a percentage of baseline values obtained from saline-treated control animals. CarbE values were determined in plasma and liver. Homogenization of the liver was performed using saline in a ratio of 1:20.

2.5. Statistics

The LD_{50} and the PR were analyzed by the method of Litchfield and Wilcoxon (1949) on Pharm/PCS statistical software. Other analyses were performed on IBM SPPS for Windows, version 18.0 (IBM SPSS Statistics, Armonk, NY). After the Kolmogorov–Smirnov test showed a non-normal distribution of data, appropriate nonparametric tests were applied: Chi-square test (or Fisher exact test) and Kruskal–Wallis test. Statistical significance level was set at $p < 0.05$.

3. Results

3.1. The LD_{50} of oximes and PRs of antidotes

The applied doses of POX, oximes, number of dead rats per group and LD_{50} are shown in Table 1. The LD_{50} of oxime K870 and obidoxime was 172.32 and 108.33 mg/kg i.m., respectively. That corresponds to the LD_{50} = 326.96 µmol/L for oxime K870 and 301.59 µmol/L for obidoxime.

The LD_{50} of POX when atropine and/or oximes were administered is shown in Figure 2. The PRs of atropine, obidoxime and oxime K870 were 2.76 (LD_{50} = 0.91), 1.79 (LD_{50} = 0.59), and 2.27 (LD_{50} = 0.75), respectively, and they were all significantly improved when compared to the saline control. Combinations of either of the oximes with atropine provided very high PRs – 125.00 (LD_{50} = 41.25) and 68.79 (LD_{50} = 22.70), for oxime K870 and obidoxime, respectively. The atropine-oxime K870 combination performed significantly better than the atropine-obidoxime, with the relative PR of 1.82.

3.2. Acetylcholinesterase activities

Inhibition of AChE in brain (separately in cerebrum, cerebellum, and brainstem) are shown in Figure 3(A–C).

After POX administration AChE activity in the cerebrum and the cerebellum decreased sharply to the minimum of 16.1% and 19.4% of the control values 45 min after POX

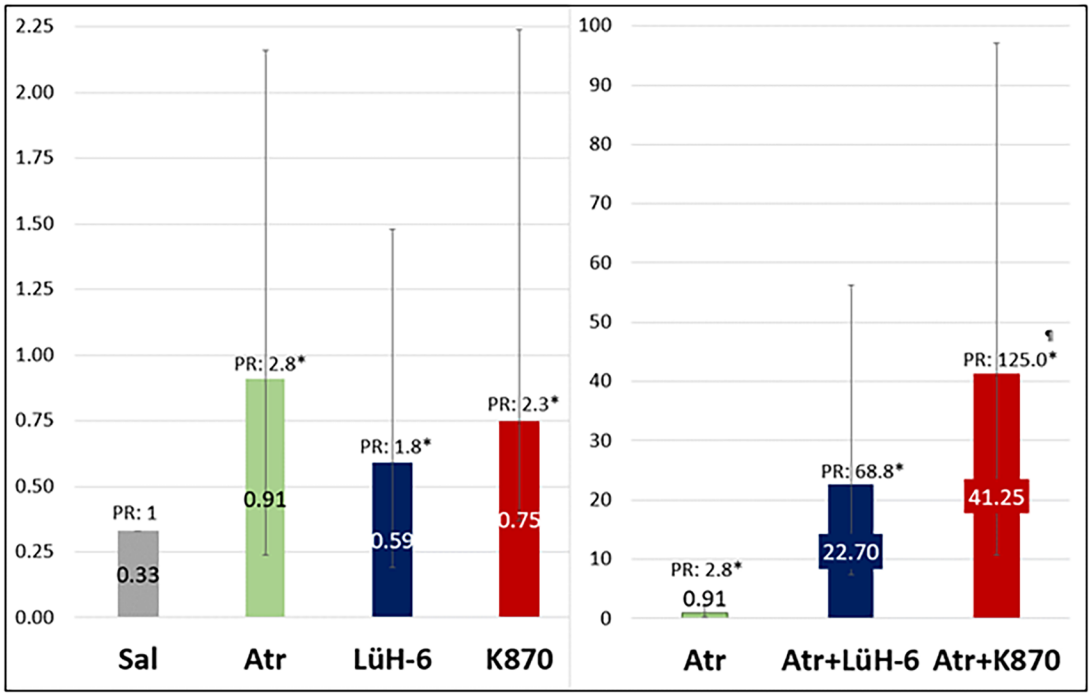

Figure 2. Median lethal dose (LD_{50}) and protective ratios (PRs) of paraoxon depending on treatment.
*Statistical significance related to Saline; ¶ statistical significance related to atropine + obidoxime (relative protective ratio 1.82); Sal: saline (1 mL/kg –i.m.); Atr: atropine (10 mg/kg i.m.); LüH-6 (obidoxime): (22 mg/kg i.m.), K870: oxime K870 (35 mg/kg im); Y axis: paraoxon (mg/kg s.c.). Bars indicate 95%-confidence intervals. Y-axis indicate dose of paraoxon (mg/kg s.c.).

administration. Spontaneous reactivation was observed thereafter, with highest value of 46.0% registered 480 min and 38.8% 960 min after POX administration in the cerebrum and the cerebellum, respectively. The final registered enzyme activity (after 24 h) in the cerebrum and the cerebellum was 43.4% and 36.3%, respectively. The AChE activities in the cerebrum in rats protected with oximes were also lowest 45 min after POX, 20.1% and 19.1% after oxime K870 and obidoxime, respectively. Similar was in the cerebellum, 24.2% and 23.8% for oxime K870 and obidoxime, respectively. The highest AChE activity value in rats protected with oxime K870 was registered 24 h after POX (55.8%) in the cerebrum and 59.9% 960 min after POX in the cerebellum, while the highest AChE activity in rats protected with obidoxime was registered 480 min (51.0%) after POX in the cerebrum and 40.4% 960 min after POX in the cerebellum. A significant difference in AChE inhibition in oxime K870-treated rats compared to saline at all-time points, both in the cerebrum and the cerebellum and obidoxime-treated rats compared to saline only at 240 and 960 min in the cerebrum and at 16, 60, and 480 min in the cerebellum. AChE values in the cerebrum in rats treated with oxime K870 compared to obidoxime were significantly higher at 60, 120, 240, and 1440 min. AChE values in the cerebellum in rats treated with oxime K870 compared to obidoxime were significantly higher at 240 and 960 min.

AChE activity in the brainstem also decreased sharply after POX administration to the minimum of 16.1% of the control values 45 min after POX administration. Spontaneous reactivation was observed thereafter, with highest value of 40.1% 960 min after POX administration. The final registered enzyme activity (after 24 h) was 36.7%. The AChE activities in rats protected with oximes were also lowest 45 min after POX, 18.3% and 18.4% after oxime K870 and obidoxime, respectively. The highest AChE activity value in rats protected with oxime K870 was registered 480 min after POX (41.4%), while the highest AChE activity in rats protected with obidoxime was registered 960 min (39.9%) after POX. Reactivation of AChE with oximes was lower than in other analyzed brain regions. Nevertheless, AChE activity was significantly higher in oxime K870 oxime-treated rats compared to saline at all time points and obidoxime-treated rats compared to saline only at 15, 45, and 60 min. AChE activities in the brainstem were statistically significantly higher in rats treated with oxime K870 compared to obidoxime at 120 and 1440 min.

Inhibition of AChE in the diaphragm is shown in Figure 4.

Lowest inhibition of AChE activity after POX administration was noted in the diaphragm. Lowest activity was noted at 45 min (19.3%), thereafter spontaneous reactivation was observed, with 48.7% of control values noted 24 h after POX administration. Inhibition of AChE was significantly lower in rats treated with both oximes in all time points. Lower AChE activities for oxime K870 and obidoxime were noted 480 min after POX administration, 43.6 and 49.9, respectively. The highest AChE activity value in rats protected with oxime K870 was registered 24 h after POX 67.2%), while the highest AChE activity in rats protected with obidoxime was registered 45 min after POX (64.4%). The difference between the degree of AChE inhibition in rats treated with obidoxime vs rats treated with oxime K870 was significant only at 15 and 30 min – AChE values were higher in rats treated with obidoxime.

Inhibition of AChE in erythrocytes is shown in Figure 5.

After POX administration AChE inhibition in erythrocytes was highest compared to the other analyzed tissues. AChE activity sharply decreased, after 15 min it was only 7.9% of

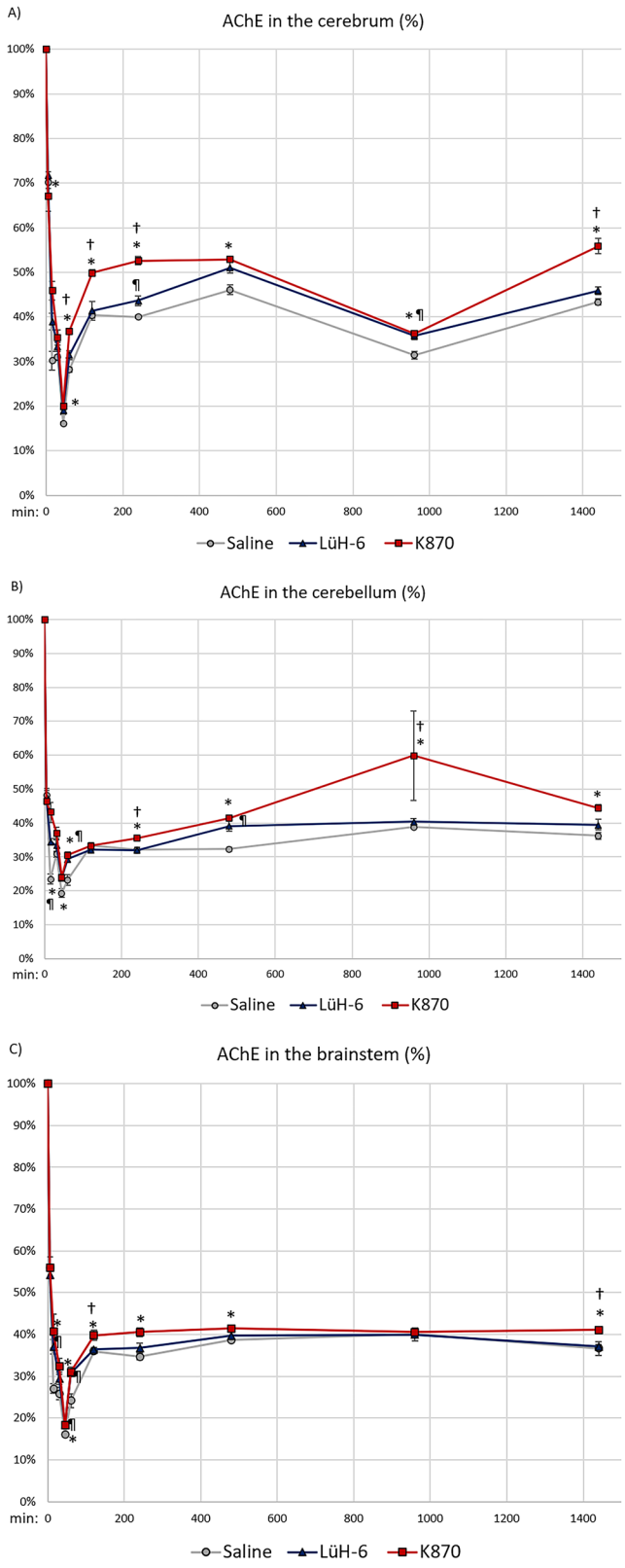

Figure 3. Time-related inhibition of acetylcholinesterase (AChE) in brain of rats poisoned by paraoxon and treated with either saline, obidoxime or oxime K870: a) in the cerebrum; b) in the cerebellum; c) in the brainstem. Administered volumes were 1 mL/kg; paraoxon (0.25 mg/kg s.c.) and 1 min later either: saline (1 mL/kg i.m.), obidoxime (22 mg/kg i.m.) or oxime K870 (35 mg/kg i.m.); Min: minutes after paraoxon administration; Kruskal–Wallis test, statistical significance: *Oxime K870 *vs.* Saline; ¶Obidoxime *vs.* Saline; †Oxime K870 *vs.* Obidoxime.

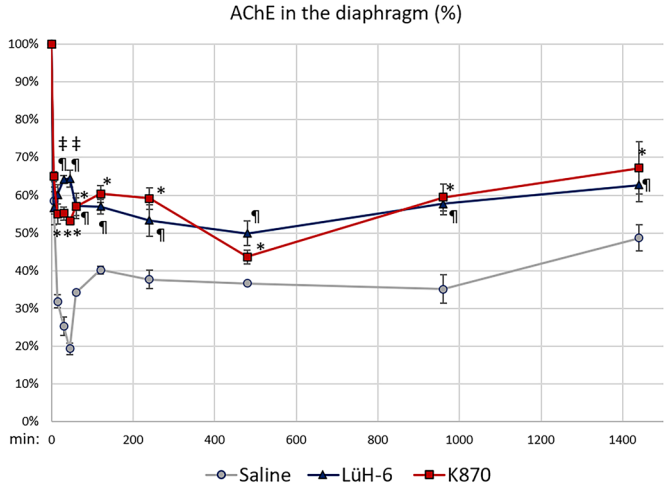

Figure 4. Time-related inhibition of acetylcholinesterase (AChE) in diaphragm of rats poisoned by paraoxon and treated with either saline, obidoxime or oxime K870.
Administered volumes were 1 mL/kg; paraoxon (0.25 mg/kg s.c.) and 1 min later either: saline (1 mL/kg intramuscularly -i.m.), obidoxime (22 mg/kg i.m.) or oxime K870 (35 mg/kg i.m.); Min: minutes after paraoxon administration; Kruskal–Wallis test, statistical significance: *Oxime K870 *vs.* Saline; ¶Obidoxime *vs.* Saline; ‡Obidoxime *vs.* Oxime K870.

control values, while minimum of 2.2% was noted 240 min after POX administration. Spontaneous reactivation slowly started thereafter, with highest value of 25.4% noted at the resulting enzyme activity (24 h after POX administration). The AChE activities in rats protected with oximes were lowest 60 min after POX administration, 7.6% and 8.2% after oxime K870 and obidoxime, respectively. The highest AChE activity values in rats protected with oxime K870 and obidoxime were registered 24 h after POX, 67.6 and 35.5, respectively. A significant difference was noted in AChE inhibition in rats treated with both oxime K870 and obidoxime compared with saline at all time points. AChE values in erythrocytes in rats treated with oxime K870 compared to obidoxime were significantly lower in the 15 min and higher in the 960 and 1440 min.

3.3. Carboxylesterase values

Percentage of CarbE inhibition in plasma of rats poisoned with POX and treated either with saline, obidoxime or oxime K870 is shown in Figure 6.

In plasma, after POX administration CarbE activity decreased sharply, after 15 min minimum of 8.8% of control values were recorded. Thereafter, spontaneous reactivation was observed, with highest value of 53.8% registered 24 h after POX application. The CarbE activities in rats protected with obidoxime was also lowest 15 min after POX (19.0%), while with oxime K870 lowest values were recorded 120 min after POX administration (27.9%). The highest CarbE activity values in rats protected with oxime K870 and obidoxime were registered 960 min after POX administration (74.3 and 96.5, respectively). *Post-hoc* analysis showed a significant difference in CarbE inhibition in rats treated with obidoxime compared to saline at 5, 15, 30, 45, 240, and 1440 min and rats treated with oxime K870 compared to saline at 5, 15, 30,

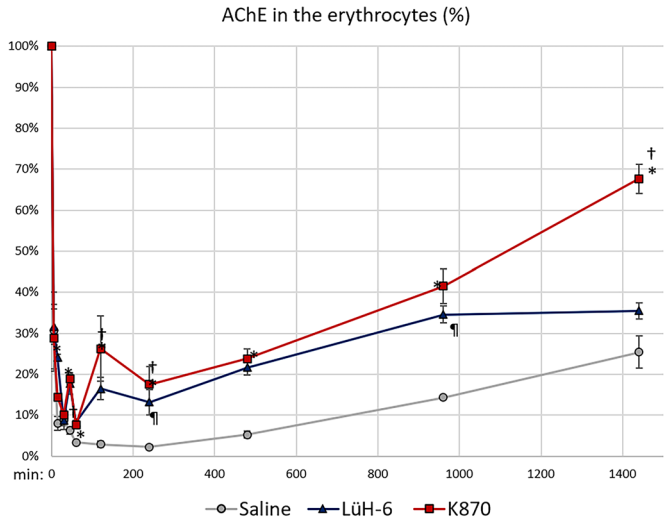

Figure 5. Time-related inhibition of acetylcholinesterase (AChE) in erythrocytes of rats poisoned by paraoxon and treated with either saline, obidoxime or oxime K870.
Administered volumes were 1 mL/kg; paraoxon (0.25 mg/kg s.c.) and 1 min later either: saline (1 mL/kg i.m.), obidoxime (22 mg/kg i.m.) or oxime K870 (35 mg/kg i.m.); Min: minutes after paraoxon administration; Kruskal–Wallis test, statistical significance: *Oxime K870 *vs.* Saline; ¶Obidoxime *vs.* Saline; †Oxime K870 *vs.* Obidoxime; ‡Obidoxime *vs.* Oxime K870.

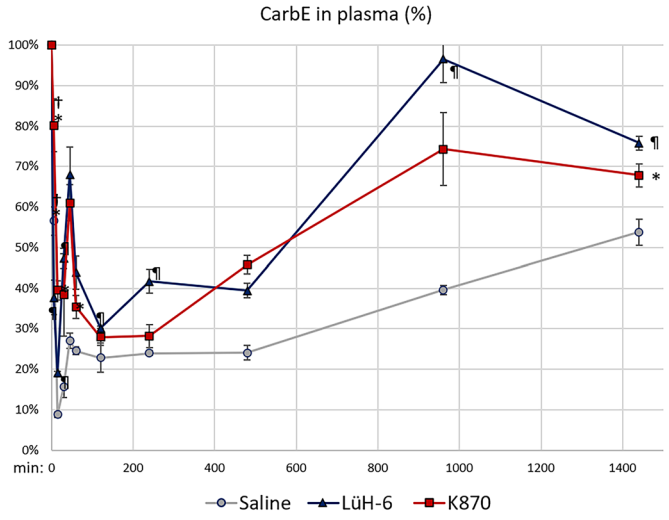

Figure 6. Time-related inhibition of carboxylesterase (CarbE) in plasma of rats poisoned by paraoxon and treated with either saline, obidoxime or oxime K870.
Administered volumes were 1 mL/kg; POX (0.25 mg/kg s.c.) and 1 min later either: saline (1 mL/kg i.m.), obidoxime (22 mg/kg i.m.) or oxime K870 (35 mg/kg i.m.); Min: minutes after POX administration; Kruskal–Wallis test, statistical significance: *Oxime K870 *vs.* Saline; ¶Obidoxime *vs.* Saline; †Oxime K870 *vs.* Obidoxime; ‡Obidoxime vs Oxime K870.

45, and 1440 min. CarbE values in plasma in rats treated with oxime K870 compared to obidoxime were significantly higher at 5 and 15 minutes and lower at 240 and 1440 min.

Inhibition of CarbE in liver is shown in Figure 7.

In the liver, CarbE activity sharply decreased to 36.8% of initial values 5 min after POX application and the minimum of CarbE activity was recorded at 45 min (30.9%). After initial decrease, slight oscillations of CarbE values were notes, with highest value of 52.0%, 24 h after POX. Similar trend was noted in rats treated with oximes. In rats treated with oxime K870 lowest CarbE activity was noted 60 min after POX

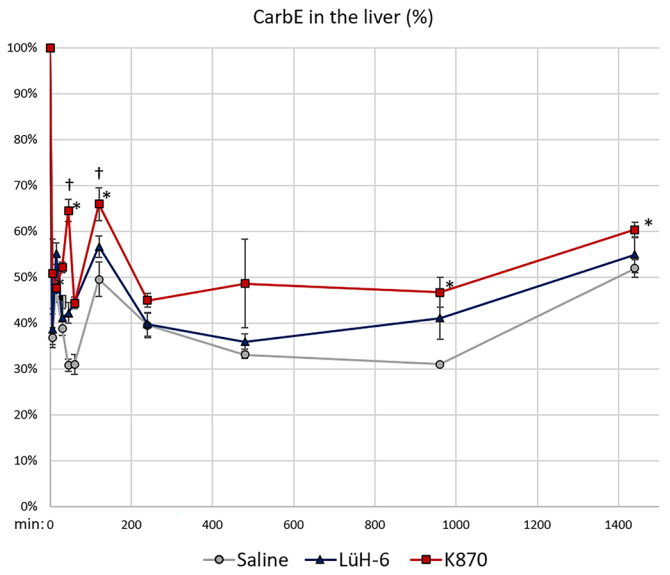

Figure 7. Time-related inhibition of carboxylesterase (CarbE) in liver of rats poisoned by paraoxon and treated with either saline, obidoxime or oxime K870.
Administered volumes were 1 mL/kg; paraoxon (0.25 mg/kg s.c.) and 1 min later either: saline (1 mL/kg i.m.), obidoxime (22 mg/kg i.m.) or oxime K870 (35 mg/kg i.m.); Min: minutes after paraoxon administration; Kruskal–Wallis test, statistical significance: *Oxime K870 *vs.* Saline; ¶Obidoxime *vs.* Saline; †Oxime K870 *vs.* Obidoxime.

(44.3%) and highest 120 min after POX (66.0%). In rats treated with obidoxime lowest CarbE activity was noted 480 min after POX (36.0%) and highest 120 min after POX (56.7%). A significant difference was noted in CarbE inhibition in obidoxime-treated rats compared to saline at 45 min and K870 oxime-treated rats compared to saline at 30, 45, 120, 960, and 1440 min. CarbE values in liver were significantly higher in rats treated with oxime K870 compared to obidoxime at 30 and 45 min.

4. Discussion

A recently published study found that the LD_{50} of POX is 0.33 mg/kg s.c. and the PR of atropine in POX poisoning is 2.73 (Maksimović et al., 2022). Krutak-Krol and Domino (1985) showed that a dose of 10 mg/kg of atropine was optimal in experimental studies. Oximes alone only slightly improve survival in OPC poisoning. In this study, the PR obidoxime and oxime K870 were 1.79 and 2.27, respectively. In poisoning with nerve agents, the PR is even lower (Stojiljković et al., 2020). The synergistic effect of atropine and oxime was also demonstrated in this study where the PR of obidoxime and oxime K870 in combination with were ~69 and 125, respectively. These findings are in accordance with the results of Žunec et al. (2015) who found PRs in male mice poisoned with POX and treated with atropine and various oximes vary from 60.0 to 100.0.

Obidoxime has been the most effective reactivator of POX-inhibited AChE to date (Stojiljković et al., 2018). Eyer et al. (2007) suggested that oxime therapy should be started as early as possible, be administered long enough and that obidoxime plasma concentration should be 10–20 µmol/L. This concentration is considered to be without major side effects, but is ineffective at very high poison loads, because

re-inhibition occurs faster than reactivation (Eyer et al., 2007). Although in principle pralidoxime is more effective with diethyl- and obidoxime with dimethyl-OPCs, obidoxime was proven to be more effective in POX poisoning (Jokanović & Stojiljković, 2006, Maksimović et al., 2023). It should be highlighted that presented results showed that oxime K870 improves survival in POX poisoning significantly better than obidoxime. As mentioned before, obidoxime is limited by its hepatotoxicity (Eyer et al., 2007), which Zdarova Karasova et al. (2022) did not found even with higher dose of oxime K870.

In vitro oxime K870 binds to both AChE and BuChE with an overall rate of reactivation for VX-inhibited human AChE that is comparable to the most effective oxime discovered to date, asoxime (HI-6) and with higher affinity than pralidoxime or oxime K203 (Kovarik et al., 2009, Zorbaz et al., 2018). Solution of oxime K870 in saline is very stable, unlike a solution in PBS (Zorbaz et al., 2019). Zandona et al. (2022) found cytotoxicity-related effects of imidazolium and chlorinated bispyridinium oximes, including oxime K870, in SH-SY5Y cells. The cytotoxic effects increased with the number of chlorine atoms and were further enhanced by the double bond in the oxime K870 linker. Degradation products of isoxazole-pyridinium chlorinated oximes are not cytotoxic to the HK-2 cell line (Handl et al., 2021), which could be considered a positive feature as the main organ for excreting oximes K870 is the kidney (Zdarova Karasova et al., 2022).

In vivo, Zdarova Karasova et al. (2022) found the maximal tolerable dose of oxime K870 to be 130 and 150 mg/kg for male and female mice. When the same dose was given to rats, necrosis, swelling and effusion at the injection site were observed. When the dose was reduced to 20% MTD, autopsy showed mild swelling and hematomas at the injection site. They found bleeding and edema at the injection site after 240 min, followed by inflammatory infiltration and degenerative changes in the myofibrils over the next 2 d. After 7 d, the acute inflammation was replaced by a round cell infiltrate, which was associated with myocyte regeneration and collagen fiber production. Similar doses (35 mg/kg) were used in the present experiment and similar local changes were observed. Kalász et al. (2020) showed that substitution with chlorine reduces the solubility and absorption of oxime K870 after its i.m. application. To the author's knowledge, no study has been published so far that determined the LD_{50} of oxime K870 alone. The results of this study show that the LD_{50} of oxime K870 is 175 mg/kg, compared to 108 mg/kg obidoxime.

The most significant limitation in the effectiveness of oximes is their poor penetration through the BBB (Voicu et al., 2010), especially when considering that the main cause of death in OPC poisoning is central respiratory depression. There are studies that claim that after OPC poisoning with convulsions the BBB becomes more permeable to oximes and that oximes then penetrate better into the brain, but there are studies that show that even after OPC poisoning, oximes do not penetrate or penetrate the brain very little (Petroianu et al., 2007). Musilek et al. (2011) analyzed the ideal characteristics that oxime should have in OPC pesticide poisoning. It was found that: oximes with a functional group at position 4 best reactivate AChE inhibited by OPC pesticides; oximes with a bisquaternary structure are more effective than monoquaternary ones; ideally, the linker should have 3–5 C-C bonds; the double bond in the linker increases both efficacy and toxicity. Besides, it was established that adding a chlorine molecule slightly increases the lipophilicity of the oxime and slightly increases the penetration of the oxime through the BBB (Zorbaz et al., 2018, 2019). Oxime K870 is a dichlorinated analogue of the oxime K203.

In this study, sharp decrease of the AChE activity was observed in the first hour as well as at 960 min compared to 480 min after POX administration. In the mentioned period, an additional release of POX from the depot and reinhibition of the enzyme probably occurred. In the study by Eyer et al. (2003) a postmortem analysis of fat tissue of a patient showed 66 times greater concentration of parathion than the one in plasma 16 d after poisoning, while the concentration in the brain was similar to the one in plasma. Overall, both obidoxime and K870 oxime statistically significantly reactivated AChE in both cerebrum and cerebellum, whereby oxime K870 was a better reactivator than obidoxime.

From the presented results, it is apparent that spontaneous reactivation of AChE occurred even in rats not treated with oximes. Numerous *in vitro* studies have shown that during the formation of the POX-AChE bond, both aging and spontaneous reactivation reactions occur simultaneously (Mason et al., 2000, Worek et al., 2011, Kousba et al., 2004, Herkert et al., 2010). Mason et al. (2000) found the half-life for aging AChE and BuChE inhibited by POX to be 46.3 and 12.6 h, respectively. Half-life of spontaneous reactivation of AChE was 49 h, with a rate of 1.4%/h. Worek et al. (2011) found that aging and spontaneous reactivation of POX-inhibited AChE followed first-order kinetics. The half-time of aging and spontaneous reactivation ranged from 30 to 43 h and from 28 to 46 h, respectively. The reactivation constant depends to a significant extent on the concentration of the inhibitor in the blood, that is, whether re-inhibition or spontaneous reactivation of AChE occurs to a greater extent (Kousba et al., 2004, Herkert et al., 2010). Eyer et al. (2003) found that the aging process of the diethyl phosphorylated AChE occurred in patients at a similar rate as *in vitro* studies.

In the brainstem, the reactivation of AChE was the lowest. At 15 min obidoxime and oxime K870 reactivated AChE by 10.0% or 13.7% and at 45 min by 2.3% or 2.2%. It is important to mention that the reactivation of AChE by oxime K870 was significant at all times except at 5 and 960 min and by obidoxime only at 15, 45, and 60 min. This is especially significant when it is known that central respiratory depression is the main cause of death in OPC poisoning and that the respiratory center is located precisely in the brainstem (Reddy et al., 2020, Herkert et al., 2010, Chuang et al., 2018). Even slight reactivation of AChE in the brain, particularly in the brainstem, can significantly improve survival.

Zdarova Karasova et al. (2022) showed that oxime K870 initially penetrates better into the brain and oxime K870 efficiently reactivates AChE in blood inhibited by POX. These results indicate that oxime K870 can enter the brain but is displaced and that total brain exposure to oxime K870 is low. This seems to be the main limitation of the oxime K870.

Kassa et al. (2021) found that neuroprotective efficacy of oxime K870 in tabun poisoning is slightly higher or similar compared to pralidoxime and HI-6, which is significant knowing that tabun-inhibited AChE is especially difficult to reactivate. In the sarin intoxication experiment, asoxime proved to be the best reactivator, followed by oxime K870 and pralidoxime, in all examined tissues (Zdarova Karasova et al., 2022). For tabun intoxication, oxime K870 was the most promising antidote over asoxime and pralidoxime in the blood and was slightly worse than asoxime in the diaphragm but showed minimal AChE reactivation in the brain. In the case of VX intoxication, all oximes showed similar reactivation in the blood, but asoxime and pralidoxime were better than oxime K870 for the reactivation of AChE in the diaphragm and brain. For POX poisoning, all oximes showed high and comparable AChE reactivation in the blood and generally low reactivation in the diaphragm (pralidoxime > asoxime > oxime K870), but oxime K870 was the only one able to reactivate AChE in the brain (Zdarova Karasova et al., 2022).

In this study, the greatest inhibition of AChE by POX as well as a much efficient reactivation of POX-inhibited AChE was found in erythrocytes. Both oximes were effective reactivators of POX-inhibited AChE and oxime K870 was more effective 960 min and more after poisoning. The highest reactivation of AChE in the blood is expected, given that the oximes themselves reach the highest concentration in the blood. The survival rate depends on the presence of an oxime in the bloodstream as soon as possible after intoxication. Oximes are more effective at reactivating AChE than BuChE, therefore more OPC bond to BuChE and other natural biological scavengers of OPC (Zorbaz et al., 2019). From this point of view, the pharmacokinetic profile of oxime K870 seems to be favorable: rapid absorption from muscle with a concentration of up to 20 mg/mL within 5 min, reaching a peak concentration of over 34 mg/mL within 20 min and an elimination half-life of 40 min. High AUCtotal in plasma (1485.78 ± 322.19 min × mg/mL) indicates a high potential to restore AChE/BuChE activity in blood in case of OPC poisoning. Kalász proposed that by increasing the dosage of oxime K870, it is possible to replace the rapid elimination of the compound with a steady-state concentration of oxime K870 in plasma (Kalász et al., 2020).

AChE inhibition in the diaphragm was lower, only in the 45 min it was at 19.3% of the baseline value. With the oximes, AChE values were maintained at more than 50% of baseline, indicating that a much greater degree of inhibition occurs in the CNS than at the neuromuscular junction, further supporting the fact that respiratory depression is of central rather than of peripheral origin (Houzé et al., 2019).

CarbE in plasma was better reactivated by obidoxime, but in the liver by oxime K870. CarbEs are esterases that are present to varying degrees in different species. Li et al. (2005) found that human plasma contains 4 esterases: BuChE, paraoxonase (PON1), albumin and only traces of AChE. Human plasma does not contain CarbE, unlike mouse, rat, rabbit, horse, cat and tiger. Mice also have, unlike humans, a significant amount of soluble AChE in their plasma (Li et al., 2005). The mouse has the highest activity of enzymes, followed by the rat, then by guinea pig (Maxwell, 1992). This is the first study that analyses reactivation of CarbE by oxime K870. Unfortunately, the reactivation of CarbE by oximes is less documented and investigated. Uncharged oximes (compounds like 2,3-butanedione monoxime (diacetylmonoxime) and monoisonitrosoacetone) were identified as effective reactivators for CarbE inhibited by a range of OPC, including phosphonates like sarin and soman. Cationic oximes (such as pyridine-2-aldoxime or the bis-pyridine aldoximes), while effective for AChE reactivation, showed poor reactivation capabilities for CarbE (Maxwell et al., 1994). Study in quails and chickens showed that oximes were good reactivators of CarbE inhibited by different OPC pesticides. HI-6 and HLö7 were superior to the DMB-4. TMB-4 and MMB-4 were weak reactivators of inhibited CarbE (Abass, 2015). The presence of CarbE affects the toxicity of the OPCs (Jokanović et al., 1996). Namely, when CarbE is inhibited, the toxicity of OPCs increases. Tri-ortho-cresyl-phosphate (TOCP) and its metabolite 2-(ortho-cresyl)-4H-1,2,3-benzodioxaphosphoran-2-one (CBDP) can inhibit CarbE (Jokanović et al., 1994). When CarbE is inhibited, OPC toxicity is multiplied (Jokanović, 1989). Therefore, the toxicity of OPC in rats should not only be observed via the inhibition of AChE, but also CarbE.

5. Conclusion

The oxime K870 is a less toxic reactivator when compared to obidoxime in rats. The reactivation effectiveness of oxime K870 was studied *in vivo* and it proved to be significantly better to obidoxime in particular in tissues after POX intoxication. Besides this, the oxime K870 generally showed a better degree of reactivation of AChE inhibited by POX. In addition, its co-administration with atropine was more effective than atropine-obidoxime combination in POX-poisoned rats. However, studies with other OPCs are needed to determine the reactivation potential of oxime K870.

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Author contributions

Conceptualization: ŽMM, KM, KK, RŠ, MPS
Data curation: ŽMM, STM, ĐĐ, NMK, SU, MD, KM, KK, DLS
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Disclosure statement

All authors declare that there is no conflict of interest.

Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

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ORCID

Žana M. Maksimović http://orcid.org/0000-0003-1190-6636
Sonja T. Marinković http://orcid.org/0000-0002-4512-9428
Đorđe Đukanović http://orcid.org/0000-0002-9887-9695
Nebojša Mandić-Kovačević http://orcid.org/0000-0003-1790-0729
Snežana Uletilović http://orcid.org/0000-0003-4219-5806
Mladen Duran http://orcid.org/0009-0004-6498-7059
Kamil Kuča http://orcid.org/0000-0001-9664-1109
Kamil Musilek http://orcid.org/0000-0002-7504-4062
Dragana Lončar-Stojiljković http://orcid.org/0000-0003-4875-3952
Ranko Škrbić http://orcid.org/0000-0002-6643-1781
Miloš P. Stojiljković http://orcid.org/0000-0001-9431-0736

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Acute organophosphate and carbamate pesticide poisonings – a five-year survey from the National Poison Control Center Of Serbia

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RESEARCH ARTICLE

Acute organophosphate and carbamate pesticide poisonings – a five-year survey from the National Poison Control Center Of Serbia

Žana M. Maksimović[a], Jasmina Jović-Stošić[b], Slavica Vučinić[b], Nataša Perković-Vukčević[b], Gordana Vuković-Ercegović[b], Ranko Škrbić[a,c] and Miloš P. Stojiljković[a,c]

[a]Center for Biomedical Research, Faculty of Medicine, University of Banja Luka, Banja Luka, Bosnia and Herzegovina; [b]National Poison Control Center, Medical Faculty, Military Medical Academy, University of Defense, Belgrade, Serbia; [c]Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine, University of Banja Luka, Banja Luka, Bosnia and Herzegovina

ABSTRACT

Pesticide poisonings, intentional as well as accidental, are common, especially in undeveloped and developing countries. The goal of this study was to analyze the clinical presentation of patients hospitalized due to acute organophosphate (OPP) or carbamate pesticide (CP) poisoning as well as to analyze the factors that potentially influenced the severity and outcome of the poisonings. A retrospective cross-sectional study was performed. The age and gender of each patient were recorded, the type of ingested pesticide, whether the poisoning was intentional or accidental, number of days of hospitalization, the severity of the poisoning, and the outcome of the treatment (i.e., whether the patient survived or not). Clinical aspects of poisonings were analyzed, as well as the therapeutic measures performed. 60 patients were hospitalized due to acute OPP or CP poisoning, out of 51 (85.00%) were cases of intentional self-poisoning. The majority of patients were poisoned by OPPs (76.67%), in one-third the causative agent was malathion, followed in frequency by chlorpyrifos and diazinon. Dimethoate poisonings were manifested with the most severe clinical picture. A 70% or lower activity of reference values of acetylcholinesterase and butyrylcholinesterase was found in 50% and 58% of patients, respectively. The most common symptom was miosis (58.33%), followed by nausea and vomiting. Pralidoxime reactivated acetylcholinesterase inhibited by chlorpyrifos or diazinon, but not with malathion or dimethoate. Impairment of consciousness and respiratory failure, as well as the degree of acetylcholinesterase and butyrylcholinesterase inhibition, were prognostic signs of the severity of poisoning. The lethal outcome was more often found in older patients ($t = 2.41$, $p = 0.019$). The type of ingested pesticide significantly affects the severity and outcome of poisoning as well as the effectiveness of antidotes.

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Introduction

Pesticides are products of chemical, biological, or natural origin whose purpose is to prevent, tackle and destroy harmful organisms and prevent their unwanted effects (Hu 2020). Pesticide poisonings, both accidental and intentional, are common, especially in undeveloped and developing countries. It is estimated that out of 3 million annual cases of pesticide poisonings, around 250 thousand are associated with lethal outcomes (WHO 2020).

Acetylcholinesterase inhibitors (AChEIs) – organophosphate (OPPs) and carbamate pesticides (CPs) constitute a significant part of all appliable pesticides. OPPs and CPs form a stable covalent bond with the serine amino acid residue at the active center of the enzyme acetylcholinesterase (AChE), which leads to its inhibition (Adeyinka *et al.* 2021). The inhibited AChE cannot perform its function – hydrolysis of the ester bond of acetylcholine (ACh) and, as a consequence, ACh accumulates in the synaptic cleft and exhibits acute cholinergic effects (Stojiljković 2019). Depending on the type of postsynaptic receptor, the following effects occur: muscarinic (bradycardia, bronchoconstriction, bronchorrhea, hypotension, increased motility of the gastrointestinal tract, abdominal cramps, miosis, hypersalivation), nicotinic (hypertension, tachycardia, fibrillations, fasciculations, skeletal muscle necrosis, diaphragm and intercostal muscle weakness progressing to peripheral respiratory failure) and central (tremor, incoordination, convulsions, central respiratory and cardiovascular depression, coma, death). The intermediate syndrome (weakness of neck flexor muscles, respiratory muscles, and muscles of the extremities) can occur 24–96 h after acute cholinergic crisis (Alahakoon *et al.* 2018), while delayed polyneuropathy can occur 2–5 weeks after the acute exposition (Jokanović *et al.* 2019). With long-term exposure to low doses of OPP, the chronic organophosphate-induced neuropsychiatric disorder can also occur. OPP and CP inhibit not only AChE but also butyrylcholinesterase (BuChE), that is, pseudocholinesterase, whose biological significance has not yet been sufficiently elucidated (Pope and Brimijoin 2018).

With energetic supportive measures (securing a breathing line), poison removal and detoxification (gastric lavage until

CONTACT Žana M. Maksimović zana.maksimovic@med.unibl.org Save Mrkalja 14, Banja Luka 78000, Bosnia and Herzegovina

obtaining clear lavage liquid, application of activated charcoal) and symptomatic therapy, specific treatment of OPP poisoning include administration of antidotes (atropine, oximes, diazepam) (Vanova *et al.* 2018, Eddleston 2019, Jokanović *et al.* 2020). Atropine, which has a 100 times stronger affinity for cholinergic receptors than ACh, alleviates the muscarinic effects but has no impact on the nicotinic ones. It is given for as long as there are signs of acute cholinergic crisis, that is until the signs of atropinization occur (Henretig *et al.* 2019). Oximes, AChE reactivators, bind to OPP already bound to AChE, which leads to the reactivation of AChE. The affinity of oximes for different organophosphorus compounds (OPCs) varies significantly. Globally, pralidoxime (PAM-2) is the oxime most frequently used for the treatment of OPP poisoning. Apart from that, in practice, obidoxime (LüH-6) is also used and it is considered the most effective oxime against OPP poisonings (Worek *et al.* 2020, Maksimović *et al.* 2021). Diazepam inhibits the excitability of the neurons in the CNS; by increasing the effect of GABA, it increases cAMP, decreases the level of cGMP, leading to the cessation of convulsions (Lundy and Magor 1978).

Unfortunately, in the Republic of Serbia, there are no comprehensive data about the annual number of pesticide poisoning. The most accurate data come from the National Poison Control Center (NPCC) of the Military Medical Academy (MMA), the only PCC in Serbia. In its 2018 Annual Report, it is stated that at the Emergency Department's Toxicology Ward 65 patients were examined based on a suspicion of acute pesticide intoxication, out of which 32 patients (49.23%) were hospitalized (Režić 2018).

The goal of this study was to analyze the clinical presentation of patients who were hospitalized with the diagnosis of acute OPP or CP poisonings at the NPCC of the MMA during the 5-year period, as well as to analyze factors that may have influenced the severity and the outcome of the poisoning.

Materials and methods

A retrospective cross-sectional study was performed. Patients who were hospitalized at the Clinic for Emergency and Clinical Toxicology, NPCC MMA (Clinic) during the 5-year period (1 September 2015 to 31 August 2020) were analyzed. This study followed the principles of the Declaration of Helsinki. Anonymity of the patients was preserved. Excel sheet was made without any personal data of patients – name, surname, initials, the exact date of birth or place of residence. Age and gender of the patient were recorded, as well as the type of the ingested pesticide, whether the poisoning was intentional or accidental, the number of days of hospitalization, the severity of the poisoning, and the outcome of treatment (whether the patient survived or not).

The following clinical aspects of poisoning on admittance were recorded: values of systolic, diastolic, and mean arterial pressure, heart rate, heart rhythm disorders, the existence of muscarinic (bronchoconstriction, bronchorrhea, vomiting, diarrhea, abdominal cramps, miosis, hypersalivation), nicotinic (fasciculations, fibrillations, rhabdomyolysis, respiratory failure) and central effects (tremor, incoordination, convulsions, disturbance of consciousness), as well as the appearance of the intermediate syndrome (weakness of neck flexor muscles, respiratory muscles, and muscles of the extremities 24–96 h after the acute cholinergic crisis) or the occurrence of the delayed polyneuropathy during the hospitalization.

Poisoning severity was determined by the Poisoning Severity Score (PSS) (Persson *et al.* 1998). PSS 0 represented poisoning without manifested symptoms and signs, PSS 1 mild, PSS 2 intermediate, PSS 3 severe, and PSS 4 lethal poisoning. The in-hospital fatality was considered a lethal outcome in this context.

Erythrocyte AChE and plasma BuChE activities were noted. Analyses were performed immediately upon admission to the Clinic, and thereafter every 6 h. Minimum enzyme activity was recorded, as well as whether the reactivation of AChE and BuChE occurred. The reference values for AChE and BuChE were 8090.6 ± 1976.7 IU/L and 14556.6 ± 4078.1 U/L, respectively (Zlatković *et al.* 2017).

Performed therapeutic measures were also recorded: antidote administration and their dosages (atropine, pralidoxime, diazepam), detoxification (lavage, activated charcoal), supportive measures (oxygenation, intubation, mechanical ventilation), as well as the administered symptomatic therapy.

The normality of data distribution was analyzed by applying the Kolmogorov-Smirnov test. Depending on the results of this test, statistical significance between the groups was analyzed primarily by appropriate parametric or nonparametric tests. Descriptive statistics consisted of expressing the data as a mean with its standard deviation (SD) and a 95% confidence interval (CI). Patients who lacked data on specific parameters were not included in the analysis. Parameters that had more than 10% of missing values were not analyzed. Categorical variables were compared using the Chi-square test, that is, Fisher exact test if one of the categories had less than 5 variables. Comparison of continual variables was performed using Student's *t*-test for two categories, that is, one-way analysis of variance for several categories for parametric data, and Man–Whitney *U*-test, or Kruskal–Wallis test for non-parametric data. Two continuous variables were analyzed using correlations – Pearson's for parametric and Spearman's for nonparametric variables. The statistical significance level was set at $p < 0.05$. Data were processed using IBM SPSS 18.0 software.

Results

During the 5-year period in the Emergency Department's Toxicology Ward, MMA a total of 345 patients were examined based on a suspicion of acute pesticide intoxication, out of which 134 were admitted to the Clinic. Out of that number, 60 patients were diagnosed with acute OPP or CP poisonings. 51 (85.00%) patients had intentional self-poisoning, 7 (11.67%) had accidental self-poisoning, and in 2 (3.33%) patients there was a suspicion of poisoning by other people. All intoxications were oral.

Gender-wise, out of 60 patients, 29 (48.33%) were male, and 31 (51.67%) were female. The average age of patients

was 52.03 ± 16.93 years, range 16–91 (95% CI: 47.66–56.41), with normal distribution.

Most poisonings were caused by OPP (76.67%), in one-third of the cases the causative agent was malathion, followed by chlorpyrifos and diazinon (Table 1). The CP poisoning was present in 15% of patients and in 5 patients (8.33%) the type of pesticide was not determined, although they presented with a typical clinical picture of OPP/CP poisoning and/or had low values of AChE/BuChE.

AChE inhibition significantly depended on the type of pesticide in question (Kruskal–Wallis test: $\chi^2 = 17.56$, $p = 0.025$), and so was the case with BuChE ($\chi^2 = 19.27$, $p = 0.013$). With further post-hoc analysis (Man–Whitney *U*-test), it was determined that dimethoate inhibited AChE significantly more than malathion or methomyl. Chlorpyrifos inhibited BuChE significantly more than methomyl and malathion. Diazinon inhibited BuChE significantly more than malathion, dimethoate, methomyl, and carbofuran ($p < 0.05$).

A 30% or higher inhibition of reference values of AChE and BuChE was found in 80% and 85% patients, respectively. A 50% or higher inhibition of reference values of AChE and BuChE was found in 67% and 70% of patients, respectively, while 70% or higher inhibition of reference values of AChE and BuChE was found in 50% and 58% of patients, respectively.

Table 1. Type of pesticide and average remaining activity of AChE and BuChE.

Pesticide	*n*	%	AChE (IU/L) (mean ± SD)	BuChE (U/L) (mean ± SD)
OPP	46	76.67	3133 ± 2682	3943 ± 4246
Malathion	19	31.67	4712 ± 2999	5994 ± 4188
Chlorpyrifos	12	20.00	2650 ± 2652	2802 ± 4622*
Diazinon	9	15.00	2790 ± 2256	794 ± 198*
Dimethoate	4	6.67	958 ± 415*	3983 ± 4529
Phorate	1	1.67	630	852
Methidathion	1	1.67	548	6775
CP	9	15.00	3523 ± 2468	5767 ± 3745
Methomyl	6	7.50	3716 ± 2961	5674 ± 4011
Carbofuran	3	3.75	3137 ± 1450	5954 ± 3975
Unknown	5	8.33	4931 ± 2848	8740 ± 5429
Total	60	100.00	3328 ± 2653	4590 ± 4386

AChE: acetylcholinesterase; BuChE: butyrylcholinesterase; OPP: organophosphate pesticide; CP: carbamate pesticide; AChE: reference values: 8090.6 ± 1976.7 IU/L; BuChE 14556.6 ± 4078.1 U/L.

*Kruskal–Wallis test AChE: $\chi^2 = 17.56$, $p = 0.025$, BuChE: $\chi^2 = 19.27$, $p = 0.013$.

Post-hoc Man–Whitney *U*-test: dimethoate inhibited AChE significantly more than malathion and methomyl. Chlorpyrifos inhibited BuChE significantly more than methomyl and malathion. Diazinon inhibited BuChE significantly more than malathion, dimethoate, methomyl and carbofuran ($p < 0.05$).

Clinical picture

Most of the patients (81.66%) were treated in hospital for 2–8 days, for a minimum of one and for a maximum of 50 days. On average, the hospital treatment lasted 6.87 ± 7.76 days (95% CI: 4.86–8.87).

Numerous symptoms and signs of AChEI poisoning are shown in Table 2. The most common symptom was miosis (58.33%) followed by nausea and vomiting, which were the only symptoms of poisoning in 4 patients. In spite of the proven exposure to AChEI (either by detecting AChEI in the blood or by finding a significant inhibition of AChE and BuChE), four patients (6.67%) showed no sign or symptom of poisoning.

The intermediate syndrome was recorded in 11 patients and it was more common in dimethoate poisoning ($\chi^2 = 7.18$, $p = 0.029$). Polyneuropathy was diagnosed in two patients who were hospitalized for longer than 15 days.

Poisoning severity and outcome

Poisoning severity was determined by the Poisoning Severity Score (PSS), which is shown in Table 3 along with the corresponding activities of AChE and BuChE.

A strong negative correlation was found between the poisoning severity and both AChE (Spearman Rho: $r = -0.589$, $p < 0.001$) and BuChE activity values ($r = -0.346$, $p = 0.010$). Although lower AChE and BuChE values were observed in lethal poisonings, the difference was not significant

Table 3. Poisoning severity and activities of cholinesterases in patients poisoned with organophosphate or carbamate pesticides.

PSS[a]	*n*	%	AChE (UI/L) Mean ± SD*	BuChE (U/L) Mean ± SD**
PSS 0	4	6.67	5825 ± 2603	8273 ± 2842
PSS 1	21	35.00	4260 ± 2080	6423 ± 4836
PSS 2	17	28.33	2923 ± 2998	3460 ± 4279
PSS 3	14	23.33	2138 ± 2355	2808 ± 2971
PSS 4	4	6.67	1027 ± 743	1329 ± 101
Total	60	100.00	3328 ± 2653	4590 ± 4386

AChE: acetylcholinesterase; BuChE: butyrylcholinesterase; AChE: reference values: 8090.6 ± 1976.7 IU/L; BuChE 14556.6 ± 4078.1 U/L.

[a]PSS: poisoning severity score, PSS 0: poisoning without symptoms and signs, PSS 1: mild, PSS 2: moderate, PSS 3: severe, PSS 4: lethal poisoning.

*Strong negative correlation between AChE values and severity of poisoning: Spearman Rho: $r = -0.589$, $p < 0.001$.

**Strong negative correlation between BuChE value and severity of poisoning: Spearman Rho: $r = -0.380$, $p = 0.004$.

Table 2. Signs of poisoning in patients poisoned with organophosphate or carbamate pesticides.

Cholinergic effects								
Muscarinic	*n*	%	Nicotinic	*n*	%	Central	*n*	%
Miosis	35	58.33	Fasciculations	17	28.33	Impairment of consciousness	22	36.67
Nausea and/or vomiting	32	53.33	Hypertension	16	26.67	Somnolence	11	18.33
Bronchoconstriction and/or bronchorrhea	22	36.67	Tachycardia	15	25.00	Stupor	4	6.67
						Coma	7	11.67
Sweating	17	28.33	Respiratory muscle weakness	8	13.33	Central respiratory depression	10	16.67
Diarrhea and/or incontinence	16	26.67				Tremor	4	6.67
Hypersalivation	12	20.00				Psychomotor anxiety	3	5.00
Hypotension	9	15.00				Seizures/ convulsions	2	3.33
Bradycardia	7	11.67						
Abdominal cramps	3	5.00	Intermediate syndrome	11	18.33	Delayed polyneuropathy	2	3.33
Lacrimation	3	5.00						

Table 4. Pesticide type and poisoning severity score.

	Poisoning severity score (PSS)* – *n* (%)					
Pesticide	0	1	2	3	4	*n* (%)
OPP	4 (8.70)	16 (34.78)	14 (30.43)	10 (21.74)	2 (4.35)	46 (76.67)
Malathion	4 (21.05)	8 (42.11)	4 (21.05)	3 (15.79)	0	19 (31.67)
Chlorpyrifos	0	6 (50.00)	4 (33.33)	1 (8.33)	1 (8.33)	12 (20.00)
Diazinon	0	2 (22.22)	6 (66.67)	1 (11.11)	0	9 (15.00)
Dimethoate	0	0	0	3 (75.00)	1 (25.00)	4 (6.67)
Phorate	0	0	0	1 (100.00)	0	1 (1.67)
Methidathion	0	0	0	1 (100.00)	0	1 (1.67)
CP	0	4 (44.44)	1 (11.11)	3 (33.33)	1 (11.11)	9 (15.00)
Methomyl	0	2 (33.33)	1 (16.67)	3 (50.00)	0	6 (7.50)
Carbofuran	0	2 (66.67)	0	0	1 (33.33)	3 (3.75)
Unknown	0	1 (20.00)	2 (40.00)	1 (20.00)	1 (20.00)	5 (8.33)
Total	4 (6.67)	21 (35.00)	17 (28.33)	14 (23.33)	4 (6.67)	60 (100.00)

*PSS 0: poisoning without symptoms and signs, PSS 1: mild, PSS 2: moderate, PSS 3: severe, PSS 4: lethal poisoning. OPP: organophosphate pesticide; CP: carbamate pesticide.

(Man–Whitney *U*-test = 30.00, $p = 0.064$ and U = 44.00, $p = 0.712$, respectively).

Age was not correlated with the severity of poisoning ($F = 1.46$, $p = 0.226$), but patients who did not survive were significantly older ($t = 2.41$, $p = 0.019$). Gender did not influence severity ($\chi^2 = 5.41$, $p = 0.262$) or poisoning outcome ($\chi^2 = 1.22$, $p = 0.346$).

Type of the OPP and CP significantly influenced the severity of poisoning ($\chi^2 = 32.32$, $p = 0.04$), but not the outcome ($\chi^2 = 9.64$, $p = 0.291$). Lethal cases were found with different OPP and CP (chlorpyrifos, dimethoate, carbofuran) (Table 4).

Malathion was the only OPP where cases without clinical symptoms/signs of poisoning were reported ($n = 4$). In a non-surviving patient poisoned by chlorpyrifos, the ingestion of the pesticide happened around 10 h before the first medical treatment was administered and severe respiratory insufficiency, nicotinic and central effects were already present. The lethal outcome in this patient was mainly due to the too long a period of time between the ingestion and treatment. Dimethoate, despite being the causative agent in only 4 patients, caused severe poisoning in all of them, with one being lethal. Intermediate syndrome occurred in 3 out of 4 patients. Lethal outcome occurred in a patient with severe hypotension, coma, and respiratory failure.

The only patient with a lethal outcome due to CP poisoning was intoxicated with carbofuran. In this patient, symptoms, and signs of acute CP poisoning have withdrawn rapidly, as a consequence of the fast spontaneous reactivation of cholinesterase, but the clinical picture was further complicated by the extensive aspiration pneumonia, perforation of peptic ulcer, and acute myocardial infarction.

Among the clinical symptoms/signs of poisoning, correlations were found only between the oxygen saturation (SpO_2) and poisoning severity ($r = -0.624$, $p < 0.001$) and state of consciousness and poisoning severity ($r = 0.729$, $p < 0.001$). Mild cases of poisoning were most often accompanied by muscarinic effects only. Nicotinic ($\chi^2 = 18.37$, $p = 0.001$) and especially CNS effects were reported in more severe poisoned patients ($\chi^2 = 40.11$, $p < 0.001$). All lethal cases were associated with CNS effects. Significantly lower values of blood pressure (U = 31.50, $p = 0.012$) and SpO_2 (U = 11.50, $p < 0.001$) were registered in lethal poisonings, as well as severe consciousness disorder (U = 22.50, $p = 0.004$). The intermediate syndrome was recorded only in severely poisoned patients ($\chi^2 = 19.35$, $p = 0.001$). In three patients, the intermediate syndrome only manifested with neck and shoulder muscle weakness, while in other patients it was accompanied by respiratory insufficiency as well, and in two patients with lethal outcome.

Table 5. Performed therapeutic measures in patients with organophosphate or carbamate pesticide poisoning.

Therapeutic measures	*n*	%
Antidotes		
Atropine	35	58.33
Pralidoxime	24	40.00
Diazepam	26	43.33
Detoxification		
Gastric lavage	35	58.33
Activated charcoal	10	16.67
Supportive therapy		
Mechanical ventilation	13	21.67
Oxygen	14	23.33
PPI	19	31.67
Symptomatic therapy		
Corticosteroids	9	15.00
Bronchodilators	3	5.00
Bicarbonates	12	20.00
Dopamine	2	3.33
Total	60	100.00

PPI: Proton-pump inhibitors.

Treatment

Performed therapeutic measures are shown in Table 5. Atropine was administered in 35 patients and the maximal dosage during the whole period of hospitalization for a single patient was 756 mg. In 22 patients atropine was administered prehospital. Pralidoxime was administered in 24, and diazepam in 26 patients. No antidote was given to 21 (35.00%) patients and all three antidotes (e.g., atropine, pralidoxime, and diazepam) to 19 (31.67%) patients. Respiratory insufficiency was in such a stage that it required intubation and mechanical ventilation in 13 patients, while hypotension required dopamine stimulation in 2 patients.

The severity of poisoning was the main factor determining the application of antidotes - atropine ($\chi^2 = 15.08$, $p = 0.003$), pralidoxime ($\chi^2 = 14.57$, $p = 0.006$), diazepam ($\chi^2 = 27.66$, $p < 0.001$) (Table 6). Also, all three antidotes were more often

Table 6. Poisoning severity score (PSS) and antidote administration.

	Atropine	Pralidoxime	Diazepam	
PSS*		*n* (%)		*n*
PSS 0	0	0	0	4
PSS 1	6 (28.57)	1 (4.76)	3 (14.29)	21
PSS 2	12 (70.59)	11 (64.71)	10 (58.82)	17
PSS 3	12 (85.71)	11 (78.57)	9 (64.29)	14
PSS 4	4 (100.00)	3 (75.00)	2 (50.00)	4
Total	34 (56.67)	24 (40.00)	24 (40.00)	60

*PSS 0: poisoning without symptoms and signs, PSS 1: mild, PSS 2: moderate, PSS 3: severe, PSS 4: lethal poisoning; *n*: number of patients.

Table 7. Pralidoxime administration and cholinesterase reactivation.

		AChE (%)				BuChE (%)			
OPP	Oxime	Nor	Inh	Reac	*p*	Nor	Inh	Reac	*p*
Malathion	No	68.75	18.75	12.50	0.296	46.67	53.33	0.00	0.124
	Yes	33.33	33.33	33.33		0.00	66.67	33.33	
Chlorpyrifos	No	50.00	25.00	25.00	0.018	50.00	50.00	0.00	0.133
	Yes	0.00	0.00	100.00		0.00	100.00	0.00	
Diazinon	No	66.67	33.33	0.00	0.012	0.00	100.00	0.00	1
	Yes	0.00	0.00	100.00		40.00	60.00	0.00	
Dimethoate	No	0.00	100.00	0.00	1	0.00	100.00	0.00	1
	Yes	0.00	100.00	0.00		33.33	66.67	0.00	
Total	No	52.78	19.44	27.78	0.001	47.06	44.12	8.82	0.138
	Yes	8.33	25.00	66.67		14.29	76.19	9.52	

%: percentage of total number of patients; OPP: organophosphate pesticide; AChE: acetylcholinesterase; BuChE: butyrylcholinesterase; Nor: values in normal range; Inh: esterases remained below normal range after treatment; Reac: reactivated esterases (values returned to normal range) after treatment.

administered in more severe cases of poisoning ($\chi^2 = 33.80$, $p < 0.001$).

AChE was more frequently reactivated in patients treated with pralidoxime ($\chi^2 = 13.36$, $p = 0.001$) (Table 7). Pralidoxime significantly improved AChE reactivation in chlorpyrifos and diazinon poisoning ($p = 0.018$ and $p = 0.012$, respectively). BuChE was not reactivated more when pralidoxime was used ($\chi^2 = 6.41$, $p = 0.138$).

Discussion

During the 5-year period, 60 patients were hospitalized due to acute poisoning with OPP or CP. Most of the patients (85%) took the pesticide intentionally, with an intention to commit suicide. Suicidal poisoning with pesticides represents a serious medical problem, especially in undeveloped countries, where it is a common means of suicide attempt and the most common method of suicide attempts with fatal outcomes (Kamaruzaman *et al.* 2020). Among the suicide attempts with fatal outcomes, 30% of them in India, 62% in China, and 71% in Sri Lanka were due to pesticide poisonings (WHO 2020). In Serbia, fatal self-poisonings with pesticides keep getting less frequent, which could be explained by the introduction of laws that had prohibited the use of most toxic pesticides in agriculture, the measure which proved efficient in other countries, as well (Rathish *et al.* 2018, Boucaud-Maitre *et al.* 2019).

In this clinical study, an equal distribution between genders was found, similarly as in earlier data obtained from the same Center (Vučinić *et al.* 2018). Studies from other countries show that in developed countries, cases of pesticide poisonings are three times higher in males than in females, while in less developed countries this ratio is close to 50:50 (Krakowiak *et al.* 2019, Tong *et al.* 2020). It is known that women attempt suicide more often than men, but the suicidal rate is higher in men because they often tend to use more lethal methods. However, when pesticide poisonings are concerned, the death rate is equal between genders (Mergl *et al.* 2015). In older patients, the lethal outcome was more frequent, which corresponds to results of other researchers (Rathish *et al.* 2018, Boucaud-Maitre *et al.* 2019, Krakowiak *et al.* 2019, Kamaruzaman *et al.* 2020).

The frequencies of specific symptoms/signs vary significantly among the studies (Amir *et al.* 2020, Reddy *et al.* 2020). The differences are to be expected when taken into consideration the vast number of variables that affect the frequency of symptoms/signs and when the availability of specific pesticides in a particular country is taken into consideration. The methodology of collecting and processing data varies significantly among the studies, from observing a specific pesticide, to monitoring all pesticides, analyzing only the acute exposure, studying only the intentional self-poisoning cases, etc.

A smaller number of cardiovascular muscarinic effects explains a relatively small percentage of patients treated with atropine (58.22%). Besides, in 35% of cases, no antidote was administered. This unexpectedly high percentage is probably due to the considerable percentage of patients with mild or no symptoms of poisoning. The maximum administered dosage of atropine to a patient was 756 mg. In most cases where atropine was given, the dose did not exceed 10 mg. Recommendations on when and how much atropine to administer vary from one guideline to another (Chuang *et al.* 2018). It is recommended that the atropinization should be achieved and maintained (heart rate above 100 per minute, systolic blood pressure above 90 mmHg, clear lung fields by auscultation). Perera *et al.* (2008) showed that *ad hoc* high dose atropine regimens are associated with more frequent atropine toxicity without any obvious improvement in patient outcome compared with doses titrated to clinical effect (1.5–3 mg of atropine with doses doubling every 5 min until atropinization is achieved). Cases of OPP poisoning have been reported where extremely high doses of atropine were given – LeBlanc *et al.* (1986) administered as much as 30 g of atropine to a patient poisoned with dimethoate, over a period of 5 weeks.

Some researchers have suggested nicotinic receptor antagonists could and should be used in the treatment of OP poisoning (Bird *et al.* 2016), but a study by Dhanarisi *et al.* (2020) has found no evidence that rocuronium in addition to standard therapy reduced the duration of intubation. It suggested that it even possibly worsened neuromuscular junction function. It is recommended that diazepam should be administered in addition to atropine. Several patients received atropine without diazepam and these were patients to whom atropine was administered prehospital. This implies that additional education on the treatment of acute OPP poisoning is necessary for doctors in emergency departments. Studies showed that diazepam should be administered to patients with more severe signs of OP poisoning. Diazepam

may be of particular benefit to patients with convulsions and fasciculations (Marrs 2003).

Although oximes have undoubtedly proved efficiency in improving survival rates in *in vivo* studies in animals, this notion was not sufficiently confirmed in clinical settings (Rahimi *et al.* 2006). Treatment with oximes is complicated by the fact that in case of intentional ingestion of pesticides, the patient often takes a dose 100 times higher than a lethal one. A high concentration of poison present in the blood constantly re-inhibits previously reactivated esterases (Buckley *et al.* 2011). In addition to the type of pesticide, the concentration of pesticides is the main factor that will affect the severity and outcome of poisoning. Numerous factors influenced the fact that the concentration of pesticides was not analyzed in this study: small sample, different types of pesticides, different samples were taken for analyses (serum, urine, liquid from gastric lavage), sometimes only the presence of pesticide in the sample was proven, etc.

More lipophilic AChEIs are being redistributed in fat tissue and they gradually reenter the blood, inhibiting esterases and prolonging their inhibition (Tsatsakis *et al.* 1998). Although it was obidoxime that turned out to be the most efficient oxime against OPPs in experimental animals, pralidoxime still remains the most commonly used and available oxime in clinical settings (Vučinić *et al.* 2018). One of the reasons is the hepatotoxicity of obidoxime that limits the use of its higher and presumably more efficient doses in humans (Balali-Mood and Shariat 1998).

An additional problem is the unequal efficacy of oximes in acute OPC poisonings. The phosphorylation occurs by the loss of the 'leaving group' of the OPC and the formation of a covalent bond with AChE through the serine hydroxyl group. Specificity of the 'leaving group' contributes to the complexity of toxic response to OPC (Antonijević *et al.* 2005). Obidoxime and pralidoxime are more efficient in reactivating AChE inhibited by dimethyl and diethyl OPPs, respectively (Jokanović and Stojiljković 2006). AChE inhibited by several OPP (dimethoate, triamiphos, profenofos, fenamiphos) resists any attempt of reactivation with any oxime, probably due to variations in the phosphoryl moiety and distribution of electronic charge (Antonijević and Stojiljković 2007). Eddleston *et al.* (2005) found that AChE inhibited by dimethoate or fenthion responds poorly to pralidoxime treatment, as compared with the chlorpyrifos-inhibited AChE.

It is recommended that every patient poisoned with OPP that needs atropine, should receive an oxime as well, whereas in CP poisoning there are no universal recommendations (Silberman and Taylor 2020). Oximes should be given for as long as there is an AChE inhibition and until there are AChEI metabolites found in urine. Recommendations are that it would be useful to develop protocols for the specific treatment of an individual OPP. Treatment of OPP poisoning is further complicated by the fact that the toxicity of OPPs varies significantly among animal species, including humans (Eddleston *et al.* 2005). The difference in toxicity of OPPs also varies, regarding the lipophilicity of OPP, its selectivity for AChE, leaving groups that are bound to phosphate.

The study that analyzed the healthy population of Serbia determined that normal values of AChE and BuChE in blood were 8090.6 ± 1976.7 IU/L and 14556.6 ± 4078.1 U/L, respectively (Zlatković *et al.* 2017). In this study, as well as in studies by other researchers, there is a clear correlation between the degree of inhibition of AChE and BuChE and the severity of the poisoning (Reddy *et al.* 2020). Although the values of cholinesterase activity strongly correlate with the clinical presentation, severity, and outcome of the poisoning, in practice significant deviations are found more often than not. For example, a patient from this study who had the lowest value of AChE, only 160.00 IU/L, had a moderately severe poisoning and was released home completely recovered. From the clinical aspect, the degree and the duration of inhibition of AChE and BuChE is much more important for the length of treatment and supervision of the patient and not for reliable prediction of the outcome of the poisoning (Soliday *et al.* 2010). There are significant individual differences in the values of AChE and BuChE. The availability of mobile devices for measuring the value of AChE (ChE mobile), would enable the measurement of values immediately in the field and better monitoring of response to therapy (Shihana *et al.* 2019). BuChE inhibition significantly varies among the different OPPs. Values of BuChE are significant only when the OPP is known.

Previous research has shown that respiratory insufficiency is the main cause of death in OPP and CP poisonings, therefore securing an airway is extremely important (Chuang *et al.* 2018, Kharel *et al.* 2020, Reddy *et al.* 2020). Basic supportive measures and symptomatic therapy are crucial for patient survival as well as adequate education of healthcare workers, especially in rural areas where pesticide poisonings most often occur (Gunnell *et al.* 2007). The appearance of intermediate syndrome further complicates the clinical picture and very often leads to respiratory failure requiring tracheal intubation and mechanical ventilation (Alahakoon *et al.* 2018, Haliga *et al.* 2018).

Delayed polyneuropathy was recorded in two patients (3.33%) who have been hospitalized for a prolonged period of time. Studies show that delayed polyneuropathy is not rare in OPP and CP poisonings and that around one-third of patients who survive acute cholinergic crisis develop neuropathy within 6 months (Pannu *et al.* 2021).

Gastric lavage was performed in 35 patients and activated charcoal was administered in 10. The discussion on whether to perform the gastric lavage or to give activated charcoal only is still ongoing (Adeyinka *et al.* 2021). Lavage can be performed if the patient contacted the medical staff within 1 h after ingestion, while others believe that OPP poisoning is an exception to that rule and that lavage can be done up to 3 h after ingestion (Olson 2018). Lavage should not be performed if the patient is unconscious and if he/she does not have a secured airway, due to the risk of aspiration (Olson 2018).

There is still no clear recommendation regarding plasma alkalinization as part of the treatment protocol for OPP poisoning, despite there being studies that showed potential benefits of such a protocol (Stefanovic *et al.* 2006, Eddleston 2019). In the present study, bicarbonates were administered in 12 patients (20.00%), but they were mainly used to treat the acid-base disorder.

Most of the poisonings were caused by OPPs, with malathion and chlorpyrifos comprising more than half of all cases. Fortunately, malathion (S-[1,2-dicarbethoxyethyl] O,O-dimethyl phosphorodithioate) is one of the least toxic OPPs. Taking into consideration that malathion is less toxic compared to other OPPs, its use is widespread in agriculture, so the patients poisoned with malathion are more frequent than those intoxicated with other OPPs (Ozsoy *et al.* 2016, Bogen and Singhal 2017, Badr 2020). Although it is registered as a pesticide, in some countries malathion is used for treating pediculosis as well (Idriss and Levitt 2009). Pralidoxime showed limited efficacy in reactivating AChE inhibited by malathion, in this study as well as in the others (Severcan *et al.* 2019).

Chlorpyrifos (0,0-diethyl 0-[3,5,6-trichloro-2-pyridinyl]-phosphorothioate) poisonings were most often of light and moderate degree, with one severe and one lethal poisoning. Chlorpyrifos is the most frequently sold OPP in the world (Burke *et al.* 2017). Liu *et al.* (2020) found lethality of 15% in chlorpyrifos-poisoned patients. Fatal outcome was found in patients with developed respiratory insufficiency, prolonged QT interval, and kidney failure. In this study, AChE inhibited by chlorpyrifos was reactivated by pralidoxime, similar to the results from other relevant reports (Eddleston *et al.* 2005).

Diazinon (O,O-diethyl O-[4-methyl-6-(propan-2-yl)pyrimidin-2-yl] phosphorothioate) is highly lipophilic and moderately toxic OPP. Hydrolyzed diazinon is more toxic and less liposoluble. For this reason, manufacturers frequently use products like soybean oil as stabilizers that prevent hydrolysis of diazinon (Moshiri *et al.* 2013). Pralidoxime successfully reactivated AChE inhibited by diazinon, in this study as well as in studies performed by others (Shlosberg *et al.* 1976).

Dimethoate, although the cause of poisoning in only 4 patients, resulted in severe poisonings in all of them, one of which was lethal. Fortunately, dimethoate poisoning was less frequent than in previous years, when dimethoate poisonings accounted for 20% of all OPP poisonings (Vučinić *et al.* 2018). Dimethoate has a much higher affinity for AChE than for BuChE, consequently a much higher degree of inhibition of this enzyme and manifestation of the full clinical picture of OP poisoning occur. Dimethoate has low-fat solubility, which results in its high concentration in blood (Eddleston *et al.* 2005). Although dimethoate exerts a similar acute toxicity profile to chlorpyrifos in animals, mortality from dimethoate is three times higher in humans. Dimethoate causes severe hypotension that is resistant to inotropic drugs (Davies *et al.* 2008). AChE inhibited by dimethoate is resistant to oxime treatment, which was the case in this study, as well.

Clinical studies show that the mortality rate associated with intentional poisonings with carbofuran is around 2.2% and that it is lower compared to other CPs and OPPs (Lamb *et al.* 2016). Methomyl poisoning was recorded in 6 patients, half of whom had severe intoxications. The majority of developed countries prohibited the usage of methomyl due to its high toxicity, but cases of methomyl poisoning are still being recorded even in countries where its usage is prohibited (Boucaud-Maitre *et al.* 2019). Sri Lanka, a country where pesticides are the most common cause of suicidal poisonings, has prohibited the use of carbofuran, carbaryl, dimethoate, and chlorpyrifos in 2014, reducing the morbidity and mortality rate of pesticide poisonings by 50% within 3 years (Rathish *et al.* 2018). Likely, other, less toxic OPPs will replace them and become more commonly used. The case fatality from OPP poisonings is higher compared to other pesticides in the same WHO Hazard Class, such as neonicotinoids, pyrethroids, and other non-cholinesterase pesticides (Rambabu *et al.* 2021). AChE inhibited by CP will be spontaneously reactivated and oxime administration is not recommended. In carbaryl intoxication, oximes even potentiate its toxicity (Faragó 1969, Lieske *et al.* 1992).

The toxicity of OPPs and CPs depends on the formulation of given preparation, taking into account that pesticides are often dissolved in organic solvents that are toxic themselves. Organic solvents lead to the depression of CNS, which manifests itself in different levels of consciousness disorder (Dick 2006, Eddleston *et al.* 2012).

Numerous factors affect both the severity and the outcome in patients with OPPs and CPs poisonings. In this study, the severity of poisoning was in relation to impairment of consciousness and respiratory failure, as well as the degree of AChE and BuChE inhibition. The lethal outcome was more often in older patients.

A major limitation of the presented study is that it is a retrospective study, where no certain cause/effect relationship can be established. Besides, the precise dose of ingested pesticide could not be determined. This study showed the potential influence of various factors on the severity and outcome of poisoning. A comprehensive prospective clinical study is needed, which will consider all factors influencing the severity and outcome of poisoning. Numerous dilemmas need to be clarified in the treatment of patients poisoned with OPPs and CPs.

Conclusion

The type of ingested pesticide significantly affects the severity and outcome of the poisoning as well as the effectiveness of antidotes. In this study pralidoxime reactivated AChE inhibited by chlorpyrifos or diazinon, but not with malathion or dimethoate. Dimethoate poisonings were more severe compared to other OPPs and CPs. A clear protocol that would take into account the causative agent of poisonings with a different type of OPPs or CPs would potentially be helpful to physicians in clinical practice.

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