Acta Veterinaria (Beograd), Vol. 51. No. 2-3, 187-196, 2001.

UDK 619:616-009

COMBINED DECONTAMINATION AND PARENTERAL THERAPY IMPROVE THE CHANCES OF SURVIVAL IN RATS PERCUTANEOUSLY POISONED WITH ORGANOPHOSPHORUS INSECTICIDES

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(Received 12. February 2001)

The effect of decontamination by alcoholate and parenteral therapy per se (oximes PAM-Cl and HI-6, cholinolytic, anticonvulsant), as well as their combination was tested in rats percutaneously poisoned with terbufos, parathion and dichlorvos. Decontamination, performed 5 minutes after exposure of the skin to the poison exerted a very high degree of efficacy in rats poisoned by the indirect inhibitors of acetylcholinesterase, terbufos (protection ratio 36.6) and parathion (protection ratio 11.3). This treatment was also effective in rats percutaneously poisoned by the direct inhibitor of acetylcholinesterase, dichlorvos, with the protection ratio of 9.3. Parenteral therapy alone, applied 4 minutes after the exposure, did not give the expected results, regardless of the oxime used, either for terbufos (protection ratio 1.1 with PAM-2 Cl, and 1.0 with HI-6, respectively), or for parathion poisoning (protection ratio 1.2 with PAM-2 Cl, and 1.0 with HI-6, respectively) Moreower, with contrary to dichlorvos posoning, the protective ratio achieved with PAM-2 Cl was 7.1, and with HI-6 6.8. The combined procedure (decontamination plus parenteral antidotes) in rats percutaneously poisoned by terbufos and parathion did not lead to any improvement over decontamination alone. For dichlorvos poisoning, however, decontamination and parenteral antidotes produced supraadditive synergism. Namely with the oxime PAM-2 Cl the protective ratio was 52.6, and with HI-6 40.6. The results of the study suggest that in percutaneous contamination by organophosphorus insecticides, timely decontamination appreciably improved the chances of survival, particularly if combined with the use of antidotes (oxime, cholinolytics, anticonvulsants).

Key words: decontamination, insecticides, parentheral therapy, percutanous poisoning

INTRODUCTION

Throughout the world organophosphorus insecticides (OP) are used to control different pest in agriculture as well as in veterinary and human medicine

(Marquis, 1986; Murphy, 1986; Ward and Glicksberg, 1971). They are also employed as herbicides and fungicides. Although synthesised in the early 19th century, it was not until 1932. that the effects were discovered (Wagner, 1989).

Some of the OPs, such as O,O-dimethyl-2,2-dichlorovinyl phosphate (dichlorvos), are administrated orally to livestock to control internal parasites such as bot larvae. Several others are used externally to control parasites on animal and human skin. Although their effects are beneficial, they put varied sections of the population at risk of exposure. Chronic poisoning is frequent and there are also accidental cases (Jeyaratman, 1990; Chaturvedi *et al.*, 1991; Reichert *et al.*, 1978; Shah and Guthrie, 1986) and suicidal occurrences (Van der Hoek *et al.*, 1998; Agarwal, 1993), as well as environmental exposure (Dancan and Griffith, 1985; Durham *et al.*, 1972; Quinby and Lemmon, 1958; Spear *et al.*, 1977). In humans and animals, OP can be absorbed through the skin, can be inhaled, or can enter the body through direct ingestion. Skin absorption is a slow process, so significant absorption occurs only after prolonged contact with the pesticide (Anonymous, 1991). Absorption is considerably faster if the skin is inflamed; thus, dermatitis could lead to much more serious poisoning than would normally occur.

OPs act through inhibition of acetylcholinesterase (AChE), causing the accumulation of acetylcholine at synaptic terminals and extreme stimulation of postsynaptic nerve endings (Murphy, 1986). Symptoms may vary with the dose and the specific type of nerve cells that are affected. Generally, the toxic effects can be broken down into three broad categories: 1) effects on smooth muscles, including the heart and endocrine glands (muscarinic receptors); 2) effects on motor nerve endings in skeletal muscles and autonomic nervous system (nicotinic signs); and 3) central nervous system effects (Timbrell, 1991; Bardin *et al.*, 1994).

Some OP, such as mevinphos, phosphamidon or dichlorvos act quickly since they are direct inhibitors of AChE, while others, such as O,O diethyl-O-(4-nitrophenyl) phosphorothionate (parathion), trichlorfon, malathion or phosphorodithionic acid, S-(tert-butylthio) methyl O,O-diethyl ester (terbufos) exert their action slowly, after metabolic conversion into active forms (Jeyaratman 1990; Chaturvedi *et al.*, 1991). Parathion exerts its action after undergoing metabolic conversion by hepatic mixed-function oxidase enzymes systems into an active, more toxic substance - paraoxon (Gage, 1953; O'Brein, 1960). They are highly lipid-soluble compounds and therefore completely absorbed by the skin (Sterling, 1983).

The aim of this study was to investigate the effects of percutaneous contamination of rats with terbufos, a broad spectrum insecticide-nematocide (Davine *et al.*, 1986), dichlorvos, a widely used ingredient of many commercial products (Kobayashi *et al.* 1983) and parathion, which although highly toxic, is still widely used for pest control throughout the world, on survival rate. These were countered by decontamination with alcoholate as well as parenteral therapy with the oximes N-methylpyridinium-2-carbaldoxime chloride (PAM-2) and 14-(aminocarbamyl)-pyridino-methoxymethyl 2(hydroxyimino) methyl pyridinium dichloride monohydrate (HI-6), combined with atropine and diazepam, *per se* or simultaneously with decontamination and the outcome was evaluated.

MATERIALS AND METHODS

Animals: White female nulliparous Wistar-strain rats, between 200 and 230 body mass were used. Before the experiment, the rats were kept 4 to a cage at room temperature in constant 12-h light-dark cycles. The rats were divided into control and treated groups, each having six animals. They were fed and watered *ad libitum*.

Poisons: Terbufos of 85% purity, dichlorvos of 95% purity and parathion of 98% purity were obtained commercially. The purity was determined before the experiments, and the stability was periodically checked using chemical (gas chromatography) and biological (LD_{50})methods.

Antidotes: Reactivators of AChE - the oximes PAM-2 and HI-6 were used in combination with cholinolitic atropine sulfate, and followed by injection of the anticonvulsant diazepam.

Decontaminant: Solution of "alcoholate", consisting of sodium alcoholate (25%), dimethylformamide (60%) and tetramethylensulfone (15%) was taken from the standard individual chemical warfare (CW) package.

Experimental and LD_{50} determination procedures: The concentrated poisons were applied with an Agla micrometer syringe (Wellcome Research Laboratories, Beckenham, England) on the back skin surface (10 cm^2) from which the hair was cut 24h before the experiment. Only rats with no macroscopically visible skin lesions were used. The rats, anesthetized i.p. with 25 mg/kg of 2.5% solution of thiopentone sodium, were fixed to a 30 X 16 cm wooden board and placed under a ventilated hood with constant negative pressure. The decontamination was performed after 5 min of exposure to the poison, by wiping with cotton pads soaked with 2 ml alcoholate. The protective efficacy of the oximes, PAM-2 and HI-6, in doses of 10 mg/kg each, regarding survival was tested by single i.m. injection, one minute before the decontamination. The oximes were injected together with 10 mg/kg of atropine sulfate and 2.5 mg/kg of diazepam, injected separately into the other thigh. LD_{50} values, based on the 24-hour mortality, were calculated by the method of Miller and Tainter (1944). Protective effects were expressed as "Protection Ratio" (PR), displaying the ratio between LD_{50} of poison with decontamination/therapy and LD_{50} of the poison without decontamination/therapy.

Statystical analyses: The results were presented as mean X ± SE, and differences between mean values were tested using Students t-test. All values of $p<0.05$ were considered statistically significant. Surviving rats were euthanased with ether after 24h.

RESULTS

Table 1. shows the protective efficacy of decontamination with alcoholate in rats percutaneously poisoned with organophosphorus insecticides. Decontamination with alcoholate was more efficacious, in terbufos poisoning (PR = 36.6), but was also effective in parathion (PR = 11.3) and dichlorvos poisoning (PR = 9.3).

The protective efficacy of parenteral treatment with the oximes, PAM-2 and HI-6, in rats percutaneously poisoned with organophosphorus insecticides are presented in table 2. PAM-2 and HI-6 were ineffective in terbufos and parathion

Table 1. The protective efficacy of decontamination[a] in rats percutaneously poisoned with organophosphorus insecticides

Insecticide	LD_{50}, mg/kg ± SE	LD_{50}, mg/kg ± SE (PR)[b]
Terbufos	2.2 ± 0.2 (1.0)	80.5 ± 5.6* (36.6)
Parathion	20.0 ± 1.9 (1.0)	226.1 ± 18.3* (11.3)
Dichlorvos	41.4 ± 7.3 (1.0)	386.4 ± 28.0* (9.3)

[a] Decontamination was performed 5 min after exposure

[b] PR = LD_{50} of poison with decontamination/therapy/LD_{50} of poison without decontamination

Table 2. The protective efficacy of oximes[a] PAM-2 and HI-6 in rats percutaneously poisoned with organophosphate insecticides

Insecticide	LD_{50}, mg/kg ± SE	LD_{50}, mg/kg ± SE (PR)	
		PAM-2	HI-6
Terbufos	2.2 ± 0.2 (1.0)	2.1 ± 0.1 (1.0)	2.6 ± 0.2 (1.1)
Parathion	20.0 ± 1.9 (1.0)	24.4 ± 4.0 (1.2)	19.9 ± 3.0 (1.0)
Dichlorvos	41.1 ± 7.3 (1.0)	293 ± 31.0* (7.1)	281 ± 21.0* (6.8)

[a] Oximes were injected i.m. 1 min. before decontamination, in doses 10 mg/kg, together with atropine sulfate (10 mg/kg), followed by separate injection of 2.5 mg/kg diazepam

*$p < 0.05$

poisoning, PRs being 1.0 and 1.1 for terbufos respectively, and 1.2 and 1.0 for parathion respectively. In dichlorvos poisoning the efficacy of both oximes was almost equal, protecting the rats against 7 LD_{50} of poison.

Table 3. shows the protective efficacy of combined decontamination and oxime therapy in rats percutaneously poisoned with organophosphorus insecticides. Oxime therapy with PAM-2 and HI-6 did not improve the outcome of decontamination in terbufos (PRs= 35.1 and 32.5 respectively) and parathion poisoning (PRs= 11.6 and 11.0 respectively), but greatly potentiated it in dichlorvos poisoning, with the PRs being 52.6 for PAM-2 and 40.6 for HI-6.

Table 3. The protective efficacy of decontamination and oxime[a] therapy in rats percuteneously poisoned with organophosphate insecticides

Poison	LD_{50}, mg/kg ± SE	LD_{50}, mg/kg ± SE (PR)	
		PAM-2 + decont.[a]	HI-6 + decont.[a]
Terbufos	2.2 ± 0.2 (1.0)	77.3 ± 3.8 (35.1)	71.1 ± 4.3 (32.5)
Parathion	20.0 ± 1.9 (1.0)	220.3 ± 15.1 (11.0)	238.1 ± 14.4 (11.6)
Dichlorvos	41.1 ± 7.3 (1.0)	2208.0 ± 294.0* (52.6)	1701.1 ± 284.0* (40.6)

[a]Oximes were injected i.m. 1 min before decontamination, in doses of 10 mg/kg, together with atropin sulfate (10 mg/kg), followed by separate injection of 2.5 mg/kg of diazepam. Decontamination was performed 5 min after exposure.
*$p<0.05$

DISCUSSION

Skin decontamination is a very important step that should never be neglected or hurried. The skin should be thoroughly washed with soap and water and ethyl alcohol, or in our case, alcoholate to prevent further absorption (Peter and Cherian, 2000).

The results of our study confirm that terbufos, applied percutaneously, although not a direct AChE inhibitor, is a very highly toxic OP compound (LD_{50}= 2.2 mg/kg), being 5 times more toxic than the nerve gas soman, applied by the same route (Kne'vić *et al.*, 1993). Moreover, terbufos was extremely toxic to birds, fish and aquatic invertebrates (Hill and Camardese, 1984; Mayer and Ellersieck, 1986). The compound has a moderate potential to accumulate in living tissues in aquatic organisms. Terbufos was expected to be extremely toxic to mammals and reptiles (Anonymous, 1988). It is nontoxic to bees. Other published data (Anonymous, 1991) have shown that lower toxicity that could be explained by sex differences.

Parathion, although not a direct AChE inhibitor, is a very highly dangerous OP insecticide if applied percutaneously (LD_{50}= 20.0 mg/kg), being nearly half as toxic as the nerve agent soman, applied by the same route (Kne'evic *et al.*, 1993). These results were well within the range of terbufos and parathion toxicity data published elsewhere (Anonymous, 1989; Gaines, 1969; Hayes, 1982; Bošković, 1989).

Although less toxic than terbufos, dichlorvos (LD_{50}= 41.4 mg/kg) was found to be much faster killer due to its direct AChE-inhibiting action (Kobayashi *et al.*, 1993). Among the organophosphates, dichlorvos is striking for its rapid metabolism and excretion by mammals (Gallo and Lauryk, 1991). It is highly toxic to birds, bees and aquatic life (Anonymous, 1988; Howard, 1991).

Regarding parenteral treatment of both terbufos and parathion poisoning, the antidotes proved to be totally ineffective, which was expected, since the peak of AChE inhibition was attained long after almost complete elimination of the oximes from the circulation, so that no reactivation was possible anyway (Reiner and Pleština, 1979; Maksimović, 1979; Simons and Briggs, 1985). It is therefore necessary to maintain the so-called therapeutic level (2.56 mg/l for PAM, and 0.72 mg/l for HI-6) of these oximes in the blood (Shiloff and Clement, 1987) by repeated injections, to achieve both AChE reactivation and the consequent protection of poisoned animals, since it is well known that oximes easily reactivate the dichlorvos-inhibited enzyme

Decontamination was very highly effective in rats percutaneously poisoned with terbufos (PR= 36.0), and less so in parathion poisoning (PR= 11.3), but much more than the parenteral therapy, for the reason explained above (Table 1). In dichlorvos poisoning both parenteral therapy and decontamination were roughly equally effective (PRs= 6.8 - 9.3), but great potentiation of the effects was registered in the combined treatment (Table 3). This could by explained by the fact that dichlorvos is known as a direct inhibitor of AChE so that the immediate oxime therapy, combined with decontamination, had shown its potential effects, because the metabolism and excretion of this compound was very rapid.

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KOMBINOVANA DEKONTAMINACIJA I PARENTERALNA TERAPIJA POBOLJŠAVAJU IZGLEDE ZA PREŽIVLJAVANJE KOD PACOVA PERKUTANO TROVANIH ORGANOFOSFORNIM INSEKTICIDIMA

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SADRŽAJ

U ovom radu su izneti rezultati ispitivanja uticaja dekontaminacije alkoholatom i perenteralnom terapijom *per se* (oksimi PAM-Cl i HI-6, holinolitik, antikonvulziv), kao i njihove kombinacije, kod pacova perkutano trovanih terbufosom, parationom i dihlorvosom. Postupkom dekontaminacije, izvršenim 5 minuta posle izlaganja ko'e otrovu, postignut je visok stepen efikasnosti kod pacova trovanih indirektnim inhibitorima acetilholinesteraze, terbufosom (zaštitni indeks 36,6) i parationom (zaštitni indeks 11,3). Ovim tretmanom dobijena je zadovoljavajuća efikasnost i kod pacova perkutano trovanih direktnim inhibitorom acetilholinesteraze, dihlorvosom uz zaštitni indeks od 9,3. Samo parenteralnom terapijom, primenjenom 4 minuta posle izlaganja kože otrovu, bez obzira na upotrebljeni oksim, nisu dobijeni očekivani rezultati ni u trovanju terbufosom (zaštitni indeks 1,1 sa PAM-2 Cl, odnosno 1,0 sa HI-6), ni parationom (zaštitni indeks 1,2 sa PAM-2 Cl, odnosno 1,0 sa HI-6), za razliku od dihlorvosa, gde je zaštitni indeks primenom oksima PAM-2 Cl bio 7,1, odnosno 6,8 sa HI-6. Kombinovanim postupkom (dekontaminacija uz parenteralnu primenu antidota) kod pacova perkutano trovanih terbufosom i parationom dobijena efikasnost

odgovara onoj koja je postignuta samo postupkom dekontaminacije. U trovanju dihlorvosom, postupkom dekontaminacije i parenteralnom primenom antidota, ostvaren je supraaditivni sinergizam - sa oksimom PAM-2 Cl zaštitni indeks bio je 52,6, odnosno 40,6 sa HI-6. Na osnovu dobijenih rezultata proističe da pri perkutanoj kontaminaciji organofosfornim insekticidima, pravovremena dekontaminacija znatno povećava izglede za preživljavanje, posebno uz istovremeno davanje antidota (oksimi, holinolitici, antikonvulzivi).