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EFFECT OF MONO- AND BIS-QUATERNARY PYRIDINIUM OXIMES ON THE ACUTE TOXICITY AND ON THE SERUMCHOLINESTERASE INHIBITORY ACTIVITY OF DIOXACARB*, CARBARYL* AND CARBOFURAN*

B. Bošković, V. Vojvodić, M. Maksimović, Azra Granov, Svetlana Besarović-Lazarev and Z. Binenfeld

Army Technical Institute, Beograd and »Bosnalijek«, Sarajevo, Yugoslavia

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The acute toxicities of insecticidal carbamates (Dioxacarb, Carbaryl and Carbofuran) were determined in mice by s. c. or i. p. injection, both in the absence and in the presence of atropine and several pyridinium oximes. Atropine had a beneficial effect on the toxicity of all the three carbamates, while the oximes varied in their effects. In the case of Carbaryl all oximes used increased its toxicity, while in Dioxacarb and Carbofuran poisoning some of them were effective. It was found that the oximes do not influence the action of carbamates on the activity of serum cholinesterase in vitro, in a way which could explain their effect on the toxicity of these compounds. It is concluded, that the use of oximes is contraindicated in cases of intoxication with Carbaryl, Dioxacarb and Carbofuran.

The inhibition of cholinesterase (ChE) following poisoning by insecticidal organophosporus compounds may be decreased or reversed, in most cases, by applying ChE reactivators such as mono-and bis-pyridinium oximes. At the same time the toxicity of these anti-ChE agents

* Carbaryl: 1-naphtyl methyl carbamate; Dioxacarb: 2-(1, 3, dioxolane-2-yl)-phenyl methyl carbamate; Carbofuran: 2,2-dimethyl-2,3-dihydro-7-benzofuranyl methyl carbamate.

decreases too. On the other hand the so called »classical antidotes« belonging to this group, such as PAM-2Cl*, TMB-4Cl$_2$* and Toxogonin are without effect in some experimental intoxications with insecticidal carbamates (1—4), in some they potentiate their toxicity (2, 4), and in some they afford protection (1, 3, 4). The growing tendency of replacing organophosphorus insecticides by carbamates because of their lower toxicity, longer persistence etc. emphasises the need for the development of effective antidotes in carbamate poisoning.

The present work describes studies in which the therapeutic efficiency of four newer pyridinium oximes (HS-3, HS6, HS-7 and »XIII«)** (5, 6) was compared with the therapeutic effect of PAM-2Cl, TMB-4Cl$_2$, Toxogonin and atropine when given separately as antidotes to Dioxacarb, Carbaryl and Carbofuran injected in mice. These three insecticidal carbamates are widely used in household and agriculture, and in the available literature we were not able to find reports of experiments designed to examine the effects of antidotes to their toxicity.

In the experimental section, data are presented which demonstrate the acute toxicity of the three carbamates and the influence of oximes or atropine on these effects, as well as on the inhibition of serum-cholinesterase in vitro by them in the presence of oximes.

MATERIAL AND METHODS

Carbamates. Carbaryl and Dioxacarb were of 98—99% purity and Carbofuran was used as 75% WP (Fig. 1). All the three were supplied by Chemical Works Chromos, Katran, Kutrilin — Zagreb. Carbaryl and Dioxacarb were dissolved in dimethylsulphoxide, and Carbofuran in propanol-2 and diluted with 0.9% solution of sodium chloride in water before use.

Oximes. All of the oximes (Fig. 1) were of 99% purity. They were synthetized in the Research Institute of »Bosna-lijek« — Sarajevo. Oximes and atropine were dissolved in 0.9% solution of sodium chloride in water.

Atropine sulphate commercially available was according to Ph. Jug. III.

* PAM-2Cl: N-methyl-(2-hydroxymininoformyl-pyridinium) chloride; TMB-4Cl$_2$: N,N'trimethylene-bis-(4-hydroximinoformyl pyridinium) dichloride; Toxogonin: N,N'dimethylether-bis-(4-hydroxyiminoformyl pyridinium) dichloride.

** HS-3: N,N'dimethylether / (2-hydroxyiminoformyl pyridinium)-4-hydroxyiminoformyl pyridinium / dichloride; HS-6: N,N'dimethylether / (2-hydroxyiminoformyl pyridinium)-3-carboxamino pyridinium / dichloride; HS-7: N,N'trimethylene / (4-hydroxyiminoformyl pyridinium)-3-carboxamino pyridinium / dibromide; »XIII«: N,N'trimethylene / (4-hydroxyiminoformyl pyridinium)-N-methyl morpholinium / dibromide.

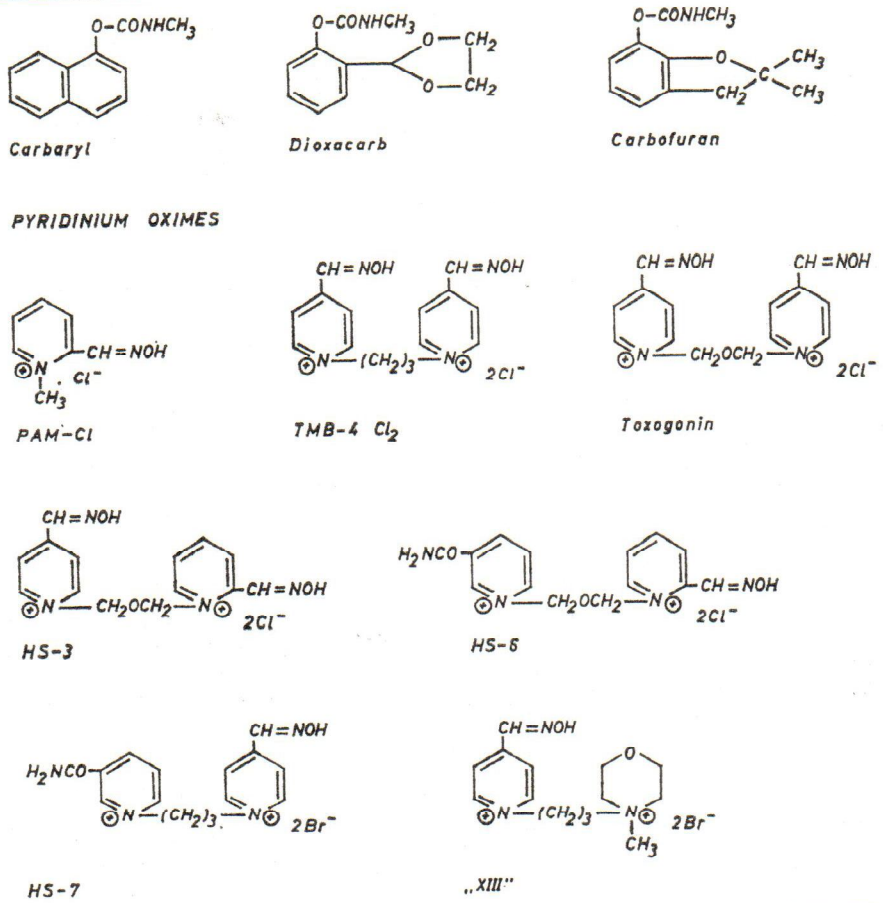

Figure 1. *Structural formulas of anticholinesterase carbamates and pyridinium oximes employed*

Acute toxicity tests. White mice of both sexes weighing from 18 to 26 g were used. The volume of the injected solution was for carbamates 5 ml/kg of body weight, and for atropine or oximes 10 ml/kg of body weight, respectively. Carbaryl was given i. p. and atropine (5 mg/kg) or oximes (10 mg/kg) s. c., while Dioxacarb and Carbofuran were given s. c. and atropine or oximes i. p. In order to avoid the possible direct interaction between carbamates and antidotes, at the site of application, the compounds were injected by varying routes. The carbamates were always injected first and immediately after them atropine or oximes. LD—50 value was calculated according to the method of *Miller* and *Tainter* (7) on the basis of 24_{hr} mortality.

In vitro inhibition of serumcholinesterase. The purified commercial preparatio of serumcholinesterase (E. C. 3.1.1.8. SChE) (*Schuhardt*) was dissolved in 0,1 M phosphate buffer (pH 7,0) in a concentration of 1 microgram/3 ml of buffer solution. The normal activity of SChE, as well as the activity in the presence of different concentrations of the inhibitor was measured for 10 minutes (at 25°C) on Ratio Recording Spectrophotometer Beckman DK-2A, with butyrylthiocholine iodide (5,4mM) as substrate (8). The carbamate concentrations causing 50% SChE inhibition (I-50) were for Carbaryl $2{,}12 \cdot 10^{-6}$ M, for Dioxacarb $8.86 \cdot 10^{-6}$ M and for Carbofuran $1.4 \cdot 10^{-6}$ M. In the experiments with oximes the enzyme was incubated with different concentrations of oximes up to $5 \cdot 10^{-4}$ M before the inhibitor was added. After that a half of the inhibitor concentration needed for I-50 was added and the enzyme activity was measured for further 10 minutes. The oxime concentrations required for a twofold reduction of carbamate concentrations which cause a 50 per cent inhibition were determined graphically: log per cent of SChE inhibition versus concentration of oxime.

RESULTS

Effect of pyridinium oximes or atropine on the LD—50 values of carbamates. Atropine showed a significant degree of protection of mice in poisoning with Dioxacarb and especially with Carbofuran, whose LD—50 value was quadrupled (Table 1). In Carbaryl poisoning the protective effect of atropine is negligible, and all the oximes significantly

Table 1.

Effect of oximes and atropine on median lethal doses of Carbaryl, Dioxacarb and Carbofuran

Substance*	LD-50 of carbamates (mg/kg ± S. E.)		
	Carbaryl (i. p.)	Dioxacarb (s. c.)	Carbofuran (s. c.)
———	63 ± 8	68 ± 6	6,9 ± 0,4
Atropine	78 ± 8,9	108 ± 10***	26 ± 2,5***
PAM-2Cl	26,4 ± 6**	102 ± 13***	9 ± 0,8***
TMB-4Cl_2	11,6 ± 2,3**	73 ± 11	10,5 ± 2***
Toxogonin	11,5 ± 1.5**	93 ± 6,7***	4,2 ± 0,8**
HS-3	21 ± 2**	40 ± 9**	4,2 ± 0,7**
HS-6	15,4 ± 2**	112 ± 11***	11,2 ± 1,6***
HS-7	26 ± 2,2**	91 ± 11***	5,8 ± 0,9
»XIII«	19 ± 1,8**	68 ± 10	3,7 ± 0,7**

* Oximes (10 mg/kg) or atropine (5 mg/kg) were given immediately after injection of carbamates.

** Significantly different from untreated group in the negative sense ($p>0{,}05$).

*** Significantly different from untreated group in the positive sense ($p>0{,}05$).

increased the toxicity of Carbaryl. So PAM—2Cl and HS—7 decreased LD—50 value about 2.5 times, HS—6 and »XIII« about four times and Toxogonin and TMB—$4Cl_2$ about six times. In Dioxacarb poisoning PAM—2Cl, HS—7 and Toxogonin had the same protective effect as atropine (about 1.5 times), TMB—$4Cl_2$ and »XIII« showed no protection, and HS—3 even increased the toxicity of Dioxacarb itself. In Carbofuran poisoning PAM—2Cl, TMB—$4Cl_2$ and HS—6 decreased its toxicity about two times, while HS—3, Toxogonin and »XIII« increased its toxicity, and HS—7 was without effect.

Effect of pyridinium oximes on the inhibition of SChE by carbamates. All the oximes, in concentrations ranging from $2.1 \cdot 10^{-6}$ M for TMB—$4Cl_2$ to $3.9 \cdot 10^{-4}$ M for PAM—2Cl, decrease the concentration of Carbaryl required for I—50 by a factor of 2 (Table 2). In the case of Dioxacarb, this effect was seen only with HS—7, TMB—$4Cl_2$ and »XIII« in concentrations between 10^{-4} M to 10^{-5} M, and for Carbofuran the only oximes to exert a similar effect in the same concentration range were HS—3, HS—7 and »XIII«. Toxogonin had to be used in a concentration of $9.1 \cdot 10^{-4}$ M.

Table 2.

Molar concentrations of pyridinium oximes required for twofold reduction of Carbamate concentrations which cause 50% inhibiton of purified horse SChE

Oxime	Concn. of oximes (M · 10^{-5})		
	Carbaryl	Dioxacarb	Carbofuran
PAM-2Cl	39.0	—*	—
TMB-$4Cl_2$	0.21	3.6	—
Toxogonin	2.9	—	91.0
HS-3	3.0	—	6.5
HS-6	1.8	—	—
HS-7	0.82	5.2	4.8
»XIII«	3.2	2.7	3.4

* (—) denotes no potentiation of inhibition in concn. of oxime up to $5 \cdot 10^{-4}$ M.

DISCUSSION

This work, although made in different experimental conditions, and on different animal species, confirms the results of *Sanderson* (3) and of *Natoff* and *Reiff* (4) in demonstrating an interaction to insecticidal carbamates and oximes. All the oximes examined increase the toxicity of Carbaryl, while in Carbofuran poisoning TMB—$4Cl_2$, HS—6 and PAM—2Cl afford some protection but to a lesser extent than atropine does. Although atropine alone is ineffective in protecting against Carbaryl poisoning it showed a marked protective effect in Carbofuran poison-

ing. In the case of Dioxacarb, most of oximes (PAM—2Cl, Toxogonine, HS—6 and HS—7) have a beneficial effect, while the others, excluding HS—3 have no influence on its toxicity. Contrary to the findings of *Natoff* and *Reiff* (4) atropine showed no protective effect in Carbaryl poisoning, while PAM—2Cl and Toxogonine increased its toxicity, as was demonstrated by *Natoff* and *Reiff* (4). These differences could be attributed to different experimental conditions, and different experimental animals, but a clear explanation is not yet available.

The increase of anti-SChe activity of Carbaryl, Dioxacarb and Carbofuran by oximes (especially of Carbaryl where all oximes showed such effect) is very significant. The experimental technique in which SChE was incubated with oximes in noninhibiting concentrations, and subsequent adding of carbamates could produce carbamoyl oximes of equal or increased anti-SChE activity as compared to Carbaryl, Dioxacarb or Carbofuran. It should be noted that only two oximes (HS—7 and »XIII«) exert such an effect in all experiments and both are bis-quaternary compounds with one oxime group in the position 4 of pyridine ring and a trimethylene chain. Although these results in vitro cannot serve as an explanation of the in vivo effects of oximes, they offer some indication for a direct interaction of carbamates and oximes in vivo.

Our results do not allow the supposition that the oximes decarbamylate ChE inhibited by carbamates or have some »ChE stimulating« properties (9). We also were not able to find such remarkable differences between Carbaryl, Dioxacarb and Carbofuran in connection with oximes, as *Natoff* and *Reiff* (4) found between Carbaryl and other carbamates. The main difference between our findings and the findings of other authors (1, 3, 4) is the fact that in our experiments the protective effect of atropine was not so pronounced compared with that of some oximes.

The results of this study show that for intoxications by Carbaryl, Dioxacarb and Carbofuran atropine is a drug of choice. The use of oximes in Carbaryl and Carbofuran poisoning may be ineffective or mainly antagonistic and the therapy with oximes is contraindicated.

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Sažetak

DJELOVANJE MONO-KVATERNERNIH I BIS-KVATERNERNIH PIRIDINIJUMSKIH OKSIMA NA AKUTNU TOKSIČNOST I ANTIHOLINESTERAZNO DJELOVANJE KARBARILA, DIOKSAKARBA I KARBOFURANA

Ispitana je akutna toksičnost karbamatnih insekticida dioksarba, karbarila i karbofurana (LD-50) s. c. ili i. p., sa simultanom primjenom atropina ili bez nje i nekoliko piridinijumskih oksima. Atropin je pokazao povoljan efekat u trovanjima sa sva tri karbamata, dok je djelovanje oksima bilo različito. U trovanjima karbarilom i karbofuranom gotovo svi oksimi su potencirali njihovo toksično djelovanje, dok su u trovanju dioksakarbom neki bili djelotvorni. Oksimi ne utiču na inhibiciju holinesteraze in vitro ovim karbamatima na način kojim bi se moglo rastumačiti njihovo in vivo djelovanje. Na temelju eksperimenata je zaključeno da je upotreba oksima u trovanju karbarilom, dioksakarbom i karbofuranom kontraindikovana.

Vojnotehnički institut, Beograd i »Bosnalijek«, Sarajevo

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