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PROTECTIVE EFFECT OF DEXETIMIDE AND HI-6 IN POISONING WITH HIGHLY TOXIC ORGANOPHOSPHORUS COMPOUNDS

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The protective effect of HI-6 in combination with atropine and dexetimide was compared in mice and rats intoxicated with soman and VX. Both combinations were equally effective. In soman poisoning the relative efficiency both in mice and rats was about 5 while the therapeutic factor in VX poisoning was about 80.

It is generally accepted that the best therapy or prevention of intoxication with highly toxic organophosphorus compounds is the application of atropine in combination with cholinesterase reactivators — oximes such as PAM-2Cl (1-methyl-2-hydroxyiminomethylpyridinium chloride), obidoxime (1,1'[bis(4-hydroxyiminomethylpyridiniummethyl)]-ether dichloride) and TMB-4 (1,3 bis[4-hydroxyiminomethylpyridinium]-propan dibromide). However, in the treatment of intoxication with soman (pinacolyl methylfluorophosphonate), this standard therapy failed (1).

Recently it has been shown that the compound HI-6 ([2-hydroxyiminomethylpyridiniummethyl) - (4'-carbamoylpyridinium-methyl)]-ether dichloride) used with atropine can counteract the effect of soman in mice and rats intoxicated with multiple lethal doses of this poison (2).

Bertram and co-workers report (3) that dexetimide ((+)-1-benzyl-4-(2,6-dioxo-3-phenyl-3-piperidyl)-piperidine. HCl), an atropine like compo und which acts both on the peripheral and central autonomic nervous systems and penetrates the blood-brain barrier more readily than

atropine, applied together with obidoxime increased extremely the protective index in mice poisoned with DFP (diisopropyl fluorophosphate). It was also very efficient in rabbits poisoned with paraoxon (diethyl 4-nitrophenylphosphate) and DFP, but inefficient in OMPA (octamethylpyrophosphoramide) poisoning. It is very important that even a single application of dexetimide to experimental animals was sufficient to completely counteract the intoxication with the organophosphorus compounds.

For these reasons it seemed of interest to study the efficiency of combined application of dexetimide and HI-6 in the poisoning with soman but also in VX (ethyl diisopropylaminoethyl methylthiophosphonate) poisoning in which the standard antidote combinations proved to be very efficient (4).

MATERIAL AND METHODS

HI-6 $\times$ *1* H_2O, m. p. 141—3° C (lit. 218° C) was synthesized according to a modified Stark's procedure (5).

PAM-2Cl was supplied by courtesy of Miss A. Granov, Bosnalijek-Saniteks, Sarajevo.

Dexetimide was supplied by courtesy of Dr. I. van Wijngaarden, Jansen Pharmaceutica, Beerse, Belgium.

Atropine sulphate puriss. Kemika, Zagreb.

Soman and *VX* were of $>$ 95.0% purity.

White male mice weighing 18—25 g, and male albino rats weighing 200—250 g were used.

Aqueous solutions of atropine, dexetimide, PAM-2Cl and propyleneglycol solutions of soman and VX were freshly prepared before use and administered to mice and rats in the amount of 2 ml/kg. Atropine (10 mg/kg), dexetimide (10 mg/kg), HI-6 (100 mg/kg) and PAM-2Cl (30 mg/kg) were injected intraperitoneally 15 min before, or 1 min and 10 min after a subcutaneous injection of the poison.

LD_{50} values based on 24 hours mortality rates were calculated according to *Thompson* (6) and *Weil* (7).

Relative efficiency is expressed as

$$\frac{LD_{50} \text{ of poison with antidote}}{LD_{50} \text{ of poison without antidote}}$$

Therapeutic factor was calculated from the highest multiple of LD_{50} of poison which could be counteracted by the antidotes intraperitoneally injected 1 min after the injection of the poison (all animals survived):

$$\text{Therapeutic factor} = \frac{\text{multiple } LD_{50} \text{ of organophosphate}}{LD_{50} \text{ controls}}$$

RESULTS AND DISCUSSION

Table 1.

The protective effect of atropine, dexetimide, PAM-2Cl and HI-6 in rats and mice poisoned by soman*

Antidote	Relative efficiency			
	Time of antidote** application			
	15 min. before	1 min. after	10 min. after	1 min. after
	mice			rats
Atropine		< 1.26		< 1.26
Dexetimide		< 1.26		1.26
PAM-2Cl		< 1.26		< 1.26
HI-6		1.40		1.50
Atropine + PAM-2Cl		< 1.26		< 1.26
Dexetimide + PAM-2Cl		< 1.26		< 1.26
Atropine + HI-6	3.90	4.40	2.80	5.00
Dexetimide + HI-6	3.80	4.70	2.50	5.60

* LD_{50} = 0.224 mg/kg (mice); LD_{50} = 0.148 mg/kg (rats).

** The antidotes were given i. p. before or after an s. c. injection of soman.

Atropine, dexetimide and PAM-2Cl applied alone or combinations of PAM-2Cl with atropine and dexetimide respectively produced no protective effects in rats and mice poisoned with soman. This is in accordance with the current knowledge of the poisoning by soman (1). HI-6 used alone provided only a weak protection, but combined with atropine or dexetimide it produced a significant protective effect. The results of our experiments in which a combination of atropine and HI-6 was given to mice fit in with the results of *Kepner* and *Wolthuis*.

However, in rats we found that the relative efficiency was lower than the protective index reported by these authors (2). The degree of protection was practically the same, when HI-6 was used with atropine or with dexetimide. In mice the antidotes used 10 min after the poisoning significantly lowered the relative efficiency, most probably because of the ageing of acetylcholinesterase inhibited by soman. This finding might have some practical significance.

Table 2.

The protective effect of atropine, dexetimide and HI-6 in mice and rats poisoned by VX***

Antidote	mice		rats	
	relative efficiency	therapeutic factor	relative efficiency	therapeutic factor
Atropine	2.0		3.9	
Dexetimide	1.8		3.4	
HI-6	5.1		5.0	
Atropine + HI-6	> 70	70	95	70
Dexetimide + HI-6	> 80	> 80	> 80	> 80

* The antidotes were given i. p. 1 min. after an s. c. injection of VX.

** LD_{50} = 0.022 mg/kg (in mice); 0.023 mg/kg (in rats).

Atropine, dexetimide and HI-6 used alone offered a significant protection in VX poisoning both in mice and rats. The protection provided by HI-6 was superior.

Used with atropine or dexetimide HI-6 showed an extremely high relative efficiency in mice and rats. Very interesting is an extremely high therapeutic factor in mice which is comparable to that obtained in rabbits poisoned with paraoxon (3). It seems that as far as the therapeutic factor is concerned dexetimide is a little superior to atropine.

We used 100 mg/kg of HI-6 because in preliminary experiments (8) the best effects were obtained with the doses between 50—100 mg/kg. This is in agreement with the published results of some other authors (2).

Our findings are different from the results of *Bertram* and co-workers (3) obtained in paraoxon and DFP poisonings where a combination of dexetimide and obidoxime was by far superior to a combination of atropine and obidoxime. Nevertheless these results are not opposite because different experimental conditions, organophosphorus poisons

and cholinesterase reactivators were applied. In *Bertram's* (3) and our experiments dexetimide in a combination with obidoxime or HI-6 was successful in counteracting poisoning with four out of five organophosphorus compounds used. Dexetimide is therefore a very promising antidote and could, perhaps, substitute atropine in the poisoning with some organophosphorus compounds. The possible influence of the solvent propyleneglycol was not taken into account in our experiments.

Our results suggest that so far HI-6 with atropine or dexetimide is the most efficient combination of antidotes in the treatment of poisoning with soman and VX. In the poisoning with soman these combinations are by far the most effective and in VX poisoning their protective value is the same as that of the so far most efficient combination of HS-3 (1-[(2-hydroxyimino-methylpyridinium-methyl)-1' (4-hydroxyimino-methylpyridinium-methyl)] ether dichloride) and atropine (4).

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Sažetak

ZAŠTITNI UČINAK DEKSETIMIDA I HI-6 PRI OTROVANJU S VRLO TOKSIČNIM ORGANOFOSFORNIM SPOJEVIMA

Ispitano je zaštitno djelovanje spoja HI-6 u kombinaciji s atropinom i deksetimidom u miševa i štakora otrovanih somanom i VX-om. Atropin i deksetimid apliciran sami nisu štitili štakore i miševe otrovane somanom. Kombinacija III-6 s atropinom odnosno deksetimidom imala je relativni učinak ~5 i u miševa i u štakora. Aplikacija antidota 10 min poslije trovanja znatno je smanjila njihovu djelotvornost u uporedbi s njihovom aplikacijom 1 minutu poslije trovanja.

U trovanju miševa i štakora VX-om, atropin, deksetimid, a naročito HI-6 dati pojedinačno pružali su izvjesnu zaštitu (relativni učinak između 3 i 5), dok je kombinacija HI-6 s deksetimidom odnosno atropinom imala relativni učinak ~80, a kombinacija deksetimida + HI-6 imala je terapijski faktor čak veći od 80.

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