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THE PROTECTIVE EFFECTS OF SOME TERTIARY AND QUARTERNARY ANTIMUSCARINIC DRUGS AND OXIME IN RATS POISONED BY LETHAL DOSES OF METHYLETHOXY-(2-DIMETHYL-AMINO ETHYLTHIO)-PHOSPHINE OXIDE

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Protective effects of atropine sulphate, 3-quinuclidynil benzilate and their quarternary derivatives were investigated in rats poisoned by lethal doses of organophosphorous anticholinesterase inhibitor methylethoxy — (2-dimethylaminoethyl thio) — phosphine oxide (33SN). It has been found that 33SN belongs to highly toxic anticholinesterase drugs and its LD_{50} value for rats (injected subcutaneously) is 0.018 mg/kg. Atropine sulphate, in a dose of 10.0 mg/kg i. p., or 3-quiniclidynil benzilate (5.0 mg/kg, i. p.) given in combination with TMB_4 (10.0 mg/kg i. p.), protected rats poisoned by 50 LD_{50} of 33SN compound. Their quarternary derivatives, however, when given either alone or in combination with TMB_4, were markedly less effective in protecting poisoned animals.

It is known that a combination of atropine with oximes represents »the therapy of choice« in the treatment of organophosphorous poisoning. In such combination atropine acts as a powerful antimuscarinic drug in both, the central and peripheral nervous system (1). Unlike atropine, oximes represent a causal therapy since they are specific reactivators of inhibited cholinesterase, mainly acting on peripheral tissues (2, 1).

Since many atropine-like drugs have been synthetised in the last 20 years, whose central and peripheral antimuscarinic activity is more potent than that of atropine (3, 4, 5), it was natural to expect that these atropine-like drugs would provide a better protective effect in organophosphorous poisoning than atropine.

The aim of the work described in this paper was to determine and compare protective effects of one of these synthetic drugs, 3-quinucli-

dynil benzilate (BZ), and atropine in rats poisoned by lethal doses of organophosphorous anticholinesterase compound 33SN (methylethoxy-(2-dimethylaminoethylthio) — phosphine oxide). Quarternary derivatives of atropine and BZ were used in order to obtain more information about the role of the central antimuscarinic activity of these drugs in organophosphate poisoning. Chemical structure of examined drugs (except for atropine and atropine methylnitrate) is given in Fig. 1.

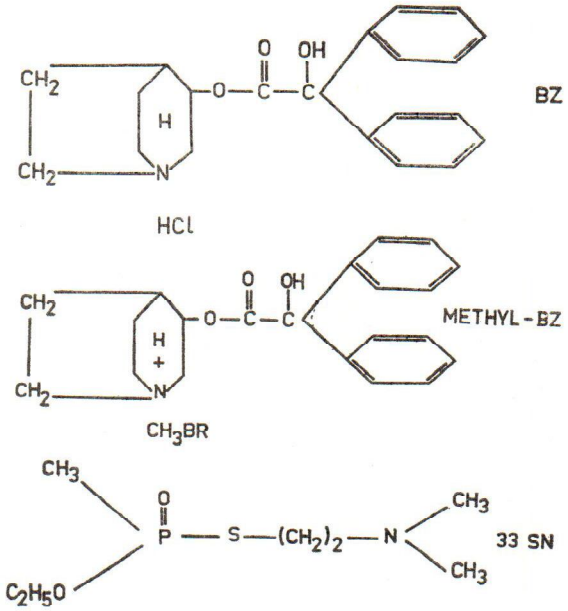

Fig. 1. *Chemical structure of BZ, methyl-BZ and 33SN compounds*

MATERIAL AND METHODS

Male albino rats from 200 to 250 g body weight were used in experiments.

Separate groups of at least 6 rats each were treated with various multiples of median lethal dose (LD_{50}) of 33SN given subcutaneously, ten minutes before intraperitoneal administration of each antimuscarinic drug or its combination with the oxime.

Atropine sulphate was injected in doses ranging from 5.0 to 25.0 mg/kg, while BZ was given in doses from 5.0 to 10.0 mg/kg. Quarternary antimuscarinic drugs, atropine methylnitrate and methyl-BZ, were used in doses of 5.0 to 10.0 mg/kg and 2.5 to 10.0 mg/kg, respectively. The oxime, N'N'-trimethylene-bis (pyridinium-4-aldoxime)-dichloride (TMB4), was injected separately in a dose of 10.0 mg/kg i.p., at the same time with antimuscarinic drugs. Water solutions of all antimuscarinic drugs and oxime

were prepared immediately before experiment. 33SN was kept in a propylene-glycol solution (1%). Final concentrations were prepared with water, daily, just before the experiment.

Protective effect of each antimuscarinic drug or its combination with TMB4 was evaluated by determining LD_{50} value of 33SN in rats pretreated with drug(s) and comparing it with controls receiving 33SN and an injection of saline instead of antimuscarinic drug. LD_{50} was calculated according to the method of *Kärber* (6). The degree of protection was expressed as a protection ratio, i.e. LD_{50} of treated: LD_{50} of untreated animals. All data were analysed for statistical differences using t-test.

RESULTS

In a series of preliminary experiments we studied the protective effects of various doses of antimuscarinic drugs which were injected to poisoned rats without oxime. This was done in order to determine a minimal protective dose of each antimuscarinic drug which provided maximal protection for poisoned animals and might be used in the main experiment with oxime. The results of these experiments showed that the least effective doses were: 10.0 mg/kg of atropine sulphate, 5.0 mg/kg of BZ, 5.0 mg/kg of methylatropine and 2.5 mg/kg of methyl-BZ.

In the main experiment the protective effects of the above selected doses of antimuscarinic drugs, given either alone or with TMB4, were studied.

The results of these experiments, which are expressed both, as LD_{50} of 33SN with and without different antidotal treatment as well as the protection ratio, are presented in Table 1.

Table 1

The protective effects of antimuscarinic drugs and oxime in rats poisoned by lethal doses of 33SN

Treatment	LD_{50}±SE (μg/kg)	Ratio*	p
1. 33SN (without antidotal treatment)	18.6± 0.2	1.0	/
2. TMB_4	51.5±11.1	2.7	ns
3. Atropine sulphate	31.6± 2.9	1.7	ns
4. Atropine sulphate + TMB_4	948.9±95.0	51.0	0.001
5. Methylatropine	18.6± 0.2	1.0	ns
6. Methylatropine + TMB_4	49.5± 8.7	2.7	ns
7. BZ	32.5± 7.5	1.7	ns
8. BZ + TMB_4	863.7±48.4	46.4	0.001
9. Methyl-BZ	18.6± 0.2	1.0	ns
10. Methyl-BZ + TMB_4	69.7±18.6	3.7	0.05

* LD_{50} treated:: LD_{50} untreated

All antidotes were given intraperitoneally, ten minutes before subcutaneous administration of 33SN.

It can be seen that both, atropine sulphate and BZ in combination with TMB4, show a high degree of protection in experimental animals, even if they were poisoned by 50 LD_{50} of 33SN. On the contrary, atropine and BZ, when given alone, failed to show satisfactory protection against 33SN poisoning. Quarternary compounds were markedly less effective in protecting poisoned animals, regardless of how they were applied — alone or in combination with TMB4. It is interesting to note that methyl BZ, when combined with oxime, was more effective than methylatropine in the same combination.

DISCUSSION

The results obtained in these experiments have confirmed the generally accepted view that a combination of atropine and oxime is a very effective therapy for most organophosphorous poisonings. In our opinion, this protection has a special value in the case of 33SN poisoning since this compound belongs to highly toxic anticholinesterase agents (7). Our results also confirm this fact because LD_{50} was found to be only 0.018 mg/kg (s. Table 1). However, it is important to point out that BZ exerted a very similar degree of protection against 33SN poisoning in rats, as was obtained by atropine and oxime. It seems, therefore, that protective effectiveness of atropine and BZ in 33SN poisoning does not correlate with their central or peripheral antimuscarinic potency, since BZ is a more potent antimuscarinic drug than atropine (3, 4).

Similar results, but with other antimuscarinic drugs and organophosphorous inhibitors were obtained by *Brimblecombe* and coworkers (8). Our results show that a combination of tertiary antimuscarinic drug and oxime is more effective in protecting animals poisoned by 33SN, than the same combination of corresponding quarternary antimuscarinic drug and oxime. These findings suggest that central actions of antimuscarinic drugs are more important for the survival of rats poisoned by organophosphorous compounds than peripheral actions of these drugs.

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Sažetak

ZAŠTITNI EFEKTI NEKIH TERCIJARNIH I KVARTARNIH ANTIMUSKARINIKA I OKSIMA U PACOVA TROVANIH LETALNIM DOZAMA METILETOKSI-(2-DIMETILAMINOETILTIO)-FOSFIN OKSIDA (33 SN)

U radu su proučavani zaštitni efekti atropin sulfata, 3-hinuklidin benzilata (BZ) i njihovih kvaternizovanih derivata u pacova trovanih višestrukim smrtonosnim dozama 33SN, antiholinesteraznog inhibitora, koji pripada grupi visokotoksičnih organofosfornih jedinjenja. Nađeno je da srednja smrtonosna doza ovog otrova pri supkutanom unošenju iznosi za pacova svega 0,018 mg/kg. Atropin sulfat u dozi od 10,0 mg/kg, ili BZ (5,0 mg/kg), kad se upotrebi u kombinaciji sa TMB_4 (10,0 mg/kg), efikasno štiti trovane životinje i posle davanja 50 srednjih smrtonosnih doza jedinjenja 33SN. Suprotno atropinu i BZ, njihovi kvaternizovani derivati (atropin metilnitrat i metil-BZ) ispoljavaju znatno slabije zaštitne efekte u trovanih pacova bez obzira na to da li su davani pojedinačno ili u kombinaciji sa oksimom.

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