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-quinuclidiny1 benzilate and
their quaternary derivatives were investigated in rats poisoned by lethal doses of organophosphorous anticholinesterase inhibi-
tor methylethoxy — (2-dimethylaminoethyl thio) — phosphine oxide
(35SN). It has been found that 35SN belongs to highly toxic anti-
cholinesterase drugs and its L.D.50 value for rats (injected subcuta-
nenously) is 0.018 mg/kg. Atropine sulphate, in a dose of 10.0
mg/kg i. p., or 3-quinuclidiny1 benzilate (5.0 mg/kg, i. p.) given in
combination with TMB, (10.0 mg/kg i. p.), protected rats poisoned
by 50 L.D.50 of 35SN compound. Their quaternary derivatives,
however, when given either alone or in combination with TMB1,
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 synthesised 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 organo-
phosphorous 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-quinucl-
dynil benzilate (BZ), and atropine in rats poisoned by lethal doses of organophosphorous anticholinesterase compound 33SN (methylethoxy-
(2-dimethylaminooethylthio) — phosphine oxide). Quaternary 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.

![Chemical structure of BZ, methyl-BZ and 33SN compounds](image)

**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 (LD₅₀) 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. Quaternary 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<sub>90</sub> 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<sub>90</sub> was calculated according to the method of Kürber (6). The degree of protection was expressed as a protection ratio, i.e. LD<sub>90</sub> of treated: LD<sub>90</sub> 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<sub>90</sub> of 33SN with and without different antidotal treatment as well as the protection ratio, are presented in Table 1.

Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LD&lt;sub&gt;90&lt;/sub&gt; ± SE (µg/kg)</th>
<th>Ratio*</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 33SN (without antidotal treatment)</td>
<td>18.6± 0.2</td>
<td>1.0</td>
<td>/</td>
</tr>
<tr>
<td>2. TMB&lt;sub&gt;4&lt;/sub&gt;</td>
<td>51.5±11.1</td>
<td>2.7</td>
<td>ns</td>
</tr>
<tr>
<td>3. Atropine sulphate</td>
<td>31.6±2.9</td>
<td>1.7</td>
<td>ns</td>
</tr>
<tr>
<td>4. Atropine sulphate + TMB&lt;sub&gt;4&lt;/sub&gt;</td>
<td>948.9±95.0</td>
<td>51.0</td>
<td>0.001</td>
</tr>
<tr>
<td>5. Methylatropine</td>
<td>18.6±0.2</td>
<td>1.0</td>
<td>ns</td>
</tr>
<tr>
<td>6. Methylatropine + TMB&lt;sub&gt;4&lt;/sub&gt;</td>
<td>49.5±8.7</td>
<td>2.7</td>
<td>ns</td>
</tr>
<tr>
<td>7. BZ</td>
<td>32.5±7.5</td>
<td>1.7</td>
<td>ns</td>
</tr>
<tr>
<td>8. BZ + TMB&lt;sub&gt;4&lt;/sub&gt;</td>
<td>863.7±48.4</td>
<td>46.4</td>
<td>0.001</td>
</tr>
<tr>
<td>9. Methyl-BZ</td>
<td>18.6±0.2</td>
<td>1.0</td>
<td>ns</td>
</tr>
<tr>
<td>10. Methyl-BZ + TMB&lt;sub&gt;4&lt;/sub&gt;</td>
<td>69.7±18.6</td>
<td>3.7</td>
<td>0.05</td>
</tr>
</tbody>
</table>

* LD<sub>90</sub> treated: LD<sub>90</sub> 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 LD₅₀ of 33SN. On the contrary, atropine and BZ, when given alone, failed to show satisfactory protection against 33SN poisoning. Quaternary 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 LD₅₀ 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 Brimblecombs 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 quaternary 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.

References

Sučetak

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

U radu su proučavani zaštitni efekti atropina sulfata, 3-himaklidin benzilata (BZ) i njihovih kvaternizovanih derivata u pacova trovanih visokom smrtnosnim dozama 33SN, antihaloperidolnog inhibitora, koji pripada grupi visokotokskićnih organofosfornih jedinjenja. Nađeno je da srednja smrtnosna doza ovog otrova pri supputanom unosu 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 upotrebili u kombinaciji sa TMH, (10,0 mg/kg), često nisu štiti trovane životinje i posle davanja 50 srednjih smrtnosnih doza jedinjenja 33SN. Suprotno atropinu i BZ, njihovi kvaternizovani derivati (atropin metilnitrat i metil-BZ) ispoljavaju znatno slabije zaštite efekte u trovanih pacova bez obzira na to da li su davanje pojedinačno ili u kombinaciji sa oksinom.

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