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TOXOGONIN IN SARIN, SOMAN AND TABUN POISONING

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Abstract—The oximes P2S, TMB-4 and Toxogonin have been tested as reactivators of Sarin and Tabun inhibited acetylcholinesterase. The protective effect of the oximes has been studied on atropinized mice in experimental Sarin, Soman and Tabun poisoning. Toxogonin is a better reactivator than P2S and comparable to TMB-4. In the presence of 10 mg/kg atropine (i.p.) and 20 mg/kg Toxogonin (i.p.) the LD_{50} of Sarin (s.c.) was raised 33 times, that of Tabun 12 times and that of Soman 1·3 times.

SO FAR, TMB-4 [N,N'-trimethylene bis(pyridinium-4-aldoxime)dibromide] is known to be the only potent reactivator of Tabun (dimethylamido ethoxy phosphoryl cyanide) inhibited cholinesterase. The oxime is also an effective antidote in Tabun poisoning.[1–3] However, TMB-4 produces some undesirable side effects in the body[4] and has a high toxicity.[5] These properties have caused hesitation when it comes to the use of TMB-4 in the treatment of organophosphorus hazards in humans.

In 1963 and 1964 a new oxime, bis(4-hydroxyiminomethyl-pyridinium (1)methyl)-ether dichloride (trade name Toxogonin, LüH6, BH6) has been described.[6–12] This oxime is able to reactivate human erythrocyte cholinesterase inhibited by Paroxon (diethyl *p*-nitrophenylphosphate), Parathion (diethyl-*p*-nitrophenylthiophosphate) and DFP (diisopropyl fluorophosphate). It is less toxic than TMB-4 and a high therapeutical index is reported.[10] It has been used in experimental intoxications of rats with Paroxon, Parathion and DFP and been found to rise the LD_{50} of these compounds when given either prophylactically, simultaneously or therapeutically.[9] As the structure of Toxogonin is identical with the structure of TMB-4 except that an ether group is substituted for a methylene group in the bridge connecting the pyridine rings it seemed reasonable to test the reactivating power of the oxime on Tabun-inhibited cholinesterase and its protective effect in Tabun poisoning. At the same time its use in Sarin (methyl isopropoxy phosphoryl fluoride) and Soman (methyl pinacolyloxy phosphoryl fluoride) poisoning was examined.

MATERIALS AND METHODS

Human erythrocytes were obtained after centrifugation of heparinized blood (blood bank) and washed three times with 0·9% sodium chloride solution. The enzyme preparation was inhibited with a 10^{-4} M solution of organophosphorus compound at 0°C for one hour. Excess of inhibitor was removed and the reactivation was studied at pH 7·4 and 37°C with an oxime concentration of $2{\cdot}5 \times 10^{-5}$ M as described earlier.[2] As substrate a final concentration of $7{\cdot}3 \times 10^{-3}$ M acetylcholine iodide was used.

The intraperitoneal LD_{50} of Toxogonin was studied with male CBA mice and the protective effect of the compound against Sarin, Soman and Tabun was compared with that of TMB-4 and P2S in the presence of atropine, 10 mg/kg. 1/5th of the LD_{50}[13] of the oximes was given. The organophosphorus compounds were injected subcutaneously. Atropine and oxime were given together and the organophosphorus compound was given 10 min later. The animals were observed for 24 hr. LD_{50} was calculated according to the method described by Miller and Tainter.[14]

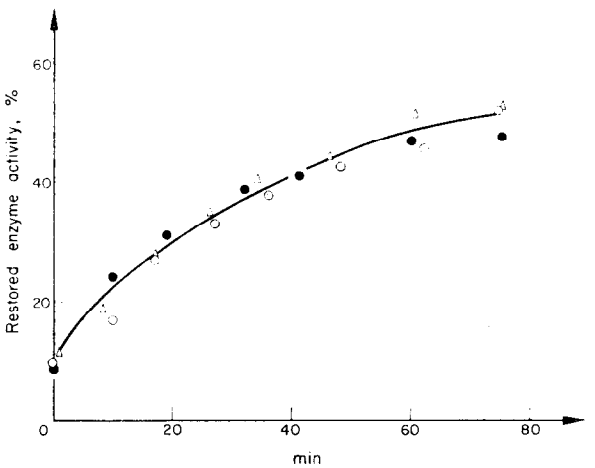

FIG. 1. Reactivation (pH 7·4, 37°C) of Sarin-inhibited erythrocyte cholinesterase expressed in per cent of the activity of uninhibited cholinesterase in the presence of the same amount of oxime ($2{\cdot}5 \times 10^{-5}$ M)
△ = TMB-4, ○ = Toxogonin, ● = P2S

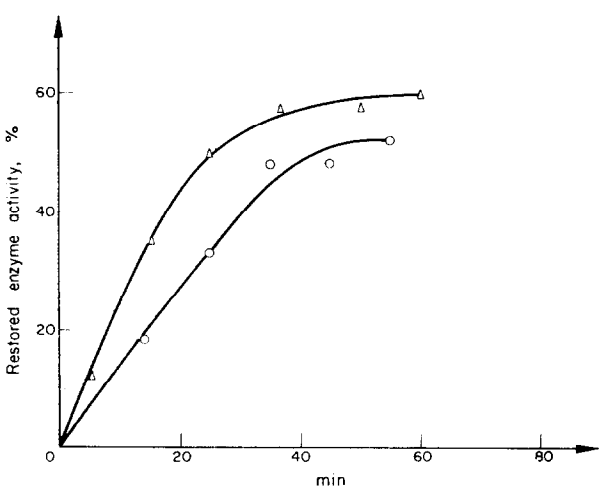

FIG. 2. Reactivation (pH 7·4, 37°C) of Tabun-inhibited erythrocyte cholinesterase expressed in per cent of the activity of unhibited cholinesterase in the presence of the same amount of oxime ($2{\cdot}5 \times 10^{-5}$ M)
△ = TMB-4, ○ = Toxogonin

RESULTS

Reactivation of inhibited erythrocyte cholinesterase

Figure 1 shows that the *in vitro* reactivating power of Toxogonin at a concentration of $2{\cdot}5 \times 10^{-5}$ M is comparable to that of P2S and TMB-4 in the case of Sarin-inhibited erythrocyte cholinesterase. Within 25 min 24 per cent of enzyme activity were recovered. With a reactivator concentration of $2{\cdot}5 \times 10^{-5}$ M reactivation reached a plateau at about 50 per cent reactivation. An oxime concentration of $8{\cdot}5 \times 10^{-3}$ M was able to restore complete enzyme activity. Tabun-inhibited erythrocytes are reactivated somewhat more rapidly with TMB-4 than with Toxogonin as seen in Fig. 2. Reactivation with P2S of the same concentration was not measurable. Toxogonin reactivates 50 per cent of the enzyme within 44 min while TMB-4 needs 26 min. At oxime concentrations of $8{\cdot}5 \times 10^{-3}$ M TMB-4 and Toxogonin restore complete enzyme activity in less than 15 min, while P2S of the same concentration restores about 30 per cent of enzyme activity during the same time.

Toxicity and protective effect of Toxogonin

The LD_{50} of Toxogonin after intraperitoneal injection into male CBA mice was found to be $102 \pm 0{\cdot}4$ mg/kg body weight. The subcutaneous LD_{50} of Tabun on untreated animals was found to be 0·34 mg/kg and that of Soman 0·14 mg/kg. For Sarin the LD_{50} of 0·31 mg/kg was used.[13] Table 1 shows the protective effect of the oximes in the presence of atropine. Under the conditions used Toxogonin is comparable to TMB-4 and 3–4 times better than P2S in Sarin poisoning and 8–10 times better than P2S in Tabun poisoning. It has no effect in Soman poisoning.

DISCUSSION

As expected the cholinesterase reactivating power of Toxogonin is comparable to that of TMB-4 in Sarin and Tabun poisoning, This effect together with the relatively low toxicity of the compound and a good resorption from intramuscular depots[10] probably makes it a better antidote in alkyphosphate poisoning than P2S. Soman inhibited acetylcholinesterase resists reactivation. Thus 10^{-4} M 2-PAM and TMB-4 reactivate only 1·4 and 8·3 per cent of the enzyme within 50 min at body temperature.[15] The reason for the failure to reactivate is probably to be found in the bulky alkoxy side chain, which may be shielding the methyl pinacolyloxy phosphorylated enzyme from the oxime and also render attraction of the oxime to the anionic site of the enzyme impossible. Another possibility is that Soman inhibited acetylcholin esterase "ages" rapidly, which would make reactivation by oximes impossible. It is further known that 2-PAM and TMB-4 (i.p.) have no prophylactic effect either alone or in combination with atropine, when Soman is injected intraperitoneally while after dermal application of Soman to mice the mortality is somewhat decreased by prior i.p. administration of atropine, oximes and especially by a mixture of both (from 90 to 30 per cent mortality after 1·3 LD_{50} of Soman).[15] This latter effect may be due to a direct action of the oximes upon the organophosphorus compound, made possible by the longer pathway of Soman in reaching its points of attack. The very slight prophylactic effect now observed after administration of atropine and Toxogonin is probable due to atropine and perhaps to an atropine-like effect of Toxogonin. Such effects have been observed with other oximes.[16]

TABLE 1. MULTIPLES OF ORGANOPHOSPHORUS COMPOUND (S.C.) TOLERATED BY MICE IN THE PRESENCE OF ATROPINE (10 MG/KG I.P.) AND OXIME (I.P.), GIVEN TOGETHER 10 MIN BEFORE THE ORGANOPHOSPHORUS COMPOUND

Name of compound	Oxime		LD_{50} of organophosphorus compound (s.c.) in the presence of oxime and atropine (10 mg/kg)					
	injected amount (i.p.)		Sarin		Tabun		Soman	
	mg/kg	multiples of LD_{50}	LD_{50} mg/kg	multiples of LD_{50} of untreated animals	LD_{50} mg/kg	multiples of LD_{50} of untreated animals	LD_{50} mg/kg	multiples of LD_{50} of untreated animals
Toxogonin (dichloride)	20	1/5	9·9 ± 0·72	33	4·0 ± 0·11	12	0·21	1·3
TMB-4 (dibromide)	14	1/5	8·2 ± 0·68	27	4·9 ± 0·11	14	—	—
P2S	24	1/5	2·6 ± 0·33	8·7	0·51	1·5	—	—

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