Mechanism of the prophylactic action of diacetylmonoxime against sarin poisoning*

Organo-phosphorus derivatives such as DFP and *iso*propylmethylphosphonofluoridate (sarin) are known to be toxic to animals by virtue of their ability to inhibit the acetylcholine esterase (true choline esterase) at vital centres in the animal. The inhibited acetylcholine esterase can be reactivated by a number of oxime and hydroxamic acid derivatives (see refs. [1-4]) and, as might be expected, potent reactivating agents (e.g., P-2-AM) are capable of protecting the animals against the toxic effects of sarin when they are administered prophylactically to atropinized animals. However, some anomalous results have been observed[1-6], notably with DAM as the prophylactic agent against sarin poisoning.

DAM, unlike P-2-AM, is not very effective in reactivating inhibited acetylcholine esterase, nor does it exert any appreciable therapeutic effect in accelerating the recovery of poisoned animals when administered some time after sarin[5]. It increases the lethal dose of sarin in atropinized mice by a factor of only 2[5,6]; yet this compound will protect atropinized rats against 50 times the normal lethal dose of sarin. These observations suggest, therefore, that in the rat DAM catalyses the destruction of sarin in the blood stream before the sarin reaches the acetylcholine esterase in the vital centres of the animal. A possible biochemical mechanism for this destruction would be a cyclic process involving the repeated inhibition of some non-essential esterase by sarin and its reactivation by DAM.

It is known that the plasma ali-esterase (tributyrinase; B-type esterase) is readily inhibited by DFP; in fact, only minute amounts of DFP appear to be utilised for the inhibition of choline esterases, while large amounts combine with the ali-esterases in the case of rat plasma[7], guinea-pig plasma[8] and bovine erythrocytes[9]. Since sarin is closely related to DFP in chemical structure and biochemical characteristics, it might be expected that similar findings would apply to sarin.

In order to determine the effects of injected sarin on various esterases, adult male rats and mice were injected subcutaneously with 0.18 mg sarin/kg in a volume of 5 ml saline/kg; this dose resulted in the death of 35–40 % of both the rats and the mice within 30 min. As expected, both the acetylcholine esterase and pseudocholine esterase in plasma and brain were strongly inhibited by this treatment. However, the plasma ali-esterase was also 80–90 % inhibited in the animals which died and 60 % inhibited in the animals which survived for 2 h. Some of the surviving animals were injected intraperitoneally with 150 mg DAM/kg 1 h after the sarin injection and killed 2 h after the sarin. This therapeutic treatment with DAM produced little reactivation of the inhibited acetylcholine esterase and only partial reactivation of the inhibited pseudocholine esterase; the inhibited plasma ali-esterases, however, were almost completely reactivated.

Similar experiments with different doses of DAM gave the results shown in Fig. 1. The inhibited plasma ali-esterase was 50 % reactivated by a dose of 3 mg DAM/kg in the rat and by a dose of 80 mg DAM/kg in the mouse, a difference of approximately 27 times in the doses required. Comparable results were obtained *in vitro* with the

Abbreviations: DFP, diisopropylphosphofluoridate; P-2-AM, pyridine-2-aldoximemethiodide; DAM, diacetyl monoxime (2-oximino-3-butanone); TOCP, tri-ortho-cresyl phosphate.

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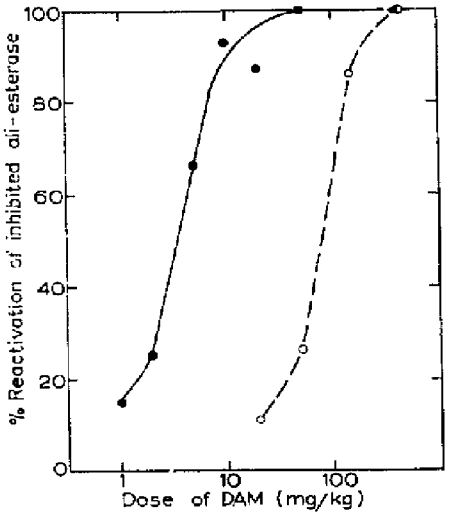

Fig. 1. Reactivation of plasma ali-esterases by various intraperitoneal doses of DAM following previous inhibition by sarin in the rat (●——●) and in the mouse (○ – – – ○).

inhibited esterases; after incubation for 1 h at pH 7.4 and 37°, the inhibited ali-esterase of rat plasma was 50 % reactivated by 1.0 mg DAM/l and that of mouse plasma by 35 mg DAM/l. Thus the plasma ali-esterases in the two species differed in their susceptibility to reactivation by DAM. Similar discrepancies between the choline esterases of the rat and mouse did not occur. Morevoer, the ali-esterases found in the erythrocytes, brain and lung of the rat and mouse were much less sensitive than the ali-esterase in plasma to reactivation by DAM. It appeared, therefore, that the difference in the prophylactic effect of DAM against sarin poisoning in rats and in mice might be due to the above difference in the effects of DAM on the plasma ali-esterase after inhibition by sarin.

In order to substantiate this hypothesis, we compared the prophylactic effects of DAM in normal rats and in rats with little plasma ali-esterase available. For this purpose, female rats were injected subcutaneously with 50 mg TOCP/kg one day previously[10]; this dose is less than one-hundredth of the lethal dose of TOCP[11] and the rats appear completely normal. The TOCP treatment caused approximately 95 % inhibition of the plasma ali-esterase without any measurable inhibition of either the

TABLE I

Prophylaxis of sarin poisoning with DAM in normal and TOCP-treated rats. Adult female rats were injected subcutaneously with various doses of sarin in a volume of 5 ml/kg and the dose required to kill 50 % of the animals (LD_{50}) was calculated from the results by the probit method[12]. Prophylactic treatment consisted of 18 mg atropine sulphate/kg and 150 mg DAM/kg, both compounds being injected intraperitoneally 15 min before the sarin was given[5].

Animals	*Prophylaxis*	*LD_{50} (mg sarin/kg)*	*95 % fiducial limits of LD_{50}*	*Increase in LD_{50} effected by DAM*
Normal	Atropine alone	0.284	(0.26–0.31)	
	Atropine + DAM	65.5	(61–70)	230 ×
TOCP	Atropine alone	0.044	(0.031–0.060)	
	Atropine + DAM	0.630	(0.41–0.98)	14 ×

pseudocholine esterase or acetylcholine esterase in plasma, brain and liver of the rats. Moreover, after this period of time, the inhibited plasma ali-esterase could not be reactivated by any dose of DAM.

Pre-treatment with TOCP increased the subcutaneous toxicity of sarin to atropinised female rats approximately 6-fold (Table I). This effect of the TOCP on the toxicity could be reproduced even when the sarin was given intravenously to non-atropinised rats, the LD_{50} being reduced from 0.064 to 0.0117 mg sarin/kg in this case. This finding substantiates the previous supposition that most of the sarin required to kill a rat is actually used up by the inhibition of the ali-esterase.

Prophylactic treatment of atropinised rats with 150 mg DAM/kg provided a striking protection against sarin poisoning (Table I)*. The protection afforded by DAM was reduced to 6 % of the control value when the rats were pre-treated with 50 mg TOCP/kg (Table I). These results support the conclusion that the prophylactic effect of DAM in rats is correlated with the reactivation of a non-essential ali-esterase which is sensitive to inhibition by sarin as well as by TOCP. For comparative purposes, parallel experiments were carried out with P-2-AM in place of DAM. P-2-AM is approximately 20 times less effective than DAM in reactivating the inhibited plasma ali-esterases of the rat and mouse but, unlike DAM, is a very potent reactivator of inhibited choline esterases (50 % reactivation after 1 h by 0.9–1.3 mg P-2-AM/l, *in vitro*). In contrast to the results obtained with DAM, the protection afforded to atropinised rats by P-2-AM was not decreased by pre-treatment with TOCP. The prophylactic effect of P-2-AM appears therefore to be due mainly to reactivation of inhibited cholinesterases.

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* The degree of protection afforded by DAM in these experiments is greater than that reported previously[5] due to differences in the volume of sarin solution injected subcutaneously into the rats. When the volume was reduced from 5 ml/kg to 0.5 ml/kg so that the sarin can be absorbed into the blood stream more rapidly, the degree of protection afforded to normal atropinised rats by 150 mg DAM/kg was reduced from 230 to 39 times the lethal dose.