SOME CHARACTERISTICS OF SOMAN, SARIN AND VX POISONING

Jiří BAJGAR
Department of Toxicology, Purkyně Military Medical Academy, Hradec Králové

Summary

Effects of sarin, soman and VX were described and discussed. The trigger mechanism of their action is inhibition of cholinesterases followed by non-specific changes. However, changes in cholinesterase molecular forms are of interest and they play an important role in mechanism of action of nerve agents, too.

KEY WORDS: Cholinesterases molecular forms sarin, soman, VX.

The threat of the use of chemical weapons not only in military conflicts but also in terroristic attacks is not excluded at present as it was clearly demonstrated in Tokyo (27) and Matsumoto (44) cities (sarin attack in the subway). The very actual group of these chemicals are organophosphates (OP) including nerve agents. The most important nerve agents are represented by sarin (O-isopropyl methylphosphonofluoridate), soman (O-pinacolyl methylphosphonofluoridate) (these two compounds belong to so called G-compounds) and VX (O-ethyl S-2-diisopropylaminoethyl methylphosphonothiolate) (V-compounds). Moreover, there are produced many organophosphorus compounds in the civilian facilities and evaluated in industry, agriculture, medicine etc. Basic mechanism of action and antidotal treatment for these compounds are in principle the same and therefore some conclusions from this paper can be applied in the civilian medicine.

From the point of pharmacodynamics and therapeutic possibilities, soman represents the most serious poison: its toxicity is comparable with that of sarin and VX (7, 8, 15, 16, 35) but therapeutic efficacy of antidotal treatment with present and perspective drugs is not good enough (14, 17, 23, 24, 29). This is probably a reason for intensive research dealing with soman intoxication and its treatment.

Soman and sarin are quickly resorbed at all routes of administration including inhalation, percutaneous and oral administration (7) and inhibit cholinesterases (preferably acetylcholinesterase, AChE, EC 3.1.1.7) in the central and peripheral nervous system. Because of high lipophility of soman, it posses high affinity to brain AChE (1, 8). Sarin is less lipophilic, however, its affinity to the brain AChE is also very high (8).

The inhibition of the brain AChE by G-compounds (sarin and soman) is very fast reaching 50% activity within minutes. For VX, there is a delay and decrease of AChE activity that was observed in more than twenty minutes, probably caused by more difficult absorption in comparison with sarin and soman. The half-lives are very dependent on the dose of the agent administered, on the species and other factors and therefore it is difficult to compare our results. In general, inhibition of AChE in vivo is faster for G-compounds in comparison with V-compounds (1, 8, 25).

Soman and sarin are detoxified in the liver, plasma (21, 37), according to some authors also in the lung (34) and therefore this part is excluded from the toxic effect. The losses of G-compounds in the organism are also caused by binding to non specific esterases and this part of soman and sarin is not able to cause toxic effect. It was assessed that only 1-3% from the dose administered of the both compounds inhibited AChE in the brain, i.e. 1-3% of the dose administered caused the basic toxic effect (7, 22, 26, 34). Another factor (up to now not very elucidated) influencing soman and sarin poisoning is an existence of a depot in the organism from it the nerve agent can be released and causes new attack of intoxication. This depot was described for the skin, erythrocytes, muscles and lung (8, 22). Bearing in mind very low portion of the dose administered causing basic toxic effect (1-3%), it is clear that releasing of a very small quantity of sarin and soman can influence significantly survival or death of intoxicated organism independently on the treatment.

On the other hand, V-compounds are not detoxified in the organism (7). Probably this is a reason of higher toxicity of V-compounds in comparison with G-compounds. The effect of V-compounds (especially VX) is prolonged in comparison with sarin and soman (42). The mechanism of action of VX is inhibition of AChE preferably in peripheral nervous system (8, 28). However, the inhibition of AChE in the brain parts was described to be selective and the most marked in the pontomedullar area of the brain (7, 8).

Mechanism of AChE inhibition for all three compounds is practically the same - it is phosphorylation of the esteratic site of AChE. However, reactivation of phosphorylated AChE by oximes is different for different nerve agents: phosphorylated but reactivatable AChE is changed to non-reactivatable complex. The half times for this

reaction described as dealkylation (18, 19) are following: for by soman inhibited AChE - cca 8-10 min, for by sarin inhibited AChE - about 10 hours and for by VX inhibited AChE this reaction was not observed within 24 hours (7, 8).

The evidence supporting AChE as the primary site of the both OP and nerve agents action has been summarized by many authors (6, 19, 28, 39). It includes the following observations: symptoms of OP poisoning are similar to those of the AChE inhibitor physostigmine; the *in vivo* LD_{50} for a variety of OP correlates well with the inhibition efficacy to AChE determined *in vitro;* and ChE reactivators (e.g. oximes), anticholinergics (e.g. atropine) and spontaneously reactivating AChE inhibitors (e.g. carbamates) can reduce OP toxicity. However, despite the model's pragmatic success, a variety of data are inharmonious with AChE inhibition as the only important biochemical event in OP intoxication.

Thus, basic mechanism of nerve agents - similarly as for other OP - is an intervention into cholinergic nerve transmission via irreversible inhibition of AChE and other hydrolases (6, 28, 39). Monitoring of cholinesterase changes - their development during the intoxication is at present the best reflexion of a severity of OP poisoning as well as a reaction to antidotal therapy.

Both enzymes (AChE and butyrylcholinesterase, BuChE, EC 3.1.1.8) exist in multiple molecular forms (2-6, 12, 25, 30, 36, 38). The activities of these forms are also influenced by many factors. The function of these forms is not known at present, however, their presence in the membrane structures at physiological conditions was demonstrated (5). There are a few data describing the changes of AChE molecular forms following intoxication with highly toxic OP (25). Some experiments were performed with relatively less toxic OP (4, 10, 30).

Molecular forms of AChE showed different sensitivity to inhibitors in vitro (4, 7, 31) and in vivo (11, 13, 30, 40, 41). Following DFP (40) and highly toxic OPs (4, 6, 25), the form with high molecular weight was the most sensitive. Intoxication with Parathion and Neguvon (less toxic OPs) caused medium inhibition of some forms of AChE (7, 13, 43).

From different results describing multiple molecular forms of AChE it can be concluded that AChE in the brain exists in molecular forms. These forms were observed also by other authors (3, 5, 12, 13, 31, 40, 43). These forms are different for various species. However, the electrophoretic mobility of AChE components from the rat, rabbit, mouse and human brains suggested that there are generally two types of AChE forms having high and low molecular weight. One BuChE and two AChE bands in the rat hippocampus after electrophoresis in polyacrylamide gel were observed (41). The distinction between the two AChE forms is difficult without electrophoresis. They differ in electrophoretic mobility and they can be well differentiated by electrophoresis.

Subcellular localization of AChE suggested that in nerve ending particles and microsomal fractions 2-4 AChE forms are present, in the mitochondrial fraction only one was detected (4). The microsomal form absent in the mitochondrial fraction is the most sensitive to OPs in vivo. From previous studies it is known that high molecular form of AChE has the lowest K_m value (2) and highest decrease in this component after deafferentation was also observed (3). These results suggested that this form of AChE would be very important for normal cholinergic nerve transmission. It arises the question on existence of the forms under physiological conditions. Using thermal denaturation, it was demonstrated that they are not artifacts formed during homogenization or other treatment of the brain tissue (5). These findings were also described by other authors (2, 5, 23, 25, 33, 41, 43). The overall data show that catalytic activity of AChE molecular forms is different and their inhibition by various inhibitors may be heterogeneous. This heterogenicity was demonstrated for AChE phosphorylating inhibitors as well as for inhibitors with different binding sites for the enzyme.

The results with another type of inhibitor - 7-methoxytacrine (7-MEOTA) fit well with our previous findings indicating a greater sensitivity of slowly migrating molecular forms separated by polyacrylamide gel electrophoresis (10). In fact, it has been demonstrated recently that slowly migrating forms of cortical AChE correspond to G_4 forms separated by sedimentation analysis (43). On the other hand, recent data indicate an almost equal sensitivity of G_4 and G_1 forms of both soluble and membrane-bound whole brain AChE to this type of inhibitor (31). It is not excluded that the reversible inhibitors such as 7-MEOTA modify their interaction with the active site resulting in a preferential inhibition of G_4 forms. It is of interest that the introduction of a heptyl group into physostigmine modified its interaction with the AChE molecular forms, heptylphysostigmine showing a stronger inhibition for G_4 than for G_4 forms while in the case of the parent compound similar inhibition of the two forms was observed (31).

The data of 7-MEOTA are different from those obtained for DFP and paraoxon showing similar IC_{50} values for G_4 and G_1 forms (43). These findings have been confirmed for membrane-bound AChE (31). This is not surprising since the interaction of OP compounds (and physostigmine) with the active site of enzymatic molecule is different from that for 7-MEOTA-type compounds. In fact, OP compounds inhibit AChE by phosphorylating the esteratic serine in the catalytic site. On the other hand, acridine derivatives bind to the

hydrophobic area close to the active site of AChE simultaneously affecting its catalytic center via an allosteric mechanism (20, 32, 38).

As regards the data on AChE molecular forms, they confirm previous findings indicating a more pronounced sensitivity of G_4 forms, as compared to G_1 forms, in brain of rats injected with paraoxon (43). Somewhat lower inhibitory effects of the same dose of paraoxon (0.25 mg/kg s.c.) as well as a somewhat lower contribution of G_4 forms to total AChE in untreated rats were observed in another experiments (11) in comparison with those reported by Volpe et al. (43) and may depend on regional differences (cerebral cortex and whole brain).

In the case of brain AChE, as it has been pointed out (43), G_4 and G_1 forms represent distinct pools in the cell, the former being mainly associated with membranes, with its catalytic site exposed to the extracellular space, and the latter confined to the intracellular compartment.

Following intoxication with nerve agents mentioned, the highest sensitivity for high molecular AChE form was observed (6). Determination of the whole AChE activity is partly misrepresenting because of different distribution of AChE molecular forms in the sample. Following determination of the whole activity, a „mean" activity containing activities of the forms is determined. It can be concluded that in studies requiring high sensitivity (e.g. the studies of antidotal action), AChE molecular forms would be of choice for more detailed information of functional stage of AChE - important marker of cholinergic nerve transmission.

This approach could lead to improvement of our knowledge of mechanisms of action of OP and soman poisoning and their treament.

Simultaneously it could contribute to better understanding of cholinergic nerve transmission and thus to pharmacology and neuropharmacology in general.

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Correspondence: Plk. doc. MUDr. Jiří Bajgar, DrSc.
Vojenská lékařská akademie J. E. Purkyně
Třebešská 1575
500 01 Hradec Králové

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