NATURAL DETOXIFICATION CAPACITY TO INACTIVATE NERVE AGENTS SARIN AND VX IN THE RAT BLOOD

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Summary: Background: The method of continual determination of the rat blood cholinesterase activity was developed to study the changes of the blood cholinesterases following different intervetions. Aims: The aim of this study is registration of cholinesterase activity in the rat blood and its changes to demonstrate detoxification capacity of rats to inactivate sarin or VX in vivo. Methods: The groups of female rats were premedicated (ketamine and xylazine) and cannulated to a. femoralis. Continual blood sampling (0.02 ml/min) and monitoring of the circulating blood cholinesterase activity were performed. Normal activity was monitored 1–2 min and then the nerve agent was administered i.m. (2× LD_{50}). Using different time intervals of the leg compression and relaxation following the agent injection, cholinesterase activity was monitored and according to the inhibition obtained, detoxification capacity was assessed. Results: Administration of sarin to the leg, then 1 and 5 min compression and 20 min later relaxation showed that further inhibition in the blood was not observed. On the other hand, VX was able to inhibit blood cholinesterases after this intervention. Conclusions: The results demonstrated that sarin can be naturally detoxified on the contrary to VX. Described method can be used as model for other studies dealing with changes of cholinesterases in the blood following different factors.

Keywords: *Sarin; VX; Detoxification; Rat; Blood; Cholinesterases*

Introduction

The toxicodynamics of nerve agents is based on irreversible acetylcholinesterase (AChE, EC 3.1.1.7) inhibition at the cholinergic synapses (4). The resulting accumulation of neuromediator acetylcholine at the cholinergic synapses overstimulates the cholinergic pathways and subsequently desensitizes the cholinergic receptor sites. Before AChE inhibition in the central and peripheral nervous system, the enzyme is inhibited in the transport system, in the blood according to the principle "first come, first serve" (6). Two enzymes in the blood are present, AChE in the erythrocytes and butyrylcholinesterase (BuChE, EC 3.1.1.8) in the plasma/serum. However, in the blood, the binding of the agent to cholinesterases is leading to the decrease of its concentration and toxic effect. Other detoxification reactions have occured, too. These characteristics are important for diagnosis, mechanism of action of nerve agents, and, especially, for prophylaxis.

These prophylactic countermeasures include i.a. AChE protection against inhibition, decrease of nerve agent level using stoichiometric, catalytic and pseudocatalytic scavengers (2, 5, 11). For review see e.g. (4, 5, 10, 12, 14, 19).

Enzymes capable of nerve agents decomposition (catalytic scavengers) are known many years. They are extensively studied with the aim to develop new ways of decontamination or to prevent nerve agent toxicity (2, 10, 12, 14, 19).

Among enzymes participating in metabolism of nerve agents, A-esterases, serum cholinesterase and carboxylesterases are involved. Their role and mechanism of action in detoxification process is different and was extensively discussed by Jokanovic (10).

Detoxification capacity was studied in rats with the aim to elucidate the action of nerve agents and, to improve prophylaxis against nerve agents and organophosphorus compounds. We developed the technique for continual monitoring of the blood cholinesterase activity (7). It allows to study the changes in the activity in the real time. The method was originally developed for the study of the blood cholinesterase changes following acute exposure with nerve agents. However, the technique can be used for modelling of effects of different factors influencing the enzyme and, in the present study, it was evaluated for the demonstration of detoxification of two nerve agents, sarin and VX.

Methodical approach

Animals: Female Wistar rats (VELAZ Prague), weighing 250–270 g, were used in this study. The animals were divid-

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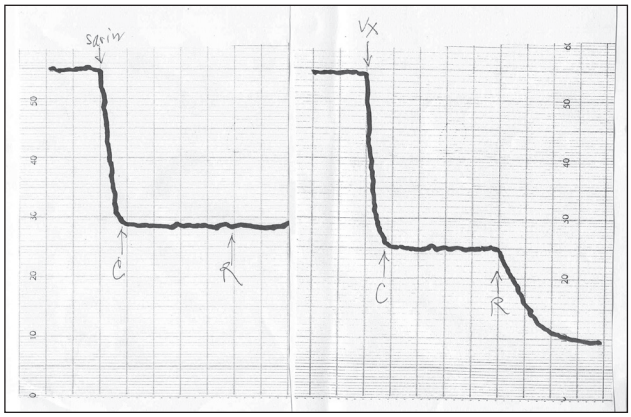

Fig. 1: Copies of original records of monitoring the rat blood cholinesterase activity. C – compression of the leg, R – relaxation of the leg.

ed into groups of 3–7 animals in each. Housing of rats was realized in the Central Vivarium of the Faculty of Military Health Sciences under veterinary control. All the experiments were performed under permission and supervision of the Ethics Committee of the Faculty of Military Health Sciences, Hradec Králové.

Chemicals: Nerve agents (sarin, VX) were obtained from Military Technical Institute of Protection (Brno, Czech Republic). They were of minimally 99% of purity and stored in glass ampullas (1 ml). The solutions in saline for experiments were prepared before use.

Continual monitoring of the rat blood cholinesterase activity: The method described by Cabal et al. (7) based on Ellman's method (8) with acetylthiocholine as substrate was used. Generally, the groups of female Wistar rats weighing 250–270 g (n = 3–7) were premedicated (ketamine and xylazine) and cannulated to a. femoralis. Continual blood sampling (0.02 ml/min) and monitoring of the circulating blood cholinesterase activity were performed (the activity represents all enzymes hydrolyzing acetylthiocholine). Normal activity was monitored 1–2 min and then the agent (sarin or VX) was administered i.m. into one leg in dose of 2× LD_{50}. Using different combinations of the leg compression and relaxation (different time intervals before/following agent injection), cholinesterase activity was monitored and according to the inhibition obtained, detoxification capacity was derived.

Results

Intoxication with sarin and VX caused strong decrease of the blood cholinesterase activity. Copies of original records of the blood cholinesterase activity is shown in Fig. 1. When administration of the agent was performed and the leg was compressed immediately (1 min) after the intoxication, the activity was decreased to 40–60% of normal activity. After relaxation 20 min later, two different changes were observed: following sarin intoxication, the inhibition was remained on the same level while following VX administration, the inhibition was continuing after relaxation (Fig. 2). When the compression was performed 5 min after the intoxication (and relaxation 20 min later), the inhibition was more expressed and continued after relaxation following VX administration while following sarin intoxication, further inhibition was not demonstrated (Fig. 3). If the compression was done later (10 min after the intoxication, then relaxation 20 min), the inhibitions by sarin and VX were not very different after relaxation in both cases (following sarin and VX administration).

Discussion

The results suggested that resorption of the agent (both sarin and VX) is very fast and 1 min after the intoxication, approximately 50% of the blood cholinesterases are inhibited. The role of compression is not significant for haemodynamic changes at the time intervals studied as it

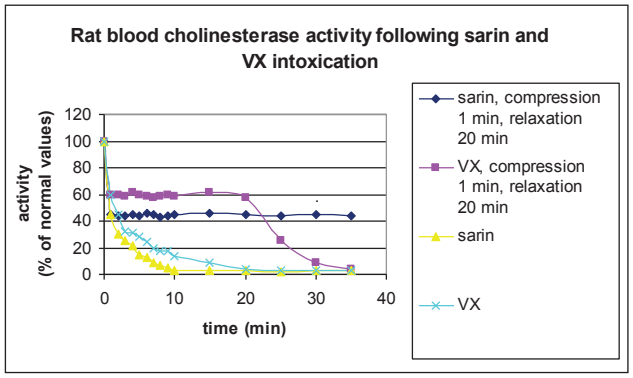

Fig. 2: Changes in the rat blood cholinesterase activity following intoxication with sarin and VX without compression and with compression 1 min after the intoxication and relaxation 20 min later [sarin (6), VX (7)]. The number in brackettes indicate the number of animals in group. The results are means only, SD were not higher than ±10%.

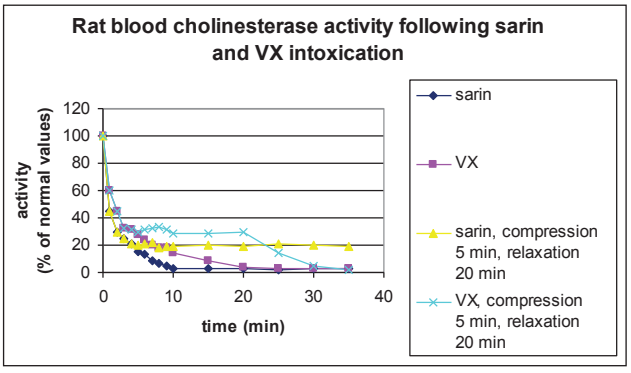

Fig. 3: Changes in the rat blood cholinesterase activity following intoxication with sarin and VX without compression [sarin (3), VX (4)] and with compression 5 min after the intoxication and relaxation 20 min later. The number in brackettes indicate the number of animals in group. The results are means only, SD were not higher than ±10%.

was described earlier: there were observed haemodynamic changes following femoral vein ligation but the changes were demonstrated after relatively long time (6 hours) after the ligation (20).

Comparison of these results is difficult because a lack of similar data in literature. The half-lives of inhibition of the blood ChE following intoxication with VX and sarin (1) can be compared with our results and the half-times of inhibition are similar. The blood cholinesterase activity on swine was monitored following percutaneous intoxication with VX (17); the inhibition was lower in comparison with our results. Toxicokinetic data with direct determination of the agents published by Schans et al. (21) show similar results as demonstrated in our experiments with inhibition efficacy in the blood.

Quite different cholinesterase changes obtained following sarin and VX intoxication (after compression and relaxation of the leg) suggested that the loss of inhibition capacity can not be scavenger effect (in that case, similar results for both agents sarin and VX would be expected). The loss of inhibition capacity is probably caused by detoxification indicating that sarin is able to be detoxified. Similar results (detoxification of nerve agents including sarin except VX) were demonstrated by Ohmori et al. (18) in decontamination study. Degradation of G-type of nerve agents was also described (15); it was caused by carboxylesterases: their amount in mice and rats was higher than in rabbits or guinea pigs leading to higher LD_{50}.

Since VX does not react with carboxylesterases, VX can not be detoxified by these enzymes (16). It could be also a reason for different cholinesterase inhibition by G- and V-nerve agents in the brain structures as demonstrated earlier (9, 3). Similar problem was solved by Wills et al. (22) using another approach: fresh frozen plasma (FFP) was used to demonstrate detoxification of G- and V-agents in vitro; the authors concluded suitability of FFP for the treatment of intoxication with G-agents but not for V-agents. Using the method of continual determination of the blood cholinesterase activity, it is possible to monitor cholinesterase activity not only for irreversible but also for reversible inhibitors used for prophylaxis against nerve agent intoxication, e.g. physostigmine, tacrine, huperzine etc. (4, 13).

Conclusions

Described methodical approach allows in real time and real conditions in vivo to monitor the changes of the blood cholinesterases following administration of different experimental interventions, e.g. agents administration, and the effect of detoxification.

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References

1. Bajgar J. Biological monitoring of exposure to nerve agents. Brit J Ind Med 1992; 49: 648–53.
2. Bajgar J, Fusek J, Kassa J, Kuca K, Jun D. Chemical aspects of pharmacological prophylaxis against nerve agent poisoning. Curr Med Chem 2009; 16: 2977–86.
3. Bajgar J, Hajek P, Kassa J, et al. Voicu VA. Combined approach to demonstrate acetylcholinesterase activity changes in the rat brain following tabun intoxication and its treatment. Toxicol Mech Meth 2012; 22, 60–66.
4. Bajgar J. The Effect of Organophosphates/Nerve Agents: Poisoning and its Treatment in Schematic Figures and Tables. Amsterdam: Elsevier: 2012.
5. Bajgar J. Prophylactic Possibilities in Case of High Risk of Exposure to Nerve Agents. In: Arora R, Arora P, editors. Disaster Management, Medical Prepardness, Response and Homeland Security. Croydon: CAB International; 2013, p. 425–45.
6. Benschop HP, de Jong LPA. Toxicokinetics of nerve agents. In: Somani SM, Romano JA, editors. Chemical Warfare Agents: Toxicity at Low Levels, Boca Raton: CRC Press: 2001, p. 25–81.
7. Cabal J, Bajgar J, Kassa J. Evaluation of flow injection analysis for determination of cholinesterase activities in biological material. Chem-Biol Interact 2010; 187: 225–8.
8. Ellman GL, Courtney DK, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961; 7: 88–95.
9. Hajek P, Slizova D, Krs O, Bajgar J. Comperison of changes in ACHE activity in the brain of the laboratory rat after soman and tabun intoxication. Biomed Papers 2004; 148: 209–11.
10. Jokanovic M. Current understanding of the mechanisms involved in metabolic detoxification of warfare nerve agents. Toxicol Lett 2009; 188: 1–10.
11. Jun D, Musilova L, Kuca K, Kassa J, Bajgar J. Potency of several oximes to reactivate human acetylcholinesterase and butyrylcholinesterase inhibited by paraoxon in vitro. Chem-Biol Interact 2008; 175: 421–4.
12. Kuca K, Jun D, Musilek K, et al. Prophylaxis and post-exposure treatment of intoxications caused by nerve agents and organophosphorus pesticides. Mini-Rev Med Chem 2013; 13: 2102–15.
13. Lallement G, Baille V, Baubichon D, et al. Review of the value of huperzine as pretreatment of organophosphate poisoning. Neurotoxicology 2002; 23: 1–5.
14. Layish I, Krivoy A, Rotman E, Finkelstein A, Tashma Z, Yehezkelli Y. Pharmacologic prophylaxis against nerve agent poisoning. Israel Med Assoc J 2005; 7: 182–7.
15. Maxwell DM, Brecht KM, O'Neill BL. The effect of carboxylesterase inhibition on interspecies differences in soman toxicity. Toxicol Lett 1987; 39: 35–42.
16. Maxwell DM. The specificity of carboxylesterase protection against the toxicity of organophosphorus compounds. Toxicol Appl Pharmacol 1992; 114: 306–12.
17. Misik J, Pavlik M, Novotny L, et al. In vivo decontamination of the nerve agent VX using the domestic swine model. Clin Toxicol 2012; 50: 807–11.
18. Ohmori T, Kawahara K, Nakayama K, et al. Decontamination of nerve agents by immobilized organophosphorus hydrolase. Forensic Toxicol 2013; 31: 37–43.
19. Patocka J, Jun D, Bajgar J, Kuca K. Prophylaxis against nerve agent intoxications. Defence Sci J 2006; 56: 775–84.
20. Revell WJ, Brooks M. Haemodynamic changes in the rat femur and tibia following femoral vein ligation. J Anat 1994; 184: 625–33.
21. Schans van der MJ, Lander BJ, van der Wiel H, Langenberg JP, Benschop HP. Toxicokinetics of the nerve agent (+/−)-VX in anesthetized and atropinized hairless guinea pigs and marmosets after intravenous and percutaneous administration. Toxicol Appl Pharmacol 2003; 191: 48–62.
22. Wille T, Thiermann H, Worek F. In vitro kinetics of nerve agent degradation by fresh frozen plasma (FFP). Arch Toxicol 2014; 88: 301–7.

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