

Original Scientific Paper

EQUINE BUTYRYLCHOLINESTERASE PROTECTS RATS AGAINST INHALATION EXPOSURE TO SUBLETHAL SARIN CONCENTRATIONS

Jiří BAJGAR, Lucie BARTOŠOVA, Josef FUSEK, Daniel JUN, and Kamil KUČA

Department of Toxicology, Faculty of Military Health Sciences, University of Defence, Hradec Kralove, Czech Republic

Received in March 2006

Accepted in August 2006

Protection experiments were conducted using different doses of equine serum butyrylcholinesterase (Eq BuChE) as pretreatment in rats. Cholinesterase activities were determined in blood [whole blood, red blood cells (RBC) acetylcholinesterase (AChE), and plasma BuChE] before and after sarin inhalation exposure in untreated rats and those pretreated with Eq BuChE. Brain AChE activity was also determined in the frontal cortex, basal ganglia and pontomedullar areas following exposure. Dose-dependent increases in plasma BuChE activity and no changes in the RBC and brain AChE activities were demonstrated following i.p. injection of different amounts of Eq BuChE. Decreases in plasma BuChE activity and RBC and brain AChE activities were observed in control rats following sarin inhalation exposure. In rats pretreated with Eq BuChE this inhibition was lower than in control animals. These results demonstrate protective effects of Eq BuChE pretreatment in rats intoxicated with sublethal concentrations of sarin by inhalation.

KEY WORDS: *acetylcholinesterase, basal ganglia, frontal cortex, plasma, pontomedullar area, red blood cells, whole blood*

Highly toxic organophosphates (OP), including nerve agents, are compounds used in chemical warfare and in terrorist attacks, as was the case with sarin in Matsumoto city attack in 1994 and Tokyo subway one year later. Protection against the OP effects (prophylaxis and treatment) is therefore of prime importance (1, 2).

Prophylaxis against nerve agents can be based on different principles: protection of cholinesterases against inhibition caused by OP/nerve agents, use of ligands binding or hydrolysing the toxic agent before its penetration to the target sites (scavenger effects) and use of different drugs to protect organisms against toxic effects (1, 3, 4).

Bioscavengers are effective prophylactics against the toxic action of nerve agents. They are very useful in exposure to both low and high doses nerve agents. In nerve agent exposure scavenger action must be

swift (because the action of nerve agents is very fast), irreversible (not allowing further exposure) and specific (reacting with toxic agent only).

The use of scavengers as prophylactics was tested many years ago (5). It is noteworthy that in our Department, in the early eighties, the use of unpurified acetylcholinesterase (AChE, EC 3.1.1.7) (rat brain homogenate) was used for the treatment of O-ethyl S-2-dimethylaminoethyl methyl phosphonothiolate (EDMM) poisoning. In experiments on rats poisoned with EDMM and treated using peritoneal dialysis with different dialysis fluids, the effect of dialysis with rat brain AChE was about one half of the effect of antidotal treatment (6). However, further development of this method was not possible.

Enzymes seem to be very promising as scavengers; they sequester nerve agents at the very beginning of the toxic action, before OPs reach their physiological

targets, and have no side effects (7-14). Among a variety of enzymes studied (12, 15-17), butyrylcholinesterase (BuChE, EC 3.1.1.8) has proven to be the most useful. The reaction between BuChE and an OP is very rapid, including nerve agents sarin, soman, and VX. BuChE has a long residence time in humans, and exerts minimal immune reaction. Thanks to these favourable features BuChE is isolated and its pharmacokinetic and pharmacodynamic properties studied (12, 13).

The aim of this study was to analyse the effects of equine BuChE (Eq BuChE) pretreatment in rats exposed to sublethal concentrations of sarin by inhalation.

MATERIALS AND METHODS

Animals

Female Wistar rats (BioTest, Konarovice), weighing 200 g to 220 g, were divided in groups of five animals each.

The efficacy of Eq BuChE in providing protection against low concentrations of sarin

Equine serum butyrylcholinesterase (Eq BuChE) was a kind gift from Dr B. P. Doctor, WRAIR, Silver Spring, Maryland, USA. Protection experiments were conducted using different doses of Eq BuChE as a pretreatment in rats. Cholinesterase activities were determined in blood [whole blood, red blood cell (RBC) AChE, and plasma BuChE] before and after sarin inhalation exposure of untreated rats and rats pretreated with Eq BuChE. Brain AChE activity was also determined in the frontal cortex (FC), pontomedullar area (PM) and basal ganglia (BG) of the brain following exposure.

Control group 1 (C) received saline (0.1 mL kg⁻¹, i.p.) and 30 min later, their blood was sampled from the tail vein (see Table 1, column "Before"). Then the animals were exposed to air (without sarin) in an inhalation chamber of our own construction (18) for 60 min. Thirty minutes later the animals were killed by decapitation and the blood and brain were collected (see Table 1, column "After").

In control group 2 (C.250) the animals were exposed 60 min to air in the inhalation chamber. Immediately after removal from the chamber their blood was sampled (see Table 1, column "Before") and then the animals received 250 mU kg⁻¹ Eq BuChE i.p. After 30 min the animals were killed and the blood and brain were collected (see Table 1, column "After").

Control group 3 (C.sa) received saline (0.1 mL kg⁻¹, i.p.) and 30 min later their blood was sampled from the tail vein (see Table 1, column "Before"). Then the animals were exposed to sarin vapours (2.6 µg L⁻¹) for 60 min, and 30 min later, the animals were killed and the blood and brain were collected (see Table 1, column "After").

Experimental groups E.125+sa, E.250+sa, E.500+sa received EqBuChE in respective doses of 125 mU kg⁻¹, 250 mU kg⁻¹ and 500 mU kg⁻¹, i.p. and 30 min later their blood was collected from the tail vein (see Table 1, column "Before") and the animals were exposed to sarin vapours (2.6 µg L⁻¹). Thirty minutes later the animals were killed and their blood and brains collected (see Table 1, column "After").

Cholinesterase determination

Cholinesterase activities were determined according to *Ellman et al.* (19) using acetyl- or butyrylthiocholine iodides (Lachema Brno) as substrates and 5,5'-

Table 1 Changes in cholinesterase activities in biological material of rats following sarin inhalation and pretreatment with Eq BuChE

Group	Whole blood		RBC		Plasma		FC	PM	BG
	Before	After	Before	After	Before	After			
C	9.98±0.6	9.94±0.49	6.96±0.34	7.00±0.27	2.98±0.16	3.00±0.16	252±14	248±12	1018±80
C.250	10.00±0.66	12.38±0.56 ^{ab}	7.00±0.19	7.04±0.13	2.94±0.11	4.22±0.16 ^{ab}	245±10	246±14	1013±50
C.sa	9.94±0.51	6.26±0.32 ^{ab}	6.94±0.73	4.24±0.17 ^{ab}	2.98±0.16	1.94±0.15 ^{ab}	128±19 ^b	102±12 ^{bc}	931±35
E.125+sa	10.92±0.36	8.88±0.56 ^{abc}	7.02±0.13	5.44±0.11 ^{abc}	3.84±0.18 ^c	2.50±0.16 ^{abc}	159±16 ^{bc}	128±10 ^{bc}	951±38
E.250+sa	12.4±0.56	10.06±0.30 ^{bc}	7.00±0.2	6.02±0.13 ^{abc}	4.56±0.3 ^c	2.76±0.11 ^{bc}	183±22 ^{bc}	146±19 ^{bc}	994±24
E.500+sa	14.06±0.24	10.32±0.35 ^{bc}	7.02±0.24	6.72±0.30 ^c	5.18±0.16 ^c	3.00±0.16 ^{bc}	208±19 ^c	162±34 ^{bc}	996±27

FC – frontal cortex, PM – pontomedullar area, BG – basal ganglia. Activities are expressed in nkat L⁻¹ or nkat g⁻¹ wet weight tissue. Values are expressed as means ±SEM per group of five rats. Each determination was made in triplicate. Values for groups C.250 After and E.250+sa Before – not statistically different

a – different (p<0.05) between Before and After values

b – different (p<0.05) from control group C

c – different (p<0.05) from sarin group C.sa

dithiobis-2-nitrobenzoic acid, DTNB (Koch-Light) as chromogene. During the assay, the final dilution of the material in cuvette was 1:200 and the final concentrations of substrates and DTNB were 10^{-4} mol L^{-1} . In RBC, the 71 % of activity was that of AChE and 21 % of BuChE (20). The activity was expressed as nkat per litre or per gram of wet weight tissue, or as percent of control values.

Statistical evaluation

Differences between groups were tested using the Student's *t*-test.

RESULTS AND DISCUSSION

The dose of sarin used in our experiments was lower than its LD_{50} value, which was determined to be $4.5 \mu g L^{-1}$ for one-hour exposure (21). The signs of poisoning were mostly muscarinic (salivation). Some fasciculations but not convulsions were also registered. Dose-dependent increases in plasma BuChE activity and no changes in the RBC or whole blood AChE activities were demonstrated following i.p. injection of different amounts of Eq BuChE. No change in AChE activity in the brain parts was observed following administration of $250 mU kg^{-1}$. Decreases in plasma BuChE activity, and RBC, whole blood and brain AChE activities were observed in control rats following sarin inhalation exposure. In rats pretreated with Eq BuChE, this inhibition was lower than in control animals.

The results are summarized in Table 1. These results also document the validity of determined values for the whole blood. It was expected that in control group, cholinesterases would not be affected (C Before, C After). Before the administration of Eq BuChE (C.250, Before), the activity should have been the same as determined in control group (C Before, C After). The activity before administration of sarin (C.sa, Before) can also be taken as control value. The results of pretreatment with Eq BuChE (E.250+sa, Before) in the same dose as with the control group (C.250, After) should also be comparable (Table 1).

Our results show that administration of Eq BuChE does not affect AChE either in RBC or in the brain. However, cholinesterase activities in plasma or in the whole blood increased depending on the dose of Eq BuChE administered. The increase in plasma activity

was expected. The increase in the whole blood could be caused by increased BuChE activity, because of percentual representation of these two enzymes in the whole blood (AChE: 71%, BuChE: 29%) (20).

We observed a decrease in cholinesterase activity in RBC, plasma, whole blood, and brain parts following sarin inhalation.

After pretreatment with Eq BuChE followed by exposure of rats to sarin inhalation, all cholinesterases determined in their blood decreased depending on the dose of Eq BuChE administered. A similar decrease was observed for AChE in the brain parts, however, with a relative resistance of AChE activity in the basal ganglia. This was also observed by Sevelova *et al.* (21). The protective effect could be higher because it takes about ten hours to reach maximum activity following cholinesterase administration (12).

Our results confirm the protective effects of Eq BuChE pretreatment in rats poisoned with sublethal concentrations of sarin by inhalation. The protective effect was observed for brain and peripheral cholinesterases (whole blood, RBC, plasma). Our results have not yet found practical application, but work in that direction is in progress (22).

CONCLUSIONS

- Administration of EqBuChE does not affect AChE activity in the brain and RBC and increases plasma BuChE.
- AChE and BuChE activities decreased following sarin inhalation.
- In rats pretreated with EqBuChE, sarin inhalation exposure led to a decrease in all cholinesterases which depended on the dose of EqBuChE.
- These results demonstrate the protective effects of EqBuChE not only in the blood, but also in different brain areas.

Acknowledgement

The authors are indebted to Mrs J. Uhlířová and M. Zechovská for skilful technical assistance and to Dr B. P. Doctor for donating cholinesterase preparation. We also acknowledge the financial support of the Ministry of Defence, under the grant "New method for prophylaxis, decontamination, diagnosis and therapy in the case of intoxication with nerve agents and sulphur mustard" (INTOX OPUOFVZ 200603).

REFERENCES

1. Bajgar J. Organophosphates/nerve agent poisoning: mechanism of action, diagnosis, prophylaxis, and treatment. *Adv Clin Chem* 2004;38:151-216.
2. Marrs TC, Maynard RL, Sidell FR. Chemical warfare agents. Toxicology and treatment. Chicester, New York, Brisbane, Toronto, Singapore: J. Wiley and Sons; 1996.
3. Bajgar J. Prophylaxis against organophosphorus poisoning. *J Med Chem Def* 2003;1:1-19.
4. Bajgar J, Fusek J, Sevelova L, Kassa J. Complex prophylaxis against nerve agent intoxication. In: Puu G, editor. Eighth International Symposium on Protection against Chemical and Biological Warfare Agents; 2-6 June 2004; Gothenburg, Sweden. Umea: FOI NBC Defence; 2004. Abstracts. p. 51.
5. Wolfe AD, Rush RS, Doctor BP, Koplovitz I, Jones D. Acetylcholinesterase prophylaxis against organophosphate toxicity. *Fund Appl Toxicol* 1987;9:266-70.
6. Bajgar J, Fusek J, Patocka J, Hrdina V. Použití peritoneální dialýzy při experimentální otravě organofosfáty [The use of peritoneal dialysis in experimental intoxications with organophosphates, in Czech]. *Sbor Ved Prac LF UK (Hradec Kralove)* 1982;25:319-26.
7. Doctor BP, Raveh L, Wolfe AD, Maxwell DM, Ashani Y. Enzymes as pretreatment drugs for organophosphate toxicity. *Neurosci Behav Rev* 1991;15:123-8.
8. Doctor BP, Saxena A, Clark MG, Bansal R, Luo C, Rosenberg Y, Lenz D, Ashani Y. Scavenger protection against organophosphates by human serum butyrylcholinesterase. In: The 4th International CB Medical Treatment Symposium; 28 April-3 May 2002; Spiez, Switzerland. Spiez: Spiez Laboratory; 2002. Abstract No 24.
9. Dunn MA, Sidell FR. Progress in medical defense against nerve agents. *JAMA* 1989;262:649-52.
10. Doctor BP, Maxwell DM, Saxena A. Preparation and characterization of bioscavengers for possible use against organophosphate toxicity. In: m-CB Medical Treatment Symposium; 26-30 May 1997; Hradec Kralove, Czech Republic. Hradec Králové: Purkyně Military Medical Academy; 1997. Abstracts p. 17-18.
11. Clark MG, Saxena A, Anderson SM, Sun W, Bansal R, Myers TM, Doctor BP. Behavioral toxicity of purified human serum butyrylcholinesterase in mice. In: The 4th International CB Medical Treatment Symposium; 28 April-3 May 2002; Spiez, Switzerland. Spiez: Spiez Laboratory; 2002. Abstract No 19.
12. Saxena A, Doctor BP, Sun W, Luo C, Bansal R, Naik RS, Fedorko JM, Koplovitz I, Maxwell DM, Lenz DE, Ross MC, HuBChE: A bioscavenger for protection against organophosphate chemical warfare agents. *US Army Med Dept J* 2004;X-XII:22-9.
13. Saxena A, Luo C, Bansal R, Sun W, Clark MG, Lenz DE, Ashani Y, Doctor BP. Scavenger protection against organophosphate agents by human serum butyrylcholinesterase. In: The meeting of NATO TG 004 Task Group on Prophylaxis and Therapy of Chemical Agents; 4-7 November 2002; Oslo, Norway. Oslo: Norwegian Defence Research Establishment; 2002. Poster No. 2.
14. Saxena A, Maxwell DM, Quinn DM, Radic Z, Taylor P, Doctor BP. Mutant acetylcholinesterases as potential detoxification agents for organophosphate poisoning. *Biochem Pharmacol* 1997;54:269-74.
15. Maxwell DM, Brecht K, Saxena A, Feaster S, Doctor BP. Comparison of cholinesterases and carboxylesterase as bioscavengers for organophosphorus compounds. In: Doctor BP, et al., editors. Structure and function of cholinesterases and related proteins. New York (NY): Plenum Press; 1998. p. 387-92.
16. Moore DH. Bioscavenger as antidotes for organophosphorus (OP) agents. In: Proceedings of the 2nd CB Medical Treatment Symposium; 7-12 July 1996; Spiez, Switzerland. Spiez: Spiez Laboratory; 1996. p. 330-49.
17. Rochu D, Renault F, Chabriere E, Masson P. Human paraoxonase as OP-scavenger: some requirements for *in vivo* safety and efficacy. In: Program/Abstracts of the International Medical Chemical Defence Conference 2005; 27-28 April 2005; Munich, Germany. Munich: Bundeswehr Institute of Pharmacology and Toxicology; 2005. p. 26.
18. Sevelova L, Vachek J, Bajgar J. Inhalation apparatus for generating sarin and soman vapors. *Acta Med (Hradec Kralove)* 2003;46:157-61.
19. Ellman GL, Courtney DK, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol* 1961;7:88-95.
20. Bajgar J. Time course of acetylcholinesterase inhibition in the medulla oblongata of the rat by O-ethyl S-(2-dimethylaminoethyl) methylphosphonothioate *in vivo*. *Brit J Pharmacol* 1972;45:368-71.
21. Sevelova L, Bajgar J, Fusek J, Saxena A, Doctor BP. Inhalation intoxication with sarin in rats: effect of pretreatment with butyrylcholinesterase. In: 22nd Xenobiochemical Symposium; 9-11 June 2003; Smolenice, Slovakia. Smolenice: Slovak Academy of Sciences; 2003. Abstracts p. 174-5.
22. Moore DH, Saunder-Price BB. Pharmaceutical countermeasures to chemical warfare agents. *ASA Newslett* 2005;05-02, No 107:1,27-9.

Sažetak

BUTIRILKOLINESTERAZA ŠTITI ŠTAKORE OD INHALACIJSKE IZLOŽENOSTI SUBLETALNIM DOZAMA SARINA

U ovome su radu na štakorima istražene mogućnosti profilakse butirilkolinesterazom iz konjskog seruma (Eq BuChE) u različitim dozama. Aktivnost kolinesteraza izmjerena je u krvi (punoj krvi, eritrocitima i plazmi) prije i nakon inhalacije sarina u štakora koji su prethodno primili Eq BuChE. Također je nakon izlaganja sarinu mjerena aktivnost acetilkolinesteraze u tkivu uzetom iz čeonog režnja mozga štakora, bazalnih ganglija i pontomedularnog područja. Nakon primjene različitih doza Eq BuChE utvrđen je porast aktivnosti butirilkolinesteraze u plazmi koji je odgovarao dozi, ali nije bilo promjena u aktivnosti acetilkolinesteraze u eritrocitima i moždanom tkivu. U kontrolnih je štakora zamijećen pad aktivnosti butirilkolinesteraze u plazmi te acetilkolinesteraze u eritrocitima i moždanom tkivu nakon njihova izlaganja sarinu inhalacijskim putem. U štakora koji su primili profilaksu Eq BuChE ova je inhibicija bila slabija. Rezultati istraživanja potvrđuju zaštitna svojstva konjske butirilkolinesteraze kao profilakse pri otrovanju štakora subletalnim dozama sarina.

KLJUČNE RIJEČI: *acetilkolinesteraza, bazalni gangliji, čeonu režanj, eritrociti, mozak, plazma, pontomedularno područje, puna krv*

REQUESTS FOR REPRINTS:

Jiří Bajgar, Ph. D.
Department of Toxicology, FVZ ÚO
Třebesska 1575, 50001 Hradec Kralove
Czech Republic
E-mail: bajgar@pmfhk.cz