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Research Article

Study of protective properties of butyrylcholinesterase in acute anticholinesterase poisoning on BChE-KO and BALB/c mice

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Abstract

Introduction: The article presents the results of studying the protective properties of recombinant human butyrylcholinesterase (rhBChE) in a model of acute anticholinesterase poisoning in mice knocked out for the BChE gene. Balb/c inbred mice were also used to demonstrate the important role of BChE.

Materials and methods: In the study, BChE-ko and Balb/c mice were used. An organophosphorus compound (OPC) paraoxon was used as a toxic agent causing acute anticholinesterase poisoning. rhBChE was used as an antidote for OPC poisoning. To obtain rhBChE, an expression system based on CHO cell lines was chosen. In order to suppress BChE in Balb/c mice, a carboxyl esterase blocker cresylbenzodioxaphosphorin oxide (CBDP) was used. Two parameters were used to study the recovery after toxicity modeling: the end time of the animal tremor and the distance covered in open-field for 3 minutes.

Results and discussion: The acute poisoning model using the CBDP blocker showed that the sensitivity of Balb/c mice increased significantly. The use of rhBChE against the background of CBDP allowed achieving 100% survival of animals with the minimum lethal dose of paraoxon. Knockout mice are expected to be more sensitive to the toxin, and the use of a biological trap in the form of rhBChE made it possible for 70% of the animals to survive with the minimum lethal dose of paraoxon. Besides, the use of rhBChE facilitated reducing the recovery time after OPC poisoning.

Conclusion: The results of the study showed that the use of rhBChE as a protective agent in acute OPC poisoning significantly increased the survival of the animals and reduced the clinical manifestations of poisoning.

Keywords

organophosphorus compound, butyrylcholinesterase, knockout mice, *in vivo* model.

Introduction

OPC is widely used both in agriculture and everyday life as a means of combating insects, rodents, and weeds. Poisoning can be seasonal and massive. The pathways of OPC in the human body are absorption by the skin, eyes and respiratory tract. Absorption can also take place through the gastric tract (self-poisoning). OPC molecules are distributed throughout the body from blood elements to organs and tissues, including natural depots, physiological targets, and excretory organs (liver and kidneys).

OPC inhibit cholinesterase enzymes, mainly acetylcholinesterase, necessary for the destruction of acetylcholine. As a result, acetylcholine accumulates, which leads to excitation, and subsequently to exhaustion and persistent paralysis of cholinergic structures (Masson et al. 2008). The main function of acetylcholinesterase (AChE) is the hydrolysis of acetylcholine and, as a result, the cessation of neurotransmission. When AChE is inhibited, the work of the diaphragm and other respiratory muscles, as well as the central rhythm generator in the brain stem, is disrupted, resulting in respiratory failure and death of the body.

Butyrylcholinesterase, also called serum cholinesterase or pseudocholinesterase, is abundant in human plasma (3 mg/L) and has a half-life of 12 days (Lockridge et al. 2005; Ostergaard et al. 2006). BChE is present in almost all body tissues – liver, intestines, pancreas, placenta, heart, in the central and peripheral nervous system, etc. (Silver 2005). Serum BChE is synthesized in the liver and from there enters the bloodstream. The physiological role of BChE is still not fully understood. Unlike AChE, BChE does not have a unique physiological function that could not be compensated by other enzymes. People without BChE activity are healthy, fertile, and live to old age (Manoharan et al. 2007). Experiments on knockout mice for the BChE gene also showed that the complete absence of BChE activity does not affect the health and fecundity of animals (Li et al. 2008; Lockridge et al. 2008). In total, in the human and mouse bodies, there is on average 10 times more BChE than AChE (Ashani and Pistinner 2005; Rudakova et al. 2011; Kurdyukov et al. 2012). The highest concentrations of BChE were found in the liver, blood plasma, skin, lungs, and small intestine, which indicate the protective role of the enzyme and its participation in the detoxification of xenobiotics that enter the body with food and air.

As said above, the physiological function of BChE is not fully understood. By hydrolyzing various compounds and binding to OPC, BChE performs primarily protective functions in the body and also participates in the metabolism of drugs (Saxena et al. 2006; Masson and Lockridge 2010). There is evidence that BChE also plays a role in the development of type 2 diabetes mellitus (Kamal et al. 2009). BChE is one of the esterases that inactivates the appetite-stimulating hormone octanoyl-ghrelin, turning it into an inactive form (pure peptide – ghrelin). The role of BChE in fat metabolism is confirmed in experiments on BChE (-/-) mice that developed obesity when receiving high-fat feed unlike wild-type animals (Lockridge et al. 1987; De Vriese et al. 2005). In (Iwasaki et al. 2007), it was shown that the activity of serum BChE correlates with an obesity degreeof patients, with the lipid profile of blood serum and with adegree of insulin resistance.

Materials and methods

Paraoxon was used as OPC. Paraoxon inhibits cholinesterase enzymes, mainly acetylcholinesterase (AChE), necessary for the destruction of acetylcholine (Terekhov et al. 2015b). As a result, acetylcholine accumulates, which leads to excitation, and subsequently to exhaustion and persistent paralysis of cholinergic structures. The function of AChE is to terminate the action of acetylcholine at the joints of various cholinergic nerve endings with their effector organs or postsynaptic sites. Organophosphorus compounds and carbamates are the most important AChE inhibitors; they are often called anticholinesterase inhibitors. In the presence of inhibitors, AChE is gradually suppressed and can no longer hydrolyze acetylcholine (Terekhov et al. 2015a, Mokrushina et al. 2017; Terekhov et al. 2017). As a result, acetylcholine does not form choline and acetic acid, which causes the accumulation of acetylcholine on cholinergic receptor sites. This leads to excessive stimulation of cholinergic receptors throughout the central and peripheral nervous systems.

To determine the effectiveness of butyrylcholinesterase as a prophylaxis of poisoning with organophosphorus compounds, a specific biomodel was used, which takes into account the difference in the esterase status of mice and humans. In human blood, there is twice as much BChE than in mouse blood (5 and 2.6 mg/L, respectively), whereas the content of AChE is 25 times smaller (0.008 and 0.2 mg/L, respectively). Unlike many animals, there is no carboxyl esterase in human plasma, which can lead to a false interpretation of the data when assessing the toxicity of OPC. In human plasma, there are two main esterases, butyrylcholinesterase (BChE, 5 mg/L) and PON1 (50 mg/L). In addition, in plasma there is a small amount of acetylcholinesterase (AChE), which practically has no contribution to the esterase activity of the blood. In order to minimize the background activity of endogenous carboxyl esterase in mice, a cresylbenzodioxaphosphorin oxide inhibitor (CBDP) was used at a dose of 1.5 mg/kg, which completely suppressed the action of this enzyme. This inhibitor was administered subcutaneously.

The study was conducted on Balb/c and BChE-ko male mice in accordance with the requirements of the current *Guidelines for the Preclinical Study of New Pharmacological Substances* and *the Rules of Laboratory Practice in the Russian Federation* (National Standard of the Russian Federation, GOST 33647-2015). The procedures with the animals were reviewed and approved of by the Bioethical Commission of the Institute of Bioorganic Chemistry of the Russian Academy of Sciences (RAS). The number of animals in the group was at least 8.

The experiment on mice of the Balb/c line was divided into 3 stages:

- At the first stage, groups of mice were injected with paraoxon intravenously at doses of 0.5; 0.55; 0.6; and 0.7 mg/kg.
- At the second stage, the CBDP blocker was injected subcutaneously, and 30 minutes later paraoxon was administered intravenously at the doses similar to those at the first stage.
- According to the results of the first two stages the doses of paraoxon from which the animals had died were selected for further research. A CBDP blocker was administered subcutaneously, then 15 minutes later, the mice were intravenously administered with BChE at a dose of 60 mg/kg, after which paraoxon was administered intravenously.

Further, the study of BChE as a prophylaxis of OPC poisoning was carried out on the knockout mice using the BChE-ko butyrylcholinesterase gene.

This part of the experiment was divided into 2 stages:

- At the first stage, a CBDP blocker was subcutaneously administered to the groups of mice, Paraoxon was administered intravenously at doses 0.4; 0.5 and 0.55 mg/kg 30 minutes later.
- According to the results of the first stage, two doses of paraoxon from which 100% and 50% of the animals had died were selected. A CBDP blocker was administered subcutaneously, then 15 minutes later the mice were intravenously administeredwith BChE at a dose of 60 mg/kg, after which paraoxon was administered intravenously.

Clinical observations

Tremor is the main clinical sign of anticholinesterase poisoning. For all the test systems, the end time of tremor was recorded. To study a locomotor activity, the open field test was used on an OptoVarimex-ATM3 unit. The observation time was 3 minutes, during which the distance covered was automatically recorded. Testing was performed 24 hours after poisoning.

Results and discussion

The first priority was to test the theory using a CBDP carboxyl esterase inhibitor. Since this esterase also acts as a natural OPC biotrap, it can affect the interpretation of the results. Figure 1 shows that a prior subcutaneous administration of CBDP significantly reduces the chances of survival in the animals treated with paraoxon. When using CBDP together with paraoxon at a dose of 0.5 mg/kg, 35% of the animals die, at a dose of 0.55 mg/kg, 85% of mice die, and a dose of 0.6 mg/kg is a lethal dose. An increase in sensitivity to OPC is associated with

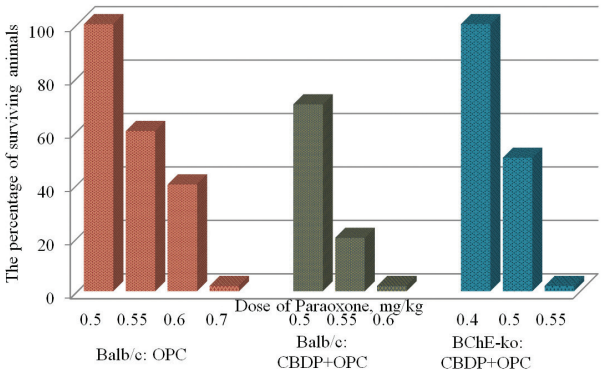

Figure 1. Animal survival in an experimental model of acute anticholinesterase poisoning. **Note:** Balb/c, BChE-ko – male mice, OPC – organophosphorus compound, CBDP – cresylbenzodioxaphosphorin oxide.

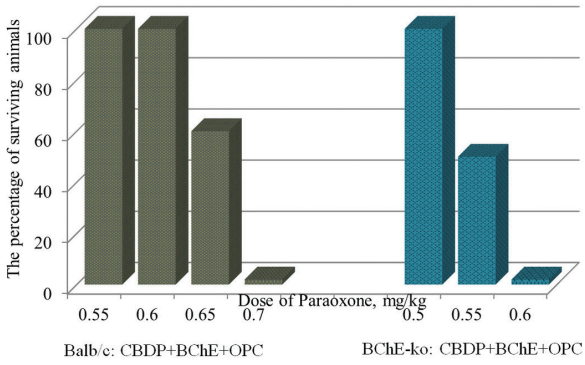

Figure 2. Survival of animals using BChE in an experimental model of acute anticholinesterase poisoning. **Note:** Balb/c, BChE-ko – male mice, OPC – organophosphorus compound, CBDP – cresylbenzodioxaphosphorin oxide, BChE – butyrylcholinesterase.

the action of the blocker.It was decided to continue a further study using CBDP.

BChE gene knockouts makes it possible to see how the absence of this esterase affects OPC poisoning. The sensitivity of the mice is increased by 30% relative to the Balb/c mice using an inhibitor. 1These results confirm the importance of this esterase.

After confirming the theory associated with the use of a carboxyl esterase inhibitor, it was time to use this experimental model to prove the protective activity of BChE in acute anticholinesterase poisoning. Work with BChE began with a paraoxon dose of 0.55 mg/kg at which 70% of the animals had died. As a result of using BChE, the entire group survived (Fig. 2). The next group was a group of animals, where, with the introduction of 0.6 mg/kg of paraoxon, the entire group died without exception. Using BChE made it possible for all the animals from this group to survive. It was further decided to continue increasing the dose of paraoxon. When using BChE together with paraoxon at a dose of 0.65 mg/kg, 40% of the animals died, whereas an increase in the dose of paraoxon to 0.7 mg/kg caused the death of the entire group.

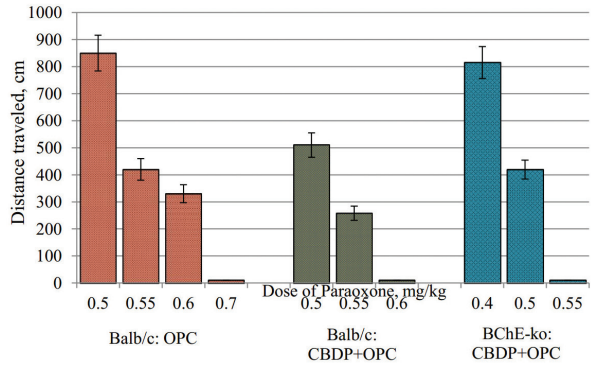

Figure 3. Locomotor activity of animals in an experimental model of acute anticholinesterase poisoning. **Note:** Balb/c, BChE-ko – male mice, OPC – organophosphorus compound, CBDP – cresylbenzodioxaphosphorin oxide.

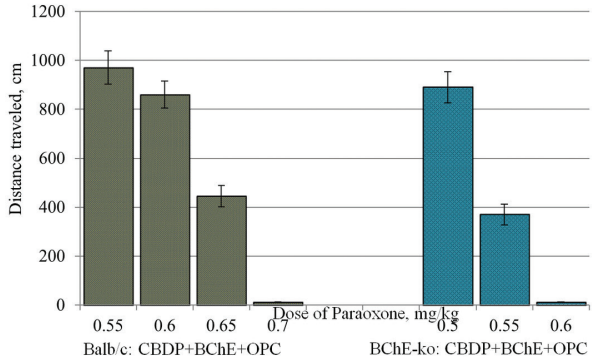

Figure 4. Locomotor activity of animals using BChE in an experimental model of acute anticholinesterase poisoning. **Note:** Balb/c, BChE-ko – male mice, OPC – organophosphorus compound, CBDP – cresylbenzodioxaphosphorin oxide, BChE – butyrylcholinesterase.

As for working with the knockout mice, 0.5 mg/kg was chosen to study the activity of BChE as the starting dose of paraoxon, in accordance with which the death of 50% of the animals from the group was observed. The use of BChE at this dose of paraoxon can increase the survival of animals up to 100%. In the experimental model, paraoxon at a dose of 0.55 mg/kg caused the death of the entire group. The use of BChE raised the survival rate to 50%. The use of BChE at a paraoxon dose of 0.6 mg/kg did not affect survival; with all the animals from this group dying.

In the death of animals, clear dose/effect dependence was observed on the administration of paraoxon. The locomotor activity showed similar results. When simulating poisoning using a CBDP inhibitor, the difference in distance in the group of animals treated with paraoxon at a dose of 0.5 mg/kg was especially noticeable (Fig. 3). One day after poisoning, the animals without carboxyl esterase were 60% less mobile than the animals in the group with this esterase. The locomotor activity implies the severity of poisoning and recovery time. As expected, the knockout mice, even 24 hours later, felt worse than the Balb/c mice.

In addition to the fact that preliminary administration of BChE at a dose of 60 mg/kg can significantly increase the chances of survival, the recovery time after poisoning is significantly reduced (Fig. 4). The Balb/c mice with BChE doubled the distance traveled. In the same way, the BChE-ko mice improved their rate by 2 times. It can be argued with confidence that the introduction of BChE will lead to a further decrease in delayed clinical signs OPC poisoning.

Conclusion

According to the results of the study, it can be argued that the intravenous use of BChE at a dose of 60 mg/kg can significantly increase the chances of survival of animals that have received a minimal lethal dose of OPC. In addition to the survival, the recovery time after poisoning is reduced, which indicates a decrease in the delayed adverse effects of poisoning. From the mechanism of action of BChE it is clear that the larger the dose, the more pronounced the effect. The question naturally arises of the maximum tolerated dose. In the future, more research is needed to study the issue of BChE activity in chronic OPC poisoningand to check the safety of this esterase.

Conflict of interest

The authors declare no conflict of interest.

References

- Ashani Y, Pistinner S (2005) Estimation of the upper limit of human butyrylcholinesterase dose required for protection against organophosphates toxicity: A mathematically based toxicokinetic model. Toxicological Sciences 77(2): 358–367. https://doi.org/10.1093/toxsci/kfh012 [PubMed]
- De Vriese C, Gregoire F, Lema-Kisoka R, Waelbroeck M, Robberecht P, Delporte C (2005) Ghrelin degradation by serum and tissue homogenates: identification of the cleavage sites. Endocrinology 145(11): 4997–5005. https://doi.org/10.1210/en.2004-0569 [PubMed]
- Iwasaki T, Yoneda M, Nakajima A, Terauchi Y (2007) Serum butyrylcholinesterase is strongly associated with adiposity, the serum lipid profile and insulin resistance. Internal Medicine Journal 46(19): 1633–1639. https://doi.org/10.2169/internalmedicine.46.0049 [PubMed]
- Kamal MA, Tan Y, Seale JP, Qu X (2009) Targeting BuChE-inflammatory pathway by SK0506 to manage type 2 diabetes and Alzheimer disease. Neurochemical Research 34(12): 2163–2169. https://doi.org/10.1007/s11064-009-0011-z [PubMed]
- Kurdyukov ID, Shmurak VI, Nadeev AD, Vojtenko NG, Prokofyeva DS, Goncharov NV (2012) "Esterase status" of the body when exposed to toxic substances and pharmaceuticals. Toxicological Bulletin [Toksikologicheskiy Vestnik] 6: 6–13. [In Russian]

- Li B, Nachon F, Froment MT, Verdier L, Debouzy JC, Brasme B, Gillon E, Schopfer LM, Lockridge O, Masson P (2008) Binding and hydrolysis of soman by human serum albumin. Chemical Research in Toxicology 21(2): 421–431. https://doi.org/10.1021/tx700339m [PubMed]
- Lockridge O, Bartels CF, Vaughan TA, Wong CK, Norton SE, Johnson LL (1987) Complete amino acid sequence of human serum cholinesterase. Journal of Biological Chemistry 262(2): 549–557. [PubMed]
- Lockridge O, Duysen EG, Voelker T, Thompson CM, Schopfer LM (2005) Life without acetylcholinesterase: the implications of cholinesterase inhibition toxicity in AChE-knockout mice. Environmental Toxicology and Pharmacology 19(3): 463–469. https://doi.org/10.1016/j.etap.2004.12.008 [PubMed]
- Lockridge O, Xue W, Gaydess A, Grigoryan H, Ding SJ, Schopfer LM, Hinrichs SH, Masson P (2008) Pseudo-esterase activity of human albumin: Slow turnover on tyrosine 411 and stable acetylation of 82 residues including 59 lysines. Journal of Biological Chemistry 283(33): 22582–22590. https://doi.org/10.1074/jbc.M802555200 [PubMed] [PMC]
- Lushchekina SV, Grigorenko BL, Nemukhin AV, Varfolomeev SD (2011) Himan Cholinesterases. Supercomputations When Studying the Mechanism of Action and the Role of Molecular Polymorphism of the Protein Molecule. In: Varfolomeev SD (Ed.) Postgenomic Research and Technology. MAX Press, Moscow, 15–102. [in Russian]
- Manoharan I, Boopathy R, Darvesh S, Lockridge O (2007) A medical health report on individuals with silent butyrylcholinesterase in the Vysya community of India. Clinica Chimica Acta 378(1–2): 128–135. https://doi.org/10.1016/j.cca.2006.11.005 [PubMed]
- Masson P, Lockridge O (2010) Butyrylcholinesterase for protection from organophosphorus poisons: Catalytic complexities and hysteretic behavior. Archives of Biochemistry and Biophysics 494(2): 107–120. https://doi.org/10.1016/j.abb.2009.12.005 [PubMed] [PMC]
- Masson P, Nachon F, Broomfield CA, Lenz DE, Verdier L, Schopfer LM, Lockridge O (2008) A collaborative endeavor to design cholinesterase-based catalytic scavengers against toxic organophosphorus esters. Chemico-Biological Interactions 175(1–3): 273–280. https://doi.org/10.1016/j.cbi.2008.04.005 [PubMed]
- Mokrushina YA, Stepanova AV, Palikov VA, Stepanov AV, Belous GI, Dyachenko IA, Knorre VD, Smirnov IV, Gabibov AG (2017) A study of the protective properties of an antibody-based antidote metabolizing organophosphorus pesticide paraoxon. Bulletin of Experimental Biology and Medicine 163(2): 218–221. https://doi.org/10.1007/s10517-017-3770-6 [PubMed]
- Ostergaard D, Viby-Moogensen J, Hanel HK, Skovgaard LT (2006) Half-life of plasma cholinesterase. Acta Anaesthesiologica Scandinavica 32(3): 266–269. https://doi.org/10.1111/j.1399-6576.1988.tb02727.x [PubMed]
- Rudakova EV, Boltneva NP, Makhaeva GF (2011) Comparative analysis of the esterase activity of human, mouse and rat blood. Bulletin of Experimental Biology and Medicine 152(7): 80–83. https://doi.org/10.1007/s10517-011-1457-y [PubMed]
- Saxena A, Sun W, Luo C, Myers TM, Koplovitz I, Lenz DE, Doctor BP (2006) Bioscavenger for protection from toxicity of organophosphorus compounds. Journal of Molecular Neuroscience 30(1–2): 145–148. https://doi.org/10.1385/JMN:30:1:145 [PubMed]
- Terekhov SS, Palikov VA, Palikova YA, Dyachenko IA, Shamborant OG, Smirnov IV, Masson P, Gabibov AG (2017) Application of tetrameric recombinant human butyrylcholinesterase as a biopharmaceutical for amelioration of symptoms of acute organophosphate poisoning. Bulletin of Experimental Biology and Medicine 163(4): 430–435. https://doi.org/10.1007/s10517-017-3821-z [PubMed]
- Terekhov S, Smirnov I, Bobik T, Shamborant O, Zenkova M, Chernolovskaya E, Gladkikh D, Murashev A, Dyachenko I, Palikov V, Palikova Y,Knorre V, Belogurov AJ, Ponomarenko N, Blackburn GM, Masson P, Gabibov A (2015a) A novel expression cassette delivers efficient production of exclusively tetrameric humanbutyrylcholinesterase with improved pharmacokinetics for protection against organophosphatepoisoning. Biochimie 118: 51–59. https://doi.org/10.1016/j.biochi.2015.07.028 [PubMed]
- Terekhov SS, Smirnov IV, Shamborant OG, Bobik TV, Ilyushin DG, Murashev AN, Dyachenko IA, Palikov VA, Knorre VD, Belogurov AA, Ponomarenko NA, Kuzina ES, Genkin DD, Masson P, Gabibov AG (2015b) Chemical polysialylation and in vivo tetramerization improve pharmacokinetic characteristics of recombinant human butyrylcholinesterase-based bioscavengers. Acta Naturae 7(4): 136–141. https://doi.org/10.32607/20758251-2015-7-4-136-141 [PubMed] [PMC]
- Silver A (1974) The Biology of Cholinesterases. Amsterdam: Elsevier, 576 pp.

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