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The influence of modulators of acetylcholinesterase on the resistance of mice against soman and on the effectiveness of antidotal treatment of soman poisoning in mice

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ABSTRACT

The potency of one reversible inhibitor of acetylcholinesterase (6-chlorotacrine), one reactivator of acetycholinesterase (K027) and their combination to increase the resistance of mice against soman and the efficacy of antidotal treatment of soman-poisoned mice was evaluated. While 6-chlorotacrine was able to markedly protect mice against acute toxicity of soman and the pharmacological pretreatment with 6-chlorotacrine increased the efficacy of antidotal treatment (the oxime HI-6 in combination with atropine) of soman-poisoned mice more than two times, the bispyridinium oxime K027 did not protect mice from acute toxicity of soman, however, the pharmacological pretreatment with this compound was able to markedly increase the efficacy of antidotal treatment of soman-poisoned mice. On the other hand, the combination of both modulators of acetylcholinesterase did not increase the prophylactic efficacy of 6-chlorotacrine alone. These findings demonstrate that pharmacological pretreatment of soman-poisoned mice can be promising and useful in the case of administration of 6-chlorotacrine while the administration of the oxime K027 did not bring any additional benefit when combined with 6-chlorotacrine.

Introduction

The highly toxic organophosphorus compounds, called nerve agents, are still considered to be the most dangerous chemical warfare agents. Their acute toxicity is based on the irreversible inhibition of the enzyme – acetylcholinesterase (AChE; EC 3.1.1.7) and subsequent accumulation of the neuromediator acetylcholine (ACh) at peripheral and central cholinergic sites. AChE plays a key role in physiological function of the cholinergic nervous system and, therefore, its inhibition is life-endangering factor. Inhibitory effect of nerve agents is based on phosphorylation or phosphonylation of serine hydroxy group at the esteratic site of the active site of the enzyme. Death occurs due to an acute cholinergic crisis, with signs and symptoms such as excessive salivation, lacrimation, urination, defecation, sweating, bronchoconstriction, neuromuscular block, generalized seizures, respiratory distress and respiratory failure (Bajgar, 2004; Delfino et al., 2009; Colovic et al., 2013).

The current standard treatment for poisoning by nerve agents is based on the combined administration of anticholinergic drugs such as atropine sulfate and AChE reactivators such as pralidoxime, obidoxime and HI-6. The anticholinergic drugs block the effects of overstimulation by accumulated ACh at muscarinic receptor sites while AChE reactivators, also called oximes, repair the biochemical lesion by dephosphonylation of AChE molecule and restoring its activity. Their effects are synergistic. Although the antidotes against nerve agents and organophosphorus insecticides have been developed based on the knowledge of above-mentioned basic mechanism of acute toxicity of nerve agents, their efficacy is limited (Jokanovic, 2012; Wilhelm et al., 2014).

Unfortunately, some nerve agents were found to be resistant to standard antidotal treatment. One of the most resistant nerve agents is soman (pinacolyl methylfluorophosphonate). Its deleterious effects are very difficult to counteract because of the existence of a rapid aging of soman-inhibited AChE. It is known that the reactivating efficacy of all oximes is very limited in the case

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of soman poisoning when administered after soman exposure due to rapid dealkylation of soman-inhibited AChE that makes the reactivation of soman-inhibited AChE impossible (Shih, 1993; Bajgar, 2004). In addition, the main action of soman is in the central nervous system where the reactivating efficacy of all oximes is low due to their limited penetration through blood-brain barrier (Lorke et al., 2008; Zdarova Karasova et al., 2010). The unsatisfactory antidotal treatment of acute nerve agent poisonings brought another approach how to protect the humans from nerve agent-induced acute lethal toxic effects – using "pharmacological pretreatment" in the case of the threat of exposure to nerve agents. The term pharmacological pretreatment generally represents the medical countermeasures applied relatively shortly before penetration of a toxic substance into the organism with the aim to protect the organism against acute toxic effects of various toxic substances and increase the effects of post-exposure antidotal treatment. Thus, the pharmacological pretreatment allows survival and increase the resistance of organisms exposed to nerve agents as previously described (Layish et al., 2005; Patocka et al., 2006; Bajgar et al., 2007, 2009).

Up to date, the most common principle of pharmacological pretreatment is the protection of AChE against nerve agent-induced irreversible inhibition that is focused on the use of reversible cholinesterase inhibitors. Among reversible inhibitors of AChE, the carbamate pyridostigmine bromide is generally accepted and commonly used for the pharmacological pretreatment of nerve agent poisonings. However, pyridostigmine is only able to protect peripheral AChE from irreversible nerve agent-induced AChE phosphonylation, while nerve agents, especially fluorophosphonates, can cross the blood-brain barrier and, thus, express their deleterious effects through their central toxic effects including centrally mediated seizure activity that can rapidly progress to *status epilepticus* and finally contribute to brain damage (Bajgar, 2004). Thus, the replacement of pyridostigmine bromide with sufficiently effective reversible inhibitors of AChE with low toxicity and ability to cross the blood-brain barrier has been an important goal for the pharmacological pretreatment of nerve agent poisonings because the small decrease of the brain AChE activity (up to 20%) was found to be beneficial for an increase in the efficacy of pharmacological pretreatment and does not affect the behavioral and neurophysiological functions of experimental animals according to our neurobehavioral research (Kassa et al., 2001). Recently, a novel reversible inhibitor of AChE – 6-chlorotacrine (6-chloro-1,2,3.4-tetrahydroacridine-9-amine hydrochloride) (Fig. 1) was synthesized at our Department of Toxicology and Military Pharmacy to improve the efficacy of pharmacological pretreatment against nerve agents and potentially for the treatment of Alzheimer's disease. From the chemical point of view, it is tacrine-related compound (Nepovimova et al., 2015).

Another commonly used principle of pharmacological pretreatment is the administration of classic antidotes (reactivators of nerve agent-inhibited AChE or anticholinergic drugs) before the exposure to nerve agents. As soman is characterized by very rapid aging of inhibitor-enzyme complex, the reactivating efficacy of all oximes is very limited and the timing of administration of oximes is very important (Bajgar, 2004). Therefore the prophylactic administration of an oxime (before exposure to soman) should bring the increase of the resistance of organisms against soman and the increase of the efficacy of post-exposure antidotal treatment due to higher reactivating capacity of pre-exposure and post-exposure administered oxime. One of the recently synthesized oximes at our Department of Toxicology and Military Pharmacy is the oxime K027 (1-[4-hydroxyiminomethylpyridinium]-3-[carbamoylpyridinium] propane dibromide) (Fig. 1) (Kuca et al., 2003). Based on the *in vitro* and *in vivo* results, the oxime

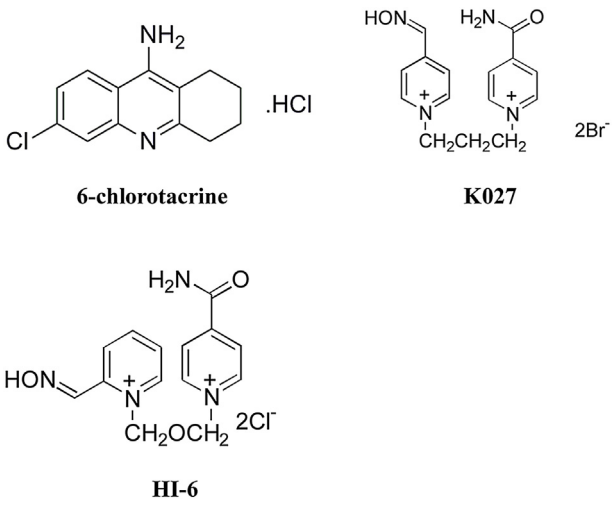

Fig. 1. Chemical structure of modulators of AChE studied.

K027 is considered to be promising reactivator of AChE inbibited by organophosphorus compounds (Kuča and Kassa, 2004; Antonijevic et al., 2016).

Recently, a synthesis of new prophylactic drugs combining in their molecule the structure able to reversibly inhibit AChE and the structure able to reactivate nerve agent-inhibited AChE (7-methoxytacrine-4-pyridinealdoxime hybrid) has been published (Nepovimova et al., 2016). Therefore, we also decided to evaluate the possible benefit of the combination of reversible inhibitor of AChE (6-chlorotacrine) and reactivator of nerve agent-inhibited AChE (the oxime K027). In the present study, the influence of 6-chlorotacrine, the oxime K027 and their combination on the resistance of soman-exposed mice and on the therapeutic efficacy of currently used antidotal treatment (the oxime HI-6 in combination with atropine) of soman-induced acute poisoning was investigated.

Materials and methods

Animals

Male NMRI mice weighing 25–30 g were purchased from VELAZ (Prague, Czech Republic). They were kept in an air-conditioned room ($22 \pm 2\,^{\circ}C$ and $50 \pm 10\%$ relative humidity, with lights from 7.00 h a.m. to 7.00 h p.m.) and allowed access to standard food and tap water *ad libitum*. The rats were divided into groups of eight animals ($N = 8$). Handling of experimental animals was done under the supervision of the Ethics Committee of the Faculty of Military Health Sciences in Hradec Kralove (Czech Republic).

Chemicals

Soman was obtained from the Military Technical Institute in Brno (Czech Republic) and was 92.0% pure. Its purity was assayed by acidimetric titration. The purity of all modulators of AChE (Fig. 1) was higher than 98%. They were synthesized earlier at the Department of Toxicology and Military Pharmacy of the Faculty of Military Health Sciences in Hradec Kralove (Czech Republic). The purity of newly synthesized modulators of AChE was analysed using HPLC (Jun et al., 2007). All other drugs and chemicals of analytical grade were obtained commercially and used without further purification. All substances were administered intramuscularly (i.m.) at a volume of 10 mL/kg body weight (b.w.).

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