8. SUMMARY

Bioscavengers as prophylactics of poisonings caused by organophosphate inhibitors of acetylcholinesterase

Nerve agents (tabun, soman, sarin, VX) and pesticides (parathion, malathion, chlorpyrifos) are highly toxic organophosphorus compounds. They irreversibly inhibit enzyme AChE. Inhibited AChE cannot perform its physiological role – hydrolysis of acetylcholine. Subsequent accumulation of acetylcholine on cholinergic synapses causes cholinergic crisis and can lead to death of intoxicated organism. Current prophylaxis of OP poisonings consists of administration reversible AChE inhibitors (pyridostigmine), central anticholinergics (benactyzine, trihexyphenidyl) and AChE reactivators (HI-6). These substances are contained in antidotal means in some armies, including Czech Armed Forces. Therapy of OP poisonings is based on administration of anticholinergics (atropine), reactivators of AChE (obidoxime, methoxime, HI-6), reversible inhibitors of AChE (pyridostigmine) and anticonvulsives (diazepam). These antidotes can protect human from death, but they don't prevent from postexposure incapacitation, convulsions and permanent brain damage. Relatively new approach in prophylaxis and therapy of OP intoxications is administration of certain enzymes as bioscavengers. Bioscavengers are capable of neutralizing of toxic OPs in the bloodstream rapidly before they can reach their natural targets (synaptic AChE) and cause intoxication. Catalytic (phosphotriesterase, paraoxonase), stoichiometric (AChE, BuChE) and pseudo catalytic (combination of cholinesterase and oxime reactivator) bioscavengers are intensively investigated for these purposes.

In our work we concerned with preparation of modified recombinant PTE which could serve as catalytic bioscavenger. PTE is an enzyme of bacterial origin and its disadvantage is relatively short half-life in the blood stream and immunogenicity after repeated administration. One way to circumvent these problems is the modification of enzyme bioscavenger by conjugation with suitable biocompatible polymer based on polyethylene glycol (pegylation). This approach can improve its circulatory stability and immunotolerance due to the decreased ability of immune system to recognize modified enzyme. We have prepared MPEG-modified PTE, we have optimized modification process and we compared properties of modified PTE and native PTE *in vitro* and subsequently *in vivo*. It was shown

that modified enzyme had significantly higher stability and longer half-life in bloodstream of the rat.

Next step of our work was design and testing of pseudo catalytic bioscavenger which consists of cholinesterase (AChE or BuChE) and suitable oxime reactivator. Oxime should have wide spectrum of reactivation efficiency (ability to reactivate different OP-inhibited AChE or BuChE). Our experiments shown that the most efficient reactivators have several common structural features: they are bisquaternary with one or two oxime groups in para position on pyridinium rings and connecting chain with 3 or 4 members. OP-inhibited BuChE was very uneasily reactivatable and values of reactivation were very low in comparison with AChE. Therefore, tested oximes are not suitable as a part of pseudo catalytic bioscavenger in combination with BuChE, but the results can be used in searching for new structures of efficient reactivators.