**Summary:** The studies dealing with mechanism of organophosphates (OP)/nerve agent action, prophylaxis and treatment of intoxications is a very hot topic at present. Though the research is very intensive, unfortunately, up to now, there is not universal or significantly better reactivator sufficiently effective against all nerve agents/OP when compared with presently available oximes (pralidoxime, methoxime, obidoxime, trimedoxime, HI-6). The use of the most effective reactivator (HI-6) using simple type of autoinjector (e.g. ComboPen) is strictly limited because of decomposition of HI-6 in solution. Thanks to better solubility it is clear that another salt of HI-6 (dimethanesulfonate, HI-6 DMS) is more convenient for the use as antidote against nerve agents in the autoinjector than HI-6 chloride (Cl). It was clearly demonstrated that reactivation potency of HI-6 DMS in comparison with HI-6 Cl in vivo was the same and bioavailability of HI-6 DMS is better than that of HI-6 Cl. Three chambered autoinjector allows administration of all three antidotes (atropine, reactivator, diazepam) simultaneously. Moreover, the content of chambers can be changed according to proposed requirements. Possible way to solve the problem of universal reactivator could be the use of two reactivators. Three chambered autoinjector is an ideal device for this purpose.

**Key words:** Anticholinergics; Reactivators; Nerve agents; Acetylcholinesterase; Treatment
store enzyme activity (reactivation) by the nucleophilic attack on phosphorylated or phosphonylated serine at the active center of the AChE molecule and liberate the free enzyme (1). This fact is limiting factor for the therapy with reactivators. The rest of the nerve agent forms a complex with the reactivator (more toxic but less stable) hydrolyzing practically immediately and not having high importance for the course of intoxication (1). The efficacy of oxime reactivation is dependent on both oxime and the conjugated phosphonate structure (1,33). Simultaneously, the microenvironment of the gorge plays a significant role in determining the selectivity of the substrate and inhibitors for cholinesterases. Depending on the structure of the inhibitor, inhibited AChE is dealkylated (aged) and the complex formed is resistant to the reactivation effect. The molecular mechanism is explained by the splitting of the complex forming the alcohol and unreactivatable enzyme (Fig. 2). This reaction, called aging or dealkylation is very fast for soman-inhibited AChE (the half-life is about 10 min) and it is less expressed for sarin (the half-life is about 10 hours), for VX-inhibited AChE this reaction was not observed within 24 hours (1,33). This is one of the reasons for difficult therapeutic interventions of soman intoxication (1,24,33,37).

The fate of poisoned organism is very dependent on early administered antidotes. Based on our knowledge of the mechanism of action, two therapeutic principles for antidotal treatment are used. The main drugs are anticholinergics (preferably atropine), that antagonize the effects of accumulated acetylcholine at the cholinergic synapses (also called symptomatic antidotes) and cholinesterase reactivators (oximes) to reactivate inhibited AChE (causal antidotes) (1,13,14,37). Their effects are synergistic. Benzodiazepines are also used to treat convulsions (anticonvulsants, usually diazepam) (1,42). Treatment of metabolic dysbalance, ions hypo/exequilibrium and support of vital functions (heart, ventilation) is necessary. The use of atropine is without any discussion; other anticholinergics may be even more efficacious. The centrally acting anticholinergics (benactyzine, biperiden) can be very useful in therapy of soman poisoning (1,20,22). Anticonvulsants (diazepam) in the treatment of OP/nerve agent poisoning are also frequently used (1,27,29). However, the use of reactivators is more complicated question.

**The choice of reactivators**

The choice of reactivators is not so simple (17,24,33,34,39). Their administration alone is not effective but simultaneous administration with atropine potentiates their antidotal effects based on AChE reactivation at the cholinergic nerve synapses. AChE reactivation at the peripheral nervous system is indisputable; however, their passing the blood-brain-barrier facilitating their central reactivation efficacy in vivo was demonstrated (2,3,26–28). Though the research is very intensive, unfortunately, up to now, there is not universal or significantly better reactivator sufficiently effective against all nerve agents/OP when compared with presently available oximes (19,31,33,41).

As it was described many times, there are only few commercially available AChE reactivators on the market – pralidoxime (2-PAM; P2S; 2-hydroxyiminomethyl-1-methylpyridinium chloride or methansulphonate), trimedoxime (Fosan®; 1,3-bis(4-hydroxyiminomethylpyridinium)-propane dichloride or dibromide), obidoxime (Toxogonin®; LüH-6; 1,3-bis(4-hydroxyiminomethylpyridinium)-2-oxapropyl chloride), methoxime (MMC-4; MMB-4; 1,1-bis(4-hydroxyiminomethylpyridinium)-3-(4-carbamoylpyridinium)-methane dichloride or dibromide) and HI-6 (1-(2-hydroxyiminomethylpyridinium)-3-(4-carbamoylpyridinium)-2-oxapropane dichloride or di-methanesulfonate) (Fig. 3). Unfortunately, none of them is able to reactivate AChE inhibited by all nerve agents/OP and it is less expressed for sarin (the half-life is about 10 hours), for VX-inhibited AChE this reaction was not observed within 24 hours (1,33). This is one of the reasons for difficult therapeutic interventions of soman intoxication (1,24,33,37).

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For example, pralidoxime, the gold standard of AChE reactivators, is from the historical point of view the most used AChE reactivator. However, potency of pralidoxime to reactivate tabun-, cyclosarin-, soman- or pesticide-inhibited AChE is poor (31). This is probably the reason for replacement of pralidoxime in the US Army by methoxime. In the literature, its use during pesticide poisoning was discussed many times. Consequently, its reactivation results are controversial (14,24,45). If pesticides are discussed, oximes and trimedoxime are the most promising reactivators (40,44). However, their potencies to reactivate cyclosarin- and soman-inhibited AChE are not good enough (33). If tabun-inhibited AChE reactivation is discussed, these reactivators are promising compared with other commercially available ones (5,8,9,10,25). On the contrary, their reactivation potency is generally not sufficient as it is expected for promising oximes. Other currently available oxime on the market – methoxime – seems to be very promising in case of cyclosarin, sarin and VX poisoning (31). Its potency to reactivate almost all nerve agent-inhibited AChE with exception of tabun (31). On the contrary, if civilian importance is considered, HI-6 is unable to reactivate AChE in case of OP pesticide-poisoning (31,43). From this short summarization of antidotal effect of reactivators against nerve agent/OP poisoning is clear that there are relatively effective oximes (trimedoxime, oximes, methoxime) but the most effective seems to be the oxime HI-6. These observations are supported by extensive reviews dealing with the topics mentioned (1,13,24,31,33,36).

In conclusion, action of reactivators as non replaceable part of antidotal therapy against OP/nerve agents intoxication is based on AChE reactivation in the different structures of the nervous system. The HI-6 appears to be very promising antidote against nerve agents including soman.

There are another oximes slightly better than HI-6 (e.g. HLö-7) but preclinical and clinical research to be done for their permission and involvement into medical practice is expensive and final result (as therapeutic effectiveness) is not sufficient (11,21). The same can be applied for oximes K-048, K201 etc. (27,33,34,41). As it was mentioned before, the action of OP (and, especially nerve agents) is very fast and, therefore, the first aid is necessary as quick as possible. Automatic administration of antidotes is solved by the use of autoinjector, however, the question of content (effective antidotes of autoinjector is more complicated.

At present, generally two types of autoinjectors (with atropine and reactivator) are mostly used, i.e. ComboPen containing atropine and oximes in one solution; it is the first choice. However, the use of more effective reactivator (HI-6) using this type of autoinjector is strictly limited because of decomposition of HI-6 in solution. This situation can be solved using wet/dry (two chambered) autoinjector containing in its chamber solution of atropine and in the second one reactivator HI-6 in powdered (lyophilized) form (HI-6 chloride). They are produced by ASTRA (Sweden) and STI (USA) (1,7,38).

Clinical use of HI-6

There is only limited experience in human poisoning by highly toxic OP/nerve agents but it is generally accepted that the persistence of clinically relevant amounts of nerve agent in blood is shorter than that of OP. However, as a result of the nerve agent-inhibited AChE, which may be very rapid following poisoning with soman, it is suggested that in the absence of clinical improvement, administration of oxime for periods in excess of 24–48 hours is unlikely to achieve further reactivating of the enzyme (for review, see, e.g. 1,14,15,36). Other studies were performed: double blind placebo controlled clinical studies of pharmacokinetics and tolerance of HI-6 administered i.m. to healthy volunteers (in combinations with atropine 2 mg) published by Clement et al. (12) and Kusic et al. (35) (blood levels, urinary excretion and tolerance) showed that changes observed following HI-6 treatment were not clinically important (44). Therapeutic effect of HI-6 as antidote against malathion and mevinfos was described Jovanovic et al. (18). Moreover, HI-6 is involved in the equipment of the Czech, Slovak, Swedish and Canadian Armies as antidote against nerve agent intoxication.

Different salts of HI-6

HI-6 in autoinjectors is used as a chloride salt. Its physical chemical properties are sufficient but there are other salts of HI-6 having these properties (especially the solubility) better than chloride as described by Kuca et al. (32): twelve salts of HI-6 were tested for solubility in water, saline and saline with atropine at different conditions (temperature) – 3.5 °C (winter condition), 25 °C (Europe condition), and 40 °C (dessert condition), respectively. The solubility of HI-6 dimethanesulfonate (HI-6 DMS) was much more better in comparison with HI-6 chloride: this property is very useful for the use of HI-6 DMS in the autoinjector. Better solubility predicts better bioavailability and simultaneously, better evaluation of the whole content of oxime in the autoinjector. Reactivation potency of HI-6 chloride and HI-6 DMS against by nerve agent inhibited AChE in vitro was the same (30,32). Simultaneously, it was clearly demonstrated that reactivation potency of HI-6 DMS in comparison with HI-6 chloride in vivo was the same or better (27,28). Thus, thanks to better solubility it is clear that HI-6 DMS is more convenient for the use as antidote against nerve agents in the autoinjector.

Two- or three-chambered autoinjector

Practical use of antidotes in the automatic devices is limited by a stability of the drug in solution contained in the autoinjector. Two autoinjectors for the first aid against
nerve agents are used in different armies – autoinjector containing atropine and reactivator (usually obidoxime) and the autoinjector with diazepam. It appears from previous analysis, obidoxime is not optimal antidote (reactivator) but the use of better oxime – HI-6 – can be not be applicable because of low stability of HI-6 in solution. For the use of HI-6, two chambered autoinjector is necessary using dichloride salt of HI-6; however, its solubility is not optimal. In all cases, it is one device for the soldier; the second one is the other autoinjector for diazepam administration. Thus, two administrations (and two manipulations) are necessary for the first aid. An idea of three-chambered autoinjector is more interesting: the content of three-chambered autoinjector is atropine in solution, the best reactivator HI-6 as a lyophilized powder and diazepam in solution. Stated differently, for administration of all types of antidotes using three-chambered autoinjector, one manipulation only is needed. Moreover, the content of chambers can be changed according to proposed requirements, e.g. adding of new anticholinergic drug like benactyzine or akineton, centrally acting anticholinergic drugs with good antidotal effect against nerve agents such as soman (1,20–23). It is not excluded the change of the chamber content by quite another drugs such as other benzodiazepines (e.g. midazolam), analgetics, antiemetics or other reactivators according to customer request. In connection with nerve agent intoxication (especially tabun exposure), the autoinjector can be used also for prophylaxis, either with classic prophylactics described (3,6) or new ones, e.g. huperzine A (4); simultaneously it could be of interest to replace diazepam with another reactivator: as it was mentioned before, universal reactivator – HI-6, two chambered autoinjector is necessary using dichloride salt of HI-6; however, its solubility is not optimal. HI-6 dimethanesulfonate (lyophilized form) is more suitable for the use in the autoinjector than HI-6 dichloride. The changes observed are not of clinical importance.

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References

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