STRATEGY FOR THE DEVELOPMENT OF NEW ACETYLCHOLINESTERASE REACTIVATORS – ANTIDOTES USED FOR TREATMENT OF NERVE AGENT POISONINGS

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The mechanism of intoxication with organophosphorus compounds, including highly toxic nerve agents, is based on the formation of irreversibly inhibited acetylcholinesterase (AChE; EC 3.1.1.7) that could be followed by a generalized cholinergic crisis. Nerve agent poisoning is conventionally treated using a combination of a cholinolytic drug (atropine mostly) to counteract the accumulation of acetylcholine at muscarinic receptors and AChE reactivators (pralidoxime or obidoxime) to reactivate inhibited AChE.

At the Department of Toxicology, the strategy of the development of new more potent AChE reactivators consists of several steps: description of the nerve agent intoxication mechanism on the molecular basis (molecular design), prediction of the biological active structure of AChE reactivators (artificial neural networks), their synthesis, *in vitro* evaluation of their potencies (potentiometric titration and Ellman's method), *in vivo* studies (therapeutic index, LD₅₀ of newly synthesized reactivators, reactivation in different tissues, neuroprotective efficacy).

INTRODUCTION

Organophosphate (OP) compounds are used as pesticides around the world and were also applied in chemical warfare agents (nerve agents, NA). NA, especially sarin, were also misused by terrorists in Japan (Matsumoto 1994, Tokyo 1995)¹.

OP poisoning leads to the formation of inhibited acetylcholinesterase (AChE, EC 3.1.1.7) followed by accumulation of acetylcholine and a generalised cholinergic crisis². Presently, a combination of an antimuscarinic agent, e.g. atropine, and an AChE reactivator (oxime) is recommended for the treatment of OP poisoning³. Atropine blocks the effects of accumulated acetylcholine induced overstimulation of peripheral muscarinic receptor sites while reactivators repair the biochemical lesion by dephosphorylating the enzymatic molecule and restoring its activity⁴.

Pralidoxime, obidoxime and HI-6 are currently used in clinical toxicology against OP pesticides and included into antidotal means against NA in several armies³. However, these oximes were shown to be rather ineffective against certain nerve agents^{5,6}. Owing to this fact, the development of new more potent AChE reactivators with sufficient efficacy to treat intoxication with broader spectra of OP pesticides and nerve agents still continues.

In this work, the strategy for the development of new AChE reactivators is outlined.

METHODS AND RESULTS

The whole developmental process consists of several approaches (Fig. 1) – prediction using artificial neural networks (ANN), molecular design, synthesis of new AChE reactivators, *in vitro* testing and *in vivo* testing. A short description of individual developmental steps is shown in Figure 1.

Prediction using Artificial Neural Networks

A biological activity of chemical compounds could be estimated using chemometric methods including ANN. On the basis of known biological activities of different substances, there is possibility to “learn” ANN without knowledge of exact interaction between a compound and organism. Due to this fact, the described method can estimate biological activity of potential antidotes, without necessity to synthesize them.

In development process described, ANN are used for the prediction of the appropriate structure of new AChE reactivators. Biological activity and structure of currently used AChE reactivators are used as input data set. Then,

Fig. 1. Description of the developmental process.

the model of relationships between chemical structure and biological activity is calculated. Afterwards, based on these models, prediction of new more potent reactivators of AChE inhibited by nerve agents is possible⁷.

Molecular Design

The methods of molecular modelling are used for study of AChE conformational changes caused by substances such as OP compounds (NA, pesticides). This study is performed by methods of molecular dynamics with possibility to calculate intramolecular energies of modified residues. Then, reconformational changes in AChE structure caused by reactivators are examined. The influence of these substances towards the enzyme is evaluated on the base of known structures and by docking method and subsequent molecular design simulations. The acquired description of interactions and their quantification got from interaction energies of model systems serves as proposition for new active more potent AChE reactivators⁸.

Synthesis of new AChE reactivators

All AChE reactivators predicted by ANN and molecular design are synthesized using methods former used for synthesis of currently used AChE reactivators. During last three years, more than twenty AChE reactivators were

synthesized⁹⁻¹². From the chemical view, all synthesized substances are mono or bis quaternary pyridinium rings connected mostly with three or four membered linkage chain. In all their structures, the oxime group is functioning as nucleophile able to split the bond between enzyme and inhibitor. The oxime group is mostly located in the position two or four on the pyridinium ring.

In vitro testing

The reactivation potency of synthesized AChE reactivators is firstly evaluated using *in vitro* experiments. Currently, two methods are used – potentiometric and Ellman¹³⁻¹⁶. The homogenates from rat, pig and human brains and rat and human erythrocytes are used as a source of AChE. Commercially available pure enzymes are also used. Results of *in vitro* experiments are used as input data for ANN prediction. According to *in vitro* results, the most potent AChE reactivators are selected for *in vivo* experiments.

In vivo testing

The toxicity of chosen newly synthesized AChE reactivators is evaluated following intramuscular administration by the calculation of their medium lethal doses (LD₅₀ values) in mice and rats. Afterwards, their therapeutic efficacy against NA is demonstrated by the evaluation of

their therapeutic index (the ratio between LD₅₀ values of treated and untreated experimental animals). The reactivating efficacy of oximes studied is investigated by the calculation of percentage of reactivation of NA-inhibited AChE in peripheral (mostly blood and diaphragm) and central (central nervous system) compartment in rats using modified Ellman method for the measurement of AChE activity. Finally, the potency of chosen oximes to eliminate NA-induced signs of neurotoxicity (neuroprotective efficacy) was evaluated using Functional observatory battery (FAB) consisting of more than 40 parameters of sensoric, motoric and autonomic nervous functions in rats^{17–23}.

Till today, two new AChE reactivators – K027 (1-(4-hydroxyiminomethylpyridinium)-3-(4-carbamoylpyridinium)propane dibromide) and K048 (1-(4-hydroxyiminomethylpyridinium)-4-(4-carbamoylpyridinium)butane dibromide) were developed^{9,10}. These oximes are according to *in vitro* and *in vivo* results very promising tabun-inhibited AChE reactivators^{14,15,24}. Owing to this fact, they are also currently under testing for their possibility to reactivate pesticides-induced AChE inhibition. First results confirm that these oximes seem to be good reactivators of paraoxon-inhibited AChE²⁵.

CONCLUSION

To conclude the whole developmental process consists of several important steps – prediction using artificial neural networks (ANN), molecular design, synthesis of new AChE reactivators, *in vitro* testing and *in vivo* testing. These steps are well connected and currently are unique for the development of new AChE reactivators.

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