Abstract and keywords

The dissertation thesis deals with problem of insufficient penetration of antidotes for treatment of organophosphorus poisonings. Methods for evaluating the ability of compounds to penetrate the central nervous system (CNS) were developed and compared to each other. These methods were subsequently used for the standard and newly synthesized potential drugs from this group to evaluate their penetration to the brain. Furthermore, the strategies that could improve CNS penetration were investigated.

Highly toxic organophosphorus compounds represent a big threat due to possible misuse in the military of for terrorist purposes. These compounds affecting cholinergic neurotransmission by inhibition of the enzyme acetylcholinesterase (AChE). For this reason, they are called nerve agents (NAs). NAs are extremely toxic, relatively easily obtainable and the therapy of intoxication is insufficiently effective. One of the major obstacles of AChE reactivators, causal antidote used in the treatment, is to overcome the blood-brain barrier in therapeutic concentration and restore the function of AChE in the CNS. Thus, research and development of new drug candidates for such antidotes requires appropriate methods for evaluation of their biological properties even *in the in vitro stage*.

Selection, validation and comparison of *in vitro* methods for evaluation blood-brain barrier (BBB) penetration was the first part of the experimental part. The PAMPA model predicts the permeation of compounds across the membrane and thus simulates BBB only on the base of physicochemical properties of compounds defining precondition to the passive diffusion. Next, the models in which the cellular membrane replaced the phospholipid membrane of PAMPA were used and by that the model was enriched with the activity of living cells. The standards drugs assesed by cellular modelsshowed the better correlation between measured and real data of CNS availability when compared with PAMPA. The best correlation was achieved by a method using an hCMEC/D3 cell line of human origin, in which the prediction of BBB permeation fully corresponded to *in vivo* situation

The second part of the experimental work dealt with the approaches to increase the penetration of AChE reactivators into the CNS. The validity of the selected approaches was verified by *in vitro* and *in vivo* methods. The evaluation of the permeation of monoquaternary and nonquaternary reactivators confirmed an important role of the quaternary nitrogen in their structure as a major obstacle in the permeation through the BBB. The use of diamond nanoparticles increased the permeation of compounds through the cell monolayer. HI-6

reactivator encapsulated in cucurbit[7]uril nanoparticles showed an improved pharmacokinetic profile by prolongation the elimination half-life from the brain in experimental animals, which resulted in overall higher bioavalability. The CACO-2 cell line was applied to assess the interaction of standard reactivators with P-glycoprotein. This model expresses this transporter, responsible for the efflux of a number of drugs from BBB back into the bloodstream. Such interaction was not confirmed for standard AChE reactivators by this method on contrary to expectations.

Thus, a complex *in vitro* tool was created by using these cellular methods for determination of the ability of newly synthesized potential antidotes for NA poisoning and for other substances targeting structures in the CNS to penetrate the BBB. The use of *in vitro* methods for the selection of hit compounds reduces the use of experimental animals and the costs of the research and development processes.

Keywords: blood-brain barrier, cell lines, in vitro, bioavailability in brain, drug, efflux mechanisms