MEDICAL PROPHYLAXIS TO MITIGATE CHEMICAL THREATS

Bifunctional Compounds Serving As Versatile Causal Antidotes Against Nerve Agents

Lukas Gorecki University Hospital Hradec Kralove, Biomedical Research Center **Vendula Hepnarova** Department of Toxicology and Military Pharmacy, Military Faculty of Medicine, University of Defence, Trebesska 1575, 500 01 Hradec Kralove, Czechia **Martina Hrabinova** Department of Toxicology and Military Pharmacy, Military Faculty of Medicine, University of Defence, Trebesska 1575, 500 01 Hradec Kralove, Czechia **Jana Zdarova Karasova** Department of Toxicology and Military Pharmacy, Military Faculty of Medicine, University of Defence, Trebesska 1575, 500 01 Hradec Kralove, Czechia **Jan Korabecny** Biomedical Research Centre, University Hospital Hradec Kralove, Sokolska 581, 500 05 Hradec Kralove, Czechia

Protection against the most toxic chemical weapons of mass destruction is still inadequate. Current causal antidotes are based on reactivation of acetylcholinesterase (AChE), which is covalently inhibited by these organophosphorus compounds. Most clinical and experimental reactivators consist of two parts. One part has an oxime group responsible for reactivation and the other part serves as an anchor to the enzyme. These properties are essential for sufficient antidotal efficacy. However, such molecule also acts as a "bipolar" agent and can have either a productive or non-productive orientation. The situation can be reversed by adding another oxime group to the molecule. For this reason, we have prepared several series of hybrids with two reactivating moieties designed to be active against both cholinesterases, AChE and butyrylcholinesterase. As a result, we also wanted to have an active ingredient that would be effective against a variety of organophosphate inhibitors. We used specific fragments known for their different selectivity and activity and followed different synthetic approaches to cover as many structural possibilities as possible.

First, we will discuss our novel library of small nucleophilic compounds. These compounds were characterized for their nucleophilicity and ability to provide nucleophilic attack to the phosphorus atom. This was a source of inspiration for us and a valuable tool for the design of true reactivators. In the following, we will discuss the synthesis of seven symmetric uncharged bis-oximes, eight asymmetric uncharged bis-oximes, and five asymmetric permanently charged compounds as reactivators to counteract organophosphorus poisoning. The structure-activity relationship is presented in detail. The in vitro reactivation abilities on both cholinesterase enzymes will be shown. We have also evaluated our compounds in human, mouse and rat enzymes. This has allowed us to better correlate in vivo experiments with potential human applications. In addition, our lead molecules were also evaluated for A-agents ("novichok") inhibition. Finally, we will present our latest in vivo data on several highlighted candidates, including MTD-estimated toxicity, pharmacokinetics, and pharmacodynamics in sarin and VX-poisoned mice. Our lead candidate has demonstrated superior efficacy over standard clinical antidotes both in vitro and in vivo according to our preliminary results.

This work has been supported by the project of Czech Science Foundation (No. 22-05318S), and by the Long-term development plan Military Faculty of Medicine, Healthcare Challenges of WMD II (DZRO-VLF22-ZHN II).