Chapter 20

Biomarkers of Nerve Agents Exposure

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Abstract. Nerve agents (OPC) could be used in terrorist acts. Investigation of longer-lived biomarkers for verification of exposure to toxic compounds is important for diagnosis and prognosis of these intoxications. We study the effects of doses of toxic agent and the times expire after intoxications on the potential biomarkers. In our studies soman and tabun applied at different single doses −1.0 LD₅₀, 0.5 LD₅₀ and 0.1 LD₅₀ were able to cause significant changes of BuChE and AChE in erythrocytes, brain and liver for a period of 10 days after the challenge. Intoxications with different doses of soman and tabun proved that brain and erythrocyte AChE are most sensitive regardless of the dose used. In the same conditions we implement a procedure for analysis of phosphorylated BuChE in plasma or serum samples, which is based on reactivation of the phosphorylated enzyme with fluoride ions (developed from [2]). The method of fluoride reactivation with further treatment and analysis by gas chromatography represented positive results for soman and tabun poisoning up to the fifth day after exposure, despite the implemented dose. We implement the method of fluoride reactivation in clinical practice. Two incidents with acute poisoning with chlorpyrifos and mixture of chlorpyrifos and diazinon have been investigated. Our studies showed that the method for fluoride reactivation is applicable in clinical practice to cases of intoxications with organophosphorus pesticides with capability to detect reactivated fluorophosphates longer than the original intoxicant and its metabolites could be detected in biological samples. The combination of routine biochemical and analytical methods with fluoride reactivation will improve the diagnosis and prognosis of intoxication with nerve (OPC) agents in case of terrorist acts with these compounds.

Keywords. Biomarkers, chemical warfare agents, diagnosis, nerve agents, fluoride reactivation

20.1. Introduction

Organophosphorus compounds (OPC) are widely used like insecticides. They were developed also as warfare nerve agents. The recent use of sarin in the terrorist acts in Matsumoto city and Tokyo underground was removed any doubts about the possibility of using chemical weapons by terrorists.
The main problem connected with the chemical terrorism is that in addition to chemical weapons, terrorists can use different toxic chemicals from chemical industry, agriculture or products released from terrorist acts on industrial facilities.

Widely used methods to diagnose and biomonitor exposure to OPC, e.g., nerve agents are measurement of enzyme activity of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) in blood and analysis of the intact poison or its degradation products in blood and/or urine.

Measurement of cholinesterase inhibition in blood does not identify the anticholinesterase and does not provide reliable evidence for exposure at inhibition levels less than 20%.

The intact poison and its degradation products can only be measured shortly after exposure. Moreover, the degradation products of pesticides may enter the body as such upon ingestion of food products containing these products [1].

Polhuijs et al. [2] developed a procedure for analysis of phosphorylated BuChE in plasma or serum samples, which is based on reactivation of the phosphorylated enzyme with fluoride ions: this converts the organophosphate moiety completely into the corresponding phosphofluoridate, which is subsequently isolated and quantitated. It can be calculated that inhibition levels ≥0.1% of inactivated BuChE (i.e., trace level exposure) should be quantifiable. Application of this method to serum samples of the victims from the Tokyo subway attack and of the Matsumoto incident yielded sarin concentrations in the range of 0.2–4.1 ng/mL serum [1].

The aim of this study was to find out conditions for implementation of the fluoride reactivation in relation to intoxications of white rats with soman and tabun and to implement this method in clinical practice in cases with OPC pesticides intoxications.

20.2. Materials and Methods

20.2.1. Experiments on Rats

Animals. Male albino rats “Wistar”, weighing between 180 and 220 g were used for the experiments.

Biochemical investigations. Biochemical investigations were carried out 1, 5 and 10 days after soman and tabune poisoning. Intoxications were caused by three doses of OPC – 1.0 LD<sub>50</sub>, 0.5 LD<sub>50</sub> and 0.1 LD<sub>50</sub>, injected s.c. in a volume of 0.1 mL/100 g body weight. For each dose of OPC used rats were divided into three groups (n = 8 in group). Control group (ten rats) was treated with 0.9% NaCl at the same experimental conditions. Biochemical observations were made 24 h, 5 and 10 days after the challenge by using Screen Master (Hospitex Diagnostics, Italia).

Biochemical parameters. The following biochemical parameters were determined: Butyryl Cholinesterase (ChE, EC 3.1.1.8.) (Kinetic method, U/L); Acetylcholinesterase (AChE, EC 3.1.1.7.) in erythrocytes, brain and liver (Ellman method, mkmol/mL/min);