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Mini-review

Current understanding of the mechanisms involved in metabolic detoxification of warfare nerve agents

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ABSTRACT

This study reviews current understanding of chemical, biochemical and toxicological aspects and mechanisms of metabolism of warfare nerve agents. Among enzymes participating in metabolism of nerve agents the role of A-esterases, serum cholinesterase and carboxylesterases is discussed. This article also discusses other aspects of metabolism of the agents such as protein binding and the role of tissue depots for these compounds.

Contents

1. Introduction

The first organophosphorus (OP) nerve agents, tabun (GA) and sarin (GB), were developed in the 1930s by Gerhard Schrader. These, and the even more toxic soman (GD), developed in 1944, are members of the so-called G-agents. Together with VX, developed after World War II in the United Kingdom, these compounds have emerged as the major nerve agents known to have been produced and weaponized. The nerve agents are alkylphosphonic acid esters. Tabun contains a cyanide group. Sarin and soman, which contain a fluorine substituent group, are methylphosphonofluoridate esters. These nerve agents contain a C–P bond that is almost unique in that it is not found in organophosphate pesticides. This C–P bond is very resistant to hydrolysis. VX contains a sulfur and is an alkylphosphonothiolate.

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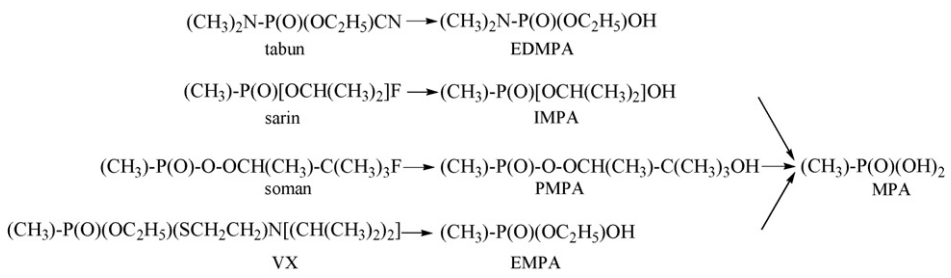

Fig. 1. Metabolic detoxification of warfare nerve agents tabun, sarin, soman and VX in mammals in vivo. Chemical names of metabolites are: EDMPA: ethyl dimethylaminophosphoric acid, IMPA: isopropyl methylphosphonic acid, PMPA: pinacolyl methylphosphonic acid, EMPA: ethyl methylphosphonic acid and MPA: methylphosphonic acid.

Very high toxicity of these agents can be attributed to the excessive cholinergic stimulation caused by inhibition of acetylcholinesterase (AChE) at neuromuscular junctions and in the central nervous system. Nerve agents react rapidly with a serine hydroxyl group in the active site of AChE with the formation of a phosphate or phosphonate ester. The phosphorylated enzyme regenerates very slowly rendering the enzyme inaccessible for its physiological substrate acetylcholine. The chirality around the phosphorus atom largely influences the toxicity of these agents as documented in the case of soman whose P(−) isomers are much more toxic than the P(+) isomers (van der Schans et al., 2007).

In the case of G-agents the intact agent is present in the organism for only several hours. An important metabolic pathway of G-agents is hydrolysis, a process mainly mediated by so-called A-esterases, and the metabolic products formed are corresponding *O*-alkyl methylphosphonic acids in the case of sarin and soman (Fig. 1). VX is a less suitable substrate for A-esterases. In addition to hydrolysis, binding reactions of nerve agents to esterases such as AChE, serum cholinesterase (ChE), carboxylesterases (CarbE) and other proteins occur. Both OP pesticides and nerve agents lose their acyl radicals when they react with AChE, ChE and CarbE. After binding to AChE and ChE the phosphoryl residues of soman, sarin, tabun and VX undergo an intramolecular rearrangement with subsequent loss of one phosphoryl group. This reaction is known as aging defined as non-enzymatic time-dependent loss of one alkyl group bound to the phosphorus which leads to a stable non-reactivatable form of phosphorylated AChE that is resistant to both spontaneous and oxime-induced reactivation (see Fig. 2). In addition, due to the reversibility of the binding reaction of sarin and soman to CarbE, it appears that CarbE are involved in metabolic detoxification of these agents to their corresponding non-toxic metabolites isopropyl methylphosphonic acid (IMPA) and pinacolyl methylphosphonic acid (PMPA) (Jokanović et al., 1996).

One of the important proofs which support the significance of detoxification reactions of nerve agents in the body was presented by Fonnum and Sterri (1981) who reported that only 5% of LD_{50} of soman in rats or about 5 μg/kg reacts with AChE causing acute toxic effects, while the remaining 95% undergoes various metabolic reactions.

The aim of this article is to review current knowledge of chemical and biochemical aspects of mechanisms involved in metabolism of warfare nerve agents. Mechanisms of biotransformation of OP pesticides are beyond the scope of this article and the interested readers are referred to other publications (Jokanović, 2001; Chambers et al., 2001; Tang et al., 2006).

2. Chemical aspects of metabolism of nerve agents

The G-agents are anticholinesterase OP nerve agents that at sufficient concentrations can be toxic or fatal by any route of exposure. Differences in volatility and water solubility result in varied degrees of persistence and variations in the likelihood of exposure by certain routes. Of the G-agents, tabun gives rise to the greatest number of degradation products. The main metabolic product of tabun is ethyl dimethylaminophosphoric acid (EDMPA) (Fig. 1). Toxicity data are available only for a limited subset of the tabun degradation products. Hydrolysis gives rise to dimethylamine, among other substances. Dimethylamine is moderately toxic in terms of acute lethality but causes human irritation of the respiratory tract (Munro et al., 1999).

Sarin is metabolized to isopropyl methylphosphonic acid (IMPA), which slowly undergoes further hydrolysis to the very stable methylphosphonic acid (MPA). IMPA also forms in the course of spontaneous reactivation of sarin-inhibited CarbE and ChEs. IMPA has low oral toxicity in rats and mice, but it produces mild skin irritation in rabbits.

In the study of Little et al. (1986) a single sublethal dose (80 μg/kg) of radiolabeled sarin was administered intravenously to mice and the tissue distribution was studied for 24 h. Within 1 min, sarin was distributed to the brain, lungs, heart, diaphragm, but the highest concentrations were found in kidneys, liver, and plasma. Thereafter, the concentrations in all tissues rapidly declined and within 15 min only trace quantities of [^{3}H]sarin were found in brain. Within the first minute, about half of the labeled sarin was associated with the major sarin metabolite IMPA. The kidneys contained the highest concentrations of sarin and its metabolites, whereas much lower concentrations of metabolite were detected in the liver suggesting a minor role for the liver in detoxification of sarin.

Shih et al. (1994) injected rats subcutaneously with a single dose of 75 μg/kg of sarin and measured excretion of the hydrolyzed metabolites, the alkylmethylphosphonic acids, including IMPA and other methylphosphonic acids. Urinary elimination was found to be quite rapid and the terminal elimination half-life of sarin metabolites in urine was 3.7 h. Most of the administered dose of sarin was retrieved from the urine in metabolite form after 2 days.

Distribution, metabolism, and elimination of sarin in humans appear to resemble findings in animals. Minami et al. (1997, 1998) detected the sarin metabolite IMPA in urine of humans after the terrorist attack in Tokyo in 1995. They found peak levels of IMPA or MPA in urine 10–18 h after exposure. The levels of IMPA in urine correlated with the clinical symptoms. They also found evidence of distribution of sarin to the human brain in 4 of the 12 people who died after exposure. IMPA and MPA were detected in patients from the Matsumoto sarin exposure (Nakajima et al., 1998).

Hydrolysis products of soman include pinacolyl methylphosphonic acid (PMPA) and MPA. No biologic data were found for PMPA. It has been shown that the toxic C(±)P(−)-isomers of soman react rapidly with covalent binding sites. The less toxic C(±)P(+)-isomers are hydrolyzed several orders of magnitude faster than the C(±)P(−)-isomers. The low toxicity of the C(±)P(+)-isomers is primarily due to a low intrinsic reactivity toward AChE and rapid hydrolysis (van der Schans et al., 2007). The levels of C(±)P(−)-isomers remain toxicologically relevant for periods of 50–100 min