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Fenamiphos is recalcitrant to the hydrolysis by alloforms PON1 Q192R of human serum

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ABSTRACT

Fenamiphos (ethyl 4-methylthio-m-tolyl isopropylphosphoramidate) is a racemic organophosphorus nematicide widely used in agriculture around the world. The paraoxonase 1 from human serum (PON1) is a phosphotriesterase (PTE) that hydrolyses several xenobiotics including drugs and organophosphorus compounds (OPs). In this work, the separation of the enantiomers of fenamiphos by HPLC using the column CHIRALCEL OJ and a mobile phase of hexane/ethanol (99/1) is presented. A liquid–liquid extraction method was implemented for the characterization of commercial nematicide hydrolysis by PON1 Q192R alloforms of human serum from children and adults. The results show a recovery of 94% for each isomer from the biological matrix. The method resulted linear response in a range concentration between 50 and 800 μM with a detection and quantification limit between 0.6 and 2 μM for the (+)-fenamiphos, and between 0.7 and 2.3 μM for the (−)-fenamiphos. The levels of the Ca^{2+}-dependent hydrolysis (residual concentration [μM]) quantified during 30 min of reaction were only just 4–14% for both fenamiphos enantiomers with the three alloforms of PON1 Q192R of the two groups of serum studied. These results demonstrate that human serum PON1 is could be involved in the detoxification of a limited number of organophosphorus insecticides.

1. Introduction

Organophosphorus compounds (OPs) have been widely employed as pesticides in the last 50 years; they represent 38% of the insecticides around the world (Singh and Walker, 2006). Their use continues being an important chemical protection in the field of agriculture and worldwide public health. However, their lack of specificity over the pests implies a risk for the human health and the ecosystems. The intoxication by OPs constitutes a worldwide health problem; there have been reported around 3 million intoxicated people and 200 thousand deaths yearly. Their main effects are acute, brought about by the covalent phosphorylation of esterases in the nervous system. The signs and symptoms of the cholinergic syndrome correlate with the inhibition of acetylcholinesterase (AChE) focused in the serine residue of the active center, with the subsequent accumulation of acetylcholine in the nerve endings. On the other hand, the ataxia and paralysis symptoms of the delayed syndrome (OPIDN), that appear 2–3 weeks after the acute exposure to the insecticide, are associated to the inhibition and aging of the esterase target of the neuropathy (NTE) in the nervous system (Johnson, 1982). Both acute neurotoxic syndromes are related to the chemical structure of the OP and in great measure to its metabolism (Jokanović, 2001).

The current therapy for the intoxication by OPs including atropine (ACh antagonist) in combination with nucleophilic agents (oximes) that reactivate the AChE (Gray, 1984). These drugs offer a limited protection in case of severe intoxication and in the prevention of long term secondary effects (Doctor and Saxena, 2005). For this reason there has been a great interest in the prophylaxis with therapies based on proteins which include the A-esterases also called OP-hydrolases (Lenz et al., 2007) or phosphotriesterases (PTEs).

The human serum paraoxonase (PON1) (EC 3.1.8.1) is an A-esterase of 355 aminoacids that is found associated to the high density lipoproteins (HDL) through the apoliprotein A-1 (Harel et al., 2004) in the bloodstream and its activity is calcium

Abbreviations: PON1, paraoxonase 1; PTE, phosphotriesterase; AChE, acetylcholinesterase; ACh, acetylcholina; HPLC, high-performance liquid chromatography; OP, organophosphorous compound; OPIDN, organophosphate-induced delayed neuropathy; NTE, neuropathy target esterase; HDL, high-density lipoprotein; EPN, ethyl 4-nitrophenyl phenylphosphonothioate; PCR, polymerase chain reaction; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol tetraacetic acid.

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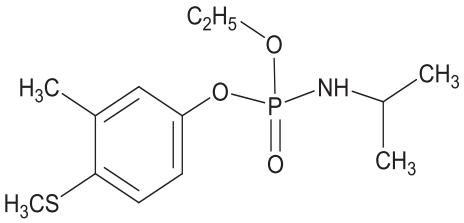

Fig. 1. The chemical structure of fenamiphos.

dependent (Furlong et al., 1991; Kuo and La Du, 1998). Its name is due to its capacity to hydrolyse paraoxon (a metabolite of the insecticide parathion). It is well known that PON1, in addition to hydrolyzing phosphoric esters, it hydrolyzes carboxylic esters and lactones. Preclinical studies employing knockout mice of PON1 have shown a greater susceptibility to intoxication by OPs (Furlong et al., 2000). Furthermore, epidemiological studies have revealed different levels of activity of the PON1 (Costa et al., 2003) attributed to the presence of the polymorphism 192 (Gln[Q] or Arg[R]), in other words the alloenzyme PON1 192R hydrolyses paraoxon faster than the alloenzyme PON1 Q192. In the meantime, other compounds such as diazinon are hydrolyzed more rapidly by the alloenzyme PON1 Q192 (Davies et al., 1996). These proteins have been considered as an alternative in the treatment of intoxication by OPs (Sogorb et al., 2004; Lenz et al., 2007).

The toxicity of the OPs depends on their conformation. One fourth of the commercial pesticides worldwide are chiral compounds (Brophy et al., 2001), which include compounds with one phosphorus chiral center. The optical properties of the stereoisomers lead to metabolic and neurotoxicity differences among the racemic OPs in biological systems. Pioneer study conducted by Johnson and Read (1987) showed that the (−)-isomer EPN inhibits and ages the NTE of the hen's brain and its oral delivery induces OPIDN. In the meantime the (+)-EPN isomer inhibits the NTE but does not induce the aging reaction of this estearase and thus it does not induce this syndrome *in vivo*. Fenamiphos (ethyl 4-methylthio-m-tolyl isopropylphosphoramidate (Fig. 1) is a racemic OP nematicide widely used in the production of fruits, vegetables, grains and tobacco among other types of crops (Wang et al., 2004), it is considered highly toxic to aquatic and land organisms (Megharaj et al., 2003). Aquatic stereoselective toxicity studies of fenamiphos showed that (+)-fenamiphos is 20 times more toxic than (−)-fenamiphos over *D. magna* (Wang et al., 2004). While other *in vitro* study with butyrylcholinesterase extracted from horse serum corroborated the toxic properties of this enantiomer (Li et al., 2010). Since fenamiphos is an OP belonging to the phosphoramidates, whose members present a high inhibiting power over cholinesterases and induce OPIDN (Johnson et al., 1991) in which the *in vivo* hydrolysis by serum A-esterases plays an important role in their stereoselective toxicity (Monroy-Noyola et al., 2007). In the present work, the metabolic evaluation of fenamiphos by the alloforms of PON1 192 from human serum of children and adults, it will be relevant to identify a possible stereoselective hydrolysis of this nematicide using a chiral HPLC method supplemented with a specific liquid–liquid extraction process.

2. Methods and materials

2.1. Reagents

The fenamiphos (4-methylthio-m-tolyl isopropylphosphoroamidate) (98% purity) was purchased from Chem. Service (West Chester, PA), HPLC grade hexane and ethanol were obtained from Burdick & Jackson (Morris Township, NJ) and the column CHIRALCEL OJ. Daicel Chem. Ind., LTD were obtained from Leacsa S.A. de CV (Mexico, DF).

2.2. Equipment employed

For the resolution, detection and quantification of the hydrolysis of the enantiomers of fenamiphos by serum samples a Waters 600 Controller HPLC equipped with a 2996 diode array detector and 717 automatic injector was used.

2.3. Sample acquisition

In this study 30 human serum samples were employed; 15 adults and 15 children. These samples were provided by an obesity and cognitive deficit project being carried out at the Facultad de Farmacia, UAEM. The 30 samples were selected on the basis of their isoforms PON1 Q192R; five samples for each isoform of each of the two groups of samples. For the diagnostic of the alloforms of PON1 Q192R, the sera were phenotyped by the method of Eckerson et al. (1983) and corroborated by PCR (Humbert et al., 1993). Both groups of samples from the clinical projects were obtained under free and informed consent.

2.4. Precautions

Due to the fact that fenamiphos is a toxic organophosphorus compound (a powerful inhibitor of cholinesterases) during this study this compound was handled employing the necessary safety measures; double gloves (nitrile), lab coat, safety glasses and mouth covers. The fenamiphos solutions were prepared inside lab fumehood. The materials employed in the preparation of the solutions and the tubing and residues from the trial reactions were treated with basic solution (2 M sodium hydroxide) and rinsed with distilled water. Finally, the wastes were labeled according to the norm NOM-052-SEMARNAT-2005.

2.5. Hydrolysis of fenamiphos

The trials of the hydrolysis of fenamiphos consisted in the incubation of 10 μL of each of the sera with an aliquot of racemic fenamiphos 400 μM (∼200 of each isomer), in the presence of Tris–HCl 10 mM, $CaCl_2$ 2.5 mM or EDTA 5 mM under physiological conditions of pH and temperature during 30 min. The reaction was stopped by adding 20 μL of 0.2 M HCl. The residual fenamiphos was extracted by a liquid–liquid extraction with 1 mL of hexane in two successive cycles, stirring for 5 min and centrifugated at 1000 x *g* during 15 min for each cycle. The upper layer was removed (2 mL) and 20 μL of the organic phase were injected into the chromatographic system. The separation and identification of the isomers of fenamiphos were carried out according to the chromatographic conditions proposed by Ellington et al. (2001). Briefly, the mobile phase was made up of hexane/ethanol 99/1 v/v, employing a UV–Visible detector at 235 nm, with a flow of 1 mL/min, injection volume 20 μL and analysis time of 30 min. Each simple was analyzed in triplicate and injected twice into the system. The quantification of the residual fenamiphos (not hydrolyzed) of each enantiomer was carried out using a calibration curve with the concentrations of 50, 100, 200, 400, 600, and 800 μM of racemic fenamiphos of standard quality (∼50% of each isomer). The hydrolysis was expressed in the residual concentration [μM] for each serum sample in the presence of calcium or EDTA. The data were corrected for their spontaneous hydrolysis.