Some genetic interventions in organophosphates and other insecticides metabolism

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Abstract. Organophosphates represent a very large class of insecticides used in the last decades, together with carbamates and pyrethroids. They act on the nervous system, with various effects, mostly cholinergic signs, but for some compounds, noncholinergic ones. Their metabolism involves esterase enzymes, including here the group of aliesterases (e.g. carboxylesterases), but also arylesterases (e.g. paraoxonase – PON1) and cholinesterases (e.g. butyrcholinesterase), both with a smaller contribution, compared to the first mentioned class. These enzymes act on a specific substrate, although in some cases interchangeable substrates were reported. The content of esterases in various species is different and inside of the individual, the site of action is particular for each enzyme. A typical way of detoxification through reactions of hydrolysis was reported for carboxylesterases, resulting products which are more polar and more easily to be excretable. Two of human carboxylesterases, hCE-1 and hiCE-2 (hCE-2) and their mammalian homologous are known to be involved in various xenobiotic detoxification processes, through this mechanism. When some insecticides, such as malathion-type compounds, are bioactivated, the further detoxification is performed by the paraoxonase (PON1), but also by the albumin and butyrcholinesterase. This paper aims to debate the main enzymes involved in the metabolism of organophosphates, carbamates and other insecticides, discussing comparative genetic aspects in animals and humans.
Key Words: esterase, pesticides, carbamates, pyrethroids.

Introduction. Four classes of pesticides are used as insecticides: organochlorides, organophosphates, carbamates and pyrethroids. Their common mechanism of action is based on damaging the insect central nervous system, at the level of nervous impulse propagation at the synaptic cleft or along the axon (Montella et al 2012). These chemicals are toxic for both insects and superior organisms, but a compensatory metabolic detoxification through the involved enzymes is naturally occurring.

Organophosphates (OPs) are one of today's most widely used classes of insecticides, comprising the malathion, parathion, diazinon, chlorpyrifos, etc., and the main nerve gases: sarin, tabun and soman (Adeyinka et al 2021; Mukherjee & Gupta 2020). They are phosphate esters in which lipophilic functional groups, such as those of alkyl or aromatic types, are substituted to a central phosphate molecule (Tucker 1979). OPs irreversibly bind the enzyme acetylcholine esterase (AChE) (Adeyinka et al 2021; Satoh 2005), whose biological function is to hydrolyze (decompose) the excess of neurotransmitter acetylcholine (ACh) into choline and acetate. As a result, an overstimulation of nicotinic and muscarinic receptors, widely distributed in the body at the level of the nervous system, occurs, causing an excessive cholinergic effect (Mukherjee & Gupta 2020). Although similar in their structure with OPs, carbamates reversibly bind AChE at the level of neuronal synapses, their effect lasting for about 24 hours (Adeyinka et al 2021). Noncholinergic effects are well-known for some OPs, which are causing an organophosphate-induced delayed polyneuropathy. This is expressed 2-3 weeks after the primary exposure, resulting in

tingling of hands and feet, subsequent sensory loss, muscular weakness, flaccidity of distal skeletal muscles and ataxia. The inhibition of phospholipase and esterase activities was reported as the biochemical mechanism behind of these clinical sings (Mukherjee & Gupta 2020).

OPs are easily absorbed through cell membranes and, to get excreted efficiently, a more polar structure is gained through the action of carboxylesterases (CEs) enzymes, preventing thus their accumulation and undesirable effects on the organisms (Tucker 1979). These enzymes are a part of the aliesterases (AliE) group, but there are also known for the intervention of arylesterases (ArE) and cholinesterases (ChE) enzymes (Augustinsson 2006).

The aim of this paper was to review the main enzymes of the esterase family, involved in the metabolism of the largest class of insecticides, the organophosphates, but also in the metabolism of the carbamates and other insecticides, comparing various genetic aspects in animals and humans.

Material and Method. This review study is based on 19 scientific papers selected for their scientific relevance for the subject matter.

Results and Discussion. As Augustinsson (2006) reviewed, there are multiple forms of esterase enzymes in vertebrates and which act on various substrates. Their location in different species is not constant in the same tissue and their intervention on the metabolism of various pesticides is peculiar. Of three classes discussed, the main contribution was reported for aliesterases (AliE), compared to arylesterases (ArE) or cholinesterases (ChE).

Carboxylesterases (CEs, CarbE, Aliesterases, EC 3.1.1.1) are an important part of the AliE group, a B-esterase family preferably acting on organic molecules with open chains, such as aliphatic molecules (Montella et al 2012). They are the main class of enzymes involved in metabolic detoxification of various xenobiotics, catalysing reactions of hydrolysis of lipophilic esters to form products which are more polar and more easily excretable. They primary act at the level of the distal carboxylic acid ester, the corresponding carboxylic acid being formed as a detoxification product and alcohol (Cashman et al 1996; Redinbo & Potter 2005). This class of enzymes was reported in a wide range of species, from bacteria to man, with a large diversity of functions and different localizations at the cellular level or types tissues (Redinbo & Potter 2005; Satoh & Hosokawa 2006).

Reported to the sites of CEs action, sensitive to the OP and carbamate insecticides, such as the liver, the skin or the respiratory tract, are important for these chemicals detoxification, along their major route of environmental exposure. Their action of hydrolytic detoxification was also reported in small intestine, testis, kidney, lung, human blood monocytes (Cashman et al 1996; Satoh & Hosokawa 2006). Redinbo & Potter (2005) reviewed a maximum expression of CEs in the organs' epithelia, as a part of their protective role against xenobiotics, but no level of activity in the human blood.

Augustinsson (2006) discussed the lack of AliEs in human, monkey, dog and pig plasmas and questioned their existence in that of ruminants. However, this class of enzymes was considered the main one in the plasma of lower vertebrates.

As Satoh & Hosokawa (2006) reviewed, while no CEs are contained by human plasma, the role of ester and amide bonds hydrolysis at this level is taken over by cholinesterases [butyrlcholinesterase (BuChE)] and arylesterases [paraoxonase (PON1, A esterase, EC 3.1.8.1)]. ArEs preferentially act on aromatic compounds and are able to hydrolyse triester OPs, although both CEs and paraoxonase are able for an interchangeable substrate of action, EC 3.1.1.1 enzymes for aryl ester compounds and EC 3.1.1.2 for open chain carboxylic esters. ChEs prefer charged substrates, such as choline esters, instead of aliphatic or aromatic esters (Montella et al 2012). ArEs were reported in high concentrations

all mammalian plasmas studied so far, but absent in the plasma of amphibians, reptiles and fish (Augustinsson 2006). BuChE is the specific pattern of ChEs in human, monkey, dog, cat, horse and guinea-pig plasmas, but not in ruminant plasmas and of very specific type in that of pig. A different sensitivity to OP compounds was reviewed for rat and rabbit ChEs, since in these species a propionylcholinesterase type was identified (Augustinsson 2006).

Li et al (2005) demonstrated differences in various species related to the content of CEs and other four esterases: BuChE, PON1, acetylcholinesterase (AChE, EC 3.1.1.7) and albumin (whose active site is Tyr 411 in humans and Tyr 410 in bovine). In contrast to the high amounts of plasma CEs found in rabbit, mouse, rat, horse, cat and tiger, neither the human plasma, nor the monkey, pig, ruminant and chicken plasmas do contain CE. BuChE is found both in lab animals and human plasma and it was reported in high amounts in diabetics. The mouse plasma contains 30-fold more CE than BuChE. AChE was found in animal plasma but as trace amounts in human plasma (25-fold higher amounts in mouse plasma than in human plasma). However, AChE was not reported to participate in esters' hydrolysis. Differences among the levels of various enzymes involved in OPs metabolism make rodent and rabbit models inappropriate for assessing their effects in humans (Li et al 2005).

The metabolism of OP and carbamate type insecticides involves both CEs and non-CEs enzymes (ArEs and ChEs). Both of them are proteins of the α,β-hydrolase-fold family. Five families of CEs were described in humans (CES1-CES5), including the isoform transcripts of each class (Holmes et al 2010). Two of human CEs, hCE-1 (also called egasyn), encoded by CES1A1 gene and primarily produced in the liver and hiCE-2, mainly expressed at the intestinal level (Bencharit et al 2003; Redinbo & Potter 2005; Satoh 2005), are involved in the metabolism of cocaine, heroin, procaine and other drugs, as reviewed by Grădinaru (2021). They are also involved in OPs and carbamate insecticide metabolism. Their different groups' preference for action was also reviewed: generally hCE-1 substitutes small groups, such as methyl and ethyl, while hiCE-2 substitutes larger ones, e.g. benzyl (Redinbo & Potter 2005; Satoh 2005). hCE-1 acts in detoxification of organophosphate chemical weapons, such as sarin, soman and tabun, therefore it is likely to be able to treat the exposure to these chemicals (Bencharit et al 2003). hCE-1 is also involved in the metabolism of another insecticide, the permethrin (from the pyrethroid family) (Redinbo & Potter 2005; Satoh 2005). Nishi et al (2006) reported that both type I (esters of primary alcohols, e.g. permethrin and bifenthrin) and type II (esters of secondary alcohols that contain a cyano group at the α-carbon of the alcohol moiety, e.g. cypermethrin, cyfluthrin, fenvalerate, γ-cyhalothrin) pyrethroids were hydrolysed by hCE-1 and hCE-2. hiCE-2 was reviewed to share a 99% amino acid sequence identity to a liver isoform hCE-2 (encoded by CES2A1 gene) and a 49% homology with hCE-1. Both are glycoproteins of ~62 kDa. Pindel et al (1997) reported a 48% sequence identity of hCE-2 with hCE-1. The highest sequence identity was observed for the rabbit liver carboxylesterase form 2 (73%) and for the hamster liver esterase AT51p (67%). The CES1 gene contains 14 exons, while the CES2 contains 12 exons, their length and that of their transcript sequences reaching 10.9 kb for CES2 and 34.8 kb, for CES1. More than 500 amino acids were found in these human variants: 567 for the product of CES1 and 559 for the product of CES2 (Holmes et al 2010). The same spanning of 30 kb and 14 small exons content were documented by Satoh & Hosokawa (2006) for both human CES1 and murine genes as a result of evolutionary conservation in these species, but with different locations, on human 16q13-q22.1 and murine chromosome 8 at 8C5, in a cluster of six carboxylesterase genes that totally spans 260.6 kb in the last case.

Other mammalian species of CEs based on their amino acid sequence homologies were established for various human CEs. For example, monkey MOCE31, dog D1, rabbit form 1, abbreviated rCE and also called rabbit liver carboxylesterase, and others for mouse

and rat, as well as rabbit form 2, hamster AT-51 or rat CE4.6 and ratICES, were included with different shares of homology with hCE-1 and hiCE-2, respectively (Satoh 2005). Loandos et al (2012) and Redinbo & Potter (2005) reviewed an 81% sequence identity among rCE and hCE-1, with the incapacity of human homolog to process the anticancer prodrug CPT-11. Bencharit et al (2003) reviewed a 71% sequence identity of guinea pig homolog of hCE-1, the gpCE.

Various mammalian CEs show a significant amino acid sequence homology, with a well-conserved active catalytic site containing Serine, Histidine and Glutamic acid. Aspartic acid was reported to substitute Glutamic acid in proteases (Loandos et al 2012; Redinbo & Potter 2005). However, the theory of catalytic triad including Ser203, Glu336 and His450 was reported through a mechanism of His450 and Ser203 protons shuttle and an oxygen nucleophile attack (by the β-OH group of Ser203) on the carbonyl carbon of the substrate (Redinbo & Potter 2005; Satoh & Hosokawa 2006). The active site of action depending on Serine is also considered for EC 3.1.1.2, but not for EC 3.1.8.1, were a cysteine residue instead of serine was discussed (Montella et al 2012). Various reports reviewed or authors of them found out different locations for the catalytic triad of the active site, such as Ser221, Glu354, His468 (Bencharit et al 2003; Holmes et al 2010), Ser221, Glu353, His467 (Loandos et al 2012), Ser202, Glu319, His431 (Pindel et al 1997), Ser203, Glu336, His450 (Satoh & Hosokawa 2006). One form or another seems to be conserved in various mammalian species.

At the cellular level, CEs associations with the endoplasmic reticulum (ER) are necessary for their enzymatic activity, regulating their flow or retention level. In fact, the general structure of CEs includes two ends: an N- and a C terminal. The N-terminal end of CEs contains a hydrophobic signal peptide which ensures their traffic through the ER. Carbohydrates attaching in an N-linkage appear to be necessary for CEs catalytic activity. The C-terminal end ensures the enzyme retention within the cell through a mechanism involving the KDEL sequence of amino acids located at this level (Lys-Asp-Glu-Leu). In fact, the consensus sequences annotated with HIEL-COOH and HTEL-COOH and discovered in two rabbit liver microsomal esterases, HVEL-COOH in rat liver CE, or HTEHK-COOH in mouse liver microsomal CE, were reported to be involved as a peptide able to be recognized by the luminal side of the KDEL receptor and to contribute to protein retention in the ER. However, the splitting of these amino acid residues may lead to the enzyme secretion (Redinbo & Potter 2005; Satoh 2005; Satoh & Hosokawa 2006).

Concerning the metabolism of OP insecticides in the liver's microsomal ER, CEs and more specifically of the egasyn (of hCE-1 family), it has been shown that these interact directly with the β-glucuronidase enzyme to remove the transferred sugar moiety in a second phase of detoxification reactions, in which glucuronide metabolites were formed to be excreted in bile and urine (Redinbo & Potter 2005; Satoh 2005). This mechanism of detoxification, involving the transfer of the glucuronyl group to the xenobiotic chemical structure, in order to increase its water solubility and to decrease its toxicity, is different of the hydrolysis mechanisms of the carboxylesterases (Cashman et al 1996). Glycosyltransferases (GTs) are involved in this process, which in eukaryotes generally takes place in the cytosol, Golgi complex, and ER. The transfer of the sugar moiety from a nucleotide sugar donor, such as uridine diphosphate (UDP), guanosine diphosphate (GDP) and cytidine monophosphate (CMP), was also reported (Nagare et al 2021). In an interesting point of view, Redinbo & Potter (2005) debated the possibility of various compounds to become a more effective substrate for another enzyme while they are already bound to one enzyme. The dissociation of β-glucuronidase enzyme from its complex, through the egasyn, leads to an increased plasma concentration, which may be used as a more accurate biomarker of OP insecticide exposure than the cholinesterase – acetylcholinesterase inhibition (Satoh 2005; Satoh & Hosokawa 2006; Soltaninejad et al

2007). Beltagy et al (2018) pointed out the serum β-glucuronidase activity as a reliable marker for the OP poisoning detection, even when measured at 24 h after the contact with the toxicant. Satoh and Hosokawa (2006) reported this metabolic pathway, found in OP and carbamate compounds, as unlikely for pyrethroid exposure since these insecticides are readily hydrolyzed by human and other mammalian carboxylesterases.

However, in the mechanism of detoxification, the distal carboxylic acid in the structure of OP compounds is the primary target of hydrolysis. In malathion-type compounds, this specific hydrolysis does not directly occur, but an intermediary oxon metabolite is formed in the process of metabolic bioactivation of these chemicals. The further detoxification is performed by paraoxonase/arylesterase or A esterase class of enzymes, including the EDTA (ethylenediaminetetraacetic acid) and the sensitive or insensitive esterase/paraoxonase, highly active in the liver and serum (Cashman et al 1996). Li et al (2005) described the paroxon (an organophosphate oxon and a metabolite of the parathion pesticide) hydrolysis by PON1 and albumin and its interaction with BuChE, but rather stoichiometric than catalytic. The authors also included the pesticide Diaoxon and the nerve agent Sarin in the PON1 sphere of action, the pesticides-insecticides Carbaryl and the organophosphorus O-Hexyl O-2,5-dichlorphenyl phosphoramidate, for albumin.

Satoh (2005) stated that the paraoxonases are not serine or cysteine esterases, but rather the mammalian paraoxonase/arylesterase (PON1), since calcium is required for their activity. The genes of all PON1, PON2 and PON3 esterases are closely aligned on the human chromosome 7 (7q21.3-22.1) and mouse chromosome 6, without amino acids similarity with carboxylesterases or cholinesterases (Mukherjee & Gupta 2020; Satoh 2005). Although these enzymes were named in the order of their discovery, it seems that PON2 is the oldest member of this family, subsequently resulting PON3 and finally PON1. All of their encoded genes have 9 exons; their amino acid sequence is well conserved and there is more than 80% identity in their structural characteristics between human, mouse and rabbit PON1 and at least 60% of the PONs 1, 2 and 3 of each of these species (Satoh 2005). PON1 exhibits a more complex activity in terms of lactonase, arylesterase and organophosphate activities, whilst PON2 has only a lactonase activity and PON3 only lactonase and arylesterase activities. The PON1 enzyme of 355 amino acids and 43-47 kDa is mainly synthesized in the liver and secreted to the plasma, but it is also found in the brain, small intestine and kidney. The sites of PON2 and PON3 are also variable, PON2 being found in the liver, lungs, placenta, testes and heart, while PON3 is localized in the liver and kidney (Mukherjee & Gupta 2020).

Conclusions. Besides the targeted benefits, the use of OPs and of many other pesticides presents risks. Neurotoxic effects depending on the substance (e.g. dose, duration and frequency of exposure, type of insecticide etc.), but also on individual factors, were reported over the last decades. Molecular mechanisms of detoxification involving various enzymes of the esterase family are the most important, although the gate of new molecular insights is still open. New biomarkers are expected to be studied and used in the evaluation of different insecticides poisoning. The enzymatic apparatus has species-related particularities, including different enzymes, showing in all cases a variable degree of amino acids sequence homology for the involved enzymes, but never 100%. A general mechanism of action was debated, with pathways of metabolic detoxification and, in particular situations, of metabolic bioactivation to toxic species. However, obtaining compounds with less toxic potential is preferable, along such an evolved but conserved metabolism, regardless of the species.

Conflict of interest. The author declares no conflict of interest.

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