Effect of structure and function of paraoxonase-1 (PON-1) on organophosphate pesticides metabolism

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Abstract: Paraoxonase-1 (PON1) is an important enzyme in various pathologies such as pesticide poisoning, diabetes, atherosclerosis, neuronal disorders, and cancer, due to its multifunctional activity since it acts on different metabolites. However, one of its main functions is the hydrolysis of organophosphate (OP) compounds from pesticides that cause fatal poisoning at the level of the central nervous system (CNS). The objective of this review was to investigate whether the structure, genetics, and function of PON1 affect the metabolism of organophosphate pesticides or other abnormalities. Information was selected from articles in the database PubMed – NCBI (https://www.ncbi.nlm.nih.gov/pubmed) with a publication date between 2011 and 2019. The enzymatic activity of PON1 can be modified depending on its chemical structure since there are different genetic polymorphisms that change PON1 morphologies or the levels of expression in the bloodstream. This leads to differences in susceptibilities to organophosphate pesticide poisoning. The results of this review reveal that phenotypic variants of PON1 have differences in affinities for OP substrates.

Introduction

The family of paraoxonases is located in continuous genes of chromosome 7 in humans (PON1, PON2, and PON3) (Arenas et al., 2018; Chistiakov et al., 2017; Moreno-Godínez et al., 2018; Nie et al., 2017). Their locus covers about 170 kb, and their protein structures are similar (Chistiakov et al., 2017; Moreno-Godínez et al., 2018).

Studies have shown that PON1 and PON3 are synthesized in the liver, and are associated with high-density lipoproteins (HDL) prior to their release into the bloodstream, although smaller amounts are also synthesized by the kidney and colon (Chistiakov et al., 2017; Grđic Rajković et al., 2011; Küçükali et al., 2015; Kulka, 2016). However, a small amount of PON1 is associated with very-low-density lipoproteins (VLDL) and postprandial chylomicrons (Grđic Rajković et al., 2011). It is known that PON2 is an intracellular enzyme synthesized in multiple tissues and organs (Chistiakov et al., 2017; Nie et al., 2017).

It is a multifunctional enzyme that acts on organophosphates (OPs), aryl esters and lactones (Küçükali et al., 2015; Nie et al., 2017; Santos et al., 2016). It is also an antioxidant enzyme that catalyzes the decomposition of oxidized phospholipids from low-density lipoproteins (LDL) (Chistiakov et al., 2017; Luo et al., 2018; Macharia et al., 2014), thereby reducing the inflammatory response in the arterial wall by decreasing the adhesion of monocytes to endothelial cells (Macharia et al., 2014). The enzyme is also able to hydrolyze oxidized low-density homocysteines (Arenas et al., 2018; Chistiakov et al., 2017). However, the name paraoxonase is due to its ability to hydrolyze the organophosphate compound paraoxon (diethyl p-nitrophenyl phosphate), a metabolite from the oxon of the pesticide organophosphate parathion (Santos et al., 2016; Sato et al., 2016).

However, there are multiple paraoxonase-1 polymorphisms that influence the enzymatic activity; therefore, they are reflected in the catabolism of organophosphorus pesticides or other metabolites of clinical interest, where an increase in their concentrations can cause different clinical abnormalities.

For example, organophosphates (OPs) are chemical compounds related to domestic and agricultural pesticides derived from phosphoric acid esters and that have a high degree of toxicity in humans, since they have a high capacity for internalization through dermal (due to their lipophilic nature), inhalation and oral routes (Burke et al., 2017; Masson and Nachon, 2017; Suratman et al., 2015;
The PON1 Gene

The PON1 gene is located in the 7q21.3 long arm and consists of 26215 bps, 9 exons and 8 introns (Eom et al., 2011; Grdić Rajković et al., 2018; Shakeri et al., 2017; Wei et al., 2016). The fourth intron contains repetitions of polymorphic AC (Grdić Rajković et al., 2018). The remote terminal unit 5′ (UTR 5′) has no TATA box (Grdić Rajković et al., 2018; Shakeri et al., 2017), and the regulatory proteins of the promoter region are sterol 2 (SREBP2) and protein specificity 1 (Sp-1), which in the presence of statins increase the expression of PON1. In addition, it has been observed that the aryl hydrocarbon receptor and the receptors activated by peroxisome proliferators (PPAR) are also regulators but the ligand sites are not yet known (Grdić Rajković et al., 2018).

Polymorphisms of PON1

The activity and expression of pon1 may be affected by non-genetic factors such as diet, alcohol, tobacco, environmental toxins, aging, pregnancy, and various pathologies, and by genetic factors such as polymorphisms in the promoter and coding regions of the pon1 gene (Ferk and Gersak, 2014). Approximately 200 single nucleotide polymorphisms (SNP) have been identified in the pon1 gene, 7 of which are 7 PNS in the promoter region, 171 SNP in the intron region, 5 in the exon region, while the rest are in the untranslated regions (Aggarwal et al., 2016). However, there are three clinically relevant polymorphisms: one in the promoter region pon1 -108 C/T (dbSNP: rs705379), and two in the coding region pon1 Q192R (dbSNP: rs662A > G) and pon1 L55M (dbSNP: rs854560A > T) (Aggarwal et al., 2016; Al-Eisa et al., 2016; Atasoy et al., 2015; Martinez-Quintana et al., 2017). In the
polymorphism of the promoter region −108 C/T, the allele −108T predisposes a lower transcriptional activity, relative to the allele −108C (Dursun et al., 2014). In the polymorphism of the coding region Q192R, there is a substitution of glutamine (Q) for arginine (R) in position 192 which causes changes in the specificity of allozymes to substrates and promotes or decreases the catalytic efficiency of the enzyme with respect to OPs and lactones (Atar et al., 2016; Bounafaa et al., 2015; Costa et al., 2013; Hernández-Díaz et al., 2016; Mackness and Mackness, 2013). Polymorphism in the coding region L55M involves changes in leucine and methionine in position 55 (Bounafaa et al., 2015; Triki et al., 2016). This polymorphism does not affect the catalytic activity of the enzyme, nor does it affect the enzyme levels in the bloodstream (Atar et al., 2016; Costa et al., 2013). Indeed, higher enzymatic activity has been found with PON1-55L allozyme than with PON1-55M allozyme, in addition to greater stability and resistance to proteolysis, as a result of the expression levels of mRNA (Androutsopoulos et al., 2011; Bajaj et al., 2014; Seow et al., 2016). There is an imbalance of link with allele 108T in the polymorphism of the promoter region (Androutsopoulos et al., 2011).

Clinical Relevance of PON1

The activity of PON1 is affected by different pathologies linked to high oxidative stress such as atherosclerosis, coronary heart disease, diabetes, cancer, and certain neuropathies (Masson and Nachon, 2017; Paul et al., 2017). In atherosclerosis (carotid and cerebral atherosclerosis), lower activities of PON1 have been observed (Costa et al., 2008; Medina-Díaz et al., 2017). Indeed, PON1 is considered an atheroprotective enzyme due to its ability to slow down LDL oxidation (the key to the pathogenesis of atherosclerosis) through hydrolysis of oxidized fatty acids, phospholipids, cholesterol and triglyceride hydroperoxides (Medina-Díaz et al., 2017). Decreased PON1 activity has also been observed in cardiovascular diseases (Luo et al., 2018). Thus, PON1 is considered a susceptibility gene for heart disease because its polymorphisms modify the oxidative status of lipoproteins (Geron et al., 2014). In diabetic patients, cardiovascular complications may occur due to inhibition and destabilization of PON1, since the enzyme may be glycated or may be affected by ligation with HDL, resulting in low efficiency in its antioxidant properties and lipid peroxidation (Paul et al., 2017). In neurological conditions, the activity of PON1 is altered, which may contribute to the development of neurological disorders (Gondo et al., 2017).
diseases such as dementia and Alzheimer’s, PON1 provides neuroprotection against environmental neurotoxins and age-linked neurodegeneration (Huen et al., 2018). Cancer is usually associated with oxidative stress. Since PON1 is an antioxidant enzyme, it may be used in assessing the risk of cancer. However, low PON1 levels and increased lipid peroxidation have been found in cancer patients, because of low antioxidants in circulation (PON1), since cancer cells take nutrients from the circulation (Grđic Rajkovic et al., 2011). However, one of the main pathologies reflected in variabilities in the enzymatic activity of PON1 is due to poisoning by organophosphate pesticides.

Based on these findings, a cutting-edge idea may be developed for the production of PON1 enzymes in pure microbial expression systems with high performance for specific substrates with the amino acid sequences necessary to enhance their activities. This will be of pharmacological significance since PON1 possesses anti-inflammatory, antioxidant, antiatherogenic, antidiabetic, antimicrobial, and OP-neutralizing properties (Millenson et al., 2017).

**Metabolism of Organophosphates by PON1**

The metabolism of pesticides is carried out in three reactions: oxidation, transfer reaction, and hydrolysis (Zhang et al., 2014). The oxidation of pesticides takes place in phase-1 of xenobiotics metabolism in the liver, which is catalyzed by the enzyme cytochrome P450 (CYP). This is a monooxygenase superfamily that catalyzes the oxidation of OP pesticides in their toxic form “oxon” (P = O) (Jusko et al., 2019; Torres-Sánchez et al., 2019; Zúñiga-Venegas et al., 2015), through an oxidative de-sulfurization reaction because OP pesticides have sulfur esters in their chemical structures (P = S; Fig. 2) (Costa et al., 2008; Paul et al., 2017; Sato et al., 2016; Zhang et al., 2014). The presence of OPs modulates the expression of cytochrome P450 (CYP) and glutathione S-transferase through the receptor X pregnenolone (PXR) (Medina-Díaz et al., 2017).

However, the reactions in the metabolism of pesticides are aimed at detoxification, which is the hydrolysis of the metabolites in the form of oxon by means of an esterase paraoxonase-1 (PON1) (Ceron et al., 2014; Costa et al., 2008; Paul et al., 2017).

Its active site is versatile as it can differentially hydrolyze multiple substrates, not just OP’s; the affinity for a particular substrate will depend upon the amino acids they surround that site, particularly the amino acid at position 192, thus regulating the affinity for a substrate and the rate of kinetic reaction (Goldsmith et al., 2016; Aggarwal et al., 2016). Another relevant change that decreases the enzymatic activity is the modification of the cysteine-free sulfhydryl group at position 283 (Kulka, 2016). Because of this, PON-1 has a high capacity to hydrolyze its various substrates through the enzyme-substrate binding that is regulated by hydrogen bonds (Blaha-Nelson et al., 2017). Moreover, this enzyme can have two active sites; one with hydrolytic activity for esterified substrates and the other to reduce hydroperoxides (Kulka, 2016).

Thus, PON1 is an enzyme capable of hydrolyzing the compounds OPs in the form of oxon, and some nerve agents (Huen et al., 2018; Küçükali et al., 2015; Naksen...
et al., 2015; Paul et al., 2017; Sato et al., 2016). Some of the major toxic metabolites are paraoxon, diazoxon, and chlorpyrifos-oxon (Costa et al., 2013). However, when these toxic metabolites are hydrolyzed by PON1, they are degraded to simpler compounds that are easily excreted in the urine such as dialkyl phosphates (DAP) and some other remnants depending on the OPs metabolized (Fig. 3) (Androutsopoulos et al., 2011; Millenson et al., 2017). The biomarker for acute exposure to OPs pesticides is DAP since its concentration is found in the urine in the last two days after exposure (Jusko et al., 2019).

The enzymatic activity of PON1 is affected by its polymorphisms that lead to differences in susceptibilities to different toxic metabolites of OPs (Naksen et al., 2015). However, although polymorphisms with 108T and 55M alleles cause a reduction in the plasma levels of PON1 (Torres-Sánchez et al., 2019), polymorphism 192 is the one that causes variabilities in the hydrolysis of some substrates. Allozyme 192R is more specific for the substrates paraoxon and chlorpyrifos-oxon, while allozyme 192Q has a higher affinity for the nerve agents soman and sarin (Torres-Sánchez et al., 2019; Zúñiga-Venegas et al., 2015).

Conclusion

The PON-1 gene has three polymorphisms of clinical relevance; two in the coding region (Q192R and L55M) and one in the promoter region: −108 (C/T). The Q192R is responsible for the variability in the catalytic activity towards substrates of OP’s, L55M and 108 (C/T) impact on the expression level of PON1 and relate to different clinical disorders. These variants, along with various endogenous factors, define a specific level of exposure that may or may not affect enzyme activity. This effect is reflected in the metabolic variability of organophosphorus pesticides, oxons, lactones, aryl esters, thioesters, thiolactones, and carbonates, resulting in differences in the degrees of susceptibility to various disorders of clinical interest.

Therefore, individual or population susceptibility to the toxic effects of organophosphorus pesticides is then subject to some aspects as (1) the particular affinity to certain substrates, (2) the level of expression of the enzymes themselves, and (3) the environmental and endogenous conditions that trigger different consequences of exposure.

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