Paraoxonase 1 – an Update of the Antioxidant Properties of High-Density Lipoproteins

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ABSTRACT
Paraoxonase 1 (PON1) belongs to a family of enzymes with related functions, being the best studied member. PON1 is a HDL-associated protein of which function is to protect LDL particles from oxidative modifications. The status of PON1 is influenced by different genetic, life style and dietary factors. This short review is aimed to present some new aspects regarding the antioxidant properties of PON1 with emphasis on the influence exerted by different factors. Also, a special attention is paid to the relationship between PON1 and low- and high-density lipoproteins in the context of atherosclerosis which affects the endothelial cells.

Keywords: paraoxonase 1, oxidative stress, HDL

INTRODUCTION
Paraoxonases (PONs) form a group of related enzymes (PON1, PON2, and PON3). The degree of amino acids sequence homology is about 60% concerning these proteins (1). In humans, the three corresponding genes (PON1, PON2, and PON3) are located on chromosome 7q21.3-22.1. Analysis of the phylogenetic data indicated that PON2 was the first gene appeared during the evolution. PON1 and PON3 appeared later through a process of gene duplication (1).

From the moment of their discovery, PONs where highly investigated. For example, using the term “paraoxonase” as a search keyword, PubMed gives back as many as 3438 results.
It is known that PONs have many biological functions, including inactivation of pro-oxidant and pro-inflammatory mediators, metabolism of certain drugs and xenobiotics, and regulation of cells proliferation (2). PONs can be described by the term “enzyme promiscuity” as they act on many structurally unrelated substrates (3). On the other hand, in spite of the great number of studies concerning the biology and biochemistry of these enzymes, their physiological substrates are still elusive.

Besides the differences with respect to their enzymatic activities, PONs differ also by the tissue distribution pattern. Thus, PON1 and PON3 are synthesized by the liver and secreted into the circulation where they become associated with HDL particles, while PON2 is an intracellular enzyme (4). It was found that the amount of high-density lipoprotein (HDL)-associated PON3 is between 50-60 and 100 times smaller than the concentration of PON1 (5). PON2 is located in the perinuclear region, the endoplasmic reticulum membrane and mitochondria. PON2 is present in cells from the vasculature relevant for the process of atherosclerosis: endothelial cells, smooth muscle cells, and adventitial fibroblasts (2).

The activity of PON1 was investigated in many pathological conditions, including cardio- and cerebrovascular diseases, preeclampsia, diabetes mellitus, metabolic syndrome (6,7).

**ENZYMATIC ACTIVITIES OF PONs**

The term “paraoxonases” is misleading, but it is still in use today because of its historical relevance. The organophosphate pesticide paraoxon was one of the first substrates used in the studies concerning these enzymes. To date, PONs are considered to have three major enzymatic activities: lactonase, paraoxonase, and arylesterase activities. The lactonase activity is regarded as being physiologically relevant and it is present in all the three members of the PONs’ family.

There is data that, besides the aforementioned activities, at least PON1 has also a lactonizing, as well as a peroxidase-like activity (8).

PONs act as lactonases, hydrolyzing a broad spectrum of lactones (9). Some physiologically relevant lactones include 5-hydroxy-icosatetraenoic acid 1,5-lactone, an oxidation product of arachidonic acid, N-acetyl-homoserine lactones, and homocysteine thiolactone.

N-(3-oxodecanoyl)-L-homoserine lactone, a key component of the quorum-sensing system used by Gram-negative bacteria like *Pseudomonas aeruginosa*, is a substrate for PON1 (10). Moreover, both in vitro and in vivo studies indicated that PON2 and PON3 offer protection against the infection with *Pseudomonas aeruginosa* by the inactivation of N-acetyl-homoserine lactone (11).

Homocysteine, a sulfur-containing amino acid, appears as an intermediate of the methionine metabolism and it is a risk factor for coronary artery disease (12). Its thiolactone results during the editing step of the protein synthesis when methionyl–tRNA synthetase incorporates homocysteine instead of methionine.

High levels of homocysteine are toxic for cells, in particular for vascular endothelial cells as they lack cystathionine β synthase, the enzyme involved in the catabolism of this compound.

The toxicity of homocysteine thiolactone arises from induction of oxidative stress as well as from the modification of different proteins through *N* - and *S*-homocysteinylination (13). As a consequence of the covalent modification proteins lose their biological properties. Among the targets of the processes of *N* - and *S*-homocysteinylation are low-density lipoprotein (LDL) and HDL apolipoproteins, albumin and the coagulation factors. Thus, homocysteine thiolactone is an important factor for the progression and development of atherosclerosis.

There is clear evidence that serum PON1 hydrolyzes homocysteine thiolactone, being thus a protective factor against atherosclerosis (14).

Also, PONs are able to hydrolyze different organophosphates (paraoxonase activity), as well as aromatic esters (arylesterase activity). PON1 has the highest paraoxonase activity, while PON3 lacks this type of enzymatic activity. Also, there are some differences with respect to the ability of these enzymes to hydrolyze the aromatic esters.

There are some studies which indicate that PONs are involved in drug metabolism. Thus, PON1 could be involved in the bioactivation of clopidogrel, while PON3 is able to hydrolyze lovastatin and spironolactone (9,15).
**PON1 – HDL RELATIONSHIP**

PON1 is a 45 kDa glycoprotein synthesized by the liver cells. It is secreted into circulation where it becomes associated mainly with HDL particles and to a lesser extent with very low-density lipoproteins (VLDL) particles and chylomicrons (16). After secretion from hepatocytes, PON1 becomes associated with HDL particles in a calcium-dependent manner and needs also apolipoproteins A-I (apoA-I) and J (apoJ).

PON1 is considered to play a major role in the control of oxidative stress and inflammatory response in the circulation and at the endothelial vascular wall.

The current concept is that HDL particles have an atheroprotective function due to (1) the reverse cholesterol transport, (2) the stimulation of cholesterol efflux from macrophages which limits the formation of foam cells, (3) antioxidative and anti-inflammatory properties, and (4) the normalisation of endothelial function (17). The HDL-associated proteome is responsible for the achievement of these functions. It consists of several proteins, including apoA-I, lecithin: cholesterol acyltransferase (LCAT), PON1, cholesteryl-ester transfer protein (CETP), phospholipid transfer protein, and lipoprotein-associated phospholipase A2 (17). Some of these proteins, including apoA-I and PON1, have direct antioxidant and anti-inflammatory functions.

PON1 protects both LDL and HDL from oxidative modifications through the catabolism of oxidized cholesteryl esters and phospholipids, like cholesteryl linoleate hydroperoxides, linoleic acid hydroperoxides and lipoprotein peroxides. The ability of PON1 to inactivate these oxidized lipids is due to a specific Cys residue (Cys284) (18). Some of these oxidized lipids, as well as some of their degradation products, have pro-inflammatory properties. They activate endothelial cells inducing the production of different adhesion molecules and chemokines (19). Expression of E- and P-selectins, vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) on the luminal surface of the endothelial cells it is one of the early stages in the atherosclerosis. On the other hand, chemokines like monocyte chemoattractant protein-1 (MCP-1) attracts monocytes into the vascular intima which is another hallmark of the early stages of atherosclerosis. Monocytes develop into macrophages which start to uptake oxidized LDL particles and become foam cells.

The antioxidant and anti-inflammatory properties of PON1 were demonstrated using PON1 KO mice (20). The absence of this enzyme from the HDL particles was associated with the dysfunction of the endothelial cells. There was an increased production of the superoxide anion by the aortic endothelial cells, as well as an increased expression of the adhesion molecules (20). On the other hand, PON1 was responsible for the decrease of lipid hydroperoxides concentration and a reduction of the MCP-1 synthesis (21).

Moreover, it was found that the loss of HDL-associated PON1 activity is associated with dysfunction of the endothelial nitric oxide synthase (eNOS) (22). This leads to further disregulation of the vascular endothelium.

In conclusion, PON1, through the aforementioned mechanisms, is able to reduce the degree of lipid oxidation in both LDL and HDL particles and to limit the progression of the atherosclerosis. Also, these data suggest that not only the amount of HDL is responsible for its antioxidant and anti-inflammatory properties, but also the composition of the HDL particles.

**FACTORS AFFECTING PON1**

Different studies indicated that, in a specific population, there is a variability of both PON1 catalytic activity (40-50 times) and concentration (13-15 times) (23). This degree of variability is a consequence of genetic as well as lifestyle and dietary factors (24).

There were identified seven major genetic polymorphisms which influence gene expression, protein synthesis or enzymatic activity of PON1 (25). Two of these polymorphisms are located into the coding region of PON1, while the other five are located into the promoter region.

The most investigated polymorphism is the one located at the position 192 in the coding region (Gln192Arg/Q192R) (24). This polymorphism gives rise to three different genotypes QQ, QR and RR respectively.

The aforementioned polymorphism influence the enzymatic activity of PON1 as well as its ability to associate with the HDL particles, but has almost no effect upon the serum concentration of the enzyme. It was found that the
HDL particles from individuals with the QQ genotype act more efficiently to protect the LDL particles against oxidative modifications (26). On the other hand, in the case of the QQ and QR genotypes it was found that PON1 has an up to three times lower affinity for the HDL particles (27). As a consequence, in individuals with QQ and QR genotypes PON1 has a lower stability, which leads to the increase of the “free” PON1 serum fraction (28). Moreover, this “free” fraction of PON1 raises in pathological conditions associated with an increased oxidative stress, like diabetes mellitus (29). Also, it is a less efficient antioxidant than the HDL-associated fraction of PON1 and has a lower antiatherogenic activity.

Finally, the Q192R influences the different enzymatic activities of PON1. Thus, individuals with the RR genotype have the highest arylesterase activity, while individuals with the QQ genotype have the highest lactonase activity.

A recent population-based cross-sectional study made on a Finnish population indicated that Q192R polymorphism was associated with enzyme’s activity (30). Moreover, a higher activity of PON1 was associated with a decreased level of oxidized lipids in LDL particles. The study detected no association between the PON1 activity and the level of oxidized lipids in HDL particles. These results are consistent with previous results from other studies.

The second most investigated genetic polymorphism of PON1 is L55M (Glu55Met) (24). This polymorphism gives rise to three different genotypes LL, LM and MM respectively.

A recent study indicated that both Q192R and L55M polymorphisms influence the response to statins in the case of patients with dyslipidemia (31). This study found that phenotypes QQ/QR and MM/ML were associated with increased chances of achieving the HDL cholesterol goal levels during statin therapy.

In conclusion, in the case of genetic factors, PON1’s status is influenced not only by the Q192R polymorphism, but also by all the other polymorphisms, as well as their interaction.

On the other hand, both the status and the enzymatic activity of PON1 are influenced by lifestyle habits as well as dietary factors.

It is well documented that antioxidant vitamins C and E, as well as the vitamin A and its provitamin β-carotene are associated with an increase of serum PON1 activity (24). In vivo studies using mice indicated that some polyphenols like quercetin and naringenin induce an increase of PON1 gene and protein expression in liver (29). Also, the polyphenols from the pomegranate juice (punicalagin, gallic acid, ellagic acid) were found to induce the expression of the liver PON1 gene (32,33).

The mechanisms through which polyphenols exert their beneficial effects upon PON1 status and activity are not completely understood. However, it was found that some of these compounds (quercetin, naringenin) are able to interact directly with the aryl hydrocarbon receptor (AhR) transcription factor (34). Also, β-carotene induces the expression of the PON1. On the other hand, it was found that the polyphenols from the pomegranate juice favour the association of the PON1 with the HDL particles (35).

The dietary fatty acids represent another important factor to influence the PON1 status. There are studies which indicate that polyunsaturated fatty acids like those from fish oil can affect negatively PON1 activity. This is a consequence of their susceptibility to peroxidative modifications. On the other hand, oleic acid supplementation has a positive effect upon PON1 status, as the oleic acid-containing glycerophospholipids from the HDL particles prevent the oxidative modifications of PON1.

In conclusion, further studies are warranted for a better understanding of the influence exerted by the dietary and genetic factors on PON1 serum activity. The aim is to find a way to preserve the activity of this enzyme in order to prevent the oxidative modifications of LDL and HDL particles. Also, there is a need to studies concerning the predictive value of the PON1 activities from a clinical point of view.

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