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Modulation of paraoxonase (PON1) activity

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Abstract

Paraoxonase 1 (PON1) is a serum enzyme closely associated with high density lipoprotein (HDL). PON1 hydrolyzes several organophosphorus compounds used as insecticides, as well as nerve agents; it metabolizes toxic oxidized lipids associated with both low density lipoprotein (LDL) and HDL; and it can hydrolyze a number of lactone-containing pharmaceutical compounds, inactivating some, while activating others. Serum PON1 activity in a given population can vary by 40-fold. Though most of this variation can be explained by polymorphisms in the coding region (Q192R) and the 5′ regulatory region (T-108C), modulation of PON1 by a variety of other factors should be taken into account, including other polymorphisms recently discovered but not yet characterized. This paper examines the major factors (environmental chemicals, drugs, smoking, alcohol, diet, age, disease conditions) that have been shown to modulate PON1 activity in either direction. As PON1 plays a protective role in organophosphate toxicity, and, because of its antioxidant capacity, in cardiovascular disease, a better understanding of how PON1 can be modulated by environmental factors has potential toxicological and clinical consequences.

1. Introduction

Paraoxonase (PON1) is a member of a family of proteins that also includes PON2 and PON3, the genes for which are clustered in tandem on the long arms of human chromosome 7 (q21.22). PON1 is synthesized primarily in the liver and a portion is secreted into the plasma, where it is associated with high density lipoproteins (HDL) (Fig. 1). PON1 received its name from paraoxon, the toxic metabolite of the insecticide parathion, which is one of its most studied substrates. PON1 hydrolyzes the active metabolites of several other organophosphorus insecticides (e.g., chlorpyrifos oxon, diazoxon), as well as nerve agents such as sarin, soman and VX [1–3]. One natural physiological function of PON1 appears to be the metabolism of toxic oxidized lipids of both low density lipoprotein (LDL) particles as well as HDL particles. Mackness et al. [3b] were the first to demonstrate that purified human PON1 could inhibit LDL oxidation in vitro. Other studies have confirmed and extended this finding, demonstrating that PON1 both prevents the formation of oxidized LDL and inactivates LDL-derived oxidized phospholipids once they are formed. PON1 also protects phospholipids in HDL from oxidation [2]. These actions suggest a role of PON1 in cardiovascular diseases and atherosclerosis. PON1 has also been shown to metabolize a number of drugs and pro-drugs via its lactonase activity [3]. PON2 and PON3 lack paraoxonase or arylesterase activities but are similar to PON1 in that both hydrolyze aromatic and long-chain aliphatic lactones [3]. PON3 in particular hydrolyzes widely used drugs such as the statin lactones lovastatin and simvastatin and the diuretic spironolactone [3]. Both PON2 and PON3 have antioxidant properties; while PON3, similarly to PON1, is predominantly expressed in the liver and is associated with HDL, PON2 is more widely distributed [3c,3d]. This commentary will focus on the modulation of PON1, so far the most studied of the three PONs.

2. PON1 polymorphisms

More than 160 polymorphisms have been described to date in PON1, some in the coding regions and others in

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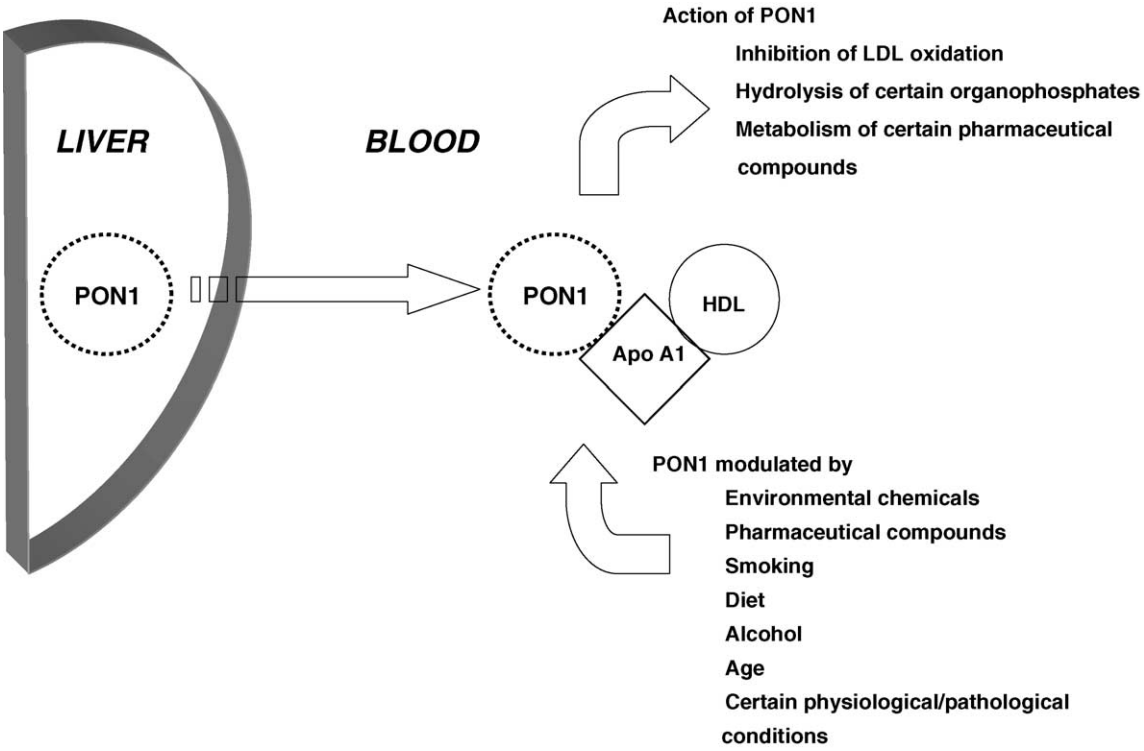

Fig. 1. Biological effects and modulation of PON1.

introns and regulatory regions of the gene. Earlier studies had indicated that the plasma paraoxonase activity in human populations exhibited a polymorphic distribution, and individuals with high, intermediate or low paraoxonase activity could be identified [4]. Gene frequencies for high or low metabolizers vary among groups of different ethnic or geographical origin [1]. The molecular basis of the paraoxonase activity polymorphisms is a missence mutation in the coding region of PON1, resulting in a glutamine (Q)/arginine (R) substitution at codon 192 [5]. The $PON1_{Q192}$ alloform hydrolyzes paraoxon much less efficiently than does $PON1_{R192}$, while the opposite is true in case of soman or sarin [6]. $PON1_{Q192}$ is also more efficient at metabolizing oxidized HDL or LDL than $PON1_{R192}$ [7].

Another coding region polymorphism, resulting in aminoacid substitution at position 55 [Leu(L)/Met(M)], has been associated with plasma PON1 protein levels, with $PON1_{M55}$ being associated with low plasma PON1; however, this appears to primarily result from linkage disequilibrium with the low efficiency –108T allele of the C-108 promoter region polymorphism. Of the five polymorphisms characterized in the promoter region, the C-108T substitution has the most significant effect on plasma PON1 levels, with the –108C allele providing levels of PON1 about twice as high as those seen with the –108T allele [8]. The other polymorphisms recently identified have for the most part not been yet characterized, but may affect splicing efficiency, message stability or efficiency of polyadenylation.

3. The importance of determining PON1 status

Most studies investigating the association of PON1 polymorphisms with diseases have examined only the nucleotide polymorphisms (Q192R, L55M, C-108T) with PCR-based assays. However, even if an individual were genotyped for all known PON1 polymorphisms, this analysis would not provide the level of plasma PON1 activity nor the phase of polymorphisms (i.e., which polymorphisms are on each of an individual's two chromosomes). A functional genomic analysis provides a much more informative approach, as measurement of an individual's PON1 function (plasma activity) takes into account all polymorphisms that might affect activity. This is accomplished through the use of a high-throughput enzyme assay involving two PON1 substrates (usually diazoxon and paraoxon) [9]. This approach, in addition to providing a functional assessment of the plasma $PON1_{192}$ alloforms, also provides the plasma level of PON1 for each individual, thus encompassing the two factors that affect PON1 levels or activity (position 192 amino acid and plasma alloform levels). This approach has been referred to as the determination of PON1 'status' for an individual [9]. Measurement of PON1 status, coupled with PCR analysis of codon 192, has been shown to detect genotype/activity discrepancies, that can be explained by the presence of recently discovered mutations in the PON1 gene [10].

Fundamental biochemical considerations dictate that it is the catalytic efficiency with which PON1 degrades toxic organophosphates and metabolizes oxidized lipids that

determines the degree of protection provided by PON1 against insults from physiological or xenobiotic toxins. In addition, higher concentrations of PON1 provide better protection. Thus, for adequate risk assessment it is important to know PON1 levels and activity. It should be noted that in a given population, plasma PON1 activity can vary up to 40-fold [4,6,9], and differences in PON1 protein levels up to 13-fold are also present within a single $PON1_{192}$ genotype [2]. Recent studies investigating the role of PON1 in cardiovascular disease have indeed provided evidence that PON1 status (encompassing genotype and activity levels) is a much better predictor of disease than PON1 genotype alone [11].

The importance of PON1 status in determining susceptibility or protection from toxicity or diseases points to the relevance of factors affecting PON1 activity and levels of expression. Though genetic determinants, such as the polymorphisms discussed above, play indeed a primary role in determining an individual's PON1 status, the contribution of other factors in modulating PON1 activity and levels may also be important [12,13]. This commentary will address the relevance of environmental, pharmacological, and life-style and dietary factors in modulating PON1, as well as the influence of age and certain disease conditions.

4. Modulation of PON1 by exogenous compounds

4.1. Environmental chemicals

PON1 activity is completely dependent upon calcium, and EDTA irreversibly abolishes its activity. Other cations, however, have been shown to have an inhibitory effect on PON1 activity. Barium, lanthanum, copper, zinc and mercurials were found to inhibit PON1 activity from rat or human liver [14]. Similar results were obtained in a subsequent study in human serum from $PON1_{Q192}$ individuals, where an inhibitory effect of manganese, cobalt, cadmium and nickel was also identified [15]. In case of mercurials and copper, further biochemical studies suggested that a free thiol group on the Cys285 residue may be the molecular target [14,15]. More recent experiments have shown that cadmium, iron, zinc and mercury are the most potent in vitro inhibitors of $PON1_{R192}$ activity, with concentrations as low as 100 nM causing greater than 80% inhibition. Overall, $PON1_{Q192}$ appears to be somewhat less sensitive to inhibition by metals, with the exception of lead [16]. Despite this robust inhibition of PON1 in vitro, in vivo exposures of mice to cadmium, methylmercury or dietary iron, leading to metal serum concentrations of greater than 1 μM, failed to alter PON1 activity in plasma and liver [16]. Thus, the inhibitory effects observed in vitro were not evident after in vivo exposure, probably because metal-binding proteins in plasma are sufficient to protect PON1 against inhibition by these metals.

Mice exposed to dichloroacetic acid, a major by-product of water disinfection by chlorination which exhibits hepatocarcinogenic effects in rodents (2 g/L in drinking water for four weeks), had a 50% lower level of PON1 mRNA in the liver [17]. Administration of the hepatotoxicant carbon tetrachloride to rats caused liver cirrhosis and >80% decrease of hepatic PON1 [18]. The authors suggest that liver PON1 may play a protective role against free radical production in the hepatic organelles. A recent study also reported a decreased level of paraoxonase and arylesterase activities in radiology workers exposed for more than five years to ionizing radiation [19].

4.2. Drugs

Most studies on the modulation of PON1 by pharmaceutical compounds have focused on lipid-lowering compounds. Such studies with statins and fibrates have yielded somewhat conflicting results. In vitro exposure of HuH7 human hepatoma cells to pravastatin, simvastatin and fluvastatin (10–100 μM) caused a 25–50% decrease in PON1 activity in the culture medium and a similar decrease in PON1 mRNA; both effects were reversed by mevalonate [20]. In the same cells, fenofibric acid (250 μM) caused a 50 and 30% increase in PON1 activity and mRNA, respectively [20]. Fenofibric acid was found to induce PON1 gene-promoter activity, while statins had an opposite effect [20]. The latter finding is in contrast with results obtained in hepatic human HepG2 cells, where simvastatin (1.5–25 μg/ml) was found to upregulate PON1 promoter activity [21]. In another in vitro study on isolated lipoproteins, two oxidized metabolites of atorvastatin (5–50 μM) and a metabolite of gemfibrozil (2–80 μM), but not the parent compounds, were found to increase HDL-associated PON1 activity [22]. A study in rats indicated that fluvastatin (20 mg/kg/day for 3 weeks) reduced both plasma and liver PON1 activity, while a lower dose (2 mg/kg/day) was only effective toward liver activity. Pravastatin (4 or 40 mg/kg/day for 3 weeks), on the other hand, was devoid of significant effects [23]. Studies in humans have provided similar contrasting results. An increase in serum-PON1 activity was found in patients treated with simvastatin and other statins, gemfibrozil and fenofibrate [21,24–26]. On the other hand, no changes in serum PON1 activity were reported by other studies in patients treated with ciprofibrate [27], bezafibrate and gemfibrozil [28].

The cholinergic muscarinic antagonist atropine was shown to inhibit human plasma and pig liver PON1 in vitro, with a K_i of 0.25 mM, well above the pharmacological levels of this compound [29]. In a cohort of aspirin users, a significant increase of plasma PON1 activity and concentration was reported [30]. Such effect may be due to the anti-inflammatory effect of aspirin, as serum PON1 levels are reduced during the inflammatory response in animal models; alternatively, aspirin may act as an anti-

oxidant [30]. Finally, the anti-inflammatory glucocorticoid dexamethasone (1 mM) caused an eight fold increase in PON1 mRNA in a mouse hepatoma cell line (Hepa cells), as well as in mice in vivo [31].

4.3. Classical inducers

A few studies have investigated whether PON1 is an inducible enzyme. Phenobarbital, a classical enzyme inducer which is particularly effective toward certain isozymes of cytochrome P450 (e.g., CYP2B), caused a modest (20–150%) increase in hepatic PON1 activity [1,32–36], with a concomitant increase in liver RNA levels [1]. However, serum PON1 activity was decreased (by 40–50%) by phenobarbital treatment [1,32,33,35]. β-Naphtoflavone, an inducer of CYP1A, did not change serum or liver PON1 [1,32,33], nor did 3-methylcholantrene in mice [1]. In rats, however, 3-methylcholanthrene was associated with increased serum and liver PON1 activity [37]. An earlier study by Main [38] indicated that administration of the organochlorine insecticide aldrin, an inducer of hepatic microsomal enzymes, caused a 50% increase in A-esterase activity in the liver, and an equal decrease in serum, similar to what was observed with phenobarbital. Overall, these findings suggest that PON1 is not sensitive to modulation by classical enzyme inducers. Rather, the observed result may be explained by the ability of phenobarbital to impair, by some unknown mechanism, secretion of PON1 from the liver to the circulation [35].

5. Modulation of PON1 by life-style factors

5.1. Smoking

Cigarette smoke extract was found to inhibit human plasma PON1 activity [39]. This effect was not reversed by antioxidants such as Vitamin E, Vitamin C, superoxide dismutase and catalase, but was antagonized by GSH, *N*-acetylcysteine, 2-mercaptoethanol, DTT and L-cysteine, suggesting that free thiols are central to the inhibitory effect. Compounds suggested to be responsible for inhibition of PON1 activity are various reactive aldehydes (acetaldehyde, formaldehyde and α,β-unsaturated aldehydes, such as acrolein and crotonaldehyde), as well as aromatic hydrocarbons [39]. Four studies in humans have confirmed that smoking is associated with reduced serum PON1 activity [13,24,40,41]. The effect appears to reverse within a relatively short time (3–24 months) [40], suggesting a direct effect of cigarette smoke on PON1 activity, as indicated by the in vitro studies [39].

5.2. Alcohol

The notion that moderate doses of ethanol exert a protective role in cardiovascular disease (a phenomenon often referred to as the 'French paradox'), has prompted studies on the possible modulation of PON1 by alcohol. Though an earlier in vitro study had indicated that human plasma PON1 was inhibited by ethanol (IC_{50} = ~100 mM) and other aliphatic alcohols [42], observations in animals and humans have shown that lower levels of ethanol increase PON1 activity and levels. Two studies in healthy middle-aged men and postmenopausal women showed an increase of plasma PON1 activity following moderate alcohol consumption as wine, beer or spirits [43,44]. In another study, light drinkers had a 395% higher, whereas heavy drinkers had a 45% lower serum plasma PON1 activity compared to non-drinkers [45]. Similarly, rats fed low doses of ethanol (resulting in a blood alcohol concentration of 39 mg/dl) showed a 20–25% increase in serum and liver PON1 activity and a 59% increase in liver PON1 mRNA, while higher doses of ethanol (BAC = 140 mg/dl) caused a 25 and 51% decrease in serum and liver PON1 activity and liver PON1 mRNA levels, respectively [45]. PON1 polymorphisms do not appear to be a factor in PON1 modulation by light and heavy drinking, and the regulation of PON1 expression may be related to effects of alcohol on protein kinase C, which may be involved in the phosphorylation of an Sp1 binding site in the promoter region of PON1 [45]. It should also be noted that no association between alcohol consumption and serum PON1 activity were found in other human studies [13,46,47].

6. Modulation of PON1 activity by diet

6.1. Fat-rich diet

Mice of the B6 strain fed an atherogenic diet (15.75% fat, 1.25% cholesterol) for 3 months showed an approximately 60% decrease in serum PON1 activity and a similar reduction in liver PON1 mRNA levels [48]. On the other hand, in CH3 mice, both parameters were slightly increased, suggesting that even in mice genetic factors contribute to PON1 gene expression. Indeed, hepatic PON1 mRNA levels were 50% lower in control CH3 mice compared to B6 mice [48]. Feeding of both normal New Zealand white rabbits and rabbits transgenic for human Apo A-I with an atherogenic diet (0.5% cholesterol) for 14 weeks, resulted in a 50% decrease in serum PON1 activity, with a partial recovery following 16 weeks of normal chow diet [49]. After consumption of a meal rich in used cooking fat, by a group of 12 healthy men, serum PON1 activity decreased by 27% for up to 8 h, but returned to normal values by 12 h [50]. Replacement of dietary saturated fat with *trans* fat was found to reduce serum PON1 activity in healthy men and women by about 6% [51].

Additional studies, in rats and humans, attempted to further address the issue of PON1 modulation by dietary fats. Groups of rats were fed control diet supplemented

with either triolein, tripalmitin or fish oil. The triolein diet significantly increased (by 46%) plasma PON1 activity, while fish oil caused a significant decrease (−39%) in PON1 activity. The tripalmitin-enriched diet had no effect [52]. These results suggest that the fatty acid composition of phospholipids may affect PON1 activity. These animal findings were in part confirmed in a human study, in which 14 individuals with type 2 diabetes received meals rich in thermally stressed olive oil or safflower oil [53]. Only the olive oil meal increased serum PON1 activity, and the effect was most pronounced in women [53]. Consumption of oleic acid from olive oil, calculated from a 12-h recall questionnaire, was found to increase serum PON1 activity in a group of 654 men, but only in those with the PON1192RR genotype [54]. An explanation for such effects may be found in the result of a recent in vitro study investigating the effects of lipids on PON1 [55]. Polyenoic fatty acids inhibited PON1 activity, with linoleic acid being the most potent. On the other hand, monoenoic acids, and in particular oleic acid, were greatly effective in protecting PON1 from oxidative inactivation. Oleic acid was also effective in stabilizing PON1 [55]. Finally, administration of an ω-3 polyunsaturated fatty acid concentrate to a group of 14 patients with familial combined hyperlipidemia for eight weeks, resulted in a modest (10%) but significant increase in plasma PON1 concentration [56].

6.2. Antioxidants

Various in vitro and in vivo studies in animals and humans have provided initial evidence that antioxidants can increase PON1 activity, possibly by protecting the enzyme from oxidative stress-induced inactivation. The antioxidant flavonoids quercetin and glabridin protected PON1 in micellar solution (isolated from other HDL components) from a loss of activity due to copper-induced oxidation [57]. Similarly, serum PON1 levels in Apo-E deficient mice fed quercetin (in alcohol) or red wine, increased by 113 and 75%, respectively, compared to ethanol-fed controls [58]. Administration of pomegranate juice, which is rich in flavonoids with antioxidant activity, to Apo-E deficient mice, was shown to increase serum PON1 activity by 26–43% [59]. Similarly, pomegranate juice consumption by a group of 13 healthy men resulted in a 20% increase in serum PON1 activity [60]. A study in 189 white men from the Pacific Northwest region of the United States found a positive correlation between the dietary and medicinal intakes of Vitamins C and E and serum PON1 activity [24]. However, two earlier studies in Finnish populations reported that high intake of vegetables, possibly rich in Vitamins E and C, was negatively correlated with serum PON1 activity [61,62a]. No associations between Vitamins C and E and β-carotene intake and serum PON1 levels was reported in yet another study in 388 men and women in Catalonia, Spain [13]. Clearly, the role of antioxidants in the diet as modulators of PON1 activity requires further investigations. Of interest is also the recent observation that oxidative stress increases macrophage PON2 while decreasing cellular PON3 activity [62b].

7. Effects of age and gender on PON1 activity

7.1. PON1 during development

Age is a major determinant of PON1 activity. Studies in rodents have shown that serum and liver PON1 activity is very low at birth and increases up to postnatal day 21, with a parallel increase in liver mRNA [63,64]. A similar increase was also seen in transgenic mice expressing either the human $PON1_{R192}$ or the $PON1_{Q192}$ alloforms [65]. Studies in humans have also shown that serum PON1 activity is very low at birth and increases over time, reaching a plateau between 6 and 15 months of age [4,65–67]. PON1 activity in the fetus may be even lower, as suggested by data indicating a 24% lower activity in premature babies (33–36 weeks of gestation) compared to term babies [67]. Low PON1 activity during development could represent a relevant risk factor for increased susceptibility to the toxicity of certain organophosphorus insecticides, as indicated by several animal studies [64,68,69].

7.2. PON1 and aging

PON1 activity is quite constant over time, once it reaches adult values. In rats, no differences were found in plasma and liver PON1 activity between 3- and 24-month-old animals [69]. Earlier studies had suggested that this holds true for humans as well [4,70]. However, more recent investigations have reported a progressive decrease in PON1 activity in elderly subjects [24,71,72]. In one study, such decline of PON1 activity levels with advancing age was found in myocardial infection patients with the 192QQ genotype [73]. This decline in PON1 activity may be related to the development of oxidative stress conditions with aging, and would have an impact on the increased incidence of atherosclerosis with age [72].

7.3. Effect of gender on PON1 activity

A study examining serum PON1 activity in different strains of mice reported that female animals had a 14–26% higher activity than males [74]. This finding was expanded by Ali et al. [31], who reported that hepatic PON1 mRNA levels in female C57BL/6J mice were 40% higher than in males. Gonadectomy resulted in an increase of PON1 mRNA in males but not in female animals. This gender dichotomy in PON1 levels manifests only within inbred strains of mice, and is readily obscured in inter-strain comparison [74]. The genetic heterogeneity in humans would obscure any gender effects on PON1 activity

[31], and indeed a slight higher mean value of serum PON1 activity in females has been found in very few studies [4].

8. PON1 in certain physiological and pathological conditions

PON1 activity can vary depending on different physiological conditions or pathological states. Pregnancy has been associated with lower PON1 activity in both rats and humans [75]. Moderate exercise was found to increase serum PON1 activity by 14%; in smokers, who have lower serum PON1 activity, physical activity 'restores' PON1 activity to the level found in nonsmokers [41]. However, in another study, no association between physical exercise and serum PON1 activity was found [13].

Plasma PON1 activity has been found to be altered, usually decreased, in a number of pathological conditions and diseases. The finding that serum PON1 activity decreased over time, by 36%, in streptozotocin-treated diabetic rats [76], has spurred several investigations on the levels of plasma PON1 activity in diabetic patients. Both insulin-dependent (type 1) and non-insulin-dependent (type 2) diabetes have been consistently associated with lower serum PON1 activity [77–79]. This low PON1 activity is independent of genotype, and may be due to altered glycation of HDL and/or PON1, lower rate of synthesis or higher rate of catabolism, or increased oxidative stress [77,79]. Postmenopausal women with type 2 diabetes had a lower serum PON1 activity than healthy postmenopausal women; hormone replacement therapy with conjugated equine estrogen and medroxyprogesterone acetate in the former group, increased their plasma PON1 activity to the levels observed in healthy women [80].

Decreased serum PON1 activity has also been found in patients with chronic renal failure undergoing hemodyalisis [81], in patients with rheumatoid arthritis [82], and in patients with Fish-Eye disease (characterized by severe corneal opacities) [83]. Hyperthyroidism was also associated with lower (−40%) serum PON1 activity; after treatment with methimazole, patients who became euthyroid had only a 14% lower serum PON1 activity than controls [84]. Patients with Alzheimer's disease and vascular dementia also displayed lower (30–40%) serum PON1 activity [85]. Following up their observations that the hepatotoxicant carbon tetrachloride caused a decrease of liver PON1 in rats [18], the same authors found a decreased serum PON1 activity in patients with chronic liver disease (chronic hepatitis and cirrhosis), which was related to the degree of liver damage [86]. They suggest that measurement of serum PON1 activity may be added to the battery of the standard biochemical liver function tests.

Animal studies have indicated that PON1 activity can be altered during the acute phase response. Administration of lipopolysaccharide (LPS), which mimics gram negative infections, to mice, caused a 50% decrease of serum and liver PON1 activity and a similar change in hepatic mRNA levels; this decrease was transient, as PON1 returned to control values within 48 h [1]. A similar study in Syrian hamster found a 67% decrease in serum PON1 activity and an 80% decrease of liver PON1 mRNA, with a partial recovery at 48 h following LPS administration [87]. In contrast, Bog-Hansen et al. [88] had reported an increase of serum A-esterase following LPS administration in mice, though this was inferred from an immunoelectrophoretic analysis and not by direct enzymatic measurements. A recent study in C57BL/6J mice found that LPS caused a 50% decrease of liver PON1 mRNA at 24 h, with a full recovery by 48 h, as shown by Costa et al. [1]. However, in female mice of the same strain, LPS caused a small increase in PON1 mRNA [31]. In neither gender was there a change in IL-2, TNF-α or IL-6 expression after LPS administration [31]. However, these same proinflammatory cytokines have been shown to upregulate (interleukin-6), or down regulate (interleukin-1β and tumor necrosis factor-α) PON1 gene expression in HepG2 human hepatoma cells [89].

9. Possible mechanisms of PON1 modulation

It is evident that a variety of factors can influence PON1 activity and/or concentration. In most studies described above, PON1 activity was measured with paraoxon or phenylacetate as a substrate, while diazoxon or chlorpyrifos oxon were used in very few occasions. Because of the Q192R polymorphism that affects the catalytic efficiency toward paraoxon and chlorpyrifos oxon, use of phenylacetate or diazoxon as substrates provides a better indication of overall PON1 activity levels. Direct measurement of PON1 protein with a PON1 antibody also provides an indication of PON1 concentration. These considerations are important, in order to correctly interpret the result of most human studies. For example, with the knowledge that low PON1 is a risk factor for cardiovascular disease, it may be surprising that a population (in Belfast) with a three fold greater incidence of coronary heart disease than in Toulose, would display higher PON1 paraoxonase activity [90]. However, since $PON1_{R192}$ metabolizes paraoxon much more efficiently than $PON1_{Q192}$, the results are consistent with the finding of a greater prevalence of the R allele in the Belfast population (R is considered a risk factor for cardiovascular disease). On the other hand, serum PON1 concentration was significantly lower in Belfast than in Toulose, as one would have predicted [90].

In some cases, the modulation of PON1 activity appears to be the result of a direct interaction with the protein, as in case of metals or smoking extracts. Thus, zinc or nickel may bind to histidine (His) -115, -134, -155 and -243 that are essential aminoacid for the esterase activity of PON1 [91]. Other metals (e.g., mercury) or smoking extract constituents may interact with cysteine (Cys) residues

on PON1. PON1 contains three Cys residues in positions 42, 284 and 353, with a disulfide bond between Cys-42 and -353 and a Cys-284 as a free thiol [91]. The disulfide-linked Cys-42 and Cys-353 are essential for PON1 hydrolase activity [91], while the Cys-284 is not, though selective covalent modification of this residue leads to inhibition of esterase activity, suggesting that Cys-284 is in or close to the active site [92]. The free Cys-284 residue is necessary for PON1 to be protective against LDL oxidation [91,93]. A calcium-binding site is also most relevant, as removal of calcium ions from PON1 by the chelating agents EGTA or EDTA leads to irreversible inactivation of PON1 esterase activity [94]. However, calcium is not required for PON1 protection against LDL oxidation [93]. Oxidation of PON1 occurs as a consequence of its interaction with oxidized lipids and/or with other oxidants (e.g., hydrogen peroxide) [93,95]. Thus, certain antioxidants may increase PON1 activity by preventing its oxidative inactivation [57]. Further understanding of the molecular mechanisms underlying the consequences of direct interactions with the PON1 protein on activity will be facilitated by the recent elucidation of a crystal structure for a recombinant PON1 [96].

Various factors have been shown to modulate PON1 concentration and mRNA levels, suggesting an interaction with elements of the PON1 promoter. This appears to be the case with lipid-lowering drugs and alcohol. In the 5′ regulatory region, a GGCGGG consensus sequence of the binding site for the transcription factor Sp1, has been shown to be the site of the C-108T mutation which affects PON1 levels of expression [8]. This site has been shown to be important in upregulation of PON1 by statins, and a possible target for ethanol [45]. In addition, a sequence in the promoter region containing the binding site for the transcription factor sterol regulatory element-binding protein-2 (SREBP-2), has been also shown to be the target for upregulation of PON1 by simvastatin [21]. An interaction between SREBP-2 and Sp-1 in binding to the promoter has also been reported [21]. Recently, in a human hepatoma cell line, the dietary polyphenol quercetin, as well as 3-methycholanthrene, were shown to increase PON1 activity and mRNA levels in an aryl hydrocarbon receptor-dependent manner, via activation of a xenobiotic responsive element-like sequence within the PON1 promoter region [97].

10. Conclusions and research needs

Investigations over the past 20 years have led to a better understanding of the role of PON1 in metabolizing organophosphorus insecticides, oxidized lipids and pharmaceutical drugs [1–3]. The characterization of major coding region and promoter region polymorphisms that affect the enzyme's catalytic abilities and levels of expression has also helped elucidating the role of genetically-determined variations of PON1 in conferring susceptibility or protection toward toxic agents or certain diseases. More recent studies have underlined the importance of determining both PON1 activity and functional alloform phenotype, over straight genotype, as opposed to analyzing any number of PON1 SNPs, when inferring associations between PON1 and disease. There is a 40-fold variation in serum PON1 activity among individuals; while a portion of this variation is explained by polymorphisms in positions 192 and −108, and possibly by several other polymorphisms yet to be characterized, modulation of PON1 by exogenous or endogenous factors also needs to be taken into account. Interest for nutritional and pharmacological positive modulators of PON1, as well as for agents that may negatively affect PON1, thereby increasing susceptibility to toxicity and/or disease, has grown substantially over the past several years; however, many issues and questions still remain. Future studies should elucidate the molecular mechanisms by which chemicals may modulate PON1 activity and expression, in order to identify new potential toxicants and, perhaps more importantly, new protective factors. Based on current findings, it appears that certain pharmacological or nutritional intervention may increase PON1 activity, but only to a limited degree. As more is learned on the molecular aspects of PON1 regulation, particularly at the promoter level, more targeted approaches would become apparent [12,97]. Substantial progress would, however, probably derive from genetic engineering approaches. The recent expression of active human PON1 in *Escherichia coli* provides the necessary breakthrough for producing recombinant variants that have catalytic efficiency sufficient for therapeutic applications [1]. Protection against organophosphorus insecticide and nerve agent poisoning and reduction of risk of cardiovascular disease in individuals that have low PON1 activity represent examples of future utilizations of this enzyme.

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