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Mini review:

HUMAN PARAOXONASE 2

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ABSTRACT

Human paraoxonase 2 (PON2), which is a member of the paraoxonase family, possesses unique properties that distinguish it from PON1 and PON3. PON2 is ubiquitously expressed in many different tissue types and is highly expressed in the vital organs, such as the heart and lungs. Early research revealed that PON2 is exclusively intracellularly found, wherein it functions as an anti-oxidative protein by reducing intracellular and local oxidative stress. Studies in the last five years have demonstrated that PON2 protects against atherosclerosis by preventing low-density lipoprotein (LDL) oxidation, reversing the oxidation of mildly oxidised LDL, inhibiting monocyte chemotaxis, and increasing cholesterol efflux. Recently, emerging evidence has proposed that PON2 is an anti-atherosclerotic and may be associated with cardiovascular disease (CVD). The number of investigations concerning the relationship between two common *PON2* polymorphisms and CVD among different ethnic groups and regions is rapidly growing. Here, we briefly review the developments in PON2 research by focusing on past and recent findings.

Keywords: antioxidant, atherosclerosis, paraoxonase 2, oxidative stress

INTRODUCTION

The paraoxonase (PON) gene family consists of three highly conserved genes (PON1, PON2, and PON3) that are located in a cluster on chromosome 7q21.3-22.1 and share a high degree of identity (La Du 1996; Primo-Parmo et al., 1996). Studies in the last five years have focused on PON2 because of its unique expression and localisation. In contrast to PON1 and PON3, which are exclusively found in the liver and are associated with HDL in the blood stream following secretion (Mackness et al., 1996), PON2 is an intracellular protein that is widely expressed in many tissues, including the liver, kidney, lung, heart, placenta, testis, stomach, spleen, pancreas, small intestine, skeletal muscle, artery wall cell, and macrophages (Ng et al., 2001, Levy et al., 2007). Although PON2 has a similar structure to that of PON1 based on high amino acid sequence homology, PON2 possesses biological functions that are distinct from PON1. PON2 cannot hydrolyse organophosphates, such as paraoxon, but, instead,

possesses hydrolase and lactonase activities (Draganov et al., 2005). Due to its anti-oxidative and anti-atherosclerotic properties, which are similar to those of PON1, PON2 has become the subject of intense investigation. Both in vivo and in vitro models have revealed that PON2 prevents low-density lipoprotein (LDL) oxidation, reverses the oxidation of mildly oxidised LDL, inhibits oxidised LDL-induced monocyte chemotaxis (Ng et al., 2001), increases cholesterol efflux (Ng et al., 2006a), and decreases the size of atherosclerotic lesions (Ng et al., 2006a, b). Furthermore, the anti-apoptotic capability of PON2 has been found to play a role in atherosclerotic protection (Horke et al., 2007).

The human PON2 gene has two common polymorphisms, which result in amino acid substitutions of an alanine (A) or glycine (G) at codon 148 (A148G) and a cysteine (C) or serine (S) at codon 311 (C311S) (Mochizuki et al., 1998). As with PON1, inconsistent data have obfuscated the relationship between these two PON2 polymorphisms and numerous pathophysiological conditions.

PON2 expression

The human PON2 gene is located on the long arm of chromosome 7q21.3 and is adjacent to PON gene family members PON1 and PON3 (Primo-Parmo et al., 1996). Based on a phylogenetic analysis, PON2 is the oldest member of this gene family (Hassett et al., 1991; Draganov and La Du, 2004). Approximately 70% and 65% of PON2 nucleotide and amino acid sequences, respectively, are similar to those of PON1 and PON3 (Primo-Parmo et al., 1996). PON2 consists of nine exons that encode a 355-amino-acid protein of approximately 43 kDa in size. The PON2 gene contains numerous transcription start sites and may be alternatively spliced, resulting in several mRNA forms of PON2 (Mochizuki et al., 1998); however, data regarding the cell types that express these various PON2 isoforms are lacking. Until now, only two PON2 protein isoforms of 40 and 43 kDa in size have been commonly observed in a variety of cell types via immunoblotting with a specific anti-PON2 antibody. These two isoforms are believed to be non-glycosylated and glycosylated, respectively, although the different functions of these isoforms have not been reported (Horke et al., 2007).

The expression profile of PON2 demonstrates two unique differences in comparison to those of PON1 and PON3. PON2 is widely expressed in many different tissues, and it was first detected in the brain, liver, kidney, and testis (Mochizuki et al., 1998). Ng and colleagues have detected higher levels of PON2 transcripts in the heart, lung, liver, placenta, and testis in comparison to other examined tissue types. PON2 expression has also been observed in primary and immortalised human endothelial cells and in human arterial smooth muscle cells (Ng et al., 2001); however, the subcellular localisation of PON2 remains ambiguous. Although early studies have claimed that PON2 is an intracellular protein, evidence obtained via confocal microscopy, biochemical cell fractionation, and the digestion of outer membrane proteins has revealed that PON2 is located in the nuclear envelope and the endoplasmic reticulum (ER) of EA.hy 926 cells (Horke et al., 2007); however, PON2 mRNA and protein has recently been detected in the human gastrointestinal tract with decreasing expression levels from the upper to the lower levels of the tract (Levy et al., 2007). Moreover, PON2 localisation to the ER and release from the brush-border membrane in Caco-2 and HT-29 cells have been observed (Shamir et al., 2005; Rothem et al., 2007). These inconsistent findings imply that the cell type in which PON2 is expressed may influence the localisation of PON2 protein; Caco-2 and HT-29 cells are human adenocarcinomas of the colon, whereas EA.hy 926 cells are human vascular endothelial cells.

Modulation of PON2

Most studies concerning the induction of PON2 expression by various stimuli have focused on oxidative stress because PON2 plays a role as an intracellular antioxidant. Both in vitro and in vivo studies have demonstrated that PON2 expression and enzymatic activity increase during oxidative stress. PON2 is up-regulated in response to oxidative stress in different cell types (HepG2 cells and macrophages), animal models (mice fed high fat diets and apoE knockout mice), and in hypercholesterolemic patients (Shih et al., 1996, 1998; Forte et al., 2002; Rosenblat et al., 2003, 2004). Mouse peritoneal macrophages (MPMs) that were treated with various agents that induce oxidative stress have demonstrated increased PON2 expression and lactonase activity (Rosenblat et al., 2003). Shiner et al. have observed an approximate seven-fold increase in PON2 expression during monocyte/macrophage differentiation that was dependent on the presence of nicotinamide adenine dinucleotide phosphate (NADPH), and this phenomenon was observed to correlate with an increase in cellular oxidative stress (Shiner et al., 2004). In addition, PON2 has been found to be inactivated during low levels of oxidative stress. Therefore, high levels of oxidative stress may induce a cellular compensatory mechanism that up-regulates PON2 expression in macrophages (Shiner et al., 2006).

PON2 expression has also been investigated in the context of common pathological conditions, such as metabolic disorders that are characterised by an increase in oxidative stress due to high cholesterol or glucose levels. An in vivo chronic exposure of mice to high-cholesterol has been observed to cause an increase in hepatic PON2 mRNA levels (Forte et al., 2002). Studies in J774A.1 mouse macrophages have provided similar results. Lysophosphatidylcholine (LPC) has been observed to increase PON2 lactonase activity in J774A.1 cells (Rosenblat et al., 2006). In another in vivo study, human monocyte-derived macrophages (HMDMs) from hypercholesterolemic patients, which were subjected to oxidative stress, were observed to demonstrate less than half of the PON2 expression levels that were detected in HMDMs that had been isolated from control patients (Rosenblat et al., 2004). A similar result was obtained in diabetic mice, which demonstrated that the up-regulation of cellular PON2 in macrophages was associated with an increase in oxidative stress (Hayek et al., 2007). Moreover, increased PON2 expression has also been observed in response to pharmaceutical compounds, such as the lipid lowering agent atorvastatin and the anti-diabetic drug rosiglitazone, among other agents. Atorvastatin or rosiglitazone can up-regulate PON2 expression, and elevated PON2 activity has been observed to result in the reduction of cellular oxidative stress in patient HMDMs and mouse macrophages, respectively (Rosenblat et al., 2004; Shiner et al., 2007b). In contrast, proinflammatory agents, such as lipopolysaccharide (LPS), have been found to decrease PON2 expression in Caco-2/15 cells and in the human intestine (Precourt et al., 2009).

Studies investigating dietary components that modulate PON2 expression are currently underway. Pomegranate juice (PJ) and extracts from different parts of the plant significantly increase PON2 expression and lactonase activity in J774A.1 cells and in MPMs that have been isolated from atherosclerotic apolipoprotein E-deficient mice (Shiner et al., 2007a; Aviram et al., 2008). Supplementation with quercetin or the methylated quercetin derivative isohamnetin has been found to up-regulate PON2 mRNA and protein levels in the RAW264.7 murine macrophage cell line; however, studies in humans have provided contrasting results. Quercetin supplementation in human volunteers had no affect on PON2 mRNA levels in human monocytes (Boesch-Saadatmandi et al., 2009).

Elucidation of the mechanism that is responsible for the modulation of PON2 requires further investigation, although recent findings have demonstrated that unfolded

proteins in the endoplasmic reticulum (ER) induce PON2 expression at both the promoter and protein levels in the three assessed cell lines (human umbilical vein endothelial cell-derived EA.hy 926 cells, primary human coronary artery smooth muscle cells [SMCs], and primary human aortic adventitial fibroblasts [AoAFs]) (Horke et al., 2007). Investigations of the signalling pathways of PON2 modulation have revealed that it may involve the urokinase plasminogen activator (uPA), extracellular signal-regulated kinase (ERK1/2), NADPH oxidase (NOX), phosphatidylinositol 3-kinase (PI3K), platelet-derived growth factor receptor-β (PDGFR-β), the tyrosine kinase cascade, the NF-kappa B pathway and lipid peroxidation, PPARγ, and AP-1 (Shiner et al., 2007a, b; Fuhrman et al., 2008, 2009; Precourt et al., 2009). Recently, a functional study of the PON2 promoter revealed a putative sterol regulatory binding protein-2 (SREBP-2) DNA element that is located between -593 bp and -575 bp of the PON2 promoter that may play a major role in PON2 regulation (Fuhrman et al., 2009; Lim and Kim, 2009).

Role of PON2 in human diseases

As with other PON family members, when PON2 was first discovered, the specific substrates and exact physiological functions of this protein were uncertain; however, the high structural similarity of PON2 to other members of the PON family have indicated that it might possess an activity that is analogous to those of PON1 and PON3. Like PON1 and PON3, PON2 very efficiently metabolises 5-hydroxy-eicosatetraenoic acid 1,5-lactone and 4-hydroxy-docosahexaenoic acid, which are the oxidation products of arachidonic acid and docosahexaenoic acid, respectively, and may also be endogenous substrates for PON2 (Draganov et al., 2005); however, unlike PON1, PON2 is incapable of hydrolysing organophosphates, such as paraoxon, although it has been demonstrated to possess a greater lactonase activity in comparison to those of PON1 and PON3 when dihydrocoumarin and some arylesters are used as substrates (Rosenblat et al., 2003; Draganov et al., 2005). Although PON2 has a lower anti-oxidative capacity than PON1 (Draganov et al., 2005), several recent investigations of this protein have been conducted due to its high expression level and widespread localisation. Studies using either purified PON2 or PON2-overexpressing cells have provided similar results. Exposure of PON2-overexpressing Hela cells to hydrogen peroxide or oxidised phospholipids has been observed to decrease intracellular oxidative stress by reducing the levels of reactive oxygen species (ROS), preventing LDL lipid peroxidation, reversing the oxidation of mildly oxidised LDL (MM-LDL), and inhibiting the ability of MM-LDL to induce monocyte chemotaxis (Ng et al., 2001). Treatment of mouse macrophages with purified recombinant PON2 has been shown to prevent LDL oxidation (Rosenblat et al., 2003).

Studies in animals have also provided similar results. PON2 has been shown to provide protection against HDL and LDL oxidation and inhibit monocyte transmigration in response to LDL oxidation in PON2-knockout (PON2KO) mice (Ng et al., 2006a). Interestingly, these PON2 knockout mice demonstrate an increased number of foam cells and lipid droplets and develop significantly larger atherosclerotic lesions in comparison to their wild-type counterparts (Ng et al., 2006b). In contrast, the atherosclerotic lesions in AdPON2-overexpressing apoE-deficient mice (apoE-/-) are significantly smaller than those in their wild-type counterparts. Furthermore, serum obtained from AdPON2-treated mice contains significantly lower levels of lipid hydroperoxides and demonstrates an enhanced capacity to induce cholesterol efflux from cholesterol-loaded macrophages. In addition to increasing cholesterol efflux, PON2 has been found to inhibit triglyceride (TG) biosynthesis and microsomal diacylglycerol acyltransferase 1 (DAGT1) activity, resulting in a decreased accumulation of TG in macrophages of PON2-deficient mice

(Meilin et al., 2009; Rosenblat et al., 2009). PON2 has been proposed to function as an antiapoptotic factor in atherosclerosis. PON2 overexpression in EA.hy 926 cells was found to protect the endoplasmic reticulum against oxidative stress-induced apoptosis by specifically preventing the generation of superoxide radicals at the inner mitochondrial membrane (Horke et al., 2008; Altenhöfer et al., 2010). Currently, based on several lines of evidence, the anti-atherosclerotic effects of PON2 have been clearly delineated.

Association of PON2 polymorphisms with human diseases

Several polymorphisms in the PON2 gene have been reported to date; however, only two common PON2 polymorphisms play a prominent role in pathophysiological conditions: codon 148 is either an alanine or a glycine (A148G), and codon 311 is either a cysteine or a serine (C311S) (Mochizuki et al., 1998). Many studies have investigated the PON2 gene polymorphism frequencies, and the results therein have revealed variations in different ethnic groups as shown in Table 1. Based on current evidence, most populations carry the A allele at codon 148 and the S allele at codon 311.

Oxidative stress is believed to be involved in human diseases. Because of the anti-oxidative and anti-atherosclerotic potential of PON2, there has been interest in examining the association of PON2 with CVD and other pathological disorders, such as diabetic mellitus, Alzheimer's disease, and sporadic amyotrophic lateral sclerosis (ALS); however, most studies have investigated the associations of PON2 polymorphisms with these diseases rather than the relationship between PON2 activity and disease. This may be because the true biological substrate of PON2 remains unknown. Currently, the activity of PON2 is determined from the hydrolysis of dihydrocoumarin (DHC), and the appropriateness of this substrate is still subject to debate. Therefore, studies investigating the association of PON2 activity with diseases have been scarce because of the lack of well-established methods. Instead, most studies emphasise the association of PON2 polymorphisms with various pathologies and diseases in different ethnic groups as shown in Table 2. The resulting data have been inconsistent because of the variations in different experimental conditions, ethnicities, and geographical locations. Hence, further investigations are required.

Table 1: Gene distributions of PON2 at codons 148 and 311

Population (n)	Allele frequencies at codon 148		Allele frequencies at codon 311		Ref.
	A allele	G allele	C allele	S allele	
Brazil (376)	0.6	0.4	0.29	0.71	Oliveira et al., 2004
Canada (324)	0.76	0.24	-	-	McKeown-Eyssen et al., 2004
China (475)	0.826	0.174	0.173	0.827	Wang et al., 2003a
United Kingdom (405)	0.73	0.27	0.26	0.74	Pasdar et al., 2006
Israel (193)	-	-	0.158	0.842	Karban et al., 2007
Italy (273)	-	-	0.198	0.802	Martinelli et al., 2004
Japan (2,210)	-	-	0.198	0.802	Yamada et al., 2003
Korea (988)	0.7825	0.2175	0.2175	0.7825	Shin, 2009
Pakistan (370)	0.51	0.49	0.612	0.388	Saeed et al., 2007
Poland (437)	-	-	0.24	0.76	Slowik et al., 2007
United States (2,553)	0.766	0.234	-	-	Ranade et al., 2005

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Table 2: Association between PON2 polymorphisms and various diseases

Polymorphism	Disease	Population	Ref.
311	associated with a risk for coronary artery disease (CAD)	Asian Indians	Sanghera et al., 1998
		Taiwan	Pan et al., 2002
		WISE study group*	Chen et al., 2003
		Chinese	Qin et al., 2006
		Iranians	Jalilian et al., 2008
	associated with coronary heart disease (CHD)	Chinese Han women	Su et al., 2005
	not associated with CHD	Chinese	Wang et al., 2003a
		NPHSII**	Robertson et al., 2003
		Meta-analysis of 43 studies	Wheeler et al., 2004
	associated with acute myocardial infarction (AMI)	Italians	Marchegiani et al., 2009
	associated with myocardial infarction (MI) in smokers but not in non-smokers	Verona Heart Project	Martinelli et al., 2004
	not associated with AMI	Spanish autonomous regions	Guxens et al., 2008
	associated with type 2 diabetes with ischemic stroke	Chinese	Wang et al., 2003b
	associated with the severity of ischemic stroke	Greece	Lazaros et al., 2010
		Polish	Slowik et al., 2007
	not associated with ischemic stroke	Japanese	Imai et al., 2000
		CARE trial***	Ranade et al., 2005
		Chinese Han	Xu et al., 2005
	associated with type 1 and 2 diabetes	Caucasian origin	Mackness et al., 2005
	associated with glycemic control in type 2 diabetes with retinopathy	England	Mackness et al., 2000
	associated with nephropathy in type 2 diabetes	Swiss	Pinizzotto et al., 2001
	associated with microalbuminuria in type 1 diabetes	Caucasians	Kao et al., 2002
	not associated with diabetic nephropathy	Russians	Voron'ko et al., 2005

Table 2 (cont.): Association between PON2 polymorphisms and various diseases

Polymorphism	Disease	Population	Ref.
	associated with Alzheimer's disease and vascular dementia	Caucasian probands	Janka et al., 2002
	associated with sporadic late-onset Alzheimer's disease	Chinese	Shi et al., 2004
	associated with sporadic amyotrophic lateral sclerosis (ALS)	Polish North Americans and Europeans	Slowik et al., 2006 Valdmanis et al., 2008
	not associated with sporadic ALS	Meta-analysis	Wills et al., 2009
	associated with familial hypercholesterolemia (FH) symptomatic	Caucasians	Leus et al., 2009
	associated with total cholesterol and LDL	Southwestern Koreans	Shin, 2009
	not associated with open-angle glaucoma (OAG)	Japanese	Inagaki et al., 2006
	associated with preterm neonate	Chinese Chinese	Liang et al., 2002a Chen et al., 2004
148	not associated with risk for CAD	Taiwanese	Pan et al., 2002
	not associated with stroke	Chinese Han	Xu et al., 2008
	not associated with risk factors for CHD	Chinese	Chi et al., 2006
	associated with total cholesterol and LDL	Canada (Oji-Cree) Southwestern Koreans Brazilians	Hegele et al., 1998 Shin, 2009 Olivera et al., 2004
	associated with fasting plasma glucose in type 2 diabetes	Canada (Oji-Cree)	Hegele et al., 1997
	not associated with plasma glucose	Alberta Hutterites	Boright et al., 1998
	associated with type 2 diabetes with renal disease	Americans	Thameem et al., 2009
	not associated with diabetic nephropathy	Russians	Voron'ko et al., 2005
	associated with low birth weight	South Asian Origin	Busch et al., 1999
	associated with preterm neonate	Chinese Chinese	Wu et al., 2003 Liang et al., 2002b

* Women's Ischemia Syndrome Evaluation (WISE) study
** Northwick Park Heart Study II
*** Cholesterol and Recurrent Events (CARE) trial

CONCLUSION

The anti-oxidative and antiatherogenic activities of PON2 have been well documented over the past five years; however, an identification of the location, biological functions, and activity of PON2 is required for further investigation. It would be of interest to explore PON2 within the context of other disorders, such as cancer or abnormalities in metabolic consumption, because of its anti-oxidative role, intracellular location, and expression in most organs at high metabolic rates and incidence in relation to distinct types of cancers, such as those associated with the lung, liver, testis, heart, and placenta. An investigation of the exogenous modulation of PON2, especially by nutrition and pharmacological means, has only recently been initiated; hence, many issues and questions still remain. Future studies should provide substantial evidence on both PON2 modulation and the mechanisms by which chemicals may modulate PON2 expression. Most studies have attempted to relate DNA sequence variants in the PON2 locus to the risk for CVD and other diseases. The controversial findings that have been obtained for the two common PON2 polymorphisms that are associated with CVD have led to equivocal conclusions. The differences in these findings may derive from individual susceptibilities that are related to many other factors that are associated with ethnic background and life style.

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