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Genetic polymorphisms and activity of PON1 in a Mexican population

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Abstract

Human paraoxonase (PON1) plays a role in detoxification of organophosphorus (OP) compounds by hydrolyzing the bioactive oxons, and in reducing oxidative low-density lipoproteins, which may protect against atherosclerosis. Some PON1 polymorphisms have been found to be responsible for variations in catalytic activity and expression and have been associated with susceptibility to OP poisoning and vascular diseases. Both situations are of public health relevance in Mexico. Therefore, the aim of this study was to evaluate PON1 phenotype and the frequencies of polymorphisms PON1 −162, −108, 55, and 192 in a Mexican population. The studied population consisted of unrelated individuals (n = 214) of either gender, 18–52 years old. Serum PON1 activity was assayed using phenylacetate and paraoxon as substrates. PON1 variants, −162, 55, and 192, were determined by real-time PCR using the TaqMan System, and PON1 −108 genotype by PCR-RFLP. We found a wide interindividual variability of PON1 activity with a unimodal distribution; the range of enzymatic activity toward phenylacetate was 84.72 to 422.0 U/mL, and 88.37 to 1645.6 U/L toward paraoxon. All four PON1 polymorphisms showed strong linkage disequilibrium (D% >90). PON1 polymorphisms −108, 55, and 192 were independently associated with arylesterase activity; whereas the activity toward paraoxon was related only with PON1 192 polymorphism, suggesting that this polymorphism is determinant to infer PON1 activity. A better understanding of the phenotype and genotypes of PON1 in Mexican populations will facilitate further epidemiological studies involving PON1 variability in OP poisoning and in the development of atherosclerosis.

Keywords: PON1; Paraoxonase polymorphism; Organophosphorus pesticides; LDL metabolism

Introduction

Human paraoxonase/arylesterase (PON1: aryldialkylphosphatase [E.C.3.1.8.1]) is a high-density lipoprotein (HDL)-associated enzyme of 354 amino acids (43 kDa) synthesized in the liver and secreted into the blood (Hassett et al., 1991; Mackness, 1989). PON1 hydrolyzes and, hence, inactivates the oxygen analogs of various commonly used organophosphorus (OP) compounds (e.g., diazoxon, paraoxon) (Geldmacher-von Mallinckrodt and Diepgen, 1988).

Human PON1 is encoded by a single gene on chromosome 7q21–22 and is a member of a multigene family (PON1, PON2, and PON3 genes) (Primo-Parmo et al., 1996). PON1 presents at least five polymorphisms in the promoter region at positions −108 (T/C), −126 (G/C), −162 (A/G), −832 (G/A), and −909 (C/G), from which −108, −162, and −909 have been related with differences in PON1 activity and expression (Brophy et al., 2001; Leviev and James, 2000; Suehiro et al., 2000). The most significant effect of these polymorphisms on PON1 activity is that at position −108, which contributes 22.8% of the activity, and that at position −909 contributes the least (<1%) (Brophy et al., 2001). Linkage disequilibrium has been reported among these polymorphisms from the regulatory region (Brophy et al., 2001). Additionally, two common polymorphisms in the coding

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region of PON1 at positions 55 and 192 have been reported. The polymorphic site at position 55 (Leu/Met) has been related with differences in PON1 activity, the 55L isoenzyme has higher enzymatic activity than the 55-M isoenzyme (Blatter-Garin et al., 1997). It has been suggested that this is due to linkage disequilibrium with the −108C allele (Brophy et al., 2001), or to an increase in the stability of the 55L isoenzyme (Leviev and James, 2000). On the other hand, PON1 polymorphism at position 192 (Glu/Arg) has two isoforms and has been described in vitro as substrate-dependent: the 192R alloenzyme hydrolyzes paraoxon faster than 192Q (Km = 0.52 vs. Km = 0.81 mM), whereas other compounds, such as diazoxon, are hydrolyzed faster by the latter alloenzyme (Davies et al., 1996; Li et al., 2000). Both isoforms hydrolyze phenylacetate at similar rates (La Du et al., 1986). In addition, linkage disequilibrium between PON1 55 and PON1 192 has also been reported (Blatter-Garin et al., 1997; Brophy et al., 2000).

Epidemiological studies have revealed a wide variation in serum PON1 activity among individuals (Furlong et al., 1988; La Du et al., 1986), showing a bimodal or trimodal distribution (Eckerson et al., 1983; Geldmacher-von Mallinckrodt and Diepgen, 1988). This variability has been attributed to the presence of polymorphisms in the PON1 gene, and people have been classified into homozygous for the low activity allele, heterozygous, or homozygous for the high activity allele (Eckerson et al., 1983; Geldmacher-von Mallinckrodt and Diepgen, 1988), giving rise to the hypothesis that individuals with low PON1 activity might be more susceptible to OP toxicity. Studies published so far have demonstrated the relevance of PON1 in modulating OP toxicity in vitro, in animal models and in epidemiological studies (review in Costa et al., 2003).

On the other hand, PON1 has been implicated in lipid metabolism, since as a HDL-associated enzyme it can prevent lipid peroxide accumulation in low-density lipoproteins that are involved in the initiation of atherosclerosis (Mackness and Durrington, 1995; Mackness et al., 1991). To this regard, some epidemiological studies reported a relationship between PON1 polymorphism and the increased risk for coronary artery disease, in which carrying the R192 allele may represent a risk factor (review in Draganov and La Du, 2004).

Considering that heart diseases are one of the most frequent (20%) causes of death in adults in Mexico (INEGI., 2003), that OP poisoning is an important public health problem in the country (Epidemiological Surveillance System/SSA, Mexico), and that PON1 may modulate the development of vascular diseases and the susceptibility to environmental pollutants, such as OP, the aim of this work was to evaluate the distribution of PON1 phenotype and genetic polymorphisms PON1 −162, PON1 −108, PON1 55, and PON1 192 in a Mexican population.

Methods

Human subjects. A convenience sample of 214 healthy and unrelated individuals who attend our Institution (Center for Research and Advanced Studies from the National Polytechnic Institute, CINVESTAV-IPN, Mexico City), were invited to participate. The study population consisted of individuals of either sex, aged 18–52 years, with no history of occupational exposure to OP. Great care was taken in selecting individuals whose parents were Mexicans. We collected blood samples through venipuncture in heparin or EDTA coated tubes to analyze PON1 activity and genotypes, respectively. All subjects signed an informed consent. This study was approved by the Ethics Committee on Human Studies from CINVESTAV-IPN.

PON1 activity. PON1 enzymatic activity was assayed as previously described, using as substrates paraoxon (paraoxonase activity) or phenylacetate (arylesterase activity) (Eckerson et al., 1983; Furlong et al., 1988). Briefly, arylesterase activity was determined by phenol production from the hydrolysis of phenylacetate (Sigma-Aldrich) using 10 mM Tris–HCl, pH 8.0, 1 mM $CaCl_2$, and 5 μM EDTA. Eserine sulfate (Sigma-Aldrich) was used to inhibit the unspecific hydrolysis due to serum albumin and serum cholinesterase, which account for about 5% of PON1 activity in plasma. The increase in A_{270} was followed for 5 min. The molar extinction coefficient of phenol is 1.31×10^3 and the arylesterase activity was expressed as U/mL. Paraoxon (ChemService) hydrolysis was performed in the presence of 1 M NaCl (salt-stimulated paraoxonase activ-

Table 1
Sequence of TaqMan Probes and primers used for real-time PCR amplification of PON1 polymorphisms

PON1 polymorphism	TaqMan probe		Forward primer/reverse primer
PON1 −162	VIC	CCGCAAGCCGCGCC	GCTGAAAGTGCTGAGCTCCT/CCGACCAGGTGCACAGAA
	FAM	CCGCAAGCCACGCC	
PON1 55	VIC	AGTATCTCCAAGTCTTC	ACAACCTGTACTTTCTGTTCTCTTTTCTG/CAGAGCTAATGAAAGCCAGTCCAT
	FAM	CAGTATCTCCATGTCTTC	
PON1 192	VIC	CCTACTTACAATCCTG	CTGAGCACTTTTATGGCACAAATGA/ACCACGCTAAACCCAAATACATCTC
	FAM	CCCTACTTACGATCCTG	

Sequence of primers and probes for the TaqMan system are from 5′ to 3′, except for PON 55 probes which are from 3′ to 5′.