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Effect of the molecular polymorphisms of human paraoxonase (PON1) on the rate of hydrolysis of paraoxon

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1 The hydrolysis of organophosphate pesticides (OP) and nerve gases by serum paraoxonase (PON1) is an important factor determining their toxicity to mammals including man. The PON1 gene contains 2 polymorphic sites at amino acid positions 55 (L→M) and 192 (G→A, classically defined as the A and B genotypes) which result in several alloenzymes of PON1 in human serum.

2 The 192 polymorphism has previously been shown to affect PON1 activity. We have investigated the effect of both polymorphisms on the hydrolysis of paraoxon by serum from 279 healthy human subjects.

3 The 55 polymorphism significantly influenced PON1 activity. MM homozygotes had over 50% less activity towards paraoxon compared to the LL and LM genotypes regardless of the 192 genotype ($P<0.001$).

4 Multiple regression analysis indicated that the 192 polymorphism, 55 polymorphism and serum PON1 concentration were responsible for 46, 16 and 13% of the variation in PON1 activity, respectively (all $P<0.001$). None of the other parameters investigated significantly affected PON1 activity.

5 Therefore both PON1 polymorphisms affect the hydrolysis of paraoxon. AA/MM and AB/MM individuals may be potentially more susceptible to OP intoxication.

6 Genotyping individuals for both PON1 polymorphisms may provide a method for identifying those individuals at most risk of OP poisoning. The effect of PON1 polymorphisms on activity may also explain why some Gulf War Veterans have developed Gulf War Syndrome and some have not.

Keywords: Paraoxonase; PON1; OP intoxication; Gulf War Syndrome

Introduction

Organophosphorus compounds are widely used as insecticides and unfortunately also as nerve gases. The hydrolysis of organophosphate (OP) insecticides and nerve gases by serum paraoxonase (aryldialkylphosphatase, E.C.3.1.8.1) is a major factor determining their toxicity to vertebrates including man (Mackness, 1989; La Du, 1992; Mackness *et al*., 1996). Human serum paraoxonase (PON1) located on high-density lipoprotein has an amino acid polymorphism at position-192 (Adkins *et al*., 1993; Humbert *et al*., 1993) which results in two alloenzymes, which differ in their hydrolytic activity towards paraoxon. Paraoxon is hydrolysed at a higher rate by the B (Arg at 192) than the A alloenzyme (Glu at 192) (La Du, 1992; Adkins *et al*., 1993; Humbert *et al*., 1993; Mackness *et al*., 1996). These alloenzymes have similar hydrolytic activity towards some other substrates, such as phenylacetate. The historical view that the B alloenzyme of PON1 was the most protective because of its higher activity towards paraoxon was recently challenged by findings showing the A alloenzyme hydrolysed diazoxon and the nerve gases sarin and soman faster than the B (Davies *et al*., 1996).

PON1 has also been shown to hydrolyse lipid-peroxides on low-density lipoprotein (LDL) which has led to the suggestion that the activity of PON1 may be important in preventing oxidative modification of LDL, which is believed to be central to the pathogenesis of atherosclerosis (Mackness *et al*., 1991a; 1993; 1996; Watson *et al*., 1995). The A alloenzyme is also more efficient at hydrolysing lipid-peroxides on low-density lipoprotein than the B alloenzyme which may be relevant to the recently proposed anti-atherogenic role of PON1 (Mackness *et al*., 1997).

A second PON1 polymorphism at position 55 involving a Leu (L)→Met (M) substitution, was not previously believed to affect PON1 activity (Adkins *et al*., 1993; Humbert *et al*., 1993; Davies *et al*., 1996; La Du, 1996). A recent study carried out in a population with non-insulin-dependent diabetes mellitus (NIDDM) has shown that the L/M polymorphism affects PON1 activity by modifying the serum concentration (Blatter-Garin *et al*., 1997). However, we have previously shown that diabetes affects PON1 activity and concentration-independently of an affect on genotype (Abbott *et al*., 1995). We now present the results of a study of the 192 and 55 polymorphisms in 279 healthy people, which clearly reveal that both have an independent effect on serum PON1 activity, but not concentration. MM homozygotes have the lowest PON1 activity regardless of their 192 genotype. The L/M polymorphism may thus make an important contribution to genetic susceptibility to OP poisoning and atheroma.

Methods

Human subjects

Blood was withdrawn by venepuncture from 279 healthy individuals (146 male, 131 female), aged 42.4 ± 12.2 years with no diseases known to affect PON1 activity such as diabetes mellitus, coronary artery disease, hepatic or renal disease. Serum and plasma were prepared by low-speed centrifugation at 3,000 r.p.m., 4°C for 15 min. White cells were removed from the buffy coat of the plasma tube. This study was approved by the Central Manchester Health Authority Research Ethics Committee.

PON1 genotype

DNA was extracted from the white cells and PON1 genotype for both the 55 and 192 polymorphisms determined by PCR amplification and restriction enzyme digestion as described (Adkins *et al*., 1993; Humbert *et al*., 1993).

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Table 1 Effect of the PON-192 and -55 polymorphisms on serum PON1 activity and concentration

	PON-192 genotype			*PON-55 genotype*		
	AA	*AB*	*BB*	*LL*	*LM*	*MM*
n	156	99	24	104	149	26
PON1 activity	121.7	288.3^{+}	396.4^{+}	254.8	212.2	77.9*
(nmol min^{-1} ml^{-1} serum)	(26.3–592.5)	(60.8–518.7)	(213.8–620.8)	(86.1–620.8)	(51.8–592.5)	(26.3–155.1)
PON1 concentration	88.2	92.7	83.0	87.7	87.4	102.8
(μg ml^{-1})	(16.8–527.4)	(25.1–333.9)	(25.1–462.4)	(16.8–490.5)	(25.1–527.4)	(43.3–248.7)
PON1 specific activity	1.39	3.05^{+}	5.87^{+}	2.63	2.17	0.82*
(nmol min^{-1} μg^{-1} protein)	(0.22–14.5)	(0.59–11.0)	(0.89–20.2)	(0.35–20.22)	(0.33–11.01)	(0.22–2.24)

PON-192 and -55 genotypes, serum PON1 activity and concentration were determined as described in the Methods section. Specific activity was calculated by dividing PON1 activity by concentration. Figures are median (range). PON1 = human serum paraoxonase. $^{+}$Significantly different from AA genotype $P<0.001$. *Significantly different from LL + LM genotypes $P<0.001$.

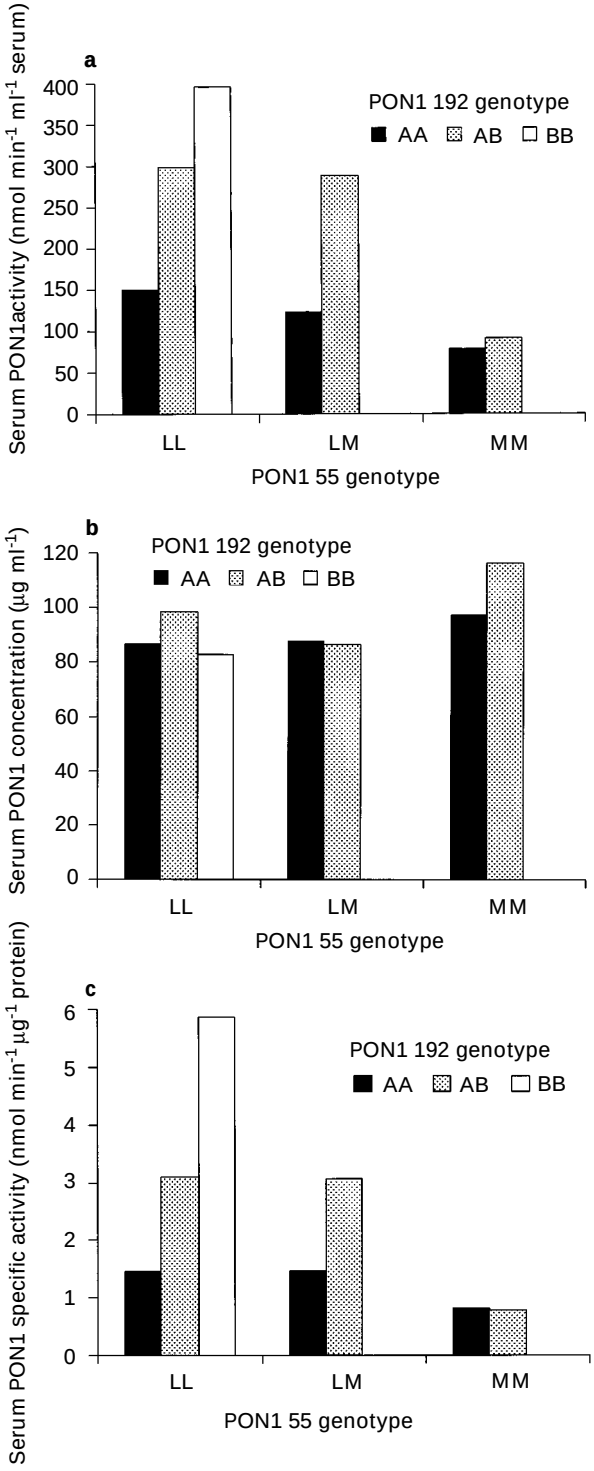

Figure 1 The effect of the PON1 polymorphism on (a) serum PON1 activity, (b) serum PON1 concentration and (c) serum PON1 specific activity. PON-192 and -55 genotypes, serum PON1 activity and concentration were determined as described in the Methods section. Specific activity was calculated by dividing PON1 activity by concentration. The height of the columns represents the median value.

PON1 activity

The rate of hydrolysis of paraoxon (5.5 mM in 100 mM Tris/HCL buffer containing 2 mM $CaCl_2$ pH 8.0) by serum was determined by a continuous spectrophotometric method at 405 nm as previously described (Mackness *et al*. 1991b).

PON1 concentration

Serum PON1 concentration was determined by our in-house ELISA method by use of rabbit anti-human PON1 monospecific polyclonal antisera as described previously (Blatter-Garin *et al*., 1994; Abbott *et al*., 1995).

Lipid, lipoprotein and apolipoprotein analysis

Total serum cholesterol and triglycerides were measured by the enzymatic cholesterol oxidase-*p*-aminophenazone (CHOD-PAP) and glycerol-3-phosphate oxidase-p-aminophenazone (GPO-PAP) methods respectively (Biostat Ltd). High-density lipoprotein (HDL) cholesterol were determined by the CHOD-PAP method after precipitation of lower-density lipoproteins with heparin/Mn^{2+}. Serum apolipoproteins B and A1 were measured by rate immunonephelometric techniques by use of the Beckman Array with antisera and standards supplied by the manufacturer.

Statistical analysis

The non-parametric Mann-Whitney U test was used to determine the significance of differences in PON1 activity, concentration and specific activity between genotypes.

Parameters responsible for the variation in the rate of hydrolysis of paraoxon were sought by step-wise Multiple Regression analysis.

Results

In 279 healthy people we found that the frequency of the position 192 alleles was A = 0.74, B = 0.26 and of the position 55 alleles was L = 0.64, M = 0.36. The PON-192 polymorphism affected paraoxon hydrolysis by serum as would be expected from previous studies (La Du, 1992; Adkins *et al*., 1993;

Humbert *et al*., 1993; Mackness *et al*., 1996; Davies *et al*., 1996; Blatter-Garin *et al*., 1997) (Table 1). The PON-55 polymorphism also influenced serum PON1 activity. The MM genotype was associated with significantly lower serum PON1 activity towards paraoxon ($P<0.001$) than the LL and LM genotypes which were similar (Table 1 and Figure 1a). Neither the 192 nor the 55 polymorphism significantly influenced serum PON1 concentration (Table 1 and Figure 1b). Differences in PON1 specific activity between the genotypes reflected the differences in PON1 activity (Table 1 and Figure 1c). No age or gender differences were observed for any of the PON1 parameters.

MM homozygotes had significantly lower rates of serum paraoxon hydrolysis than did the LL and LM genotypes, regardless of whether they were AA homozygotes or AB heterzygotes (Figure 1a). Neither LM nor MM were represented in the BB homozygotes. The effect of the 55 polymorphism on PON1 activity was marked, being 50% less in MM homozygotes compared to the LL and LM genotypes in AA homozygotes and 60% less in the MM genotype compared to LL and LM genotypes in AB heterozygotes (both $P<0.001$) (Figure 1a).

Discussion

A previous study of 72 subjects showed the 192 polymorphism to be responsible for 75% of the variation in paraoxon hydrolysis (Nevin *et al*., 1996). However, neither the 55 polymorphism nor the serum PON1 concentration were determined. In our study population multiple regression analysis, which included as covariates the 55 and 192 polymorphisms, serum PON1 concentration, apolipoprotein A1 and B, HDL-cholesterol, total serum cholesterol and triglyceride concentrations, age, gender and BMI, indicated that the 192 polymorphism, 55 polymorphism and serum PON1 concentration were responsible for 46, 16 and 13% of the variation in serum paraoxon hydrolysis, respectively (all $P<0.001$). None of the other parameters significantly affected serum paraoxon hydrolysis.

Thus the genetic control of PON1 activity towards paraoxon involves both the polymorphisms at 192 and 55. Neither polymorphism significantly influenced PON1 concentration. This latter finding differs from those from a previous study in NIDDM (Blatter-Garin *et al*., 1997). However, we have previously shown that the presence of diabetes severely affects both PON1 activity and concentration-independently of a genetic effect on the PON1 phenotypic distribution (Abbott *et al*., 1995). MM homozygosity has a particularly marked effect in depressing PON1 activity. Further studies are required to determine whether this is unique to paraoxon hydrolysis or whether it is true for other substrates. However, if true, AA/MM and AB/MM individuals will be potentially more susceptible to OP intoxication than other individuals. It has been suggested that genotyping for the 192 polymorphism may provide a basis for determining an individual's susceptibility to OP poisoning (Costa & Manzo, 1995). However, our results would indicate that genotyping for both polymorphisms may be necessary for identifying those individuals at most risk of OP poisoning, which should be valuable for agricultural workers who may be at risk of OP poisoning and which results in a large number of deaths annually (WHO, 1986; 1990). Genetic testing of Gulf War Veterans for the PON1 polymorphisms may also help to explain why some have developed Gulf War Syndrome (in which OP intoxication is implicated (Haley *et al*., 1997a, b; Haley & Kurt, 1997; The Iowa Persian Gulf Study Group, 1997)) and some have not, despite apparently similar rates of exposure. The mechanism(s) by which the PON1 55 polymorphism effects PON1 activity is unclear. However, it does not appear to be an effect on synthesis and secretion or clearance, as serum PON1 concentration is unaffected by this polymorphism. It is possible that there is an effect on protein conformation or binding to HDL altering its substrate specificity. Further investigations are also required on the role of the PON1 polymorphisms in coronary artery diseases and their effects on the anti-atherogenic properties of PON1, such as its ability to hydrolyse lipid-peroxides and to protect low-density lipoprotein against oxidative modification. Such studies are currently underway in our laboratory (Mackness *et al*., 1997).

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