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Unexpectedly higher diazoxon hydrolysis by serum paraoxonase-1 in coronary heart disease

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ABSTRACT

Objectives: Low serum PON1 activities (paraoxon, phenyl-acetate or lactone substrates) are associated with coronary heart disease (CHD). We investigated the rate of diazoxon hydrolysis by PON1 in a population with CHD.

Design & methods: Case- control study of 410 subjects with CHD and 274 controls. PON1 activity towards paraoxon and diazoxon, PON1 serum concentration and the PON1–55 and 192 polymorphisms were determined.

Results: There were no differences in the distribution of the PON1–55 or PON1–192 genotypes between the CHD and controls, however, PON1 activity towards diazoxon (DIAZ) was significantly (+160%) higher in CHD. In the control population, DIAZ was significantly different between the PON1–192 genotypes in the order QQ > QR > RR ($P < .001$). However, in CHD the order was QQ > QR = RR. In CHD DIAZ was significantly higher in all the PON1–192 and 55 genotypes compared to controls. In both populations DIAZ was significantly different between the PON1–55 genotypes in the order LL > LM > MM ($P < .001$).

Conclusion: If this result can be replicated in other studies and/or with other PON1 substrates, there may be major diagnostic and mechanistic implications for the relationship of PON1 and CHD.

1. Introduction

Human serum paraoxonase-1 (PON1) is a Ca^{2+} dependent lipolactonase/esterasehydrolysing a large number of different substrate groups such as lactones and thiolactones (including a number of drugs), arylesters, and organophosphate pesticides and nerve gases amongst others [1]. PON1 is synthesised predominantly by the liver and secreted into the blood where it associates with high-density lipoprotein (HDL) [2].Immunohistochemical studies in several mammalian species including humans have indicated the presence of PON1 in a wide variety of tissues [3,4], probably transported there by HDL. PON1 is a major antioxidative component of HDL, preventing the oxidation of lipids in lipoproteins and cell membranes which is a proinflammatory process. PON1 therefore contributes to the antiatherosclerotic function of HDL [5,6].

Previous prospective, case-control and meta-analysis studies have largely indicated that serum PON1 activity and atherosclerosis development are inversely correlated. PON1 activity is low in patients with CHD compared to matched controls using a variety of PON1 substrates including paraoxon, phenyl-acetate, lactones and thiolactones (reviewed in [5,7]), being on average 19% lower in patients with CHD [8]. Recent studies has shown that low PON1 activity (paraoxon or phenyl-acetate) could predict the severity of coronary stenosis [9,10] and the occurrence of adverse outcomes in patients with chronic heart failure [11]. These results have led to the theory that PON1 is causally related to CHD development and suggestions that PON1 activity could be used as a diagnostic for CHD [5,7–11].

Here we report an unexpected increase in the rates of hydrolysis of diazoxon by PON1 in subjects with CHD, which if confirmed by other studies and or substrates, may change the way we think of the relation of PON1 to atherosclerosis.

2. Materials and methods

2.1. Subjects

The study population was 410 subjects with angiographically proven CHD recruited as described previously [12]. The control population was 274 healthy individuals attending a routine health check. The demographic details of the populations and blood biochemistry have been published previously [12]. The study was approved by the Central Manchester NHS Trust Research Ethics Committee (REC 01/030) and all subjects gave written informed consent.

Blood was obtained by venepuncture, after an overnight fast, and

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serum, plasma and buffy coat prepared by low speed centrifugation as described previously [12].

2.2. Biochemical analyses

Serum PON1 activity towards paraoxon and its serum concentration were determined by spectrophotometry and in-house ELISA respectively [12]. Serum PON1 hydrolysis of diazoxon was determined by adding 5 μl of serum to 1 ml of TRIS/HCl buffer pH 8.0 containing 2 mM $CaCl_2$ and 500 μM diazoxon and measuring the increase in absorbance at 270 nm as described [13].

For the determination of PON1 SNPs DNA was extracted from leukocytes using the Puregene DNA isolation kit (Gentra systems, Milwaukee, USA) and the PON1–55 and 192 polymorphisms were determined by our RFLP protocols [12].

2.3. Statistical analyses

Student's unpaired *t*-test was used to test for differences between groups after logarithmic transformation of non-gaussian parameters. Chi squared test was used to analyse differences in PON1 gene frequency.

3. Results

As we have previously published [12], both serum PON1 activity towards paraoxon and PON1 concentration were significantly lower in the CHD patients compared to controls (Table 1). However, diazoxon hydrolysis was 160% higher in the CHD patients than controls ($P < .0001$) (Table1) and was normally distributed in both populations.

There were no differences in the distribution of the PON1–192 or PON1–55 genotypes between the CHD population and the controls (Table 2). Diazoxon hydrolysis was significantly higher in all genotypes of the CHD population (Table 2). In the control population activity towards diazoxon (DIAZ) was in the order QQ > QR > RR for the PON1–192 polymorphism (the reverse of the order for activity towards paraoxon (POX)) as has been previously reported [14]. However, in the CHD population the order was QQ > QR = RR (Table 2). For the PON1–55 polymorphism DIAZ was LL > LM > MM for both populations, the same as POX [15]. Using haplotype analysis we could find no confounding effect of the PON1–55 polymorphism on PON1–192 activity or *vice versa*.

4. Discussion

We have found a 160% increase in diazoxon hydrolysis by PON1 in a population with CHD. To say our finding was unexpected is somewhat of an understatement. The vast majority of previously published studies have found low PON1 activity to be associated with CHD presence or susceptibility [5–7,16], regardless of population ethnicity or the PON1 substrate used. One previous study to report on DIAZ, showed a 16% lower DIAZ in 106 patients with carotid artery disease compared to 106 controls, however whether this difference was statistically significant is not reported by the authors [17]. The reasons for this difference between studies are unclear, both studies included almost identical proportions of smokers and subjects on lipid lowering and antihypertensive medication. However, the study of Jarvik *et al* [17] was much smaller and included a large proportion of people with type 2 diabetes in both study groups. Type 2 diabetes was an exclusion in our study because it reduces serum PON1 activity [18]. A study of Japanese patients with type 2 diabetes found DIAZ, POX and arylesterase activities of PON1 all to be lower than in controls [19]. In addition all our patients received aspirin which is known to increase serum PON1 [20]. Further studies in this area are clearly warranted.

Table 1
Serum PON1 concentration (PON1c) and activities in the control and CHD populations.

	Control	CHD
N	274	410
PON1c (μg/ml)	89.1 (16.8–527.4)	71.6 (11.4–489.3)$^{+}$
POX (nmol/min/ml)	214.6 (26.6–620.8)	122.8 (36.3–802.8)$^{+}$
DIAZ (μmol/min/ml)	10.13 ± 0.26	(16.72 ± 0.27)*

Figures are mean ± SD or median (range).
Significantly different to control $^{+}P < .001$, $^{*}P < .0001$.

Table 2
DIAZ activity of PON1 according to 192 and 55 genotypes DIAZ activity = μmol/min/ml.

	Control		CHD	
	n	Activity[1]	n	Activity[1]
192QQ	155	1.04 ± 0.36^{+}	206	17.60 ± 0.37**
QR	97	9.54 ± 0.93	172	16.45 ± 0.43**
RR	22	8.81 ± 0.41	32	2.99 ± 0.87^{**+}
55MM	26	8.73 ± 0.76	45	13.79 ± 0.66^{**+}
LM	146	9.67 ± 0.36	219	16.28 ± 0.37**
LL	102	11.14 ± 0.45^{+}	146	16.95 ± 0.47**

Significantly different from control $^{**}P < .0001$ or other genotypes $+P < .0001$.

It is now well established that the structure and function of HDL are altered in CHD [21,22] and that these alterations affect the structure and/or binding of the PON1 protein effecting its activity [23,24] usually resulting in a lowering of PON1 activity towards such substrates as paraoxon and phenyl-acetate. However, it is entirely possible that due to the versatility of the PON1 active site, alterations to the structure or binding of PON1 could also result in increased activity towards certain substrates which could profoundly alter our view of the role of PON1 in CHD development. Studies into the effect of CHD on other PON1 substrates are needed.

Previously it was believed that the lower PON1 activity would result in the slower detoxication of some, unknown, proatherosclerotic metabolite, increasing susceptibility to CHD development. It now seems equally plausible that increased metabolism of some unknown compound by PON1 could result in the production of a proatherosclerotic metabolite, or conversely, increased metabolism by PON1 of an anti-atherosclerotic compound, resulting in increased susceptibility to CHD development. While the nature of these putative PON1 substrates is unknown, they are likely to be lactones [25] and there is an urgent need to identify them to further elucidate the role of PON1 in atherosclerosis development.

5. Conclusion

In conclusion, we have shown a 160% increase in diazoxon hydrolysis by serum PON1 in a population with CHD. If confirmed by other studies and or substrates, this may change the way we think of the relationship of PON1 to atherosclerosis.

Conflict of interest

There are none.

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