Presence of paraoxonase in human interstitial fluid

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Abstract Human serum paraoxonase (PON1) is postulated to have anti-atherosclerotic properties through its ability to prevent lipid peroxide generation on LDL. However, in order to perform this role it must be present in interstitial fluid, to prevent LDL oxidation which takes place in the sub-intimal space of the artery wall. The PON1 activity in interstitial fluid was 15.7 (2.3–183.0) (median (range)) nmol/min/ml compared to 105.3 (74.6–323.9) nmol/min/ml in serum. The PON1 concentration in interstitial fluid was found to be 20.2 (1.1–78.1) μg/ml (median (range)) compared to 109.6 (11.1–485.7) μg/ml in serum. Interstitial fluid PON1 concentration was dependent on the interstitial fluid apo AI concentration ($r = 0.690$, $P < 0.005$) indicating PON1 remained associated with HDL. However, the ratio of PON1 concentration to apo AI was lower in interstitial fluid (0.60 ± 0.20) than in the serum (0.95 ± 0.18) ($P < 0.001$) indicating sequestration of PON1 in the sub-intimal space. Therefore, PON1 is present and active in interstitial fluid where it can perform its anti-atherosclerotic function.

Key words: Paraoxonase; PON1; Apolipoprotein AI; High-density lipoprotein

1. Introduction

Human serum paraoxonase (aryldialkylphosphatase, E.C.3.1.8.1, PON1) is located on high-density lipoprotein (HDL) and is associated with its anti-oxidative activity [1–3]. PON1 can prevent lipid peroxide accumulation on low-density lipoprotein (LDL) [1,2,4] by a mechanism which is postulated to involve the hydrolysis of LDL phospholipid hydroperoxides [2,4,5]. The oxidative modification of LDL by lipid peroxidation is a pro-inflammatory reaction believed to be centrally involved in the initiation and propagation of atherosclerosis [6]. It is therefore believed that PON1 is an important determinant of the anti-inflammatory/anti-atherosclerotic action of HDL [1–5].

We have previously shown that PON1 accumulates in the human artery wall as atherosclerosis progresses [7]. LDL oxidation is believed to occur on particles sequestered in the sub-intimal space of the artery wall where there is a deficiency in anti-oxidant defences [8]. HDL is present in the interstitial fluid at a much higher concentration than LDL [9] and is therefore, in an ideal position to protect LDL (and perhaps cell membranes) from oxidative modification through the action of its associated enzymes including PON1 [10]. The purpose of the present investigation was to determine whether PON1 was present in the interstitial fluid of healthy subjects and what factors determined its concentration.

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2. Material and methods

2.1. Subjects

Sixteen healthy subjects (7 female, 9 male) aged 25–50 years took part in the study. All subjects gave informed consent and the study was approved by the Central Manchester Health Authority Research Ethical Committee.

Tissue fluid was obtained by a suction blister technique, previously described in detail and shown to be a good surrogate for interstitial fluid [11]. Venous blood was taken at the same time and serum and plasma prepared by centrifugation at $3000 \times g$, 4°C for 15 min. White cells were taken from the buffy coat of the plasma tube. Interstitial fluid and serum were stored in aliquots at −80°C for subsequent analysis.

2.2. Lipid and apolipoprotein analysis

VLDL, LDL and HDL were isolated from plasma by sequential ultracentrifugation [12].

Total cholesterol, VLDL, LDL and HDL cholesterol were determined by the CHOD-PAP enzymatic method (Biostat, Stockport, UK). Triglycerides were determined by the GPO-PAP enzymatic method (Biostat, Stockport, UK). Free cholesterol was analysed by an enzymatic method (Boehringer-Mannheim, Lewes, Sussex). Apolipoproteins (apos) AI and B were determined by rate immunonephelometric techniques on the Beckman Array, using standards and controls provided by the manufacturer (Beckman Instruments, Palo Alto, USA).

2.2.1. 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 [13,14].

2.2.2. 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) was determined by a continuous spectrophotometric method at 405 nm as previously described [15].

2.2.3. PON1 concentration. Serum PON1 concentration was determined by an ELISA method utilising rabbit anti-human PON1 monospecific polyclonal antisera as described previously [16,17].

2.2.4. Statistical analysis. The statistical significance of differences between parameters with a gaussian distribution were sought by Student's *t*-test and between parameters with a non-gaussian distribution by the Mann-Whitney U test. Pearson's *r* was used to determine the strength of correlation between parameters.

3. Results

The serum lipid and lipoprotein concentrations of the subjects are shown in Table 1. In all cases, as expected, the concentration in the interstitial fluid was much less than in the serum. The concentration of both apo AI and apo B in the interstitial fluid were related to their serum concentrations, $r = 0.656$, $P < 0.01$ for apo AI and $r = 0.504$, $P < 0.05$ for apo B. However, there was no such relationship between serum and interstitial fluid total cholesterol, free cholesterol and triglyceride.

The PON1 activity in interstitial fluid was 15.7 (2.3–183.0) nmol/min/ml (median (range)) which was 15% of that found in serum (105.3 (74.6–323.9) nmol/min/ml) (Table 1). PON1 activity in interstitial fluid was not correlated with any of the

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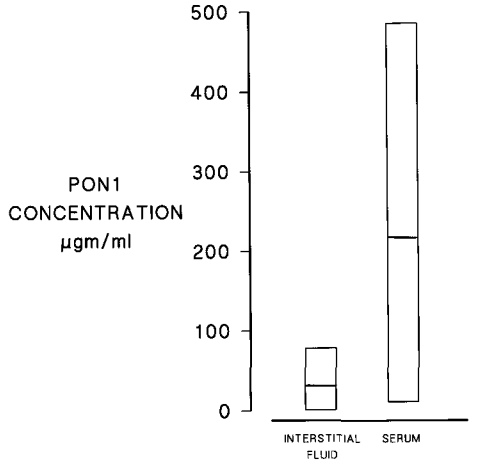

Fig. 1. PON1 concentration in interstitial fluid (IFPON) and serum (SEPON). PON1 concentration was determined in interstitial fluid and serum by our specific ELISA as described in Section 2. Length of boxes indicates the minimum and maximum values. The bar indicates the mean value.

other interstitial fluid or serum parameters investigated. The PON1 concentration in serum was related to the concentration of apo AI ($r=0.727$, $P<0.001$). The PON1 concentration in interstitial fluid (20.2 (1.1–78.1)) μg/ml (median (range)) was 20% of that found in serum (109.6 (11.1–485.7)) μg/ml ($P<0.001$) (Fig. 1) and the two concentrations were correlated ($r=0.560$, $P<0.025$) (Fig. 2a). PON1 concentration in interstitial fluid was also related to the concentration of apo AI in the interstitial fluid ($r=0.690$, $P<0.005$) (Fig. 2b) but not to the concentration of serum HDL cholesterol or PON1. However, the ratio of PON1 concentration to apo AI was lower in interstitial fluid (0.60 ± 0.20) than in the serum (0.95 ± 0.18) ($P<0.001$). The interstitial fluid:serum ratio of the concentration of apo AI was 0.29 ± 0.05 and was significantly higher than that of PON1 which was 0.18 ± 0.04 ($P<0.05$) (Fig. 3).

The PON1 genotype distributions in the study subjects were 44% AA and 56% AB for the 192 polymorphism and 6% MM, 56% LM and 38% LL for the 55 polymorphism. Some studies have indicated a relationship between the genetic polymorphisms of PON1 and plasma lipids and lipoproteins. However, none of the interstitial fluid parameters measured were affected by either of the polymorphisms.

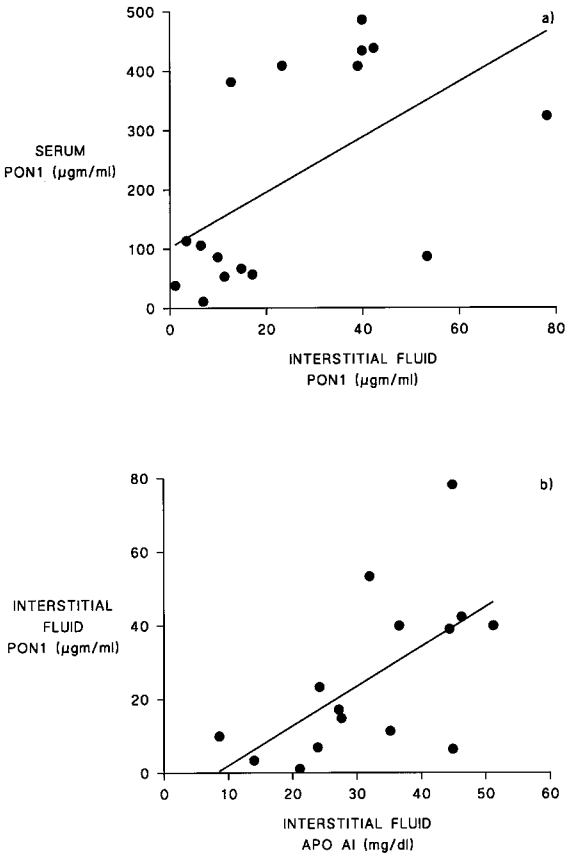

Fig. 2. a: Correlation between PON1 concentration in interstitial fluid (IFPON) and serum (SEPON). b: Correlation between interstitial fluid PON1 (IFPON) and Apo AI (IFAPOAI) concentrations.

Multiple regression analysis to determine which factors determine the interstitial fluid concentration of PON1 and which included as co-variables all the lipid and lipoprotein parameters listed in Table 1 and additionally serum PON1 concentration, age, gender and the PON-55 and 192 genotypes indicated that the concentration of interstitial fluid apo AI was responsible for 37% of the variation in interstitial fluid PON1 concentration ($P<0.025$). None of the other parameters significantly accounted for variation in the PON1 concentration in interstitial fluid.

Table 1
Serum lipid, lipoprotein and apolipoprotein concentrations in serum and interstitial fluid of the study subjects

	Serum	Interstitial fluid
Total cholesterol (mmol/l)	5.61 ± 1.32	0.92 ± 0.22*
Triglyceride (mmol/l)	1.57 ± 0.76	0.19 ± 0.08*
Free cholesterol (mmol/l)	1.45 ± 0.50	0.23 ± 0.10*
VLDL cholesterol (mmol/l)	0.40 ± 0.24	ND
LDL cholesterol (mmol/l)	4.37 ± 0.82	ND
HDL cholesterol (mmol/l)	1.74 ± 0.52	ND
Apolipoprotein AI (mg/dl)	115.8 ± 22.2	33.8 ± 11.3*
Apolipoprotein B (mg/dl)	91.7 ± 29.8	2.2 ± 0.08*
Paraoxonase activity (nmol/min/ml)	105.3 (74.6–323.9)	15.7* (2.3–183.0)

Serum and interstitial fluid lipids and lipoproteins were analysed in the 16 subjects as described in Section 2.
*Significantly different from serum concentration $P<0.001$.
ND = Not determined.

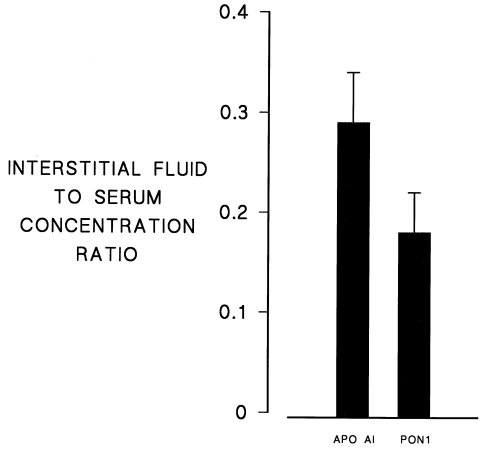

Fig. 3. Ratio of concentration of Apo AI and PON1 in interstitial fluid and serum. Values are mean and error bars represent the standard deviation.

4. Discussion

PON1 was first shown by us to reduce LDL lipid peroxidation in vitro [1,2] a finding which has subsequently been confirmed and extended by others [4,5]. This has led to a postulated role for PON1 as an anti-atherosclerotic agent in vivo. It is proposed that the pro-atherogenic modifications of LDL take place in the sub-intimal space of the artery wall in areas poor in anti-oxidant defences [8]. HDL is present in the artery wall at a much higher concentration than LDL [9] and should, therefore, be ideally placed to protect LDL from oxidative modification. However, in order for PON1 to be anti-atherogenic it must retain its association with HDL on passing from the blood to the interstitial fluid, where it can protect LDL from pro-atherogenic modifications. In this investigation we have shown that PON1 is present in interstitial fluid in an active form. We have previously shown that the PON1 genotype influences activity towards paraoxon [18] and lipid peroxides [19]. In this study, the amino acid substitutions did not affect the transfer of PON1 to interstitial fluid or is sequestration in the sub-intimal space.

PON1 in serum is associated with a specific HDL sub-species also containing apo AI and clusterin [20]. It has been suggested that this particle may play a major role in protecting cell membranes and lipoproteins from oxidative damage due to the presence of PON1 and clusterin [10]. Clusterin has been postulated to protect cell membranes from oxidative damage by binding to and removing damaged membrane lipids [21]. Using immunohistochemical techniques we have previously shown that PON1, apo AI and clusterin are present in the normal human artery wall and that immunostaining for PON1 greatly increases as atherosclerosis develops [7] providing circumstantial evidence for the involvement of the PON1-HDL particle in atherogenesis, perhaps as a reaction to the oxidative element in the process. In the present study, the ratio of the concentrations of PON1 to apo AI fell from 0.95 in serum to 0.60 in the interstitial fluid. This indicates either a loss of PON1 from HDL particles in the interstitial fluid or sequestration of the minor sub-fraction of HDL which contains PON1. Using crossed immunoelectrophoresis PON1 was only associated with mature α migrating HDL and not pre-β HDL (result not shown). The proportion of apo AI present in pre-β HDL is greater in interstitial fluid than in serum but this difference is probably not large enough to account for the change in the PON1:apo AI ratio found in this investigation. An alternative explanation is provided by our earlier findings using immunohistochemistry of the human artery wall which indicated that a significant amount of PON1 staining could be found on the membranes and within lysosomes of smooth muscle cells [7]. This would indicate that a significant amount of the PON1-HDL has a physical association with tissue (where it could be protecting cell membranes and/or undergoing degradation) and will not be detected in the tissue fluid. This may also explain why there was no relationship between PON1 concentration in interstitial fluid and that in the serum.

In conclusion the results presented here indicate that PON1 is present in the interstitial fluid and that a major determinant of its concentration is the interstitial fluid apo AI concentration. It is therefore evident that the association between PON1 and HDL found in serum [22] remains at least as HDL passes into the interstitial fluid and that PON1 in interstitial fluid is available to prevent the oxidative modification of LDL and perform its postulated anti-atherogenic and anti-inflammatory role.

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References

[1] Mackness, M.I., Arrol, S. and Durrington, P.N. (1991) FEBS Lett. 286, 152–154.
[2] Mackness, M.I., Arrol, S., Abbott, C.A. and Durrington, P.N. (1993) Atherosclerosis 104, 129–135.
[3] Mackness, M.I., Abbott, C.A., Arrol, S. and Durrington, P.N. (1993) Biochem. J. 294, 829–835.
[4] Watson, A.D., Berliner, J.A., Hama, S.Y., La Du, B.N., Fault, K.F., Fogelman, A.M. and Navab, M. (1995) J. Clin. Invest. 96, 2882–2891.
[5] Navab, M., Hama-Levy, S., Van Lenten, B.J., Fonarow, G.C., Gardinez, C.J. and Castellani, L.W. et al. (1997) J. Clin. Invest. 99, 2005–2019.
[6] Steinberg, D., Parthasarathy, S., Carew, T.E., Khoo, J.C. and Witztum, J.L. (1989) New Engl. J. Med. 320, 915–924.
[7] Mackness, B., Hunt, R., Durrington, P.N. and Mackness, M.I. (1997) Arterioscl. Thromb. Vasc. Biol. 17, 1233–1238.
[8] Steinberg, D. (1997) Circulation 95, 1062–1071.
[9] Eisenberg, S. (1984) J. Lipid. Res. 25, 1017–1058.
[10] Mackness, M.I. and Durrington, P.N. (1995) Atherosclerosis 115, 243–253.
[11] Kiistala, U. (1968) J. Invest. Dermatol. 50, 129–137.
[12] Mackness, M.I. and Durrington, P.N. (1992) in: Lipoprotein Analysis: A Practical Approach (Converse, C.A. and Skinner, E.R., Eds.), pp. 1–42, IRL Press, Oxford, UK.
[13] Adkins, S., Gan, K.N., Mody, M. and La Du, B.N. (1993) Am. J. Hum. Genet. 52, 598–608.
[14] Humbert, R., Adler, D.A., Disteche, C.M., Hassett, C., Omiecinski, C.J. and Furlong, C.E. (1993) Nature Genet. 3, 73–76.
[15] Mackness, M.I., Harty, D., Bhatnagar, D., Winocour, P.H., Arrol, S., Ishola, M. and Durrington, P.N. (1991) Atherosclerosis 86, 193–199.
[16] Abbott, C.A., Mackness, M.I., Kumar, S., Boulton, A.J.M. and Durrington, P.N. (1995) Arterioscler. Thromb. Vasc. Biol. 15, 1812–1818.
[17] Blatter Garin, M.-C., Abbott, C., Messmer, S., Mackness, M.I., Durrington, P.N., Pometta, D. and James, R.W. (1994) Biochem. J. 304, 549–554.

[18] Mackness, B., Mackness, M.I., Arrol, S., Turkie, W. and Durrington, P.N., Br. J. Pharmacol., in press.
[19] Mackness, M.I., Arrol, S., Mackness, B. and Durrington, P.N. (1997) Lancet 349, 851–852.
[20] Blatter, M.-C., James, R.W., Messmer, S., Barja, F. and Pometta, D. (1993) Eur. J. Biochem. 211, 871–879.
[21] Jordan-Starck, T.C., Witte, D.P., Aronow, B.J. and Harmony, J.A.K. (1992) Curr. Opin. Lipidol. 3, 75–85.
[22] Mackness, M.I., Mackness, B., Durrington, P.N., Connelly, P.W. and Hegele, R.A. (1996) Curr. Opin. Lipidol. 7, 69–76.