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Paraoxonase activity in human pericardial fluid: its relationship to coronary artery disease

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Summary. Pericardial fluid paraoxonase activity was compared with 3 biochemical markers of atherosclerosis (HDL, LDL/HDL ratio and Apolipoprotein A-I) and a significant association was found. When the paraoxonase activity in pericardial fluid samples was separated into 2 groups according to the degree of coronary atherosclerosis (slight and severe), most of the cases showing low levels of paraoxonase activity also showed severe coronary atherosclerosis. In addition, paraoxonase activity in pericardial fluid was found to be statistically correlated with HDL levels, which agrees with the results reported in serum.

Key words: "A"-esterases – Paraoxonase – Pericardial fluid – Atherosclerosis

Zusammenfassung. Die Paraoxonase-Aktivität in Perikard-Flüssigkeit wurde mit drei biochemischem Markern der Arteriosklerose (HDL, LDL/HDL-Verhältnis und Apolipoprotein A-I) vergleichen. Hierbei fand sich eine signifikante Assoziation. Wenn die Paraoxonase-Aktivität in Perikardsflüssigkeitsproben in zwei Gruppen unterteilt wurde, entsprechend dem Grad der koronaren Atherosklerose (leicht und schwer), zeigten die meisten der Fälle mit geringen Spiegeln von Paraoxonase-Aktivität gleichzeitig eine schwere koronare Atherosklerose. Zusätzlich wurde gefunden, daß die Paraoxonase-Aktivität in Perikardflüssigkeit statistich korreliert mit den HDL-Spiegeln, was mit den für das serum berichteten Ergebnisse übereinstimmt.

Schlüsselwörter: "A"-esterasen – Paraoxonase – Perikardflüssigkeit – Atherosklerose

Introduction

Paraoxonase (E.C. 3.1.1.2) is the "A"-esterase enzyme capable of hydrolysing the organophosphate paraoxon (O,O-diethyl-O-p-nitrophenyl phosphate). Recently, various reports indicated that serum "A"-esterase activity is associated with the high-density lipoprotein (HDL) (Mackness et al. 1985; Mackness 1989a). HDL is a molecule containing apolipoprotein A-I and is able to remove cholesterol from the arterial walls. Therefore, it protects against the development of atherosclerosis.

Low concentrations of HDL and apolipoprotein A-I in plasma have been connected with premature coronary artery disease so that it could be possible to use serum paraoxonase as an indicator of susceptibility to develop atherosclerosis (Mackness 1989b). In addition, it is of considerable interest for the possible relationship between serum paraoxonase and coronary artery disease and, consequently, between the enzyme and myocardial infarction.

Various biochemical parameters (CK, CK-MB, myoglobin) are currently measured in serum for the clinical diagnosis of myocardial infarction. Several studies (Luna et al. 1983; Hougen et al. 1992; Valenzuela et al. in press) have established that these parameters can be investigated in pericardial fluid for the postmortem diagnosis of both the degree of coronary atherosclerosis and myocardial infarction.

The main aim of this study was to see whether there is a correlation between paraoxonase activity in the pericardial fluid and the degree of coronary atherosclerosis. In addition, we have also studied the possible relationship between other biochemical parameters of coronary atherosclerosis (HDL, LDL/HDL ratio and apolipoprotein A-I) and paraoxonase activity measured in pericardial fluid. The reliability of this new biochemical parameter for such a purpose was studied after establishing the identity of serum and pericardial fluid paraoxonases as previously reported (Hernandez et al. 1992).

Materials and methods

Pericardial fluid from 87 corpses autopsied at the Institute of Forensic Pathology of the University of Copenhagen was taken by pericardial puncture using a sterilized syringe. All samples were centrifuged at

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1000 g for 10min, and stored at –40°C until analysis. All corpses were cases of death caused by brain damage, multiple trauma, myocardial infarction and other natural deaths of which, 42 had slight coronary atherosclerosis and 45 were diagnosed as severe coronary atherosclerosis. Among all cases with severe atherosclerosis, 34 (75%) suffered a myocardial infarction diagnosed both microscopically and biochemically. From 42 cases with slight atherosclerosis only 13 (31%) were diagnosed as myocardial infarction.

The degree of stenosis of the coronary arteries is critically important in determining the functional significance of atherosclerotic disease (Buja and Willerson 1991). The areas of maximal narrowing were noted by specifying the degree of reduction of the cross-sectional area of the lumen (Virmani et al. 1991). A case was considered as slight atherosclerosis when the reduction of all coronary arteries was less than 75% of the lumen. This group included both the cases with slight and moderate degrees of atherosclerosis (as described by Virmani et al. 1991). Cases included in the group with severe atherosclerosis showed a reduction of the lumen greater than 75% of one or more coronary arteries.

Paraoxonase activity was measured by a modification of the method of Reiner and Radic (1985) recording the 4-nitrophenol cleaved from the hydrolysis of paraoxon. The substrate solution (0.95ml 0.1*M* Tris-HCl buffer containing 1m*M* $CaCl_2$ and 2m*M* paraoxon) was preincubated at 37°C. The reaction was started by adding 0.05ml of sample and the change in absorbance at 405nm was recorded for 2min at 37°C, using a Shimadzu UV-160 spectrophotometer. Non-enzymic hydrolysis was measured under the same assay conditions using 0.05ml of buffer instead of sample.

HDL-cholesterol was determined by the enzymatic method (Lipid Research Clinic Programs, 1974). Concentrations of LDL-cholesterol were estimated according to the equation of Friedewald et al. (1972) and apolipoprotein A-I was quantified by radio-immunoassay (RIA, Ventrex Labs. Inc.).

The diagnosis of myocardial infarction was carried out in all cases studied and included the use of both morphological and biochemical methods. The former consisted of a macroscopical study of the myocardium and the 4 major epicardial coronary arteries (left main, left anterior descending, left circumflex and right coronary arteries) as well as a histological examination of sections of the heart stained with hematoxylin-eosin (H&E) and van-Gieson. Biochemical analysis included the determination of the K/Na ratio in myocardial tissue, creatin kinase activity and its isoenzymes and myoglobin in pericardial fluid according to methods previously described (Hougen et al. 1992). Samples for microscopical and biochemical studies were taken from 4 specific sites of the myocardium: the anterior wall of the left ventricle (A), the posterior wall of the left ventricle (B), the interventricular septum (C) and the lateral wall of the right ventricle (D).

Biochemically, a case was regarded as positive for infarction if the K/Na ratio was equal to or less than 0.7 in at least 2 different areas of the myocardium (A, B, or C), or if two out of three of the following parameters were present: K/Na ratio equal to or less than 0.7 in only one of the same 3 above-mentioned areas, positive myoglobin in pericardial fluid, or CK-MB equal to or higher than 15% of total CK activity in pericardial fluid (Hougen et al. 1992).

Statistical studies were performed by Chi square test and linear regression.

Results and discussion

The paraoxonase activity in pericardial fluid in cases of slight atherosclerosis ranged from 6.1 to 171mU/ml (mean 41.7 ± 43.5; $n = 42$) and in the severe atherosclerosis group from 4.4 to 133mU/ml (mean 26.4 ± 25.0; $n = 45$).

Figure 1 shows the values of paraoxonase activity in pericardial fluid classified into 2 groups: slight and severe atherosclerosis. Most of the cases with a diagnosis of severe coronary atherosclerosis showed low values of paraoxonase activity (58% of severe atherosclerosis had paraoxonase activity lower than 30 mU/ml). On the contrary, among all the cases with slight atherosclerosis 60% showed paraoxonase activity higher than 30 mU/ml.

Furthermore, 3 biochemical markers of atherosclerosis (HDL, LDL/HDL ratio and apolipoprotein A-I) (de Baker et al. 1982) were also compared with the paraoxonase activity in pericardial fluid. Significant statistical correlations were found between the LDL/HDL ratio and paraoxonase activity ($r = -0.273$; $p < 0.05$; $n = 59$). It is an inverse correlation since this enzyme activity is associated with HDL. Therefore low values of paraoxonase activity correspond to low values of HDL and, consequently, with higher values of the LDL/HDL ratio. Moreover, paraoxonase activity is significantly correlated to a third biochemical marker of the degree of atherosclerosis, apolipoprotein A-I ($r = 0.246$; $p < 0.05$; $n = 59$), the protein carrier of the high density lipoproteins (HDL).

Figure 2 shows the correlation between HDL and paraoxonase. Values of paraoxonase activity below 30 mU/ml together with a HDL below 40 mg/dl are suggestive of severe atherosclerosis. A total of 31 cases from 59 were in-

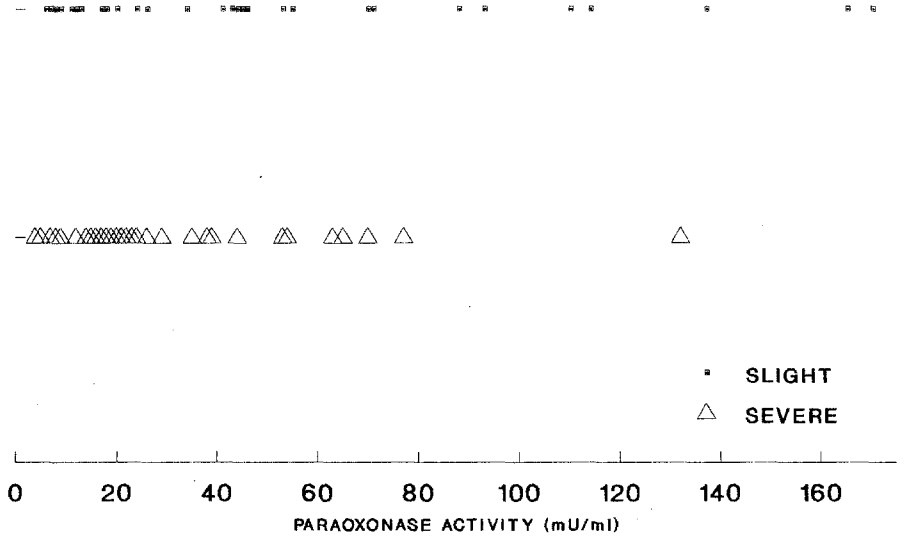

Fig. 1. Paraoxonase activity in pericardial fluid in two groups of coronary atherosclerosis: slight < 75% of coronary occlusion and severe > 75%

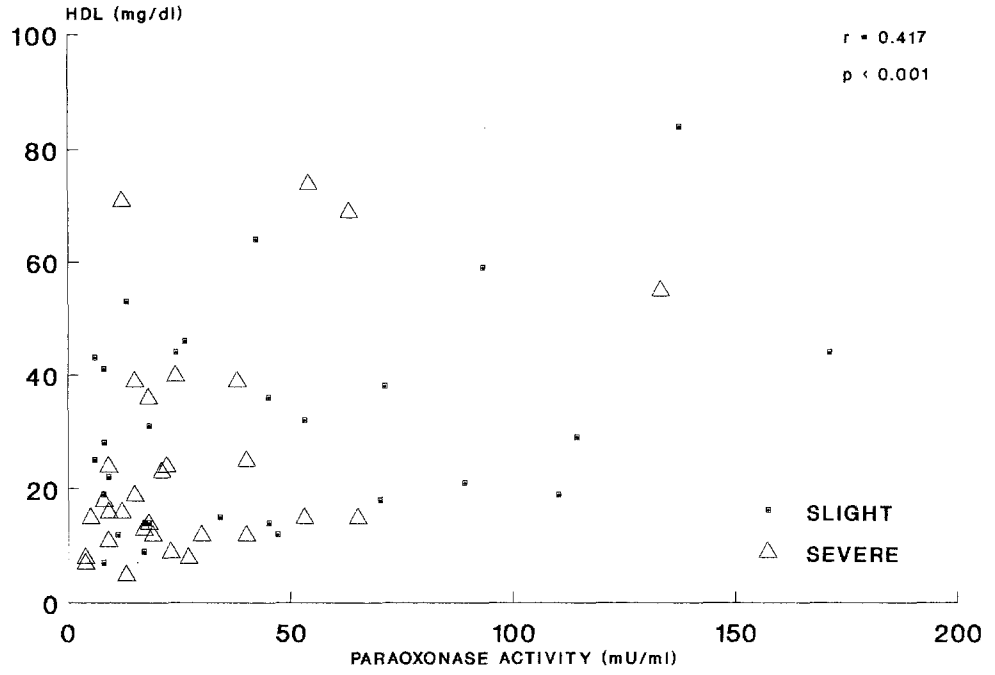

Fig. 2. Correlation between paraoxonase activity and HDL levels in pericardial fluid (■ slight atherosclerosis, △ severe atherosclerosis)

Table 1. Comparison between paraoxonase activity in pericardial fluid, degree of coronary atherosclerosis and myocardial infarction

Atherosclerosis	Myocardial infarction	Paraoxonase (mU/ml)	
		< 30	⩾ 30
Slight	Yes	9	4
	No	15	14
Severe	Yes	23	11
	No	10	1

cluded in that group, and of these, 21 cases (68%) had severe atherosclerosis while 10 (32%) had slight atherosclerosis. These results show that the combination of the paraoxonase activity to the HDL levels could be used to assist the postmortem biochemical diagnosis of atherosclerosis.

These results are in accordance with the possibility suggested by Mackness (1989b) that paraoxonase could be an indicator of susceptibility to the development of coronary atherosclerosis.

As is shown in Table 1, more cases of myocardial infarction were included in the group of lower paraoxonase activity and severe degree of atherosclerosis, although no significant differences were found when the different groups were compared.

The lower paraoxonase activity in the myocardial infarction group was expected taking into account that this esterase activity is associated with HDL and HDL is inversely correlated to coronary atherosclerosis (Miller and Miller 1975; Castelli et al. 1977). Similar low values of paraoxonase were previously reported for serum of myocardial infarction patients (McElveen et al. 1986).

Based on the results obtained it could be concluded that paraoxonase activity in pericardial fluid is a new postmortem biochemical marker of coronary atherosclerosis. The fact that low levels of paraoxonase activity are also found in cases of myocardial infarction points to the possibility of an atherosclerotic origin for these infarctions.

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