Volume 286, number 1,2, 152–154 FEBS 09936 July 1991

ADONIS 001457939100647T

Paraoxonase prevents accumulation of lipoperoxides in low-density lipoprotein

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Received 7 May 1991

Oxidative modification of low-density lipoprotein (LDL) enhances its uptake by macrophages in tissue culture and in vivo may underly the formation of arterial fatty streaks, the progenitors of atheroma. We investigated the possible protection which high-density lipoprotein (HDL) affords against LDL oxidation. The formation of lipoperoxides and thiobarbituric acid reactive substances when LDL was incubated with copper ions was significantly decreased by HDL. The enzyme, paraoxonase (E.C. 3.1.8.1), purified from human HDL, had a similar effect and thus may be the component of HDL responsible for decreasing the accumulation of lipid peroxidation products.

Lipoprotein oxidation; High-density lipoprotein; Low-density lipoprotein; Paraoxonase

1. INTRODUCTION

Oxidation of low-density lipoprotein (LDL) by redox metals, macrophage, smooth muscle or endothelial cells in tissue culture modifies its structure so that it binds to the acetyl low-density lipoprotein receptor of monocyte-derived macrophages [1,2]. Its subsequent avid uptake by these cells in vitro leads to the formation of foam cells. These resemble the main cell type of the arterial fatty streak, the progenitor of atheroma. It has been suggested that similar events may occur in vivo leading to atherogenesis. Epidemiological evidence has revealed that whereas the LDL concentration in the plasma related directly to the risk of developing coronary heart disease, high-density lipoprotein (HDL) is inversely related to risk [3]. However, the mechanism for the apparently protective effect of HDL remains a mystery [4]. It is known that the oxidative damage to low-density lipoproteins proceeds via the formation of lipoperoxides of phospholipids containing unsaturated fatty acids. These break down to release reactive aldehydes and thence through a phospholipase A_2 reaction to lysophospholipids [5]. Here, we report the prevention of lipoperoxide generation during the Cu^{2+}-induced oxidation of low-density lipoprotein, by high-density lipoproteins and one of their component enzymes, paraoxonase (EC 3.1.8.1) [6].

2. MATERIALS AND METHODS

2.1. *Preparation of lipoproteins*

Low-density lipoproteins (LDL), high-density lipoprotein (HDL) and its subfractions HDL_2 and HDL_3 were prepared by sequential ultracentrifugation [7] from the serum of 7 normolipidaemic volunteers, one patient with heterozygous familial hypercholesterolaemia (FH) and one subject with homozygous FH. After exhaustive dialysis against phosphate-buffered saline (PBS) (0.05 M, pH 7.3) lipoproteins were stored at 4°C under nitrogen until used.

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2.2. *Purification of paraoxonase*

Paraoxonase was purified from the serum of a female donor as described [8] which results in a single band on SDS-PAGE. The resulting preparation was 900-fold purified with respect to serum.

2.3. *Oxidation of LDL by Cu^{2+}*

LDL was oxidised as described [9]. Either 0.1 mg of LDL protein (in experiments to determine the amount of thiobarbituric acid reactive substances (TBARS)), or 1.5 mg of LDL protein (for lipoperoxide determination) was incubated in EDTA-free PBS with 5 μM $CuSO_4$ in the presence or absence of HDL, HDL_2, HDL_3, paraoxonase or Tris buffer in a total volume of 1.0 ml. Blanks consisted of PBS plus $CuSO_4$. Control incubations were performed omitting $CuSO_4$. Oxidation was terminated by the addition of 24 μM EDTA and 20 μM butylated hydroxytoluene. Incubation periods varied from 0 to 24 h.

2.4. *Analytical procedures*

Lipid peroxidation in the incubations was estimated by two methods. Firstly, as the fluorescent reaction with thiobarbituric acid (TBARS) using tetramethoxy-propane as a standard [10] and secondly as the reaction with CHOD-iodide to determine lipoperoxides [11].

3. RESULTS AND DISCUSSION

HDL protected against lipoperoxide formation during Cu^{2+} oxidation of LDL (Fig. 1), but did not influence the concentration of reactive aldehydes (TBARS) (Table I). HDL was itself resistant to oxidation compared to LDL. Both HDL_2 and HDL_3 gave protection against lipoperoxide formation and a slight, but non-significant decrease in detectable aldehydes (Table I), although the latter effect varied widely be-

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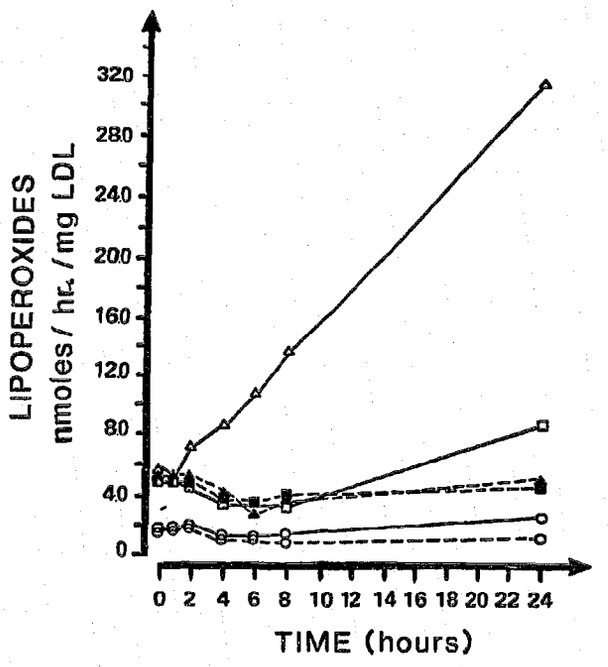

Fig. 1. The effect of HDL on Cu^{2+}-catalysed LDL oxidation. 1.5 mg of LDL and/or HDL protein was incubated at 37°C in the presence or absence of 5 μM Cu^{2+} in a total volume of 1 ml (made up with PBS) for various times before assay for lipoperoxides as described [11]. Blanks consisted of PBS + Cu^{2+} only. (△—△) LDL with Cu^{2+}, (▲---▲) LDL without Cu^{2+}, (○—○) HDL with Cu^{2+}, (○---○) HDL without Cu^{2+}, (□—□) LDL plus HDL with Cu^{2+}, (■---■) LDL plus HDL without Cu^{2+}. The figure shown represents a typical experiment. Points are the mean of duplicate determinations.

tween individual preparations of LDL. This difference between protection against lipoperoxide and aldehyde formation may have been due to the hydrophobic environment of the lipoproteins. The TBARS formed during LDL oxidation are partially water-soluble. They may therefore have escaped into the aqueous environment and have been unavailable to HDL or its subfractions whereas the lipoperoxides remained in the lipid environment where they could interact with HDL. Since the level of TBARS was less than that of the lipoperoxides, the lack of effect of HDL on TBARS was not indicative of its potential to protect against oxidative modification of LDL. Indeed it has previously been reported that HDL prevents the changes in LDL electrophoretic mobility caused by oxidation [12] and that HDL will prevent LDL subjected to oxidising conditions from being taken up by cultured macrophages [13].

In our experiments the abolition of lipoperoxide generation by HDL was not confined to any single subfraction (Table I). Overall, as in the case of whole HDL, neither HDL_2 or HDL_3 significantly altered the production of TBARS. However, there was a greater tendency for the subfractions to do so which was more obvious with some LDL preparations. Although an unexplained similar phenomenon has previously been observed when HDL was immunologically fractionated [12]. Paraoxonase prevented both TBARS and lipoperoxide formation (Table I), only 1 μg of paraoxonase protein was required to produce the observed effect. The activity of the paraoxonase preparation was 4126 nmol paraoxon hydrolysed/min/mg protein compared to a serum activity for healthy individuals (n =

Table I

The effect of HDL_2, HDL_3 and paraoxonase on Cu^{2+}-catalysed LDL oxidation

Incubation mixture	Lipoproteins		TBARS	
	nmol/mg LDL protein/h	% of LDL response	nmol malondialdehyde equivalents/mg LDL protein/h	% of LDL response
LDL	19.3 ± 1.7	100	0.39 ± 0.06	100
LDL + Tris buffer	19.5 ± 2.1	101	0.363 ± 0.063	93.1
LDL + HDL	2.89 ± 0.15	14.9**	0.24 ± 0.031	62.5
LDL + HDL_2	0.46 ± 0.06	2.4**	0.25 ± 0.026	64.1
LDL + HDL_3	0.11 ± 0.02	0.6**	0.227 ± 0.027	58.2
LDL + paraoxonase	15.3 ± 2.3	79.3****	0.08 ± 0.017	20.5***

For lipoperoxide determination: 1.5 mg of LDL protein was incubated (i) alone, (ii) plus Tris buffer*, (iii) plus 1.5 mg HDL, (iv) plus 1.5 mg HDL_2 protein, (v) plus 1.5 mg HDL_3 protein, (vi) plus 1 μg paraoxonase. Blanks consisted of PBS + Cu^{2+} only. Incubation was at 37°C in the presence of 5 μM Cu^{2+}. Lipoperoxides were measured as described [11]. For TBARS determination: 50 μg of LDL protein was incubated (i) alone, (ii) plus Tris buffer, (iii) plus 50 μg HDL, (iv) plus 50 μg HDL_2, (v) plus 50 μg HDL_3, (vi) plus 1 μg paraoxonase. Blanks consisted of PBS + Cu^{2+} only. Incubation was at 37°C in the presence of 5 μM Cu^{2+}. TBARS were measured as described [10]. Figures are mean ± SE of the mean of 9 determinations. * Tris buffer = 0.025 M Tris/HCl, pH 8.0, containing 0.005 M $CaCl_2$, 5 μM EDTA, 20% (v/v) glycerol and 0.2% (v/v) Emulgen 911 and is the buffer in which paraoxonase is purified. ** Statistically significant from LDL response $P < 0.001$. *** Statistically significant from LDL response $P < 0.005$. **** Statistically significant from LDL response $P < 0.01$

79) of 119 nmol (+117, −59) paraoxon hydrolysed/min/ml serum (geometric mean +1 SD, −1 SD) [9]. In the circulation more than 85% of paraoxonase activity is located on HDL and the rest is not associated with lipoprotein [6]. The specific activity of paraoxonase on HDL is approximately 50 nmol/min/mg HDL protein. Our experiments tested the effect of 1.5 mg of HDL protein which thus contained 75 nmol/min of paraoxonase activity. The greater effect of HDL in preventing lipoperoxidation is probably therefore explicable because it was compared with an activity of 4 nmol/min in the experiments with purified enzyme.

Paraoxonase is known to hydrolyse organophosphate insecticides and nerve gasses [14]. However, its physiological role is unknown. It is possible that a biological action of paraoxonase is to hydrolyse lipid peroxides formed during the oxidation to harmless carboxylic acids. This possibility is being investigated in detail in our laboratory. This action may form the basis by which HDL protects against the development of coronary artery disease by preventing lipid peroxidation in LDL and ultimately, foam cell formation. It has not escaped our attention that HDL, which is present in tissue fluids at concentrations which even in the human are 10-fold greater than those of LDL [4], may have a more general role in protecting against oxidative damage in biological systems.

Other HDL enzymes such as lecithin cholesterol acyltransferase (LCAT) (EC2.3.1.43) and protease (EC 3.4.4.7) could also be involved in the protective function of HDL. However, the paraoxonase preparation used in these experiments was not contaminated with either LCAT or protease activity. Final proof of the role of paraoxonase could be obtained by the use of specific inhibitors of the enzyme. However, its two competitive inhibitors, which are highly toxic [15], are currently unavailable.

In the context of the potential of paraoxonase to protect against oxidative modification of LDL, it is interesting to note that serum paraoxonase activity is decreased in both diabetes and familial hypercholesterolaemia [16], two diseases in which there is a greatly increased risk of coronary heart disease and its serum activity is also decreased in myocardial infarction survivors [17]. The diversity of diseases, in the aetiology of which free radicals have been imputed, emphasises the cogent need for further examination of the biological role of HDL and paraoxonase.

Acknowledgements: This work was supported by grants from the British Heart Foundation, Medical Research Council and North Western Regional Health Authority.

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