Volume 257, number 2, 435–438 FEB 07809 November 1989

Protective effect of lipoproteins containing apoprotein A-I on Cu^{2+}-catalyzed oxidation of human low density lipoprotein

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Received 28 August 1989; revised version received 25 September 1989

Two apoprotein A-I (apoA-I)-containing lipoproteins, one containing apoA-I and apoA-II (LpA-I/A-II) and the other containing only apoA-I (LpA-I), were examined for their effect on Cu^{2+}-mediated oxidation of low density lipoprotein (LDL). The presence of LpA-I or LpA-I/A-II prevented LDL oxidation when assessed by the electrophoretic mobility, apoprotein B fragmentation and amounts of thiobarbituric acid-reactive substances. The protection of LDL oxidation by these lipoproteins was effective for up to 6 h, with LpA-I being more active than LpA-I/A-II. Results from these in vitro model experiments raise a possibility that LpA-I may play a role in protecting LDL from Cu^{2+}-mediated oxidation.

Lipoprotein oxidation; High density lipoprotein; Apolipoprotein A-I; Apolipoprotein A-II

1. INTRODUCTION

ApoA-I-containing lipoproteins isolated by immunological methods most probably reflect a more native state than conventionally available HDL because ultracentrifugation steps are not involved in the isolation procedures [1,2]. We isolated and characterized two different species of apoA-I-containing lipoproteins: lipoprotein containing apoA-I and apoA-II (LpA-I/A-II) and lipoprotein containing apoA-I but no apoA-II (LpA-I) [3–5]. Age- and sex-related changes in LpA-I were higher in females than in males, both during and after puberty, whereas LpA-I/A-II levels remained constant. Thus, we suggested that the sex difference in the incidence of coronary heart disease [5] is accounted for by the change in LpA-I levels. However, less is known about the functional difference of these lipoproteins at a molecular level. The protective role of HDL in atherosclerosis is generally attributed to acceleration of cholesterol efflux from cholesteryl ester-laden cells (foam cells), whereas no information is available on its direct role in intracellular accumulation of cholesteryl esters. The scavenger receptor for chemically modified LDL has been proposed as a potential mechanism for foam cell formation [6–9]. Recent studies focus on the identification of a natural ligand for this receptor, and an oxidative process which occurs in vivo to LDL is proposed as a major potential candidate [10–14]. As a first step to understand the role of HDL in the atherogenic process, we examined the effect of HDL and apoA-I-containing lipoproteins on Cu^{2+}-mediated oxidation of LDL in vitro.

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Abbreviations: LpA-I, lipoprotein containing apoprotein A-I with no apoprotein A-II; LpA-I/A-II, lipoprotein containing apoprotein A-I and A-II; HDL, high density lipoprotein; LDL, low density lipoprotein; PBS, phosphate-buffered saline; TBARS, thiobarbituric acid reactive substances

2. MATERIALS AND METHODS

2.1. *Preparation of lipoproteins*

LDL (d = 1.019–1.063) and HDL (d = 1.063–1.21) were isolated from human plasma by conventional ultracentrifugation [15]. Two species of apoA-I-containing lipoprotein were prepared by the combination of anti-apoA-I and anti-apoA-II immunosorbent columns as described previously [3–5]. Briefly, fresh human plasma was applied to an anti-apoA-I immunosorbent column. After washing with 0.01 M Tris-HCl, pH 7.5/0.5 M NaCl/1 mM EDTA (buffer A), the column was eluted with 0.1 M acetic acid/1 mM EDTA (pH 3.0). Each effluent was immediately adjusted to pH 7.4 with 1 M Tris solution, followed by dialysis against 0.15 M NaCl/1 mM EDTA (pH 7.4). The lipoprotein fraction containing apoA-I was then concentrated and applied to an anti-apoA-II immunosorbent column to isolate LpA-I and LpA-I/A-II. The column was washed with buffer A to obtain the unbound fraction (LpA-I). The bound fraction corresponding to LpA-I/A-II was eluted with 0.1 M acetic acid/1 mM EDTA (pH 3.0). To examine the effect on Cu^{2+}-mediated LDL oxidation, LpA-I and LpA-I/A-II were dialyzed against PBS to remove EDTA.

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

LDL was oxidized as described [16]. Briefly, 0.1 mg of LDL was incubated at 37°C in 1.0 ml of EDTA-free PBS with 5 μM $CuSO_4$ in the presence or absence of LpA-I, LpA-I/A-II, HDL or human serum albumin (HSA). Control incubations were done in the presence of 0.2 mM EDTA but no $CuSO_4$. Oxidation was terminated by refrigeration and the addition of 0.2 mM EDTA and 40 μM butylated hydroxytoluene. Incubation periods were varied between 3 and 12 h.

2.3. *Analytical procedures*

Agarose gel electrophoresis of lipoproteins was performed using a

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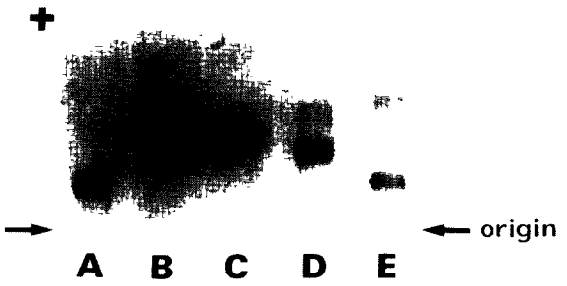

Fig.1. Effect of LpA-I and LpA-I/A-II on Cu^{2+}-mediated oxidation of LDL. LDL (0.1 mg) was incubated, in a final volume of 1.0 ml of PBS, with 5 μM $CuSO_4$ at 37°C for 3 h in the absence (B) or presence of 150 μg/ml of HDL (C), LpA-I/A-II (D), or LpA-I (E). As a control, LDL was incubated in parallel without $CuSO_4$ (A). Each sample was run on agarose gel electrophoresis. Arrows show the origin of electrophoresis.

Pol-E film system (Corning) at pH 8.6, followed by staining with Fat Red 7B. Sodium dodecyl sulfate gradient gel electrophoresis (4–30%) was performed as described [3–5] and stained by Coomassie brilliant blue R-250. Protein was measured as described in [17]. In this study, lipoprotein (LDL, HDL, LpA-I/A-II and LpA-I) concentrations were expressed in mg protein/ml. Lipid peroxidation was estimated as the fluorescent reaction with thiobarbituric acid using freshly diluted tetramethoxypropane as a standard [18].

3. RESULTS

LDL (100 μg) was mixed with 150 μg/ml of LpA-I/A-II, LpA-I or HDL in a total volume of 1.0 ml of PBS. The oxidation was initiated by the addition of 10 μl of 0.5 mM $CuSO_4$, followed by incubation at 37°C for 3 h. Upon agarose gel electrophoresis (fig.1), LDL increased its electrophoretic mobility by oxidation with Cu^{2+} (lane B). Under the conditions, the presence of LpA-I (lane E), LpA-I/A-II (lane D) or HDL (lane C) significantly prevented the increase in the electrophoretic mobility of LDL, suggesting the protective effect of these lipoproteins on LDL oxidation by $CuSO_4$. We extended the incubation time from 3 to 12 h. The results obtained were almost identical or similar for up to 6 h-incubation, whereas these protective effects of LpA-I/A-II, LpA-I or HDL were no longer observed after 12 h-incubation (data not shown). Therefore, the following data represent those obtained by 3 h-incubation.

In our next attempt, the same oxidation reaction was performed in the presence of LpA-I/A-II, LpA-I, HDL, or HSA at three different concentrations (30, 100 and 200 μg/ml). Extents of LDL oxidation were then assessed both by the increase in the electrophoretic mobility and the change in TBARS levels. As shown in fig.2, the increase in electrophoretic mobility was prevented by the presence of LpA-I/A-II, or LpA-I or HSA in a dose-dependent manner (from 30 to 200 μg/ml, $P < 0.005$). LpA-I was more effective than LpA-I/A-II at the concentration of 200 μg/ml, but similar to HSA. Although a protective effect reached a maximum level at 100 μg/ml, HDL itself was also pro-

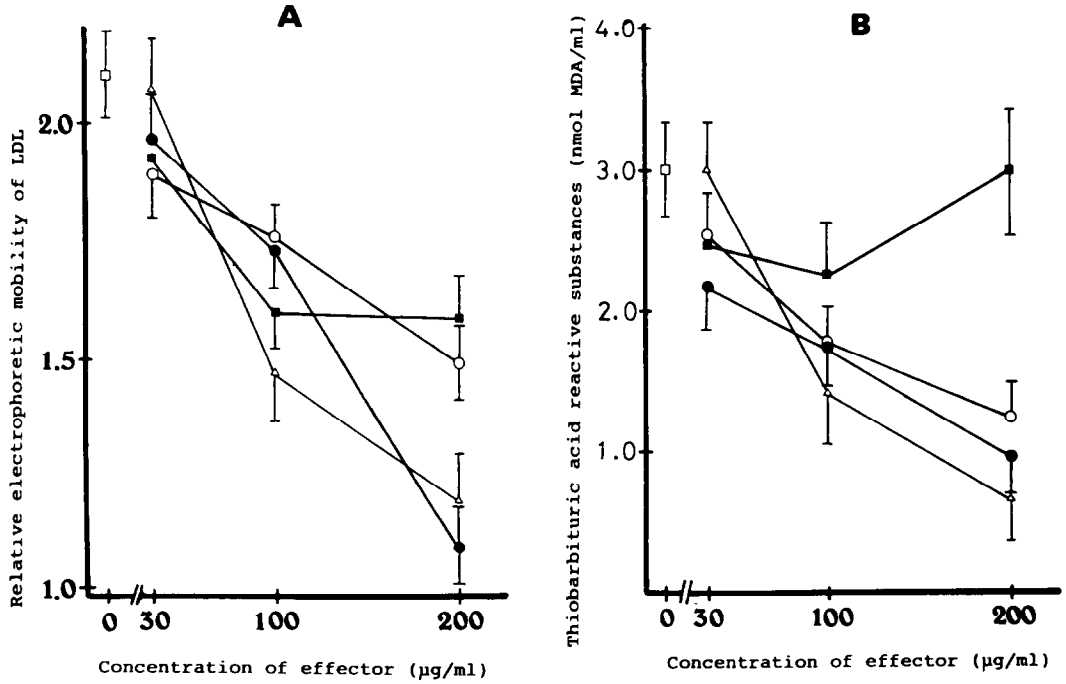

Fig.2. Dose-dependent effects of ApoA-I-containing lipoproteins on LDL oxidation. Each reaction mixture contained, in a 1.0 ml of PBS, 5 μM $CuSO_4$ and three different concentrations of LpA-I (●), LpA-I/A-II (○), HDL (■), HSA (△) and control LDL with no effector (□). After incubation at 37°C for 3 h, a portion of each sample was run on agarose gel electrophoresis and stained. The increase in electrophoretic mobility of each LDL sample was expressed as relative ratio to that of unmodified LDL (A). Another portion of the sample was determined for TBARS levels as described in section 2 (B). Each value was the mean of triplicate experiments and bars show the range.

tective. Similar to these effects on the electrophoretic mobility, LpA-I/A-II, LpA-I and HSA decreased dose-dependently TBARS levels to a similar extent (from 30 to 200 μg/ml, $P < 0.05$). The presence of HDL did not decrease TBARS levels, and when effects of HDL at 100 μg/ml were compared with those at 200 μg/ml, amounts of TBARS appeared to increase. But no statistical significance was observed.

The fragmentation of apoprotein B is known to occur to Cu^{2+}-mediated oxidation of LDL [16]. Our LDL contained apoB-100, apoB-48 and a few subspecies (lane B), but all protein bands disappeared after oxidation by Cu^{2+} (lane C). LpA-I contained unknown protein of 54 kDa which is now under investigation. Therefore, the protein band (54 kDa) seen in lane E was not the fragment of apoB. As shown in fig.3, apoprotein B fragmentation (lane C) was virtually prevented by the presence of LpA-I/A-II (lane D), LpA-I (lane E) or HDL (lane F). It was also noted that apoA-I of these three lipoproteins seemed intact during oxidation, suggesting that apoA-I itself was resistant to Cu^{2+}-mediated oxidation.

4. DISCUSSION

In the present study, we showed the protective effect of LpA-I, LpA-I/A-II and HDL on Cu^{2+} catalyzed oxidation of human LDL. As shown in fig.2, the protective effect was not specific for these lipoproteins; HSA also could prevent Cu^{2+}-mediated oxidation of LDL. Therefore, it seems unlikely that apoA-I-containing lipoproteins might play an exclusive role in preventing LDL oxidation under normal conditions. However, under pathological conditions such as the atherosclerotic process where the generation of reactive oxygen molecules is increased [19], these lipoproteins could play some role in preventing LDL oxidation.

Fig.3. Effect of ApoA-I-containing lipoproteins on apoprotein B fragmentation. LDL (0.1 mg) was incubated for 3 h at 37°C in 1.0 ml of PBS with 5 μM $CuSO_4$ in the absence (C) or presence of 150 μg/ml of LpA-I/A-II (D), LpA-I (E), or HDL (F). Each sample was run on sodium dodecyl sulfate gradient gel electrophoresis. (A) Molecular weight standards in ascending order are 14400, 20100, 30000, 43000, 67000 and 94000. (B) Native LDL.

Hinsberg et al. [20] reported the function of HDL as an anti-oxidant. When determined by an electrophoretic mobility of modified LDL preparations, HDL effectively prevented LDL from modification by endothelial cells, but had only a partial effect on Cu^{2+}-mediated LDL oxidation, whereas in both cases TBARS levels were not altered upon addition of HDL. Although they did not test the effect of subspecies of HDL on LDL oxidation, these data were consistent with our present data; the increase in the electrophoretic mobility of Cu^{2+}-mediated LDL was slightly prevented by HDL while TBARS levels were not affected at all under the same conditions (figs 1 and 2).

It is of interest that the effects of HDL and affinity purified lipoproteins on the oxidation of LDL differed, particularly in terms of the amount of TBARS. In our preliminary study, HDL was found to consist of LpA-I/A-II (60–65%) and LpA-I (35–40%), suggesting that 200 μg of HDL comprised approx. 125 μg of LpA-I/A-II and 75 μg of LpA-I. At this concentration range of these apoA-I-containing lipoproteins (75–125 μg/ml), TBARS levels of HDL were much higher than those of LpA-I and LpA-I/A-II (see fig.2). Thus, it seems likely that the lipid composition of HDL might be altered during ultracentrifugation steps to be more susceptible than the affinity purified lipoprotein to Cu^{2+}-induced lipid peroxidation.

According to the current concept of LDL oxidation, chemically active aldehydes such as malondialdehyde or 4-hydroxynonenal generated by lipid peroxidation from polyunsaturated fatty acids of LDL phospholipids might selectively modify lysine residues of LDL apoprotein B, thus increasing a net negative charge of LDL [21,22]. We observed the different effects of LpA-I/A-II and LpA-I on the oxidation of LDL. Since LpA-I/A-II and LpA-I generated the similar amount of malondialdehyde (see fig.2B), LpA-I might act by preventing apoprotein B from its interaction with malondialdehyde more effectively than LpA-I/A-II. However, since we did not measure 4-hydroxynonenal, it would also be possible that the presence of LpA-I/A-II may increase the concentration of 4-hydroxynonenal more so than does LpA-I.

With respect to a functional difference between LpA-I/A-II and LpA-I, Barbaras et al. [23] reported that only LpA-I was active in promoting cholesterol efflux from fat cells. However, in our preliminary ex-

periments with cholesteryl ester-laden rat peritoneal macrophages which were induced by acetylated LDL, cholesterol efflux from these cells was effectively enhanced either by LpA-I/A-II or by LpA-I to a similar extent. The reason for the discrepancy between their data and ours is not clear. The property of these lipoproteins could differ. They first isolated HDL by conventional ultracentrifugation and used affinity chromatographies to prepare LpA-I and LpA-I/A-II, while LpA-I/A-II and LpA-I used in the present study were isolated directly from the plasma. Differences in the cells used for the efflux assay (fat cells versus macrophages) may also contribute in part to the discrepancy.

From age- and sex-related changes in LpA-I and the constancy of LpA-I/A-II levels, we proposed that LpA-I might be responsible for the sex difference in the incidence of coronary heart disease [3,5]. If the oxidation of LDL in an extravascular space could be one of the initial events in atherogenesis [24,25], the present finding that LpA-I is more active than LpA-I/A-II in protecting LDL against its in vitro oxidation by Cu^{2+} would be consistent with our contention. Thus, one can assume that the anti-oxidant activity of apoA-I-containing lipoproteins might account in part for a protective role of HDL in atherogenesis.

Acknowledgements: This work was supported by Uehara Memorial Foundation to T.O. and Byotai-Taisha Foundation to S.H. We thank Ms M. Ohara for helpful comments.

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