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HIGH-DENSITY LIPOPROTEIN ANTAGONIZES THE INHIBITORY EFFECTS OF OXIDIZED LOW-DENSITY LIPOPROTEIN AND LYSOLECITHIN ON SOLUBLE GUANYLYL CYCLASE

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Oxidatively modified LDL (LDLox) reduces the response of soluble guanylyl cyclase to nitrovasodilators. We now demonstrate that this desensitization can be antagonized by HDL. Similar to its protective effect against LDLox, HDL also inhibited the lysolecithin-induced desensitization of soluble guanylyl cyclase. Since the lysolecithin content of LDLox correlated with the amount of lysolecithin necessary to diminish stimulation of soluble guanylyl cyclase, our data support the hypothesis that lysolecithin may be responsible for the inhibitory effect of LDLox on smooth muscle relaxation and provide evidence that the antagonistic effect of HDL against desensitization of soluble guanylyl cyclase by atherogenic compounds could be responsible for the protective role of HDL in atherosclerosis.

While oxidatively modified forms of LDL contribute to the development of atherosclerosis (for review see ref. 1), HDL is discussed to antagonize this process (2-4). One major characteristic of atherosclerosis, namely the impaired response of blood vessels to vasodilators, can be mimicked in vitro by exposing isolated smooth muscle strips to LDLox obtained either by oxidation with $CuCl_2$ (5-8) or by incubation with endothelial cells (9). We previously demonstrated that LDLox reduces the response of soluble guanylyl cyclase to vasodilators (10-12) which indicates that the impaired relaxant response of atherosclerotic arteries may be,

Abbreviations used: LDL, low-density lipoprotein; LDLox, oxidized low-density lipoprotein; HDL, high-density lipoprotein; PLA_2, phospholipase A_2.

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at least partially, due to a desensitization of smooth muscle guanylyl cyclase. Since phospholipase A_2-treated LDL (6,8) and lysolecithin (6,8,9) were both found to antagonize the relaxation of vascular smooth muscle in a similar manner as LDLox, lysolecithin could be responsible for the atherogenic properties of LDLox. We, therefore, investigated whether lysolecithin also accounts for the inhibitory effect of LDLox on soluble guanylyl cyclase and whether the anti-atherogenic effect of HDL could be due to a reduced desensitization of this enzyme.

Material and Methods

Preparation of lipoproteins. Native lipoproteins (LDL: density 1.025 - 1.063 g/ml; HDL_3: density 1.125 - 1.210 g/ml) were isolated from human plasma by stepwise ultracentrifugation in the presence of sodium azide (1 mg/ml) and EDTA (1 mg/ml) as described (13). LDL was oxidized in the presence of 16 µM $CuCl_2$ for 18-20 hours at room temperature as previously described (10). Phospholipase A_2 modified LDL was obtained by incubating 100 µl of native LDL (≤ 3 mg total cholesterol/ml) with 50 µl containing 150 U/ml phospholipase A_2 (from bee venom), 3 mM $CaCl_2$, and 150 mM triethanolamine (pH 7.4) for 30 minutes at room temperature. The reaction was terminated by adding 150 µl of 100 mM EGTA. To exclude any non-enzymatic reactions of phospholipase A_2, control incubations were performed in the presence of 30 mM EGTA.

Preparation of soluble guanylyl cyclase from bovine platelets. Soluble guanylyl cyclase was isolated from bovine platelets as previously described (10) and stored as 50% ammonium sulfate precipitation in 50 mM imidazole/HCl buffer (pH 7.4) containing 2 mM DTT and 1 mM EDTA at 4 °C. Preparations were used within 2 weeks. Prior to the experiment, aliquots of the ammonium sulfate suspension were dialysed against 50 mM imidazole/HCl buffer (pH 7.4) containing 2 mM glutathione and 1 mM EDTA for 2 hours at 4° C. Protein concentration of the dialysate was adjusted to about 1-2 mg/ml and bovine serum albumin was added in a concentration of 1 mg/ml.

Guanylyl cyclase assay. 30 µl of the enzyme solution were preincubated at 37 °C with 10 µl of a solution of LDLox, phospholipase A_2 modified LDL or lysolecithin in the absence or presence of 10 µl of a solution of HDL. After 10 minutes, 100 µM sodium nitroprusside, 1 mM $MgCl_2$, 1 mM cGMP, 1 mM 3-isobutyl-1-methyl-xanthine, 0.15 mM GTP (containing ~ 300,000 cpm [α-^{32}P]GTP), and 50 mM tri-ethanolamine/HCl, pH 7.4 in a total volume of 50 µl were added and incubated for 10 further minutes. [α-^{32}P]cGMP was separated from [α-^{32}P]GTP by ZnCO3-precipitation and chromatography over Al_2O_3-columns (14).

Data evaluation. Each experiment was performed in triplicate with at least three different enzyme preparations. IC_{50}-values were extrapolated from individual concentration-activity curves by the means of non linear curve-fitting and are expressed as geometric means with 95% confidence limits.

Materials. Lysolecithin (L-α-Lysophosphatidylcholine from egg yolk containing mainly palmitic and stearic acid) and phospholipase A_2 (lyophilized salt-free powder from bee venom) were purchased from Sigma, Deisenhofen (F.R.G.). [α-^{32}P]GTP was obtained from DuPont de Nemours, Bad Homburg (F.R.G.), buffer salts of analytical grade from Merck, Darmstadt (F.R.G.) and all other

biochemicals from Sigma, Deisenhofen (F.R.G.). Solutions of nitric oxide and S-nitrosoglutathione were prepared as previously described (10).

Results

Preincubation of soluble guanylyl cyclase with increasing concentrations of LDLox almost completely abolished the stimulatory effect of sodium nitroprusside (Fig. 1 A). The mean IC_{50}-value and 95% confidence limits were 22 (13 - 39) µg/ml LDLox. $CuCl_2$ itself, which was used to oxidize LDL, did not affect guanylyl cyclase activity up to 10 µM (not shown) The inhibitory effect of LDLox was partially antagonized when preincubation was performed in the presence of HDL. As shown in Fig. 1 A, HDL in a concentration of 200 µg/ml produced a rightward shift of the dose-response curve of LDLox by about one order of magnitude. Under these conditions, the highest LDLox concentration used (250 µg/ml) reduced the stimulatory effect of sodium nitroprusside only by about 40%, whereas under control conditions (i.e. in the absence of HDL) the stimulatory effect was almost completely abolished. HDL itself did neither affect basal (not shown) nor stimulated guanylyl cyclase activity.

A protective role of HDL was also observed when soluble guanylyl cyclase was preincubated with lysolecithin, which is discussed to be responsible for the inhibitory effects of LDLox. Similar to the oxidized lipoprotein, preincubation of guanylyl cyclase with lysolecithin resulted in a dose dependent inhibition of the stimulatory effect of sodium nitroprusside with an IC_{50}-value of 61 (50 - 75) µM

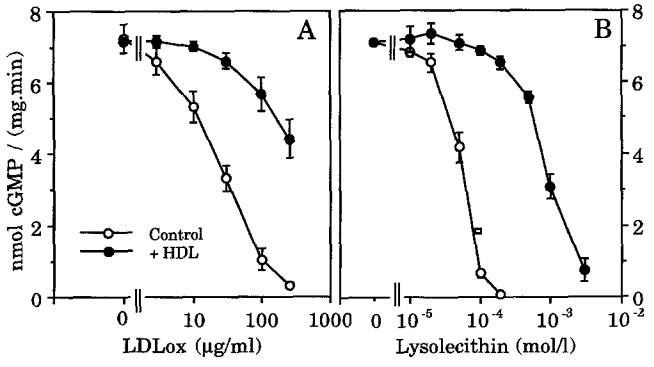

Fig. 1. Effect of LDLox and lysolecithin on guanylyl cyclase activity. Native LDL was oxidized as described under materials and methods. Soluble guanylyl cyclase was preincubated with increasing concentrations of LDLox (panel A) or lysolecithin (panel B) in the absence or presence of 200 µg/ml HDL. After 10 minutes, 100 µM sodium nitroprusside and the remaining components of the guanylyl cyclase assay were added and incubated for further 10 minutes. Guanylyl cyclase activity was determined in triplicates and is given as the mean ± SEM from 4 different experiments. The concentrations of LDLox and HDL are expressed as µg total cholesterol/ml.

TABLE 1

Effect of lysolecithin on soluble guanylyl cyclase activity

Incubation	Pretreatment		
	Control	Lysolecithin (50 μM)	Lysolecithin (200 μM)
	nmol cGMP x mg^{-1} x min^{-1}		
Control	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
Nitroprusside 10 μM	5.20 ± 0.10	2.55 ± 0.07	0.07 ± 0.01
Nitroprusside 100 μM	8.86 ± 0.19	2.87 ± 0.16	0.09 ± 0.01
GSNO 10 μM	5.07 ± 0.08	1.96 ± 0.24	0.07 ± 0.01
GSNO 100 μM	10.67 ± 0.08	4.03 ± 0.23	0.06 ± 0.01
Nitric oxide 100 μM	9.57 ± 0.16	3.33 ± 0.09	0.08 ± 0.01

Soluble guanylyl cyclase was preincubated in the absence or presence of lysolecithin. After 10 minutes the remaining components of guanylyl cyclase assay were added and the enzyme incubated for further 10 minutes in the absence or presence of sodium nitroprusside, S-nitrosoglutathione (GSNO) or nitric oxide. Guanylyl cyclase activity was determined in triplicates and is given as the mean ± SEM from 4 different experiments.

(Fig. 1 B). In the presence of 200 μg/ml HDL, the IC_{50} for lysolecithin was increased to 861 (729 - 1017) μM, which clearly indicates the antagonism between lysolecithin and HDL. To compare the effects of LDLox and lysolecithin on a molar basis, we determined the lysolecithin content of LDLox by measuring inorganic phosphorus in the lysolecithin fraction and obtained a value of 30 μg Pi/mg LDLox. Thus, a maximal active concentration of LDLox (250 μg/ml) contains about 125 μM lysolecithin, a concentration which has been found to be sufficient to abolish the stimulatory effect of sodium nitroprusside on guanylyl cyclase (Fig. 1 B). A similar effect of lysolecithin was obtained when guanylyl cyclase was stimulated with S-nitrosoglutathione or nitric oxide (Table 1). In contrast to the stimulated enzyme, lysolecithin did not affect basal guanylyl cyclase activity.

Similar to oxidation of LDL with $CuCl_2$, incubation of LDL with phospholipase A_2 also results in the conversion of lecithin to lysolecithin. To prove whether this modification of LDL is sufficient to gain its inhibitory properties, we preincubated guanylyl cyclase with PLA_2-treated LDL. As evident from Fig. 2 A, PLA_2-LDL reduced the activity of the sodium nitroprusside stimulated guanylyl cyclase with an IC_{50}-value of 41 (20 - 81) μg/ml in a similar manner as observed with LDLox or lysolecithin. HDL also diminished this inhibitory effect of PLA_2-LDL, however to lesser extent than those of LDLox or lysolecithin. Since treatment of LDL with phospholipase A_2 also results in the formation of unsaturated fatty acids, we investigated the effects of arachidonic, linoleic and oleic acid on guanylyl cyclase

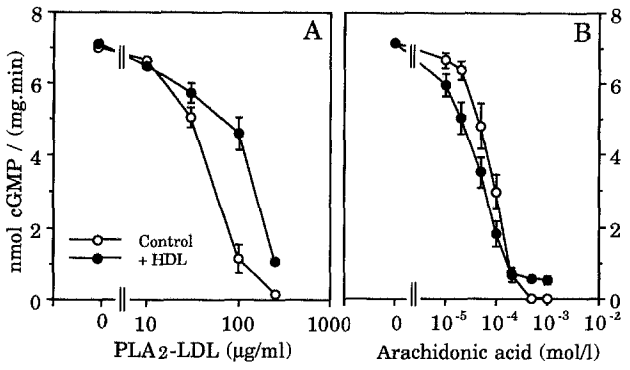

Fig. 2. Effect of phospholipase A_2 treated LDL and arachidonic acid on guanylyl cyclase activity. Native LDL was incubated with phospholipase A_2 as described under materials and methods. Soluble guanylyl cyclase was preincubated with increasing concentrations of PLA_2-LDL (panel A) or arachidonic acid (panel B) in the absence or presence of 200 µg/ml HDL. After 10 minutes, 100 µM sodium nitroprusside and the remaining components of the guanylyl cyclase assay were added and incubated for further 10 minutes. Guanylyl cyclase activity was determined in triplicates and is given as the mean ± SEM from 4 different experiments. The concentrations of LDLox and HDL are expressed as µg total cholesterol/ml.

activity. As shown in Fig. 2 B, arachidonic acid abolished the stimulatory effect of sodium nitroprusside with an IC_{50} of 74 (60 - 90) µM. In contrast to lysolecithin, the inhibitory effect of arachidonic acid could not be reduced by HDL. Similar to these results, HDL also failed to antagonize the inhibitory effects of linoleic acid (IC_{50} = 81 (69 - 95) µM) or oleic acid (IC_{50} = 73 (43-123) µM) which clearly separates the inhibitory effects of LDLox and lysolecithin from those of unsaturated fatty acids.

Discussion

Although HDL has been found to reduce the risks of atherosclerosis and coronary heart disease (2-4), little is known regarding the mechanism of its protective effect. Reverse cholesterol transport (15), the inhibition of oxidative modifications of LDL (16) or the protection of cells against LDLox-induced cytotoxicity (17) are some of these possibilities. In recent studies we demonstrated that LDLox inhibits the stimulation of soluble guanylyl cyclase by vasodilators (10-12) which could explain the impaired relaxant response of arteries with severe atherosclerotic lesions to nitrovasodilators (18,19). Our results showing that HDL protects soluble guanylyl cyclase against desensitization by LDLox may provide another explanation for the anti-atherogenic properties of HDL. The mechanism of this protective effect is unclear, but one possibility could be an uptake of LDLox-derived atherogenic compounds into the HDL-molecule so that an interaction between these compounds and guanylyl cyclase is prevented. An unspecific binding of

LDLox to the protein component of HDL appears to be unlikely since bovine serum albumin in concentrations up to 4 mg/ml did not antagonize the effect of LDLox (unpublished observations).

There is recent evidence that lysolecithin may be responsible for the inhibitory effect of LDLox on smooth muscle relaxation. First, LDLox has an about 10-fold higher lysolecithin content than native LDL (6,20), second, phospholipase A_2-treated LDL which also exhibits enhanced levels of lysolecithin, inhibits smooth muscle relaxation very similarly to LDLox (6), and third, the inhibitory effect of LDLox can be mimicked by using synthetic 1-palmitoyl-lysolecithin (8,9). In agreement with these results obtained by studies using isolated smooth muscle strips, we now demonstrate that lysolecithin may also account for the inhibitory effects of LDLox on soluble guanylyl cyclase. This conclusion is not only drawn from our results that lysolecithin and phospholipase A_2-treated LDL antagonized the stimulation of soluble guanylyl cyclase in the same concentration range as LDLox, but also from the findings that the inhibitory effects of all three compounds were antagonized by HDL. In contrast to LDLox or lysolecithin, however, the protective effect of HDL against desensitization of soluble guanylyl cyclase by PLA_2-treated LDL was less pronounced. This diminished effect of HDL is probably due to the PLA_2-induced formation of unsaturated fatty acids which were found to produce an HDL-insensitive desensitization of guanylyl cyclase, so that the inhibitory effect of PLA_2-treated LDL probably results from both, the release of free fatty acids and lysolecithin. Unsaturated fatty acids, however, most likely do not contribute to the effect of LDLox because oxidation of LDL with $CuCl_2$ for 18 hours completely metabolizes these fatty acids (21). Various possible degradation products, such as malonaldehyde, 4-hydroxypentenal, 4-hydroxyhexenal, 4-hydroxyoctenal or 4-hydroxynonenal (22) did not affect guanylyl cyclase activity (unpublished observations), but we cannot exclude, that in addition to lysolecithin, some other products of fatty acid oxidation may be involved in the observed effects of LDLox on guanylyl cyclase.

In conclusion, we suggest that the protective role of HDL against the risks of coronary heart disease may be due to a protective effect of HDL against desensitization of guanylyl cyclase by atherogenic compounds.

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