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Matsuda, Y

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HIGH DENSITY LIPOPROTEIN AND LOW DENSITY LIPOPROTEIN ATTENUATE THE INHIBITORY EFFECTS OF OXIDIZED LOW DENSITY LIPOPROTEIN ON ENDOTHELIUM-DEPENDENT ARTERIAL RELAXATION

Yuichi MATSUDA

First Division, Department of Internal Medicine,
Kobe University School of Medicine

INDEXING WORDS

endothelium; endothelium-dependent relaxation; high density lipoprotein; low density lipoprotein; oxidized low density lipoprotein

SYNOPSIS

We have recently reported that oxidized low density lipoprotein (ox-LDL) inhibits endothelium-dependent arterial relaxation through its increased lysophosphatidylcholine (LPC). In this study we examined whether high density lipoprotein (HDL) as well as native low density lipoprotein have any effects on the inhibition of endothelium-dependent relaxation by ox-LDL in isolated strips of rabbit thoracic aorta. Both LDL and HDL were isolated from normal human plasma and LDL was oxidized by exposure to copper. Preincubation of arterial strips with ox-LDL (0.1-0.5 mg protein/ml) inhibited endothelium-dependent relaxation to acetylcholine (ACh) in a concentration-dependent manner. HDL (1 mg protein/ml) by itself had no effect on the relaxation to ACh. In the presence of HDL, the inhibition by ox-LDL was markedly reduced. In addition, native LDL also attenuated the inhibition of endothelium-dependent relaxation by ox-LDL. Thus, HDL and native LDL may have salutary effects against the impairment of endothelium-mediated vasodilation in atherosclerotic arteries.

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INTRODUCTION

Atherosclerotic arteries are known to exhibit hyperreactivity in response to specific agonists such as ergonovine and serotonin.[16, 44] In addition, endothelium-dependent relaxation is markedly reduced in atherosclerotic arteries.[5, 11, 12, 33] These two characteristic alterations in vascular responsiveness in atherosclerosis may cooperatively predispose atherosclerotic coronary arteries to coronary spasm.[40, 43]

Oxidized low density lipoprotein (ox-LDL) has been implicated as an important cause of atherosclerosis and its presence in atherosclerotic lesion has been demonstrated.[42] Recently, we and other group have demonstrated that ox-LDL inhibits endothelium-dependent arterial relaxation through its increased lysophosphatidylcholine (LPC).[25, 45] LPC is an amphiphilic compound and its concentration is increased manyfolds in atherosclerotic arteries.[9, 29] Since LDL is oxidatively modified in intima and ox-LDL accumulates in atherosclerotic lesion, it is speculated that increased LPC in ox-LDL acts on endothelium and inhibits endothelium-dependent arterial relaxation.

High density lipoprotein (HDL) is considered to exert its antiatherogenic effect through promoting reverse cholesterol transport.[14, 35] In addition, HDL is demonstrated to exhibit protective effect against a variety of unfavorable actions of LDL and ox-LDL.[2, 4, 17, 24, 28] Since HDL, like most of the plasma macromolecules, is able to pass through the vascular endothelium and reach the subendothelial space of intima,[34] there might be an interaction between HDL and ox-LDL. These findings lead us to carry out an investigation to examine whether HDL may have any protective effect against the inhibition of endothelium-dependent relaxation by ox-LDL.

MATERIALS AND METHODS

Preparation of lipoproteins

LDL (d = 1.020 - 1.063 g/ml) and HDL (d = 1.063 - 1.210 g/ml) were isolated by sequential ultracentrifugation from fresh human plasma drawn from healthy normolipidemic donors who had been fasted for 14 h according to the method of Havel et al.[15]

Native LDL (1 mg protein/ml) was oxidatively modified by exposure to 5 μM $CuSO_4$ in phosphate-buffered saline without Ca^{2+} and Mg^{2+} (PBS-) for 24 h at 37 °C according to the method of Quinn et al.[30] Oxidative modification of LDL was confirmed by agarose gel electrophoresis.

Measurement of endothelium-dependent relaxation

Japanese white rabbits (2.5-3.5 kg) were anesthetized with pentobarbital sodium (30 mg/kg body wt,iv) and the descending thoracic aortas were isolated and cleaned of surrounding tissue. Aortic rings approximately 3-mm wide were cut and opened. For isometric force measurements, transverse aortic strips were suspended in 5 ml-organ baths containing Krebs-bicarbonate buffer of the following composition (values in mM): NaCl 118, KCl 4.0, $CaCl_2$ 1.5, $MgSO_4$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 25, and glucose 5 [44], and equilibrated at 37 °C with a 95%O_2-5%CO_2 gas mixture. Final pH was approximately 7.38. One end of the strip was attached to the bottom of the chamber, and the other end was attached to a Statham 4C-2 force transducer (Gould, Inc., Glen Burnie, Md.), which was connected to a Nihonkoden amplifier/recorder system (Nihonkoden, Tokyo, Japan). An initial preload of 1.5 g was applied, and the strips were allowed to stabilize for 90 min. A test contraction was induced by raising KCl concentration to 40 mM. When the developed tension attained its peak value, the strips were relaxed by rinsing with the buffer. Then the strips were pre-contracted with 0.3 μM phenylephrine (PE) and subsequently relaxed by a cumulative addition of acetylcholine (ACh). After washing with the buffer and equilibration, the strips were preincubated with selected concentration of ox-LDL, HDL, LDL for 30 min and then the contraction/relaxation cycle was repeated. In some experiments, the contraction/relaxation cycle was produced by a cumulative addition of nitroglycelin. Relaxation values were expressed as percent decreases of the PE (0.3 μM)-induced constrictor tone.

Materials

The following drugs were used: L-phenylephrine hydrochloride, acetylcholine chloride (Sigma Chemical Co., St. Louis, Mo.), nitroglycerin (Nihonkayaku, Tokyo, Japan). The drugs were

dissolved in distilled water and then diluted in the buffer. All concentrations are expressed as final concentrations.

Determinations

Protein content of the lipoproteins was determined by the method described by Bradford[6] with bovine serum albumin as a standard.

Statistical Analysis

Data are expressed as mean±SEM. The significance of the difference between group means was analyzed by oneway analysis of variance and the Bonferroni test for samples. Probability values <0.05 were taken as statistically significant.

RESULTS

Effect of oxidized LDL on endothelium-dependent relaxation to acetylcholine

Figure 1 (top panel) shows representative tracings of the responses to ACh in rabbit aortic strips preincubated with ox-LDL (0.5 mg protein/ml) for 30 min. We confirmed previous observation that ox-LDL by itself altered neither the resting tension nor PE-elicited contraction. Strips showed reduced relaxation to ACh after preincubation with ox-LDL. Strips showed the same dilatation to ACh after preincubation with the buffer for 30 min as compared to the control run (data not shown). The preincubation of strips with ox-LDL (0.1 and 0.5 mg protein/ml) significantly inhibited endothelium-dependent relaxation to ACh in dose-dependent manners (Figure 2). In contrast, endothelium-independent relaxation to nitroglycerin was little affected by preincubation with ox-LDL, as reported previously.[45] (data not shown).

Effect of HDL on the inhibition by oxidized LDL of endothelium-dependent relaxation to acetylcholine

Representative tracings of strips preincubated with ox-LDL in the presence of HDL are demonstrated in Figure 1 (bottom panel). In the presence of HDL (1 mg protein/ml), the inhibitory effect of ox-LDL (0.5 mg protein/ml) on the endothelium-dependent relaxation to ACh was markedly reduced. HDL (1 mg protein/ml) slightly

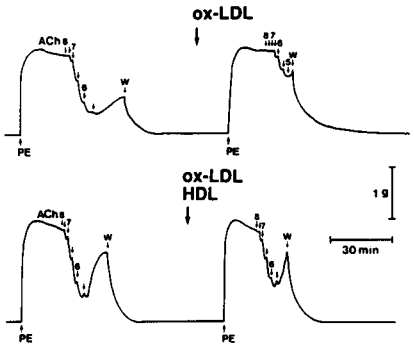

FIGURE 1. Representative tracings of responses to acetylcholine (ACh) in rabbit aortic strips preincubated with oxidized LDL (ox-LDL) (0.5 mg protein/ml) (top) or ox-LDL (0.5 mg protein/ml) plus HDL (1 mg protein/ml) (bottom). Strips were pre-contracted with 0.3 μM phenylephrine and subsequently endothelium-dependent relaxation was induced by cumulative addition of ACh. After incubation with a selected concentration of lipoproteins for 30 minutes, the contraction/relaxation cycle was repeated. Concentrations of ACh are expressed as negative logarithms. PE, phenylephrine (0.3 μM) W, washout

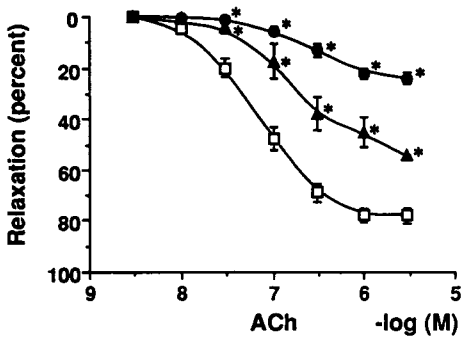

FIGURE 2. Cumulative concentration-response curves to acetylcholine (ACh) in strips preincubated with Krebs buffer (control, —□—) and strips preincubated with oxidized LDL 0.1 (—▲—) and 0.5 mg protein/ml (—●—) for 30 min. Horizontal axis represents the final concentrations of ACh (-log M) in the organ bath and vertical axis represents the percent decrease in tension from the contraction evoked by 0.3 μM phenylephrine. Each point is the mean of 5-6 observations. Vertical bars indicate SEM. *p<0.05 vs. control.

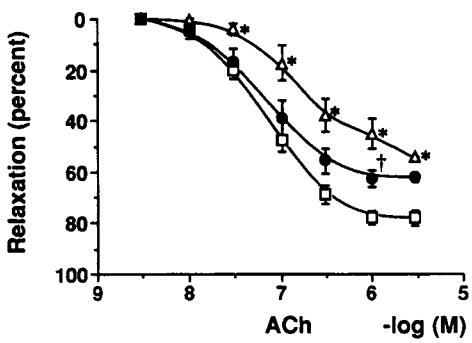

FIGURE 3. Effect of HDL on the inhibition by lower dose of oxidized LDL (ox-LDL) of response to acetylcholine (ACh). Cumulative concentration-response curves to ACh in strips preincubated with Krebs buffer (control, —□—), strips preincubated with ox-LDL (0.1 mg protein/ml) (—▲—), and strips preincubated with ox-LDL (0.1 mg protein/ml) plus HDL (1 mg protein/ml) (—●—) for 30 min. The horizontal axis represents the final concentrations of ACh (-log M) in the organ bath and the vertical axis represents the percent decrease in tension from the contraction evoked by 0.3 μM phenylephrine. Each point is the mean of 6 observations. Vertical bars indicate SEM. *p<0.05 vs. control. †p<0.05 vs. ox-LDL.

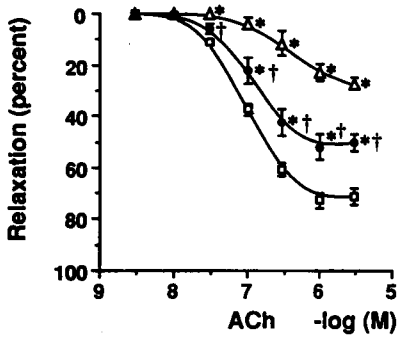

FIGURE 4. Effect of HDL on the inhibition by higher dose of oxidized LDL (ox-LDL) of response to acetylcholine (ACh). Cumulative concentration-response curves to ACh in strips preincubated with Krebs buffer (control, —□—), strips preincubated with ox-LDL (0.5 mg protein/ml) (—△—), and strips preincubated with ox-LDL (0.5 mg protein/ml) plus HDL (1 mg protein/ml) (—●—) for 30 min. The horizontal axis represents the final concentrations of ACh (-log M) in the organ bath and the vertical axis represents the percent decrease in tension from the contraction evoked by 0.3 μM phenylephrine. Each point is the mean of 6 observations. Vertical bars indicate SEM. *p<0.05 vs. control. †p<0.05 vs. ox-LDL.

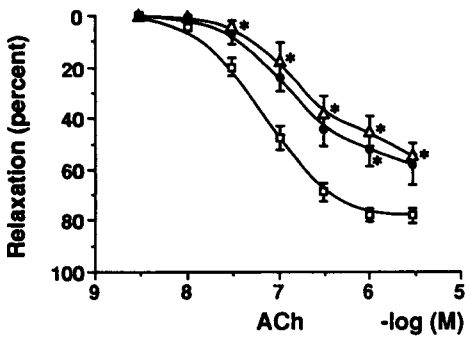

FIGURE 5. Effect of native LDL on the inhibition by oxidized LDL (ox-LDL) of response to acetylcholine (ACh). Cumulative concentration-response curves to ACh in strips preincubated with Krebs buffer (control, —□—), strips preincubated with ox-LDL (0.1 mg protein/ml) (—△—), and strips preincubated with ox-LDL (0.1 mg protein/ml) plus native LDL (1 mg protein/ml) (—●—) for 30 min. The horizontal axis represents the final concentrations of ACh (-log M) in the organ bath and the vertical axis represents the percent decrease in tension from the contraction evoked by 0.3 μM phenylephrine. Each point is the mean of 6 observations. Vertical bars indicate SEM. *p<0.05 vs. control. †p<0.05 vs. ox-LDL.

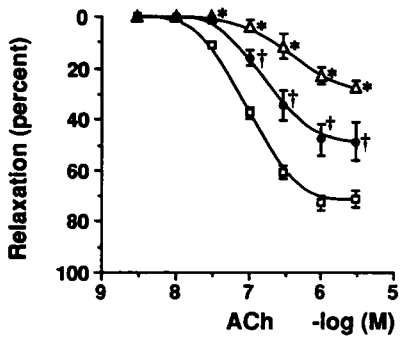

FIGURE 6. Effect of native LDL on the inhibition by oxidized LDL (ox-LDL) of response to acetylcholine (ACh). Cumulative concentration-response curves to ACh in strips preincubated with Krebs buffer (control, —□—), strips preincubated with ox-LDL (0.5 mg protein/ml) (—△—), and strips preincubated with ox-LDL (0.5 mg protein/ml) plus native LDL (1 mg protein/ml) (—●—) for 30 min. The horizontal axis represents the final concentrations of ACh (-log M) in the organ bath and the vertical axis represents the percent decrease in tension from the contraction evoked by 0.3 μM phenylephrine. Each point is the mean of 6 observations. Vertical bars indicate SEM. *p<0.05 vs. control. †p<0.05 vs. ox-LDL.

attenuated the inhibitory effect of ox-LDL (0.1 mg protein/ml) as shown in Figure 3. As shown in Figure 4, HDL (1 mg protein/ml) significantly attenuated the inhibitory effect of ox-LDL (0.5 mg protein/ml). HDL (1 mg protein/ml) by itself had no effect on the resting tension, PE-induced contraction, and endothelium-dependent relaxation to ACh (data not shown).

Effect of native LDL on the inhibition by oxidized LDL of endothelium-dependent relaxation to acetylcholine

To examine whether the attenuation of the inhibition by oxidized LDL of endothelium-dependent relaxation is specific to HDL, we investigated the effect of native LDL.

Native LDL (1 mg protein/ml) had little effect on the inhibitory effect of ox-LDL (0.1 mg protein/ml) as shown in Figure 5. However, the inhibitory effect of ox-LDL (0.5 mg protein/ml) was significantly attenuated in the presence of native LDL (1 mg protein/ml) as shown in Figure 6. Native LDL (1 mg protein/ml) by itself had no effect on the resting tension, PE-induced contraction, and endothelium-dependent relaxation to ACh (data not shown).

DISCUSSION

Previous studies have shown that lipoproteins including oxidized LDL inhibit endothelium-dependent relaxation.[3, 13, 22, 25, 37-39, 45] In this study HDL had preventive effect on the ox-LDL-induced inhibition of endothelium-dependent relaxation in rabbit aortic strips. In addition, native LDL had the similar effect on the inhibitory effect of ox-LDL.

HDL is considered to exert its antiatherogenic effect through promoting reverse cholesterol transport.[14, 35] In addition, HDL may directly inhibit binding and uptake of LDL by endothelial cells.[2] HDL also prevents LDL aggregation which leads to rapid uptake of LDL by macrophage.[24] HDL ameliorates cytotoxic effect of ox-LDL on vascular smooth muscle and endothelial cells,[17] and prevents oxidative modification of LDL.[28] Furthermore, HDL inhibits enhanced platelet aggregation evoked by ox-LDL.[4] Thus, HDL possesses protective effect against a variety of unfavorable actions of LDL and ox-LDL. The present study shows that HDL also

has protective effect on the impairment of endothelium-dependent relaxation by ox-LDL.

Since HDL as well as LDL readily passes through endothelium and accumulates in the subendothelial space of intima,[34] it is possible that HDL and LDL interact with ox-LDL in the subendothelial space of atherosclerotic lesion. Although the real concentrations of ox-LDL accumulated in the vascular wall are unknown, we used the concentration of HDL compatible with the value calculated from normolipidemic human plasma HDL in this study.

The protective effect was not specific to HDL, but it was also observed with native LDL. Native LDL had similar protective effect as HDL in same protein concentration. The concentration of native LDL that significantly attenuated the inhibition by ox-LDL was approximately two fold above the value calculated from normolipidemic human plasma LDL. In vitro, high concentration of native LDL itself can exert beneficial effect on the impairment of endothelium-dependent relaxations by ox-LDL, but, on the other hand, native LDL is the source of ox-LDL and hence its protective role in vivo is unknown.

Recently, we have demonstrated that LPC extracted from ox-LDL inhibits endothelium-dependent relaxation and that the inhibition of endothelium-dependent relaxation by ox-LDL is due to its increased LPC content.[45] LPC is an amphiphilic compound and its concentration is increased in atherosclerotic arteries[9, 29] as well as ox-LDL.[36] LPC alters physical properties of plasma membrane such as membrane fluidity,[10] leading to change in the function of cell membrane, i.e., the kinetics of transmembrane ion transport,[31] the activity of membrane-bound enzymes,[1, 23, 27, 32] and ligand-receptor coupling.[7] We have recently reported that ox-LDL and LPC inhibit both inositol 1,4,5-trisphosphate (IP_3) formation and an increase in intracellular calcium evoked by bradykinin, an endothelium-dependent vasodilator, in cultured bovine aortic endothelial cells (BAECs).[18, 20, 21] In addition, we have shown that ox-LDL inhibits the production and/or release of endothelium-derived relaxing factor (EDRF, NO) from cultured BAECs using bioassay experiment.[19] Thus, these findings allow us to speculate that ox-LDL, via increased LPC, inhibits the signal transduction which links the receptor stimulation to the biosynthesis of the second messenger in endothelial cells, leading

to the impaired production and/or release of EDRF. Although we can not elucidate the mechanisms in which HDL and native LDL reduce the inhibitory effect of ox-LDL from this study, we speculate that HDL and native LDL may prevent LPC in ox-LDL from acting on endothelium by removal of LPC from ox-LDL and attenuate its effect. The investigation of this mechanism is now in progress.

Endothelium-dependent relaxations are shown to be impaired in coronary arteries from hypercholesterolemic or atherosclerotic pigs and man.[5, 8, 12, 41] If ox-LDL is responsible for this impairment, HDL may play a beneficial role. Recently Kuhn and coworkers have reported that there is a positive correlation between HDL cholesterol and normal ACh-induced coronary vasoreactivity in patients undergoing coronary arteriography,[26] implying the relevance of our findings.

In conclusion, this study demonstrates that HDL and native LDL attenuate the ox-LDL-induced impairment of endothelium-dependent relaxation. HDL, aside from its antiatherogenic potential, may have salutary effect against the impaired endothelium-mediated vasorelaxation in atherosclerotic artery and contribute to reducing the susceptibility of atherosclerotic coronary arteries to vasospasm.

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