LDL Impairs Endothelium-Dependent Relaxation especially in Hyperlipidemic Porcine Coronary Arteries*


Introduction

It has been reported that hyperlipidemia, a major risk factor for atherosclerosis1), can lead to functional and morphologic changes in endothelial cells2,3). Endothelial cellular dysfunction may play a major role in the etiology of atherosclerosis4). The endothelium may contribute in several ways to the local regulation of vascular function, since endothelial cells produce prostaglandin I2 (prostacyclin) as well as endothelium-derived relaxing factor(s)5,6). The impairment of endothelium-dependent relaxations in atherosclerotic arteries has been reported both in experimental animals and humans7,8). Information regarding the impairment of endothelium-dependent relaxation in hypercholesterolemia is inconsistent9,10). It was reported that human LDL inhibits endothelium-dependent relaxation in normal rabbit aorta11). This study was therefore designed to examine whether a mild degree of hypercholesterolemia might alter porcine coronary artery reactivity, and how atherogenic lipoprotein would affect the vasoactivity of the endothelium of coronary arteries exposed to hypercholesterolemia.

Materials and Methods

1) Source of normal and atherosclerotic blood vessels

Twelve male Land-Yorkshire pigs, about three months old and weighing 20–25 kg were divided into three groups. Four animals were fed a high-cholesterol diet (semisynthetic diet containing 3.2% cholesterol and 20% lard) for 4 weeks (3.8±0.4 weeks) and four animals received the diet for 9 weeks (8.6±0.7 weeks). Four control pigs were fed regular pig mash for an average of 4.8±1.2 weeks. On the day of study for coronary artery relaxation, the animals were sedated with ketamine (500 mg i.m.), anesthetized with pentobarbital (12.5 mg/kg i.v.) and then exanguinated.

2) Evaluation of fatty streak formation

A cross-section of the left descending coronary artery just cranial adjacent to the each segment used in the experiments on contractility was examined histologically by hematoxylin-eosin staining, and by van Gieson’s elastic staining.

3) Lipoproteins

Pig LDL (density, 1.019–1.063 g/ml) was isolated from the plasma by differential ultracentrifugation12). LDL was dialyzed for 24 h against at least two changes of modified Krebs Ringer’s solution (content below), and identified by agarose gel electrophoresis13), and measuring thiobarbituric acid-reacting substances (TBAR)14). Protein content was determined by Lowry’s method15).

4) Organ Chamber Experiments

The left descending coronary artery was excised from each animal and trimmed free of adherent fat and connective tissue. Transverse strips and rings of 2 mm in width were cut from the arteries using scissors. Intact rings and transverse strips were fixed vertically between hooks in an organ bath containing 20 ml of modified Krebs solution. It contained (in mM): NaCl, 118; KCl, 4.8; CaCl2, 2.5; MgSO4, 1.2; NaHCO3, 24; KH2PO4, 1.2;
Na-EDTA, 0.06; and dextrose, 11. The bathing solutions were maintained at 37°C and bubbled with a mixture of 95% O₂-5% CO₂. The upper end of the strip was connected to the lever of a force-displacement transducer (TB-612T, Nihon Kohden Kogyo Co., Tokyo, Japan) by a silk thread. Strips were then progressively stretched until the contractile response evoked by 20 mM KCl was maximal (optimal tension)\(^1\). After equilibration for 90 minutes, preparations were precontracted with PGF\(_2\alpha\) and then relaxed by cumulative concentrations of bradykinin (BK), substance P, serotonin (5HT in the presence of 5-HT\(_2\) serotoninergic antagonist, ketanserin \(10^{-6}\) M), the Ca\(^{2+}\) ionophore A23187, and nitroglycerin. After washing, the rings were incubated for 30 min with LDL in the presence of indomethacin \(5 \times 10^{-6}\) M. Relaxation was then evaluated during the contraction induced by PFG\(_2\alpha\).

**Statistical Analysis**

Data were expressed as means±SEM. Statistical evaluation utilized the Student’s t test and the analysis of variance (ANOVA procedure, SAS statistical programs, version 5, SAS Institute, Cary, North Carolina, USA).

**Source of Agents Tested**

The following pharmacological agents were used: bradykinin, substance P, 5-HT creatinine sulfate (serotonin), Ca\(^{2+}\) ionophore A23187, prostaglandin F\(_2\alpha\) (Tris salt), indomethacin (all from Sigma Chemical, St. Louis, Missouri, USA), nitroglycerin (Nippon Kayaku, Tokyo, Japan), ketanserin bitartrate (Janssen-Kyowa, Tokyo, Japan). Solutions were prepared fresh daily using distilled water.

**Results**

1) **Lipid Profile**

There were no significant differences in body weight and serum total protein among the three groups. The total cholesterol level and the LDL cholesterol level were significantly higher in the C4 and C9 groups than that in the normal group. No significant alteration in HDL cholesterol or triglyceride was observed (Table 1).

2) **Baseline Characteristics**

The optimal resting tensions did not differ significantly in the normal vessels as compared to those of C4 and C9 animals (1.8 g). Contractions caused by KCl (10-40 mM), and PGF\(_2\alpha\) (0.1-2.7 \(\mu\)M) did not differ significantly among the 3 groups (Data not shown).

3) **Effect of Hypercholesterolemia on Relaxation**

The response to bradykinin in the presence of indomethacin in the vessels from the normal control and hypercholesterolemic pigs appears in Figs. 1 and 2. Bradykinin produced a concentration-dependent relaxation in vessels with endothelium. In contrast to the normal vessels, the response to bradykinin was attenuated in the hypercholesterolemic arteries. A typical response to bradykinin is shown in Fig. 1 a, b, and c. There was no relaxation in those vessels without endothelium. The average max % relaxation to bradykinin in the C9 vessels was less than in normal vessels. The relaxation in C4 vessels was significantly attenuated only at a high concentration of bradykinin compared with the normal control vessels (Fig. 2).

<table>
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<th>Table 1 Baseline data in control and cholesterol groups</th>
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<td><strong>Normal</strong></td>
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<td><strong>Cholesterol</strong></td>
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<td>Phospholipid (mg/dl)</td>
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<td>TBARS (nomol/ml)</td>
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\(\#p<0.05\)  \(\#\#p<0.001\)

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**Fig. 1** Relaxation in response to bradykinin in coronary arteries from normal (N) and cholesterol-fed (C4, C9) pigs in the presence of indomethacin (5 μM). Strips were first contracted with prostaglandin F2α. Wo and dots, wash-out with Krebs-Ringer bicarbonate solution.

**Fig. 2** Cumulative concentration-response curves to bradykinin, substance P, and serotonin during contraction evoked by prostaglandin F2α in control and cholesterol-fed groups. Relaxation is expressed as percent decrease in tension from the contraction evoked by prostaglandin F2α. Data shown as means ± SEM. *Significant difference (p<0.05).

The cumulative relaxation response to 5HT, Substance P, A23187, and nitroglycerin in vessels from each group appears in Figs. 2 and 3. The relaxation induced by substance P or 5HT was attenuated in C9 vessels. The relaxation induced by the Ca ionophore A23187 and by nitroglycerin was similar in the three groups.

**4) Morphology**

In vessels examined by light microscopy, there were limited small areas of fragmentation of internal elastic lamina induced by a high cholesterol diet for 4 weeks. Limited small areas (2.6%) of intimal thickening and fragmentation of internal elastic lamina were observed in the animals given the high cholesterol diet for 9 weeks.
Fig. 3 Cumulative concentration-response curves to A23187 and nitroglycerin during contraction evoked by prostaglandin F$_2$α in control and cholesterol-fed groups. Data shown as means $\pm$ SEM. *Significant difference (p<0.05).

Fig. 4 Cumulative concentration-response curves to bradykinin and serotonin with or without preincubation of LDL during a contraction evoked by prostaglandin F$_2$α in control and cholesterol-fed groups. Data shown as means $\pm$ SEM. *Significant difference (p<0.05).
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5) Effect of Atherogenic Lipoprotein on Relaxation

A typical effect of LDL treatment (75 mg protein per dl) on the relaxation induced by bradykinin is shown Fig. 1d and e. Though LDL inhibited endothelium-dependent relaxation, the contraction response to PGF$_{2\alpha}$ by intact or endothelium-denuded preparations was unaltered by exposure to LDL. Fig. 4 shows the dose-response curves to cumulative concentrations of bradykinin and serotonin on intact rings precontracted with 2.7 μM PGF$_{2\alpha}$ following after preincubation with LDL at a concentration of 25 or 75 mg protein per dl. A concentration of 75 mg protein per dl of LDL produced a shift to the right and a decrease in relaxation by bradykinin in three groups, 25 mg protein per dl produced a right shift in especially in C9 arteries. In the case of serotonin, 75 mg protein per dl of LDL significantly attenuated relaxation especially in the cholesterol-fed pig coronary arteries. LDL significantly inhibited arterial relaxation in the C9 group induced by substance P similarly. In the case of the Ca$^{2+}$ ionophore A23187, LDL attenuated endothelium-dependent relaxation like bradykinin. In the case of nitroglycerin, LDL did not produce any effect in arterial relaxation.

Discussion

This study was designed to evaluate the effect of hyperlipidemia on the vascular responsiveness of isolated porcine coronary arteries. It was demonstrated in this study that a mild degree of hypercholesterolemia impairs the endothelium-dependent relaxations induced by exposure to bradykinin, serotonin and substance P but that hypercholesterolemia must be induced in pigs by feeding a high cholesterol diet longer than 4 weeks; 9 weeks. The relaxation response to A23187 or nitroglycerin was not impaired. We studied the effects of five pharmacological agents: bradykinin, serotonin, and substance P stimulate the release of EDRF by receptor-mediated mechanisms, A23187 stimulates the release of EDRF by nonreceptor-mediated mechanisms, and nitroglycerin is an endothelium-independent vasodilator$^{17,18}$. Our results suggest that there is no change in the capacity to release of EDRF at very early stage of atherosclerosis, but rather a decrease of endothelial receptor affinity for such vasodilators as bradykinin, serotonin, or substance P.

We found intimal change and fragmentation of internal elastic lamina in only a very limited area. These morphological changes do not seem to become a barrier for diffusion of EDRFs or a cause of decrease of EDR, but morphological changes may precede or correlate with a decrease of EDR$^{19}$. To evaluate the role of lipoprotein in the regulation of vascular tonus, we investigated the direct effect of atherogenic lipoprotein on vascular responsiveness. A physiological concentration of LDL did not alter the contraction by KCl or prostaglandin F$_{2\alpha}$ in control or hypercholesterolemic porcine arteries. 75 mg protein per dl LDL also inhibited the response to bradykinin in normal arteries, but the decrease was greater in hypercholesterolemic arteries. As an inhibitory effect of LDL was also observed in the response to A23187, LDL might affect the formation and release of EDRF. This idea is consistent with the report that LDL inhibits two steps in the response of EDRFs$^{20}$. We previously observed that β VLDL attenuates EDR especially in the atherosclerotic aortas of rabbits$^{21}$. Recently it was reported that lyssolecithin in oxidized LDL impaired EDR of rabbit aorta$^{22}$. It was confirmed in our experiment that LDL was not oxidized by incubation for 30 minutes in Krebs Ringer solution aerated with a mixture of 95 % O$_2$-5 % CO$_2$ in this experiment. Though it was reported that the half life of EDRF is shortened by O$_2$- or free radicals, the mechanism of the inhibitory effect of LDL seems to differ from that of the destruction of EDRF released by natural oxidation. Recently heat and acid labile factor in LDL was reported to inhibit EDR in porcine coronary arteries$^{23}$. Additional study is required to evaluate the mechanism of the inhibitory effect for EDR by LDL.

Lastly we want to think about the course of atherosclerosis relevant to EDR. The regression of atherosclerosis has been reported$^{24}$. Strict control of the serum cholesterol level is currently recommended to allow the lesion to regress$^{25}$. Our experimental results would support these reports to some extent, since the lowering of LDL levels could promote circulation by vasodilation and by a decrease in aggregability of platelet, if the endothelium-dependent relaxation exists in vivo.
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Summary

We evaluated the effect of a low pathophysiological level of hyperlipidemia as well as the effect of in vitro exposure to LDL on the vascular responsiveness of isolated porcine coronary arteries. First, we fed pigs a cholesterol-rich diet for 4 weeks (C4 pigs) and 9 weeks (C9 pigs). The serum cholesterol level in the C9 pigs reached 218.5 ± 32.9 mg/dl compared with 85.5 ± 8.4 mg/dl in the controls. The Endothelium-dependent relaxation (EDR) caused by bradykinin, substance P, and serotonin were significantly reduced in the C9 pigs, but the relaxation induced by the Ca²⁺ ionophore, A23187 or nitroglycerin (NG) did not alter. Second, the direct effect of LDL in the presence of indomethacin was studied. The pre-incubation of LDL inhibited the EDR caused by bradykinin and A23187 especially in cholesterol-fed arteries, and also inhibited the EDR induced by substance P or serotonin in only cholesterol-fed arteries, not the control ones. The LDL did not affect the relaxation induced by the NG. These results suggest that EDR is attenuated by hyperlipidemia, and that the LDL directly attenuated the EDR especially in cholesterol-fed coronary arteries.

Key words: endothelium-dependent relaxation, hyperlipidemia, LDL, atherosclerosis, endothelium-derived relaxation factor.