Oxidized Low-Density Lipoprotein and High-Density Lipoprotein Cholesterol Modulate Coronary Arterial Remodeling: An Intravascular Ultrasound Study

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Summary

Background: Oxidized low-density lipoprotein (oxLDL) not only plays an important role in plaque formation, but also impairs the endothelium-dependent relaxation. Constrictive remodeling rather than intimal hyperplasia mainly contributes to restenosis after balloon angioplasty. Probucol (powerful antioxidant) reduced restenosis rate by improving constrictive remodeling. Thus, oxLDL may modulate coronary arterial remodeling.

Hypothesis: The study was designed for using intravascular ultrasound to test the hypothesis that arterial constrictive remodeling (CR) was associated with oxLDL in patients with coronary artery disease.

Methods: Intravascular ultrasound was performed in 36 patients with de novo atherosclerotic coronary. Remodeling was defined and evaluated as follows: remodeling index (RI) = lesion vessel area (VA)/(proximal reference VA + distal reference VA)/2. Constrictive remodeling (CR) was defined as remodeling index (RI) < 0.9. Neutral and expansive remodeling (NER) was defined as RI ≥ 0.9. The level of plasma ox-LDL was measured by sandwich ELISA using the monoclonal antibody (DLH3)-recognized oxidatively modified lipoproteins and the antihuman apoprotein B monoclonal antibody.

Results: Neutral and expansive remodeling was found in 24 lesions, and CR in 12 lesions. Remodeling index was significantly lower in the CR group than in the NER group (0.8 ± 0.1 vs. 1.0 ± 0.1 , $p < 0.001$). The level of oxLDL in the CR group was significantly higher than that in the NER group (24.0 ± 12.1 vs. 16.4 ± 6.2 U/ml, $p < 0.05$). The level of high-density lipoprotein-cholesterol (HDL-C) in the CR group was significantly lower than that in the NER group (40.5 ± 4.8 vs. 46.2 ± 10.6 mg/ml, $p < 0.05$). There was a statistically significant correlation between the value of HDL-C/ox-LDL and the RI ($r = -0.48$, $p < 0.005$).

Conclusions: Oxidized LDL and HDL-C were associated with arterial remodeling in de novo atherosclerotic lesions.

Key words: remodeling, oxidized low lipoprotein, risk factors, ultrasonics

Introduction

Compensatory arterial enlargement of diseased coronary arteries has been described as an early adaptive process of lumen loss. This “adaptive or expansive” remodeling was originally described by Glagov *et al.*¹ In recent studies, so-called paradoxical and constrictive remodeling (CR), defined as the shrinkage of internal elastic lamina in response to atherosclerotic plaque, was observed histologically^{2,3} and with intravascular ultrasound (IVUS) examinations.^{4–6} Thus, arterial remodeling is a heterogeneous process. The mechanism leading to arterial remodeling of diseased coronary arteries has not been fully elucidated.

Constrictive remodeling plays a pivotal role in lumen narrowing of restenosis after balloon angioplasty.⁷ A Multivitamins and Probucol Study group⁸ has reported that the antioxidant probucol reduced lumen loss by improving CR after balloon angioplasty. Oxidized low-density lipoprotein (oxLDL) is not only related to plaque formation and progression but also induces impairment of endothelium-dependent

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vasodilation. High-density lipoprotein cholesterol (HDL-C) has protective effects against unfavorable actions of oxLDL⁹ and is associated with expansive remodeling.¹⁰ This present study was designed to use IVUS to test the hypothesis that arterial CR was associated with oxLDL in patients with coronary artery disease.

Methods

Study Patients

The Japanese Ministry of Health and Welfare requires us to evaluate and validate an oxLDL measurement kit before commercialization. During the study period for obtaining approval, 150 healthy volunteers and 150 patients with coronary artery disease were enrolled for study in our hospital. Each patient provided written informed consent. Of the 150 patients with coronary artery disease, 36 consecutive patients underwent IVUS examination during diagnostic cardiac catheterization or when coronary interventions were under consideration. Angiographic and IVUS entry criteria were (1) significant (> 50% diameter) narrowing of a de novo lesion, (2) lesion between two major side branches, (3) lesion without a calcium arc > 60°, and (4) proximal and distal reference sites within 10 mm proximal or 10 mm distal to the target lesion. Vessels showing spasm were not included in this study. The patients in whom the IVUS catheter occluded antegrade flow in the artery were excluded because this factor could influence the reference segment size. All patients had antegrade flow while the IVUS catheter was in place.

Patient characteristics were obtained via chart review. Patients were classified as tobacco users if they had smoked habitually within the last 3 years. Systemic hypertension was defined as any requirement for antihypertensive medication or a resting blood pressure > 140/90 mmHg. Patients who were given probucol before this study were excluded.

Body size and blood chemistry measurements: Height and weight were recorded to the nearest 0.1 cm and 0.1 kg; body mass index was calculated as weight divided by height.² Venous blood samples were taken from patients after overnight fasting after admission. Blood samples were allowed to clot for 1 h at room temperature; serum was then separated by low-speed centrifugation at 3,000 rpm. Serum total cholesterol, triglyceride, and HDL-C concentrations in fresh serum were measured with automated enzymatic procedures. Serum apolipoprotein A I, B, and E concentrations were measured with an automated immunoturbidimetric method. Serum lipoprotein(a) [Lp(a)] concentration was measured with an enzyme-linked immunosorbent assay (Biopool, Umea, Sweden). Low-density lipoprotein cholesterol (LDL-C) was calculated with the Friedewald equation: $LDL-C = \text{total cholesterol} - HDL-C - \text{triglyceride}/5$.

Measurement of plasma oxLDL concentration: Plasma oxLDL concentration was calculated by measurement of the apolipoprotein B-containing lipoproteins that were transporting oxidized phosphatidylcholine (PC); this was done with a

sandwich enzyme-linked immunosorbent assay (Kyowa Med-ex, Tokyo, Japan) according to a method developed by Itabe *et al.*^{11,12} In this method, a mouse monoclonal antibody, FOH1a/DLH3, is used as the first antibody. The second antibody is goat antihuman apolipoprotein B polyclonal antibody. The concentrations of plasma oxLDL are expressed in units/ml.

Angiographic Method

Cardiac catheterization and percutaneous transluminal coronary angioplasty (PTCA) were performed according to a standard femoral technique. The technical aspects of the procedure, including balloon choice, inflation duration, and pressure were decided by individual operators. Coronary angiograms were obtained in multiple projections after intracoronary injections of 3 mg isosorbide dinitrate. The optimal views of the target lesions from among all technically suitable angiograms were analyzed by quantitative coronary angiography with a Kontron Cardio 500 (Kontron Elektronik, Everett, Mass., USA). All angiographic analyses were performed by two observers who were blinded to the results of blood chemistry and IVUS measurements and had no access to clinical data. Quantitative measurements were calibrated with the guiding catheter as the reference dimension. The absolute values for the minimal lumen diameter as well as the reference vessel diameter were measured.

Intravascular Ultrasound System and Imaging Procedure

After completion of the diagnostic catheterization or before coronary interventions, studies were performed with a commercially available IVUS imaging catheter (Sonicath, 3.5 F, 30 MHz, Boston Scientific Corporation, Waterstone, Mass., USA) and a Sonos Intravascular System imaging console (Hewlett-Packard [Philips Medical Systems/Agilent Technologies], Andover, Mass., USA). Intravascular ultrasound imaging was performed after administration of 2 mg of intracoronary isosorbide. After the imaging catheter was advanced across the lesion to the distal portion of the vessel under fluoroscopic guidance, imaging was performed during slow manual retraction of the imaging catheter (approximately 0.5 mm/s). The dimensional images were displayed on the IVUS imaging console and recorded on a 0.5" high-resolution S-VHS videotape for off-line analysis.

Imaging Analysis and Definition of Coronary Remodeling

The off-line IVUS images were analyzed with the Cardio 500 system. Qualitative (plaque morphology and intraluminal structure) and quantitative (cross-sectional) analysis of the ultrasound images was performed by two investigators who were blinded to the angiographic and clinical results. Single images of the lesion sites were selected for quantitative and qualitative analysis. The external elastic lamina of a vessel was defined as the outer border of the sonolucent zone.¹³ The area within the external elastic lamina was measured as the vessel area (VA; mm²). The plaque area (PA; mm²) was defined and

calculated by subtracting the lumen area (LA; mm²) from the VA. Remodeling index was defined and calculated as follows:

Remodeling index (RI) = $2 \times \text{lesion VA} / (\text{proximal reference VA} + \text{distal reference VA})$

Arterial remodeling was classified into two remodeling groups: CR and neutral or expansive remodeling (NER). Constrictive remodeling was defined as RI < 0.9, and NER was defined as RI ≥ 0.9.^{6,14} Since calcium deposits obscure peripheral structure, the outer border of the plaque was measured only in the presence of a calcium arc < 60°.

Statistical Analysis

Statistical analysis was performed with Statview 4.5 (Berkeley, Calif., USA). All values are expressed as mean ± standard deviation (SD). Comparison of continuous variables between the two groups was performed by the Mann-Whitney's U-test, and categorical variables were tested with the chi-square test. Probability values of < 0.05 were considered statistically significant.

Results

No significant differences were found in baseline patient and lesion characteristics between the NER and the CR groups (Tables I and II). Quantitative IVUS findings are presented in Table III. No significant differences were found between proximal VA, proximal LA, distal VA, distal LA, lesion VA, lesion LA, and lesion PA. Remodeling index was significantly lower in the NER group than in the CR group; RI distribution showed a unimodal rather than a bimodal shape (Fig. 1).

The levels of oxLDL, other lipid profiles, and HbA1c are shown in Table IV. The mean values of total cholesterol, triglycerides, LDL-C, apolipoprotein A I, apolipoprotein B,

apolipoprotein E, Lp(a), and HbA1c were very similar between the two remodeling groups. The mean value of oxLDL in the CR group was significantly higher than that in the NER group (24.0 ± 12.1 vs. 16.4 ± 6.2 U/ml, $p < 0.05$). The mean value of HDL-C in the CR group was significantly lower than that in the NER group (40.5 ± 4.8 vs. 46.2 ± 10.6 mg/dl, $p < 0.05$). Individual values of oxidized LDL and HDL-C are shown in Figure 2. The mean value of the ratio of HDL-C to oxLDL (HDL-C/oxLDL) was significantly lower in the CR group than in the NER group (2.1 ± 1.0 vs. 3.4 ± 1.9 , $p < 0.02$). There was a statistically significant correlation between the value of HDL-C/oxLDL and the RI ($r = -0.48$, $p < 0.05$; Fig. 3).

Discussion

Arterial remodeling in response to atherosclerotic plaque was described originally by Glagov *et al.*¹ They reported that

TABLE II Lesion characteristics

	NER	CR	p Value
Number of lesions	24	12	
Coronary artery			
RCA	6	4	0.6
LAD	16	6	0.33
LCx	2	2	0.45
Calcified lesions	6	3	0.13
MLD (mm)	0.8 ± 0.58	0.93 ± 0.54	0.64
% diam. stenosis (%)	67 ± 21	69 ± 15	0.99
Lesion length (mm)	12.95 ± 5.54	13.60 ± 6.97	0.87

Abbreviations: RCA = right coronary artery, LAD = left anterior descending artery, LCX = left circumflex artery, MLD = minimum lumen diameter. Other abbreviations as in Table I.

TABLE I Patient characteristics

	NER	CR	p Value
Number of patients	24	12	
Age (years)	57.5 ± 10.0	62.6 ± 5.7	0.18
BMI (kg/m ²)	23.6 ± 2.1	24.4 ± 3.5	0.25
Male	17	11	0.16
Prior MI	8	2	0.29
Prior CABG	1	1	0.61
Multivessel disease	13	8	0.47
Hypertension	13	5	0.48
Current smoker	17	9	0.79
Use of statins	7	5	0.45

Abbreviations: NER = neutral or expansive remodeling group, CR = constrictive remodeling group, BMI = body mass index, MI = myocardial infarction, CABG = coronary artery bypass graft.

TABLE III Intravascular ultrasound measurements

	NER	CR	p Value
Proximal reference			
VA (mm ²)	16.4 ± 3.8	18.4 ± 3.7	0.13
LA (mm ²)	9.6 ± 3.4	10.2 ± 3.4	0.36
Distal reference			
VA (mm ²)	13.4 ± 5.1	15.8 ± 3.5	0.11
LA (mm ²)	8.6 ± 3.8	9.7 ± 2.1	0.08
Target lesion			
VA (mm ²)	15.2 ± 4.3	13.8 ± 3.5	0.38
LA (mm ²)	4.4 ± 1.2	4.7 ± 2.0	0.73
PA (mm ²)	10.8 ± 3.9	9.0 ± 2.9	0.23
Remodeling index	1.02 ± 0.10	0.80 ± 0.10	$p < 0.001$

Abbreviations: VA = vessel area, LA = lumen area, PA = plaque area. Other abbreviations as in Table I.

FIG. 1 Remodeling index distribution. The superimposed curve represents the normal probability density function based on the parameters of the overall population. The value of the remodeling index was distributed almost normally (skewness = -0.287, kurtosis = 1.162). CR = constrictive remodeling, NER = neutral or expanding remodeling. □ = CR, ■ = NER.

arterial lumen is maintained by adaptive arterial expansion in a stenosis of up to 30 to 50% that is accompanied by increases in plaque size. The lumen area begins to decrease progressively beyond this threshold. They called this phenomenon adaptive (expansive) arterial remodeling. But Pasterkamp *et al.* reported that CR contributes to luminal narrowing and that arterial remodeling in response to plaque formation varies among individuals. The results of many studies^{3, 5, 6, 15} support the proposal notion that arterial remodeling is a heterogeneous process, including the previously mentioned expansive and constrictive arterial remodeling. When arterial remodeling is distributed normally, CR and expansive remodeling are at the opposite ends of the normal distribution curve showing the response to plaque formation.^{5, 15}

The mechanism of arterial remodeling is uncertain, but several hypotheses have been proposed. One hypothesis is related to flow-mediated vasodilation.^{2, 16, 17} As plaque size increases and lumen area decreases, coronary flow and shear stress in-

FIG. 2 Individual values of oxidized low-density lipoprotein (oxLDL) and high-density lipoprotein cholesterol (HDL-C) in the two groups. Each bar indicates the mean of each group. (A) The individual levels of oxLDL. (B) The individual levels of HDL-C. Abbreviations as in Figure 1. (○) = Individual value of NER, (●) = individual value of CR.

crease, which results in arterial enlargement. Another hypothesis is that the remodeling results from impaired endothelial function.¹⁸ Oxidized LDL is known to inhibit endothelium-dependent vasodilation by increased lysophosphatidylcholine (LPC) in oxLDL.^{19, 20} Since LDL-C is oxidatively modified in the arterial wall and oxLDL accumulated in a local atherosclerotic lesion, increased LPC in oxLDL seems to inhibit endothelium-dependent vasodilation.

An IVUS study⁷ showed that CR played a more important role than intimal thickening in restenosis. Probucol is one of the strongest antioxidant agents and has lipid-lowering effects.²⁰ Cote *et al.*⁸ reported that probucol reduced the restenosis rate after percutaneous old balloon angioplasty by improving CR. Thus, oxLDL may contribute to CR.

The association of expansive remodeling with HDL-C has already been reported,¹⁰ although the mechanism by which HDL-C influences arterial remodeling is still unknown. High-density lipoprotein cholesterol is known to have its antiatherogenic effect by promoting reverse cholesterol transport;²¹ it can pass through the endothelium and enter deep into the intima; it reverses the oxLDL-induced impaired endothelium-dependent vasodilation by removing LPC from oxLDL and

TABLE IV Lipid profile and Hb A1c levels

	NER	CR	p Value
Ox LDL (U/ml)	16.4 ± 6.2	24.0 ± 12.1	0.04
Total-C (mg/dl)	204 ± 19	212 ± 42	0.95
LDL-C (mg/dl)	125 ± 23	141 ± 41	0.36
HDL-C (mg/dl)	46 ± 11	41 ± 5	0.05
HDL-C/oxLDL	3.4 ± 1.9	2.1 ± 1.0	0.02
Triglyceride (mg/dl)	162 ± 72	148 ± 53	0.72
Apolipoprotein AI (mg/dl)	115 ± 22	123 ± 21	0.17
Apolipoprotein B (mg/dl)	115 ± 16	121 ± 22	0.49
Apolipoprotein E (mg/dl)	5.0 ± 1.4	4.4 ± 1.0	0.48
Lp(a) (mg/dl)	27.7 ± 35.7	25.5 ± 16.9	0.79
Hb A1c (%)	6.1 ± 1.9	5.4 ± 0.7	0.42

Abbreviations: Ox LDL = oxidized low-density lipoprotein, Total-C = total cholesterol, LDL-C = low-density lipoprotein cholesterol, HDL-C = high-density lipoprotein cholesterol, Lp(a) = lipoprotein a, Hb A1c = hemoglobin A1c. Other abbreviations as in Table I.

FIG. 3 Relation between the ratio of HDL-C and oxLDL and remodeling index. Abbreviations as in Figure 2. (○) = Individual value of neutral or expanding remodeling, (●) = individual value of constrictive remodeling.

preventing LPC from inhibiting the endothelium-dependent vasodilation.⁹

We found a weak but significant correlation between the individual value of HDL-C/oxLDL and RI in this study. The RI curve of coronary arterial remodeling, CR, and expansive remodeling seemed to shift according to the balance between HDL-C and oxLDL levels. Increases in oxLDL resulted in the index shifting toward CR, whereas increases in HDL-C resulted in the index shifting toward expansive remodeling.

The reduction in oxLDL level and the increase in HDL-C level are known to lead to regression of atherosclerosis, but they also seemed to have a beneficial effect on maintaining the lumen cross-sectional area, in part by expansive remodeling.

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