Elevated Homocysteine Levels Might Be Associated with Coronary Artery Remodeling in Patients with Stable Angina: An Intravascular Ultrasound Study

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Summary

Background: The relationship between plasma biologic markers and coronary artery remodeling is unknown.

Hypothesis: Plasma biologic markers are associated with coronary artery remodeling.

Methods: Preintervention intravascular ultrasound images were obtained in 44 patients with chronic stable angina. Plasma samples were collected 24 h before coronary intervention. The biologic markers included total cholesterol, low-density lipoprotein (LDL) cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, lipoprotein(a) [LP(a)], C-reactive protein (CRP), and homocysteine. The remodeling index (RI) was defined as a ratio of the (lesion/proximal reference) external elastic membrane cross-sectional area. Positive remodeling was defined as an RI > 1.05, negative remodeling as an RI < 0.95, and intermediate remodeling as an RI between 0.95 and 1.05.

Results: Total cholesterol level ($r = 0.092$, $p = 0.557$), LDL cholesterol level ($r = 0.123$, $p = 0.426$), triglyceride level ($r = 0.020$, $p = 0.901$), HDL cholesterol level ($r = 0.042$, $p = 0.789$), LP(a) level ($r = 0.062$, $p = 0.729$), and CRP level ($r = 0.266$, $p =$

0.089) did not significantly correlate with the RI. However, the plasma homocysteine level positively correlated with the RI ($r = 0.398$, $p = 0.008$). The plasma homocysteine level was significantly lower in the lesions with negative remodeling and higher in the lesions with positive remodeling (10.8 ± 0.7 $\mu\text{mol/l}$ in negative remodeling, 13.1 ± 0.6 $\mu\text{mol/l}$ in intermediate remodeling, and 18.1 ± 2.8 $\mu\text{mol/l}$ in positive remodeling, $p = 0.021$).

Conclusions: Elevated homocysteine levels might be associated with coronary artery remodeling in patients with stable angina.

Key words: homocysteine, coronary artery disease, intravascular ultrasound

Introduction

Arterial remodeling is commonly observed in human atherosclerosis and recently has been recognized as a major determinant of vessel lumen size. It may be a heterogeneous response, positive, intermediate, and negative remodeling.^{1–4} Positive remodeling of the arterial wall delays the development of lumen narrowing that results from the accumulation of atherosclerotic plaque.^{1–5} On the contrary, several studies using intravascular ultrasound (IVUS) have emphasized that negative remodeling leads to lumen narrowing in certain lesions. Recent IVUS studies have suggested that positive remodeling is associated with unstable clinical presentation.⁶ Other IVUS studies reported that the preintervention pattern of remodeling is an independent predictor of clinical restenosis after coronary intervention.^{7,8}

Epidemiologic data have demonstrated that several biologic markers are associated with increased risk of cardiovascular disease or unstable clinical presentation. These included hyperlipidemia, elevated levels of lipoprotein(a) [LP(a)] and homocysteine, elevations in cell adhesion molecules, and elevations in molecules associated with infection or inflammation.^{9–11} However, the relationship between these biologic

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markers and the pattern of coronary artery remodeling are unknown. The objective of the current study was to evaluate the relationship between these biologic markers and the pattern of coronary artery remodeling.

Materials and Methods

Study Population

Preintervention IVUS images of 44 nonstent de novo lesions of native coronary artery (mean lesion length: 10.2 ± 0.3 mm) were obtained in 44 consecutive patients with chronic stable angina. The lesions had simple characteristics of morphology: type A in 18 lesions and type B1 in 26 lesions by American Heart Association/American College of Cardiology (AHA/ACC) classification.¹² The complex lesions, including ulcerative lesions, thrombus-containing lesions, and severe calcific lesions were not included in this study. All patients had objective evidence of myocardial ischemia and $\geq 50\%$ angiographic diameter stenosis by a visual estimate. Patients were studied after having given written informed consent as part of ongoing protocols approved by our Institutional Review Board.

Measurement of Biologic Markers

Plasma samples in the fasting state were collected 24 h before coronary intervention. The biologic markers included total cholesterol, LDL cholesterol, triglyceride, HDL cholesterol, LP(a), C-reactive protein (CRP), and homocysteine. Total cholesterol, triglyceride, and HDL cholesterol were measured by the enzymatic method on Hitachi 747-200 instruments (Hitachi Co., Tokyo, Japan). Low-density lipoprotein cholesterol was calculated as follows: LDL cholesterol = total cholesterol – (HDL cholesterol + triglyceride/5). The LP(a) and CRP were analyzed by a 1-step sandwich enzyme immunoassay on an Immunozymp LP(a) kit (Immuno A.G., Vienna, Austria) and a turbidimetric assay on a Cobas Integra (Roche Diagnostics, Indianapolis, Ind., USA), respectively. The homocysteine was determined by a fluorescence polarization immunoassay on an IMx system (Abbott Diagnostics, Abbott Park, Ill., USA). Hyperhomocysteinemia was defined as fasting plasma homocysteine levels $\geq 16 \mu\text{mol/l}$.¹¹

Intravascular Ultrasound Imaging Protocol

A preintervention IVUS study was performed after intracoronary administration of 0.2 mg nitroglycerin. The ultrasound catheter was advanced approximately 10 mm beyond the target lesion, and an imaging run was performed from beyond the target lesion to the aorto-ostial junction. The study was performed with a commercially available system (Boston Scientific Corporation/ Cardiovascular Imaging System, Inc., San Jose, Calif., USA) that used a 30 MHz single-element beveled transducer mounted on the end of a flexible shaft and rotated at 1,800 rpm within a 3.2 F short monorail imaging sheath. With this system, the transducer was withdrawn automatically at 0.5 mm/s to perform the imaging sequence. Ultra-

sound studies were recorded on a half-inch high-resolution s-VHS tape for off-line analysis.

Quantitative Intravascular Ultrasound Measurements

Validation of cross-sectional area (CSA) measurements of external elastic membrane (EEM), lumen, and plaque + media (P+M) by IVUS has been reported previously.^{13, 14} The EEM CSA (that represents total arterial CSA) was measured by tracing the leading edge of the adventitia. The lumen CSA was measured by tracing the interface between the lumen and the leading edge of the intima/plaque. The P+M CSA (that represents atherosclerotic plaque) was calculated as EEM CSA minus lumen CSA. The plaque burden was measured as P+M CSA divided by EEM CSA. The target lesion and both the proximal and distal reference segments were assessed quantitatively. The preintervention lesion site was the image slice with the smallest lumen CSA. Among image slices with the same minimum lumen CSA, the one with the largest plaque burden (and therefore, the largest P+M CSA) was measured. The proximal and distal reference segments were the most normal-looking segments within 5 mm proximal and distal to the lesion. At each image slice, EEM and lumen CSA were measured with a commercially available program for computerized planimetry.

Crossing a tight stenosis with an IVUS catheter may underperfuse the distal vessel, make identification of distal reference segment difficult and cause the CSA measurement to be unreliably small.⁴ Therefore, the remodeling index (RI) was defined as the (lesion/proximal reference segment) preintervention EEM CSA in this study. Positive remodeling was defined as an RI > 1.05 , negative remodeling as an RI < 0.95 , and intermediate remodeling as an RI between 0.95 and 1.05.⁶

Statistical Analysis

Statistical analysis was performed with an SPSS software program (Statistical Package for Social Sciences, Chicago, Ill., USA). Categorical data are presented as frequencies. Continuous data are presented as mean $\pm$ standard error. Comparison of continuous data was performed with an unpaired *t*-test for two groups and an analysis of variance (ANOVA) for more than two groups. A linear regression analysis was performed to evaluate the relationship between plasma biologic markers and coronary artery remodeling. A *p* value of < 0.05 was considered statistically significant.

Results

The baseline clinical and angiographic characteristics of 44 patients are presented in Table I. The EEM CSA of the lesion and proximal reference segment in 44 lesions was 14.1 ± 0.6 and $15.2 \pm 0.6 \text{ mm}^2$, respectively. The lumen CSA was 1.9 ± 0.1 and $8.7 \pm 0.4 \text{ mm}^2$, and plaque burden was 86 ± 1 and $43 \pm 1\%$, respectively. The RI was 0.94 ± 0.02 . The IVUS findings and the level of biologic markers according to the pattern of coronary remodeling are shown in Table II. The plasma homocysteine level was significantly lower in the le-

TABLE I Baseline clinical and angiographic characteristics

Number of patients	44
Age (years)	60 ± 1
Males (%)	33 (75)
Prior myocardial infarction (%)	1 (2)
Hypertension (%)	15 (34)
Diabetes mellitus (%)	5 (11)
Hypercholesterolemia (≥ 240 mg/dl) (%)	12 (27)
Cigarette smoking (%)	19 (43)
Number of disease vessel	
One (%)	28 (64)
Two (%)	10 (23)
Three (%)	6 (14)
Coronary artery dilated	
Left anterior descending (%)	24 (55)
Left circumflex (%)	10 (23)
Right coronary (%)	10 (23)

sions with negative remodeling and higher in the lesions with positive remodeling (10.8 ± 0.7 $\mu\text{mol/l}$ in negative remodeling, 13.1 ± 0.6 $\mu\text{mol/l}$ in intermediate remodeling, and 18.1 ± 2.8 $\mu\text{mol/l}$ in positive remodeling, $p = 0.021$). Total cholesterol level ($r = 0.092$, $p = 0.557$), LDL cholesterol level ($r = 0.123$, $p = 0.426$), triglyceride level ($r = 0.020$, $p = 0.901$), HDL cholesterol level ($r = 0.042$, $p = 0.789$), LP(a) level ($r = 0.062$, $p = 0.729$), and CRP level ($r = 0.266$, $p = 0.089$) did not significantly correlate with the RI. However, the plasma homocysteine level correlated positively with the RI ($r = 0.398$, $p = 0.008$) (Fig. 1). The RI was 1.04 ± 0.04 in the lesions ($n =$

FIG. 1 The plasma homocysteine level correlated positively with the remodeling index ($r = 0.398$, $p = 0.008$).

7) with hyperhomocysteinemia and 0.92 ± 0.03 in the lesions ($n = 37$) with a normal homocysteine level ($p = 0.034$).

Discussion

Histopathologic examination of human coronary artery specimens has shown that atherosclerotic arteries tend to undergo a compensatory enlargement during the early phase of plaque accumulation.⁵ On average, lumen compromise is delayed until the atherosclerotic lesion occupies more than an estimated 40 to 50% of the potential area within the internal elastic lamina (40 to 50% cross-sectional narrowing).^{4,5} An early study using IVUS underlined the concept of positive or compensatory remodeling with adaptive vessel enlargement.¹⁵

TABLE II Intravascular ultrasound findings and level of biologic markers according to the pattern of coronary remodeling

	Negative	Intermediate	Positive	p Value
Number of lesions	23	11	10	
IVUS findings				
Proximal reference segment				
EEM CSA (mm^2)	15.7 ± 0.7	15.7 ± 1.7	13.6 ± 1.1	0.349
Lumen CSA (mm^2)	9.1 ± 0.4	8.9 ± 0.9	7.6 ± 0.6	0.208
Plaque burden (%)	42 ± 2	42 ± 2	44 ± 1	0.625
Lesion segment				
EEM CSA (mm^2)	13.1 ± 0.7	15.4 ± 1.6	15.0 ± 1.5	0.245
Lumen CSA (mm^2)	1.9 ± 0.1	2.2 ± 0.2	1.7 ± 0.1	0.068
Plaque burden (%)	85 ± 1	84 ± 2	87 ± 2	0.315
Remodeling index	0.83 ± 0.02	0.99 ± 0.01	1.15 ± 0.03	0.001
Biologic markers				
Total cholesterol (mg/dl)	194.3 ± 7.8	201.1 ± 10.6	187.3 ± 14.7	0.722
Triglyceride (mg/dl)	164.5 ± 17.5	188.6 ± 22.4	176.6 ± 32.5	0.740
LDL cholesterol (mg/dl)	121.0 ± 6.8	123.7 ± 9.7	109.6 ± 13.2	0.607
HDL cholesterol (mg/dl)	40.4 ± 1.7	39.7 ± 2.5	42.4 ± 2.3	0.735
LP (a) (mg/dl)	37.3 ± 5.6	31.9 ± 6.4	26.8 ± 5.5	0.550
CRP (mg/dl)	0.8 ± 0.2	0.4 ± 0.1	0.3 ± 0.1	0.183
Homocysteine ($\mu\text{mol/l}$)	10.8 ± 0.7	13.1 ± 0.6	18.1 ± 2.8	0.021

Abbreviations: CRP = C-reactive protein, CSA = cross-sectional area, EEM = external elastic membrane, IVUS = intravascular ultrasound, LDL = low-density lipoprotein, HDL = high-density lipoprotein, LP (a) = lipoprotein (a).

However, recent studies, primarily using IVUS in vivo, have emphasized that in an important minority of lesions, inadequate or negative arterial remodeling contributes to lumen compromise.²⁻⁴ The exact mechanism of negative remodeling is not clear. Possible explanations for negative remodeling are (1) that positive remodeling may fail from the outset, (2) that early positive remodeling may be followed by late negative remodeling, and (3) that certain types of plaque elements (i.e., calcium) may limit the extent of positive remodeling.⁷ A recent IVUS study suggested that positive remodeling was more frequent in unstable than in stable angina (51.8 vs. 19.6%), whereas negative remodeling was more frequent in stable angina (56.5 vs. 31.8%) ($p = 0.001$).⁶ The results of the current study (negative remodeling in 52%, intermediate remodeling in 25%, and positive remodeling in 23%) were similar to those of the previous study.

Homocysteine is a sulphhydryl-containing amino acid derived from the metabolic demethylation of dietary methionine that is abundant in animal protein. The prevalence of hyperhomocysteinemia has been estimated to be 5% in the general population, and 13–47% among patients with symptomatic atherosclerotic vascular disease.^{16,17} The prevalence of hyperhomocysteinemia was 15.9% (7/44 patients) in this study. Emerging evidence from epidemiologic studies supports positive association between plasma homocysteine levels and increased cardiovascular risk factors. Cross-sectional and retrospective case-control studies suggested that there was a graded relation between homocysteine level and vascular risk.¹⁰ A 5 $\mu\text{mol/l}$ increase in homocysteine level was associated with an increase in vascular risk of about one-third,¹⁸ and with a relative risk of atherosclerotic vascular disease of 1.35 in men and 1.42 in women.¹⁹ However, the results of prospective cohort studies that evaluated the association between elevated homocysteine levels and cardiovascular disease remain inconclusive.¹⁰

Several in vitro experimental studies have suggested that homocysteine promotes atherogenesis by facilitating oxidative arterial injury, damaging the vascular matrix, and augmenting the proliferation of vascular smooth muscle;^{10,20} however, little is known about the in vivo contributions of hyperhomocysteinemia to atherosclerosis. Therefore, despite numerous epidemiologic and clinical data, the pathophysiologic mechanism by which homocysteine might be involved in atherosclerosis is not clearly understood. Likewise, the exact mechanism of positive remodeling in patients with hyperhomocysteinemia in the current study is not definitely clarified. A recent in vivo animal study showed that diet-induced hyperhomocysteinemia exacerbates neointimal formation in rat carotid arteries after balloon injury.²¹ A clinical study revealed that asymptomatic adults with thickened intimal-medial carotid walls had significantly higher plasma homocysteine levels than controls.²² Therefore, the positive remodeling in patients with hyperhomocysteinemia in the current study might be partly explained by Glagov's hypothesis⁵ of vascular remodeling (enlargement of the coronary artery in relation to the plaque area to delay functionally important lumen stenosis). The positive remodeling occurred more frequently in pa-

tients with hyperhomocysteinemia to delay lumen compromise by plaque accumulation and to maintain the adequate coronary perfusion; however, positive remodeling was more frequent in unstable than in stable angina.⁶ Those findings support the concept of the remodeling paradox that positive remodeling prevents luminal narrowing on one hand, but may be associated with vulnerable plaques on the other hand.²³ This study shows an adequate clinical example of the remodeling paradox that an elevated homocysteine level may be one of the predictors of poor long-term clinical outcome and may be associated with positive remodeling.

Limitations

This study has several limitations. First, the number of patients was too small to suggest clinical implications. The plasma homocysteine level correlated moderately ($r = 0.398$, $p = 0.008$), not highly, with RI. Therefore, larger numbers of patients are needed to draw a definite conclusion between homocysteine and coronary artery remodeling, and to achieve a statistically clearer significance. Second, the lesions with severe calcifications were excluded in this study because calcium interferes with the exact evaluation of EEM. Therefore, the findings of this study could not be applied to severe calcific lesions. Third, the duration of elevated biologic markers, including homocysteine (whether it is a short-term or long-term effect on the coronary artery remodeling), was not evaluated in this study.

Conclusions

Elevated homocysteine levels might be associated with coronary artery remodeling in patients with chronic stable angina.

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