High Remnant Lipoprotein Levels in Patients with Variant Angina

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Summary

Background: Dyslipidemia with increased oxidative stress but without elevation of low-density lipoprotein cholesterol has been recently implicated in the pathogenesis of coronary vasospasm.

Hypothesis: Disordered triglyceride-rich lipoprotein metabolism may be linked to the genesis of coronary artery spasm.

Methods: Both serum remnant lipoprotein (RLP) and α -tocopherol levels were determined in 18 patients with the active stage of variant angina (VA), in 16 patients with the inactive stage of variant angina (IVA), and in 19 control subjects (CONTROL).

Results: The RLP levels were significantly ($p < 0.05$) higher in VA (6.4 ± 2.7 mg/dl) than in IVA (4.4 ± 1.5 mg/dl). In contrast, α -tocopherol levels were significantly lower in VA than that in CONTROL. Serum triglyceride levels were not significantly different among the study groups, although serum high-density lipoprotein cholesterol levels were significantly lower in VA than in CONTROL. Smoking was significantly ($p < 0.05$) more prevalent in VA (72%) than in IVA (25%) and CONTROL (37%). Serum RLP levels correlated positively with triglyceride levels ($R = 0.73$) and correlated inversely with α -tocopherol levels ($R = -0.31$) significantly in all study subjects.

Conclusions: Patients with active stage of variant angina had higher RLP levels than inactive patients with variant angina and lower α -tocopherol levels than control subjects. Disordered triglyceride-rich lipoprotein metabolism with increased ox-

idative stress appears to be linked to the activity of coronary vasospasm, suggesting a possible role in its pathogenesis.

Key words: coronary vasospasm, variant angina, remnant lipoproteins, α -tocopherol

Introduction

Lipid disorders have been recognized as an important risk factor for atherosclerotic coronary artery disease.^{1,2} Dyslipidemia in patients with coronary vasospasm has been characterized by a low level of high-density lipoprotein (HDL) cholesterol as well as apolipoprotein A-I, without elevation of low-density lipoprotein (LDL) cholesterol, distinct from patients with organic coronary artery disease.^{3,4} A low plasma level of antioxidant vitamin E concomitant with increased oxidative susceptibility of LDL was demonstrated in patients with variant angina (VA).^{5,6}

Recently we have reported that the “midband lipoprotein,”⁷ which is observed between LDL and very low-density lipoprotein (VLDL) by polyacrylamide gel electrophoresis, was frequently detected in patients with coronary vasospasm, suggesting that dyslipidemia with disordered triglyceride-rich lipoprotein metabolism may be linked to the genesis of coronary vasospasm.⁸ The “midband lipoprotein” mainly consisted of VLDL remnant lipoproteins, which are known to be atherogenic.^{9,10} Recent reports showed that endothelium-dependent vasodilation was impaired in patients with high remnant lipoprotein (RLP) levels, and the increase in oxidative stress may partly contribute to endothelial vasomotor dysfunction in patients with high RLP levels.^{11–13}

In the present study, we tried to clarify whether RLP is linked to the genesis or activity of coronary artery spasm by determining its serum levels in patients with active stage of VA compared with those in the inactive stage of the disease and also control subjects.

Methods

Study Patients

Consecutive 18 patients with VA who had had at least two episodes of angina in the preceding week, and in whom electrocardiographic ST-segment deviation (≥ 0.1 mV) was con-

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firmed during anginal attacks, were subjected to the RLP assay. In all patients, coronary artery spasm was angiographically demonstrated in at least one of the major coronary arteries during spontaneous attacks or those induced by intracoronary injection of acetylcholine.¹⁴ None had significant (> 75% luminal narrowing) organic coronary arterial stenosis, although mild lesions or irregularity were angiographically detected in most cases. Another 16 patients with inactive stage of variant angina (IVA), who had had no anginal attack during the preceding 6 months while receiving calcium entry blocking agents, were also included in the study. For control, 19 age- and gender-matched subjects without any significant systemic disease, who had negative treadmill exercise stress test results with the standard Bruce protocol (CONTROL), were selected. Patients receiving antioxidative agents, such as vitamins E and C, or probucol, and also patients receiving 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors were excluded from the study. No patient was receiving niacins, fibrates, or adrenergic beta-receptor blockers. Clinical and angiographic characteristics of the study groups are listed in Table I. Written informed consent was obtained from all study patients, and the study protocol was approved by the ethics committee of our institution.

Serum Analysis

Venous samples were obtained from the study subjects in the overnight fasting state. Remnant lipoprotein levels were measured using an immunoaffinity gel assay as reported previously.^{12, 13} Briefly, serum was added to an immunoaffinity-mixed gel suspension containing anti-apo-B-100 and anti-apo-A-I monoclonal antibodies (Japan Immunoresearch Labora-

tories, Takasaki, Japan). Cholesterol was measured in the unbounded fraction containing apo E-enriched lipoproteins by the enzymatic method. The LDL cholesterol concentration was calculated according to Friedwald's formula.¹⁵ The total lipid level in serum was calculated as total cholesterol plus triglyceride levels. The serum α -tocopherol levels were determined by high-performance liquid chromatography as reported previously.⁵

Assessment of Coronary Risk Factors

Current smoking was considered to be a factor if the patient had smoked > 10 cigarettes/day in the year preceding the procedure. Diabetes mellitus was diagnosed either clinically or by 75 g oral glucose tolerance test. Impaired glucose tolerance was diagnosed by 75 g oral glucose tolerance test, based on World Health Organization (WHO) diagnostic guidelines.¹⁶ Hypertension was defined as a persistent resting blood pressure exceeding 140 (systolic) or 90 (diastolic) mmHg.

Statistical Analysis

Values are presented as mean $\pm$ standard deviation. Comparisons of serum lipid levels and age among the three groups were performed with one-way analysis of variance (ANOVA) followed by Scheffé's test. The clinical characteristics and electrophoretic profiles of the study groups were compared by chi-square test with Yates' correction. The Student's *t*-test was used to determine the significance of the difference between the two groups. The level of significance was set as $p < 0.05$.

Results

Results are summarized in Table I and Figures 1–4. As shown in Table I, smoking was found to be significantly ($p < 0.05$) more prevalent in VA (72%) than in CONTROL (37%)

TABLE I Clinical characteristics and serum lipid levels of the study groups

Characteristics	CONTROL	VA	IVA
No. of patients	19	18	16
Age	57 $\pm$ 15	59 $\pm$ 11	60 $\pm$ 11
Male/female	15/4	15/3	13/3
Smoking (%)	37	72 ^a	25
Hypertension (%)	0	39	44
Diabetes mellitus (%)	0	17	13
Serum lipid (mg/dl)			
Total cholesterol	198 $\pm$ 22	183 $\pm$ 30	193 $\pm$ 29
LDL cholesterol	119 $\pm$ 21	108 $\pm$ 24	121 $\pm$ 22
Triglycerides	113 $\pm$ 47	145 $\pm$ 69	114 $\pm$ 48
HDL cholesterol	57 $\pm$ 13	45 $\pm$ 17 ^b	49 $\pm$ 15
RLP	4.8 $\pm$ 2.0	6.4 $\pm$ 2.7 ^c	4.4 $\pm$ 1.5
Serum α -tocopherol (mg/g)	3.9 $\pm$ 0.7	3.1 $\pm$ 0.7 ^b	3.6 $\pm$ 0.6

^a $p < 0.05$ vs. others.

^b $p < 0.05$ vs. CONTROL.

^c $p < 0.05$ vs. IVA.

Abbreviations: VA = variant angina, IVA = inactive stage of variant angina, LDL = low-density lipoprotein, HDL = high-density lipoprotein, RLP = remnant lipoprotein.

FIG. 1 Plots of serum remnant lipoprotein (RLP) levels in study groups. The mean level was significantly higher in patients with active stage of variant angina (VA) than in patients with inactive stage of variant angina (IVA). CONTROL = control subjects.

FIG. 2 Plots of serum α -tocopherol levels in study groups. The mean level was significantly lower in VA than in CONTROL and IVA. Abbreviations as in Figure 1.

and IVA (25%). The incidence of hypertension or diabetes mellitus did not differ significantly between VA and IVA.

Serum Lipid Levels

Serum lipid levels in the study patients are shown in Table I. Compared with CONTROL, serum HDL cholesterol levels were significantly lower in VA. No significant differences were noted in serum total cholesterol, LDL cholesterol, or triglyceride levels among the groups, although the mean serum triglyceride level was somewhat higher in VA than in the other two groups.

Serum Remnant Lipoprotein Levels

As shown in Table I and Figure 1, the mean serum RLP level was significantly higher in VA than in IVA. No significant difference was noted in the mean RLP levels between CONTROL and VA or IVA. In the total study subjects, the mean RLP level was significantly ($p < 0.01$) higher in smokers (6.3 ± 2.5 mg/dl, $n = 24$) than in nonsmokers (4.4 ± 1.8 mg/dl, $n = 29$).

Serum α -Tocopherol Level

As shown in Table I and Figure 2, the mean serum α -tocopherol level corrected by the total lipid level was found to be significantly lower in VA than in CONTROL as well as in IVA. In the total study subjects, the mean α -tocopherol level tended to be lower in smokers (3.4 ± 1.0 mg/g lipids, $n = 24$) than in nonsmokers (3.7 ± 0.5 mg/g lipids, $n = 29$), but the difference was not significant.

Correlation between Remnant Lipoprotein and Triglyceride or α -Tocopherol Levels

The correlation between serum RLP and triglyceride or α -tocopherol levels was analyzed in all 53 study patients. Pos-

FIG. 3 Correlation between serum remnant lipoprotein (RLP) and triglyceride levels in all study subjects. A significant positive correlation was found between them.

FIG. 4 Correlation between serum remnant lipoprotein (RLP) and α -tocopherol levels in all study subjects. A significant inverse correlation was found between them.

itive correlation of serum RLP levels with the triglyceride levels ($R = 0.73$, $p < 0.001$) (Fig. 3), was significant, as was negative correlation of serum RLP levels with the α -tocopherol levels ($R = -0.31$, $p < 0.05$) (Fig. 4).

Discussion

High Remnant Lipoprotein Levels and Disordered Triglyceride-Rich Lipoprotein Metabolism

Triglyceride-rich lipoproteins, including chylomicrons and VLDLs, are hydrolyzed by lipoprotein lipase and then hepatic triglyceride lipase. Since VLDL remnants are the intermediate products of VLDL catabolism, and most RLPs are cleared rapidly from the circulation by hepatic uptake in normolipidemic subjects, high RLP levels suggest an impairment of triglyceride-rich lipoprotein catabolism.¹⁰ Besides abnormalities in lipoprotein lipase or hepatic triglyceride lipase activity, and excessive triglyceride intake, overexpression of dysfunctional apoE mutants also leads to accumulation of VLDL and remnant particles in the plasma, as the endocytosis of triglyceride-

rich lipoproteins by hepatocytes occurs via the interaction of apoE with receptors present on these cells.¹⁷ Both high RLP levels and low HDL cholesterol levels are generally observed in association with hypertriglyceridemia because production of HDL cholesterol appears to be coupled with the process of the degradation or lipolysis of triglyceride-rich lipoproteins.¹⁸

Oxidative Stress and Remnant Lipoproteins

Triglyceride-rich lipoproteins may contribute to lesion progression, plaque rupture, and clinical coronary events by a variety of mechanisms.¹⁹ Triglyceride-rich lipoproteins are susceptible to peroxidative damage, can be taken up by macrophages directly without oxidative modification to produce foam cells, and are intimately associated with the clotting and fibrinolytic pathways, thus linking atherosclerosis and thrombosis.¹⁰ Recently, RLPs have been shown to impair endothelium-dependent arterial relaxation in rabbit aortic preparations.¹¹ In addition, RLP levels were reported to be independently associated with abnormal endothelium-dependent vasomotor function in humans, suggesting that a triglyceride-rich lipoprotein metabolic disorder may cause endothelial dysfunction.^{12, 20} In the present study, a weak but significant inverse correlation was noted between serum RLP and α -tocopherol levels. In patients with high RLP levels, the prolonged retention of RLPs in the circulation, as a result of the delayed hepatic uptake and/or the increased hepatic secretion of VLDL, may augment susceptibility of remnants to oxidative modification in the arterial intima, leading to an increase in the injurious effects of remnants on endothelial function.¹² During incubation of endothelial cells with RLPs, endothelium-derived reactive oxygen species may initiate and propagate a chain of free radical reactions, especially in polyunsaturated fatty acids in RLPs, leading to the production of highly reactive intermediates.¹² Doi *et al.* reported that RLPs increased intracellular oxidant levels in the cultured cells, and that α -tocopherol and N-acetylcysteine suppressed RLP-induced increases in mRNA and protein expression of intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and tissue factor.²¹

High Remnant Lipoprotein Levels Associated with Smoking and Impaired Glucose Metabolism

Disordered triglyceride-rich lipoprotein metabolism is frequently associated with cigarette smoking and glucose intolerance.^{22–24} Cigarette smokers have a high level of cholesterol-rich VLDL as well as a low level of HDL cholesterol.²⁴ Topping *et al.* reported that clearance of remnants in smokers would be delayed and their concentrations would rise concomitantly.²⁵ High smoking prevalence appeared to be an important contributing factor in high RLP levels in patients with VA. Indeed, smoking is known to be a major risk factor for coronary spasm.²⁵

Recent reports demonstrated that patients with coronary vasospasm exhibited a high incidence of impaired glucose tolerance with late hypersecretion of insulin.²⁶ We have reported

that nonobese Japanese type 2 diabetic patients are characterized by high RLP levels.²⁷ Dyslipidemia secondary to diabetes mellitus, hyperinsulinemia, or glucose intolerance causes impairment of catabolism of triglyceride-rich lipoprotein, lowers lipoprotein lipase activity, and increases RLP levels.

Remnant Lipoprotein in the Pathogenesis of Coronary Artery Spasm

In the present study, patients with the active stage of VA had a higher level of RLPs and a lower level of antioxidant vitamin E, suggesting increased oxidative stress with disordered triglyceride-rich lipoprotein metabolism. Recently Sakata *et al.* reported that RLP is a major risk factor for myocardial infarction in patients with vasospastic angina.²⁸

As vascular smooth muscle hypercontraction based on endothelial dysfunction or damage is postulated as the pathogenesis of coronary artery spasm, dyslipidemia with abnormal triglyceride-rich lipoprotein metabolism causing enhancement of coronary tone, via endothelial dysfunction, may predispose patients to coronary spasm.²⁹ One additional potential mechanism for the effects of the RLPs is that they may cause a dysfunction of Gi protein-dependent signal transduction in vascular smooth muscle cells, as has been proposed for LDL and modified LDL.³⁰

Although it is possible that triglyceride-rich RLPs play a key role as a substrate for oxygen free radical reactions in the pathogenesis of coronary artery spasm, it cannot be excluded that impaired triglyceride-rich lipoprotein metabolism may be caused by the lipid oxidative stress from the frequently repeated spasm-induced ischemia/reperfusion. It is also conceivable that RLPs may represent some form of acute phase reaction rather than the cause of coronary spasm. Further studies for multivariate analysis in a large population of patients are needed to clarify the causal relation between high RLP levels and coronary vasospasm.

Conclusion

Although the precise mechanisms by which disordered triglyceride-rich lipoprotein metabolism relates to the genesis of coronary spasm remain to be elucidated, RLP levels are high in patients with coronary spasm and seem to be intimately related to disease activity.

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