

Significance of the lipid profile and endothelium-dependent vasodilatation in the pathogenesis of microvascular angina

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Abstract

Background: *To investigate the significance of lipid disorders and endothelial dysfunction in the pathogenesis of microvascular angina.*

Methods: *Levels of plasma lipids, lipoproteins and apolipoproteins were assessed in 21 patients with microvascular angina and 24 healthy subjects as controls. Also, the endothelium-dependent vasodilatation function was determined with high-resolution ultrasound in both groups.*

Results: *Levels of serum total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), apolipoprotein B₁₀₀ (ApoB₁₀₀) and lipoprotein(a) [Lp(a)] in microvascular angina group were significantly higher than those in healthy subjects (each $p < 0.05$). The flow-mediated dilatation (FMD) in brachial arteries in patients with microvascular angina declined significantly as compared with that in control subjects ($4.7 \pm 1.9\%$ vs. $12.8 \pm 3.7\%$, $p < 0.001$). However, no significant difference was observed in response to nitroglycerin between groups ($19.7 \pm 8.1\%$ vs. $21.2 \pm 6.6\%$; $p > 0.05$). Linear correlation analysis revealed a significant negative correlation between the FMD of brachial arteries and the serum levels of LDL-C and Lp(a) in the microvascular angina group ($r = -0.5125$ and -0.4271 , respectively, both $p < 0.001$). Subsequently, all subjects were pooled and divided into two groups (groups A and B) according to the degree of FMD in brachial arteries ($A \leq 4\%$ and $B > 4\%$). The serum LDL-C level was found to be significantly higher in group A than in group B (4.09 ± 0.65 mmol/L vs. 2.59 ± 0.49 mmol/L; $p < 0.05$).*

Conclusions: *Plasma lipid disorders and vascular endothelial dysfunction may play important roles in the development of microvascular angina. The dysfunction of endothelium-dependent vasodilation was mainly associated with anomalies in LDL-C and Lp(a), and myocardial endothelial dysfunction was aggravated by lipid abnormalities in patients with microvascular angina. (Cardiol J 2008; 15: 324–328)*

Key words: lipid profile, vasodilatation, endothelium, vascular, ultrasonography, microvascular angina

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Introduction

Microvascular angina has recently been discovered as a new type of angina. Patients with this condition have symptoms of typical exertional angina with a positive exercise test along with normal coronary angiography and left ventricular function, but without large coronary artery spasm, extracardiac factors or established heart disease. Recent research has shown that anomalous blood flow reserve of the coronary arteries is an important feature, but mechanisms for this have not been fully defined though there may be a close association with lipid disorders and coronary endothelial dysfunction [1, 2]. In this study, we observed changes in blood lipid levels and changes in vascular endothelial function by high-resolution ultrasound in patients with microvascular angina to explore the significance of the two indexes in the pathogenesis of microvascular angina.

Methods

Subjects

A total of 21 patients [8 males and 13 females, aged 38–59 years (44 ± 8)] were recruited for the microvascular angina group. These patients had been diagnosed with microvascular angina and admitted to our hospital during the period from May 2003 to October 2007. All patients were selected according to the following criteria: history of typical exertional angina pectoris; positive results from an exercise test, Dobutamine stress test or Holter monitoring over 24 hours; sparse radioactive areas on movement and partial or complete refilling of radioactivity at rest as shown by ^{201}Tl myocardial imaging, without radioactive defects; normal coronary angiography and left ventricular function, with the exclusion of large coronary artery spasm by negative ergonovine provocation testing in 16 patients; exclusion of angina possibly caused by hypertension and/or hypertensive heart disease, cardiac valve disease, myocardioathy, pericarditis, etc. or non-cardiogenic chest pain caused by diseases of breast, lung, oesophagus, etc. or myocardial ischemic factors associated with anemia, diabetes mellitus, peripheral vascular lesions, etc. The control group consisted of 24 healthy volunteers, comprising 10 men and 14 women aged 35–60 years (46 ± 10), who underwent history and physical examination, electrocardiogram and tests for blood sugar and uric acid, and heart, kidney and liver function tests. None of the subjects were permitted to take medicines for 48 hours prior to testing.

The study was approved by the local bioethical committee and all patients gave their informed consent.

Measurement of blood lipid

Venous blood was collected from subjects after 12 h of fasting. Serum was immediately separated by centrifugation and stored at -70°C for later use. Serum total triglyceride (TG) and total cholesterol (TC) were measured by enzymatic methods on an automatic biochemical analyzer. Serum high-density lipoprotein-cholesterol (HDL-C) was tested by magnesium tungstate phosphate sedimentation and the concentration of low-density lipoprotein-cholesterol (LDL-C) was calculated by the Friedewald formula as follows: $\text{LDL-C (mmol/L)} = \text{TC} - \text{HDL-C} - \text{TG}/2.2$ ($\text{TG} < 4.5 \text{ mmol/L}$). Concentrations of apolipoprotein A₁ (ApoA₁) and B₁₀₀ (ApoB₁₀₀) were determined by circular immunoprecipitation. The concentration of lipoprotein(a) [LP(a)] was measured by sandwich ELISA assay.

Examination of vascular endothelial function

All tests were performed by an experienced ultrasonic doctor without prior knowledge of this study. Using the methods described previously [3, 4], we examined the brachial artery flow-mediated dilation (FMD) using an Acuson 128XP/10 colour Doppler sonograph (Acuson, Mountain View, CA, USA) with a 7.0 MHz array detecting head at an exploration depth of 4 cm; simultaneous electrocardiograph readings were recorded. At the time of measurement, subjects were in supine position with right upper extremities abducted 15 degrees and palms facing upward. The brachial artery 2–15 cm above the elbow was scanned by two-dimensional ultrasound imaging and longitudinal sections were observed. When the arterial intima of both anterior and posterior walls were most clearly defined, amplification was adjusted to satisfactorily identify lumen interfaces. At the ventricular diastasis (ECG showing R wave), the distance between the anterior and posterior intima of the brachial artery was measured for three different cardiac cycles and the average was calculated.

Brachial arterial calibre at rest and its changes in response to reactive hyperemia and sublingual nitroglycerin were measured in all subjects. Before the reactive hyperemia test, subjects were allowed to rest for 10 min and the baseline value of arterial calibre (D_0) was determined. A sphygmomanometer cuff was placed below the elbow, inflated to 300 mm Hg and deflated quickly after 4 min. The brachial arterial calibre (D_1) was measured within

60–90 s of the deflation. Vascular calibre was allowed to return to baseline status after resting for 10 min. Then the subjects were given 0.5 mg sublingual nitroglycerin, and brachial arterial calibre (D_2) was measured after 4 min. All calibre measurements were performed at the same site. Calibre changes of the brachial artery in response to reactive hyperemia and sublingual nitroglycerin were expressed as percentages of D_0 . $(D_1 - D_0) / D_0 \times 100\%$ represents endothelium-dependent, flow-mediated vasodilation; $(D_2 - D_0) / D_0 \times 100\%$ represents nitroglycerin-mediated, non-endothelium-dependent vascular relaxation.

Statistical analysis

SPSS10.0 for Windows statistical software was utilized to analyze the data and all results expressed as the mean \pm standard deviation. Student *t* test was employed to determine differences between the groups. The relationship between plasma lipids and brachial arterial calibre was determined by linear correlation analysis and multivariate linear stepwise regression analysis. $P < 0.05$ was regarded as statistically significant.

Results

General information

No significant differences were found between the microvascular angina group and control group with regard to sex and age, systolic and diastolic pressure, body mass index, number of cigarette smoking subjects, baseline heart rate, blood sugar and uric acid, hemorheology, etc. (all $p > 0.05$).

Levels of blood lipids

For fasting levels of TC, LDL-C, ApoB₁₀₀ and Lp(a), the microvascular angina group was significantly higher than the control group (each $p < 0.05$). However, there were no differences in serum TG, HDL-C and ApoA₁ between the groups ($p > 0.05$, Table 1).

Changes in brachial arterial calibre

There was no significant difference in the baseline values of brachial arterial calibre between the microvascular angina and control groups ($p > 0.05$). Calibre changes in response to reactive hyperemia in microvascular angina patients had significantly decreased as compared with those in healthy controls ($p < 0.001$). Administration of sublingual nitroglycerin led to obvious dilatation of the brachial artery with no significant difference observed between the groups ($p > 0.05$, Table 2).

Table 1. Comparison of blood lipids between the microvascular angina and control groups.

	Microvascular angina (n = 21)	Control group (n = 24)
TC [mmol/L]	5.42 \pm 0.53*	4.69 \pm 0.61
TG [mmol/L]	1.45 \pm 0.39	1.46 \pm 0.52
LDL-C [mmol/L]	3.98 \pm 0.42*	2.56 \pm 0.54
HDL-C [mmol/L]	1.43 \pm 0.30	1.44 \pm 0.47
ApoA ₁ [g/L]	1.21 \pm 0.36	1.23 \pm 0.25
ApoB ₁₀₀ [g/L]	1.01 \pm 0.37*	1.19 \pm 0.18
Lp(a) [mg/L]	282.4 \pm 220.3*	161.8 \pm 116.5

*Comparison with the control group: $p < 0.05$; all values are presented as mean \pm SD

Table 2. Comparison of changes in brachial artery calibre between the microvascular angina and the control groups.

	Microvascular angina (n = 21)	Control group (n = 24)
Baseline calibre [mm]	3.79 \pm 0.63	3.81 \pm 0.50
Flow-mediated dilation (%)	4.7 \pm 1.9*	12.8 \pm 3.7
Nitroglycerin-mediated dilation (%)	19.7 \pm 8.1	21.2 \pm 6.6

*Comparison with control group: $p < 0.001$; all values are presented as mean \pm SD

Association between lipid levels and FMD

Univariate correlation analysis indicated that brachial artery FMD was inversely related to LDL-C and Lp(a) levels ($r = -0.5125$ and -0.4271 , respectively, $p < 0.001$); multivariate stepwise regression analysis revealed that the plasma level of LDL-C was the major lipid component influencing FMD. According to the degree of FMD of the brachial artery ($\leq 4\%$ and $> 4\%$), all subjects ($n = 45$) from both groups were pooled and then divided into two groups: group A ($n = 25$) and group B ($n = 20$). The mean FMD for groups A and B were $1.41 \pm 1.20\%$ and $8.35 \pm 3.02\%$, respectively. It was found that the lipid disorders in group A were dominated by the increase in LDL-C (4.09 ± 0.65 mmol/L) and by the decrease in HDL-C in group B (2.59 ± 0.49 mmol/L). LDL-C levels differed significantly between the two groups ($p < 0.05$), while no significant differences were observed in the other lipid components (all $p > 0.05$).

Discussion

Microvascular angina, characterized mainly by a reduction in coronary artery dilatation reserve, differs from coronary artery disease; however, intra-coronary artery ultrasound and Doppler reveal early changes of coronary atherosclerosis in some patients displaying focal or eccentric lesions with pathologically-confirmed fatty deposits, infiltration of foam cells of macrophage origin, microarterial fibromuscular hyperplasia, vascular wall thickening, endothelial degeneration, etc. [5, 6]. The results of the present study indicated that lipid disorders in patients with microvascular angina mainly featured increased levels of TC, LDL-C, ApoB₁₀₀ and Lp(a), while TG, HDL-C and ApoA₁ were notably unchanged, in accordance with previous reports [7, 8]. Lipid disorders may result in fatty deposits in the vascular wall including microvessels. As an essential structural protein of lipoprotein, apolipoproteins are metabolic procedural agents which encode and regulate lipoprotein metabolism and balance cholesterol in the body. ApoB₁₀₀ exerts a strong effect to stimulate the esterization of cholesterol in microphages, which promotes foam cell formation. An elevated Lp(a) level is an independent risk factor for atherosclerosis. Its unique apolipoprotein, Apo(a), for the most part outside of the cell, is able to penetrate through arterial endothelium into the intima and bind to glycoprotein and collagen fibres to impair vascular endothelium, which, together with aberrant lipids, lead to eventual endothelial dysfunction [9, 10].

Endothelium-dependent vasodilation refers to vascular relaxation caused by endothelium-derived relaxing factor (EDRF) released by endothelial cells in response to physiological stimulations (e.g. reactive hyperemia) or drugs (e.g. acetylcholine), and is dependent on the complete structure and normal function of the vascular endothelium [11, 12]. In contrast, non-endothelium-dependent vasodilatation refers to that caused by nitric oxide (NO) directly released by drugs, e.g. nitroglycerin and sodium nitroprusside. In this study, we used calibre changes of the brachial artery in response to reactive hyperemia to reflect changes in endothelium-dependent vasodilation. Studies have demonstrated that endothelium-dependent dilation changes in peripheral vessels not only reflect endothelial function of peripheral vessels, but also correlate well with coronary endothelial function; therefore, brachial artery FMD could indirectly reveal coronary endothelial function in patients with microvascular angina [13, 14]. Results of this study have demon-

strated marked impairment in endothelium-dependent vasodilation of peripheral brachial arteries in patients with microvascular angina, with unapparent changes in non-endothelium-dependent vasodilation, indirectly reflecting the dysfunction of the coronary endothelium. With the exclusion of large coronary artery spasm by negative Ergonovine provocation test, endothelial dysfunction may lead to a decrease in the reserve of coronary artery dilation and dynamic abnormalities which play an important role in the development of microvascular angina.

Conclusions

In the study, LDL-C and Lp(a) levels in serum were demonstrated to be negatively associated with endothelium-dependent dilation, and LDL-C was shown to be the major contributing factor determined by multivariate analysis. Also, the grouping study based on the degree of brachial artery FMD revealed that an elevated LDL-C level was a major lipid component resulting in the impairment of endothelium-dependent dilation function. These results suggest that lipid disorders in microvascular angina aggravate endothelial impairment and have a role in the pathogenesis of microvascular angina. Therefore, positive monitoring of the lipid profile and endothelium-dependent dilation is valuable in early prophylaxis and diagnosis of microvascular angina. Balancing plasma lipid levels and protecting vascular endothelial function are critical in the prevention and cure of microvascular angina.

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