Impaired Endothelium-Dependent Vasodilation of Coronary Resistance Vessels in Hypercholesterolemic Patients

Nobuo Shiode, Masaya Kato, Akito Hiraoka, Togo Yamagata, Hideo Matsuura and Goro Kajiyama

To determine the relationship between hypercholesterolemia and the endothelial function of coronary resistance vessels, we studied the changes in coronary blood flow (CBF) in response to acetylcholine, an endothelium-dependent vasodilator, and adenosine, an endothelium-independent vasodilator, in patients with hypercholesterolemia (n=17) and in control patients (n=17). All patients had normal epicardial coronary arteries. Serial 2-min infusions of acetylcholine, at 3 μg/min and 30 μg/min, caused a dose-dependent increase in CBF in each group. The acetylcholine-induced maximal increases in CBF were inversely correlated with the serum cholesterol level (r=-0.55, p<0.01), and were significantly smaller in the hypercholesterolemic patients than in control patients. However, the adenosine-induced increases in CBF were similar in the two groups. These results suggest that the endothelium-dependent vasodilation of resistance vessels is lessened in patients with hypercholesterolemia even before the formation of atherosclerotic stenotic lesions in epicardial coronary arteries, and that hypercholesterolemia impairs endothelium-dependent vasodilation of coronary resistance vessels.

(Key words: acetylcholine, hypercholesterolemia, endothelium-derived relaxing factor (EDRF)

Introduction

The endothelium plays a major role in modulating vascular smooth muscle tone by synthesizing and metabolizing vasoactive substances (1), including endothelium-derived relaxing factor (EDRF) (2). Recent studies have demonstrated that acetylcholine infusion causes angiographically normal coronary arteries to dilate and arterial segments with atherosclerotic lesions to constrict (3–6). Acetylcholine also increases coronary blood flow, and atherosclerosis and coronary risk factors are associated with an impaired coronary blood flow response to acetylcholine (7–10). These findings suggest that coronary risk factors are responsible for endothelial dysfunction of both the epicardial and resistance coronary arteries.

Hypercholesterolemia, a primary risk factor for coronary atherosclerosis, impairs endothelium-dependent vasorelaxation of the large coronary artery before the formation of atherosclerotic lesions (6, 8, 11). Hypercholesterolemia is also associated with blunted endothelium-dependent vasodilation of the coronary microcirculation in animals and humans (9, 11–13). The present study was designed to determine the relationship between hypercholesterolemia and endothelial function of the coronary resistance vessels.

Patients and Methods

Patients

Thirty-four Japanese patients undergoing diagnostic arteriography for investigation of atypical chest pain were classified into two groups: 17 patients with hypercholesterolemia (fasting total cholesterol levels >220 mg/dl) and 17 controls without hypercholesterolemia (Table 1). All patients had angiographically normal, smooth epicardial coronary arteries, normal left ventricular function (contrast ventriculogram ejection fraction, ≥50%), and normal coronary flow reserve. Serum total and high density lipoprotein (HDL) cholesterol and triglycerides were determined by the enzyme assay method. Low-density lipoprotein (LDL) was calculated as follows: total cholesterol-HDL cholesterol-(triglyceride/5).

The hypercholesterolemic group (n=17) consisted of 7 men and 10 women, age range 48 to 74, mean 62±2 years. None of the patients were receiving cholesterol lowering agents prior to...
the study. Those with a family history of hypercholesterolemia, thickening of the Achilles tendon, or xanthoma were excluded from this study. The control group (n=17) consisted of 14 men and 3 women, age range 44 to 74, mean 58±2 years. All control patients had a total cholesterol level of <220 mg/dl.

None of the study participants had hypertension (defined as systolic and/or diastolic blood pressure >160 and/or 95 mmHg) or diabetes mellitus. Two of the hypercholesterolemic patients and 2 of the control patients were smokers (>10 cigarettes/day), but they were asked to refrain from smoking at least 72 hours before the study until completion. Written informed consent was obtained from all patients prior to the diagnostic angiogram, and all study methods were approved by the Human Investigation Ethical Committee of the University of Hiroshima.

**Design**

Anti-anginal therapy was discontinued 48 hours prior to catheterization, except for the unrestricted use of sublingual nitroglycerin, which was withheld 1 hour prior to catheterization. Patients were brought to the catheterization laboratory in the fasting state following premedication with hydroxyzine (25 mg, i.m.) and promethazine hydrochloride (25 mg, i.m.).

Diagnostic right and left heart catheterization and coronary angiography were performed using the standard percutaneous femoral approach. Once vascular access was obtained, 10,000 units of heparin were administered intravenously. A 6F guide catheter was introduced into the left main coronary artery. A 0.014-inch Doppler flow guidewire (FloWire, Cardiometrics, Mountain View, CA) then was advanced through the guide catheter into the proximal segment of the left anterior descending coronary artery.

**Protocol**

After diagnostic catheterization was completed, the following interventions were performed:

1) infusion of saline (1 ml/min for 2 minutes); 2) serial infusions of intracoronary acetylcholine, at 3 and 30 µg/min (1 ml/min, 2 minutes each); 3) infusion of 100 µg/min adenosine (1 ml/min for 2 minutes).

All drugs were infused into the left coronary ostium through the guide catheter. Infusions of adenosine and acetylcholine were administered at 5-min intervals. Coronary arteriography was performed just after the end of each infusion. Heart rate, arterial pressure, coronary blood flow velocity, and electrocardiogram were monitored continuously throughout each infusion, as well as under steady-state conditions.

**Quantitative coronary arteriography**

Coronary cineangiograms were recorded on 35-mm cinefilm (30 frames/sec) using a Siemens cineangiographic system with the view that allowed the best visualization of the left anterior descending coronary artery (LAD). Nonionic contrast medium (7–10 ml) was injected into the left coronary artery at a rate of 5–7 ml/sec. A power injector (Medrad, Pittsburgh, PA) was used to optimize the quality and reproducibility of the opacification (14). To avoid the possible effects of respiration, patients held their breath while angiograms were obtained (15). The segment of LAD 2 to 3 mm distal to the tip of the Doppler flow guide wire was selected for quantitative analysis. The lumen diameter of the segment was measured quantitatively with the aid of a computer-assisted coronary angiography analysis system. The arterial segments under study were video-digitized at end-diastole and then stored in a cardiac image analysis system (Cardio 500, Kontron Instruments, Munich). Automated counter detection was performed using a geometric edge differentiation technique similar to that described by Reiber et al (16). The diameter of the segment of interest was measured, and the averaged value from triplicate measurements was used for later analysis. A 6F Judkins catheter was used to calibrate the arterial diameter in millimeters. The arterial diameter measurements were taken without knowledge of the patients’ clinical characteristics.

**Measurements of coronary blood flow velocity and estimation of coronary blood flow**

A Doppler guidewire, with a 12 MHz piezo-electric transducer at its tip (FloWire), was used to measure the coronary artery blood flow velocity (17). Continuous flow velocity profiles using a 12 MHz pulsed Doppler velocimeter (FloMap, Cardiometrics), along with simultaneous electrocardiograms and aortic pressures, were displayed on a video monitor and recorded continuously on a 0.5-inch VHS videotape. Changes in coronary blood flow induced by the vasoactive agents were estimated from the product of the mean coronary blood flow velocity and the cross-sectional area 2 to 3 mm distal to the tip of the Doppler flow guidewire, because this Doppler transducer has a range gate depth of 4.2 mm.

**Drug preparation**

Acetylcholine (Daiichi Pharmaceutical Corp., Tokyo) was dissolved in physiological saline, immediately prior to use, 3 µg/ml or 30 µg/ml. Adenosine (Sigma Chemical Co., St. Louis, MO) was dissolved in physiological saline at a concentration of 100 µg/ml. Each drug was administered with an infusion pump (CFV 3000, Nihonkoden, Tokyo), at a rate of 1 ml/min.
Statistical analysis

Data are expressed as mean values±SEM. Changes in lumen diameter are expressed as the percent change from the baseline control value. For comparison of the dose-related responses to acetylcholine between the groups, two-way ANOVA for repeat measurements was used. Serial changes in blood flow, epicardial coronary artery diameter and hemodynamic variables in response to acetylcholine at the graded doses were compared using one-way ANOVA. If ANOVA showed a significant difference between the means, the level of significance was determined by contrast. The effects of adenosine infusion were compared with control conditions using paired t tests. A value of p<0.05 was considered statistically significant.

Results

Clinical characteristics and hemodynamic variables

Clinical characteristics are shown in Table 1. No significant changes in mean blood pressure, heart rate, or rate-pressure product occurred during the serial infusion of acetylcholine and adenosine in either group (Table 2).

Effects of acetylcholine on epicardial coronary arteries and coronary blood flow

In control patients, acetylcholine, at 3 µg/min, significantly increased the diameter of the epicardial coronary arteries (Fig. 1, Table 3), but it had no effect on the epicardial artery diameter at 30 µg/min. Acetylcholine caused a dose-dependent increase in coronary blood flow (Fig. 2, Table 4).

In patients with hypercholesterolemia, acetylcholine, 30 µg/min, produced a decrease in the diameter of epicardial coronary arteries, whereas 3 µg/min had no effect on coronary artery diameter (Fig. 1, Table 3). Acetylcholine caused a dose-dependent increase in coronary blood flow, but the percent increase was significantly lower in hypercholesterolemic patients than in control patients (Fig. 2, Table 4).

The acetylcholine-induced maximal increase in coronary blood flow was inversely correlated with total serum chole-

Table 2. Hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>ACh (µg/min)</th>
<th>Adenosine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>HR (n=17)</td>
<td>63±2</td>
<td>64±3</td>
<td>65±3</td>
</tr>
<tr>
<td>NC</td>
<td>98±3</td>
<td>99±3</td>
<td>98±3</td>
</tr>
<tr>
<td>mBP</td>
<td>8,450±538</td>
<td>8,794±607</td>
<td>8,864±623</td>
</tr>
<tr>
<td>RPP</td>
<td>8,600±578</td>
<td>8,670±578</td>
<td>8,600±578</td>
</tr>
<tr>
<td>HR (n=17)</td>
<td>63±2</td>
<td>64±2</td>
<td>64±2</td>
</tr>
<tr>
<td>HC</td>
<td>101±3</td>
<td>103±3</td>
<td>98±3</td>
</tr>
<tr>
<td>mBP</td>
<td>9,197±314</td>
<td>9,517±394</td>
<td>9,340±421</td>
</tr>
<tr>
<td>RPP</td>
<td>9,005±452</td>
<td>9,005±452</td>
<td>9,005±452</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SEM, ACh: acetylcholine, HC: patients with hypercholesterolemia, NC: control patients, HR: heart rate (beats/min), mBP: mean blood pressure (mmHg), RPP: rate-pressure product (mmHg beats/min).

Figure 1. Dose-dependent effects of acetylcholine (ACh) on epicardial coronary artery diameter (percent change from baseline) in patients with hypercholesterolemia (HC) and control patients (NC). Data are mean±SEM. *p<0.01 compared with baseline.

Table 3. The Percent Changes in Coronary Diameter

<table>
<thead>
<tr>
<th></th>
<th>HC (n=17)</th>
<th>NC (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline diameter</td>
<td>2.43±0.14 mm</td>
<td>2.51±0.13 mm</td>
</tr>
<tr>
<td>Acetylcholine 3 µg/min</td>
<td>-3.1±1.5</td>
<td>+6.3±1.3*</td>
</tr>
<tr>
<td>30 µg/min</td>
<td>-6.4±2.0*</td>
<td>+1.2±2.2</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SEM, HC: patients with hypercholesterolemia, NC: control patients. *p<0.01 compared with baseline.

Figure 2. Changes in coronary blood flow (CBF) response to serial infusions of acetylcholine (ACh) in patients with hypercholesterolemia (HC) and control patients (NC). Data are mean±SEM. *p<0.05, **p<0.01 compared with hypercholesterolemic patients.
sterol values (Fig. 3. The endothelium-dependent increase in coronary blood flow (percent of blood flow response to acetylcholine compared with that to adenosine) was also inversely correlated with serum cholesterol (Fig. 4).

**Effect of adenosine on coronary blood flow**

Adenosine significantly increased the coronary blood flow, and this increase was similar in both groups of patients (Fig. 2, Table 4).

**Discussion**

The primary finding was that coronary blood flow responses to intracoronary infusions of acetylcholine, an endothelium-dependent vasodilator, were reduced in patients with hypercholesterolemia, whereas coronary blood flow responses to adenosine, an endothelium-independent vasodilator, resembled those of control patients. The acetylcholine-induced maximal increases in coronary blood flow were inversely correlated with total serum cholesterol values. These results suggest that endothelium-dependent vasodilation of resistance vessels is reduced in hypercholesterolemic patients before the formation of atherosclerotic stenotic lesions in epicardial coronary arteries, and that hypercholesterolemia impairs the endothelium-dependent vasodilation of coronary resistance vessels.

Previous clinical studies in humans have demonstrated dilatation of angiographically normal epicardial coronary arteries and constriction of arterial segments with atherosclerotic lesions following acetylcholine infusion (3-5). Hypercholesterolemia has also been found to impair the endothelium-dependent vasorelaxation of the large coronary arteries before formation of atherosclerotic lesions (6). In the present study, 3 µg/min of acetylcholine increased the epicardial coronary artery diameter in control patients, but no such dilation was observed in patients with hypercholesterolemia. In contrast, 30 µg/min of acetylcholine reduced the epicardial coronary artery diameter in patients with hypercholesterolemia, but not in control patients. These results are consistent with those previously reported (3, 6, 18).

In this study, acetylcholine-induced increases in coronary blood flow were impaired in patients with hypercholesterolemia, whereas adenosine-induced increases in coronary blood flow were similar in the two patient groups. Our findings support the hypothesis that the endothelium-dependent vasodilation of resistance coronary vessels is impaired in patients with hypercholesterolemia (9, 12).

**Clinical implications**

Zeihler et al (19) recently reported that impaired endothelium-dependent vasodilation of the coronary resistance vessels is associated with the exercise-induced myocardial ischemia in patients without hemodynamically significant epicardial artery lesions. Endothelial vasodilator dysfunction extending into the coronary resistance vessels may play a role in the development of coronary artery disease in hypercholesterolemia.

Egashira et al (20) demonstrated that cholesterol-lowering therapy with pravastatin improves endothelium-dependent vasomotion of both large epicardial coronary arteries and...
resistance vessels in hypercholesterolemic patients. Examination of the endothelial function of coronary resistance vessels during cardiac catheterization in hypercholesterolemic patients may be a valuable method for detecting endothelial dysfunction and determining the effect of cholesterol-lowering therapy.

**Limitations**

In this study, the mean age of the patients in each group did not differ significantly, but the hypercholesterolemic patients tended to be older than the controls. Several studies (10, 21) have shown that advanced age is a significant independent predictor of impaired endothelium-dependent dilation of the coronary resistance vessels, regardless of the presence or absence of epicardial artery atherosclerosis. Therefore, the impairment of coronary blood flow response to acetylcholine seen in the present hypercholesterolemic patients may have been influenced by their age.

Here, the patients with hypercholesterolemia exhibited a significant decrease in epicardial coronary artery diameter in response to 30 μg/min. Constriction of epicardial coronary arteries may have influenced the coronary blood flow response to acetylcholine. However, no patients demonstrated >50% angiographic constriction at the highest dose of acetylcholine, indicating that the attenuated coronary blood flow response to acetylcholine was not caused by excessive vasoconstriction of the large epicardial coronary arteries.

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**References**