The Role of Nitric Oxide in Bradykinin-Induced Dilation of Coronary Resistance Vessels in Patients with Hypercholesterolemia


Object. In hypercholesterolemic patients, acetylcholine- and substance P-mediated endothelium-dependent dilation of the coronary resistance vessels is impaired due to decreased nitric oxide production. However, it is not clear if bradykinin-induced coronary vasodilation is impaired in these patients. We investigated whether the endothelium-dependent dilation of coronary resistance vessels mediated by bradykinin is impaired in patients with hypercholesterolemia and, if so, whether this impairment is caused by a decreased production of nitric oxide. Methods. We examined the coronary vascular responses to acetylcholine and bradykinin. The vascular responses to bradykinin were also assessed after N\textsuperscript{G}-monomethyl-L-arginine was infused to inhibit nitric oxide production. Drugs were infused into the left coronary ostium and coronary blood flow (CBF) and coronary vascular resistance were evaluated by quantitative angiography and Doppler flow velocity measurements. Patients. Twelve hypercholesterolemic patients and 11 control patients with angiographically normal coronary arteries were studied. Results. The vasodilator responses to acetylcholine and bradykinin were reduced in hypercholesterolemic patients compared with control patients (p<0.005 and p<0.04, respectively, by two-way analysis of variance (ANOVA)). The CBF responses to acetylcholine and bradykinin were significantly correlated (r=0.56; p<0.01). Bradykinin-induced dilation was similar in hypercholesterolemic patients and control patients after inhibition of nitric oxide. Conclusion. These results suggest that the bradykinin-mediated endothelium-dependent dilation of coronary resistance vessels may be impaired due to depressed nitric oxide production in patients with hypercholesterolemia. (Internal Medicine 38: 394-400, 1999)

Key words: endothelial function, acetylcholine, Doppler guidewire

Introduction

The vascular endothelium is important in the modulation of vascular smooth muscle tone via the production and release of vasoactive factors (1–4). These factors are produced in response to a variety of pharmacologic stimuli, including acetylcholine and bradykinin (1–9). Human coronary endothelial function is generally investigated by stimulating the production of endothelium-dependent vasorelaxing agents with acetylcholine (11–15). Impaired endothelium-dependent coronary vasodilation in response to acetylcholine has been demonstrated in patients with various coronary risk factors, including hypercholesterolemia which is a primary risk factor for coronary atherosclerosis (15–17).

Bradykinin, another endothelium-dependent vasoactive agent, is a potent vasodilator that acts through vascular endothelial B\textsubscript{2} kinin receptors using a different signal transduction pathway than acetylcholine (18–20). Bradykinin has been shown to induce endothelium-dependent relaxation of large and small coronary vessels in humans (21–25).

We investigated whether the impairment of endothelium-dependent dilation of human coronary resistance vessels is selective for endothelium-dependent agents by evaluating the vascular responses to acetylcholine and bradykinin in patients with hypercholesterolemia. Furthermore, to determine whether this impairment reflects a decreased bioavailability of nitric oxide, we examined the responses to bradykinin before and after infusion of N\textsuperscript{G}-monomethyl-L-arginine (l-NMMA), an
inhibitor of nitric oxide synthesis.

Methods

Study population
We studied 12 patients with hypercholesterolemia (fasting total serum cholesterol level >220 mg/dl without antihypercholesterolemic drugs) and 11 patients without hypercholesterolemia (total serum cholesterol level ≤210 mg/dl) who had atypical chest pain, normal left ventricular function (contrast ventriculogram ejection fraction, ≥50%), and normal coronary flow reserve (Table 1). These patients had no angiographic evidence of significant stenosis in the epicardial coronary arteries. Serum levels of total and high-density lipoprotein (HDL) cholesterol and triglycerides were determined by enzyme assays. The serum level of low-density lipoprotein (LDL) cholesterol was calculated as follows: total cholesterol-HDL cholesterol-(triglyceride/5).

The hypercholesterolemic group contained more females than the control group. However, only one hypercholesterolemic female was pre-menopausal and no patients were receiving estrogen supplements. None of the hypercholesterolemic patients had a family history of hypercholesterolemia. None of the study patients had hypertension (defined as a systolic and/or diastolic blood pressure >160 and/or 95 mmHg) or diabetes mellitus. Patients with a previous history of myocardial infarction, cardiomyopathy, valvular heart disease, or heart failure were excluded. Patients with coronary spastic angina who showed angiographically documented coronary spasm (≥50% luminal narrowing) after intracoronary injection of acetycholine were also excluded. Smokers were asked to refrain from smoking for the 72 hours preceding the study. Written informed consent was obtained from each patient prior to the study, and the protocol for this study was approved by the Human Investigation Ethics Committee of Hiroshima University.

Study design
Antianginal, antihypercholesterolemic, and antihypertensive drugs, including angiotensin-converting enzyme inhibitors, were discontinued at least 48 hours prior to the study. Patients were brought to the catheterization laboratory in the fasting state following premedication with hydroxyzine (25 mg administered intramuscularly) and promethazine hydrochloride (25 mg administered intramuscularly). Diagnostic catheterization was performed by the standard percutaneous femoral approach. Heparin (10,000 U) was administered intravenously just before catheterization. After completion of diagnostic coronary angiography, a 6-Fr guide catheter was introduced into the left main coronary artery. A 0.014-inch Doppler flow guidewire (Flowire, Cardiometrics Inc, Mountain View, CA) (26, 27) was then advanced into the proximal segment of the left anterior descending coronary artery, and carefully positioned in a straight segment of the vessel to permit an adequate flow velocity signal. The vessel could be visualized within 1 cm of the wire tip without overlap from other vessels to allow for quantitative measurements of the luminal diameter.

Study protocol
After baseline measurements were obtained, acetylcholine was infused at rates of 3 and 30 μg/min for 2 minutes each. After a 15-minute interval, baseline measurements were obtained again, and bradykinin was infused at rates of 0.5, 1.5, and 2.5 μg/min for 2 minutes at each dose. Our preliminary study indicated that 2.5 μg/min of bradykinin induced the maximal increase in endothelium-dependent coronary blood flow (CBF) (data not shown). Adenosine was then infused at a rate of 100 μg/min for 2 minutes to evaluate the coronary flow reserve (28). To assess the bioavailability of nitric oxide, serial doses of bradykinin were infused after infusion of l-NMMA (60 μmol/min for 3 minutes) in 8 hypercholesterolemic patients and 9 control patients.

All drugs were infused directly into the left main coronary artery through a catheter with an infusion pump (CFV 3000, Nihonkoden, Tokyo) at a rate of 1 ml/min. We determined the baseline coronary diameter at least 5 minutes after infusion of each drug. Coronary angiography was performed under control conditions and just after the end of each drug administration. Arterial pressure (at the distal end of the catheter), heart rate, and cardiac rhythms (via 12-lead electrocardiograms) were monitored continuously and recorded on a multichannel recorder (Nihonkoden Polygraph System, Nihonkoden, Tokyo).

Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Hypercholesterolemic Patients (n=12)</th>
<th>Control Patients (n=11)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>55 ± 5</td>
<td>53 ± 10</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>256 ± 21</td>
<td>192 ± 18</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>135 ± 65</td>
<td>133 ± 29</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>55 ± 20</td>
<td>47 ± 8</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>174 ± 24</td>
<td>119 ± 15</td>
</tr>
<tr>
<td>Current smoker (&gt;10 cigarettes/day)</td>
<td>2</td>
<td>6</td>
</tr>
</tbody>
</table>

Data are expressed as the mean value±SD.
Quantitative coronary angiography

Quantitative coronary angiography was performed according to a previously described method (29). Coronary cineangiograms were recorded on 35-mm cinefilm (30 frames/s) using a cineangiographic system (Siemens Corp., Munich, Germany). Nonionic contrast medium was injected into the left coronary artery at a rate of 5 to 10 ml/s up to a total of 7 to 10 ml using a power injector (Medrad, Pittsburgh, PA). An end-diastolic frame was selected on the cineprojector, and the left anterior descending artery was scanned with a videocamera. Images were digitized and analyzed with a videodensitometric analysis system (Cardio-500, Kontron Instruments, Munich, Germany). The internal diameter of the vessel at the sampling site (2 to 3 mm distal to the wire tip) was measured by the geometric edge differentiation technique described in detail by Reiber et al (30). A 6-Fr Judkins catheter was used for calibration. The average of triplicate measurements of the luminal diameter was used for subsequent analysis.

Estimation of the CBF and coronary vascular resistance

CBF was calculated as the product of the CBF velocity and diameter using the following formula: \( \pi \times \text{average peak velocity} \times 0.125 \times \text{diameter}^2 \) (27). Coronary vascular resistance (CVR) was calculated as the mean arterial pressure divided by the CBF.

Preparation of drugs

Acetylcholine (Daiichi Pharmaceutical Corp., Tokyo), bradykinin (Sigma Chemical Co. St. Louis, MO, USA), adenosine (Sigma Chemical Co. St. Louis, MO, USA), and L-NMMA (Sigma Chemical Co. St. Louis, MO, USA) were dissolved in physiological saline at appropriate concentrations. Bradykinin, adenosine, and L-NMMA were sterilized at the Pharmacy Department of Hiroshima University Hospital.

Statistical analysis

Data are expressed as the mean ± SD. Changes in CBF and CVR are expressed as the percent change from the control value. Serial changes in hemodynamic variables, and the percent changes in CBF and CVR in response to acetylcholine and bradykinin were compared by one-way analysis of variance (ANOVA). If ANOVA showed a significant difference between means, the level of significance was determined by contrast. The difference in serial percent changes in CBF and CVR between groups was compared by two-way ANOVA. Patient characteristics for noncontinuous variables were compared by the chi-square test. Paired and unpaired data were compared using the Student’s t-test, as appropriate. All p values are two-tailed. A level of p<0.05 was considered statistically significant.

Results

Hemodynamic variables

Neither the intracoronary administration of acetylcholine, bradykinin, and adenosine nor the infusion of L-NMMA altered the baseline mean arterial pressure, heart rate, or rate-pressure product in either group.

Vasodilator effects of acetylcholine on coronary resistance vessels

Acetylcholine increased the CBF and decreased the CVR in a dose-dependent manner in both the hypercholesterolemic and control groups (p<0.001) (Table 2), but the effects of acetylcholine were significantly smaller in the hypercholesterolemic patients than in the control patients (Fig. 1).

Vasodilator effects of bradykinin on coronary resistance vessels

The second control CBF and CVR values were similar to the first control values in both groups. Bradykinin increased the CBF and decreased the CVR in a dose-dependent manner in both groups (p<0.001) (Table 2), but the effects of bradykinin were significantly smaller in the hypercholesterolemic patients than in control patients (Fig. 2). The increase in the CBF and the decrease in the CVR in response to 30 µg/min of acetylcholine were significantly correlated with those in response to 2.5 µg/min of bradykinin (r=0.56, p<0.006 for CBF; r=0.57, p<0.005 for CVR) (Fig. 3).

Table 2. Change in Coronary blood Flow and Coronary Vascular Resistance in Response to Acetylcholine and Bradykinin before L-NMMA

<table>
<thead>
<tr>
<th></th>
<th>Control 1</th>
<th>Acetylcholine (µg/min)</th>
<th>Control 2</th>
<th>Bradykinin (µg/min)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>3 30</td>
<td></td>
<td>0.5 1.5  2.5</td>
<td></td>
</tr>
<tr>
<td>Hypercholesterolemic Patients (n=12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBF (ml/min)</td>
<td>29 ± 12</td>
<td>37 ± 14** 53 ± 19*</td>
<td>28 ± 11</td>
<td>36 ± 13* 43 ± 20* 50 ± 22*</td>
</tr>
<tr>
<td>CVR (mm Hg x ml^-1 x min)</td>
<td>4.4 ± 2.9</td>
<td>3.3 ± 2.1* 2.1 ± 0.8* 4.3 ± 2.5</td>
<td>3.1 ± 1.3** 2.6 ± 0.8** 2.3 ± 1.0*</td>
<td></td>
</tr>
<tr>
<td>Control Patients (n=11)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBF (ml/min)</td>
<td>34 ± 11</td>
<td>60 ± 28* 107 ± 61*</td>
<td>35 ± 12</td>
<td>61 ± 17* 73 ± 22* 87 ± 37*</td>
</tr>
<tr>
<td>CVR (mm Hg x ml^-1 x min)</td>
<td>3.6 ± 1.7</td>
<td>2.2 ± 1.0* 1.3 ± 0.7* 3.4 ± 1.5</td>
<td>1.8 ± 0.4* 1.5 ± 0.4* 1.3 ± 0.4*</td>
<td></td>
</tr>
</tbody>
</table>

CBF: coronary blood flow, CVR: coronary vascular resistance, L-NMMA: Nω-monomethyl-L-arginine. *p<0.01 versus control, **p<0.05 versus control.

Internal Medicine Vol. 38, No. 5 (May 1999)
Bradykinin-Induced Coronary Vasodilation

Figure 1. The percent changes in coronary blood flow (CBF) and coronary vascular resistance (CVR) in response to acetylcholine in patients with hypercholesterolemia (solid circles) and control patients (open circles).

Figure 2. The percent changes in coronary blood flow (CBF) and coronary vascular resistance (CVR) in response to bradykinin in patients with hypercholesterolemia (solid circles) and control patients (open circles).
Kato et al

\[ y = 0.42x + 1.16, \ r = 0.56, \ p < 0.006 \]

\[ y = 0.50x + 0.27, \ r = 0.58, \ p < 0.005 \]

Figure 3. Correlation between the percent changes in coronary blood flow (CBF) and coronary vascular resistance (CVR) in response to 30 \( \mu \text{g/min} \) of acetylcholine and to 2.5 \( \mu \text{g/min} \) of bradykinin.

Table 3. Change in Coronary blood Flow and Coronary Vascular Resistance in Response to Bradykinin after \( 1\text{-NMMA} \)

<table>
<thead>
<tr>
<th>Bradykinin (( \mu \text{g/min} ))</th>
<th>0.5</th>
<th>1.5</th>
<th>2.5</th>
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<tbody>
<tr>
<td>Hypercholesterolic Patients (n=8)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CBF (ml/min)</td>
<td>20 ± 8</td>
<td>27 ± 11*</td>
<td>37 ± 14*</td>
</tr>
<tr>
<td>CVR (mm Hg • ml(^{-1})• min)</td>
<td>6.4 ± 3.9</td>
<td>4.7 ± 2.8*</td>
<td>3.2 ± 1.3**</td>
</tr>
<tr>
<td>Control Patients (n=9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBF (ml/min)</td>
<td>24 ± 8</td>
<td>34 ± 10*</td>
<td>46 ± 14*</td>
</tr>
<tr>
<td>CVR (mm Hg • ml(^{-1})• min)</td>
<td>5.2 ± 2.1</td>
<td>3.6 ± 1.1*</td>
<td>2.5 ± 0.7*</td>
</tr>
</tbody>
</table>

CBF: coronary blood flow, CVR: coronary vascular resistance, \( 1\text{-NMMA} \): N\(^\circ\)-monomethyl-L-arginine. *p<0.01 versus control, **p<0.05 versus control.

Bradykinin-induced dilation of coronary resistance vessels after nitric oxide inhibition

\( 1\text{-NMMA} \) reduced the CBF by 32±13% in 8 hypercholesterolemic patients and by 28±6% in 9 control patients (p<0.001). \( 1\text{-NMMA} \) increased the CVR by 50±22% in hypercholesterolemic patients and by 43±12% in control patients (p<0.001). Bradykinin increased the CBF and decreased the CVR in a dose-dependent manner after \( 1\text{-NMMA} \) infusion (Table 3), and the effects of bradykinin were similar in both groups (Fig. 4).

Vasodilator effect of adenosine on coronary resistance vessels

There were no significant differences in the changes in the CBF (228±113% vs 317±115%, p=NS) and the CVR (−68±10% vs −74±7%, p=NS) in response to adenosine between the hypercholesterolemic and control groups.

Discussion

Both the acetylcholine-induced and the bradykinin-induced dilation of coronary resistance vessels were impaired in patients with hypercholesterolemia. The coronary flow reserve in response to adenosine was similar in hypercholesterolemic and control patients. These results suggest that endothelium-dependent dilation of coronary resistance vessels is generally impaired in patients with hypercholesterolemia. Impairment of bradykinin-induced dilation of coronary resistance vessels may be caused by reduction in nitric oxide production.

Acetylcholine-induced coronary vasodilation in hypercholesterolemia

Human coronary endothelial function is generally examined
Bradykinin-Induced Coronary Vasodilation

Figure 4. Percent changes in coronary blood flow (CBF) and coronary vascular resistance (CVR) in response to bradykinin after nitric oxide inhibition by N\(^\text{\textsuperscript{G}}\)-monomethyl-L-arginine infusion in patients with hypercholesterolemia (solid circles) and control patients (open circles).

by stimulating the production of endothelium-derived relaxing factors with acetylcholine (11–17). Previous studies (14, 16) have demonstrated that acetylcholine-induced dilation of human coronary resistance vessels is impaired in patients with hypercholesterolemia. The present results are consistent with these previous studies.

Comparison of the effects of acetylcholine and bradykinin in patients with hypercholesterolemia

Bradykinin, another endothelium-dependent vasoactive agent, does not directly induce coronary vascular smooth muscle contraction under normal conditions, but it is a potent vasodilator that acts through vascular endothelial B2 kinin receptors to induce the release of endothelium-derived relaxing factors. Bradykinin uses a different signal transduction pathway than acetylcholine for this purpose (18, 20). Bradykinin also induces dilation of human forearm vessels (18) and coronary resistance vessels (22–24). Gilligan et al (18) reported that bradykinin-induced dilation of human forearm resistance vessels was preserved, whereas acetylcholine-induced dilation was impaired, in hypercholesterolemic patients and suggested that impaired endothelial vasodilator function of the forearm vessels in patients with hypercholesterolemia is related to an abnormality at the level of the muscarinic receptor or its signal transduction pathway. In the present study, bradykinin-induced dilation of coronary resistance vessels was impaired, suggesting that impairment of endothelial vasodilator function of coronary resistance vessels depends on the location of the vascular bed.

**Nitric oxide release at rest and in response to bradykinin**

Quyyumi et al (14) have suggested that acetylcholine-induced coronary resistance vasodilation is at least partly due to the stimulation of production of nitric oxide and that the decreased release of nitric oxide is responsible for the difference in dilation between patients with and without risk factors for atherosclerosis. Bradykinin-mediated endothelium-dependent dilation of human coronary resistance vessels is also at least partly due to the production of endothelium-derived nitric oxide (24). The present results suggest that impaired bradykinin-induced dilation of coronary resistance vessels is associated with the decreased production of nitric oxide in patients with hypercholesterolemia.

Our previous study (23) suggested that endothelium-derived nitric oxide may not be an important contributor to bradykinin-induced dilation of human coronary resistance vessels. We observed a significant bradykinin-induced dilation even after nitric oxide inhibition. Endothelium-derived hyperpolarizing factor (7, 8) and prostacyclin (9, 10) are the most likely bradykinin-induced coronary resistance vasodilating agents. The present results suggest that endothelium-derived agents other than nitric oxide may be preserved in patients with hypercholesterolemia.

**Study limitations**

It is possible that the pharmacologic effect of acetylcholine influenced the subsequent effect of bradykinin. However, the effect of acetylcholine disappears rapidly (31), and we allowed a 15-minute interval before bradykinin infusion which should...
have been sufficient to permit full recovery of the physiologic properties of epicardial coronary arteries after administration of acetylcholine. The basal CBF and CVR values were similar in the first baseline condition and the second baseline condition.

Conclusions

The present study demonstrated that impairment of endothelium-dependent dilation of coronary resistance vessels characterized of hypercholesterolemic patients was not related to a specific defect of the muscarinic receptor and that bradykinin-induced dilation was also impaired. This abnormality in the coronary hemodynamic response to bradykinin may have been caused by a decreased release of nitric oxide.

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