Tetrahydrobiopterin Improves Endothelial Dysfunction in Coronary Microcirculation in Patients Without Epicardial Coronary Artery Disease
Soko Setoguchi, MD, Masahiro Mohri, MD, P1D, Hiroaki Shimokawa, MD, P1D, Akira Takeshita, MD, P1D
Fukuoka, Japan

OBJECTIVES
We aimed to determine whether intracoronary supplementation with nitric oxide (NO) synthase co-factor tetrahydrobiopterin (BH4) improves NO-dependent coronary microvascular dilation in patients with coronary risk factors but no significant organic stenosis.

BACKGROUND
Impaired coronary microvascular dilator reserve attributable to endothelial dysfunction plays an important role in the regulation of coronary blood flow (CBF).

METHODS
Fifteen patients were measured for CBF (Doppler-wire and quantitative coronary angiography). Stimulated release of NO in the coronary microcirculation was evaluated by percent increase in CBF (%ΔCBF) at graded doses of intracoronary acetylcholine (1, 3, 10 and 30 μg/min). Measurements were repeated after intracoronary co-infusion of BH4 (4 mg/min) and acetylcholine.

RESULTS
The patients were divided into two groups on the basis of CBF responses to acetylcholine: those with “diminished” (%ΔCBF <300%, n = 8) and “normal” (%ΔCBF >300%, n = 7) flow responses. Tetrahydrobiopterin significantly (p < 0.0001) improved acetylcholine-induced increases in CBF in patients with diminished flow responses, but exerted no effect in those with normal flow responses. Among the 15 studied patients, the magnitude of flow improvement by BH4 was inversely correlated with baseline flow responses (p < 0.02). Microvascular dilator response to direct NO donor (isosorbide dinitrate) was not affected by BH4.

CONCLUSIONS
We demonstrated for the first time that intracoronary BH4 improved acetylcholine-induced microvascular dilator responses in patients with endothelial dysfunction in vivo. Thus, supplementation with BH4 may be a novel therapeutic means to increase NO availability for patients with coronary microvascular disease. (J Am Coll Cardiol 2001;38:493–8) © 2001 by the American College of Cardiology
had no flow-limiting (>75%) coronary artery stenosis in large epicardial coronary arteries. Maximal diameter stenosis as measured at the conclusion of the whole-study protocol was 21 ± 19% (range 0 to 56). Patients with a history of myocardial infarction, congestive heart failure, hypertrophic cardiomyopathy or end-stage renal disease were excluded.

The clinical characteristics of the studied patients are summarized in Table 1. As coronary risk factors, we specified diabetes mellitus (fasting glucose >120 mg/dl, HbA1c >6.0% or treatment with hypoglycemic agents or insulin), hypertension (arterial pressure >140/90 mm Hg or treatment with antihypertensive medication), hypercholesterolemia (total cholesterol >220 mg/dl, low-density lipoprotein cholesterol >140 mg/dl), current smoking, family history of premature CAD, age >45 years for men and age >55 years and/or being postmenopausal for women. Two patients (13%) were diabetic, nine (60%) were hypertensive, three (20%) had hypercholesterolemia, nine (60%) were current smokers or had smoked in the previous year and 13 (87%) had age risk factors. None had family history of premature CAD. The average number of risk factors was 2.5.

**Study protocol.** The Institutional Review Committee on Human Research, Faculty of Medicine, Kyushu University, approved the study protocol. We obtained written informed consent from each patient before the study.

Cardiac catheterization was performed with patients in the fasting state after 5 mg oral diazepam. All cardiovascular medications were discontinued at least 24 h before the study. Right and left heart catheterization was performed via the femoral approach. The following protocols were performed after the diagnostic catheterization.

To evaluate endothelium-dependent vasodilation of coronary microvessels, we measured coronary blood flow (CBF) responses to intracoronary acetylcholine, as reported previously (9). Briefly, a 0.014-inch Doppler-tipped guide-wire (FloWire, Cardiometrics, Mountain View, California) was advanced through a 6F Judkins catheter and the tip of the wire was placed at the proximal segment of the left anterior descending coronary artery. Isosorbide dinitrate (1 mg, intracoronary bolus) was given before the guidewire placement to avoid epicardial coronary vasospasm. Blood flow velocity was continuously recorded throughout the study. First, four graded doses of acetylcholine (1, 3, 10 and 30 μg/min) were infused directly into the left coronary artery (LCA) through the Judkins catheter for 1 min at each dose. Physiological saline was co-infused with acetylcholine as a vehicle. Second, after the systemic and coronary hemodynamics returned to the baseline values, BH4 (BH4, Clinalfa, Läufelfingen, Switzerland) at 4 mg/min was infused directly into the LCA for 2 min. This dose yields BH4 concentration of 20 μM in the coronary circulation if resting CBF of the LCA is assumed to be 100 ml/min. We adopted this dosing because it has been shown that endothelial NO production was maximized at BH4 concentrations of >10 μM (15,22). While the infusion of BH4 continued at 4 mg/min, acetylcholine was co-infused at 1, 3, 10 and 30 μg/min in the same manner. In a separate group of five patients, effect of BH4 on endothelium-independent coronary microvascular dilation was examined by measuring CBF responses to intracoronary administration of isosor-

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**Table 1. Clinical Background and CBF Responses in the Studied Patients**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/Gender (y)</th>
<th>HTN</th>
<th>DM</th>
<th>T. Chol (mg/dl)</th>
<th>Smoking (pack/year)</th>
<th>Family History</th>
<th>No. of RFs</th>
<th>%CBF at ACh 30 μg/min With Saline</th>
<th>%CBF at ACh 30 μg/min With BH4</th>
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<td>1</td>
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<td>183 (pack-year)</td>
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<td>4</td>
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<tr>
<td>2</td>
<td>49/M</td>
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<td>174</td>
<td>225</td>
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<td>225</td>
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<td>3</td>
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<td>+</td>
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<td>245</td>
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<td>3</td>
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<tr>
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<td></td>
<td>250</td>
<td>—</td>
<td>—</td>
<td>2</td>
<td>380</td>
<td>310</td>
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</table>

ACh = acetylcholine; BH4 = tetrahydrobiopterin; %CBF = coronary blood flow (% from baseline); DM = diabetes mellitus; HTN = hypertension; RFs = risk factors; T. Chol = total cholesterol.
bide dinitrate. Isosorbide dinitrate was infused at 2 mg/min with saline or BH4 (4 mg/min).

**Measurements.** Quantitative coronary angiography was performed with a Siemens cineangiographic system (Bicor and Hicor, Erlangen, Germany). An appropriate view was selected that allows clear visualization of the vessel under study. Throughout the study, the angle of projection, the distance from the X-ray focus to the object, and the distance from the object to the image intensifier were kept constant. An end-diastolic frame of the coronary angiogram was selected and the luminal diameter of the segment distal to the Doppler guidewire tip was determined. The accuracy and precision of our quantitative angiographic system were validated with precision-drilled models, as previously reported (23). Measurements were made three times, and the averaged value was used for analysis.

Coronary blood flow velocity was measured with a 0.014-inch Doppler guidewire and an on-line spectral analyzer (FloMap, Cardiometrics, Mountain View, California) and was recorded on a multichannel recorder. Volumetric CBF was calculated with the formula validated by Doucette et al. (23,24).

**Statistical analysis.** Data are expressed as mean ± standard deviation unless otherwise indicated. Differences between means were compared by paired or unpaired Student t test, as appropriate. Effects of BH4 on CBF responses to graded doses of acetylcholine were compared by two-way analysis of variance with appropriate interactions. All p-values were two-tailed; a value <0.05 was considered statistically significant.

**RESULTS**

**Effect of BH4 on baseline hemodynamics.** Intracoronary infusion of BH4 did not change baseline heart rate (63 ± 14 vs. 62 ± 13 beats/min) or mean aortic pressure (97 ± 14 vs. 97 ± 13 mm Hg). After 2-min infusion of BH4, baseline CBF was significantly increased by 16% from 146 ± 65 to 169 ± 85 ml/min (p < 0.02).

**CBF responses to acetylcholine.** Before BH4, acetylcholine increased CBF dose-dependently in all 15 patients. However, we recognized two distinct subsets of patients based on the degree of responses to acetylcholine (Fig. 1). We previously demonstrated (25) that CBF was increased to 345 ± 78% of baseline from 146 ± 65 to 169 ± 85 ml/min (p < 0.02).

**Figure 1.** Changes in coronary blood flow (CBF) in response to acetylcholine. Baseline CBF is expressed as 100%. *p < 0.0001 for acetylcholine (two-way analysis of variance) in both groups. Data, means ± SEM. See text for detail. Ach = acetylcholine.

**Figure 2.** Effects of tetrahydrobiopterin (BH4) on acetylcholine-induced increases in coronary blood flow in normal response group (A) and diminished response group (B). *p < 0.0001 for treatment (BH4) by two-way analysis of variance. Ach = acetylcholine.

Coronary blood flow responses after BH4 in the diminished response group were not significantly different from those in the normal response group.

Figure 4 shows the relationship between maximal increases in CBF in response to acetylcholine alone and the magnitude of improvement by BH4 in CBF after BH4 in all 15 studied patients from both groups. There was a significant inverse correlation between the two variables (r = −0.65, p < 0.01). Thus, patients with most blunted blood flow response to acetylcholine at baseline had the greatest improvement with BH4 supplementation.
isosorbide dinitrate plus BH4. The difference was not statistically significant.

DISCUSSION

This is the first study demonstrating that intracoronary BH4 improves stimulated release of NO as estimated by flow responses to acetylcholine in the coronary microcirculation in patients with coronary risk factors but no significant organic stenosis. Vasodilator effect of direct NO donor was not affected by BH4. Furthermore, the magnitude of flow improvement by BH4 was inversely correlated with baseline flow responses to acetylcholine. These data suggest that endothelial dysfunction in these patients is caused at least in part by suboptimal availability of BH4.

Previous studies. It has been suggested that BH4 deficiency reduces NO production by affecting dimerization and electron transfer of NO synthase and impairs NO-dependent vasodilation in vitro (19,26,27). Furthermore, supplementation with BH4 was shown to improve NO-mediated vasodilation in the forearm circulation of patients with hyperlipidemia (15) and smokers (17,18). However, the effect of BH4 in the coronary circulation in patients with endothelial dysfunction and normal epicardial coronary arteries has not been examined. Recently, Maier et al. reported (28) that BH4 improved dilator response of large epicardial coronary arteries to acetylcholine in patients undergoing angioplasty. In their study, microvascular dilator response to acetylcholine was not improved by BH4. The discrepancy between their results and ours may be due, at least in part, to the difference in patient characteristics. The
patients in the Maier study had more advanced CAD and required angioplasty. Furthermore, these investigators measured vascular reactivity after angioplasty, which is known to alter systemic and coronary oxidative stress and, therefore, vascular reactivity (29–31).

The present study. In the present study, we enrolled patients with coronary risk factors and no flow-limiting fixed coronary stenosis. Thus, our patients had a relatively early stage of coronary atherosclerosis. We found that BH4 increased basal CBF and augmented flow responses to intracoronary acetylcholine. Importantly, the effect of BH4 was not uniform among the studied patients, being most prominent in those having the most seriously impaired NO-dependent microvascular dilator responses. Furthermore, BH4 did not affect endothelium-independent microvascular dilator responses. However, we previously demonstrated (33) that acetylcholine dilates coronary resistance vessels largely via NO in humans. Furthermore, in forearm circulation it has been shown that beneficial effects of BH4 were abolished by NO synthase inhibitor.

Conclusions. We demonstrated that intracoronary supplementation with NO synthase co-factor BH4 improved acetylcholine-induced microvascular dilator responses in patients with endothelial dysfunction and no obstructive CAD. Future studies are warranted to elucidate the long-term effect of BH4 supplementation in this population.

REFERENCES
