Tetrahydrobiopterin Improves Coronary Endothelial Function, But Does Not Prevent Coronary Spasm in Patients With Vasospastic Angina

Yukihiro Fukuda, MD; Hiroki Teragawa, MD; Keiji Matsuda, MD; Togo Yamagata, MD; Hideo Matsuura, MD; Kazuaki Chayama, MD

Reduced bioavailability of tetrahydrobiopterin (BH4), an essential cofactor for nitric oxide (NO) synthase, and the resulting decrease in NO in the coronary circulation may be involved in the pathogenesis of coronary spasm. The present study investigated the effects of BH4 on the vascular response to acetylcholine (ACh) in 28 patients with vasospastic angina (VA) using quantitative angiography. After recording the vascular responses to ACh (3 and 30μg/min), either BH4 (1 mg/min) or saline was infused into the coronary artery for 2 min before and during a subsequent infusion of ACh. With the 3μg/min dose of ACh, BH4 attenuated the ACh-induced decrease in coronary diameter in both the nonspastic segments (–1.1±2.2% ACh vs 6.0±2.8% ACh+BH4) and spastic segments (–6.3±2.7% ACh vs 2.9±2.7% ACh+BH4), but did not influence the ACh-induced coronary spasm at 30μg/min (–57.3±2.4% ACh vs –55.3±2.4% ACh+BH4). In the control patients, saline did not influence either the spastic or nonspastic vasoconstrictor responses to ACh. Acute administration of BH4 improves coronary endothelial function, but does not prevent coronary spasm in patients with VA. (Circ J 2002; 66: 58–62)

Key Words: Coronary spasm; Endothelial function; Nitric oxide; Tetrahydrobiopterin

Coronary spasm is believed to play an important role in the pathogenesis of vasospastic angina (VA) and can cause acute myocardial infarction or ischemic sudden death! However, the precise pathophysiology of coronary spasm has yet to be elucidated, although coronary endothelial dysfunction is thought to be one of the mechanisms.

Tetrahydrobiopterin (BH4) is an essential cofactor for nitric oxide (NO) synthase in both endothelial and vascular smooth muscle cells and also serves as a scavenger of oxygen-derived free radicals, thereby decreasing NO breakdown! Therefore, BH4 is considered essential for the regulation of NO bioavailability in endothelial cells and for the maintenance of cardiovascular homeostasis. In support of this concept, recent studies have shown that supplementation of BH4 improves impaired endothelial function in humans with various pathologic states, including hypercholesterolemia, smoking and coronary artery disease.

Because coronary endothelial dysfunction is commonly observed in patients with VA, the depletion of BH4 and resultant reduction in NO bioavailability in the coronary circulation may be involved in the pathogenesis of coronary spasm. However, the effects of BH4 supplementation on coronary spasm have not been evaluated and so we investigated the effects of BH4 on the coronary vasoconstrictor response to acetylcholine (ACh) in patients with VA using quantitative coronary angiography.

Methods

Study Population

We enrolled 28 Japanese patients with VA (21 men, 7 women; mean age, 55 years; range, 38–70) who fulfilled the following inclusion criteria: (1) spontaneous chest pain associated with ST-segment elevation or depression at rest on 12-lead ECG or ambulatory ECG; (2) coronary spasm (≥50% reduction in arterial diameter during coronary angiography) in the left coronary artery associated with ST-segment changes and/or typical chest pain after intra-coronary injection of ACh (Daiichi Pharmaceutical Co, Tokyo, Japan); and (3) no angiographic organic stenosis in the coronary arteries. Patients with severe left ventricular dysfunction and those with valvular heart disease were excluded. Written consent was obtained from each patient, and the protocol was approved by the Hiroshima University School of Medicine Ethics Committee.

Study Design

The study design has been described previously in detail. Briefly, anti-anginal therapy was withheld for at least 48 h before cardiac catheterization, except for unrestricted use of sublingual nitroglycerin (NTG). Diagnostic right and left heart catheterization and coronary angiography were performed using the standard percutaneous femoral approach. A 6Fr guide catheter was introduced into the left main coronary artery, and a 5Fr temporary pacing electrode catheter (Bard, Tewksbury, MA, USA) was placed in the right ventricular apex via the right jugular vein and connected to a temporary pacemaker set at a rate of 50 beats/min.

Study Protocol

After baseline conditions were established, incremental
Table 1  Patient Characteristics

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Data are expressed as the mean±SD. BMI, body mass index; EF, ejection fraction; LAD, left anterior descending coronary artery; LCX, left circumflex coronary artery; pro, proximal segment; mid, mid-segment; dis, distal segment.

doses of ACh (3 and 30μg/min) were infused at 5-min intervals into the left coronary artery over a 2-min period until the induction of coronary spasm. Once coronary spasm was induced, the ACh infusion was ceased. Coronary spasm resolved spontaneously within 2 to 3 min without the use of NTG, allowing for further studies in almost all the patients. Patients with prolonged coronary spasm and/or unstable hemodynamics during the ACh infusion received an intracoronary infusion of NTG (Nihonkayaku Co, Tokyo, Japan) to relieve the coronary spasm and were excluded from further study.

After 15 min, when baseline conditions had been reestablished, BH4 (Sigma Chemical Co, St Louis, MO) (1 mg/min; intracoronary concentration 3.3×10⁻⁵ mol/L) was infused into the left coronary artery over a 2-min period in 20 patients (BH4-treated patients: 15 men, 5 women; mean age 55 years, range 40–70). Saline (0.9%) served as a placebo and was infused in the same manner as BH4 in the remaining 8 patients (control patients: 6 men, 2 women; mean age 55 years, range 38–69). Subsequently, incremental doses of ACh (3 and 30μg/min) were simultaneously infused with BH4 (1 mg/min) for 2 min. Finally, NTG (200 μg) was given as an intracoronary injection. All of the drugs were infused with an infusion pump (Terufusion, Terumo, Tokyo, Japan) at a rate of 1 ml/min.

Coronary angiograms were performed at baseline and at the end of each drug infusion. Arterial pressure, heart rate, and ECG were monitored continuously and recorded using a multichannel recorder (Polygraph 1600, NEC, Tokyo, Japan).

Quantitative Coronary Angiography
Coronary angiograms were acquired and analyzed using digital image acquisition (HICOR X-ray system, Siemens, Forchheim, Germany) and analysis systems (CAAS II QCA system, Pie Medical, Maastricht, The Netherlands). The average of 3 luminal diameter measurements was used for analysis. Changes in coronary diameter in response to the various drugs were expressed as the percent change from the baseline measurements on the angiogram taken prior to infusion.

Diameters of the spastic and nonspastic segments were measured in each patient. Spastic segments were defined as sites with ≥50% reduction in coronary artery diameter from baseline with ACh infusion. When coronary spasm occurred diffusely from the proximal to the distal segments of a coronary artery, the diameter of both segments of the spastic artery was measured. Nonspastic segments were defined as the proximal and distal segments of the left anterior descending artery (LAD) and left circumflex coronary artery (LCX) demonstrating less than 30% reduction in coronary diameter with ACh infusion. A strong correlation for intraobserver measurements was noted (r=0.996 for nonspastic segments; and r=0.995 for spastic segments during coronary spasm). Analysis of interobserver measurements also showed high reproducibility (r=0.987 for nonspastic segments; and r=0.978 for spastic segments during coronary spasm).

Assessment of Chest Pain and ECG Parameters
Chest pain severity was semi-quantified using a subjective scale ranging from 0 (no pain) to 10 (unbearable pain) for chest symptoms produced during the ACh provocation test; these symptoms were similar to the usual chest symptoms. ECG changes during coronary spasm were assessed by the sum of the ST segment deviations in 12 leads of the ECG (sigma ST delta). The ST segment shift was measured at a point 80 ms after the nadir of the S wave.

Drug Preparations
BH4 was sterilized at the Pharmacy Department of Hiroshima University Hospital. All drugs were dissolved in oxygen-free saline immediately before use.

Statistical Analysis
Data are expressed as the mean±SEM, unless otherwise indicated. Differences in categorical variables between the 2 groups were assessed by chi-square analysis and goodness of fit tests. Changes in chest pain severity and the sigma ST delta before and after BH4 or placebo infusion were compared using the Mann-Whitney U test and the Wilcoxon signed-rank test, respectively. Serial changes in hemodynamic variables and coronary diameter in response to various drugs were compared using a one-way analysis of variance (ANOVA). Serial percent changes in coronary diameter with BH4 or placebo infusion and with ACh infusion before and after the administration of BH4 or placebo were compared using a 2-way ANOVA. If the ANOVA showed a significant difference between the mean values, the level of statistical significance was determined by
The present study demonstrated that intracoronary infusion of BH4 attenuated the nonspastic vasoconstriction of coronary arteries elicited by ACh, but did not attenuate the spastic vasoconstriction (ie, ACh-induced coronary spasm).
in patients with VA.

BH4 and Endothelial Function

In this study, intracoronary infusion of BH4 alleviated the vasoconstrictor responses to ACh in the nonspastic and spastic segments in patients with VA, indicating that BH4 may enhance endothelium-dependent vasorelaxation in epicardial arteries. Although the intracellular BH4 status in patients with VA remains to be determined, it is possible that oxidative stress influences it, leading to a net reduction in the bioavailability. The BH4 supplementation presumably restored the vasorelaxant response to ACh by increasing NO bioavailability and/or decreasing NO breakdown by oxygen-derived free radicals. This has been confirmed by in vitro observations in which vitamin C, an antioxidant, increased endothelial NO synthase activity by increasing intracellular BH4 levels.18

BH4 alone did not change the coronary diameter or blood flow in either group, which is consistent with recent observations in the peripheral and coronary circulations, suggesting that BH4 does not influence the basal NO bioavailability of endothelial cells.

Failure of BH4 to Prevent Coronary Artery Spasm

In this study, short-term administration of BH4 did not prevent ACh-induced coronary spasm, nor did it elicit changes in chest pain severity and ST segment deviation during spasm. There are 3 possible reasons for this. First, the etiology of coronary spasm is multifactorial and factors other than endothelial dysfunction, including a change in autonomic tone,19,20 enhanced β-adrenergic receptor activity,21 magnesium deficiency22 and hyperactivity of coronary smooth muscle23,24 have been implicated. Second, acute improvement in endothelial function might not prevent coronary spasm in patients with VA, as shown by 4 months of treatment with eicosapentaenoic acid, which improved coronary spasm in patients with VA, as indicated by 4 months improvement in endothelial function might not prevent endothelial cells at concentrations of 10–5 to 10–4 mol/L.25

This study demonstrated that in patients with VA intracoronary delivery of BH4 attenuates the nonspastic vasoconstriction of coronary arteries elicited by ACh, but does not prevent ACh-induced coronary spasm. This is because the intracellular BH4 status in patients with VA remains to be determined, and requires further research. Third, the amount of bioavailable BH4 in this study may have been insufficient to prevent ACh-induced coronary spasm. A previous study in patients with coronary artery disease reported a vasodilatory effect in the coronary microcirculation of 4 mg/min BH4 as an intracoronary infusion11 but we used a much lower dose of BH4 (1 mg/min), which we based on that required to dilating human forearm vessels8 and a previous study that demonstrated maximal NO bioavailability in endothelial cells at concentrations of 10–8 to 10–4 mol/L.25

Our preliminary study showed that infusion of BH4 at 10 mg/min tended to decrease the baseline coronary diameter and that 1 mg/min significantly increased the concentration of BH4 in the coronary sinus from 2.5±0.3 to 232.4±3.7 ng/ml, so we expected that this dose of BH4 would be sufficient to increase NO bioavailability in coronary endothelial cells. Although other investigators have used oxygen-free saline for in vivo studies, it may not completely inhibit BH4 oxidation, so it is possible that BH4 dissolved in some other solution will prevent coronary spasm in patients with VA.

Study Limitations

Measuring the diameter of the arterial segment involved during coronary spasm was difficult, especially when spasm occurred distally, so we excluded these segments from the data analysis because of poor reproducibility.

We did not examine the effect of L-NG-monomethyl-L-arginine on the BH4-mediated enhancement of the vascular response to ACh, nor did we compare the effect of tetrahydrobiopterin, another reduced pteridine, with that of BH4. Therefore, we could not ascertain whether endothelium-derived NO contributes to the BH4-induced improvements in coronary endothelial dysfunction. Similarly, we did not measure markers of oxidative stress in the coronary circulation and so were unable to determine the direct antioxidant contribution of BH4 to improved endothelial dysfunction in patients with VA.

ACh has a direct vasoconstrictor effect on vascular smooth muscle in addition to a vasodilator effect mediated by the endothelium.26,27 Although attenuation of the vasoconstrictor response to ACh might not be equal to the improvement in endothelial function, studies of human coronary endothelial function have largely relied on stimulating NO bioavailability with ACh.28–30 Therefore, we estimated the BH4-induced improvement in coronary endothelial function by measuring the change in coronary diameter in response to ACh.

Conclusion

This study demonstrated that in patients with VA intracoronary infusion of BH4 attenuates the nonspastic vasoconstriction of coronary arteries elicited by ACh, but does not prevent ACh-induced coronary spasm. We conclude that reduced bioavailability of BH4 is in part involved in the pathophysiology of endothelial dysfunction, but further studies are needed to clarify the correlation between BH4 deficiency and coronary spasm.

Acknowledgments

We are grateful to Nobuo Shiode, MD, Yukihito Higashi, MD, Hidekazu Hirao, MD, Fumiharu Miura, MD and Kenya Sakai, MD of the First Department of Internal Medicine, Hiroshima University School of Medicine for their technical assistance and helpful comments. We thank Mitsu Yoko Omura for her assistance with the manuscript. We also thank Dr Shigeaki Arai and Dr Masahiko Sakai for the preparation of BH4 and oxygen-free saline.

References