TETRAHYDROBIOPTERIN IMPROVES ENDOTHELIAL FUNCTION IN HUMAN SAPHENOUS VEINS

Subodh Verma, MD, PhD^a
Fina Lovren, PhD^b
Aaron S. Dumont, MD^a
Kieren J. Mather, MD^a
Andrew Maitland, MD^c
Teresa M. Kieser, MD^c
Christopher R. Triggie, PhD^b
Todd J. Anderson, MD^a

Objectives: Diminished production of nitric oxide has been linked to saphenous vein endothelial dysfunction. Tetrahydrobiopterin is an obligate cofactor for the oxidation of L-arginine by nitric oxide synthase in the production of nitric oxide by endothelial cells. The objective of the present study was to examine whether the exogenous addition of tetrahydrobiopterin improves endothelial function in saphenous veins from patients undergoing coronary artery bypass graft operations.

Methods: Vascular segments of saphenous veins were obtained from 17 patients undergoing elective coronary artery bypass grafting, and in vitro endothelium-dependent and endothelium-independent responses to acetylcholine and sodium nitroprusside were assessed. Isometric dose-response curves were constructed in precontracted rings in the presence and absence of tetrahydrobiopterin (0.1 mmol/L) with the use of the organ bath apparatus. The percentages of maximum relaxation and sensitivity were compared between interventions.

Results: Acetylcholine caused dose-dependent endothelium-mediated relaxation in saphenous veins. In the presence of tetrahydrobiopterin, acetylcholine-induced relaxation was significantly augmented (percentage maximum relaxation, $16.8\% \pm 2.9\%$ vs control $7.5\% \pm 1.8\%$; $P = .003$) without an effect on agonist sensitivity. These effects were endothelium-specific because endothelium-independent responses to sodium nitroprusside were preserved.

Conclusions: These data uncover beneficial effects of acute tetrahydrobiopterin addition on endothelial function in human vessels. Because endothelial dysfunction has been implicated in the development of graft failure, studies aimed at chronic delivery of tetrahydrobiopterin would be useful in determining the contribution of this cofactor toward saphenous vein atherosclerosis. (J Thorac Cardiovasc Surg 2000;120:668-71)

From the Divisions of Cardiology,^a Pharmacology,^b and Cardiac Surgery,^c Faculty of Medicine, The University of Calgary, Calgary, Alberta, Canada.

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Address for reprints: Todd J. Anderson, MD, FRCPC, Associate Professor of Medicine, Division of Cardiology, Foothills Hospital, 1403 29th St, NW, Calgary, Alberta, T2N 2T9 Canada (E-mail: todd.anderson@CRHA-health.ab.ca).

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Endothelial dysfunction is believed to predispose the vascular smooth muscle to increased tone, decreased vasomotion, altered reactivity, changes in structure and geometry, enhanced platelet aggregation, and eventual atherosclerosis or graft failure. Elucidating the mechanisms of endothelial dysfunction in bypass conduits has implications in terms of vessel spasm, graft patency, and the outcome of coronary artery bypass graft (CABG) operations. A strong body of evidence supports the notion that nitric oxide (NO) is one of the most important antiatherosclerotic agents produced by the endothelium.¹ Indeed, the resilience of the internal thoracic artery and its resistance to atherosclerosis has been attributed in part to the potent endothelial NO system in these vessels.² In contrast, the increased rates of atherosclerotic changes in saphenous veins has been

Table I. Demographic characteristics

No. of patients	17*
Risk factors	
Age (y)	59 ± 4
Smoking, n (%)	8 (47)
Hypertension, n (%)	7 (42)
Diabetes, n (%)	4 (23)
Obesity (BMI ≥27), n (%)	8 (48)
Family history, n (%)	10 (59)
Hypercholesterolemia, n (%)	11 (65)
Total cholesterol (mmol/L)	6.3 ± 0.5
Drugs	
ACE inhibitors, n (%)	6 (35)
Calcium antagonists, n (%)	7 (41)
Antioxidant vitamins, vitamin B6, folate, B12; n (%)	3 (18)
β-Blockers, n (%)	10 (58)
Nitroglycerin, n (%)	10 (58)

BMI, Body mass index; ACE, angiotensin-converting enzyme.

*The group comprised 11 men and 6 women.

linked to diminished production of endothelium-derived NO in these conduits.² Hence, targeting the pathways of NO synthesis and production in the endothelium of venous grafts has important ramifications. The results of one such approach with tetrahydrobiopterin (BH₄) are described in this article. BH₄ is an absolute cofactor for endothelial NO synthase (NOS).³ Because low levels of BH₄ may lead to endothelial dysfunction through decreasing NO production or enhancing its breakdown, the present study was aimed at examining the effects of this cofactor on in vitro endothelium-dependent and endothelium-independent vascular responses in human saphenous veins.

Methods

We obtained saphenous vein segments (2-3 cm) from 17 patients undergoing CABG operations after obtaining written informed consent. The vessels were cut into rings (~5 mm) and suspended in an isolated tissue bath (under 4g tension) containing oxygenated Krebs buffer (37°C) to record isometric dose-response curves. After 60 minutes of equilibration, the vessels were stimulated according to the following protocol: (1) cumulative dose-response curves to phenylephrine (10⁻⁸ to 10⁻⁵ mol/L); (2) cumulative dose-response curves to acetylcholine in rings precontracted with the effective dose causing 75% of the maximum contraction to this agonist (ED₇₅) of phenylephrine; (3) cumulative dose-response curves to acetylcholine in the presence and absence of BH₄ (0.1 mmol/L for 20 minutes); and (4) cumulative dose-response curves to sodium nitroprusside in the presence and absence of BH₄. After the initial dose-response curve to phenylephrine (step 1), the dose that evoked 75% of the maximum obtained tension response was used to precontract the vascular segments for the relaxation studies (steps 2-4). The percentages of maximum relaxation (%Emax) and agonist

Fig 1. Endothelium-dependent vascular relaxation to acetylcholine (ACh) in saphenous veins in the absence (control) and presence of 0.1 mmol/L of tetrahydrobiopterin (20 minutes). Data are represented as means ± SE. Vascular relaxation is expressed as the percentage of maximum relaxation in rings precontracted with the ED₇₅ of phenylephrine. Saphenous vein segments were collected from 17 patients undergoing elective CABG operations and cut into two rings for determination of isometric dose-response curves to acetylcholine in the absence and presence of BH₄. Because of limitations in vessel length, in 5 of 17 patients, only one ring could be prepared. In the presence of BH₄, endothelium-dependent responses to acetylcholine were significantly augmented. Weak endothelium-dependent relaxation is typical of saphenous veins.

sensitivity (pEC₅₀) were compared between interventions. The %Emax values were calculated manually from each dose-response curve tracing, and results were averaged and quoted as means ± SE. pEC₅₀ values are an index of agonist sensitivity and represent -logEC₅₀. Nonlinear analysis of the individual dose-response curves was performed, and the obtained pEC₅₀ values were averaged and presented as means ± SE. %Emax values were compared by means of a 2-tailed *t* test. The dose-response curve was compared by means of repeated-measures analysis of variance followed by a Newman-Keuls test for post hoc comparisons.

Results

Patient characteristics. Table I depicts the characteristics and risk factor profiles of the patient population. The distribution of coronary artery disease risk factors in this study population was comparable with that found in other studies with similar methods.⁴

Vascular relaxation. In precontracted arteries acetylcholine caused dose-dependent endothelium-mediated relaxation (%Emax, 7.5% ± 1.8%; pEC₅₀, 6.52% ± 0.06). This effect was endothelium specific

Fig 2. Saphenous vein relaxation to the endothelium-independent vasodilator sodium nitroprusside (SNP) in the presence and absence of BH₄. Sodium nitroprusside-induced vascular relaxation was not augmented by BH₄, suggesting that the action of this cofactor was specific for the endothelium.

because it was abolished in vessels denuded of the endothelium (not shown). The effects of BH₄ on acetylcholine- and sodium nitroprusside-mediated relaxation are depicted in Figs 1 and 2. In the presence of BH₄, endothelium-mediated relaxation to acetylcholine was improved significantly (%Emax, $16.8 \pm 2.9\%$ vs control; $P = .003$) without an effect on agonist sensitivity (pEC_{50} , 6.57 ± 0.06 vs control; $P = .2$). The beneficial effects were endothelium specific because the responses to the endothelium-independent vasodilator (sodium nitroprusside) were preserved (Fig 2).

Discussion

These data uncover the ability of BH₄ to augment endothelium-mediated vasodilation in saphenous veins from patients undergoing CABG operations. In the presence of BH₄, acetylcholine responses improved approximately 2-fold, an effect specific for the endothelium. Because endothelial dysfunction and diminished NO production are key determinants of atherogenicity,¹ this observation may have theoretic implications for vein graft atherosclerosis.

The effects of BH₄ on endothelial function are an area of much current interest in the field of cardiovascular and cerebrovascular research.^{5,6} In the landmark article by Higman and colleagues,⁶ exogenous BH₄ addition was demonstrated to improve endothelial function in saphenous veins recovered from smokers without other endothelial or coronary risk factors. Data from the present study serve to confirm, complement, and extend the observations made by Higman and colleagues⁶ by demonstrating the beneficial effects of this cofactor on endothelial function in the face of multiple

risk factors. Because patients undergoing CABG operations often have a clustering of endothelial risks, these observations closely mimic those of the cohort of patients undergoing coronary revascularization. However, the contribution of individual risk factors toward endothelial dysfunction and the observed effects of BH₄ cannot be made. All major risk factors for atherosclerotic vascular disease have been associated with impaired NO activity.⁷ Hence it is plausible that the beneficial effects of BH₄ may extend non-specifically to any process that dampens endothelial function through affecting NO production, release, or availability.

The primary role of BH₄ appears to be as an obligate cofactor for the enzyme NOS III in the endothelium.^{3,7} NOS III consists of a flavin-containing reductase domain and a heme-containing oxidase domain. Reduced nicotinamide adenine dinucleotide phosphate reduces the flavin component of the reductase domain; however, electron transfer to heme does not occur until calcium-calmodulin is present. In the presence of calcium-calmodulin, an electron transfer from reduced nicotinamide adenine dinucleotide phosphate to heme occurs, and with L-arginine present, electrons can flow to the heme moiety to reduce oxygen, which in turn is used to oxidize L-arginine to NO (5 electron transfer).^{3,7} BH₄ is an essential cofactor for the proper flow of electrons to oxidize L-arginine, and hence endothelial NO production is highly dependent on the presence of adequate amounts of this agent.³ BH₄ has also been suggested to induce a change in the NOS conformation to the high-affinity state (allosterism) and increase the affinity of NOS for L-arginine. Therefore the addition of BH₄ may improve saphenous vein endothelial function through enhancing or restoring the ability of NOS III to produce NO. On the other hand, low levels of BH₄ shift the balance between NO and oxygen-derived free radicals, such that oxygen becomes an electron acceptor (vs L-arginine) and produces superoxide anions and hydrogen peroxide, potent inducers of endothelial dysfunction. Although BH₄ substitution may correct this imbalance in saphenous veins, this mechanism seems unlikely given the presence of ascorbic acid in the buffer. Finally, recent data suggest that BH₄ synthesis is associated with parallel stimulation of superoxide dismutase in the vascular endothelium.³ Thus, BH₄ may enhance NO production-stabilization and improve endothelial function through a direct antioxidant effect.

In human saphenous veins the production of constricting prostanoids and thromboxane A₂ have been linked to endothelial dysfunction.⁸ In addition, data

from our laboratory suggest a role of endothelin-1 as a potent modulator of endothelial function in human saphenous veins.⁹ Whether these contracting factors are primarily increased in saphenous veins or become important as a result of diminished NO production remains unknown. Whatever the exact mechanism, it is apparent that restoring or augmenting NO production may serve to counteract the actions of these vasoconstrictors and improve endothelial function.

The increased rates of saphenous vein graft atherosclerosis (vs the internal thoracic artery) are related, at least in part, to the poor production and release of endothelium-derived NO.² The weak responses to acetylcholine in our study are consistent with those of previously published reports. The current literature, including this study, raises an important and central question regarding saphenous vein endothelial function per se. Why is endothelium-dependent vasomotion so severely depressed in these vessels? Does this relate to significant endothelial damage during harvesting and distention? Is it related to decreased activity of the endothelial NO system or are endothelium-derived vasoconstrictors the culprit? Although pharmacologic modulation (with BH₄, indomethacin [INN: indometacin], thromboxane, and endothelin antagonists) improves saphenous vein endothelial function, it is important to realize that the percentage of maximum dilation to acetylcholine remains poor in the 20% to 30% range. Clearly, understanding and probing other mechanisms of saphenous vein endothelial dysfunction is needed to shed light on this issue.

Endothelial dysfunction is believed to predispose to graft atherosclerosis and failure; hence, it is tempting to speculate that long-term administration of BH₄ may serve as a useful tool in impeding this process. Results from the present study depict an acute and single-dose experiment that provides very limited information about graft atherosclerosis. Devising a means of long-term delivery of BH₄ in conjunction with morphologic

and pathologic assessment of saphenous veins is needed to specifically answer this question.

In summary, this study demonstrates that pharmacologic modulation of NOS by exogenous BH₄ addition exerts beneficial effects on endothelial function in human saphenous veins used for CABG operations. Understanding and improving endothelial function is a key factor in promoting saphenous vein thromboresistance.

REFERENCES

1. Luscher TF, Noll G. The pathogenesis of cardiovascular disease: role of the endothelium as target and mediator. *Atherosclerosis* 1995;188:S81-90.
2. Luscher TF, Diederich D, Siebenmann R, Lehmann K, Stulz P, von Segesser L, et al. Differences between endothelium-dependent relaxation in arterial and venous coronary bypass grafts. *N Engl J Med* 1988;319:462-7.
3. Cosentino F, Lusher TF. Tetrahydrobiopterin and endothelial function. *Cardiovasc Res* 1999;43:274-8.
4. Huraux C, Makita T, Kurz S, Yamaguchi K, Szlam F, Tarpey MM, et al. Superoxide production, risk factors, and endothelium-dependent relaxations in human internal mammary arteries. *Circulation* 1999;99:53-9.
5. Tsutsui M, Milstien S, Katusic ZS. Effect of tetrahydrobiopterin on endothelial function in canine middle cerebral arteries. *Circ Res* 1996;79:336-42.
6. Higman DJ, Strachan AMJ, Buttery L, Hicks RCJ, Springall DR, Greenhalgh RM, et al. Smoking impairs the activity of endothelial nitric oxide synthase in saphenous vein. *Arterioscler Thromb Vasc Biol* 1996;16:546-52.
7. Cosentino F, Luscher TF. Tetrahydrobiopterin and endothelial function. *Eur Heart J* 1998;19(Suppl G):G3-8.
8. Yang Z, von Segesser L, Bauer E, Stulz P, Turina M, Lusher TF. Different activation of endothelial L-arginine and cyclooxygenase pathway in human internal mammary artery and saphenous vein. *Circ Res* 1991;68:52-60.
9. Verma S, Lovren F, Mather KJ, Maitland A, Kieser T, McNeill JH, et al. Novel endothelial protective effects of bosentan in internal mammary arteries (IMA) and saphenous veins (SV) from patients undergoing bypass surgery [abstract]. *Circulation* 1999;100(Suppl):I-830.