Myocardial Protection and Vascular Biology

Enhanced Nitric Oxide–Mediated Vascular Relaxation in Radial Artery Compared With Internal Mammary Artery or Saphenous Vein

Oz M. Shapira, MD; Aiming Xu, MD; Gabriel S. Aldea, MD; Joseph A. Vita, MD; Richard J. Shemin, MD; John F. Keaney, Jr, MD

Background—The superior long-term patency of internal mammary artery coronary bypass grafts compared with venous grafts has been attributed in part to increased endothelium-derived nitric oxide (NO) production. Interest in the radial artery as an alternative bypass conduit has recently been revived; however, its biological characteristics remain incompletely defined. The purpose of this study was to compare the NO-mediated vasomotor properties of the radial artery to those of the internal mammary artery and saphenous vein.

Methods and Results—Matched segments of radial artery, internal mammary artery, and saphenous vein (n=24 patients) were examined by use of organ-chamber methodology. Endothelium-dependent and -independent vasomotor responses were assessed by dose-response curves to acetylcholine, \( \text{N}^{\text{G}} \)-nitro-L-arginine methyl ester (L-NAME), 8-bromo-cyclic 3',5'-guanosine monophosphate (8-bromo-cGMP), and nitroglycerin. Maximum NO-mediated radial artery relaxation in response to acetylcholine (86±10%) was significantly greater than internal mammary artery (56±9%) or saphenous vein (11±5%, both \( P<0.0001 \)). Similarly, acetylcholine-stimulated cGMP accumulation in radial artery (9.1±1.7 pmol/mg protein) was also greater than internal mammary artery (6.2±0.3 pmol/mg protein) or saphenous vein (1.4±0.2 pmol/mg protein, both \( P<0.05 \)). Estimated basal endothelial NO production, assayed as the percent maximum contraction in response to L-NAME, was greater in radial artery (39±5%) than internal mammary artery (23±6%) or saphenous vein (5±2%, both \( P<0.05 \)). Maximum relaxation of all vessels to nitroglycerin was similar, although the sensitivity of radial artery to nitroglycerin was greater (EC50=33±7 mmol/L) than the internal mammary artery (203±32 mmol/L) or saphenous vein (97±12 mmol/L, both \( P<0.05 \)). Vascular cGMP in response to 0.1 \( \mu \)mol/L nitroglycerin was significantly higher in the radial artery (8.3±1.4 pmol/mg protein) compared with the internal mammary artery (3.5±1.3 pmol/mg protein) or saphenous vein (1.4±0.3 pmol/mg protein, both \( P<0.0001 \)). Relaxation to 8-bromo-cGMP was identical for all 3 conduits.

Conclusions—These data indicate that NO-dependent relaxation of radial artery is greater than that of internal mammary artery or saphenous vein. This difference is related to endothelial production of NO and/or vessel sensitivity to NO. Such favorable physiological characteristics of radial artery could conceivably contribute to improved long-term patency of this conduit compared with saphenous vein. (Circulation. 1999;100[suppl II]:II-322–II-327.)

Key Words: arteries - veins - nitric oxide - endothelium - vessels

Arterial conduits are increasingly preferred for CABG because of their improved long-term patency rates compared with venous conduits.1 This difference in patency between arterial and venous the conduits persists even when these grafts supply the same coronary bed.2 This observation suggests that specific biological properties of the grafts themselves may account for the difference in long-term patency between arterial and venous conduits.3,4 One inherent difference between arterial and venous conduits is endothelial production of nitric oxide (NO).

The importance of NO in vascular homeostasis has become increasingly apparent.5 NO contributes to resting vascular tone,6 impairs platelet activation,7,8 prevents leukocyte adhesion to the endothelium,9 and inhibits the migration10 and proliferation11 of vascular smooth muscle cells. These effects of NO on the vessel wall are thought to afford protection against thrombosis and atherosclerosis.3 Using endothelium-dependent relaxation as an index of NO production, several investigators have found that endothelium-derived NO production in the internal mammary artery is more pronounced than that of the saphenous vein.3,4 One proposed consequence of increased endothelial NO production is superior short- and long-term patency rates of the internal mammary artery grafts compared with saphenous vein grafts.3,4

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The radial artery has recently been rejuvenated as a bypass conduit with encouraging early and intermediate-term results. Some of this success has been attributed to improved harvesting techniques and prolonged administration of antispasmodic agents, 13-15 in keeping with the tendency of the radial artery for vasospasm. 16 However, -NO-mediated relaxation of the radial artery compared with normal mammary artery and saphenous vein is not yet defined. Therefore, the purpose of this study was to evaluate -NO-mediated relaxation of the radial artery in relation to that of the normal mammary artery and saphenous vein.

Methods

Patients

Matched segments of the radial artery, internal mammary artery, and saphenous vein that would otherwise have been discarded were obtained from patients (n=24) undergoing CABG at Boston Medical Center. Subjects were included in this study only if segments of all 3 conduits could be obtained. The study was approved by the Boston Medical Center Institutional Review Board. There were 21 men and 3 women with a mean age of 58±7 years (range, 39 to 73 years). The clinical profile of these patients is depicted in Table 1. All patients were being treated with aspirin.

Materials

Physiological salt solution (PSS) contained (in mmol/L) NaCl 118.3, KCl 4.7, CaCl2 2.5, MgSO4 1.2, KH2PO4 1.2, NaHCO3 25, glucose 11.1, and Na2EDTA 0.026. Nitroglycerin was obtained from Baxter Healthcare Inc. Acetylcholine, U46619, Nω-nitro-L-arginine methyl ester (L-NAME), 3-isobutyl-1-methylxanthine (IBMX), and 8-bromo-cGMP (8-bromo-cGMP) were purchased from Sigma Chemical Co. Kits for cGMP determination by ELISA were obtained from Cayman.

Organ-Case Methodology

Vessels were harvested with the accompanying fat pedicle, immediately placed in ice-cold PSS, and prepared for studies of vascular function. The vessels were carefully dissected from their surrounding fat tissue and cut into 2 to 3 segments measuring 4 mm in length. Vessel segments were placed in organ chambers (37°C) containing 20 mL PSS, suspended between 2 tungsten stirrups for measurement of isometric tension as described 17-19 and constantly aerated with 95% O2/5% CO2. Each vessel was then progressively stretched in 1-g increments to its optimal resting tension that produced a maximal response to 80 mmol/L KCl. Vessels were then allowed to equilibrate for 1 hour before the introduction of vasoactive drugs as described. 17 Relaxation studies were performed after vessels were contracted with 0.1 to 1 mmol/L of U46619 so that contraction was 50% to 60% of the maximal KCl-induced contraction. All experiments were done in the presence of 10 mmol/L indomethacin to inhibit prostaglandin synthesis. Responses to acetylcholine were evaluated in vessels with and without endothelium. In addition, vessel response to acetylcholine was assessed in the presence (300 μmol/L) or absence of the NO synthase (NOS) inhibitor L-NAME. 20,21 The dose-response curves to nitroglycerin were obtained in vessels in which the endothelium was removed. For estimation of basal endothelial NO production, the contractile response to L-NAME was assessed. Briefly, vessel segments were contracted with 1 to 10 mmol/L of U46619 to 20% of the maximal KCl-induced contraction and exposed to increasing doses of L-NAME. The contractile response was recorded as the percent of maximum contraction produced by 80 mmol/L KCl. Because vascular relaxation in response to nitrovasodilators is due in part to increased smooth muscle cGMP content, 22 we examined cGMP-dependent vascular relaxation in all 3 conduits with the cell-permeable cGMP analog 8-bromo-cGMP.

Tissue cGMP

Segments of the radial artery, mammary artery, and saphenous vein with or without endothelium were incised in organ chambers for 90 minutes as described above without tension. To inhibit phosphodiesterase activity, 0.1 mmol/L IBMX was added 20 minutes before 1 mmol/L acetylcholine, 0.1 mmol/L nitroglycerin, or vehicle. Three minutes after the agents were added, vessels were immediately snap-frozen in liquid nitrogen. Rings were homogenized in 1 mL of 6% trichloracetic acid at 4°C, and cGMP levels were measured as described. 23

Histology and Scanning Electron Microscopy

To assess vascular morphology and endothelial integrity in our experiments, we subjected samples of the radial artery, internal mammary artery, and saphenous vein obtained from 7 randomly selected patients to histological examination and scanning electron microscopy. Segments were fixed with a solution of 10% formalin in PBS, pH 7.4, for 20 minutes. Segments were then cleaned, washed in cacodylate-sucrose buffer (10.26 g sucrose in 150 mL of 0.1-mol/L cacodylate) for 5 minutes, and further fixed in glutaraldehyde-cacodylate solution (3% glutaraldehyde, 0.1 mol/L cacodylate) for 24 hours. Samples prepared in this manner were sectioned (0.2 mm), dehydrated, and embedded in paraffin as described. 24 Sections were stained with resorcin fuchsin (for elastin) and subjected to morphometric analysis of intimai and medial areas with an automated videomicroscopy system (Image Technology Corp). Fixed tissues were also prepared for scanning electron microscopy by postfixation in 1% osmium tetroxide and dehydration with graded ethanol exposure. Sections were dried in hexamethyldisilazane, coated with gold and palladium, and observed in an AMR 100-nm scanning electron microscope (AMRAY).

Statistical Analysis

Unless otherwise specified, all data are expressed as mean±SEM. Vessel relaxation is expressed as percent reduction in tension induced by U46619. Emax represents the drug concentration producing 50% of maximum relaxation determined by sigmoidal curve fitting with the use of commercially available software (Origin, Microcal Inc). Dose-response curves for acetylcholine, nitroglycerin, and L-NAME were compared among groups by use of 2-way ANOVA for repeated measures. Comparisons of cGMP and tension were performed with a 1-way ANOVA and appropriate post hoc test (Neuman-Keuls’ or Dunnet’s as appropriate).

Results

Vascular Contraction and Endothelial Morphology

The contractile responses of the radial artery, internal mammary artery, and saphenous vein are given in Table 2. Raw contractions of the radial artery and saphenous vein to 80 mmol/L KCl were comparable, and both were greater than the internal mammary artery. In contrast, contractions of all conduits to 80 mmol/L KCl were similar if corrected for wall thickness (Table 2.) Representative scanning electron micro-

<table>
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<th>TABLE 1. Clinical Profile of the 24 Study Patients</th>
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<td>Age, y</td>
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<tr>
<td>Sex, M/F</td>
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<tr>
<td>Hypertension, n (%)</td>
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<td>Diabetes mellitus, n (%)</td>
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<tr>
<td>Peripheral vascular disease, n (%)</td>
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<tr>
<td>Intravenous nitroglycerin, n (%)</td>
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<td>Grafts per patient, n</td>
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graphs of harvested vessels for this study are given in Figure 1. As shown, we observed an intact endothelial cell layer with the harvesting technique used in this study.

**Endothelium-Dependent Vascular Relaxation**

We observed dose-dependent relaxation of radial artery, saphenous vein, and internal mammary artery in response to acetylcholine, a known stimulus for endothelial NO production (Figure 2). Maximum relaxation of the radial artery to acetylcholine (86±6%) was significantly greater than that of either the internal mammary artery (56±9%, P<0.0001) or saphenous vein (11±5%, P<0.0001). This difference between the radial artery and other conduits was evident throughout the dose-response curve to acetylcholine (Figure 2). In 6 experiments, we found that saphenous vein relaxation to acetylcholine was inhibited 92±6% in the presence of 300 μmol/L L-NAME (data not shown). The corresponding values for the radial and internal mammary arteries were 96±6% and 95±4%, respectively. In the absence of endothelium, no conduit demonstrated any relaxation to acetylcholine (data not shown).

**Estimated Basal Endothelial NO Production**

We observed dose-dependent contraction of all conduits in response to the NOS inhibitor L-NAME (Figure 3). Maximum contraction of the radial artery (30±5%) was significantly greater than either the saphenous vein (5±2%, P<0.05) or internal mammary artery (23±6%, P<0.05), suggesting a greater basal NO production in radial artery endothelium than other vessels. We observed no contraction to L-NAME in conduits devoid of endothelium (data not shown).

**Endothelium-Independent Vascular Relaxation**

In vessels devoid of endothelium, the response to nitroglycerin is as shown in Figure 4. Maximum relaxation to 1 μmol/L nitroglycerin was similar for the radial artery (108±4%), internal mammary artery (96±3%), and saphenous vein (100±4%, all P=NS). In contrast, we found that the radial artery was significantly more sensitive to nitroglycerin (EC50=33±7 mmol/L) than either the saphenous vein (97±12 mmol/L, P<0.05) or internal mammary artery (203±32 mmol/L, P<0.05). To assess cGMP-mediated vascular relaxation, we examined dose-dependent relaxation to 8-bromo-cGMP; these data are given in Figure 5. We found no differences in dose responses of all 3 conduits to 8-bromo-cGMP over a concentration range of 0.1 to 100 μmol/L.

### Table 2. Contractile Response of Vessel Segments to KCl

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<th>RA</th>
<th>IMA</th>
<th>SV</th>
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<tr>
<td>Absolute contraction, g</td>
<td>10.4±1.1</td>
<td>5.3±0.6*</td>
<td>9.4±1.0</td>
</tr>
<tr>
<td>Normalized contraction, g/mm</td>
<td>25.1±4.0</td>
<td>24.9±4.8</td>
<td>32.0±5.1</td>
</tr>
</tbody>
</table>

Matched sets of human radial artery (RA), internal mammary artery (IMA), and saphenous vein (SV) were suspended in organ chambers as described in Methods and exposed to KCl (80 mmol/L). Contractile force either was recorded as absolute grams of tension or was corrected for wall thickness on the basis of histological measurements as described in Methods. Data represent mean±SEM derived from 11 patients.

*P<0.002 for IMA vs RA and SV.
Vessel cGMP levels

As shown in Figure 6A, baseline vascular cGMP was greater in radial artery (1.8±0.4 pmol/mg protein) and internal mammary artery (1.6±0.7 pmol/mg protein) than saphenous vein (0.3±0.3 pmol/mg protein, P<0.05 versus both by 1-way ANOVA). Similarly, cGMP in response to 1 μmol/L acetylcholine was also greater in the radial artery (9.1±1.7 pmol/mg protein) than either the internal mammary artery (6.2±0.3 pmol/mg protein) or saphenous vein (1.4±0.2 pmol/mg protein, P<0.05 versus both by 1-way ANOVA).

Vascular tissue levels of cGMP after exposure to 0.1 μmol/L nitroglycerin are depicted in Figure 6B. The radial artery produced significantly more cGMP (8.3±1.4 pmol/mg protein) than either the internal mammary artery (3.5±1.3 pmol/mg protein, P<0.001 by 1-way ANOVA) or saphenous vein (1.4±0.3 pmol/mg protein, P<0.001 by 1-way ANOVA) in response to nitroglycerin.

Discussion

The superior patency rate of the internal mammary artery compared with saphenous vein is well documented and has triggered interest in the use of other arterial conduits. Luscher and colleagues were the first to link the superior internal mammary artery graft function to enhanced in vitro production of NO. They found that both receptor-mediated (acetylcholine-induced) and receptor-independent (calcium ionophore-induced) endothelium-dependent relaxation of the internal mammary artery was significantly greater than the saphenous vein. These responses could be prevented through mechanical removal of the endothelium, guanylyl cyclase inhibition, or NO scavenging with oxyhemoglobin. Pearson and coworkers confirmed these observations and demon-
strated that internal mammary artery relaxation to acetylcholine was attenuated by the NOS inhibitor L-NMMA. These in vitro properties of the internal mammary artery appear operative in vivo. Nishioka and colleagues have demonstrated greater acetylcholine-induced -NO production (as nitrite accumulation) in internal mammary artery grafts compared with saphenous vein grafts in post-CABG patients. The concept that enhanced graft endothelium-derived -NO production may translate into superior long-term patency has prompted speculation that transfection of saphenous vein with the endothelial NOS gene may enhance vein graft patency.

Our study extends these observations to another arterial conduit, the radial artery. We observed that endothelium-dependent relaxation in the radial artery was superior to other conduits. We believe that these observations indicate that acetylcholine-stimulated -NO production in radial artery is enhanced compared with the internal mammary artery or saphenous vein. The role of -NO is supported by previous data, our own observations that acetylcholine-mediated conduit relaxation was inhibited >90% by the NOS inhibitor L-NAME, and the fact that no relaxation was observed in the absence of endothelium. Moreover, we also observed a greater degree of cGMP accumulation in response to acetylcholine in the radial artery compared with other conduits, again pointing to -NO as the species responsible for our observations. Because our vascular relaxation studies were performed in the presence of indomethacin, there is also no evidence for involvement of prostacyclin in the data presented here.

Our studies also support the idea that basal -NO production from radial artery endothelium is enhanced compared with the saphenous vein and comparable to the internal mammary artery. We observed that dose-dependent NOS inhibition with L-NAME produced a greater degree of contraction in the radial artery than either the internal mammary artery or saphenous vein (Figure 3). This indirect assessment of basal -NO production has been used successfully in other studies but is limited because we cannot exclude the possibility that some contribution of the contractile response is due to U46619. However, we also observed increased basal levels of cGMP in the radial and internal mammary artery segments compared with the saphenous vein (Figure 6), consistent with greater basal endothelium-derived -NO production in these arterial conduits. Thus, our data support relatively greater endothelial NOS activity in both the basal and stimulated states in the radial and internal mammary arteries compared with the saphenous vein.

Our observations of enhanced endothelium-dependent relaxation of the radial artery are in contrast to prior studies reporting comparable responses of the radial and internal mammary arteries. Several factors may account for these differences among studies. In the present study, both the internal mammary and radial arteries were harvested with the accompanying fat and veins with a minimum of mechanical manipulation. Grafts were not flushed with any solution (avoiding chemical injury), and hydrostatic or mechanical dilation of the vessel was strictly avoided. These precautions were designed to minimize endothelial trauma. Consequently, we observed an intact endothelial cell layer in all vessels by electron microscopy (Figure 1) and a uniform response (100%) of the vessels to acetylcholine in the organ chamber. In contrast, Chardigny and colleagues observed endothelium-dependent relaxation in only 62% and 40% of radial and internal mammary artery segments, respectively. In the study of He and Yang, endothelium-dependent relaxation was not observed in all radial and internal mammary artery segments. There were also considerable differences in the stimuli for endothelium-derived -NO in these studies. For example, we stimulated endothelium-derived -NO with a dose response to acetylcholine. In contrast, He and Yang used substance P and calcium ionophore to induce endothelium-dependent relaxation. Although Chardigny and colleagues used acetylcholine, they assessed relaxation by response to a single dose only.

An unexpected and new finding in our study was the greater sensitivity of the radial artery to nitroglycerin (Figure 4). This enhanced sensitivity to nitrovasodilators was also associated with greater tissue levels of cGMP in response to nitroglycerin in the radial artery compared with the internal mammary artery or saphenous vein (Figure 6). These findings suggest that smooth muscle cells in the radial artery may contain more guanylyl cyclase (or a greater specific enzymatic activity) than the smooth muscle in either the internal mammary artery or saphenous vein. To date, there is limited information on the -NO sensitivity of vascular beds with respect to cGMP production. Papapetropoulos and colleagues investigated the response of human and animal vascular tissues to sodium nitroprusside. They found that vascular endothelium and smooth muscle cells obtained from different vascular beds had distinct cGMP responses to sodium nitroprusside. One explanation offered by those investigators was a difference in guanylyl cyclase gene expression based on the vascular bed. Our study results are consistent with this contention. Alternatively, we cannot exclude the possibility that our observations reflect some difference in nitroglycerin metabolism among the 3 conduits. The exact mechanisms underlying this phenomenon and its clinical significance warrant investigation.

The long-term patency rate of the radial artery is presently unknown. However, excellent clinical and angiographic results with a follow-up of up to 5 years have recently been reported. In this study, we documented enhanced endothelium-dependent, -NO-mediated vascular relaxation and greater sensitivity to nitroglycerin in the radial artery compared with the internal mammary artery or saphenous vein. From the known bioactivity of -NO as a vasodilator and antiatherogen, one might expect that these properties of the radial artery protect against vasoconstriction and graft atherosclerosis and thus translate into improved long-term patency of this conduit.

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References

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