DIFFERENTIAL ACTION OF ANGIOTENSIN II AND ACTIVITY OF ANGIOTENSIN-CONVERTING ENZYME IN HUMAN BYPASS GRAFTS

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Objective: The activity of the renin-angiotensin system may be important in determining the performance of coronary artery bypass grafts. We have examined the activity of tissue angiotensin-converting enzyme and the effects of angiotensin II in vessels used as bypass grafts. **Methods:** Organ bath studies were used to determine the vasoactive effect of angiotensin II. The activity of the angiotensin-converting enzyme was assessed by metabolism of a specific synthetic substrate. **Results:** The saphenous vein produced greater maximum responses to angiotensin II than did the internal thoracic artery. This response was not modified by inhibition of nitric oxide synthase, cyclooxygenase, or by an endothelin receptor antagonist in either vessel. Losartan, an AT₁ receptor antagonist, inhibited the vasoconstrictor response in both blood vessels. Homogenates of saphenous vein and internal thoracic artery displayed tissue angiotensin-converting enzyme activity, which was inhibited by captopril. Enzyme activity was threefold greater in the vein. Both the contractile response to angiotensin II and the enzyme activity were retained in venous grafts removed up to 20 years after coronary bypass surgery. **Conclusions:** These data demonstrate that marked differences exist in angiotensin-converting enzyme activity and AT₁ receptor responses in the saphenous vein compared with the internal thoracic artery. These findings may have important implications for the performance of the vein when used as a coronary artery bypass graft and may have clinical implications for the use of angiotensin-converting inhibitors and AT₁ receptor antagonists in the prevention and treatment of vein graft disease. (J Thorac Cardiovasc Surg 1998;116:206-12)

Coronary artery bypass grafting offers patients with ischemic heart disease a significant improvement in quality of life and longevity. However, these clinical benefits are gradually lost and deterioration of graft function results in recurrence of clinical symptoms.¹ The limitation of coronary artery operations is widely attributed to the poor

performance of the saphenous vein (SV) when used as a bypass conduit.²

Morphologic changes develop in the vessel wall as a result of loss of endothelium-derived mediators and stimulation of smooth muscle cell growth and contraction, resulting in deterioration of the function of the graft and its eventual failure.³ Many of these changes have the capacity to be mediated by angiotensin II. Animal models of atherosclerosis and bypass grafting have demonstrated the clinical benefits of angiotensin-converting enzyme (ACE) inhibitors to improve the prognosis of patients with left ventricular dysfunction, as well as to reduce smooth muscle cell proliferation and improve endothelial function in grafted vessels.⁴⁻⁶

Differences in the biologic properties of smooth muscle cells and endothelial cells between the SV and the internal thoracic artery (ITA) may influence the regulation of vessel tone and the control of intimal smooth muscle cell growth.⁷ SV graft function is inferior to that of the ITA, possibly because

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Fig. 1. Measurements of ACE activity in human SVs ($n = 25$), ITAs ($n = 27$), and old SVs ($n = 16$). Values are means $\pm$ standard error of the mean. $**p < 0.001$ in the SV and old SV when compared with the ITA.

of specific differences in receptors on smooth muscle cells, the release of endothelium-derived relaxing factors, the actions of mitogens, and the response of the vessel wall to mechanical trauma.⁸⁻¹⁰ The responses of endothelial and smooth muscle cells to local ACE and angiotensin II release may influence their differing abilities to perform as bypass conduits.

The involvement of angiotensin II in these pathophysiological processes remains largely unexplored and requires investigation. Thus we have examined the local tissue ACE activity, as well as the response of the vessel wall to angiotensin II.

Methods

Undistended SVs ($n = 44$), ITAs ($n = 41$), and old vein grafts, (old SVs, $n = 22$) were obtained from patients (aged 40 to 78 years, 87 male and 20 female) undergoing coronary artery bypass grafting or cardiac transplantation. Vessel specimens were immediately placed in Hanks balanced salt solution or frozen in liquid nitrogen for ACE activity studies or immunohistochemical staining of the endothelium. All patients from whom samples were obtained were receiving a number of different drug combinations. Only 17% of patients were prescribed ACE inhibitor therapy prior to surgery, while no patient had received an AT₁ receptor antagonist. No difference in ACE activity or angiotensin II response was seen in those patients receiving ACE inhibitors.

Fig. 2. Contractile effect of angiotensin II in isolated segments of SV (■, $n = 18$), ITA (●, $n = 16$), and old SV (▲, $n = 9$) bypass grafts. Values are means $\pm$ standard error of the mean. $*p = 0.004$ in the SV when compared with the ITA and $p = 0.03$ in the SV compared with the old SV graft.

Determination of tissue ACE activity. ACE activity was determined in frozen samples of vessel specimens by measurement of the conversion of ³H-benzoyl-phenylalanine-alanine-proline (³H-BPAP) a synthetic substrate for ACE.¹¹ The ³H-BPAP is broken down by ACE in the tissue homogenate to ³H-benzoyl-phenyl and alanine-proline. The tritiated product was separated from the unchanged substrate by its dissolution into a toluene-containing solution. The extracted ³H-BPAP product was then assayed by liquid scintillation counting. ACE activity was measured in the presence and the absence of the ACE inhibitor captopril (10^{-6} mol/L) and expressed as units of activity per milligram of wet weight of tissue. Results were expressed as the mean $\pm$ standard error of the mean and the 95% confidence interval (CI).

In vitro organ bath studies. Isolated vessels were mounted in organ baths containing modified Tyrode's solution composed of (mmol/L) 136.9 NaCl, 11.9 NaHCO₃, 2.7 KCl, 0.4 NaH₂PO₄, 2.5 MgCl₂, 2.5 CaCl₂, 11.1 glucose, and 0.04 disodium ethylenediaminetetraacetic acid (BDH, Poole, United Kingdom). Vessel segments were stretched to their optimum tension for smooth muscle contractility. The vasoactive effect of angiotensin II (Sigma, Poole, United Kingdom) was assessed by the addition of cumulative concentrations of the peptide (10^{-10} – 10^{-6} mol/L) in half log₁₀ units. The means of the absolute responses were expressed in millinewtons (mN) $\pm$ standard error of the mean and the 95% CI.

To examine the receptor subtypes responsible for the effects mediated by angiotensin II, vessel segments of both

Fig. 3. Contractile effect of angiotensin II in the absence (■, $n = 6$) and the presence of 10^{-4} mol/L L-NMMA (●, $n = 6$), 10^{-5} mol/L bosentan (▲, $n = 6$), or 10^{-6} mol/L indomethacin (★, $n = 6$) in either (A) isolated segments of SV or (B) isolated segments of ITA. Values are means $\pm$ standard error of the mean.

Table I. Effect of angiotensin II on maximum contractile response in the SV and ITA*

	L-NMMA	Indomethacin	Bosentan	Control
SV (mN)	51.1 $\pm$ 11.8	46.0 $\pm$ 11.9	39.8 $\pm$ 9.4	48.2 $\pm$ 13.4
95% CI (mN)	20.9–104.4	15.6–76.5	15.7–63.9	13.8–82.6
<i>p</i> Value	>0.2	>0.2	>0.2	
ITA (mN)	12.1 $\pm$ 1.4	19.7 $\pm$ 3.1	9.3 $\pm$ 4.2	12.3 $\pm$ 4.5
95% CI (mN)	8.5–15.6	11.8–27.5	–4.9–21.7	4.8–19.8
<i>p</i> Value	>0.2	>0.2	>0.2	

*No significant effect compared with control segments.

SV and ITA were incubated for 30 minutes with one of three concentrations of either the AT₁ receptor antagonist losartan (10^{-8} , 10^{-7} , and 10^{-6} mol/L) (gift from Merck, Rahway, N.J.) or the AT₂ receptor antagonist PD123319 (10^{-8} , 10^{-7} , and 10^{-6} mol/L) (gift from Parke Davis, Ann Arbor, Mich.) before the addition of angiotensin II.¹² Comparative responses between the blood vessels were made by expressing the maximum constrictions as a percentage of the response to a 90 mmol/L concentration of KCl. Results were expressed as mean $\pm$ standard error of the mean.

The contribution of endothelium-derived vasoactive substances was assessed with prior incubation of the vessel segments for 30 minutes with either the nitric oxide synthase inhibitor, *N*^G-monomethyl-L-arginine (L-NMMA) (10^{-4} mol/L) (gift from Dr. S. Moncada, UCL, London, United Kingdom), the cyclooxygenase inhibitor indomethacin (INN: indometacin) (10^{-6} mol/L), (Sigma), or the mixed endothelin-1 (ET-1) receptor (ET_A/ET_B) antagonist bosentan (10^{-5} mol/L) (gift from RPR, Daganham, Kent, United Kingdom). The means of the absolute responses were expressed in millinewtons $\pm$ standard error of the mean, and the 95% CI.

Immunohistochemistry. Frozen sections, 6 μ m thick, were cut from vessels of each of the three groups. With the use of an immunohistochemical technique employing streptavidin biotin peroxidase (Dako Ltd., High Wycombe, Bucks, United Kingdom), the vessel endothelium was labeled with a rabbit polyclonal antibody to endothelial nitric oxide synthase (eNOS) (0.001 mg/ml dilution). eNOS antibody was purchased from Transduction Laboratories, Lexington, Kentucky.

Statistics. Statistical analysis was carried out using the Primer Statistical Program (McGraw Hill Inc., New York, N.Y.). Control and experimental groups were compared by means of a one-way analysis of variance followed by a Bonferroni *t* test.

Results

ACE activity. Human SVs ($n = 25$), ITAs ($n = 27$), and old SVs ($n = 16$) all displayed tissue ACE activity (Fig. 1). The ACE activity in the SV was significantly enhanced, 5.9 ± 0.5 units/mg of wet weight (95% CI, 4.9 to 6.9 units/mg of wet weight,

$p < 0.001$), in comparison with the ITA, 2.1 ± 0.3 units/mg of wet weight (95% CI, 1.4 to 2.8 units/mg of wet weight). Old SV grafts retained similar levels of ACE activity, 6.8 ± 1.5 units/mg of wet weight (95% CI, 3.5 to 10.1 units/mg of wet weight) when compared to the native vein. There was no correlation between ACE activity and the age of the graft. The ACE inhibitor captopril (10^{-6} mol/L) was able to completely inhibit ACE activity in all vessel types.

Angiotensin II responses. Angiotensin II was capable of inducing contractions in segments of SV ($n = 18$), ITA ($n = 16$), and old SV ($n = 9$) (Fig. 2). Angiotensin II was approximately sevenfold more efficacious in the native vein than in the ITA or the old SV graft. The maximum effects in these vessels was in the order SV > old SV = ITA. The maximum contractile effect of angiotensin II was significantly greater in the SV, 50.6 ± 11.9 mN (95% CI, 25.4 to 75.8 mN), than in the ITA, 10.3 ± 2.2 mN (95% CI, 5.5 to 15.0 mN) ($p = 0.004$), and old SV graft, 11.3 ± 2.7 mN (95% CI, 5.2 to 17.4 mN) ($p = 0.03$).

Role of endothelium-derived vasoactive factors. There was a lack of modulating effects of nitric oxide, prostanoids, or endothelin-1 on the response to angiotensin II in both the SV and the ITA (Fig. 3). Compared with control segments, angiotensin II had no significant effect on the maximum contractile response in the SV and the ITA when incubated with L-NMMA, indomethacin, or bosentan (Table I).

Sections of SV and ITA displayed similar endothelial coverage of the vessel shown by positive staining for eNOS. The endothelial layer, although present, was not continuous. There were fewer positive staining cells for eNOS in the old SV grafts studied (Fig. 4).

Role of AT₁ and AT₂ receptors. The effects mediated by angiotensin II could be inhibited by antagonism of the AT₁ receptor but not the AT₂ receptor. The AT₁ receptor antagonist losartan caused a concentration-dependent rightward shift of the angiotensin II concentration response curve and a marked depression in the maximum effect achieved with increasing concentrations of the antagonist in both the SV and the ITA (Figs. 5 and 6). In contrast, the response to angiotensin II in both vessel types was unaffected by incubation with the AT₂ receptor antagonist PD123319.

Discussion

This study has demonstrated that both the activity of ACE and the contractile effect of its product,

Fig. 4. Photomicrographs of cross sections of (A) SV, (B) old SV graft, and (C) ITA, showing positive staining for eNOS (arrows). Scale bar represents 100 μ m.

angiotensin II, are significantly greater in the SV than in the ITA. In addition, veins that have served as bypass conduits for up to 20 years retain similar levels of ACE activity when compared with the native vein.

The SV showed a threefold greater ACE activity than that of the ITA. This result may help explain the findings of Yang and associates,¹³ who demonstrated the ability of ACE inhibitors to significantly augment the dilator response of bradykinin which is inactivated by ACE, in the SV but not in the ITA. Enhancement of tissue ACE activity via gene transfer into rat carotid arteries increases vascular smooth muscle cell hypertrophy, an effect mediated by AT₁ receptors.¹⁴ The greater ACE activity in the

Fig. 5. Contractile effect of angiotensin II in the absence (■, $n = 7$) and the presence of 10^{-8} mol/L (●, $n = 7$), 10^{-7} mol/L (▲, $n = 7$), or 10^{-6} mol/L (★, $n = 7$) of either (A) losartan or (B) PD123319 in isolated segments of SV. Values are means $\pm$ standard error of the mean.

Fig. 6. Contractile effect of angiotensin II in the absence (■, $n = 4$) and the presence of 10^{-8} mol/L (●, $n = 4$), 10^{-7} mol/L (▲, $n = 4$), or 10^{-6} mol/L (★, $n = 4$) of either (A) losartan or (B) PD123319 in isolated segments of ITA. Values are means $\pm$ standard error of the mean.

vein suggests an associated enhancement in local angiotensin II production, which may predispose the SV to the growth-promoting actions of this peptide.

We have also demonstrated that the SV produces a greater maximum constriction in response to angiotensin II when compared to the ITA. The reduced contractile response of the ITA to angiotensin II may have been due to greater endothelial protection in this vessel.¹⁵ Despite similar endothelial coverage and expression of eNOS, we were unable to enhance the degree of vasoconstriction in

segments of ITA by inhibition of nitric oxide synthase or cyclooxygenase. Angiotensin II has also been shown to be capable of promoting increased expression of the gene for, and the release of, endothelin-1 from vascular endothelial cells, which could potentiate the contractile effects of angiotensin II.¹⁶ However, in this study we were unable to demonstrate an association between angiotensin II induced vasoconstriction and an effect mediated by endothelin-1 at either ET_A or ET_B receptors.

We have also demonstrated that in both the ITA

and the SV, contractions to angiotensin II were mediated via the AT₁ receptor but not the AT₂ receptor. The AT₁ receptor is present in many human and animal organs and on smooth muscle cells in vascular tissues, where it generally predominates over the AT₂ receptor and has been shown to be responsible for mediating the contractile and mitogenic effects of angiotensin II.^{12, 17} In contrast, the AT₂ receptor is localized to specific regions of the kidney and brain, as well as in the developing fetus. Although studies have demonstrated a functional activity associated with the AT₂ receptor, a physiologic role for these receptors has not been adequately defined.^{12, 17}

Many human and animal *in vitro* studies have shown regional variations in the response to angiotensin II, which are suggested to be the result of differences in AT₁ receptor density and distribution.^{18, 19} Thus the difference in the ability of the vascular smooth muscle to respond to angiotensin II in these two blood vessels may be due to the existence of varying receptor densities or differences in signal transduction pathways. The precise mechanisms that underlie the differences in the response of angiotensin II in these blood vessels requires further investigation.

We have demonstrated that ACE activity is retained in SV bypass grafts irrespective of the age of the graft, despite damage to the endothelium. The retention of ACE activity may be important, since the progression of vein graft disease in animal models of carotid artery bypass has been shown to benefit from treatment with ACE inhibitors,²⁰ which have been shown to produce a 40% reduction in intimal hyperplasia,²¹ as well as restoration of endothelium-dependent relaxation to acetylcholine of grafts.²² It has been demonstrated that local ACE activity is increased and contractile response to angiotensin II is enhanced in experimental models of vein graft disease.^{6, 23} However, we observed a reduction in the contractile response of angiotensin II in old vein grafts. A generalized loss of contractile mechanisms caused by vascular stiffness, rather than a loss of receptor function after bypass grafting, has been reported.²⁴ The retention of contractile receptor function and endothelial dilator mechanisms in bypass grafts suggest that vascular tone may still be amenable to pharmacologic regulation.^{24, 25} The continued activity of ACE and subsequent production of angiotensin II may also be important with respect to the mitogenic and/or pro-oxidant effects of angiotensin II.

The wall of the vein graft is exposed to both uncontrolled stretch before grafting and pulsatile stretch after its exposure to arterial pressures. Increased tension has been shown to stimulate proliferation of cultured smooth muscle cells from the SV but not the ITA.²⁶ Furthermore, in some cell types increased stretch can up-regulate the proliferative effect of angiotensin II and possibly enhance the sensitivity of the vessel wall to other growth-promoting factors.^{27, 28} The role of ACE and angiotensin II in smooth muscle cell growth in response to stretch has not been addressed. While *in situ*, the vessel wall of the vein graft may also be acted on either in the short term by enhanced levels of angiotensin II during and after cardiopulmonary bypass or in the long term by locally released angiotensin II.^{6, 29} This, combined with the long-term positioning of the SV into an arterial circulation, may allow the effects of locally released angiotensin II to override the control mechanisms that limit intimal smooth muscle cell growth under normal circumstances.

In conclusion, this study has demonstrated that the SV exhibits significantly greater angiotensin II-mediated contractions than the ITA, with enhanced ACE activity, suggesting its greater potential for development of the changes associated with graft failure. Our findings suggest the need for a clinical trial of ACE inhibitors and AT₁ receptor antagonists for prevention of vein graft disease.

REFERENCES

1. Lytle BW, Loop FD, Taylor PC, Simpfordorfer C, Kramer JR, Ratliff NB, et al. Vein graft disease: the clinical impact of stenoses in saphenous vein bypass grafts to coronary arteries. *J Thorac Cardiovasc Surg* 1992;103:831-40.
2. Nwasokwa ON, Bergamini TM, Towne JB, Bandyk DF, Seabrook GR, Schmitt DD. Coronary artery bypass graft disease: experience with *in situ* saphenous vein bypasses during 1981 to 1989—determinant factors of long-term patency. *Ann Intern Med* 1991;13:137-47.
3. Lüscher TF. Vascular biology of coronary bypass grafts. *Curr Opin Cardiol* 1991;6:868-76.
4. Aberg G, Ferrer P. Effects of captopril on atherosclerosis in cynomolgus monkeys. *J Cardiovasc Pharmacol* 1990;15(suppl 5):565-72.
5. Pfeffer MA, Braunwald E, Moya LA, Basta L, Brown EJ, Cuddy TE, et al. Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction: results of the survival and ventricular enlargement trial. The SAVE Investigators. *N Engl J Med* 1992;327:669-77.
6. O'Donohue MK, Davies MG, Radic ZS, Mikat EM, Hagen PO. Increased concentrations of angiotensin-converting enzyme in the intimal hyperplasia of experimental vein grafts. *J Cardiovasc Pharmacol* 1994;23:594-601.
7. Yang Z, Diederich D, Scheider K, Siebenmann R, Stulz P,

- von Segesser L, et al. Endothelium-derived relaxing factor and protection against contractions induced by histamine and serotonin in the human internal mammary artery and in the saphenous vein. *Circulation* 1989;80:1041-8.
8. Yang Z, von Segesser L, Bauer E, Stulz P, Turina M, Lüscher TF. Different activation of the endothelial l-arginine and cyclooxygenase pathway in the human internal mammary artery and saphenous vein. *Circ Res* 1991;68:52-60.
9. Lüscher TF. Endothelial control of vascular tone and growth. *Clin Exp Hypertens A* 1990;12:897-902.
10. Angelini GD, Christie MI, Bryan AJ, Lewis MJ. Surgical preparation impairs release of endothelium-derived relaxing factor from human saphenous vein. *Ann Thorac Surg* 1989;48:417-20.
11. Catravas JD, Gillis CN. Metabolism of [³H] benzoyl-phenylalanyl-alanyl-proline by pulmonary angiotensin converting enzyme in vivo: effects of bradykinin, SQ 14225 or acute hypoxia. *J Pharmacol Exp Ther* 1981;217:263-70.
12. Timmermans PB, Wong PC, Chiu AT, Herblin WF, Benfield P, Carini DJ, et al. Angiotensin II receptors and angiotensin II receptor antagonists. *Pharmacol Rev* 1993;45:205-51.
13. Yang Z, Arnet U, von Segesser L, Siebenmann R, Turina M, Lüscher TF. Different effects of angiotensin-converting enzyme inhibition in human arteries and veins. *J Cardiovasc Pharmacol* 1993;22:S17-22.
14. Morishita R, Gibbons GH, Ellison KE, Lee W, Zhang L, Yu H, et al. Evidence for direct local effect of angiotensin in vascular hypertrophy: in vivo gene transfer of angiotensin converting enzyme. *J Clin Invest* 1994;94:978-84.
15. Toda N, Ayaziki K, Okamura T. Modifications by endogenous prostaglandins of angiotensin II-induced contractions in dog and monkey cerebral and mesenteric arteries. *J Pharmacol Exp Ther* 1990;252:374-79.
16. Webb ML, Dickinson KE, Delaney CL, Liu EC, Serafino R, Cohen RB, et al. The endothelin receptor antagonist, BQ-123, inhibits angiotensin II-induced contractions in rabbit aorta. *Biochem Biophys Res Commun* 1992;185:887-92.
17. Griendling KK, Murphy TJ, Alexander RW. Molecular biology of the renin-angiotensin system. *Circulation* 1993;87:1816-28.
18. Myers PR, Katwa LC, Tanner M, Morrow C, Guarda E, Parker JL. Effects of angiotensin II on canine and porcine coronary epicardial and resistance arteries. *J Vasc Res* 1994;31:338-46.
19. Chen L, McNeill JR, Wilson TW, Gopalakrishnan V. Heterogeneity in vascular smooth muscle responsiveness to angiotensin II: role of endothelin. *Hypertension* 1995;26:83-8.
20. Powell JS, Clozel JP, Muller RK, Kuhn H, Hefti F, Hosang M, et al. Inhibitors of angiotensin-converting enzyme prevent myointimal proliferation after vascular injury. *Science* 1989;245:186-8.
21. O'Donohoe MK, Schwartz LB, Radic ZS, Mikat EM, McCann RL, Hagen PO. Chronic ACE inhibition reduces intimal hyperplasia in experimental vein grafts. *Ann Surg* 1991;214:727-32.
22. Cross KS, el Sanadiki MN, Murray JJ, Mikat EM, McCann RL, Hagen PO. Functional abnormalities of experimental autogenous vein graft neoendothelium. *Ann Surg* 1988;208:631-8.
23. O'Donohoe MK, Radic ZS, Stein AD, Schwartz LB, McCann RL, Hagen P-O. Loss of tachyphylaxis and increased sensitivity to angiotensin II in experimental vein grafts. *Vasc Surg* 1991;25:281-7.
24. O'Neil GS, Chester AH, Schyns CJ, Tadjkarimi S, Borland JA, Yacoub MH. Effect of surgical preparation and arterialization on vasomotion of human saphenous vein. *J Thorac Cardiovasc Surg* 1994;107:699-706.
25. Ku DD, Caulfield JB, Kirklin JK. Endothelium-dependent responses in long-term human coronary artery bypass grafts. *Circulation* 1991;83:402-11.
26. Predel HG, Yang Z, von Segesser L, Turina M, Buhler FR, Lüscher TF. Implications of pulsatile stretch on growth of saphenous vein and mammary artery smooth muscle. *Lancet* 1992;340:878-9.
27. Yang Z, Noll G, Lüscher TF. Calcium antagonists differently inhibit proliferation of human coronary smooth muscle cells in response to pulsatile stretch and platelet-derived growth factor. *Circulation* 1993;88:832-6.
28. Bishop JE, Kiernan LA, Butt RP. Mechanical load enhances the stimulatory effect of angiotensin II on human cardiac fibroblast procollagen synthesis. *Circulation* 1996;94:1657.
29. Taylor KM, Bain WH, Russell M, Brannan JJ, Morton JJ. Peripheral vascular resistance and angiotensin II levels during pulsatile and no-pulsatile cardiopulmonary bypass. *Thorax* 1979;34:594-8.