Pharmacologic inhibition of vein graft neointimal hyperplasia

Thomas Schachner, MD

Dr Schachner

Although arterial conduits are widely used and have improved the long-term results of coronary artery bypass grafting, vein grafts remain important additional conduits in coronary surgery. Newer studies show a saphenous vein graft patency of 60% or more at 10 years postoperatively. The pathology of vein graft disease consists of thrombosis, neointimal hyperplasia, and vein graft atherosclerosis, which limit graft longevity. Therapeutic strategies to prevent vein graft disease include external stenting, pharmacotherapy, and gene therapy. The potential benefits of a pharmacologic approach are as follows: (1) Drugs with a broad clinical experience can be used; (2) side effects of systemic application can be minimized by local therapy; and (3) no vascular injury, such as pressurizing the vein for a viral transfection approach, is necessary. The different sites for pharmacotherapy in vein graft disease are reviewed in this article.

In addition to the use of arterial bypass conduits, vein grafts still play an important role in coronary surgery. Newer studies show a saphenous vein graft patency of 60% or more at 10 years postoperatively.¹⁻³ There seems to be an equal or even increased patency over decades despite the generally increased age and comorbidity of the patient population. One can hypothesize that this is the result of an increased awareness of vein graft disease. In addition, a more refined technical handling, influenced by the knowledge in arterial grafting, may have occurred. Furthermore, better postoperative care (eg, early aspirin, statins, cessation of smoking, blood pressure lowering) may contribute to increased vein graft longevity. Angiographic follow-up studies demonstrated equal long-term patency rates of vein grafts to the right coronary artery compared with internal thoracic artery grafts, especially when the target vessel stenosis was less than 70%.⁴⁻⁶

Neointimal hyperplasia (NIH) is a typical pathologic feature of vein grafts during the first weeks after arterialization. It is thought that NIH eases vein graft atherosclerosis, which occurs during several years after grafting and leads to stenosis and occlusion of the vein grafts (Figure 1).⁷⁻⁹

Reasons for an immediate injury of the vein are surgical trauma, ischemia, endothelial injury, and increased wall stress after arterialization. After an early (first 3–5 days) medial smooth muscle cell (SMC) loss (caused by apoptosis), a consecutive increase of total vascular wall thickness occurs.^{10,11}

NIH results from cellular proliferation (SMC, myofibroblasts), cell migration (SMC from the media, myofibroblasts from the periadventitia, macrophages from luminal and adventitial side, platelets from luminal side), extracellular matrix deposition, and inflammatory processes (leukocytes, platelets, growth factors, cytokines).

The source of cells in the hyperplastic vein graft is of importance in the attempt to prevent vein graft disease. In LacZ-expressing mice, Zhang and colleagues¹² found that approximately 60% of cells in the vein graft neointima developed from graft-extrinsic precursors. Another experiment using decellularized vein grafts demonstrated that vein graft-extrinsic cells can form a neointima.¹³

Hu and colleagues¹⁴ used SM-LacZ transgenic mice (which express beta galactosidase in SMCs) and found that 40% of SMCs in vein grafts were graft-extrinsic

From the Department of Cardiac Surgery, Innsbruck Medical University, Innsbruck, Austria.

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Address for reprints: Thomas Schachner, MD, Anichstrasse 35, 6020 Innsbruck, Austria (E-mail: Thomas.Schachner@uibk.ac.at).

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Abbreviations and Acronyms

ACE	= angiotensin-converting enzyme
CABG	= coronary artery bypass graft
ET	= endothelin
ICAM-1	= intercellular adhesion molecule-1
MMP	= matrix metalloproteinase
NIH	= neointimal hyperplasia
NO	= nitric oxide
ODN	= oligodeoxynucleotide
PDGF	= platelet-derived growth factor
PGI ₂	= prostacyclin
SMC	= smooth muscle cell
TIMP	= tissue inhibitor of metalloproteinase
VSMC	= vascular smooth muscle cell

cells and that 60% were graft-intrinsic cells at 8 weeks postoperatively.¹⁴ In another study,¹⁵ the potential role of adventitial progenitor cells for vein graft NIH was demonstrated. In another mouse model,¹⁶ predominantly recipient origins of endothelial cells (with one third of endothelial cells of vein grafts derived from bone marrow progenitor cells) were found in vein grafts.

The adventitia also has a clear role in the development of NIH. For example, one third of periadventitial cells were positive for proliferating cell nuclear antigen early after arterialization in a pig model.¹⁷ In addition, Shi and colleagues¹⁸ demonstrated labeled fibroblasts migrating from the periadventitial space into the vein graft wall.

Convincing evidence exists for the role of migrating monocytes/macrophages on the inflammatory features of

vein graft disease.¹⁹ Cross and colleagues²⁰ found the presence of mast cells (an important source of cytokines such as histamine, leukotriene C₄, and platelet-activating factor) in rabbit vein grafts 4 weeks postoperatively, whereas no mast cells were found in ungrafted veins.²⁰

Intercellular adhesion molecule (ICAM)-1 is a counter-receptor that is engaged in leucocyte adhesion and transmigration, and that is abundantly present on the endothelial surface of mouse vein grafts 4 weeks after surgery. Zou and colleagues²¹ observed a decreased leucocyte amount and a 40% reduced neointimal thickness in ICAM-1 knockout mice compared with wild-type animals. Crook and colleagues²² found an increase in ICAM-1 in human saphenous veins cultured for 14 days compared with freshly isolated veins. In the same study the ICAM-1 amount was dramatically increased after a 24-hour incubation with tumor necrosis factor- α , interleukin-1 α , and interferon- γ .²²

In low-flow vein grafts (poor distal runoff), the neointimal thickness was markedly increased and the messenger ribonucleic acid expression of the proinflammatory interleukin-1 β was greater than in high-flow vein grafts.^{23,24} The increased vein graft patency in good runoff areas is also documented clinically. Goldman and colleagues² found a 10-year patency of saphenous vein graft of 88% in target vessels greater than 2.0 mm diameter versus 55% in vessels with diameters 2.0 mm or less.²

Vein graft atherosclerosis occurs after several years and is difficult to achieve in animal models. However, it is in part possible to find features of graft atherosclerosis in vein grafts of animals with hyperlipidemia. Atheromas with infiltrating mononuclear cells, foam cells, cholesterol crystals,

Figure 1. Different grades of saphenous vein graft atherosclerosis in the same patient 9 years after CABG. Parts without calcifications (A), intramural calcifications (B), superficial calcifications (C), and calcifications involving large parts of the vascular wall (D). *Areas of calcification (hematoxylin-eosin stain).

Figure 2. Overview of pharmacotherapeutic approaches to prevent vein graft neointimal hyperplasia. *bFGF*, Basic fibroblast growth factor; *cAMP*, cyclic adenosine monophosphate; *cGMP*, cyclic guanosine monophosphate; *ERK*, extracellular signal-regulated kinase; *IGF*, insulin-like growth factor; *IL*, interleukin; *JNK*, c-jun N-terminal kinase; *MAPK*, mitogen-activated protein kinase; *mTOR*, mammalian target of rapamycin; *NF*, nuclear factor; *PCNA*, proliferating cell nuclear antigen; *TNF*, tumor necrosis factor.

and calcified necrotic cores and areas of necrosis in the vascular wall have been found in vein grafts of apolipoprotein E-deficient (hypercholesterolemic) mice.²⁵ In hypercholesterolemic rabbits, NIH was markedly increased and contained lipid vacuoles, and foam cells compared with those of normocholesterolemic rabbits at 4 weeks after operation.^{26,27} Grafts of hypercholesterolemic rabbits exhibited a significantly increased (contractile) sensitivity to serotonin and decreased (vasodilatory) sensitivity to sodium nitroprusside compared with vein grafts of normocholesterolemic rabbits.²⁸

The prevention of vein graft NIH starts with careful "no touch" harvesting and a short ischemic time. In arterialized mouse jugular vein patches, Sakaguchi and colleagues²⁹ demonstrated an increased neointimal area in vein patches stored for 2 hours before grafting compared with immediately grafted vein patches. Furthermore, the increased storage time was associated with a decreased cyclic adenosine monophosphate amount in the veins.²⁹

Vein graft disease can be studied clinically or experimentally (SMC culture, saphenous vein organ culture, and animal models). Experimentally, veins have been treated with external stenting, pharmacologic therapy, or gene therapy to attenuate neointimal formation. However, only a few methods for prevention of vein graft failure, such as the use of aspirin, lipid-lowering drugs (especially statins), and cessation of smoking, have reached clinical practice.

Promising results have been achieved with the use of E2F decoy in vascular surgery, but thus far no benefit has been shown in patients who underwent coronary artery bypass grafting (CABG).³⁰ The potential benefits of a phar-

macologic approach are as follows: (1) Drugs with a broad clinical experience can be used, (2) side effects of systemic application can be minimized with local therapy, and (3) no vascular injury such as pressurizing the vein for a viral transfection approach is necessary. Hu and colleagues³¹ demonstrated that local application of adenovirus vector resulted in enhanced inflammatory response and NIH in mouse vein grafts at 4 and 8 weeks postoperatively. With a pharmacologic approach there is no transfection virus-mediated inflammatory response in the vascular wall.

One of the major disadvantages of local pharmacotherapy is the limited duration of action, which may be improved by suitable carriers.

Special Sites of Pharmacologic Intervention to Prevent Neointimal Hyperplasia Thrombosis

In mouse vein grafts, mural fibrin deposits and thrombus formation (with a maximum at 1-3 days postoperatively) were found during the first week after arterialization (Figure 2).^{32,33} Thrombomodulin, by binding thrombin (which thereby loses its fibrinogen cleaving capacity and gains protein C-activating ability), is important for the endothelial thromboresistance. Kim and colleagues³⁴ demonstrated a decrease of thrombomodulin in rabbit vein grafts of less than 10% of the amount of ungrafted veins within the first 2 weeks postoperatively. Tissue factor is a transmembrane glycoprotein that activates factor VII and leads to increased thrombin production. Channon and colleagues³⁵ found an increased amount of tissue factor in rabbit vein grafts, which was spatially associated with infiltrating leukocytes early after arterialization. The same group, how-

ever, noticed no benefit of locally applied antitissue factor antibody regarding the development of NIH.³⁶ Both local and systemic treatment with aspirin reduced thrombus formation and NIH in animal experiments of vein graft disease.^{33,37} C-type natriuretic peptide inhibits vascular smooth muscle cell (VSMC) proliferation and shows anti-inflammatory and antithrombotic effects. Local application of C-type natriuretic peptide decreased NIH in mouse vein grafts. In the same study the amount of adventitial CD8+ leukocytes was decreased in C-type natriuretic peptide-treated vein grafts compared with controls.³⁸ In addition to antithrombotic properties, heparin inhibits SMC proliferation in vitro and inhibits neointimal thickening in injured arteries. However, heparin treatment of rats (800 U/12 h for 3 days subcutaneously) failed to suppress NIH at 2 weeks postoperatively.³⁹

Reactive Oxygen Species

Reactive oxygen species (eg, superoxide anion, peroxide, hydroxyl radical) are constantly generated in aerobic life, but unusually high amounts are found in activated leukocytes and after tissue hypoxia. Malondialdehyde, a marker for lipid peroxidation, was more than 5-fold increased in vein grafts 3 days postoperatively compared with ungrafted veins. Antioxidative treatment with polyethylene glycolated superoxide dismutase (vein immersed in polyethylene glycol-superoxide dismutase for 15 minutes and perivascular application in Pluronic gel) reduced both malondialdehyde concentrations in grafts at 3 days postoperatively and NIH at 4 weeks postoperatively.⁴⁰ Treatment of rabbits with the antioxidant methylaminochroman (10 mg · kg · d by mouth) showed an increased preservation of vein graft vasorelaxation after acetylcholine and sodium nitroprusside exposure at 4 weeks postoperatively compared with controls. Morphologically the vein grafts of methylaminochroman-treated animals exhibited a reduced NIH compared with controls.⁴¹

Nitric Oxide and Prostacyclin

Nitric oxide (NO) and prostacyclin (PGI₂) exhibit antithrombotic, vasodilatory, and antiproliferative (PGI₂) properties. Early after arterialization, NO, PGI₂, and their second messengers cyclic guanosine monophosphate and cyclic adenosine monophosphate are reduced in porcine vein grafts.^{42,43} Onohara and colleagues^{44,45} demonstrated a decreased stimulated (with arachidonic acid) PGI₂ production in canine vein grafts with a poor runoff and low shear stress compared with veins grafted into a good runoff area. The flow dependency of vein graft disease was confirmed by the fact that vein grafts with poor distal runoff showed increased (approximately doubled) NIH compared with control vein grafts. Rabbit vein grafts instilled for 15 minutes with 100 μmol/L L-arginine show increased NO levels, and at 4 weeks postoperatively they exhibit a reduced neointi-

Figure 3. Influence of pharmacologic intervention in the endothelin system on vein graft NIH. ECE, Endothelin-converting enzyme.

mal formation.⁴⁶ A similar attenuation of NIH can be obtained by perivascular application of spermine/NO as NO donor.⁴⁷ In hypercholesterolemic rabbits, L-arginine (2 g/kg, by mouth) treatment led to a 24% reduction of neointimal thickness.⁴⁸

After precontraction with phenylephrine, human saphenous veins did not relax with aspirin, but they did relax with nitroaspirins and sodium nitroprusside (NO donor). Nitroaspirins and sodium nitroprusside increased the tissue cyclic guanosine monophosphate amount, whereas aspirin did not increase cyclic guanosine monophosphate in fresh human saphenous vein organ cultures.⁴⁹

Endothelin

Endothelin (ET)-1 is a 21 amino acid peptide with vasoconstrictile and VSMC mitogenic properties (Figure 3). ET-1 is produced as inactive precursor big ET-1 (38 amino acids) in vascular endothelial cells, which is activated by cleavage by the metalloproteinase endothelin-converting enzyme. Porter and colleagues⁵⁰ demonstrated that nonselective ET receptor antagonism with bosentan and ET receptor B antagonism with BQ788 reduced NIH in human saphenous vein cultures, whereas the selective ET receptor A antagonist BQ123 failed to reduce neointimal formation.⁵⁰ The same group found in another study⁵¹ that the neutral endopeptidase inhibitor CGS 24592 increases NIH by approximately 95% in cultured saphenous veins. This augmentation of NIH was associated with an increased amount of ET-1 (by blocking its degradation). On the other hand, treatment with CGS 26303, an inhibitor of both neutral endopeptidase and endothelin-converting enzyme, had no effect on neointimal thickness. Wan and colleagues⁵² demonstrated a reduced neointimal thickness in porcine vein grafts by oral treatment with the ET-1A receptor antagonist BSF 302146. Dashwood and colleagues⁵³ found ET-A receptors abundantly present in ungrafted porcine saphenous veins, which was even higher than in grafted veins 4 weeks after implantation. ET-B receptors were found to a lesser extent in the media but more frequently in the adventitial microvasculature. This suggests a susceptibility of the vein for VSMC mito-

genic effects of ET 1, which is increased after mechanical wall stress.

Platelet-Derived Growth Factor

From various growth factors, platelet-derived growth factor (PDGF) (which is released by activated platelets, monocytes, and SMCs) is thought to play a key role in restenosis of injured arteries.⁵⁴ In a pig model, Francis and colleagues⁵⁵ found higher messenger ribonucleic acid levels for PDGF in vein grafts than in ungrafted veins. PDGF and other cytokines (basic fibroblast growth factor, interleukin-1, and tumor necrosis factor- α) are increased during the development of NIH in rat vein grafts.^{56,57} Huang and colleagues⁵⁸ demonstrated a dose-dependent augmentation of NIH in saphenous veins when PDGF was added to the culture medium. Receptors for PDGF, endothelial growth factor, and basic fibroblast growth factor exhibit an intrinsic ligand-stimulated tyrosine kinase activity. Local treatment of rabbit vein grafts with tyrphostin AG51, a tyrosine kinase inhibitor, led to an approximately 50% reduction of neointimal thickness 4 weeks postoperatively. Tyrphostin-treated vein grafts showed no contractile response to norepinephrine, whereas control vein grafts contracted.⁵⁹ Suramin is a growth factor (especially PDGF) receptor antagonist. In a mouse model, local treatment of veins with suramin led to more than a 50% reduction of neointimal thickness 4 and 8 weeks postoperatively compared with controls. In the same study suramin treatment was associated with a decreased amount of PDGF α and PDGF β receptors in neointimal SMCs compared with control vein grafts.⁶⁰

Angiotensin

Angiotensin II is generated from angiotensin I by angiotensin-converting enzyme (ACE) and chymase, which are present in the vascular tissue (Figure 4). Yuda and colleagues⁶¹ demonstrated a 10-fold chymase activity and a doubled ACE activity in grafted veins compared with control veins 4 weeks postoperatively. In animal experiments,⁶² angiotensin II accelerated atherosclerosis and even aneurysmal disease. Systemic treatment of rabbits with the ACE inhibitor captopril (10 mg · kg · d, by mouth) reduced NIH by 40% at 4 weeks postoperatively.⁶³ However, the effects of ACE inhibitors are influenced by 2 important factors: (1) the vascular chymase activity and (2) the tissue penetration of the ACE inhibitors, which vary from low (eg, enalapril) to high (eg, quinapril).^{61,64} Local therapy of veins with the chymase inhibitor Suc-Val-Pro-Phe-(OPh)₂ for 20 minutes has been shown to inhibit neointimal formation and total angiotensin II-forming activity until 3 months after operation in dogs.⁶⁵ In 2 studies the systemic treatment (10 mg · kg · d, by mouth) with the angiotensin II antagonist L-158,809 led to a significant decrease of NIH.^{61,66} Local treatment of rabbit vein grafts with L158809 reduced neo-

Figure 4. Potential pharmacologic attenuation of vascular angiotensin II effects.

intimal thickness by 33%, whereas the medial thickness was not different from controls at 4 weeks postoperatively. There was a contractile response to angiotensin of controls and locally treated vein grafts (at 4 weeks postoperatively), whereas there was no contractile reaction after angiotensin stimulation in systemically L158809 treated vein grafts.⁶⁶

Matrix Metalloproteinases

Matrix metalloproteinases (MMPs) are zinc-dependent enzymes that mediate matrix degradation and deposition. For the development of NIH, SMC hyperplasia and migration are key events that require the degradation of matrix proteins and the elastic laminae.⁶⁷ In 2 days of organ culture segments of porcine veins 1 to 4 weeks after bypass grafting produced significantly more (pro-) MMPs (pro-MMP9, pro-MMP2, active MMP2) compared with ungrafted veins as measured by zymography. Immunohistochemically, MMP2 was found in the neointima 1 to 4 weeks postoperatively, and MMP9 was localized in the neointima and media starting from day 2 (and increased until day 28) after bypass grafting.⁶⁸ The physiologic antagonists of the MMPs are serine proteases and specific tissue inhibitors of metalloproteinases (TIMPs). Treatment with the broad-spectrum MMP inhibitor marimastat led to a reduced NIH in cultured human saphenous veins that was paralleled by a significant reduction in the levels of MMP-2 and MMP-9 in the tissues.⁶⁹ Treatment of cultured veins with doxycycline (10 μ g/mL) also led to a reduction of NIH that was associated with a reduced MMP-9 amount (gelatin zymography). However, there was no difference in the amounts of MMP-2, TIMP-1, and TIMP-2 between doxycycline-treated veins and controls.⁷⁰

Statins inhibit 3-hydroxy-3 methylglutaryl coenzyme A reductase and thereby decrease mevalonic acid. The consequences are not only a cholesterol-lowering effect but also so-called pleiotropic effects (eg, inhibition of SMC migration and proliferation, increase of NO and decrease of

ET-1 in endothelial cells, inhibition of MMPs).⁷¹ Simvastatin attenuated NIH in cultured human saphenous veins, which was reversed with additional application of mevalonate. Furthermore, simvastatin treatment led to a reduction of MMP-9, but not MMP-2, in the vascular tissue.⁷²

Calcium

Calcium is important for a number of cellular events including platelet aggregation, PDGF release, and SMC proliferation. Calcium-channel blockers have been reported to interfere with PDGF-induced VSMC proliferation in vitro. Verapamil, applied in a periadventitial matrix, reduced NIH and balanced the increased sensitivity to serotonin in hypercholesterolemic rabbit vein grafts.⁷³ El-Sanadiki and colleagues⁷⁴ observed a significant reduction of NIH in vein grafts of rabbits treated systemically with verapamil (1.25 mg/d intravenously) at 4 weeks postoperatively compared with controls. The vein grafts of verapamil-treated animals showed an increased contractile sensitivity to norepinephrine and histamine.

G Proteins

G proteins bind the guanine nucleotides GDP and GTP with the G- α subunit that carries the effector site (eg, stimulation or inhibition of adenyl cyclase, activation of phospholipase C).

Davies and colleagues⁷⁵ found a continuous increase of G protein- α subunits 4 weeks after vein graft arterialization. Monoterpenes exert a cytostatic effect by inhibiting post-translational isoprenylation of guanine nucleotide binding proteins. Perillyl alcohol (200 mg · kg · d, by mouth) treatment led to a 22% reduction of neointimal thickness at 4 weeks postoperatively compared with controls.⁷⁶

Mitogen-Activated Protein Kinases

Mitogen-activated protein kinases are ultimate mediators of extracellular stimuli between the cytoplasm and the nucleus of a cell. Saunders and colleagues⁷⁷ found that extracellular signal-regulated kinase-1/2 and p38 mitogen-activated protein kinase (but not c-jun N-terminal kinases) were activated in dog jugular vein grafts during the first postoperative days compared with contralateral ungrafted jugular veins. Yamashita and colleagues⁷⁸ demonstrated the activation of extracellular signal-regulated kinase-1/2, c-jun N-terminal kinase-1, and p38, which were doubled at 4 to 7 days postoperatively compared with ungrafted veins.

Transcription and Translation

Growth factor-mediated cellular stimulation is typically followed by activation of immediate early genes. Suggs and colleagues⁷⁹ found a 5-fold increase of c-fos protein and a 10-fold increase of c-jun protein 15 minutes after vein graft arterialization, which peaked after 2 to 6 hours and de-

creased during the following days. Local treatment of porcine veins with antisense oligonucleotides complementary to the messenger ribonucleic acid of c-myc showed a significant reduction of neointimal thickness 3 months postoperatively.⁸⁰ Suggs and colleagues showed that local treatment of the rat veins with c-fos and c-jun antisense oligodeoxynucleotide (ODN) (in pluronic gel) decreased NIH, which was unfortunately associated with an aneurysmal dilatation of 4 of 20 vein grafts. Both transcription factors nuclear factor-kappa B and E2F were successfully targeted by decoy ODNs to prevent vein graft NIH. In addition, the proliferating cell nuclear antigen amount was reduced in transcription factor decoy ODN-treated vein grafts compared with controls.^{81,82} In E2F decoy ODN-treated vein grafts of hypercholesterolemic rabbits, the atherosclerotic lesions (which were common in controls) were inhibited in treated grafts.⁸²

The antiproliferative properties of rapamycin are partially caused by an inhibition of the serine/threonine protein kinase mammalian target of rapamycin. We demonstrated a dose-dependent reduction of neointimal thickness with perivascularly applied rapamycin in mouse vein grafts compared with controls.⁸³ Another study showed that treatment with rapamycin was associated with an increased apoptotic rate in grafted veins.⁸⁴

Future Perspectives

Pharmacologic inhibition of in-stent restenosis is commonly used in drug-eluting coronary stents.^{85,86} All of these substances used as a stent coating are of potential interest in the prevention of vein graft NIH. In general, all antiproliferative drugs (including immunosuppressive agents) have to be considered for vein graft pharmacotherapy. Systemic treatment with statins, ACE inhibitors, and angiotensin II receptor antagonists could be optimized with regard to the individual pleiotropic effects and tissue permeability of the substances.

References

1. Shah PJ, Gordon I, Fuller J, Seevanayagam S, Rosalion A, Tatoulis J, et al. Factors affecting saphenous vein graft patency: clinical and angiographic study in 1402 symptomatic patients operated on between 1977 and 1999. *J Thorac Cardiovasc Surg.* 2003;126:1972-7.
2. Goldman S, Zadina K, Moritz T, Ovitt T, Sethi G, Copeland JG, et al; VA Cooperative Study Group #207/297/364. Long-term patency of saphenous vein and left internal mammary artery grafts after coronary artery bypass surgery: results from a Department of Veterans Affairs Cooperative Study. *J Am Coll Cardiol.* 2004;44:2149-56.
3. Arima M, Kanoh T, Suzuki T, Kuremoto K, Tanimoto K, Oigawa T, et al. Serial angiographic follow-up beyond 10 years after coronary artery bypass grafting. *Circ J.* 2005;69:896-902.
4. Dion R, Glineur D, Derouck D, Verhelst R, Noirhomme P, El Khoury G, et al. Complementary saphenous grafting: long-term follow-up. *J Thorac Cardiovasc Surg.* 2001;122:296-304.
5. Sabik JF, Lytle BW, Blackstone EH, Houghtaling PL, Cosgrove DM. Comparison of saphenous vein and internal thoracic artery graft patency by coronary system. *Ann Thorac Surg.* 2005;79:544-51.

6. Shah PJ, Bui K, Blackmore S, Gordon I, Hare DL, Fuller J, et al. Has the in situ right internal thoracic artery been overlooked? An angiographic study of the radial artery, internal thoracic arteries and saphenous vein graft patencies in symptomatic patients. *Eur J Cardiothorac Surg.* 2005;27:870-5.
7. Motwani JG, Topol EJ. Aortocoronary saphenous vein graft disease: pathogenesis, predisposition, and prevention. *Circulation.* 1998;97:916-31.
8. Shuhaiber JH, Evans AN, Massad MG, Geha AS. Mechanisms and future directions for prevention of vein graft failure in coronary bypass surgery. *Eur J Cardiothorac Surg.* 2002;22:387-96.
9. Bonatti J, Oberhuber A, Schachner T, Zou Y, Hammerer-Lercher A, Mittermair R, et al. Neointimal hyperplasia in coronary vein grafts: pathophysiology and prevention of a significant clinical problem. *Heart Surg Forum.* 2004;7:72-87.
10. O'Brien JE, Ormont ML, Shi Y, Wang D, Zalewski A, Mannion JD. Early injury to the media after saphenous vein grafting. *Ann Thorac Surg.* 1998;65:1273-8.
11. Rodriguez E, Lambert EH, Magno MG, Mannion JD. Contractile smooth muscle cell apoptosis early after saphenous vein grafting. *Ann Thorac Surg.* 2000;70:1145-53.
12. Zhang L, Freedman NJ, Brian L, Peppel K. Graft-extrinsic cells predominate in vein graft arterIALIZATION. *Arterioscler Thromb Vasc Biol.* 2004;24:470-6.
13. Fogelstrand P, Osterberg K, Mattsson E. Reduced neointima in vein grafts following a blockage of cell recruitment from the vein and the surrounding tissue. *Cardiovasc Res.* 2005;67:326-32.
14. Hu Y, Mayr M, Metzler B, Erdel M, Davison F, Xu Q. Both donor and recipient origins of smooth muscle cells in vein graft atherosclerotic lesions. *Circ Res.* 2002;91:e13-e20.
15. Hu Y, Zhang Z, Torsney E, Afzal AR, Davison F, Metzler B, et al. Abundant progenitor cells in the adventitia contribute to atherosclerosis of vein grafts in ApoE-deficient mice. *J Clin Invest.* 2004;113:1258-65.
16. Xu Q, Zhang Z, Davison F, Hu Y. Circulating progenitor cells regenerate endothelium of vein graft atherosclerosis, which is diminished in ApoE-deficient mice. *Circ Res.* 2003;93:e76-86.
17. O'Brien JE, Shi Y, Fard A, Bauer T, Zalewski A, Mannion JD. Wound healing around and within saphenous vein bypass grafts. *J Thorac Cardiovasc Surg.* 1997;114:38-45.
18. Shi Y, O'Brien JE, Mannion JD, Morrison RC, Chung W, Fard A, et al. Remodeling of autologous saphenous vein grafts. The role of perivascular myofibroblasts. *Circulation.* 1997;95:2684-93.
19. Zou Y, Dietrich H, Hu Y, Metzler B, Wick G, Xu Q. Mouse model of venous bypass graft arteriosclerosis. *Am J Pathol.* 1998;153:1301-10.
20. Cross KS, el-Sanadiki MN, Murray JJ, Mikat EM, McCann RL, Hagen PO. Mast cell infiltration: a possible mechanism for vein graft vasospasm. *Surgery.* 1988;104:171-7.
21. Zou Y, Hu Y, Mayr M, Dietrich H, Wick G, Xu Q. Reduced neointima hyperplasia of vein bypass grafts in intercellular adhesion molecule-1-deficient mice. *Circ Res.* 2000;86:434-40.
22. Crook MF, Newby AC, Southgate KM. Expression of intercellular adhesion molecules in human saphenous veins: effects of inflammatory cytokines and neointima formation in culture. *Atherosclerosis.* 2000;150:33-41.
23. Jiang Z, Wu L, Miller BL, Goldman DR, Fernandez CM, Abouhamze ZS, et al. A novel vein graft model: adaptation to differential flow environments. *Am J Physiol Heart Circ Physiol.* 2004;286:H240-5.
24. Jiang Z, Berceci SA, Pfahnl CL, Wu L, Goldman D, Tao M, et al. Wall shear modulation of cytokines in early vein grafts. *J Vasc Surg.* 2004;40:345-50.
25. Dietrich H, Hu Y, Zou Y, Huemer U, Metzler B, Li C, et al. Rapid development of vein graft atheroma in ApoE-deficient mice. *Am J Pathol.* 2000;157:659-69.
26. Klyachkin ML, Davies MG, Kim JH, Barber L, Dalen H, Svendsen E, et al. Postoperative reduction of high serum cholesterol concentrations and experimental vein bypass grafts: effect on the development of intimal hyperplasia and abnormal vasomotor function. *J Thorac Cardiovasc Surg.* 1994;108:556-66.
27. Davies MG, Fulton GJ, Huynh TTT, Barber L, Svendsen E, Hagen PO. Combination therapy of cholesterol reduction and L-arginine supplementation controls accelerated vein graft atheroma. *Ann Vasc Surg.* 1999;13:484-93.
28. Brauner R, Laks H, Drinkwater DC, Chaudhuri G, Shvarts O, Drake T, et al. Controlled periaortocoronary administration of verapamil inhibits neointimal smooth muscle cell proliferation and ameliorates vasomotor abnormalities in experimental vein bypass grafts. *J Thorac Cardiovasc Surg.* 1997;114:53-63.
29. Sakaguchi T, Asai T, Belov D, Okada M, Pinsky DJ, Schmidt AM, et al. Influence of ischemic injury on vein graft remodeling: role of cyclic adenosine monophosphate second messenger pathway in enhanced vein graft preservation. *J Thorac Cardiovasc Surg.* 2005;129:129-37.
30. Mann MJ, Whittemore AD, Donaldson MC, Belkin M, Conte MS, Polak JF, et al. Ex-vivo gene therapy of human vascular bypass grafts with E2F decoy: the PREVENT single-centre, randomized, controlled trial. *Lancet.* 1999;354:1493-98.
31. Hu Y, Baker AH, Zou Y, Newby AC, Xu Q. Local gene transfer of tissue inhibitor of metalloproteinase-2 influences vein graft remodeling in a mouse model. *Arterioscler Thromb Vasc Biol.* 2001;21:1275.
32. Shi C, Patel A, Zhang D, Wang H, Carmeliet P, Reed GL, et al. Plasminogen is not required for neointima formation in a mouse model of vein graft stenosis. *Circ Res.* 1999;84:883-90.
33. Torsney E, Mayr U, Zou Y, Thompson WD, Hu Y, Xu Q. Thrombosis and neointima formation in vein grafts are inhibited by locally applied aspirin through endothelial protection. *Circ Res.* 2004;94:1466-73.
34. Kim AY, Walinsky PL, Kolodgie FD, Bian C, Sperry JL, Deming CB, et al. Early loss of thrombomodulin expression impairs vein graft thromboresistance implications for vein graft failure. *Circ Res.* 2002;90:205-12.
35. Channon KM, Fulton GJ, Davies MG, Peters KG, Ezekowitz MD, Hagen PO, et al. Modulation of tissue factor protein expression in experimental venous bypass grafts. *Arterioscler Thromb Vasc Biol.* 1997;17:1313-9.
36. Annex BH, Davies MG, Fulton GJ, Huynh TTT, Channon KM, Ezekowitz MD, et al. Local delivery of a tissue factor antibody reduces early leukocyte infiltration but fails to limit intimal hyperplasia in experimental vein grafts. *J Surg Res.* 1998;80:164-70.
37. McCann RL, Hagen PO, Fuchs JC. Aspirin and dipyridamole decrease intimal hyperplasia in experimental vein grafts. *Ann Surg.* 1980;191:238-43.
38. Schachner T, Zou Y, Oberhuber A, Mairinger T, Tzankov A, Laufer G, et al. Perivascular application of C-type natriuretic peptide attenuates neointimal hyperplasia in experimental vein grafts. *Eur J Cardiothorac Surg.* 2004;25:585-90.
39. Cambria RP, Ivarsson BL, Fallon JT, Abbott WM. Heparin fails to suppress intimal proliferation in experimental vein grafts. *Surgery.* 1992;111:424-9.
40. Huynh TTT, Davies MG, Trovato MJ, Barber L, Safi HJ, Hagen PO. Reduction of lipid peroxidation with intraoperative superoxide dismutase treatment decreases intimal hyperplasia in experimental vein grafts. *J Surg Res.* 1999;84:223-32.
41. Davies MG, Dalen H, Barber L, Svendsen E, Hagen PO. Lazaroid therapy (methylaminochroman: U83836E) reduces vein graft intimal hyperplasia. *J Surg Res.* 1996;63:128-36.
42. Jeremy JY, Dashwood MR, Timm M, Izzat MB, Mehta D, Bryan AJ, et al. Nitric oxide synthase and adenylyl and guanylyl cyclase activity in porcine interposition vein grafts. *Ann Thorac Surg.* 1997;63:470-6.
43. Jeremy JY, Izzat MB, Birkett SD, Knight DM, Bryan AJ, Angelini GD. Reduced prostacyclin and increased leukotriene B4 synthesis in porcine venous-arterial grafts. *Ann Thorac Surg.* 1996;61:143-8.
44. Onohara T, Okadome K, Yamamura S, Komori K, Ishii T, Odashiro T, et al. Impaired endothelial prostacyclin production of the canine vein graft in a poor distal runoff limb. *Surgery.* 1993;113:700-8.
45. Onohara T, Okadome K, Ishii T, Yamamura S, Komori K, Sugimachi K. The reversibility of impaired prostacyclin production of the vein graft. *J Surg Res.* 1993;55:344-50.
46. Kown MH, Yamaguchi A, Jahncke CL, Miniati D, Murata S, Grunenfelder J, et al. L-Arginine polymers inhibit the development of vein graft neointimal hyperplasia. *J Thorac Cardiovasc Surg.* 2001;121:971-80.

47. Chaux A, Ruan XM, Fishbein MC, Ouyang Y, Kaul S, Pass JA, et al. Perivascular delivery of a nitric oxide donor inhibits neointimal hyperplasia in vein grafts implanted in the arterial circulation. *J Thorac Cardiovasc Surg.* 1998;115:604-14.
48. Davies MG, Dalen H, Kim J, Barber L, Svendsen E, Hagen PO. Control of accelerated vein graft atheroma with the nitric oxide precursor: L-arginine. *J Surg Res.* 1995;59:35-42.
49. Shukla N, Angelini GD, Ascione R, Talpahewa S, Capoun R, Jeremy JY. Nitric oxide donating aspirins: novel drugs for the treatment of saphenous vein graft failure. *Ann Thorac Surg.* 2003;75:1437-42.
50. Porter KE, Olojugba DH, Masood I, Pemberton M, Bell PR, London NJ. Endothelin-B receptors mediate intimal hyperplasia in an organ culture of human saphenous vein. *J Vasc Surg.* 1998;28:695-701.
51. Porter KE, Dickinson T, London NJM. Inhibition of neointima formation in an organ culture of human saphenous vein: a comparison of dual endothelin-converting enzyme/neutral endopeptidase and selective neutral endopeptidase inhibition. *J Vasc Surg.* 2001;34:548-54.
52. Wan S, Yim APC, Johnson JL, Shukla N, Angelini GD, Smith FCT, et al. The endothelin 1A receptor antagonist BSF 302146 is a potent inhibitor of neointimal and medial thickening in porcine saphenous vein-carotid artery interposition grafts. *J Thorac Cardiovasc Surg.* 2004;127:1317-22.
53. Dashwood MR, Mehta D, Izzat MB, Timm M, Bryan AJ, Angelini GD, et al. Distribution of endothelin-1 (ET) receptors (ETA and ETB) and immunoreactive ET-1 in porcine saphenous vein-carotid artery interposition grafts. *Atherosclerosis.* 1998;137:233-42.
54. Levitzki A. PDGF receptor kinase inhibitors for the treatment of restenosis. *Cardiovasc Res.* 2005;65:581-6.
55. Francis SE, Hunter S, Holt CM, Gadsdon PA, Rogers S, Duff GW, et al. Release of platelet-derived growth factor activity from pig venous arterial grafts. *J Thorac Cardiovasc Surg.* 1994;108:540-8.
56. Sterpetti AV, Cucina A, Lepidi S, Randone B, Corvino V, D'Angelo LS, et al. Formation of myointimal hyperplasia and cytokine production in experimental vein grafts. *Surgery.* 1998;123:461-9.
57. Sterpetti AV, Cucina A, Randone B, Palumbo R, Stipa F, Proietti P, et al. Growth factor production by arterial and vein grafts: relevance to coronary artery bypass grafting. *Surgery.* 1996;120:460-7.
58. Huang B, Dreyer T, Heidt M, Yu JC, Philipp M, Hehrlein FW, et al. Insulin and local growth factor PDGF induce intimal hyperplasia in bypass graft culture models of saphenous vein and internal mammary artery. *Eur J Cardiothorac Surg.* 2002;21:1002-8.
59. Huynh TTT, Davies MG, Barber L, Svendsen E, Hagen PO. Local inhibition of tyrosine kinase activity markedly attenuates the development of intimal hyperplasia in experimental vein grafts. *J Surg Res.* 1998;77:104-11.
60. Hu Y, Zou Y, Dietrich H, Wick G, Xu Q. Inhibition of neointima hyperplasia of mouse vein grafts by locally applied suramin. *Circulation.* 1999;100:861-8.
61. Yuda A, Takai S, Jin D, Sawada Y, Nishimoto M, Matsuyama N, et al. Angiotensin II receptor antagonist, L-158,809, prevents intimal hyperplasia in dog grafted veins. *Life Sci.* 2000;68:41-8.
62. Daugherty A, Cassis L. Angiotensin II-mediated development of vascular diseases. *Trends Cardiovasc Med.* 2004;14:117-20.
63. O'Donohue 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.
64. Tsikouris JP, Suarez JA, Simoni JS, Ziska M, Meyerrose GE. Exploring the effects of ACE inhibitor tissue penetration on vascular inflammation following acute myocardial infarction. *Coron Artery Dis.* 2004;15:211-7.
65. Tsunemi K, Takai S, Nishimoto M, Yuda A, Jin D, Sakaguchi M, et al. Lengthy suppression of vascular proliferation by a chymase inhibitor in dog grafted veins. *J Thorac Cardiovasc Surg.* 2002;124:621-5.
66. Fulton GJ, Davies MG, Barber L, Svendsen E, Hagen PO. Localized versus systemic angiotensin II receptor inhibition of intimal hyperplasia in experimental vein grafts by the specific angiotensin II receptor inhibitor L158,809. *Surgery.* 1998;123:218-27.
67. Aguilera CM, George SJ, Johnson JL, Newby AC. Relationship between type IV collagen degradation, metalloproteinase activity and smooth muscle cell migration and proliferation in cultured human saphenous vein. *Cardiovasc Res.* 2003;58:679-88.
68. Southgate KM, Mehta D, Izzat MB, Newby AC, Angelini GD. Increased secretion of basement membrane-degrading metalloproteinases in pig saphenous vein into carotid artery interposition grafts. *Arterioscler Thromb Vasc Biol.* 1999;19:1640-9.
69. Porter KE, Loftus IM, Peterson M, Bell PR, London NJ, Thompson MM. Marimastat inhibits neointimal thickening in a model of human vein graft stenosis. *Br J Surg.* 1998;85:1373-7.
70. Porter KE, Thompson MM, Loftus IM, McDermott E, Jones L, Crowther M, et al. Production and inhibition of the gelatinolytic matrix metalloproteinases in a human model of vein graft stenosis. *Eur J Vasc Endovasc Surg.* 1999;17:404-12.
71. Werba JP, Tremoli E, Massironi P, Camera M, Cannata A, Alamanni F, et al. Statins in coronary bypass surgery: rationale and clinical use. *Ann Thorac Surg.* 2003;76:2132-40.
72. Porter KE, Naik J, Turner NA, Dickinson T, Thompson MM, London NJM. Simvastatin inhibits human saphenous vein neointima formation via inhibition of smooth muscle cell proliferation and migration. *J Vasc Surg.* 2002;36:150-7.
73. Brauner R, Laks H, Drinkwater DC, Chaudhuri G, Shvarts O, Drake T, et al. Controlled periadventitial administration of verapamil inhibits neointimal smooth muscle cell proliferation and ameliorates vasomotor abnormalities in experimental vein bypass grafts. *J Thorac Cardiovasc Surg.* 1997;114:53-63.
74. El-Sanadiki MN, Cross KS, Murray JJ, Schuman RW, Mikat E, McCann RL, et al. Reduction of intimal hyperplasia and enhanced reactivity of experimental vein bypass grafts with verapamil treatment. *Ann Surg.* 1990;212:87-96.
75. Davies MG, Barber L, Dhanraj DN, Gettys TW, Ramkumar V, Hagen PO. The temporal sequence of G-protein expression in intimal hyperplasia. *J Surg Res.* 1996;63:115-22.
76. Fulton GJ, Barber L, Svendsen E, Hagen PO, Davies MG. Oral monoterpene therapy (perillyl alcohol) reduces vein graft intimal hyperplasia. *J Surg Res.* 1997;69:128-34.
77. Saunders PC, Pintucci G, Bizakis CS, Sharony R, Hyman KM, Saponara F, et al. Vein graft arterialization causes differential activation of mitogen-activated protein kinases. *J Thorac Cardiovasc Surg.* 2004;127:1276.
78. Yamashita A, Hanna AK, Hirata S, Dardik A, Sumpio BA. Antisense basic fibroblast growth factor alters the time course of mitogen-activated protein kinase in arterialized vein graft remodeling. *J Vasc Surg.* 2003;37:866-73.
79. Suggs WD, Olson SC, Madnani D, Patel S, Veith F. Antisense oligonucleotides to c-fos and c-jun inhibit intimal thickening in a rat vein graft model. *Surgery.* 1999;126:443-9.
80. Mannion JD, Ormont ML, Magno MG, O'Brien JE, Shi Y, Zalewski A. Sustained reduction of neointima with c-myc antisense oligonucleotides in saphenous vein grafts. *Ann Thorac Surg.* 1998;66:1948-52.
81. Shintani T, Sawa Y, Takahashi T, Matsumiya G, Matsuura N, Miyamoto Y, et al. Intraoperative transfection of vein grafts with the NFkappaB decoy in a canine aortocoronary bypass model: a strategy to attenuate intimal hyperplasia. *Ann Thorac Surg.* 2002;74:1132-8.
82. Ehsan A, Mann MJ, Dell'Acqua G, Dzau VJ. Long-term stabilization of vein graft wall architecture and prolonged resistance to experimental atherosclerosis after E2F decoy oligonucleotide gene therapy. *J Thorac Cardiovasc Surg.* 2001;121:714-22.
83. Schachner T, Zou Y, Oberhuber A, Tzankov A, Mairinger T, Laufer G, et al. Local application of rapamycin inhibits neointimal hyperplasia in experimental vein grafts. *Ann Thorac Surg.* 2004;77:1580-5.
84. Schachner T, Oberhuber A, Zou Y, Tzankov A, Ott H, Laufer G, et al. Rapamycin treatment is associated with an increased apoptosis rate in experimental vein grafts. *Eur J Cardiothorac Surg.* 2005;27:302-6.
85. Sousa JE, Serruys PW, Costa MA. New frontiers in cardiology: drug-eluting stents: Part I. *Circulation.* 2003;107:2274-9.
86. Sousa JE, Serruys PW, Costa MA. New frontiers in cardiology: drug-eluting stents: Part II. *Circulation.* 2003;107:2383-9.