Pharmacologic inhibition of vein graft neointimal hyperplasia

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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.
cells and that 60% were graft-intrinsic cells at 8 weeks postoperatively. In another study, the potential role of adventitial progenitor cells for vein graft NIH was demonstrated. In another mouse model, 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. In addition, Shi and colleagues 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. Cross and colleagues 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.

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 colleagues observed a decreased leucocyte amount and a 40% reduced neointimal thickness in ICAM-1 knockout mice compared with wild-type animals. Crook and colleagues 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-1α, and interferon-γ.

In low-flow vein grafts (poor distal runoff), the neointimal thickness was markedly increased and the messenger ribonucleic acid expression of the proinflammatory interleukin-1β was greater than in high-flow vein grafts. The increased vein graft patency in good runoff areas is also documented clinically. Goldman and colleagues 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.

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,
and calcified necrotic cores and areas of necrosis in the vascular wall have been found in vein grafts of apolipoprotein E-deficient (hypercholesterolemic) mice. 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. Grafts of hypercholesterolemic rabbits exhibited a significantly increased (contractile) sensitivity to serotonin and decreased (vasodilatory) sensitivity to sodium nitroprusside compared with vein grafts of normocholesterinemic rabbits.

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 colleagues 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.

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). 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 with local therapy, and (3) no vascular injury such as pressurizing the vein for a viral transfection approach is necessary. Hu and colleagues 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). 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 colleagues demonstrated a decrease of thrombomodulin 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·db y mouth) treatment led to a 24% reduction of neointimal thickness.41

**Nitric Oxide and Prostacyclin**

Nitric oxide (NO) and prostacyclin (PGI2) exhibit antithrombotic, vasodilatory, and anti proliferative (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 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 colleagues44,45 demonstrated a reduced 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 neointima-
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. In a pig model, Francis and colleagues 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. Huang and colleagues 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. 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 PDGFα and PDGFβ receptors in neointimal SMCs compared with control vein grafts.

**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 colleagues demonstrated a 10-fold chymase activity and a doubled ACE activity in grafted veins compared with control veins 4 weeks postoperatively. In animal experiments, 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. 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). 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. 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. Local treatment of rabbit vein grafts with L158809 reduced 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.

**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. 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) compared with ungrafted veins. 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. 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. Treatment of cultured veins with doxycycline (10 μg/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.

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). 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. 

**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. El-Sanadiki and colleagues 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 colleagues found a continuous increase of G protein-α subunits 4 weeks after vein graft arterialization. Monoterpenes exert a cytostatic effect by inhibiting posttranslational isoprenylation of guanine nucleotide binding proteins. Perillyl alcohol (200 mg·kg⁻¹·d) by mouth treatment led to a 22% reduction of neointimal thickness at 4 weeks postoperatively compared with controls. The vein grafts of verapamil-treated animals showed an increased contractile sensitivity to norepinephrine and histamine.

**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 colleagues 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 colleagues 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 colleagues 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 decreased 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. 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. In E2F decoy ODN-treated vein grafts of hypercholesterolemic rabbits, the atherosclerotic lesions (which were common in controls) were inhibited in treated grafts.

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. Another study showed that treatment with rapamycin was associated with an increased apoptotic rate in grafted veins.

**Future Perspectives**
Pharmacologic inhibition of in-stent restenosis is commonly used in drug-eluting coronary stents. 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**


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