Intimal hyperplasia, the obstacle in bypass grafts

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CHAPTER 2

An Englishman in New York; Pathobiology of intimal hyperplasia. A review.

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ABSTRACT

Improvements in surgical technique and development of vascular grafts have permitted the reconstruction of obstructed small diameter arteries. Although most bypass procedures result in open reconstructions directly after the implantation procedure, they may later fail. An important reason of late graft failure is the development of intimal hyperplasia, particularly in vein and synthetic vascular grafts. On the contrary, the autologous internal mammary artery is very successful as bypass graft. However, due to the anatomic position and the short length of the internal mammary artery the use of this graft material is limited. Due to this limited availability of autologous arterial grafts it is unlikely that arterial grafts will expel the vein graft and synthetic graft as bypass graft conduits. It is therefore a challenge to improve the performance of vein grafts and synthetic grafts making it as successful as autologous arterial grafts. Control of the formation of intimal hyperplasia in vein graft and synthetic grafts is hereby crucial.

INTRODUCTION

Intimal hyperplasia in a historical perspective

Intimal hyperplasia was first described in 1906. Carrel and Guthrie observed macroscopically that within a few days after vein graft implantation into the arterial circulation the anastomotic stitches became covered with a glistening substance similar in appearance to the normal endothelium.[1] Since that time intimal hyperplasia has been reported in vein grafts implanted into the arterial circulation in many mammals including mice, rats, dogs, sheep, monkeys, and humans.[2]

Intimal hyperplasia is recognized as intrinsic obstructive lesion after many other forms of arterial vascular wall injury as well. For instance in coronary and superficial femoral artery angioplasty, endarterectomy, arteriovenous fistulae for haemodialyis and organ transplantation.[3] Intimal hyperplasia occurs physiologically in closure of the ductus arteriosus after birth.[4]

In 1949 the autologous vein was the first conduit to be used to bypass an obstructed artery in man.[5] About 30-50% of the patients with vein grafts have recurrent ischemic complaints caused by vein graft occlusion after 5 to 10 years. An important cause of vein graft occlusion is the formation of intimal hyperplasia in the vein graft.[6] Other types of bypass grafts were introduced in the hope to improve the bypass graft patency and to lower the incidence of recurrent ischemic complaints. From its introduction in 1968 the autologous internal mammary artery is a successful alternative bypass graft instead of vein grafts.[7] Patients with internal mammary artery bypass grafts have hardly recurrent ischemic complaints. Due to its anatomic location and short length internal mammary arteries are only used for coronary bypass grafting.

A much successful alternative bypass graft is the synthetic graft. In 1954 the synthetic graft was introduced to bridge arterial defects in man.[76] The synthetic graft has the advantage that it is not limited in length and is always available. The disadvantage of synthetic bypass graft with a diameter lesser than 6 mm is the high incidence of occlusion due to the rapid formation of intimal hyperplasia.

The challenge for the future is to improve the patency of autologous vein grafts by controlling the formation of intimal hyperplasia. To improve the performance of vein grafts the pathobiology of intimal hyperplasia in these grafts must be studied in detail.

Several aspects of intimal hyperplasia in vein grafts are discussed in this review including cellular kinetics, molecular mechanisms, pathophysiological triggers, and strategies to control intimal hyperplasia.

Intimal hyperplasia in the autologous vein graft

Cell biology of the vessel wall.

The histology of a vein reflects its capacity to transport large volumes of blood at a low pressure. The wall of a vein consist of three layers: intima, media, and adventitia. The intima is composed of a monolayer of endothelial cells lying on a fenestrated basement membrane. The basement membrane is a specialized extracellular matrix and consists of type IV collagen, laminin, and heparan sulphate proteoglycans, such as perlecan and syndecans.[8,9]. In the media, the smooth muscle cells are arranged in an inner longitudinal and an outer circumferential pattern with collagen and fine elastic fibrils interlaced. The elastic fibrils appear to be orientated predominantly in a longitudinal direction and do not form an internal elastic lamina. The adventitia forms the outer layer and contains fibroblasts, loose extracellular matrix, and small unmyelinated nerves.[10] The interaction between extracellular matrix and smooth muscle cells maintain the smooth muscle cell in a quiescent, contractile status. Participants in this type of growth arrest are heparin, heparin-like molecules and transforming growth factor β. These molecules are synthesized by endothelial cells. Heparin inhibits smooth muscle cell proliferation, reduces smooth muscle cell migration, and alters the production of extracellular matrix by smooth muscle cells.[11,12] It has been suggested that heparin exerts its effect by displacing growth factors from the extracellular matrix. Heparin may bind fibroblast growth factor and neutralize its smooth muscle cell mitogenic activity.[13] Heparin may also inhibit the production of tissue plasminogen activator and collagenases at the level of transcription.[14] Transforming growth factor β can inhibit smooth muscle cell proliferation and migration. Furthermore, it stabilizes the extracellular matrix by increasing the formation of plasminogen activator inhibitor, and decreasing the formation of protease inhibitors.[15]

The endothelial cells maintain a delicate balance in veins and arteries between smooth muscle cell growth promotion and inhibition, blood cell adhesion to the endothelium, and anticoagulation or procoagulation. In these ways the endothelium controls the vascular structure and regulates the vasomotor tone. To exercise these functions the endothelial cells synthesize crucial products like prostacyclin and nitric oxide.

Prostacyclin and nitric oxide are able to relax smooth muscle cells resulting in vasodilatation, inhibit platelet adhesion and aggregation, endothelium-leucocyte interaction and inhibit smooth muscle cell proliferation.[16]

Autonomic nerves stimulate the proliferation of arterial smooth muscle cells.[77] The influence of the autonomic nerves on the

development of intimal hyperplasia in vein grafts is not clear. Two studies reported that experimental vein grafts became hyperinnervated after implantation into the arterial circulation.[78,79] Unfortunately, These studies failed to correlate the degree of innervation with the development of intimal hyperplasia in the vein grafts.

It is important to realize that saphenous veins demonstrate a spectrum of pre-existing pathological conditions ranging from significantly thickened walls to post phlebitic changes and varicosities at the time of harvest.[10] Up to 12% of the saphenous veins can be considered as "diseased''. These diseased veins have a patency rate half of that of "non-diseased,, veins. The etiology of the venous diseases observed are multifactorial in origin and at the present time without gross morphological evidence of disease there is no clear prognostic indicator to discriminate those veins which should be rejected as grafts.[17]

Cellular kinetics of vein graft intimal hyperplasia

First phase: smooth muscle cell proliferation in the media.

Implantation of a vein graft into the arterial circulation leads to endothelial denudation, particularly at the perianastomotic sides, which is immediately followed by deposition of platelets and leucocytes. Besides damage to the endothelium, the implantation procedure, high arterial pressure and flow cause damage to the medial layer of the vein graft. Approximately 6 hours after implantation polymorphonuclear leucocytes infiltrate the vein graft media. The medial layer of the vein graft is to a certain extent damaged resulting in death of smooth muscle cells. Both dead and injured endothelium and medial smooth muscle cells are able to release growth factors. One of these released growth factors is basic fibroblast growth factor (bFGF) which stimulates the proliferation of endothelial cells and smooth muscle cells.[2,6,18]

A part of the viable remaining smooth muscle cells in the media will change from contractile to synthetic phenotype. Before implantation into the arterial circulation, the vascular smooth muscle cells are well differentiated, characterized by an abundance of contractile proteins, predominantly smooth muscle actin and myosin, but little rough endoplasmic reticulum. After implantation, smooth muscle cells lose their differentiated state and they acquire abundant endoplasmic reticulum and start synthesis of extracellular matrix. Both clinical and experimental studies demonstrated that within 24 hours after implantation smooth muscle cells in the media of the vein graft start to proliferate, reaching a peak at 2 days, and finally return to normal at 14 days.[2,19,20] Smooth muscle cell proliferation in the media, which is normally less than 1%

increases to 17% within 48 hours. The fraction of smooth muscle cells proliferating does not change after 3 days. Besides proliferation, the synthetic smooth muscle cells produce extracellular matrix resulting in medial thickening.[2,6]

Second phase: smooth muscle cell migration from the media to the intima.

Injury to the vessel wall induces the production of tissue type plasminogen activator and urokinase-type plasminogen activator which degrade the extracellular matrix and activate matrix metalloproteases.[21] Some of these metalloproteases have the capacity to degrade the extracellular matrix allowing smooth muscle cells to migrate from the media to the neo intima.[22]
The integrity of the vein graft wall is further affected by the release of lysosomal enzymes and oxygen radical production from the deposited leucocytes.[6,23] Both lysosomal enzymes and radicals have the ability to degenerate the extracellular matrix. Growth factors promote also the migration of smooth muscle cells from the media to the intima.[23] Smooth muscle cells start one week after implantation to migrate from the media to the intima and continue to migrate to the intima up to three weeks.[2] About 30% of the medial smooth muscle cells may migrate from the media to the intima.[23] Also smooth muscle cells from the adventitia have been shown to proliferate and migrate to the intima as well.[25,26]

Third phase: intimal thickening

Strictly, intimal hyperplasia signifies an increase in the number of cells in the intima. It is, however, also accompanied by an increase in the amount of extracellular matrix, and these processes together result in the generally known intimal thickening.[24] About 50% of the migrated smooth muscle cells in the intima continue to proliferate up to 4 weeks after implantation.[2,23] After 4 weeks the smooth muscle cell proliferation returns to near quiescent levels, and the continued increase in intimal thickening is related to the accumulation of extracellular matrix.[2] The proportional contribution of smooth muscle cell and extracellular matrix volume to the overall intimal volume remains constant after 12 weeks implantation, and is 20% and 80% respectively.

It is hypothesized that intimal hyperplasia serves as a substrate for the development of vein graft atherosclerosis. This accelerated form of intimal hyperplasia increases the chance of graft occlusion.[23] Intimal hyperplasia in vein grafts in the arterial circulation *increases* significantly in conditions of hyperlipidemia.[23, 27] Smoking, another important risk factor for the development of atherosclerosis, is also an evident risk factor for the development of intimal hyperplasia. Cigarette smoking increases the development of intimal hyperplasia after vascular injury.[28, 29]

Intimal hyperplasia in vein grafts *de-*

creases when the vein graft is taken out the arterial circulation and implanted back into the venous circulation.[30,31,32] This reversal of intimal hyperplasia occurs only when a vein graft does not stay longer than 2 weeks in the arterial circulation. This indicates that successful strategies to reduce intimal hyperplasia must at least begin in the first 2 weeks after operation. One of the first steps to reduce intimal hyperplasia in man are likely to be correction of hyperlipidemia and to stop smoking.

Molecular pathways in intimal hyperplasia in vein grafts

Pathways of proliferation

Smooth muscle cells are quiescent stable cells which can be induced to re-enter the cell cycle by appropriate stimuli.[6,24] The pathways for proliferation can be divided temporally into segments of the cell cycle, which comprises Gap (G)1, DNA (S), G2 and mitosis (M). When smooth muscle cells are stimulated by growth factors then leave the Go phase of the cell cycle and traverse a series of the G1 phase before reaching the restriction point. After the restriction point, smooth muscle cells will continue to DNA synthesis, G2 and mitosis phases without the continued presence of growth factors.[33]

Cell growth is initiated by the binding of a signaling agent, most commonly a growth factor. Growth factors are besides important regulators of proliferation, regulators of smooth muscle cell and endothelial cell migration. The most potent growth factors for smooth muscle cells are peptides that bind to receptors with intrinsic tyrosine kinase activity.[34] Receptors with intrinsic tyrosine kinase activity couple indirectly to a variety of signalling pathways, which include the Ras, Raf, MAP kinase cascade, the phosphoinositol 3-kinase, protein kinase B pathway and the diacylglycerol, protein C pathway.[35]

Two of the best analyzed growth factors are platelet derived growth factor and basic fibroblast growth factor. Platelet derived growth factor was originally identified from human platelets.[36] Also smooth muscle cells and endothelial cells are capable to release platelet derived growth factor as well. Platelet derived growth factor is a 27 to 31 kD glycoprotein and is composed of 2 polypeptide chains (A and B) and acts on two distinct receptors: alpha and beta.[37] The development of intimal hyperplasia in balloon injured rat carotid arteries was reduced by 40% after administration of an antibody to platelet derived growth factor.[38]

Fibroblast growth factors are a family of heparin binding growth factors. The best characterized are basic fibroblast growth factor and acidic fibroblast growth factor. Basic fibroblast growth factor is 30 to 100 fold more powerful than the acidic type.[39] Both the basic and acidic type

share the same receptors. The proliferation of medial smooth muscle cells after balloon injury of rat carotid arteries was reduced by 80% after administration of an antibody to basic fibroblast growth factor.[40] These findings suggest that endogenous fibroblast growth factor is an important growth factor for proliferation of medial smooth muscle cells and that platelet derived growth factor is important for the migration of smooth muscle cells from the media to the intima.

In an experimental study it was demonstrated that vein grafts implanted into the arterial circulation produce a large quantity of platelet derived growth factor and basic fibroblast growth factor. When vein grafts were taken out from the arterial circulation and re-implanted into the venous circulation the production of these growth factors ceased.[32]

Early response genes, which include nuclear transcription factors are among the immediate down stream targets of the initial transduction pathways. The nuclear transcription factors stimulate new protein synthesis that causes progression to the next stage of the cell cycle.[24] Certain cyclins are directly induced in response to the early transduction pathways. For example, cyclin D1 is induced by MAP kinase activation.[41]

A family of cyclin-dependent protein kinases and their activator subunits, the cyclins, are of particular importance. Cyclin-dependent protein kinases and cyclins act in a cascade to multiply phosphorylate. Due to hyperphosphorylation the transcription factor E2F is disrupted from retinoblastoma protein. The transcription factor E2F causes induction of DNA polymerase and initiates the S phase.[33]

Pathophysiological triggers for intimal hyperplasia

The triggers for the formation of intimal hyperplasia that have been defined are injury, circulating blood components, and haemodynamics.[3,6,10,18,23,24]

Injury

The assumption that growth factor action alone can stimulate intimal hyperplasia is a simplification. Infusion of basic fibroblast growth factor does not stimulate intimal hyperplasia in intact arteries.[42] Platelet derived growth factor appears only to stimulate intimal hyperplasia after injury of carotid arteries.[43] The degree of intimal hyperplasia that develops after injury of a blood vessel depends on the depth of this injury. When the media is not injured minimal intimal hyperplasia occurs. When the media is injured intimal hyperplasia increases in proportion to the depth of injury.[6,23] These observations suggest that injury does more than simply cause a release of growth factors.

The vein graft undergoes different types of injury when it is implanted into the ar-

terial circulation. Injury to the endothelium occurs at different time points. At the time of harvest injury is due to inevitable surgical trauma, due to ischemia during ex vivo preservation, storage conditions, and distention prior to anastomosis.[18,44] Injury to the endothelium after implantation into the arterial circulation is furthermore caused by exposure to the arterial pressure and flow.

The viability of endothelium partly depends on the composition of the storage solution, the pH of the storage solution, and the time of storage.[45] When a vein is harvested and stored in heparinized crystalloid solutions or in heparinized blood a rapid denudation of the endothelial cells is observed together with a decrease of the ability to produce nitric oxide.[46] After 4 hours of storage, especially in hypothermic storage solutions veins loose the ability to produce nitric oxide. Vein grafts stored in heparinized solutions containing papaverine and maintained at physiological pH levels 7.3-7.4 protect the endothelium better than vein grafts stored in for example warmed saline solutions.[47]

A new physiologic storage solution GALA(Hank's balanced salt solution modified with gluthathione, ascorbic acid and L-arginine) demonstrated that endothelial and smooth muscle cells remained viable even 24 hours after storage.[46] Clinical trials will be needed to verify if this new GALA solution is optimal protection for vein grafts.

Prior to anastomosis vein grafts are usually distended to check for leakage. Distention of the vein above physiological pressure leads to denudation and damage to the media. Due to endothelial dysfunction nitric oxide and prostacyclin are not longer produced at the time the vein is implanted into the arterial circulation.[18] The loss of endothelium and the loss of above mentioned endothelial products lead to adhesion and activation of platelets and leucocytes and activation of the coagulation system.

Platelets

Endothelial denudation exposes the subendothelial matrix and leads to platelet adhesion and aggregation. The subendothelium is completely covered by platelets immediately after denudation. Platelet *adhesion* requires the interaction platelet receptor Gp1b, plasma von Willebrand factor, and fibronectin. Platelet *aggregation* requires tissue factor, fibronectin, von Willebrand factor, vibronectin, and platelet receptor GpIIb-IIIa. The adhered platelets release adenosine diphosphate and activate the arachidonic acid synthesis pathway to produce thromboxane A2.[6] Thromboxane A2 is a potent chemo attractant and smooth muscle cell mitogen and leads to further platelet recruitment.[6] Once activated, platelets release platelet derived growth factor and constituents of their granules. Monoclonal antibodies to platelets were used in an experimental study to induce

thrombocytopenia. This study reported that thrombocytopenia reduced intimal hyperplasia but was not abolished. It appeared that the smooth muscle cells in these thrombocytopenic laboratory animals have a normal proliferative response but fail to migrate from the media to the intima.[48]

After denudation also thrombin can bind specifically to the subendothelial matrix, where it remains active and protected from its circulating inhibitor antithrombin III. Thrombin is important in the coagulation cascades. Furthermore, thrombin is a potent mitogen for smooth muscle cells. Thrombin upregulates platelet derived growth factor, platelet derived growth factor receptor, and urokinase receptor expression. This expression comes throughout the intima of the injured bloods vessel within 2 weeks after the initial injury.[6]

Leucocytes

Leucocytes can contribute in different ways in the development of intimal hyperplasia.

After endothelial injury leucocytes adhere to the injured endothelium and denuded areas of the vein graft. Leucocytes will release a number of inflammatory products including chemotactic factors, growth factors, and complement components.

Another mechanism involves the production of lysosomal degradation enzymes. Activated leucocytes elaborate several potent proteases capable of degrading collagen and other structural extracellular matrix and other extracellular components for example the basement membranes. Heparanases can remove heparan sulphate proteoglycans from the cell surface and diminish their inhibition on cell proliferation.[23]

Moreover, leucocytes may also act at sites of endothelial injury through the production of oxygen free radicals. Polymorphonuclear neutrophils can produce oxygen free radicals capable to injure remaining viable endothelium leading to an ongoing stimulation of inflammatory injury.[6,23]

Trauma caused by the surgical procedure and the exposure of the arterial environment will lead to disturbance of the architecture of the vein resulting in loss of its defensive mechanisms. Due to the loss of the defensive mechanisms, adhering platelets and leucocytes are able to release growth factors and proteases capable of damaging the unprotected medial smooth muscle cells and loosing the extracellular matrix. The sum of trauma, release of proteases and growth factors results in smooth muscle cell proliferation.

After two weeks of implantation into the arterial circulation the endothelial layer of the vein graft is restored. However, the endothelium of the vein graft is no longer capable to produce nitric oxide nor prostacyclin.

Haemodynamics

Haemodynamic alterations which take place in a vein when it is taken out from the low pressure and low flow venous environment and placed in a high pressure and high flow arterial environment are implicated in the formation of intimal hyperplasia.

When a vein is implanted into the arterial circulation it immediately suffers from the high pressure and flow leading to direct damage of the vein graft leading to some degree of endothelial denudation. The surviving endothelial layer is elevated by intimal edema. Furthermore, edema to the medial layer and injury to the medial smooth muscle cells are reported.[18]

In an experimental animal study the association between altered local haemodynamics and intimal hyperplasia was carefully evaluated. Nine mechanical deformations and stresses to which vein grafts are subjected after implantation into the arterial circulation were systematically evaluated. Medial thickening in vein grafts was best associated with increased circumferential deformation. The formation of intimal hyperplasia was highest in the presence of *low flow*.[49]

This finding was confirmed in two other experimental studies evaluating the formation of intimal hyperplasia under different flow conditions. Vein grafts developed significantly more intimal hyperplasia under low flow conditions in arteries with poor distal runoff. Intimal hyperplasia in these vein grafts decreased when the vein grafts were re-implanted into arteries with normal flow parameters.[50,51] Flow velocity is directly related to blood-vessel wall shear stress. Shear stress is an important factor determining the probability and duration of adhesion of blood borne elements like platelets and leucocytes onto the vessel wall. Low blood flow leads to low shear stress. Low shear stress augments proliferation of smooth muscle cells, possibly mediated by the release of growth factors from adhered platelets and/ or leucocytes. Low shear stress in vein grafts also significantly increases platelet derived growth factor and basic fibroblast growth factor mRNA levels in endothelial cells.[52] The flow velocity in the internal mammary graft is about three times higher than in vein grafts. This higher flow velocity in the internal mammary graft is possibly one of the reasons for the low intimal hyperplasia observed in this arterial graft.[53]

From these observations it is evident that a vein graft in the arterial circulation does not develop the characteristics of an artery. Implantation of a vein graft into the arterial circulation leads to morphological and functional abnormalities in such a manner that a vein in the arterial circulation behaves like “an Englishman in New York„.[80]

Strategies to control the formation of intimal hyperplasia

Control of the development of intimal hyperplasia is considered to be the key to maintain vein graft patency. So far, no clinical study has reported control of the formation of intimal hyperplasia in vein grafts in man.[3]

Strategies designed to control intimal hyperplasia in vein are shown in table I.

Pre operative strategies

Intimal hyperplasia in vein grafts in the arterial circulation *increases* significantly in conditions of smoking and hyperlipidemia.[23, 27, 29] Smoking decreases the patency rate after femoro popliteal bypass grafting in man.[28] Important pre operative steps to control intimal hyperplasia are to stop smoking and to correct serum hyperlipidemia. The number of patients who stop smoking is low, ranging from 5 to 26 %.[54] The Dutch consensus working group for treatment of peripheral arterial disease considers the urgent advise to stop smoking as a key stone in the treatment of peripheral arterial disease.[54]

Per operative strategies

The formation of intimal hyperplasia is only reversible in the first 2 weeks after implantation into the arterial circulation.[30,31,32] Strategies to reduce intimal hyperplasia must at least begin in the first 2 weeks after operation. The degree of intimal hyperplasia depends on the degree of injury of the vein graft. Minimizing the injury to the vein graft appears to be an effective per operative step in the control of intimal hyperplasia.

The degree of injury can be minimized by per operative protecting the integrity of the vein graft especially by protecting the endothelium. A better preserved vein graft will attract a smaller number of platelets and leucocytes, important contributors to intimal hyperplasia.
The integrity of the vein graft seems to benefit from per operative storage media containing Hank's balanced salt sodium solution modified with glutathione, ascorbid acid and L-arginine or desferrioxamine manganese.[44,66] The architecture of the vein is also preserved by preventing overstretching of the vein before implantation into the arterial circulation. Furthermore, the "no touch,, technique of the vein should be used during harvesting avoiding grasping the vein graft by means of a forceps.[18]

Some degree of trauma to the vein graft is inevitable. Trauma and influence of the arterial environment initiate smooth muscle cell proliferation in the vein graft. Many pharmacological strategies were designed to reduce smooth muscle cell proliferation, the central cellular event in the formation of intimal hyperplasia.

Acetyl salicylic acid was the first tested

Table I.

Strategy Application	Agent
Anti platelet aggregation systemic	acetyl salicylic acid/dipyridamole[55, 56]
Heparin systemic	heparin[57-59]
Mechanical local external support	PTFE/ p-urethane[60, 61]
Diet, unsaturated fatty acids systemic	eicosapentaenoic acid[62]
Calcium channel blocker systemic	verapamil[63]
ACE inhibitor systemic	captopril[64]
Immunosuppression systemic	cyclosporine[65]
Antioxidant local	desferrio manganese[66]
Receptor antagonists systemic	ketanserin, L158,809[67,68]
Endothelial cell modulation systemic	L-arginine[69]
Growth factor antagonist local	polycyclodextrin- sulfate[70]
Cytotoxic local	radiation[75]
Gene therapy local	
Antisense oligonucleotides	anti-proliferating cell nuclear antigen, anti-c-fos/ c-jun, anti-c-myc[83, 71,75,84]
Gene transfer	endothelial cell/ inducible nitric oxide synthase[72,74]

drug to control the formation of intimal hyperplasia in experimental vein grafts. Immediately after implantation of the vein graft platelets adhere to the exposed subendothelial matrix. Simultaneous with platelet adherence growth factors are released from the platelets alpha granules. In 1974, a platelet dependent serum factor was discovered that stimulated smooth muscle cell proliferation in vitro.[81] The discovery of the later called platelet derived growth factor triggered the research on the effect of antiplatelet therapies on the formation of intimal hyperplasia in the eighties. An experimental study confirmed a role for platelets in the formation of intimal hyperplasia by inducing thrombocytopenia.[48] In these thrombocytopenic animals no migration of smooth muscle cells occurred after injury of an artery. Because platelets are crucial in achieving hemostasis, thrombocytopenia and blockade of the platelet adherence are no therapeutic options.

The most common platelet aggregation inhibitor is acetyl salicylic acid. Acetyl salicylic acid and other non steroid anti inflammatory drugs irreversibly acetylate a serine residue in the active side of cyclo oxygenase, which prevents the formation of thromboxane. Acetyl salicylic acid has no capacity to block the release of platelet derived growth factor nor the capacity to block the first wave of ADP induced platelet aggregation.[82] So the effect of acetyl salicylic acid is partial. In experimental models of vein grafting, conflicting results are presented on the reduction of intimal hyperplasia using platelet aggregation inhibitors acetyl salicylic acid and dipyridamole. [55,56]

In 1977, Clowes et al discovered that systemic delivery of heparin suppresses the formation of intimal hyperplasia after injury of carotid arteries in rats.[11] Later studies revealed that heparin inhibits proliferation and migration of smooth muscle cells probably by interfering with growth factors and independently from its anticoagulant properties.[6,12] Systemic administration of heparin has yielded conflicting results with respect to its effect on intimal hyperplasia in experimental vein grafts.[57-59]

Various types of anti hypertension drugs including calcium channel blockers and angiotensin converting enzyme inhibitors have been proven to be effective to control the formation of intimal hyperplasia in models of arterial injury. These agents were later successful to inhibit intimal hyperplasia in vein graft models.[63,64,67,68] However, all of these pharmacological agents require chronic therapy making these agents less attractive strategies.

In the late eighties a shift from systemic therapies towards local therapies occurred. To minimize systemic effects, therapeutic agents were applied locally during the operation.

In 1995 the first genetic therapy to con-

trol intimal hyperplasia in experimental vein grafts was reported.[83] Gene therapy is one of the latest strategies to reduce intimal hyperplasia in experimental vein grafts. Gene therapy includes methods to block the cell cycle and methods of gene transfer into vein graft wall.

Implantation of a vein graft into the arterial circulation leads to an immediate increase of growth factors in the vein graft wall. The growth factors interact with their receptors on the medial smooth muscle cells. The growth factor – receptor interaction leads eventually to smooth muscle cell proliferation. Because of the instant increase of growth factors, intervention at a post receptor level where growth factors convey is likely to be effective. For example, proliferation may be interrupted in cells that have already entered the cell cycle. Proliferation can be interrupted using antisense oligonucleotides against different phases in the G1 of the cell cycle. Antisense oligonucleotides against the immediate early gene c-fos and c-jun and the late G1 cell cycle dependent genes as c-myc and proliferating cell nuclear antigen successfully reduced intimal hyperplasia in experimental vein grafts.[83,71,73,84]

Nitric oxide is a potent vasodilator and inhibits smooth muscle cell proliferation, leucocyte-endothelial interactions, platelet adhesion and platelet aggregation. After implantation of a vein into the arterial circulation the vein graft looses its capability to produce nitric oxide. Implantation of a vein into the arterial circulation leads thus to a disbalance in the organization of the vessel wall. The oral administration of L-arginine, a precursor of nitric oxide formation, reverses defects in nitric oxide activity and reduces the development of intimal hyperplasia in experimental vein grafts.[69] Systemic administration of drugs may, however, give rise to unwanted side effects.

The next logical step is to substitute the loss of nitric oxide where it is actually needed in the vein graft it self. Two experimental studies reported successful transfection of respectively endothelial cell nitric oxide synthase and inducible nitric oxide synthase in the vein graft wall resulting in reduction of intimal hyperplasia.[72,74]

Post operative strategies

Ongoing atherosclerosis of the native vasculature and in the vein graft itself will undermine the blood flow to the organs. No smoking advice and control of the serum lipids is the policy after the operation.

CONCLUSION

Intimal hyperplasia is still the obstacle in arterialized vein grafts. The broad spectrum of therapeutical agents capable to inhibit intimal hyperplasia emphasize that intimal hyperplasia has a multifactorial

etiology and a complex pathobiology.

A combination therapy including pre, per, and post operative measurements is likely to be the best strategy to control intimal hyperplasia and to maintain a patent vein graft. Examples of pre and post operative measurements are stop smoking and control of hyperlipidemia. Per operative measurements to minimize the degree of implantation injury are a second step to control intimal hyperplasia. Local strategies to inhibit smooth muscle cell proliferation or to substitute loss of native line of defensive in the vein graft is a next logical step to control intimal hyperplasia.

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