

Biology of arteriovenous fistula failure

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ABSTRACT

Although arteriovenous (AV) fistulae are the preferred form of dialysis access, they continue to have significant problems with both early and late failures. Despite the magnitude of the clinical problem, however, there are unfortunately no effective therapies for AV fistula failure. We believe that this inability to intervene is partly due to a lack of understanding about the pathology and pathogenesis of AV fistula failure. Therefore, in the current review we will initially explore novel concepts about the pathology and pathogenesis of AV fistula failure. This information will then be used to suggest potential therapeutic interventions for this important, yet unmet clinical need. Finally, we will end with a brief description of some state-of-the-art clinical trials that are attempting to apply some of these novel therapeutic concepts to the recalcitrant clinical problem of AV fistula failure.

Key words: *Arteriovenous fistula, Primary failure, Vascular access outcomes, Risk factors*

THE CLINICAL PROBLEM

Hemodialysis vascular access dysfunction is currently a huge clinical problem, which results in very significant clinical morbidity and economic burden within the end-stage renal disease (ESRD) population (1-5). Although the arteriovenous (AV) fistula is the preferred form of permanent dialysis access, it has significant problems with both early and late failures (6-11). At a clinical level, early failure has been defined as an AV fistula that never develops adequately for dialysis (failure to mature), or which fails within 3 months of starting dialysis (12). The recently published Disease Outcome Quality Initiative (DOQI) guidelines have also attempted to provide some anatomical and functional parameters for an "adequate" AV fistula (13). This has been defined as an AV fistula that has a flow of greater than 600 ml/min, a diameter of greater than 0.6 cm and which is approximately 0.6 cm from the skin surface (13). All other clinical settings of AV fistula failure are categorized as late failures. It is currently estimated that between 23%-46% of all AV fistulae (both in Europe and the United States) have problems with early failure or failure to mature, resulting in a 1 year primary patency of 60%-65% (7-10, 14). In addition, some authors believe that the aggressive institution of a "fistula first" policy has resulted in an increased incidence of early AV fistula failure due to fistulae being placed in patients with clinical risk factors and small vessels (6). At a radiological level, both early and late failures are characterized by vascular stenosis (12, 15). These often occur within the first few centimeters of the anastomosis, although they can occur at other sites as well (e.g., cephalic arch stenosis in the setting of brachiocephalic fistulae).

PATHOLOGY

The classical histological lesion that appears to be associated with AV fistula failure is neointimal hyperplasia. We and others have documented that this comprises smooth muscle cells, myofibroblasts and endothelial cells within microvessels (16, 17). Specifically, Stracke et al have documented the presence of transforming growth factor β and insulin-like growth factor I within these neointimal lesions (17), while other studies have described the coexpression of transforming growth factor- β (TGF- β) and markers of oxidative stress (18). Our own group has also performed a detailed cellular phenotyping analysis of the stenotic venous segment of failed AV fistulae and have shown that the predominant cell type is the SMA+ve, vimentin+ve, desmin-ve myofibroblast (16).

However, in the setting of early failure, it is still unclear as to whether aggressive neointimal hyperplasia or adverse vascular remodeling (vasoconstriction or an inability to dilate adequately) plays the major role. To try and answer this question, we have performed a detailed histological analysis of the venous segment in a small number of AV fistulae with early failure. Interestingly, our results have documented the presence of significant neointimal hyperplasia as early as 3.5 months after AV fistula placement (19). However, it is important to point out that adverse vascular remodeling could still be playing an important role in AV fistula failure. In particular, the combination of early and aggressive neointimal hyperplasia together with adverse vascular remodeling could result in an aggressive early stenosis.

NORMAL PHYSIOLOGY OF AV FISTULA MATURATION

Basic physiology (wall shear stress)

In 1983, Zarins et al (20) demonstrated that intimal thickening and atherosclerosis at the carotid bifurcation occurred in regions of low wall shear stress with flow separation and non-unidirectional flow. Since then, numerous studies in different vascular models (21-23) have confirmed this linkage between low flow, low wall shear stress and intimal thickening. Shear stress is the frictional force exerted by blood on the vessel wall and is defined mathematically by the formula $4\eta Q/\pi r^3$ where η = blood viscosity, Q = blood flow and r = vessel radius (24). Thus the magni-

tude of shear stress is directly linked to blood flow. Under most conditions, an increase in shear stress usually results in endothelial quiescence and survival, orientation of endothelial cells in the direction of flow and the secretion of antiinflammatory and anticoagulant mediators (24). At a physiological level, this generally results in a dilatation of the vessel with a reduction in neointimal hyperplasia (beneficial vascular remodeling) (23, 25). These vascular responses also tend to return shear stress levels back toward their baseline. In contrast, a reduction in blood flow and shear stress is associated with endothelial cell activation and proliferation, cellular shape change and the release of inflammatory and procoagulant substances. At a physiological level, this manifests as vascular constriction and increased neointimal hyperplasia (26, 27). In addition to absolute magnitude, the specific pattern of shear stress also appears to play an important role in the vascular response; with physiological (laminar) shear stress resulting in endothelial stability and appropriate dilatation, while oscillatory shear stress often results in a proinflammatory state with increased cellular proliferation and matrix metalloproteinase (MMP) up-regulation (27-29).

Basic physiology (transmural or circumferential pressure)

Transmural or circumferential pressure is the second major hemodynamic parameter that is likely to influence the natural history of an AV fistula. Transmural pressure is defined as the pressure that is generated within the vessel, with numerous studies demonstrating that an increase in pressure results in smooth muscle cell activation, increased cytokine expression and an increase in extracellular matrix components (30-32). These pathways invariably result in vessel wall thickening, which results in a reduction of transmural pressure back toward the basal level.

Based on the above, it is likely that neointimal hyperplasia in the setting of an AV fistula is due to an abnormal wall shear stress profile, while medial hypertrophy is due to the increase in transmural pressure.

Experimental studies

Data from rabbit, and more recently mouse, models of AV fistula placement, clearly demonstrate a very significant increase in arterial flow and consequent hemodynamic shear stress, following the creation of an AV fistula (33-35). The increase in hemodynamic shear stress results in

dilatation of the artery, which tends to return shear stress back to normal over a period of weeks to months. This flow-mediated arterial vasodilatation is primarily a result of the release of nitric oxide. Thus inhibition of nitric oxide activity with L-NAME (33) or endothelial denudation, significantly attenuates this vascular dilatation. More recently it has been shown that reactive oxygen species also play a key role in flow-mediated vasodilatation (36). Thus an increase in shear stress increases superoxide ($O_2^{\cdot-}$) production, which combines with nitric oxide to form peroxynitrite. Peroxynitrite then up-regulates MMP production, which enhances vascular dilatation by fragmenting the internal elastic lamina. Proof for the validity of this pathway comes from recent elegant studies performed by Castier et al (35) in which mice lacking the p47phox-dependent NADPH oxidase gene (and therefore unable to generate superoxide ions and downstream peroxynitrite and MMP) had markedly attenuated arterial dilatation following AV fistula placement.

However, it needs to be emphasized that while reactive oxygen species may play a beneficial role in arterial remodeling in some situations, the overall role of oxidative and nitrosative stress in AV fistula success or failure is likely to be far more complex. For example, production of reactive oxygen species in the absence of adequate local nitric oxide production could result in tissue injury rather than the beneficial cascade of peroxynitrite and MMP formation.

Finally, it is important to emphasize that all of the above studies focus on the feeding artery. There is currently no information on the role of hemodynamic shear stress, oxidative stress, nitric oxide or endothelial denudation on vascular remodeling and intimal hyperplasia of the venous segment. *We believe that this is an important unmet clinical and research need, in view of the fact that AV fistula failure is primarily as a result of venous segment stenoses.*

Clinical studies

There is unfortunately very little hard scientific data in humans to validate some of the mechanistic information that has been derived from the above experimental models. In an important study (albeit in only 6 patients with AV fistulae), Corpataux et al (37) studied the impact of hemodynamic parameters on the remodeling of a newly created arteriovenous fistula. Using echo-tracking and Doppler ultrasound techniques, vessel diameter, cross-sectional wall thickness, blood pressure and blood flow were measured immediately after surgery and at 1 and 3 months.

Within the first week, the blood flow increased to 539 ml/min (range 325-990 ml/min) resulting in an increase in the mean shear stress to 24.5 dyne/cm² from its normal value of 5-10 dyne/cm². This was accompanied by an increase in the internal diameter of the cephalic vein from its preoperative value of 2,370-4,430 μ m at week 1, of 5,041 μ m at week 4 and 6,620 μ m at week 12. This increase in cephalic vein diameter resulted in a decrease in shear stress (inversely linked to vessel diameter) to within the normal range (18.1 dyne/cm² at 4 weeks; 10.4 dyne/cm² at 12 weeks). These changes were accompanied by a progressive increase in the wall cross-sectional area of the cephalic vein, from 4.4 mm² at 1 week to 5.3 mm² at 4 weeks and 6.9 mm² at 12 weeks ($p < 0.028$), indicating an increase in vascular mass. However, because of the increase in cephalic vein diameter (positive remodeling), the increase in wall cross-sectional area did not result in luminal stenosis. The blood pressure in the AV fistulae remained unchanged throughout the study.

Based on the above physiological, experimental and clinical data, the sequence of events that results in the rapid maturation of an AV fistula appears to be as follows: (a) increased arterial and venous flow which results in an increase in arterial and venous shear stress; (b) increase in arterial and venous shear stress initiates a molecular pathway that includes nitric oxide, reactive oxygen species and MMP which results in arterial and venous dilatation; (c) in addition to increased flow, the creation of an AV fistula also results in an increase in pressure within the venous segment, which results in medial hypertrophy. Unfortunately, the above sequence of events has become the exception rather than the rule with a 23%-46% incidence of maturation failure and a primary patency at 1 year of only 60%-65% (14). The following paragraphs will address some of the pathogenetic reasons for the increasing incidence of AV fistula failure.

PATHOGENESIS OF AV FISTULA FAILURE

Demographic and clinical factors

A number of clinical studies have attempted to identify risk factors for AV fistula failure. Thus Miller et al (38) have shown that increasing age, female gender and the presence of diabetes were associated with a poorer prognosis for forearm AV fistulae. Lok et al (39) looked specifically at maturation failure and found that Caucasian race, increasing age and the presence of peripheral and coronary vas-

cular disease were predictive of these early failures. AV fistula success has also been associated with the skills of the operating surgeon, suggesting that issues such as vessel handling, torsion, kinking and the degree of endothelial injury play an important role in AV fistula failure (40-42). More recently, Van der Linden et al (43) have shown that patients with greater preoperative venous distensibility measured by plethysmography tended to have a better chance of successful maturation. This suggests that vessel quality and/or a baseline genetic predisposition (see below) for flow-mediated dilatation could play an important role in the development of a successful AV fistula. Finally, although it has been shown that very small vessels (arterial sizes of less than 1.6 mm and venous diameters of less than 2.5 mm) are associated with poor success rates, there is surprisingly no continuous correlation between larger vessel diameters and better AV fistula survival (44). This suggests that it could be the ability of the vessel to dilate, rather than the initial vessel size that determines AV fistula success.

Upstream events

Abnormal hemodynamic shear stress profiles

As described earlier, high levels of laminar shear stress tend to be associated in experimental models with appropriate vascular dilatation and a relative lack of neointimal hyperplasia. This is probably as a result of endothelial quiescence, high levels of nitric oxide release and low levels of inflammatory cytokines. In contrast, low shear stress values, especially in the context of oscillatory shear stress, tend to be associated with a lack of vascular dilatation and an increase in neointimal hyperplasia. This is probably as a result of endothelial activation, low levels of nitric oxide and the release of inflammatory mediators that predispose to vascular stenosis. Pilot studies in our own laboratory and data from other groups clearly document different patterns of shear stress in different experimental and clinical settings (45-47). Based on these data, we speculate that AV fistulae failure could be due to the presence of a "bad" hemodynamic profile following access surgery: i.e. regions of low flow and oscillatory shear stress within the venous segment. Such a hemodynamic profile could result not only in aggressive neointimal hyperplasia but also in a failure of venous dilatation. In this context, it is important to emphasize that the final amount of luminal stenosis in an AV fistula is determined by the balance between the amount of vascular dilatation or con-

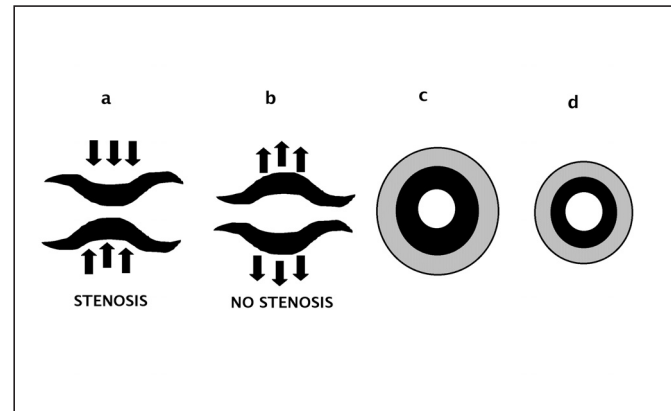


Fig. 1 - Vascular remodeling: The degree of luminal stenosis is dependent upon both the magnitude of neointimal hyperplasia and the degree of vasodilatation or vasoconstriction. With the same amount of neointimal hyperplasia, vascular constriction (a) results in luminal stenosis, while vasodilatation (b) prevents the occurrence of luminal stenosis. A similar situation is described in (c, d), where the white area is the lumen. The area in black is the neointima, which is bordered on the outside by the internal elastic lamina and on the inside by the lumen. The hatched area comprises the adventitia and the media. Note that the luminal (white) area in both (c) and (d) are identical, despite (d) having much less neointima (black area). The reason for this is vasoconstriction in (d), which has resulted in a decrease in the area enclosed by the internal elastic lamina. This latter parameter is a good indicator of the amount of vascular or adventitial remodeling.

striction and the amount of neointimal hyperplasia and medial thickening. *Thus even a very significant amount of neointimal hyperplasia and medial hypertrophy will not result in luminal stenosis in the presence of adequate dilatation. In contrast, even a small amount of intimal hyperplasia and medial hypertrophy can cause a tight stenosis in the absence of venous dilatation* (Fig. 1).

Other upstream injury pathways

While hemodynamic shear stress is likely to be the most important upstream factor responsible for AV fistula failure, other factors such as surgical injury (42, 48, 49) and insertion of dialysis needles (needle infiltrations (50)), are also likely to play a role. In addition to direct injury/infiltration, it has been shown that dialysis needles can result in an increase in turbulence up to 4 cm downstream of the site of needle placement (51). Finally, our own bias is that even though angioplasty is a treatment for AV fistula stenosis, the actual procedure can cause

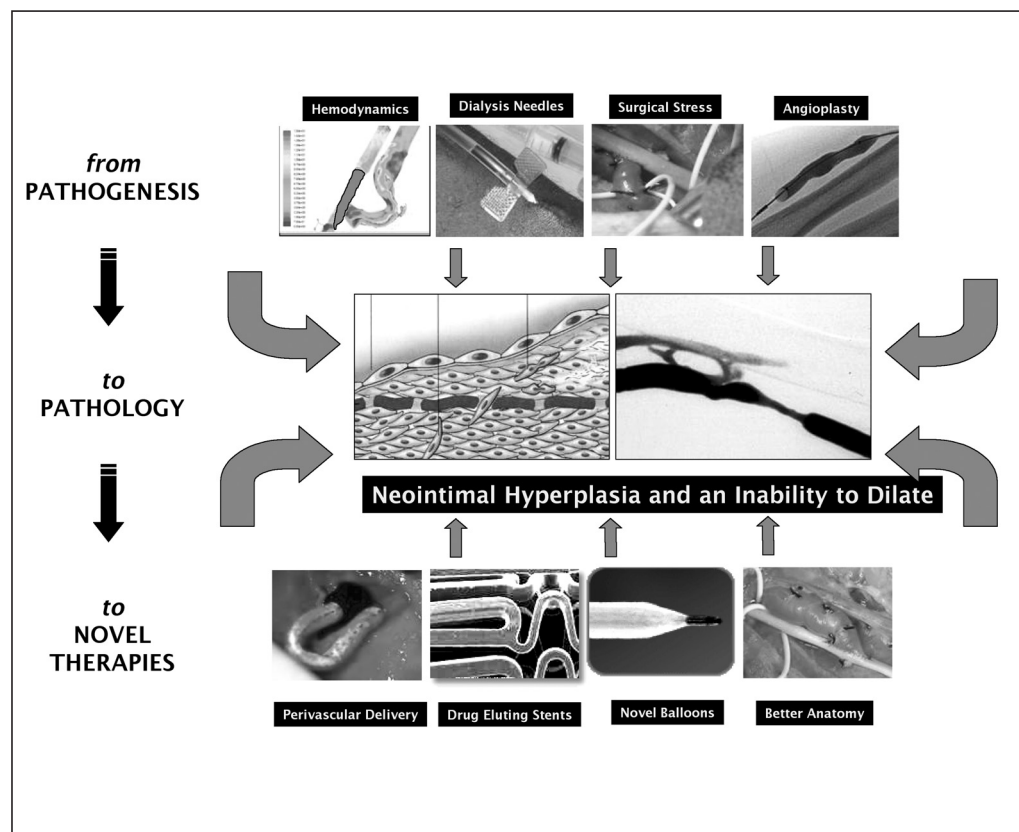


Fig. 2 - From pathogenesis to pathology to novel therapies. The pathogenetic factors responsible for venous stenosis include hemodynamic and surgical stressors, inflammatory stimuli from dialysis needles and the unavoidable vascular injury that occurs at the time of angioplasty (see “Upstream events” for a more detailed discussion). Novel therapeutic modalities include perivascular drug delivery (example from a graft model), drug eluting stents, novel balloons (the “novel balloon” is a Conquest balloon, courtesy Bard Peripheral Vascular) and better final surgical anatomy (see “Clinical trial concepts” for a more detailed discussion). Adapted with permission from (4) (Roy-Chaudhury P, Sukhatme VP, Cheung AK. Hemodialysis vascular access dysfunction: a cellular and molecular viewpoint. *J Am Soc Nephrol* 2006; 17: 1112-27).

significant endothelial and smooth muscle cell injury which could result in an exacerbation of the restenotic lesion. In support of this hypothesis, Chang et al (52) have demonstrated increased cellular proliferation and a shorter time to stenosis in AV fistulae subjected to an angioplasty compared with those with a primary stenosis.

Regardless of which form of upstream injury has the most significance, we believe that aggressive efforts to limit these different types of injury could result in the development of novel therapies for AV fistula failure. Some of these approaches could include (a) identification of an optimal anatomical configuration for AV fistula placement which generates a “good” hemodynamic profile, (b) the design of better dialysis needles that cause less injury and turbulence (c), evidence-based guidelines for performing angioplasty on failing fistulae and (d) minimization of angioplasty and surgical injury through the local application of antiproliferative or prodilatory agents (see “Clinical trial concepts,” below).

Downstream events

Paradigms for neointimal hyperplasia

Medial origin for neointimal cells (traditional paradigm)

According to the “traditional theory” of neointimal hyperplasia, endothelial and smooth muscle injury (hemodynamic stress, surgical injury, dialysis needles) results in the migration of smooth muscle cells and myofibroblasts from the media into the intima, where they proliferate and form the lesion of venous neointimal hyperplasia (Fig. 2). This process of injury followed by migration and proliferation is orchestrated by a large number of mediators which include the cell cycle regulators (p27 and p16, retinoblastoma protein, p38 MAP kinase), cytokines (PDGF, bFGF and TNF- α), chemokines (MCP 1 and RANTES), vasoactive molecules (nitric oxide and endothelin), adhesion molecules (ICAM-1 and P-selectin) and molecules such as osteopontin, apolipoprotein E, MMP-2 and human hepatocyte growth factor (1). It should be emphasized, howev-

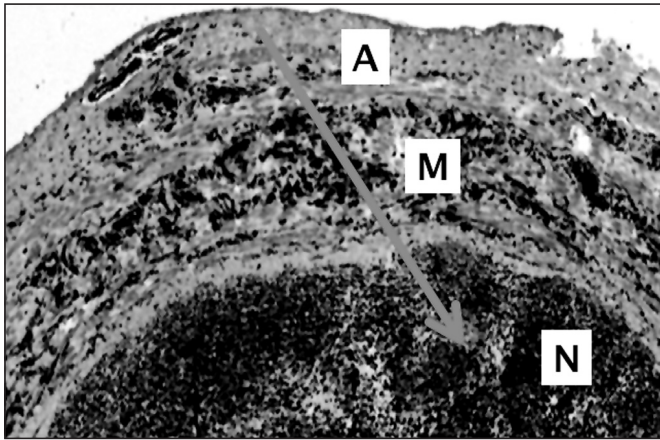


Fig. 3 - Adventitial cells migrate to the intima: High-power view of the stenotic venous limb (Vimentin, $\times 800$) which has been used to depict a cartoon representation of the migration of adventitial fibroblasts from the adventitia (A), through the media (M) and into the intima (I) where they can acquire the phenotype of myofibroblasts or smooth muscle cells. Adapted with permission from (4) (Roy-Chaudhury P, Sukhatme VP, Cheung AK. Hemodialysis vascular access dysfunction: a cellular and molecular viewpoint. *J Am Soc Nephrol* 2006; 17: 1112-27).

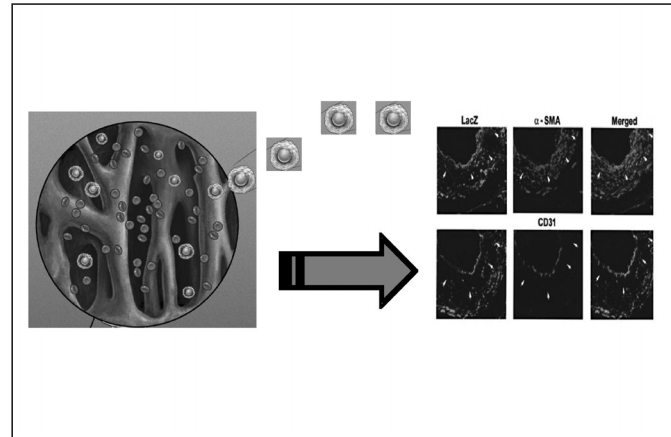


Fig. 4 - Smooth muscle progenitor cells contribute to neointimal hyperplasia: Smooth muscle progenitor cells have been shown to contribute significantly to total neointimal volume. LacZ = beta galactosidase; α -SMA = α -smooth muscle actin. Adapted with permission from (4) (Roy-Chaudhury P, Sukhatme VP, Cheung AK. Hemodialysis vascular access dysfunction: a cellular and molecular viewpoint. *J Am Soc Nephrol* 2006; 17: 1112-27).

er, that most of this information is derived from arterial angioplasty models rather than from experimental or clinical settings of arteriovenous stenosis. The only available studies in the setting of human AV fistulae demonstrate the presence of transforming growth factor- β , insulin-like growth factor I, ICAM-1 and MMP-9 within the venous segments of failed AV fistulae (17, 53).

Inhibition of a panel of these mediators in a synergistic fashion through the local application of cell-, gene- or drug-based therapies, at the time of surgery or angioplasty, could significantly improve AV fistula survival. A classic example of the efficacy of this approach comes from the reduction of in-stent coronary restenosis to less than 10% through the use of drug-eluting stents (54, 55) (notwithstanding the recent problems with late thrombosis).

Adventitial origin for neointimal cells

Recent studies have demonstrated that following experimental coronary angioplasty or saphenous vein grafting, there is a migration of fibroblasts from the adventitia, through the media and into the intima, where these cells acquire the phenotype of myofibroblasts (express smooth muscle α actin) and contribute to final neointimal volume (Fig. 3) (56-58). A similar paradigm has also been suggested in the setting of polytetrafluoroethylene (PTFE) graft stenosis (59). In addition, our own studies in the set-

ting of human AV fistulae have documented the presence of fibroblasts within the media of stenotic AV fistulae (16), suggesting that there is a migration of these cells inwards from the adventitia. These results emphasize the fact that the adventitia is *not* an innocent bystander in the pathogenesis of neointimal hyperplasia. Rather, these concepts make a strong case for the development of therapeutic interventions that (i) focus on the adventitia and (ii) target multiple cell types (fibroblasts, myofibroblasts and smooth muscle cells) instead of only the differentiated contractile smooth muscle cell (see "Clinical trial concepts," below).

Bone marrow origin for neointimal cells

Recent data also suggest a role for bone marrow-derived smooth muscle progenitor cells in the pathogenesis of neointimal hyperplasia (Fig. 4). Thus Sata et al (60) have shown that up to 60% of both endothelial and smooth muscle cells within the lesion of neointimal hyperplasia following femoral angioplasty are bone marrow-derived cells. Bone marrow-derived cells that have acquired a smooth muscle phenotype have also been identified in a mouse model of venous neointimal hyperplasia (61). A recent study in a chimeric mouse model of AV fistula stenosis, however, did not demonstrate the presence of bone marrow-derived smooth muscle cells (36).

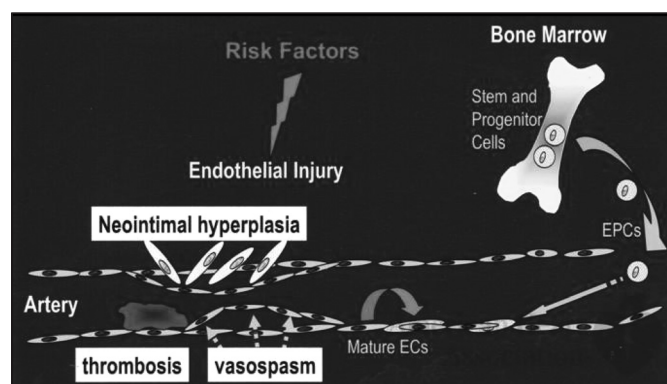


Fig. 5 - Endothelial progenitor cells: Endothelial progenitor cells (EPCs) are produced in the bone marrow and can be mobilized by a number of different factors including granulocyte colony-stimulating factor. This is a diagrammatic representation of EPCs binding to injured endothelium in order to possibly prevent vasospasm, thrombosis and neointimal hyperplasia. EC = endothelial cell. Adapted with permission from (4) (Roy-Chaudhury P, Sukhatme VP, Cheung AK. Hemodialysis vascular access dysfunction: a cellular and molecular viewpoint. *J Am Soc Nephrol* 2006; 17: 1112-27).

Endothelial progenitor cells and vascular repair

Endothelial progenitor cells (EPCs) are circulating bone marrow-derived cells that express both hematopoietic (CD34) and endothelial cell markers (vascular endothelial growth factor receptor 2 [VEGFR-2]) (62, 63). In addition to promoting angiogenesis, an important role of EPCs appears to be the rapid endothelialization of regions of vascular injury (Fig. 5) (64, 65). For example, the infusion of EPCs in the setting of angioplasty or surgical graft placement results in enhanced endothelialization, which translates into a reduction in neointimal hyperplasia (66, 67). This is in keeping with previous data which demonstrate an inhibitory effect of endothelialization and endothelial-derived factors such as nitric oxide on smooth muscle cell proliferation and migration (68-70). Most importantly, there are a number of agents that can enhance the mobilization of EPCs from the bone marrow (statins, erythropoietin, granulocyte colony-stimulating factor and MMP-9) (64). Indeed, Kong et al (71) have demonstrated that granulocyte colony-stimulating factor therapy results in an increase in endothelialization *and* a reduction in neointimal hyperplasia in a mouse angioplasty model. Whether or not EPCs play a role in AV fistula success or failure is unknown at the present time.

Endothelial dysfunction in uremia

A number of studies have demonstrated that flow-mediated vasodilatation (a marker for endothelial function) is reduced in uremic patients compared with normal controls (72). The reasons for this could include increased oxidative stress (73, 74), the presence of inhibitors of nitric oxide such as asymmetric dimethylarginine (75-77) and a reduced number and function of endothelial progenitor cells (78, 79). Since endothelial dysfunction is at the heart of adequate AV fistula function, attempts to modulate the above factors could result in improved AV fistula function. In particular, we believe that the magnitude of endothelial dysfunction in uremic patients makes this group of patients more likely to respond to local or systemic interventions that improve endothelial function. A similar analogy comes from published data which demonstrate that antioxidants are successful in reducing cardiovascular events in hemodialysis patients (with high baseline levels of oxidative stress (80, 81)) but not in nonuremic individuals (82).

Preexisting vascular abnormalities

Increasing attention is now being focused on preexisting arterial and venous abnormalities in uremic patients. Thus, uremic patients tend to have increased vascular stiffness which could be due to increased deposition of collagenous material (83-85), and also the impact of vascular calcification (86, 87). In addition, Kim et al (88) have documented that intimal thickening in the radial artery prior to surgical creation of AV fistulae correlates with poorer fistula survival. Finally Wali et al (89) have shown that preexisting neointimal hyperplasia is present in the cephalic vein of uremic patients undergoing AV fistula placement. These data suggest that attempts to improve the overall vascular health of uremic patients might result in improved AV fistula survival. Alternatively, local therapies to reduce arterial and/or venous stiffness applied at the time of AV fistula placement could be extremely effective in enhancing AV fistula maturation. Such an approach is currently being investigated by Proteon Therapeutics through the use of perivascular elastase (see "Clinical trial concepts," below).

Genetic polymorphisms

The response to injury that results in neointimal hyperplasia and AV fistula failure has been shown to be linked to

genetic polymorphisms for methylene tetrahydrofolate reductase (90), TGF- β (91) and heme oxygenase-1 (92) gene products. While the exact magnitude of the impact of these genetic polymorphisms is unclear, they could function as important screening tools to identify patients at high risk of AV fistula failure. Alternatively, manipulation of the levels of the gene products of these genes could result in the development of novel therapies for AV fistula stenosis.

CLINICAL TRIAL CONCEPTS

The above paragraphs have attempted to summarize those pathogenetic processes that could play an important role in AV fistula failure and also to emphasize how manipulation of some of these pathways could result in future therapies for AV fistula failure. In the final section of this review we will focus on innovative therapeutic approaches that are currently being evaluated in clinical trials or which are likely to be studied in the very near future.

Currently available systemic agents

There has been a lot of interest in the use of currently available agents that have the potential to block smooth muscle cell proliferation and migration and/or thrombosis, in the setting of hemodialysis vascular access dysfunction. Unfortunately, much of this data is anecdotal and involves a very small number of patients (93). In the 2 randomized clinical trials published to date, dipyridamole (94) and fish oil (95) were both shown to prevent stenosis and thrombosis in PTFE dialysis grafts (primary prevention). Angiotensin-converting enzyme inhibitors in contrast have been shown to be of benefit only in retrospective registry analyses (96). The National Institutes of Health (NIH)-sponsored Dialysis Access Consortium (DAC) is currently conducting a large multicenter, randomized prospective, primary prevention study on the use of Plavix to improve patency in new AV fistulae (97). Two additional oral agents that have been shown to have potent inhibitory effects on vascular stenosis in experimental models are sirolimus (98) and the PPAR gamma agonist rosiglitazone (99). In addition to blocking smooth muscle cell migration and proliferation, both sirolimus and rosiglitazone appear to modulate the relative number of smooth muscle and endothelial cell progenitors in experimental models (100, 101), emphasizing the importance of these alternative

mechanisms in the pathogenesis of neointimal hyperplasia. Preliminary clinical studies demonstrate a reduction in in-stent restenosis following coronary angioplasty, with the oral administration of sirolimus (102) and rosiglitazone (103). The role of these agents in the clinical setting of hemodialysis vascular access dysfunction, however, is unknown at present.

Cell-based therapies

A potential disadvantage of using a single agent to attenuate a complex process such as AV fistula stenosis is that biological systems tend to be redundant (i.e., many different mediators can perform the same function). This suggests that single-agent therapy may not be effective in certain clinical settings. An exciting alternative approach has been pioneered by the Edelman group in which Gelfoam wraps are loaded with endothelial cells. The wraps are then placed around the arteriovenous anastomosis of AV fistulae at the time of surgery. The rationale behind this approach is that these endothelial cells could then produce an array of mediators that would hopefully mimic the 2 main functions of endothelial cells (i.e., promote vascular dilatation *and* inhibit neointimal hyperplasia). Initial studies in a pig model of AV fistula stenosis have demonstrated a reduction in neointimal hyperplasia using this approach (104, 105). Based on these data a multicenter phase II/III study using these cellular implants is currently in progress in the United States (Pervasis Vascugel Study).

Drug-based therapies

Perivascular wraps could also be loaded with specific antiproliferative agents. Thus experiments in our laboratory have documented a marked attenuation of venous neointimal hyperplasia at the graft-vein anastomosis following the placement of paclitaxel-loaded perivascular wraps in a pig model of AV graft stenosis (106). A large multicenter clinical trial comparing paclitaxel-loaded wraps with control wraps will be initiated in the United States within the next 2 months. Whether such an approach will also be effective in the clinical setting of AV fistulae is currently unknown.

Gene-based therapies

This could become an effective means of local therapy for neointimal hyperplasia in AV fistulae (107), especially if

improvements continue to be made in the safety and efficacy of delivery techniques (108). Currently, inhibition of neointimal hyperplasia in experimental angioplasty models has been achieved by the gene transfer of endothelial and inducible nitric oxide synthase, cyclin-dependent kinase inhibitors, retinoblastoma protein, hepatocyte growth factor and transcription factors such as E2F (1). A phase II trial of the E2F decoy in arteriovenous grafts was unfortunately discontinued in view of the lack of efficacy of this therapy in the setting of saphenous vein bypass grafting in the coronary circulation (PREVENT IV) (109) and in peripheral vein bypass grafting (PREVENT III) (110). The PREVENT studies, however, clearly contributed to the development of innovative pressure-based perivascular and endovascular delivery methods into venous conduits. More recently, Ark Therapeutics (111) has initiated a phase II/III study of adenoviral-based gene therapy for vascular endothelial growth factor D (VEGF-D) in PTFE dialysis access grafts, using a special perivascular delivery device that fits around the proximal vein just beyond the graft-vein anastomosis (112).

Local perivascular delivery through an endovascular balloon

The previously described perivascular delivery systems, however, have a disadvantage in that each delivery system has been developed for a specific intervention. The delivery systems are therefore not interchangeable between different therapies. In marked contrast, Mercator Med has developed an innovative endovascular balloon, which when inflated at the site of required "agent" delivery, extrudes a microsyringe through the vessel wall into the adventitia through which drugs, cells or genes could be injected (113)! Thus this device could potentially be used to deliver different concentrations of different agents to the adventitia of the venous segment at different time points during the natural history of AV fistula development.

CONCLUSIONS

Despite the clinical complexities associated with the placement of functional AV fistulae in an elderly patient population with significant comorbidities, this is an exciting time for dialysis access. We believe that this is so because of (a) a better understanding of the biological processes involved in both AV fistula success and failure, and (b) unprecedented advances in the fields of molecular

biology, drug delivery and device technology. The task before us therefore is not to increase the availability of novel therapies to improve AV fistula survival, but rather to realize the applications that are needed to take these novel therapeutic concepts from the bench to the bedside!

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