Review

Prevention of vein graft failure: potential applications for gene therapy

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Received 5 February 1997; accepted 14 April 1997

Abstract

The use of gene therapy in the clinical setting is believed to be a realistic option for the future. Many clinical trials are underway for treatment of disorders as diverse as cancer, peripheral vascular disease, and numerous monogenic diseases. However, gene therapy for vein graft failure may be more distant due to the highly complex, multifactorial aetiology of the disease. Although many of the cellular mechanisms involved in vein graft failure have been reported, important barriers still need to be overcome before gene therapy could become a clinical reality. Further understanding of the molecular mechanisms involved in graft failure will lead to the identification of appropriate therapeutic genes. Moreover, limitations in the current delivery systems need to be overcome to allow efficient, safe delivery and expression of transgenes for the required length of time in vivo. However, currently available gene delivery vectors are extremely useful tools to help in our understanding of vein graft failure. In this review, we address the issues surrounding gene therapy with particular emphasis on its future potential to ameliorate long term vein graft occlusion. © 1997 Elsevier Science B.V.

Keywords: Vein graft; Gene therapy; Adenovirus; Smooth muscle cells; Endothelium

1. Introduction

Much attention has focused on the evaluation of gene transfer technology in the treatment of cardiovascular diseases such as atherosclerosis, arterial thrombosis, and post-angioplasty restenosis. Its application to the phenomenon of vein graft failure has however been largely neglected. This is surprising given that over 400,000 coronary artery bypass procedures are performed each year in the USA alone [1], with autologous saphenous vein representing over 70% of grafts used [2]. The poor long term patency of vein grafts however, remains the Achilles heel of this procedure. Despite a trend towards increased use of arterial conduits (internal mammary, gastroepiploic, radial arteries) with their superior patency rates [3], it is the ready availability, ease of harvesting and favourable surgical handling of saphenous vein that has ensured its continued use. The clinical and economic consequences of late graft failure and recurrent angina has made the search for an effective treatment of vein graft failure a matter of some urgency.

1.1. Pathophysiology of vein graft failure

Observations from clinical angiographic studies [4], human pathological specimens [5], animal models and in vitro systems have all contributed to the understanding of the events underlying vein graft occlusion. Vein grafts inserted into the arterial circulation undergo a characteristic sequence of adaptive pathological changes. Early failure, within one month, occurs in 8–18% of saphenous vein grafts [6] and results from acute thrombosis consequent upon vessel wall injury during preparation and implantation. Late vein graft failure is characterized by progressive medial thickening and neointima formation that begins within days of implantation, continues over subsequent months, and is followed after 1–2 years by the development of superimposed atherosclerotic changes [7]. Thus, one year after implantation the cumulative graft attrition rate is some 25%, increasing with the development of atherosclerotic changes to over 50% after 10 years [4].

The cellular mechanism underlying graft wall thickening is well established. Briefly, soon after implantation...
proliferation of vascular smooth muscle cells (SMC) within the graft media is observed followed by their migration through the internal elastic lamina into the intima, where further proliferation is accompanied by a prolonged period of extracellular matrix protein synthesis and deposition [5,7,8]. Although atherosclerotic changes appear somewhat later, intimal accumulation of biologically active oxidised lipids is seen within days of implantation, promoting monocyte adhesion, infiltration into the intima, and differentiation into macrophages [9].

Early endothelial and medial injury from mobilisation, preparation, and storage prior to implantation provokes release of a host of substances from adherent platelets, monocytes and SMC within the vessel wall, that are mitogenic and promigratory for SMC. These include specific growth factors, vasoconstrictors (released by activated platelets) [10], coagulation factors [11], and leucocytic release products. Furthermore, endothelial injury is associated with a decline in prostacyclin (PGI2) and nitric oxide (NO) release [12], resulting in loss of tonic antiproliferative influence on local SMC, and suppression of local fibrinolytic activity [13] thus favouring platelet activation and thrombus formation.

Mechanical forces such as arterial perfusion pressure, increased tangential wall stress, and high shear stress provide further independent stimuli for growth factor synthesis and release by both endothelial cells and SMC [14,15]. We have found that exposure of experimental vein grafts to high shear stress is associated with upregulation of endothelial nitric oxide synthase (NOS) activity, but loss of receptor-mediated NO release [16], suggesting either a breakdown in receptor-NOS coupling or rapid degradation of NO by increased levels of superoxide species [17]. Shear stress also induces expression of cellular adhesion molecules that play a key role in early monocyte binding and migration into the vessel wall [18].

Despite identification of an increasing number of growth factors implicated in SMC migration and proliferation in arterial injury models [19,20], current evidence exists only for the role of platelet-derived growth factor (PDGF) in vein graft intimal hyperplasia [21,22]. In this respect, gene transfer has considerable potential as a tool for determining the involvement of other growth factors in vein graft failure.

It is now well established that alterations in matrix composition occur at sites of vessel wall injury, perhaps releasing SMC to increased proliferation, migration and intima formation. The family of matrix metalloproteinases (MMPs) can degrade components of the extracellular matrix, and in this respect, elevated synthesis and/or extracellular activation of gelatinase A (MMP-2) and gelatinase B (MMP-9) have been observed following arterial injury in the rat and porcine models of restenosis [23–25]. Additionally, MMP-2 and MMP-9 have been implicated in the pathogenesis of vein graft stenosis in both human organ culture and porcine interposition grafts [26,27]. It is believed that the tight extracellular regulation of MMPs by their endogenous inhibitors, the tissue inhibitor of metalloproteinases (TIMPs) in the vessel wall is critical to the maintenance of vascular cells in a quiescent phenotype in vivo. Vessel wall injury leading to release and activation of MMPs may result in a shift in the MMP:TIMP balance producing net matrix destruction.

2. Models of vein graft failure

An essential requirement for the development of gene therapy for vein graft failure is the availability of suitable experimental models, both in vitro and in vivo. Since the aetiology of vein graft failure is complex, appropriate models are required for assessing experimental gene transfer strategies and candidate genes.

2.1. The human saphenous vein organ culture model

The organ culture model of human saphenous vein (HSV) has been the main choice for in vitro analysis within our laboratory and has been extensively characterized [28,29]. In the presence of luminal endothelial cells, exogenous growth factors supplied by 30% foetal calf serum stimulate smooth muscle cell proliferation and migration. This leads to the formation of a neointima, containing smooth muscle cells below an endothelial cell monolayer following 14 days of culture (Fig. 1) [30]. Surgically prepared vein segments develop a more profound neointima compared to freshly isolated samples, presumably due to the higher degree of medial smooth muscle cell damage [31] (Fig. 1).

This model has been used to identify the specific roles of a number of genes in neointimal development including platelet-derived growth factor (PDGF) and its receptors, metalloproteinases (MMPs) and tissue inhibitor of metalloproteinases (TIMPs) [22,26,32]. The model provides a unique opportunity to assess the factors that are involved in growth factor induced proliferation and migration of SMC residing within their native extracellular matrix. It has clear advantages as an experimental tool for assessing gene therapy strategies. First, the ability of transgenes to alter SMC proliferation and migration can be assessed directly. Second, as the vast majority of transgenes cloned into expression systems to date are human, no species barriers exist and therefore expression of non-functional transgenes will not occur. Third, with the use of viral delivery systems, as discussed below, there is a host immune response in vivo that can result in the premature elimination of transgene expression. This is clearly not problematic with this in vitro model. However, the relative simplicity of this model can be disadvantageous due to the absence of flow and peripheral blood cell involvement. Additionally, the accelerated neointimal formation here may not accurately reflect all aspects of vein graft intimal formation in vivo.
With respect to gene delivery, there is ample time in which to deliver genes to the HSV in vitro, either prior to culturing or for the duration of the culture procedure. To demonstrate this, we have used a replication defective adenovirus capable of expressing the bacterial lacZ gene under control of the high output cytomegalovirus major immediate early promoter (CMV IEP) to produce high level gene transfer. In surgically prepared segments of human saphenous vein, exposure of cells at the luminal surface to the viral suspension prior to culture resulted in high level gene expression occurred throughout the 14 days of culture (Fig. 2). Using this gene transfer protocol, adenoviral infection occurs only at the luminal surface in both exposed SMC and residual endothelial cells. Adenoviral infection of adventitial cells was not detected. Therefore, high levels of candidate genes can be expressed from the luminal surface of human saphenous vein and this will provide a useful experimental tool in vitro.

2.2. The porcine model of venous interposition grafting

A number of in vivo experimental models have been used in the study of vein graft disease, including rabbit, canine, sheep, porcine, and non-human primate species [33]. Technical challenges associated with coronary artery grafting in small animals has led to models based on end-to-end or end-to-side interposition of vein segments into peripheral arterial sites such as the carotid or iliofemoral arteries. The donor vein is usually external jugular, cephalic, femoral, or the saphenous vein. In our laboratory, we have favoured the use of the porcine model, in which bilateral saphenous vein-into-carotid artery interposition grafts are performed using end-to-end anastomoses [34]. Physiologically, the animal shares similar coagulation profiles and lipoprotein metabolism with humans, and is susceptible to spontaneous and diet-induced atherosclerosis [33]. Of the other animal groups, only non-human primates show such similarities, but their use is limited by their higher cost and special handling requirements.

The behaviour of vein grafts in this model has been characterised histologically over periods as long as one year [35], and has been found to be readily reproducible. Medial thickening and neointima formation are present at seven days, and are well-established by one month. The impact of topical agents such as transgene applied to the graft prior to implantation can thus be assessed within relatively short periods of time. Cell and organ culture-based studies have confirmed that porcine SMC behave very similarly to their human counterparts. The obvious advantage of the in vivo model is exposure of the graft to the physiological environment of arterial haemodynamics and circulating blood products (cells, hormones, coagulation factors etc.). Furthermore, aspects such as systemic dissemination of transgene through washout from the impregnated graft, and immune responses to expressed viral antigens can be readily assessed (see later).

Fig. 1. Neointimal formation in HSV organ cultures. Histochemical staining of HSV cultures with Miller’s elastic van Gieson. (a) Day 0 control, (b) day 14, freshly isolated vein, (c) day 14, surgically prepared vein. Arrows indicate the intimal medial boundary. N = neointima, M = media. The scale bar represents 25 μm.

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3. Gene delivery systems

There are many available methods that can be utilized to deliver genes into cells and these can be divided into two main categories, non-viral and viral. Non-viral methods include calcium phosphate, dithyl-aminooethyl (DEAE)-dextran and liposome mediated gene transfer. Viral methods of gene delivery including retroviruses, adenoviruses, herpes viruses, lentiviruses and parvoviruses have now been developed and many are suited to specific applications. However, in the case of vein graft failure, the major limitation associated with a number of these methods is their inability to induce high efficiency gene delivery in vivo. The number of gene transfer studies in the context of vein grafting are limited. High level gene transfer of a lacZ reporter gene into porcine vein grafts was achieved using the adenoviral delivery system [36]. Additionally, biotinylated adenoviral particles linked to transferrin/polylysine-DNA complexes have been shown to induce high level transfection of genes into the rabbit jugular vein to carotid artery model of vein grafting [37].

It is believed that recombinant adenoviruses are currently the most efficient delivery system in which high level transient gene expression can be achieved in vitro and in vivo. Replication defective adenoviruses have a number of advantages over other systems such as retroviruses. These include the ability to infect both dividing and non-dividing cells, the lack of viral DNA integration into the host genome resulting in transient recombinant gene expression, and the ease at which genetic manipulations and high titre viral stocks can be obtained. Since the majority of cells in the vein graft are non-dividing at the time of gene delivery, high level infection can be achieved using the adenoviral system [36,37]. Additionally, many studies have also shown the relative ease in which both smooth muscle cells and endothelial cells can be infected with recombinant adenoviruses in vitro and in vivo [38]. High level infection of endothelial cells has been shown in uninjured arteries in vivo [39,40] although another study has reported lower level infection of endothelial cell in vivo [41]. The ability to infect both smooth muscle cells and endothelial cells presumably reflects cell surface expression of the putative adenovirus type 5 receptor and αvβ3/αvβ5 integrins required for the efficient binding and internalisation of the adenovirus particles [42].

However, the major problem in the use of the first generation adenoviral gene delivery systems (i.e., E1 and E1/E3 deleted) [43] is the host inflammatory response to infected cells induced in vivo [44,45]. With replication competent adenoviruses, the E1a gene located at the left-hand end of the adenoviral genome is responsible for the initiation of transcription of viral structural proteins following infection (Fig. 3). Although the production of structural (late) adenoviral proteins is highly reduced in E1 defective viruses compared to the wild type adenoviruses, ‘leaky’ transcription occurs. This is thought to be partially responsible for the cellular and humoral immune reactions observed in vivo [44,46]. This can lead to elimination of infected cells in vivo and hence a rapid decline in the level of transgene production. Additionally, if the recombinant virus expresses a gene not originating from the species under study, an immune response can also occur against the transgene [47]. Further manipulations of the adenoviral genome have been performed to try and eliminate or substantially reduce the immune response evoked (Fig. 3). Construction of second generation adenoviral vectors containing an E2a temperature sensitive mutation in addition to the E1 deletion resulted in diminished late viral protein synthesis at the non-permissive temperature, prolonged transgene expression and reduction of the inflammatory response in infected mouse livers [48]. However, significant late viral gene expression and T-cell response was detected when the vector was used to infect airway epithelium of cotton rats [49].

Development of adenoviral vectors with an E4 deletion in addition to E1 and subsequent generation of permissive cells lines has resulted in systems that remain stable for longer in vivo, induce prolonged transgene expression and reduced organ damage in vivo [50–53]. Deletion of the E4 region is additionally important due to the recent demonstration of the oncogenic potential of the E4orf6 protein [54]. As an alternative strategy to deleting single additional regions of the adenoviral genome, third generation adenoviral vectors deleted of all viral open reading frames have now been developed [55–57]. In this instance, helper virus is used to propagate infectious adenoviral recombinants which are separated from the helper by density centrifugation. Further development and evaluation of these systems in vivo will determine their applicability for use in clinical gene therapy.

4. Gene selection strategies

Of the various pharmacological agents evaluated in experimental models of vein graft failure [33] only lipid lowering therapy has shown any benefit, albeit modest, in preventing late graft failure [58]. The disappointing results from clinical studies, both of restenosis and vein graft failure, has spawned a search for alternative strategies. Indeed, much has now been published on the potential application of gene therapy in the prevention of post-angioplasty restenosis [59], where emphasis has rested on inhibition of SMC proliferation as a key aetiological factor. It is now apparent however, that unlike models of arterial injury, the role of SMC proliferation in clinical restenosis may be somewhat overstated, and that other mechanisms such as constrictive remodelling of the vessel wall may be responsible for the majority of luminal loss [60,61]. The failure to demonstrate angiographic benefit in clinical trials of many pharmacological agents that have targeted SMC proliferation would seem to support this finding [33]. The clinical setting of coronary artery surgery
STRUCTURE OF THE ADENOVIRUS GENOME

Fig. 3. Structure of the adenoviral genome and development of gene transfer vector systems. The 36 Kb genome is divided into 100 map units and gene transcription occurs in both directions. Early region gene expression occurs from regions E1 to E4 while production of late proteins is initiated from the major late promoter (MLP) located at 16 map units. First generation adenoviruses are deleted at E1 alone or E1 plus E3 while second generation systems have an additional mutation in the E2a or E4 genes. Third generation vectors are deleted of all viral open reading frames.

Transduction efficiency can be further optimised through gap junctions [65]. This system has the advantage that transduced cells are also rendered sensitive to ganciclovir TK converts the pro-drug into a toxic metabolite capable of inducing cell death [62]. This has been shown to be effective in reducing restenosis in different models [63,64]. Neighbouring (non-transduced) cells are also rendered sensitive to ganciclovir through gap junctions [65]. This system has the advantage over ‘infected cell only’ strategies by the virtue that lower viral loads can be used to evoke the desired effect resulting in a decrease in viral-mediated immune reactions in vivo. Cytostatic gene methods are theoretically more problematic, as higher concentrations of viral vectors should be required to produce infection of enough cells to induce the required response in vivo. However, efficient inhibition of restenosis has been observed using this strategy [66–68]. The effect of antiproliferative strategies remains to be tested in the human vein organ culture model.

However, a staged procedure, involving temporally separate harvesting and implantation procedures is unlikely to gain surgical acceptance. The feasibility of ‘single-stage’ graft harvesting, incubation, and implantation can be readily determined by assessing the speed of gene transduction in the human vein organ culture model.

Given the pathophysiology of vein graft failure, strategies for gene transfer could potentially be designed to target mediators of early or late graft failure. Thus, expression of factors inhibiting platelet activation or adhesion may reduce early graft failure, while strategies modulating SMC or mononuclear activity may influence the intimal hyperplasia and atherosclerosis involved in late failure (see below). A point of particular interest is the transient duration of gene expression provided by most vectors. Whereas this might be sufficient in prevention of early thrombotic occlusion, it is unknown whether early transient gene expression can provide long term benefit, such as the prevention of late graft failure. Unlike restenosis, where injury is brief and response occurs relatively rapidly, stimuli for SMC proliferation in vein grafts (shear stress, tangential wall stress, etc.) persists long after graft implantation, and thus may require prolonged expression of a given therapeutic gene. This hypothesis remains to be tested.

The application of antiproliferative gene transfer technology to the prevention of this pathology seems entirely appropriate, but has so far been greatly under-investigated. Targeting smooth muscle cells has been the method of choice for a number of groups investigating gene therapy for restenosis. Interestingly, different strategies for altering the function of the smooth muscle cells have been successful. These include cytotoxic, cytostatic and diffusible inhibitor strategies. The cytotoxic approach involves transfer of the viral thymidine kinase (TK) gene and systemic delivery of the pro-drug ganciclovir. TK converts the pro-drug into a toxic metabolite capable of inducing cell death [62]. This has been shown to be effective in reducing restenosis in different models [63,64]. Neighbouring (non-transduced) cells are also rendered sensitive to ganciclovir through gap junctions [65]. This system has the advantage over ‘infected cell only’ strategies by the virtue that lower viral loads can be used to evoke the desired effect resulting in a decrease in viral-mediated immune reactions in vivo. Cytostatic gene methods are theoretically more problematic, as higher concentrations of viral vectors should be required to produce infection of enough cells to induce the required response in vivo. However, efficient inhibition of restenosis has been observed using this strategy [66–68]. The effect of antiproliferative strategies remains to be tested in the setting of vein graft disease. Diffusible in-
hibitor strategies for the prevention of SMC proliferation and migration have also shown great promise for therapeutic intervention in restenosis. Gene transfer of the endothelial form of nitric oxide synthase complexed to the Sendai virus-liposome complex reduced restenosis by 70% in the rat carotid injury model [69]. This strategy, like that of viral TK, has the advantage of the requirement for a lower transduction efficiency than cytostatic strategies as the nitric oxide produced from transduced cells will produce the bystander effect on non-transduced cells. Additionally, Tzeng et al. [70] showed that retroviral mediated gene transfer of the inducible form of nitric oxide synthase (iNOS) to balloon injured pig femoral arteries in vitro resulted in complete inhibition of injury induced myointimal hyperplasia. However, the effect of elevating NO levels in vein sections following autologous grafting remains to be determined.

Inhibition of extracellular matrix degradation by MMPs is a further potential area for intervention. Elevation of TIMP expression by gene transfer may limit the migration and proliferation of SMCs through inhibition of MMP activity. Adenoviral mediated gene transfer of TIMPs to the vessel wall will be a useful system to investigate this [71]. Recently, retroviral mediated overexpression of balloon TIMP-1 in the rat carotid model of angioplasty restenosis led to reduced smooth muscle cell proliferation and neointima formation using a vascular cell seeding method [72]. Due to the extensive loss of endothelium from the vein wall prior to graft interpositioning, promotion of endothelial cell regeneration may limit neointimal formation as has been observed for restenosis [73]. The anti-thrombotic approach may also be beneficial. Indeed, adenoviral mediated gene transfer of COX-1 into injured porcine arteries has proved successful by augmenting prostaglandin I2 synthesis [74]. Gene transfer of a soluble vascular cell adhesion molecule (sVCAM) has also been suggested to block monocyte binding to the vascular endothelium through competitive inhibition of binding to the wild type, cell surface associated VCAM [36].

5. Conclusions

The ability to selectively manipulate in vivo the expression of a specific gene within the graft wall, either by inhibition or overexpression, represents a very powerful tool both in furthering our understanding of vascular biology and pathophysiology and in the development of novel therapies. Moreover, the ability to transfer therapeutic genes into the vein wall ex vivo prior to graft interpositioning provides a unique opportunity to alter the pathogenesis of vein graft failure.

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