

Editorial Focus

Ex-vivo thrombolytic gene therapy for vein graft patency: The frontier for development of selective, localised therapeutic approaches

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Targeted delivery of drugs and localised treatment is known to increase efficacy and decrease systemic side effects. This principle has been applied for a long time utilising sophisticated methods in cancer therapy (1). However, only recently have targeted therapeutic approaches been evaluated in the field of thrombosis and atherosclerosis. Localised gene therapy and antibody (ligand)-mediated drug delivery are emerging technologies. They hold the promise of powerful, localised anti-thrombotic and anti-atherosclerotic actions with a reduction in systemic effects, in particular with less bleeding complications (2, 3).

One eminent field for targeted therapy is autologous saphenous vein graft failure after arterial bypass surgery. Despite increasing use of percutaneous interventional techniques, this surgical procedure remains a standard therapy for occlusive arterial diseases. This operation is performed more than 500,000 times each year worldwide. It is effective in relieving symptoms of angina and prolongs life, especially in patients with multiple-vessel disease. However 10% of grafts fail early because of thrombotic occlusions, another 15% of grafts fail in the following five years because of intimal thickening, and a further 25% fail later on because of atheroma (4). As a result repeated operations are very common, accounting for around 20% of all procedures (5). These repeat procedures are associated with significantly higher rates of operative mortality and morbidity due to the surgical challenges posed by scarring. Without doubt, there is a clear need to develop new effective therapies preventing vein graft failure.

Early graft failure is often a result of mechanical damage during graft preparation and exposure to arterial pressure after implantation. This results in a procoagulant state characterised by fibrin deposition and platelet adhesion. Anticoagulation and thrombolytic therapy is able to reduce the risk of early thrombosis (6, 7), but this intervention is unable to completely avoid graft failure (8). Non-targeted systemic anticoagulation and thrombolysis is often associated with serious side effects such as bleed-

ing complications (9, 10). One way of avoiding these complications is targeting drugs to the injured vessel wall, providing strong localised action without systemic side effects (11). Several targeted fusion proteins with anticoagulation (12, 13) and fibrinolytic properties (3, 14–16) have been developed in recent years. In these studies antibody-targeted delivery of thrombin and factor Xa inhibitors as well as thrombolytic drugs showed strong anticoagulation and effective thrombolysis with minimal adverse effects in experimental models of thrombosis, pulmonary embolism and stroke.

Gene therapy has emerged as another potential site-specific therapeutic approach. An ideal opportunity for localised gene delivery is available during the harvesting and preparation of veins for implantation. This technique allows the removal of the transduced gene after exposure avoiding washout into the circulation, thus minimising systemic effects. Preclinical studies have demonstrated reduction in vein graft failure based on the expression of a variety of transgenes. For example, reduced nitric oxide production in grafts was altered by nitric oxide synthase transfer leading to higher nitric oxide levels, decreased platelet aggregability and strong suppression of adhesion molecules such as VCAM-1 and ICAM-1 (17). The direct competition with these receptors by over-expressing soluble adhesion molecules has also been proven successful (18). A novel way of blocking the adhesion molecule VCAM-1 by adenoviral gene transfer of inert competing fusion molecules has been recently described but not yet been tested in the setting of graft failure (19). Another study shows that reduced thrombomodulin expression in grafts causing enhanced local thrombin generation can be successfully attenuated by thrombomodulin gene transfer *in vivo* (20). Others have over-expressed natural matrix metalloproteinase inhibitors to reduce cell migration-mediated vessel remodelling (21).

In their study reported in this issue of *Thrombosis and Haemostasis*, Thomas et al. successfully developed a pig model allowing continuous measurement of blood flow and the production of flow-restricting thrombi (cyclic flow reductions; CFR) in

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autologous saphenous vein grafts (2). This new model was then used to test adenoviral overexpression of tissue plasminogen activator (tPA). As tPA is the primary intravascular activator of plasminogen promoting fibrinolysis, and decreased tPA expression was observed in vein grafts, this approach might be useful to keep the graft in an anti-thrombotic state thereby reducing early graft thrombosis, increasing graft blood flow and avoiding graft failure. It has been shown previously that cultured endothelial cells transduced with tPA and then seeded onto collagen-coated vascular graft have less platelet deposition and fibrin accumulation than non-transduced control grafts. This provided initial clues that higher tPA release may contribute to a local enhancement of thrombolysis in grafts (22). Furthermore, it has been shown that platelet aggregation promotes neointima formation and superimposed atherogenesis (23). Timely inhibition of thrombosis after implantation has the potential to reduce late complications by inhibiting the pro-inflammatory triggers of early thrombosis.

Thomas et al. found that non-transduced grafts examined on the day of surgery developed repeated CFR. Adenovirus transduction caused tPA expression one day after engraftment and

was not only expressed in endothelial cells but also in underlying smooth muscle cells. This is of importance as endothelial cells are damaged or removed from the graft upon exposure to the arterial environment, resulting in the loss of endogenous tPA expression leaving the graft vulnerable to thrombotic incidents. Blood flow was significantly increased in tPA-transduced grafts after one day and CFR were less severe. A high blood flow during the initial graft perfusion is crucial in limiting the risk of thrombosis and graft failure.

Overall, this therapeutic approach of local targeted gene therapy with tPA is very promising as it can be directly transferred into the clinical setting. It is important that beyond the initial proof of concept, long-term animal studies and hopefully clinical studies will follow, looking at the impact of the early intervention on late stage graft failure. Recent advances in vascular imaging will be valuable for the monitoring of the impact of early interventions on the progression of vein graft disease. Localised pharmacological or gene therapeutic approaches are important new developments that will allow strong localised effects where needed, without creating systemic side effects.

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