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Gene Therapy for Restenosis
Current Status

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Abstract

Atherosclerosis is a major cause of morbidity and mortality in Western world. Vascular occlusion caused by atherosclerosis usually requires invasive treatment, such as surgical bypass or angioplasty. However, bypass graft failure and restenosis limit the usefulness of these procedures, with 20% of patients needing a new revascularisation procedure within 6 months of angioplasty. Numerous pharmacological agents have been investigated for the prevention of restenosis but none has shown undisputed efficacy in clinical medicine.

Gene transfer offers a novel approach to the treatment of restenosis because of easy accessibility of vessels and already existing gene delivery methods. It can be used to overexpress therapeutically important proteins locally without high systemic toxicity, and the therapeutic effect can be targeted to a particular pathophysiological event. Promising results have been obtained from many pre-clinical experiments using therapeutic genes or oligonucleotides to prevent restenosis. Early clinical trials have shown that plasmid- and adenovirus-mediated vascular gene transfers can be conducted safely and are well tolerated. *Ex vivo* gene therapy with E2F-decoy succeeded in reducing graft occlusion rate after surgical bypass in a randomised, double-blind clinical trial.

In the future, further development of gene delivery methods and vectors is needed to improve the efficacy and safety of gene therapy. Also, better knowledge of vascular biology at the molecular level is needed to find optimal strategies and gene combinations to treat restenosis. Provided that these difficulties can be solved, gene therapy offers an enormous potential for clinical medicine in the future.

Despite significant advances in prevention, atherosclerosis remains the leading cause of death in the Western world. Vascular occlusion caused by atherosclerosis in coronary or lower limb arteries is often treated with surgical revascularisation or percutaneous transluminal (coronary) angioplasty, PT(C)A. Since first introduced in 1970s, PTCA/PTA has become a well established technique in the treatment of myocardial and lower limb ischaemia. However, the usefulness of PTCA/PTA is limited by restenosis that occurs in 20 to 30% of patients despite the successful procedure and 20% of patients need a new revascularization procedure within a 6 months.[1,2] Restenosis rates may be decreased by using stents in carefully selected lesions, but in-stent restenosis remains a notable clinical problem.[2] Numerous agents have been used to prevent restenosis and encouraging preclinical

results have been obtained.[1] Despite this intensive research and a large number of clinical trials, no pharmacological therapy against restenosis has yet been useful in clinical medicine.

Blood vessels are among the most feasible targets for gene therapy because of ease of access, and because in most cases only a temporary expression of the transfected gene may be required to achieve a therapeutic effect.[3] Therefore, gene therapy provides a new promising approach for the prevention of restenosis. It has several advantages compared with traditional pharmacological therapy. The effect of gene therapy can be aimed directly to a specific event in the cell cycle, the local expression of protein coded by transgene is high without elevated systemic concentration, and a long therapeutic effect can be achieved with a single treatment. In this review we discuss the current stage of gene therapy for the treatment of restenosis.

1. Vascular Gene Transfer

Gene transfer against restenosis can be conducted using viral or non-viral vectors through intravascular or extravascular routes. Furthermore, an *ex vivo* strategy meaning the transfection of autologous cells *in vitro* and their subsequent installation into vessel wall or adventitia can be used. The effectiveness of gene therapy is determined by the entry of the new genetic material into cells and the expression of the transfected gene in the target tissue. It is often limited by the compromised efficiency of the biological and physical targeting methods. Even with powerful viral vectors, gene transfer efficiency in the target tissue is often low and there is a risk of transfecting ectopic organs not affected by the disease. Therefore, new strategies to target vectors to the vessel wall have recently been introduced.[4-8] However, much basic research is still needed to develop efficient and regulated vectors and methods for gene transfer before gene therapy can be used as a standard clinical treatment in cardiovascular diseases.

1.1 Vectors

If naked plasmid DNA is used for gene transfer, only a small number of cells will be transfected because of the poor uptake of plasmid into the cell. Therefore, carrier molecules and virus vectors are used to increase the transfection efficiency (table I). An ideal vector for gene therapy would be one that entails efficient transfection and long-term, stable and regulated transgene expression in target tissue without the risk of biodistribution and adverse effects, such as inflammation, immunogenicity or host cell mutagenesis. It is clear that such a vector does not yet exist and is unlikely to be developed in the near future.

1.1.1 Viral Vectors

In viral vectors, the sequences essential for replication are replaced by DNA sequences from the gene to be transferred making the virus replication deficient, that is, it cannot spread any infection in the treated patient. Although the gene transfer efficiency remains relatively low, it should be sufficient for the treatment of restenosis or at least to evaluate the therapeutic potential of the treatment gene.

Adenoviruses are currently the most widely used viral vectors for gene transfer in the vascular system.[3,9-11] Adenoviruses enter cells via specific receptors, and after entering the nucleus the transgene remains extrachromosomal and does not integrate into the host genome.[12] Therefore, first generation adenoviruses cause only transient gene expression, usually lasting from a few days to 2 weeks depending on the target tissue.[13] For the treatment of restenosis a local, temporary 2- to 3-week expression of the transfected gene is preferred, as a systemic, longer expression time may cause pathological consequences.[3,14]

Adenoviruses can be produced in high titres and they have an ability to transfect both proliferating and non-proliferating cells. Their efficiency is dependent on the presence of Coxsackie-adenovirus receptor (CAR) which is expressed to varying degrees in most human tissues.[15,16] In addition, up-regulation of vascular cell adhesion molecule

Table I. Vectors used for vascular gene transfer

Vector	Advantages	Disadvantages
Naked plasmid DNA	Easy to produce	Very low transfection efficiency
	Safer than viral vectors	Transient expression
Adenovirus	High transfection efficiency	Immunological and inflammatory reactions
	Relatively high DNA-capacity (<37kb)	Transient expression
	Transient expression	Cytotoxic effects at high concentrations
	Easy to produce in high titres	
	Transfects proliferative and quiescent cells	
Adeno-associated virus (AAV)	Long transgene expression	Limited DNA capacity (4-5kb)
	Low inflammatory and immune responses	Difficult to produce
	High virus titres	
	Transfects proliferative and quiescent cells	
Baculovirus	Relatively high transfection efficiency	Transient expression
	High DNA-capacity	Immunological and inflammatory reactions
	Transient expression	
	Easy to produce in high titres	
	Rapid construction of recombinant baculoviruses	
Herpes simplex virus (HSV-1)	High transfection efficiency	Unable to transduce non-dividing cells
	High DNA capacity (<150kbp)	Cytotoxicity and neurotoxicity
	Easy to produce in large quantities	
	Low pathogenicity	
Epstein-Barr virus	High DNA-capacity (<150kbp)	Unable to transduce non-dividing cells
	High transfection efficiency	Difficult to construct viral mutants
	Persistence in the host	
	Extrachromosomal replication	
Lentivirus	High DNA-capacity	Low transfection efficiency
	Transfects proliferative and quiescent cells	Difficult to produce
	Stabile gene expression	Low titres
	No immune response	Non-specific integration in the chromosome
Retrovirus	Stabile gene expression	Low virus titres
	Relatively easy to produce	Low transfection efficiency
		Transfects only dividing cells
		Limited DNA capacity

(VCAM-1) in endothelial cells of atherosclerotic lesions favours adenovirus-mediated gene transfer.[17] This makes adenovirus a promising vector for gene therapy against restenosis.

The problems with the use of first-generation adenoviral vectors are related to immunological and inflammatory reactions they cause. These complications should be reduced with second- and third-generation vectors that have larger deletions of viral genome, or with tissue specific adenoviruses with modified attachment receptors.[12] Adenovirus infection is not associated with malignancies and oral adenoviral vaccines have been used in humans for decades. These circumstances, in addition to the fact that adenovirus-mediated gene transfer leads only to a temporary expression of transgene, favour its use in human gene therapy. Although adenoviral gene transfer in vascular system also transfects many unwanted organs and peripheral blood monocytes, it has been found feasible and well tolerated in clinical trials.[10,11,13]

Retroviruses have also been used for gene transfer in the vascular system.[18] They enter the cells via specific receptors after which genomic RNA is reverse transcribed to DNA which integrates into the host genome. Thus, retroviral gene transfer

leads to a long lasting gene expression. Retroviruses can transfect only proliferating cells and they can be produced only with relatively low titres, and therefore their transfection efficiency remains low.[18] This quality makes the practical use of retroviruses difficult in other than extravascular or *ex vivo* approaches. However, the development of new pseudotyped retroviruses with increased virus titres will probably generate better transfection efficiency. In addition, extracellular matrix targeted retroviruses that deliver the transgene into the target area more efficiently have been developed.[7]

Baculoviruses, adeno-associated viruses, herpesviruses, Epstein-Barr virus and lentiviruses have also been used in pre-clinical gene transfer studies but their usefulness for vascular gene therapy need to be further evaluated before planning therapeutic approaches.[3,19-21]

1.1.2 Non-Viral Vectors

The most widely used non-viral vectors for vascular gene therapy are plasmid DNA with or without carrier molecules.[18,22,23] The gene delivery efficiency of plasmid DNA to cytoplasm can be improved with liposome complexes but it still remains quite low.[18] Only a small fraction of plasmid DNA enters the nucleus, where it remains extrachromosomal and directs a transient transgene expression that lasts for a few weeks.[22,23] Plasmid DNA with cationic polymers are more efficient for vascular gene therapy but their suitability for clinical trials needs to be further evaluated.[23] In addition, antisense oligonucleotides can be used for gene transfer.[24] Because of constant blood flow, any intravascular approach requires an efficient vector. Therefore, the use of non-viral vectors in the treatment of restenosis is practically limited to extravascular and *ex vivo* strategies.

Safety concerns, such as immunogenicity and oncogenic properties, are less important with non-viral plasmid DNA gene transfer. Even high doses of naked vascular endothelial growth factor (VEGF) plasmid in human ischaemic legs has not lead to any toxic effects.[25] Furthermore, plasmids are easier to produce and purify in large quantities than viral vectors. Because of the safety aspects and relatively easy ability to process these vectors, there is a great interest in the development of targeted non-viral gene delivery methods.

1.2 Methods

In the treatment of restenosis, an intravascular approach using a catheter is the most commonly used gene delivery method (figure 1a).[10,11,26,27] Various types of catheters, such as microporous, hydrogel-coated and channel balloon catheters, are available for gene transfer into vessel wall.[27,28] These can be used simultaneously with the PTCA/PTA operation, thus avoiding extra operations and unnecessary risks for patients. For example, a perfusion-infusion catheter that allows a long infusion time in arteries as a result of non-disrupted blood flow into the distal myocardium or skeletal muscle, has been used in clinical trials in heart and lower limbs.[10,11,26] Unfortunately, the efficiency of intravascular gene transfer through human atherosclerotic lesions and lipid-rich atheroma is low.[10] The lesions are frequently rich in connective tissue and contain only a limited number of transfectable cells. Therefore, needle catheters that perform the injection from the inside of the vessel lumen through the atherosclerotic lesions have been designed for a direct delivery of genes into the artery wall.

Gene transfer vectors can also be delivered to the artery adventitially with a biodegradable collar, biodegradable gel or by direct injection into the adventitia (figure 1b).[13,18,22,23] Although perivascular gene delivery is mainly useful during surgical procedures, such as by-pass operations, anastomosis, prosthesis placement and endarterectomies, it may also be useful to prevent restenosis. Even though the majority of transgene expression is located in adventitia, secreted gene products seem to have a biological effect in the media and intima layers of the vessels.[22] Furthermore, with adventitial delivery the leakage to the systemic circulation is less than delivery via the intravascular route.[13]

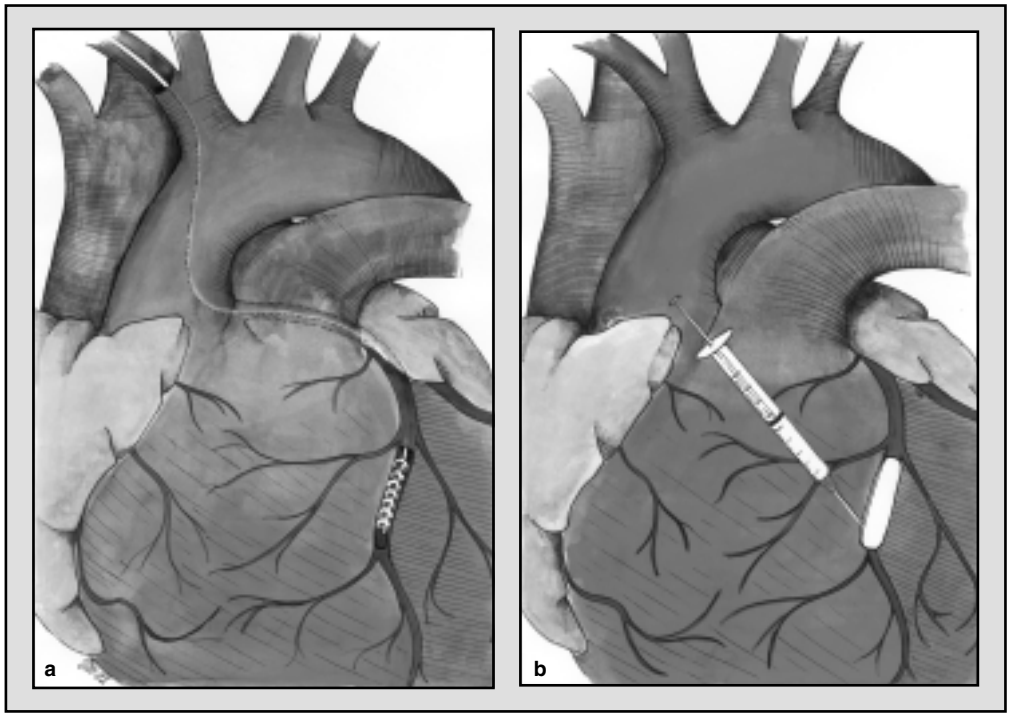

Fig. 1. (**a**) Intra-arterial catheter mediated gene transfer to coronary artery. (**b**) Adventitial gene transfer to coronary artery.

Ex vivo gene transfer can be used to deliver transgenes to their target. In *ex vivo* gene therapy, cells or other parts of organs are isolated and transfected and then implanted back to the same individual. Thus, the transfection is made outside the patient and no gene transfer vehicles are transferred into the body. This makes it a very safe method of gene therapy but it is difficult to perform in practice, which limits its usefulness in clinical practice.

2. Targets of Gene Therapy for Restenosis

Restenosis is defined as the shrinkage of vessel lumen cross-sectional area after vascular intervention. The understanding of events leading to this treatment failure has increased substantially during past years. Restenosis is a process involving platelet activation, thrombosis, leukocyte adhesion and recruitment, vessel vasoconstriction and remodeling, and proliferation and migration of neointimal cells and their participation in matrix formation.[1,29] In response to arterial injury, medial smooth muscle cells (SMC) migrate, proliferate and produce extracellular matrix, leading to the development of neointimal thickening known as neointimal hyperplasia. SMC proliferation and migration are key factors in the development of restenosis and most gene therapy strategies are directed towards these targets.[3] However, all aspects of restenosis need to be considered when searching for optimal gene treatment. The growth factors and cytokines that reduce restenosis *in vivo* are listed in table II.

2.1 Smooth Muscle Cell Migration and Proliferation

Platelet derived growth factor (PDGF) is one of the most potent chemoattractants of vascular

Table II. Growth factors and cytokines that reduce restenosis *in vivo*

Target	Treatment gene
Intimal hyperplasia	VEGF/VEGF-A, VEGF-C, VEGF-D, Retinoblastoma, βARKct eNOS, iNOS, NF-κB and E2F decoys, TIMP-1, TIMP-2, COX, TK, *c-myb*, *c-myc*, *cdk-2*, *cdc-2*, Gax, CyA, p16, ras, bcl, Gβγ, GATA-6, p21, p27, p53, PCNA antisense oligonucleotides, Dominant-neg. H-ras, Hirudin, Fas ligand, sdi-1, blocking PDGF or TGF-β expression, IFN-β, CNP, HSV-tk, ATF.BPTI, HGF, EC-SOD
Arterial cytoprotection	VEGF/VEGF-A, VEGF-C, eNOS, iNOS, COX
Thrombosis	Hirudin, tPA, thrombomodulin, COX, TFPI
Vascular remodelling	HK

ATF.BPTI = amino-terminal fragment linked to bovine pancreas trypsin inhibitor; **βARKct** = C-terminal β-adrenergic receptor kinase; **COX** = cyclooxygenase; **CNP** = C-type natriuretic peptide; **CyA** = cytosine deaminase; **EC-SOD** = extracellular superoxide dismutase; **eNOS** = endothelial NOS; **GATA** = gata-binding protein; **Gax** = growth arrest-specific homeo box; **HGF** = hepatocyte growth factor; **HK** = human tissue kallikrein; **HSV-tk** = herpes simplex virus thymidine kinase; **IFN-β** = interferon-β; **iNOS** = inducible NOS; **NF-κB** = nuclear factor-κB; **NOS** = nitric oxide synthase; **PCNA** = proliferating cell nuclear antigen; **PDGF** = platelet-derived growth factor; **sdi-1** = senescent cell-derived inhibitor; **TFPI** = tissue factor pathway inhibitor; **TGF-β** = transforming growth factor β; **TIMP** = tissue inhibitor of metalloproteinase; **TK** = thymidine kinase; **tPA** = tissue plasminogen activator; **VEGF** = vascular endothelial growth factor.

SMC.[30] PDGF is composed of separate polypeptide chains -A, -B, -C, and -D, which are regulated independently and are expressed as homo- or heterodimers.[31-37] Corresponding receptor dimers α and β belong to a closely related family of tyrosine kinase receptors. PDGFs mediate neointimal growth after vascular injury in animal models and are expressed in human atherosclerotic lesions.[38,39] (Rutanen et al. Unpublished data). In addition, they stimulate cell cycle regulating genes, chemotaxis, induction of matrix formation and protection from apoptosis.[31-37] Restenosis caused by neointimal growth could be blocked by inhibition of PDGF expression with antibodies against PDGF or its receptors.[40-42] However, clinical use of antibodies carries the risk of immunoreactivity and thus gene therapy approaches with PDGF-receptor decoys or antisense oligonucleotides against PDGF mRNA have been introduced.[24,43]

Matrix metalloproteinases (MMPs) are involved in migration of SMCs from the medial layer and can be inhibited by overexpressing tissue inhibitors of metalloproteinases (TIMPs). Gene transfer of TIMP-1 and TIMP-2 has been shown to reduce neointimal growth in animal models (Puhakka et al. Unpublished data).[44]

Early markers of SMC activation that lead to growth factor stimulation, such as nuclear oncogenes, are rapidly detectable after arterial injury.[1] Antisense oligonucleotides directed against these proto-oncogenes and cell cycle regulators, for example *c-myb, c-myc, cdk-2*, nuclear factor kappa B (NF-κB) and E2F have been used to decrease neointimal thickening in animal models.[45,46] In addition, the ability of gene transfer to affect these and many other cell cycle regulators (table I) has been evaluated in various pre-clinical studies.

Since intravascular manipulation causes damage to the endothelium, it is hypothesised that rapid re-endothelialisation of the arterial wall after balloon dilation should reduce restenosis. Vascular endothelial growth factor (VEGF) is a growth factor family that induces endothelial cell proliferation, regrowth and migration.[47] VEGF-A is expressed as five isoforms consisting of different polypeptides which differ in their extracellular matrix binding properties. Other members of the VEGF family are VEGF-B, -C, -D and -E, and placental growth factor. VEGF-A and -C gene therapy after arterial injury has been shown to attenuate neointimal growth.[22,48,49] In a controlled clinical study, VEGF-A gene transfer has been used safely and shown to be feasible in conjunction with coronary and lower limb angioplasty.[11,26] Hepatocyte growth factor is another gene that can reduce neointimal hyperplasia through re-endothelialisation.[50]

Nitric oxide (NO) inhibits SMC migration and proliferation. Thus, long-term NO production at a lesion site may prevent restenosis. It has been

shown that overexpression of NO synthase has a preventive effect on neointimal growth[51-53] and the therapeutic effect of VEGF-A gene therapy is attenuated in the presence of the NO inhibitor L-nitro arginine methyl ester (L-NAME).[22] Thus, some of the effects of VEGF are probably due to increased NO production. VEGF and NO are also cytoprotective compounds because they stimulate endothelial cell re-growth, repair and survival. However, NO can also participate in apoptosis and superoxide radical formation, and may have less favourable effects in advanced lesions.[54]

It is suggested that a cytotoxic 'suicide' gene therapy approach could prevent intimal hyperplasia and herpes simplex virus thymidine kinase (HSV-tk) gene transfer with ganciclovir treatment has been shown to reduce restenosis in animal models.[55,56] However, careful evaluation of safety and biodistribution is needed before these approaches can be approved for clinical use.

2.2 Thrombosis

Thrombosis is recognised as a major step in initiating restenosis.[1] Intravascular interventions cause damage to the endothelium and media exposing thrombogenic molecules (collagen, lipids from plaque), which activate platelets and the thrombotic cascade. Acute thrombosis has been managed with antiplatelet agents in clinical practice for decades but thrombosis is still a concern for long-term patency of the arteries. Gene transfer of antithrombotic agents, such as tissue plasminogen activator (tPA), hirudin or tissue factor pathway inhibitor, may prevent restenosis by protecting the vessel from thrombus formation shortly after vascular manipulations.[57,58]

2.3 Vascular Remodelling

Although the majority of gene therapy interventions are directed against neointimal hyperplasia, it is suggested that a long-term success of angioplasty is determined by vascular remodelling rather than intimal hyperplasia.[59-61] In addition, changed shear stress conditions may induce neointimal hyperplasia.[62,63] Therefore, genes that have an effect on negative remodelling in addition to intimal hyperplasia, such as VEGF or NO synthases may be very useful in the prevention of restenosis. Prevention of negative vascular remodelling with human tissue kallikrein gene transfer has been shown to reduce neointimal hyperplasia in an animal model.[64]

3. In-Stent Restenosis

In-stent restenosis is the result of thrombus formation, acute inflammation and neointimal growth.[65-67] As negative remodeling is mostly prevented mechanically by stent struts, neointimal growth is the most prominent factor in occluding the treated vessel and should be seen as the main target for the treatment.

Stents themselves can be used as gene delivery devices. Stents coated with polymers could release gene transfer vectors or oligonucleotides in a controlled fashion.[68] Also, an *ex vivo* approach using genetically engineered endothelial cells attached to stent surface could be a possibility.[69] In-stent restenosis is still a relatively new problem and gene therapy against it is just taking its first steps. Importantly, promising clinical results in preventing in-stent restenosis have recently been obtained with sirolimus-eluting stents.[70]

4. Clinical Trials

In spite of the promising results aimed to prevent neointimal hyperplasia with gene therapy in animal experiments, only a few clinical trials have taken place so far (table III). On the way to the clinic, vascular gene transfer faces the same problems as pharmacological therapy: how to transfer positive pre-clinical results to clinically successful therapy. The relationships between animal models and clinical circumstances need to be carefully evaluated in order to recognise the best mechanisms to prevent restenosis. In addition, larger double-blinded, placebo-controlled studies are needed before the usefulness of gene therapy for restenosis can be evaluated.

Laitinen et al.[10] have demonstrated the safety and feasibility of intravascular gene therapy to

Table III. Clinical gene therapy and recombinant protein trials to prevent restenosis

Investigator/company	Location	Disease	Delivery route	Treatment	Vector or protein	Patients
Isner JM et al. [71]	St. Elizabeth's Medical Center, Boston, MA, USA	PAD, post PTA-DA restenosis	Hydrogel-coated balloon catheter after angioplasty	VEGF-A	Naked DNA	28
Ylä-Herttuala S. et al. [10]	Kuopio University Central Hospital, Kuopio, Finland	PAD	Infusion-perfusion catheter after angioplasty	LacZ	Adenovirus	10
Ylä-Herttuala S. et al. [11]	Kuopio University Central Hospital, Kuopio, Finland	PAD, post PTA restenosis	Infusion-perfusion catheter after angioplasty	VEGF-A	Liposome/ Adenovirus	54
Ylä-Herttuala S. et al. [26]	Kuopio University Central Hospital, Kuopio, Finland	Coronary heart disease, post-PTCA restenosis	Infusion-perfusion catheter after angioplasty	LacZ / VEGF-A	Liposome / Adenovirus	108
Mann M, et al. [72]	Multicentre, USA	Vein-graft stenosis, infrainguinal by-pass surgery	Pressure *ex vivo* delivery	E2F Decoy	Oligonucleotide	41[a]
Ark Therapeutics Ltd. (unpublished data)	University Central Hospitals of Kuopio, Oulu and Tampere, Finland	Severe PAD	Adventitial delivery with biodegradable collar	VEGF-A	Plasmid/ Liposome	6[a]

a Currently enrolling patients.

PAD = peripheral arterial disease; **PTA** = percutaneous transluminal angioplasty; **PTA-DA** = PTA-directional atherectomy; **PTCA** = percutaneous transluminal coronary angioplasty; **VEGF** = vascular endothelial growth factor.

human peripheral arteries with high adenovirus titres from 1×10^8 to 4×10^{10} plaque-forming units, resulting in a maximum of 5% transfection efficiency in arterial cells. In a controlled study of catheter mediated VEGF-A gene transfer with plasmid or adenovirus to infra-inguinal arteries after PTA, increased vasculature was registered in the VEGF treatment groups in the follow-up angiography.[11] In addition, catheter-mediated plasmid/liposome VEGF-A gene transfer to human coronary arteries in conjunction with PTCA was well tolerated and safely used in a randomised, double-blinded, placebo-controlled study. However, the study failed to show any therapeutic effect of gene transfer.[26]

To date, there is only one report of a clinical trial successful in preventing neointimal hyperplasia: the restenosis-related problem of bypass grafts failure was reduced with an E2F decoy by using an *ex vivo* gene transfer method.[72]

5. Ethics and Safety of Vascular Gene Therapy

Although the aim of vascular gene therapy is to achieve a local transfection of somatic cells, there is a risk of transfecting cells in testicles and ovaries when using effective non-targeted vectors.[13,14,73] In addition, the use of cell cycle regulators and growth factors involved in vascular development and growth includes the risk of unwanted angiogenesis in other vital organs. This effect is involved in tumour growth, diabetic proliferative retinopathy, macular degeneration, rheumatoid arthritis, osteomyelitis and neovascularization of atherosclerotic plaques.[14] Therefore, a short term or controlled expression of transgenes is preferred.

Although systemically administered recombinant proteins may produce adverse effects,[74] or even acceleration of atherosclerosis,[75,76] local gene therapy with the same growth factors using plasmids, adenovirus and retrovirus vectors has been well tolerated in early clinical studies.[10,11,26] The only adverse effects seen are a transient increase in serum C-reactive protein and a transient decrease in platelet number.[11,26]

Provided that safety and efficacy of gene therapy are established, there should be no fundamental ethical issues that prevent the use of transient gene transfer methods. However, it must be kept in mind that like any other drug, too large a dose of a

viral vector may lead to serious consequences or even death, especially in immunocompromised patients.[77]

6. Conclusion

Gene therapy offers a promising new approach for the treatment of post-angioplasty restenosis. Several genes and proteins have been tested in preclinical studies to prevent restenosis and methods of gene delivery to the target tissue already exist. First clinical trials have shown that vascular gene transfer is well tolerated and can be used safely, and some promising results have been obtained. However, we are still at the beginning of making gene therapy a useful treatment method in everyday clinical medicine. Several theoretical and practical difficulties need to be solved and larger double-blind clinical trials are needed before gene therapy can really answer the needs of clinicians.

One of the most important difficulties in gene therapy is the low transfection efficiency in the target tissue. Wide biodistribution occurs often in connection with good transfection efficiency. These difficulties will be solved with more efficient and specific gene delivery methods and tissue specific vectors. On the other hand, efficiently secreted cytokines, such as VEGF, may lead to therapeutic response even with less effective gene transfer methods.

Pre-clinical studies have only investigated single gene treatments to prevent restenosis. Considering the complex pathophysiology, it is unlikely that any single gene will solve the problem. Therefore, further characterisation of the vascular biology at the molecular level is needed to identify and optimise new therapeutic strategies. New molecular technologies, such as the laser capture microscope, provide more precise approaches to study the pathogenesis of restenosis and atherosclerosis. With DNA-array technology and through the human genome project more therapeutic factors will be identified to provide optimal 'gene cocktails' to prevent restenosis more efficiently.

The development of gene therapy in the vascular system is proceeding rapidly because of the great importance and easy accessibility of the blood vessels to gene-based treatments. Although only a few clinical trials have been performed, it is clear that in the future gene therapy may provide us with a novel treatment against post-angioplasty restenosis.

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