

Angiogenesis of the heart.

Kutryk MJ, Stewart DJ.

Despite continued advances in the prevention and treatment of coronary artery disease, there are still a large number of patients who are not candidates for the conventional revascularization techniques of balloon angioplasty and stenting, or coronary artery bypass grafting (CABG). Therapeutic angiogenesis, in the form of the administration of growth factor protein or gene therapy, has emerged as a promising new method of treatment for patients with coronary artery disease. The goal of this strategy is to promote the development of supplemental blood conduits that will act as endogenous bypass vessels. New vessel formation occurs through the processes of angiogenesis, vasculogenesis, and arteriogenesis, under the control of growth factors such as those that belong to the vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and angiopoietin (Ang) families of molecules. Preclinical studies have suggested that such an approach is both feasible and effective; however many questions remain to be answered. This review will address the elements of pharmacologic revascularization, focusing on gene and protein-based therapy. The important growth factors, the vector (for gene therapy), routes of delivery, the desired therapeutic effect, and quantifiable clinical end points for trials of angiogenesis will all be addressed.

Gene therapy in cardiovascular diseases.

Tamirisa KP, Mukherjee D.

Established modalities of treatment for obstructive coronary artery disease include medical therapy, bypass surgery and percutaneous coronary intervention. Similarly, conventional treatment of congestive heart failure is also limited to medical therapy, temporary assist devices and transplantation. A significant subset of patients with severe symptomatic coronary artery disease and end stage heart failure is not eligible for these traditional methods of treatment. In spite of maximal medical and revascularization therapy, these patients may not get adequate symptomatic benefit. After a decade of investigations, gene therapy has emerged as a promising therapeutic option for this group of patients. This review discusses newer modalities of therapy for this subset, including therapeutic angiogenesis with growth factors and cell transplantation.

Vascular growth factors for coronary angiogenesis.

Lee CH, Smits PC.

Coronary artery disease not amendable to conventional revascularization poses a significant medical problem. Advances in the understanding of blood vessel growth have given rise to efforts to develop novel therapeutic approaches for these "no-option" patients. Therapeutic angiogenesis makes use of the administration of angiogenic growth factor protein or gene to promote the development of endogenous collateral vessels in ischemic myocardium. Among the growth factors that play a role in blood vessel growth and development, vascular endothelial growth factors (VEGFs) and fibroblast growth factors have been the most extensively studied. Various methods of delivery have been used to enhance localization and persistence. Preliminary animal experiments have been promising with evidence of capillary formation at the target myocardium after growth factor administration. Initial phase I and II clinical trials have been undertaken. Preliminary information on efficacy is beginning to become available, raising hopes and questions about the future direction and potential success of therapeutic angiogenesis as a clinical approach to the treatment of myocardial ischemia. Although the initial clinical results are encouraging, real efficacy has still to be proven and the potential side-effects of these potent angiogenic growth factors remain a concern. Large-scale, randomized, and placebo-controlled studies will be required to demonstrate the true clinical benefit of this novel therapeutic treatment for ischemic heart disease.

The T(-786)C endothelial nitric oxide synthase genotype is a novel risk factor for coronary artery disease in Caucasian patients of the GENICA study.

Paolo Rossi G, Cesari M, Zanchetta M, Colonna S, Maiolino G, Pedon L, Cavallin M, Maiolino P, Pessina AC.

We investigated the association of polymorphisms in the promoter region and exon 7 endothelial nitric oxide synthase (eNOS) gene with coronary artery disease (CAD). Endothelial dysfunction foretells cardiovascular events and can be genetically determined. We genotyped for the promoter (T(-786)C) and exon 7 (Glu298Asp, G(894)T) polymorphisms in 1,225 subjects; 1,106 were consecutive patients undergoing coronary angiography and 119 control subjects without any cardiovascular risk factors. Genotyping was performed with melting curve analysis of polymerase chain reaction products from allele-specific acceptor and donor probes that were 5'- and 3'-end labeled with LCRed640 and fluorescein, respectively; CAD was assessed by quantitative coronary angiography. We performed multiple logistic regression analysis for the effect of the T(-786)C, the missense Glu298Asp variant, and other coronary risk factors on two- and three-vessel CAD. The overall genotype distribution of T(-786)C (CC = 17.7%, CT = 40.4%, and TT = 41.9%) and Glu298Asp (GG = 43.3%, GT = 37.0%, and TT = 19.7%) was consistent with the Hardy-Weinberg equilibrium. The regression analysis showed that the T(-786)C, but not the missense Glu298Asp variant, significantly predicted CAD, independent of other risk factors. Compared with TT homozygous, subjects carrying the C allele had a significant ($p = 0.002$) increase in the odds ratio of harboring two- or three-vessel CAD of 1.672 (95% confidence interval, 1.062 to 2.527). A subgroup analysis confirmed this effect of the T(-786)C polymorphism in men ($p = 0.007$), cigarette smokers ($p = 0.001$), subjects older than 60 years of age ($p = 0.007$), with hypercholesterolemia ($p = 0.011$), low high-density lipoprotein cholesterol ($p = 0.006$), and overweight or with obesity ($p = 0.041$). The C allele at the T(-786)C endothelial nitric oxide synthase polymorphism is associated with a higher risk of multivessel CAD in Caucasians.

Oxidized LDL receptor gene (OLR1) is associated with the risk of myocardial infarction.

Tatsuguchi M, Furutani M, Hinagata J, Tanaka T, Furutani Y, Imamura S, Kawana M, Masaki T, Kasanuki H, Sawamura T, Matsuoka R.

Lectin-like oxidized low-density lipoprotein receptor (LOX-1/OLR1) has been suggested to play a role in the progression of atherogenesis. We analyzed the OLR1 gene and found a single nucleotide polymorphism (SNP), G501C, in patients with ischemic heart disease from a single family, which resulted in the missense mutation of K167N in LOX-1 protein. We compared the group of patients with myocardial infarction (MI) (n=102) with a group of clinically healthy subjects (n=102), and found that the MI group had a significantly high frequency of 501G/C+501C/C (38.2%) compared with the healthy group (17.6%; $p < 0.002$). The odds ratio for the risk of MI associated with the 501G/C+501C/C genotype was 2.89 (95% CI, 1.51-5.53). These findings suggest that OLR1 or a neighboring gene linked with G501C SNP is important for the incidence of MI. Manipulating LOX-1 activity might be a useful therapeutic and preventative approach for coronary artery disease, especially for individuals with the G501C genotype of OLR1.

Novel LPL mutation (L303F) found in a patient associated with coronary artery disease and severe systemic atherosclerosis.

Saika Y, Sakai N, Takahashi M, Maruyama T, Kihara S, Ouchi N, Ishigami M, Hiraoka H, Nakamura T, Yamashita S, Matsuzawa Y.

BACKGROUND: Patients with lipoprotein lipase (LPL) deficiency had been generally thought to be spared accelerated atherosclerosis in spite of a marked elevation of plasma triglyceride levels. However, it has been recently reported that some heterozygous and homozygous LPL-deficient patients are associated with premature atherosclerosis. In this paper, we report a 55-year-old type I hyperlipidaemic patient with a novel missense mutation in the LPL gene. **PATIENT AND RESULTS:** The patient had suffered from coronary artery disease, abdominal aortic aneurysm, and stenoses of the bilateral renal arteries and superficial femoral arteries. Sequencing of the genomic DNA revealed that the patient was a homozygote for the mutation, a G to C transition at nucleotide position 1069 in the exon 6, resulting in an amino acid substitution of Phe for Leu303 (L303F). Approximately approximately 6% and approximately 40% of normal LPL activity and LPL mass, respectively, were detected in the patient's postheparin plasma. An in vitro expression study demonstrated that COS7 cells transfected with L303F mutant cDNA produced a 40% amount of LPL protein in cell lysates compared with normal cDNA, but no protein was detected in the media. Lipoprotein lipase activity was completely absent in both lysates and media of the cells transfected with the mutant cDNA, suggesting that this mutation in the LPL gene results in the production of a functionally inactive protein. **CONCLUSION:** This case suggests that the LPL missense mutation (L303F), which impairs lipolysis but preserves the LPL mass, is proatherogenic.

Therapeutic angiogenesis with vascular endothelial growth factor in peripheral and coronary artery disease: a review.

Kusumanto YH, Hospers GA, Mulder NH, Tio RA.

Therapeutic angiogenesis constitutes an alternative treatment for patients with extensive tissue ischaemia in whom primary vascular reconstruction procedures are not feasible or have previously failed. At present vascular endothelial growth factor (VEGF) has been the most widely used angiogenic factor in experimental and human clinical trials. Early clinical data provide evidence that gene transfer of the VEGF gene can achieve beneficial angiogenesis, with minimal side-effects. Ongoing phase III clinical studies will reveal definitive efficacy.

Association between the severity of angiographic coronary artery disease and paraoxonase gene polymorphisms in the National Heart, Lung, and Blood Institute-sponsored Women's Ischemia Syndrome Evaluation (WISE) study.

Chen Q, Reis SE, Kammerer CM, McNamara DM, Holubkov R, Sharaf BL, Sopko G, Pauly DF, Merz CN, Kamboh MI; WISE Study Group.

Paraoxonase (PON), a high-density lipoprotein-associated enzyme, is believed to protect against low-density lipoprotein oxidation and thus affects the risk of coronary artery disease (CAD). Three polymorphisms in the PON1 (Leu55Met and Gln192Arg) and PON2 (Ser311Cys) genes have been shown to be associated with the risk of CAD in several European or European-derived populations. In the present study, we examined the associations between these three markers and the severity of CAD as determined by the number of diseased coronary artery vessels in 711 subjects (589 whites and 122 blacks) from the Women's Ischemia Syndrome Evaluation (WISE) study. WISE is a National Heart, Lung, and Blood Institute-sponsored multicenter study designed to address issues related to ischemic-heart-disease recognition and diagnosis in women. Subjects were classified as having normal/minimal CAD (<20% stenosis), mild CAD (20%-49% stenosis), and significant CAD (\geq 50% stenosis). The women who had \geq 50% stenosis were further classified into groups with one-, two-, or three-vessel disease if any of the three coronary arteries had diameter stenosis \geq 50%. No significant association was found between the PON polymorphisms and stenosis severity in either white or black women. However, among white women, when data were stratified by the number of diseased vessels, the frequency of the PON1 codon 192 Arg/Arg genotype was significantly higher in the group with three-vessel disease than in the other groups (those with one-vessel and two-vessel disease) combined (17.02% vs. 4.58%; $P=0.0066$). Similarly, the frequency of the PON2 codon 311 Cys/Cys genotype was significantly higher in the group with three-vessel disease than in the other groups combined (15.22% vs. 4.61%; $P=0.018$). The adjusted odds ratios for the development of three-vessel disease were 2.80 (95% confidence interval 1.06-7.37; $P=0.038$) for PON1 codon 192 Arg/Arg and 3.68 (95% confidence interval 1.26-10.68; $P=0.017$) for PON2 codon 311 Cys/Cys. Our data indicate that the severity of CAD, in terms of the number of diseased vessels, may be affected by common genetic variation in the PON gene cluster, on chromosome 7.

Gene and cell therapy for heart disease.

Graham RM, Bishopric NH, Webster KA.

HHeart disease is the most common cause of morbidity and mortality in Western society and the incidence is projected to increase significantly over the next few decades as our population ages. Heart failure occurs when the heart is unable to pump blood at a rate to commensurate with tissue metabolic requirements and represents the end stage of a variety of pathological conditions. Causes of heart failure include ischemia, hypertension, coronary artery disease, and idiopathic dilated cardiomyopathy. Hypertension and ischemia both cause infarction with loss of function and a consequent contractile deficit that promotes ventricular remodeling. Remodeling results in dramatic alterations in the size, shape, and composition of the walls and chambers of the heart and can have both positive and negative effects on function. In 30-40% of patients with heart failure, left ventricular systolic function is relatively unaffected while diastolic dysfunction predominates. Recent progress in our understanding of the molecular and cellular bases of heart disease has provided new therapeutic targets and led to novel approaches including the delivery of proteins, genes, and cells to replace defective or deficient components and restore function to the diseased heart. This review focuses on three such strategies that are currently under development: (a) gene transfer to modulate contractility, (b) therapeutic angiogenesis for the treatment of ischemia, and (c) embryonic and adult stem cell transfer to replace damaged myocardium.

Angiogenic gene therapy strategies for the treatment of cardiovascular disease.

Rasmussen HS, Rasmussen CS, Macko J, Yonehiro G.

Coronary artery disease (CAD) and peripheral vascular disease (PVD) are significant medical problems worldwide, and arguably the biggest medical problems in the developed world. Although substantial progress has been made in prevention as well as in the treatment of these diseases, particularly of CAD, there are a large number of patients, who despite maximal medical treatment, have substantial symptomatology, and who are not candidates for mechanical revascularization. Therapeutic angiogenesis represents a novel, conceptually appealing, treatment option for these patients. Consequently, there are several different products in clinical trials, looking at various angiogenic growth factors. A number of small, mostly open-labeled phase I or phase I/II studies have been conducted with adeno- and plasmid-based vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) gene constructs in CAD and PVD. Although these studies have provided intriguing indications that new vessel formation is possible, and that these new vessels could be functional, these studies have been too small to allow conclusions to be drawn about potential efficacy. A number of proof-of-concept studies are presently underway or planned with four different constructs Ad(GV)VEGF121.10 (BioByPass; GenVec Inc), ph-VEGF (St Elizabeth's Medical Center of Boston Inc), Ad5-FGF4 (Collateral Therapeutics Inc/Schering Inc) and NV1FGF (Aventis Pharma AG/Aventis Gencell), and should, upon completion, provide a better indication as to the potential therapeutic role of these treatment modalities in the armamentarium against atherosclerotic disease. This exciting new field is reviewed, with special emphasis on clinical trials.

Angiogenic Gene Therapy (AGENT) Trial in Patients With Stable Angina Pectoris

Cindy L. Grines, MD; Matthew W. Watkins, MD; Greg Helmer, MD; William Penny, MD; Jeffrey Brinker, MD; Jonathan D. Marmur, MD; Andrew West, MD; Jeffery J. Rade, MD; Pran Marrott, MRCP, MSc; H. Kirk Hammond, MD; Robert L. Engler, MD

Background: The angiogenic response to myocardial ischemia can be augmented in animal models by gene transfer with the use of a replication defective adenovirus (Ad) containing a human fibroblast growth factor (FGF) gene. Methods and Results: The objectives of the Angiogenic GENE Therapy (AGENT) trial were to evaluate the safety and anti-ischemic effects of 5 ascending doses of Ad5-FGF4 in patients with angina and to select potentially safe and effective doses for subsequent study. Seventy-nine patients with chronic stable angina Canadian Cardiovascular Society class 2 or 3 underwent double-blind randomization (1:3) to placebo (n=19) or Ad5-FGF4 (n=60). Safety evaluations were performed at each visit and exercise treadmill testing (ETT) at baseline and at 4 and 12 weeks. Single intracoronary administration of Ad5-FGF4 seemed to be safe and well tolerated with no immediate adverse events. Fever of <1-day duration occurred in 3 patients in the highest-dose group. Transient, asymptomatic elevations in liver enzymes occurred in 2 patients in lower-dose groups. Serious adverse events during follow-up (mean, 311 days) were not different between placebo and Ad5-FGF4. Overall, patients who received Ad5-FGF4 tended to have greater improvements in exercise time at 4 weeks (1.3 versus 0.7 minutes, P=NS, n=79). A protocol-specified, subgroup analysis showed the greatest improvement in patients with baseline ETT 10 minutes (1.6 versus 0.6 minutes, P=0.01, n=50). Conclusions: Results show evidence of favorable anti-ischemic effects with Ad5-FGF4 compared with placebo, and it appears to be safe. Angiogenic gene transfer with Ad5-FGF4 shows promise as a new therapeutic approach to the treatment of angina pectoris.

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Local Delivery of Plasmid DNA Into Rat Carotid Artery Using Ultrasound

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Background: Although viral vector systems are efficient to transfect foreign genes into blood vessels, safety issues remain in relation to human gene therapy. In this study, we examined the feasibility of a novel nonviral vector system by using high-frequency, low-intensity ultrasound irradiation for transfection into blood vessels. Methods and Results: Luciferase plasmid mixed with or without echo contrast microbubble (Optison) was transfected into cultured human vascular smooth muscle cells (VSMC) and endothelial cells (EC) with the use of ultrasound. Interestingly, luciferase activity was markedly increased in both cell types treated with Optison. We then transfected luciferase plasmid mixed with Optison by means of therapeutic ultrasound into rat artery. Two days after transfection, luciferase activity was significantly higher in carotid artery transfected with luciferase gene with Optison and ultrasound than with plasmid alone. In addition, we transfected an anti-oncogene (p53) plasmid into carotid artery after balloon injury as a model of gene therapy for restenosis. Two weeks after transfection, the intimal-to-medial area ratio in rats transfected with wild-type p53 plasmid complexed with Optison by means of ultrasound was significantly decreased as compared with control, accompanied by a significant increase in p53 protein. No apparent toxicity such as inflammation could be detected in blood vessels transfected with plasmid DNA with ultrasound and Optison. Conclusions: Overall, we demonstrated that an ultrasound transfection method with Optison enhanced transfection efficiency of naked plasmid DNA into blood vessels without any apparent toxicity. Transfection of p53 plasmid with the use of this method should be useful for safe clinical gene therapy without a viral vector system.

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Gene Therapy Strategy for Long-Term Myocardial Protection Using Adeno-Associated Virus-Mediated Delivery of Heme Oxygenase Gene

Luis G. Melo, PhD; Reitu Agrawal, PhD; Lunan Zhang, MD; Mojgan Rezvani, PhD; Abeel A. Mangi, MD; Afshin Ehsan, MD; Daniel P. Griese, MD; Giorgio Dell'Acqua, PhD; Michael J. Mann, MD; Junichi Oyama, MD, PhD; Shaw-Fang Yet, PhD; Matthew D. Layne, PhD; Mark A. Perrella, MD; Victor J. Dzau, MD

Background: Ischemia and oxidative stress are the leading mechanisms for tissue injury. An ideal strategy for preventive/protective therapy would be to develop an approach that could confer long-term transgene expression and, consequently, tissue protection from repeated ischemia/reperfusion injury with a single administration of a therapeutic gene. In the present study, we used recombinant adeno-associated virus (rAAV) as a vector for direct delivery of the cytoprotective gene heme oxygenase-1 (HO-1) into the rat myocardium, with the purpose of evaluating this strategy as a therapeutic approach for long-term protection from ischemia-induced myocardial injury. Methods and Results: Human HO-1 gene (hHO-1) was delivered to normal rat hearts by intramyocardial injection. AAV-mediated transfer of the hHO-1 gene 8 weeks before acute coronary artery ligation and release led to a dramatic reduction (>75%) in left ventricular myocardial infarction. The reduction in infarct size was accompanied by decreases in myocardial lipid peroxidation and in proapoptotic Bax and proinflammatory interleukin-1 β protein abundance, concomitant with an increase in antiapoptotic Bcl-2 protein level. This suggested that the transgene exerts its cardioprotective effects in part by reducing oxidative stress and associated inflammation and apoptotic cell death. Conclusions: This study documents the beneficial therapeutic effect of rAAV-mediated transfer, before myocardial injury, of a cytoprotective gene that confers long-term myocardial protection from ischemia/reperfusion injury. Our data suggest that this novel "pre-event" gene transfer approach may provide sustained tissue protection from future repeated episodes of injury and may be beneficial as preventive therapy for patients with or at risk of developing coronary ischemic events.

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Anti-Monocyte Chemoattractant Protein-1 Gene Therapy Limits Progression and Destabilization of Established Atherosclerosis in Apolipoprotein E-Knockout Mice

Shujiro Inoue, MD; Kensuke Egashira, MD; Weihua Ni, MD; Shiro Kitamoto, MD; Makoto Usui, MD; Kisho Otani, MD; Minako Ishibashi, MD; Ken-ichi Hiasa, MD; Ken-ichi Nishida, PhD; Akira Takeshita, MD

Background: Monocyte infiltration into the arterial wall and its activation is the central event in atherogenesis. Thus, monocyte chemoattractant protein-1 (MCP-1) might be a novel therapeutic target against atherogenesis. We and others recently reported that blockade or abrogation of the MCP-1 pathway attenuates the initiation of atheroma formation in hypercholesterolemic mice. It remains unclear, however, whether blockade of MCP-1 can limit progression or destabilization of established lesions. **Methods and Results:** We report here that blockade of MCP-1 by transfecting an N-terminal deletion mutant of the MCP-1 gene limited progression of preexisting atherosclerotic lesions in the aortic root in hypercholesterolemic mice. In addition, blockade of MCP-1 changed the lesion composition into a more stable phenotype, ie, containing fewer macrophages and lymphocytes, less lipid, and more smooth muscle cells and collagen. This strategy decreased expression of CD40 and the CD40 ligand in the atherosclerotic plaque and normalized the increased chemokine (RANTES and MCP-1) and cytokine (tumor necrosis factor, interleukin-6, interleukin-1 β , and transforming growth factor β 1) gene expression. These data suggest that MCP-1 is a central mediator in the progression and destabilization of established atheroma. **Conclusions:** The results of the present study suggest that the inflammatory responses mediated by MCP-1 are important in atherosclerosis and its complications.

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Adenovirus-Mediated Extracellular Superoxide Dismutase Gene Therapy Reduces Neointima Formation in Balloon-Denuded Rabbit Aorta

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Background: Restenosis is a frequent problem after invasive treatment of atherosclerotic vessels and is associated with intimal hyperplasia, which is primarily a result of proliferation and migration of smooth muscle cells, leading to the formation of neointima. Because there is no effective conventional medication for restenosis, gene therapy is a potential new treatment to prevent neointima formation. Methods and Results: In the present study, we analyzed the effects of adenovirus-mediated extracellular superoxide dismutase (EC-SOD) gene transfer (3×10^9 pfu/kg AdEC-SOD versus AdLacZ control virus) on neointima formation in balloon-denuded rabbit aortas. Local catheter-mediated gene transfer to the arterial wall reduced restenosis ($P < 0.001$) and decreased the number of macrophages in the transduced segment ($P < 0.001$) 2 weeks and 4 weeks after the gene transfer compared with AdLacZ controls. Transgene expression was detected in the arterial wall by RT-PCR 2 weeks after the procedure, and the production of superoxide anion was reduced after the gene transfer. Recovery of the endothelial layer was enhanced in EC-SOD-transduced rabbits compared with LacZ controls ($P < 0.001$) 2 weeks after the gene transfer. The therapeutic effect was found to be extended, affecting the gene transfer site and flanking aortic segments from the renal arteries to the bifurcation. However, systemic AdEC-SOD gene transfer to liver did not have any effects on restenosis. Conclusions: The results suggest that EC-SOD gene transfer reduces restenosis and may be useful for the prevention of intimal hyperplasia after vascular manipulations.

Angiogenic Gene Therapy for Experimental Critical Limb Ischemia Acceleration of Limb Loss by Overexpression of Vascular Endothelial Growth Factor 165 but not of Fibroblast Growth Factor-2

Ichiro Masaki*, Yoshikazu Yonemitsu*, Akihisa Yamashita, Shihoko Sata, Mitsugu Tanii, Kimihiro Komori, Kazunori Nakagawa, Xiaogang Hou, Yoshiyuki Nagai, Mamoru Hasegawa, Keizo Sugimachi, Katsuo Sueishi

Recent studies suggest the possible therapeutic effect of intramuscular vascular endothelial growth factor (VEGF) gene transfer in individuals with critical limb ischemia. Little information, however, is available regarding (1) the required expression level of VEGF for therapeutic effect, (2) the related expression of endogenous angiogenic factors, including fibroblast growth factor-2 (FGF-2), and (3) the related adverse effects due to overexpression of VEGF. To address these issues, we tested effects of overexpression of VEGF165 using recombinant Sendai virus (SeV), as directly compared with FGF-2 gene transfer. Intramuscular injection of SeV strongly boosted FGF-2, resulting in significant therapeutic effects for limb salvage with increased blood perfusion associated with enhanced endogenous VEGF expression in murine models of critical limb ischemia. In contrast, VEGF165 overexpression, 5-times higher than that of baseline on day 1, also strongly evoked endogenous VEGF in muscles, resulting in an accelerated limb amputation without recovery of blood perfusion. Interestingly, viable skeletal muscles of either VEGF165- or FGF-2 treated ischemic limbs showed similar platelet-endothelial cell adhesion molecule-1 positive vessel densities. Maturation of newly formed vessels suggested by smooth muscle cell actin positive cell lining, however, was significantly disturbed in muscles with VEGF. Further, therapeutic effects of FGF-2 were completely diminished by anti-VEGF neutralizing antibody in vivo, thus indicating that endogenous VEGF does contribute to the effect of FGF-2. These results suggest that VEGF is necessary, but should be delicately regulated to lower expression to treat ischemic limb. The therapeutic effect of FGF-2, associated with the harmonized angiogenic effects seen with endogenous VEGF, provides important insights into therapeutic angiogenesis.

Hypoxia Inducible Double Plasmid System for Myocardial Ischemia Gene Therapy

Yi Tang; MaShira Jackson; Keping Qian; M. Ian Phillips

Coronary artery disease frequently involves repeated bouts of myocardial ischemia. To automatically up-regulate the cardioprotective transgenes under hypoxic ischemia, a "vigilant vector" gene therapy system was developed and tested in a rat embryonic myocardial cell line (H9c2). In the vigilant vector, a hypoxia response element-incorporated promoter was used as a switch to turn on the gene expression in response to hypoxic signal. Furthermore, a novel double plasmid system was designed to elevate the potency of the vigilant vector. Instead of putting the promoter and the reporter gene in the same plasmid (single plasmid system), we separated them into two plasmids: the transactivator plasmid and reporter plasmid (double plasmid system). The hypoxia response element (HRE)-incorporated promoter increased the expression of a chimeric transcription factor consisting of the yeast GAL4 DNA binding domain and the human nuclear (transcription) factor- κ B (NF- κ B) p65 activation domain. The powerful chimeric regulator binds specifically to the upstream activating sequence for GAL4 in the reporter plasmid and activates the transcription of the transgene. Our experiments showed that the HRE-mediated expression could quickly increase 2.08 ± 0.75 -fold within 6 hours of hypoxia and further augmented 7.12 ± 1.52 -fold when the hypoxia condition was prolonged to 24 hours. The hypoxia-inducible double plasmid system dramatically amplified the transgene expression under both hypoxia and normoxia by 412.79 ± 185.27 -fold and 205.35 ± 65.44 -fold, respectively, relative to the single plasmid system. From these results, we concluded that this hypoxia inducible double plasmid system could be used therapeutically to switch on genes that have proven beneficial effects in myocardial ischemia.

GENE THERAPY

1. Angiogenesis of the heart.

Kutryk MJ, Stewart DJ.

Microsc Res Tech 2003 Feb 1;60(2):138-58

2. Gene therapy in cardiovascular diseases.

Tamirisa KP, Mukherjee D.

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Lee CH, Smits PC.

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4. The T(-786)C endothelial nitric oxide synthase genotype is a novel risk factor for coronary artery disease in Caucasian patients of the GENICA study.

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6. Novel LPL mutation (L303F) found in a patient associated with coronary artery disease and severe systemic atherosclerosis.

Saika Y, Sakai N, Takahashi M, Maruyama T, Kihara S, Ouchi N, Ishigami M, Hiraoka H, Nakamura T, Yamashita S, Matsuzawa Y.

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7. Therapeutic angiogenesis with vascular endothelial growth factor in peripheral and coronary artery disease: a review.

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8. Association between the severity of angiographic coronary artery disease and paraoxonase gene polymorphisms in the National Heart, Lung, and Blood Institute-sponsored Women's Ischemia Syndrome Evaluation (WISE) study.

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11. Angiogenic Gene Therapy (AGENT) Trial in Patients With Stable Angina Pectoris.

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Marmur, Andrew West, Jeffery J. Rade, Pran Marrott, H. Kirk Hammond, and Robert L. Engler

Circulation Mar 2002;105:1291-1297

12. Local Delivery of Plasmid DNA Into Rat Carotid Artery Using Ultrasound.

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Circulation. 2002;105:1233.

13. Gene Therapy Strategy for Long-Term Myocardial Protection Using Adeno-Associated Virus-Mediated Delivery of Heme Oxygenase Gene

Luis G. Melo, Reitu Agrawal, Lunan Zhang, Mojgan Rezvani, Abeel A. Mangi, Afshin Ehsan, Daniel P. Griese, Giorgio Dell'Acqua, Michael J. Mann, Junichi Oyama, Shaw-Fang Yet, Matthew D. Layne, Mark A. Perrella, and Victor J. Dzau

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14. Anti-Monocyte Chemoattractant Protein-1 Gene Therapy Limits Progression and Destabilization of Established Atherosclerosis in Apolipoprotein E⁰ Knockout Mice

Shujiro Inoue, Kensuke Egashira, Weihua Ni, Shiro Kitamoto, Makoto Usui, Kisho Otani, Minako Ishibashi, Ken-ichi Hiasa, Ken-ichi Nishida, and Akira Takeshita

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15. Adenovirus-Mediated Extracellular Superoxide Dismutase Gene Therapy Reduces Neointima Formation in Balloon-Denuded Rabbit Aorta

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16. Angiogenic Gene Therapy for Experimental Critical Limb Ischemia: Acceleration of Limb Loss by Overexpression of Vascular Endothelial Growth Factor 165 but not of Fibroblast Growth Factor-2

Ichiro Masaki, Yoshikazu Yonemitsu, Akihisa Yamashita, Shihoko Sata, Mitsugu Tanii, Kimihiro Komori, Kazunori Nakagawa, Xiaogang Hou, Yoshiyuki Nagai, Mamoru Hasegawa, Keizo Sugimachi, and Katsuo Sueishi

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17. Hypoxia Inducible Double Plasmid System for Myocardial Ischemia Gene Therapy

Yi Tang, MaShira Jackson, Keping Qian, and M. Ian Phillips

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Cardiac gene delivery with cardiopulmonary bypass.

Davidson MJ, Jones JM, Emani SM, Wilson KH, Jagers J, Koch WJ, Milano CA.

BACKGROUND: Cardiac gene therapy offers the possibility of enhancing myocardial performance in the compromised heart. However, current gene delivery techniques have limited myocardial transgene expression and pose the risk of extracardiac expression. Isolation of the coronary circulation during cardiac surgery may allow for more efficient and cardiac-selective gene delivery in a clinically relevant model. **Methods and Results?** Neonatal piglets (3 kg) underwent a median sternotomy and cardiopulmonary bypass, followed by aortic cross-clamping with 30 minutes of cardioplegic arrest. Adenoviral vectors containing transgenes for either beta-galactosidase (adeno-beta-gal, n=11) or the human beta(2)-adrenergic receptor (adeno-beta(2)-AR, n=15) were administered through the cardioplegia cannula immediately after arrest and were allowed to dwell in the coronary circulation during the cross-clamp period. After 1 week, the animals were killed, and their heart, lungs, and liver were excised and examined for gene expression. Analysis of beta-galactosidase staining revealed transmural myocardial gene expression among animals receiving adeno-beta-gal. No marker gene expression was detected in liver or lung tissue. beta-AR density in the left ventricle after adeno-beta(2)-AR delivery was 396 \pm 85% of levels in control animals ($P<0.01$). Animals receiving adeno-beta(2)-AR and control animals demonstrated similar beta-AR density in both the liver (114 \pm 8% versus 100 \pm 9%, $P=NS$) and lung (114 \pm 7% versus 100 \pm 9%, $P=NS$). There was no evidence of cardiac inflammation. **CONCLUSIONS:** By using cardiopulmonary bypass and cardioplegic arrest, intracoronary delivery of adenoviral vectors resulted in efficient myocardial uptake and expression. Undetectable transgene expression in liver or lung tissue suggests cardiac-selective expression.

Circulation ,2001;104(21):2595-601

Adenovirus-mediated gene transfer of a secreted transforming growth factor-beta type II receptor inhibits luminal loss and constrictive remodeling after coronary angioplasty and enhances adventitial collagen deposition.

Kingston PA, Sinha S, David A, Castro MG, Lowenstein PR, Heagerty AM.

BACKGROUND: Extracellular matrix (ECM) remodeling is central to the development of restenosis after coronary angioplasty (PTCA). As a regulator of ECM deposition by vascular cells, substantial evidence implicates transforming growth factor-beta1 (TGF-beta1) in the pathogenesis of restenosis. We investigated the effects of intracoronary expression of a transgenic antagonist of TGF-beta1 on luminal loss after PTCA. **METHODS AND RESULTS:** Porcine coronary arteries were randomized to receive a recombinant adenovirus expressing a secreted form of TGF-beta type II receptor (Ad5-RIIs), an adenovirus expressing beta-galactosidase (Ad5-lacZ), or vehicle only by intramural injection at the site of PTCA. Computerized morphometry 28 days after angioplasty revealed a greater minimum luminal area in Ad5-RIIs-injected arteries (1.71 ± 0.12 mm²) than in the Ad5-lacZ (1.33 ± 0.13 mm²) or vehicle-only (1.08 ± 0.17 mm²; $P=0.010$ by ANOVA) groups. This was accompanied by greater areas within the internal ($P=0.013$) and external ($P=0.031$) elastic laminae in Ad5-RIIs-treated vessels. Adventitial collagen content at the site of injury was increased in the Ad5-RIIs group, in contrast to decreases in the Ad5-lacZ and vehicle-only groups ($P=0.004$). **CONCLUSIONS:** Adenovirus-mediated antagonism of TGF-beta1 at the site of PTCA reduces luminal loss after PTCA by inhibiting constrictive remodeling. Antagonism of TGF-beta1 stimulates the formation of a dense collagenous adventitia, which prevents constrictive remodeling by acting as an external scaffold. These findings demonstrate the potential of gene therapy-mediated antagonism of TGF-beta1 as prophylactic therapy for restenosis.

Am Coll Cardiol ,2002;39(2):281-7

Local intracoronary administration of antisense oligonucleotide against c-myc for the prevention of in-stent restenosis: results of the randomized investigation by the Thoraxcenter of antisense DNA using local delivery and IVUS after coronary stenting (ITALICS) trial.

Kutryk MJ, Foley DP, van den Brand M, Hamburger JN, van der Giessen WJ, deFeyter PJ, Bruining N, Sabate M, Serruys PW; ITALICS Trial.

OBJECTIVE: This study was designed to determine whether antisense oligodeoxynucleotides (ODN) directed against the nuclear proto-oncogene c-myc could inhibit restenosis when given by local delivery immediately after coronary stent implantation. **BACKGROUND:** Failure of conventional pharmacologic therapies to reduce the incidence of coronary restenosis after percutaneous revascularization techniques has prompted interest in the use of agents that target intracellular central regulatory mechanisms. **METHODS:** Eighty-five patients were randomly assigned to receive either 10 mg of phosphorothioate-modified 15-mer antisense ODN or saline vehicle by intracoronary local delivery after coronary stent implantation. The primary end point was percent neointimal volume obstruction measured by computerized analysis of electrocardiogram-gated intravascular ultrasound (IVUS) at six-month follow-up. Secondary end points included clinical outcome and quantitative coronary angiography analysis. **RESULTS:** Analysis of follow-up IVUS data was performed on 77 patients. In-stent volume obstruction was similar between groups (44 +/- 16% and 46 +/- 14%, placebo vs. ODN; $p = 0.57$; 95% confidence interval: -1.13 to 0.85). Minimum luminal diameter increased from 0.84 +/- 0.36 and 0.90 +/- 0.45 ($p = 0.55$) to 2.70 +/- 0.37 and 2.80 +/- 0.37 ($p = 0.28$) after stent implantation, which decreased to 1.50 +/- 0.61 and 1.50 +/- 0.53 ($p = 0.98$) by six months, yielding similar loss indexes (placebo vs. ODN, respectively). There were no differences in angiographic restenosis rates (38.5 and 34.2%; $p = 0.81$; placebo vs. ODN) or clinical outcome. **CONCLUSIONS:** Treatment with 10 mg of phosphorothioate-modified ODN directed against c-myc does not reduce neointimal volume obstruction or the angiographic restenosis rate in this patient population

Nature ,2002;415(6868):234-9

Myocardial gene therapy.

Isner JM.

Gene therapy is proving likely to be a viable alternative to conventional therapies in coronary artery disease and heart failure. Phase 1 clinical trials indicate high levels of safety and clinical benefits with gene therapy using angiogenic growth factors in myocardial ischaemia. Although gene therapy for heart failure is still at the pre-clinical stage, experimental data indicate that therapeutic angiogenesis using short-term gene expression may elicit functional improvement in affected individuals

Circulation 2002;105(5):602-7

Gene therapy strategy for long-term myocardial protection using adeno-associated virus-mediated delivery of heme oxygenase gene.

Melo LG, Agrawal R, Zhang L, Rezvani M, Mangi AA, Ehsan A, Griese DP, Dell'acqua G, Mann MJ, Oyama J, Yet SF, Layne MD, Perrella MA, Dzau VJ.

BACKGROUND: Ischemia and oxidative stress are the leading mechanisms for tissue injury. An ideal strategy for preventive/protective therapy would be to develop an approach that could confer long-term transgene expression and, consequently, tissue protection from repeated ischemia/reperfusion injury with a single administration of a therapeutic gene. In the present study, we used recombinant adeno-associated virus (rAAV) as a vector for direct delivery of the cytoprotective gene heme oxygenase-1 (HO-1) into the rat myocardium, with the purpose of evaluating this strategy as a therapeutic approach for long-term protection from ischemia-induced myocardial injury. **METHODS AND RESULTS:** Human HO-1 gene (hHO-1) was delivered to normal rat hearts by intramyocardial injection. AAV-mediated transfer of the hHO-1 gene 8 weeks before acute coronary artery ligation and release led to a dramatic reduction (>75%) in left ventricular myocardial infarction. The reduction in infarct size was accompanied by decreases in myocardial lipid peroxidation and in proapoptotic Bax and proinflammatory interleukin-1beta protein abundance, concomitant with an increase in antiapoptotic Bcl-2 protein level. This suggested that the transgene exerts its cardioprotective effects in part by reducing oxidative stress and associated inflammation and apoptotic cell death. **CONCLUSIONS:** This study documents the beneficial therapeutic effect of rAAV-mediated transfer, before myocardial injury, of a cytoprotective gene that confers long-term myocardial protection from ischemia/reperfusion injury. Our data suggest that this novel "re-event" gene transfer approach may provide sustained tissue protection from future repeated episodes of injury and may be beneficial as preventive therapy for patients with or at risk of developing coronary ischemic events.

Circulation 1998 ;98: 2081-2087.

Direct Intramuscular Injection of Plasmid DNA Encoding Angiopoietin-1 but not Angiopoietin-2 Augments Revascularization in the Rabbit Ischemic Hindlimb

Kou-Gi Shyu, Orit Manor, Meredith Magner, George D. Yancopoulos, and Jeffrey M. Isner

Background-Angiopoietin-1 (Ang1) and angiopoietin-2 (Ang2) have recently been identified as ligands for the endothelial cell-specific Tie2 receptor. Little is known regarding the impact of these Tie2 ligands on postnatal neovascularization. Accordingly, we tested the hypothesis that gene transfer of plasmid DNA encoding Ang1 and Ang2 could modulate collateral vessel development in a rabbit model of hindlimb ischemia.

Methods and Results-pAng1* (n=15), pJFE control (no Ang1* insert) (n=9), pAng2 (n=9), pcDNA3 control (no Ang2 insert) (n=10), or saline (n=5) was injected intramuscularly into the rabbit ischemic hindlimb. Collateral vessel development and limb perfusion were assessed before and 30 days after treatment. Calf blood pressure ratio (ischemic to normal hindlimb) was increased 30 days after Ang1* gene transfer versus controls (Ang1*, 0.90 ± 0.02 ; pJFE, 0.76 ± 0.05 ; saline, 0.77 ± 0.03 ; $P < 0.05$). Angiographic score was higher ($P < 0.05$) in the pAng1* group (0.63 ± 0.02) than in the pJFE (0.51 ± 0.03) or saline (0.52 ± 0.02) group. Maximal (postpapaverine) blood flow in the ischemic limb was higher ($P < 0.05$) after pAng1* (67.8 ± 4.9 mL/min) than pJFE (51.2 ± 4.4 mL/min) or saline (52.9 ± 4.9 mL/min). Capillary density and capillary/muscle fiber ratio ($242 \pm 12/\text{mm}^2$ and 0.89 ± 0.06 , respectively) were higher ($P < 0.01$) with pAng1* than pJFE ($172 \pm 11/\text{mm}^2$ and 0.64 ± 0.05) or saline ($166 \pm 10/\text{mm}^2$ and 0.67 ± 0.05). Neovascularization was not enhanced with pAng2.

Conclusions-Ang1 but not Ang2 gene transfer produces anatomic and physiological evidence of enhanced collateral vessel formation. Ang1 may modulate neovascularization in adult animals and thus represents a feasible therapeutic strategy for patients with tissue ischemia. The role of Ang2 in postnatal neovascularization remains to be clarified.

Circulation ,1998; 98: 2108-2116

Vascular Endothelial Growth Factor (VEGF) Expression in Human Coronary Atherosclerotic Lesions : Possible Pathophysiological Significance of VEGF in Progression of Atherosclerosis

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Background-Vascular endothelial growth factor (VEGF) is an important angiogenic factor reported to induce migration and proliferation of endothelial cells, enhance vascular permeability, and modulate thrombogenicity.

VEGF expression in cultured cells (smooth muscle cells, macrophages, endothelial cells) is controlled by growth factors and cytokines. Hence, the question arises of whether VEGF could play a role in atherogenesis.

Methods and Results-Frozen sections from 38 coronary artery segments were studied. The specimens were characterized as normal with diffuse intimal thickening, early atherosclerosis with hypercellularity, and advanced atherosclerosis (atheromatous plaques, fibrous plaques, and totally occlusive lesions). VEGF expression as well as the expression of 2 VEGF receptors, flt-1 and Flk-1, were studied with immunohistochemical techniques in these samples at the different stages of human coronary atherosclerosis progression. The expression of VEGF mRNA was also studied with reverse transcription-polymerase chain reaction. Normal arterial segments showed no substantial VEGF expression. Hypercellular and atheromatous lesions showed distinct VEGF positivity of activated endothelial cells, macrophages, and partially differentiated smooth muscle cells. VEGF positivity was also detected in endothelial cells of intraplaque microvessels within advanced lesions. In totally occlusive lesions with extensive neovascularization, intense immunostaining for VEGF was observed in accumulated macrophages and endothelial cells of the microvessels. Furthermore, VEGF mRNA expression was detected in atherosclerotic coronary segments but not in normal coronary segments. The immunostainings for flt-1 and Flk-1 were detected in aggregating macrophages in atherosclerotic lesions and also in endothelial cells of the microvessels in totally occlusive lesions.

Conclusions-These results demonstrate distinct expression of VEGF and its receptors (flt-1 and Flk-1) in atherosclerotic lesions in human coronary arteries. Considering the multipotent actions of VEGF documented experimentally in vivo and in vitro, our findings suggest that VEGF may have some role in the progression of human coronary atherosclerosis, as well as in recanalization processes in obstructive coronary diseases.

Circulation, 1998;97: 1114-1123

Constitutive Expression of phVEGF165 After Intramuscular Gene Transfer Promotes Collateral Vessel Development in Patients With Critical Limb Ischemia

Iris Baumgartner, Ann Pieczek, Orit Manor, Richard Blair, Marianne Kearney, Kenneth Walsh, and Jeffrey M. Isner

Background-Preclinical studies have indicated that angiogenic growth factors can stimulate the development of collateral arteries, a concept called "therapeutic angiogenesis." The objectives of this phase 1 clinical trial were (1) to document the safety and feasibility of intramuscular gene transfer by use of naked plasmid DNA

encoding an endothelial cell mitogen and (2) to analyze potential therapeutic benefits in patients with critical limb ischemia.

Methods and Results-Gene transfer was performed in 10 limbs of 9 patients with nonhealing ischemic ulcers (n=7/10) and/or rest pain (n=10/10) due to peripheral arterial disease. A total dose of 4000 µg of naked plasmid DNA encoding the 165-amino-acid isoform of human vascular endothelial growth factor (phVEGF165) was injected directly into the muscles of the ischemic limb. Gene expression was documented by a transient increase in serum levels of VEGF monitored by ELISA. The ankle-brachial index improved significantly (0.33 ± 0.05 to 0.48 ± 0.03 , $P=.02$); newly visible collateral blood vessels were directly documented by contrast angiography in 7 limbs; and magnetic resonance angiography showed qualitative evidence of improved distal flow in 8 limbs. Ischemic ulcers healed or markedly improved in 4 of 7 limbs, including successful limb salvage in 3 patients recommended for below-knee amputation. Tissue specimens obtained from an amputee 10 weeks after gene therapy showed foci of proliferating endothelial cells by immunohistochemistry. PCR and Southern blot analyses indicated persistence of small amounts of plasmid DNA. Complications were limited to transient lower-extremity edema in 6 patients, consistent with VEGF enhancement of vascular permeability.

Conclusions-These findings may be cautiously interpreted to indicate that intramuscular injection of naked plasmid DNA achieves constitutive overexpression of VEGF sufficient to induce therapeutic angiogenesis in selected patients with critical limb ischemia.

Circulation, 1998 ;97: 147-154.

Plasma Activity and Insertion/Deletion Polymorphism of Angiotensin I-Converting Enzyme : A Major Risk Factor and a Marker of Risk for Coronary Stent Restenosis

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Background-Tissue proliferation is almost invariably observed in recurrent lesions within stents, and ACE, a factor of smooth muscle cell proliferation, may play an important role. Plasma ACE level is largely controlled by the insertion/deletion (I/D) polymorphism of the enzyme gene. The association among restenosis within coronary stents, plasma ACE level, and the I/D polymorphism is analyzed in the present prospective study.

Methods and Results-One hundred seventy-six consecutive patients with successful, high-pressure, elective stenting of de novo lesions in the native coronary vessels were considered. At follow-up angiography,

recurrence was observed in 35 patients (19.9%). Baseline clinical and demographic variables, plasma glucose and serum fibrinogen levels, lipid profile, descriptive and quantitative angiographic data, and procedural variables were not significantly different in patients with and without restenosis; mean plasma ACE levels (\pm SEM) were 40.8 ± 3.5 and 20.7 ± 1.0 U/L, respectively ($P < .0001$). Diameter stenosis percentage and minimum luminal diameter at 6 months showed statistically significant correlation with plasma ACE level ($r = .352$ and $-.387$, respectively $P < .001$). Twenty-one of 62 patients (33.9%) with D/D genotype, 13 of 80 (16.3%) with I/D genotype, and 1 of 34 (2.9%) with I/I genotype showed recurrence; the restenosis rate for each genotype is consistent with a codominant expression of the allele D.

Conclusions-In a selected cohort of patients, both the D/D genotype of the ACE gene, and high plasma activity of the enzyme are significantly associated with in-stent restenosis. Continued study with clinically different subsets of patients and various stent designs is warranted.

Circulation 1998; 98: 2800-2804

Gene Therapy for Myocardial Angiogenesis : Initial Clinical Results With Direct Myocardial Injection of phVEGF165 as Sole Therapy for Myocardial Ischemia

Douglas W. Losordo, Peter R. Vale, James F. Symes, Cheryl H. Dunnington, Darryl D. Esakof, Michael Maysky, Alan B. Ashare, Kishor Lathi, and Jeffrey M. Isner

Background-We initiated a phase 1 clinical study to determine the safety and bioactivity of direct myocardial gene transfer of vascular endothelial growth factor (VEGF) as sole therapy for patients with symptomatic myocardial ischemia.

Methods and Results-VEGF gene transfer (GTx) was performed in 5 patients (all male, ages 53 to 71) who had failed conventional therapy; these men had angina (determined by angiographically documented coronary artery disease). Naked plasmid DNA encoding VEGF (phVEGF165) was injected directly into the ischemic myocardium via a mini left anterior thoracotomy. Injections caused no changes in heart rate (pre-GTx= 75 ± 15 /min versus post-GTx= 80 ± 16 /min, $P = \text{NS}$), systolic BP (114 ± 7 versus 118 ± 7 mm Hg, $P = \text{NS}$), or diastolic BP (57 ± 2 versus 59 ± 2 mm Hg, $P = \text{NS}$). Ventricular arrhythmias were limited to single unifocal premature beats at the moment of injection. Serial ECGs showed no evidence of new myocardial infarction in any patient. Intraoperative blood loss was 0 to 50 cm³, and total chest tube drainage was 110 to 395 cm³. Postoperative cardiac output fell transiently but increased within 24 hours (preanesthesia= 4.8 ± 0.4 versus postanesthesia= 4.1

± 0.3 versus 24 hours postoperative= 6.3 ± 0.8 , $P=0.02$). Time to extubation after closure was 18.4 ± 1.4 minutes; average postoperative hospital stay was 3.8 days. All patients had significant reduction in angina (nitroglycerin [NTG] use= 53.9 ± 10.0 /wk pre-GTx versus 9.8 ± 6.9 /wk post-GTx, $P<0.03$). Postoperative left ventricular ejection fraction (LVEF) was either unchanged ($n=3$) or improved ($n=2$, mean increase in LVEF=5%). Objective evidence of reduced ischemia was documented using dobutamine single photon emission computed tomography (SPECT)-sestamibi imaging in all patients. Coronary angiography showed improved Rentrop score in 5 of 5 patients.

Conclusions-This initial experience with naked gene transfer as sole therapy for myocardial ischemia suggests that direct myocardial injection of naked plasmid DNA, via a minimally invasive chest wall incision, is safe and may lead to reduced symptoms and improved myocardial perfusion in selected patients with chronic myocardial ischemia.

Circulation, 1999 ;100: 547-552

Interindividual Heterogeneity in the Hypoxic Regulation of VEGF : Significance for the Development of the Coronary Artery Collateral Circulation

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Background-The coronary artery collateral circulation may be beneficial in protecting against myocardial ischemia and necrosis. However, there is a tremendous interindividual variability in the degree of new collateral formation in patients with coronary artery disease. The basis for this interindividual heterogeneity is not understood. In this study we test the hypothesis that failure to generate collateral vessels is associated with a failure to appropriately induce with hypoxia or ischemia the angiogenic factor, vascular endothelial growth factor (VEGF).

Methods and Results-We correlated the VEGF response to hypoxia in the monocytes harvested from patients with coronary artery disease with the presence of collaterals visualized during routine angiography. We found that there was a highly significant difference in the hypoxic induction of VEGF in patients with no collaterals compared with patients with some collaterals (mean fold induction 1.9 ± 0.2 versus 3.2 ± 0.3 , $P<0.0001$). After subjecting the data to ANCOVA, using as covariates a number of factors that might influence the amount of collateral formation (ie, age, sex, diabetes, smoking, hypercholesterolemia), patients with no collaterals still

have a significantly lower hypoxic induction of VEGF than patients with collaterals.

Conclusions-This study provides evidence in support of the hypothesis that the ability to respond to progressive coronary artery stenosis is strongly associated with the ability to induce VEGF in response to hypoxia. The observed interindividual heterogeneity in this response may be due to environmental, epigenetic, or genetic causes. This interindividual heterogeneity may also help to explain the variable angiogenic responses seen in other conditions such as diabetic retinopathy and solid tumors.

Circulation, 1999;100:468-474

Angiogenesis Gene Therapy

Phase I Assessment of Direct Intramyocardial Administration of an Adenovirus Vector Expressing VEGF121 cDNA to Individuals With Clinically Significant Severe Coronary Artery Disease

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Background

Therapeutic angiogenesis, a new experimental strategy for the treatment of vascular insufficiency, uses the administration of mediators known to induce vascular development in embryogenesis to induce neovascularization of ischemic adult tissues. This report summarizes a phase I clinical experience with a gene-therapy strategy that used an E1-E3- adenovirus (Ad) gene-transfer vector expressing human vascular endothelial growth factor (VEGF) 121 cDNA (AdGVVEGF121.10) to induce therapeutic angiogenesis in the myocardium of individuals with clinically significant coronary artery disease.

Methods and Results

AdGVVEGF121.10 was administered to 21 individuals by direct myocardial injection into an area of reversible ischemia either as an adjunct to conventional coronary artery bypass grafting (group A, n=15) or as sole therapy via a minithoracotomy (group B, n=6). There was no evidence of systemic or cardiac-related adverse events related to vector administration. In both groups, coronary angiography and stress sestamibi scan assessment of wall motion 30 days after therapy suggested improvement in the area of vector administration. All patients reported improvement in angina class after therapy. In group B, in which gene transfer was the only therapy,

treadmill exercise assessment suggested improvement in most individuals.

Conclusions

The data are consistent with the concept that direct myocardial administration of AdGVVEGF121.10 to individuals with clinically significant coronary artery disease appears to be well tolerated, and initiation of phase II evaluation of this therapy is warranted.

Journal of the American College of Cardiology, 1999;34:1:246-254

Catheter-based myocardial gene transfer utilizing nonfluoroscopic electromechanical left ventricular mapping

Peter R. Vale, Douglas W. Losordo, Tengiz Tkebuchava, Donghui Chen, Charles E. Milliken, Jeffrey M. Isner

OBJECTIVES

This study investigated the feasibility and safety of percutaneous, catheter-based myocardial gene transfer.

BACKGROUND

Direct myocardial gene transfer has, to date, required direct injection via an open thoracotomy.

METHODS

Electroanatomical mapping was performed to establish the site of left ventricular (LV) gene transfer. A steerable, deformable 7F catheter with a 27G needle, which can be advanced 3 to 5 mm beyond its distal tip, was then directed to previously acquired map sites, the needle was advanced, and injections were made into the LV myocardium.

RESULTS

In two pigs in which methylene blue dye was injected, discretely stained LV sites were observed at necropsy in each pig, corresponding to the injection sites indicated prospectively by the endocardial map. In six pigs in which the injection catheter was used to deliver plasmid using cytomegalovirus promoter/enhancer, encoding nuclear-specific LacZ gene (pCMV-nlsLacZ) (50 µg/ml) to a single LV myocardial region, peak beta-galactosidase activity after five days (relative light units [RLU], mean $135,333 \pm 28,239$, range = 31,508 to 192,748) was documented in the target area of myocardial injection in each pig. Percutaneous gene transfer of pCMV-nls LacZ (50 µg/ml) was also performed in two pigs with an ameroid constrictor applied to the left circumflex coronary artery; in each pig, peak beta-galactosidase activity after five days (214,851 and 23,140 RLU) was documented at the injection site. All pigs survived until sacrifice, and no complications were observed with either the mapping or the injection procedures.

CONCLUSIONS

Percutaneous myocardial gene transfer can be successfully achieved in normal and ischemic myocardium without significant morbidity or mortality. These findings establish the potential for minimally invasive cardiovascular gene transfer.

Am Heart J, 1999 ;138(2 Pt 2):132-41

Gene therapy for myocardial angiogenesis.

Losordo DW, Vale PR, Isner JM

In patients in whom antianginal medications fail to provide sufficient symptomatic relief, additional interventions such as angioplasty or bypass surgery may be required. Although both types of intervention have been shown to be effective for various types of patients, a certain group of patients may not be candidates for either intervention because of the diffuse nature of their coronary artery disease. Moreover, there are many patients in whom recurrent narrowing and/or occlusion of bypass conduits after initially successful surgery has left the patient again symptomatic with no further angioplasty or surgical option. Ischemic muscle represents a promising target for gene therapy with naked plasmid DNA. Intramuscular transfection of genes encoding angiogenic cytokines, particularly those naturally secreted by intact cells, may constitute an alternative treatment strategy for patients with extensive tissue ischemia in whom contemporary therapies (antianginal medications, angioplasty, bypass surgery) have previously failed or are not feasible. This strategy is designed to promote the development of supplemental collateral blood vessels that will constitute endogenous bypass conduits around occluded native arteries, a strategy termed "therapeutic angiogenesis." Preclinical animal studies from our laboratory have established that intramuscular gene transfer may be used to successfully accomplish therapeutic angiogenesis. More recently, phase 1 clinical studies from our institution have established that intramuscular gene transfer may be used to safely and successfully accomplish therapeutic angiogenesis in patients with critical limb ischemia. The notion that this concept could be extrapolated to the treatment of chronic myocardial ischemia was demonstrated in our laboratory by administering recombinant human vascular endothelial growth factor (VEGF) to a porcine model of chronic myocardial ischemia. Recent experiments performed in this same porcine model of myocardial ischemia have shown that direct intramyocardial gene transfer of naked plasmid DNA encoding VEGF (phVEGF(165), the identical plasmid used in our previous animal and human clinical trials) can be safely and successfully achieved through a

minimally invasive chest wall incision. Finally, initial results have supported the concept that intramyocardial injection of naked plasmid DNA encoding VEGF can achieve therapeutic angiogenesis, as demonstrated by clinical improvement in patient symptoms and improved myocardial perfusion shown by single-photon emission computed tomography-sestamibi imaging.

Summary

1. Phase 1 clinical studies established that intramuscular gene transfer may be used to safely and successfully accomplish therapeutic angiogenesis in patients with critical limb ischemia.
2. Initial results have supported the concept that intramyocardial injection of naked plasmid DNA encoding VEGF can achieve therapeutic angiogenesis

Journal of the American College of Cardiology, 36:3:717-722

Prevalence of factor v leiden and prothrombin variant g20210a in patients age <50 years with no significant stenoses at angiography three to four weeks after myocardial infarction

Neil S. Van de Water, John K. French, Mayanna Lund, Thomas A. Hyde, Harvey D. White, Peter J. Browett

OBJECTIVES

We sought to determine the frequencies of factor V Leiden and prothrombin variant G20210A in patients age <50 years with no significant coronary stenoses three to four weeks after myocardial infarction (MI).

BACKGROUND

Factor V Leiden and prothrombin variant G20210A occur frequently in patients with venous thromboembolism. However, the contribution of these mutations to the development of MI requires clarification.

METHODS

The frequencies of factor V Leiden and prothrombin variant G20210A were determined in 41 patients age <50 years who had normal coronary arteries (no stenosis >50%) at angiography three to four weeks after MI (the study group) and compared with those in 114 patients who had at least one angiographic stenosis >50% after MI (the control group). Patients age 50 years with, or without, stenoses were also studied.

RESULTS

The frequency of factor V Leiden was 14.6% in patients age <50 years in the study group compared with 3.6% in patients in the control group (odds ratio [OR] 4.7 [95% confidence interval (CI) 1.3-17.7], $p = 0.02$). The

frequency of the prothrombin variant G20210A was 7.3% in the study group compared with 1.8% in the control group (OR 4.4 [95% CI 0.7-27.5], $p = 0.12$). One or both mutations were present in 8 of the 41 patients (19.5%) age <50 years in the study group compared with 6 of the 114 patients (5.5%) in the control group (OR 4.4 [95% CI 1.4-13.5], $p = 0.01$). In all 271 patients (irrespective of age) with normal arteries, the frequency of factor V Leiden was 11.7% (7/60) compared with 4.3% (9/211) in patients with at least one >50% stenosis (OR 2.9 [95% CI 1.1-8.3], $p = 0.04$), and the frequency of prothrombin variant G20210A was 6.7% (4/60) compared with 1.4% (3/211) (OR 4.9 [95% CI 1.1-22.8], $p = 0.04$), respectively.

CONCLUSIONS

The frequencies of factor V Leiden and/or prothrombin variant G20210A are increased in patients age <50 years with normal or near normal coronary arteries after MI.

N Engl J Med, 2000;343:774-80.

Polymorphisms in the Factor VII Gene and the Risk of Myocardial Infarction in Patients with Coronary Artery Disease

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Background. High plasma levels of coagulation factor VII have been suggested to be predictors of death due to coronary artery disease. Since polymorphisms in the factor VII gene contribute to variations in factor VII levels, such polymorphisms may be associated with the risk of myocardial infarction, which is precipitated by thrombosis.

Methods. We studied a total of 444 patients, 311 of whom had severe, angiographically documented coronary atherosclerosis. Of these 311 patients, 175 had documentation of a previous myocardial infarction. As a control group, 133 patients with normal coronary arteriograms were also included. We measured the levels of activated factor VII and assessed three polymorphisms in the factor VII gene, one involving the promoter (A1 and A2 alleles), one involving the catalytic region (R353Q), and one involving intron 7.

Results. Each of the polymorphisms influenced factor VII levels. Patients with the A2A2 and QQ genotypes had the lowest levels of activated factor VII (66 percent and 72 percent lower, respectively, than the levels in patients with the wild-type genotypes). The frequencies of the various genotypes in the patients free of coronary artery disease were similar to those in the entire population of patients with coronary artery disease. In the latter

group, there were significantly more heterozygotes and homozygotes for the A2 and Q alleles among those who had not had a myocardial infarction than among those who had had an infarction (P=0.008 for the presence of the promoter polymorphism and P=0.01 for the presence of the R353Q polymorphism by chi-square analysis). The adjusted odds ratio for myocardial infarction among the patients with the A1A2 or RQ genotype was 0.47 (95 percent confidence interval, 0.27 to 0.81).

Conclusions. Our findings suggest that certain factor VII genotypes have a role in protection against myocardial infarction. This may explain why some patients do not have myocardial infarction despite the presence of severe coronary atherosclerosis.

Circulation 2000 ;101: 1519-1526.

IL-8 Is an Angiogenic Factor in Human Coronary Atherectomy Tissue

Americo Simonini, Mauro Moscucci, David W. M. Muller, Eric R. Bates, Francis D. Pagani, Marie D. Burdick, and Robert M. Strieter

Background-Interleukin-8 (IL-8), a CXC chemokine that induces the migration and proliferation of endothelial cells and smooth muscle cells, is a potent angiogenic factor that may play a role in atherosclerosis. Previously, IL-8 has been reported in atherosclerotic lesions and circulating macrophages from patients with atherosclerosis. Therefore, we sought to determine whether IL-8 plays a role in mediating angiogenic activity in atherosclerosis.

Methods and Results-Homogenates from 16 patients undergoing directional coronary atherectomy (DCA) and control samples from the internal mammary artery (IMA) of 7 patients undergoing bypass graft surgery were assessed for IL-8 content by specific ELISA, immunohistochemistry, and in situ hybridization for IL-8 mRNA. The contribution of IL-8 to net angiogenic activity was assessed using the rat cornea micropocket assay and cultured cells. IL-8 expression was significantly elevated in DCA samples compared with IMA samples (1.71 ± 0.6 versus 0.05 ± 0.03 ng/mg of total protein; $P < 0.01$). Positive immunolocalization of IL-8 was found exclusively in DCA tissue sections, and it correlated with the presence of factor VIII-related antigen. In situ reverse transcriptase polymerase chain reaction revealed the expression of IL-8 mRNA in DCA tissue. Corneal neovascular response, defined by ingrowth of capillary sprouts toward the implant, was markedly positive with DCA pellets, but no constitutive vessel ingrowth was seen with IMA specimens. Neutralizing IL-8 attenuated both the in vivo corneal neovascular response and the in vitro proliferation of cultured cells.

Conclusions-The results suggest that, in human coronary atherosclerosis, IL-8 is an important mediator of angiogenesis and may contribute to plaque formation via its angiogenic properties.

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Reduced procedural risk for coronary catheter interventions in carriers of the coagulation factor VII-Gln353 gene

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OBJECTIVES

We have focused on the role of coagulation factor VII (FVII) Arg353Gln polymorphism as a risk predictor of complications following percutaneous transluminal coronary angioplasty (PTCA), directional coronary atherectomy (DCA), and stenting.

BACKGROUND

The FVII Arg353Gln mutation decreases FVII activity, and presence of the Gln353 allele could be protective against thrombus formation during catheter interventions.

METHODS

A total of 666 consecutive patients with coronary artery disease who had undergone PTCA (n = 280), DCA (n = 104), or stenting (n = 282) were followed up for a 30-day composite end point, which included need for target vessel revascularization, myocardial infarction, and death. The Arg353Gln polymorphism of FVII was determined by PCR/RFLP assay.

RESULTS

Carriers of the Gln353 allele had significantly lower levels of total FVII activity (FVIIc, -20.7%, $p < 0.001$) and of activated circulating FVII (FVIIa, -32.7%, $P = 0.03$) compared with Arg353/Arg353. The composite end point occurred in 43 patients: 4 were heterozygous Arg353/Gln353, and 39 were homozygous Arg353/Arg353. The incidence of the composite end point was 2.5% in carriers of the Gln353 allele and 7.7% in Arg353/Arg353 homozygotes ($p = 0.013$). This corresponds to a 72% risk reduction in carriers of the Gln353 allele (relative risk: 0.28; 95% confidence interval: 0.09-0.81; $P = 0.02$).

CONCLUSIONS

The Gln353 allele of FVII is associated with substantial risk reduction in adverse events that complicate

coronary catheter interventions. With the perspective of active site-blocked activated FVII (FVIIai) as conjunctive medication, the results suggest that the FVII genotype should be taken into due consideration in assessment of FVIIai medication and of its dosage.

Circulation, 2000;101:454

Delivery Strategies to Achieve Therapeutic Myocardial Angiogenesis

Ran Kornowski, MD; Shmuel Fuchs, MD; Martin B. Leon, MD; Stephen E. Epstein, MD

Abstract-The use of recombinant genes or growth factors to enhance myocardial collateral blood vessel function may represent a new approach to the treatment of cardiovascular disease. Proof of concept has been demonstrated in animal models of myocardial ischemia, and clinical trials are underway. Currently, it is unknown which is the safest and most effective delivery strategy to induce clinically important therapeutic angiogenic responses in ischemic myocardium. Most strategies for transcatheter delivery of angiogenic factors have used an intracoronary route, which may have limitations because of imprecise localization of genes or proteins and systemic delivery to noncardiac tissue. The effect of direct intraoperative intramyocardial injection of angiogenic factors on collateral function has been reported in experimental models, and angiogenesis is being studied after direct intramyocardial injection of angiogenic peptides or plasmid vectors during open heart surgery in patients. Catheter-based transendocardial injection of angiogenic factors may provide equivalent benefit without the need for surgery. Intrapericardial delivery of angiogenic factors may offer a theoretical advantage of prolonged exposure of either coronary or myocardial tissue to the administered drug as result of a reservoir function of the pericardium. In this article, we review the different modes of administration for therapeutic myocardial angiogenesis therapy.

Circulation, 2000;102:e73

Clinical Trials in Coronary Angiogenesis: Issues, Problems, Consensus

An Expert Panel Summary

Abstract-The rapid development of angiogenic growth factor therapy for patients with advanced ischemic heart disease over the last 5 years offers hope of a new treatment strategy based on generation of new blood supply in the diseased heart. However, as the field of therapeutic coronary angiogenesis is maturing from basic and preclinical investigations to clinical trials, many new and presently unresolved issues are coming into focus. These include in-depth understanding of the biology of angiogenesis, selection of appropriate patient populations for clinical trials, choice of therapeutic end points and means of their assessment, choice of therapeutic strategy (gene versus protein delivery), route of administration, and the side effect profile. The present article presents a summary statement of a panel of experts actively working in the field, convened by the Angiogenesis Foundation and the Angiogenesis Research Center during the 72nd meeting of the American Heart Association to define and achieve a consensus on the challenges facing development of therapeutic angiogenesis for coronary disease.

Table 1. Three Types of Neovascularization Ang indicates angiopoietin; PDGF, platelet-derived growth factor.

Table 2. Gene vs Protein Therapy

Circulation, 2000;102:2262.

Intravascular Adenovirus-Mediated VEGF-C Gene Transfer Reduces Neointima Formation in Balloon-Denuded Rabbit Aorta

Mikko O. Hiltunen, MD; Marja Laitinen, MD; Mikko P. Turunen, PhD; Michael Jeltsch, PhD; Juha Hartikainen, MD; Tuomas T. Rissanen, MD; Johanna Laukkanen, MD.

Background-Gene transfer to the vessel wall may provide new possibilities for the treatment of vascular disorders, such as postangioplasty restenosis. In this study, we analyzed the effects of adenovirus-mediated vascular endothelial growth factor (VEGF)-C gene transfer on neointima formation after endothelial denudation in rabbits. For comparison, a second group was treated with VEGF-A adenovirus and a third group with lacZ adenovirus. Clinical-grade adenoviruses were used for the study.

Methods and Results-Aortas of cholesterol-fed New Zealand White rabbits were balloon-denuded, and gene transfer was performed 3 days later. Animals were euthanized 2 and 4 weeks after the gene transfer, and intima/media ratio (I/M), histology, and cell proliferation were analyzed. Two weeks after the gene transfer, I/M in the lacZ-transfected control group was 0.57 ± 0.04 . VEGF-C gene transfer reduced I/M to 0.38 ± 0.02 ($P < 0.05$ versus lacZ group). I/M in VEGF-A-treated animals was 0.49 ± 0.17 ($P = \text{NS}$). The tendency that both VEGF

groups had smaller I/M persisted at the 4-week time point, when the lacZ group had an I/M of 0.73 ± 0.16 , the VEGF-C group 0.44 ± 0.14 , and the VEGF-A group 0.63 ± 0.21 (P=NS). Expression of VEGF receptors 1, 2, and 3 was detected in the vessel wall by immunocytochemistry and in situ hybridization. As an additional control, the effect of adenovirus on cell proliferation was analyzed by performing gene transfer to intact aorta without endothelial denudation. No differences were seen in smooth muscle cell proliferation or I/M between lacZ adenovirus and 0.9% saline-treated animals.

Conclusions-Adenovirus-mediated VEGF-C gene transfer may be useful for the treatment of postangioplasty restenosis and vessel wall thickening after vascular manipulations.

Figure 3. I/M in study groups after balloon denudation and gene transfer (mean \pm SEM). A, I/M 2 weeks after gene transfer. *P <0.05. B, I/M 4 weeks after gene transfer. n=6 in each study group.

Figure 5. I/M in study groups after gene transfer without balloon denudation (mean \pm SEM). a, I/M 2 weeks after gene transfer. b, I/M 4 weeks after gene transfer. n=3 in each study group.

Lancet, 2000;356(9241);1581

Gene-coated stents herald hope for restenosis prevention

Morris, Kelly

High hopes are held for gene therapy to prevent restenosis of unblocked coronary arteries. But so far, gene delivery has been impractical for therapeutic use. Now, the development of a stent that releases DNA in a controlled way brings gene therapy for coronary-artery disease closer.

Robert Levy (Children's Hospital of Philadelphia, PA, USA) and colleagues made DNA-eluting stents by coating standard stents with layers of polymer-containing green fluorescent protein (GFP) plasmid DNA.

Initially, the team used cell culture to show transfection of the gene without the need for transfection-enhancing agents; GFP was expressed in about 8% of rat aortic smooth muscle cells. Subsequently, six pigs had both a DNA polymer-coated stent and a control polymer-coated stent placed into separate coronary arteries (Nat Biotech 2000; 18: 1181-84).

After 5-7 days, GFP expression was found in about 1% of cells in the DNA-stented arteries but not in control arteries. "Our findings show that spread is actually limited chiefly to the site of stenting, with some DNA

detected downstream in the same artery, but not in other coronaries in the same animal”, explains Levy. The DNA was found to diffuse particularly to the arterial media.

Although some DNA was detected in the lungs of two pigs at necropsy, the lack of DNA in other organs “is encouraging from the safety point of view”, says Levy.

Mayo Clinic Proceedings. 2000;75(8):831-834,

Gene Therapy for Atherosclerotic Cardiovascular Disease: A Time for Optimism and Caution

Cardiovascular disease is the leading cause of death in the Western world, and gene therapy approaches to several cardiovascular disorders have been proposed. One of the major stumbling blocks to be overcome before widespread clinical use of this technology is how to deliver DNA efficiently and safely to cells in vivo. While delivery of DNA alone is inefficient, use of viral vectors may overcome this problem. Adenoviral vectors are most commonly used in cardiovascular gene delivery, but toxicity related to these vectors remains a concern. In addition, duration of gene expression with use of these vectors is limited, which may be advantageous in settings in which transient expression is satisfactory to obtain a therapeutic effect. Gene therapy has been suggested as an approach to multiple conditions, including restenosis after angioplasty, therapeutic neovascularization, and bypass graft restenosis. Phase 1 clinical trials were recently reported. While proof of principle has been established in preclinical animal models, convincing efficacy data in humans do not yet exist. Improvements in vector technology and methods of catheter-mediated vascular gene delivery are needed before widespread clinical application of this therapy.

Lancet, 2000;355(9199):213-222

Cardiovascular gene therapy.

Yla-Herttuala, Seppo; Martin, John F

Vascular gene transfer potentially offers new treatments for cardiovascular diseases. It can be used to overexpress therapeutically important proteins and correct genetic defects, and to test experimentally the effects of various genes in a local vascular compartment. Vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) gene transfers have improved blood flow and collateral development in ischaemic limb and myocardium. Promising therapeutic effects have been obtained in animal models of restenosis or vein-graft thickening with the transfer of genes coding for VEGF, nitric-oxide synthase, thymidine kinase, retinoblastoma, growth arrest homeobox, tissue inhibitor of metalloproteinases, cyclin or cyclin-dependent kinase inhibitors, fas ligand and hirudin, and antisense oligonucleotides against transcription factors or cell-cycle regulatory proteins. First experiences of VEGF gene transfer and decoy oligonucleotides in human beings have been reported. However, further developments in gene-transfer vectors, gene-delivery techniques and identification of effective treatment genes will be required before the full therapeutic potential of gene therapy in cardiovascular disease can be assessed.

Panel. Gene-therapy tool box Treatment genes by therapeutic target

Gene therapy

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