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Gene Therapy for Therapeutic Angiogenesis

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1. Introduction

Cardiovascular diseases still represent the leading cause of death in the western world. Coronary artery disease (CAD) affects over 5% of the US population and is responsible for nearly 7 millions of in-patient procedures every year (1). Peripheral arterial disease (PAD), with a prevalence of 3-30%, also is a very common disease (2). PAD can be classified according to the severity of clinical symptoms into Fontaine-stages I-IV. In Fontaine-stage I patients are clinically asymptomatic and this stage is the most common form of PAD (70-80%). Patients with Fontaine II (10-20%) suffer from intermittent claudication that might be life-style limiting and require therapy like percutaneous transluminal angioplasty (PTA). A smaller portion (3-5%) of PAD patients have critical limb ischemia (CLI) characterized by rest pain (Fontaine III) or ulcer (Fontaine IV). The incidence of CLI is estimated to be 500-1000 per 1 Million but prognosis is very bad. One year after diagnosis only 45 % of patients are alive without major amputation and effective revascularization with relieve of symptoms can only be achieved in 25% of patients. Therefore, new therapeutic strategies are urgently needed for these patients.

2. Preclinical data

Generation of new blood vessels can be achieved by sprouting of new vessels out of the pre-existing capillary plexus (angiogenesis), by generation of new arteries (arteriogenesis) or by circulating endothelial progenitor cells (vasculogenesis) (3). Several factors have been characterized which induce growth of new blood vessels, the most prominent being vascular endothelial growth factor (VEGF) and members of the fibroblast growth factor (FGF) family. In animal models of hindlimb and myocardial ischemia beneficial effects on blood perfusion and blood vessel density of these (and other) factors as well as of progenitor cells could be demonstrated (4) (5). This therapeutic concept was named "therapeutic angiogenesis" and application of angiogenic factors via gene therapy vectors like plasmids or adenoviruses was superior to protein application probably due to longer lasting expression of respective cytokines.

3. Therapeutic angiogenesis: gene therapy trials in PAD patients

Due to promising data in preclinical studies the concept of therapeutic angiogenesis was tested in clinical trials in PAD and CAD patients. While first phase-1 studies in PAD patients were promising phase-II studies in patients with intermittent claudication were negative

(see summary for clinical trials in PAD patients in table 1). Obviously especially patients with CLI respond to therapy with angiogenic factors and gene therapy seems to have a benefit over therapy with respective proteins.

Trial	Factor	Patients	Effects	Reference
Phase-1	VEGF-165 plasmid i.m.	n=6; CLI (Buerger's)	Increase ABI, collaterals; improvement ulcer, pain	Isner et al 1998 (6)
Phase-1	VEGF-165 plasmid i.m.	n=9; CLI	Increase ABI, collaterals; improvement ulcer, pain, walking time	Baumgartner et al 1998 (7)
PREVENT I Phase-1	E2F decoy, bypass graft ex-vivo	n=41; bypass OP	Reduction bypass-stenosis, -occlusion and -revision	Mann et al 1999 (8)
Phase-1	FGF-2 protein i.a.	n=13; claudication	Increase calf blood flow	Lazarous et al 2000 (9)
Phase-1	FGF-2 protein i.v.	n=24; claudication	No improvement of walking time, proteinuria	Cooper et al 2001(10)
Phase-1	FGF-1 Plasmid i.m.	n=66; CLI	Improvement TcPO2, ABI, pain, ulcer	Comerota et al 2002 (11)
TRAFFIC Phase-2	FGF-2 protein i.a.	n=195; claudication	Improvement walking time, ABI day 90, not 180	Lederman et al 2002 (12)
RAVE Phase-2	VEGF-121 adenovirus i.m.	n=105; claudication	No improvement of walking time	Rajagopalan et al 2003 (13)
Phase-1	VEGF-165 plasmid i.m.	n=21; CLI	Improvement ABI, collaterals, ulcer, pain	Shyu et al 2003 (14)
Phase-1/2	FGF-4 adenovirus i.m.	n=13, CLI	Improvement pain	Matyas et al 2005(15)
PREVENT III Phase-3	E2F decoy, bypass graft ex-vivo	n=1138 bypass operation	Secondary bypass patency improved; primary endpoint (time to bypass occlusion) negative	Conte et al 2006(16)

Trial	Factor	Patients	Effects	Reference
Phase-1/2	HGF plasmid i.m.	n=6; CLI	Improvement pain, ABI, TcPO2, ulcer	Morishita et al 2006(17)
Phase-1/2	FGF-2 gelatine-hydrogel	n=7; CLI	Improvement walking time, TcPO2, ABI, pain	Marui et al 2007(18)
DELTA-1 Phase-2	Del-1 plasmid i.m.	n=105; claudication	No improvement walking time, ABI	Grossman et al 2007(19)
Phase-1	HIF-1α/VP16 adenovirus i.m.	n=41; CLI	Improvement pain, ulcer	Rajagopalan et al 2007(20)
WALK Phase-2	HIF-1α/VP16 adenovirus i.m.	n=289 claudication	No difference in walking time	ACC 2009

Abbreviations: CLI, critical limb ischemia; ABI, ankle/brachial index; E2F, transcription factor E2F; HGF, hepatocyte growth factor; Del-1, developmentally regulated endothelial locus 1; HIF-1 α, hypoxia inducible factor-1 α ; Buerger's, thrombangitis obliterans Winiwater-Buerger; i.m., intra-muscular; i.v., intra-venous; i.a., intra-arterial; TcPO2, transcutaneous oxygen tension

Table 1. Therapeutic angiogenesesis in PAD.

Trial	Factor	Patients	Outcome
VEGF PVD Mäkinen et al (22)	VEGF-165 adenovirus or plasmid/liposome i.a. after PTA	n=54; claudication, CLI	Increase of vascular density
Groningen Kusumanto et al (23)	VEGF-165 plasmid i.m.	n=54; CLI	Improvement ABI, ulcers
TALISMAN Nikol et al (24)	FGF-1 plasmid i.m.	n=112; CLI	Reduction of amputations; primary endpoint (healing of ulcers) not reached
HGF-STAT Powell et al (25)	HGF plasmid i.m.	n=106; CLI	Improvement TcPO2
TAMARIS, Phase 3 AHA 2010	FGF-1 plasmid i.m.	n=525; CLI	Primary endpoint (major amputation or death) not reached

Abbreviations: ABI, ankle/brachial index; i.m., intra-muscular; i.v., intra-venous; i.a., intra-arterial; TcPO2, transcutaneous oxygen tension

Table 2. Therapeutic Angiogenesis in PAD:-larger placebo-controlled, double-blinded trials.

The last years several placebo-controlled double-blinded trials have been published which showed beneficial effects in CLI patients after i.m.plasmid gene therapy with VEGF, FGF1 or hepatocyte growth factor (HGF) (Tab. 2). Especially the TALISMAN study could demonstrate a reduction in amputation rate. Regarding potential adverse effects these studies did not show evidence of increase of cancer rates or proliferative retinopathy. (21)

The positive results of the TALISMAN study on reduction of amputation rate and mortality in CLI patients by FGF1 gene therapy was the basis for a large phase 3 study. Over 500 CLI patients were treated with FGF1 gene therapy versus placebo. The primary outcome after 12 months was a combined endpoint of major amputation above the ankle or death. The results of this trial, called TAMARIS, were presented at the AHA meeting, November 2010, in Chicago, USA. There was no difference in mortality and major amputation between FGF1 gene therapy and placebo. Also secondary endpoints were not different and there was no increase in occurrence of malignant diseases or proliferative retinopathy. The difference between the positive results in phase 2 (TALISMAN) and negative results in phase 3 (TAMARIS) were explained by a type-1 error (finding by chance) in the phase-2 study. It will be interesting to see the publication of the TAMARIS trial to further discuss the reasons for this negative trial and the different results of this trial and phase 2 TALISMAN.

4. Therapeutic angiogenesis: gene therapy trials in CAD patients

Several angiogenic cytokines (especially VEGF-A and FGF4) were tested in patients with severe chronic CAD in whom revascularization by angioplasty or bypass surgery was no further option and who suffered from severe angina and limited exercise tolerance (for recent excellent reviews please also see (26, 27). As observed in PAD-patients phase-1 and phase-2 studies showed feasibility of these therapies and signs of bioactivity. Specifically, gene therapy (adenovirus, administered intra-coronary) with FGF4 showed a trend toward increase in exercise time in the AGENT (Angiogenic Gene Therapy) trial and the subsequent phase-2 AGENT 2 trial showed reduction in reversible perfusion defect size (however not statistically significant due to one outlier in the placebo group). The phase-3 AGENT 3 and AGENT 4 trials were stopped early when an interim analysis of the AGENT 3 cohort indicated that the primary endpoint (change in exercise treadmill test after 12 weeks) was unlikely to differ between FGF4 and placebo. A pooled analysis of AGENT 3 and 4 however revealed that women and patients >65 years with severe angina had statistically significant improvement in angina class and exercise test. A subsequent gene therapy trial in women with CAD was stopped, apparently due to slow enrollment.

Also VEGF gene therapy was tested in CAD patients in randomized studies. In the Kuopio Angiogenesis Trial (KAT) no difference in restenosis rate (primary endpoint) was observed after intra-coronary VEGF gene therapy (plasmid liposome or adenovirus), however after 6 months increased myocardial perfusion was found after adenoviral VEGF application. In the Euroinject One study VEGF plasmid was injected intra- myocardial into regions with perfusion defects. The primary endpoint, improvement of myocardial perfusion was not reached, however, VEGF improved regional wall motion score.

For summary of controlled trials on therapeutic angiogenesis in CAD patients see table 3.

5. Future perspectives

The negative results of phase-3 trials AGENT and TAMARIS raise important question about therapeutic angiogenesis and gene therapy. What is the reason that therapeutic angiogenesis with factors like VEGF or FGF did improve outcomes in a variety of animal models but failed to improve human disease? One explanation is that often young animals were used

Trial	Factor	Patients	Effects	Reference
Phase-1/2	VEGF-2 plasmid i.myoc.	n=19; CCS3-4, RA, NR	Improvement angina class	Losordo et al 2002(28)
AGENT Phase-1/2	Adenovirus-FGF4; i.coro.	n=79; CCS2-3	Trend toward increase in exercise time	Grines et al 2002 (29)
AGENT 2 Phase-2	Adenovirus-FGF4; i.coro.	n=52; CCS2-4, RA, NR	Improvement of perfusion defects by SPECT (not sign.)	Grines et al 2003 (30)
VIVA Phase-2	VEGF protein i.coro., i.v.	n=178; RA, NR	Improvement angina class, no effect on exercise time	Henry et al 2003(31)
KAT Phase-2	VEGF-165 adenovirus or plasmid/liposome i.coro.	n=103; stable angina	Improvement in myocardial perfusion, no effect on restenosis	Hedman et al 2003(32)
EUROINJECT-ONE Phase-2	VEGF-165 Plasmid i.myoc.	n=80; CCS3-4, RA, NR	Improvement wall motion, no effect on myocardial perfusion	Kastrup et al 2005(33)
REVASC Open label	Adenovirus VEGF-121 i.myoc. (thoracotomy)	n=65; CCS2-4, RA, NR	Improvement in exercise time at 26 weeks, not at 12 weeks	Stewart et al 2006(34)
AGENT3/4 Phase-3	Adenovirus-FGF4; i.coro.	n=532; CCS2-4, RA, (AGENT4: NR)	Enrollment stopped after interim analysis, primary endpoint negative. Improvement angina and exercise time in women, older patients with severe symptoms	Henry et al 2007(35)

Abbreviations: CCS, Canadian cardiovascular society; i.coro., intra-coronary; i.myoc., intra-myocardial; i.v., intra-venous; NR, nonrevascularizable; RA, refractory stable angina;

Table 3. Controlled trials on therapeutic angiogenesis in CAD patients.

whereas in humans usually patients of older age and a variety of co-morbidities are affected. Additionally, transfection efficacy of gene therapy vectors, even of adenoviruses, is lower in humans than in animals and precise dosing of vectors is not possible due to the fact that transgene expression cannot be precisely quantified. Another open question is the selection of gene therapy vectors-adenoviruses usually have adverse effects, especially immunogenicity, whereas plasmid vectors are safe but have low transfection efficacy. Dose and duration of therapy is another question. One dose of a vector that expresses the transgene for days to weeks might not be sufficient to treat a disease that evolved over the time-course of many years. Also patient selection might have been a problem: usually "no-option" patients were included in these studies, e.g. patients with large ischemic ulcers in the case of CLI (Rutherford class 6). Maybe patients with less severe disease, like patients with Rutherford class 5 or patients who would be treated additionally with revascularization procedures would benefit more from therapeutic angiogenesis. Endpoint selection is another critical point as some functional outcome measurements like severity of angina are subjective and might be affected by the placebo effect. Cell-based therapies have shown positive effects in CAD and PAD (36, 37)-maybe a combined therapeutic strategy consisting of cell application and gene therapy with angiogenic factors would result in better outcome.

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Gene Therapy - Developments and Future Perspectives
Edited by Prof. Chunsheng Kang

ISBN 978-953-307-617-1
Hard cover, 356 pages
Publisher InTech
Published online 22, June, 2011
Published in print edition June, 2011

The aim of this book is to cover key aspects of existing problems in the field of development and future perspectives in gene therapy. Contributions consist of basic and translational research, as well as clinical experiences, and they outline functional mechanisms, predictive approaches, patient-related studies and upcoming challenges in this stimulating but also controversial field of gene therapy research. This source will make our doctors become comfortable with the common problems of gene therapy and inspire others to delve a bit more deeply into a topic of interest.

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