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Therapeutic angiogenesis for revascularization in peripheral artery disease

Anna Grochot-Przeczek [a,b], Jozef Dulak [a], Alicja Jozkowicz [a,*]

[a] Department of Medical Biotechnology Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland
[b] Jagiellonian Centre for Experimental Therapeutics, Jagiellonian University, Krakow, Poland

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ABSTRACT

Therapeutic angiogenesis for peripheral artery disease (PAD), achieved by gene and cell therapy, has recently raised a great deal of hope for patients who cannot undergo standard revascularizing treatment. Although pre-clinical studies gave very promising data, still clinical trials of gene therapy have not provided satisfactory results. On the other hand, cell therapy approach, despite several limitations, demonstrated more beneficial effects but initial clinical studies must be constantly validated by larger randomized, multi-center, double-blinded, placebo-controlled trials. This review focuses on previous and recent gene and cell therapy studies for limb ischemia, including both experimental and clinical research, and summarizes some important papers published in this field. Moreover, it provides a short comment on combined gene and cell therapy approach on the example of heme oxygenase-1 overexpressing cells with therapeutic properties.

1. Introduction

Disorders in neovascularization are at the root of many diseases in which impaired formation of blood vessels is the cause of disease progress and setbacks in the therapy. An impairment in function of endothelium and inhibition of neovascularization are the main factors for development of peripheral artery disease (PAD) (Behrendt and Ganz, 2002; Ferrara, 2002). The clinical implications of this condition, caused by atherosclerotic changes within peripheral arteries, are the mostly intermittent claudication and critical limb ischemia leading to pain, tissue damage, and ulceration. Mortality at one year in patients with critical limb ischemia is estimated to be about 25%, with a further 30% of patients requiring amputation (Norgren et al., 2007). Currently, surgical (bypass or endarterectomy) or endovascular (angioplasty, stents, intra-arterial thrombolysis) methods appear to be the best treatment option for such patients. Unfortunately, only 50% of them can be treated with this kind of therapy, and the beneficial effects are visible only in about 25% of the patients (Kawamoto et al., 2009). Therefore stimulation of collateral vessel formation seems to be a good alternative for therapy of PAD. Research on therapeutic angiogenesis for PAD has been mostly directed on administration of proangiogenic growth factors as recombinant protein (protein-based therapy) or by gene delivery (gene therapy), as well as on injection of cells participating in blood vessel formation (cell therapy).

2. Protein-based therapy

Among numerous growth factors exerting proangiogenic effects, several have been demonstrated to induce neovascularization in experimental studies of therapeutic angiogenesis for PAD. As expected, administration of recombinant vascular endothelial growth factor (VEGF) protein caused increase in formation of collateral vessels and capillary density, improvement of muscle function, as well as limited limb necrosis in rabbit model of hind limb ischemia (Bauters et al., 1995; Takeshita et al., 1994a, 1994b; Walder et al., 1996). Similar results, were observed for recombinant basic fibroblast growth factor (bFGF) in rats and rabbits (Baffour et al., 1992, 2000; Bush et al., 1998; Chleboun et al., 1992; Yang et al., 1996, 2000), including diabetic or aged animals (Stark et al., 1998; Yang and Feng, 2000). Noteworthy, high doses of bFGF inhibited angiogenesis and collateral circulation (Baffour et al., 2000). It was also shown that combined administration of VEGF and bFGF proteins has a synergistic effect on angiogenesis *in vivo* (Asahara et al., 1995). Also hepatocyte growth factor (HGF) and placental growth factor (PlGF) recombinant proteins exerted

Abbreviations: AdVEGF, adenoviral vector encoding vascular endothelial growth factor; aFGF, acidic fibroblast growth factor; Ang-1, angiopoietin 1; bFGF, basic fibroblast growth factor; BMDCs, bone marrow-derived cells; BVR, biliverdin reductase; Del-1, developmental endothelial locus-1; EPCs, endothelial progenitor cells; G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte monocyte-colony stimulating factor; GPx-1, glutathione peroxidase 1; HGF, hepatocyte growth factor; HIF-1α, hypoxia inducible factor-1α; HO-1, heme oxygenase-1; IL-1β, interleukin 1β; IL-6, interleukin 6; IL-10, interleukin 1; MCP-1, monocyte chemotactic protein-1; MnSOD, manganese superoxide dismutase; MSCs, mesenchymal stem cells; PAD, peripheral artery disease; PBDCs, peripheral blood-derived cells; PDGF, platelet-derived growth factor; PlGF, placental growth factor; SDF-1, stromal cell-derived factor-1; SnPP, tin protoporphyrin; TNFα, tumor necrosis factor α; VEGF, vascular endothelial growth factor; VEGF-R1, vascular endothelial growth factor receptor 1; VEGF-R2, vascular endothelial growth factor receptor 2; vWF, von Willebrand factor.

* Corresponding author at: Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, 30-387 Krakow, Poland. Tel.: +48 12 664 6411; fax: +48 12 664 6918.
E-mail address: alicja.jozkowicz@uj.edu.pl (A. Jozkowicz).

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beneficial effects on revascularization, such as increase in collateral response, angiographic score and angiogenesis, in experimental models of PAD (Luttun et al., 2002; Morishita et al., 1999). Other growth factors and cytokines that have been shown to induce therapeutic angiogenesis *in vivo* include acidic FGF (aFGF) (Pu et al., 1993, 1995; Rosengart et al., 1997), platelet-derived growth factor (PDGF) (Martins et al., 1994), monocyte chemotactic protein-1 (MCP-1) (Ito et al., 1997) and granulocyte macrophage-colony stimulating factor (GM-CSF) (Takahashi et al., 1999). Administration of the abovementioned proteins enhanced collateral and capillary development (Ito et al., 1997; Pu et al., 1995; Takahashi et al., 1999), improved perfusion (Martins et al., 1994; Pu et al., 1995; Takahashi et al., 1999), prevented necrotic incidents (Morishita et al., 1999), and increased mitogenic activity of vascular cells (Rosengart et al., 1997). Importantly, some experimental studies regarding protein-based therapy reported adverse effects of protein administration, especially in case of bFGF treatment, such as renal toxicity, anemia and thrombocytopenia (Lazarous et al., 1995, 1996).

Clinical trials of protein-based therapy for PAD have been limited to administration of bFGF (Table 1). The results of the first clinical trial have been published in 2000. The patients suffering from atherosclerotic PAD with intermittent claudication underwent intra-arterial infusion of bFGF protein. The study showed that bFGF administration is safe and free of significant adverse effects at short-term follow-up (including no neovascularization in retina) (Lazarous et al., 2000). The next clinical trial performed on patients with PAD and intermittent claudication had to be stopped prematurely because of severe proteinuria which was noticed after administration of bFGF. Interestingly, such a strong adverse effect was observed, although the total dose of injected bFGF was lower in comparison to the first trial. There have been no positive results observed in this study at the time of cessation (Cooper et al., 2001). The third and the biggest clinical trial in patients with intermittent claudication was executed by intra-arterial injection of recombinant bFGF (TRAFFIC study). Primary outcome was a 90-day change in peak walking time. Secondary outcomes included ankle-brachial pressure index and safety. Both investigated outcomes significantly increased at 90 days in bFGF treated group in comparison to the placebo counterpart. bFGF administration did not increase mortality, cardiac or cerebrovascular events. Moreover, there was no evidence of tumorigenesis or toxic effects on the retina related to bFGF. However, proteinuria was more frequent in patients treated with bFGF than in those given placebo (Lederman et al., 2002).

The results of clinical studies related to bFGF recombinant protein administration have been generally not as efficient as expected (Table 1). Systemic infusion of bFGF led to some improvement in clinical symptoms of PAD, nevertheless the efficacy of such therapy was rather poor and likely caused by insufficient level of growth factor in the ischemic tissue. Still a significant nephrotoxicity was observed after bFGF administration. Therefore, local gene therapy, giving sustained over-expression of therapeutic factor precisely in the targeted tissue, became an interesting alternative to the protein-based therapy for patients with PAD.

3. Gene therapy

Delivery of therapeutic nucleic acids into somatic cells has been believed to be a promising strategy for treatment of diseases related to tissue ischemia. This approach provides several important advantages: therapeutic gene can be delivered to the cells present exactly at the site of tissue hypoxia, giving the transient local expression of proangiogenic protein without increase in systemic concentration. Moreover, it is possible to deliver two or more therapeutic genes, in bicistronic or polycistronic vectors, to achieve a more pronounced effect on blood vessel formation, including the genes playing a role in processes directly influencing angiogenesis, such as inflammation or oxidative stress. The proof of concept of gene therapy for cardiovascular diseases sounds hopeful and pre-clinical studies gave very good results, but it occurred that translation of gene therapy approach from bench to bedside is not that easy, and the main problems are related to transduction efficiency, choice of route of administration, type of vector and its dose.

Numerous experimental studies evaluated the efficiency of proangiogenic gene therapy. VEGF has been the most intensively investigated gene. It was shown that adenoviral-mediated transfer of VEGF gene protects against acute vascular occlusion in the setting of pre-existing ischemia in rats (Mack et al., 1998). In another study, intramuscular or intra-arterial transfer of naked VEGF plasmid or VEGF plasmid delivered with fibrin scaffold augmented collateral development and tissue perfusion in rabbit model of hind limb ischemia (Dulak et al., 2002a; Gowdak et al., 2000; Jozkowicz et al., 2003a; Takeshita et al., 1996a, 1996b, 1998; Tsurumi et al., 1996). Similar results have been obtained for intramuscular adenoviral vector-mediated VEGF (AdVEGF) transduction (Rivard et al., 1999). However, edema and excess non-physiological growth of capillaries were detected as adverse effects of the AdVEGF gene therapy (Vajanto et al., 2002). There was also an attempt to use bFGF as a therapeutic gene in treatment of experimental limb ischemia. In opposite to VEGF, bFGF lacks a secretory signal sequence, therefore bFGF gene has to be modified by adding this sequence prior to transfection. Interestingly, it was possible to obtain an increased collateral response with such a strategy in adenoviral-mediated *ex vivo* bFGF gene transfer in a rabbit model of hind limb ischemia (Ohara et al., 2001). HIF-1α (hypoxia inducible factor-1), a transcription factor regulating expression of genes in response to hypoxia, has been also shown to provide significant improvement in perfusion of rabbit ischemic limb when administered as a naked DNA (Vincent et al., 2000). Several other genes have been investigated in experimental models of limb ischemia and gave increase in collateral vessels and angiogenesis (angiopoietin-1 (Ang-1) (Huang et al., 2009; Shyu et al., 1998), Ang-1 and VEGF combination therapy (Chae et al.,

Table 1
Protein-based therapy for peripheral artery disease — clinical trials.

Trial	Treatment	Patients (treated/control)	Follow up	Side effects	Endpoints	Results	Reference
Phase I Double-blind Placebo-controlled Dose-escalation	rh bFGF Intra-arterial 1 × 10–1 × 30–2 × 30 μg/kg	4–5–4/6	6 months	None	Calf blood flow	Increase	Lazarous et al. (2000)
Phase II Double-blind Placebo-controlled	rh bFGF Intravenous 6 × 2 μg/kg	16/8	Prematurely stopped	Severe proteinuria	Peak walking time Functional status	None at the time of cessation	Cooper et al. (2001)
TRAFFIC Phase II Double-blind Placebo-controlled	rh bFGF Intra-arterial 1 × 30–2 × 30 μg/kg	66–61/63	3 months	Proteinuria	Peak walking time Ankle-brachial index	Increase Increase	Lederman et al. (2002)