

Gene and cell therapy of critical leg ischemia.

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Critical limb ischemia (CLI) is a severe disease associated with a high risk of amputation and mortality. In patients who cannot be revascularized several medical options have been tested, including the use of prostanoids, spinal cord stimulation and lumbar sympathectomy. None of these treatments have demonstrated a benefit on the amputation rate after 6-month of follow-up, and they cannot be recommended for CLI treatment in patients without any surgical option. In this setting gene therapy and cell therapy to stimulate angiogenesis have been tested mainly in phase I and II clinical trials and will be reviewed.

Gene therapy

Gene-therapy with the goal to increase or stimulate angiogenesis used mainly gene coding for growth-factors such as vascular endothelial growth factor(VEGF)₁₂₁, VEGF₁₆₅, VEGF-C, fibroblast growth factor (FGF)-1 or hypoxia-inducible factor 1 (HIF-1). Initially, intra-arterial gene-therapy was used but systemic dilution of the gene-therapy product and diffusion of the atherosclerotic occlusion limit efficient gene transfer to the vicinity of the ischemic cells. For these reasons direct intra-muscular injection is hitherto the usual approach in PAD. It was demonstrated that intramuscular injection of naked plasmids is feasible, and that the plasmids remain in a non replicative, unintegrated form that is thus unlikely to be complicated by insertional mutagenesis. Using this approach, plasmid expression could remain active for 2 months. Main results of gene therapy in CLI are summarized in table 1.

It remains impossible to affirm that gene therapy is efficient in limb salvage in CLI patients. To date no study was randomized versus placebo and if increased collaterals seems to be observed it does not mean that it is sufficient enough to reverse severe ischemia. The only randomized study TALISMAN has compared placebo to 4 intramuscular gene transfers within 2 months using non-viral FGF-1 plasmid DNA in patients with CLI and ulcers or gangrenes. Results are expected at the end of this year. Another potential pitfall of gene therapy to stimulate angiogenesis is that we cannot exclude that the increase amount of one single growth factor such as VEGF or FGF-1 is not sufficient enough to durably stimulate angiogenesis, as it was nicely shown in a model of angiogenesis in cornea and rat and rabbit ischemic hind limb models. In human, no therapeutic trial was done using a combination of two or more genes.

Cell therapy

Endothelial progenitor cells derived from bone marrow circulate in the peripheral blood and are implicated in regeneration of injured endothelium and neoangiogenesis after tissue ischemia. These cells were first reported by Asahara who demonstrated their ability to contribute to new vessel formation. The origin of progenitor cells really involved in this vascular replenishment in case of vascular damage or ischemia is still a matter of debate, as it

clearly appears that two types of cells have been used in pre-clinical trials : early endothelial progenitor cells from monocytic origin (CD14⁺) and late or outgrowth endothelial cells (CD14⁻). Both types of cells are able to induce angiogenesis in animal models, with a synergistic effect obtained by mixed transplantation of early and late endothelial progenitor cells. If different types of cells, from distinct origins, can induce angiogenesis *in-vivo* in animal models, to date only bone-marrow mononuclear cells (BMMNCs) and peripheral blood mononuclear cells (PBMNCs), after stimulation by granulocyte colony-stimulating factor (G-CSF), have been used in clinical trials. These studies are summarized in table 2.

Finally, from an evidence based medicine point of view none of the gene- or cell-therapy studies published still give a definite answer concerning the efficacy and safety of the pro-angiogenic approach of CLI. They just demonstrated the feasibility of such a therapy but definite answer will need larger randomized studies using amputation rate as major endpoint. Furthermore, the approach undertaken to date used either a single gene or at odds multiple crude cells from bone-marrow or mobilized peripheral blood. It remains to analyze if the administration of several genes, combination of gene and cell therapy, or the use of an optimized and more specific cell therapy product could achieve a more potent stimulation of angiogenesis. Several other major questions remain unanswered : which patients should be considered best for cell or gene therapy, what is the best route of delivery and is it necessary to perform additional transplantations or gene injections, what is the optimum number of cells or yield of plasmids to inject, and is it safe to stimulate angiogenesis in the long term ? All these questions demonstrate, if necessary, that we still are in the prehistoric age of this fascinating and promising development of new armamentarium in cardiology, using tools hitherto mainly developed in hematology.

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