

The Role of Gene Therapy for Critical Limb Ischemia

Angiogenesis may offer a means of treatment for patients with CLI who are not candidates for surgery or endovascular therapy, but further study is still needed.

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Critical limb ischemia (CLI) is a severe peripheral vascular disease that encompasses patients with nonischemic rest pain, ulcer, or gangrene, as well as patients with impending tissue loss.^{1,2} CLI is considered to be the most severe manifestation of peripheral arterial disease, having a Fontaine classification of 3 to 4 and a corresponding Rutherford classification of 4 to 6.³ Patients with CLI have low ankle-brachial indexes (ABIs; < 0.4) and ankle pressures < 40 mm Hg. Limb loss will occur if patients with CLI are not revascularized in a timely fashion. The main cause of CLI is atherosclerosis followed by thromboembolization, atheroembolization, trauma, vasculitis, and thromboangiitis obliterans.^{1,4}

The burden of CLI in the United States affects approximately 1.7 million people and close to 3 million in developing countries worldwide. The incidence of CLI is expected to rise especially in developed countries due to increasing age, diabetes mellitus, and smoking. Surgical bypass and amputation used to be the mainstay of treatment for patients with CLI. However, the last decade has witnessed a paradigm shift in the management of CLI. This change is mainly in the field of endovascular therapy and medical management for risk factor reduction. These innovative revascularization techniques diminish ischemic pain, improve patient function, and prevent limb loss by restarting perfusion of the distal tissue.⁵

Despite advances in medical treatment, endovascular approaches, and vascular surgery techniques, approximately 10% to 20% of patients with CLI still have no option for

treatment and will eventually need to undergo amputation. Patients who undergo amputation will have poor physical function and quality of life. These patients are at high risk for severe morbidity and increased cardiovascular mortality (myocardial infarction and stroke).^{3,5} The concern associated with the limitation of medical treatments available has created significant incentives to find alternative medical therapies. Gene therapy is believed to be an effective alternative for patients who are not candidates for revascularization by endovascular or surgical approaches. Therapeutic neovascularization using cell- and gene-based therapy is a tool that continues to evolve as an alternative method of therapy in the treatment of CLI.

Gene therapy seeks to address the overexpression of proangiogenic factors through the insertion of genetic material into the DNA of specific cells using viral and nonviral vectors, which initiate the development of new capillaries and blood vessels and restore the irrigation of the affected area.^{6,7}

PATHOPHYSIOLOGY OF ANGIOGENESIS

Angiogenesis, also known as *neovascularization*, is the creation of new capillaries based on preexisting blood vessels through the orchestration of different cells and growth factors, which, all together, allow the branching and extension of new blood vessels.⁸

The initial physiologic response to the ischemic process is the induction of angiogenesis and arteriogenesis (expansion of preexisting collaterals). These mechanisms

attempt to restore perfusion of the distal tissue. Despite this effort, the compensatory mechanism fails to return the blood flow required in the critical ischemic limb.⁴

Hypoxia induces angiogenesis and arteriogenesis through the production of hypoxia inducible factor-1 (HIF-1), which is a heterodimeric protein that has one subunit that is regulated by oxygen. Therefore, the absence of oxygen generates the activation of the HIF-1.⁹ The activated HIF-1 then induces the production of other proangiogenic cytokines, including vascular endothelial growth factor (VEGF).

These growth factors generate the proliferation, differentiation, migration, and survival of circulating angiogenic cells (CACs). The CACs include the endothelial progenitor cells, myeloid cells, and hematopoietic and mesenchymal stem cells.^{8,10} The CACs migrate from the bone marrow to the tissue guided by the chemotactic effect of the growth factors, where they start the process of endothelialization of the new vascular implants.⁸

Some articles suggest that certain proangiogenic factors, such as fibroblast growth factor (FGF), act indirectly by inducing the activation of the VEGF.⁴

GENE THERAPY FOR CLI

Several studies conducted on CLI and gene therapy have resulted in a large number of growth factors being tested. The most recent studies were conducted with hepatocyte growth factor (HGF), FGF, and HIF-1 alpha.

Hepatocyte Growth Factor

A recent trial including 22 patients who were treated with an intramuscular injection of naked HGF plasmid found a significant increase in ABI after 6 months of follow-up. It also showed an improvement in rest pain, ulcer diameter, and maximum walking distance.¹¹

In another trial, HGF plasmid was administered intramuscularly to 21 patients, and six patients received placebo. This trial found that patients receiving HGF had a significant improvement in toe-brachial index and lower extremity pain after 6 months. However, it could not establish a significant difference in the wound healing process, amputation rate, or mortality rate between the two groups.¹²

Fibroblast Growth Factor

The TALISMAN 201 trial was performed with the intramuscular administration of a plasmid with FGF (NV1FGF). In this trial, 107 patients were evaluated and showed no difference in the ulcer healing process in either of the ABI and toe-brachial index groups. Nevertheless, the investigators found a twofold reduction in the amputation risk ($P = .015$). The 12-month amputation-free survival rate of the patients receiving placebo was 48% compared with 73% in patients treated with FGF.¹³

In the TAMARIS trial, 525 patients were enrolled: 259 patients were assigned to the NV1FGF group and 266 to the placebo. This study, contrary to TALISMAN, could not show a difference between the two groups in terms of major amputation or death.¹⁴

Hypoxia Inducible Factor-1 Alpha

An adenoviral vector with an active form of HIF-1 was used to treat diabetic mice that were subjected to unilateral ligation of the femoral artery. Three days after ligation, the limb perfusion significantly improved. The mice treated with HIF-1 also showed less neurological deficits and tissue damage.¹⁰

META-ANALYSES

Since 2008, two meta-analyses have been conducted of gene therapy for the treatment of patients with peripheral arterial disease; one of those evaluated a subgroup of patients with CLI.

The first meta-analysis, performed in 2008, analyzed five different trials. Three trials included VEGF, one FGF, and one developmental endothelial locus-1. The meta-analysis could not find a significant difference in ABI, peak walking time, or claudication onset time.¹⁵

The second meta-analysis was published in 2009. It analyzed six trials, four of which had already been analyzed in the 2008 meta-analysis. However, this study found that peak walking time, ulcer healing, rest pain relief, and limb salvage improved in the treated patients. Specifically, the investigators found a higher rate of ulcer healing and pain relief in patients with CLI who were treated with gene and cell therapy.¹⁶

CONCLUSION

There is a need to find a clinical therapy for CLI patients who are not candidates for surgical or endovascular intervention, and gene therapy could be a possible future solution for these patients. However, gene therapy as a treatment for CLI is still a controversial topic due to varying results in clinical trials. Therefore, it is essential to perform new trials that study different doses, methods of administration, and growth factors that can help clarify the effects of this therapy. ■

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