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Therapeutic Angiogenesis Another Passing Phase?

Gregg L. Semenza

Efforts to stimulate tissue vascularization in patients with ischemia resulting from coronary or peripheral arterial disease (therapeutic angiogenesis^{1,2}) have passed through several scientific phases over the last dozen years, based on our increased understanding of the molecular mechanisms underlying vascular homeostasis (Table). In the first phase, a single angiogenic growth factor, or its cognate DNA sequence, was injected into ischemic tissue.^{2,3} In the case of angiogenic growth factor gene therapy, the goal was to augment production of the angiogenic factor by the patient's own cardiac or skeletal muscle cells. The angiogenic factor, as exemplified by vascular endothelial growth factor (VEGF), was believed to act by binding to receptors that were expressed on the surface of endothelial cells, activating the cells to migrate, proliferate, and form new or large vessels, thus allowing increased perfusion of tissue surrounding the injection site. The administration of these factors worked well in accelerating the recovery of perfusion in healthy young laboratory animals subjected to an acute interruption of coronary or femoral arterial blood flow. Encouraging results were also obtained in early clinical trials that lacked control populations. However, the double-blind placebo-controlled VIVA study of VEGF in patients with myocardial ischemia showed no significant objective benefit of therapy but instead revealed a placebo effect that was remarkable in its magnitude.⁴

The second phase of therapeutic angiogenesis began with the identification of circulating bone marrow–derived cells, which expressed markers of both hematopoietic progenitor cells (CD34, c-kit) and endothelial cells (VEGFR2) and thus were given the name endothelial progenitor cells (EPCs) and shown to participate in ischemia-induced angiogenesis.^{5,6} In this phase, the cells, which were mobilized from the bone marrow and recruited to sites of ischemia, were thought to differentiate into the additional endothelial cells and pericyte/smooth muscle cells that were required for new blood vessel formation. In cardiac infarction models, even more amazing feats of transdifferentiation were ascribed to these cells, leading to the regeneration of myocardium by cells which

were purported to have only recently arrived from the marrow.^{7,8} Many of the “angiogenic growth factors” (such as VEGF) that were identified in the first phase were shown to function as “vasculogenic cytokines” in the second phase.^{3,6} A hybrid between the first and second phases of therapeutic angiogenesis involved the administration of cytokines, such as granulocyte-macrophage colony-stimulating factor (GM-CSF), that promote the mobilization of bone marrow cells. The START trial, in which patients with limb ischemia were treated with GM-CSF, failed to demonstrate efficacy.⁹

In the third phase, careful cell labeling experiments using confocal microscopy confirmed that bone marrow–derived cells were indeed recruited to sites of ischemia in laboratory animals, but indicated that these cells took up residence in a perivascular location where, it was suggested, their participation in tissue vascularization was limited to an instructive role, through the production of “paracrine factors”^{10,11} corresponding to the “vasculogenic cytokines” of the second phase and the “angiogenic growth factors” of the first phase. Similarities between the functional and structural characteristics of EPCs and peripheral blood mononuclear cells (PB-MNCs) were also reported.^{12,13} Despite continued uncertainty regarding the mechanisms by which these cells promote vascularization after administration to laboratory animals, the TOPCARE-AMI trial, which was performed in Europe and involved autologous transplants of bone marrow MNCs or circulating progenitor cells, demonstrated encouraging results.¹⁴ Double-blind placebo-controlled studies have not yet definitively established whether this approach is efficacious.

We may have entered another phase with the publication of an interesting study by Tateno et al in this issue of *Circulation Research*.¹⁵ The authors enrolled 29 patients with critical limb ischemia, the majority of whom (21 of 29) were facing amputation as the only therapeutic option, in a study designed to explore the utility of autologous PB-MNC transplantation. Approximately 10 billion PB-MNCs were collected from each patient by peripheral blood apheresis which, unlike bone marrow collection, does not require general anesthesia. The PB-MNCs were transplanted into the ischemic leg divided among ≈100 injection sites that were spaced several centimeters apart. The procedure was repeated approximately one month later. Patient status was assessed 2, 9, and 12 months after treatment. Maximum walking distance, healing of ischemic ulcers, and ankle:brachial blood pressure ratios were increased, whereas rest pain and amputation were decreased, with 21 of 29 patients manifesting improvement in at least one of these categories. The results reported by Tateno et al¹⁵ stand in contrast to the results of a study by Tateishi-Yuyama et al in which injection of PB-MNCs was performed as a negative control in the contralateral limb of patients injected

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Molecular Mechanisms of Angiogenesis: A Brief Scientific History

Phase 1	Tissue vascularization is controlled by the ischemia-induced production of angiogenic growth factors
Phase 2	Bone marrow–derived progenitor cells are recruited to sites of ischemia and contribute to tissue vascularization and repair by differentiating into endothelial cells, pericyte/smooth muscle cells, and other cell types
Phase 3	Bone marrow–derived mononuclear cells are recruited to sites of ischemia and contribute to tissue vascularization by producing paracrine factors
Phase 3a	Mononuclear cells are recruited to sites of ischemia and stimulate myocytes to produce angiogenic cytokines and growth factors

with bone marrow MNCs.¹⁶ The establishment of clinical efficacy will require double-blind studies in which patients are randomly assigned to receive PB-MNCs or placebo, a design which should be facilitated by the relative ease with which PB-MNCs, as compared with bone marrow cells, can be collected.

To understand the basis for what appeared to be a dramatic therapeutic effect in their patients, Tateno et al¹⁵ performed a detailed comparison of the clinical characteristics of responders and non-responders. This analysis revealed that after cell implantation, the serum levels of C-reactive protein, which is a marker of inflammation that is induced by cytokines, were higher in responders than in non-responders, as were the peak levels of interleukin (IL) 1 β , IL-6, and VEGF. To investigate the role of IL-1 β , the authors performed experiments with wild-type and IL-1 β -deficient mice. PB-MNCs from donor mice of either genotype promoted the recovery of blood flow and the production of IL-1 β after femoral artery ligation, whereas implantation of PB-MNCs from donor mice of either genotype into IL-1 β -deficient recipient mice had little effect. Further studies suggested that transplanted PB-MNCs promoted tissue vascularization by stimulating resident skeletal muscle cells in the ischemic limb to increase the production of IL-1 β and other angiogenic cytokines.

Has therapeutic angiogenesis come full circle to a renewed focus on stimulating the production of angiogenic cytokines/growth factors? Well, yes and no. First, IL-1 β was shown to be necessary but not sufficient to elicit the vascular response that was elicited by PB-MNCs. This result is consistent with many other studies indicating that multiple angiogenic factors are required for physiological vascularization. But the authors suggest another important difference in the Discussion, where they state that the implantation of PB-MNCs increased the number of regenerating myocytes, which were a major source of IL-1 β . If this observation is confirmed, then the elucidation of molecular mechanisms by which PB-MNCs stimulate muscle regeneration in the ischemic mouse limb, and investigations as to whether this process is relevant to critical limb ischemia in humans, appear to represent the next steps in what may be an interesting new phase of circulation research and therapeutic angiogenesis.

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