Hotline Editorial

Angiogenesis for revascularization of ischaemic tissues

Bypass surgery and percutaneous revascularization have, for the past 30 and 20 years respectively, provided a direct means of augmenting blood flow to ischaemic tissues. Many patients with symptomatic coronary and/or peripheral artery disease, however, are not suitable candidates for either of these interventions due to the extensive nature of the vascular obstructions; this often includes recurrent obstruction of the grafts or angioplasty sites themselves. Therapeutic angiogenesis constitutes a novel treatment option for such patients.

The strategy which we and others have employed in this regard borrows from earlier studies which have established the potential for endothelial cells to break free from their basement membrane and surrounding extracellular matrix, migrate, proliferate, and remodel (i.e. form a lumen), thus generating sprouts from the parent vessel. Such a paradigm for post-natal development of new blood vessels has been termed angiogenesis, and presumably accounts for some, if not most, collateral vessels which constitute 'auto-bypass' conduits in patients with vascular occlusive disease.

Growth factors which have been recognized to promote angiogenesis share in common the potential to act as mitogens for endothelial cells. Vascular endothelial growth factor is distinguished from other known angiogenic cytokines by two features: (1) it is a mitogen only for endothelial cells (as opposed to smooth muscle cells and fibroblasts), and (2) it includes at its amino terminus a signal sequence that permits vascular endothelial growth factor to be naturally secreted by intact cells.

We have exploited these features of vascular endothelial growth factor to develop clinically applicable strategies for therapeutic angiogenesis employing either recombinant human vascular endothelial growth factor protein (rhVEGF) or the gene encoding vascular endothelial growth factor (phVEGF). Because the protein is not yet available for human application, we initiated in December 1994 clinical trials of human gene therapy involving percutaneous arterial gene transfer of phVEGF for patients with critical limb ischaemia. The gene encoding vascular endothelial growth factor is delivered as so-called 'naked DNA', i.e. DNA unassociated with other vectors, including viruses of liposomes. The solution of plasmid DNA is applied to the hydrogel coating of an angioplasty balloon; the polymer acts as a 'sponge' to retain DNA until the balloon is inflated at the site of gene transfer at which time DNA is transferred to the arterial wall.

Using a dose-escalating design, treatment was initiated with 100 µg of phVEGF. Three patients presenting with rest pain (but no gangrene) and treated with 1000 µg were subsequently shown at 1-year follow-up to have improved blood flow to the ischaemic limb and remain free of rest pain. We considered the possibility that vascular endothelial growth factor could produce flow augmentation in these patients simply as a result of its ability to act as a potent stimulus for the release of nitric oxide; this explanation, however, seemed unlikely in view of the demonstration that augmented flow was documented on serial studies performed well beyond the time (21–30 days) that the transferred gene is actively expressed. With the increase in dose of phVEGF to 2000 µg, angiographic and histological evidence of new blood vessel formation became apparent.

These findings have thus established proof of principle for two concepts. The first is the potential for the administration of angiogenic growth factors to promote development of new collateral blood vessels in human patients. While not yet sufficient to prevent distal limb amputation in patients with advanced gangrene, use of higher doses, multiple applications, and/or alternative delivery routes, viz intramuscular injection, of the gene or protein may yield sufficient neovascularity to make this goal a reality.

The second concept is the feasibility of arterial gene transfer of naked DNA. The use of naked DNA is admittedly inefficient, permitting successful transfection of <1% of target smooth muscle cells. In the case of vascular endothelial growth factor, there are several aspects of the gene, protein, and target tissue which may have contributed to modulation of the host phenotype (increased vascularity and flow) despite a low transfection efficiency. First, vascular endothelial growth factor, as noted above, is actively secreted by intact cells; previous studies in our laboratory have documented that genes which encode for secreted proteins — as opposed to proteins which remain intracellular — may yield meaningful...
biological outcomes due to paracrine effects of the secreted gene product.

Second, heparin-avidity of the intermediate and longer vascular endothelial growth factor isoforms, VEGF$_{165}$ and VEGF$_{189}$ respectively, promotes binding to cell surface and matrix heparan sulfates that may create a biological reservoir of the secreted protein, enhancing the temporal opportunity for bioactivity.

Third, while endothelial cells were previously viewed solely as the target for vascular endothelial growth factor, it is now clear that endothelial cells subjected to hypoxia can synthesize vascular endothelial growth factor as well.[10] This autocrine feature of vascular endothelial growth factor created the opportunity for amplifying the effects of even a small amount of exogenous vascular endothelial growth factor, as endothelial cell proliferation in the ischemic territory creates additional potential cellular sources of vascular endothelial growth factor synthesis and secretion.

Fourth, vascular endothelial growth factor inhibits apoptosis[11], apparently by upregulating endothelial cell expression of fibronectin and avf$eta_3$, thus preserving the survival signal generated by attachment of endothelial cells to their extracellular matrix. Such reduction in endothelial cell apoptosis would be expected to complement the mitogenic effect of vascular endothelial growth factor, resulting in a further net increase in endothelial cells viability.

Fifth, with regard to the target of gene therapy, it has been noted[5,6,8] that vascular endothelial growth factor-induced angiogenesis is not indiscriminate or widespread but is instead restricted to sites of ischaemia. This appears to result from paracrine upregulation of the principal high-affinity vascular endothelial growth factor receptor (Kdr) in response to factors released from hypoxic skeletal myocytes.[12] Receptor upregulation on endothelial cells within the region of lower limb or myocardial ischaemia thus enables these cells to act as magnets for any vascular endothelial growth factor secreted into the ischaemic milieu.

These considerations underscore the notion that the success of gene therapy is not solely a function of vectors or transfection efficiency. While it is clear that better vectors with which to augment transfection efficiency should remain a principal goal of gene therapy, features of the gene and target may independently increase or decrease the likelihood of a favourable outcome. Modifications in any of these respects, including the use alone or in combination of other angiogenic growth factors, will receive intensive scrutiny in the near future to optimize angiogenesis as a useful treatment option for lower extremity and myocardial ischaemia.

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References


