A shot in the leg to treat blocked arteries

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Atherosclerotic disease of blood vessels in the leg is a common cause for suffering in the elderly. The arteries usually get narrowed due to fatty deposits and fibrous plaques. Occlusion of limb arteries and consequent reduction in blood flow to the limb can lead to disabling clinical symptoms such as pain at rest, ulcers in the skin and even suppuration of the toes. Smokers and patients with diabetes mellitus are more prone to severe affliction and in them, the disease tends to progress rapidly.

Medical treatment is ineffective when blood through the arteries is critically reduced. Operative treatment to re-establish blood flow consists of either opening up the artery and removing the offending atherosclerotic plaque (end arterectomy) or creating a bypass using venous or synthetic grafts. Currently, there are several other forms of treatment as well. The atherosclerotic plaque can be burnt off with laser or the obstructing lesion can be shaved off with a rotary cutting device. Narrowed arteries can also be dilated by introducing a balloon to the site of obstruction and suddenly inflating the balloon (balloon angioplasty). Arteries can be kept in the dilated state by inserting metallic or polymeric stents. These techniques are relatively safe and have acceptable success rates. However, in a significant number of patients, the arteries get occluded again. Moreover, the techniques are invasive and involve puncture of arteries at accessible sites and threading of catheters to the sites of lesion.

A novel approach to treatment of locally reduced blood supply is to induce controlled growth of new blood vessels in the region of ischaemia[1]. In recent years, considerable progress has been made in identifying and characterizing substances which regulate mechanisms of blood vessel formation (angiogenesis). In experimental animals as well as in patients, local application of angiogenic factors has been found to be promising to treat conditions with localized hypovascularity. Attempts are now being made to introduce or transfer genes encoding angiogenic factors to the arterial wall. Direct gene transfer to the arterial wall involves invasive techniques which are difficult to be performed in patients with extensive and diffuse vascular disease. Extensive calcification, which accompanies atherosclerosis also precludes gene transfer to smooth muscle cells in the arterial wall.

Jeffrey Isner's group at Tufts University School at Boston, has been in the forefront of angiogenic gene therapy for a number of years. They are engaged in identifying appropriate genes for targeting, developing suitable biological vectors for efficient gene transfer and also finding effective delivery techniques for gene therapy in peripheral vessel disease.

Isner and colleagues (Tsurami *et al.*), in a recent issue of *Circulation,* report a simple gene transfer approach for inducing angiogenesis[2]. They have demonstrated blood vessel growth in experimental animals, after injection of naked DNA encoding vascular endothelial growth factor (VEGF) into skeletal muscles in ischaemic limbs.

There have been previous reports on the feasibility and efficacy of direct intramuscular gene transfer of plasmid DNA with a variety of reporter genes[3]. The possibility of intramuscular gene transfer of naked DNA to achieve expression of angiogenic factors in the treatment of peripheral vascular disease is demonstrated for the first time.

Naked plasmid DNA encoding the 165 amino acid secreted form of human vascular endothelial growth factor (hVEGF-165) was injected at multiple sites into three major thigh muscles of hind limbs of New Zealand white rabbits. The limbs were earlier made ischaemic by excising the femoral arteries in the thighs. Thirty days after injection, increased number of capillaries, collateral blood vessel formation and improved blood flow and tissue perfusion could be demonstrated in the injected limb. VEGF mRNA was detected in the skeletal muscle from day 3 after transfection and evidence of gene expression at protein level was seen at 5 days after transfection.

Vascular endothelial growth factor was discovered by Senger and co-workers[4] in the early 1980s. At that time, it was identified as a vascular permeability factor (VPF) which increases leakage of cells, fluids and proteins across the vessel wall. Later, this heat stable 46 kD dimeric protein was found to have the ability to stimulate the growth of endothelial cells and promote angiogenesis[5]. The biological activities of VEGF are mediated by two transmembrane receptor tyrosine kinases, which are expressed on vascular endothelial cells and angioblasts, their embryonic precursors. During embryonic development these receptors play an important role in the development of blood vessels[6]. VEGF was cloned and characterized eight years ago.

Isner's group had shown earlier that VEGF can stimulate the development of collateral arteries in animal models of both peripheral and myocardial ischaemia[7,8]. They also demonstrated recently, that angiogenesis can be induced in patients by transferring ph VEGF gene directly to proximal regions of occluded arteries[9].

The demonstration of viability of intramuscular gene transfer of naked DNA to achieve local delivery of an angiogenic factor and the observation that transfection leads to angiogenesis and improved tissue perfusion are important for cardiovascular gene therapy in general. The technique may be employed for trans endocardial delivery of angiogenic factor in patients with coronary artery disease.

A disadvantage in using naked plasmid DNA is the low efficiency of gene transfer which results in low levels of gene expression. However, acute or chronic hypoxia associated with ischaemia has been shown to be a strong stimulus for upregulation of VEGF receptor gene expression and endothelial cells of ischaemic tissues are likely to be more responsive to VEGF[10]. Hypoxic skeletal muscle in comparison with normal muscle, has been shown to have increased uptake and expression of exogenous plasmid DNA[11]. Hence, Tsurumi *et al.*'s results are encouraging.

M. W. Majesky cautions that VEGF may cause leakage of vasoconstrictors and coagulation factors from vessels, resulting in constriction of arteries and clot formation, further reducing blood flow through ischaemic vascular bed[12]. He also draws attention to the potential for elevated levels of VEGF in blood to initiate latent tumour growth or exacerbate diabetic retinopathy.

Be that as it may, the results reported from the laboratory of Isner are indeed exciting because of the wide spectrum of potential clinical application. The important question is whether the increase in perfusion achieved by angiogenesis induced by intramuscular transfer of VEGF will last long.

Early this year, yet another advance was reported, strategically significant for therapeutic angiogenesis[13]. From human blood, using magnetic beads coated with antibody to CD34, mononuclear blood cells which differentiated and proliferated under tissue culture conditions have been harvested. These cells stained positively for markers such as Factor VIII, endothelial constitutive nitric oxide synthase and E-Selectin. Thus, they have been identified as endothelial cell progenitors or angioblasts. In animal models with hind limb ischaemia, when these cells were administered into the tail vein, the cells integrated into capillary vessel walls. New blood vessels were observed on histological examination of ischaemic limb, 1–6 weeks later.

These findings imply that angioblasts isolated from the blood of patients, themselves may be used to promote vascular growth and also as vectors to deliver angiogenic factors.

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Birth and growth of early continental crust

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The present configuration of Earth's continents is the result of plate tectonic and mantle activities operating over past 4.5 billion years. While we have authentic records for the past 4 billion years, no readily observable crustal growth details in the form of vestigial bits of land or any indirect evidences for earlier continental segments for the first half-billion years are available for geochemists to reconstruct this chapter of Earth's history. The latter, therefore, had remained somewhat enigmatic till discoveries of 4.2 b.y. detrital zircon grains in Australia a few years back, which confirmed the existence of an earlier granitic crust[1,2]. Quite understandably therefore, many of the earlier ideas about continental growth in this span of Earth's evolution were speculative. Finds of such relict minerals or other geologic remnants indicating existence of early crusts were rare, or, even if found, conclusions derived from some of them were found to be rather unreliable owing to imprints of later geologic events they carried which were masking the pristine history. It is obvious from such a scenario that most of the crust that formed between 4.5 and 3.9 billion years ago, must have perished soon; whether they were engulfed or resorbed by the mantle, or whether they piled up into thicker and denser masses only to sink soon back into the mantle in an unceasing cyclic process of creation and destruction, is difficult to say now[3–5].

The earliest well-preserved continental crusts, just a little less than 4.0 b.y., are the Acasta gneisses in Canada and slightly younger rocks in western Greenland, North America, Antarctica, China and western Australia[3,6]; in India, such ancient crusts dated between 3.5 and 3.8 b.y. or older have been reported from the Older Metamorphic Group (OMG rocks) in the Singbhum batholith in Bihar, and the Grey gneiss in Rajasthan; and from Karnataka and Orissa, younger examples are reported[7–9]. Today questions for which earth scientists are trying to seek answers are: how much continental crust formed early out of the mantle; whether the extent or spread of the continents continued to be the same ever since they fractionated early or did they grow gradually with fresh increments of crustal slices?[10].

Two contemporary models on this subject of crustal growth are current today, but they present diametrically opposite view points. One of them considers that the total volume of continental crust had increased steadily with progress of geologic time. This view draws support from (a) the global spread of continental crust and their eroded equivalents which are found to vary through different ages; (b) the observed changes in the chemical composition of mantle extract forming the upper crust which varies from sodium-rich crust of the Archaean times (tonalite–trondjemite–granodiorite or TTG) to potassium-rich crust of younger periods[5]. This model, however, is considered inadequate, by some, inasmuch as the early Archaean melt is not basaltic, as one would expect, but of TTG composition[5,11,12]. According to the second model, the