NEW HORIZONS

Angiogenesis And Gene Therapy State-of-the-Art

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The ever-continuing search for treatment of Coronary Artery Disease(CAD) has encompassed medical therapy with nitrates, beta-blockers, thrombolytic agents, trans-catheter interventions, Laser, Coronary Artery Bypass Surgery (CABG and many other modes of revascularization. Anti-anginals like nitrates and beta-blockers give symptomatic relief without reducing the structural blocks in the coronaries. Fifty percent of patients of CAD do not have suitable anatomy for conventional re-vascularisation procedures due to diffuse multiple blocks. Those with suitable anatomy who undergo re-vascularisation may again develop re-stenosis at the stent or anastomotic sites or develop critical blocks in the grafts or develop new blocks distal to re-vascularised sites. For finding alternative treatment for such patients research in vascular biology has brought focus on vascular growth factors. Therapeutic Angiogenesis has emerged as a helpful modality in these patients as documented by improvement in clinical status and angiographic findings.

Definition : Angiogenesis is the process of stimulating 'new blood vessel formation' from pre-existing capillary beds to produce more capillaries for improving blood flow in ischemic tissues.

The agents used for creating angiogenesis are :

- Recombinant angiogenic growth factors e.g. Vascular Endothelial Growth Factor (V-EGF) and basic Fibroblast Growth Factor (bFGF).
- Gene therapy i.e. in Vivo transfection of angiogenic growth factor G, Intra muscular gene transfer of naked DNA encoding human VEGF.

Vascular Endothel Growth Factor (VEGF) is an angiogenic peptide - which is primarily mitogenic for endothchal cells. It can stimulate collateral vessel development in the ischemic myocardium.

Fibroblast Growth Factor (FGP) is mitogenic for vascular endothelial cells and smooth cells. The biochemical structure of FGF-1 was recognised in 1985 (1). It was isolated from human brain tissues in 1986 (2). Successful technique of gene transfer to introduce the information for expressing FGF-1 into apathogenic Escherichia coli was reported in 1991 (3).

Angiopoietin-1 - mediates the recruitment of smooth muscles cells to the wan of neo-vessels (4).

Angiopoietin-2 - prevents smooth muscle apposition to the walls of microvessels (5).

- Growth Factor protein (Naked DNA)

Routes of delivery : These agents can be delivered by Intra-venous, Intra-muscular, intra-myocardial or Intra-Coronary routes.

The **concept** of angiogenesis developed in seventies (6) based on the observation by cancer research workers

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that tumors grow because they develop their own blood vessels - the process of Neo-vascularisation.

Transmyocardial Laser Revascularization (TMLR) showed reduction of symptoms in patients with chronic ischemic heart disease. It is now believed that angiogenesis is stimulated around the intra-rnyocardial channels created by the laser beam.New capillaries thus formed improve local perfusion in the ischemic myocardial territories (7).

Local gene therapy aims at over expressing proteins that (1) Regulate the cell cycle of vascular smooth muscles cells (VSMC) (2). Inhibit VSMC migration (3). Endow the endothelium with its vasoprotective properties (4). Stimulate growth of endothelium and angiogenesis alternatively. Some approaches tend to suppress gene expression of proteins believed to promote VSMC proliferation and migration.In contrast to drug therapies local gene therapy limits expression of the beneficial agents to the injured vascular site.The clinical potential of this approach has led to the initiation of trials that currently evaluate gene therapy approaches to the attenuation of peripheral and myocardial ischaemia and the prevention of vein graft disease (8-9).

Over-View : Research so far has shown promising outcome : In one study recombinant human fibroblast growth factor-1 (FGF-1) was tested in vitro and vivo. FGF-1 was obtained from the strains of Escherichia coli by genetic engineering, isolated and highly purified (10). Initially FGF-1 was tested and proved successful on animal models. Later it was used on 20 post-CABG patients with native triple vessel disease.FGF-1 was directly injected in the myocardium adjacent to left anterior descending artery (LAD) and distal to the anastomotic site with left internal mammary artery during surgery and after completion of anastomosis. A three-month post procedural angiographic follow-up revealed coronary artery neo-vascularisation spreading firom the injection site of FGF-1, creating a capillary network from the proximal to the distal segment of the stenosed blood vessel producing a "capillary-to-capillary by-pass". In the comparable CABG control group of 20-patients who received inactivated FGF-1 by same technique there was no such neo-vascularisation at 3-months.

Further research has led to bioassays for angiogenesis, purification of the angiogenic factors (11-14), discovery of angiogenesis inhibitors (15-17). and use of Interferon-alfa (18-20) for treatment of haemangiomas.

Angiographically the growth of new blood vessels after administration of VEGF and fibrin composite has been demonstrated (21). Fibrin glue ensures slower release and prolonged availability of VEGF,thus sustaining angiogenesis, which leads to improved oxygenation of ischemic tissues in patients with critical limb ischemia.

MRI mapping has demonstrated the benefits of VEGF induced myocardial angiogenesis. Studies (22-23) in the porcine model of chronic ischemia , that closely mimics the human pathophysiology of progressive coronary occlusion, have demonstrated that with VEGF treatment the ischaemic zone become smaller leading to better ejection fraction and improved regional wall thickening.

Downside

Agents which stimulate angiogenesis can also stimulate plaque angiogenesis and secondary plaque growth in the coronary arteries thus worsening the disease. Angiogenesis involves sprouting of capillaries - new vessel formation at capillary level.These capillary tubes lack vascular smooth muscles cells, they are fragile and prone to rupture. They do not become remodelled and are unable to sustain proper circulation as they cannot adopt to changes in physiological demands of blood supply.The new capillaries sprouting from already diseased atheroselerotic vessels may not have a sufficient native flow to provide required blood supply distally to the ischemic tissue (24).

Future

The treatment of CAD by the end of the first decade of this century is likely to be guided by the advancement in Angiogenesis and the gene therapy. A long term followup will determine whether there will be sustained and significant symptomatic and functional improvement.

Genetic Engineering is expected to alter the vulnerability of certain communities to coronary atheroselerosis.Techniques for myocardial tissue regeneration are also on the anvil. The ravages of end stage CAD producing heart failure are likely to be countered by inducing a new myocardial cell formation by gene therapy.

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