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Angiogenesis: Protein, Gene, or Cell Therapy?

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INTRODUCTION

Cardiac surgeons often encounter patients with ischemic but viable myocardium who are not candidates for direct revascularization due to diffuse coronary atherosclerosis that prohibits angioplasty or surgery. Although incomplete revascularization is occasionally an option for these challenging patients, the long-term risk of additional adverse events is high. Therapeutic angiogenesis, a strategy designed to amplify the native angiogenic process and enhance the reperfusion of ischemic tissues, may represent a novel approach to revascularization of these high-risk patients. Independent efforts have focused on achieving therapeutic angiogenesis by way of protein, gene, and cell-based therapies, all of which have been used clinically. The purpose of this review is to highlight the current progress, controversies, and challenges in the development of protein, gene, and cell-based approaches for clinical therapeutic angiogenesis.

Angiogenesis as a Therapeutic Target

The process of angiogenesis is a complex, tightly regulated cascade of events involving numerous growth factors, such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF), resulting in the proliferation of endothelial cells to form a new capillary network. Angiogenesis can be induced by inflammation or ischemia but the contribution of these new capillaries to overall effective perfusion may be minimal. The collateral vessels associated with chronic coronary occlusions are the result of arteriogenesis, which is equally complex and involves a wider variety of cell types and growth factor signals. In contrast, vasculogenesis is the formation of fully formed vessels from endothelial cell precursors, a process that does not occur in developed (postembryonic) tissues according to Simons and colleagues [Simons 2000]. The larger, media-rich vessels of arterio- and vasculogenesis would contribute considerably to effective perfusion and may represent a more relevant clinical target.

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MATERIALS AND METHODS

Approaches to Therapeutic Angiogenesis

In animal models of myocardial ischemia, administration of angiogenic growth factors, either as recombinant protein or by gene transfer, can improve the perfusion of the ischemic region by inducing angiogenesis. While many growth factors have angiogenic activity, the best studied are VEGF and FGF. Both of these proteins can induce angiogenesis in vivo in ischemic tissues, but the relative advantage of either factor is unclear. Gene transfer to the myocardium has been used as an alternative strategy to achieve sustained, local expression of angiogenic proteins. Common vectors for delivery include naked cDNA plasmids and viral vectors, such as replication-deficient adenovirus, adeno-associated virus, and retrovirus [Dzau 2001]. The relative advantages and disadvantages of the vectors employed include the size of the inserted gene, the site of nuclear incorporation, the duration of expression, the efficiency of transfection, and the degree of host immune response [Hamawy 1999]. Plasmid vectors have no limit on the size of the inserted gene and induce a minimal host immune response, which results in sustained transgene expression. The disadvantage of plasmid vectors is their low transfection efficiency. In contrast, viral vectors are very efficient in transferring therapeutic genes into target cells but induce a significant host immune response, which limits transgene expression over time. Viral transfection of cells in vivo may present additional issues of concern, especially if inappropriate doses are administered. Gene-based therapy may result in unregulated and variable protein expression. Continuous expression of VEGF has been associated with the formation of intramural vascular tumors and decreased survival in animal models [Lee 2000].

Vector Delivery

The effective delivery of growth factors, whether protein or gene, is a considerable challenge. Systemic administration (intravenous or intracoronary) is inefficient and has the potential for serious systemic complications, including progression of occult tumors, exacerbation of retinopathy, angioma formation, neointimal proliferation, and coronary plaque rupture. Intrapericardial infusion has been successful but is impractical in most clinical settings. Intramyocardial injection of proteins, genes, or cells, either by direct transepicardial injection or by percutaneous transendocardial routes, holds the most promise. In a recent comparison, adenovirus-mediated gene delivery achieved similar results with either

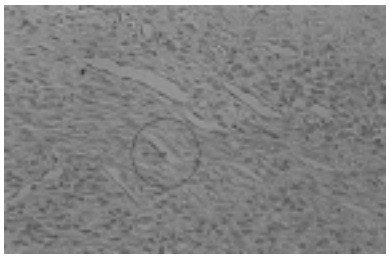

Figure 1. Cell transplantation can stimulate angiogenesis in ischemic myocardium. Unlike protein and gene therapies, cell transplantation can provide the cellular substrate for angiogenesis by incorporating into newly forming blood vessels [Circle: BrdU-labeled endothelial cells form the endothelium of a new blood vessel after cell transplantation in an ischemic region of myocardium].

transendocardial percutaneous catheter injection or direct surgical transepicardial injection [Post 2000]. However, endovascular delivery is "blind" and may not be as effective as epicardial injections under direct vision. Cell therapy, however, may be limited to direct epicardial injection, as the integrity of the cells may be compromised by shear stresses resulting from catheter delivery. Additionally, cell transplantation requires prior harvesting, culture, and preparation of the cells for injection, with the potential for contamination throughout the process. Therefore, cell therapy is more cumbersome. The combination of cell and gene therapy permits the ex vivo transfection of cells with angiogenic genes before transplantation into the ischemic region.

RESULTS

Results of Protein and Gene Therapy for Therapeutic Angiogenesis

Data obtained from successful animal studies of protein and gene-based therapeutic strategies has resulted in the inception of numerous clinical trials to evaluate the angiogenic potential of these developing techniques. Phase I trials of protein and gene-based approaches, designed to establish safety and feasibility, have been completed. Various forms of FGF and VEGF were administered by direct epicardial injection or by intra-coronary or intravenous infusion in patients with end-stage coronary artery disease [Losordo 1998, Laham 1999, Rosengart 1999a, Rosengart 1999b, Symes 1999, Vale 2000, Epstein 2001]. These small, uncontrolled therapeutic angiogenesis trials revealed significant improvements in angina symptoms and exercise duration. In addition, they provided objective evidence of improved regional perfusion and left ventricular function. However, results of the initial Phase II-III trials of protein-based treatments are less encouraging. The two large, randomized, double-blinded and controlled protein studies involving VEGF (VIVA Trial) [Henry 1999], and basic FGF (FIRST Trial) [Kleiman 2000] did not show benefits over a placebo in terms of the primary end-point of treadmill exercise performance. However, recent three-year data from a Phase II protein-based assessment of basic FGF combined with CABG is promising [Selke 2001]. While the Phase I studies for gene-based approaches are favorable and suggest that the approach is safe, it should be emphasized that the benefit of angiogenic gene therapy for myocardial ischemia has not yet been established by a single, randomized, placebo-controlled trial. The inconsistent results of these clinical trials has called for a reevaluation of current approaches to therapeutic angiogenesis, including the underlying biological principles, choice of angiogenic agents, validity and interpretation of animal model data, translation of animal data to clinical trials, and the means of delivery [Simons 2001].

In summary, both protein and gene-based approaches appear safe for clinical use. The magnitude of benefit, type of angiogen, and optimal mode of delivery remain to be established and are the subject of several ongoing trials. The beneficial effects of protein therapies appear to be inconsistent, and the gene-based approach may be limited by the inflammatory host response induced by the viral vectors, the transient nature of the intervention, and the lack of controlled gene expression.

Results of Cell Therapy for Therapeutic Angiogenesis

The concept of transplanting autologous cells to the myocardium to induce angiogenesis is intriguing. Autologous cells, in theory, would be well tolerated by the host myocardium and should lead to sustained, controlled, and regionally delivered growth factor expression. Evidence obtained from animal models of myocardial injury supports the concept of cellular cardiomyoplasty in the setting of coronary insufficiency. Heart cells [Li 2000], skeletal muscle cells [Taylor 1998], smooth muscle cells [Li 1999], bone marrow stem cells [Wang 2000], and endothelial cells [Kim 1998; see Figure 1, ◎] have been implanted into ischemic myocardium. The transplanted cells survived and engrafted within the injured region, promoting regional perfusion, wall thickening and motion, and global cardiac function. These animal studies suggest that cell transplantation has considerable therapeutic potential, but the mechanism of benefit is unclear. There is evidence from myocardial infarction experiments to suggest that enhanced angiogenesis with increased perfusion to surviving but hibernating native cardiomyocytes results in improved regional contractility [Tomita 2000]. Augmented elasticity of the infarct region after cell transplantation may afford independent benefits [Taylor 1998]. Both the altered elastic properties in the engrafted region and the enhanced angiogenesis may have prevented cardiac remodeling, avoiding cardiac thinning, dilatation, and congestive heart failure. Given the favorable prognostic implications of preventing remodeling and preserving ventricular function in ischemic heart disease, these data are encouraging.

The optimal cell type to induce therapeutic angiogenesis has not been established. While a variety of cell types have shown functional benefits, bone marrow mesenchymal cells may offer the best promise for cell transplantation-induced angiogenesis. Transplanted autologous bone marrow cells have been shown to induce angiogenesis, improve left ventricular function, and decrease infarct expansion in cryoinjured rats [Tomita 1999]. Importantly, the bone marrow-derived endothelial cells were incorporated into the newly formed capillaries, suggesting that cell transplantation not

only induced angiogenesis but also provided some of the necessary cellular substrate for new vessel formation. In a porcine model of coil-induced occlusion of the left anterior descending artery (LAD) and myocardial infarction, autologous porcine bone marrow cell transplantation induced local angiogenesis, which improved regional perfusion and global ventricular function [Tomita 2000]. Recent evidence suggests that therapeutic vasculogenesis can also be stimulated by bone marrow cell transplantation. Transplantation of autologous bone-marrow mononuclear cells (endothelial progenitor cells) in a rabbit model of leg ischemia provided a quantitative improvement in collateral vessel development [Shintani 2001]. These exciting observations have been confirmed in the ischemic myocardium. The use of cytokine-mobilized, autologous, human bone-marrow-derived angioblasts for revascularization of infarcted myocardium induced new blood vessel formation in the infarct-bed (vasculogenesis) and proliferation of preexisting vasculature (angiogenesis) after experimental myocardial infarction. The neoangiogenesis resulted in decreased apoptosis of hypertrophied myocytes in the peri-infarct region, long-term salvage and survival of viable myocardium, reduction in collagen deposition, and sustained improvement in cardiac function [Kocher 2001].

The combination of gene transfer and cell transplantation strategies may enhance the angiogenic and vasculogenic response elicited by the implanted cells while increasing the survival of cells transplanted into a hypoperfused infarct zone. Initial studies of transplantation of VEGF-transfected heart cells in rat hearts have demonstrated increased vascular densities and regional blood flow compared to hearts transplanted with untransfected cells [Yau 2000].

The first clinical experience with myoblast transplantation for ischemic heart disease was recently reported [Menasché 2001]. The encouraging results from this case report of combined coronary bypass grafting with myoblast transplantation demonstrates the clinical feasibility of cell therapy for patients with advanced ischemic cardiomyopathy. Clinical trials of cell transplantation as a sole therapy should perhaps be accelerated in light of the relative advantages of that approach and the encouraging animal studies.

In summary, cell transplantation may afford significant advantages over protein and gene-based strategies. First, transplanted cells may provide sustained and regulated delivery of multiple growth factors directly into the target region. Second, the transplanted cells can serve as a cellular substrate for the formation of new vessels. Third, cellular therapy with bone marrow stromal cells can induce vasculogenesis (as well as angiogenesis), producing more effective perfusion of the target region. Fourth, the ability to deliver specific angiogens to the ischemic myocardium, if desired, can be achieved with ex vivo gene-transfection of the transplanted cells. Lastly, the transplanted cells seem to afford functional benefits beyond those attributed to angiogenesis alone.

CONCLUSION

Fundamental to the success of protein, gene, or cell therapy in the clinical setting is the development of appropriate trial designs for their evaluation. There are significant challenges and dilemmas in this regard, as outlined in a recent expert panel summary [Simons 2000]. Clinical studies are hampered by a lack of consensus on the optimal method of delivery, the selection and appropriate evaluation of patients, the difficulty and ethics of blinding (especially with invasive procedures), and the difficulty of measuring "effective" perfusion and new vessel formation. There is a need for consensus on what is a "clinically meaningful" improvement in results of trials of this nature as well as the ability to distinguish the relative contribution of the experimental therapy with that of bypass grafting in combined procedures. Adequate trial design will require a better understanding of the process of angiogenesis and collateral vessel formation.

The optimal approach for effective therapeutic angiogenesis has not been established. The results from Phase II-III trials of gene-based approaches are anticipated with interest. The prospect of autologous cell transplantation during a combined revascularization procedure holds promise for delivering both the cellular substrate and the specific signaling factors necessary to effect a significant and sustained myocardial revascularization. A clinical trial of cell transplantation as a sole therapy may also be warranted. Therapeutic angiogenesis, either through protein, gene, cell, or combined approaches, may emerge from continued rigorous investigation as a valuable tool for cardiac surgeons to provide complete and definitive revascularization for our most challenging patients.

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