Therapeutic Angiogenesis: The effect of intramyocardial injections of growth factor containing Vascular Endothelial Growth Factor and Basic Fibroblast Growth Factor on new blood vessel formation in patients with end-stage ischemic cardiomyopathy requiring a Left Ventricular Assist Device.

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A. Study Purpose and Rationale

One of the major problems facing cardiology today is how to more effectively treat the patient with occlusive coronary disease who has incapacitating and refractory symptoms and who has the type of extensive coronary disease that precludes currently available revascularization procedures. Despite continued advances in the treatment of ischemic heart disease, a large population of individuals with diffuse coronary artery disease exists for whom conventional therapies such as percutaneous angioplasty and coronary bypass surgery provide little or no benefit. Therapeutic angiogenesis, the development of collateral blood vessels supplying ischemic tissues, either endogenously or in response to administered growth factors, may be applied in such situations. Angiogenesis is a complex process involving endothelial and smooth muscle proliferation and migration, formation of new capillaries, breakdown of existing extracellular matrix and formation of the new one. It is likely that a coordinated action of several mitogens is needed to achieve this result. Myocardial ischemia is a potent stimulus and a number of growth factors have been isolated from the ischemic myocardium, suggesting that these molecules may play a role in the ischemia-induced angiogenesis. Among these growth factors, fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) constitute the most widely studied group.

The therapeutic implications of angiogenic growth factors were identified by the pioneering work of Folkman and colleagues more than 2 decades ago. (1) Their work documented the extent to which tumor development was dependent upon neovascularization and suggested that this relationship might involve angiogenic growth factors that were specific for neoplasms. Over the past several years major advances have been made in sequencing and cloning agents involved in normal angiogenic processes. Of these agents, the most understood and most potent angiogenic growth factors are Vascular Endothelial Growth Factor (VEGF) (2-4) and basic Fibroblast Growth Factor (bFGF) (5,6). Recent observations suggest that VEGF is actually a family of several growth factors, which interact with different receptors to induce endothelial mitogenesis (3,4). Of these VEGF-13 appears to be highly expressed in cardiac tissues. Several animal studies have shown that administration of these angiogenic growth factors are sufficient to augment blood delivery in myocardial ischemia. Baffour et al administered bFGF in daily intramuscular doses of 1 or 3 ug to rabbits with acute hindlimb ischemia; at the completion of 14 days of treatment, angiography and necropsy measurement of capillary density showed evidence of augmented collateral vessels in the lower limb compared with controls (7). Pu et al. used FGF to treat rabbits in which the acute effects of surgically induced hindlimb ischemia were allowed to subside for 10 days before beginning a 10-day course of daily 4mg IM injections; at the completion of 30 days follow-up, both angiographic and hemodynamic evidence of collateral development was superior to ischemic controls treated with IM saline(8). Yanagisawa-Miwa et al likewise demonstrated the feasibility of bFGF for salvage of infarcted myocardium. In this study, two intracoronary injections of bFGF were given immediately following induction of acute myocardial infarction in dogs. Examination of the hearts two weeks later demonstrated a remarkable reduction in the infarct size as well as an increase in the number of capillaries and arterioles in the bFGF treated group (9). The functional significance of bFGF-induced angiogenesis has been demonstrated in pigs with chronic ischemia treated with bFGF delivered by an

extravascular slow-release heparin-alginate polymer, applied to the adventitial side of the occluded vessel. This study found that treated animals demonstrated not only increased vessel density and improved coronary flow in the collateral region but also improved global and regional left ventricular function 6 weeks after initiation of bFGF therapy (10). Evidence that VEGF stimulates angiogenesis in vivo had been developed in experiments performed on rat and rabbit cornea, the chorioallantoic membrane, and the rabbit bone graft model (11, 12,13). The finding that VEGF could be employed to achieve angiogenesis that was therapeutic was first demonstrated by Takashita et al who administered VEGF to the internal iliac artery of rabbits in which the ipsilateral femoral artery was excised to induce unilateral hindlimb ischemia. Doses of 500 to 100 ug of VEGF produced statistically significant augmentation of angiographically visible collateral vessels and histologically identifiable capillaries; consequent amelioration of the hemodynamic deficit in the ischemic limb was significantly greater in animals receiving VEGF than in nontreated controls (14). Similar to bFGF , chronic intracoronary or extracoronary infusion of VEGF resulted in a significant improvement in coronary flow and function of the compromised myocardium (15,16). Recently Mack et al. demonstrated that an AD vector expressing VEGF cDNA induced collateral vessel development in ischemic myocardium. of pigs and resulted in significant improvement in both myocardial perfusion and function. They concluded that such a strategy may be useful in patients with ischemic heart disease in whom complete revascularization is not possible (17). Important to the validity of our hypothesis is the fact that studies also have shown a potent synergistic effect of VEGF and bFGF on angiogenesis in culture and in animal models (18,19). Currently underway are several phase I studies evaluating the clinical benefits of bFGF and VEGF in humans with ischemic heart disease (20). At this institution a project is being conducted in which a protein growth factor milieu (GFm) containing VEGF and bFGF is directly injected into the ischemic area of the porcine myocardium. This study will aid in the assessment of this growth factor and its effect on myocardial tissue.

During the past five years 128 patients have received a left ventricular assist device (LVAD) as a bridge to heart transplantation at Columbia Presbyterian Hospital. Not only does Presbyterian Hospital conduct the largest number of these procedures, but this year an 40 LVADs are expected to be issued to patients with end stage heart failure. The LVAD is a battery powered device that is surgically implanted alongside the natural heart to take over the pumping action of the nonfunctioning left ventricle. The device is connected between the natural heart and the aorta. Blood is then pumped from the heart into the device and the LVAD then pumps blood throughout the body. The most common reasons for initiating mechanical support with the LVAD are postcardiotomy left ventricular failure, cardiogenic shock with a cardiac index < 2.0 L/min/m2 and left atrial pressure > 20 mmHg, or deterioration from chronic heart failure.

In the clinical trial outlined in this proposal, we plan to inject GFm(VEGF/bFGF) directly into the myocardium of patients receiving the LVAD. This group of patients, 40% of which have ischemic cardiomyopathy, represent an optimal study group

The goal of the present study is to determine the effect of GFm on the human myocardium and to assess its ability to stimulate angiogenesis and enhance collateral vessel formation.

B. Study Design and Statistical Analysis

This is to be a randomized placebo-controlled double-blind study in which the effects of an angiogenic growth factor on myocardial tissue will be assessed both by nuclear imaging and histologically. The patients, all of whom will fit the criteria for requiring the LVAD as a bridge to cardiac transplantation will be randomized to one of two treatment arms: one receiving intramyocardial injections of protein growth factor and the other receiving placebo by the surgeon in a blinded fashion. The study is planned as follows: All patients, who qualify for LVAD placement and who have ischemic cardiomyopathy will undergo either PET scan imaging prior to LVAD placement in order to assess myocardial viability/perfusion. The results will be reviewed by an expert in the field in a blinded fashion. In the pre-op assessment all areas of ischemia as defined by PET will be specified in each of the patients

so that during placement of the LVAD the injections will be done in these site-specific regions. Prior to injection of the growth factors, random biopsies will be taken of the untreated myocardium. All patients will then be randomized during placement of the LVAD to receive either protein growth factor or placebo. They will then be allowed to recover and managed as is currently accepted with inotropic support and afterload reduction. While waiting for heart transplant the patients will again undergo diagnostic evaluation with PET three weeks after LVAD placement/intramyocardial injection (a study is currently being conducted on PET scans post-LVAD placement). The goal of the imaging portion of this study is to provide an assessment of changes in perfusion and viability after induction of angiogenesis. At this point in time we are unable to provide a measurable outcome as we are in the process of evaluating the effects of the LVAD on heart physiology and PET scans. All diagnostic studies will be reviewed by the same expert in a blinded fashion. All patients who are still on LVAD by the seventh week post-op will undergo repeat PET scan if deemed feasible. After cardiac transplantation or after expiration, which can occur at any time ranging from 2 to 249 days post-op, each heart will be prepared and histologically examined in a blinded fashion. The goal of the histologic portion of our study is to provide a quantitative assessment of the various observations by determining vascular density (number of vessels with at least one layer of smooth muscle cell/cm2) and proliferating cell density (positive BrdU and positive proliferating cell nuclear antigen vascular smooth muscle or endothelial cells /cm2) in several areas of each heart. At no time will either the patients or the investigators know whether a patient received protein growth factor versus placebo. Although there is no planned crossover between the arms: if at any time a benefit is shown than all patients will receive the protein and conversely if at any time it is shown there is a detrimental effect of protein injection then the study will be terminated. The statistical measures for a sample calculation of PET scan results will be offered at a later time. Currently there is a study underway evaluating the PET scan in patients with a LVAD. The results will give the information needed to calculate a sample size. For the histologic portion of this protocol an analysis of covariance will be used to assess power. The baseline measures of vascular density and proliferating cell density will be used as the covariant measures and the post injection vascular density and proliferating cell density will be the dependent measures in our analysis.

C. Study Procedures

Pre-treatment evaluation, prior to LVAD placement, will include the placement of Swan Ganz catheter to determine the cardiac pressures, cardiac output and cardiac index among other parameters. All patients will undergo various studies as deemed necessary by his/her cardiologist to best manage his/her particular cardiac illness. For this study however all patients will undergo PET scan before and after the injections as outlined above. Otherwise the only deviation from normal clinical practice during the surgery will be that intramyocardial injections of Gfm will be performed at the conclusion of the normal surgical procedures. The investigation is expected to last approximately 12 months. This period will allow 8 months to recruit subjects and -4 months for the last enrolled patient to undergo heart transplantation. The duration of each patient's participation will equal the duration of his/her LVAD support, which averages between 3 and 4 months at our institution.

D. Study Drugs

(To be provided by the biotech company)

Gfm is an investigational drug. Rationale for use of Gfm (including information about safety and efficacy in previous clinical or experimental experience) Method and route of administration and whether this differs from standard use. The dosage regimen and whether this differs from standard use. Known side effects and their expected frequency.

E. Medical Devices

Although not being studied in this protocol, the Left Ventricular Assist Device (LVAD) has been utilized at this institution for more than five years mainly as a bridge to transplantation. Included is a description of the LVAD. It is an investigational device currently being reviewed by the FDA for approval as a bridge to transplantation. Nevertheless, we have a large clinical experience with this device at our institution. It is implanted in approximately 30 patients per year. It is used in patients with severe heart failure meeting specific hemodynamic criteria who are not expected to survive for 48 hours without the device. In comparison to the expected high mortality rate, the device successfully bridges -70% of patients to heart transplantation. However, the device is associated with an -20% perioperative mortality because of the severity of the patients' underlying heart failure condition.

F. Study Questionnaires:

This study will not involve any questionnaire.

G. Study Subjects/Recruitment

Inclusion criteria for subjects will be those who are accepted to receive the LVAD based on criteria mentioned above, those with cardiomyopathy secondary to coronary artery disease as documented by findings on coronary angiography and perfusion imaging, have not received any protein growth factors any time prior to this study, from whom written consent has been obtained, are a minimum of 18 years of age but no older than 70 years of age. Excluded will be anyone who does not meet any of the above criteria, anyone who has already undergone cardiac transplantation. Also excluded will be those patients in whom Gfm is contraindicated as provided by the biotech company.

H. Confidentiality of Study Data

All study data is to be coded with a unique number for each subject and stored in a secure location accessible only to the investigators.

I. Potential Conflict of Interest

Neither the investigators nor the University has a proprietary interest in the drug or devices under investigation.

J. Location of Study

The study will be conducted at Columbia Presbyterian Hospital where it is expected a sufficient number of subjects will be recruited.

K. Potential Risks

The potential risks of the study include those risks involved in placement of the LVAD for which there are no other options available to those meeting the criteria. The potential risks associated with injecting the growth factors include possibility of nonspecific mitogenesis (carcinogenic effects), potentiation of angiogenesis-driven diseases such as diabetic retinopathy and certain tumors. VIEGF, by virtue of its effect on vessel permeability, may lead to exacerbation or initiation of local edema and inflammatory reactions. Of note, in limited clinical experience, plasmidmediated VEGF gene therapy has led to the development of extensive extremity edema and telangiectasia in a patient. Administration of bFGF in high dosages is also associated with a number of side effects including anemia, thrombocytopenia, membranous nephropathy, and hyperostosis. Finally, a theoretical concern is that VEGF and FGF may exacerbate plaque angiogenesis and thus may adversely affect progression of

coronary disease or plaque stability. At the moment the biotech company is intensely scrutinizing the risks of Gfm.

The potential risks associated with LVAD placement include -20% perioperative mortality rate, stroke or peripheral embolisms, right heart failure, bleeding, infection, end-organ failure, arrhythmias, pain and discomfort.

L. Potential Benefits

The potential benefits include the ability to treat the many patients who suffer from ischemic heart disease in whom either PTCA or CABG are contraindicated and in whom medical therapy is ineffective. Furthermore, by gaining an understanding of how these growth factors work in the human myocardium we will be able to further our understanding of a process whose mysteries carry the potential to eradicate a great number of diseases that afflict mankind, especially cancer and ischemia

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M. Alternative Therapies

Transmyocardial Laser Revascularization has been shown to improve symptoms of patients with angina symptoms refractory to medical management but this procedure is still under investigation.

N. Compensation to Subjects

There will be no monetary compensation to subjects.

O. Costs to Subjects

There will be no additional costs to subjects. The patient and their insurance agency will be responsible for the costs associated with the implantation of the LVAD and no additional charges will be added by the performance of the growth factor procedure.

P. Minors and Research Subjects

Not applicable

Q. Radiation or Radioactive Substances

Not applicable

R. References

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