Therapeutic Angiogenesis with Autologous Transplantation of Bone Marrow Cells for Intermittent Claudication: A Randomized Controlled Trial

Farooq Sheikh

A. Introduction

Peripheral artery disease (PAD) affects up to 15% of adults over the age of 55 years (1). Approximately one third of patients with PAD have intermittent claudication. Despite optimal medical management, most of these patients continue to have symptoms (2). Revascularization for focal disease or aorto-iliac disease is a viable option as a definitive treatment modality, however, for the patient with diffuse or infra-inguinal PAD, medical treatment (such as cilastazol, pentoxifylline) has proven to be less than stellar. New therapeutic modalities must be sought in hopes of altering the natural history of PAD.

Therapeutic angiogenesis (TA) has emerged as a potential novel treatment for PAD. TA seeks to improve tissue perfusion through the growth and proliferation of blood vessels (3). Results of preclinical studies have shown that angiogenic cytokines promote development of collateral vessels (4). Initial phase II clinical trials have aimed at delivering pro-angiogenic growth factors such as fibroblast growth factor-2 (FGF-2) or vascular endothelial growth factor (VEGF) to sites of inadequate tissue perfusion via direct cytokine injection or even gene-delivery (3, 5). The results have proven to be only mildly successful as technical challenges with the gene therapy techniques (as well as the mode of cytokine delivery) have marred early clinical trials.

Recently, a new approach to therapeutic angiogenesis has given the field a renewed sense of hope. The discovery and isolation of a population of bone marrow- derived endothelial progenitor cells (EPCs) has opened the door to yet another avenue of investigation in the field of angiogenesis: cellular therapy (6). EPCs in the CD34+ primitive cell fraction of adult human peripheral blood are known to take part in neovascularization after mobilization from the bone marrow (7, 8). Preclinical studies have established that the implantation of bone marrow primitive mononuclear cells (including EPCs) into ischemic limbs increases collateral vessel formation (8, 9). In light of these preclinical findings, I wish to investigate whether primitive bone marrow mononuclear cells can slow the progression of peripheral arterial disease and ameliorate one of PAD's dreaded symptoms: intermittent claudication.

B. Hypothesis

The direct injection of autologous bone marrow-derived CD34+ primitive mononuclear cells (including EPCs) into the femoral arteries of patients with known peripheral arterial disease (PAD) will lead to improvements in signs and symptoms of PAD through the achievement of therapeutic angiogenesis.

C. Methods

a. Design

This trial will be a randomized, double-blind, placebo-controlled study comparing the effects of intra-arterial injection (femoral) of bone marrow-derived CD34+ mononuclear cells into ischemic legs (treatment group) with the intra-arterial injection of peripheral blood mononuclear cells (placebo). Peripheral mononuclear cells serve as a more accurate placebo than saline as peripheral blood is partially

contaminated during the bone marrow aspiration process (4) and the number of CD34+ cells in peripheral blood is known to be approximately 500-fold lower than the bone marrow (4, 10).

b. Study Outcomes

The primary outcome of this study will be change in peak walking time from baseline to 24 weeks. Exercise performance will be measured with the standard Gardner treadmill protocol. The Gardner protocol involves walking on a treadmill at a constant speed with a change in grade (2% rise) every 2 minutes (11). Each patient will have one session to become familiar with the treadmill. Each patient will be asked to fast at least 2 hours before the measurement. Patients will be instructed to take all regular scheduled medications except beta-blockers that must be discontinued 24 hours before the test. The treadmill study will be done at baseline and it will be repeated at 12 weeks and 24 weeks. Each test will be performed at the same time of the day. The treadmill test will record time to the onset of muscle pain, ache, cramps, numbness, or fatigue in either leg (5). This time will be defined as the "claudication onset time" (5). Patients will be asked to exercise until they can not continue any further. This time will be defined as "peak walking time" (5). Patients will perform a second treadmill test between 24 hours and 1 week after the first test. If the difference between the two peak walking times is greater than 20% of his mean, the patient will be excluded.

Secondary outcomes for this study will include change in claudication onset time, ankle-brachial index (ABI) change from baseline to 12 and 24 weeks, quality of life change from baseline to 12 weeks and 24 weeks, and change in collateral vessel formation as evidence by angiography. ABI will be measured in the standard fashion (2). Quality of life will be measured by 2 questionnaires that have been validated by previous studies of PAD: the Walking Impairment Questionnaire and the SF-36 (12). Each patient will have digital subtraction angiography 1 week before and 12 weeks after intra-arterial injection. Variables such as amount of contrast, force of injection, and catheter tip position will be fixed (4). Three radiologists, who will be blinded to the study groups, will assess for collateral vessels. New collateral vessels will be assessed on a number scale previously described: 0 for no collaterals, 1 for slight, 2 for moderate, 3 for rich (4).

c. Statistical Analysis

A sample size of 30 patients in each treatment arm is estimated to provide 80% power to detect a mean difference of 1.5 minutes in change in peak walking time between the treatment group and the placebo, assuming a standard deviation of 2 minutes. An unpaired t-test was used to make this calculation. Data will ultimately be analyzed by use of an intention-to-treat analysis.

D. Subject Selection

Men over the age of 40 are eligible for this study if exercise is limited by moderate to severe claudication and not be arthritis, angina, dyspnea, or other symptoms (5). There will be a screening period (estimated 30 to 60 days) where patients will need to have 2 Gardner treadmill examinations that are reproducible (i.e., within 20% of each other). The peak walking time must be between 1 and 12 minutes. Other inclusion criteria will include documented infra-inguinal obstructive atherosclerosis ($\geq$ 70% stenosis of the femoral, popliteal, or tibial arteries on angiography), an ABI (resting) of <0.8 on at least one of the lower extremities (5). Patients will receive optimal medical management with risk-factor modification and the administration of drugs considered first-line for PAD (cilastazol, pentoxifylline, etc.).

Exclusion criteria include women, any history of malignancy (except successfully resected basal-cell or squamous cell carcinoma) within the past 10 years, renal insufficiency (Cr $\geq$ 2.0), retinopathy, evidence of any inflammatory or fibrotic illness, and poorly uncontrolled diabetes mellitus as ascertained by a hemoglobin A1C $\geq$ 8 or evidence of retinopathy (5). These exclusion criteria will require a detailed initial physical exam that will include screening for occult malignancies.

The exclusion of women in this initial clinical trial of bone marrow cells for PAD is required to exclude the possibility that significant differences in basal levels of angiogenesis may attribute to positive findings in this study. It is well known that women of child bearing age who menstruate have increased levels of pro-angiogenic factors that may contribute to superior collateral vessel formation (13).

E. Miscellaneous

a. Study procedures

Using conscious sedation, each patient will have a bone marrow aspirate of about 500-750cc ml taken from the ileum. The sample will then be used to perform FACS analysis to sort for CD34+ mononuclear cells to a concentration of approximately 50cc that will ultimately be used for the intra-arterial injection (4, 7, 8). The autologous CD34+ cells will be injected into both femoral arteries via femoral artery puncture with eventual iliac crossover to the contralateral femoral artery (5). The autologous bone marrow injection will occur on the same day as the bone marrow aspirate. The placebo will consist of 40-50cc of peripheral blood obtained at the time of aspirate as well.

All patients must grant informed written consent. This study will follow the standard Declaration of Helsinki rules for scientific investigation. A potential difficulty in the recruitment of patients for this trial will be concern for unknown, adverse effects of cellular therapy. Patients will be given a packet of information detailing and describing all clinical trials involving bone marrow mononuclear cells for both peripheral arterial disease and coronary artery disease thus far, with full disclosure of any side effects that have been discovered.

There will be no financial compensation for this clinical trial. Patients will be recruited with the promise of receiving free optimal medical management with the cost of such care to be absorbed by the host institution.

F. References

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