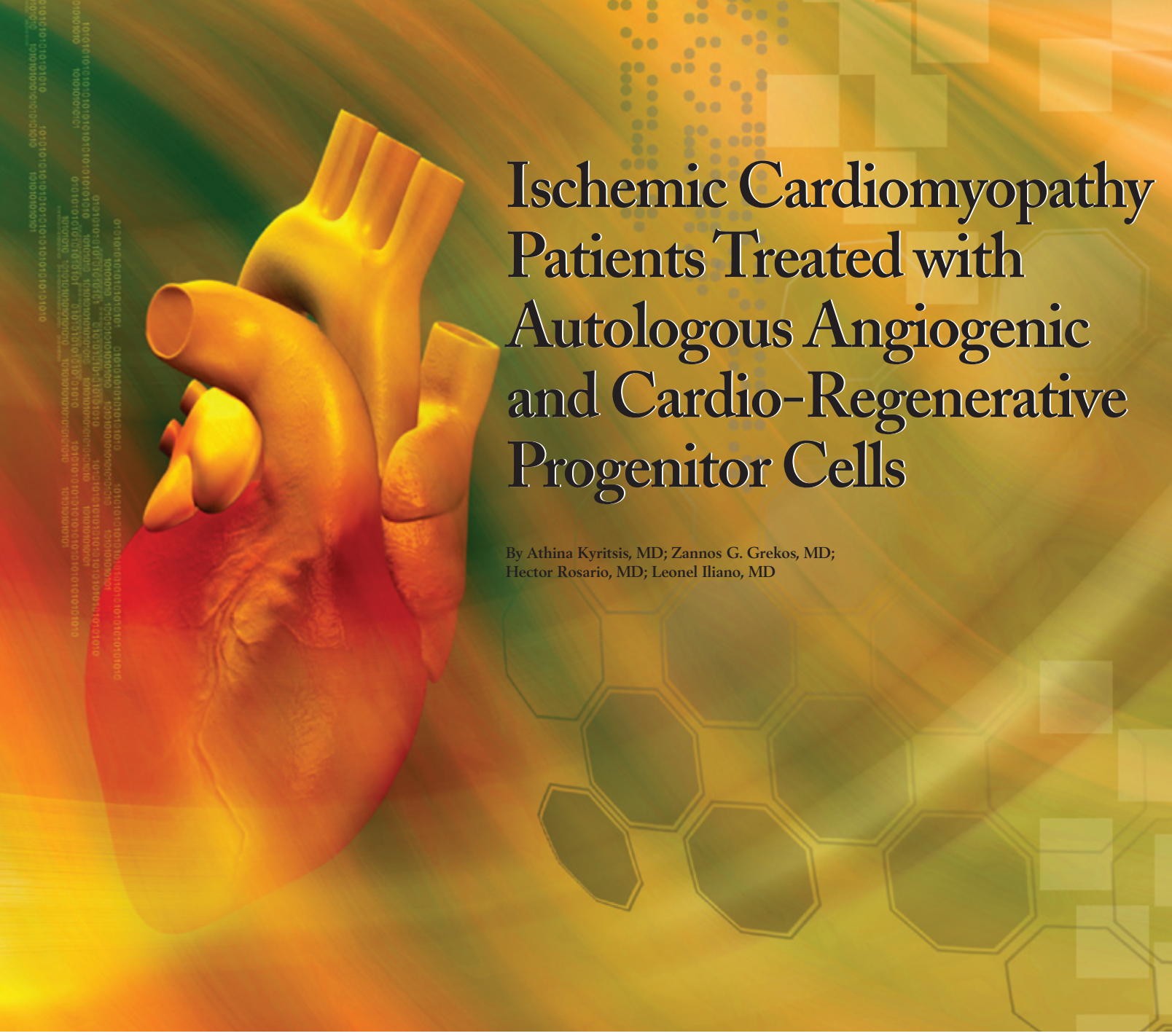

Ischemic Cardiomyopathy Patients Treated with Autologous Angiogenic and Cardio-Regenerative Progenitor Cells

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Objective; The goal of this study is to investigate the feasibility, safety, and clinical outcome of patients with Ischemic Cardiomyopathy treated with Autologous Angiogenic and Cardio-Regenerative Progenitor cells (ACP's) in a prospective fashion.

Background; In numerous human trials there is evidence of improvement in the ejection fractions of Cardiomyopathy patients treated with ACP's. Animal experiments, not only show improvement in cardiac function, but also engraftment and differentiation of ACP's into cardiomyocytes as well as neo-vascularization in infarcted myocardium. In our clinical experience the process has shown to be safe as well as effective.

Methods; We conducted a prospective, non-randomized study evaluating the effects of ACPs (*ex vivo* expanded and differentiated peripheral blood stem cells) implanted in sixteen patients with chronic ischemic cardiomyopathy (Ejection fractions < 45%) with congestive heart failure symptoms of at least NYHA class II. ACP's where implanted via either intra-myocardial injection or intra-coronary infusion. Patients where optimized medically prior to ACP's therapy with standard medical therapy for CHF as well as revascularization and upgraded to Bi-ventricular defibrillators when indicated. Ejection fractions where recorded at baseline then at 3 and 6 months using MUGA at rest as well as at stress (dobutamine protocol). The primary end points were changes in rest and stress ejection fractions.

Results; We found treated patients exhibiting a significant increase in cardiac ejection fraction from baseline. The increases in ejection fraction were

21 points (75% increase) at rest and 28.5 points (80% increase) at stress.

Conclusion; This study exemplifies that ACP's can improve the ejection fraction in patients with severely reduced cardiac function with benefits sustained to six months. These patients will continue to be followed in a similar fashion to determine long term outcomes. Other secondary outcomes will also be followed including cardiac events, hospitalizations, mortality, functional class, cardiac dimensions.

INTRODUCTION

Despite significant advances in the new therapeutic modalities and prevention, cardiac disorders are very prevalent all over the world. The magnitude of the problem will increase considerably in the future due to increasing life expectancy and the prevalence of diabetes. In spite of considerable advances in medical therapy and improvements in revascularization procedures for coronary artery disease, a substantial proportion of patients who suffer from angina pectoris and heart failure are not responsive to maximal medical and surgical treatment modalities. Importantly *Cardiovascular Disease* is at the top of the list for medical expenditures in the United States of America. With the majority of dollars spent on hospitalizations for congestive heart failure. Consequently, effective alternative therapies for these patients would have far reaching benefits.

Regenocyte's therapeutic strategy collects blood samples from patients, isolates peripheral blood mononuclear cells, and grows these cells in conditions that will cause a significant increase of the number of progenitor cells as well as partially differentiate these cells into a population specifically targeted at cardiac regeneration.

Following this culturing stage, the ACPs are harvested, packaged, and transported to the treatment center to be injected into the coronary vessels and myocardium of the patients. The final cell product is known as Regenocytes.

Regenocyte therapy treats patients suffering from angina pectoris or cardiomyopathy, not responsive to maximal drug treatment or not willing or without option of undergoing coronary artery bypass graft (CABG) surgery or PCI. The use of ACPs promotes the formation of neo-vascularization and viable myocardial tissue.

SCIENTIFIC BACKGROUND CELLULAR BIOLOGY

Angiogenic Cell Precursors (ACPs) or Endothelial progenitor cells (ECPs) possess the ability to differentiate into endothelium, the layer of cells involved in both the forming of blood vessels (neovascularization) and the lining of their lumen (endothelialization). These functions of the ACPs enable the development of new therapies that aim to use these cells for the treatment of severe vascular disorders.

The first evidence indicating the presence of ACPs in the adult circulation was obtained when mononuclear blood cells from healthy human volunteers were shown to acquire an endothelial cell–like phenotype *in vitro* and to incorporate into capillaries *in vivo* [11]. These putative ACPs were characterized via expression of CD34 and vascular endothelial growth factor receptor-2 (VEGFR-2), two antigens shared by embryonic endothelial progenitors, and hematopoietic stem cells. In addition to CD34, early hematopoietic progenitor cells express CD133 (AC133), which is not expressed after differentiation. Currently, the widely accepted definition of ACPs in circulation is, for practical purposes, $CD34^+/VEGFR\text{-}2^+$ or $CD133^+/VEGFR\text{-}2^+$ cells.

The fact that ACPs can take part in the formation of new blood vessels was first observed by Bhattacharya and colleagues who showed the formation of capillary-like structures from hematopoietic stem cells or *ex-vivo* expanded ACPs [12,13]. The contribution of bone marrow-derived cells, mainly ACPs, to neovascularization after ischemic injury *in vivo*, was shown in experiments using labeled populations of stem cells to reconstitute lethally irradiated mice. The cells or their progeny were shown to migrate into ischemic cardiac muscle and blood vessels, differentiate to cardiomyocytes and endothelial cells, and contribute to the formation of functional tissue [14]. Other work, involving a mouse retinopathy model, demonstrated the important role that the recruitment of endothelial precursors to sites of ischemic injury plays in neovascularization [15].

The majority of ACPs reside in the bone marrow in close association with Hematopoietic Stem Cells (HSCs) and bone marrow stromal cells that provide the microenvironment for hematopoiesis. ACPs have been shown to mobilize (i.e. migrate in increased numbers from the bone marrow into circulation) in patients with vascular trauma or Acute Myocardial Infarction (AMI) [16, 17], or in response to Administration of VEGF via gene transfer [18, 19].

The sources of Autologous Angiogenic Cell Precursors that can be used for treatment varies and include bone marrow, peripheral blood and different mesenchymal organs. The use of cells from peripheral blood has the advantage of being more uniform easier to characterize and control and that their collection is easier (without anesthesia). The disadvantages are the relative small number of Angiogenic Cell Precursors in peripheral blood which requires a relatively large volume of blood and the time consuming process of augmentation.

The use of Angiogenic Cell Precursors promotes the formation of neo-vascularization as well as new myocardial cells in the failing heart and as a consequence attenuates congestive failure.

CLINICAL TRIALS OF STEM CELL THERAPY FOR CARDIAC DISEASE

Considerable work has been carried out to elucidate the mechanisms behind ACP's mobilization, localization and function. Progress has also been achieved in establishing therapeutic protocols for treating a variety of conditions, such as peripheral limb ischemia, acute myocardial ischemia and infarction by using progenitor cells.

The last few years have seen significant progress being achieved by clinical trials using therapeutic protocols for treating a variety of vascular conditions, such as peripheral limb ischemia, acute myocardial ischemia and infarction by using stem and progenitor cells. Clinical trials have been performed to test the safety and potential efficacy of several types of cells [8-24].

The trials showed considerable potential at alleviating these conditions with no serious adverse effects directly related to the cells administered. These studies demonstrated the potential safety of the administration of other peripheral blood–derived cells in humans suffering from myocardial and vascular diseases and the potential for enhancing myocardial function with associated improvement in symptoms as manifested in the patients' physical condition and in objective cardiac function tests.

Methods of cell administration were intracoronary injection while performance of angiography, intramuscular injection at CABG operation or intramuscular injection.

The parameters of heart performance were improvements in left ventricular ejection fraction (LVEF), improvement in cardiac perfusion and in angina score. The results of these trials are in general promising after follow-up of 4-16 months. Adverse effects were minimal and were not related to administration of the ACPs.

However, most studies have the disadvantage of having been small series, conducted as open label trials and only some of them included a control group. When considering the benefit of stem cell treatment there is wide agreement that these treatments are safe and carry minimal risk to patients, as supported by "*The Consensus Of The Task Force Of The European Society Of Cardiology Concerning The Clinical Investigation Of The Use Of Autologous Adult Stem Cells For Repair Of The Heart*" [30]

METHODS AND PROCEDURES

Sixteen patients were selected based on the following guidelines.

INCLUSION CRITERIA:

1. Patients with ischemic cardiomyopathy on maximal medical therapy.
2. Ejection Fraction less than 45%.
3. Age 18 to 80 years
4. Male or non-pregnant, non-lactating female
5. Informed consent obtained and consent form signed

EXCLUSION CRITERIA:

1. Patients who received blood transfusions during the previous 4 weeks (to exclude the potential of non-autologous ACPs in the harvested blood).
2. Inability to communicate (that may interfere with the clinical evaluation of the patient)
3. After heart transplantation
4. Renal failure
5. Hepatic failure
6. Anemia (lower than 10mg/dl.hemoglobin for female and lower than 11 mg/dl for male)
7. Abnormal coagulation tests [platelets, PT (INR), PTT]
8. Malignancy
9. Concurrent chronic or acute infectious disease
10. Severe concurrent medical disease (e.g., septicemia, HIV-1,2/HBV/HCV infections, systemic lupus erythematosus)
11. Chronic immunomodulating or cytotoxic drug treatment
12. Patients who have rectal temperature above 38.40C for 2 consecutive days
13. Patient unlikely to be available for follow-up

Evaluation Parameters:

The following tests were performed at baseline and at 3 and 6 month follow-up visits to measure subjective and objective parameters of the treatment:

1. Physical exam
2. Blood pressure, heart rate and ECG
3. Blood tests
4. Hematology: RBC; Hemoglobin; Hematocrit; WBC; Neutrophils; Lymphocytes; Monocytes; Platelet count.
5. Blood Chemistry: Glucose; Blood urea nitrogen (BUN); Serum creatinine; Serum chloride; Serum potassium; Serum sodium; HgA, C, C-Peptide, CRP, P-BNP
6. CCS (Canadian Cardiovascular Society) grading for Angina
7. NYHA (New York Heart Association) grading for congestive heart failure
8. Assessment of cardiovascular drug types and doses
9. Echocardiography
10. Dobutamine Stress MUGA
11. Bruce exercise nuclear perfusion test
12. Number of hospitalizations
13. Mortality
14. Cardiovascular events

CELL PRODUCT

The Final Cell Product (Regenocytes) consisted of Autologous Angiogenic Cells Precursors isolated from the patient's blood and then expanded and partially differentiated *ex vivo* under sterile conditions. The cells were divided into 3 syringes suspended in 15 ml sterile cell culture medium. The product was sterile and pyrogen-free.

BIOLOGICAL ACTIVITY ANALYSES

Acceptable biological parameters as assessed by microscopy and flow cytometry that were in accordance with the following specifications:

1. Cell viability of greater than 75%
2. Appropriate Morphology – spindle-shaped, large cells forming long thread-like structures.
3. Minimum subpopulations of cells staining positive for the CD34 and CD 31 markers (assessed by flow cytometry).

The final cell product was also tested for safety based on the following:

1. Sterility
2. Gram stain
3. Bacterial Endotoxin
4. Mycoplasma contamination
5. Bacterial culture

TREATMENT ADMINISTRATION OF REGENOCYTES

Patients were transferred to the cardiac catheterization laboratory approximately one hour before the anticipated arrival of the cells. Coronary angiography was performed to define the artery or arteries planned to be used for the cell injection. The administration was performed intracoronary utilizing an over-the-wire balloon catheter and following a specific delivery protocol or by intra-myocardial injection.

SAFETY

There were no adverse events associated with the ACP's. No cardiac events occurred. There was one severe adverse event. One patient suffered a CVA during one of the cardiac catheterizations and was therefore excluded from the group.

RESULTS

We found treated patients exhibiting a significant increase in ejection fraction from baseline that was sustained to the six month time period. Baseline average resting EF (measured by MUGA) was 28% (range; 14% to 42%), with an average stress (dobutamine) EF at 36% (range; 19% to 52%). At the three month mark resting EF had increased to 40% and the stress EF was at 50% and at six months the resting EF had reached 49% (range; 38% to 56%), and the stress EF was at 64.5% (range; 56% to 67%).

DISCUSSION

Heart failure is estimated to affect 4 to 5 million Americans, with 550,000 new cases reported annually.[31] In the past 3 decades, both the incidence and prevalence of heart failure have increased. [31-33] Factors that have contributed to this increase are the aging US population and improved survival rates in patients with cardiovascular disease due to advancements in diagnostic techniques and medical and surgical therapies.[32-36] Heart failure is a chronic, progressive disease that is characterized by frequent hospital admissions and ultimately high mortality rates. Because of its high medical resource consumption, heart failure is the most costly cardiovascular illness in the United States.[37]

Advances in the treatment of heart failure and early intervention to prevent decompensation may delay disease progression and improve survival. However the natural course of the disease is progressive deterioration. Despite increasing success in comprehensive treatment by conventional medical therapy refractory congestive heart failure continues to pose a difficult medical and economic problem.

The results of this study suggest that intracoronary and intramyocardial injection of autologous, peripheral blood-derived cell population enriched in Angiogenic Cell Precursors (ACPs) in patients with congestive heart failure is a safe and effective alternative treatment for patients who have exhausted other therapeutic options. In this study, such treatment resulted in significant increases in ejection fraction with a concomitant decrease in_symptoms.

These results reflect the high potential of this cellular treatment as a novel adjunctive therapy for congestive heart failure. ◆

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