საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, 175, №2, 2007
BULLETIN OF THE GEORGIAN NATIONAL ACADEMY OF SCIENCES, 175, №2, 2007

Medical Sciences

Intramyocardial and Extramyocardial Autologous Endothelial Cell Transplantation for Treatment of Ischemic Cardiomyopathy (Experimental Investigation)

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ABSTRACT. Regional myocardial perfusion and function may improve after cell transplantation. The objective of the study was to investigate the feasibility and efficacy of autologous endothelial cell implantation into myocardium or transplantation onto myocardium using a fibrin matrix.

An ameroid constrictor was placed on the circumflex artery of 48 adult sheep to induce ischemic cardiomyopathy. Four weeks later, the animals were divided into the following six groups (n=8 in each group): cardiomyoplasty alone without cell transplantation (CMP), cardiomyoplasty with EC added to a fibrin platform and placed between the myocardium and latissimus dorsi muscle (CMP+EC), autologous EC transplantation (AEC), saline injection with added denaturated cells (SAL), fibrin sealant injection without EC (FS) or a control (CON) group. Eight weeks after treatment the left ventricular function was investigated and the animals were sacrificed. Myocardial blood flow and capillarization were evaluated.

Eight weeks after injection (AEC, SAL, FS groups), or cardiomyoplasty (CMP, CMP+EC groups) or control (CON) groups, ventricular function was markedly improved in the CMP, CMP+EC and AEC groups ($p<0.05$ versus baseline, SAL, FS and CON), but had deteriorated in the SAL, FS and CON groups ($p<0.05$ versus baseline). Myocardial blood flow was also deteriorated after ameroid constrictor placement, but 8 weeks later was increased in the EC-group.

Myocardial blood flow was also deteriorated after ameroid constrictor placement ($p<0.05$ in all groups vs. baseline), but 8 weeks later was increased in the EC and CMP+EC groups ($p<0.05$ vs. before treatment). In the CMP, SAL, and CON groups myocardial blood flow continued to deteriorate for the next 8 weeks. In the FS group myocardial blood flow had a tendency to increase after treatment, but statistically non-significant. Histology and electron microscopy revealed extensive neovascularization and improved myocardial appearance after endothelial cell implantation into myocardium and cell transplantation between myocardium and latissimus dorsi muscle. In the other groups capillary density was considerably deteriorated.

These results suggest that intramyocardial transplantation of autologous EC within a fibrin matrix and application of EC to the myocardium during cardiomyoplasty enhances neovascularization, increases myocardial blood flow, and improves the left ventricular function. *© 2007 Bull. Georg. Natl. Acad. Sci.*

Key words: *intramyocardial transplantation, ischemic cardiomyopathy.*

Introduction

Today, hundreds of thousands of patients who have been diagnosed with heart failure or either pre-end or end stage ischemic cardiomyopathy are not helped by the available medical and surgical treatment interventions. Despite the current treatment options of coronary artery bypass surgery and angioplasty with or without stent implantation, this patient population, which includes those individuals who have severe diffuse coronary artery disease or small vessel disease, continues to suffer severe anginal pain with undiminished high risk to viable myocardial cells. Two unavoidable processes lead these patients to their inevitable demise: aggravation of atherosclerotic ischemic coronary artery disease and the inability of the ventricles in a highly ischemic heart to support adequate hemodynamics. Heart transplantation is an answer for only a few due to a lack of available donor organs.

Thus the challenge for present day cardiologists is how to treat patients with advanced ischemic cardiomyopathy, who have a limited chance at heart transplantation or who have refused other surgical treatment options. One of the most promising new approaches for treatment in such patients is neovascularization of ischemic tissue or the border zones through local stimulation of angiogenesis [1, 2]. It has been shown [2-4] that ischemic tissue injury causes the release of special endogenous biochemical agents, including growth factors, which stimulate angiogenesis through collateralization of the available vessels. Unfortunately the process of collateralization of the available capillaries and arteries requires more time than does vascular narrowing or occlusion. This angiogenic response is often inadequate to prevent clinical manifestation of ischemia. Therefore, processes needed to be found, which accelerated and artificially enhanced the development of the new vessels before irreversible tissue damage took place.

The concept of promoting new vessel growth using growth factor therapy is intriguing. This approach differs from previous revascularization techniques and may provide a means to treat patients who are not candidates for current standard treatment options. Direct application of vascular growth factors or DNA encoding for such factors have been tried [5, 6], but it was difficult to prove whether these agents remained at the target long enough to be specifically active. The demonstration of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) to elicit an angiogenic response via their own endothelial receptors has led to subsequent experimental and clinical investigations to study their use in myocardial revascularization. However clinical results have ranged from merely questionable to speculative [7-12].

Another area of research involves utilization of mature ECs and EC-precursor cells [13-15]. In many ways, the magnitude of risk is equal for both harvesting bone marrow and for mobilizing and collecting EC-precursors from peripheral blood vessels. It is unknown, however, whether heterotopic EC transplantation will produce enough vascular cell proliferation to form a functional capillary network in order to revascularize ischemic tissue.

It also was necessary to devise a method for the prolonged release of endothelial cells into the ischemic tissue. Studies from several laboratories [16, 17], including our own [18], have shown that fibrin can be used as a carrier for the application and prolonged delivery of agents. A fibrin network is critical for effective wound healing, and is biodegradable through routine tissue fibrinolysis. Since the fibrin sealant is slowly lysed, it can serve as a vehicle to deliver agents that may act to help heal wounds [19-24] to promote new vessel growth [16, 25] or to store and slowly release any therapeutic agent [19]. In preliminary investigations, we used fibrin sealant to deliver aprotinin and pyrrolostatin to ischemic skeletal muscle in order to accelerate local revascularization [26, 27]. We also used the fibrin platform to endothelialize vascular grafts and cell transplantation [28, 29].

We have hypothesized that a fibrin platform could be used to support the viability of applied or transplanted EC, direct the morpho-functional process of capillary formation, and accelerate compensatory myocardial revascularization in ischemic myocardium. In this chapter for the first time feasibility and efficacy of autologous mature EC application or transplantation in a fibrin matrix is described in a large animal model. The strategy of mature EC transplantation used in this study contrasts with approaches used before, including GF therapy, bone marrow cell transplantation and *ex vivo* expanded EC progenitor transplantation.

Materials and Methods

An ameroid constrictor was placed on the circumflex artery of 48 adult sheep to induce ischemic cardiomyopathy. Four weeks later, the animals were divided into the following six groups (n=8 in each group): cardiomyoplasty alone without cell transplantation (CMP), cardiomyoplasty with EC added to a fibrin platform and placed between the myocardium and latissimus dorsi muscle (CMP+EC), autologous EC transplantation (AEC), saline injection with added denaturated cells (SAL), fibrin sealant injection without EC (FS) or a control (CON) group. Eight weeks after treatment, left ventricular function was investigated and animals were sacrificed. Myocardial blood flow and capillarization were evaluated.

Preparation of autologous endothelial cells. Endothelial cells were cultivated from the endothelium of the jugular veins of sheep using standard [30]. After removal, the vessels were immersed in Hanks solution supplemented with penicillin/streptomycin and fungizon

and immediately transported to the cell culture laboratory, where they were opened longitudinally. The vein was placed on a Petri dish, irrigated with a few drops of 0.2% solution of collagenase (type 1A, Sigma), and incubated at 37°C for 15 minutes. Next, it was washed with a medium containing serum and centrifuged at 600 rpm for 5 min. The pellet was resuspended in Dulbecco's modified Eagle's medium (DMEM), supplemented with serum, and placed into a T25 culture flask.

Preparation of fibrinogen. Standard cryoprecipitate technique was used to prepare autologous fibrinogen from sheep plasma collected one week before EC seeding. Whole blood was collected in 50cc polypropylene centrifuge tubes (Fischer Scientific, Pittsburgh, PA) containing citrate-phosphate dextrose anticoagulant solution and centrifuged at 4° C at 1,750 rpm for 20 min. The plasma was then separated from red cells, frozen, and stored at –20° C for 18 h to 24 h before further processing. The frozen plasma was thawed in a 4° C cold room for 4 h and centrifuged at 2° C at 2,500 rpm for 40 min, then drained, leaving 3–4 ml of concentrated fibrinogen, approximately 20 mg/ml).

Sources of thrombin. In all experiments bovine thrombin (Johnson and Johnson Medical, Inc. Arlington, TX) was used. Lyophilized thrombin was solubilized in complete cell culture medium and diluted to an activity 3 U/ml.

Experimental animals. The animals were cared for according to the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health.

1) General Anesthesia and Antibiotic Therapy

All animals underwent general anesthesia for each surgical procedure. The animals were premedicated with Valium (Elkins-Sinn, Cherry Hill, NJ; 5 mg/kg IV) and anesthetized with sodium Pentothal (Abbot Laboratories, North Chicago, IL; 20-25 mg/kg IV). They were then intubated, placed on a Draeger (North American Draeger, Telford, PA) ventilator, and maintained on Halothane gas anesthesia (1-2% with 4.0 L O_2). Oxygen saturation levels and heart rate were monitored via a pulse oximeter placed on the animal's tongue. Strict sterile technique was followed at all times to reduce the potential for infection. The animals were started on a postoperative regimen of antibiotic therapy (amoxicillin, 15 mg/kg IM once a day for 5-7 days), and monitored twice daily for signs of infection.

2) Surgical Procedures

a) Creation of a model of myocardial ischemia (all groups). A model of myocardial ischemia was created. A branch of the left anterior descending coronary artery was isolated. An ameroid constrictor was placed around the arterial branch. During the ensuing two months, the constrictor gradually tightened and created a stenotic lesion in the coronary artery, and thus a model of chronic ischemia.

b) Subtotal latissimus dorsi muscle mobilization for CMP (CMP group). With the animal in the lateral position, a left 25 cm cutaneous incision was made at the level of the lateral border of the scapula, from the axillary region to the intersection between the iliac crest and paravertebral muscles. The LDM was dissected from the iliac crest, vertebral, inferior scapular angle, and 9th to 12th rib attachments. Collateral blood vessels arising from intercostal arteries were dissected. The muscle flap was freed of its distal attachments with its neurovascular pedicle carefully preserved. A 4 cm segment of the anterior portion of the second rib was then resected and the LDM flap transposed into the anterior mediastinal space. The window in the rib was carefully sutured closed.

c) Lateral dynamic CMP (CMP group). CMP was performed through a left anterolateral thoracotomy. The pericardium was opened widely. The flap of the LDM was not sutured directly to the myocardium; all sutures were anchored to the pericardium. The first two sutures were placed close to left branches of the pulmonary artery and the inferior vena without elevation of the heart. The apex of the left ventricle was then elevated 2-3 cm, and the LDM flap placed under the heart. The heart was immediately returned to its normal position and any ECG changes were noted. If no severe cardiac dysarrhythmias were noted, the next suture was placed as deep as possible at a level of right ventricle. ABG with EC was administered between the LDM and myocardium. The distal portion of the LDM flap was then sutured to the proximal portion of the LDM, thus completely covering both ventricles.

d) Intramyocardial administration of ABG (AEC group). A left thoracotomy was performed and the pericardium opened. Fibrinogen (0.5 ml) containing EC and thrombin (0.5 ml) were injected into the ischemic zone of the myocardium at less than 2 atmospheres of pressure four weeks after placement of an ameroid constrictor. The solution was injected at a rate of 0.5 ml/min over a period of 120 seconds, using a 27 gauge needle. Three injections were made in each animal. When mixed together at these concentrations, thrombin causes fibrinogen polymerization in 40-90 seconds. Such polymerization times induce the formation of a vascular platform at the site of intervention. In SAL animals, saline only was injected in the same fashion. The CON group served as a control (chest was opened but no injections were performed).

e) Cardiosynchronized electrical stimulation after cardiomyoplasty (Medtronic protocol – CMP group). The electrical stimulation protocol was begun 14 days after LDM mobilization and heart wrap with a cardiosynchronization ratio of 1:2, with an amplitude of 5 V and a frequency of 10 Hz. Stimulation was begun with single impulses for two weeks, followed by double im-

pulses (two weeks), triple impulses (two weeks), and finally two months after CMP, trains of impulses (30 Hz, burst of six impulses, pulse width of 210 msec) synchronized 1:1 with the cardiac cycle (the number of impulses and frequency changed) were given.

Histology

1. Biopsies for light microscopy, immunohistochemistry and transmission electron microscopy were taken from the LDM before and after subtotal mobilization on the days designated in the experimental protocol. At the termination of experiments, the heart/muscle complex after CMP was carefully excised. Samples from the different areas (proximal, middle and distal) of the LDM, and the apical, middle and base regions of the heart were taken for analysis. Samples (3x4 mm) for light microscopy and immunohistochemistry were placed in 10% Formalin and taken to the hospital's pathology department for embedding and sectioning. Samples (3 x 4 mm) for transmission electron microscopy (TEM) were placed in Karnovsky's fixative.

2. Light microscopy. For histological examination, samples were taken from each area and fixed as described above. Transverse sections were made for conventional histological (Hematoxylin & Eosin) staining and for subsequent evaluation. Multiple slides were made of each biopsy sample. Histological data were submitted for interpretation to an independent observer. Particular attention was paid to evidence of muscle regeneration, thickness and composition of the reparative response, the density of neovascularization, the presence of large bore vessels, and margination of leukocytes.

3. Transmission electron microscopy (TEM). Biopsies of the LDM (approximately 3x4 mm) for transmission electron microscopy were placed into Karnovsky's fixative (2% formaldehyde, 2.5% glutaraldehyde in 0.1 M Sodium Cacodylate buffer, pH 7.2) and then sliced into smaller (1-2 mm) pieces. The biopsies were postfixes in a 1% Osmium Tetroxide, dehydrated through a series of graded alcohols and acetone, and embedded in Spurr resin. Preliminary thick (1 mM) sections were cut and stained with 0.1% Toluidine blue. Longitudinal areas of muscle were selected for ultra-thin sectioning; thin sectioning (60-90 nm) was done with a Reichert Ultra-Cut microtome. The thin sections were stained with 5% Uranyl Acetate and Reynolds's Lead stain (3.5% lead citrate, 2% lead nitrate). Examination and photography of the thin sections were done with a Philips 400T transmission electron microscope at an accelerating voltage of 60 kV on Kodak SO-163 image film. Five electron micrographs of adjacent areas in each of 2 regions were taken on each biopsy at a magnification of 6000x. A 2800x magnification of the two regions was also included as an overview. Special attention was paid to the morphology of capillaries, pericytes and smooth muscle cells.

4. Conventional indirect immunofluorescent en-face staining (immunohistochemistry [IH]). In order to assess angiogenesis, conventional indirect immunoperoxidase staining was used after fixation and proteolytic predigestion of formalin-fixed tissue followed by incubation with von Willebrand Factors (vWF) as angiogenic markers. This analysis yielded information as to overall angiogenesis and vascularization in the ABG, as well as the adjacent skeletal muscle and myocardium. The degree of vascularization was evaluated by counting the number of vessels per unit area.

Blood Flow Analysis [Fluorescent Microspheres]

After completing the thoracotomy, one temporary catheter was placed into the femoral artery and one in the left atrial appendage. A temporary occluder was placed on a previously isolated coronary artery, and blood flow was occluded for 3 minutes during which time 15 million fluorescent microspheres (15 μm) were injected into the left atrium. A reference blood sample was simultaneously withdrawn from the femoral artery at a fixed rate (Harvard Apparatus Withdrawal/Infusion Pump). Blood flow to the area at risk through the innate intramyocardial collateral system was able to be determined. Both temporary catheters and the temporary occluder were removed, and blood flow restored. On day 60, catheters were again placed into the left atrium and femoral artery. In animals with cardiomyoplasties (series IV), an additional catheter was placed in the thoracodorsalis artery as well. Fifteen million fluorescent microspheres of a different color were injected into the left atrium, and a reference blood sample taken. Blood flow representing the flow delivered by the innate intracoronary and the extramyocardial collaterals from the LDM were able to be determined. In animals from Series IV, seven and a half million microspheres of a third fluorescence were then injected directly into the thoracodorsalis artery to determine collateral blood flow from the LDM. From this injection the ratio of microspheres found in the heart in relation to those found in the LDM could be determined. After euthanization, the heart/muscle complex was removed. Samples of the heart and LDM were taken to determine the blood flow.

Data Analysis

Histological data was submitted to one of the hospital's pathologists for interpretation and evaluation. Hemodynamic data and capillary numbers were collected and tabulated. Values are reported as mean plus or minus standard deviation of the mean. Data was compared using an ANOVA and a subsequent Student Newman-Keuls t-test if applicable. A p value of less than 0.05 was considered statistically significant.

Results

I. Animals without cardiomyoplasty (AEC, SAL and CON groups)

1. Assessment of left ventricular function. Baseline LVEF was 0.68 ± 0.03, LVESV was 26.4 ± 6.1 ml, and LVEDV 53.4 ± 10.4 ml. At four weeks after ameroid placement, LVEF decreased to 0.49 ± 0.05 (p=0.0000000004). LVEDV increased to 68.1 ± 9.9 ml (p=0.0019) and LVESV to 42.4 ± 7.5 ml (p=0.00006).

Two months after treatment, in animals subjected to EC transplantation (AEC group), LVEF increased to 0.56 ± 0.04 (p=0.029 vs. four weeks after ameroid placement). In the control group (no treatment), mean LVEF decreased to 0.40 ± 0.09 (p=0.013 vs. EC groups); and in SAL group EF decreased to 0.39± 0.05 (p=0.0017 vs. AEC group).

LVEDV increased in all three groups: to 81.3 ± 9.9 ml in the CON group and to 76.1 ± 11.6 ml in the SAL group. Although we observed an increase in LVEDV in the EC group to 60.4 ± 2.7 ml, this parameter was significantly lower than in the control groups (p=0.006 vs. CON group).

LVESV in control animals increased to 51.1 ± 7.5 ml and to 53.8 ± 6.2 ml in the SAL group. However, LVESV was significantly lower after EC transplantation (30.1 ± 4.1, p=0.002 vs. CON group and p=0.0007 vs. SAL group).

2. Myocardial Blood Flow. Myocardial blood flow (MBF) before placement of the ameroid constrictor was 0.59 ± 0.05 ml/min/gm. One month after ameroid constrictor placement, blood flow in the ischemic part of the myocardium decreased to 0.41± 0.07 ml/min/gm (p=0.0000009). At the time of sacrifice, MBF decreased to 0.15 ± 0.03 in the CON group (p=0.00001 vs. four weeks) and to 0.18 ± 0.05 in the SAL group (p=0.0006 vs. four weeks). In AEC animals, MBF increased to 0.66 ± 0.10 ml/min/gm (p=0.0009 vs. CON group and p=0.0001 vs. SAL group).

3. Immunostaining. In normal nonischemic myocardium, in all three groups, 5.6 ± 0.4% of the area was occupied by capillaries. Three months after ameroid constrictor placement, in the CON group this percentage decreased to 3.3 ± 0.3% (p=0.003 vs. baseline) and to 3.4 ± 0.6% in SAL group (p=0.009 vs. baseline). In the EC group, however, the area occupied by capillaries increased to 8.4 ± 1.3% (p=0.0004 vs. baseline, p=0.00027 vs. control group and p=0.00016 vs. SAL group).

4. Transmission Electron Microscopy. In both CON and SAL animals, the typical pattern of ischemic damage (cytoplasm folding and projection into the capillary lumen) was seen in the EC in ischemic vasculature. However, in the border areas of ischemic zone a few ECs appeared normal and had no signs of damage or injury. Perhaps these cells were able to give rise to new capillaries and arterioles. In contrast, tissue samples obtained from animals in the AEC group had EC that appeared normal with significantly enhanced vascularization and no signs of significant ischemic lesions.

II. Animals with Cardiomyoplasty (CMP group)

1. Assessment of left ventricular function

LVEF increased after cardiomyoplasty to 0.59 ± 0.03 (p=0.037 vs. 4 weeks after ameroid placement). LVEDS was 28.0 ± 5.1 ml and LVEDV was 56.1 ± 4.7 ml (p<0.05 vs. SAL and CON groups but p>0.05 vs. the AEC group).

2. Myocardial Blood Flow

MBF was 0.60 ± 0.08 ml/min/gm. It was better than in the CON group (0.15 ± 0.03, p=0.0004) and in the SAL group (0.18 ± 0.05, p=0.008), but worse than in the AEC group (0.66 ± 0.10, p=0.077). Blood flow in the latissimus dorsi muscle before mobilization was 0.36 ± 0.04 ml/min/gm and 0.15 ± 0.04 ml/min/gm after mobilization (p=0.0019). After EC application blood flow increased from 0.15 ± 0.04 to 0.41 ± 0.06 ml/min/gm (p=0.0003).

3. Immunostaining

The area occupied by capillaries increased from 5.6 ± 0.43% to 7.7 ± 0.8% (p=0.0027). It was considerably better than in the control series (p=0.00091) and SAL series (p=0.00058), but worse than in the AEC group (p=0.036).

4. Transmission electron microscopy

Transmission electron microscopy revealed new capillary formation in the fibrin glue between the myocardium and the LDM, and well-preserved capillaries in the ischemic part of the myocardium.

Discussion

Transplantation of mature cells has been proposed as a strategy for organ replacement or tissue repair (neomorphogenesis) for a variety of therapeutic needs, e.g., generation of bioengineered skin, blood vessels, liver, nerves, bone, and cartilage [31-34].

A crucial component of transplantation procedure is forming the three-dimensional biodegradable matrix in which neomorphogenic processes take place. These matrices are generally used for cell anchorage, guided migration, proliferation, and differentiation. The ones currently used in cardiovascular bioengineering are potentially immunogenic; they show toxic degradation and inflammatory reactions and act primarily as passive scaffolding for cell manipulation in culture or for implantation as a film.

Rather than a passive matrix, for EC transplantation, we needed a matrix that had two essential characteristics: morphogenically active to direct implanted cells to form vessel-like structures and an injectable liquid to polymerize and solidify after administration into target territory. Fibrin-based materials have these and many other characteristics beneficial for bioengineering a vascular bed.

We also needed to be able to generate a specific cellular signal that would direct the morphogenesis of implanted cells to form a neovascular network that would be connected to existing vessels. The idea of using fibrin as an angiogenesis substance either alone or with

the addition of proteins belongs to Fasol et al. [16], who demonstrated in a rat model that significant site-directed formation of new blood vessel structures could be induced by using a modified fibrin glue implant that contains the angiogenic growth factors HBFG-1.

In previous experiments we found that a fibrin-based sealant becomes vascularized [18, 35], thus capable of delivering some of the plasma proteins needed to perform some functions and extracellular matrix to anchor EC to the vessel wall [28]. We also demonstrated clinically in patients with PAD that VEGF and a fibrin-based sealant accelerated angiogenesis [36, 37].

The present study is the first to show that EC confined in a 3-dimensional fibrin matrix will form capillaries in ischemic myocardium. In microvascular ECs, we previously [38] found two morphogenically distinct types of growth. Cultured in a two-dimensional matrix, ECs quickly form a cobblestone monolayer that has a density 2 – 2.5 fold higher than in controls, i.e., those cultivated on a single-plane tissue culture surfaces. We hypothesized that the ionic strength of the ratio of these components enabled the cells to "recognize" minor spatial changes in fibrin-meshwork architecture. Cultured in a three-dimensional matrix, ECs formed "true" capillaries while other vascular cells trapped in this matrix underwent apoptosis [39].

Because the direction in which capillaries form is generally determined by the orientation of the administered angiogenic mixture, we needed to insure that the EC would grow in the desired direction. On the other hand, ischemic tissue itself produces many chemotactic factors that attract EC or EC-precursors and will support capillary growth, suggesting that plasma proteins can perform some of the functions of the extracellular matrix involved in anchoring EC to the myocardium.

Because it is well known that ischemia interrupts local circuit neurons, previous studies showing that fibrin enhances nerve regeneration [40, 41] point to another mechanism for improving LV function after EC/fibrin intramyocardial injection [42].

During our investigation we also showed that application of EC in biological glue when added to ischemic LDM increased blood flow to the muscle more than two fold, compared with non-treated mobilized muscle.

Application of glue and EC between the LDM and myocardium (during CMP or injection of EC directly into the ischemic myocardium) creates strong adhesions between the two layers, provides for capillary growth, and increases revascularization of the ischemic and nonischemic myocardium.

In the clinical setting our proposed strategy would require that the patient donate autologous EC and reagents for the fibrin matrix, thus also avoiding potential immunological rejection. As has been clearly demonstrated, transplantation of allogenic cells, no matter how freshly prepared or viable, provokes strong conflict with a spectrum of likely immunological and inflammatory events that lead to rejection [43]. Thus, autologous cell transplantation is a reasonable, if not mandatory, step, but this raises the problem of how to apply this laboratory technique clinically, namely, what source should be used for autologous EC.

Certainly, patients who have cardiovascular disease cannot be autologous donors of EC derived from jugular, cephalic or saphenous veins. Adipose tissue has been actively explored as an alternate source of EC because of its abundance in the body, easy access, and its composition of primarily two cell types, i.e., adipocytes and capillary EC. In culture, these EC demonstrate many of the functional and morphologic characteristics seen in EC in large vessels, hence, large quantities of adipose tissue could be removed with minimal risk to the patient and the inherent difference in density between adipocytes and capillary fragments means that they can be easily separated by simple centrifugation.

Once a human adipose tissue EC procurement protocol is developed, its use for high density seeding has to be validated by successive successful studies of culturing of EC derived from patients with cardiovascular diseases. One such study of harvesting adipose tissue EC by means of liposuction has already reported 100% success in 140 patients [44].

Finally we think that cell transplantation is more potent than GF therapy. Although it was previously demonstrated that intramyocardial transplantation of bone marrow cells induce neovascularization of ischemic tissue [13, 14], it remains to be proven that these cells indeed differentiate into mature EC and not into other types of cells including fibroblasts, osteoblasts, etc.

Recently Kawamoto et al. [15] demonstrated the therapeutic potential of *ex vivo* expanded endothelial progenitor cells in a small animal model. However, the safety of *ex vivo* culture expansion needs to be established.

Conclusions

Although endothelial cell transplantation induced capillary growth in the myocardium and improved circulation in the ischemic muscle in a sheep model, clinical trials are needed to investigate the efficacy of this intriguing technique. We suggest that this technique or a similar one that uses EC application (in the case of cardiomyoplasty) to accelerate the formation of compensatory collateral vasculature may be an answer to one of the most challenging issues in contemporary cardiology: how to treat small vessel pathology and disseminated lesions. Future approaches may consist of delivering a "cocktail" of angiogenic agents laced simultaneously with vasculogenic precursors and mature vascular cells in a temporally, spatially coordinated fashion.

სამედიცინო მეცნიერებანი

იშემიური კარდიომიოპათიის მკურნალობა აუტოლოგიური ენდოთელური უჯრედების ექსტრამიოკარდიული და ინტრამიოკარდიული ტრანსპლანტაციით (ექსპერიმენტული კვლევა)

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მიოკარდიუმის რეგიონული პერფუზია და მისი ფუნქცია შესაძლებელია გაუმჯობესდეს უჯრედების ტრანსპლანტაციის შედეგად. კვლევის მიზანს წარმოადგენდა მიოკარდიუმში აუტოლოგიური ენდოთელური უჯრედების ან ფიბრინული მატრიქსის იმპლანტაციის გავლენის შესწავლა გულის კუნთის ფუნქციურ მდგომარეობასა და ვასკულარიზაციის პროცესზე. ექსპერიმენტში იშემიური კარდიომიოპათიის განვითარების მიზნით, ამეროიდის კონსტრიქტორი შემომხვევ კორონარულ არტერიაზე თავსდებოდა. კვლევა ტარდებოდა 48 ცხოველზე (მოზრდილი ცხვარი). ოთხი კვირის შემდეგ, კვლევის დიზაინით გათვალისწინებული იყო შემდეგი ექვსი ჯგუფის (თითოეულში 8 ცხვარი) გამოყოფა: ჯგუფი I – ცხოველები ენდოთელური უჯრედების ტრანსპლანტაციის გარეშე; ჯგუფი II – კარდიომიოპლასტიკა ენდოთელური უჯრედების ფიბრინის სუბსტრატზე დამატებით და მიოკარდიუმში ტრანსპლანტაციით; ჯგუფი III – აუტოლოგიური ენდოთელური უჯრედების ტრანსპლანტაცია; ჯგუფი IV - სელინის ინექციისა და დენატურირებული უჯრედების დამატებით; ჯგუფი V - სელინის ინექციით ენდოთელური უჯრედების გარეშე და ჯგუფი VI - საკონტროლო ჯგუფი. მკურნალობის რვა კვირის შემდეგ შეისწავლებოდა მარცხენა პარკუჭის ფუნქციური მდგომარეობა, მიოკარდიუმში სისხლის ნაკადი და მიოკარდიუმის კაპილარიზაცია. მარცხენა პარკუჭის ფუნქციური მდგომარეობა საგრძნობლად გაუმჯობესდა I, II და III ჯგუფებში ($p<0.05$ საწყის მაჩვენებლებთან შედარებით), გაუარესდა IV, V და VI ჯგუფებში ($p<0.05$ საწყის მაჩვენებლებთან შედარებით). ასევე, აღინიშნა მიოკარდიუმში სისხლის ნაკადის გაუარესება ყველა ჯგუფში საწყის მაჩვენებლებთან შედარებით ($p<0.05$), თუმცა 8 კვირის შემდეგ სისხლის ნაკადის გაუმჯობესება აღინიშნა III და II ჯგუფებში. I, IV და VI ჯგუფებში აღინიშნა მიოკარდიუმში სისხლის ნაკადის პროგრესული გაუარესება შემდეგი რვა კვირის განმავლობაში. V ჯგუფში შეიმჩნეოდა მიოკარდიუმში სისხლის ნაკადის გაუმჯობესების სტატისტიკურად არასარწმუნო ტენდენცია. ჰისტოლოგიურმა და ელექტრონული მიკროსკოპით ჩატარებულმა კვლევებმა ნეოვასკულარიზაციის მნიშვნელოვანი ტენდენცია გამოავლინეს ენდოთელური უჯრედების იმპლანტაციისა და ტრანსპლანტაციის შემდგომ. სხვა ჯგუფებში აღინიშნა კაპილარების რაოდენობის მნიშვნელოვანი გაუარესება.

კვლევის შედეგების გათვალისწინებით, მიოკარდიუმში აუტოლოგიური ენდოთელური უჯრედების ტრანსპლანტაცია ფიბრინული მატრიქსის გამოყენებით და ამჟე უჯრედების მიოკარდიუმზე აპლიკაცია კარდიოპლასტიკასთან ერთად, მნიშვნელოვნად აუმჯობესებს მარცხენა პარკუჭის ფუნქციურ მდგომარეობას, ხელს უწყობს ნეოვასკულარიზაციის პროცესს და ზრდის მიოკარდიუმში სისხლის ნაკადს.

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Received January, 2007