Journal of the American College of Cardiology
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Published by Elsevier Inc.

Vol. 52, No. 23, 2008
ISSN 0735-1097/08/$34.00
doi:10.1016/j.jacc.2008.06.052

MINI-FOCUS: CELL-BASED THERAPY

Controlled Delivery of Basic Fibroblast Growth Factor Promotes Human Cardiosphere-Derived Cell Engraftment to Enhance Cardiac Repair for Chronic Myocardial Infarction

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Objectives This study was designed to determine whether controlled release of basic fibroblast growth factor (bFGF) might improve human cardiosphere-derived cell (hCDC) therapy in a pig model of chronic myocardial infarction.

Background Current cell therapies for cardiac repair are limited by loss of the transplanted cells and poor differentiation.

Methods We conducted 2 randomized, placebo-controlled studies in immunosuppressed pigs with anterior myocardial infarctions. Four weeks after coronary reperfusion, 14 pigs were randomly assigned to receive an intramyocardial injection of placebo medium with or without bFGF-incorporating hydrogel implantation. As a second study, 26 pigs were randomized to receive controlled release of bFGF combined with or without hCDCs or bone marrow–derived mesenchymal stem cell transplantation 4 weeks after reperfusion.

Results Controlled release of bFGF in ischemic myocardium significantly augmented the formation of microvascular networks to enhance myocardial perfusion and contractile function. When combined with cell transplantation, the additive effects of bFGF were confined to hCDC-injected animals, but were not observed in animals receiving human bone marrow–derived mesenchymal stem cell transplantation. This was shown by increased donor-cell engraftment and enhanced cardiomyocyte differentiation in the transplanted hearts, resulting in synergistically improved ventricular function and regional wall motion and reduced infarct size.

Conclusions Controlled delivery of bFGF modulates the post-ischemic microenvironment to enhance hCDC engraftment and differentiation. This novel strategy demonstrates significant functional improvements after myocardial infarction and may potentially represent a therapeutic approach to be studied in a clinical trial in human heart failure. (J Am Coll Cardiol 2008;52:1858–65) © 2008 by the American College of Cardiology Foundation

Stem cell therapies offer tremendous possibilities for curative approaches toward restoring lost myocardium and cardiac function; however, recent studies have indicated that effective cardiac muscle regeneration might be hindered by poor cell engraftment and inefficient cardiomyocyte differentiation of the transplanted cells in the absence of integration with the host myocardial environment after infarction (1). Although prior studies (2–5)

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Manuscript received March 7, 2008; revised manuscript received June 4, 2008, accepted June 10, 2008.

including our report have suggested human cardiac stem/progenitor cells as an attractive cell source for cardiac repair, the beneficial effects of these cells in large animal models have yet to be investigated.

The basic fibroblast growth factor (bFGF) is a pluripotent mitogen and possesses properties to promote stem cell differentiation, proliferation, and survival (3,6,7). Biodegradable gelatin is a useful delivery modality to circumvent the short half-life of recombinant bFGF in vivo. We have designed a controlled-release system for bFGF composed of acidic gelatin, which forms a poly-ion complex with bFGF (8). Biodegradable hydrogels display excellent biocompatibility demonstrated by the absence of rejection and inflammation and achieve a controlled release of bFGF in vivo as a result of hydrogel degradation within 3 weeks (9). Controlled release of bFGF has been shown to effectively enhance neoangiogenesis in human ischemic limbs (10).

This study was conducted to test whether the cell engraftment, survival, and differentiation potential of human cardiosphere-derived cells (hCDCs) could be promoted by controlled delivery of bFGF-incorporating hydrogel in response to experimental myocardial infarction, ultimately leading to improved performance in cardiovascular regeneration.

Methods

Isolation and expansion of hCDCs from human heart samples. Human samples were obtained from 10 male patients undergoing cardiac surgery, in conformity with the guidelines of the Kyoto University Hospital and Ministry of Education, Culture, Sports, Science, and Technology, Japan. Samples were excised, minced, and digested with 0.2% type II collagenase and 0.01% DNase I (Worthington Biochemical Corp., Lakewood, New Jersey) to obtain single cell suspensions to generate cardiospheres as described previously (3). Cardiospheres were mechanically selected from the cultures and expanded in Dulbecco's Modified Eagle Medium (DMEM)/F12 medium containing 10% fetal bovine serum, 2% penicillin and streptomycin, and 40 ng/ml human recombinant bFGF (Promega Corp., Madison, Wisconsin). Six independent human bone marrow–derived mesenchymal stem cells (hBMCs) were purchased from the RIKEN Cell Bank (RIKEN Bioresource Center, Ibaraki, Japan) (11). The hBMCs were plated in DMEM containing 10% fetal bovine serum, 2% penicillin, streptomycin, and 4 ng/ml bFGF. Cells were harvested at passage 2, frozen at −80°C, and were thawed to process the third rounds of passage 3 weeks before the transplantation. The expanded hBMCs were characterized by a fluorescence-activated cell sorter using CD29, CD105, CD71, and CD90.

Generation of gelatin hydrogel sheet. The gelatin was isolated by an alkaline process from bovine bone with an isoelectric point of 5.0 as previously described (10). The water content of gelatin hydrogel was prepared to 94% by chemical cross-linking at 140°C for 72 h. The gelatin was reinforced by polytetrafluoroethylene (W. L. Gore and Associates, Inc., Flagstaff, Arizona) pericardial sheet (12) to provide strength against the beating heart. Human recombinant bFGF (Kaken Pharmaceutical Co., Tokyo, Japan) was incorporated into the gelatin hydrogel by impregnation for 3 h before implantation.

Abbreviations and Acronyms

bFGF = basic fibroblast growth factor
DMEM = Dulbecco's Modified Eagle Medium
FISH = fluorescent in situ hybridization
hBMC = human bone marrow–derived mesenchymal stem cell
hCDC = human cardiosphere-derived cell
LV = left ventricle/ventricular
LVEF = left ventricular ejection fraction
MRI = magnetic resonance imaging
SPIO = superparamagnetic iron oxide
SRS = systolic radial strain

Animal models and study protocol. Based on computer-generated random allocation, we performed 2 randomized studies of chronically instrumented animals (Fig. 1). Myocardial infarction was created in 60 female Yorkshire pigs by inflating the balloon at the left ascending coronary artery for 90 min, followed by reperfusion. Thirteen of the study pigs died in the early postoperative period. We excluded 7 pigs with an ejection fraction <35% or >45% determined by transthoracic echocardiography using the Teichholz method before randomization. Animals were assigned for randomization 1 week after the creation of myocardial infarction, and then cells were grown in culture for 3 weeks to prepare for transplantation. Four study pigs died within 1 week after randomization but before the treatment due to heart failure.

In study 1, the eligible pigs (n = 14) were randomized to receive DMEM intramyocardial injection with or without 200 μg bFGF-incorporating gelatin hydrogel implantation 4 weeks after reperfusion. In study 2, we randomly assigned eligible pigs (n = 26) to receive bFGF hydrogel sheet implantation with intramyocardial injections of either DMEM, 2.0×10^7 hBMCs, or 2.0×10^7 hCDCs. All animals in both studies were immunosuppressed with cyclosporine A (Novartis Pharmaceuticals, East Hanover, New Jersey) 5 mg/kg daily from 5 days before transplantation until the time for sacrifice (13). Transplantation was performed by a 3-ml injection at 30 different sites along the border zone and in the center of the scar area.

Fluorescence-activated cell sorter analysis. Single cell suspensions were stained with the following antibodies: phycoerythrin-conjugated antibodies against CD29, and fluorescein isothiocyanate–conjugated antibodies against CD45 (all from BD Biosciences, San Jose, California) or CD105 (Ancell, Bayport, Minnesota). Antibody against mouse anti-human CD90 was detected by phycoerythrin-conjugated gout anti-mouse immunoglobin G (BD Biosciences). Samples were analyzed by FACSCalibur flow cytometer (BD Biosciences).

Generation of retroviral vectors. The *LacZ* reporter gene was subcloned into a human cardiac troponin I promoter