Adipose-derived stem cell sheet transplantation therapy in a porcine model of chronic heart failure

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Adipose-derived stem cells (ASCs) are a promising resource for cell transplantation therapy for damaged heart tissue. Cell death in the graft early after transplantation represents the main cause of unsatisfactory therapeutic efficacy, but tissue-engineered cell sheets grown in temperature-responsive cell culture dishes may enable improved engraftment of transplanted cells. We investigated the therapeutic potential of this method in chronic myocardial ischemia in swine. We created a porcine model of chronic heart failure by implanting an ameroid constrictor around the main trunk of the left anterior descending artery, just distal to the circumflex branch. Simultaneously, ASCs were obtained from a piece of subcutaneous adipose tissue and expanded to form ASC sheets using temperature-responsive dishes. Four weeks after ameroid constrictor placement, triple-layered ASC sheets were transplanted onto the area of the ischemic myocardium (sheet group, n = 7). Controls (n = 7) received no sheet. Just before and 4 weeks after transplantation, left ventriculography (LVG) and coronary angiography (CAG) were performed. LVG revealed a significant improvement in the left ventricular ejection fraction of the sheet group compared with controls (47.6 ± 2.9% vs 41.4 ± 2.8%, $P < 0.05$). Furthermore, development of collateral vessels was only detected in the sheet group with right CAG. Histologic analysis demonstrated that engrafted ASC sheets grew to form a thickened layer that included newly formed vessels. ASC sheet transplantation therapy is an intriguing therapeutic method for ischemic heart failure. (Translational Research 2015;165:631–639)

Abbreviations: ASCs = adipose-derived stem cells; ECs = endothelial cells; vWF = von Willebrand factor

INTRODUCTION

Heart failure continues to pose significant burdens on healthcare systems worldwide.[1] Over the past decade, cell transplantation therapy for the treatment of heart failure has emerged as an alternative to cardiac transplantation, and a large body of experimental evidence has accumulated.[2] Adult stem cells such as skeletal myoblasts and endothelial progenitor cells have been proposed as promising sources for cardiac regeneration.[3,4] However, recent clinical

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AT A GLANCE COMMENTARY

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Background

Cell transplantation therapy for the treatment of heart failure has emerged as an alternative to cardiac transplantation over the past decade and a large body of experimental evidence has been accumulated. However, recent clinical studies have shown unsatisfactory therapeutic results.

Translational Significance

In this article, we demonstrated, for the first time, adipose-derived stem cell sheet transplantation therapy is feasible and effective in a large animal heart failure model. We believe this article will pave the way for the clinical application of this efficient cell transplantation therapy for heart failure.

studies have shown unsatisfactory therapeutic results.[5] One possible reason for this may be cell death in the graft early after injection of cells because of hypoxia, inflammatory cytokines, and proapoptotic factors.[6] Alternative transplantation methods involving the use of tissue engineering techniques are therefore essential for achieving more effective cell delivery.[7]

Our colleagues have developed an original method to create sheets of cells without using a scaffold.[8] Using a culture surface coated with the covalently linked temperature-responsive polymer poly-(*N*-isopropylacrylamide), cells can be harvested as intact sheets by a simple change in temperature.[9-11] Furthermore, because of the presence of preserved adhesion proteins and because enzymatic digestion is not needed, thick, cell-dense tissues are constructed by layering these cell sheets.[11-13] Transplantation of cell sheets onto damaged heart tissue improves heart function in several animal models.[14-18]

Adipose-derived stem cells (ASCs) are multipotent cells that are a promising source of cells for a range of therapeutic applications.[19,20] We have previously reported that ASC sheets transplanted onto scarred myocardium in rats grow to form a thick stratum that includes newly formed vessels, undifferentiated cells, and small numbers of cardiomyocytes.[18] ASC sheets also act through paracrine pathways to trigger angiogenesis and improve cardiac function in rats with myocardial infarction.[18] The aim of our present study was to investigate the therapeutic potential of ASC sheet transplantation therapy on chronic myocardial ischemia in a preclinical setting.

MATERIALS AND METHODS

Experimental animals. The present study used male domestic pigs (weight, 15–20 kg; Keari, Osaka, Japan). All experimental procedures were approved by the Animal Care Committee at the National Cardiovascular Center. The study protocol included 3 stages: (1) induction of chronic heart failure; (2) ASC sheet transplantation; and (3) final evaluation.

Animal model. All surgical procedures in this study were performed under general anesthesia and continuous monitoring of the heart rate, 3-lead electrocardiography, and oxygen saturation. A porcine model of chronic heart failure was created.[21] After induction of anesthesia with intramuscular injection of ketamine (750 mg) and xylazine (150 mg), animals underwent endotracheal intubation and were connected to a mechanical ventilator, followed by continuous intravenous injection of propofol (5 mg/kg/h) during the procedure. A left thoracotomy was made through the third intercostal space, and the pericardium was opened. The left anterior descending (LAD) artery was ligated distal to the second diagonal branch, followed by implantation of an ameroid constrictor (2.5 mm diameter; Research Instruments SW, Escondido, California) around the main trunk of the LAD artery, just distal to the circumflex branch. A lidocaine injection (1 mg/min, continuous intravenous infusion) was used to avoid arrhythmia. After stabilization, the pericardium was approximated, and the thorax was closed.

Isolation and expansion of ASCs. ASCs were obtained from a piece of subcutaneous adipose tissue (about 1–2 g) that was collected from the iliac area during ameroid constrictor placement. After mincing the tissue into small pieces, adipose tissue was digested with collagenase type I (25 mg/mL in phosphate-buffered saline) at 37°C with constant shaking for 2 hours. After filtration through 100-μm polyethylene mesh and centrifugation, isolated cells were suspended in an α-minimum essential medium supplemented with 10% fetal calf serum, plated onto a 100-mm dish, and incubated at 37°C in 5% CO_2. Plastic-adherent spindle-shaped cells became apparent 3–4 days later and were isolated with trypsinization after reaching 80%–90% confluence.[18,20]

Characterization of ASCs. Identity of the isolated ASCs was determined by assessing their surface markers using fluorescence-activated cell sorting (FACScan flow cytometer; Becton Dickinson, Mountain View, California) using the following antibodies for porcine