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Epicardial delivery of collagen patches with adipose-derived stem cells in rat and minipig models of chronic myocardial infarction

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ABSTRACT

Although transplantation of adipose-derived stem cells (ADSC) in chronic myocardial infarction (MI) models is associated with functional improvement, its therapeutic value is limited due to poor long-term cell engraftment and survival. Thus, the objective of this study was to examine whether transplantation of collagen patches seeded with ADSC could enhance cell engraftment and improve cardiac function in models of chronic MI. With that purpose, chronically infarcted Sprague–Dawley rats ($n = 58$) were divided into four groups and transplanted with media, collagen scaffold (CS), rat ADSC, or CS seeded with rat ADSC (CS-rADSC). Cell engraftment, histological changes, and cardiac function were assessed 4 months after transplantation. In addition, Göttingen minipigs ($n = 18$) were subjected to MI and then transplanted 2 months later with CS or CS seeded with autologous minipig ADSC (CS-pADSC). Functional and histological assessments were performed 3 months post-transplantation. Transplantation of CS-rADSC was associated with increased cell engraftment, significant improvement in cardiac function, myocardial remodeling, and revascularization. Moreover, transplantation of CS-pADSC in the pre-clinical swine model improved cardiac function and was associated with decreased fibrosis and increased vasculogenesis. In summary, transplantation of CS-ADSC resulted in enhanced cell engraftment and was associated with a significant improvement in cardiac function and myocardial remodeling.

1. Introduction

While transplantation of stem cells in models of myocardial infarction (MI) has been associated with functional benefit, the results obtained in clinical studies have not been conclusively coincident with those observed in animal models [1,2]. It is generally accepted that the beneficial effects of this procedure result from indirect mechanisms, such as the release of growth factors and chemokines, and are to a certain degree, independent of the stem cell type [3]. Adipose tissue represents an attractive source of stem cells for cardiac cell therapy. This is due to the fact that

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adipose-derived stem cells (ADSC) (i) can be obtained from patients through an easy procedure with low morbidity, (ii) can be efficiently isolated and cultured, (iii) exert a potent paracrine effect involved in tissue revascularization and reduction of myocardial remodeling, and (iv) show immunomodulatory properties [4]. The efficacy of ADSC has already been demonstrated in animal models of MI [5–7], and clinical trials have recently been completed (APOLLO and PRECISE) exploring the safety and feasibility of ADSC transplantation in MI patients.

Efficacy of cell therapy, in general, is still hampered by limitations, such as the high degree of cell attrition that occurs within a few hours after transplantation [8]. Indeed, several approaches have been explored in order to enhance stem cell engraftment, including genetic manipulation of cells to increase homing and survival [9] as well as tissue engineering strategies involving scaffolds made of natural (extracellular matrix components) or synthetic polymers [10]. Among natural materials that could be used to generate scaffolds for cardiac cell therapy, collagen represents an optimal candidate as it is the most abundant heart matrix component, shows a remarkable biocompatibility with host tissues, induces low immunogenicity after injection [11–13], and provides sufficient mechanical stability [14].

In the current study, we have determined the long-term benefit of epicardial collagen patches seeded with ADSC in rat and minipig models of chronic MI. As hypothesized, the use of natural scaffolds seeded with ADSC led to increased stem cell engraftment, thus improving the efficacy of therapy and providing rationale for future application of this technique in clinical studies.

2. Material and methods

Please refer to the Online methods section for further details.

2.1. Isolation, infection and characterization of ADSC

Adipose-derived stem cells (ADSC) were isolated from 8 to 10 weeks-old Sprague–Dawley eGFP-rats and from 30 months-old Göttingen minipigs. Adipose pads were digested with collagenase-I and the derived stromal vascular fraction (SVF) cultured at a density of 7500 cells/cm^2. Five days after isolation, cells were infected with GFP-expressing lentivirus and after 3–5 passages, characterized by flow cytometry.

2.2. Collagen scaffold

A non-cross-linked collagen type-I scaffold of bovine origin (20 μm thickness) was provided by Viscofan SA company. Scaffold features were previously reported [15]. ADSC were seeded at a density of 500 × 10^3 cells/cm^2 onto the scaffolds, and kept in culture 24 h before the implantation. Scaffolds of 1 × 1 cm size were used for rat experiments, and 10 × 10 cm size for minipig experiments.

2.3. Adhesion and apoptosis assays

Biocompatibility of the CS with rADSC has been previously confirmed [15]. The optimal ADSC density was established by seeding 100, 250, and 500 [×10^3] cells/scaffold and counting with a NucleoCounter (Chemometec, Denmark), after 24 h. Adhesion and distribution of rat ADSC on the CS were determined by fluorescence microscopy after nuclear and vimentin staining with a monoclonal anti-vimentin primary antibody (dilution 1:100, Dako) and anti-mouse IgG conjugated with Alexa Fluor-594 (1:500). For nuclear staining, DAPI (Vector Laboratories) was added to the mounting media. To determine the cell patch thickness formed on scaffolds, the CS-ADSC were stained with TOPRO-3 (Molecular Probes) to label nuclei. Image acquisition was performed using the AIM 4.2 (Zeiss) program with the LSM 510 META (Zeiss) confocal microscope.

For cell apoptosis assays, rat ADSC were seeded onto the CS at a density of 100, 250, and 500 [×10^3] cells/scaffold, and cultured for 24 h. Apoptosis was quantified by TUNEL technique (In Situ Cell Death Detection Kit; Roche) and image acquisition performed using an AxioCam MR3 (Zeiss) camera connected to a Zeiss AxioImager M1 (Zeiss) microscope.

2.4. Ultrastructural analysis

For electron microscopy studies, CS-ADSC were fixed, post-fixed, dehydrated, and embedded in araldite (Durcupan, Fluka). Semi-thin sections were cut and stained lightly with 1% toluidine blue. Ultra-thin sections were cut with a diamond knife, stained with lead citrate (Reynolds solution), and examined under a FEI Tecnai G2 Spirit transmission electron microscopy.

2.5. Gene array

An array for extracellular matrix constitution and cell adhesion gene expression profile was performed (Quiagen). Rat-ADSC were seeded onto the CS or cell culture plates at a density of 100 × 10^3 cells/scaffold or plastic and cultured 24 h. Cell pellets were stored, RNA was isolated with the RNeasy Mini Kit (Quiagen), and cDNA was obtained with the RT^2 First Strand Kit (Quiagen). Real-time PCR was performed according to the RT^2 Profiler Array System user manual (Quiagen).

2.6. Rat and minipig in vivo models

All experiments were performed in accordance with 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" prepared by the Institute of Laboratory Animal Resources, Commission on Life Science, National Research Council, and published by the National Academy Press, revised 1996. All animal procedures were approved by the University of Navarra Institutional Committee on Care and Use of Laboratory Animals.

A total of 98 female Sprague–Dawley rats (Harlan Iberica) underwent permanent left coronary artery ligation as previously described [16]. Only those animals with a left ventricular ejection fraction (LVEF) below 45% at 1 month post-MI ($n = 58$) received the CS cellularized with rat ADSC (350 × 10^3 cells), the CS alone, ADSC alone or media. Four months after transplantation, functional analysis were performed by echocardiography and animals sacrificed for mechanical and histological analyses.

A total of 18 Göttingen minipigs were implanted with ADSC-seeded or non-seeded CS 2 months after induction of MI as previously described [7]. Animals were followed, and functional and histological analyses were performed 3 months after transplantation.

2.7. Echocardiographic studies

Rat cardiac functional analyses were performed by transthoracic two-dimensional echocardiography, M-mode recordings, and Doppler ultrasound measurements as previously described [16,17]. The left ventricular ejection fraction (LVEF) was determined according to Teichholz in parasternal short axes [18] and left ventricular remodeling was analyzed by measuring end-systolic and end-diastolic volumes and diameters according to the American Society of Echocardiography [19]. Echocardiographic studies were performed before treatment and 4 months post-transplantation.