Transplanted fetal cardiomyocytes as cardiac pacemaker

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Abstract

Background: While morphologic integration of transplanted fetal cardiomyocytes into the ventricular myocardium is a well-known fact, no studies have yet shown transplanted cells to coherently contribute to contraction and electrical excitation of the host myocardium. The aim of this study was to prove the hypothesis that by transplanting cardiomyocytes with a higher intrinsic rhythmic rate into the myocardium of the left ventricle, these cells could act as an ectopic pacemaker by functional coupling with host cardiomyocytes.

Methods and Results: Dissociated fetal canine atrial cardiomyocytes including sinus nodal cells were delivered into the free wall of the left ventricle of adult canine X-linked muscular dystrophy dogs (n = 2). These dogs fail to express Dystrophin in both cardiac and skeletal muscle. In the control group (n = 2) fetal skin fibroblasts were used for grafting. A total of 3–4 weeks after transplantation the dogs underwent catheter ablation of the atrioventricular node (AV-node) and subsequent electrophysiological mapping studies. Transplanted cells were identified by Dystrophin immunoreactivity, indicating survival and morphological integration in the recipient heart. The expression of Connexin 43 between donor and recipient cells suggested formation of gap junctions between injected and host cardiomyocytes. After catheter ablation of the AV-node, a ventricular escape rhythm emerged driving the pace of the heart and originating from the labeled transplantation site. This effect could not be observed in the control group (n = 2).

Conclusions: The results constitute the first observation of phenomena indicating electrical and mechanical coupling between allogeneic donor cardiomyocytes and recipient myocardium in-vivo. Further experiments are necessary to evaluate the technique as a potential therapy for atrioventricular block.

Keywords: Pacemakers; Pacing; Cell grafting; Micro-transplantation

1. Introduction

Adult hearts lack stem cells and cardiomyocytes loose their ability to divide in the neonatal period [1]. The heart muscle therefore has limited mechanisms for regeneration later in life. Chronic heart failure develops, when a critical number of cardiomyocytes are lost, whether from ischemic, immune mediated, or other injury. The primary problem is the critical loss of myocytes that triggers the disease. Therefore any intervention increasing the number of cardiomyocytes in patients could reverse the disease process. While morphologic integration of transplanted cardiomyocytes into the ventricular myocardium is a well-known fact [2–5], no studies have yet shown transplanted cells to coherently contribute to contraction and electrical excitation of the host myocardium in-vivo. The aim of this study was to prove the hypothesis that by transplanting cardiomyocytes with a higher intrinsic rhythmic rate into the myocardium of the left ventricle, these cells could act as an ectopic pacemaker.

2. Methods

All animal experiments were conducted upon the supervision of the animal ethics council of Hannover Medical School and the state government of Lower Saxony.

2.1. Cell grafting

Third trimester fetuses of mongrel dogs were delivered by Caesarian-section. Skin flaps and atria, including the sinus node, were dissected and dissociated, using 0.1% Collagenase A (Boehringer Mannheim Corp.). Cardiomyocytes and skin fibroblasts were isolated. Cardiomyocytes (2 x 10⁶) were injected into the free wall of the left ventricle of adult canine X-linked muscular dystrophy (CXMD) dogs...
(these dogs fail to express Dystrophin in both cardiac and skeletal muscle) via anterolateral thoracotomy (n = 2). Additionally, an epimyocardial pacemaker system with a minimum rate of 30 beats per minute (VVI-mode) was implanted to allow for survival after atrioventricular node (AV-node) ablation. In the control group (n = 2), the same procedure was performed using fetal skin fibroblasts of the same donor animals for grafting. For fluoroscopic detection, the injection site was labeled using a prolene suture with attached titanium clips. Immunosuppression was ensured by daily administration of cyclosporine (15 mg/kg) and corticosteroids (2.5 mg/kg).

2.2. Immunohistologic analysis

After sacrifice engrafted hearts were dissected and snap frozen in liquid nitrogen. For each specimen conventional Hematoxylin and Eosin staining was made and serial sections were used for immunohistochemical staining. Slides were fixed in acetone (−20°C; 10 min), blocked with normal rabbit serum (RT; 15 min) and then incubated with the primary monoclonal mouse antibodies. Anti-Dystrophin clone NCL-Dys 1 1:5 (Novocastra Lab., UK) and anti-Connexin 43 clone MAB 3068 1:40 were each incubated at 37°C for 1 h. After incubation of biotinylated secondary antibody, detection was performed by a Streptavidin-Alkaline-Phosphatase-complex. Neufuchsin served as substrate and Hemalum for counterstain. For each antibody appropriate positive and negative control stains were carried out.

2.3. Electrophysiological studies and radiofrequency catheter ablation

A total of 3–4 weeks after transplantation dogs were anesthetized and introducer sheaths were placed in the right femoral artery and vein. A 6F hexapolar catheter was placed at the His bundle region and a 7F steerable ablation catheter was positioned at the presumed compact AV-node based on anatomical and electrophysiological guidance. After 1–3 temperature-controlled radiofrequency applications, total AV-block was induced. Detailed activation – and pace-mapping of the left ventricle were performed using a steerable mapping catheter.

3. Results

The Dystrophin gene product was used to follow the fate of engrafted cardiomyocytes [3]. Immunohistologic analyzes using anti-Dystrophin antibodies demonstrated the presence of Dystrophin-positive donor cardiomyocytes within the Dystrophin-negative recipient myocardium at

Fig. 1. (A) HE staining (200x) of canine CXMD heart 3 weeks after injection of fetal atrial cardiomyocytes (FCM). FCM (arrow heads) lie in and around the injection channel. Focally dystrophic calcifications were found (arrow). RCM: Recipient cardiomyocytes. (B) Dystrophin staining (630x). Only FCM are dystrophin-positive (red). No expression in RCM. (C) Connexin 43 staining (630x). Strong linear staining pattern between RCM (internal method control) and FCM (arrow). (D) HE staining of canine CXMD heart, transplanted with fibroblasts (Control heart). Fibroblasts engrafted well around the injection channel.
3–4 weeks post engraftment. Visualization of the sections including the engrafted region revealed a large number of Dystrophin-positive cardiomyocytes around the injection channel. In contrast, no Dystrophin signal was detected in the recipient myocardium (Figs. 1A,B). Engrafted regions were also examined for the expression of Connexin 43. This molecule is the major constituent of cardiac gap junctions, which enable transmission of electric excitation and with that contraction from cell to cell [6]. Connexin 43 immunoreactivity was visible at junctional complexes between donor and recipient cells, suggesting morphological coupling as a result of gap junction formation (Fig. 1C). In the control group fibroblasts also engrafted well around the injection channel (Fig. 1D).

A total of 3–4 weeks after transplantation dogs were subjected to detailed electrophysiologic studies. All tests were performed under X-ray control. First, temperature-controlled radiofrequency catheter ablation of the AV-node was performed. Subsequently, an escape rhythm with narrow QRS complexes (non-ventricular in origin; mean rate: 35 ± 5 beats per minute) was observed. After AV-node ablation a faster than the former stable escape rhythm emerged within 5–30 min with broad QRS-complexes (ventricular in origin; right bundle-branch-block configuration, RBBB) in all dogs transplanted with fetal cardiomyocytes (mean rate 70 ± 25 beats per minute, Fig. 2). The control dogs (engrafted with fibroblasts) underwent the same procedure but did not develop a ventricular escape rhythm despite provocation tests with atropine, orciprenaline and rapid ventricular pacing even after 60 min. For survival, the control animals were totally dependent on the implanted artificial pacemaker with a programmed stimulation rhythm of 30 beats per minute during the experiment. To further evaluate the origin of the ventricular escape rhythm (only in the dogs engrafted with cardiomyocytes) detailed mapping was performed in the left ventricle using a 7F steerable mapping catheter. Using pace-mapping analysis with a match of 6/6 surface electrocardiograms (Figs. 3A,B), a QS-pattern at the unipolar electrogram and the earliest site of its origin being 10 ms before QRS onset of the bipolar electrogram were obtained at the endocardial site in very close vicinity to the labeled epicardial transplanta-

Fig. 2. Pacemaker ECG from engrafted cells after AV node ablation. Six surface ECGs and two intracardiac electrograms (ABL dist and ABL unpo) are given showing RBBB morphology of the escape rhythm and earliest site of activation at the mapping catheter (ABL) being at the labeled site of the endocardial free wall of the left ventricle where cells were injected. The ECG shows total AV block with an escape rhythm of 70 bpm.
The findings of our study demonstrate, that dogs transplanted with fetal atrial cardiomyocytes exhibit an escape rhythm originating from the site where the myocytes were injected into the left ventricular myocardium, indicating functional coupling between donor and recipient cells resulting in the ability of engrafted cells to generate and maintain a stable ventricular escape rhythm. The site of origin of the ventricular escape rhythm was clearly identified on the basis of activation mapping and pace mapping analysis. Myocyte transplantation is increasingly being discussed for a potential therapy of irreversible loss of heart muscle cells as a result of injury or disease [2–5]. Although a few studies suggest that this procedure may improve left ventricular function [5,7,8], it is not clear whether this improvement is a result of changed left ventricular geometry after grafting or based upon active coherent contraction of transplanted cells. Therefore it is imperative to prove the ability of transplanted cells to actively contribute to myocardial contraction. As the generation of an action potential in the cardiomyocyte simultaneously induces cell contraction [9], the observation of electrical coupling between donor and recipient cells would automatically prove mechanical coupling of engrafted cells in the host myocardium. Other groups have demonstrated the ability of skeletal myocytes and cardiomyocytes to electrically couple in-vitro [10,11], but no study has been able to demonstrate in-vivo coupling. In our opinion the only way...
to demonstrate electrical coupling in-vivo is to show, that transplanted cardiomyocytes dictate the pace of the heart and thus are functionally connected with the recipient myocardium. Therefore atrial cardiomyocytes including sinus nodal cells, which have a higher intrinsic rhythmic rate than ventricular myocytes and thus may function as pacemaker in the ventricle, were chosen for grafting. After transplantation, to be regarded as ‘pacemaker’, the cells needed to fulfill three electrophysiologic criteria: (1) activation mapping must show the epicardially labeled site (for fluoroscopy) to be the source of the escape rhythm; (2) pacing at the injection site must generate QRS-complexes with the morphology of QRS-complexes of the escape rhythm (without pacing); and (3) a QS pattern at the unipolar electrogram, indicating the onset of the cardiac electrical activity which moves away from the labeled site. In this study all criteria were met by the engrafted cells (Fig. 3). The histological findings constitute a morphological correlate to the electrophysiologic result, showing survival of grafted cells and suggesting formation of gap junctions between donor and recipient cells. Although these experiments so far have been performed in a small number of animals, the results were very explicit.

Transplanting cardiomyocytes as cardiac pacemaker also may open a new perspective for the treatment of cardiac arrhythmia such as atrioventricular block (AV-block), ranging from infants and premature babies with congenital AV-block (incidence: one in 20,000 live births; Refs. [12,13]) who might be small for the treatment with artificial pacemakers, to patients with acquired AV-block. If cardiomyocyte engraftment proves to be of therapeutic value, this study could initiate further research aiming at generation of autologous cardiomyocytes or cardiomyocyte-like cells, preferably from pluripotent embryonic or adult stem cells or by achieving controlled proliferation of adult cardiomyocytes. Additionally, the potential ability of engrafted cardiomyocytes to act as pacemaker and inotropic support or to cause arrhythmia must be tested in long-term studies including mapping experiments at various time points and with a larger cohort of animals using reporter genes as cell marker, as the number of available CXMD dogs is strongly limited.

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References