International Journal of Cardiology 168 (2013) 3747–3751

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Autologous biological pacing function with adrenergic-responsiveness in porcine of complete heart block

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ARTICLE INFO

Article history:
Received 9 September 2012
Received in revised form 12 April 2013
Accepted 15 June 2013
Available online 5 July 2013

Keywords:
Mesenchymal stem cells
HCN4
Autograft
Complete heart block

ABSTRACT

Aims: To assess the efficacy of autologous biological pacing function by autograft of gene-transferred mesenchymal stem cells in a porcine model of complete heart block.
Methods and results: Fourteen healthy young male pigs were randomized into active group (n = 8) and control group (n = 6). Porcine MSCs were transfected with Ad.HCN4 or Ad.Null. The pacemaker function of transfected MSCs was studied by whole-cell patch clamp. The CHB model of porcine was created with transthoracic ablation technique and the transfected MSCs were autografted into the free wall of right ventricle. The pacing function was studied by ECG and ambulatory Holter recording weekly. The adrenergic responsiveness was evaluated by the variation of heart rate after isoprenaline infusion or food provision following an overnight fasting. HCN4-MSCs expressed a robust time-dependent inward current (If) and the current density of If was 4.3 ± 0.6 pA/pF at −105 mV. In week 2 after autograft, the heart rate of active group became significantly higher than control (53 ± 5 bpm vs. 38 ± 4 bpm, $P < 0.05$) and the percent of pacing beats in active group was higher than control (69 ± 10% vs. 28 ± 8%, $P < 0.05$). By infusion of isoprenaline, the heart rate was increased significantly in both groups. However, there was a significant increase of heart rate when presenting food for active group ($P < 0.05$) while not in control.
Conclusions: Our findings demonstrated that autografted HCN4-MSCs could increase the heart rate by providing an adrenergic-responsive biological pacing function, indicating a promising approach without immunological or ethical issues for the treatment of complete heart block.

1. Introduction

Complete heart block (CHB) is one of severe diseases of cardiac conduction system. Implantation of electronic pacemaker is still the mainstay therapy for this disease [1], despite the shortcomings such as imbedding of foreign matter, limited life of battery and lack of autonomic responsiveness [2,3]. In our previous studies, we have demonstrated the feasibility of treating CHB by implantation of autologous sinus nodal cells [4,5]. Considering the restriction of the limited number of sinus nodal cells, we explored the biological pacing function of autograft of gene-transferred MSCs for the treatment of CHB in the present study.

2. Methods

The study conforms to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health. Approval for protocols was granted by the Animal Care and Use Committee of Changhai Hospital.

2.1. MSC preparation and transfection

Primary porcine MSC cultures were established following bone marrow aspiration from healthy young male pigs (20–30 kg) in the active group (n = 8) and the control group (n = 6). The MSCs were harvested with gradient isolation techniques [6] and cultured in Dulbecco's Modified Eagle Media (D-MEM) with 20% fetal bovine serum (Gibco; USA), 50 U/ml penicillin, 50 μg/ml streptomycin sulfate, and 2 mM L-glutamine (Invitrogen; USA). The adenoviral construct of human HCN4 (GenBank accession no. NC_000015) was prepared following previously published methods [7]. Briefly, the HCN4 cDNA was derived from the plasmid pcDNA1.1/HCN4 and subcloned to the multiple cloning sites (Sal I/Hind III) of the shuttle plasmid, pShuttleCMV, thereafter co-transformed into *Escherichia coli* BJ5183 cells with adenoviral backbone plasmid pAdEasy-1 for homologous recombination. Recombinant plasmid was subsequently transferred to HEK293 cells. The adenoviral construct Ad.HCN4 was finally harvested and purified. For transfection, the MSCs in the fourth passage were exposed to Ad.HCN4 for the active group or Ad.Null for the control group for 24 h, then rinsed in PBS and returned to basal media.

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http://dx.doi.org/10.1016/j.ijcard.2013.06.012

2.2. Patch clamp study

At the 72nd hour following transfection, samples of transfected MSCs were placed in a heated (35 °C) bath and superfused with a solution of following composition (in mM): NaCl 140, KCl 5.4, $CaCl_2$ 2, $MgCl_2$ 1, and HEPES 5, glucose 10, pH 7.4. Only single cell was chosen for measurement. Membrane potential and current were recorded by whole cell patch techniques using an Axopatch-1D amplifier (Axon instruments) and a computer equipped with pCLAMP software (versions 8; Axon Instruments). Borosilicate glass pipettes were filled with a solution of following composition (in mM): aspartic acid 90, NaCl 10, CsOH 100, CsCl 30, $MgCl_2$ 2, EGTA 5, $CaCl_2$ 2, and HEPES 10, pH 7.2. Pipette resistance was 3–5 MV. The membrane capacity was measured by applying a voltage-clamp step.

2.3. CHB model creation and cell transplantation

Animals were anesthetized with an injection of ketamine (8 mg/kg, IM) followed by propofol (1 mg/kg, IV), then intratracheally intubated and ventilated. Anesthesia was maintained with 1.0% isoflurane. Thereafter, the heart was exposed through an anterior right-sided thoracotomy at the fourth intercostal space. An electronic pacing wire was sutured to the epicardium of right ventricle and connected to a portable electronic pacemaker set at VVI 35 bpm as a "safe net" for the heart rate. Via a small incision within a preset purse-string suture in right atrium, a radiofrequency ablation catheter assembled within a 7-F sheath (Fig. 1A) was stabbed into the right atrium and manipulated to locate the His-bundle by detecting the intervening His-bundle potential. Once the potential presented stably, radiofrequency was discharged persistently until atrial–ventricular dissociation occurred and a rhythm of CHB was stabilized (Fig. 1B). Subsequently, the region for injection was marked by two sutures in the free wall of right ventricle and paced for seconds via an epicardial electrode (Fig. 1C and D) for identifying the origin of idioventricular rhythm during follow-up. Afterwards, a purse-string suture was made in the marked region and the transfected MSCs suspension ($800–1000 \times 10^3$transfected MSCs in 0.5 ml PBS solution) was injected subepicardially within the purse suture (Fig. 1E). Once the injection finished, the needle was withdrawn and the purse suture was tied to avoid reflux of the cell suspension. Complete hemostasis was achieved and the chest wall was closed in layers. All animals were treated carefully for recover during follow-up period.

2.4. Intact animal study of heart rhythm and adrenergic responsiveness

One week after autograft, the cardiac temporary pacemaker was removed. ECG, 24-hour Holter recording and pacemaker log record were performed for both groups weekly over 4 weeks after autograft. Biological pacing beats were defined as the beats with similar QRS configurations with the pace-mapped beats originating from the injection site. According to the analysis of Holter records, the average heart rate and the percent of biological pacing beats were calculated. The adrenergic responsiveness of the biological pacing rhythm was evaluated by the variability of heart rate to adrenergic agonist and sympathetic stimulation. In detail, the heart rate was monitored by ECG when isoprenaline was administered with step-wise doses (ranged from 0.01 to 0.1 μg/kg/min) for 10 min in week 3. After an overnight fasting, animals were fed with food and water and the heart rate was also recorded.

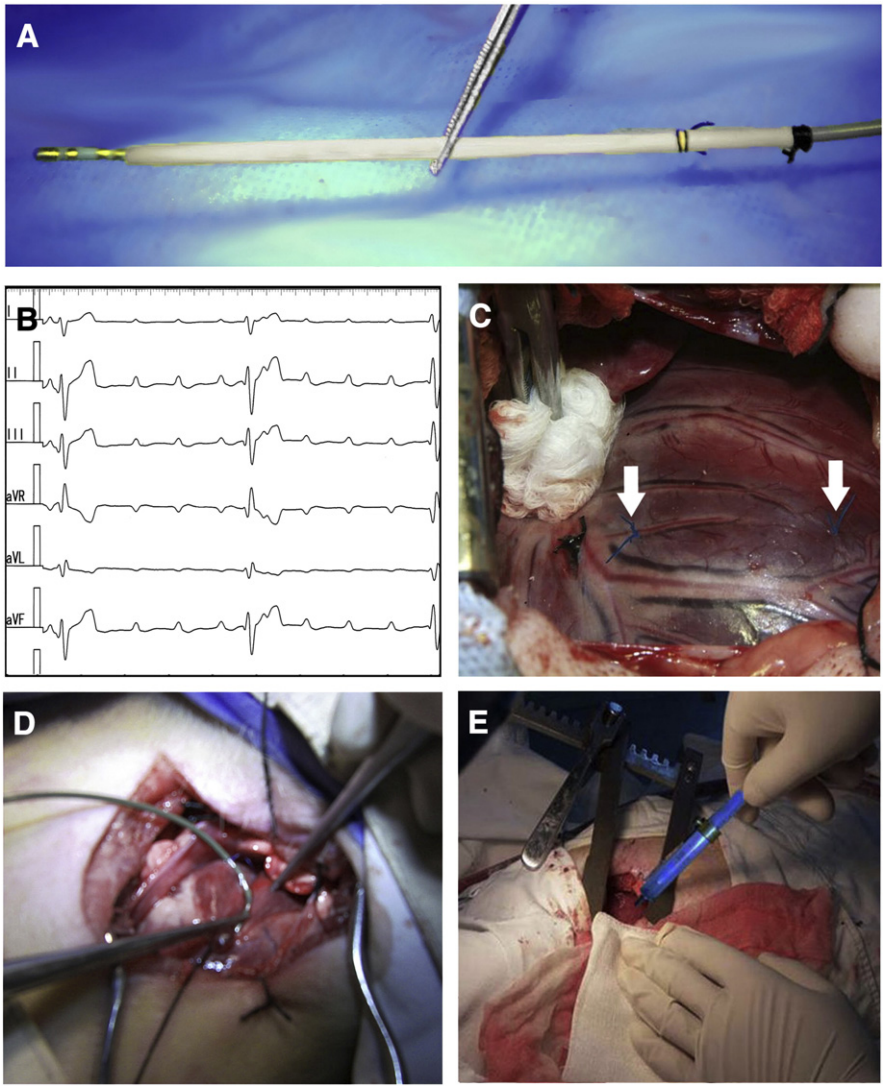

Fig. 1. (A) The radiofrequency ablation catheter deployed in the present study. (B) Representative ECG of complete heart block after the ablation of His-bundle. (C) The injection site was located at the midpoint of two prolene sutures (white arrows). (D) The injection site was electronically pace-mapped for identification of QRS configuration. (E) The MSCs were then injected into the site subepicardially.