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Mesenchymal stem cell injection ameliorates the inducibility of ventricular arrhythmias after myocardial infarction in rats

Deguo Wang, Fengxiang Zhang, Wenzhi Shen, Minglong Chen, Bing Yang, Yuzhen Zhang, Kejiang Cao *

Department of Cardiology, The First Affiliated Hospital of Nanjing Medical University, PR China

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ABSTRACT

Background: Mesenchymal stem cell transplantation is a promising new therapy to improve cardiac function after myocardial infarction (MI). The electrophysiological consequences of MSC implantation has not been systematically studied.

Methods: We investigated the electrophysiological and arrhythmogenic effects of mesenchymal stem cells (MSCs) therapy in experimental infarction model. Rats were subjected to MI operation by LAD ligation and randomly allocated to receive intramyocardially injection PBS (MI-PBS) or 5×10^5 EGFP labeled MSCs (MI-MSCs). Electrophysiological study, histological examination, and western blotting were performed 2 weeks after cell transplantation.

Results: Programmed electrical stimulation (PES) showed a significant reduced inducible ventricular tachycardias (VTs), raised ventricular fibrillation threshold (VFT) and prolonged ventricular effective refractory period (VERP) in MSC-treated rats compared to PBS-treated animals. MSC implantation led to markedly longer action potential duration (APD) and shorter activation time (AT) in infarcted border zone (IBZ) of left ventricular epicardium compared with PBS-treated hearts. Histological study revealed that fibrotic area and collagen deposition in infarcted region were significantly lower in MI-MSC group than in MI-PBS group. Abnormal alterations of Connexin 43 including reduction and lateralization were significantly attenuated by MSC treatment.

Conclusions: This study provide strong evidence that MSC implantation ameliorates interstitial fibrosis and the remodeling of gap junction, attenuates focal heterogeneity of reporlarization and conduction and reduces vulnerability to VTs. The results suggest that MSC transplantation might emerge as a new preventive strategy against VAs besides improving cardiac performance in ischemic heart disease.

1. Introduction

Ventricular remodeling after myocardial infarction (MI) not only causes failure of pump function, but also promotes generating a proarrhythmogenic substrate [1]. Therefore, new approaches to heart failure therapy should aim at reducing vulnerability to arrhythmia as well as improving hemodynamic function. Cell transplantation is a promising new therapy to improve cardiac function after MI. However, concern that cell replacement therapy may be accompanied by an increased arrhythmic risk has been repeatedly raised [2,3].

Mesenchymal stem cells (MSCs) have several advantages available for real-time transplantation soon after a patient presents with MI. These advantages include rapidly expansion in culture, lack of allogeneic immunorejection [4] and safety to be cryopreserved without lost their protective effects [5].

The electrophysiological consequences of MSC implantation has not been systematically studied. Earlier experimental studies suspected that MSC therapy is proarrhythmic. Through a MSC/neonatal coculture model, Chang et al. [6] deduced that adequate survived MSCs in heart tissue would result in reentry arrhythmias. Pak et al. [7] founded that MSC injection induces cardiac heterogeneously distributed sympathetic nerve sprouting in a swine model of MI, which may trigger ventricular arrhythmia. Price et al. [8]observed a shortened epicardial effective refractory periods (ERPs) after intravenous MSC transplantation. In contrast, Mills et al. [9] recently demonstrated that MSC therapy tended to reduce arrhythmia inducibility which linked with enhanced electrical viability and impulse propagation in the border zone. More recently, a randomized, double-blind, placebo-controlled clinic trials revealed that intravenous adult human MSCs after acute MI lead to four-fold reduction of arrhythmias [10]. Therefore, whether cell therapy cause proarrhythmic risk is still uncertain.

It is well known that the most important mechanism of arrhythmogenesis associated with MI is electrophysiological heterogeneity due to coexistence of infarcted, border zone, and viable myocardial tissue [1]. The proarrhythmogenic substrate may

* Corresponding author. Department of Cardiology, The First Affiliated Hospital of Nanjing Medical University, Guangzhou Road 300#, Nanjing, 210036, PR China. Tel./fax: +86 25 83717168.
E-mail address: kJcao@njmu.edu.cn (K. Cao).

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involved in altered ionic currents, action potentials, cardiac fibrosis and cell-to-cell coupling. In this study, we attempt to investigate electrophysiological alterations after MSC transplantation in a rat model with MI and focus on the vulnerability to VTs, focal heterogeneity of repolarization and conduction, and possible underlying mechanisms.

2. Materials and methods

2.1. Animal care

Sprague–Dawley rats were obtained from the experimental animal center of Nanjing Medical University. All rats were housed in stainless steel cages with sawdust bedding. They were kept at 23 ± 1 °C, humidity 55 ± 5%, under a 12 h dark/light cycle and were allowed unlimited food and water. All experimental procedures were in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health, and were approved by the Experimental Animal Ethics Committee of Nanjing Medical University, China.

2.2. Cell isolation, culture and label

MSCs were obtained from the bone marrow of 4-week old male Sprague–Dawley rats and expanded according to a previously reported protocol [11]. The MSCs were cultured in low-glucose Dulbecco's Modified Eagle Medium (DMEM, Invitrogen) containing 10% fetal bovine serum (Gibco), penicillin (100 U/ml) and streptomycin (100 μg/ml). MSCs were transfected by purified commercial adenovirus (Genechem. Co. Shanghai) expressing enhanced green fluorescent protein (EGFP) reporter gene [9] and used in cell transplantation.

2.3. Myocardial infarction model and cell implantation

A total of 55 healthy, 8-week old SD female rats were randomized into the following groups: MI-PBS (20), MI-MSCs (20) and control (15). The left anterior descending artery (LAD) was ligated to induce MI [12]. The animals were quickly anesthetized using an intraperitoneal (i.p.) injection of pentobarbital (50 mg/kg body weight; Roche). A left thoracotomy was performed under artificial ventilation with air. The LAD was ligated with a silk suture, approximately midway between the left atrium and the apex of the heart. The control animals contained a loose ligature and no further injection was performed. MI model animals received MSCs or PBS injections intramyocardially. A suspension of 5×10^5 MSCs in PBS, or an equivalent volume (50 μl) of PBS alone, was injected into five different sites in the anterior wall of the left ventricle. Two weeks after transplantation, all animals were subjected to Programmed Electrical Stimulation (PES) and then sacrificed for histological examination.

2.4. Electrophysiological study in vivo

Electrocardiogram (ECG) and Monophasic Action Potentials (MAP): Two weeks after coronary ligation, the chest of all rats was reopened for electrophysiological study of the in situ heart. For ventricular pacing, the paired hook electrodes were positioned 2-mm apart at the non-infarcted epicardial base of the left ventricular anterior wall. ECG was recorded from 3 needle electrodes at both upper limbs and the left leg. The ECG signals were filtered out below 10 Hz and above 100 Hz. MAPs were recorded from three different location of left ventricular epicardium at normal sinus rhythm [13]. These locations included non-infarcted zone (above ligation), border zone of the infarct (between ligation and infarcted scar) and infarcted zone (infarcted scar) (Fig. 1a). Each of the electrodes was connected to a computer through an analog–digital converter for monitoring and later analysis of surface recordings. MAP signals were amplified using dedicated MAP amplifiers. A commercially available biosignal analysis software (RM6240, Chengdu, China) was used to digitalize, store and analyze MAP signals. The software was applied to analyze action potential durations (APD) at 90% of repolarization (Fig. 1b). Ventricular activation time (AT) was defined as the interval between stimulus peak and fastest MAP upstroke at rapid ventricular stimulation and was measured manually [14] (Fig. 1c).

Programmed Electrical Stimulation (PES): After a train of 8 stimuli at 120-ms drive cycle length, single, double and triple extra stimuli were applied using a standard PES protocol. The coupling interval of the last extra stimulus was decreased in 2 ms steps beginning at 80 ms and finishing at the ventricular effective refractory period (VERP). VT was defined as equal to or more than 3 consecutive premature ventricular beats. Sustained VT was defined as a fast ventricular rhythm extending over 15 or more beats, according to Lambeth conventions [15].

Ventricular fibrillation threshold (VFT): Briefly, a graded, 100-Hz stimulation was delivered to the heart via the punctate electrode located in the peak of right ventricle, initially with a voltage level of 1 V (peak-to-peak) for 1 s. If the termination of current was not followed by VF, the voltage level was increased by 0.5 V for the next delivery after 5 s interval. The VF state was easily recognized by the irregular rapid electrical potentials following the end of ventricular stimulation [16].

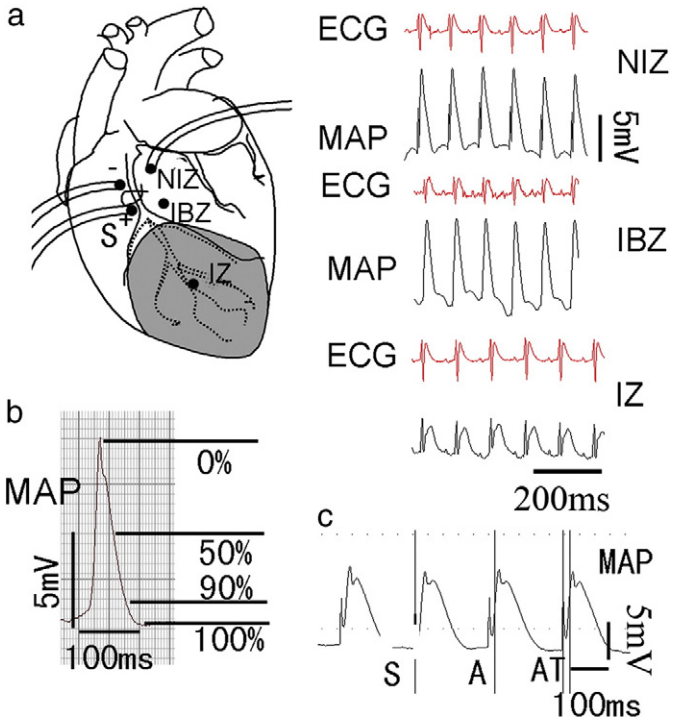

Fig. 1. Schematic representation of the recording of the epicardial monophasic action potential (MAP) from non-infarcted zone (NIZ), infarct border zone (IBZ) and infarcted zone (IZ) of the left ventricular anterior wall. (a) Schematic drawing of the heart representing the anatomical positions of the electrodes of pacing for programmed stimulation and recording for MAP. Representative MAP recorded from NIZ, IBZ and IZ and body surface lead II electrocardiogram simultaneously recording at normal sinus rhythm. 'MAP' recorded from the infarcted area shows ECG-like morphology rather than typical MAP.(b) APD_{90} was action potential durations (APD) at 90% of repolarization. (c) Activation time was the interval between stimulus peak and fastest MAP upstroke at a cycle length 150 ms ventricular stimulation.

2.5. Western blotting

The protein concentrations of the samples were determined by the BCA method. Protein (20 μg) was resolved on 14% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a PVDF membrane. Primary antibody (Cx43, 1:1000 dilutions) was added after blocking the membrane and incubated and cultured overnight at 4 °C. The housekeeping protein GAPDH was used as a loading control. Antibody binding was detected by horseradish peroxidase (HRP) conjugated secondary antibody (1:2000; Sigma) and visualized by an ECL kit. Cx43 band intensity was quantified by densitometry and normalized to GAPDH (Chemicon).

2.6. Immunofluorescence analysis

Fixed tissue sections in acetone were used for Immunofluorescence. Non-specific antibody binding sites were blocked by incubating with 5% FBS for 60 min. Tissue sections were labeled for 16 h at 4 °C with antibodies (Collagen I, 1:300 dilutions, Abcam; Cx43, 1:200 dilutions, Abcam; cTnI, 1: 200, Santa Cruz). Anti-rabbit and anti-mouse IgG conjugated with FITC and TRITC were used for detection. Images were recorded by confocal microscopy (LSM510, Zeiss). For Cx43, all images were recorded using the same settings and the pixel intensities were quantified using ImageJ software.

2.7. Measurement of fibrosis

Fixed hearts were embedded in paraffin and LV cross sections from apex, mid-LV, and base were stained with Trichrome-Masson. Image of LV area of each slide was taken by an microscope equipped with CCD camera. Additionally, heart sections were stained with Masson-Trichrome stain to distinguish areas of connective tissue. The extent of fibrosis in the infarct region of each heart was measured as previously described [17]. The percentage of blue staining, indicative of fibrosis, was measured (10 fields randomly selected from the infarct area on each section) from the infarct area on two sections from each heart and averaged. The value was expressed as the ratio of Trichrome-stained fibrosis area to total infarct area.

2.8. Statistical analysis

Data are presented as mean ± SD and frequencies. Continuous variables were compared using Student's unpaired t-test or one-way ANOVA as appropriate. If the F