Journal of Molecular and Cellular Cardiology 47 (2009) 41–48

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

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

Original article

Bone marrow mesenchymal stem cells upregulate transient outward potassium currents in postnatal rat ventricular myocytes

Cai Benzhi [1], Zhao Limei [1], Wang Ning, Liu Jiaqi, Zhu Songling, Meng Fanyu, Zhou Hongyu, Lu Yanjie, Ai Jing, Yang Baofeng *

Department of Pharmacology and the State-Province Key Laboratory of Biomedicine and Pharmaceutics, Harbin Medical University, Baojian Road 157, Nangang District, Harbin, Heilongjiang Province 150081, PR China

ARTICLE INFO

Article history:
Received 19 June 2008
Received in revised form 15 February 2009
Accepted 5 March 2009
Available online 12 March 2009

Keywords:
Bone marrow mesenchymal stem cells
Neonatal rat ventricular myocytes
Transient outward potassium current
Basic fibroblast growth factor
Insulin growth factor-1

ABSTRACT

Bone marrow mesenchymal stem cell (BMSC) transplantation has been shown to effectively improve cardiac function in experimental animals and patients with myocardial infarction and heart hypertrophy. BMSCs exert potent effects on cardiomyocytes through the inhibition of cardiac apoptosis, the attenuation of cardiac inflammation, etc. However, novel biological actions of BMSCs on cardiomyocytes remain to be explored. The present study was designed to investigate whether BMSCs affect electrophysiological features of neonatal rat ventricular myocytes (NRVMs). BMSCs and NRVMs were indirectly co-cultured at a ratio of 1:10 with a semi-permeable membrane. We found that compared with mono-cultured NRVMs, co-cultured NRVMs exhibited an obvious increase of transient outward potassium current (I_{to}), accompanied by significant changes in activation, inactivation and recovery of I_{to}. Meanwhile, $K_V4.2$ mRNA which encodes the channel carrying I_{to} was more abundant in co-cultured NRVMs than mono-cultured NRVMs. The increases in basic fibroblast growth factor (bFGF) and insulin growth factor-1 (IGF-1) levels were observed in culture medium of BMSCs. bFGF but not IGF-1 upregulated the $K_V4.2$ mRNA expression and enhanced I_{to} currents. Taken together, we conclude that BMSCs upregulate I_{to} of NRVMs, at least partially, by secreting bFGF that in turn upregulates $K_V4.2$ expression and alters the kinetics of I_{to}.

1. Introduction

A large body of evidence has indicated that the transplantation of bone marrow mesenchymal stem cells (BMSCs) play a strongly therapeutic role in many heart diseases, such as myocardial infarction and cardiac hypertrophy [1–3]. Some progress has been made on the understanding of therapeutic mechanisms of BMSC transplantation in these heart diseases. For example, BMSCs could differentiate into vascular endothelial cells and cardiomyocytes, and contribute to regeneration of myocardium and angiogenesis in ischemic hearts [2,4–6]. Additionally, large amounts of protective cytokines secreted by BMSCs, functioned as the limitation of inflammation, the inhibition of apoptosis, the stimulating of myoangiogenic differentiation, and so on [5–8]. However, exploration of novel biological roles of BMSCs in regulation of cardiac myocyte function will expand our understanding of biological function of BMSCs.

Numerous studies showed that aberrant electrical remodeling was involved in many heart diseases, such as cardiac arrhythmias, myocardial ischemia, heart failure, etc. [9–15]. Ventricular myocytes from canine ischemic cardiomyopathy exhibit prolonged APD and more pronounced APD dispersion, and I_{to} was simultaneously reduced along with an acceleration of the slow phase of recovery from inactivation [9]. The I_{to} reduction and APD prolongation were also observed in cardiac myocytes isolated from hypertrophic and failing hearts, which may contribute to the occurrence of abnormal repolarization and sudden death in patients with heart failure [10]. The hearts of transgenic mice expressing a dominant-negative amino-terminal fragment of $K_V4.2$ not only presented the primary reduction of I_{to} and the prolonged APD, but also easily suffered from congestive heart failure, along with hypertrophy, chamber dilatation, and interstitial fibrosis [11]. The reduction of I_{to} in heart diseases may provide the substrate for arrhythmogenesis and aggravates the electrical and structural remodeling [10–12]. Thus, it is suggested that restoration of I_{to} in cardiomyocytes may prevent or ameliorate the electrical and structural remodeling in heart diseases. Though BMSC transplantation has been shown to effectively improve cardiac functions in these heart diseases, whether BMSCs affect electrophysiological property in cardiomyopathy is completely unknown. Therefore, we hypothesized that BMSCs regulate I_{to} in cardiac myocytes. The present study was designed to investigate the effects of BMSCs on the electrophysiological properties of neonatal rat cardiomyocytes.

* Corresponding author. Tel.: +86 451 86671354; fax: +86 451 86675769.
E-mail addresses: meilizhao@163.com (Z. Limei), yangbf@ems.hrbmu.edu.cn (Y. Baofeng).
[1] Contributed equally to this work.

0022-2828/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.yjmcc.2009.03.002

2. Materials and methods

2.1. Animal care

Sprague–Dawley rats were purchased from Shenyang Shuangyi Laboratorial Animal Institute, China. All rats were housed in stainless steel cages with sawdust bedding. They were kept at a room with 23 ± 1 °C, humidity of $55 \pm 5\%$, 12 h dark/light cycle and allowed food and water unlimited. All experimental procedures were in agreement with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health, and has been approved by the Experimental Animal Ethic Committee of Harbin Medical University, China.

2.2. Isolation and culture of BMSCs

Bone marrow mesenchymal stem cells (BMSCs) were isolated just as described by Markino et al. [16] with some modification. In brief, femurs and tibias from SD rats (male, weighing 100 ± 20 g) were removed. Muscle and extraosteal tissues were trimmed. Bone marrow cells were flushed into a beaker and then transferred into culture flasks with Basal Medium for Mesenchymal Stem Cells (Stem Cell Technologies Inc.) supplemented with 20% Mesenchymal Stem Cell Stimulatory Supplements (Stem Cell Technologies Inc.), and penicillin (100 U/ml)/streptomycin (100 U/ml) at 37 °C in humid air with 5% CO_2. After being incubated for 48 h, BMSCs adhered to the bottom of culture plates, and hematopoietic cells remained suspended in the medium. Fresh medium was changed every 3 days. The subconfluent cells in the cultures were removed from the flasks by 0.25% Trypsin (Sigma) treatment after the initial plating of 8–12 days. They were labeled as passage 1 and continued to culture until passage 3. All experiments were performed using cells of the 3rd passage.

2.3. Isolation and culture of NRVMs

Neonatal rat ventricular myocytes (NRVMs) were isolated from the hearts of neonatal SD rats (1–3 days old). Briefly, neonatal rats were killed and their hearts were rapidly removed. Both ventricles were cut into l to 2 mm^3 and dissociated in 0.25% trypsin at 37 °C for 10 min. Cell suspensions were discarded. Residual tissues were trypsinized 4–6 times and cell suspensions were collected. Then all suspensions were pelleted by centrifugation at 1500 rpm for 145 s. The isolated cells were then resuspended in DMEM (Hyclone Laboratories) supplemented with 10% fetal bovine serum (Gibco) and penicillin (100 U/ml)/streptomycin (100 U/ml), transferred into culture flask and cultured at 37 °C in humid air with 5% CO_2. After 90 min for fibroblast adherence, cell suspension were plated into 6-well plate at 3×10^5 cells per well. After the 48-hour culture, NRVMs were co-cultured with BMSCs.

2.4. The establishment of NRVMs co-culture with BMSCs

BMSCs and NRVMs were indirectly co-cultured at the ratio of 1:10 with the DMEM medium supplemented with 10% fetal bovine serum in two chambers separated by a semi-permeable membrane of Transwell (pore size 3 μm) (Corning Company) which allows the diffusion of secreted factors but prevents cell transport between the two cell populations. NRVMs were cultured in the lower chamber and BMSCs in the upper. NRVMs cultured alone in the same DMEM medium were taken as control group. Fresh medium was changed every 3 days.

2.5. The whole-cell patch-clamp recording

Patch-clamp techniques have been previously described in detail [17]. The outward potassium currents were recorded in the whole-cell voltage-clamp mode with an Axopatch-200B amplifier (Axon Instrument). Borosilicate glass electrodes had tip resistances of 2–4 MΩ when filled with the internal pipette solution. The pipette solution for K^+ currents and AP recordings contained (mM): KCl 20, potassium aspartate 110, $MgCl_2$ 1.0, HEPES 5.0, EGTA 10, Na_2-ATP 5.0 (pH adjusted to 7.2 with KOH). The extracellular (normal Tyrode's) solution for K^+ and AP recording contained (mM): NaCl 136, KCl 5.4, $MgCl_2$ 1.0, $CaCl_2$ 1.8, NaH_2PO_4 0.33, glucose 10, and HEPES 10 (pH adjusted to 7.4 with NaOH). Experiments were conducted at room temperature. Junction potentials were zeroed before formation of the membrane–pipette seal. Series resistance and capacitance were compensated and leak currents were subtracted. The voltage protocols for current recordings are shown in the insets of the respective figures. The currents were all recorded immediately after membrane rupture and series resistance compensation in order to minimize the possible time-dependent rundown, run-up, or negative shift of currents. The amplitude of I_{to} was measured as the difference between the initial peak of I_{to} and the current level remaining at the end of the pulse.

2.6. The detection of $K_V4.2$ and $K_V4.3$ mRNA by reverse-transcription PCR

The first-strand cDNA was obtained by reverse transcriptase kit (Invitrogen, USA) according to the manufacturer's instructions. The $K_V4.2$, $K_V4.3$ and β-actin were amplified using specific primers obtained from Integrated DNA Technologies (Takara, China) as follow: $K_V4.2$ F: 5-ATT TCT TTG ACC GTG ACC CA-3; R: 5-ATG AAG AAC CCG GTC ACA TAC-3; $K_V4.3$ F: 5-TGT GGC CAA AAC AGG GAG CT-3; R: 5-CAG GTT GGA GTT GGG CAG GT-3; β-actin F: 5-ACT ATC GGC AAT GAG CG-3′; R: 5-GAG CCA GGG CAG TAA TCT-3. The length of the amplified products for $K_V4.2$, $K_V4.3$ and β-actin was 336 bp, 394 bp and 220 bp respectively. The reverse-transcription PCR reaction was carried out in a thermal cycler for 35 cycles, programmed to 94 °C 30 s, 56 °C 60 s, 72 °C 60 s, and followed by an additional 10 min at 72 °C to complete cDNA synthesis. PCR products were resolved on 1.5% agarose gels.

2.7. ELISA assay for bFGF and IGF-1

The 3rd passage BMSCs and NRVMs were separately plated on 60 mm dishes. After BMSCs and NRVMs were separately cultured in serum-free culture medium for 48 h, their culture medium were collected and centrifuged at 10,000 *g* at 4 °C for 5 min. Supernatants were stored at −20 °C. The bFGF and IGF-1 level were measured by the ELISA kits (Purchased from, respectively, R & D systems and Wuhan Boster Co. Ltd) according to the manufacturer's instructions. The antibody specific for bFGF and IGF-1 was used, and ELISA values were calibrated by total cell protein.

2.8. Data analysis

Group data are expressed as mean ± S.E.M. Statistical comparisons among groups were performed by ANOVA and *t*-test. A two-tailed $p<0.05$ was taken to indicate a statistically significant difference. Nonlinear least square curve fitting was performed with Clampfit in GraphPad Prism 4.0.

3. Result

3.1. BMSCs increased I_{to} of NRVMs

Fig. 1A displayed the representative traces of I_{to} elicited by the 150-ms depolarizing pulses between −40 and +50 mV from a holding potential of −70 mV, in NRVMs after 9 days culturing. I_{to} was significantly greater in co-cultured NRVMs than mono-cultured NRVMs. The current–voltage (*I–V*) relationship of I_{to} in mono-cultured NRVMs and co-cultured NRVMs on days 3, 6, and 9 is illustrated