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Therapeutic value of bone marrow mesenchymal stem cell transplantation incorporated with milrinone on restoring cardiac function

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Abstract

To explore the potential effect of bone marrow mesenchymal stem cell (BMSCs) transplantation combined with milrinone on the myocardial cell survival and cardiac function in heart failure. After isolation and culture of rat BMSCs and establishment of rat model of heart failure through intraperitoneal injection of adriamycin, the animals were administered with BMSCs through tail vein injection and/or with milrinone through the jugular vein injection. After that, echocardiography and Doppler images were used to evaluate cardiac function, and ELISA determined the milrinone concentration before and after intervention. The expression of myocardial specific proteins GATA-4, Cx43 and cTnI was determined. Compared with other treatments, administration BMSCs combined with milrinone more significantly improved cardiac function ($p < 0.05$). The BMSCs treatment had similar effect on the heart as milrinone ($p > 0.05$). The combined treatment obtained significantly increased expression of BNP levels and the highest expression levels of GATA-4, Cx43 and cTnI, compared to the BMSCs group. Combined treatment with BMSCs and milrinone effectively increase the expressions of GATA-4, Cx43, cTnI, and enhances healing of damaged myocardial cells, reducing lung resistance and plasma volume, and heart afterload. This treatment restores systolic and diastolic functions of the heart.

Keywords: BMSCs; milrinone; heart function; BNP; myocardial specific protein.

Practical Application: Our study shows that combined treatment with BMSCs and milrinone effectively enhances healing of damaged myocardial cells and restores systolic and diastolic functions of the heart. However, whether it exerts the same role in patients with cardiovascular disease remains to be further investigated.

1 Introduction

Despite the significant advances in treatments (Fornasini et al., 2010), heart diseases remain a major threat to human's life, such as acute myocardial infarction (AMI) (Roger et al., 2011; Thygesen et al., 2007). In AMI, almost half of all cases appear ventricular dysfunction (Esposito et al., 2010), usually complicated with acute heart failure (AHF). AHF imposes a burden to the medical care industry and society due to its high morbidity, high mortality, high readmission rate (Chen et al., 2013; Hunt et al., 2009; Najafi et al., 2008). Therefore, even after overcoming the challenge by AMI, AHF is still a difficult task. Reducing cardiac contractility effectively functions in systolic AHF, leaving positive muscle strength an important part of treatment (Partovian et al., 2012). Milrinone, could be used to alleviate of adenosine monophosphate-camp degradation, which accumulates calcium ion and improves myocardial contractility (Alousi & Johnson, 1986; Shipley & Hess, 1995) possibly by increasing the concentration of cAMP (Silver et al., 1989), which in turn causes cell relaxation and vasodilation. Milrinone has been widely applied to cases of severe AHF and acute exacerbation of chronic heart failure (HF) to facilitate circulation (Jessup et al., 2009). However, it is not appropriate for AHF following AMI because of concerns that it may cause arrhythmias (DiBianco et al., 1989). Some previous studies have assessed the effectiveness and safety of milrinone in AHF patients after AMI, but still no trial with large sample size could support a clear conclusion.

Mesenchymal stem cells (MSCs) have been used for tissue regeneration. This kind of cells has low immunogenicity and is not prone to rejection. MSCs could differentiate into cardiomyocytes in vitro and have unique immunomodulatory effects. The therapeutic potential of MSCs for HF has been frequently reported and the effectiveness has been confirmed (Jessup et al., 2009). Therefore, this study intended to examine their potential to alleviate heart damage. Compared with other stem cells like embryonic stem cells, MSCs own potentials of self-renewal, proliferation, and differentiation (Caplan, 2009; Caplan & Correa, 2011). They also have low immunogenicity and rarely express MHC II and T cell stimulating molecules (Jiang et al., 2008), indicating that they are immunologically inert. Easy collection, and less sensitivity to genetic mutations and proliferative capacity leaves MSCs available for cell therapy. Animal experiments have demonstrated that MSC transplantation would enhance neovascularization, increase cell survial and restore heart functions in AMI (Amado et al., 2005; Nagaya et al., 2005; Schuleri et al., 2009; Silva et al., 2005; Toma et al., 2002; Valina et al., 2007). Additionally, MSCs can repair DNA damage (Sugrue et al., 2013). In a recent study using two cloned mouse MSC lines MS5 and ST2, MSCs essentially

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express DDR proteins, such as ATM to enhance healing of strand breaks and improve survival after heavy ion irradiation (Yu et al., 2018). MSCs effectively improve DNA DSB through activation of signaling pathways.

Bone mesenchymal stem cells (BMSCs) have attracted much attentions from researchers because of their great differential potentials and rare immune and rejection reactions (Gnecchi et al., 2012; Hatzistergos et al., 2010; Wei et al., 2012). In recent years, animal experiments have confirmed that BMSC transplantation obtained improved heart function (Li et al., 2012; Tokunaga et al., 2010; Vassalli & Moccetti, 2011; Wen et al., 2011). However, the efficacy of BMSC transplantation in previous clinical studies is not satisfactory. BMSCs used for transplantation are not purely cultured; therefore, less transplanted cells survive to differentiate (Hilfiker et al., 2011; Menasche, 2011). Therefore, many studies on the treatment of heart disease are trying to improve cell survial and affect differentiation into cardiomyocytes. In this study, we established a rat HF model and administered milrinone and BMSCs to the animals, and then examined the effect of combined treatment on heart function. Assays were carried out to assess cell survial and examine the level of myocardial proteins, providing new ideas for the treatment of HF.

2 Materials and methods

2.1 Animals

Forty male (6-7 weeks old, 200-220 g) were purchased to establish a model of HF as previous description (13). Briefly, the animals received intraperitoneal injection of adriamycin 2.5 mg/kg 3 times in one week. After an interval of two weeks, doxorubicin was given for another week with total dosage of 15 mg/kg. This study followed the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health and obtained approval by the IACUC of the First Affiliated Hospital.

2.2 Cell culture

The rat femur and tibia were dissected, and the epiphyseal plates were exposed. Following rinsing the bone marrow with PBS, the marrow was blown into the cell suspension and centrifuged at 1500 rpm. The pellet was incubated in DMEM and prepared into the cell suspension, and placed it in a culture flask. When the confluency reached > 80%, the cells were digested and passaged, as the cell morphology was detected by a phase-contrast microscope, and cells were labeled. The cells were seeded in a 25 mm petri dish. When the confluence reached 60%, bromodeoxyuridine was added to the cells (10 mol/L). After 48 h of incubation, the mixture was subjected to centrifugation and the cells were kept in ice.

2.3 Grouping

With some healthy rats untreated (normal group) and HF rats treated with normal saline (HF group), other rats were administered with BMSCs through tail vein injection (BMSCs group) and/or 15 g/kg/day of milrinone (milrinone group and BMSCs + milrinone group) through the jugular vein injection (n = 8) for 4 weeks. .

2.4 Echocardiogram

An 11.4 MHz high-frequency ultrasound examination was performed along the chest wall with an l0S probe, with depth of 2.0 cm and total dose of intraperitoneal injection 800 mg/kg (20%). Two-dimensional ultrasound and m-mode ultrasound were used to detect LVSD, LVDD, LVSV, and LVDV. Each parameter was measured three times under a continuous full heartbeat cycle and the average value was calculated. Simpson method measured LVEF and LVFS.

2.5 Detection of heart function

Under anesthesia, the catheter was inserted into the left ventricle after separation of the right common carotid artery. A Doppler recorder examined the LVSP, LVDP, heart rate and left ventricular pressure (dp/dtmax).

2.6 Detection of serum BNP

Blood sample was collected from the tail vein of fasting rats in the morning before and after 4 weeks of treatment. The sample was centrifuged and the serum was collected. The serum BNP level was detected by ELISA (Boster Company, Wuhan, China).

2.7 Western blot

Proteins extracted from tissues were subjected to Western blot analysis with primary antibodies against cTnI (1:3000), Cx43 (1:300) and GATA-4 (1:200) (Wuhan Bobst Company).

2.8 Statistical analysis

Data were analyzed by SPSS 16.0 software. The measurement data were analyzed by paired-sample Student's t test and Spearman rank test assessed the correlation between the data. $p < 0.05$ indicates significant difference.

3 Results

3.1 Animal model

Of all rats, only 34 ones survived for further experiments and 4 rats died suddenly of unknown reasons, and the other 2 died of massive ascites. The echocardiography revealed insignificant difference on LVDD and LVSD before and after modeling ($p \geq 0.05$) as well as LVEF and LVFS with significant difference. According to the standard of HF model, LVEF and LVFS shall decrease by 20-30%. The ventricles of the 5 rats did not show significant dilation, and their LVEF value did not decrease significantly, so these animals were excluded. 29 rats meeting the criteria survived.

3.2 Comparison of cardiac function

The echocardiogram of the heart failure group confirmed the impaired left ventricular function with increased LVSD and LVDD, and decreased LVFS and LVEF. Treatment with either BMSCs, milrinone or the combination of them resulted in significant differences in LVSD, LVDD, LVEF, LVFS and

HF ($p < 0.05$), improving the ventricular function. Among the three groups, the BMSCs + Milrinone group most significantly improved the contractile function ($p < 0.05$). However, the difference between the BMSCs group and the milrinone group did not reach significance ($p \geq 0.05$; Table 1).

3.3 Hemodynamic index

After 4 weeks of treatment, hemodynamic indicators varied among the groups ($p < 0.05$). The BMSCs, milrinone and BMSCs + milrinone groups all had increased LVSP and dp/dtmax, and decreased LVDP value (P<0.01), indicating improvements in the heart function, but there is no significant improvement compared with the normal group ($p \geq 0.05$). Of all groups, the dp/dtmax of the BMSCs with milrinone group was the highest, as the difference between the BMSCss group and the milrinone group was not significant ($p \geq 0.05$; Table 2)

3.4 Changes in serum BNP levels

The BNP level of each group decreased, and it restored significantly after treatments. Before treatment, the BNP levels of the BMSCs group, the milrinone group, and the BMSCs + milrinone group did not vary significantly ($p > 0.05$). After the intervention, the level of BNP increased ($p < 0.05$) with lowest level indicated in the BMSCs + milrinone group ($p < 0.05$) (Table 3).

3.5 Specific proteins

After 4 weeks, we checked up the level of proteins. Treatment with BMSCs and/or milrinone restored the expressions of GATA-4, cTnI, and Cx43 ($p < 0.05$), especially BMSCss + milrinone group having the highest expressions ($p < 0.05$). The expression level of GATA-4 in the BMSCs group increased more significantly ($p < 0.05$; Figure 1).

3.6 Discussion

Heart function changes following cardiac diseases are essentially caused by degeneration and necrosis of myocardial cells. Conventional treatments for heart failure can only alleviate the symptoms instead of preventing the progression. Considering the insufficient donors and transplant rejection, heart transplantation is not available enough. Myocardial tissue to replace the damaged tissues has a broader application prospect. Therefore, repair and replacement of cardiomyocytes and stem cells is expected as a promising option (Arnous et al., 2012; Hoover-Plow & Gong, 2012; Oh et al., 2012).

Table 1. Echocardiographic analysis of heart function in rats with heart failure 4 weeks after injection (mean ± sd).

Group	n	LVDD (mm)	LVSD (mm)	LVEF (%)	LVFS (%)
Normal group	6	4.21 ± 0.09	1.03 ± 0.21	74.21 ± 1.49	44.21 ± 1.78
HF group	6	6.75 ± 0.42[a]	4.25 ± 0.12[a]	47.75 ± 2.42[a]	20.15 ± 1.04[a]
BMSC group	8	5.56 ± 0.71[b]	2.26 ± 0.91[b, c]	55.56 ± 2.73[b, c]	25.26 ± 1.91[b, c]
BMSC + Milrinone group	8	5.32 ± 0.65[b, c]	2.32 ± 0.06[b, c]	65.32 ± 4.35[b-d]	29.12 ± 1.05[b-d]
Milrinone Group	7	6.23 ± 0.61	3.33 ± 0.41	56.65 ± 3.61[b]	24.23 ± 1.61

LVDD = left ventricular end-diastolic diameter; LVSD = left ventricular end-systolic diameter; LVEF = left ventricular ejection fraction; LVFS = left ventricular shortening rate; a = $p < 0.05$ vs. normal group; b = $p < 0.05$ vs. HF group; c = $p < 0.05$ vs. milrinone group; d = $p < 0.05$ vs. BMSC group.

Table 2. Hemodynamic index of rats with HF 4 (mean ± sd).

Group	n	LVSP (kPa)	LVDP (kPa)	+dp/dtmax (kPa/sec)	-dp/dtmax (kPa/sec)
Normal group	6	21.24 ± 1.09	21.24 ± 1.09	974.21 ± 71.39	974.21 ± 71.39
HF group	6	14.35 ± 2.62	14.35 ± 2.62	14.35 ± 2.62	14.35 ± 2.62
BMSCs group	8	16.56 ± 2.73[a]	16.56 ± 2.73[a]	16.56 ± 2.73[a]	16.56 ± 2.73[a]
BMSCs + Milrinone group	8	18.02 ± 3.85[ab]	18.02 ± 3.85[ab]	18.02 ± 3.85[ab]	18.02 ± 3.85[ab]
Milrinone Group	7	16.03 ± 3.31[a]	16.03 ± 3.31[a]	16.03 ± 3.31[a]	16.03 ± 3.31[a]

LVSP = left ventricular systolic pressure; LVDP = left ventricular diastolic pressure; dp/dtmax = the maximum change in left ventricular pressure; a = $p < 0.05$ vs. normal group or HF group; b = $p < 0.05$ vs. milrinone group or BMSC group.

Table 3. Serum BNP levels (ng/L, mean ± sd) 4 weeks after injection.

Group	n	Pre-injection	Pre-injection
Normal group	6	75.04 ± 7.28	75.04 ± 7.28
HF group	6	554.15 ± 32.60	554.15 ± 32.60
BMSC group	8	566.86 ± 19.53	566.86 ± 19.53[a, b]
BMSC + Milrinone group	8	571.62 ± 13.67	571.62 ± 13.67[a-c]
Milrinone Group	7	402.71 ± 15.76[a, b]	402.71 ± 15.76[a, b]

a = $p < 0.05$ vs. normal group or HF group; b = $p < 0.05$ vs. pre-injection group; c = $p < 0.05$ vs. Milrinone group or BMSC group.

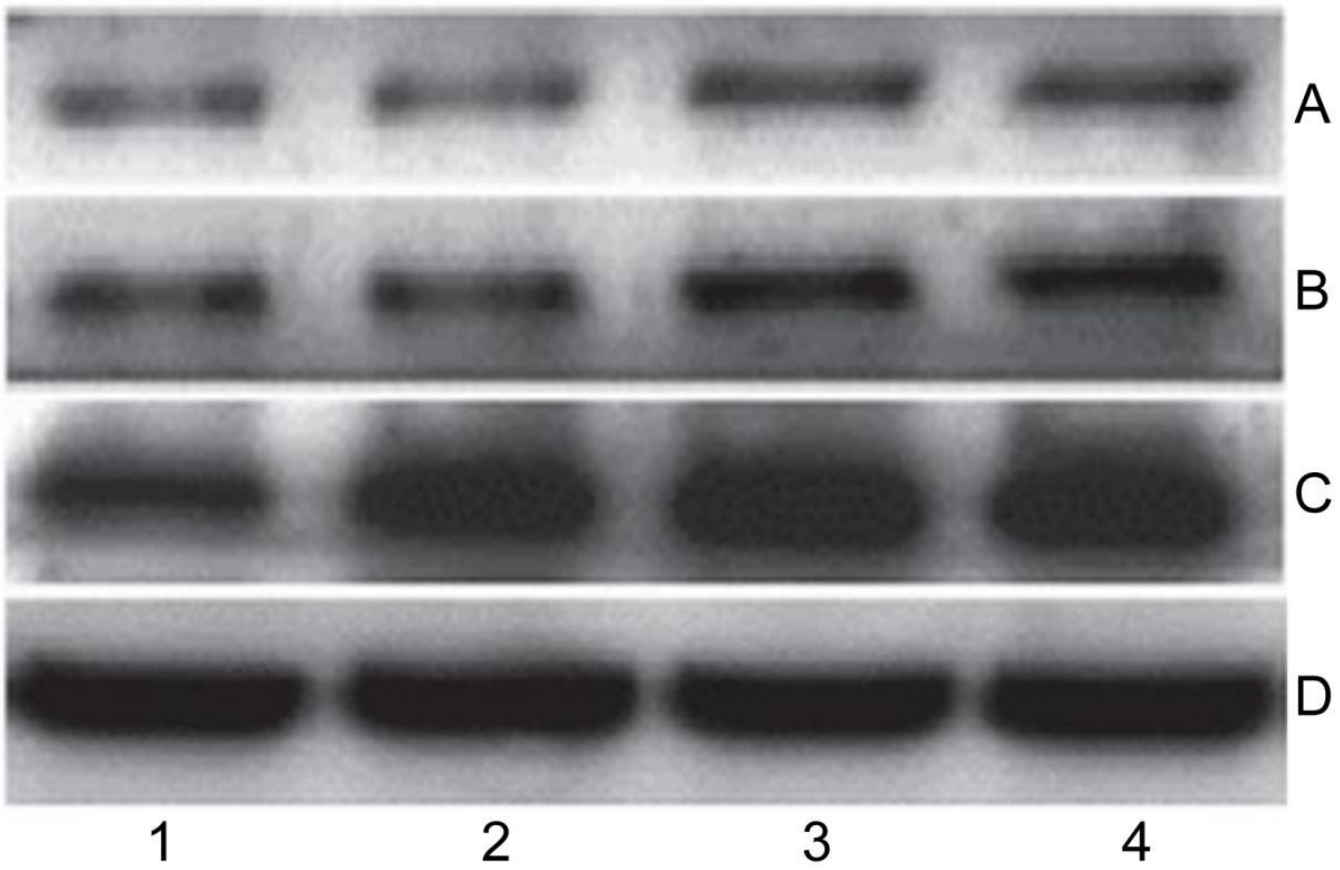

Figure 1. Western blot detection of rat GATA-4, Cx43 and cTnI protein expression. (A) GATA-4 protein; (B) Cx43 protein; (C) cTNI protein; (D) β-actin. Group 1: HF group; group 2: milrinone group; group 3: BMSC group; group 4: BMSC + milrinone group.

Though Milrinone does not damage heart, it might accumulate cGMP relaxing smooth muscle cells and decreasing pressures on vessels and heart, thereby improving cardiac function. Additionally, milrinone hinders the reabsorption of sodium relieving load through enhancement of filtration (Bocchi et al., 2013; Iglesias et al., 2006; Mills et al., 1999). Milrinone impacts the blood pressure, volume, and electrolyte balance, restoring cardiac function. With no positive inotropic effect, milrinone hardly imposes oxygen consumption.

Dobutamine mainly improves cardiac contractility and mitigates resistance through agonistic effect with significantly cardioprotective effect. Nevertheless, in the case of increased cardiac contractility, administration of high-dose dobutamine might accelerate the ventricular rate and blood pressure will increase, which might affect the condition of AMI.

4 Conclusion

Rats with a 20-30% reduction in LVEF were selected as experimental models. In this study, our results indicated that BMSCs transplantation could restore heart function, but the degree was limited. BMSCs exerted similar effect on the heart function as milrinone with no significant difference. Compared with simple cell transplantation, BMSCs transplantation incorporated with milrinone more significantly improved HF.

A controversial issue on cell transplantation is the possibility of transplanted cells to be induced into cardiomyocytes. BMSCs transplantation is confirmed to exert protective effect on heart. But this approach hardly cures the condition and its long-term efficacy remains elusive. Few evidences indicated the potential of BMSCs to differentiate into cardiomyocytes, but it has been acceptable that cell transplantation can angiogenesis and resist myocardial apoptosis through paracrine instead of differentiation. In this study, BMSCs transplantation induced increased expression of GATA-4 in myocardial tissue which correlates to cardiomyocyte differentiation. The lower level of GATA-4 expression in HF model rats treated with milrinone may be due to the presence of cell division. In the presence of BMSCs and milrinone, the expression of GATA-4 as well as Cx43 and cTnI increased. Whether the above changes result from enhancing the differentiation of BMSCs into cardiomyocytes, or enhancing BMSC survival and whether the efficacy of combined treatment is the sum of the two approaches or the synergistic effect deserves further analysis.

Conflict of interest

None.

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