Cardiovascular Drugs and Therapy 20 85–91 2006

DOI: 10.1007/s10557-006-7652-9

BASIC RESEARCH

Granulocyte Colony-Stimulating Factor Reduces Cardiomyocyte Apoptosis and Improves Cardiac Function in Adriamycin-Induced Cardiomyopathy in Rats

Xu Wei Hou [1], Ja Son [2], Yang Wang [1], Yong Xin Ru[1], Qing Lian [3], Wuliya Majiti [3], A. Amazouzi[3], Yu Ling Zhou [1], Pei Xian Wang [2], and Zhong Chao Han[1]

[1] *State Key Laboratory of Experimental Hematology, Institute of Hematology, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin 30020, China;* [2] *Tianjin Medical College, Tianjin 30020, China;* [3] *European Hospital of George Pompidou of Paris, France, Rue Leblanc 75908, Paris Cedex 15, France*

Published online: 22 April 2006

Summary. Background: **Cardiomyocyte apoptosis reportedly participates in the occurrence and progression of dilated cardiomyopathy (DCM). Recent studies have shown that granulocyte colony-stimulating factor (G-CSF) enhances bone marrow cells migration to the damaged heart in the DCM model and improves the ultrastructure of the cardiomyocyte in adriamycin (ADR) induced DCM. However, its influence on cardiac pump function and cardiomyocyte apoptosis has not been studied.**

Methods and materials: **Wistar Rats were randomly grouped into control, ADR, ADR + PBS, ADR + G-CSF group (n = 10). ADR (2.5 mg/kg, 6 times for 2 weeks) was administered intraperitoneally in all rats except the control group. After 2 weeks, the rats in ADR + G-CSF group were injected with G-CSF (50 microg/kg/day for 8 days) subcutaneously. Cardiac function was evaluated by echocardiogram and cardiac catheterization after 4 weeks. Cardiomyocytes apoptosis and apoptosis-related protein Fas were detected by in situ terminal deoxynucleotidyl transferase assay (TUNEL method) and Western blot, respectively.**

Results: **The ADR and ADR + PBS groups showed significant deteriorations of left ventricular functions and high cardiomyocyte apoptosis index, as well as high Fas expressions. Meanwhile, the ADR + G-CSF group showed significant improvement in LV function, inhibition of cardiomyocyte apoptosis compared with the ADR and ADR + Phosphate-Buffered Saline PBS group. The Fas protein expression was remarkably attenuated as well.**

Conclusion: **Our results suggest that administration of G-CSF inhibited cardiomyocyte apoptosis and Fas protein expression and contributes to improving cardiac pump function *in vivo* in ADR induced DCM rat model.**

Key words. **dilated cardiomyopathy, bone marrow mobilization, granulocyte colony-stimulating factor, apoptosis**

Introduction

Non-ischemic dilated cardiomyopathy (DCM) accounts for almost one half of new cases of heart failure encountered in clinical practice [1,2]. Although the mechanism of DCM remains unclear, evidence is accumulating that cardiomyocyte apoptosis is involved in loss of myocytes in DCM [3,4]. Animal experiments have shown that inhibition of cardiomyocyte apoptosis contributes to the improvement of cardiac function in DCM [5].

Currently, satisfactory therapy for DCM is lacking. Cell transplantation is a promising therapy for end-stage heart failure. Its feasibility and efficiency have been proven in ischemic cardiomyopathy [6–11] and may also offer a new hope for non-ischemic DCM [12–15].

Bone marrow mobilization, namely mobilizing endogenous stem cell with certain cytokines, such as granulocyte colony-stimulating factor (G-CSF) alone or combined with stem cell factors, was firstly proposed by Orlic et al. in 2001 as a new method for post-infarction heart repair in mice [16]. Using this strategy, Tomita et al. reported that G-CSF administration prompted the migration of bone marrow cells to the damaged heart and improved the ultrastructure of cardiomyocytes in adriamycin (ADR) induced DCM rats [13]. This implies that

Address for correspondence: Zhong Chao Han, State Key Laboratory of Experimental Hematology, Institute of Hematology, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin 30020, China.

bone marrow mobilization with G-CSF might offer a cardioprotective effect in DCM at a cellular level. However, its influence on the *in vivo* cardiac function in this DCM model remains unknown.

The effect of bone marrow mobilisation on cardiomyocyte apoptosis has also attracted interest. Previous studies indicated that G-CSF administration has an anti-apoptosis effect on human neutrophils in pathological conditions [17–19]. In post-infarction hearts, Harada et al. found that G-CSF inhibited apoptotic death of cardiomyocytes [20]. However, its effect on cardiomyocyte apoptosis in ADR induced DCM model has not been studied yet.

In a spinal cord injury model, Ha et al. recently reported that bone marrow mobilisation with granulocyte-macrophage colony-stimulating factor rescued neuronal cells from apoptosis and improved neurological function [21]. Accordingly, we hypothesize that in the DCM model, G-CSF may rescue the cardiomyocytes from apoptosis, thus enhancing *in vivo* cardiac function. In the present study, we used the ADR induced cardiomyopathy rat model to test this hypothesis.

Materials and methods

Animal preparation

Adult male Wistar rats (body weight, 220 ± 10 g) were divided randomly into 4 groups: Control, ADR, ADR + Phosphate-Buffered Saline (PBS) and ADR + G-CSF group ($n = 10$). Heart failure was induced with Adriamycin (Sigma Chemical; St. Louis, MO) as described before [22,23]. After 2 weeks of wash-out period, rats in the ADR + G-CSF group were injected with the G-CSF (50 microgram/kg/day for 8 days) subcutaneously [13] and rats in the ADR + PBS group were injected with same volume of PBS. All rats were maintained on normal rat chow for 4 weeks. Body weight was measured 2 times per week.

Echocardiography

Echocardiography was performed after the final ADR injection and 4 weeks after the G-CSF administration. Rats were anesthetised with pentobarbital sodium (60 mg/kg, IP). From M mode tracings, LV dimensions (end-diastolic diameter [LVDd] and end systolic diameter [LVDs]) were measured by echocardiogram with an HP SONOS 1000 with a 7.5-MHz transducer (Hewlett-Packard Co, USA). The percent of fractional shortening (%FS) of the LV was calculated from the following formula: $\%FS = [(LVDd–LVDs)/LVDd] \times 100\%$.

Hemodynamic study

The hemodynamic parameters were measured immediately after echocardiography. A carotid artery was isolated and cannulated with a 3-F high-fidelity microtip catheter connected to a pressure transducer (Millar Instruments, Houston, USA). The catheter was advanced into the left ventricle. After an equilibration period of 20 min, LV systolic pressures (LVSP) and end-diastolic pressures (LVEDP), the maximum rate of LV systolic pressure rise (dP/d*t*max) were monitored. All hemodynamic parameters were recorded continuously for 15 min and were computerized with the MP100-Acknowledge software (Biopac Systems, Inc, USA). All rats were sacrificed after the hemodynamic study. The hearts were isolated rapidly and the left ventricles were cut into 2 pieces. One piece was fixed with 4% paraformaldehyde and the other stored in liquid nitrogen.

In situ terminal deoxynucleotidyl transferase assay (TUNEL assay)

The TUNEL method is based on the specific binding of terminal deoxynucleotidyl transferase (TdT) to the 3′-OH ends of DNA and the ensuing synthesis of a polydeoxynucleotide polymer. The TUNEL method was applied to 4% paraformaldehyde-fixed, paraffin-embedded sections 3 μm thick with an apoptosis Detection Kit (Boehringer Mannheim, German) according to the manufacturer's instructions

Quantification of apoptotic cells

Myocardial nuclei of 3 μm horizontal sections of the LV were labelled by the TUNEL method. The nuclei were counted, and the apoptotic index (number of myocardial nuclei labelled by the TUNEL method/number of total myocardial nuclei) was calculated.

Western blot analysis

Proteins were extracted from freshly frozen LV myocardium. Homogenized myocardial tissue was lysed in a solution containing 50 mM Tris-HCl (pH 7.2), 50 mM NaCl, 1% Nonidet P-40, 1% sodium deoxycholic acid, and 2% SDS with protease inhibitor cocktail. 20 mg of each protein preparation was electrophoretically separated on a 15% SDS-PAGE gel and transferred to a nitrocellulose membrane. The membrane was incubated with anti-Fas antigen antibody (ViroGen Corporation, U.S.A., diluted at 1:2500) for 1 h at room temperature. Western blots were exposed to X-ray film with an enhanced chemiluminescence kit (BD Biosciences, U.S.A.). Bands were identified by comparison with standards purchased from the same companies that supplied the antibody.

Ultrastructural studies

For ultrastructural studies, 3 hearts in each group were processed as described [24,25]. Hearts were washed in cold 0.1 mol/L sodium phosphate buffer (pH 7.4). Tissue samples, 4 to 6 mm in size, were taken from four different areas of the free left ventricular wall between the mid-region and apex of the heart. The tissue pieces were immersed for 15 min in 0.1 mol/L phosphate buffer (pH 7.4) containing 3% glutaraldehyde. This briefly fixed tissue was further cut into pieces smaller than 1-mm^3 cubes. Aldehyde fixation was continued for a total duration of 2 h. The tissues were

washed for 1 h in the above phosphate buffer containing 0.05 mol/L sucrose. Post-fixation was done in 2% OsO_4 for 1.5 h, after which the tissue pieces were dehydrated in graded alcohol series. Tissue embedding was done in epon. Ultrathin sections were placed on Formvar-coated grids and stained with uranyl acetate and lead citrate. The ultra-thin sections were mounted on Veco-R-300 grids, and examined under a Hitachi H-600 electron microscope operating at 100 kV.

Statistics

All data were expressed as mean ± SEM. Statistical significance of differences was ascertained by the One-Way ANOVA and a p value <0.05 was considered as significant.

Results

Overall conditions and body weights

No rats died before sacrifice. The rats in ADR group and ADR + PBS group presented dyspnea, scruffy fur and red exudate developed around the eyes and noses. 5 of 10 in ADR group and 6 of 10 in ADR + PBS group presented enlarged abdomen caused by ascites. The rats in ADR and ADR + PBS were subjected to significant body weight losses (182 ± 14 g and 179 ± 19 g, respectively). The body weights of control rat increased to 288 ± 13 g. The ADR + G-CSF group showed better overall conditions (less dyspnea, scruffy fur and no red exudate around the eyes and noses) and gained body weight during this period (273 ± 11 g; $p < .05$ compared to ADR and ADR + PBS groups) (see Fig. 1).

LV performance

Echocardiography after the final ADR injection showed significant deteriorations of % FS in all ADR rats (ADR group: 39.4 ± 3.3%, ADR + PBS group: 38.1 ± 2.6%, ADR + G-CSF group: 38.4 ± 3.7%) compared to control rats (51.2 ± 4.7%). The %FS in the ADR + G-CSF group was significantly enhanced by G-CSF treatment (53.3 ± 4.6%) whereas the % FS in the ADR and ADR + PBS group remain at low level (41.9 ± 3.3% and 40.7 ± 4.4%, respectively) (see Fig. 2).

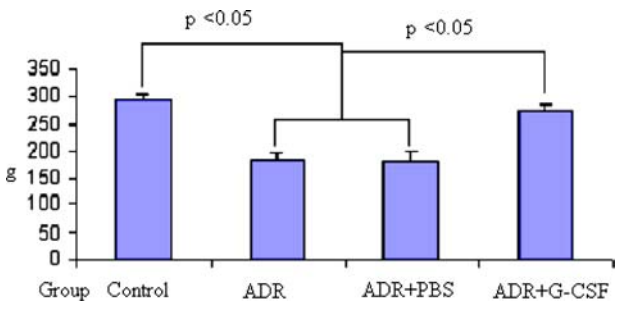

Fig. 1. *The body weights of each group 4 weeks after G-CSF administration.*

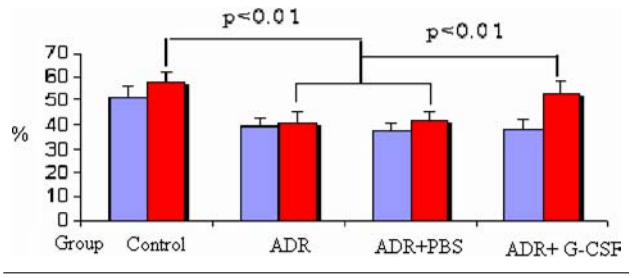

Fig. 2. *The % FS of each group before (blue) and after (red) G-CSF administration.*

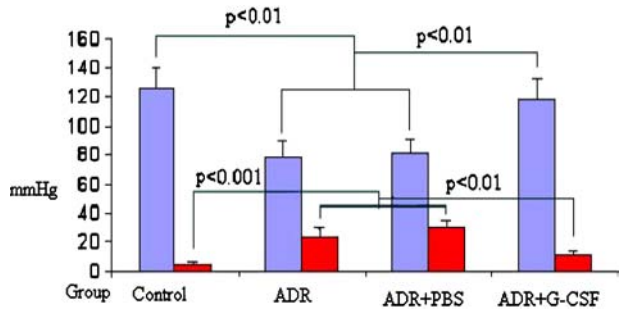

Fig. 3. *The LVSP (blue) and LVEDP (red) of each group after G-CSF administration.*

Hemodynamics compared with the ADR and the ADR + PBS group, the ADR + G-CSF group showed a significant higher LVSP (119 ± 16.8 mmHg as compared to 84 ± 10.3 mmHg in the ADR + PBS group and 83 ± 11.2 mm Hg in the ADR group and significant lower LVEDP (11 ± 3.4 mmHg compared to 30 ± 5.2 mmHg in the ADR + PBS group and 27 ± 4.4 in the mmHg ADR group) In the control group, LVSP and LVEDP were 121 ± 15.1 mmHg, respectively (Fig. 3).

Apoptosis

Cardiomyocyte apoptosis was induced by ADR administration in the ADR and ADR + PBS group (apoptotic index 13.64 ± 2.46% and 11.24 ± 2.51%, respectively, both $p < 0.001$, vs. control apoptotic index 2.88 ± 0.72%). Cardiomyocyte apoptosis in the ADR + G-CSF group was significantly inhibited by G-CSF treatment (apoptotic index 3.75 ± 0.84%, $p < 0.01$, vs. both ADR and ADR + PBS groups) (Figs. 4 and 5).

Expression of Fas protein

The pro-apoptosis protein, Fas was highly expressed in the samples of ADR and ADR + PBS groups and was markedly attenuated in the ADR + G-CSF group (Fig. 6).

Ultrastructure

The ADR and ADR + PBS groups presented morphological changes typical for ADR induced cardiomyopathy

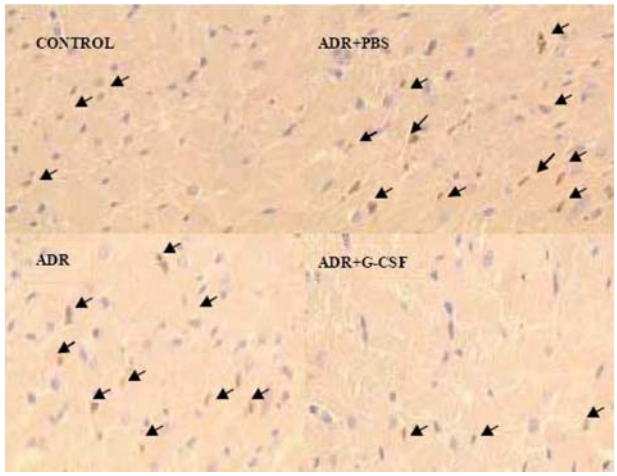

Fig. 4. *The apoptotic cells in each group. The nuclear of the apoptotic cells were stained brown by TUNEL (arrow heads), non-apoptotic cell nuclear were blue. TUNEL assays showed that ADR administration caused a significant increase in the apoptosis in ADR group (13.64 ± 2.46%) and ADR + PBS group (11.24 ± 2.51%). G-CSF inhibited ADR induced apoptosis in ADR + G-CSF group (3.75 ± 0.84).*

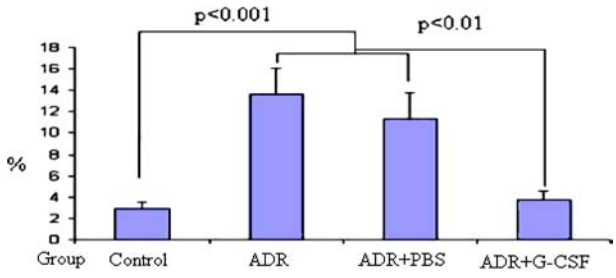

Fig. 5. *The apoptosis index of cardiomyocytes of each group.*

Fig. 6. *The protein expression of Fas in each group compared with the control. The Fas protein expression was high in the samples of the ADR and ADR + PBS group, and was remarkably attenuated in the ADR + G-CSF group.*

including swelling of mitochondria, vacuolization of the cytoplasm, and dilation of the sarcotubular system. The ultrastructure of hearts from the ADR + G-CSF group was significantly different from that of ADR and ADR + PBS groups and showed regular myofibril arrangement, maintained sarcotubular system, and preserved mitochondria (Fig. 7)

Discussion

Our study showed that subcutaneous injection of G-CSF improved cardiac performance and inhibited cardiomyocyte apoptosis in the ADR-induced DCM model in rats. Previous experimental and clinical studies have found that G-CSF administration improves LV function in infarcted hearts [26–29]. Harada et al. reported that post-infarction administration of G-CSF decreased apoptotic cell death both *in vivo* and *in vitro* and improved cardiac function by activating the Jak-Stat pathway [20]. Minatoguchi et al. found that short term, small dose of G-CSF administration (10 microgram/kg/day for 5 days, even smaller than our dose) improved the heart function and prevented left ventricular remodelling [30]. Our study showed, for the first time, that even short-term administration of G-CSF (50 microgram/kg/day for 8 days), improved cardiac function in the ADR-induced DCM rat model. This finding extends the beneficial effect of G-CSF to a non-ischemic heart failure model.

Since Hamamoto et al. and Fukuhara et al. reported that endogenous bone marrow-derived stem cells contribute to only a small proportion of regenerated myocardium in the acute infarction model [31, 32]; we doubt that improvement in LV function can solely be explained by bone marrow mobilisation. In this study, we hypothesised that other cardioprotective mechanisms, such as the inhibition of apoptosis might participate in this process. In fact, the TUNEL assay confirms our hypothesis and provides *in vivo* evidence that G-CSF significantly inhibits ADR-induced apoptosis (Figs. 5 and 6).

Observations on the percentages of apoptotic cells in the failing heart are currently controversial: 0.86 ± 0.11% in ADR induced DCM by Nakamura et al. [5]; 0.23 ± 0.20% in the failing human heart by Olivetti et al. [4] and 5 to 35.5% in patients with end-stage cardiomyopathy by Narula et al. [3]. Our data show a relatively high percentage of apoptotic cells in ADR group (13.64 ± 2.46%), consistent with the data of Narula's.

Apoptotic cardiomyocytes have also been detected in ADR induced DCM rats, rapid pacing-induced DCM dogs and biopsy samples from DCM patients [33–35]. An apoptosis related protein, Fas, was reported to play an important role in inducing cardiomyocyte apoptosis in DCM. Its expression is in correlation with cardiac dysfunction in DCM and anti-Fas antibody significantly inhibits cardiomyocyte apoptosis, leading to better LV performance [5,35]. In our study, the Fas protein was highly expressed in ADR and ADR + PBS groups, and was markedly attenuated by G-CSF. Based on these results, we suggest that G-CSF inhibited cardiomyocyte apoptosis, at least in part via Fas pathway, contributing to a better LV performance.

Compared with the ADR and ADR + PBS groups, the G-CSF group showed better overall conditions in our study. However, whether these physical

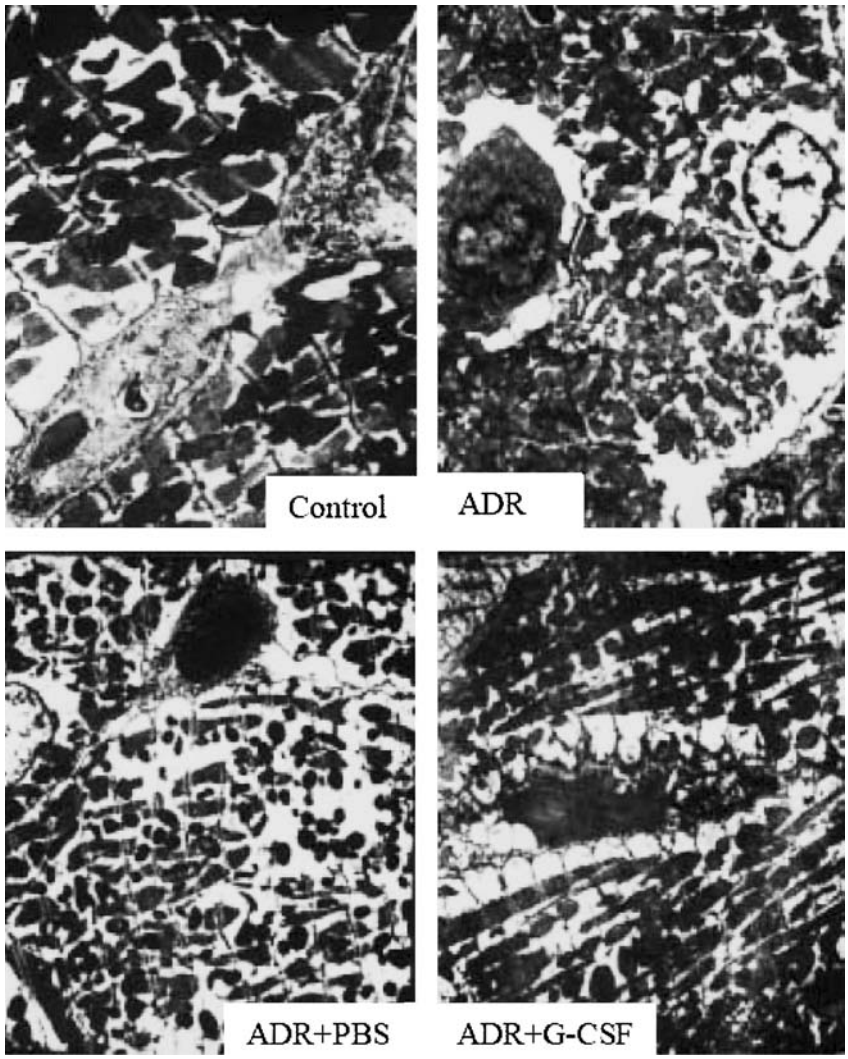

Fig. 7. *The electron-microscope ultrastructure showed significant differences among the 4 groups. ADR caused typical morphological changes in ADR and ADR + PBS including swelling of mitochondria, vacuolization of the cytoplasm, and dilation of the sarcotubular system, while the ultrastructure of hearts in ADR + G-CSF group were ameliorated: regular myofibril arrangement, maintained sarcotubular system, and preserved mitochondria.*

improvements were attributed solely to the amelioration of heart function is question worth exploring. It is well known that the ADR leads to general toxicity, not only in heart, but also in liver and bone marrow [36,37], which can be attenuated by G-CSF administration as well [38,39]. So we cannot exclude the possibility that G-CSF exerted a systematic effect on the DCM rat, including enhancing the damaged liver and bone marrow function, thus contributing to better overall conditions. However, these factors were not studied in our study.

Our study suggests that subcutaneous administration of G-CSF might serve as an alternative treatment for the ADR induced DCM. Its feasibility and safety need being explored further before clinical x application.

Acknowledgment

This work was supported by the grants of project 863(2002AA223354, 2003AA205060) from the Ministry of Science and Technology of China.

References

1. Codd MB, Sugrue DD, Gersch BJ, et al. Epidemiology of idiopathic dilated and hypertrophic cardiomyopathy: A population-based study in Olmsted Country, Minnesota, 1975–1984. Circulation 1989;80:564–572.
2. Dec GW, Fuster V. Idiopathic dilated cardiomyopathy. N Engl J Med 1994;331:1564–1575.
3. Narula J, Haider N, Virmani R, et al. Apoptosis in myocytes in end-stage heart failure. N Engl J Med 1996;335:1182–1189.

4. Olivetti G, Abbi R, Quaini F, et al. Apoptosis in the failing human heart. N Engl J Med 1997;336:1131–1141.
5. Nakamura T, Ueda Y, Guan Y, et al. Fas-mediated apoptosis in adriamycin-induced cardiomyopathy in rats: *in vivo* study. 2000;102(5):572–578.
6. Orlic D, Kajstura J, Chimenti S, et al. Bone marrow cells regenerate infracted myocardium. Nature 2001;410:701–705.
7. Kocher AA, Schuster, Szabolcs MJ, et al. Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. Nat Med 2001;7:430–436.
8. Strauer BE, Brehm M, Zeus T, et al. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. Circulation 2002;106:1913–1918.
9. Assmus B, Schachinger V, Teupe C, et al. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI). Circulation 2002;106:3009–3017.
10. Britten MB, Abolmaali N, Assmus B, et al. Infarct remodeling after intracoronary progenitor cell treatment in patients with acute myocardial infarction (TOPCARE-AMI): mechanistic insights from serial contrast-enhanced magnetic resonance imaging. Circulation 2003;108:2212–2218.
11. Schächinger V, Assmus B, Britten MB, et al. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction. Final one-year results of the TOPCARE-AMI Trial. J Am Coll Cardiol 2004;44:1690–1699.
12. Ishida M, Tomita S, Nakatani T, et al. Bone marrow mononuclear cell transplantation had beneficial effects on doxorubicin-induced cardiomyopathy. J Heart Lung Transplant 2004;4:436–445.
13. Tomita S, Ishida M, Nakatani T, et al. Bone marrow is a source of regenerated cardiomyocytes in doxorubicin-induced cardiomyopathy and granulocyte colony-stimulating factor enhances migration of bone marrow cells and attenuates cardiotoxicity of doxorubicin under electron microscopy. J Heart Lung Transplant 2004;23:577–584.
14. Scorsin M, Hagege AA, Dolizy I, et al. Can cellular transplantation improve function in doxorubicin-induced heart failure? Circulation 1998;98(Suppl III):III-51–III-55.
15. Yoo KJ, Li RK, Richard D, et al. Heart cell transplantation improves heart function in dilated cardiomyopathic hamsters. Circulation 2000;102:III–20.
16. Orlic D, Kajstura J, Chimenti S, et al. Mobilized bone marrow cells repair the infarcted heart, improving function and survival. Proc Natl Acad Sci USA 2001;98:10344–10349.
17. Maianski NA, Mul FP, Van Buul JD, et al. Granulocyte colony-stimulating factor inhibits the mitochondria-dependent activation of caspase-3 in neutrophils. Blood 2002;99:672–679.
18. Sakamoto C, Suzuki K, Hato F, et al. Anti-apoptotic effect of granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, and cyclic AMP on human neutrophils: protein synthesis-dependent and protein synthesis-independent mechanisms and the role of the Janus kinase-STAT pathway. Int J Hematol 2003;77:60–70.
19. Hasegawa T, Suzuki K, Sakamoto C, et al. Expression of the inhibitor of apoptosis (IAP) family members in human neutrophils: up-regulation of cIAP2 by granulocyte colonys-timulating factor and overexpression of cIAP2 in chronic neutrophilic leukemia. Blood 2003;101:1164–1171.
20. Harada M, Qin Y, Takano H, et al. G-CSF prevents cardiac remodeling after myocardial infarction by activating the Jak-Stat pathway in cardiomyocytes. Nat Med 2005;11:305–311.
21. Ha Y, Kim YS, Cho JM, et al. Role of granulocyte-macrophage colony-stimulating factor in preventing apoptosis and improving functional outcome in experimental spinal cord contusion injury. J Neurosurg Spine 2005;2:55–61.
22. Siveski-Iliskovic N, Kaul N, Singal PK, et al. Probucol promotes endogenous antioxidants and provides protection against adriamycin-induced cardiomyopathy in rats. Circulation 1994;89:2829–2835.
23. Timao Li, Singal P K, et al. Adriamycin-induced early changes in myocardial antioxidant enzymes and their modulation by probucol. Circulation 2000;102:2105.
24. Singal PK, Segstro RJ, Singh RP, et al. Changes in lysosomal morphology and enzyme activities during the development of adriamycin-induced cardiomyopathy. Can J Cardiol 1985;1:139–147.
25. Tong J, Ganguly PK, Singal PK, et al. Myocardial adrenergic changes at two stages of heart failure due to adriamycin treatment in rats. Am J Physiol 1991;260:H909–H916.
26. Valgimigli M, Rigolin GM, Cittanti C, et al. Use of granulocyte-colony stimulating factor during acute myocardial infarction to enhance bone marrow stem cell mobilization in humans: clinical and angiographic safety profile. Eur Heart J 2005;26:1838–1845.
27. Wang Y, Tagil K, Ripa RS, et al. Effect of mobilization of bone marrow stem cells by granulocyte colony stimulating factor on clinical symptoms, left ventricular perfusion and function in patients with severe chronic ischemic heart disease. Int J Cardiol 2005;100:477–483.
28. Fukuhara S, Tomita S, Nakatani T, et al. G-CSF promotes bone marrow cells to migrate into infarcted mice heart, and differentiate into cardiomyocytes. Cell Transplant 2004;13:741–748.
29. Sugano Y, Anzai T, Yoshikawa T, et al. Granulocyte colony-stimulating factor attenuates early ventricular expansion after experimental myocardial infarction. Cardiovasc Res 2005;65:446–456.
30. Minatoguchi S, Takemura G, Chen XH, et al. Acceleration of the healing process and myocardial regeneration may be important as a mechanism of improvement of cardiac function and remodeling by post-infarction granulocyte colony-stimulating factor treatment. Circulation 2004;109:2572–2580.
31. Hamamoto M, Tomita S, Nakatani T, et al. Granulocyte-colony stimulating factor directly enhances proliferation of human troponin I-positive cells derived from idiopathic dilated cardiomyopathy through specific receptors. J Heart Lung Transplant 2004;23:1430–1437.
32. Fukuhara S, Tomita S, Nakatani T, et al. Endogenous bone-marrow-derived stem cells contribute only a small proportion of regenerated myocardium in the acute infarction model. J Heart Lung Transplant 2005;24:67–72.
33. Eikan K, Yosimichi U,Tsuneyuki N, et al. Apoptosis in young rats with Adriamycin-induced cardiomyopathy—Comparison with Pirarubicin, a new anthracycline derivative. Pediatric Res 2002;51:256–259.
34. Heinke MY, Yao M, Chang D, et al. Apoptosis of ventricular and atrial myocytes from pacing-induced canine heart failure. Cardiovasc Res 2001;49:127–134.
35. Yamamura T, Nakamura H, Yamamoto T, et al. Fas expression and apoptosis correlate with cardiac dysfunction in patients with dilated cardiomyopathy. Jpn Circ J 1999;63:149–154.
36. Bagchi D, Bagchi M, Hassoun EA, et al. Adriamycin-induced hepatic and myocardial lipid-peroxidation and DNA-damage, and enhanced excretion of urinary lipid metabolites in rats. Toxicology 1995;95:1–9.

37. Papadopoulou, LC,Tsiftsoglou, AS, et al. Effects of hemin on apoptosis, suppression of cytochrome C oxidase gene expression and bone marrow toxicity induced by doxorubicin (adriamycin). Biochem Pharmacol 1996;52:713–722.
38. Mizushima Y, Morikage T, Kuwahara T, et al. Effects of granulocyte colony-stimulating factor on hematopoietic injury induced by anticancer drugs in mice. J Biol Response Mod 1990;9:576–583.
39. Ohtani K, Na nya T, Aoyama Y, et al. Recombinant human granulocyte colony-stimulating factor accelerates regeneration after T-2 toxin-induced hemopoietic injury and lessens lethality in mice. J Toxicol Sci 1993;18:155–166.