Effect of Stem Cell Therapy on Amiodarone Induced Fibrosing Interstitial Lung Disease in Albino Rat

Somaya Saad Zagloul¹, Maha Baligh Zickri¹, Dalia Hussein Abd El Aziz²,
Doaa Mabrouk³, Hala Gabr Metwally⁴

Histology Departments, Faculty of Medicine, ¹Cairo University, ²Beni-Suef University, ³Misr University of Science and Technology, ⁴Clinical Pathology Department, Faculty of Medicine, Cairo University, Egypt

Background and Objectives: The fibrosing forms of interstitial lung disease (ILD) are associated with significant morbidity and mortality. ILD may be idiopathic, secondary to occupational, infection, complicate rheumatic diseases or drug induced. Efficacy of antifibrotic agents is as far as, limited and uncertain. No effective treatment was confirmed for pulmonary fibrosis except lung transplantation. The present study aimed at investigating the possible effect of human cord blood mesenchymal stem cell (MSC) therapy on fibrosing ILD. This was accomplished by using amiodarone as a model of induced lung damage in albino rat.

Methods and Results: Seventeen adult male albino rats were divided into 3 groups. Rats of amiodarone group were given 30 mg/kg of amiodarone orally 6 days/ week for 6 weeks. Rats of stem cell therapy group were injected with stem cells in the tail vein following confirmation of lung damage and left for 4 weeks before sacrifice. Obstructed bronchioles, thickened interalveolar septa and thickened wall of pulmonary vessels were found and proved morphometrically. Reduced type I pneumocytes and increased area% of collagen fibers were recorded. All findings regressed on stem cell therapy.

Conclusions: Cord blood MSC therapy proved definite amelioration of fibrosing interstitial lung disease provided therapy starts early in the development of the pathogenesis.

Keywords: Mesenchymal stem cells, Lung, Amiodarone, Cord blood, Interstitial lung disease

Introduction

The fibrosing forms of interstitial lung disease (ILD) are often incurable and are associated with significant morbidity and mortality. ILD may be idiopathic (1), secondary to occupational, environmental exposures, complicate rheumatic diseases (2) or secondary to infection (3). Drug induced pathogenesis has to be taken into account in the differential diagnosis of ILD. Anticancer drugs (4)

and amiodarone (5) are considered potential causes.

The efficacy of high doses of pirfenidone, a novel anti-fibrotic oral agent was proved in the treatment of idiopathic pulmonary fibrosis. However, photosensitivity was a well-established side-effect (6). Promising results were documented by using an initial trial pirfenidone, but further studies were needed to confirm the findings. However, it was reported that no effective treatment was confirmed for pulmonary fibrosis except lung transplantation (7).

Mesenchymal stem cells (MSCs) are stromal cells that have the ability to self-renew and exhibit multilineage differentiation. MSCs can be isolated from a variety of tissues, such as umbilical cord, bone marrow and adipose tissue. The multipotent properties of MSCs make them an attractive choice for possible development of clinical applications (8).

Accepted for publication September 19, 2011

Correspondence to **Maha Baligh Zickri**

Histology Departments, Faculty of Medicine, Cairo University, 10 street 24 Maadi, Cairo, Egypt

Tel: +2/01223955078, Fax: +0020235381183/+00202353811760

E-mail: mahakaah@yahoo.com

Umbilical cord blood transplantation (CBT) offers several advantages over traditional stem cell sources, such as immediate availability, absence of risk for donors and lower risk of acute graft-versus-host disease. Recent studies suggest that CBT is a safe and effective strategy for adult patients lacking a suitable related or unrelated donor (9). CBT has been widely used as an alternative source of mesenchymal cell support for stem cell transplant patients (10).

The present study aimed at investigating the possible effect of human cord blood mesenchymal stem cell therapy on fibrosing interstitial pulmonary disease. This was accomplished by using amiodarone as a model of induced lung damage in albino rat.

Materials and Methods

Drug

Amiodarone (Cordarone): Used as ampoules 150 mg/3 ml dissolved in tween 80 (Sanofi Corporation).

Animals

Seventeen adult male albino rats weighing 150~200 g were divided into 3 groups. Each group was kept in a separate cage under good hygienic conditions, fed ad libitum and allowed for free water supply in Histology Department Animal House, Faculty of Medicine, Cairo University. The rats were treated in accordance with guidelines approved by the Animal Use Committee of Cairo University.

Control group: 4 rats, two for each experimental group. Each rat was given 0.5 ml tween 80 (solvent of amiodarone) daily orally six days/week for 6 weeks. The latter being water insoluble at room temperature.

Group A (Amiodarone group): 8 animals were used in this group. Each received 30 mg/kg of amiodarone orally (11) 6 days/week for 6 weeks. 3 animals were sacrificed for microscopic confirmation of lung damage. The remaining 5 animals were left for 4 weeks without therapy (12). A stock solution of the drug was prepared weekly and kept at 4°C. The required dose for each rat was introduced into the mouth using a syringe with a metal tube instead of the needle.

Group S (Stem cell therapy group): 5 rats were given amiodarone in the same dose, by the same route and for the same duration (6 weeks) as in group A. Following confirmation of lung injury, the animals were injected each with 0.5 ml of cultured and labeled human mesenchymal stem cells (HMSCs) suspended in phosphate buffer saline (PBS) on two successive days in the tail vein (13). Stem cells were isolated from cord blood (14). Cord blood col-

lection was performed at Gynaecology Department, Faculty of Medicine, Cairo University. Stem cell culture and labeling were performed at Hematology Unit, New Kasr El Aini Teaching Hospital, Cairo University. The animals were sacrificed 4 weeks following therapy (15).

Cord blood collection (16)

The storage and transport temperature was 15~22°C, transport time was 8~24 hours, sample volume was 65~250 ml, and no sample had signs of coagulation or hemolysis.

Mononuclear cell fraction isolation and propagation (16)

The mononuclear cell fraction (MNCF) was isolated by loading 30 ml of whole blood onto 10 ml of Ficoll density media (Healthcare Bio-Sciences) in 50 ml polypropylene tubes, centrifuge for 30 minutes at room temperature. The interphase collected after aspirating and discarding the supernatant. The interphase was washed with 20 ml PBS and centrifuged at room temperature. The supernatant was aspirated and the cells were washed with PBS a second time. The cells were re-suspended in the isolation media and transferred to culture dishes. The isolation media was low-glucose DMEM (Dulbecco's modified Eagles medium) (Cambrex Bio Science) supplemented with low dexamethazone (10^{-7} M) (Sigma-Aldrich), penicillin (100 IU/ml) (Invitrogen), streptomycin (0.1 mg/ml) (Invitrogen), and ultraglutamine (2 mM) (Cambrex Bio-Science). Incubation was at 38.5°C in humidified atmosphere containing 5% CO₂.

Culture (16)

The isolation media were replaced after overnight incubation (12~18 hours) in order to remove non-adherent cells. The media were replaced every 3 days until MSC colonies were noted. The cultures were inspected daily for formation of adherent spindle-shaped fibroblastoid cell colonies. Sub-culturing was done by chemical detachment using 0.04% trypsin. Later, when cell numbers allowed, expansion was done in 25 cm² or 75 cm² tissue culture flasks.

Labeling (17)

Mesenchymal stem cells were labeled by incubation with ferumoxides injectable solution (25 microgramFe/ml, Feridex, Berlex Laboratories) in culture medium for 24 hours with 375 nanogram/ml poly L lysine added 1 hour before cell incubation. Labeling was histologically assessed using Prussian blue. Feridex labeled HMSCs were washed

in PBS, trypsinized, washed and resuspended in 0.01 Mol/L PBS at concentration of $1 \times 1,000,000$ cells/ml.

Cell viability analysis

Cell viability was done using trypan blue dye exclusion test. This method is based on the principle that viable cells do not take up certain dyes, whereas dead cells do.

Flow cytometry (18)

Flow cytometric analyses were performed on a Fluorescence Activated Cell Sorter (FACS) flow cytometer (Coulter Epics Elite, Miami, FL, USA). HMSC were trypsinized and washed twice with PBS. A total number of 1×10^5 HMSC were used for each run. To evaluate the HMSC marker profile, cells were incubated in $100 \mu\text{l}$ of PBS with $3 \mu\text{l}$ of CD105-FITC for 20 min at room temperature. Antibody concentration was 0.1 mg ml^{-1} . Cells were washed twice with PBS and finally diluted in $200 \mu\text{l}$ of PBS. The expression of surface marker was assessed by the mean fluorescence. CD105 (mesenchymal stem cell marker), CD133 (early hematopoietic & endothelial progenitor stem cell marker) and CD45 (panleucocytic marker) were also used. The percentage of cells positive for CD 105 was determined by subtracting the percentage of cells stained non-specifically with isotype control antibodies.

Histological study

The animals were sacrificed using lethal dose of ether. Lung specimens were removed and fixed in 10% formal saline for 24 hours. Paraffin blocks were prepared and $5 \mu\text{m}$ thick sections were subjected to hematoxylin and eosin (H&E) (19) and Masson's trichrome stain (20).

Fig. 1. A bronchiole (B), an arteriole (ar), alveoli (a), alveolar sacs (A) and interalveolar septa (I) (H&E, $\times 100$).

Histochemical study

Lung sections were stained with Prussian blue stain (21) for demonstration of iron oxide labeled therapeutic stem cells.

Immunohistochemical study

CD105 immunostaining (22) the marker for human mesenchymal stem cells. 0.1 ml prediluted primary antibody (CD105) rabbit polyclonal Ab (ab27422) and incubate at room temperature in moist chamber for $30 \sim 60$ minutes. Tonsil used as positive control specimens. Cellular localization is the cell membrane. On the other hand, one of the lung sections was used as a negative control by passing the step of applying the primary antibody.

Morphometric study

Using Leica Qwin 500 LTD image analysis, assessment of the thickness of the interalveolar septa and the pulmonary vessels (indicated by the distance parameter) was performed in H&E stained sections using interactive measurements menu. The measurements were done in 10 low power fields in control and experimental groups. In Masson's trichrome stained sections, the area percent of collagen fibers was measured using binary mode. The measurements were done in 10 high power fields in control and experimental groups.

Statistical analysis

Quantitative data were summarized as means and standard deviations and compared using one-way analysis-of-variance (ANOVA). p -values < 0.05 were considered statistically significant. Calculations were made on SPSS

Fig. 2. Alveoli (a), alveolar sacs (A) and interalveolar septa (I) exhibiting pulmonary capillaries (c) (H&E, $\times 400$).

Fig. 3. Higher magnification of the previous figure showing alveoli (a) lined mainly by flat nuclei (arrows) (H&E, $\times 1,000$).

Fig. 4. 2 small bronchioles with partial obliteration of the lumen (*) by shed epithelial cells, markedly thickened wall of an arteriole (arrow) and thickened wall of a small pulmonary arteriole (arrow-head) (H&E, $\times 100$).

software (23).

Results

Haematoxylin and eosin (H&E) stained sections

Lung sections of control rats demonstrated bronchioles, alveoli, alveolar sacs, interalveolar septa and pulmonary arterioles (Fig. 1). Closer observation revealed the interalveolar septa exhibiting pulmonary capillaries. The cells mainly lining the alveoli showed flat nuclei (pneumocyte type I) (Figs. 2, 3).

In amiodarone group, some fields showed small bronchioles with partial obliteration of the lumen by shed epi-

Fig. 5. Marked thickening of interalveolar septa (*) with collapsed alveoli. Cellular debris is seen in the lumen of some alveoli (arrows). Note adjacent normal alveoli (H&E, $\times 100$).

Fig. 6. Higher magnification of the previous figure showing multiple fibroblasts and fibrocytes (arrows) in the interalveolar septa (I) (H&E, $\times 400$).

thelial cells. Marked thickening of the wall of small and large pulmonary arterioles was also noticed (Fig. 4). Markedly thickened interalveolar septa with collapsed alveoli existed in some fields. Cellular debris was found in the lumen of few alveoli (Fig. 5). Closer observation demonstrated multiple fibroblasts and fibrocytes in the interalveolar septa and in the lumen of the alveoli. No lining alveolar epithelium was detected (Figs. 6, 7).

In stem cell therapy group, no congestion or thickening of the wall of pulmonary arterioles and venules were noted in multiple fields (Fig. 8). On the other hand, accidental fields revealed pulmonary vessels with thickened wall (Fig. 9). There were few infiltrating cells in the inter-

Fig. 7. Higher magnification of the previous figure showing no alveolar epithelium. Note fibroblasts and fibrocytes (arrows) in the lumen of the alveoli (H&E, $\times 1,000$).

Fig. 9. Normal bronchiole (B), normal alveoli (a) and alveolar sacs (A). Note wall thickening of a pulmonary vessel (arrow) (H&E, $\times 100$).

Fig. 8. Normal bronchiole (B), normal pulmonary venules (V), normal alveoli (a) and alveolar sacs (A) (H&E, $\times 100$).

Fig. 10. Interalveolar septa with few infiltrating cells (arrowheads) (H&E, $\times 400$).

alveolar septa (Fig. 10). Alveoli with multiple lining cells exhibiting flat nuclei were found in comparison to amiodarone group (Fig. 11).

Masson's trichrome stained sections

Minimal collagen fibers were found in the interalveolar septa of control rats (Fig. 12). While in amiodarone group, extensive collagen fibers existed in the interalveolar septa and some collagen fibers in the alveoli (Fig. 13). In stem cell therapy group, minimal collagen fibers were seen in the interalveolar septa and no collagen fibres in the alveoli appeared (Fig. 14).

Prussian blue stained sections

Sections in the lung of control rats showed negative staining with Prussian blue in the alveoli, alveolar sacs and interalveolar septa (Fig. 15). The stem cell therapy group showed few spindle and occasional cuboidal Prussian blue positive (+ve) cells in some interalveolar septa (Fig. 16). Few spindle +ve cells were found in the adventitia of small bronchioles existing near blood vessels (Fig. 17).

CD105 immunostained sections

Sections in the lung of control rats showed negative immunostaining with CD105 in the alveoli, alveolar sacs and interalveolar septa (Fig. 18). The stem cell therapy group

Fig. 11. Higher magnification of the previous figure showing alveoli with multiple lining flat nuclei (arrows) (H&E, $\times 1,000$).

Fig. 12. A control rat showing minimal collagen fibers (arrows) in the interalveolar septa.

showed some spindle and few cuboidal CD105 +ve cells, detected in the interalveolar septa (Fig. 19). Closer observation showed +ve cytoplasmic reaction in the spindle and cuboidal cells (Fig. 20). Some spindle and few cuboidal +ve cells were detected in the lining epithelium and adventitia of some bronchioles (Fig. 21).

Morphometric results

The mean thickness of the interalveolar septa and the mean thickness of the wall of pulmonary vessels were significantly increased in amiodarone group compared to control and stem cell therapy groups. Similar significance was recorded as regards the mean area% of collagen fibers (Table 1, Figs. 22~24).

Fig. 13. A rat in group A showing extensive collagen fibers (arrows) in the interalveolar septa and collagen fibers (arrowheads) in the alveoli.

Fig. 14. A rat in group S showing minimal collagen fibers (arrows) in the interalveolar septa and no collagen fibres in the alveoli.

Discussion

The current study demonstrated modulating effect of cord blood stem cell therapy on amiodarone induced fibrotic ILD in albino rat. This was evidenced by histological, histochemical, immunohistochemical and morphometric studies.

By examination, amiodarone administration resulted in partial obliteration of the lumen of some small bronchioles by shed epithelial cells. Choi et al. (24) reported lower apoptosis in amiodarone-treated rats evidenced by fewer TUNEL+ve alveolar epithelial cells. Increased mRNA levels of bax and caspase-3 were found in a human lung epithelial cell line treated with amiodarone, with in-

Fig. 15. A control rat showing negative staining in the alveoli (a), alveolar sacs (A) and interalveolar septa (I).

Fig. 17. A rat in group S showing few spindle (s) Prussian blue positive cells in the adventitia of a small bronchiole near a blood vessel (v).

Fig. 16. A rat in group S showing two spindle (s) and a cuboidal (cu) Prussian blue positive cells in an interalveolar septum.

Fig. 18. A control rat showing negative immunostaining with CD105 in alveoli (a), alveolar sacs (A) and interalveolar septa (I) ($\times 400$).

creased number of cells exhibiting apoptotic features. Wolkove and Baltzan (25) reported patchy bronchiolitis obliterans in amiodarone pulmonary toxicity.

Marked thickening of the wall of small and large pulmonary arterioles was also noticed and confirmed by a significant increase in the mean thickness of the wall of pulmonary arterioles. This can be referred to the development of pulmonary hypertension. It was stated that the latter condition is characterized by smooth muscle proliferation and consequent arteriolar wall thickening (26).

Markedly thickened interalveolar septa, which was confirmed morphometrically existed with collapsed alveoli in some fields. Multiple fibroblasts and fibrocytes were detected in the interalveolar septa and in the lumen of the alveoli. In addition, increased area% of interalveolar colla-

gen fibers was proved. Concomitantly, it was documented that the histopathologic appearance of amiodarone pneumonitis include septal thickening, interstitial oedema, non-specific inflammation and fibrosis (27). Amiodarone was found to upregulate angiotensinogen messenger RNA and angiotensin II was shown to promote fibrosis through stimulation of transforming growth factor- $\beta 1$ (28).

In amiodarone group, the previously described fields revealed no lining alveolar epithelium. It was postulated that amiodarone induce apoptosis of alveolar epithelial cells in daily and cumulative dosages due to decreased intracellular anti-apoptotic proteins (29).

In stem cell therapy group, no congestion or thickening

of the wall of pulmonary arterioles and venules were noted in multiple fields. This was proved morphometrically. On the other hand, accidental fields revealed pulmonary vessels with thickened wall. Weiss et al. (30) reported that adult tissue-derived stem cells can participate in the re-

generation and repair of diseased adult organs including the lungs. It was added that MSCs express intermediate to low levels of human leucocytic antigen (HLA) class I, low levels of HLA class II, and low levels of co-stimulatory molecules allowing the MSCs to escape alloreactive

Fig. 19. A rat in group S showing some spindle (s) and few cuboidal (cu) CD105 positive cells in the interalveolar septa ($\times 400$).

Fig. 21. A rat in group S showing some spindle (s) and few cuboidal (cu) CD105 positive cells in the lining epithelium and adventitia of a bronchiole ($\times 400$).

Fig. 20. Higher magnification of the previous figure showing +ve cytoplasmic reaction in CD105 positive cells (arrows) ($\times 1,000$).

Fig. 22. Thickness of interalveolar septa.

Table 1. Mean $\pm$ standard deviation (SD) of the thickness of interalveolar septa, thickness of the wall of pulmonary vessels and area % of collagen fibers in control and experimental groups

Groups	Thickness of interalveolar septa	Thickness of the wall of pulmonary vessels	Area % of collagen fibers
Control group	26.3720 $\pm$ 6.9345	6.0750 $\pm$.8602	1.1900 $\pm$ 0.2519
Group A (amiodarone group)	185.1300 $\pm$ 12.4240 ^a	78.0340 $\pm$ 12.7697 ^b	24.1320 $\pm$ 3.4653 ^c
Group S (stem cell therapy group)	25.5720 $\pm$ 3.8328	15.5920 $\pm$ 0.9710	2.7200 $\pm$ 0.4530

^aSignificant compared to control and stem cell therapy groups, ^bsignificant compared to control and stem cell therapy groups, ^csignificant compared to control and stem cell therapy groups.

Fig. 23. Thickness of the wall of pulmonary vessels.

Fig. 24. Area% of collagen fibers.

recognition. These properties result in modulation of the immune response by MSCs and have been the basis for clinical trials of allogeneic MSC administration to patients (31).

In the present study, few infiltrating cells were seen in the interalveolar septa, the latter thickness was comparable to control group. Similarly was the assessed area% of interalveolar collagen fibers. In addition, Prussian blue +ve and CD105 +ve cells were found in the septa. It was postulated that following systemic administration, lung injury results in increased localization and/or retention of cells in lung. The timing of cell administration after lung injury can influence phenotypic conversion of donor-derived cells. Early administration of MSCs resulted in engraftment of cells as epithelial and vascular endothelial cell while administration of cells at later time points resulted in engraftment as interstitial cells (32). Recently, the exogenous administration of MSCs was proved to attenuate bleomycin-induced lung injury by downmodulating the inflammatory responses and ameliorating fibrotic effects (33).

Alveoli with multiple lining cells exhibiting flat nuclei were found in stem cell therapy group compared to amiodarone group. MSCs derived from bone marrow and umbilical cord blood were documented to differentiate into alveolar epithelial cells, albeit at a very low frequency (34).

Few spindle Prussian blue +ve cells were found in the adventitia of small bronchioles existing near blood vessels. In addition, some spindle and cuboidal CD 105 +ve cells were detected in the lining epithelium of some bronchioles. It was reported that to restore functions of airway, epithelium has to rapidly repair the injuries and regenerate its structure and integrity. Stem cell populations are heterogeneous and comprise subpopulations capable of either

multipotent or unipotent differentiation leading to the restoration of a completely differentiated airway epithelium (35).

It could be concluded that amiodarone induced ILD that correlate to progressive deterioration of the respiratory function and the development of pulmonary hypertension. Cord blood mesenchymal stem cell therapy proved definite amelioration of morphological changes associating induced fibrosing ILD provided therapy starts early in the development of the pathogenesis.

Potential conflict of interest

The authors have no conflicting financial interest.

References

- Castelino FV, Varga J. Interstitial lung disease in connective tissue diseases: evolving concepts of pathogenesis and management. *Arthritis Res Ther* 2010;12:213
- Kinder BW, Shariat C, Collard HR, Koth LL, Wolters PJ, Golden JA, Panos RJ, King TE Jr. Undifferentiated connective tissue disease-associated interstitial lung disease: changes in lung function. *Lung* 2010;188:143-149
- Vanfleteren LE, Linssen CF. Role of microorganisms in interstitial lung disease. *Curr Opin Pulm Med* 2010;16:489-495
- Peerzada MM, Spiro TP, Daw HA. Pulmonary toxicities of biologics: a review. *Anticancer Drugs* 2010;21:131-139
- Freise J, Wehmeier P, Welte T. Drug-induced pulmonary interstitial disease. *Dtsch Med Wochenschr* 2010;135:450-454
- Taniguchi H, Ebina M, Kondoh Y, Ogura T, Azuma A, Suga M, Taguchi Y, Takahashi H, Nakata K, Sato A, Takeuchi M, Raghu G, Kudoh S, Nukiwa T; Pirfenidone Clinical Study Group in Japan. Pirfenidone in idiopathic pulmonary fibrosis. *Eur Respir J* 2010;35:821-829

7. O'Brien K, Troendle J, Gochuico BR, Markello TC, Salas J, Cardona H, Yao J, Bernardini I, Hess R, Gahl WA. Pirfenidone for the treatment of Hermansky-Pudlak syndrome pulmonary fibrosis. *Mol Genet Metab* 2011;103:128-134
8. Ding DC, Shyu WC, Lin SZ. Mesenchymal stem cells. *Cell Transplant* 2011;20:5-14
9. Bashir Q, Robinson SN, de Lima MJ, Parmar S, Shpall E. Umbilical cord blood transplantation. *Clin Adv Hematol Oncol* 2010;8:786-801
10. Maurer MH. Proteomic definitions of mesenchymal stem cells. *Stem Cells Int* 2011;2011:704256
11. Kolettis TM, Agelaki MG, Baltogiannis GG, Vlahos AP, Mourouzis I, Fotopoulos A, Pantos C. Comparative effects of acute vs. chronic oral amiodarone treatment during acute myocardial infarction in rats. *Europace* 2007;9:1099-1104
12. Osman FRO, Aboul Fotouh GI, Zickri MB, El Deeb D. Histological and ultrastructural study on the effect of some antiarrhythmic drugs on the thyroid gland of albino rat. M.D Thesis. 2004
13. Lee Y, Hwang J, Park S, Shin I, Kang S, Seo K, Lee Y, Kang K. Reduction of liver fibrosis by xenogeneic human umbilical cord blood and adipose tissue-derived multipotent stem cells without treatment of an immunosuppressant. *Tissue Engineering and Regenerative Medicine* 2008;5:613-621
14. Stocum DL, Zupanc GK. Stretching the limits: stem cells in regeneration science. *Dev Dyn* 2008;237:3648-3671
15. Dua HS, Joseph A, Shanmuganathan VA, Jones RE. Stem cell differentiation and the effects of deficiency. *Eye (Lond)* 2003;17:877-885
16. Koch TG, Heerkens T, Thomsen PD, Betts DH. Isolation of mesenchymal stem cells from equine umbilical cord blood. *BMC Biotechnol* 2007;7:26
17. Kraitchman DL, Heldman AW, Atalar E, Amado LC, Martin BJ, Pittenger MF, Hare JM, Bulte JW. In vivo magnetic resonance imaging of mesenchymal stem cells in myocardial infarction. *Circulation* 2003;107:2290-2293
18. Haasters F, Prall WC, Anz D, Bourquin C, Pautke C, Endres S, Mutschler W, Docheva D, Schieker M. Morphological and immunocytochemical characteristics indicate the yield of early progenitors and represent a quality control for human mesenchymal stem cell culturing. *J Anat* 2009;214:759-767
19. Kiernan JA. *Histological and Histochemical Methods: Theory and Practice*. 3rd ed. London, New York and New Delhi: Arnold Publisher: 2001.111-162
20. Bancroft JD, Gamble M. *Connective tissue stains*. In: *Theory and Practice of Histological Techniques*. 6th ed. London, Oxford, New York, Philadelphia, St Louis, Sydney and Toronto: Elsevier Health Sciences, Churchill Livingstone, Edinburgh; 2008.150
21. Ellis R. *Perls Prussian blue Stain Protocol*, Pathology Division. South Australia: Queen Elizabeth Hospital; 2007
22. Yagi H, Soto-Gutierrez A, Navarro-Alvarez N, Nahmias Y, Goldwasser Y, Kitagawa Y, Tilles AW, Tompkins RG, Parekkadan B, Yarmush ML. Reactive bone marrow stromal cells attenuate systemic inflammation via sTNFR1. *Mol Ther* 2010;18:1857-1864
23. Emsley R, Dunn G, White IR. Mediation and moderation of treatment effects in randomised controlled trials of complex interventions. *Stat Methods Med Res* 2010;19:237-270
24. Choi IS, Kim BS, Cho KS, Park JC, Jang MH, Shin MC, Jung SB, Chung JH, Kim CJ. Amiodarone induces apoptosis in L-132 human lung epithelial cell line. *Toxicol Lett* 2002;132:47-55
25. Wolkove N, Baltzan M. Amiodarone pulmonary toxicity. *Can Respir J* 2009;16:43-48
26. Sood BG, Wykes S, Landa M, De Jesus L, Rabah R. Expression of eNOS in the lungs of neonates with pulmonary hypertension. *Exp Mol Pathol* 2011;90:9-12
27. Schwaiblmair M, Berghaus T, Haeckel T, Wagner T, von Scheidt W. Amiodarone-induced pulmonary toxicity: an under-recognized and severe adverse effect? *Clin Res Cardiol* 2010;99:693-700
28. Papiris SA, Triantafyllidou C, Kolilekas L, Markoulaki D, Manali ED. Amiodarone: review of pulmonary effects and toxicity. *Drug Saf* 2010;33:539-558
29. Kapatou E, Skyras A, Agelaki MG, Pantos C, Kolettis TM, Malamou-Mitsi V. Amiodarone attenuates apoptosis, but induces phospholipidosis in rat alveolar epithelial cells. *J Physiol Pharmacol* 2010;61:671-677
30. Weiss DJ, Kolls JK, Ortiz LA, Panoskaltis-Mortari A, Prockop DJ. Stem cells and cell therapies in lung biology and lung diseases. *Proc Am Thorac Soc* 2008;5:637-667
31. Lanzoni G, Roda G, Belluzzi A, Roda E, Bagnara GP. Inflammatory bowel disease: Moving toward a stem cell-based therapy. *World J Gastroenterol* 2008;14:4616-4626
32. Sueblinvong V, Weiss DJ. Cell therapy approaches for lung diseases: current status. *Curr Opin Pharmacol* 2009;9:268-273
33. Malgieri A, Kantzari E, Patrizi MP, Gambardella S. Bone marrow and umbilical cord blood human mesenchymal stem cells: state of the art. *Int J Clin Exp Med* 2010;3:248-269
34. Moodley Y, Atienza D, Manuelpillai U, Samuel CS, Tchongue J, Ilancheran S, Boyd R, Trounson A. Human umbilical cord mesenchymal stem cells reduce fibrosis of bleomycin-induced lung injury. *Am J Pathol* 2009;175:303-313
35. Chistiakov DA. Endogenous and exogenous stem cells: a role in lung repair and use in airway tissue engineering and transplantation. *J Biomed Sci* 2010;17:92