Bone marrow-derived myocyte-like cells and regulation of repair-related cytokines after bone marrow cell transplantation

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Abstract

Objective: Whether bone marrow cells injected following acute myocardial infarction (MI) transdifferentiate into cardiomyocytes remains controversial, and how these cells affect repair-related cytokines is not known.

Methods: Autologous bone marrow-derived mononuclear cells (BM-MNCs) labeled with DiI, 1,1-V-dioctadecyl-1 to 3,3,3-V-tetramethylindocarbocyanine perchlorate, or saline were intravenously injected into rabbits 5 h following a 30-min ischemia and reperfusion protocol, and cardiac function and the general pathology of the infarcted heart were followed up 1 and 3 months post-MI. To search for regenerated myocardium, electron microscopy as well as confocal microscopy were performed in the infarcted myocardium 7 days post-MI. Expression levels of repair-related cytokines were evaluated by immunohistochemistry and Western blotting.

Results: Improvements in cardiac function and reductions in infarct size were observed in the BM-MNC group 1 month and 3 months post-MI. Using electron microscopy 7 days after infarction, clusters of very immature (fetal) and relatively mature cardiomyocytes undergoing differentiation were identified in the infarcted anterior LV wall in the BM-MNC group, though their numbers were small. These cells contained many small and dense DiI particles (a BM-MNC marker), indicating that cardiomyocytes had regenerated from the injected BM-MNCs. The expression of both transforming growth factor-β, which stimulates collagen synthesis and matrix metalloproteinase-1, a collagenase, were both down-regulated 7 days and 1 month post-MI in the BM-MNC group. Stromal cell-derived factor-1, which is known to recruit BM-MNCs into target tissues, was overexpressed in the infarcted areas of BM-MNC hearts 7 days post-MI.

Conclusions: Intravenous transplantation of BM-MNCs leads to the development of BM-MNC-derived myocyte-like cells and regulates the expression of repair-related cytokines that facilitate repair following myocardial infarction.

Keywords: Myocardial regeneration; Ultrastructure; Cardiac repair; Bone marrow; Cytokines

1. Introduction

Transplantation of bone marrow-derived mononuclear cells (BM-MNCs), including hematopoietic and mesenchymal stem cells, following acute myocardial infarction (MI) diminishes left ventricular (LV) remodeling, improves LV function, and reduces the size of old infarcts in post-MI hearts [1–3]. Nevertheless, the presence of BM-derived cardiomyocytes in infarcted myocardium remains controversial because although they are readily detectable in some cases [1], they are undetectable in others [4–6]. Contributing to this discrepancy may be an overestimation or underestimation of the numbers of BM-derived cardiomyo-
cytes as a result of technical problems with the methods used. For instance, autofluorescence can interfere with the confocal microscopic analysis of immunofluorescence. In addition, earlier studies of post-MI regeneration were carried out using models that involved the permanent occlusion of coronary arteries, though clinical treatment for acute MI generally involves recanalization of infarct-related coronary arteries by thrombolysis or percutaneous angioplasty. Bearing these issues in mind, one of our aims in the present study was to use electron microscopy to determine whether intravenously injected autologous BM-MNCs can transdifferentiate into cardiomyocytes during post-MI repair in a rabbit ischemia–reperfusion model.

We also recently reported that the beneficial effects of granulocyte colony-stimulating factor are likely associated with the expression of repair-related cytokines as well as with cardiomyocyte regeneration [7], which suggests that BM-MNCs may also exert an effect on the expression of repair-related cytokines. Therefore, the second aim of this study was to determine whether expression levels of transforming growth factor (TGF)-β, a mediator stimulating collagen synthesis; matrix metalloproteinase (MMP)-1, a collagenase; or stromal cell-derived factor (SDF)-1, a chemoattractant known to recruit BM-MNCs into target tissues, correlate with post-MI repair after BM-MNC treatment.

2. Materials and methods

All the rabbits received humane care in accordance with the Guide for Care and Use of Laboratory Animals, published by the U.S. National Institutes of Health (NIH publication 8523, revised 1985). The study protocol was approved by the Ethics Committee of Gifu University School of Medicine, Gifu, Japan.

2.1. Autologous BM-MNCs

Male Japanese white rabbits weighing 1.9–2.5 kg were anesthetized by intravenous injection with 30 mg/kg of sodium pentobarbital. Approximately 10 ml of iliac BM was aspirated and suspended in 20 ml of RPMI-1640 medium (Sigma) containing 2000 U of heparin sodium. BM-MNCs were then isolated by centrifugation on a Ficoll gradient (JIMRO, Takasaki, Japan), and approximately 1.0 × 10⁸ BM-MNCs were suspended in 2 ml of phosphate-buffered saline.

2.2. Ischemia–reperfusion infarct model and injection of BM-MNCs

One hour after the aspiration of BM, a 30-min ischemia and reperfusion protocol was carried out as previously described [7]. The rabbits were randomly assigned to either a BM-MNC or saline group, which were respectively administered the autologous BM-MNCs or 2 ml of saline via an ear vein after 5 h of reperfusion.

2.3. Protocol I: cardiac function and pathology

2.3.1. Echocardiography

The 90 rabbits in the BM-MNC and saline groups received trans-thoracic echocardiography (Aloka SSD4000) using a 7.5-MHz sector scan probe 7 days, 1 month and 3 months after the 30-min ischemia (n = 15 in each group). LV anterior wall thickness (AWT, mm), posterior wall thickness (PWT, mm), body weight-corrected LV end-diastolic dimension (EDD/BW, mm/kg), and LV ejection fraction (EF, %) were measured. All measurements were made by two examiners (Y.M. and M.A.) blinded to the conditions.

2.3.2. General pathology

Following the echocardiography, each of the 90 rabbits was sacrificed with an overdose of pentobarbital after heparinization (500 U/kg), after which body weight and LV weight were measured. The LV was then fixed in 10% buffered formalin and sliced into 7 transverse sections parallel to the atrio-ventricular ring from the apex to the base. The slices were embedded in paraffin, cut to a thickness of 4 μm, and stained with hematoxylin and eosin (HE) and Sirius red. For transversely sliced preparations with infarction, the LV wall areas, infarcted areas, non-infarcted areas, and Sirius red-positive collagen areas were calculated using an image analyzer connected to a light microscope (LUZEX-F, NIRECO, Tokyo) and expressed as mm²/slice/body weight (kg). Comparisons were made by two persons (Y.M. and M.A.) blinded to the conditions.

2.3.3. Immunohistochemistry

Using the indirect immunoperoxidase method, immunohistochemical staining was carried out with the following monoclonal antibodies (mAbs) that all cross-react with rabbit tissues: mouse anti-rabbit sarcomeric actin mAb (1:75; DAKO), mouse anti-human endothelial cell CD31 mAb (1:100; DAKO), mouse anti-human α-smooth muscle actin mAb (1:250; DAKO, 1A4), mouse anti-macrophage mAb (1:100; DAKO, RAM11), mouse anti-human MMP-1 mAb (1:500; Daiichi Fine Chemical, F-67), mouse anti-human TGF-β mAb (1:2000; Oxford Biotechnology,) and mouse anti-human/mouse SDF-1 mAb (1:120; R&D Systems). Morphometric analyses were carried out by two persons (Y.M. and G.T.) blinded to the conditions.

2.4. Protocol II: Dil-labeling of BM-MNCs, electron microscopy, laser scanning confocal microscopy and Western blotting

BM-MNCs were isolated from 12 rabbits as described above and then incubated for 30 min at 37 °C. To label the
cells with 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate dye (DiI, Molecular Probes), 25 μl of a 2-mg/ml solution of Dil in dimethyl sulfoxide (Wako, Japan) was added to the BM-MNCs from 6 of the rabbits [8,9]. Thereafter, those 12 rabbits plus 6 in a saline group were subjected to the 30-min ischemia and reperfusion protocol, and after 5 h of reperfusion, the autologous BM-MNCs, with or without DiI, or 2 ml of saline (6 rabbits in each group) were injected via an ear vein. Seven days post-MI, the rabbits were sacrificed under anesthesia, and the hearts were immediately excised and placed in iced PBS (less than 4 °C) and cut transversely at the center of the infarcted anterior LV wall. The upper half was subsequently used for electron microscopy and laser-scanning confocal microscopy, and the lower half was used for Western blot analysis.

Immediately after sacrifice (within 1 min), myocardial tissue from the upper infarcted anterior LV wall (AW-MI) and non-infarcted posterior LV wall (PW-NMI) of each heart was cut into 1-mm cubes, fixed in 2.5% glutaraldehyde in 0.1 mol/L phosphate buffer (pH 7.4) at 4 °C, and post-fixed in 1% buffered osmium tetroxide for 2 h. The samples were then dehydrated through a graded ethanol series and embedded in epoxy resin. One serial thin section (80 nm) was conventionally double-stained with uranylacetate for 10 min and lead citrate for 4 min and used for an electron microscopic (H-800, Hitachi) detection of ultrastructure. Another thin section stained with lead citrate for 10 min was used for the detection of Dil particles at magnifications greater than 20,000×.

In addition, other tissue samples obtained from the upper AW-MI and PW-NMI (three samples each, approximately 3 x 3 x 2 mm) were embedded in OCT compound (Miles Scientific), snap-frozen in liquid nitrogen and cut into 6-μm-thick sections using a cryostat for immunohistochemical analysis. Immunofluorescence microscopy was carried out using mouse anti-troponin I mAb (1:5000; DAKO) and mouse anti-human α-smooth muscle actin (a specific marker of smooth muscle cells) and microvessels positive for CD31 (a specific endothelial marker) were significantly larger in the BM-MNC group than the saline group (Fig. 2a and b), as were numbers of macrophages positive for RAM 11 (a specific marker of macrophages).

### 2.5. Protocol III: in vitro study

BM-MNCs and cultured adult cardiomyocytes [10] were left untreated or pretreated with Dil for 30 min at 37 °C, after which they were incubated for 7 days at 37 °C and processed for electron microscopy (fixed in 2.5% glutaraldehyde in 0.1 mol/L phosphate buffer [pH 7.4] for 30 min, post-fixed in 1% buffered osmium tetroxide for 1 h, dehydrated through graded ethanol and embedded in epoxy resin).

### 2.6. Statistical analysis

All values are presented as means±S.D. Differences between the saline and BM-MNC groups were assessed by two-way repeated-measures analysis of variance (ANOVA), followed with a post hoc Tukey-Kramer’s test. Linear regression techniques were used to evaluate the correlation between pathological/echocardiographic parameters and the expression of cytokines. Values of p<0.05 were considered significant.

### 3. Results

#### 3.1. Mortality

All rabbits in the saline and BM-MNC groups survived until sacrificed 7 days, 1 month or 3 months post-MI.

#### 3.2. Echocardiography

LV anterior wall thickness 1 and 3 months after MI was significantly greater in the BM-MNC group (2.2±0.2 and 2.3±0.2 mm, respectively) than the saline group (1.6±0.2 and 1.8±0.2 mm, respectively), but there was no significant difference in posterior wall thickness between the two groups at any time point as shown in Fig. 1a and b. In the saline group, additionally, EFs were significantly lower and EDD/BWs were significantly higher 1 month and 3 months post-MI than before infarction (Fig. 1c and d). These adverse effects were significantly attenuated in the BM-MNC group, indicating an improved LV function and reduced remodeling (Fig. 1c and d).

#### 3.3. General histology

Seven days post-MI, the infarcted areas showed mostly granulation with numerous myofibroblasts and small vessels in both the saline and BM-MNC groups. In addition, residual necrotic areas were observed in the centers of the granulated tissues. The numbers of small arteries positive for α-smooth muscle actin (a specific marker of smooth muscle cells) and microvessels positive for CD31 (a specific endothelial marker) were significantly larger in the BM-MNC group than the saline group (Fig. 2a and b), as were numbers of macrophages positive for RAM 11 (a specific marker of macrophages).
marker of macrophages) and multinuclear giant cells (Fig. 2c). On the other hand, the respective transverse diameters of the cardiomyocytes in the AW-MI and PW-NMI were similar in the saline and BM-MNC groups, though the size of the cells was significantly greater in the AW-MI than the PW-NMI in each group (Fig. 3a).

One and three months post-MI, LV weight and LV wall area were also similar between the BM-MNC and saline groups (Fig. 3b and c), and the infarcted areas showed scar tissue comprised of collagen and fatty tissue in both the saline and BM-MNC groups. When the LV walls were stained with HE and the collagen with Sirius red, however, the old infarcts were found to be significantly smaller in the BM-MNC group than in the saline group (Fig. 3d and e). Conversely, the non-infarcted areas were significantly larger in the BM-MNC group (Fig. 3f). In both groups, the numbers of CD31-positive capillaries, α-smooth muscle actin-positive small arteries, and RAM 11-positive macrophages were markedly smaller than the numbers seen 7 days after infarction and were similar (Fig. 2a–c).

### 3.4. Electron microscopy

An extensive search using electron microscopy revealed unfamiliar mononuclear cells in the AW-MI of BM-MNC hearts 7 days post-MI. Differing from normal adult cardiomyocytes with mature sarcomeres (Fig. 4a), these cells were relatively large and electron-lucent, and their cytoplasm lacked cross-striations, but was loosely or tightly packed with myofilaments, the size of which suggested they were thin filaments (Fig. 4b1). Although reminiscent of myofibroblasts or smooth muscle cells, these cells had several features that were specific to immature cardiomyocytes: first, they were connected to one another by intercalated discs, a specific structure of striated muscle cells, including cardiomyocytes, and the intercalated discs contained distinct desmosomes and gap junctions (Fig. 4b2, c2, e and f). In intercalated discs, we did not detect clear fascia adherence; the absence is probably due to not-well-developed myofilaments in the cytoplasm; and second, Z disc-like structures were scattered among the myofilaments (Fig. 4 c, c1 and d). There were small structures that appeared like myofilaments tied up into bundles in the vicinity of intermingling thick filaments, a configuration similar to the developing Z discs observed in fetal hearts [11]. Other, apparently more differentiated, cell types were also found, which contained better developed, but still not complete, Z discs (Fig. 4f). Such cells formed occasional clusters (Fig. 4b and c). Taken together, these findings suggest the presence of myocyte-like cells undergoing differentiation in the AW-MI of BM-MNC hearts, though their numbers were small: on average, the number of observed myocyte-like cells was 4.6±3.9/heart in the BM-MNC group and 0±0/heart in the saline group.

To determine whether these myocyte-like cells were derived from bone marrow cells, we first examined cultured BM-MNCs and isolated adult cardiomyocytes that had been incubated with DiI. We detected no DiI in specimens conventionally double-stained with uranyl acetate and lead citrate for electron microscopic examination (Fig. 5a).
However, when specimens were stained for only a short time with lead citrate alone, DiI was detected as 5-nm particles that accumulated within the cells and were scattered in the cytoplasm, mitochondria and lysosomes (Fig. 5b–d). These particles were not observed in cells that were not incubated with DiI. When DiI-labeled BM-MNC and control hearts were examined using the same method, DiI particles were detected in a total of 20 myocyte-like cells in the BM-MNC hearts (Fig. 5e–f) and were also occasionally found in pericytes present in the infarcted area (Fig. 5g and h). In addition, there was no evidence of DiI particles in BM-MNC and saline hearts not labeled with DiI.

### 3.5. Confocal microscopy

Within tissue sections obtained from the AW-MI of Dil-labeled BM-MNC hearts 7 days after MI, 0.14±0.13% of troponin I-positive (green) cells were also Dil-positive (red), and these cells formed a cluster (Fig. 6b). In addition, 0.05±0.04% of the cells positive for α-smooth muscle actin...
and 0.3 ± 0.3% of cells positive for CD31 were also positive for Dil.

3.6. TGF-β, MMP-1 and SDF-1

Immunohistochemical analysis showed TGF-β to be expressed in endothelial cells, smooth muscle cells and myofibroblasts (Fig. 7 and Supplementary data 1). In the saline groups, the level of expression within the infarcted areas was highest 7 days and 1 month post-MI (2.2 ± 0.3 and 2.4 ± 0.3, respectively) but had declined by 3 months (1.6 ± 0.8) (Fig. 8a). There was significantly less expression of TGF-β in the BM-MNC group 7 days and 1 month post-MI (1.4 ± 0.7) compared to the saline group (1.6 ± 0.8).

MMP-1 was expressed in cardiomyocytes located at the border between the infarcted area and salvaged myocardial tissues (Fig. 7). In the saline group, the level of MMP-1 expression was highest 7 days and 1 month post-MI (2.6 ± 0.4 and 2.4 ± 0.6, respectively), but dropped significantly by 3 months (1.4 ± 0.8) (Fig. 8b). As was seen with TGF-β, there was significantly less expression of MMP-1 in the BM-MNC group 7 days and 1 month post-MI (1.1 ± 0.5 and 1.3 ± 0.8, respectively).

Expression of SDF-1 was observed in endothelial cells, vascular smooth muscle cells, myofibroblasts and interstitial fibrous tissues within the infarcts 7 days post-MI. (Fig. 7 and Supplementary data 1) The level of SDF-1
Fig. 4. Ultrastructure of myocyte-like cells in the infarcted anterior LV wall of the BM-MNC group 7 days post-MI. (a) Normal adult cardiomyocyte showing characteristic striations and mature sarcomeres. (b) Relatively large and electron-lucent immature cells; (b1 and b2) highly magnified images of the boxed portions in panel (b). Note the thin filaments filling the cytoplasm (b1), and that the cells were perpendicularly connected to one another by well-developed intercalated discs (ID) (b2). Small triple arrowheads indicate gap junctions (b2). (c) Z disk-like structures in myocyte-like cells. (c1 and c2) Highly magnified photographs of the boxed portions of panel (c); the cells contain Z disc-like structures (arrows) scattered in the cytoplasm (c1). An arrowhead indicates desmosome-containing intercalated discs (c2). (d and e) Myocyte-like cells; High magnification of Z disc-like structures (d) and desmosomes within an intercalated disc (e). (f) More developed Z discs (double arrows) connected to one another by intercalated discs (ID). Black and white arrowheads indicate, respectively, desmosomes and gap junction in ID.
expression was significantly higher in the BM-MNC group (2.6±0.3) than in the saline group (0.8±0.3) (Fig. 8c). No expression was detected in either group 1 and 3 months post-MI.

As shown in Fig. 9, Western blot analysis 7 days post-MI showed that the enhanced expression of TGF-β in the LV anterior wall with infarction and the LV posterior wall in the saline group was significantly down-regulated in the BM-MNC group. Pro-MMP-1 expression was enhanced in the LV anterior wall of the saline group and the BM-MNC group. The enhanced expression of SDF-1 in the LV anterior wall 24 h after MI disappeared 7 days later in the saline group. In the BM-MNC group, however, SDF-1 was markedly expressed in both the LV anterior and posterior walls 7 days post-MI.

3.7. Correlation between cytokine expression, LV function and remodeling, and old infarct size

One month after infarction, levels of TGF-β and MMP-1 were negatively and significantly correlated with AWT/PWT ratios and EFs. They were also positively and significantly correlated with EDD and old infarct size (Fig. 8a and b).

4. Discussion

4.1. Injected BM-MNC-derived myocyte-like cells and pericytes—limitation of electron microscopic analysis

Our electron microscopic analysis suggests that very immature (fetal) as well as relatively mature mononuclear cardiomyocytes formed clusters in the infarcted area of the anterior LV myocardium in the hearts of rabbits receiving BM-MNCs following acute MI. These cells differed from cardiomyocytes fused with stem cells which retain the fine ultrastructure and cross-striations of mature cardiomyocytes and are multinuclear [6,12–14]. Each of the myocyte-like cells was positive for Dil particles (a marker of injected BM-MNCs), and Dil particles were observed in pericytes, as well. This finding is consistent with the idea that intravenously injected BM-MNCs are able to transdifferentiate into both myocyte-like cells and pericytes. As BM-MNCs include both hematopoietic and mesenchymal stem cells, it is unclear from which of these cell types the BM-MNC-derived myocyte-like cells are derived.

However, a limitation of electron microscopic analysis is that only very small areas of the tissue samples are actually observed. For that reason, we were unable to precisely determine the total numbers of BM-MNC-derived cells present in infarcted hearts. For the same reason, we cannot rule out the possible existence of cardiomyocytes fused with BM-MNCs. Conversely, negative electron microscopic findings do not imply that the cells are absent.

In the present study, our confocal light microscopic analysis of Dil fluorescence as well as electron microscopic analysis indicated that the incidence of BM-MNC-derived cardiomyocytes was very low in post-MI hearts. However, recent observations reported from several laboratories indicate that following i.v. injection [15], only a very small percentage of cells are actually retained in the heart. Thus, the very small percentage of the regenerated cells is not surprising. However, this observation does not prove that a higher degree of myocyte regeneration cannot be achieved following intramyocardial or intracoronary injection of bone marrow cells. Nor can this observation be extrapolated to imply that bone marrow cell therapy leads to minimal regeneration of cardiomyocytes.

4.2. Up-regulation of SDF-1

Up-regulation of SDF-1 expression was seen in myocardial tissues in the BM-MNC group 7 days post-MI. Given that SDF-1 is able to recruit circulating bone marrow-derived cells into targeted tissues [16–18], this up-regulation may be related to the appearance of BM-MNC-derived cardiac cells.

4.3. Mechanisms of improved LV function and reduced infarct size

A reduction in infarcted area via the formation of less scar tissue could only be supported by an increase in size of the preexisting myocytes and/or the formation of new myocyte, since the total LV area and weight were similar. In the present study, this reduction was equivalent to approximately 10% of the LV area. First, it is clear that the number of regenerated cardiomyocytes within infarcted areas was too small to explain the 10% increase in non-scarred areas. Second, the increase in LV area approximately 10% corresponds to the increase of approximately 3% in the transverse size of cardiomyocytes. Although a significant difference in the transverse size of cardiomyocytes was not observed between the BM-MNC and saline groups in the present study, there is no reliable method of detecting precisely the minimum increase in the transverse size of cardiomyocytes at present. Therefore, the increase in non-scarred areas would be considered to be due to a minimum hypertrophy of preexisting myocytes rather than the regeneration of cardiomyocytes. Further investigation is warranted.

Because the mediators contributing to cardiac development and remodeling are expressed only transiently and may thus be absent from healed and/or newly formed cardiac tissue [19], we assessed their levels at several points post-MI, with the aim of evaluating the causal relationship between changes in cytokine levels and LV function. Expression of TGF-β and MMP-1 is reportedly up-regulated following MI [20–22]. Similarly, in the
saline group, their expression was up-regulated 7 days and 1 month post-MI. However, we found that this up-regulation was significantly attenuated by administration of BM-MNCs. That the improvement in LV function was not seen in the BM-MNC group until 1 month post-MI means that the down-regulation of cytokines in the BM-MNC group 7 days post-MI preceded the improved LV function. Moreover, the reduction in TGF-$\beta$ and MMP-1 levels seen 1 month after infarction correlated significantly with the improvement in indicators of LV function (EDD, EF and AWT/PWT ratios), which is consistent with the earlier finding that inhibition of TGF-$\beta$ or MMP improves cardiac function in failing hearts [21,23,24]. We therefore suggest that the down-regulation of TGF-$\beta$ and MMP-1 likely contributed to the improved LV function seen in the BM-MNC-treated animals.

Finally, the old infarcts were smaller in the hearts from rabbits administered BM-MNCs than in those from rabbits administered saline, which is indicative of a reduction in the amount of collagen present at the infarct site. This reduction in infarct size is also consistent with the lower levels of TGF-$\beta$ expressed in BM-MNC hearts, given the ability of TGF-$\beta$ to induce collagen synthesis. Also, inhibition of apoptosis by a paracrine effect of BMT would be a possible mechanism for the beneficial effect, because the presence has been reported [25].

4.4. Transient increases in vessel density

Generally, vascular regeneration during the chronic stage of MI is enhanced by the injection of BM-MNCs [26]. In the present study, however, the increase in vascular density seen at the subacute stage (7 days post-MI) in the BM-MNC group disappeared during the chronic stage (3 months post-MI). This likely reflects the fact that we used a model of ischemia–reperfusion, which produced moderate infarctions, and differed from the models used in most earlier studies, which involved permanent occlusion.
and produced large infarctions. In the permanent occlusion model, ischemia within the risk areas continues even during the chronic stage; consequently, neovascularization in the form of collaterals is important. On the other hand, in hearts that are reperfused after ischemia, increased neovascularization may be beneficial for the absorption of necrotic tissues and formation of granulation tissue at the subacute stage, but its importance may be reduced at the chronic stage, when the major histological feature is scarring with few cells.
Fig. 8. BM-MNC treatment significantly down-regulated TGF-β and MMP-1 expression 7 days and 1 month post-MI, and up-regulated SDF-1 expression 7 days post-MI (a1, b1 and c1). Levels of TGF-β and MMP-1 were negatively correlated with AWT/PWT ratios and EFs and positively correlated with EDD/BW ratios and infarct size 1 month post-MI (a2–5 and b2–5).
Fig. 9. Western blot analysis 7 days after infarction. Note the up-regulation of TGF-β in both the AW and PW is up-regulated in the Saline group, but down-regulated in the BM-MNC group. Note also that the expression of Pro-MMP-1 in the AW is up-regulated in the Saline, but down-regulated in the BM-MNC group. Finally, note the marked up-regulation of SDF-1 expression in both AW and PW of the BM-MNC group.
4.5. Clinical implications

The clinical implications of the present findings are related to the fact that (1) our model of ischemia–reperfusion corresponds more closely to standard strategies for the treatment of human acute MI than earlier models utilizing permanent occlusion; and that (2) intravenous injection of autologous BM-MNCs is less invasive than direct or intracoronary injection and removes some ethical and technical barriers, such as donor–recipient mismatch and demand–supply imbalance.

In the present study, injected bone marrow cells were aspirated before infarction. However, Dimmeler’s group reported that bone marrow mononuclear cells derived from patients with ischemic heart disease showed impaired colony-forming capacity and migratory response to SDF-1 and VEGF. As bone marrow cells after the onset of infarction are available in the clinical setting, our findings based on the bone marrow cells obtained before infarction have limitations.

5. Conclusion

Intravenous transplantation of BM-MNCs leads to the development of BM-MNC-derived myocyte-like cells and regulates the expression of repair-related cytokines that facilitate repair in the post-MI heart.

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Appendix A. Supplementary data


References


