Recently, stem cell transplantation has emerged as a promising therapeutic method for acute myocardial infarction (AMI). However, its efficacy is limited by low survival rates of implanted cells. Therefore, to achieve successful stem cell therapy, it is important to protect the implanted stem cells against the harmful microenvironment in the injured heart. Our previous studies have demonstrated that short-term administration of low-dose statins can effectively increase the survival and differentiation of implanted bone marrow-derived mesenchymal stem cells (MSCs), although the underlying mechanisms behind the process have not been explored.

The Janus kinase (JAK)-signal transducers and activators of the transcription (STAT) pathway participate in the regulation of stress responses of the myocardium to various insults. This study aimed to detect whether rosuvastatin (ROSU) facilitates the survival, engraftment, and differentiation of allogeneic bone marrow-derived MSCs in the post-infarct heart via the JAK-STAT signaling pathway.

Methods and Results: Female Sprague-Dawley rats were randomized into 5 groups: AMI (control), ROSU gavage (group R), MSCs transplantation (group M), MSCs and ROSU (group M+R), or MSCs, ROSU and a JAK2 inhibitor AG-490 (group M+R+AG). MSCs from male rats were injected into the myocardium 1 week after AMI. Cardiac function and histology, as well as the expression of Y-chromosomal genes and JAK-STAT signaling proteins, were examined at 4 weeks after transplantation. Better functional recovery, increased survival and differentiation of MSCs occurred in group M+R. Furthermore, phosphorylation of JAK2 and STAT3 was higher in group M+R. The effects of ROSU, as well as of activated JAK-STAT proteins, could be attenuated by AG-490.

Conclusions: ROSU treatment improves the efficacy of stem cell transplantation in infarcted hearts by activation of the JAK2-STAT3 signaling pathway. (Circ J 2011; 75: 1476–1485)

Key Words: JAK-STAT; Mesenchymal stem cells; Myocardial infarction; Rosuvastatin
Methods

Animals and Experimental Design
MSCs isolated from male Sprague-Dawley rats weighing 60 g (23 weeks old) were transplanted into female Sprague-Dawley rats weighing 200–220 g (12–16 weeks old).

The recipient Sprague-Dawley rats were randomized into 5 groups (n=40 for each group): (1) AMI alone (group control), (2) ROSU treatment (8 mg/kg on the day before MI, and thereafter 4 mg·kg⁻¹·day⁻¹ orally for 5 weeks; group R), (3) MSCs transplantation (group M), (4) combined therapy of MSCs and ROSU (group M+R), or (5) MSCs, ROSU and a JAK2 inhibitor AG-490 (CalBiochem; 4 mg·kg⁻¹·day⁻¹ i.p. for 5 weeks; group M+R+AG). All animals received humane care in compliance with The Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health, USA. The experimental protocol was approved by the Care of Experimental Animals Committee of Fuwai Hospital.

Models of MI and MSC Transplantation
MI models in rats were established as described previously. Briefly, rats were anaesthetized using 10% chloral hydrate (4 ml/kg i.p.). After endotracheal intubation and initiation of ventilation (room air, rate 60 cycles/min, tidal volume 1 ml/100 g of body weight. Harvard Apparatus Rodent Ventilator, model 683), the heart was exposed through a left thoracotomy at the 5th intercostal space, and the left anterior descending coronary artery (LAD) was permanently ligated with a 6-0 polyester suture 1 mm from tip of the normally positioned left auricle. Successful ligation of the LAD was verified by myocardial blanching and abnormal movement of the anterior wall.

One week later, rats were re-anesthetized and their hearts re-exposed (as described above) for intramyocardial injections of 70 μl basal IMDM without cells (group control and R) or containing 2×10⁶ MSCs (group M, M+R and M+R+AG). The injections were performed at 2 sites around the infarct border zone and 1 site within the infarction zone with a 28-gauge needle.

Echocardiographic Assessment of Cardiac Function and Ventricular Dimensions
Transthoracic echocardiography (Sonos 7500; Phillips; equipped with a 12-MHz phased-array transducer) was performed to investigate the change in cardiac function before MSC transplantation (baseline) and 4 weeks after transplantation (endpoint) as previously described. The hearts (n=8 per group) were imaged in 2D and M-mode, and recordings were obtained from the parasternal long-axis view at the papillary muscle level. Left ventricular end-systolic dimension (LVEDd) and end-diastolic dimension (LVEDd) were measured for at least 3 consecutive cardiac cycles. Indices of left ventricular (LV) systolic function, including LV fractional shortening (LVFS) and LV ejection fraction (LVEF) were calculated using the following equations:

\[
LVFS (\%) = (LVEDd - LVESd)/LVEDd \times 100\% \quad \text{and} \quad LVEF (\%) = [(LVESd)^3 - (LVESd)^2]/(LVEDd)^2 \times 100\%.
\]

Histology
All rats were killed 4 weeks after MSCs transplantation. The heart tissue below the occlusion site was collected and embedded in OCT for the preparation of frozen sections (8 μm), or fixed in 10% formalin for the preparation of paraffin sections (5 μm) (n=6 per group, respectively).

For investigation of cardiomyogenic differentiation of engrafted MSCs, immunostaining for cTnl (Santa Cruz) was carried out in sections of the hearts with cell implantation. The MSC graft was identified by DAPI in the frozen sections. Immunofluorescence was detected with a laser scanning confocal microscope (Leica, Inc). To evaluate the vascular density, sections were immunostained with anti-rat CD31 (Santa Cruz). The capillaries that were positive for CD31 were counted using the Image-Pro-Plus software (version 6.0; NIH) in at least 5 high power fields (HPF, ×400) in the infarct and peri-infarct regions, which were randomly selected and counted from at least 3 sections from each animal (n=4 animals per group).

Masson’s trichrome stain was used to quantify the extent of fibrosis and infarct size in the LV. The fibrotic area and total area of the LV on each image were measured using the Image-Pro-Plus software, and the fibrotic area as a percentage of the total LV area was calculated as (fibrotic area/total LV area)×100%. For routine histopathology, heart sections were stained with hematoxylin–eosin (H&E). At least 5 thin sections from each heart were stained.

Analysis of Donor Cell Marker and Survival
Representing a male stem cell marker the Y-chromosomal DNA contents were determined by qRT-PCR at 4 weeks after cell transplantation. Briefly, DNA of the whole hearts from groups M, M+R, and M+R+AG (n=4–6 animals per group) was extracted using the Genomic DNA Purification Kit (Promega), and the concentration of the purified DNA was determined by spectrophotometry. The specific primers were designed according to the GeneBank sequences of the sex-determining region of Y-chromosome (SRY) gene of the rat (sense 5’-CATCGAAGGGTTAAAGTGCCA-3’, anti-sense 5’-ATA- GTGTTAGTTGTGGTCC-3’). The genomic DNA taken from male MSCs was used to obtain a standard curve. Real-time PCR was run with the ABI 7300 Sequence Detection System (Applied Biosystems). The primary curve method was used to calculate the threshold cycle (Ct), which is defined as the cycle at which the fluorescence level reaches a predetermined threshold. Ct was measured for each reaction and used to calculate the fold change of each experimental sample.

In addition, the number of surviving male donor MSCs within the female recipient hearts was calculated by counting the DAPI-positive cells under the fluorescence microscope. The morphology of the DAPI-stained nuclei was assessed and apoptotic figures were determined.

Western Blot Analysis
The expressions of JAK2, phosphorylation of JAK2 (p-JAK2), STAT1, phosphorylation of STAT1 (p-STAT1), STAT3, and phosphorylation of STAT3 (p-STAT3) proteins were analyzed by Western blot with specific antibodies. Total protein and nuclear extracts were prepared from the peri-infarct myocardial tissue. The concentrations of proteins were determined by BCA protein assay (Pierce, Rockford, IL, USA). Equal amounts of protein (40 ng protein/lane) were subjected to 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis in a Tris/HCl buffer system, and sequentially electrophoretic...
Table. LV Function and Geometry Parameters by Echocardiography

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>R</th>
<th>M</th>
<th>M+R</th>
<th>M+R+AG</th>
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<tr>
<td>LVEDd (mm)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>7.1±0.21</td>
<td>6.9±0.27</td>
<td>6.6±0.18</td>
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<tr>
<td>Endpoint</td>
<td>8.6±0.24</td>
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</tr>
<tr>
<td>∆</td>
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<td>1.3±0.20*</td>
<td>1.5±0.24*</td>
<td>0.2±0.12**</td>
<td>2.1±0.34**</td>
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<td></td>
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<td></td>
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<tr>
<td>Baseline</td>
<td>5.9±0.12</td>
<td>5.7±0.25</td>
<td>5.3±0.19</td>
<td>5.6±0.21</td>
<td>5.3±0.26</td>
</tr>
<tr>
<td>Endpoint</td>
<td>6.9±0.17</td>
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<td>6.4±0.24</td>
<td>5.2±0.29</td>
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<td>1.1±0.14*</td>
<td>−0.4±0.18**</td>
<td>1.3±0.23**</td>
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<td>LVEF (%)</td>
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</tr>
<tr>
<td>Baseline</td>
<td>51.6±2.0</td>
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<td>52.0±2.1</td>
<td>55.0±3.3</td>
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<tr>
<td>Endpoint</td>
<td>48.3±2.7</td>
<td>52.2±2.5</td>
<td>54.1±3.0</td>
<td>65.4±2.9</td>
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<td>1.8±1.1*†</td>
</tr>
<tr>
<td>LVFS (%)</td>
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</tr>
<tr>
<td>Baseline</td>
<td>21.5±2.2</td>
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<td>21.8±3.0</td>
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<td>1.1±0.9†</td>
<td>4.9±0.7**</td>
<td>−0.8±0.8*†</td>
</tr>
</tbody>
</table>

All values expressed as means±SEM.
Baseline=1 week after infarction; Endpoint=4 weeks after cell transplantation; ∆=change in values between baseline and endpoint.
*P<0.05 vs control; **P<0.001 vs all other groups; †P<0.05 vs control; n=8 for each group.
LV, left ventricular; LVEDd, LV end-diastolic dimension; LVESd, LV end-systolic dimension; LVEF, LV ejection fraction; LVFS, LV fractional shortening.
cally transferred to a polyvinylidene difluoride microporous membrane (Millipore, Billerica, MA, USA). After blocking with 5% non-fat dry milk in Tris-buffered saline (TBS) with 0.1% Tween 20 (TBST) for 1 h at room temperature, membranes were washed 3 times for 5 min in TBST. Next, blotting was conducted according to standard procedures with primary antibody: monoclonal rabbit anti-JAK2 (1:1,000), polyclonal rabbit p-JAK2 (Tyr1007/1008) (1:1,000), polyclonal rabbit STAT1 (1:1,000), polyclonal rabbit p-STAT1 (Tyr701) (1:1,000), monoclonal mouse STAT3 (1:1,000), polyclonal rabbit p-STAT3 (Tyr705) (1:1,000, all from Cell Signaling Technology), followed by incubation with a secondary goat anti-mouse or goat anti-rabbit antibodies conjugated to horseradish peroxidase (1:2,000, Immunology Consultants Laboratories). The specific bands of target protein were visualized by chemiluminescence. Membranes were then stripped and rebotted with monoclonal mouse anti-actin, beta (1:2,000, ProteinTech Group). Target signals were normalized to actin signal and analyzed semiquantitatively with the Quantity One software.

Statistical Analysis
All data are presented as means±SEM. Differences among multiple groups were analyzed using 1-way analysis of variance (ANOVA) followed by the Bonferroni/Dunnett test or unpaired t-test. P<0.05 was considered statistically significant. Statistical analysis was completed by SPSS15.0 (SPSS Science, Chicago, IL, USA).

Results
Of 200 rats, 37 (18.5%) died during the MI procedure, another 29 rats (14.5%) died during the first week after MI and during the injection procedures. The dead animals were excluded from statistical analysis.

Effect of ROSU+MSC Implantation on Functional Recovery
Figure 1 illustrates the echocardiography performed in the various treatment groups at baseline (1 week after AMI) and endpoint (4 weeks after MSCs transplantation). Baseline echocardiography revealed no significant difference between treated and control groups (Table, Figure 1). However, at 4 weeks after MSC transplantation, the LVEDd and LVESd in group M+R (P<0.001) were significantly different from the control group. But there was no difference between groups R, M and M+R+AG as compared with control (Table, Figure 1). LVEF and LVFS showed significant improvement in groups M (P<0.05) and M+R (P<0.001). The improvement was most...
striking in group M+R compared with group M (P<0.001) (Table). These results suggested that, although MSC transplantation alone led to a modest improvement in cardiac function, the combined therapy of ROSU and MSCs significantly promoted functional recovery of the heart and inhibited cardiac remodeling, which could be reversed by AG-490.

Effect of ROSU+MSC Implantation on Myocardial Infarct Size and Fibrosis
Although Masson’s trichrome staining showed transmural...
Rosuvastatin and JAK-STAT Signaling Pathway

infarction in all groups (Figure 2 A1), there was more surviving myocardium, less extracellular matrix accumulation and less fibrosis in the peri-infarct region in group M+R than in the other groups (Figure 2 A2). The LV fibrotic area was markedly reduced in group M+R (8.4±1.8%) as compared with the control group (18.3±2.8%, P<0.001), and no remarkable changes were observed in groups R (17.7±2.1%, P>0.05), M (16.1±2.0%, P>0.05) and M+R+AG (16.5±1.9%, P>0.05). The percent fibrotic area in group M+R was also significantly decreased as compared with groups M (P<0.001) and M+R+AG (P<0.001) (Figure 2 A3).

H&E staining showed the development of inflammation with the infiltration of numerous leukocytes in the infarcted hearts. Reduced inflammatory cell infiltration was observed in groups R and M+R as compared with the other groups (Figure 2B).

Effect of ROSU on the Survival of Implanted MSCs

We used 2 methods of evaluating the survival of implanted MSCs. Although qPCR analysis of SRY-specific genes showed that most donor cells did not survive 4 weeks after cell transplantation, significantly increased expression of SRY genes occurred in group M+R (8.90±0.74%, P<0.001, respectively) as compared with groups M (2.31±0.20%) and M+R+AG (3.89±0.33%) (Figure 3 A3).

On the other hand, there were more DAPI-labeled cells observed in group M+R (290.2±41.6, P<0.001) compared with group M (96.4±27.2). Notably, after treatment with AG-490, the number of DAPI-labeled cells was significantly reduced to 115.0±36.3 in group M+R+AG (Figures 3 B1, B2). Thus, both methods similarly indicated the survival tendency of implanted MSCs among groups. These results indicated that treatment with a combination of ROSU and MSCs increased the survival of implanted MSCs in post-infarction hearts.

Effect of ROSU on Vascularization and Myogenesis of Implanted MSCs

The capillary density was determined by anti-CD31 immunostaining of heart tissue sections from the infarct and peri-infarct areas (Figure 4A). Capillary density was expressed as the number of capillaries per HPF (×400). Quantitative analysis demonstrated that capillary density in the peri-infarct regions was significantly increased in group M+R (21.72±1.40; P<0.005 vs. control, R and M; P<0.001 vs. M+R+AG) than in the control (8.54±0.61), R (13.90±1.01), M (9.28±0.80) and M+R+AG (9.57±0.82) groups (Figure 4B). Similarly, the density in the infarct regions was also significantly increased in group M+R (19.40±1.12; P<0.005 vs. control, R and M; P<0.001 vs. M+R+AG) compared with the control (5.20±0.51), R (11.13±0.9), M (7.22±0.63) and M+R+AG (6.20±0.45) groups. However, the capillary density in both the peri-infarct and infarct regions of groups R,
M and M+R+AG were not significantly different compared with the control (P>0.05, respectively). These results indicated that the combined therapy of ROSU and MSCs was more favorable for vascularization than ROSU or MSCs alone, and the effects could be reversed by the addition of AG-490.

Immunofluorescent analyses showed that DAPI-labeled cells expressed the cardiac-specific protein c-Tnl. The efficiency of DAPI-labeled cell differentiation into cardiac myocytes was higher in group M+R than in groups M and M+R+AG (Figure 5).
Effect of ROSU on Phosphorylation of JAK-STAT Signaling Proteins

The expression of JAK2, STAT1 and STAT3, as well as p-JAK2 (Tyr1007/1008), p-STAT1 (Tyr701) and p-STAT3 (Tyr705), at the protein level was detected in the peri-infarct regions. JAK2, STAT1 and STAT3 were phosphorylated after MI in all groups (Figure 6A). The phosphorylation ratios of JAK2, STAT1 and STAT3 were significantly augmented in groups R and M+R compared with the control (P<0.01, respectively), and there was no difference in group M (P>0.05) as compared with the control. Treatment with AG-490 resulted in reduced p-JAK2, but did not change the expression of JAK2. Meanwhile, p-STAT1 and p-STAT3, downstream molecules of the JAK2 cascade, were almost completely inhibited by AG-490 treatment as indicated in group M+R+AG when compared with the control group (P<0.01, respectively) (Figure 6B).

Discussion

In the present study we used a rat model of AMI to investigate the therapeutic effects and underlying mechanisms of ROSU on the infarcted heart implanted with or without MSCs. The major findings are: (1) combined therapy of ROSU and MSCs significantly improved the regenerative effects of MSCs; (2) the JAK-STAT signaling pathway played an essential role in the ROSU-mediated effect on MSCs.

Previous studies have documented that MSC transplantation immediately after AMI did not significantly improve cardiac perfusion and function because of the poor survival rate of implanted cells in the post-infarct environment, which is rich in cytotoxic factors. Therefore, protecting engrafted cells from acute death in the ischemic myocardium is extremely important for exerting and maintaining the therapeutic effects of stem cell transplantation. Up to date, there have been several methods used to improve the fate of implanted cells, such as Akt, bcl-2, survivin and CXCR4 gene engineering. These methods are based on the levels of donor cells, but they may not be suitable for clinical application. Therefore, interventions aimed at improving the quality of local micro-environments to facilitate survival and biological behavior of implanted cells must be both effective and clinically practicable. Statins have been demonstrated to exert pleiotropic effects in the cardiovascular system, including anti-inflammatory and immunomodulatory properties, improving endothelial function, increasing the bioavailability of nitric oxide (NO), and antioxidant activity. ROSU is relatively hydrophilic, and thus has reduced access to many cell types throughout the body by diffusion across cell membranes in contrast to lipophilic statins, such as simvastatin and atorvastatin. Although simvastatin and atorvastatin can have protective effects on transplanted stem cells, they may not be suitable for clinical application. In the present study, we used a clinically relevant dose of ROSU to facilitate its effects on MSC transplantation. Our present results indicate that combined therapy of MSC and ROSU significantly enhanced the engraftment, survival and differentiation of MSCs, and improved cardiac function after AMI. However, ROSU alone provided limited benefits after AMI. Taken together, it is clear that the main effect of ROSU was to enhance the survival and regeneration efficacy of the implanted MSCs.

The JAK-STAT pathway has recently been shown to be an integral part of the response of the myocardium to various cardiac insults, including AMI, ischemia–reperfusion (I/R) injury, oxidative damage, hypertrophy and remodeling, in addition to having a prominent role in cardiac-protective therapies such as ischemic preconditioning ( IPC) and post-conditioning. Cardiac stress induces the formation of reactive oxygen species (ROS) and the release of cytokines and growth factors that bind to their cognate receptors. Subsequently, JAK2 tyrosine kinases recruit and activate downstream proteins such as STAT1 and STAT3. Activated STAT proteins then translocate into the nucleus, where they bind to the promoter region and regulate the transcription of target genes.

The majority of the available information about STAT activity in the heart is confined to the actions of STAT1 and STAT3. In a previous study, the JAK2-STAT3 pathway was rapidly activated in rat heart in the first 24 h after MI. Pretreatment AG-490 inhibited STAT3 activation, increased Bax expression and caspase-3 activation and thus resulted in augmented myocardial apoptosis in the infarcted heart. In another study, tyrosine phosphorylation of nuclear STAT1 and STAT3 was transiently increased after IPC, peaking at 30 min after IPC and subsiding almost completely by 2 h. All this evidence only shows that the JAK-STAT pathway is short-term activated and mediates cardioprotection efficacy in the early phase after cardiac stress. However, in the present study, we observed that JAK-STAT proteins were activated and persisted for at least 5 weeks (the whole study interval), although with a low activation ratio (phosphorylation protein/total protein) in the control group. Administration of ROSU further activated JAK-STAT proteins in the myocardium, whereas MSC transplantation alone had a limited effect. Furthermore, our results indicated that treatment with AG-490 significantly suppressed almost all the phosphorylation of JAK2, STAT1 and STAT3. This is probably that continuous activation of JAK-STAT signaling mediated the facilitated effects of ROSU.

The local microenvironment in the infarcted myocardium, mainly containing cardiac fibroblasts and cardiac myocytes, is affected by various factors, such as hypoxia, inflammatory responses and mechanical stretching. Cardiac fibroblasts play a central role in the maintenance of the extracellular matrix in the normal heart and as mediators of inflammatory and fibrotic myocardial remodeling in the injured heart. Obviously, not only cardiac myocytes but also cardiac fibroblasts would be a possible cellular source for STAT3 activation in the infarcted heart. Further in-depth work is necessary to identify which cells mediate the mobilization process of JAK-STAT signaling. In addition, previous studies have indicated that cross talk exists between the JAK-STAT and other pathways such as ischemic preconditioning (IPC) and post-conditioning. In a previous study, tyrosine phosphorylation of nuclear STAT1 was rapidly activated in rat heart in the first 24 h after MI. Pretreatment AG-490 inhibited STAT3 activation, increased Bax expression and caspase-3 activation and thus resulted in augmented myocardial apoptosis in the infarcted heart. In another study, tyrosine phosphorylation of nuclear STAT1 and STAT3 was transiently increased after IPC, peaking at 30 min after IPC and subsiding almost completely by 2 h. All this evidence only shows that the JAK-STAT pathway is short-term activated and mediates cardioprotection efficacy in the early phase after cardiac stress. However, in the present study, we observed that JAK-STAT proteins were activated and persisted for at least 5 weeks (the whole study interval), although with a low activation ratio (phosphorylation protein/total protein) in the control group. Administration of ROSU further activated JAK-STAT proteins in the myocardium, whereas MSC transplantation alone had a limited effect. Furthermore, our results indicated that treatment with AG-490 significantly suppressed almost all the phosphorylation of JAK2, STAT1 and STAT3. This is probably that continuous activation of JAK-STAT signaling mediated the facilitated effects of ROSU.

AG-490 is a specific JAK inhibitor with an IC50 of 10 μmol/L and a maximal effect at 50–100 μmol/L. Previous investigations have documented that AG-490 inhibits tyrosine phosphorylation of JAKs and subsequent STAT proteins. Pretreatment with AG-490 at a dosage of 5 mg·kg−1·day−1 can significantly block JAK-STAT activation in rats. In the present study, we chose a dosage of 4 mg·kg−1·day−1 based on significant inhibition of JAK2 phosphorylation in our preliminary experiment (data not shown) and previous reports. Our results demonstrated that AG-490 effectively blocked the activation of the JAK-STAT axis after MI, as evidenced by the fact that treatment with AG-490 sup-
pressed not only the tyrosine phosphorylation of JAK2 but also the tyrosine phosphorylation and nuclear translocation of STAT1 and STAT3. AG-490 has also been shown to have variable effects on infarct development. Xuan et al showed that AG-490 pretreatment did not affect infarct size before I/R. Negoro et al reported that AG-490 resulted in an attenuation of myocardial viability, whereas Mascarenos et al demonstrated an infarct size reduction following AG-490 pretreatment. These variable effects of AG-490 are likely dependent on multiple factors, including the length of time AG490 is applied and signaling pathway variability between specific conditions, in addition to the downstream modification of other signaling pathways such as PI3K–Akt and MAPK. Our findings also cannot exclude that the dose of AG490 used may have had nonspecific effects in the rat heart; however, the inhibitory effect of AG-490 on ROSU and JAK-STAT proteins is definite.

Research indicates that STAT3 activation elicits a protective response, whereas STAT1 is a mediator of cardiac myocyte apoptosis and myocardial injury. Cardiac-specific STAT3-knockout mice suffer from decreased LV capillary density, larger infarct size and a greater number of caspase-3 and TUNEL-positive cells following reperfusion compared with wild-type controls. STAT1 phosphorylation increases in cardiac myocytes following I/R injury and exacerbates cardiac damage through induction of pro-apoptotic effectors such as caspase-1, FAS and FASL. Moreover, STAT3 might counteract the pro-apoptotic effect of STAT1 in fibroblasts and cardiac myocytes. Cotransfection of increasing amounts of STAT3 with STAT1 in cardiac myocytes leads to a dose-dependent reduction in STAT1-induced apoptosis following ischemia. Intriguingly, although STAT1 and STAT3 are activated by distinct ligands, in the absence of STAT3, IL-6 results in prolonged activation of STAT1 and induces interferon-gamma-like effects. Conversely, STAT3 phosphorylation is enhanced and prolonged by interferon-gamma in STAT1-deficient cells. These findings suggest a model whereby STAT1 and STAT3 might inhibit each other’s activity under certain circumstances. Their relative abundance, which may vary substantially in different cell types, under different conditions is likely to have a major impact on how cells behave in response to different cytokines. As shown in the present study, STAT1 phosphorylation also existed in the infarcted myocardium and was suppressed by AG490. Considering that STAT1 and STAT3 might inhibit each other’s activity, it is probably that ROSU treatment activated STAT3 more prominently than STAT1, as indicated by the protection effects on MSCs. Modulation of the functional balance between STAT3 and STAT1, with preferential activation of STAT3 over STAT1, represents a mechanism by which ROSU improve MSC survival after transplantation.

Study Limitations

Firstly, we demonstrated the impact of statins on JAK-STAT signaling; however, downstream signaling of JAK-STAT was not investigated. Secondly, the observation time was not long enough to enable the evaluation of the long-term effects of ROSU-facilitated MSC transplantation. In conclusion, ROSU effectively facilitated the regenerative effects of stem cell transplantation by modification of the environment of the post-infarct heart, which might be expected via the JAK2-STAT3 signaling pathway. Modulation of the JAK-STAT pathway could be a potentially feasible method for increasing and maintaining the therapeutic effects of stem cell transplantation.

Acknowledgments

We appreciate Dr Fei-Feng Jin’s contributions to the MI models and MSC transplantation.

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