In vivo monitoring of magnetically labelled mesenchymal stem cells administered intravascularly in rat acute renal failure

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Summary

Questions under study: Many studies have demonstrated that mesenchymal stem cells (MSCs) contribute to the recovery of acute renal failure (ARF). The purpose of the present study was to evaluate in vivo tracking of MSCs intravascularly administered in a rat model of ARF for cellular therapy using a 1.5 T MRI system.

Methods: Fe2O3-PLL nanoparticle-labelled and unlabelled MSCs were injected into the abdominal aortas by transcatheterisation of 20 ARF rats, whose renal failure was induced by intramuscular injection of glycerol, while phosphate-buffered saline (PBS) was injected in 20 control rats. Magnetic resonance (MR) images of the kidneys were obtained, before injection of MSCs, after 1 hour, after 1, 2, and 4 days respectively. The MR imaging findings were correlated with the distribution of transplanted MSCs. The kidney injury was histologically evaluated, and the expression of proliferating cell nuclear antigen (PCNA) protein was examined. The Renal function was estimated by quantitative analysis.

Results: In the rat model of ARF the labelled MSCs showed a bilateral loss of signal intensity in the outer zone of the renal cortex on T2*-weighted MR images, which was visible up to 4 days after transplantation. Labelled MSCs were detected in glomerular capillaries by histological examination, the corresponding areas where signal intensity decreased in MR images. Compared to the control group, those ARF rats with MSCs injection had less renal injury, more enhanced tubular cell proliferation and better renal function.

Conclusions: MR imaging visualises those intravascularly administered MSCs in vivo, which promoted recovery of ARF.

Key words: magnetic resonance imaging; stem cells; acute renal failure; animal model

Introduction

Acute renal failure (ARF) characterised by a sudden deterioration in renal function may result from one or several factors such as ischaemic or toxic insults etc. Dysfunction or necrosis of renal tubular epithelial cells is the most common pathological change in ARF. Despite technical advances in renal replacement therapy and supportive therapy during the past decades, ARF remains a common intractable clinical problem [1].

Over the past few years, cellular therapy using...
stem cells has shown great therapeutic promise in the promotion of recovery of organ failure or injury, owing to the cells’ innate capability of regenerating damaged tissues and organs [2]. Among the various sources of stem cells, bone marrow-derived mesenchymal stem cells (MSCs) have been the most attractive recently. Bone marrow MSCs have the advantages of easy collection, rapid repopulation in vitro and in vivo, and are free of societal, ethical and legal disputes, etc. The plasticity of bone marrow MSCs has been widely investigated over the past years. Many studies have demonstrated that MSCs are able to form functional components of other tissues, such as the heart, liver, etc. [2, 3]. Although it is evident that MSCs can differentiate into renal tubular epithelial cells and contribute to the renal repair of acute tubular epithelial injury [4], the repop-tection obtained with MSCs is primarily mediated via complex paracrine actions instead of their differentiation into target cells in an investigation [5]. Therefore, this situation warrants further study to reveal the mechanism of stem cell therapy in ARF.

In order to shed light on the mechanism by which stem cell therapy works, it is essential for the fate of the transplanted cells to be tracked live in vivo following their migration, proliferation, differentiation and restoration. In the past few years, MR imaging depicting cells using a cell labelling technique with superparamagnetic iron oxide (SPIO) has become such a valuable technique [6, 7]. MR imaging using SPIO has been confirmed to be practicable for in vivo stem cell tracking [8]. In order to show the mineralisation, while adipogenic differentiation was visualised by means of oil red O staining of lipid vacuoles in the adipocytes differentiated from MSCs.

Methods

Cell culture and identification

This study was approved by our institutional Animal Use and Care Committee. MSCs were generated from the bone marrow of adult Sprague-Dawley rats aged 6 weeks weighing 120–150 g. The bone marrow cells were obtained by flushing the rat femurs with phosphate-buffered saline (PBS). The monocyte layer was selected and washed with PBS. The bone marrow cells obtained with MSCs was primarily mediated via complex paracrine actions instead of their differentiation into target cells. The bone marrow MSCs were incubated continuously for 12 h at 37 °C in a 95% air per 5% CO2 incubator.

The cells were harvested by removing the free Fe3O4-PLL washed four times with PBS. For the purpose of Prussian blue staining to identify the cell profile and intracellular iron particles, the cells were stained with nuclear fast red for 3 minutes. To measure the iron concentration within the cells, the cell suspension was adjusted to 1 × 106/ml of the culture solution, which was adjusted to 1 × 106/ml of the culture solution, which was adjusted to

Cell labelling and identification of the labelled cells

The Fe3O4-PLL nanoparticles were presented by Pro. N Gu (Laboratory of Molecular and Biomolecular Electronics, Southeast University, Nanjing, China). The physico-chemical properties of this SPIO and its efficacy and safety in vitro and in vivo of labelling on stem cells had been investigated previously in our laboratory [13]. The MSCs grew in 25 cm² flasks. The cells growing at Passage (P) were transferred to the culture media containing Fe3O4-PLL for labelling. The concentration of 20 µg/ml iron was used for culture based on our preliminary tests. The MSCs were incubated continuously for 12 h at 37 °C in a 95% air per 5% CO2 incubator.

The expression of membranous antigen on MSCs was detected by cytofluorimetric analysis with a flow cytometer [Becton Dickinson, San Jose, CA]. The primary mouse anti-rat monoclonal antibodies of anti-CD29, anti-CD45 (Becton Dickinson, San Jose, CA) were employed. The multipotency of MSCs was confirmed by induction of osteogenic and adipogenic differentiation with specific differentiation media [12]. Osteogenic differentiation was assessed by using von Kossa staining, a way to show the mineralisation, while adipogenic differentiation was visualised by means of oil red O staining of lipid vacuoles in the adipocytes differentiated from MSCs.

The purpose of the present study was to evaluate in vivo magnetically labelled bone marrow MSCs injected via trans catheterisation into abdominal aortas using a 1.5 T MRI system and to correlate the cellular trafficking to their therapeutic effect in rat model of ARF. To the best of our knowledge, this is the first study of in vivo MR tracking of the distribution of MSCs following local injection into abdominal aortas in an ARF model induced by intramuscular injection of glycerol, which has similar characteristics to clinical ARF, to evaluate the effects reported in the present study.
glutaraldehyde for 1 h, followed by 1% osmium tetroxide for 2 h. The samples of these cells were examined with a transmission electron microscope (H600, Hitachi, Japan).

Figure 1
Under an inverse microscopy (×100), Culture bone marrow mesenchymal stem cells (MSCs) (P3) derived from rat appear as uniform fibroblast-like cells with slim bodies.

Figure 2
Flow cytometric analysis shows that the mesenchymal stem cells (MSCs) are homogeneous on P3, uniformly positive for the surface proteins of CD29 and CD90, while they are negative for CD45.

Rat model of ARF and MSCs transplantation
A model of ARF was induced by intramuscular injection of glycerol in male SD rats, aged 7 weeks and weighing 200–300 g, based on a standard technique [14]. After anaesthesia with intraperitoneal injection of Pentobarbital (Shanghai Bioengineering Co., Shanghai, China) at 40 mg/kg, 50% glycerol (Shanghai Bioengineering Co., Shanghai, China) in normal sodium at dose of 8 ml/kg was injected into both posterior thigh muscles of the rats. Before glycerol injection, the animals were allowed to eat freely, but deprived of drinking water for 17 h. After successful induction of ARF 2 days after glycerol injection, the rats were randomly divided into three groups: group A (transplanted with labelled cells, n = 20), group B (transplanted with unlabelled cells, n = 20) and group C (control group, PBS injected only, n = 20). All the rats survived after the glycerol injection before the ending of this study.

Labelled and unlabelled MSCs were trypsinised, centrifuged, and washed with PBS, which were counted in a microcytometer chamber and resuspended in DMEM. A modified catheter from PE-50 line was inserted into the retrorenal aorta via the right femoral or iliac artery exposed surgically. One ml of DMEM containing 2×10^6 labelled or unlabelled cells, or 1 ml PBS was injected into the abdominal aorta via the catheter.

MR imaging
MR images of the kidneys of the recipients were obtained before injection of MSCs, and 1 h, 1, 2, and 4 days post transplantation, respectively. MR imaging was performed with a 1.5 T MRI system (Eclipse, Philips Medical Systems) using a 12.7 cm receiver with surface coil only. The MR imaging sequence was T2*-weighted gradient-echo (620/15.7; flip angle, 35°) sequence. Images were taken with a matrix size of 256 × 256, section thickness of 2.5 mm, and field of view of 8 × 6 cm, two measurements were acquired.

Mean signal intensities at four interesting regions in each kidney were evaluated by one of the authors (S.H.J.), an experienced radiologist practicing MR image analysis for 14 years, who was blinded to the transplanting procedure. Signal-to-noise ratio (SNR) of the outer zone of the renal cortex on T2*-weighted MR imaging calculated by dividing signal intensity by the background noise [10, 15] was obtained before and 1 hour, 1, 2, 4 days after transplantation, respectively.

Evaluation of renal function
Serum creatinine (SCr) and blood urea nitrogen (BUN) in blood samples collected from the tail vein of the rats were measured by an autoanalyser (7150, Hitachi, Japan) immediately after the MRI examinations before and 1, 2, 4 days post transplantation, respectively.

Histopathological and immunohistochemical analysis
Five rats selected randomly in each group were euthanised at 1h, 1, 2, and 4 days, respectively post cell transplantation following the MR examination. The histological examinations of the kidneys were performed immediately after sacrifice. After perfusion and fixation in 4% buffered paraformaldehyde for 24 h at least, 5 μm-thick sections were cut and stained with haematoxylin/eosin (H&E stain).

The degree of tubular injury was scored by a previously reported approach [5, 16] in random (using a simple random sampling table) cortical fields using a graduated grid with 25 squares with a ×20 lens. One hundred intersections between tubular profiles and the grid were examined in each kidney. A score for each tubular cross...
section per intersection was assigned as follows: 0 = normal histology; 1 = tubular cell swelling, loss of brush border, nuclear condensation, up to one-third of tubular cross section showing nuclear loss; 2 = same as for score 1, except for greater than one-third and less than two-thirds of nuclear loss per tubular cross section; and 3 = greater than two-thirds of tubular cross section shows nuclear loss. The total score for each kidney was calculated by the addition of all 100 scores (the higher the score, the more serious the tubular injury). The total injury score for each kidney was calculated by one of the authors (Z.L.M.) with 10 years’ experience of histopathological analysis who was blinded to the transplanting procedure.

Figure 3
Mesenchymal stem cells (MSCs) (P3) exhibit nodular aggregation on the von Kossa staining mineral (arrows) after osteogenic induction.

Figure 4
Intracytoplasmic lipid vesicles (arrows) are observed on the oil red O staining after adipogenic induction.

Figure 5
Intracytoplasmic blue particles in most mesenchymal stem cells (MSCs) (P3) incubated with ferric oxide nanoparticles and coupling with poly-L-lysine (FeO3-PLL) are clearly visible with Prussian blue staining (×400).

Figure 6
The endosomal vesicles (arrows) containing superparamagnetic iron oxide (SPIO) particles appear inside the mesenchymal stem cells (MSCs) (P3) in the transmission electron micrograph (original amplification: x15000).

Figure 7
Transverse T2*-weighted MR image of the rat kidney obtained before mesenchymal stem cells (MSCs) injection shows mild signal intensity decrease around the renal pelvis (arrows).

Figure 8
Coronal T2*-weighted MR image of the rat kidney obtained 1 h after injection with the labelled mesenchymal stem cells (MSCs) shows a signal intensity decrease (arrows) in the outer zone of the renal cortex.
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Signal intensity loss in the outer zone of the renal cortex (arrows) is detected on transverse T2*-weighted MR image of the kidney obtained 1 h after injection with the labelled mesenchymal stem cells (MSCs).

Figure 10

On the 4th day after labelled mesenchymal stem cell (MSC) transplantation, the signal intensity decrease (arrows) is still visible on the transverse T2*-weighted MR image.

Most cells positive for Prussian blue staining (arrow) are located in the glomeruli in outer zone of renal cortex in the kidneys of group A (transplanted with labelled cells), which corresponds to the signal intensity loss observed in MR image. (Prussian blue staining, ×100)

Table 1

Renal function parameters of the rats in 3 groups at different time points before and after mesenchymal stem cells (MSCs) or phosphate-buffered saline (PBS) administration.

<table>
<thead>
<tr>
<th>Groups</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>95% confidence intervals (CI) C-AB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum creatinine (SCr) (μmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before injection (n = 5)</td>
<td>519 (55)</td>
<td>490 (46)</td>
<td>510 (57)</td>
<td>–114–126</td>
</tr>
<tr>
<td>1 day post injection (n = 5)</td>
<td>499 (49)</td>
<td>437 (38)</td>
<td>483 (17)</td>
<td>–94–125</td>
</tr>
<tr>
<td>2 days post injection (n = 5)</td>
<td>194 (10)</td>
<td>211 (18)</td>
<td>331 (52)*</td>
<td>39–219</td>
</tr>
<tr>
<td>4 days post injection (n = 5)</td>
<td>91 (14)</td>
<td>85 (22)</td>
<td>154 (15)*</td>
<td>29–101</td>
</tr>
<tr>
<td>Blood urea nitrogen (BUN) (mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before injection (n = 5)</td>
<td>49 (6)</td>
<td>55 (6)</td>
<td>53 (9)</td>
<td>–17–18</td>
</tr>
<tr>
<td>1 day post injection (n = 5)</td>
<td>46 (7)</td>
<td>49 (7)</td>
<td>47 (6)</td>
<td>–13–11</td>
</tr>
<tr>
<td>2 days post injection (n = 5)</td>
<td>23 (4)</td>
<td>21 (4)</td>
<td>36 (4)*</td>
<td>6–24</td>
</tr>
<tr>
<td>4 days post injection (n = 5)</td>
<td>9 (2)</td>
<td>10 (3)</td>
<td>17 (2)*</td>
<td>3–13</td>
</tr>
</tbody>
</table>

Note: – Values are expressed as mean (SD).
Significant difference as compared with group A or B (P <0.05, two-tailed).
Prussian blue staining was performed to assess the localisation of the iron-loaded MSCs. Dual staining of Prussian blue and CD68 (immunohistochemistry) were used for identifying infiltrating macrophages that might be iron-positive. Immunohistochemistry assay of CD68 was performed on paraffin sections, stained with a mouse anti-rat monoclonal antibody of anti-CD68 (Biolegend, San Diego, CA).

Proliferative cell nuclear antigen (PCNA) was also determined on paraffin sections using a rabbit polyclonal antibody (Biolegend, San Diego, CA). The PCNA positive nuclei in the renal medulla and cortex were quantified under a light microscope at a magnification of 400 times and were expressed as mean values on day 4 post MSC administration based on the examination of 50 fields in each experimental animal by an author (Z.L.M.) who was blinded to the treatment of each rat according to a previously reported method [17].

Statistical analysis
Statistical analyses were carried out with the SPSS software (SPSS for Windows, version 11.0, 2001; SPSS, Chicago, Ill). Values were expressed as mean (standard deviation, SD). A consistent notation was adopted when reporting means and confidence intervals (CI). Differences of the means among the groups were analysed using one-way analysis of variance (ANOVA) followed by Dunnett’s or LSD test. A $p$ value (two-tailed) of less than 0.05 was considered as statistically significantly different.

Results
Characterisation of MSCs
When they were passed to $P_3$, the purified MSCs remained in the pattern of uniform fibroblast-like cells (fig. 1). These MSCs, as a single phenotypic population, were able to be further cultured and passed, and proliferated rapidly. The homogeneity of the cells reached 99%. The surface proteins, the characteristics of MSCs, such as CD29 and CD90, were uniformly positive, while CD45 as an indicative marker for haematopoietic lineage was negative (fig. 2). MSCs showed positive with von Kossa staining for the mineralisation nodules after osteogenic induction (fig. 3). Intracytoplasmic lipid vesicles were demonstrated by oil red O staining after adipogenic induction (fig. 4). The cells used in the subsequent experiments were those characterised by confirmation of their multipotency and the specific markers of the surface proteins.

In vitro cell labelling
Blue particles were clearly shown within almost every labelled cell on the Prussian staining slides (fig. 5), however they were not found in the unlabelled cells. The iron quantification per cell measured by atomic absorption spectrometer was 12.6 (2.6) picograms. The SPIO particles located in the endosomal vesicles of the cells were confirmed by transmission electron microscopy (fig. 6).

In vivo MR imaging of the labelled MSCs
Before injection of the labelled cells into the suprarenal aorta, a mild signal intensity loss was shown around the renal pelvis on T2*-weighted MR images (fig. 7). In group A, signal intensity loss in the outer zone of the renal cortex was demonstrated on T2*-weighted MR images 1 h after transplantation (fig. 8, 9). Since then, the remarkable visual regional signal intensity loss lasted for 4 days following the transplantation (fig. 10). Signal intensity loss was not detected in group B. Before and 1 hour, 1, 2 and 4 days after injection, the outer zone of renal cortex SNRs were 11.5 (1.5), 3.2 (0.8), 3.3 (0.8), 3.0 (0.9), 2.9 (0.8) respectively for group A, and 10.1 (1.2), 10.3 (1.8), 9.0 (1.2), 9.4 (1.7), 10.1 (1.8) for group B. Injection of labelled cells caused a significant SNR decline in renal cortex for group A at 1 hour, 1, 2, and 4 days after injection compared to group A rats before transplantation or group B control rats (ANOVA, $P < 0.001$; Dunnett’s test, $P < 0.001$, respectively, 95% CI: 6.4–8.7). For the control group B, there was no statistically significant difference in SNR at each time point compared to pretransplantation (ANOVA, $P = 0.799$, 95% CI: –3.1–1.9) (fig. 11).
Renal function of rats with ARF after MSC transplantation

Before the experiment of the cell transplantation, ARF model of the rats had been confirmed by a significant increase of SCr and BUN concentrations 2 days after glycerol administration, which reached the peak concentrations on the 2nd day following glycerol injection.

The measurements of renal function were shown by SCr and BUN changes of group A, B and C at different time points after transplantation (table). The Rats in group A and B had a significantly better renal function at 2 and 4 days, respectively post MSC administration than that in group C, as demonstrated by SCr (ANOVA, $P = 0.012$; LSD test, $P = 0.006$, $P = 0.011$, respectively, at 2 days; ANOVA, $P = 0.005$; LSD test, $P = 0.004$, $P = 0.003$, respectively, at 4 days) and BUN levels (ANOVA, $P = 0.006$; LSD test, $P = 0.006$, $P = 0.003$, respectively, at 2 days; ANOVA, $P = 0.011$; LSD test, $P = 0.006$, $P = 0.009$, respectively, at 4 days), while there were no significant differences of SCr (LSD test, $P = 0.637$, $P = 0.705$, respectively, at 2 days and 4 days) and BUN (LSD test, $P = 0.598$ $P = 0.771$, respectively, at 2 days and 4 days) levels between group A and B. There were no significant differences of SCr (ANOVA, $P = 0.800$) and BUN (ANOVA, $P = 0.572$) levels among three groups before MSC or PBS injection. There also were no significant differences of SCr (ANOVA, $P = 0.210$) and BUN (ANOVA, $P = 0.699$) levels among three groups on day 1 after MSC or PBS injection.

Histological analysis

In group A, positive Prussian blue staining, the characteristic phenotype of the iron-labelled MSCs, was revealed in the glomerular capillaries in the outer zone of the renal cortex, which corresponded well with the signal extinction observed on MRI, however it was not detected in the rats injected with unlabelled cells (fig. 12). None of the iron-loaded cells were positive for CD68 in the dual staining of CD68 and Prussian blue in kidney tissues (fig. 13), which was considered as a protein characteristic of macrophages. Few iron-labelled MSCs were found in the interstitial tissues of the kidney, and no iron-labelled MSCs were detected in the renal tubules.

Tubular epithelial injury was evident on day 2 post glycerol injection. The morphological changes observed in animals with glycerol-treated ARF included tubular hyaline cast formation and widespread necrosis of tubular epithelial cells (fig. 14). The tubular lesions of the rats in group A and B were less severe on the 4th day after MSC injection than in group C. The renal injury scores of the three groups were 103(27), 110(30), 201(30), respectively. The scores of group C were significantly higher than in group A and B (ANOVA, $P < 0.001$; LSD test, $P < 0.0001$, respectively, 95% CI C-AB: 57–133), while there was no significant difference in the renal injury scores between group A and B (ANOVA, $P < 0.001$; LSD test, $P = 0.660$, 95% CI C-AB: −42–27).

The numbers of PCNA positive tubular cells detected at day 4 after MSC or PBS injection in group A, B and C were 33(6), 34(6), 16(4)/HPF $< 0.0001$, respectively. The proliferation of tubular cells positive for PCNA staining was significantly higher in group A and B compared to group C (ANOVA, $P < 0.001$; LSD test, $P < 0.0001$, respectively, 95% CI C-AB: 15–20), While there was no significantly different between group A and B (ANOVA, $P < 0.001$; LSD test, $P = 0.76$, 95% CI C-AB: −2–3).

Discussion

ARF, which is associated with high morbidity and mortality, occurs frequently in hospitalised patients [18]. A series of circumstances, including primary renal diseases, impaired renal perfusion, hypovolaemia, sepsis, vascular occlusion and drug toxicity etc, leading to irreversible injury of the glomerular and tubular cells, culminates in acute renal failure [1]. Stem cell transplantation in kidneys was investigated in the past few years due to its potential capability for repairing various diseased organs and tissues [2, 3]. Successful differentiation into glomerular mesangial cells from the bone marrow-derived stem cells in rats has been reported [19]. Moreover, it has been demonstrated by several groups that transplantation of MSCs improved renal function, and MSCs reconstituted the renal epithelium by transdifferentiation [20]. However, the mechanisms by which renoprotection arises following MSC transplantation are disputed. Togel and colleagues [5, 21] indicated that the effectiveness of renoprotection obtained with MSCs for ARF induced by ischaemic injury was likely to be mediated primarily via complex paracrine actions instead of the MSCs' differentiation into target cells, because there was no evidence of transdifferentiation of the grafted MSCs into renal cells up to 3 days after implantation. The mechanism of glycerol induced ARF, which had been used in the present research, is different from the ischaemic induction of ARF mentioned above. The intramuscular administration of hypertonic glycerol induces rhabdomyolysis with a lot of myoglobin being released into the circulation with three major pathophysiological effects: renal vasoconstriction, direct cytotoxicity, and cast formation. Hypovolaemia and
metabolic acidosis facilitate tubular precipitation of myoglobin, besides causing tubular obstruction, may contribute to tubular epithelial cell injury through a probable haem-iron-mediated lipid peroxidation mechanism. This latter process further compromises the tubular fluid flow dynamics, and interferes with the complete clearance of tubular haem proteins, thus initiating the vicious cycle ultimately leading to ARF [14]. These are similar characteristics to clinical ARF.

During recent years, several noninvasive in vivo tracking molecular imaging techniques including nuclear medicine, optical imaging and MR imaging have been developed [22–24]. MR technique holds promising advantages due to its properties of wide imaging window, high temporal and spatial resolution, and good contrast but without ionic radiation. Recently, great progress in cell labelling techniques using SPIO has been achieved [13, 25]. SPIO particles nanometric in size have several advantages described previously, which include properties of biological degradation, ease of metabolism and integration into the serum Fe pool. These are similar characteristics to clinical ARF. Furthermore, the infusion of MSCs enhanced tubular proliferation and regeneration indicated by increased PCNA expression, and better renal function compared to those in the control group. Therefore, we are assuming that the renoprotective effect of MSC transplantation during the early stages of ARF induced by nephrotoxic injury is not mainly mediated by transdifferentiation mechanism but via complex paracrine actions. The finding that no significant differences of renal function and histological findings between the groups with labelled or unlabelled MSCs indicate that SPIO labelling has little influence on the cells’ biological properties in vivo.

In conclusion, this study demonstrates that the cells throughout both kidneys with a single perfusion. Therefore, we developed a new approach of injecting MSCs into the abdominal aorta above the renal arteries. It is expected that this new technique might replace the two approaches mentioned above. The results of this study indicate that ARF for 72 h, and the infusion of MSCs enhanced recovery of renal injury [21]. No Prussian blue staining-positive tubules and vascular endothelial cells were demonstrated in 4 days after cell injection, which suggests that the transdifferentiation process from the grafted MSCs into the renal tubular epithelial cells was uncertain up to 4 days post cell transplantation. However, the animals administered with MSCs had less severe tubular injury, more enhanced tubular proliferation and regeneration indicated by increased PCNA expression, and better renal function compared to those in the control group. Therefore, we are assuming that the renoprotective effect of MSC transplantation during ARF induced by nephrotoxic injury is not mainly mediated by transdifferentiation mechanism but via complex paracrine actions. The finding that no significant differences of renal function and histological findings between the groups with labelled or unlabelled MSCs indicate that SPIO labelling has little influence on the cells’ biological properties in vivo.

There were several limitations in this study. Firstly, the number of animals used in this experiment was small, which decreased the statistical power of our results although it was sufficiently large to obtain the main conclusion. Secondly, the long-term fate of the MSCs in the rat model of ARF was not observed due to the short-term follow-up of 4 days. Thirdly, the MR imaging, for its limited spatial resolution, failed to reveal the detailed anatomical structures of the kidney, which might reflect the cellular therapeutic outcomes, and therefore it was impossible to correlate the MRI findings with the histopathological findings of the renal injury.

In conclusion, this study demonstrates that...
MSCs intravascularly injected via abdominal aorta mainly distribute within the renal glomerular capillaries. The effectiveness of renoprotection is confirmed in a nephrotoxic ARF rat model. However, our study suggests that the mechanism of this renoprotective effect may not mainly depend on the transdifferentiation from the transplanted stem cells into the target renal cells. Our results indicate that MSCs’ vitality is not influenced by SPION labelling in vivo.

There is no doubt that cellular therapy has promising potential in renal disease, especially for end-stage renal failure, ARF or chronic acute failure, etc. Apparently, this in vivo monitoring technique is useful for future investigation of cell therapy in the kidney, especially in depicting in vivo cells administered by various approaches into kidneys and for studying the renoprotective mechanisms of cell therapy.

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References


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