Administered mesenchymal stem cells enhance recovery from ischemia/reperfusion–induced acute renal failure in rats

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Mesenchymal stem cells are renoprotective in ischemia/reperfusion–induced acute renal failure in rats.

Background. Adult stem cells are promising for the development of novel therapies in regenerative medicine. Acute renal failure (ARF) remains a frequent clinical complication, associated with an unacceptably high mortality rate, in large part due to the ineffectiveness of currently available therapies. The aim of this study was, therefore, to evaluate the therapeutic effectiveness of bone marrow–derived mesenchymal stem cells in a rat model of ischemia/reperfusion (I/R) ARF.

Methods. We used a common I/R model in rats to induce ARF by clamping both renal pedicles for 40 minutes. Mesenchymal stem cells were iron-dextran–labeled for in vivo tracking studies by magnetic resonance imaging (MRI) and kidneys were imaged for mesenchymal stem cells immediately after infusion and at day 3 after ARF. Renal injury was scored on day 3 and cells were additionally tracked by Prussian blue staining.

Results. We show in I/R-induced ARF in rats, modeling the most common form of clinical ARF, that infusion of mesenchymal stem cells enhances recovery of renal function. Mesenchymal stem cells were found to be located in the kidney cortex after injection, as demonstrated by MRI. Mesenchymal stem cells–treated animals had both significantly better renal function on days 2 and 3 and better injury scores at day 3 after ARF. Histologically, mesenchymal stem cells were predominantly located in glomerular capillaries, while tubules showed no iron labeling, indicating absent tubular transdifferentiation.

Conclusion. We conclude that the highly renoprotective capacity of mesenchymal stem cells opens the possibility for a cell-based paradigm shift in the treatment of I/R ARF.

Methods

All animal work was approved by the Institutional Animal Control and Utilization Committee. I/R ARF was induced in anesthetized (ketamine/xylazine), adult male Sprague-Dawley rats, weighing 300 to 400g

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examined.

A minimum of 16 fields of each kidney were disrupted, markedly attenuated, or missing tubular brush and more severe in the deep cortex. The mean number of parts of the cortex, since the injury was inhomogeneous was done to assure a representative sampling of different the medulla (the first field containing no glomeruli). This examined along these lines from the cortical surface to on the slides perpendicular to the cortical surface, and ally examined for disruption or loss of the tubular brush method as previously reported [14, 16], and addition-(PAS)–stained sections were scored by a standard iron-positive. The kidneys were formalin-fixed and blue to identify infiltrating macrophages that may be stained for CD68 (immunohistochemistry) and Prussian staining (Fig. 2E) in animals injected with free iron-dextran. Further details of the MRI data will be reported elsewhere (manuscript in preparation).

RESULTS

Iron-dextran incubation resulted in effective labeling of mesenchymal stem cells (Fig. 1A and B). Forty minutes of ischemia led to severe renal damage in control ARF animals, causing significant increases in serum creatinine levels (Fig. 1C) from a common baseline for both groups of 0.48 ± 0.13 mg/dL to 2.3 ± 0.3 mg/dL, 4.0 ± 1.1 mg/dL, and 2.7 ± 1.0 mg/dL at days 1, 2, and 3, respectively (Fig. 1C). Animals with ARF who received mesenchymal stem cells immediately post-reflow had a significantly better renal function on days 2 and 3 after ARF (Fig. 1C). Serum creatinine on day 1 was 2.4 ± 0.3 mg/dL, on day 2, 2.2 ± 1.1 mg/dL, and on day 3, 1.3 ± 0.7 mg/dL). Obtained serum creatinine data were qualitatively paralleled by changes in BUN levels (not shown), thereby further validating the utility of using serum creatinine as a marker of renal function in rats.

MRI 30 to 120 minutes after reflow showed loss of outer renocortical signal, consistent with the presence of iron-labeled mesenchymal stem cells in the kidney (Fig. 2A and B). This pattern persisted in all mesenchymal stem cells–treated rats until day 3, together suggesting the continued intrarenal presence of injected mesenchymal stem cells during this observation period. At no time point did infusion of free iron-dextran particles after ARF result in changes in kidney signal intensity (not shown), thereby excluding significant intrarenal accumulation of free iron-dextran. Histologically, on day 3, iron-labeled mesenchymal stem cells of characteristic phenotype (Fig. 1) were found predominantly in glomerular capillaries (Fig. 2C to E) at a frequency of 2 to 32 (average 17.6 cells per section) or approximately 30,000 cells per kidney, as calculated by volume, together corresponding well with the signal extinction observed on MRI. The ratio of cells found in glomeruli versus interstitial locations was 1.1. Mononuclear cell infiltration peaks at days 3 to 5 following ischemic ARF. As we did not detect a signal loss on MRI or iron staining (Fig. 2E) in animals injected with free iron-dextran only, it is unlikely that the signal in the kidneys is derived from infiltrating monocyes that have phagocytosed contrast material [15]. This possibility was further excluded by double-staining kidneys for CD68 and with Prussian blue, both of which failed to detect any iron-loaded macrophages (Fig. 2E). Renal injury was scored (0, intact kidney; 300, cortical necrosis) in all animals in blinded fashion by a standard method [14, 16].

Data are expressed as means ± SD. Differences between data means were analyzed by analysis of variance (ANOVA) or Student t test using Prism software (GraphPad, San Diego, CA, USA). A P value of less than 0.05 was considered significant.

(mesenchymal stem cells–treated group, N = 7) (control group, N = 6) by clamping both renal pedicles for 40 minutes. Reflow was visually confirmed, and, to reduce abdominal air, 4 mL warm normal saline was given intraperitoneally before abdominal closure.

Rat mesenchymal stem cells were generated from the bone marrow of adult Sprague-Dawley rats by standard procedures [12, 13]. Their phenotype and purity were confirmed by their ability to differentiate into adipocytes, chondrocytes and osteocytes as well as exclusion, by fluorescence-activated cell sorter (FACS) analysis, of contaminating CD45-positive cells, respectively. Furthermore, mesenchymal stem cells used in this study were cultured for more than 10 passages, which practically excludes hematopoietic cell contamination. Cultured mesenchymal stem cells were prelabeled for 24 hours with carboxy-dextran-coated iron oxide nanoparticles (Resovist) (Schering, Berlin, Germany), diluted 1:30 in media. Before trypsinization mesenchymal stem cells were washed with phosphate-buffered saline (PBS) to eliminate extracellular iron-dextran. After reflow, 1.5 × 10⁶ mesenchymal stem cells in 1 mL of normal saline were infused into the thoracic aorta via a carotid artery, controls received vehicle. Renal function was monitored by measurement of serum creatinine and blood urea nitrogen (BUN), using an autoanalyzer. The serum creatinine levels obtained in this fashion were confirmed by high-performance liquid chromatography (HPLC) (data not shown) and found to be identical. At 72 hours after ARF, animals in both groups were studied by MRI for in vivo tracking of administered mesenchymal stem cells, then sacrificed to obtain kidney injury scores and for localization of administered mesenchymal stem cells. Sequential kidney sections were stained with hematoxylin and eosin for injury scoring, with Prussian blue for tracking of iron-loaded mesenchymal stem cells, and double-stained for CD68 (immunohistochemistry) and Prussian blue to identify infiltrating macrophages that may be iron-positive. The kidneys were formalin-fixed and paraffin-embedded and 4 µ thick periodic acid-Schiff (PAS)–stained sections were scored by a standard method as previously reported [14, 16], and additionally examined for disruption or loss of the tubular brush border as a measure of tubular injury. Lines were drawn on the slides perpendicular to the cortical surface, and contiguous high power fields (0.41 mm diameter) were examined along these lines from the cortical surface to the medulla (the first field containing no glomeruli). This was done to assure a representative sampling of different parts of the cortex, since the injury was inhomogeneous and more severe in the deep cortex. The mean number of disrupted, markedly attenuated, or missing tubular brush borders per field were counted in a blinded fashion on coded slides. A minimum of 16 fields of each kidney were examined.
Mesenchymal stem cells–treated animals had a significantly lower ($P = 0.03$) injury score of 23 ± 4 compared to that of 88 ± 46 in controls.

Additionally, assessing brush border preservation in proximal tubules, a highly sensitive marker for surviving, functional proximal tubular cells (i.e., the site where injury due to I/R is most intense) showed on day 3 in mesenchymal stem cells–treated animals 47 ± 5.9% preservation of proximal tubular brush border was preserved and only 33 ± 7.4% in control animals ($P = 0.019$).

**DISCUSSION**

Labeling of cells with iron-dextran has been shown to be a nontoxic and long-lasting method for in vivo tracking of cells, including stem cells [17–19], as was shown in myocardial infarction models in which labeled mesenchymal stem cells were directly injected into the myocardium [20, 21]. In addition, their canonical ability to differentiate into adipocytes and osteocytes is preserved. Mesenchymal stem cells have also been shown to enhance recovery after myocardial infarction through mechanisms other than transdifferentiation into myocytes [6], and they were found to promote angiogenesis through paracrine mechanisms [22].

We demonstrate here that iron-dextran labeled mesenchymal stem cells injected after ARF can be histologically located in the kidney, significantly enhance the recovery of renal function, and that they can be tracked in the kidney using MRI. Because of the absence of iron-stained tubules and vascular endothelial cells, the mostly glomerular location of labeled mesenchymal stem cells, and their early effects on improving renal function on days 2 and 3, we hypothesize that transdifferentiation of administered mesenchymal stem cells into renal cells, a process that requires more than 3 days, is not their primary renoprotective mechanism. Whether the prolonged presence of mesenchymal stem cells in glomeruli is the result of specific homing or random entrapment remains to be elucidated. Whatever the mechanism for the localization of mesenchymal stem cells in the kidney turns out to be, the administration of mesenchymal stem cells in rats with ARF is associated with a faster recovery of renal function. In a cisplatinum model of renal failure, occasional transdifferentiation of administered mesenchymal stem cells into tubular cells was observed 28 days after cell infusion [8]. However, it is unclear if this mechanism contributed significantly to the renoprotection that was seen much earlier in the course of this toxic form of ARF (i.e., at a time when the authors did not quantitate the frequency of transdifferentiation events) [8]. In further support of our hypothesis that the obtained early recovery is affected by transdifferentiation-independent mechanisms are data reported using vascular endothelial cells and muscle-derived stem cells. In these, renoprotection in ARF was mediated by nitric oxide release and other transdifferentiation-independent mechanism [23, 24]. However, the exact mechanisms
underlying the better organ recovery of kidneys treated with mesenchymal stem cells in I/R ARF remain to be investigated.

In summary, we have shown that adult bone marow–derived mesenchymal stem cells can be detected in the kidney after I/R-induced ARF, and that they significantly enhance the recovery after renal failure by mechanisms distinct from transdifferentiation into renal tubular or vascular endothelial cells. We conclude that their robust renoprotective actions, likely mediated by various paracrine mechanisms, have substantial promise for the development of a novel, cell-based approach to the treatment of clinical ARF. In this fashion, we suggest, vascular, inflammatory and tubular injury components
of this largely treatment resistant complication can be simultaneously targeted. Since mesenchymal stem cells can be readily obtained from a patient at high risk for or with established ARF, and since they can be efficiently culture expanded, purified, and administered prophylactically, their use in clinical ARF may be most practical. Finally, the ease with which mesenchymal stem cells can be iron-dextran labeled and monitored by MRI in vivo provides a useful and safe tool to monitor the renal presence of administered mesenchymal stem cells and allows for the in vivo correlation of obtained renoprotection with the quality and duration of renal signal extintion by a given number of administered mesenchymal stem cells (i.e., providing a measure whereby the renoprotective effectiveness of a single or repeated cell dose can be gauged).

CONCLUSION
Our results demonstrate the effects of mesenchymal stem cells on enhanced recovery of renal function in I/R ARF and provide the basis for a new therapeutic concept for the treatment of ARF.

NOTE ADDED IN PROOF
Using 2 photon laser confocal microscopy and Y-chromosome FISH, we recently confirmed that the renoprotective actions of mesenchymal stem cells in acute renal failure are mediated by differentiation-independent mechanisms (Am J Physiol Renal Physiol 289:F31–F42, 2005).

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REFERENCES
22. KINNAIRD T, STABLE E, BURNETT MS, et al: Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms. Circ Res 94:678–685, 2004