Research Paper

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Contribution of Stem Cells to Renal Ischemia Repair

Biochemistry

KEYWORDS : Mesenchymal Stem Cells (MSCs)-Acute kidney Injury (AKI)-ischemia/reperfusion (I/R)-Acute renal failure (ARF)

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ABSTRACT

Background: The potential role of stem cells in the repair of glomerular and tubular injury is under intensive investigation. Several studies have addressed the role of endogenous bone-marrow (BM)-derived stem cells (SC) in the repair of renal injury.

Materials and Methods: MSCs were cultured and rats were used as a common I/R model to induce Acute renal failure (ARF) by clamping both renal pedicles for 45 minutes then stem cells were transplanted.Ki-67 was used as marker for regeneration of renal tubulointerstitial injury.

Results: shows increase in regeneration in renal tubulointerstitial in treated animals with MSCs more than untreated animals (control +ve). Conclusion: MSCs may be useful in treatment of renal ischemia and it can use as therapy of AKI.

INTRODUCTION

Acute renal failure continues to be associated with significant morbidity and mortality, despite increased understanding of its pathogenesis, novel therapeutic approaches, and the development of technical advances in therapeutic delivery [(1)]. Ischemia/reperfusion (I/R) injury, one of the most important causes of acute renal failure, cannot be avoided in some situations, including renal artery surgery, renal transplantation, atheroembolic disease, interventional radiologic manipulations to the renal artery, cardiac arrest, hypotensive states and shock. Injury following I/R depends not only on the duration of ischemia, but also on the reperfusion that follows [(2), (3)].

Stem cells play fundamental roles in the self-renewal of adult tissues throughout life. Bone marrow–derived hematopoietic stem cells have been discovered to transdifferentiate into cells of different germ layers [(5)]. MSCs are also present in the kidney and might be derived from the embryonic tissue or bone marrow. Bone marrow cells can migrate to the kidney and participate in normal tubular epithelial cell turnover and repair after AKI [(4)].

Based on this background, the objective of the present study was to test the therapeutic potential of mesenchymal stem cells, administered to rats following induction of ARF by ischemia/reperfusion (I/R) and showed the time that MSCs took to start the regeneration of renal tubulointerstitial injury that marked by the pathological change and uses of marker Ki-67 that shows if the regeneration occurred.

Materials and Methods:

The study included male Sprague–Dawley rats (body weight 200–250 g, 4–6 months old) that were bred in the animal house, faculty of science, Zagazig University, Egypt. The rats were randomly divided into three equal groups: (1) sham, where rats were subjected to right nephrectomy and exposure of the left renal pedicle with no ischemia; (2) positive control groups, subjected to right nephrectomy and left renal ischemia for 45 min; (3) treated groups as the control group, but were injected with MSCs after ischemia. Experimental animals were killed after 15 min, 3 days, 5 days, and 7 days from operation. I/R (ARF) were induced in anesthetized (ketamine/valpam) adult male Sprague Dawley rats by clamping both renal pedicles for 45 minutes. Then animals were injected with 4 ml warmed normal saline gave intraperitoneally before abdominal closure [(6)].

Cell culture and transplantation

Rat mesenchymal stem cells were generated from the bone marrow of adult Sprague-Dawley rats by standard procedures [(7), (8)]. Mesenchymal stem cells used in this study were cultured for more than 3 passages, which practically excludes hematopoietic cell contamination.

After 3 days of isolation non-adherent cells were removed by two to three washes with PBS and adherent cells further cultured incomplete medium. The medium was changed every 3 days until the monolayer of adherent cells reach 70-80% confluence. Trypsinization was made for cell splitting by Trypsin/EDTA solution (0. 25%, lonza, USA) for passage 1 .Number of cells were evaluated by Homocytometer and cellular viability by the Trypan Blue exclusion test. Each 250-300 × 10^3 cells were inoculated in 75 Cm2 culture flask that were incubated at 37°C and 5% CO_2. Cell cultivation was maintained up to the 3rd passage. After reflow, 2 × 10^6mesenchymal stem cells in 0.5 mL of complete medium were infused into the renal vein [(9)].

Histopathology study:

a) Histology

Explanted kidneys were bisected along the long axis and were fixed in 10% formalin solution for 24 h. After automated dehydration through a graded-alcohol series, transverse kidney slices were embedded in paraffin, sectioned at 5µ thick, and stained with haematoxylin and eosin (H&E). For the histopathological assessment of ischemic tubular injury [(10)].

b) Imunohistochemstry

Other sections were stained with Ki-67 imunohistochemstry marker to show the regeneration in renal tubulointerstitial cells according to a previously published guideline [(12)], and the staining protocol according a previously published guideline [(11)].

Results:

Cell culture

Attachment of spindle-shaped cells to tissue culture flask was observed after 1 day of culture BMSC. After 9 days, spindle shaped cells reached 80% confluency. Morphology of cells changed gradually with passage number. Cells become more flat-shape with increasing in passage number. BMSCs showed the ability to form multilayer after confluent **(Fig.1)**.

Histopathology study

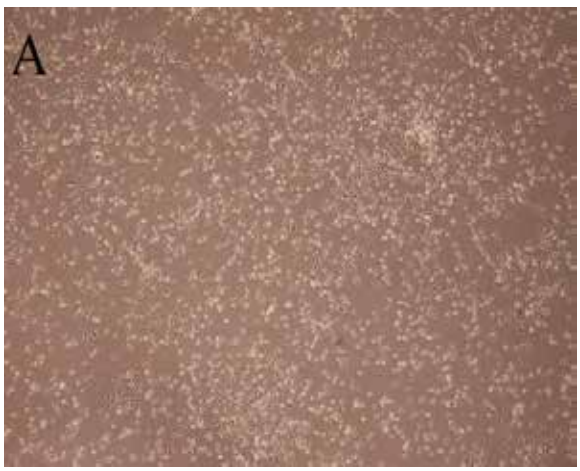
α) Histology

Forty five minutes of ischemia led to severe renal damage in positive control ARF animals. Three morphologic changes were assessed: (1) causing glomeruli shrinkage of vascular tuft, (2) in acute tubular necrosis (ATN) and tubular atrophy, (3) tubular regeneration **(Fig. 2, 3, 4 and 5)**.Treated animals with ARF that received mesenchymal stem cells immediately three morphologic changes were assessed: (1) decrease in ATN, (2) glomeruli

may be returned to its normal shape gradually, (3) increasing in regeneration in renal tubulointerstitial **(Fig.6, 7, 8 and 9).**

b) Imunohistochemstry

By using Ki-67 imunohistochemstry marker showed increased in regeneration in renal tubulointerstitial in treated animals more than untreated animals (control +ve) **(Fig.10, 11, 12 and 13)**.



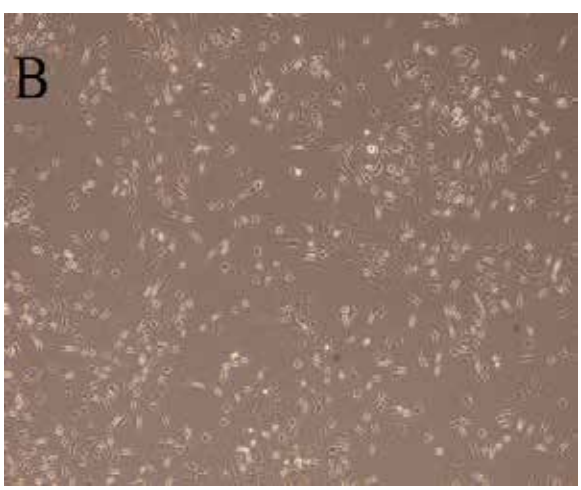


Fig.1. (A) shows Attachment of BMSCs in passage 1 Scale bar=100 µm, (2) shows Attachment of BMSCs in passage 2 Scale bar=100 µm, (3) shows Attachment of BMSCs spindle-shaped cells to tissue culture flask in passage 3 Scale bar=100 µm

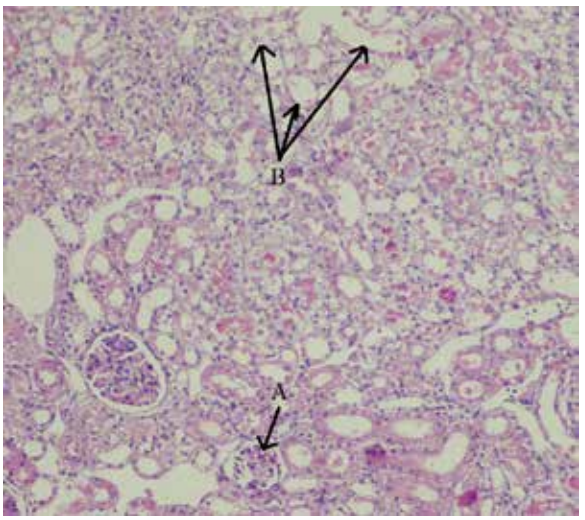


Fig.2. (A) Shows –glomeruli shrinkage of vascular tuft, (B) Tubules marked ATN and increase tubules necrosis, no regeneration occurred in untreated (control +ve) animals after 15 min of ARF Scale bar=100 µm.

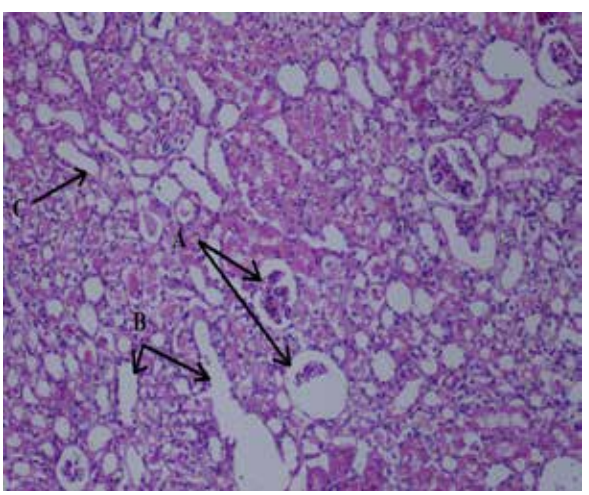


Fig.3. (A) Shows –glomeruli shrinkage of vascular tuft, (B) Tubules moderate tubular atrophy and ATN and (C) regeneration about 10% in untreated (control +ve) animals after3days of ARF Scale bar=100 µm.

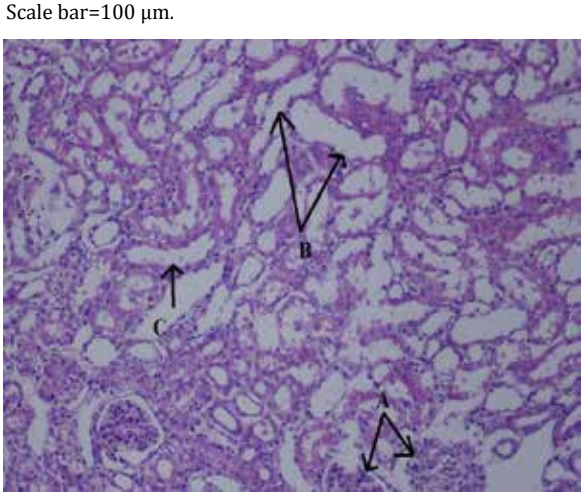


Fig.4. (A) Shows glomeruli congested capillaries, (B) Tubules marked ATN and increase tubules injury and (C) regeneration about 30%in untreated animals (control +ve) after 5 days of ARF Scale bar=100 µm.

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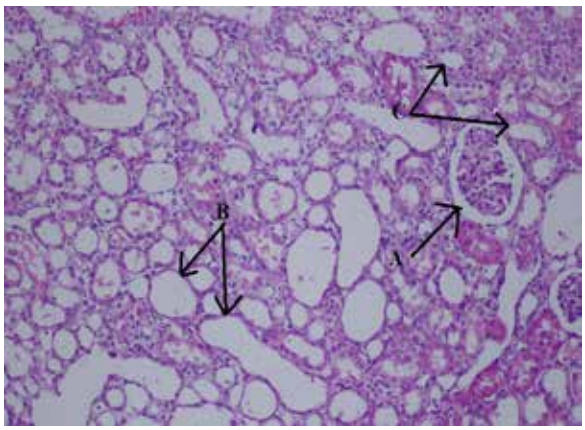

Fig.5. (A) Shows glomeruli normal, (B) Tubules marked ATN, tubules atrophy and marked dilatation and (C) regeneration about 40% untreated animals (control +ve) after 7 days of ARF Scale bar=100 µm.

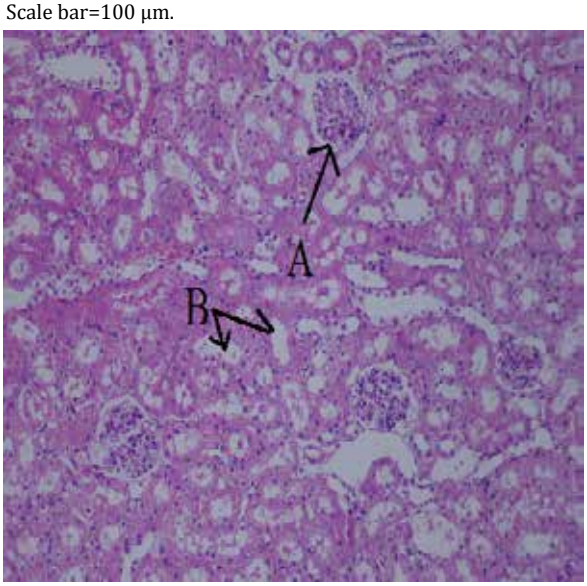

Fig.6. (A) Showed –normal glomeruli, (B) very mild ATN and congested blood vessels, little regeneration occurred in treated animals after 15 min of ARF Scale bar=100 µm.

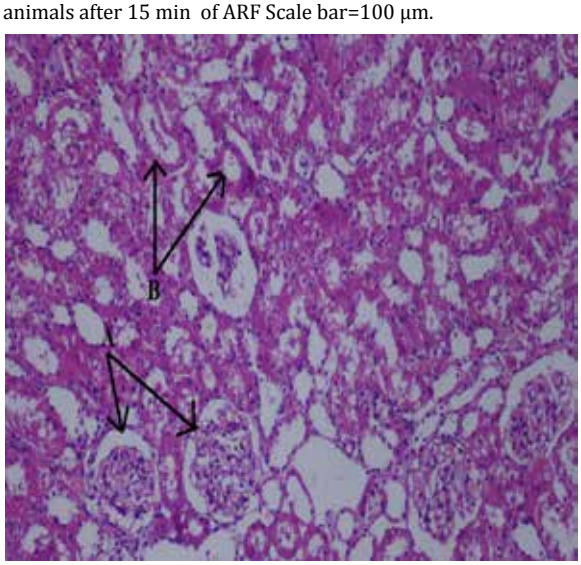

Fig.7. (A) Shows glomeruli mostly normal, (B) Tubules decrease in ATN and increase in regeneration and regeneration about 25% in treated animals after 3 days of ARF Scale bar=100 µm.

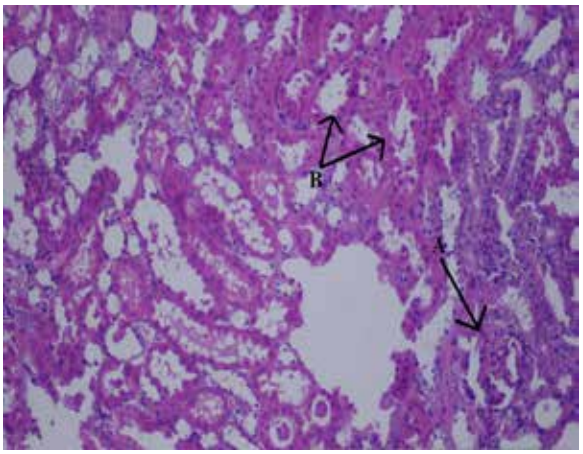

Fig.8. (A) Shows glomeruli congested capillaries, (B) more increase in regeneration and decrease ATN and regeneration about 50%in treated animals after 5 days of ARF Scale bar=200 µm.

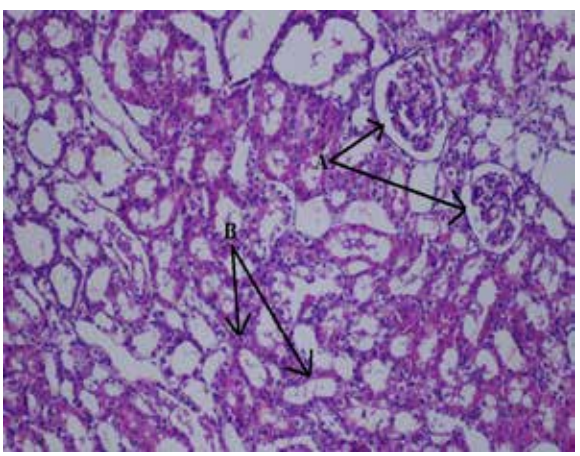

Fig.9. (A) shows glomeruli normal, (B) more increase in regeneration and decrease mild ATN in and regeneration about 75% treated animals after 7 days of ARF Scale bar=100 µm.

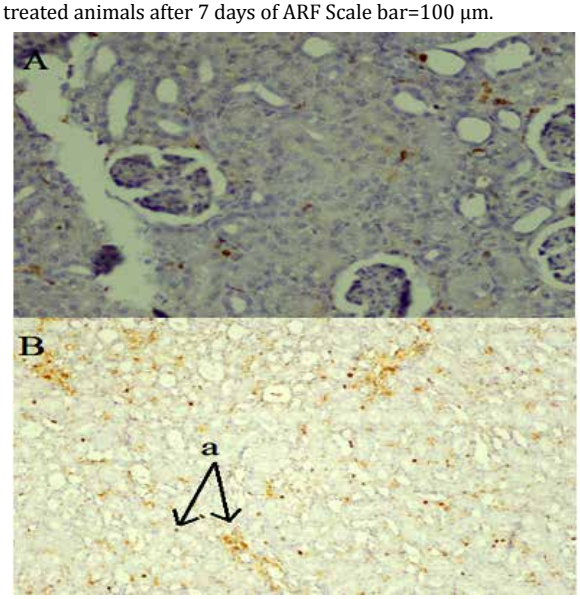

Fig.10. shows the difference between (A) untreated animals (control +ve) Scale bar=200 µm where there no in regeneration of renal tubulointerstitial cells and (B) treated animals after 15 min of ARF with Ki-67 marker which showed increase in regeneration of renal tubulointerstitial cells (a) in animals which injected with MSCs. Scale bar=100 µm

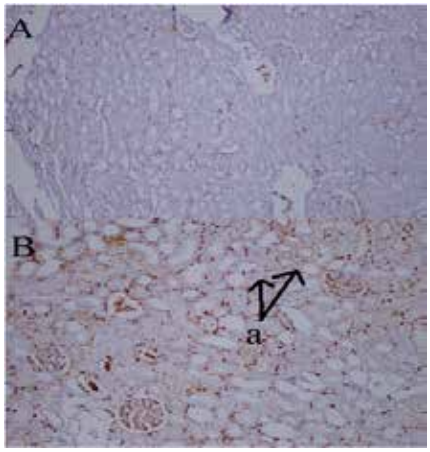

Fig.11. shows the difference between (A) untreated animals (control +ve) and (B) treated animals after 3 days of ARF with Ki-67 marker which showed increase in regeneration of renal tubulointerstitial cells (a) in animals which injected with MSCs Scale bar=100 μm.

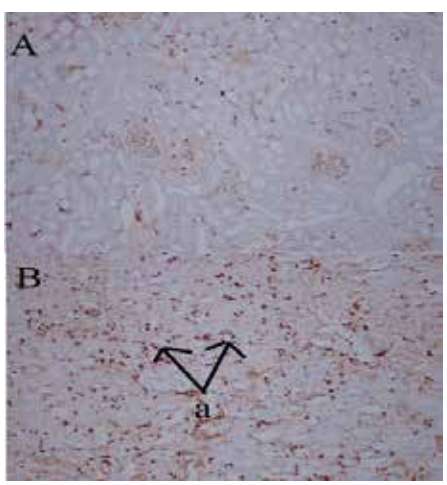

Fig.12. shows the difference between (A) untreated animals (control +ve) and (B) treated animals after 5 days of ARF with Ki-67 marker which showed more increase in regeneration of renal tubulointerstitial cells (a) in animals which injected with MSCs Scale bar=100 μm.

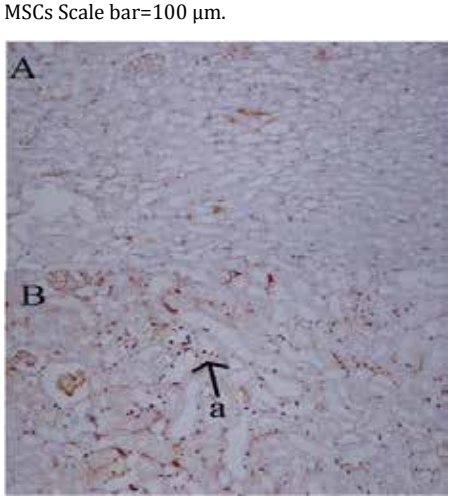

Fig.13. shows the difference between (A) untreated animals (control +ve) and (B) treated animals after 7 days of ARF with Ki-67 maker which showed more increase in regeneration of renal tubulointerstitial cells (a) in animals which injected with MSCs Scale bar=100 μm

Discussion:

In the present study, Mesenchymal stem cells were injected into the renal vein with 2×10^6 in 0.5mL of complete medium, and this agree with [9].

Histopathology study showed that sections were stained with Hematoxylin and Eosin and recorded that glomeruli shrinkage, tubules marked ATN, increase tubules necrosis and no regeneration in case of positive control untreated animals after 15 min occurred but in treated animals with MSCs recorded after 15 min recorded that normal glomeruli, very mild ATN, congested blood vessels and little regeneration occurred and this agree with[14], who reported that Ipost caused a significant improvement in renal function after renal I/R injury and in the other days showed that renal damage in positive control ARF animals, causing glomeruli shrinkage of vascular tuft and increase in acute tubular necrosis (ATN) and tubular atrophy but animals with ARF who received mesenchymal stem cells immediately post-reflow had a significantly decrease in ATN and glomeruli returned to its normal shape gradually with increasing in regeneration in renal tubulointerstitial cells, as time increase the regeneration increase, and this agree with [6], [14].

Bone marrow was isolated from rats from femurs and tibiae by flushing with DMED containing 10% FBS and 1% penicillin/streptomycin, and then was cultured for 24 hour after that the product was washed with FBS to remove adherent cells. The cells adhered to the flask and constituted a rapidly expanding into spindle shaped cells and fibroblast like cells and this agree with [15], and this in contrast to [16] who excluded BMSCs from his comparative study owing to their low growth rate. The low growth rate may be due to the unsuitable method used in the isolation.

Ki-67 imunohistochmistry marker which is expressed in proliferating cells was used to record the regeneration tubulointerstitial; in the present study as the time increased the regenerated increased and this marked by Ki-67 imunohistochmistry stain, and this agree with [13].

Conclusion:

MSCs may be useful in treatment of renal ischemia and it can use as therapy of AKI.

Acknowledgments:

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