

Bone Marrow Derived Mesenchymal Stem Cell Therapy in Induced Acute Renal Injury in Adult Male Albino Rats

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Abstract

Background and objectives: Acute renal injury (ARI) is a serious clinical problem without established regimen for treatment. Mesenchymal stem cells (MSCs) are undifferentiated cells that can differentiate and give rise to other cell types. The aim of the present work is to study the possible therapeutic role of MSCs in the treatment of the deleterious changes occurred in renal cortex and medulla of adult male albino rat model of ischemia/reperfusion (I/R) ARI.

Material and methods: Forty adult male albino rats were used in this study. Animals were divided into four groups, 10 animals for each group. Group I is control sham-operated group. Three experimental groups were all subjected to I/R injury by clamping both renal pedicles for 40 min. These animals were further divided into three subgroups. Group II is non-MSC treated group. Group III (MSC treated group (local injection)): I/R animals received bromodeoxyuridine labeled BM-MSCs locally in renal cortex immediately after removal of the clamps and confirmation of reflow. Group IV (MSC treated group (systemic injection)): I/R animals that received single intravenous injection of bromodeoxyuridine labeled BM-MSCs in tail vein immediately after removal of the clamps and confirmation of reflow. Animals were sacrificed after 3 days of intervention. Serological measurements included serum urea and creatinine. Kidney specimens were processed for H&E, PCNA and Caspase-3. Real time RT-PCR study was done for TNF α and IL-6 gene expression.

Results: Cortical and medullary renal tissue exhibited marked improvement histologically and serologically after MSCs treatment. The improvement was more obvious in case of systemic treatment.

Conclusion: The present study shows the ability of MSCs to repair the structural and functional renal damage after I/R, Systemic route is more beneficial than local route. This may pave the future for therapeutic use of MSCs in the treatment of acute renal injury.

Keywords: Acute renal injury; Ischemia reperfusion; Mesenchymal stem cells; Proliferating cell nuclear antigen

Introduction

The kidney is a very specialized organ; histological structure of the kidney shows more than thirty different cell types. Each cell type has its own proliferation rate and regenerative capacity [1]. Epithelial Cells lining inner layer of the Bowman's capsule "podocytes", have no proliferative capacity [2]. If these cells are lost, they cannot be replaced [3]. Cells of the proximal tubules have a low physiological turnover capacity. Upon pathological damage, these tubular cells respond with diffuse proliferation [1].

Acute kidney injury is a medical emergency characterized by rapid deterioration of renal functions as a result of kidney damage, with accumulation of the nitrogenous compounds (urea and creatinine) and non-nitrogenous waste products that are normally eliminated in urine [4]. In severe cases, metabolic acidosis hyperkalemia, and changes in body fluid balance may be observed. Oliguria or anuria is the most common symptom [5].

Acute renal failure is mostly the end result of Ischemia-reperfusion (I/R) injury of the renal tissue. Its pathogenic events include acute tubular necrosis, apoptosis, glomerular injury and inflammation. It is characterized by sharp decline of glomerular filtration rate (GFR), is a very common complication in hospitalized patients and particularly in patients with multiorgan failure. Although it develops most frequently in multimorbid patients, its occurrence increases the risk of death by 10 to 15 folds [6]. This unacceptable situation warrants the urgent development of new treatment modalities.

Mesenchymal stem cells of Bone marrow-derived are multipotent stromal cells, which can give rise to osteocytes, chondrocytes, and adipocytes. Recent studies showed their capacity to give rise endothelial, myocardial [7], liver [8], renal [9], and pulmonary epithelial cells [10].

Mesenchymal stem cells have also been shown to have reparative, regenerative and immunomodulatory properties [11] and express growth factors known to be renoprotective in experimental ARF [12]. Stem cell-based approaches have enormous potential for the development of future therapies [13]. In recent years, many studies have demonstrated the therapeutic capacity of different stem cell populations.

Because of their easy accessibility and manipulation as well as their capacity to differentiate into a wide variety of other cell types; Bone marrow derived mesenchymal stem cells are promising for clinical applications as they are ideal vehicles for cellular gene transfer [14].

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Aim of the present work is to study the possible reno-protective effects of BM-MSCs on induced acute kidney injury in a rat model of ischemia reperfusion, and to find out if there any difference in the outcome between local and systemic route of administration, using histological, immunohistochemical and biochemical methods.

Materials and Methods

Experimental animals

This study was conducted on 40 adult male spargue-dawley albino rats. They were 12 weeks old, weighing 150-200 g. The animals were housed in hygienic plastic cages and kept in clean well-ventilated room, with food and water ad-libitum. All animals' procedures were done according to the recommendation of El- Minia University Ethics committee for proper care and use of experimental animals.

Animals were divided into four groups, each group has 10 animals as follows

1) Group I (control sham-operated): 10 rats were subjected to sham operation, and then received intravenous saline.

2) Experimental group: 30 rats were subjected to renal ischemia-reperfusion (I/R) injury by clamping both renal pedicles for 40 min.

Rats were divided as follows:

A) Group II (untreated group): After removal of the clamps and visual confirmation of reflow. The animals received intravenous saline injection, and they were sacrificed after 3 days.

B) Group III: 10 rats received single intravenous injection of bromodeoxyuridine labeled BM-MSCs locally in renal cortex just after removal of the clamps and visual confirmation of reflow. They were sacrificed 3 days after removal of clamps.

C) Group IV (systemic treated group): 10 rats received single injection of MSCs in tail vein just after removal of the clamps and visual confirmation of reflow. They were sacrificed 3 days after MSCs injection.

Preparation of Bone Marrow Derived Mesenchymal Stem cells

The animals were anesthetized by halothane, and then the skin was sterilized with 70% ethyl alcohol before cutting the skin. The femurs and tibia were carefully dissected from adherent soft tissues. Then they were placed into sterilized beaker containing 70% ethyl alcohol for 1-2 min. The bones were put in Petri dish contain Phosphate buffer saline 1X PBS (Hyclone, USA) for wash. The bones were taken to laminar air flow (unilab biological safety cabinet class II, china) to extract the BM. The two ends of the bones were removed using sterile scissors.

Bone marrow was collected by rapid infusion of the tibiae and femurs of 12 weeks adult male albino rats with Dulbecco's modified Eagles medium (DMEM) (lonza, Belgium) supplemented with 10% fetal bovine serum (FBS) (lonza, Belgium) and 1% Antibiotic, Antimicotic (penicillin streptomycin) (lonza, USA). To isolate the nucleated cells density gradient ficol/paquet (pharmacia fine chemicals) was used. The isolated cells were cultured in 20 ml complete media and incubated at 37°C in 5% humidified CO₂ incubator (shellab, USA) for 7-10 days as primary culture or upon formation of large colonies. MSCs in tissue culture are fusiform in shape and adhesive to the polystyrene dish. Adherent MSCs were washed with phosphate buffered saline and dislodged from the cultured dish using Trypsin/EDTA solution, then

centrifuged and re-suspended in cultured media enriched with serum [15,16].

Labeling of stem cells with bromodeoxyuridine dye

MSCs were harvested during the 2nd passage and were labeled with bromouridine fluorescent linker dye [17].

Counting of cells

Washed cells were resuspended in 1 ml of appropriate media. From this cell suspension, 10 µl was removed for counting. Depending on the estimated (using a microscope) cell number, a dilution factor between two and ten was used to count cells. Test the cell viability 10 µl of cells was add to 10 µl of Trypan blue 0.4% (lonza, USA) and mixed well. 10 µl of the mixture was taken and put on hemocytometer (Neubauer, Germany) and cells were counted under ordinary microscope (Olympus CX31, USA). Then use this equation:

$$\text{NO of cells/ml} = \text{average of count cells} \times \text{dilution factor} \times 10^4$$

Injection of MSCs

Immediately after reflow, rats of group III received 0.5 ml labeled MSCs (2×10^5) diluted with 1 ml of saline, loaded in a 1ml sterile syringe and injected *via* tail vein for each rat [18]. Group IV received 2×10^5 labeled MSCs diluted with 1 ml of normal saline, loaded in a 1 ml sterile syringe and injected systemically *via* tail vein for each rat [19].

Induction of ischemia-reperfusion acute kidney injury

Animals were anesthetized and Midline abdominal incision was done. Kidneys were exposed and renal pedicles were bilaterally clamped for 40 min to induce renal ischemia [20]. The clamps were then removed to allow kidney reperfusion. Kidneys were observed to ensure reflow. The incision was closed followed by topical application of antibiotic cream.

Sham operation: Sham-operated animals are subjected to the same steps without applying the clamps [21].

Laboratory investigations

Blood samples were collected from retro-orbital veins using capillary tubes. Urea and creatinine were measured for all rats, before and after the operation.

Total RNA extraction and real time RT-PCR for TNF α and IL-6 gene expression

Total RNA was isolated from renal tissue using RiboZol solution (Amresco, Solon, USA) according to the manufacturer's instructions. The relative purity of the isolated RNA was assessed spectrophotometrically, and the ratio of A260-A280 nm exceeded 1.8 for all preparations. Real-time PCR was performed using 5 µL total RNA in a 25 µL reaction containing 70 nM of each primer and 12.5 µL Sybr Green Real-time RT-PCR MasterMix (Thermo Scientific one step kits). Cycling conditions were 50°C for 2 min, 5 min at 95°C, followed by 40 cycles of 30 s at 95°C, 30 s at 58°C, and 30 s at 72°C, and a final incubation for 5 min at 72°C.

The primers for TNF α were 5'-TCC CAA CAA GGA GGA GAA GT-3' and 5'-TGG TAT GAA GTG GCA AAT CG-3', IL-6-primers were 5'-TGA TGG ATG CTT CCA AAC TG-3' and 5'-GAG CAT TGG AAG TTG GGG TA-3', and β -actin primers were 5'-GAG ACC TTC AAC ACC CCA GCC-3' and 5'-TCG GGG CAT CGG AAC CGC TCA-3'.

Values are expressed relative to a reference sample (calibrator): samples from the Sham animals. A mathematical model was used for the relative quantification of *TNF α* and *IL-6* genes [22].

Histological examination

Light microscopic studies: At the end of the experiment, renal tissue specimens were cut into small pieces 2 mm each, fixed in 10% formalin solution for 48 h, dehydrated in ascending grades of alcohol and embedded in paraffin. Serial sections of 5-6 μ m thickness were cut, mounted on glass slides and subjected to the following techniques:

1. Examination of bromodeoxyuridine labelled MSCs using Fluorescent Microscope.
2. H&E staining for histological examination.
3. Immunohistochemical staining for:-
 - a) Mouse monoclonal antibody for PCNA (proliferating cell nuclear antigen).
 - b) Mouse monoclonal antibody for Caspase-3 (for detection of apoptosis).

Results

BM-MSCs localization into the renal tissue

Sections from treated groups examined with fluorescent microscopy showed bromodeoxyuridine labelled cells homing into the renal tissue cortex and medulla (Figure 1).

Hematoxylin and eosin-stained sections

In the present study, H&E stained sections of groups I (the control group) showed normal histological appearance of renal tissue, with renal corpuscles formed of tuft of glomeruli, Bowman's capsule including Bowman's space, proximal (PCT) and distal convoluted tubules (DCT). The lumina of the proximal convoluted tubules were narrow and were lined with pyramidal cells with basal striations, apical brush border and basal pale nuclei. The DCTs showed wider lumina and were lined with cubical cells with rounded central nuclei. Lining cells of DCTs ill-defined basal striations. Examination of renal medullary sections revealed normal histological architecture of loop of

Henel formed of thick descending limb similar in structure to PCT, thin descending and thin ascending limb lined with simple squamous epithelium and thick ascending limb similar in structure as DCT (Figure 2).

Examination of renal tissue of untreated group (II) showed degeneration of renal corpuscles with glomerular capillary congestion. Cytoplasm of the renal corpuscular cells showed vacuolations. In some cortical areas widening of Bowman's capsule could be observed. Tubular lining cells showed vacuolated cytoplasm and deeply stained nuclei. Peritubular capillary dilatation and congestion were observed in some cortical sections with inflammatory cell infiltration in the interstitial tissue. Acidophilic casts, and desquamated epithelial were detected in tubular lumina (Figure 3).

Sections of the renal tissue treated with MSCs through local injection showed decrease degeneration of renal corpuscle with decrease glomerular capillary congestion and slight dilatation of Bowman's space. Renal tubules tubular lining cells shows less vacuolated cytoplasm and less hyaline casts with decrease in peri-tubular capillary congestion.

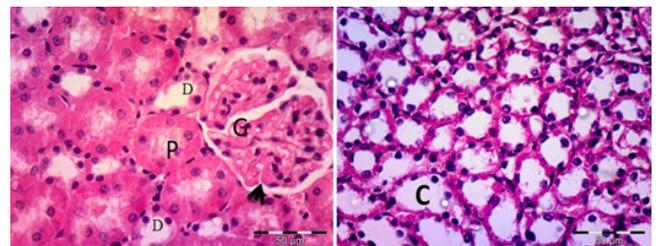


Figure 2: A photomicrograph of rat renal tissue of the group I (control sham-operated) showing Malpighian renal corpuscle containing glomerulus (G) surrounded by Bowman's space (arrow head). PCT (P) is lined with high cuboidal cells having rounded basal nuclei. DCT (D) is lined with cubical cells having rounded central nuclei. Collecting tubule (C) is lined with low cubical cells having rounded central nuclei (H and E X400).

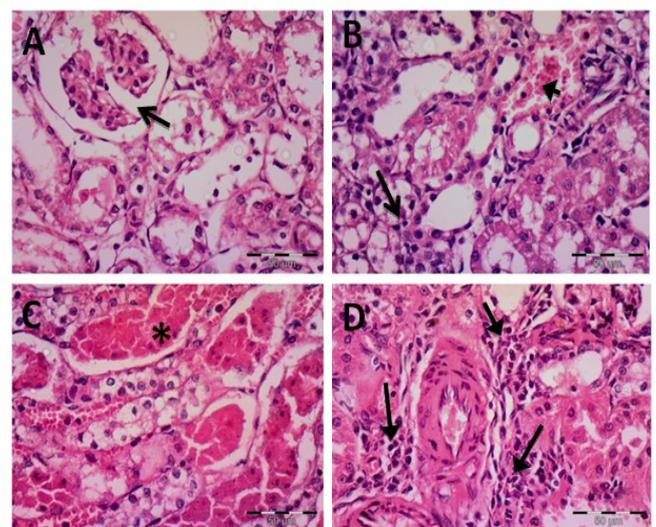


Figure 3: A photomicrograph of rat renal tissue of the untreated group showing: A) Degeneration of renal corpuscles with widening of Bowman's space (arrow). B) Vacuolated cytoplasm & darkly stained nuclei of tubular cells (arrow) Notice peritubular capillary dilatation and congestion (arrowhead). C) Tubular Lumina with desquamated epithelial cells and acidophilic hyaline casts (*). D) Inflammatory cell infiltration in the interstitial tissue (arrow) (H and E X400 Scale bar 50 μ m).

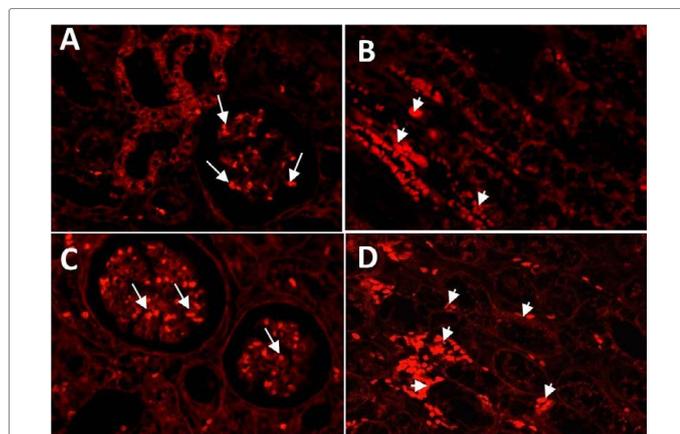


Figure 1: A photomicrograph of rat renal tissue of the treated groups showing: (A and B) group III MSCs labeled with bromodeoxyuridine are present within renal corpuscular cells (arrows) and among medullary lining tubular cells (arrowhead). (C and D) group IV (systemic injection) more labeled MSCs are present within renal corpuscular cells (arrow) and medullary lining tubular cells (arrowhead) (fluorescent microscopy X400).

Sections from the renal tissue treated with MSCs through systemic injection showed marked improvement in tissue architecture represented by well-formed renal corpuscles with normal glomerular tuft of capillaries, and nearly normal Bowman's space. The tubules showed absence of acidophilic casts and epithelial shedding from their Lumina (Figure 4).

Immune histochemistry results

A) Caspase-3

Immunohistochemistry for Caspase-3 showed negative expression in the normal renal tissue (Figure 5). Untreated group showed strong expression for Caspase-3 in the cytoplasm and nuclei of cells in both renal cortex and medulla. There is a significant decrease in Caspase-3 expression after local treatment with MSCs, while faint and nearly negative expression is noticed in systemically treated group (Figure 6).

B) PCNA

Immunohistochemistry for PCNA showed some immune-positive nuclei in normal renal tissue (Figure 7). Both Untreated sections and MSCs locally treated sections showed some PCNA positive nuclei as well. While systemic MSCs treatment revealed marked increase in the number of PCNA positive nuclei (Figure 8).

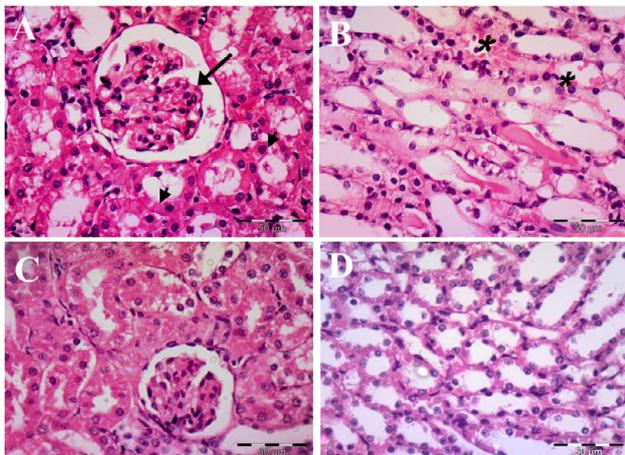


Figure 4: A photomicrograph of the rat renal tissue treated with MSCs (A and B) group III local injection showing improvement of histological structure in both cortex and medulla. Improvement in histological appearance of renal corpuscle & decrease glomerular capillary congestion (arrow), less cytoplasmic vacuolation of tubular lining cells (arrowhead). Notice: regression of intertubular congestion and inflammation (*). (C and D) Systemic injection with MSCs group IV showing nearly normal renal structure of both cortex and medulla. (H&E X400 Scale bar 50 μ m).

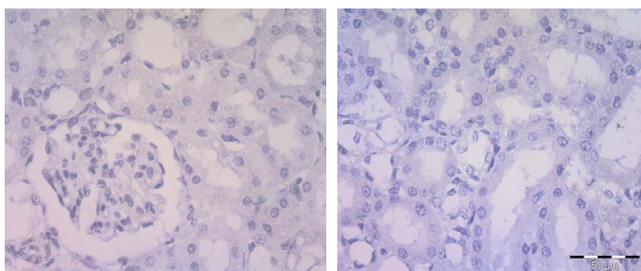


Figure 5: A photomicrograph of the rat renal tissue of control group showing negative Caspase-3 immunoreactivity in cytoplasm and nuclei of the renal corpuscular cells and medullary tubular lining cells. (Scale bar 50 μ m).

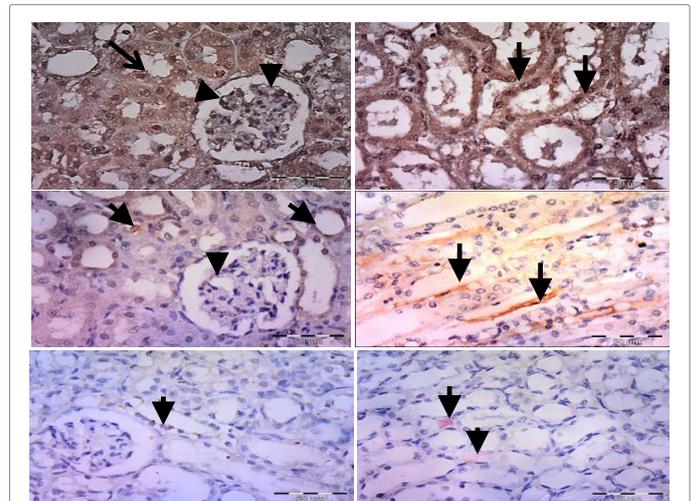
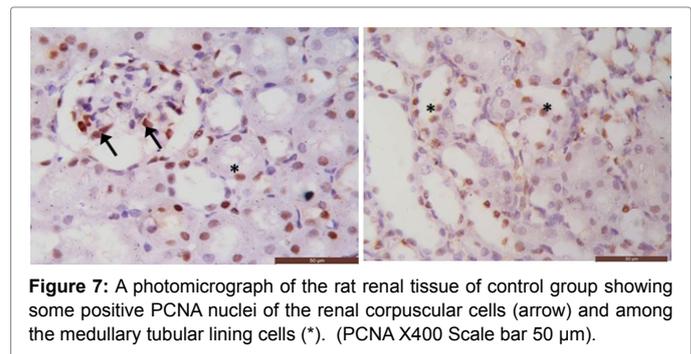


Figure 6: A photomicrograph of the rat renal tissue (A and B) group II shows strong Caspase-3 immunoreactivity in cytoplasm and nuclei of the renal corpuscular cells (arrowhead) and tubular lining cells in cortex and medulla (arrow). (C and D) group III showing mild Caspase-3 immunoreactivity in the cytoplasm of renal corpuscular cells (arrowhead) and tubular lining cells (arrow). Notice negative nuclear expression. (E and F) group IV showing very few immunoreactive.



Results of real time RT-PCR for *TNF α* and *IL-6* gene expression

The fold changes in the gene expression for *TNF α* and *IL-6* were detected by Real-Time PCR and the concentration for each practical test was normalized to its β -actin reference gene. Each test also was done in triplicate for the all samples. The mean values and standard errors were calculated and showed in Chart 1.

In the 3rd day of operation *TNF α* and *IL-6* genes were expressed less in the IR with local stem cells (2 ± 0.3 and 1.6 ± 0.87), respectively and IR with systemic stem cells group (1.6 ± 0.43 and 1 ± 0.77) respectively than IR (6.9 ± 0.93 and 6.5 ± 0.77) respectively.

Biochemical results

Serum levels of urea and creatinine showed significant increase in untreated group, while treated groups (local and systemic with MSCs) showed marked improvement in these biochemical parameters. In the systemic treated group urea was nearly back to normal (Tables 1 and 2).

Quantitative morphometric results

Mean number of PCNA positive nuclei (\pm SD) in the studied groups: No significant increase in the number of PCNA positive nuclei in group III (local treatment with MSCs) compared with group

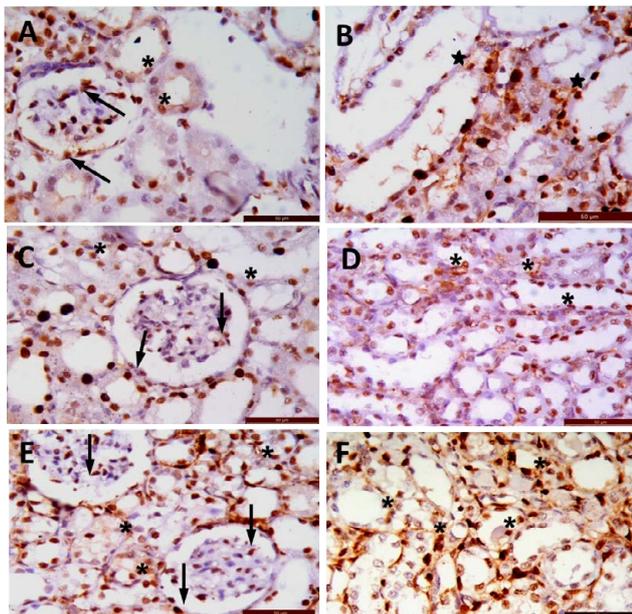


Figure 8: A photomicrograph of the rat renal tissue (A and B) group II showing some PCNA immuno- reactive nuclei in the renal corpuscular cells (arrow) and among the tubular lining cells in cortex and medulla (*). (C and D) group III showing some PCNA immuno-reactive nuclei in renal corpuscles (arrow) and among tubular lining cells (*). (E and F) group IV showing multiple PCNA positive nuclei in renal corpuscles (arrow) and among tubular lining cells (*). (PCNA X400 Scale bar 50 μ m).

Control group (group I)	Untreated group (group II)	locally Treated group (group III)	Systemically treated group (group IV)
32.3 \pm 2	90.1 \pm 2	42 \pm 3	33.2 \pm 2

Table 1: A Significantly different $p < 0.05$.

Control group (group I)	Untreated group (group II)	locally Treated group (group III)	Systemically treated group (group IV)
0.81 \pm 0.2	3.46 \pm 0.7	2.21 \pm 0.5	1.1 \pm 0.3

Table 2: A Significantly different $p < 0.05$.

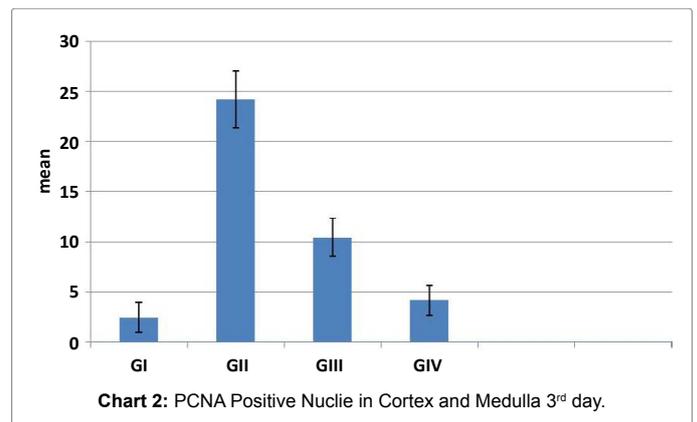


Chart 2: PCNA Positive Nuclei in Cortex and Medulla 3rd day.

Discussion

Ischemic-reperfusion (I/R) injury is the most common cause for acute renal failure, warrants the urgent development of new treatment modalities. Recent studies introduce bone marrow derived cells (MSCs) as a new therapeutic rationale for acute renal injury. In the present study we used ischemic reperfusion method to induce acute renal injury in rats, MSCs labeled with bromodeoxyuridine fluorescent linker dye injected through two different routes (systemic and cortical injection) to evaluate their therapeutic effect biochemically and histologically.

Biochemical results of untreated group (II) showed marked impairment of the renal function with high plasma level of urea and creatinine. Histological sections of the same group showed widening of the capsular space, shrinkage of glomerular tuft and inter-glomerular hemorrhage. Tubular cells showed vacuolated cytoplasm, pyknotic nuclei, and hyaline casts. Desquamated epithelial cells were observed in tubular lumen of both cortex and medulla. Acute renal injury is manifested by both serological and histological findings in renal cortex and medulla [23-25]. In our study homing of MSCs to the damaged renal tissue in the treated groups (III & IV) was confirmed by examination with the fluorescent microscopy, labeled cells appeared immediately in the cortical corpuscles and in the peri-tubular spaces. In rat model of AKI through 40 min bilateral clamping of renal pedicle, fluorescence-labeled MSC were detected early after injection in glomeruli, and at microvasculature sites [26]. In mice animal model of acute renal ischemia, MSCs transplanted into mice selectively grafted onto damaged area [27].

In the present study MSCs treated groups showed marked improvement in renal function parameters especially in systemic injected group; in which the plasma level of urea and creatinine nearly back to normal level 3 days after injection. In clinical trial conducted on 30 patients with acute kidney injury, serum urea and creatinine decreased significantly after MSCs injection, and renal functions had

Comparison among different groups

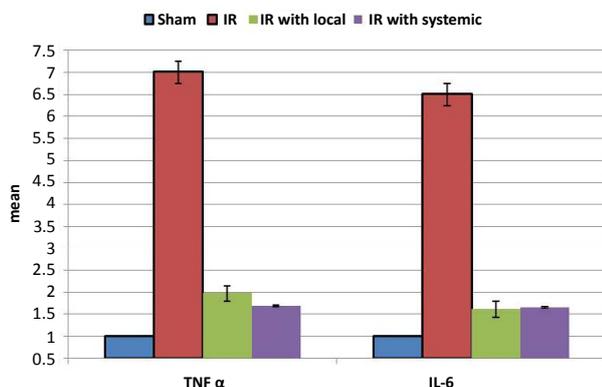


Chart 1: Representing the differences between studied groups in pro-inflammatory Cytokines production gene ($p < 0.05$).

II (untreated group). The highest value for PCNA positive nuclei was recorded in group IV (MSCs systemically treated group) with high statistically significant increase in the number of PCNA positive nuclei in this group compared to group II & III ($p < 0.05$) (Table 3 and Chart 2).

Mean area% of Caspase-3 positive cells (\pm SD) in the studied groups

Mean area% of Caspase-3 immuno-reactivity in cortical and medullary tubular cells of groups II and III showed a significant decrease ($p < 0.05$) when compared to control. There is significant difference between group III and group IV (Table 4).

PCNA positive nuclei	2 ± 1.2	4.4 ± 1.9	5.4 ± 1.5	8.6 ± 1.3
P-value	0.001*			
	I vs. II	I vs. III	II vs. III	I vs. IV
	0.3	0.01*	0.7	0.001*
				II vs. IV
				0.003*
				III vs. IV
				0.02*

Table 3: Mean number of PCNA positive nuclei (± SD) in the studied groups.

Cortex and medulla 3rd day	Control	Untreated	Local treatment	Systemic treatment
Caspase3 positive cells	2.5 ± 1.51	24.2 ± 2.85	10.5 ± 1.87	4.2 ± 1.47
P-value	0.001*			
	I vs. II	I vs. III	II vs. III	I vs. IV
	0.001*	0.001*	0.001*	0.1
				II vs. IV
				0.001*
				III vs. IV
				0.03*

Table 4: Mean area% of Caspase-3 positive cells (± SD) in the studied groups.

been restored [28]. Other results stated that MSCs administration to rats with acute kidney injury significantly restored renal functional parameters and hastened functional recovery [27,29,30].

Histological manifestations of renal damage showed marked improvement after MSCs injection. The histological improvement was more evident in group (IV) which was treated with systemic administration of MSCs. Less tubular necrosis, less tubular casts and fewer desquamated cells within tubular lumen. In cisplatin-treated rats MSCs infusion prevented occurrence of AKI through down regulation of tubular cell apoptosis and enhancing proliferation [31].

The Peri-tubular dilatation and congestion observed in the untreated group; showed improvement after local infusion of MSCs and completely disappeared after their systemic infusion. This was explained with the pro-angiogenic ability of MSCs and their ability to induce five folds increase in the level of vascular endothelial cell growth factors (VEGF) when administrated to the mice with acute renal ischemia [32,33]. MSCs can induce angiogenesis by supporting the new vasculature and maintain renal perfusion or by producing growth factors associated with the angiogenesis process as vascular endothelial growth factor (VEGF) insulin-like growth factor (IGF), and platelet-derived growth factor (PDGF) [29].

In our study inflammatory cell requirement to the renal tissue showed marked regression after MSCs infusion, furthermore systemic infusion showed complete absence of inflammatory cells from the examined sections of the treated group (IV).

The same findings were described in others work who studied the anti-inflammatory/immunomodulatory properties of MSCs in AKI and stated that inflammatory cells as macrophages, neutrophils and T cells can enhance the development of AKI [34]. MSCs move to sites of inflammation and release trophic growth factors to modulate the immune system, modulate macrophages activity and provide renal protection and repair [35].

Our results showed improvement of renal function was associated with reduced expression of pro-inflammatory cytokines (IL-6, TNF-α) in 3rd day after local and systemic MSCs application (P<0.001). Regarding TNF-α expression, systemic treatment was significantly lower than local injection MSCs application resulting in down-regulation of pro-inflammatory cytokines [36].

MSCs have strong immune-modulatory action on immune cells through cell-to-cell contacts through production of cytokines, chemokines and growth factors [37]. In allogeneic co-culture

assay MSCs inhibit the ability of dendritic cells to stimulate T cell proliferation and reduce B cell activity, it is well known that both immune cells (dendritic and B cell) have an important role in the development of AKI [38]. MSCs have numerous possible immune regulatory functions enable them to ameliorate kidney inflammation and improve the outcome of AKI [39].

In our study, immunohistochemical staining for Caspase-3 as an apoptotic marker was done to detect confirm apoptosis in renal cells in the kidney sections. The results showed that the number of Caspase-3 positive cells in the kidney tubules in cortex and medulla markedly increased in group II while in group III and IV the infusion of BM-MSCs significantly decrease the number of apoptotic cells. Other studies also observed down-regulation of Caspase-3 in MSCs treated groups [25].

In this study, immuno-histochemical staining for PCNA was done to measure cellular proliferation and renal tissue regeneration. BM-MSCs significantly increased the number of PCNA-positive cells, suggesting that BM-MSCs stimulate cell proliferation of renal tubular cells.

After an ischemic injury to the kidney in group II, sections of renal cortex showed many positive PCNA immuno-reactive nuclei of renal corpuscular cells and also among renal tubular lining cells in renal cortex and medulla when compared with control group. This agrees with other studies which stated that MSC injection help the recovery of renal function by stimulation of tissue proliferation [33].

The number of immuno-positive nuclei for PCNA was increased after MSCs administration especially in systemic group. This is in agreement with recent studies which explained the renal tubular protective effect of BM-MSCs by its potential ability to Trans-differentiate [40]. The same results are in disagreement with other studies stated that BM-MSCs moved into injured renal tissue have no chance to differentiate into renal cells; as they were mainly localized in peri-tubular areas, not within the tubules. Large number of undifferentiated cells is localized within the normal renal tissue [41]. Renal papillae, epithelium of the tubules, and parietal layer of Bowman's capsule are the main sites for renal progenitor cells [42-44].

The normal progenitor cells within the renal tissue markedly increase in number following renal injury suggesting their important role in renal repair. After restoration of normal renal function the number of renal progenitor cells back to its normal ratio [45,46]. MSCs release extracellular microvesicles (MSC-EVs) with small RNAs content, miRNA is expected to have a reno-protective effect [47-49].

The RNA content of MSCs extracellular microvesicles is small non coding RNA of 21-25 nucleotides in length. This molecule is directed to the damaged cells and can regulate their gene expression pattern towards regeneration [35,50].

The present study provided evidence that BM-MSCs when administered systemically have a better protective effect on the renal tissue than locally administrated cells. More experimental studies are still needed for better understanding of the different mechanisms through which MSCs induce their therapeutic effects on the damaged renal tissue. Controlled clinical trials should be done in parallel.

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