

Hyaluronic Acid Potentiates the Renoprotective Effects of Wharton Jelly –Derived Mesenchymal Stem Cells In a rat Model of Renal Ischemia Reperfusion Injury

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Abstract

The present study investigated the effects of combination of hyaluronic acid (HA) and Wharton Jelly-derived mesenchymal stem cells (WJ-MSCs) on renal ischemia/reperfusion (I/R) injury in rats. 120 male Sprague Dawley rats were divided into 5 equal groups; a) sham operated, b) I/R (45 min left renal ischemia), c) HA group (0.04-0.08 ml of HA, 2-3 injections on lateral aspect of left kidney just after renal ischemia, d) WJ-MSCs-treated group (as control with 1×10^6 MSCs suspended in 0.15 ml complete medium on lateral aspect of left kidney after ischemia time) and e) WJ-MSCs + HA group (as control with HA and WH-MSCs). WJ-MSCs group showed significant decrease in serum creatinine, urinary proteins and kidney morphology with significant increase in creatinine clearance ($p < 0.05$). Moreover, HA +WJ-MSCs group showed more significant decrease in serum creatinine, urinary proteins and kidney morphology with more significant increase in creatinine clearance than WJ-MSCs group ($p < 0.05$). We concluded that HA potentiates the renoprotective effect of WJ-MSCs against renal I/R injury probably by increasing its homing in injured tissues.

Keywords: WJ-MSCs, renal ischemia/reperfusion, Hyaluronic acid

Introduction

Renal ischemic injury is a common clinical problem during systemic hypo perfusion (e.g. shock and acute myocardial infarction) and temporary discontinuation of renal blood supply e.g. in renal transplantation (1), partial nephrectomy, renal vascular surgery, enucleation of renal cell carcinoma (2) and aortic cross-clamping (juxta-renal or suprarenal aortic cross clamping for abdominal aortic aneurysms) (3).

Renal ischemia in kidney transplantation is a common cause of renal cell death, renal failure, delayed graft function (4) and renal graft rejection (5).

The primary non-function is also a significant problem (occurs at an unacceptable rate ranging between 10 and 50 % (6).

Also, renal ischemia contributes to the considerable morbidity associated with surgery and anesthesia, where renal dysfunction occurs in up to 50% of patients undergoing

aortovascular surgery e.g. for supra or juxtarenal abdominal aortic aneurysms (7-8).

Also, renal ischemia/reperfusion injury is a major cause of acute renal failure (ARF) (9).

The current management of acute kidney injury (AKI) is non-specific and associated with limited supportive care; thus, the need for more novel approaches is necessary (10).

Most of the therapeutic approaches to kidney regeneration are based on administration of cells proven to enhance healthy function of the kidney. Endogenous or exogenous cells of different sources were tested in models of ischemia/reperfusion (I/R), acute kidney injury, or chronic disease (11).

The translation to clinic is at the moment focused on the role of mesenchymal stem cells. Allogeneic stem/progenitor populations and their derived bio products demonstrating therapeutic effects in kidney repair upon injury (11).

Cell therapy, as a blanket term covering the regenerative medicine, tissue, and bioengineering, is dependent on cell and tissue culture methodologies to expand specific cells to replace important differentiated functions lost or deranged in various disease states. Central to the successful development of cell-based therapeutics is the question of cell sourcing, and advances in stem cell research have a vital impact on this problem (12).

MSCs can be isolated virtually from every tissue in the body. In experimental animal, sources of MSCs that have been utilized for expansion and clinical therapy include bone marrow, adipose, testicular and ovarian tissue salvaged from routine sterilization procedures, and fetal membrane tissues discarded from pregnant and ovariectomy (13-17). As the tissue source with the highest MSC proliferation potential appears to vary from species to species (1819).

A recent study in cats compared the proliferative capacities of MSCs from different sources (15), In addition to a relatively easier collection procedure, adipose-derived MSCs were found to be superior in proliferative potential than bone marrow-derived MSCs (Bm MSCs) and were considered therefore to be the preferred source for MSC therapy in cats (15).

Wharton jelly of umbilical cord derived mesenchymal stem cells, a subpopulation of multipotent cells, are known to secrete growth factors and anti-inflammatory cytokines. In addition, these cells are easy to collect; present higher proliferation and self-renewal rates compared with other adult stem cells (ASCs), and are suitable for banking (20).

Consequently, umbilical cord-MSCs might represent a promising source of stem cells for regenerative therapies in kidney ischemia. Recently, (21) studied the role of CD44-hyaluronic acid (HA) in the homing and improving renal function of systemically transplanted MSCs in chronic renal

failure (5/6Nx). They found that CD44-HA has the potential to induce MSCs homing to injured tissue, while its effect on the ability of MSCs, improving tissue function, is not significant. We hypothesized that addition of HA to WJ-MSCs could increase its homing in kidney tissues and increase its potential renoprotective effects against renal IR injury. So, the present study was designed to examine the repair of the renal ischemic damage by using mesenchymal stem cells (MSCs) derived from Wharton jelly tissue of umbilical cord as well as to investigate the effect of HA in homing of WJ-MSCs in renal tissues and its effects on kidney function and morphology.

Materials and Methods

Experimental animals

One hundred and twenty of male Sprague-Dawley (SD) rats, aged 4-6 months and weighing ~250-300 g, were housed and bred in the animal house at the Urology and Nephrology Center, Mansoura University. The temperature was kept at 20 °C, and the animals were fed a standard diet with free access to water and live in 12h in dark and light.

Ethical considerations

Experiments were performed according to the Guide for the Care and Use of Laboratory Animals (ILAR 1996). All protocols were approved by the ethical committee of Mansoura University, Faculty of Medicine (code # MS/15.08.94).

Isolation, extraction and characterization of Wharton Jelly (WJ)-MSCs

According to the policy approved by the local Ethical Committee, all tissue samples were collected after informed consent from mothers at Mansoura university hospital. About 15-20 cm of the term umbilical cord after caesarian sections were rapidly collected and rinsed in normal saline and placed in a container with medium (DMEM) and penicillin and streptomycin and transported to Mansoura Research Center For Cord Stem Cells (MARC_CSC) at medical college in Mansoura University. The cells were extracted according to the technique of (22).

Cells isolated by using 10 ml of collagenase type one (sigma Aldrich-c1639) preheated at 37°C for 60 min at 37°C and 5 ml of trypsin EDTA (Sigma Aldrich T4049) was added and shaking was done again for 30 min.

The fibroblast-like cells cultures were harvested by treatment with 25% trypsin-EDTA (Sigma Aldrich T4049). By add of 5ml of pre wormed trypsin 25% EDTA for wash removed it rapidly then add again 5ml of trypsin 25% EDTA wait for 3-5 min in incubator and see the cells were it separated from each other

and detachment from flask wall then remove it and add 5ml of complete medium and take 2.5 ml of medium in flask were 10^6 fibroblast cells there.

And incubated $50 \mu\text{g}/10^6$ cells with antibodies conjugated with different fluorescent probes for 30 min at room temperature in the dark. The antibodies used were: CD105, CD73, CD90, CD14, CD34, and CD45). For CD105 and CD90 Add 5ul and for CD73, CD14, CD34 and CD45 add 20 ul. After that, washing with stain buffer 2ml and centrifugation for 10min 2000 rpm. Discard the supernatant then dissolve the pellet with 500um of stain buffer. The cells ready for analyzed on a flow cytometer (FACS caliber, Becton Dickinson) by collecting 10,000 events and the data analyzed using the cellquest Software (Becton Dickinson).

Stem cell subculture and expansion of the cell:

We used in these processes complete medium (DMEM low glucose (Sigma Aldrich D5523), streptomycin, penicillin and fetal bovine serum (High Clone sv30160.03). The medium was changed every 3 days for 14 days. Cells were allowed to adhere to culture flask and non-adherent cells were removed by changing medium when the adherent cells reached to covering 70% to 80% of flask, the cells washed by medium then add 10ml of 0.25% trypsin-EDTA (sigma Aldrich-T4049), for 10 min then add the medium, were cells suspend in medium and transferred to new culture flask at 250×10^3 cells/cm². For future passage the above procedure done again.

Treatment of cells by hyaluronic acid:

One of these flask pretreated the media with hyaluronic acid (sigma Aldrich-5747) 1ml/ml of media for 14 days by concentration of 1g/L of medium for 14 day (23).

Experimental design:

Rats were randomly allocated to the following five equal groups:

- 1. Sham group** (n= 24) : rats underwent right nephrectomy without left renal ischemia.
- 2. Control group (I/R injury) group** (n= 24): rats were subjected to right nephrectomy and left renal ischemia for 45 minutes.
- 3. Hyaluronic acid (HA) group** (n= 24): as IR group with administration of 0.04-0.08 ml of HA (2-3 injections) on lateral aspect of left kidney (23).
- 4. Wharton Jelly-Mesenchymal stem cell (WJ-MSCs) group** (n= 24): as IR group with administration of 1×10^6 MSCs suspended in 0.15 ml complete medium. (23).
- 5. WJ-MSCs +HA groups** (n= 24): as IR group with administration of MSCs and HA in the same previous doses (23).

Then each group was subdivided into 4 subgroups (each 6 rats) according to the time of sacrifice;

- a) 24 hrs subgroup:** rats were sacrificed after 24 hrs.
- b) Day 3 subgroup:** rats were sacrificed after 3 days.
- c) Day 5 subgroup:** rats were sacrificed after 5 days .
- d) Day 7 subgroup:** rats were sacrificed after 7 days .

Experimental Model:

The animal model of 45-min left renal ischemia with right nephrectomy was done according to (24).

Blood and urine samples collections:

The blood sample was collected via cardiac puncture at the time of sacrifice at 24 hrs, day 3, day 5 and day 7 after IR operation. The rat was anaesthetized using halothane inhalation and by 5ml syringe tapping in heart. The blood was centrifuged and serum was stored at - 20 °C till the time of biochemical analysis. The rats were placed in a metabolic cage for 24 hours in order to collect 24-hour urine. The urine volume was measured, and then a sample was taken for estimation of urine and creatinine (25).

Harvesting of kidney specimen:

The animal was anaesthetized again by sodium thiopental (12 mg/kg BW) intraperitoneally (25), then the abdomen was opened and the left kidney was perfused briefly with phosphate-buffered saline (PBS) through a cannula inserted into the abdominal aorta to rinse out the blood. The kidney was removed rapidly and cut into two equal halves by a scalpel. One half of the kidney was rapidly placed in a container containing 10% neutral buffered formalin for histopathological examination and immunostaining and the other half was rapidly frozen in liquid N₂, and stored at - 72 °C until biochemical assay.

Assessment of kidney functions:

Measurement of serum and urine creatinine and proteins:

Serum levels of creatinine were determined using the Architect c4000 system (Abbott Diagnostics, Wiesbaden, Germany). Also, examination of urine sample involved calculation its 24 hr volume and measurement of its creatinine and total protein using the Architect c4000 system (Abbott Diagnostics, Wiesbaden, Germany).

Calculation of creatinine clearance:

The creatinine clearance was measured from the following equation (26);

$$\text{Ccr} = \frac{\text{Urine creatinine (mg/dl)} \times \text{urine volume (ml/24hr)}}{\text{Serum creatinine (mg/dl)} \times 1440 \text{ (minutes)}}$$

Histopathological examination:

After removal of the left kidney, its half was immediately fixed in 10% neutral buffer formalin. The kidney specimens were processed for paraffin blocks and sections of 4- μm thickness were made and stained with haematoxylin and eosin stain. The light microscopy study was reviewed by a

pathologist blinded to experimental conditions. For light microscopy the pathologist divided the kidney into 2 main parts cortex and medulla and 10 fields were examined by high power field. In each part, the glomeruli were observed for hypercellularity and changes in the juxtaglomerular apparatus and renal tubules were examined for apoptosis, dilated tubules, loss of epithelial borders, intratubular casts and tubular atrophy. Regeneration indicators were mitosis and solid sheets and prominent nucleoli. Also, the interstitium was examined for fibrosis and cellular infiltrate.

Scoring of the degeneration and regeneration:

Damage was scored as follow; for necrosis 1= 1 to 3 necrotic tubules per hpf, 2= 4 to 5, 3=5 to 10 and 4=more than 10, for dilated tubules 1= 1 to 3 dilated tubules per hpf, 2= 4 to 5, 3 = >5 , loss of brush border 1= in 1 to 3 tubules per hpf, 2 = 4 to 5, 3=5 to 10 and 4=more than 10, casts 1= 1 to 3 casts, 2= 4 to 5, 3=>5 and tubular atrophy was scored as 1=1 to 5 atrophic tubules, 2=6 to 10 and 3=more than 10. Interstitial fibrosis either 0= absent and 1= present and interstitial inflammatory cell infiltrate was scored as follow 1= 1 to 5 inflammatory cells per hpf, 2= 6 to 10, 3=11 to 20 and 4=more than 20. Kidney regenerative capacity was scored as 1= 1 or 2 mitotic figures per 10 hpf, 2= 3 to 5, 3= 6-10 and 4= 11-20 and 5 = more than 20 . It was also scored as 1=1 or 2 solid sheets per hpf, 2= 3 to 5 and 3= more than 5 (27)

Statistical analysis

The data are given as the mean (SD) and analyzed by an unpaired T-test for comparing two quantitative variables, or a one-way ANOVA to compare more than two quantitative variables. If there were differences among the means, a post hoc Scheffe’s test was used to determine which means differed, with differences considered significant at $P < 0.05$.

Results

Characterization of WJ-MSCs

Attachment of spindle-shaped cells to tissue culture plastic flask was observed after 3 days of culture. After 14 days, spindle-shaped cells reached 80% confluency. After third passage, cultures were composed of a homogenously fibroblastic cell monolayer (fig. 1a-b).

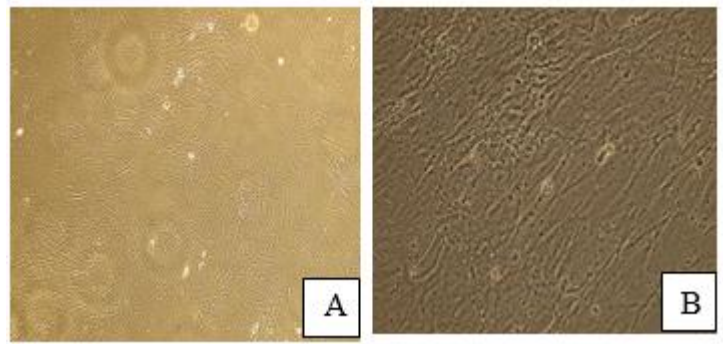


Figure (1): Showing images of WJMSCs alone in passage 3 (a) and after treating with HA (b) with 80% confluency.

Immunophenotypic characterization:

Cultures of WJMSCs were analyzed for expression of cell-surface markers. WJ-MSCs were negative for the hematopoietic markers CD45, CD34 and CD14 with percentage 99.39%, 64.81 % and 87.68% respectively and positive for CD105, CD73 and CD90 with percentage 20 %, 33.87% and 88.21% respectively (fig 2-4)

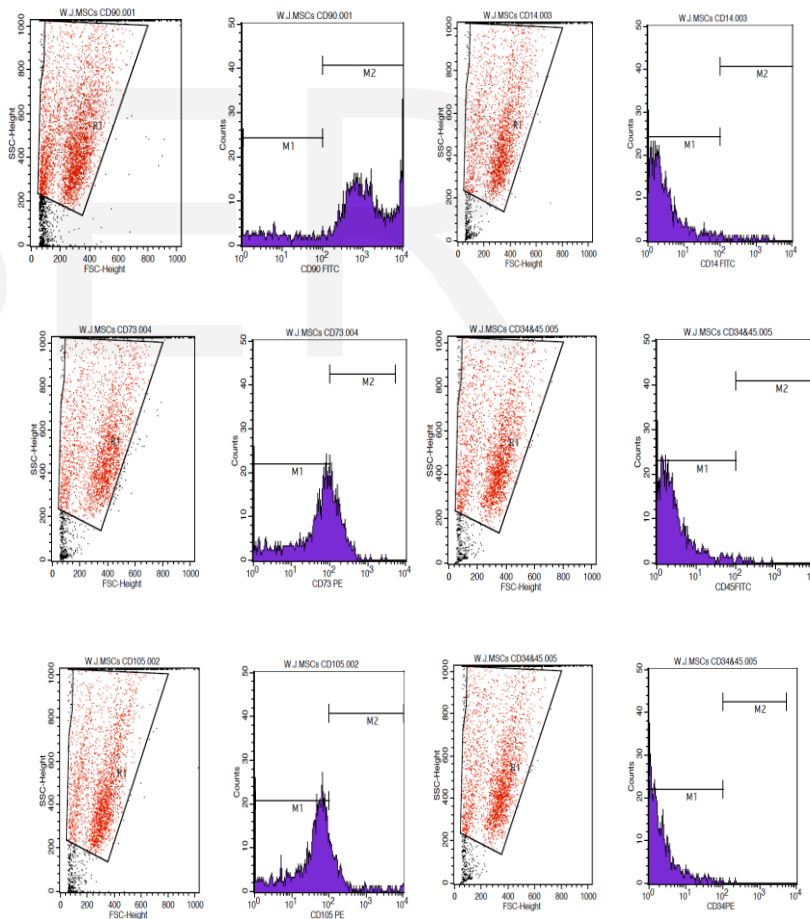


Fig. (2): Immunophenotypic analysis of WJ-MSCs. (a and b), and histogram representing the flow cytometry performed on the WJ-MSCs (c and d) for CD 14 and CD34, CD45, CD73, CD90, CD105.

Effects of WJ-MSCs and HA on serum creatinine

Basal values of serum creatinine were comparable among different subgroups. Test values of serum creatinine in IR, HA and WJ-MSCs groups were significantly higher than sham group at 24 hrs, day 3 and day 5 time intervals ($p < 0.05$), while in day 7, compared to sham group, IR and HA groups showed statistical significant higher values of serum creatinine and WJ-MSCs group showed statistical lower values ($p < 0.05$). On other hand, WJ-MSCs +HA group showed statistical significant lower values of serum creatinine compared to IR, HA, WJ-MSCs groups at 24 hrs, day 3, day 5 and day 7 ($p < 0.05$) (fig. 3).

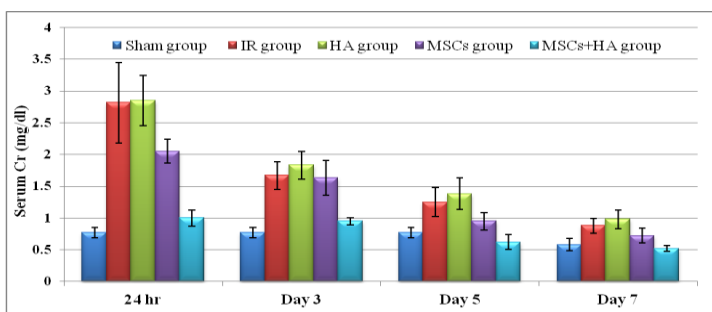


Fig. (3): Mean \pm SD of test values of serum creatinine (mg/dl) in different groups at different time intervals.

Effects of WJ-MSCs and HA on creatinine clearance

Test values of creatinine clearance were statistically significant lower than their basal values of the same group at different times ($p < 0.05$). Test values of creatinine clearance in IR and HA and WJ-MSCs groups were significantly lower than sham group at 24 hrs, day 3, day 5 and day 7 time intervals ($p < 0.05$). Also, compared to sham group, WJ-MSCs + HA group showed statistical significant higher values at day 5 and 7 ($p < 0.05$) (fig. 4).

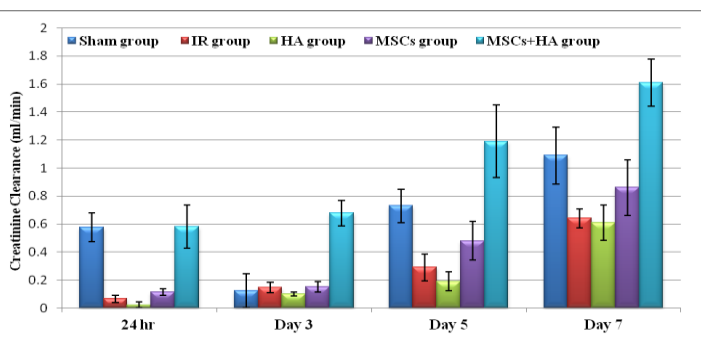


Fig. (4): Mean \pm SD of test values of creatinine clearance (ml/min) in different groups at different time intervals

Effects of WJ-MSCs and HA on urinary proteins (mg/24 hrs)

Test values of urinary proteins were statistically significant higher than their basal values of the same group at different times except WJ-MSCs + HA group ($p < 0.05$). Test values of urinary proteins in IR and HA and MSCs groups were significantly higher than sham group at 24 hrs, day 3, day 5 and day 7 time intervals ($p < 0.05$). Also, compared to WJ-MSCs + HA group, HA group and WJ-MSCs group showed statistical significant higher values at 24 hrs, day 3, day 5 and day 7 ($p < 0.05$) (fig. 5).

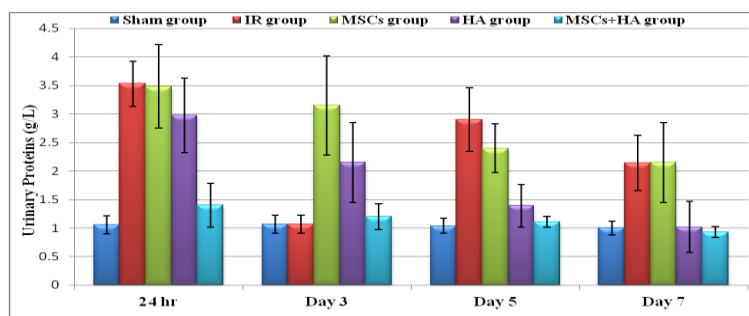


Fig. (5): Mean \pm SD of test values of urinary proteins (mg/24 hrs) in different groups at different time intervals

Effects of WJ-MSCs and HA on degeneration and regeneration scores

Regarding degeneration scores in different group, they were 0 in all items of degeneration at different times in sham group. While, IR group showed high necrosis score (1 - 2) and interstitial inflammatory cells (2 - 4) and the scores were 1 for dilated tubules, casts and loss of brush borders and tubular casts and 0 for interstitial fibrosis and atrophy. Also, HA group showed high score of necrosis (1-2) and inflammatory cell infiltrate (2-3) with the same scores regarding other parameters as IR group. On other hand, WJ-MSCs and WJ-MSCs + HA groups showed low scores of necrosis (± 1), dilated tubules (± 1), tubular casts (± 1) and zero scores for atrophy, fibrosis and loss of brush borders. While, the inflammatory cell infiltrate still high (2-3 in WJ-MSCs group and 2-4 in WJ-MSCs +HA group).

Regarding regeneration scores, mitosis scores were 0 in sham group, 0-3 in IR and HA groups and 1-4 in WJ-MSCs and WJ-MSCs +HA groups. Scores of solid sheets of cells were 0 in sham group, 0-1 in IR and WJ-MSCs groups and 1 in HA and WJ-MSCs + HA groups. Fig.6 is representative sample from different groups showing signs of degeneration and regeneration in kidney tissues.

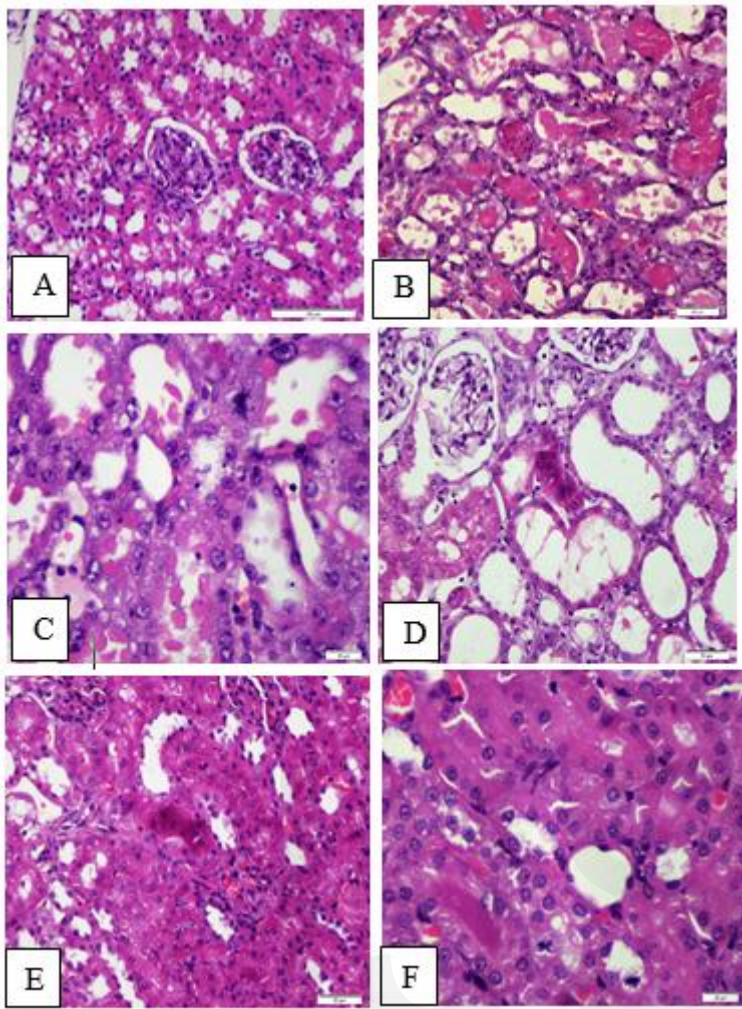


Fig (6): Kidney specimens showing a) normal structure of kidney tissues (sham group), b) tubular casts, loss of brush border, necrosis and dilated tubules (IR group), c) solid sheets of tissues and dilated necrotic tubules (HA group), d) mitotic figures (MSCs group), e) mitotic figures (MSCs + HA group) and f) masses of solid tissues (MSCs + HA group) (H&E, 200x).

Discussion

Renal warm ischemia/reperfusion injury is a complex inflammatory process that in clinical practice can occur as a consequence of both systemic hypoperfusion (e.g. shock) with subsequent circulatory resuscitations and local renal hypoperfusion following renal transplantation, partial nephrectomy or renal vascular surgery(1). It is a common cause of renal cell death, renal failure, delayed graft function (4), and renal graft rejection (5) in renal transplantation. Although a special care in donor management, the introduction of better preservation solutions in clinical practice and shortening cold ischemic times have allowed to minimize this lesion, ischaemia-reperfusion injury remains as a major problem (28). Recovery of

kidney functions after ischaemic injury involves the entry of differentiated, quiescent tubule cells into the cell cycle in a rapid, closely regulated fashion. This process depends not only on the replacement or regeneration of injured cells but also on protection from programmed cell death (apoptosis) (29-31). Moreover, there is no specific medication in clinical use for acute kidney injury arising from IRI (32-35). Therefore, our intention hypothesis in the present study was to investigate Wharton jelly stem cells in prevention of I/R injury as well as to investigate the role of HA which enhance the homing of MSCs in injured tissues in improving WJ-MSCs homing in kidney, thus potentiate its renoprotective effect.

The present study showed that, renal ischemia caused significant increase in significant increase in serum creatinine with significant reduction in UOP and creatinine clearance at the different times of follow up suggesting significant impairment of glomerular function. Also renal I/R resulted in a significant increase in urinary proteins, creatinine and serum Na^+ at the different times of follow up after ischemia suggesting significant impairment of tubular function. These findings confirm that I/R injury of the kidney causes both glomerular and tubular dysfunctions and are in agreement with those reported by others (36,9,3)

The impairment of glomerular functions in renal I/R injury might be due to glomerular endothelial cell injury which results in a local imbalance of vasoactive substances with enhanced release of vasoconstrictors such as endothelin and decreased abundance of vasodilators such as endothelium-derived nitric oxide (NO) (37-39) or tubular obstruction by tubular casts (cells, blebs, and Tamm Horsfall protein), which reduce the outward driving forces for the filtrate formation (40). On other hand, the impairment of tubular functions might be due to reduction in aquaporins (AQPs) expression in the collecting duct and the proximal tubule, as well as disruption of the actin-based cytoskeleton resulting in dissociation of $\text{Na}^+\text{-K}^+$ ATPase from its basolateral border (41-42).

At the level of morphology, we demonstrated that the kidneys obtained from ischemic groups showed increase in renal tubular necrosis, loss of brush borders, intratubular casts and interstitial inflammatory cells which explain impairment of glomerular and tubular functions of the kidneys and confirm the development of acute tubular necrosis which is the pathognomonic feature of renal ischemia/reperfusion injury. Also, we demonstrated minimal regenerative capacity in kidney tissues in the form mitotic figures, prominent nucleoli and solid masses of sheets especially at 7 days. Previous studies reported and demonstrated significant derangement in kidney morphology at the level of tubules and interstitium such as (31, 43,24) which demonstrated morphological changes of acute tubular necrosis (ATN) such as tubular dilatation,

cellular vacuolization, necrosis, intratubular detachment of cells with loss of integrity of brush border cell membrane in ischemic kidney.

Hyaluronan (hyaluronic acid/HA) is a natural, unbranched polysaccharide with molecular weight (MW) range from 1 kDa to above 2 MDa. It is part of a group of polysaccharides typically observed in the epithelial, connective, and nerve tissues of vertebrates which are designated as a glycosaminoglycan (44). (45) Demonstrated that HA enhanced the repair of myocardium from ischemic myocardial infarction by stem cell therapy. On other hand, (26) demonstrated that HA improve the myocardial injury in case of MI without stem cell transplantation. So, we tried to investigate the effect of HA alone on renal ischemia. The present study demonstrated that treatment with HA alone did not cause any significant improvement in markers of kidney functions including serum creatinine, creatinine clearance and urinary proteins with no changes in kidney morphological changes of degenerative and regenerative capacities. These findings are the first, upto the best of our knowledge, findings reported regarding the effect of HA alone in case of renal ischemia suggesting exogenous HA has no effect of kidney function and morphology in renal ischemia.

The 2nd aim of the present study was to investigate the impact of WJ-MSCs on the outcomes of renal ischemia. Renal I/R injury is a common problem during kidney transplantation and may results in rejection of the transplanted kidney and WJ-MSCs were superior than other stem cells because they possess immunomodulatory and immunosuppressive capabilities (47), so they can inhibit the immune response caused by T lymphocytes in allogenic transplantation (48), that is why we chose WJ-MSCs in the present study. We reported significant improvement in renal function parameters including serum creatinine, creatinine clearance and urinary proteins in WJ-MSCs group compared to I/R group suggesting and confirmed the renoprotective effect of WJ-MSCs on renal I/R injury. However, the degree of improvement was not marked. Moreover, good signs of regenerations in renal tubules were demonstrated in WJ-MSCs group with minimal tubular and interstitial damage in histopathological examination for kidney tissues. These findings are in agreement with previous experimental studies that demonstrate the effectiveness of using stem cell therapy (49-56, 24) against renal I/R injury. (54-55) investigated the effects of human Wharton's jelly-derived mesenchymal stromal cells (WJ-MSC) on acute and chronic kidney injury induced by ischemia-reperfusion injury (IRI) and its possible underlying mechanisms. They found that WJ-MSCs ameliorated the renal injury at the levels of kidney functions and morphology and inhibited renal tubular apoptosis, inflammatory reaction and fibrosis. They reported

that hepatocyte growth factor (HGF) may play a role in the renoprotective action of WJ-MSCs against renal I/R injury. Also, (57) demonstrated the renoprotective effects of human WJ-MSCs against renal I/R injury by suppressing CX3CL1.

This renoprotective effect for human WJ-MSCs was demonstrated in other experimental kidney injury models. (58) Showed that human umbilical cord mesenchymal stem cells (hucMSCs) significantly improved the kidney function and morphology in cisplatin acute and chronic toxicity. huMSCs reduced collagen deposit, the ratio of Bax to Bcl-2 and transforming growth factor β mRNA expression and prevented the epithelial-mesenchymal transition (EMT) in injury renal tissues. Also, (59) demonstrated that human WJ-MSCs protect the kidney in a model of sepsis in rats and improved the glomerular filtration rate, improved tubular function, decreased expression of nuclear factor κ B and of cytokines, increased expression of eNOS and of Klotho, attenuated renal apoptosis, and improved survival.

The last point investigated in the present study was the effects of WJ-MSCs that treated with HA on renal I/R injury. The present study demonstrated significant improvement in markers of kidney functions including serum creatinine, Na and K, urinary proteins and creatinine clearance more than WJ-MSCs that were not pretreated with HA. In line with these findings, histopathological examination of kidney tissues showed significant attenuation in acute tubular necrosis score with significant increase in regenerative capacity of renal tubules other than WJ-MSCs alone. These findings suggest renoprotective effect for this combination and HA might improve the homing of WJ-MSCs in the injured tissues which enhance its protective potency in the injured tissues. These findings, upto the best of our knowledge, are the first findings that demonstrated the potentiating effects for HA on WJ-MSCs against renal I/R injury. Also, in line with these findings, (45) demonstrated that HA potentiated the repairing effects of stem cell on myocardial ischemic injury.

Conclusion

Although, the present study demonstrated for the first time the enhancing effect for HA on the renoprotective effects of WJ-MSCs against renal I/R injury, some limitations for the present study are encountered. The most important limitation of the present study is the lack of the studying the underlying mechanisms for this effects. We recommend further studies to investigate the possible underlying mechanisms for this potentiating effect of HA.

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