Effect of Different Times of Intraperitoneal Injections of Human Bone Marrow Mesenchymal Stem Cell Conditioned Medium on Gentamicin-Induced Acute Kidney Injury

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Purpose: This study examined the effect of mesenchymal stem cells' conditioned media on the severity of acute kidney injury.

Materials and Methods: Acute kidney injury was induced in male rats with 100 mg/kg of gentamicin for six consecutive days intraperitoneally. After inducing the standard model of acute kidney injury, the conditioned medium of 5×10^6 cells was calculated for each kilogram of body weight of the rats. Then, it was injected in three different injection patterns other than the baseline injection of gentamicin. The rats were randomly divided into four groups: control group (n = 18) that did not receive any treatment, gentamicin group (n = 18) that received gentamicin at a dosage of 100 mg/kg for six consecutive days intraperitoneally, sham group (n = 54) that received gentamicin for six consecutive days, and an experimental group (n = 54) that received gentamicin for six consecutive days. Serum biochemical analysis and histological changes were studied and analyzed in all groups.

Results: Although human mesenchymal stem cells' conditioned media did not improve serum and tissue markers in the treatment groups, a relative improvement was observed in some indicators of tissue damage.

Conclusion: Secretory factors of human mesenchymal stem cells can be partly protective against gentamicin-induced nephrotoxicity.

Keywords: acute kidney injury; gentamicin; mesenchymal stem cells; conditioned culture media; secretory factors.

INTRODUCTION

As a common and grave illness with a high mortality rate, acute kidney injury is caused by toxic or ischemic insult from chemotherapy, antibiotics, or shock occurring from infection or major surgery. Acute kidney injury can lead to dysfunction and apoptosis/necrosis of renal tubular epithelial cells, in addition to a loss of renal endothelial cells.[1] Acute kidney injury occurs in 1% of hospital admissions. Up to 7% of hospitalized patients develop acute kidney injury.[2] Moreover, around 25% of patients in the intensive care unit develop acute kidney injury and 5% of them require kidney replacement therapy.[2,3]

Despite the use of modern dialysis techniques, such as intermittent or continuous kidney replacement therapy and kidney transplantation,[4,5] the syndrome still has a high mortality and morbidity rate.[4] However, kidney transplantation is hampered by shortage of donors. Dialysis is lifesaving and the main treatment in these patients, but it has several limitations. It is not a complete kidney replacement therapy and is associated with several socio-economic problems for the patients. Hence, it is imperative to accelerate the understanding of underlying causes of acute kidney injury and to develop new interventional and therapeutic modalities.[6]

In recent years, much attention has been focused on the plasticity of bone marrow-derived mesenchymal stem cells (MSCs).[7-9] However, systemic administration of MSCs has resulted in remarkable functional improvements in injured tissues without either long-term engraftment or differentiation in many clinical and experimental situations.[10] Moreover, improvements of injured tissues take place too rapidly to be explained by differentiation of MSCs. The emerging evidences suggest that most of the beneficial effects could be explained by secretion of therapeutic factors that have multiple effects, including modulation of inflammatory and immune reactions, protection from cell death, and stimulation of endogenous progenitor cells.[11] Moreover, it has been shown that MSCs secrete a large number of cytokines under normal culture conditions.[12] More importantly, they can be activated to express high levels of additional therapeutic factors by cross-talk with injured cells or microenvironments.[13]

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Received September 2015 & Accepted April 2016

Table 1. The study groups and their treatments.

Group Name	Subgroups	No. of Animals	Procedure of Injection
Control group	-----	18	No injection
Gentamicin group	-----	18	Gentamicin was injected at a dosage of 100 mg/kg for six consecutive days
Non-conditioned media group	A. Sham 1 B. Sham 2 D. Sham 3	54	(Gentamicin was injected at a dosage of 100 mg/kg for six consecutive days) + (NCM injection for three consecutive days with three of different injection patterns than the injection of gentamicin)
Experimental group	A. Experimental B. Experimental 2 D. Experimental 3	54	(Gentamicin was injected at a dosage of 100 mg/kg for six consecutive days) + (conditioned medium for three consecutive days with three of different injection patterns than the injection of gentamicin)

Abbreviation: NCM, non-conditioned medium.

Based on these evidences, it has been hypothesized that for protection against kidney failure, direct transplantation of stem cells is not necessary and administration of the conditioned media of the cells may also be effective.[14] Gentamicin as an aminoglycoside drug can induce renal tubular cell injury such as derangement of lysosomal, mitochondrial, and plasma membrane structure. It has been shown that gentamicin-induced nephrotoxicity is characterized by direct tubular necrosis, which is localized mainly in the proximal tubule. In this study we evaluated the therapeutic effects of conditioned media derived from human MSCs (hMSCs) in animal models of gentamicin-induced kidney failure.

MATERIALS AND METHODS

A total number of 144 adult male Wistar Albino rats weighting 180-220 grams were housed under standard laboratory conditions (12 hours of light/dark cycles) in a room with controlled temperature (24 ± 3°C) during the experiment. They were provided from a local veterinary research institute. The rats were housed in plastic cages under standard conditions with free access to drinking water and basic diet. All experimental procedures were conducted in accordance to the guide of care and use of laboratory animals.

Human MSCs were isolated from aspirate samples in the laboratory according to Fiedler and Fickert's procedure using Ficoll (Sucrose concentration gradient). The isolated bone marrow MSCs were transferred to Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum and 1% penicillin-streptomycin. After the incubation time, the cell supernatant was used for the treatment of animals. Each animal was injected per kilogram of body weight with conditioned medium derived from 5×10^6 cells intraperitoneally. These amounts were injected at three equal volumes for three consecutive days.

Design Model of Acute Kidney Injury

Firstly, an experiment was designed to achieve a standard animal model. A group of six rats received no injection as the control group. Ten rats (with six times

Table 2. The CM-hMSC impact on changes of renal tissue on the third day of review.

Groups	Acute Cell Swelling	Necrosis Tubules	Aggregation Inflammatory Cells	Glomerular Injury	Hyaline Cast
Control	-	-	-	-	-
Genta (Models group)	+++	+++	+	+	++
Genta + Medium (A)	+++	+++	+	+	++
Genta+ CM (A)	+++	+++	+	-	-
Genta+ Medium (B)	+++	+++	+	+	++
Genta+ CM (B)	++	++	+	-	-
Genta+ Medium (D)	+++	+++	+	+	++
Genta+ CM (D)	++	+++	+	+	++

Abbreviations: CM, conditioned medium; hMSC, human mesenchymal stem cell
- Parietal cells of tubules are normal and there is no injury.
+ There is acute kidney injury in less than one-third of the tubules.
++ There is acute kidney injury in one-third to two-thirds of tubules.
+++ There is acute kidney injury in more than two-thirds of the tubules.

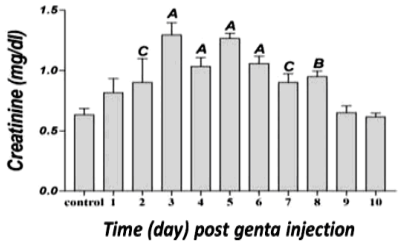

Figure 1. Gentamicin significantly increased the concentration of serum Cr (mg/dL) on the second, third, fourth, fifth, sixth, seventh and eighth days after injection compared with the control group. (A: $P < .001$) (B: $P < .01$) (C: $P < .05$) (n = 6).

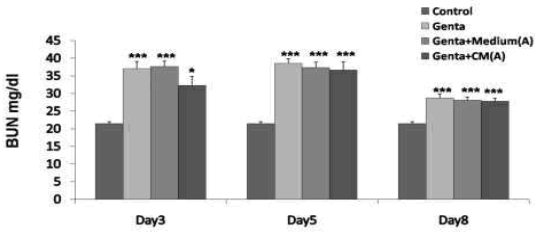

Figure 3. Effect of CM-hMSC on the amount of serum concentrations BUN (mg/dL) in the experimental group (A) compared with the model group. (***$P < .001$) (*$P < .05$) (n = 6).
Abbreviations: CM, conditioned medium; hMSC, human mesenchymal stem cell.

repetition) were in the second group. They received 100 mg/kg of gentamicin for six consecutive days intraperitoneally. Every day one rat was anesthetized for blood collection from the heart. Creatinine and blood urea nitrogen levels were studied for ten days by obtained peak day of the gentamicin effect in the period. The highest proportion of urea in respect to creatinine was on the 3rd and 5th days to determine the peak day of gentamicin effect. For further investigate, the 8th day was added to the study.

Experimental Design

The animals were randomly divided into four groups in order to study the effect of conditioned medium-derived MSCs on the disease process. Finally, on the 3rd, 5th and 8th days after gentamicin injection, kidney and blood samples of all rats were collected for histological and biochemical analysis.

Study Groups

The study groups were as follows (**Table 1**):

1) control group (n = 18) that did not receive any treatment,

2) gentamicin group (n = 18) that received gentamicin at a dosage of 100 mg/kg for six consecutive days intraperitoneally,

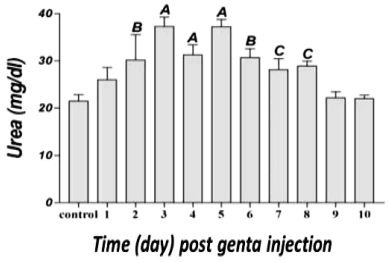

Figure 2. Verification induction of the animal model in acute renal disease through the analysis amount of changes in concentration BUN (mg/dL) of blood serum ten days after gentamicin injection compared with the control group. (A: $P < .001$) (B: $P < .01$) (C: $P < .05$) (n = 6).

3) sham group that received gentamicin for six consecutive days as well as non-conditioned medium in equal injection volume to the experimental group intraperitoneally for three consecutive days. This group was divided into three subgroups as follows (n = 18 for each sub-group):

A. Sham 1: non-conditioned medium injection began simultaneously with gentamicin injection.

B. Sham 2: non-conditioned medium injection began one day before gentamicin injection.

D. Sham 3: non-conditioned medium injection began one day after gentamicin injection.

4) experimental group that received gentamicin for six consecutive days as well as hMSC conditioned media intraperitoneally for three consecutive days. This group was also divided into three subgroups as follows (n = 18 for each sub-group):

A. Experimental 1: hMSC conditioned media injection began simultaneously with gentamicin injection.

B. Experimental 2: hMSC conditioned media injection began one day before gentamicin injection.

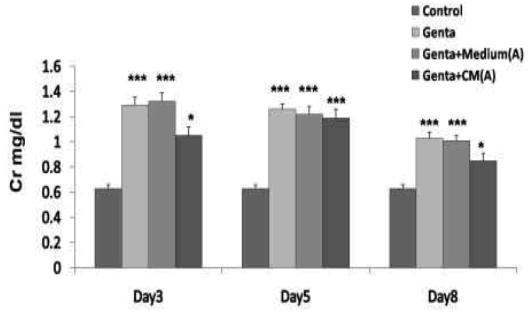

Figure 4. Effect of CM-hMSC on the amount of serum concentrations Cr (mg/dL) in the experimental group (A) compared with the model group. CM-hMSC had no significant decrease is in the amount of serum concentrations Cr (mg/dL) in animals treated on the third, fifth, and eighth days in the experimental group (A) compared with the model group. (***$P < .001$) (*$P < .05$) (n = 6).
Abbreviations: CM, conditioned medium; hMSC, human mesenchymal stem cell.

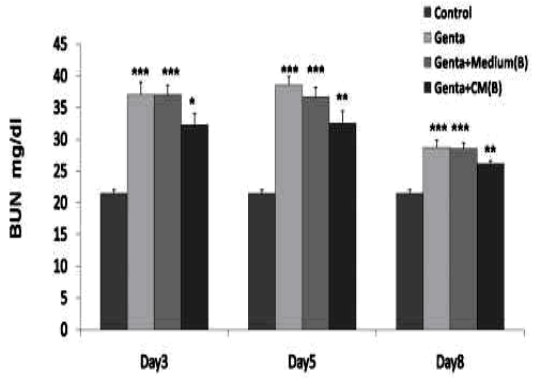

Figure 5. Effect of CM-hMSC on the amount of serum concentrations BUN (mg/dL) in the experimental group (B) compared with the model group. (***$P < .001$) (**$P < .01$) (*$P < .05$) (n = 6).
Abbreviations: CM, conditioned medium; hMSC, human mesenchymal stem cell.

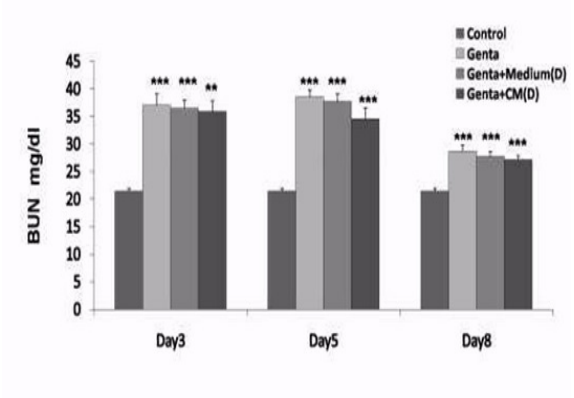

Figure 7. Effect of CM-hMSC on the amount of serum concentrations BUN (mg/dL) in the experimental group (D) compared with the model group. CM-hMSC had no significant decrease is in the amount of serum concentrations BUN (mg/dL) in animals treated on the third, fifth, and eighth days in the experimental group (D) compared with the model group (***$P < .001$) (n = 6).
Abbreviations: CM, conditioned medium; hMSC, human mesenchymal stem cell.

D. Experimental 3: hMSC conditioned media injection began one day after gentamicin injection.

Measuring Kidney's Functional Indices

On the 3rd, 5th and 8th days after gentamicin injection, all groups were anesthetized with a solution of ketamine-xylazine. Blood samples were taken from the heart and kidneys after dissecting the body. They were washed with saline and placed in 10% formalin. The collected blood samples were centrifuged and the serum was separated and analyzed for serum creatinine and blood urea nitrogen.

Four-micron sections were prepared from kidney samples and stained with hematoxylin and eosin. In the prepared slides, acute cell swelling, necrosis tubules, aggregation inflammatory cells, glomerular injury, and hyaline cast were evaluated by light microscopy and all tissue injuries were scored from (-) to (+++) for each kidney.[14]

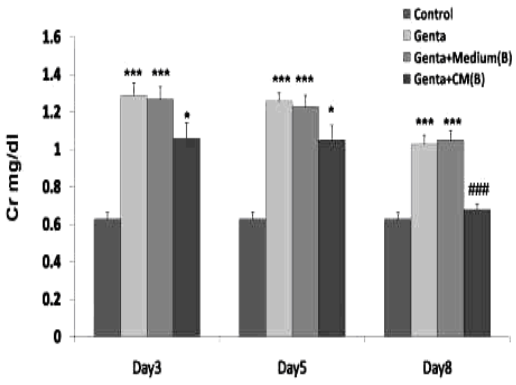

Figure 6. Effect of CM-hMSC on the amount of serum concentrations Cr (mg/dL) in the experimental group (B) compared with the model group. CM-hMSC had no significant decrease in the amount of serum concentrations BUN (mg/dL) on the third and fifth days in the experimental group (B) compared with the model group. (***$P < .001$) (*$P < .05$) (# # #$P <$ .001) (n = 6).
Abbreviations: CM, conditioned medium; hMSC, human mesenchymal stem cell.

Briefly, random cortical and medulla fields were analyzed using a × 40 objective light microscopy. To evaluate the degree of acute cell swelling and necrosis tubules, 150 tubules were counted in the cortical of each kidney randomly: (-) = parietal cells of the tubules were normal and there were no acute cell swellings and necrosis tubules; (+) = there were acute cell swelling and necrosis tubules in less than one-third of the tubules; (++) = there were acute cell swelling and necrosis tubules in one-third to two-thirds of tubules; (+++) = there were acute cell swelling and necrosis tubules in more than two-thirds of the tubules.

For scoring the degree of glomerular injury, 15 glomeruli were counted in the cortical of each kidney randomly: (-) = glomeruli were normal and there were no swelling, enlargement and lower urinary space; (+) = there were swelling, enlargement and lower urinary space in less than one-third of the glomeruli; (++) = there were swelling, enlargement and lower urinary space in one-third to two-thirds of the glomeruli. (+++) = there were swelling, enlargement, and lower urinary space in more than two-thirds of the glomeruli.

To evaluate the aggregation of inflammatory cells, 10 microscopic fields were counted in the cortical of each kidney randomly: (-) = There were no aggregated inflammatory cells in the space of between the tubules;

Table 3. The CM-hMSC impact on changes of renal tissue on the fifth day of review.

Groups	Acute Cell Swelling	Necrosis Tubules	Aggregation Inflammatory Cells	Glomerular Injury	Hyaline Cast
Control	-	-	-	-	-
Genta (Models group)	++	++	+	+	+
Genta + Medium (A)	++	++	+	+	+
Genta+ CM (A)	++	++	+	+	+
Genta+ Medium (B)	++	++	+	+	+
Genta+ CM (B)	+	++	+	-	-
Genta+ Medium (D)	++	++	+	+	+
Genta+ CM (D)	++	++	+	+	+

Abbreviations: CM, conditioned medium; hMSC, human mesenchymal stem cell.
- Parietal cells of tubules are normal and there is no injury.
+ There is acute kidney injury in less than one-third of the tubules.
++ There is acute kidney injury in one-third to two-thirds of tubules.
+++ There is acute kidney injury in more than two-thirds of the tubules.

(+) = there were aggregated inflammatory cells in less than one-third space between the tubules; (++) = there were aggregated inflammatory cells in one-third to two-thirds space between the tubules; (+++) = there were aggregated inflammatory cells in more than two-thirds space between the tubules. Finally, to evaluate hyaline casts, 10 microscopic fields were counted in the medulla of each kidney randomly: (-) = there was no casts in the tubules; (+) = there were casts in less than one-third of the tubules; (++) = there were casts in one-third to two-thirds of the tubules. (+++) = there were casts in more than two-thirds of the tubules.

Statistical Analysis

The statistical analysis was carried out using Statistical Package for the Social Science (SPSS Inc, Chicago, Illinois, USA) version 16.0. The differences between groups were analyzed by one-way variance analysis (ANOVA) and Dunnett's test. P value less than .05 was considered as statistically significant.

RESULTS

Creatinine and blood urea nitrogen were the most important serum markers in diagnosis of acute kidney injury. Biochemical serum analysis of the studied animals showed that creatinine and blood urea nitrogen increased significantly in rats that were treated with 100 mg/kg dosage of gentamicin for six days. Gentamicin significantly increased the concentration of serum creatinine and blood urea nitrogen in the 2nd to 8th days after injection. The most significant difference ($P < .001$) was on the 3rd and 5th days compared to the control group (**Figures 1 and 2**). Considering that the of

Table 4. The CM-hMSC impact on changes of renal tissue on the eighth day.

Groups	acute cell swelling	Necrosis tubules	Aggregation Inflammatory cells	Glomerular injury	Hyaline cast
Control	-	-	-	-	-
Genta (Models group)	++	+++	+	+	++
Genta + Medium (A)	++	+++	+	+	++
Genta+ CM (A)	+	++	+	+	++
Genta+ Medium (B)	++	++	+	+	++
Genta+ CM (B)	-	-	-	-	-
Genta+ Medium (D)	++	+++	+	+	++
Genta+ CM (D)	++	+++	+	+	++

Abbreviations: CM, conditioned medium; hMSC, human mesenchymal stem cell.
- Parietal cells of tubules are normal and there is no injury.
+ There is acute kidney injury in less than one-third of the tubules.
++ There is acute kidney injury in one-third to two-thirds of tubules.
+++ There is acute kidney injury in more than two-thirds of the tubules.

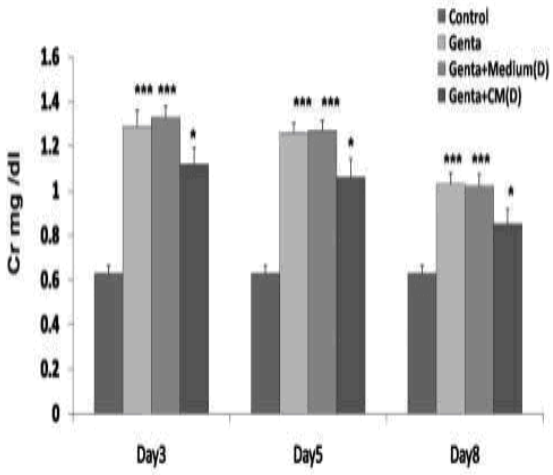

Figure 8. Effect of CM-hMSC on the amount of serum concentrations Cr (mg/dL) in the experimental group (D) compared with the model group. (***$P < .001$) (*$P < .05$) (n = 6).
Abbreviations: CM, conditioned medium; hMSC, human mesenchymal stem cell.

highest levels of blood urea nitrogen, creatinine and the ratio between the two were three days after injection of gentamicin during the ten-day period of study, this day was the acute phase of the disease.

Blood urea nitrogen and creatinine are the most important pathological indicators of acute kidney injury. Measuring serum concentration of waste products is their most common detection method. Hence, biochemical analysis of this material was done on collected serum of the studied animals.

Our results showed that after intraperitoneal injection of hMSC conditioned media for three consecutive days and simultaneous with the beginning of gentamicin injection, no significant decrease was seen in the 3rd, 5th and 8th days in blood urea nitrogen and creatinin(mg/dL) levels of the rats in the sub-group A of the experimental group compared to the gentamicin group (**Figures 3 and 4**).

Intraperitoneal injection of hMSC conditioned media for three consecutive days and 24 hours before injecting gentamicin had no significant effect in the 3rd, 5th and 8th days in the experimental group (sub-group B) compared to gentamicin (**Figure 5**). No significant decrease was seen in the creatinine of blood serum in the 3rd and 5th days in blood urea nitrogen levels of the rats in the experimental group (sub-group B) compared to the gentamicin group. But on the 8th day the level of creatinine (0.68 mg/dL) decreased significantly ($P < .001$) in sub-group B of the experimental group compared to the gentamicin group (0.63 mg/dL) (**Figure 6**).

Intraperitoneal injection of hMSC conditioned media for three consecutive days and 24 hours after injection of gentamicin had no significant effect on the amount of blood serum concentrations of blood urea nitrogen (mg/dL) and creatinine (mg/dL) in the 3rd, 5th and 8th days in sub-group D of the experimental group compared to gentamicin group (**Figures 7 and 8**).

Histological Study of the Kidney

Histological analysis is very efficient for evaluating kidney damage. Damage induced by gentamicin occurs mainly in the proximal tubule[15] which is the golden key in the diagnosis of acute kidney injury. In this study, the accuracy of inducing acute kidney injury was examined with preparing sections from the tested kidneys of the

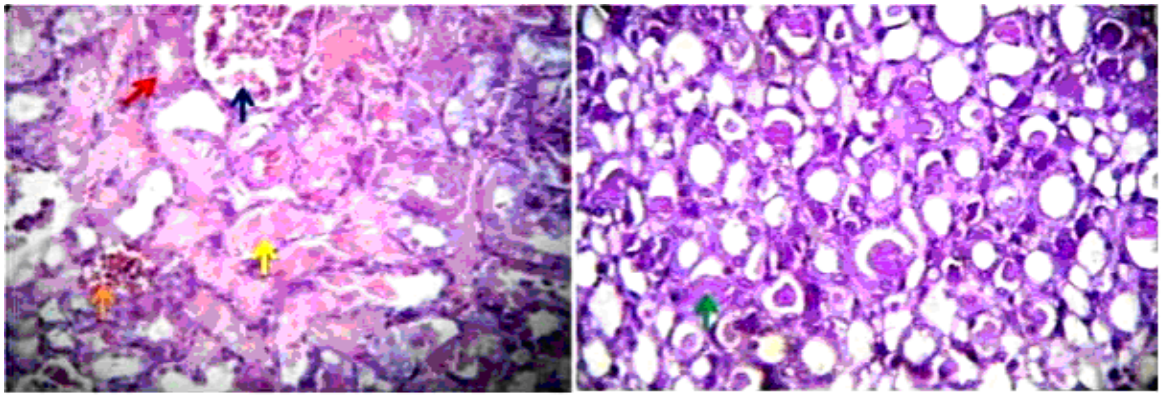

Figure 9. Verify the induction of acute kidney injury by histological analysis of the kidney of tested animals: Light microscopic image of kidney tissue of animals receiving gentamicin (model), renal tubular necrosis (yellow marker), acute cellular swelling (red marker), accumulation of inflammatory cells (orange marker) and glomerular injury (black marker) In the renal cortical (left figure), and also hyaline cysts (green marker) be clearly visible in the renal medulla (right figure).

animals. The results showed that a dosage of 100 mg/kg of gentamicin for six days causes renal tubular necrosis, acute cellular swelling, accumulation of inflammatory cells, hyaline cysts glomerular injury, all of which are symptoms of acute kidney injury (**Figure 9**). In contrast, the kidney tissue of control animals was normal and free from damage.

The Impact of hMSC Conditioned Media on the Changes of Kidney Tissue on the 3rd Day

To assess the protective effect of hMSC conditioned media on the 3rd day, the kidney tissues were examined in all groups three days after the gentamicin injection. Microscopic analysis (with H & E staining) showed that the kidneys of animals in the control group were completely normal and there was no tissue damage. However, kidneys in the gentamicin group had acute cellular swelling, renal tubular necrosis, and accumulation of inflammatory cells in the space between the tubules, hyaline cysts, and glomerular injury. The sub-group A of the experimental group showed no change in the rate of renal tubular necrosis, acute cellular swelling, and accumulation of inflammatory cells. However, glomerular damage and hyaline cysts was reduced. The amount of acute cellular swelling, renal tubular necrosis, hyaline casts, and glomerular injury was reduced in sub-group B of the experimental group compared to the model group. But no change occurred in the rate of accumulation of inflammatory cells. Evaluation of tissue damage in sub-group D of the experimental group only showed a slight reduction in the acute cellular swelling. No further reduction was visible in the amount of tissue damages in this group compared to the model group (**Table 2**).

The Impact of hMSC Conditioned Media on the Changes of Kidney Tissue on the 5th Day

To assess the protective effect of hMSC conditioned media on the 5th day, the kidney tissues were examined in all groups five days after gentamicin injection and staining with H & E. The kidneys of the control group were normal like the 3rd day and there was no tissue damage. However, kidneys in the gentamicin group had acute cellular swelling, renal tubular necrosis, and accumulation of inflammatory cells in the space between the tubules, hyaline cysts, and glomerular injury. The results of sub-groups A and D of the experimental group did not show any changes in the amount of tissue damage compared to the model group. In the sub-group B of the experimental group a significant reduction was seen in the amount of acute cellular swelling, glomerular damage and hyaline cysts in urine-collecting tube compared to the model group (**Table 3**).

The Impact of hMSC Conditioned Media on the Changes of Kidney Tissue on the 8th Day

To assess the protective effect of hMSC conditioned media on the 8th day, the kidney tissues were examined in all groups eight days after the gentamicin injection. Microscopic analysis (with H & E staining) showed that the kidneys of animals in the control group were completely normal. However, kidneys in the gentamicin group had acute cellular swelling, renal tubular necrosis, accumulation of inflammatory cells in the space between the tubules, hyaline cysts, and glomerular injury. The sub-group A of the experimental group showed a slight reduction in the rate of acute cellular swelling and no improvement was observed in other cases of tissue damage. In sub-group D of the experimental group all signs of tissue injuries were observed without any improvement in the kidney tissue. This showed that hMSC conditioned media has no protective effect against acute kidney injury. The results of sub-group B of the experimental group showed a significant decrease in the extent of tissue injury criteria measured in this study, indicating hMSC conditioned media has protective effects against acute kidney injury (**Table 4**).

DISCUSSION

According to some previous studies the culture of MSCs have protective effects on animal models with acute kidney injury.[16] Although the culture of MSCs led to restoration of some tissue damage parameters in the days after the injection of gentamicin in the experimental group, almost no significant effect was observed in reducing serum chemical biomarkers on the studied days (except in sub-group B of the experiment group on the 8th day).

Aminoglycoside antibiotics are widely used in treating many infections produced by Gram-negative bacteria and bacteria endocarditis.[17] A relatively large amount (about 10%) of the intravenously administered dosage is accumulated in the kidney, whereas little distribution of aminoglycosides into other tissues is observed.[18] The tubular toxicity of gentamicin has two aspects: (i) the death of tubular epithelial cells associated with a very important inflammatory component and (ii) the nonlethal, functional alteration of key cellular components involved in water and solute transport. Indeed, the gentamicin-induced necrosis and apoptosis in the tubular epithelial cells, decreases tubular function, and dysfunctional reabsorption process of water and electrolytes.

Growth factors derived from MSC contain high levels

of vascular dilation. Prostaglandins lead to vasodilatation and increased kidney blood and glomerular filtration rate. Therefore, MSCs can increase glomerular filtration rate, and consequently decrease the creatinine and urea levels.[19] In our study, no significant reduction was seen in serum biomarkers (except on the 8th day in sub-group B of the experimental group).

In this study we could see some improvement in histological parameters. Sub-group B of the experimental group showed the highest restoration rate compared to the other two groups. Also, the simultaneous injection of hMSC conditioned media with gentamicin was more effective than the injection of hMSC conditioned media one day after starting gentamicin injection. Parekkadan and colleagues showed the presence of vascular endothelial and hepatocyte growth factors and other anti-apoptotic molecules in MSC conditioned medium.[20] They stated that bioactive molecules can lead to increased survival of the recipient animals.[20] Gheisari and colleagues assessed the therapeutic effect of conditioned media of mouse bone marrow MSCs and human umbilical cord blood somatic stem cells on acute kidney injury.[14] Indeed they could not confirm the beneficial effects of MSC conditioned medium in decreasing blood urea nitrogen and creatinine in acute kidney injury.

Some previous studies have identified that administration of growth factors before and after kidney damage in animal models improves the damage and its healing. This might be because of its anti-apoptotic effects.[21,22] Human MSCs conditioned media activate the pathway of phosphatidylinositol protein kinase B signaling that reduces apoptosis and increases the survival of proximal tubular epithelial cells.[19] Protein kinase B provides survival signals by several independent mechanisms. Protein kinase B directly phosphorylates and inhibits proapoptotic factors such as Bcl-2-associated death and others.[23]

In our study some improvements were seen in the histological parameters. Sub-group B of the experimental group showed the highest restoration rate compared to the other two groups. Hence, we assumed this was attributed to the protective effect of hMSC conditioned media injection one day before gentamicin injection. This is because if factors necessary for the repair at the time of injury should stay in place they can be more helpful. Also, the simultaneous injection of hMSC conditioned media with gentamicin was more effective than the injection of hMSC conditioned media one day after the start of gentamicin injection. In most previous studies, administration of hMSC conditioned media had been done after the induction of acute kidney injury.

In a study in which injections of stem cell and granulocyte-macrophage colony-stimulating factors was done simultaneously with the beginning of the induction of acute tubular necrosis, Zhang and colleagues stated that stem cell and granulocyte-macrophage colony-stimulating factors effectively mobilized bone marrow cells. This partially led to creating the treatment effects in acute tubular necrosis induced by gentamicin.[18] Also, in another example in which the injections of growth factors was done before the beginning of the induction of acute kidney injury, Morin and colleagues showed that epidermal growth factor accelerates repair in a rat model of gentamicin nephrotoxicity.[24]

There are some problems in using hMSC conditioned media for treating acute kidney failure. For examples, each bioactive molecule works in a defined concentration and in some cases they show different functions in different concentrations.[2] It is possible that the concentration of mediators secreted by stem cells in the culture is not in the appropriate range for a renoprotective effect. Also, many researchers assume that stem cells secrete defined sets of mediators in a temporally and spatially regulated manner in response to injury.[25] In fact, when stem cells reach the location of injury, they secrete special factors based on the specific signals received from the injury microenvironment.[14]

Today, it is not clear that what other factors that are secreted by MSC can offer kidney protection. Identification and purification of these factors would provide new avenues for pharmacological therapy of acute kidney injury and avoid injection of a large volume of hMSC conditioned media.[26] Despite all the information that is obtained in different studies there is no final conclusion that whether the effects of MSCs are involved in kidney repair directly by cellular differentiation or are due to fusion between bone marrow cells and injured cells or indirectly by various paracrine-endocrine effects created by MSCs on tubular cells.[27-29] If the protective effect is mediated in an endocrine manner, then injection of the cells themselves would not be required. Rather the factors that those cells secrete could be provided immediately at the time of kidney injury.[9] The results of this study showed that the protective effect of MSCs might be due to their endocrine function which is in agreement with a number of previous studies described above.

CONCLUSIONS

Although conditioned media derived from humans in an animal model of gentamicin-induced kidney failure did not improve all of serum and tissue markers in the treat-

ment groups, relative improvement in some indicators of tissue damage was observed (especially in the hMSC conditioned media injection one day before gentamicin injection). It can be assumed that the injection time of hMSC conditioned media has a role in injury recovery. In addition to the injection time of hMSC conditioned media, other factors such as injections dosages, longevity factor, etc. can have a role in achieving positive and negative results. However, these findings suggest that secretory factors of hMSCs can be partially protective against gentamicin-induced nephrotoxicity.

ACKNOWLEDGMENTS

This study was funded by the faculty of Biological Sciences of Kharazmi University in Tehran. The authors would like to thank Seyed Muhammed Hussein Mousavinasab for his sincere cooperation in editing this text.

CONFLICT OF INTEREST

None declared.

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