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Effect of mesenchymal stem cells transplantation in treatment of amikacin induced- kidney injury in male mice

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Abstract:

Acute kidney injury (AKI) is a word that refers to a loss of renal function that occurs suddenly and accompanied with an elevated creatinine levels in the blood (a renal sign) and decreased urinary output (oliguria). Since Stem cell has immense potential in regenerative cellular therapy. Mesenchymal stem cells (MSCs) can become a potential attractive candidate for therapy due to its remarkable ability of self-renewal and differentiation. Stem cell holds tremendous promises in the field of tissue regeneration and transplantation for disease treatments. The project aimed to evaluate the role of bone marrow-mesenchymal stem cells (BM-MCSs) as an anti-inflammatory, anti-oxidant agent and therapy for amikacin - induced kidney injury. For the kidney injury induction, 100 mg/kg amikacin were daily intraperitoneally injected for ten days to mice (group II to III). Fifteen mice were divided into three groups (5 mice/group); group I: healthy negative control mice, group II: untreated amikacin induced-kidney injured mice (positive control), group III: amikacin induced-kidney injured mice treated with BM-MSCs. Following the induction of kidney injury two million BM-MSCs suspended in Dulbecco's modified Eagles medium (DMEM) were intravenously injected to mice (group III). BM-MSCs were successfully transplanted in mice kidney leading to a reduction in the elevated levels of kidney function parameters (urea and creatinine), oxidative stress (MDA) and inflammatory cytokines (IL-1β and IL-6). BM-MSCs succeeded in ameliorating the amikacin-induced nephrotoxicity in balb/c mice. MSCs exerted antioxidant and anti-inflammatory effects during treatment, where BM-MSCs decreased cytokines levels and elevated the antioxidant enzyme levels in kidney tissue homogenates.

Keywords: kidney injury, amikacin, MSCs, cytokines.

Introduction

Kidneys are essential for preserving homeostasis through the regulation of fluid and electrolyte balance, hormone secretion, acid-base balance, metabolism, gluconeogenesis, arterial pressure, and excretion. Kidneys modify excretion rate to correspond with the consumption of various substances. They control long-term arterial pressure by regulating sodium and water levels, short-term pressure by secreting hormones and vasoactive substances, and acid levels by excreting acids and preserving body buffers (Costa, 2021). The kidneys also produce the vitamin D's active form (calcitriol or 1,25-dihydroxyvitamin D3), aid in the production of red blood cells in bone marrow by releasing erythropoietin, and produce glucose during prolonged fasting periods (gluconeogenesis) (Costanzo, 2021).

Acute kidney injury (AKI) is a multifaceted syndrome marked by a decline in renal function, with various causes and underlying mechanisms (Susantitaphong et al., 2013; Hoste et al., 2018). Commonly diagnosed in hospitalized patients, AKI is linked to worse short- and long-term outcomes and higher healthcare costs (Chertow et al., 2005). The incidence of AKI has risen in recent years (Chertow et al., 2005; Susantitaphong et al., 2013); although reported incidence rates vary due to differences in population characteristics, causes of AKI, and diagnostic criteria (Chertow et al., 2005; Lameire et al., 2006; Susantitaphong et al., 2013; Hoste et al., 2018). Amikacin, a powerful antibiotic in the aminoglycoside family, can cause kidney injury through its nephrotoxic effects. Amikacin-induced nephrotoxicity arises from its accumulation in the renal tubules, triggering oxidative stress and inflammation. Jiang et al. (2017) described how amikacin disrupts cellular homeostasis in renal tubular cells. Oxidative stress and inflammation significantly contribute to tubular damage and reduced filtration capacity, leading to impaired kidney function.

Mesenchymal stem cells (MSCs) are noted for their distinctive immunomodulatory and regenerative capabilities, which make them promising candidates for kidney injury therapy (Morigi et al., 2021). Their ability to self-renew and differentiate into multiple cell types enhances their appeal in regenerative medicine (Chen et al., 2023). MSCs show significant potential in repairing damaged tissues and improving cellular functions, positioning them as a viable option for treating AKI.

Therefore, the study aimed to evaluate the effect of bone marrow mesenchymal stem cells (BM-MSCs) in treatment of amikacin-induced kidney injury. This was performed by measuring the kidney function parameters (urea and creatinine) and inflammatory cytokines in all experimental groups.

Materials and methods

Bone marrow-mesenchymal stem cells

The mouse BM-MSCs cells (sixth passage) were purchased from cyagen, USA (MUBMX-01001). BM-MSCs were obtained from the healthy bone marrow of C57BL/6 mouse which showed a potent capability for differentiation and proliferation. A long with the manufacturer's instructions the results showed that CD117 and CD31 were negative (<5%). On the other hand, Sca-1, CD29 and CD44 were positive (>70%). Cells were mixed with an equal volume of the Paul Karl Horan (PKH26) red Fluorescent Cell Linker Kit (Sigma-Aldrich, USA) prior to mice injection (4 nM) and incubated for five minutes at 25 °C (Haas et al. 2000). Induction of kidney injury.

For the kidney injury induction, according to Batoo et al. (2018), 100 mg/kg amikacin were daily intraperitoneally injected for ten days to mice (group II to III).

Experimental design

Male BALB/c mice were purchased from the animal house colony, National Research Centre, Cairo, Egypt; and kept under standard experimental condition (22 ± 2oC, 12 h dark/light cycle and 50 ± 2% humidity) for one week for acclimatization. Fifteen mice were divided into three groups (5 mice/group); group I: healthy negative control mice, group II: untreated amikacin induced-kidney injured mice (positive control), group III: amikacin induced-kidney injured mice treated with BM-MSCs. Animals received standard mouse chow and water ad libitum, throughout the experimental time; and all experimental procedures were accomplished according to the ARRIVE guidelines for the reporting of animal experiments. The study was approved by the institutional animal care and use committee of Cairo University (CU-IACUC) under the number (CUIF6122). Following the induction of kidney injury two million BM-MSCs suspended in Dulbecco's modified Eagles medium (DMEM) were intravenously injected to mice (group III). Animals were anaesthetized by 50 mg/kg of sodium pentobarbital at the end of the experiment. For preparation of serum samples, the blood was collected by cardiac puncture and left to clot then centrifuged for 10 minutes at 1500 rpm. Serum samples were divided into aliquots and kept at -80oC. For preparation of kidney tissue homogenates, kidneys from all experimental groups were collected. According to Farid et al. (2023), one gram of kidney was homogenized with cold Tris-HCl solution (10 mmol, pH = 7.4) followed by centrifugation at 1500 rpm for 15 minutes. The supernatant was separated and used in the measurement of oxidative stress and inflammation.

Analysis of Kidney function

In the serum samples, urea and creatinine were measured by Colorimetric kits (ab83362 and ab65340, respectively; abcam, USA). In urea Assay

protocol, enzymes acted on urea to produce a product that reacted with a probe to generate a color (ODmax=570nm). The absorbance measured was directly proportion to the urea concentration in the solution. The materials required for the assay included a 96-well clear plate for colorimetric assay, orbital shaker, microcentrifuge, pipette and pipette tips, a colorimetric microplate reader, MiliQ water or double-distilled water (ddH2O), phosphate buffered saline (PBS), urea assay buffer, OxiRed probe in DMSO, lyophilized enzyme mix, 100mM urea standard, developer, and converting enzyme. To each well, 50 µL of standard dilutions and 25 µL of samples were added. Subsequently, 50 µL of reaction mix was added to each standard and sample well, while 50 µL of background reaction mix was added to the background control wells. The plates were incubated at 37°C for 60 minutes, protected from light. Finally, the plates were measured colorimetrically on a microplate reader at OD 570 nm using a microplate reader.

The principle of the serum creatinine assay involved the conversion of creatinine to creatine by creatininase, followed by the conversion of creatine to sarcosine. This sarcosine was then oxidized to produce a product that reacted with a probe, generating a red color with a maximum wavelength of 570 nm. The materials required for this assay included creatinine assay buffer, lyophilized creatinase and creatininase, a creatinine probe in DMSO, creatinine enzyme mix, a 10 µmol creatinine standard, and a microplate reader capable of measuring absorbance at OD 570 nm. Additionally, MilliQ water or double distilled water, tubes for reagent preparation, pipettes (including a multi-channel pipette), a 96-well plate with a clear flat bottom, and assorted glassware for reagent and buffer solution preparation were needed. To each well, 50 µL of standard dilutions and 50 µL of samples were added. This was followed by adding 50 µL of reaction mix to each standard and sample well, and 50 µL of background reaction mix to the background control sample wells. The plates were incubated at 37°C for 60 minutes, protected from light, and then measured colorimetrically on a microplate reader at OD 570 nm.

Oxidative stress measurements

Oxidative stress was assessed by measuring malondialdehyde (MDA) and superoxide dismutase (SOD) levels in kidney tissue homogenates using ELISA kits (MBS741034 and MBS2707323, respectively; MyBioSource, USA).

Immunological measurements

Inflammatory markers (IL-1β and IL-6) were measured in kidney tissue homogenates using ELISA kits (ab197742 and ab222503, respectively; Abcam, UK). ELISA procedures followed the manufacturer's guidelines and precautions.

ELISA procedures

The principle behind ELISA involves detecting and quantifying proteins secreted or released from cells. Target-specific capture antibodies or antigens against viruses, bacteria, and other materials were immobilized onto an ELISA plate with high protein binding capacity, enabling the capture of the target protein. A protein-specific biotinylated antibody detected the captured protein. The target protein was quantified through a colorimetric reaction facilitated by avidin-horseradish peroxidase bound to the biotinylated detection antibody, measured as optical density (O.D.) using an ELISA plate reader. Washing buffer (PBS/T) was diluted 1:20 in distilled water. Only the required number of precoated ELISA 96 well plate wells was used, while the rest were resealed in the pouch with a desiccant. Standards were diluted in assay diluent at 1:2 serial dilutions, and samples were diluted to the appropriate concentration in assay diluent. The detection antibody was reconstituted and diluted in assay diluent to a concentration of 0.3 μg/ml (1:20), and the Streptavidin-HRP conjugate was diluted 1:20 in assay diluent. The procedure began with washing the plate three times using 300 μl/well of washing solution. After the final wash, the plate was inverted to remove residual solution on a paper towel. 100 μl of standard or sample was added to each well in duplicate, followed by covering the plate with a sealer and incubating it at room temperature for 2 hours. The plate was then washed four times with washing buffer. 100 μl/well of the diluted detection antibody (0.3 μg/ml) was added, the plate was covered and incubated at room temperature for 2 hours, and then washed four times with washing buffer again. 100 μl of the diluted color development enzyme (1:20) was added to each well and incubated at room temperature for 30 minutes. After washing the plate four times, 100 μl/well of the color development solution was added and incubated at room temperature for 8-18 minutes. Finally, 100 μl/well of stop solution was added to halt the reaction, and the plate was read at a wavelength of 450 nm using a microplate reader.

Fluorescence examination

Segments of kidney tissue were fixed in 10% buffered formalin. For embedding, the fixed segments were washed in tap water, dehydrated sequentially in ethanol (70%, 90%, 95%, and 100%), cleared in xylene, and embedded in paraffin wax at 55°C. During sectioning, 5 sections of 4 μm thickness were cut from each segment. Kidney sections were examined by fluorescent microscope (Olympus, Tokyo, Japan).

Statistical analysis

For statistical analysis, SPSS version 20.0 (SPSS Inc., Chicago, IL, USA) was utilized to analyze the results. Data were presented as means ± standard deviations (SD) and subjected to one-way analysis of variance (ANOVA). Differences between means were assessed using the Tukey post hoc test, with results considered statistically significant if the p value was less than 0.05.

Results

Kidney function:

Amikacin administration led to an increase in kidney function parameters (urea and creatinine) in untreated group II when compared to control group I. MSCs transplantation has significantly decreased this elevation in treated group III when compared to untreated group II (figure 1).

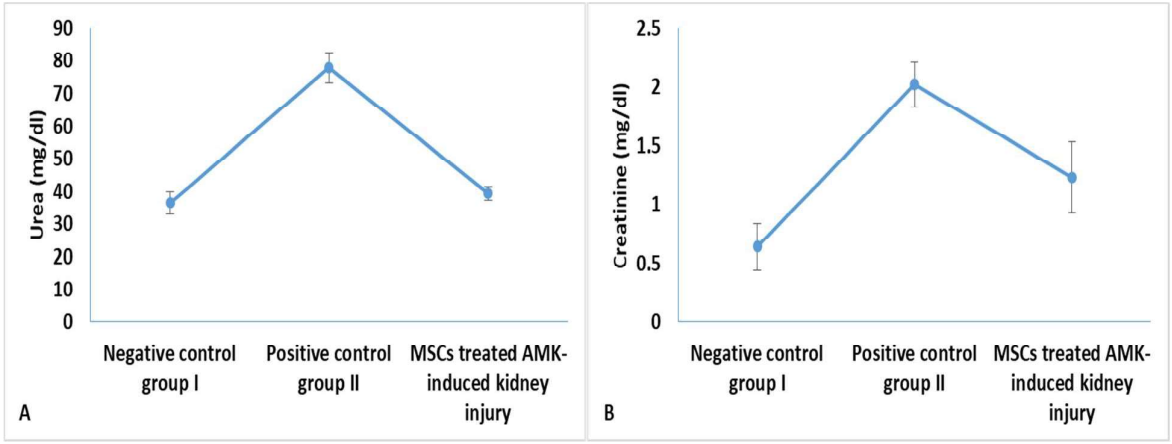

Figure 1: kidney function parameters in different experimental groups.

Oxidative stress

Amikacin induced nephrotoxicity resulted in an increase in MDA level and a decrease in SOD level in untreated group II. MSCs transplantation amolerated this disturbance in treated group III (figure 2).

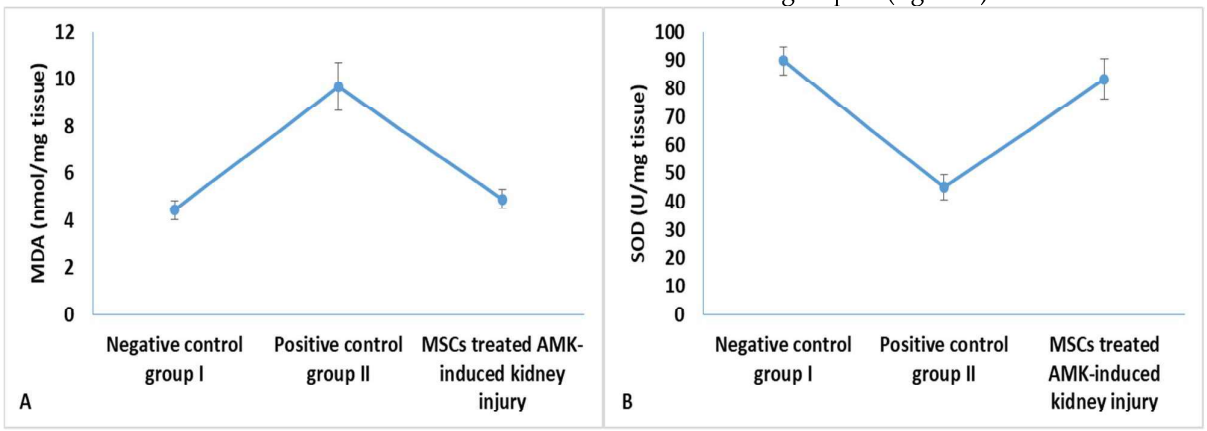

Figure 2: oxidative stress parameters in different experimental groups.

Immunological markers

Amikacin induced nephrotoxicity resulted in an increase in cytokines levels in kidney tissue homogenates in untreated group II in comparison to those of control group I. MSCs transplantation decreased this elevation in treated group III, where cytokine levels were similar to those of control group I (figure 3).

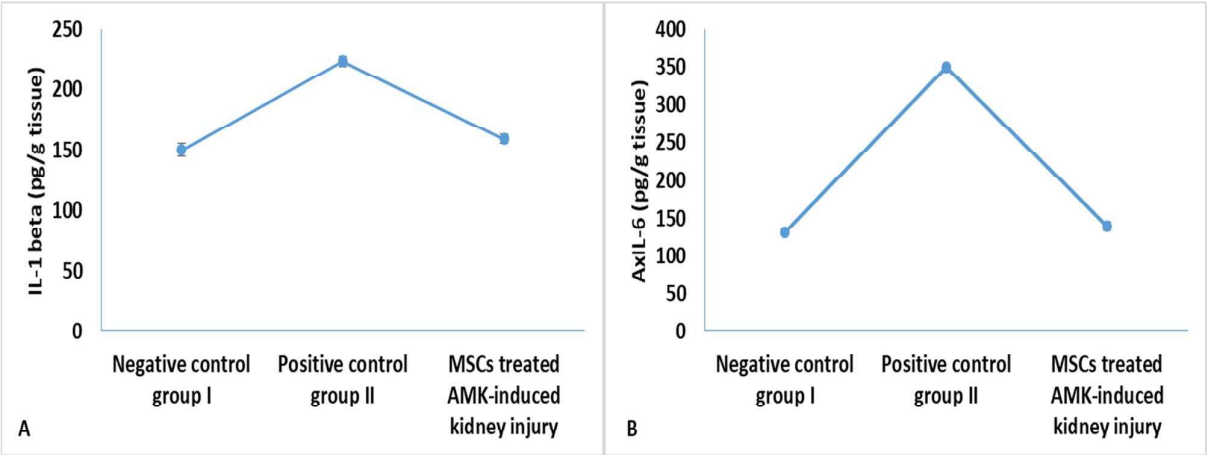

Figure 3: cytokines levels in different experimental groups.

Histopathological examination

Fluorescent microscopic images of mice kidney sections showed the red fluorescence of PKH26 labeled-BM-MSCs in group III (figure 4).

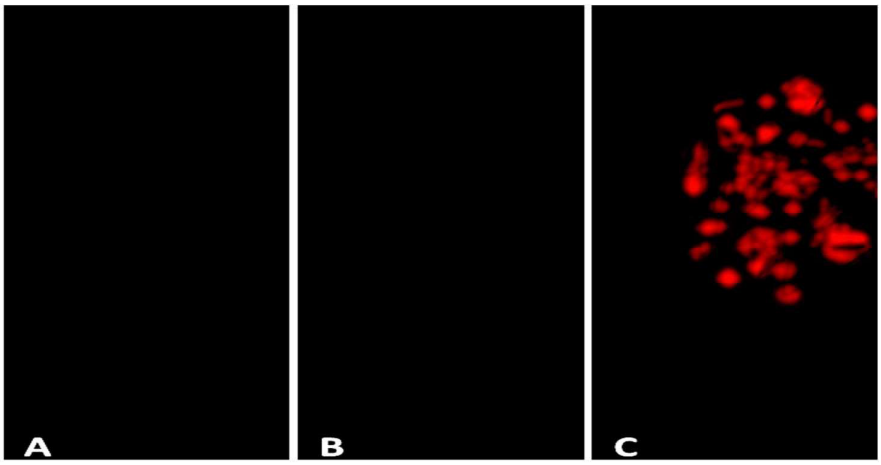

Figure 4: Fluorescent microscopic images of mice kidney sections of negative control group I (A), positive control group II (B) and BM-MSCs treated group III (C).

Discussion

AKI is a condition characterized by the rapid decline of renal function over a period of hours or days, caused by various factors. The primary pathological changes include reduced renal perfusion, renal tubular injury, tubulointerstitial inflammation, and a decreased glomerular filtration rate (Kellum et al., 2013). AKI is a significant global health issue, affecting approximately 14 million patients and resulting in 1.7 million deaths annually (Mehta et al., 2015). Additionally, 20% of hospitalized

patients develop AKI, with half requiring renal replacement therapy (Levey and James, 2017). Even mild and reversible AKI can lead to severe outcomes such as death (Hoste et al., 2006; Uchino et al., 2006). The long-term effects of AKI include the progression and exacerbation of chronic kidney disease and end-stage renal disease (Ishani et al., 2009; Chawla et al., 2014). Despite advancements in understanding the pathogenesis of AKI, clinical treatment options remain limited. Various treatment strategies, including antioxidants, diuretics, dopamine, and reducing exposure to nephrotoxic drugs, do not alter the disease's course (Benoit and Devarajan, 2018).

According to Sutherland et al. (2016), aminoglycosides constitute a class of antibiotics that continue to be extremely successful versus Gram-negative bacteria in vitro. However, due to their concomitant nephrotoxicity, these antibiotics have limited clinical utility (Mingeot-Leclercq and Tulkens, 1999). According to a recent study of Oliveira et al. (2009), 58% of critically sick patients in intensive care units who needed such medication experienced aminoglycoside-associated nephrotoxicity. Because of their unfavorable side effect characteristics, aminoglycosides are primarily employed as a last resort after all other choices have been exhausted. Medically, aminoglycoside-associated nephrotoxicity is a substantial cause of death in patients in hospitals (Paquette et al., 2015). Research has demonstrated that the length of therapy and dosage are related factors in aminoglycoside-associated nephrotoxicity (Murry et al., 1999). Additionally, it is well recognized that a number of risk factors, including concomitant antibiotic treatment, underlying heart disease, and preexisting renal illness, are linked to a higher risk of kidney injury (Paquette et al., 2015). Thus, reducing the nephrotoxicity brought on by aminoglycoside medication may greatly enhance patient outcomes. Previous study has demonstrated preferential absorption and storage of aminoglycosides in the kidneys, which then produces major physiological alterations predominantly in the proximal tubule cells. This sets off a number of biological processes that eventually result in the death of kidney cells (Mingeot-Leclercq and Tulkens, 1999). It is thought that interactions between the highly polar aminoglycosides and lipid-based cellular components are what cause these cascades. The aforementioned cascades comprise modifications to the architecture of lysosomes that impact filtration effectiveness, interactions with phospholipid membranes that result in aggregation, shedding, and lesion development, loss of lipid transport proteins and enzymes, and disruption of mitochondrial activities. When taken as a whole, these cascades are linked to cell necrosis, which lowers renal function and can occasionally cause irreversible kidney damage.

Recent advances in regenerative medicine have offered promising therapeutic strategies for preventing or treating AKI. Among these, MSCs are mesoderm-derived stem cells capable of self-renewal and

multidirectional differentiation. Their diverse sources, easy isolation, high migration capacity, expansion rate, mild immune rejection, and fewer ethical concerns make them a research hotspot (Almalki and Agrawal, 2016; Peired et al., 2016). MSCs exhibit renal protective effects primarily through anti-inflammation, promoting angiogenesis, mobilizing endogenous stem cells, anti-apoptosis, anti-fibrosis, anti-oxidation, and promoting cell reprogramming (de Almeida et al., 2013). Studies have shown that bone marrow-derived stem cells can differentiate into various inherent kidney components, including renal tubular epithelial cells, podocytes, mesangial cells, and capillary endothelial cells (Poulsom et al., 2001; Li et al., 2006).

According to Dominicici et al. (2006), MSCs have fibroblast-like morphology, are plastic-adherent in conventional tissue culture conditions, and express the CD105, CD90, and CD73 antigens on their surface. Because MSCs are immune-privileged due to their lack of expression of major histocompatibility complex class II, co-stimulatory molecules (CD40 and CD86), and hematopoietic markers (CD45 and CD14), allogenic transplantation of MSCs is a safe and effective therapeutic option (Wise and Ricardo, 2012). Moreover, MSCs do not result in the production of teratomas, in contrast to other progenitor cells as induced pluripotent stem cells and embryonic stem cells (Yamaoka and Mahara, 2011). BM-MSCs, are the most studied type of MSCs. They are simple to produce on a large scale, have low immunogenicity, and can be readily extracted from young, healthy donors.

Therefore, the study aimed to evaluate the effect of BM-MSCs in treatment of amikacin-induced kidney injury. This was performed by measuring the kidney function parameters (urea and creatinine), oxidative stress and inflammatory cytokines in all experimental groups. Fifteen mice were divided into three groups (5 mice/group); group I: healthy negative control mice, group II: untreated amikacin induced-kidney injured mice (positive control), group III: amikacin induced-kidney injured mice treated with BM-MSCs. For the kidney injury induction, according to Batoo et al. (2018), 100 mg/kg amikacin were daily intraperitoneally injected for ten days to mice (group II to III). Following the induction of kidney injury two million BM-MSCs suspended in DMEM were intravenously injected to mice (group III). Animals were anaesthetized at the end of the experiment by 50 mg/kg of sodium pentobarbital. Blood samples were collected by cardiac puncture for preparation of serum. For preparation of kidney tissue homogenates, kidneys from all experimental groups were collected. According to Farid et al. (2023), cold Tris-HCl solution (10 mmol, pH = 7.4) was used for the preparation of kidney tissue homogenates.

Our results showed that amikacin administration led to an increase in kidney function parameters (urea and creatinine), MDA level and

cytokines levels (IL-1beta and IL-6) in untreated group II when compared to control group I. MSCs transplantation has significantly decreased this elevation in treated group III when compared to untreated group II, where cytokine levels were similar to those of control group I. Kidney sections proofed the transplantation of BM-MSCs by the appearance of red fluorescence.

Recent research has demonstrated that the renoprotective effects of exogenously delivered BM-MSCs occur mainly in a paracrine manner, via the production of trophic factors and by immune modulation (Payne et al., 2013). This is in spite of the fact that it has been found that the BM-MSCs can directly engraft and trans-differentiate to regenerate the damaged kidney tubular epithelial cells (Yun and Lee, 2019). Many surface chemokine receptors on BM-MSCs, such as the chemokine receptor, may interact with the chemokines generated under pathogenic circumstances and help the cells migrate to inflammatory areas (Wise and Ricardo, 2012). When BM-MSCs arrive at the site of damage, they produce a wide range of regulating chemicals known as the MSC secretome, which includes growth factor, proteinase, hormone, cytokine, and chemokine (Eleuteri and Fierabracci, 2019).

Therapies utilizing MSCs may address current limitations due to their low immunogenicity, immunological privilege, and immunomodulatory properties (Mohib et al., 2010). MSCs have the ability to differentiate into various cell types, which can be used to replace damaged tissues and restore their function after local engraftment (Aggarwal & Pittenger, 2005). Preclinical studies have demonstrated the therapeutic potential of MSCs in treating kidney injury, and early-phase clinical trials are currently in progress (Chen et al., 2023). The regenerative ability of MSCs and their tendency to migrate toward damaged tissue have been explored in AKI animal models. Preclinical studies demonstrate that MSCs can secrete cytokines such as IL-6, IL-10, TGF-β, and other cytokines to counteract the early inflammatory environment of AKI (Humphreys and Bonventre, 2008; Wang et al., 2012), differentiate into pericyte-like cells, and promote angiogenesis and renal vascular perfusion by secreting vascular endothelial growth factor, insulin-like growth factor-1, and hepatocyte growth factor (Ball et al., 2007; Imberti et al., 2007; Au et al., 2008; Sanz et al., 2008). Additionally, they can promote the regeneration of injured renal cells by migrating to the kidney and differentiating into renal parenchyma cells (Humphreys and Bonventre, 2008).

In conclusion, MSCs transplantation succeeded in amolerating the amikacine-induced nephrotoxicity in balb/c mice. MSCs exerted an antioxidant and anti-inflammatory effects during treatment, where MSCs decreased cytokines levels and elevated the antioxidant enzyme levels in kidney tissue homogenates.

Conflict of Interest

None to declare.

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