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RESEARCH ARTICLE

Cell-based Therapy: A Good Option for Retrieving Nephropathy Induced by Cyclosporine

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Abstract

Objective: The main goal is to address the role of mesenchymal stem cells derived from bone marrow (BM-MSCs) and adipose tissue (AD-MSCs) in mitigation of cyclosporine A (CsA)-induced nephropathy in rats. Methods: Isolation and propagation of MSCs from both sources were performed and the cells were recognized by the microscopic appearance and estimation of specific surface antigens (CD90, CD105 and CD45) using flow cytometry. In this study 40 adult male rats were distributed into 4 groups; (1) control group, (2) CsA group, (3) BM-MSCs group and (4) AD-MSCs group. Two month after stem cell infusion, urea and creatinine serum levels were estimated. Also, serum levels of MCP-1, NF-κB, nephrin and NAG activity were evaluated. Also, histopathology of kidney sections was carried out. Results: Microscopic photo documentation and CD surface markers proved that the isolated cells have typical characteristics of MSCs. The outcomes of the *in vivo* study indicate the beneficial effect of BM-MSCs and AD-MSCs in kidney restoration as manifested by the significant blunting in serum urea, creatinine, MCP- 1, NF-κB levels and the significant inhibition of serum NAG paralleled by the significant increase of serum nephrin level and GFR. These findings were also appreciated by the histopathological observation of kidney tissue sections as the cell therapy elicited marked betterment in the structural organization of kidney tissue. Conclusion: This study provides great evidence favoring the significant role of MSCs, particularly isolated from AD, in retrieving experimental nephropathy induced by cyclosporine. The efficiency of MSCs could be ascribed to their immunomodulatory effect, anti-inflammatory and anti-apoptotic property.

Keywords: *Mesenchymal stem cells, Nephropathy, Inflammation, Apoptosis, Rats.*

Introduction

Cyclosporin a (CsA) is a valuable immunosuppressive candidate which is extensively applied for avoiding rejection after organ transplantation. Also, it has extensively revealed significant clinical value in the treatment of autoimmune ailments. However, chronic employment of CsA may lead to a number of adverse events; the most frequent and clinically important one is CsA nephropathy.

The precise mechanism of this complication is not clear, although many probable mechanisms have been suggested. Chronic CsA nephropathy is described by cumulative renal dysfunction, afferent arteriolopathy, inflammatory cell influx and elevated intrarenal immunogenicity. Moreover, tubulointerstitial fibrosis and tubular cell apoptosis have been also reported as clinical manifestations for long term use of CsA [1]. It is intractable to retrieve kidney employment once it has become seriously destroyed. While kidney transplantation is a curative therapy, donor numbers remain restricted [2].

Hence, searching for a substitution therapy that is clinically effective and safe enough is deemed necessary. Scientific research spanning over the last two decades has confirmed the significant role of stem cells in various aspects of biology. Stem cells are unspecialized or undifferentiated cells that

are able to dividing and renewing themselves for long intervals of time, thus giving rise to specialized cell types *in vivo* and eventually helping in the functional reconstitution of a given tissue with flexibility in the timing of differentiation [3]. Different types of stem cells have been discovered and broadly they can be assigned into embryonic stem cells and adult stem cells. Adult stem cells are derived from niches in postnatal tissues including (but not limited) adipose tissue, bone marrow, brain, heart, liver, pancreas, intestine and skin [4].

Adult stem cells are categorized into hematopoietic stem cells, epithelial stem cells and mesenchymal stem cells. Such cells have been found to engage in both repair and regeneration of organs. Multitude of studies have shown that the therapeutic influences of mesenchymal stem cells (MSCs) not only depend on their differentiation capability to restore deteriorated tissue, but also relay on their power to modify local environment, stimulate endogenous progenitor cells and release various factors [5].

Adipose-derived mesenchymal stem cells (AD-MSCs) are not only multipotent and plastic, but also abandoned as they can be easily harvested with minimally invasive surgical techniques. This makes AD-MSCs conductive for clinical applications [6]. This approach aimed to appraise the potent role of BM-MCSs and AD-MSCs in regaining the functional and structural entities of kidney in cyclosporine-induced nephropathy in rats and to recognize the possible mechanisms by which they operate.

Material and Methods

Derivation and Expansion of MSCs

BM-MSCs

Bone marrow was harvested by flushing the tibiae and femurs of 6-week-old male albino *Wistar* rats with Dulbecco's modified Eagle's medium (DMEM; Lonza, Belgium) supplemented with 30% fetal bovine serum (Biowest, France). Nucleated cells were isolated with a density gradient [Ficoll/Paque (Pharmacia, Sweden)] and re-suspended in complete culture medium supplemented with 1% penicillin–streptomycin (Biowest, France). Cells were incubated at 37 °C in 5% humidified CO_2 incubator for 12-14 days as primary culture or upon formation of large colonies [7].

AD-MSCs

Adipose tissue was excised from both the omentum (i.e. abdominal) and the inguinal fat pad (i.e. subcutaneous) of male albino *Wistar* rats under general anesthesia according to Tomiyama et al [8]. The adipose tissue was resected and placed into a sterile tube containing 15 mL of a phosphate-buffered saline (PBS; Biowest, France). Enzymatic digestion was performed using 0.075% collagenase II (Serva Electrophoresis GmbH, Mannheim, Germany) in Hank's balanced salt solution for 60 min at 37 °C with shaking.

Digested tissue was filtered then centrifuged and erythrocytes were removed by treatment with erythrocyte lysis buffer. The cells were transferred to tissue culture flasks containing DMEM (Lonza, Belgium) supplemented with 30% fetal bovine serum (FBS; Biowest, France) and after an attachment period of 24 hours, non-adherent cells were removed by PBS wash. Attached cells were cultured in DMEM media supplemented with 30% FBS, 1% penicillin-streptomycin (Biowest, France) and then expanded *in vitro*.

When large colonies of both BM-MSCs and AD-MSCs developed (80–90% confluence), cultures were washed twice with PBS and the cells were trypsinized with 1X trypsin/EDTA (Biowest, France) for 5 min at 37 °C. After centrifugation, cells were re-suspended with serum-supplemented medium and incubated (at 37 °C in 5% humidified CO_2 incubator) in 25 cm^2 culture flasks. The resulting cultures were referred to as first-passage cultures [7].

Identification of MSCs

Morphological Appearance

To ensure the identity of the resulting cell culture, the MSCs were characterized morphologically by inverted microscope examination.

Surface Markers Determination

Flow cytometry analysis was used for detection of CDs cell surface markers of MSCs (CD 45, CD 90 and CD 105) to confirm whether the isolated BM-MSCs and AD-MSCs maintain their phenotype after expansion in culture. The FITC conjugated-CD 45 antibody was procured from

Immunotech SAS, France, whereas, the PE-conjugated CD 90 and PE-conjugated CD 105 antibodies were purchased from R & D systems, UK and Germany respectively. Briefly, the cells were incubated with the antibody against each of the surface markers for 30 min at 4 ºC in case of CD 45 and 10 min at 4 ºC for CD 90 and CD 105 followed by flow cytometry analysis (Beckman Coulter Elite XL, USA).

Animals

Adult male albino rats of *Wistar* strain weighing 130-150g were obtained from the Animal House Facility of the National Research Centre, Giza, Egypt, and housed in a well-ventilated area with a 12 hour light/ dark cycle at the ambient temperature (25 ± 1 °C) and humidity (55 %) throughout the experimental period. Rats were allowed to adapt to their environment for at least 10 days before the initiation of the experiment. Standard rodent diet and fresh clean drinking water were supplied *ad-libitum*. Rats were cared according to the guidelines for animal experiments which were approved by the Ethical Committee of Medical Research at the National Research Centre, Giza, Egypt.

Induction of Nephropathy

Nephropathy was induced by a daily subcutaneous injection of 0.5 ml/rat of cyclosporine A (CsA) (Novartis Pharma, Basel, Switzerland) dissolved in olive oil in a dose of 15 mg/kg/day for 28 days [9].

Animal Grouping

Forty adult male albino *Wistar* rats were enrolled in this study and randomized into five experimental groups (10 rats/group) as follows: (1) control group received 0.5 ml/rat olive oil *via* subcutaneous injection, (2) CsA group; nephropathy-induced group, (3) BM-MSCs-treated group in which nephropathy-induced rats were infused with a single dose of undifferentiated BM-MSCs (3×10^6 cells/rat) intravenously and sacrificed after two months of cell transplantation and (4) AD-MSCs-treated group in which nephropathy-induced rats were inculcated with a single dose of undifferentiated AD-MSCs (3×10^6 cells/rat) intravenously and sacrificed after two months of cell inoculation.

Samples Collection

At the end of the intervention period, all animals were fasted for 12 hours and the blood samples were withdrawn from retro-orbital venous plexus under diethyl ether anesthesia. The blood samples were left to clot and the sera were separated using cooling centrifugation (4 ºC) at 1800 xg rpm for 10 min and then preserved immediately at -20 ºC in clean plastic Eppendorf tube until analysis. After that, the rats were sacrificed by cervical dislocation and the kidneys were dissected and fixed in formalin saline solution (10%) for histopathological procedure.

Biochemical Evaluations

Serum creatinine and urea levels were estimated colorimetrically following the methods of Brod and Sirota [10] and Chaney and Marbach [11] respectively, using kit purchased from Biosystem, Barcelona, Spain. Glomerular filtration rate (GFR) was calculated as: eGFR= 186.3 × (Serum creatinine level)$^{-1.154}$ X 1.21 according to Levey et al [12]. Serum monocyte chemoattractant protein-1(MCP-1), nuclear factor kappa B (NF-κB) and nephrin (neph) levels determined using ELISA kits procured from Glory Science Co. LTd "USA" under the guidance of the manufacturer's protocol. Serum N-acetyl-ß-D-Glucosaminidase (NAG) activity, was assessed using kit obtained from Diazyme Laboratories Co., USA according to the method described by Price and Whiting [13].

Histopathological Procedure

After fixation of kidneys obtained from rats in the different studied groups in 10 % formal saline for twenty four hours, washing was done in tap water and then serial dilutions of alcohol were applied for dehydration. After that, kidney specimens were cleared in xylene and embedded in paraffin wax at 56 degree in hot air oven for twenty four hours. Paraffin wax tissue blocks were prepared for sectioning at 4 microns by rotary microtome. The obtained tissue sections were collected on glass slides deparaffinized and stained by hematoxylin and eosin (H&E) stains for histopathological examination under the electric light microscope.

Statistical Analysis

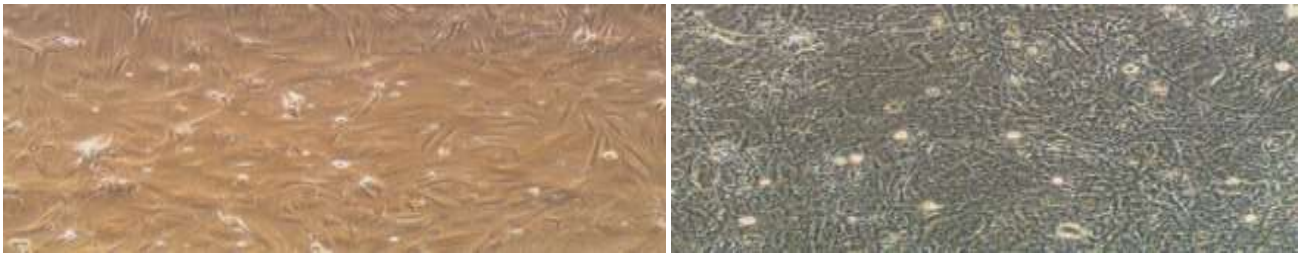
In the present study, all results were expressed as Mean ± standard error (S.E) of the mean. Data were analyzed by one way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) program, version 17 followed by least significant difference (LSD) to compare significance between groups. Difference was considered significant when P value was < 0.05.

Results

In Vitro Study

Microscopic Follow up and Photo-documentation of MSCs

The photomicrographs presented in Fig. (1) Show the morphology of cells isolated from rat bone marrow (a) and adipose tissue (b) at the third passage. Images indicate that MSCs derived from rat bone marrow and adipose tissue revealed obvious characteristic of spindle-shaped as fibroblastic-like morphology cells.

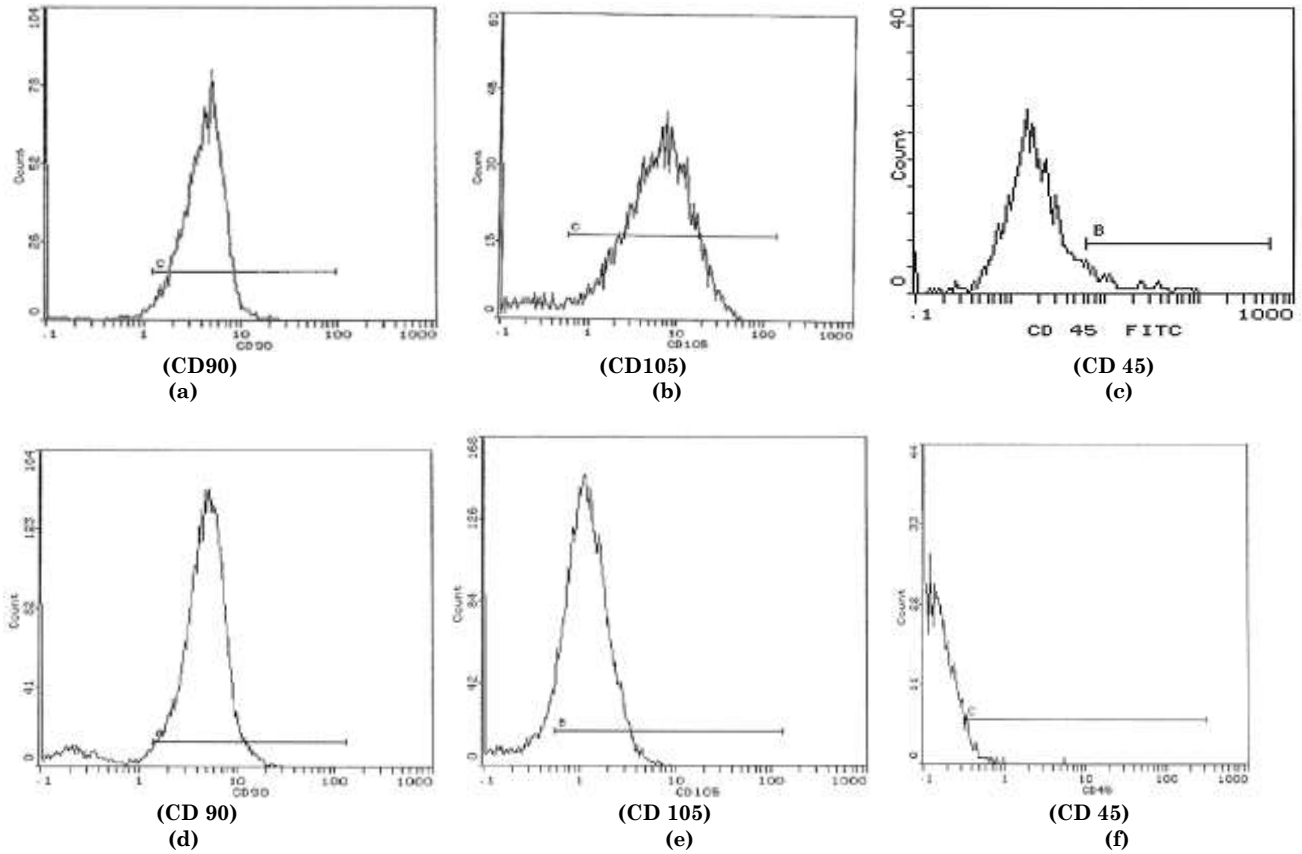


(A) (B)

Fig.1: Morphological aspects of isolated MSCs from rat bone marrow (a) and adipose tissue (b) at third passage

Surface Markers Characterization of MSCs by Flow Cytometry

Flow cytometry analysis revealed that the isolated BM-MSCs are positive for CD 90 (85.9 %), CD 105 (81.7%) and negative for CD 45 (11.4%) (Fig. 2a, b, c).Moreover, the results of flow cytometry analysis showed that the isolated AD-MSCs are positive for CD 90 (81.6%), CD 105 (81.1 %), and negative for CD 45 (0.94 %) (Fig.2 d, e, f).



(CD90) (a) (CD105) (b) (CD 45) (c)

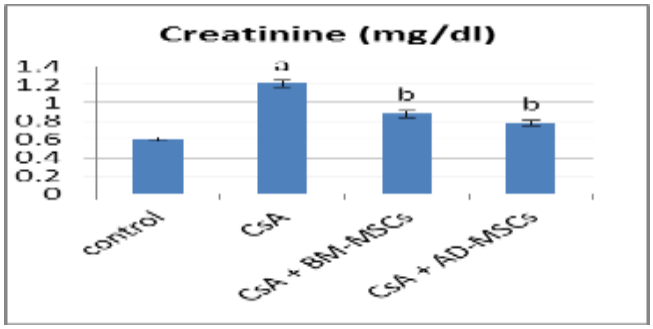
(CD 90) (d) (CD 105) (e) (CD 45) (f)

Fig.2: Flow cytometry analysis of BM-MSCs after staining with PE- conjugated-CD 90 and CD 105 (a, b) and FITC conjugated-CD45 (c) antibodies [CD 90 (85.9%), CD 105 (81.7%) and CD 45 (11.4%)] and flow cytometry analysis of AD-MSCs after staining with PE- conjugated- CD 90, CD 105 (d,e) and FITC conjugated-CD45 antibodies (f) [CD 90 (81.6%) and CD 105 (81.1%), CD 45 (0.94%)]

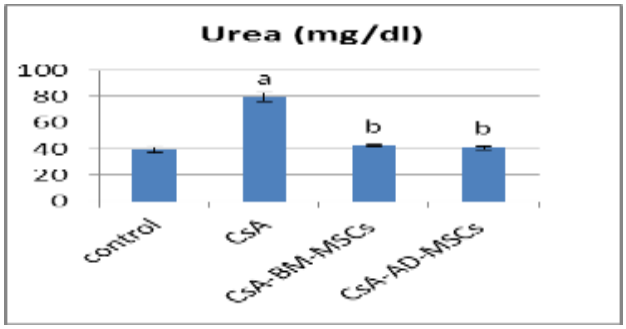
In *Vivo* Study

Biochemical Determinations

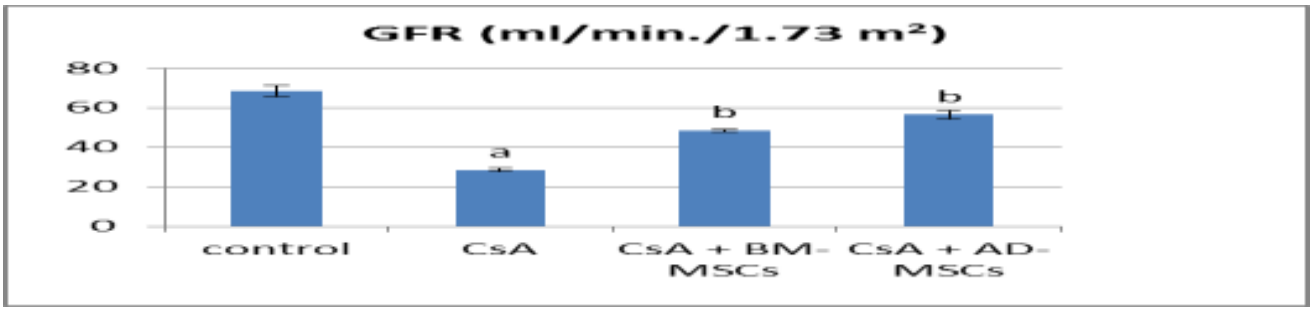
Serum creatinine level showed significant elevation ($P < 0.05$) in CsA group with respect to the control group. On the contrary, the treatment with either BM-MSCs or AD-MSCs brought about significant decline ($P < 0.05$) in serum creatinine level as compared to CsA group (Fig. 3a).The results in Fig. (3b) indicated that urea level is significantly increased ($P < 0.05$) in CsA group relative to the control group. While the treatment with either BM-MSCs or AD-MSCs resulted significant depletion ($P < 0.05$) in serum urea level versus CsA group. The findings of GFR evidenced that the value of GFR is significantly dropped ($P < 0.05$) in CsA group when compared with the control group. However, the treatment with BM-MSCs or AD-MSCs yielded significant enhancement ($P < 0.05$) in GFR values relative to CsA group (Fig. 3c).



(a)



(b)

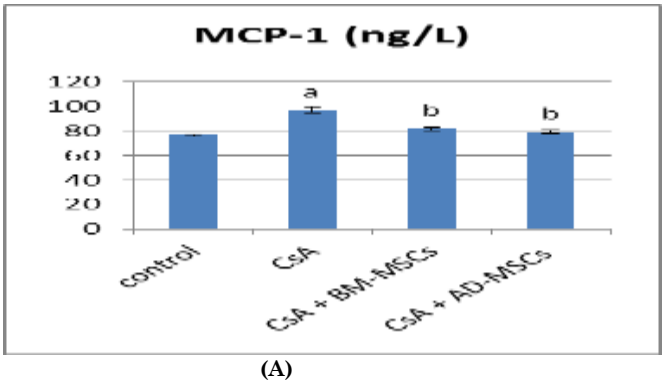


(c)

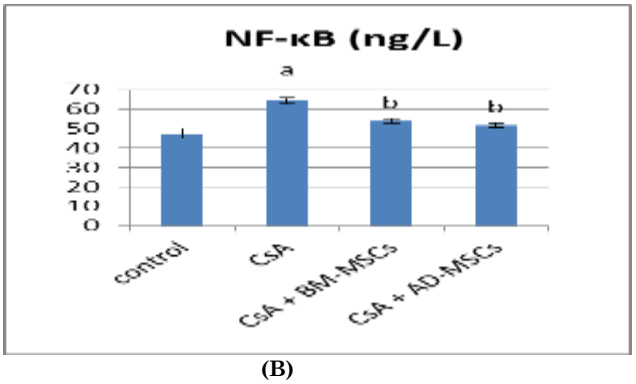
Fig.3: Influence of treatment with BM-MSCs and AD-MSCs on kidney function tests including: (a) serum creatinine level, (b) serum urea level and (c) GFR value in CsA-induced nephropathy in rats after two months A: Significant change at P< 0.05 in comparison with the control group. B: Significant change at P< 0.05 in comparison with CsA group

The results in Fig. (4a) indicated that MCP-1 serum level is significantly elevated ($P < 0.05$) in CsA group relative to the control group. In contrast, the treatment with either BM-MSCs or AD-MSCs evoked significant reduction ($P < 0.05$) in MCP-1serum level as compared to CsA group.

Serum level of NF-κB revealed significant amplification ($P < 0.05$) in CsA group versus the control group. On the opposite side, the treatment with BM-MSCs or AD-MSCs elicited significant depletion ($P < 0.05$) in serum NF-κB level as compared to CsA group **(Fig. 4b)**.



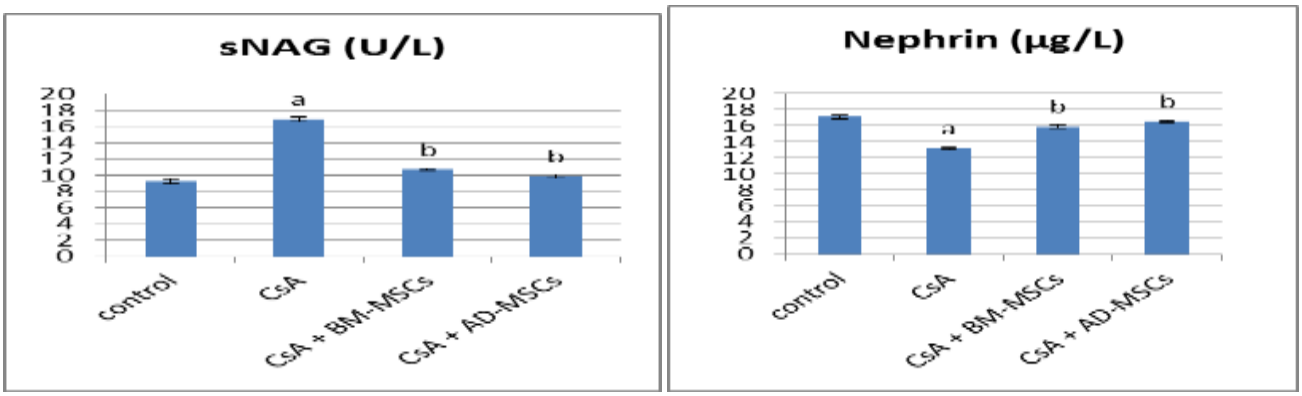
(A)



(B)

Fig. 4: Influence of treatment with BM-MSCs and AD-MSCs on: (a) serum MCP-1 level and (b) serum NF-κB level in CsA-induced nephropathy in rats after two months A: Significant change at P< 0.05 in comparison with the control group. B: Significant change at P< 0.05 in comparison with the CsA group

The data in Fig. (5a) illustrated that serum NAG activity is significantly upregulated (P < 0.05) in CsA group when compared with the control group. On the opposite hand, the treatment with either BM-MSCs or AD-MSCs produced significant inhibition (P < 0.05) in serum NAG activity with respect to CsA group. Serum nephrin level showed significant drop (P < 0.05) in CsA group relative to the control group. Conversely, the treatment with either BM-MSCs or AD-MSCs induced significant rise (P < 0.05) in serum nephrin level versus CsA group (Fig. 5b).

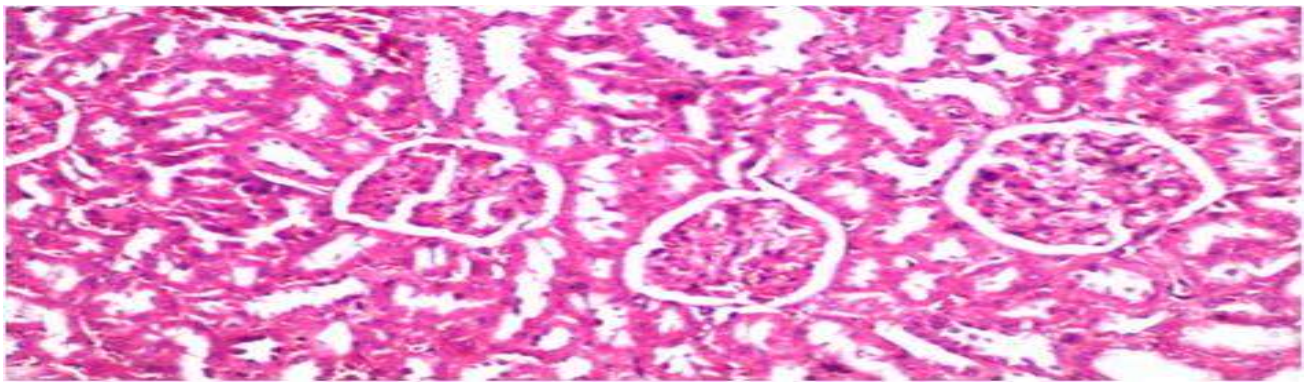


(A) **(B)**

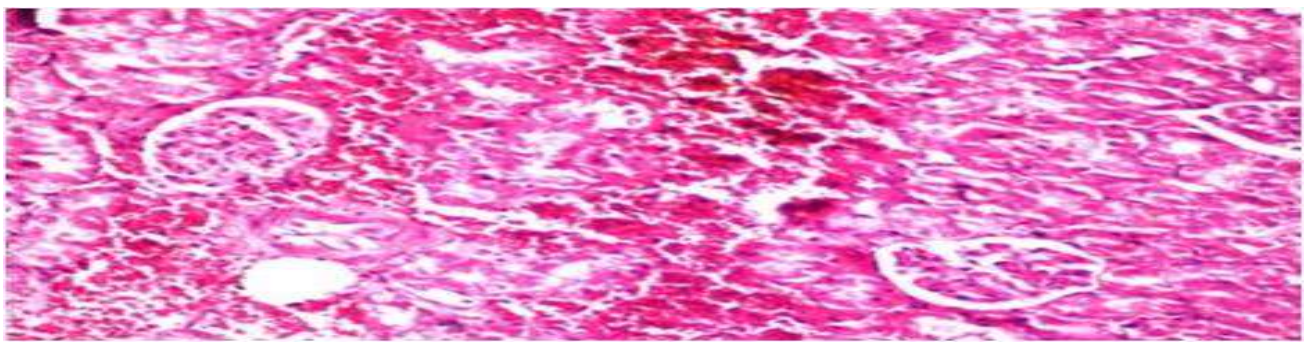
Fig. 5: Influence of treatment with BM-MSCs and AD-MSCs on: (a) serum sNAG activity and (b) serum nephrin level in CsA-induced nephropathy in rats after two months. A: Significant change at P< 0.05 in comparison with the control group. B: Significant change at P< 0.05 in comparison with CsA group

Histopathological Description

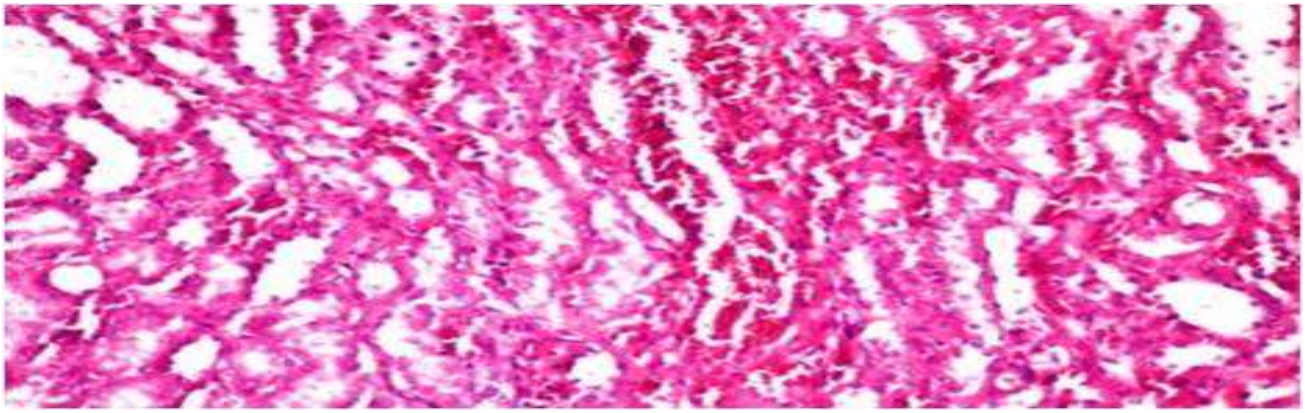
Optical micrograph of the cross-sectioned kidney tissue of rat in the control group showed normal histological structure of the glomeruli and tubules of the cortex (Fig. 6a). Optical micrograph of the cross-sectioned kidney tissue of rat in CsA group showed focal hemorrhage in between the degenerated tubules of the cortex with congestion in the cortical blood vessels and perivascular inflammatory cells infiltration (Fig. 6b). Optical micrograph of the cross-sectioned kidney tissue of rat in the BM-MSCs-treated group after 2 months showed congestion in the cortical blood vessels (Fig. 6c). Optical micrograph of the cross-sectioned kidney tissue of rat in the AD-MSCs-treated group after 2 months showed normal histological structure of the glomeruli and tubules (Fig. 6d).



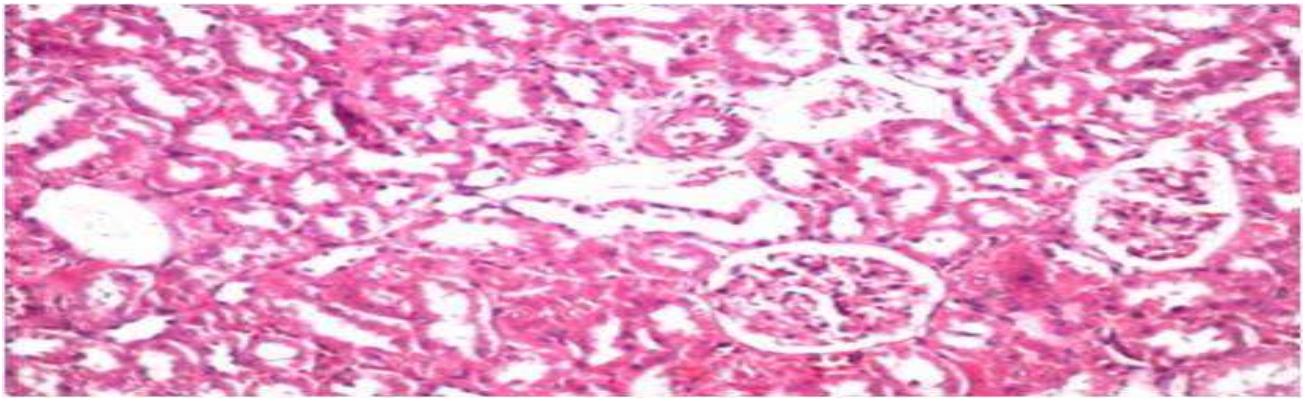
(A): Control group showing normal histological structure of the glomeruli and tubules of the cortex (H&E X100)



(b): CsA group showing focal hemorrhage in between the degenerated tubules of the cortex with congestion in the cortical blood vessels and perivascular inflammatory cells infiltration (H&E x100)



(c): BM-MSCs-treated group after two months showing congestion in the cortical blood vessels (H&E X100)



(d): AD-MSCs-treated group after 2 months showing normal histological structure of the glomeruli and tubules (H&E X100)

Fig.6: Photomicrographs of kidney tissue sections in the different studied groups

Discussion

The rational of the present work was to address the potential outcome of mesenchymal stem cells against nephropathy induced by cyclosporine (CsA) in adult rats and to elucidate the probable mechanisms by which they can offer their effects. In the current study, the morphological appearance of the isolated stem cells from either bone marrow or adipose tissue indicated that the cells are spindle-shaped as fibroblastic-like cells. These observations match those noticed by Masoud et al [14].

Who reported that the isolated MSCs are elongated, fusiform and spindle-shaped cells with fibroblast-like morphology. Mesenchymal stem cells are also recognized by their CD surface antigens (positive for CD 90 and CD 105 and negative for CD 45) as shown in the present study. These findings echo those of Ghaneialvar et al. [15] regarding the characterization of BM-MSCs and those of Mildmay-White and Khan [16] concerning the identification of AD-MSCs. The present biological investigation revealed significant rise in creatinine and urea levels in serum in association with the significant drop of GFR after CsA administration. These findings denoted the onset of severe renal impairment [17]. Long-term intake of CsA quickly suppresses glomerular filtration rate (GFR) and renal blood flow by promoting vasoconstriction or elevating intra-renal vascular resistance, which finally leads to low-grade ischemic lesion. The underlying mechanism for vasoconstriction is partly linked to stimulation of the intra-renal renin angiotensin system (RAS) and dissymmetry in prostaglandins and thromboxane [18]. The current finding revealed that CsA administration elicits significant elevation in serum MCP-1 level.

The underlying molecular mechanism for chronic CsA nephropathy is multifactorial and since the interstitial inflammatory consequences precede ongoing fibrosis, the amplification of chemoattractants and subsequent inflammatory cell infiltration are believed to play essential roles in this situation. Benigni et al. [19] have observed an intense staining of tubular cells for vasoactive and inflammatory peptides such as MCP-1, RANTES in biopsy of kidney transplant recipients receive CsA. In view of the present data, CsA administration produced significant enhancement in serum

NF-κB level. Transcription factors such as NF-κB act on genes for proinflammatory cytokines, chemokines adhesion molecules and matrix proteins involved in inflammation, immunologic responses, cell differentiation and the control of growth [20]. These investigators suggested that the activation of NF-κB is implicated in the transcription of MCP-1 and TGF-ß1 in the kidney. In the present setting, CsA induced significant amplification in serum NAG activity. NAG is accounted as an indicator of the operable status of the renal tubules as well as tubular damage [21].

Additionally, because of NAG is one of the enzymes involved in the decay of one common components of extracellular matrix constituting the tubular structure of the kidney, the elevation of such enzyme activity can add more to the devastation of renal tubules producing more nephrotoxicity. This finding emphasizes the burden of CsA administration on kidney tubules. In the current experimental set-up, CsA administration evoked significant suppression in serum nephrin level. Nephrin is a protein molecule expressed in podocytes. Previous study of Fornoni et al [22].

Demonstrated that CsA induces apoptosis of podocytes in a dose and time-dependent manner and leads to the reduced Bcl-xl levels in these cells. These findings may explain the decreased serum nephrin level in CsA-administered rats in the present study. Mesenchymal stem cells have been the focus of many researches in the last two decades because of their potent effect in tissue repair and regeneration. The well-characterized curative effect of transplanted mesenchymal stem cells has been mainly attributed to their homing and subsequent differentiation for the repair and regeneration of damaged tissue [6].

The treatment with MSCs in rats submitted to chronic CsA administration in the present attempt elicited significant modulation of renal dysfunction as manifested by the significant decline in serum creatinine and urea levels paralleled by the significant enhancement of GFR. The decrease in creatinine and urea serum levels together with the increase in the GFR value in rats treated with BM-MSCs or AD-MSCs indicate that MSCs indeed are promising candidates for mitigation of CsA-induced nephropathy.

It has been mentioned that MSCs enhance renal reparative processes through improvement of glomerulonephritis, hemodynamic and tubular cell dysfunction [23]. The potency of MSCs to suppress the liberation of pro- inflammatory cytokines and secrete a variety of trophic growth factors that induce angiogenesis, mitogenesis, and proliferation whilst reducing apoptosis may collectively mediate the repairment effect in the kidney of laboratory rodents [24].

The current results indicated that serum level of MCP-1 is significantly blunted in rats subjected to CsA administration and treated with BM-MSCs or AD-MSCs. It has been demonstrated that MSCs inhibit LPS-induced rat peritoneal macrophage activation *via* the down-regulation of inflammatory-related cytokines mRNA expression levels such as IL-6, MCP-1, TNF-α and IL-1ß in *ex vivo*. Also, *in vivo* experiment, the treatment of rats bearing diabetic nephropathy with MSCs brought about marked attenuation of MCP-1 and ICAM-1 expression paralleled by remarkable reduction in the accumulation of macrophages in the glomeruli [25]. These findings indicated that MSCs are able to improve the inflammatory environment of diabetic nephropathy.

The data in the present work indicated that the infusion of BM-MSCs or AD-MSCs in rats underwent chronic CsA administration caused significant reduction in serum NF-κB level. Recent study of Song et al [26]. Proved that *in vitro*, MSCs suppress oxidative stress related molecules, inflammatory cytokines and NF-κB transcription in renal tubular epithelial cells. Also, these investigators demonstrated that MSCs ameliorate ADR-induced nephropathy in rats by minimizing oxidative stress and inflammation *via* down-regulation of NF-κB. In the present research, significant inhibition in serum NAG activity was detected in rats subjected to chronic CsA administration and treated with MSCs.

This result comes in line with that obtained by Liu et al. [27] who mentioned that the subcapsular transplantation of metanephric mesenchymal cells in rats underwent gentamicin-induced acute tubular necrosis ameliorates renal functions as evidenced by the significant reduction in serum NAG activity. Serum nephrin level showed significant rise in rats submitted to chronic CsA and treated with MSCs in the current

attempt. Zoja et al. [28] cited that MSCs by virtue of their tropism for injured kidney and capability to import a local pro-survival environment may constitute a beneficial strategy to maintain podocyte viability and decrease glomerular inflammation and sclerosis. Our results are in great agreement with those of Wang et al. [29] who demonstrated that MSCs treatment in diabetic nephropathy rat model reduces the loss of podocytes and loss of glomerular nephrin and podocin.

These investigators suggested that the protective effect of MSCs may be mediated in part by elevating BMP-7 secretion. Moreover, Li et al. [30] stated that AD-MSCs can reduce podocytic apoptosis in a dose-dependent manner, decrease the expression of podocytic cleaved caspase-3 and prevent the reduced expression and maintain the normal arrangement of podocytic synaptopodin and nephrin. These scientists commented that AD-MSCs prevent podocytic apoptosis and injury induced by high glucose, mainly through secreting epithelial growth factor.

Furthermore, it has been reported that BM-MSCs translation in nephrosis rat model increases nephrin mRNA and protein expression compared with the untreated counterparts [31]. These authors suggested that BM-MSCs can repair glomerular podocytes in nephrosis rat model and the changes of nephrin expression may be involved in the process. Histopathological examination of kidney tissue section of rat in CsA group showed focal hemorrhage in between the degenerated tubules of the cortex with congestion in the cortical blood vessels and perivascular inflammatory cells infiltration.

These findings echo those observed by Sattarinezhad et al. [17]. Improvement in the histopathological feature of kidney tissue section of rat treated with BM-MSCs after two month has been noticed as represented by the presence of congestion in the cortical blood vessels only. These observations fit those obtained by Lee et al. [32] documenting the capability of BM-MSCs in regenerating kidney tissues. The complete recovery of the histopathological changes in the kidney tissue of rat treated with AD-MSCs has been observed in the present study as the photomicrograph of the kidney tissue section of rat in this group showed normal histological organization of the glomeruli and tubules. These findings match those obtained by Zhang et al. [33] suggesting the highly reparative action of AD-MSCs in kidney tissues. Noteworthy, the present study demonstrated that AD-MSCs have superior effect in improving kidney functions promoting kidney repair and modifying the tissue microenvironment in the rat model of nephropathy.

These findings could be ascribed to the biological advantages of AD-MSCs in proteins secretion like (basic fibroblast growth factor, insulin-like growth factor-1 and interferon-γ), proliferative capacity, and immunomodulatory actions more than BM-MSCs [34]. The secretory function of AD-MSCs has been regarded as primary mediator of MSCs-based therapy [6].

Conclusively, the outcome of the present study offer a clear evidence for mesenchymal stem cells, particularly those procured from adipose tissue, in stimulating functional and structural kidney repair in animal model of nephropathy. The mechanisms behind these effects seem to be linked with the anti-inflammatory, immunomoduatory and anti-apoptotic properties of MSCs. The results delivered in this study pave the way for the development of cellular therapeutics which represents an opportunity to fundamentally change the approach in kidney diseases treatment.

Statement on the Welfare of Animals

All procedures performed in this study were in accordance with the ethical standards of our institution at which the study was conducted and ethical approval was obtained from Ethical Committee of Medical Research at the National Research Centre, Giza, Egypt (Approval no.14 036).

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