Bone Marrow-Derived Mesenchymal Stem Cells Modulate Apoptosis and Angiogenesis in Cyclophosphamide-Induced Spleen Injury in Adult Female Rats

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Original Article

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ABSTRACT

Introduction: Bone marrow-derived mesenchymal stem cells (BM-MSCs) are multipotent cells that have been examined for their potential role in cell therapy. BM-MSCs displayed repairing and cytoprotective functions against many injured tissues. Furthermore, they exerted trophic effects mediated by different growth factors and cytokines. For severe spleen-associated diseases, stem cell therapy could be considered for its possible use in targeting and repairing damaged tissues and organs. Therefore, the aim of the present study is to investigate, on molecular basis, the cytoprotective and healing capacity of BM-MSCs in cyclophosphamide (CP)-induced spleen damage in rats.

Materials and Methods: A total number of Forty Sprague Dawley adult female rats were divided into two groups: the normal control group (NC) and CP-treated group. Normal control (NC) group that was treated once daily with sterile saline, and a CP-treated group that was injected intraperitoneally by CP at the concentration of 70 mg/kg for three successive days. The latter group was further subdivided into three subgroups: - the (CP-treated group) was sacrificed one week after CP treatment, the (BM-MSCs treated group), in which BM-MSCs were injected by a single dose of Male BM-MSCs 5x106/Kg BW via intraperitoneal injection on the tenth day after CP treatment, and the (CP auto-healing group) was left without treatment. Spleen was dissected from all animals and prepared for histological examination. The expression of genes and proteins was estimated by real-time qPCR and immunohistochemistry.

Results: BM-MSCs restored the normal architecture of the damaged spleen. They regained the mRNA levels of p53, caspase3, bcl2 more/less similar to the normal control group. The expression of VEGF was upregulated after BM-MSCs injection. Also, BM-MSCs relatively retained the normal expression of CD14, CD21, Akt and PI3K proteins after CP-induced toxicity.

Conclusion: This study revealed the potential therapeutic role of BM-MSCs in severe damaged spleen and explained the underlying mechanism on molecular basis.

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Key Words: Angiogenesis; apoptosis; cyclophosphamide; MSCs; spleen.

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INTRODUCTION

Mesenchymal stem cells (MSCs) are multipotent cells that have been examined extensively for their potential role in cell therapy[1-3]. It was indicated that MSCs do not arouse an immune response, instead, they decrease inflammation and modulate the immune system[4]. Bone marrow is considered a rich source for different adult pluripotent stem cells such as endothelial progenitor cells (EPCs), hematopoietic stem cells (HSCs), and MSCs. Bone marrow-derived mesenchymal stem cells (BM-MSC) have shown self-renewal, plastic-adherence and fibroblast-like population along with differentiation characteristic in *vitro* and in *vivo* into numerous cell types of mesodermal lineage, such as adipocytes, osteoblasts, and chondrocytes[5,6]. Numerous reports suggested the ability of BM-MSCs to differentiate into endodermal and ectodermal lineage type of cells, such as skin, lung, hepatocyte, pancreatic islets, retinal pigment and other collagenous connective tissue[7-11]. BM-MSCs can sense and target injured organs, migrate to the site of damage and undergo differentiation. However, factors affecting

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the stemness and differentiation of MSCs are not well understood yet[12-15]. The protective capacity of BM-MSCs have been previously demonstrated against injured lung[16], acute kidney injury[17], myocardial infarction[18-20], liver injury[21,22], diabetes[23], and neurological disorder[24].

Additionally, the repairing capacity of MSCs is not dependent on the differentiation ability and immunosuppressive activity alone. They showed trophic effects mediated by different growth factors and cytokines[25-27]. Several reports from different pathological diseases indicated that BM-MSCs exhibit powerful protective, anti-apoptotic and pro-mitotic action through the secretion of different mediators[28]. Consequently, BM-MSCs hold great potentials in tissue and organ healing and restoration, which put them in the lead for tissue repair, immune-intervention and cell therapy[29].

Cyclophosphamide (CP) is an antineoplastic cytotoxic chemotherapeutic drug which has been used as a therapy for a wide range of tumors, in addition to other autoimmune diseases. CP is an anti-cancer alkylating agent. Alkylating agents are most active in the resting phase of the cell cycle; they halt cell division by damaging DNA. CP works through the addition of alkyl group in the guanine base of DNA, which results in base pairing between cytosine-thymine and initiation of DNA repair system to remove the alkylated guanine, thus, triggering apoptosis[30,31]. CP was reported to induce multi-organ toxicity upon injection, it induced liver fibrosis and necrosis, lung lesions, kidney injury and spleen damage[32,33].

For severe spleen injury-associated diseases, stem cell therapy could be considered for its possible use in targeting and repairing damaged tissues and organs. Therefore, the aim of the present study is to investigate, on molecular basis, the repairing and healing capacity of BM-MSCs in cyclophosphamide-induced spleen damage in rats.

MATERIALS AND METHODS

Mesenchymal stem cells isolation and characterization

Rat male BM-MSCs were isolated and cultured as described before[34]. In brief, BM-MSCs were isolated and collected from femurs and tibias of 4-weeks old rat (60-80g), the cells were cultured in Iscove's modified Dulbecco's medium (IMDM) culture medium (Sigma-Aldrich, Inc) with L-glutamine mixed with 10% fetal bovine serum (Sigma-Aldrich, Inc) on tissue culture flasks and incubated in humidified atmosphere of 5% CO2 at 37 °C. After 48 h, non-adherent cells were removed and adherent cells were washed with PBS and re-cultivated in fresh complete culture medium, which was changed every 3 to 4 days until 80-90% confluent. Then cells were trypsinised and passaged until the second passage. Cells were washed and suspended in PBS to be ready for use. The mesenchymal population was identified based on its plastic adherence to the bottom of the flask and morphology and characterized for the expression of the cell surface markers, MSC markers (CD90 and CD31) and hematopoietic markers (CD45), by flow cytometry as described before[2].

Animals and the experimental model

Animal care and study protocols were preceded according to the guidelines established by The Experimental Animal Center and Research Ethics Committee, Minia University, Minia, Egypt. A total number of ten male rats and Sprague Dawley adult 40 female rats (10 weeks old and about 150-180gms weight) , the female rats were randomly assigned into 2 groups: - normal control (NC) group that was treated once daily with sterile saline for 3 consecutive days, and a CP-treated group that was injected intraperitoneally by CP at the concentration of 70 mg/kg for three successive days. The latter group was further subdivided into three subgroups: - the first subgroup (CP-treated group) was sacrificed one week after CP treatment, and the second subgroup (BM-MSCs treated group), in which BM-MSCs were injected with a single dose of Male BM-MSCs 5x106/Kg BW via intraperitoneal injection on the tenth day after CP treatment, and the third subgroup (CP auto-healing group) was left without treatment. The last two subgroups were sacrificed one week later. Spleen was dissected from all animals and divided into three parts, two of them were put in neutral buffered formaldehyde (10%) to be used for histological and immunohistochemical studies, then (stored at -80°C), the third part of samples were stored at – 80° C directly for further RNA isolation and quantitative RT-PCR analysis.

RNA isolation and real-time qPCR assay

Total RNA extraction was performed for all tissue homogenate using TRIzol (In*vitro*gen, CA, USA) according to the manufacturer's instructions. The RNA samples were then treated with DNase I (In*vitro*gen); cDNA synthesis was achieved with High-Capacity cDNA Reverse Transcription Kit (ThermoFisher Scientific, Inc) according to the manufacturer's instructions. The expression of P53, bcl-2, caspase3, vascular endothelial growth factor (VEGF) and SRY genes was assessed by real-time qPCR. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as internal control[35]. The sequences of the primers, which were obtained from National Center for Biotechnology Information (NCBI), are mentioned in (Table 1). The quantification of mRNA was achieved on an Applied Biosystems ABI 7500 Fast real-time PCR system (Applied Biosystems, Foster City, CA, USA). Triplicate RT-PCR reactions were performed for each sample. Cycle threshold (Ct) was determined for each sample, and the average Ct was calculated. In order to exclude the generation of non-specific compounds and to characterize the obtained amplified mixture with the avoidance of contamination, a melting curve analysis was achieved between 60– 95˚C at 1˚C intervals with the Rotor-Gene 6000 Series Software 1.7 using the SYBR Green fluorescent dye.

Table 1: Sequences of the primers

Primer	Sequence of the primer
P53	Forward: 5´- GTTCCGGAGCTGAATGAGG -3' Reverse: 5' – TTTTATGGCGGGACGTAGAC -3'
Bcl2	Forward: 5′-TGAACCGGCATCTGCACAC-3 Reverse: 5′-CGTCTTCAGAGACAGCCAGGAG-3′
Caspase3	Forward: 5′- CGGAGCTTGGAACGCGAAGA-3' Reverse: 5'- ACACAAGCCCATTTCAGGGTAA-3'
VEGF	Forward: 5'- GTGCACTGGACCCTGGCTTT-3' Reverse: 5'- CCCTTCTGTCGTGGGTGCAG-3'
SRY	Forward: 5′-AGGGTTAAAGTGCCACAGAGGA-3′ Reverse: 5′-GCTTTT CTGGTTCTTGGAGGAC-3′.
GAPDH	Forward: 5′-AACCTGCCAAGTATGATGACATCA-3′ Reverse: 5′-TTCCACTGATATCCCAGCTGCT-3′

Histopathological analysis

Spleen tissue sections were fixed with 10% formaldehyde, then, dehydrated in ascending grades of ethanol and embedded in paraffin. Then, the sections were stained with hematoxylin-eosin (H&E) and observed for histopathological changes using the optical microscope (Leica DMRBE) with the DP Controller software.

Immunohistochemistry

Spleen tissue sections were de-paraffinized and stained with anti-cluster of differentiation 14 (anti-CD14) (1:400) (Abcam), anti-follicle dendritic cell markers (anti-CD21) (1:400) (Abcam), anti-protein kinase B (anti-Akt) (1:400) (Abcam), and anti-phosphoinositide 3-kinases (anti-PI3K) (1:400) (Abcam) antibodies according to manufacturer's instructions, using the Horseradish peroxidase/DAB (ABC) detection immunohistochemistry kit (Abcam, Cambridge, UK). The assayed sections were examined by microscopy. The antigen-positive area is brown. Ten random microscopic fields were captured per sample, and signal intensity was then semi-quantified using The Image Processing and Analysis Java (ImageJ) program, as prescribed previously[36].

Statistical analysis

At least three independent experiments were used to obtain the results. Data were expressed as mean±standard deviation. Student's t-test was used to analyze differences after one-way analysis of variance (ANOVA), with the use of GraphPad Prism 9 statistical software (GraphPad, La Jolla, CA, USA) and Excel software (Microsoft, Redwood, WA, USA). Differences were considered significant when the probability values (P) were less than 0.05.

RESULTS

Cell surface markers expression

Flowcytometric analysis revealed the immunophenotyping of MSCs based on the following phenotype: CD90, CD31 and CD45. The expression of CD45 (hematopoietic markers) was negative while the expression of CD31 and CD90 (MSCs markers) was positive in cultured MSCs (second passage) isolated from rat bone marrow, as shown in (Figure 1).

Expression of p53, caspase3, bcl2, VEGF and SRY genes

In the present study, the expression of p53, caspase3, bcl2, VEGF and SRY genes was amplified and normalized to the internal control GAPDH by using quantitative real-time PCR, as shown in Figure 2. In comparison to CP-treated group, the expression of pro-apoptotic genes such as p53 and caspase3 was reduced (P<0.01) and (P<0.001) in auto-healing group, respectively. While after treatment with BM-MSCs, the reduction (P<0.001) increased to 2.72±0.33 and 2.08±0.62 fold change, respectively. The mRNA levels of anti-apoptotic gene, bcl2, increased (P<0.05) and (P<0.001) in auto-healing group and BM-MSCs treated group, respectively, when compared to CP-treated group. The Expression of VEGF increased (P<0.01) and (P<0.001) in auto-healing group and BM-MSCs treated group, respectively, relatively to CP-treated group. The expression of SRY gene was noted in BM-MSCs treated group, when compared to normal control group.

Histopathological investigations

Splenic tissues for rats were examined for histopathological changes using hematoxylin-eosin staining. For NC group, histopathological investigations of the spleen revealed normal architecture with a uniform marginal zone (MZ) and a more pronounced marginal sinus region (MS). Granulocytes, erythropoietic cells, lymphocytes, and hemosiderin-laden macrophages are present amid the splenic cords, as shown in (Figure 3A). In CP-treated group, lymphocytes necrosis was revealed in lymphatic follicles and periarterial lymphoid sheaths (PALS) of white pulp areas (Figure 3B) along with focal splenic hemorrhage occurred with necrosis and lymphocytic depletion, as shown in (Figure 3C). Also, a dilation of the intercellular spaces with massive infiltration of red cells in red pulp has been revealed (Figure 3D). Regarding CP auto-healing group, the white pulp showed improvements, while the magnified picture of the white pulp showed necrosis and lymphocytic depletion (Figure 3E) with dilation of the intercellular spaces and infiltration of red cells in red pulp area (Figure 3F). Also, the examination revealed lymphocytes necrosis in lymphatic follicles and PALS of white pulp areas. After BM-MSCs injection to CP-treated injured group, clear improvement in the white and red pulp has occurred, spleen sinuses are relatively well loaded with red blood cells and lymphoid cells appear healthy with no necrotic changes, as shown in (Figure 3G) and H. While the spleen follicle regained normal appearance in the white pulp, yet they did not display a germinative center.

Expression of CD14 protein

The expression of macrophage-derived monocyte marker, CD14 was examined within the splenic tissues in CP-treated group and with/without BM-MSCs treatment in

the present study. In normal control group, white pulp and red pulp of the spleen showed few positive cells of CD14, but after CP-treatment, macrophages subpopulations exhibited signs of cellular activation such as an increase in size and number of the cell. There was numerous positive CD14 cells in the white pulp within the marginal zone and red pulp of the spleen. In CP auto-healing group, CD14 positive cells were less (P<0.001) and (P<0.05) observed within the white and red pulp of the spleen, respectively, when compared to CP-treated group. After BM-MSCs injection, the white and the red pulp of the spleen showed less (P<0.001) positive cells of CD14, when compared to CP-treated group, but more or less similar to the normal control group, as shown in (Figure 4).

Expression of CD21 protein

The expression of follicular dendritic cell marker, CD21 was examined within the splenic tissues in CP-treated group and with/without BM-MSCs treatment in the present study. In normal control group, there was numerous positive CD21 branched cells within the white and red pulp of the spleen. After CP treatment, the white pulp showed some scattered positive CD21 cells in marginal zone with negative cells in PALS, the red pulp exhibited scattered positive CD21 cells. During auto-healing phase, the white pulp of the spleen showed negative CD21 cells, while the red pulp showed few scattered positive CD21 cells with non-significant difference (P>0.05) to CP-treated group. After BM-MSCs treatment, white pulp of the spleen showed few positive CD21 cells with non-significant difference (P>0.05) to CP-treated group, while the red pulp showed multiple (P<0.01) scattered positive CD21 cells, when compared to CP-treated group, as shown in (Figure 5).

Expression of Akt and PI3K

The expression of Akt and PI3k was examined within the splenic tissues in CP-treated group and with/without BM-MSCs treatment in the present study. Normal control group showed multiple positive Akt cells in PALS and germinal center of the white pulp of the spleen, as shown in (Figure 6A). After cyclophosphamide treatment, there was observed reduction in Akt positive cells. During the auto-healing phase, Akt protein expression exerted no significant difference (P>0.05) with CP-treated group. After BM-MSCs injection, Akt positive cells was increased (P<0.01) relatively to CP-treated group (Figure 6C).

Regarding PI3K, white and red pulp of the spleen exhibited positive PI3k cells in normal control group. There was relative reduction in PI3K positive cells after treatment with CP. PI3K protein expression showed non-significant difference (P>0.05) in auto-healing group when compared to CP-treated group. After BM-MSCs treatment, there was relative increase (P<0.05) in PI3K positive cells when compared to CP-treated group, as shown in (Figures 6B, Figure 6C).

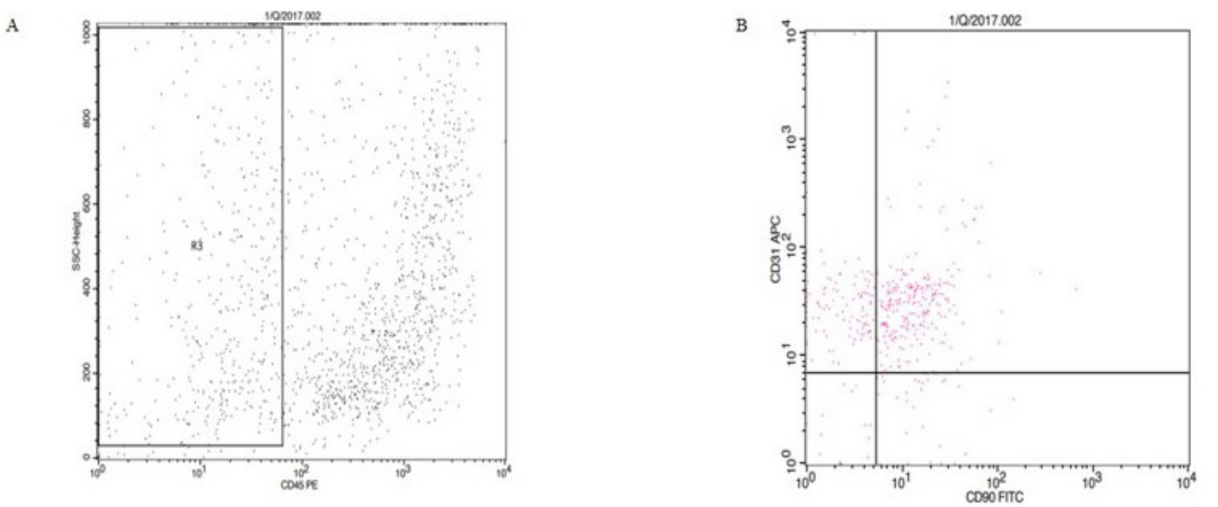

Fig. 1: Cell surface markers expression of in *vitro* cultured BM-MSCs at the second passage. Representative dot plots of flowcytometric analysis against (A) CD45 (hematopoietic markers), (B) CD31 and CD90 (MSCs markers) expression.

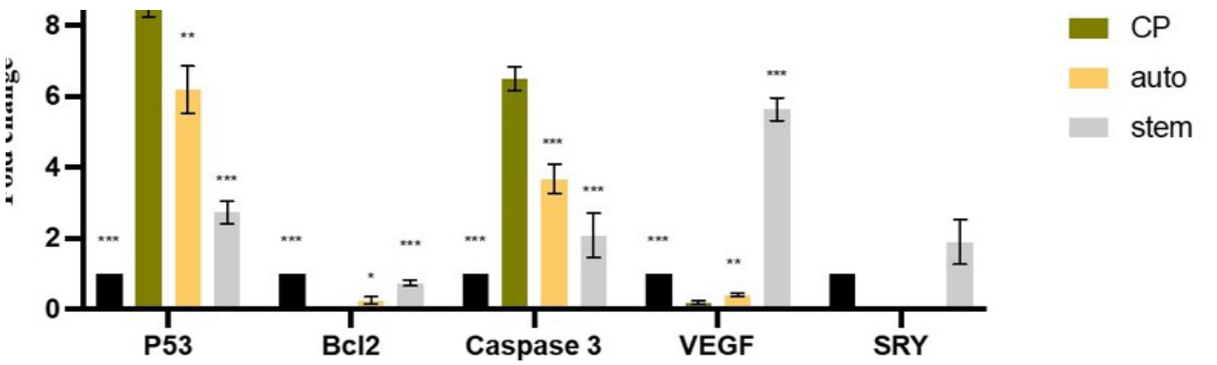

Fig. 2: Expression of p53, caspase3, bcl2, VEGF and SRY genes was determined by quantitative real-time PCR. Data represent fold change relative to the normal control group expression after normalization to GAPDH. Bars represent mean±SD. Significant difference was analyzed by one-way ANOVA. Where, *; $p < 0.05$, **; $p < 0.01$, ***; $p < 0.001$, compared to CP-treated group.

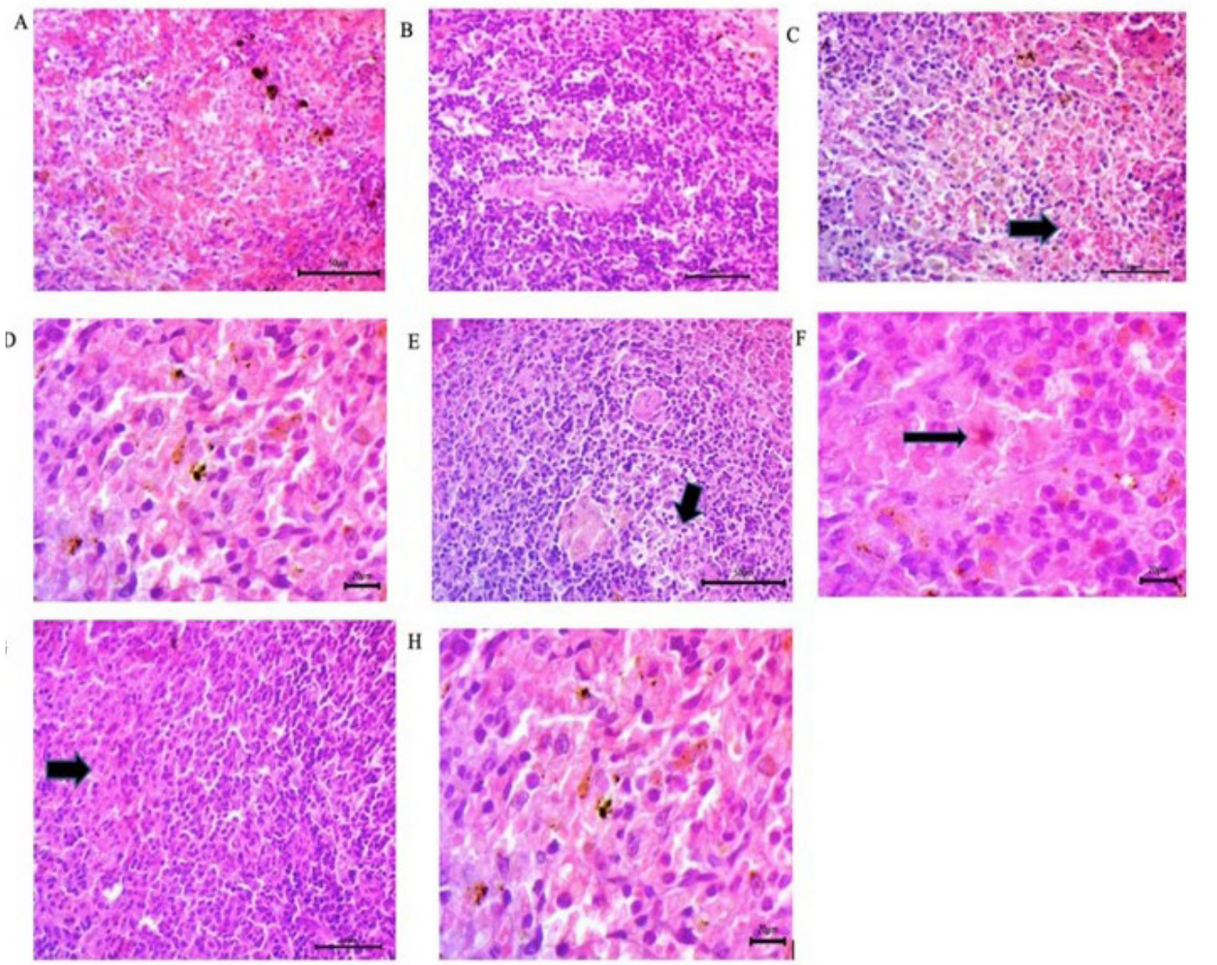

Fig. 3: Histological examination of splenic tissues for rats of different groups. Splenic tissues were stained with hematoxylin-eosin staining. (A) Normal control group, revealed normal architecture with a uniform marginal zone and a more pronounced marginal sinus region. Granulocytes, erythropoietic cells, lymphocytes, and hemosiderin-laden macrophages are present amid the splenic cords, In CP-treated group, lymphocytes necrosis was revealed in lymphatic follicles and periarterial lymphoid sheaths (PALS) of white pulp areas (3B). Focal splenic hemorrhage occurred with necrosis and lymphocytic depletion, also, a dilation of the intercellular spaces with massive infiltration of red cells in red pulp has been revealed (C) and (D) CP-treated group, Regarding CP auto-healing group, the white pulp showed improvements, while the magnified picture of the white pulp showed necrosis and lymphocytic depletion with dilation of the intercellular spaces and infiltration of red cells in red pulp area E and F . Also, the examination revealed lymphocytes necrosis in lymphatic follicles and PALS of white pulp areas. After BM-MSCs injection to CP-treated injured group, clear improvement in the white and red pulp has occurred, spleen sinuses are relatively well loaded with red blood cells and lymphoid cells appear healthy with no necrotic changes, as shown (G and H.) While the spleen follicle regained normal appearance in the white pulp, yet they did not display a germinative center.

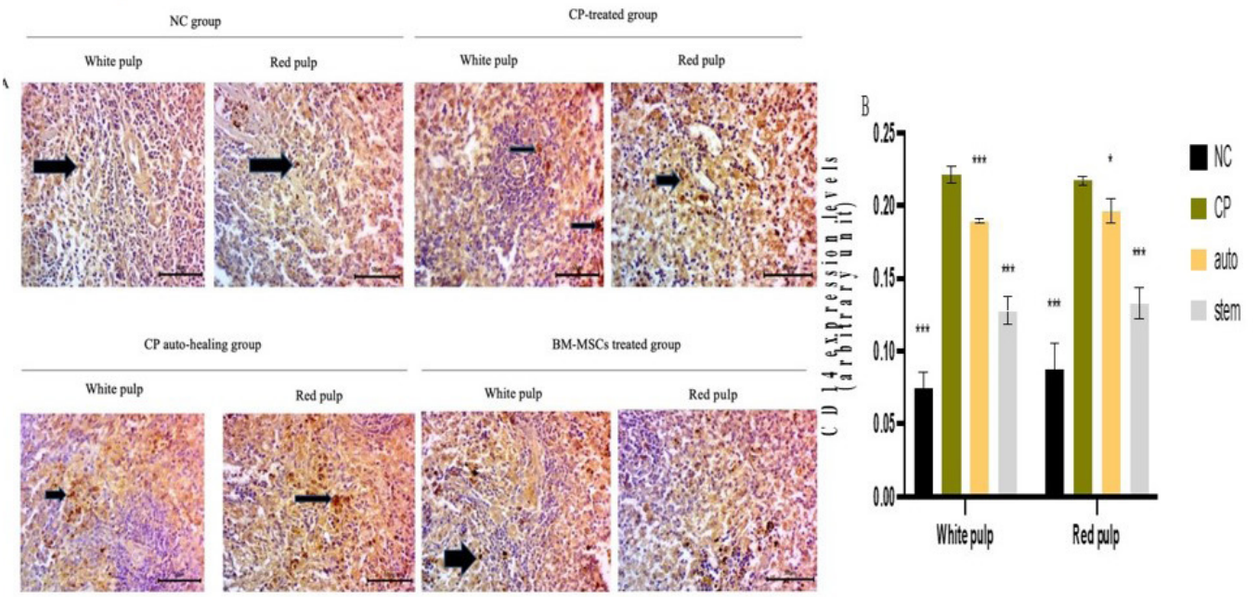

Fig. 4: Expression of CD14 protein. (A) Representative photos of CD14 staining in splenic tissues, red pulp and white pulp (arrow) where antigen positive area is brown. ,(arrow) (B) Relative protein expression of CD14. ,(arrow) Bars represent mean ± SD. Significant difference was analyzed by one-way ANOVA test, *; $p<0.05$, ***; $p < 0.001$, compared to CP-treated group

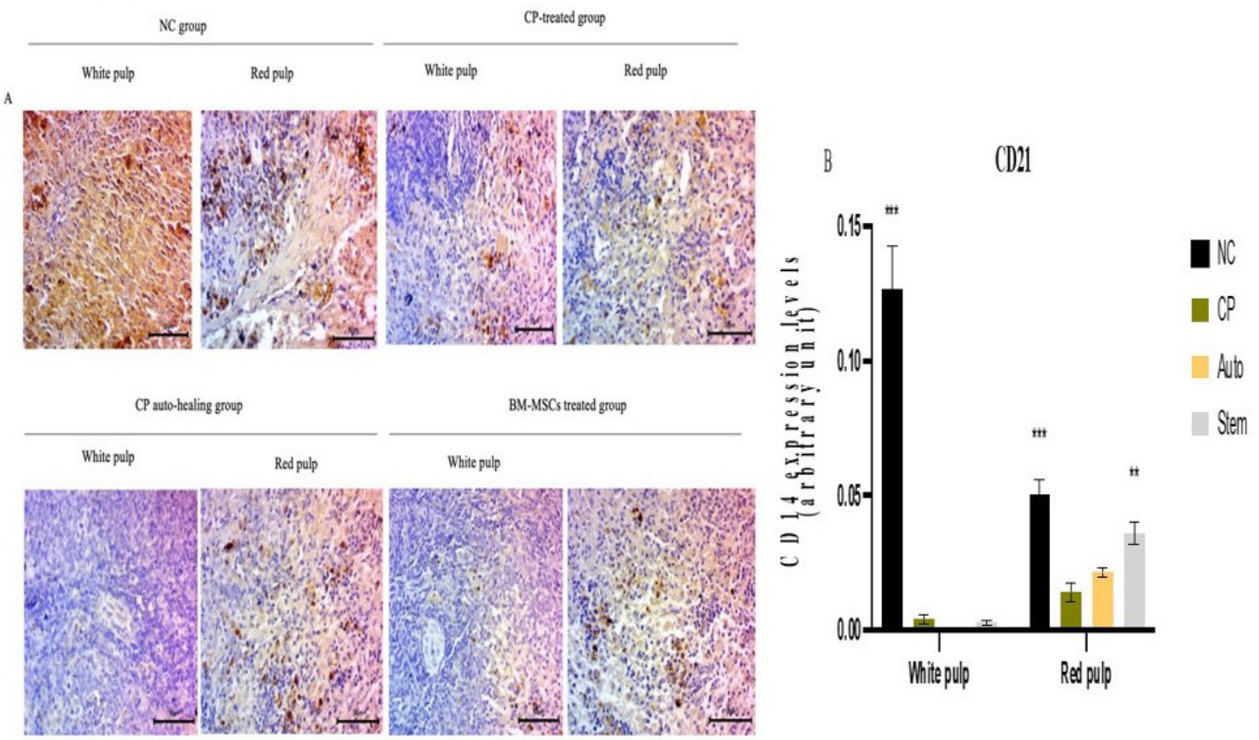

Fig. 5: Expression of CD21 protein. (A) Representative photos of CD21 staining in splenic tissues, red pulp and white pulp (arrow) where antigen positive area is brown. (B) Relative protein expression of CD21. Bars represent mean ± SD. Significant difference was analyzed by one-way ANOVA test, **; $p<0.01$, ***; $p < 0.001$, compared to CP-treated group.

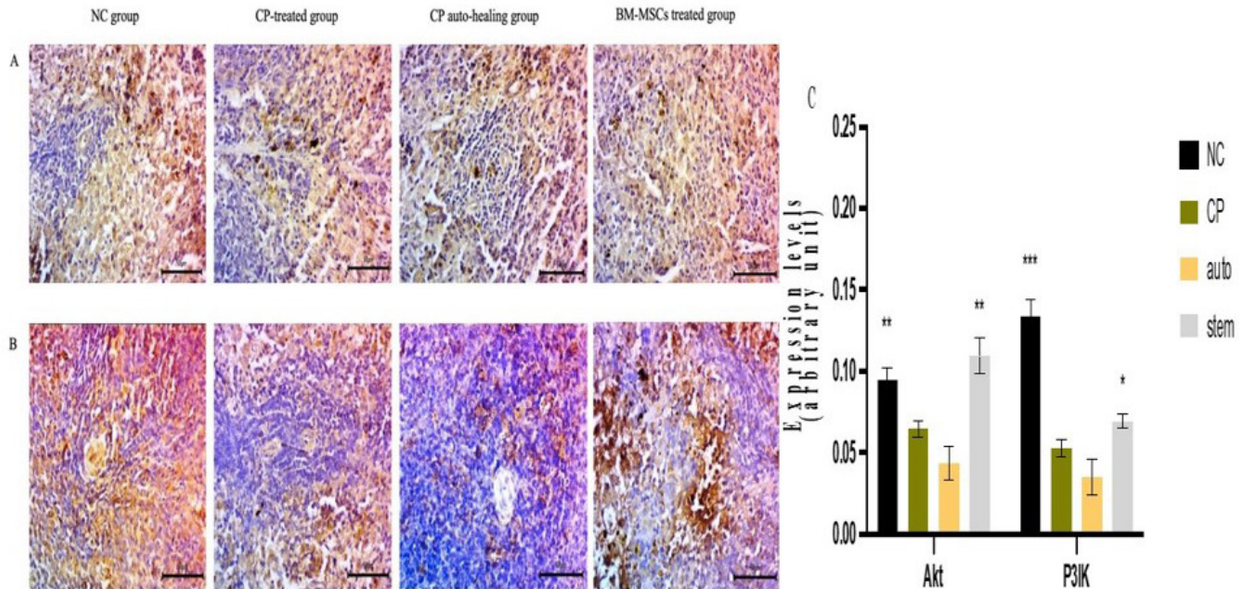

Fig. 6: Expression of Akt and PI3k proteins. (A) and (B) Representative photos of Akt and PI3K staining in splenic tissues, red pulp and white pulp (arrow) respectively, where antigen positive area is brown. (C) Relative protein expression of Akt and PI3k. Bars represent mean ± SD. Significant difference was analyzed by one-way ANOVA test, *; $p<0.05$, **; $p<0.01$, ***; $p < 0.001$, compared to CP-treated group.

DISCUSSION

Cytotherapy has got a great attention in various disorders[37-39] and looking for new therapeutic abilities of existed[40-42] or new candidates[43-45] becomes a remarkable approach[46-50]. BM-MSCs displayed many potential therapeutic mechanisms including tissue repair, cryoprotection, proliferation, anti-apoptosis, pro-vascularization, and anti-fibrosis effects[51]. The paracrine action of BM-MSCs through cell-cell signaling and secretion of different soluble factors including cytokines and growth factors have been proposed to be the main functional benefit of MSCs transplantation rather than the trans-differentiation activity, since many reports indicated that MSCs differentiation is challenging and requires cell manipulation[52,53].

In the present study, Cyclophosphamide (CP) was used to induce spleen damage and toxicity in rats. This was supported by our findings in the histopathological sections where CP-treated group showed necrosis in the lymphatic follicles along with focal splenic hemorrhage. Upon the injection of BM-MSCs into CP-induced spleen damage in rats, there was clear improvement in the white and red pulp with enhanced vascularization inside the spleen sinuses.

For tracing male BM-MSCs injected inside our female rat models[54], SRY gene expression was investigated in spleen tissue samples, our Real-time findings reported the expression of SRY gene within BM-MSCs treated group which proof the hypothesis that BM-MSCs actively react to the damaged tissue, migrate to the injured site in a similar manner to the immune system response to foreign bodies, they home to the damaged inflamed tissue and exert cytoprotective, angiogenic, repairing, remodeling and anti-apoptotic activity toward damaged tissues.

Modulation of angiogenic, oxidative stress and apoptotic pathways plays an important role in tissue disorders and restoration[55-60]. In accordance with our mRNA quantification of VEGF, rats injected with BM-MSCs exhibited obvious increase in VEGF expression, which explains the paracrine pro-angiogenic activity exerted by BM-MSCs. It was reported that MSCs was able to restore blood flow in injured organs, also stimulate the host cells to produce VEGF, which further initiates damaged tissue to start the restoration process[61,62]. However, CP was able to cause cell death in apoptotic and necrotic fashion inside the spleen in treated group, which was supported by our Real-time findings, where the mRNA levels of pro-apoptotic genes, p53 and caspase3, were upregulated and the mRNA of anti-apoptotic genes, bcl2, was downregulated in CP-treated group. CP auto-healing group showed little improvement, while BM-MSCs injection to CP-induced spleen injury group inhibited the apoptotic activities induced by CP toward splenic cells where the expression of p53, caspase3 was substantially reduced and the expression of bcl2 was increased, when compared to CP-treated group, indicating that the splenic cells started to grow and proliferate in a normal ordinarily manner.

CD14 is a glycoprotein that can exist in a membrane-anchored form or soluble form, it is expressed mainly in monocytes and macrophages. CD14 play crucial step on how macrophages interact and engulf apoptotic cells. The role of macrophages in apoptosis is to rapidly clear up dying cells which prevent the release of intracellular materials that could attract inflammatory mediators. The mediated interaction between CD14 and macrophages was found to be specific to cells undergoing apoptosis,

and there was no interaction with viable cells. There is a growing evidence about an interaction between CD14 and phosphatidylserine on the outer layer of an apoptotic cell which get the dying cell to be recognized by macrophages. Also, the apoptotic capacity by macrophages was suppressed when removing CD14 from macrophages by cleaving glycosylphosphatidylinositol ether[63]. It has been found that, CD14 is integrated with the deleterious systemic inflammatory response associated with sepsis, and a decrease in CD14 was associated with increase survival rate. Our findings displayed the expression of CD14 protein in normal control group, while in CP-treated group, the white pulp and the red pulp of the spleen showed numerous CD14 positive cells, which indicate the activation of macrophages and induction of apoptosis. In CP auto-healing group, the white and red pulp showed numerous CD14 positive cells less than those observed in CP-treated group which indicate the little improvement in the spleen condition. While after BM-MSCs injection to CP-injured rats, CD14 positive cells were notably less observed in the white and red pulp of the spleen relatively to the CP-treated group and more similar to those in the control group, which supports our previous Real-time findings that BM-MSC suppressed the apoptotic activity induced by CP.[63]

Spleen is the largest secondary lymphoid organ that contribute to B cell maintenance and maturation, it serves as reservoir for B cells[64]. CD21 is a follicular dendritic cell marker which is essential for B cell activation and response in lymphoid tissues. CD21 is crucial for follicular retention and survival within germinal centers of lymphoid compartment[65]. Abnormal splenic B cell development can lead to increased marginal zone and decreased follicular B cell numbers[66]. Several studies reported that B cells lacking CD21 contributes to chronic immune activation[67,68]. In the present study, CD21 was present in the normal control group within the red pulp and white pulp of the spleen, while CP-treated group and CP auto-healing group showed negative results for CD21 positive cells within the white pulp, only the red pulp of the spleen showed few scattered positive cells. After BM-MSCs injection to CP-injured rats, CD21 positive cells were regained in the red pulp of the spleen with no effect on CD21 within the white pulp of the spleen. This indicates the healing capacity of BM-MSCs to retain the normal physiology of the spleen when compared to CP-treated group and CP auto healing group.

PI3K-Akt pathway is an intracellular signaling pathway that regulate cell physiology, proliferation, growth, cell survival, metabolism and angiogenesis[38,69]. Akt can promote cell survival by increasing the degradation of p53 through activation of Mouse double minute 2 (MDM2), thus, inhibiting p53-mediated apoptosis, another suggested mechanism that Akt negatively regulates pro-apoptotic proteins through various transcription factors[70]. In the present study, PI3k and Akt proteins were expressed in multiple cells in PALS and germinal centers in NC group, while CP-treated group and CP auto healing group displayed obvious reduction in PI3k and Akt proteins within the splenic cells. Thus, the downregulation of this pathway could explain the induction of apoptosis in splenic cells. After the injection of BM-MSCs to CP-injured rats, there was relative increase in cells which express PI3k and Akt within the spleen, which supports our theory that BM-MSCs could restore the normal function of cyclophosphamide-induced spleen damage and promote survival of the cells.

CONCLUSION

BM-MSCs displayed many potential therapeutic mechanisms in cell therapy, the ability of MSCs to normally repair endogenously damaged tissues has been thought-provoking. BM-MSCs can sense the injury signals, migrate to the damaged site through peripheral circulation and start the healing process via paracrine action or direct differentiation. In this study we investigated the protective and healing capacity of BM-MSCs against CP-injured spleen on molecular basis. Our findings revealed that BM-MSCs upregulated growth factors such as VEGF and promoted angiogenesis. Moreover, they targeted and regained the normal level of p53, caspase, bcl2, CD21, CD14, Akt and PI3k which promoted cell survival, proliferation and growth. Altogether, these findings may be helpful for BM-MSCs-based cell therapy applications in severe spleen-injury associated diseases. Future clinical studies are required to increase our knowledge for their therapeutic mechanisms.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

تأثير الخلايا الجذعية الوسيطة المشتقه من نخاع العظم علي موت الخلايا المبرمج وتولد الأوعية الدموية في الطحال الناجم عن عقار سيكلوفوسفاميد في إناث الجرذان البالغة

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الخلفية / الهدف: الخلايا الجذعية الوسيطة المشتقة من نخاع العظام خلايا متعددة القدرات تم فحصها لدورها المحتمل في العلاج بالخلايا. وظائف الإصلاح والحماية الخلوية ضد العديد من الأنسجة المصابة. علاوة على ذلك توجد تأثيرات غذائية بوساطة عوامل النمو المختلفة والسيتوكينات. اما بالنسبة للأمراض الشديدة المرتبطة بالطحال، يمكن النظر في العلاج بالخلايا الجذعية لاستخدامه المحتمل في استهداف وإصلاح الأنسجة والأعضاء التالفة لذلك فإن الهدف من هذه الدراسة هو التحقق من قدرةالخلايا الجذعية، على أساس جزيئي في الشفاء من الامراض الطحال الناجم عن في.

المواد والأساليب: تم تقسيم الجرذان إلى مجموعتين: المجموعة الضابطة العادية و المعالجة بالسيكلوفوسفاميد. مجموعة التحكم الطبيعي التي عولجت مرة واحدة يوميا (NC) بمحلول ملحي معقم، ومجموعة معالجة بالسيكلوفوسفاميد تم حقنها داخل الصفاق بتركيز ٧٠ مجم / كجم لمدة ثلاثة أيام متتالية. كما تم تقسيم المجموعة الأخيرة إلى ثلاث مجموعات فرعية: - تم التضحية ب (المجموعة المعالجة بالسيكلوفوسفاميد) بعد أسبوع واحد من العلاج، الجرذان بالخلايا الجذعية مجموعة اخري من وتم حقن الحقن في اليوم العاشر المجموعه الفرعية الثالثة حتي وتركت للشفاء التلقائي دون علاج. تم تشريح الطحال من جميع الحيوانات، وتم تقييم الأقسام النسيجية والكيمياء النسيجية المناعية.

النتائج: بعد العلاج بالخلاياالجذعية: استعادت البنية الطبيعية للطحال التالف كما تم تقدير التعبير عن الجينات والبروتينات بواسطة عدة دلالات. استعادوا مستويات mRNA من p٥٣، caspase٣، bcl٢ بطريقه مماثلة الي حد ما لمجموعة التحكم العادية. أيضا تم تنظيم التعبير عن VEGF بعد حقن الخلايا الجذعية، كما تم استعادت المستوي الطبيعي أيضا لبعض الدلالات مثل: CD١٤ و CD٢١ و Akt و PI٣K

الاستنتاج: كشفت هذه الدراسة عن الدور العلاجي المحتمل للخلايا الجذعية المستخلصه من نخاع العظام في الطحال التالف بشدة وشرحت الآلية الأساسية على أساس جزيئي.