The Potential Ameliorative Effect of Bone Marrow Derived Mesenchymal Stem Cells on Cyclophosphamide Injured Lung in Adult Female Albino Rats

Original Article

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ABSTRACT

Background: Mesenchymal stem cells particularly those derived from bone marrow (BM-MSCs) exhibit self-renewal as well as trilineage differentiation capabilities. These cells are considered for cell therapy in several medical disorders. Cyclophosphamide is a well-known immunosuppressive drug, it has a potential pulmonary damage effect in humans and animals. Therefore, the aim of this study is to investigate the immunomodulatory effects of BM-MSCs in cyclophosphamide (CP)-induced lung damage of rats.

Material and Methods: A total number of 40 female rats were divided into 4 groups (A, B, C &D). Group (A) served as a control group, this group was administered intraperitoneal sterile normal saline for 10 d, (10 animals). Thirty rats were treated with intraperitoneal cyclophosphamide at 70 mg/kg BW/d for 3 d, then equally subdivided into three subgroups (B, C, D): Group B (sacrificed after three days). Group C (Auto healing) was left without treatment for ten days. Group D (MSCs treated) was treated on the 4th and 10th days with male BM-derived MSCs in a dose of 3X106/KG BW, by intraperitoneal injection. After ten days animals were sacrificed, lung tissue was obtained and processed for light microscopy exam, and samples were taken to -80 for RNA extraction. The genes expression was estimated by real-time qPCR and the proteins were detected by immunohistochemistry.

Results: BM-MSCs ameliorated the damaged lung. They reverted the mRNA levels of p53, caspase3, band cl2 more/less similar to those of the control group. Upregulation of the mRNA level of VEGF was noticed after BM-MSCs injection. Also, BM-MSCs exerted significant down-regulation of CD14, CD21, Akt and PI3K proteins expression after CP-induced upregulation of these proteins.

Conclusion: This study confirmed that MSCs were ameliorating pulmonary inflammatory and fibrotic changes through their immunomodulatory effects, thus they are considered to be very promising pharmacological therapy for CP-induced lung toxicity.

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Key Words: Akt, Cd14, cyclophosphamide, Lung, MSCs.

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INTRODUCTION

Cyclophosphamide is commonly used as an immunosuppressive drug either in autoimmune diseases or in malignancy therapies in combination with other drugs[1]. Cyclophosphamide can produce lung injury in both humans and animals[2,3]. Pulmonary toxicity is commonly encountered after treatment with cyclophosphamide, sometimes cyclophosphamide can be used in the treatment of interstitial lung disease of autoimmune type, and sometimes it is so difficult to differentiate between the pathology of lung disease and drug-related pulmonary toxicity[4]. Acute pneumonitis or pneumonitis with late-onset are the most frequently encountered syndromes after cyclophosphamide therapy and these conditions are associated usually with progressive pulmonary fibrosis[5]. Mesenchymal stem cells (MSCs) have the characteristics of self-renewal and have the ability to differentiate into different lineage mesoderm, ectoderm and endoderm[6], MSCs can be derived from adipose tissue, bone marrow, umbilical cord, and others[7]. MSCs have the ability to treat a variety of diseases such as inflammatory, myocardial ischemia, systemic lupus erythematosus, end-stage liver disease, and ischemic stroke in addition to its periodontal regeneration effect[8,9,10,11]. Moreover, several previous studies discussed the regulatory effects of stem cell factors on different immune cells including dendritic cells DCs, macrophages, natural killer cells NK neutrophils, B cells, and T cells[12,13]. Several studies were done to understand of interaction between stem cell secreted factors and the immune system, which might raise attention to the potential new strategies to render stem cell therapies more effective. The phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) signaling pathway plays a critical role in the proliferation and differentiation of MSCs[14,15,16]. It has been found that overexpression of PI3K/AKT in MSCs might

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lead to increase MSCs survival after their transplantation in the hearts of rats[17].

The aim of this study was to determine if bone marrow derived MSCs could be beneficial to be used as a substitution for anti-inflammatory and /or immunosuppressive drugs per se and whether they affect the expression of lung damage as well as immune markers by using therapeutic doses of cyclophosphamide in the rat. Monitoring restorative effects and or/ immunomodulatory effects of MSCs were done by using different tools and several markers.

MATERIALS AND METHODS

Animals

2 types of rats were used in this experiment:

1. Five adult male Wister rats weighing up to 120± 20 g were used for the extraction of BM-MSCs.

2. 40 adult female albino rats weighing up to 180± 20 g were used for studying the beneficial effect of BM-MSCs on lung injury.

Animals were acquired from the Laboratory Animal House of the Faculty of Medicine, Assiut University, Egypt. Animals were hosted under standard circumstances of temperature, light and dark series, and humidity, fed with a standard diet. The generally accepted regulations and guidelines in the Laboratory Animal Research Institute were followed for the work of experimental animals after review by the Medical Ethical Committee, Faculty of Medicine, Assiut University Assiut University. IRB 17300339.

Experimental Model and Treatment Protocols

A total number of 40 female rats were divided into 4 groups (A, B, C &D).

• Group (A) served as a control group, this group was administered sterile normal saline IP for 10 d, (10 animals)

• Thirty rats were treated with intraperitoneal cyclophosphamide at 70 mg/kg BW/d for 3 d, then equally subdivided into three subgroups (B, C, D):

- Group B (sacrificed after three days)

- Group C (Auto healing) was left without treatment for ten days,

- Group D (MSCs treated) was treated on the 4th and 10th days with male BM-derived MSCs in a dose of 3X106/ KG BW, by intraperitoneal injection.

After ten days animals were sacrificed, lung tissue was obtained and processed for light microscopy exam, and samples were taken to -80 for RNA extraction[18].

Histological Analysis

Lungs were cut into small pieces and located in 10% buffered formalin for 24 h. Then, the lung specimens were put in different concentrations of alcohol and embedded in paraffin. Sectioning and staining were performed then examination was performed by light microscope.

Separation and culture of Rat BM-MSCs

Eight weeks old rats are sacrificed and placed in a 100-mm cell culture dish, Then bone was isolated and replaced in a 100-mm sterile culture dish with 10 mL extreme α-MEM medium. The bones were washed with PBS having 5% Penicillin Streptomycin /Amphotericin B, then the skeletons were replaced into a fresh 100-mm sterile culture dish with 10 mL whole α-DMEM medium. A 3 mL syringe is used to flush bone marrow cavities several times until the bones became pale. The dish is hatched at 37°C in a 5% CO2 hatchery. Within 7-8 d, MSCs achieve 70–90% confluence. Cells were cleaned two time with PBS and trypsinized[19].

Immunohistochemistry

Tissue pieces were hatched at 60°C for 40 min and then de-paraffinization, washing with dH2O and PBS-Thermo Fisher Scientific - USA) for 4 min were done. Sections were incubated in 3% H2O2 (K31355100, Merck, Darmstadt, Germany) for 4 min. They were incubated with appropriate primary antibodies (anti-CD14) (1:500) (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 using the producer's guidelines, applying the Horseradish peroxidase/DAB (ABC) recognition immunohistochemistry kit (Abcam, UK). The antigen-positive area was brown. The sectors were hatched with secondary antibody (85-9043; Invitrogen, Camarillo, CA, USA) overnight at 4°C, and then with streptavidin (85-9043; Invitrogen, Camarillo, CA, USA) in PBS for 25 min each. Then triple cleaning with PBS, and incubation of slices with diaminobenzidine (ACK125, ScyTek DAB Chromogen, Logan Utah, USA) was done for 10 min. Then counterstaining with Mayer's hematoxylin (MHS16 Sigma-Aldrich USA,) was performed. Then sections were dried, cleared, and mounted with growing solution (EverBrite™ Mounting Medium)[20]. Mean area percentage was assessed in all groups after immunohistochemical staining of four markers. One-way ANOVA test, was used to study significant differences in all groups in comparison to Cyclophosphamide - treated group. *; $p<0.05$, **; $p<0.01$, ***; $p < 0.001$.

Real-time quantitative RT-PCR

RNA extraction by TRIzol (Invitrogen, CA, USA) was performed using the producer's instructions. CDNA creation was done by the Extra-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Inc) using the producer's guidelines. The representation of P53, BCL-2, caspase3, vascular endothelial growth factor (VEGF) and SRY genes was assessed by RT-qPCR. SRY gene is considered a (Y chromosome-linked gene), it was used for tracking of male-derived MSCs in kidney tissue derived from female rats that help in confirmation of homing of MSCs. GAPDH (housekeeping gene) was used as an internal control. Primer sequences were mentioned in (Table 1). The RT-quantitative PCR was done on (Step one, Functional Biosystems, Foster City, CA) as observed: 1) 94°C for 2 min and 2) magnification over 40 cycles at 94°C for 15 s and at 58–62°C (based on the primer put) for 30 s. Data were evaluated by Sequence Revealing Software version 1.3.1 (Applied Biosystems)[21].

Table 1: Sequences of the primers

Primer	The primer's Sequence
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′

RESULTS

Quantitative PCR analyses demonstrated a significant increase in Caspase3, and VEGF (*p0.0004*) in animals treated with Cyclophosphamide, while a decrease in Bcl2 and P53 expression compared to the control group. After MSCs treatment, a significant reduction in the expression levels of, caspase3, these markers were more/less similar to those in the control group. The expression of VEGF , p53 ,and bcl2, were upregulated after MSCs treatment). The observed significant up-regulation of mRNA expression of SRY gene (Y chromosome-linked gene) in MSCs treated group, was denoting the presence of male-derived MSCs in the lung tissue derived from female rats ($P < 0.05$) (Figure 1).

General histological examination: Hx& E stained sections from control group (A) animals demonstrated normal lung tissue with normal alveoli separated by thin interalveolar septa. Animals treated with cyclophosphamide Group (B), showed acute pulmonary congestion with red blood cells, mononuclear inflammatory cell infiltration in the thickened interstitial spaces and collapsed alveoli. In (Auto-healing, Group C) animals showed thick congested pulmonary blood vessels, mononuclear inflammatory cell infiltration, and collapsed alveoli. Animals treated with MSCs Group (D) showed restoration of the majority of lung structures, including airways remodeled alveolar septa with absent mononuclear cell infiltration (Figure 2).

Immunohistochemical staining of phosphorylated AKT distribution in lung tissue revealed its expression in alveoli of control animals group (A), significant upregulation of AKT expression after cyclophosphamide treatment (group B), relative protein expression of AKT in Auto healing group was noticed (group C) and significant downregulation after MSCs treatment (group D). (Figure 3).

Representative photos of PI3K staining in lung tissues revealed expression of PI3K in the alveoli of the control animals group (A). Significant upregulation of PI3K was noticed in group (B). Relative protein expression of PI3K in Auto healing group(C). There was significant down-regulation in group D after treatment by MSCs. (Figure 4).

Cd 14 marker was expressed in lung tissue of the control group (A) with its significant upregulation in cyclophosphamide group (B) was noticed. In the Auto healing group(C), relative protein expression of was revealed with significant down-regulation in group D after treatment by MSCs. (Figure 5).

Representative photos of Cd21 marker staining in lung tissues revealed expression of Cd21 in the alveoli of the control animals in group (A), and significant up-regulation of Cd21 marker expression in the group (B). Relative protein expression of Cd21 in the Auto healing group was noticed (C). There was significant down-regulation in group D after treatment by MSCs (Figure 6).

Mean area percentage of the previous four markers was assessed, there was significant upregulations were observed in AKT, PI3K, CD14 and Cd21protein expressions after cyclophosphamide treatment, whereas significant down-regulations were observed in all markers after MSCs treatment. Bars showing average ± SD. (Figure 7).

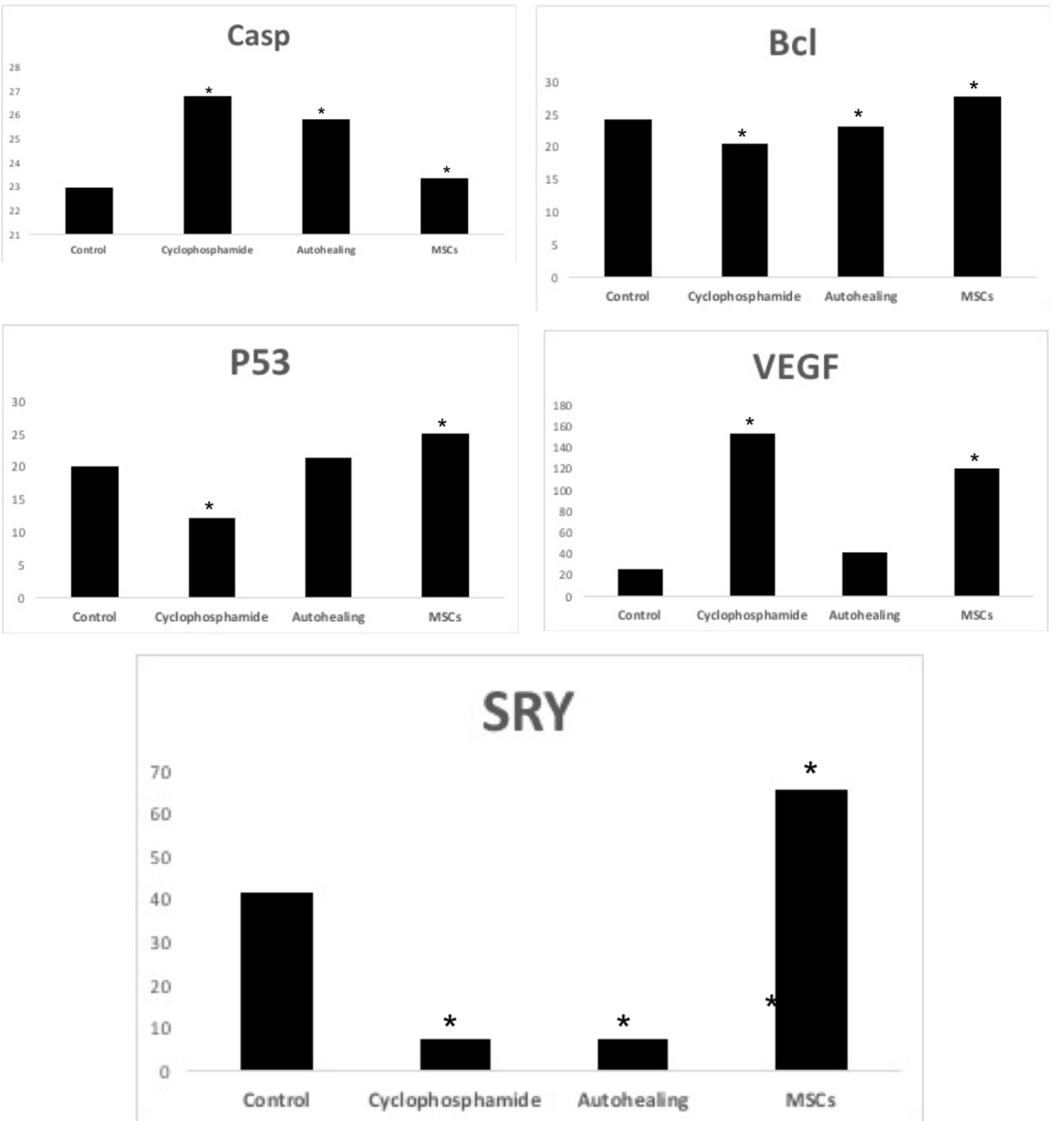

Fig. 1: Quantitative PCR analyses showed a significant increase in Caspase3, and VEGF (*p0.0004*) in animals treated with Cyclophosphamide, Meanwhile there was a decrease in Bcl2 and P53 expression compared to the control group. After MSCs treatment, a significant reduction in the expression levels of, caspase3, these markers were more/less similar to those in the control group. The expression of VEGF, p53, and bcl2, were upregulated after MSCs treatment, There was significant up-regulation of mRNA expression of SRY gene (Y chromosome-linked gene) in MSCs treated group.

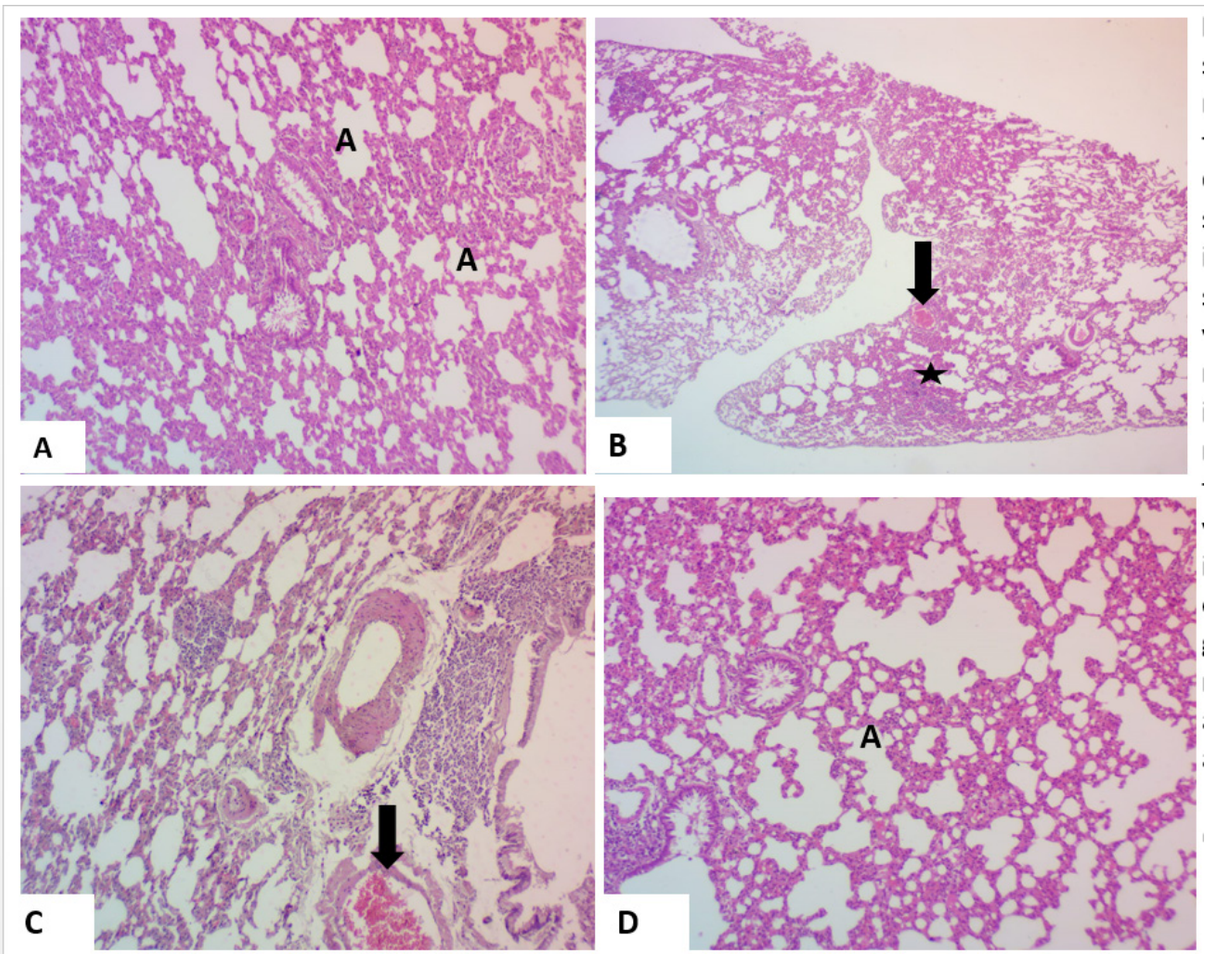

Fig. 2: Photomicrographs of lung sections. (A) Healthy control with normal alveoli (A) are separated by thin interalveolar septa. (B) Cyclophosphamide treated group shows mononuclear inflammatory cell infiltration in the thickened interstitial spaces (star) and congested blood vessels (arrow) surrounded by mononuclear inflammatory cell infiltration. (star), collapsed alveoli are noticed (C) Auto-healing shows thick congested pulmonary blood vessels (arrow), mononuclear inflammatory cell infiltration, and collapsed alveoli. (D) MSCs treated group shows restoration of the majority of lung structures, including airways remodeled alveolar septa with absent mononuclear cell infiltration. (Haematoxyline & Eosin original magnification × 100).

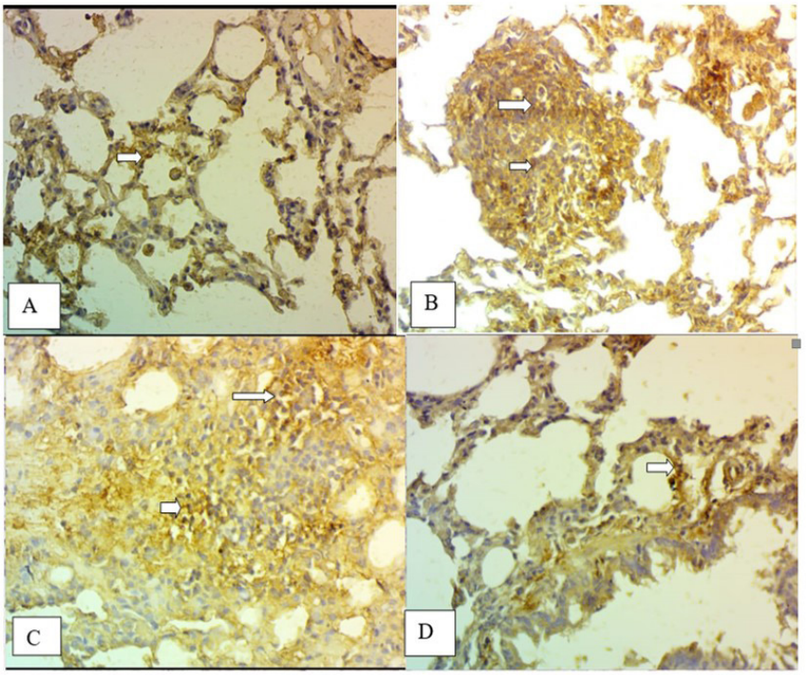

Figure 3. Expression of AKT protein. (A) Representative photos of AKT staining in lung tissues, lung alveoli where an antigen-positive area is brown. ,(arrow) (B) Significant upregulation of AKT expression ,(arrow) and in Auto healing group(C) Relative protein expression of AKT, Significant downregulation in group D. **(original magnification × 100)**

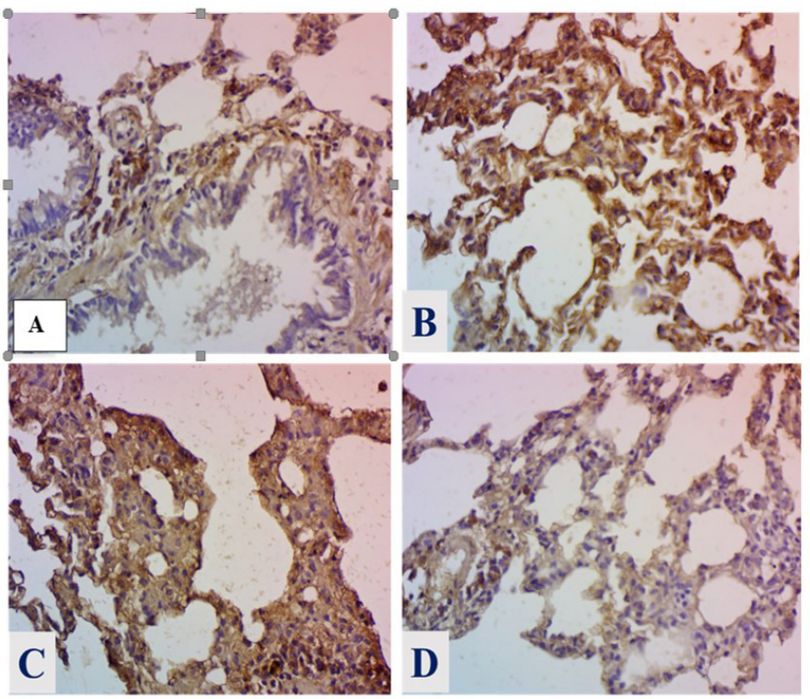

Figure 4. Expression of PI3K protein. (A) Representative photos of PI3K staining in lung tissues, alveoli where the antigen-positive area is brown. (B) Significant upregulation of PI3K. In Auto healing group(C) Relative protein expression of PI3K. Significant downregulation in group D .

(original magnification × 100)

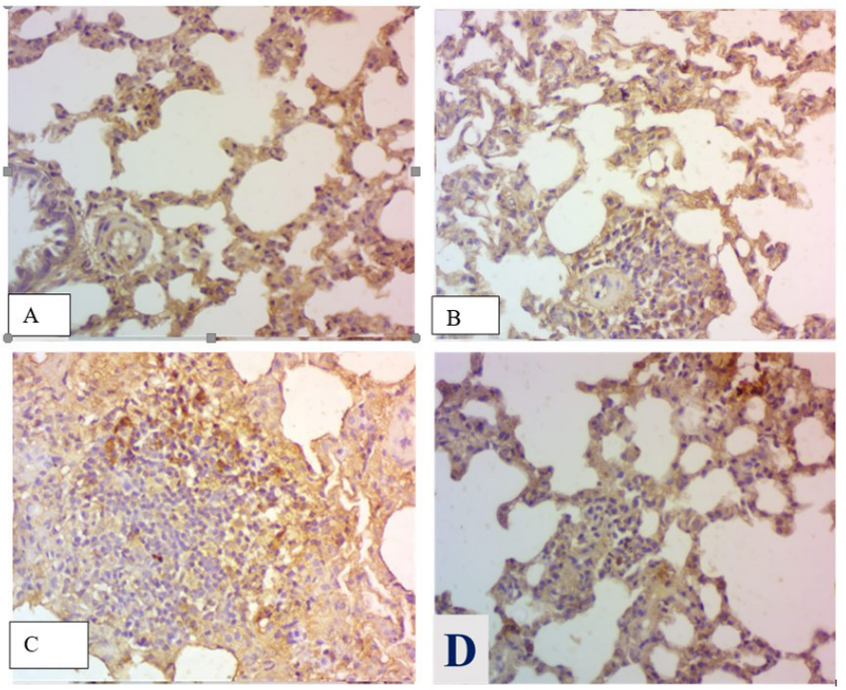

Figure 5. Representative photos of Cd14 marker staining in lung tissues, alveoli Expression of Cd14. (A) , Significant upregulation of Cd14 Expression in group (B) In Auto healing group(C) Relative protein expression of Cd14. Significant down-regulation in group D .

(original magnification × 100)

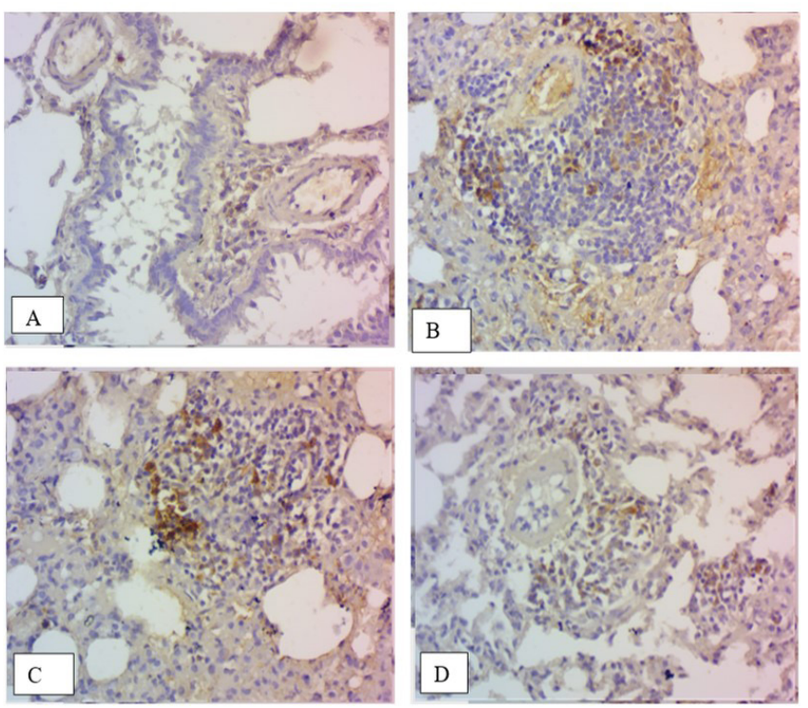

Figure 6. Representative photos of Cd21 marker staining in lung tissues, Expression of Cd21 in alveoli (A), and Significant upregulation of Cd21 marker expression in the group (B). In Auto healing group (C) Relative protein expression of Cd21. Significant downregulation in group D .

(**original magnification × 100)**

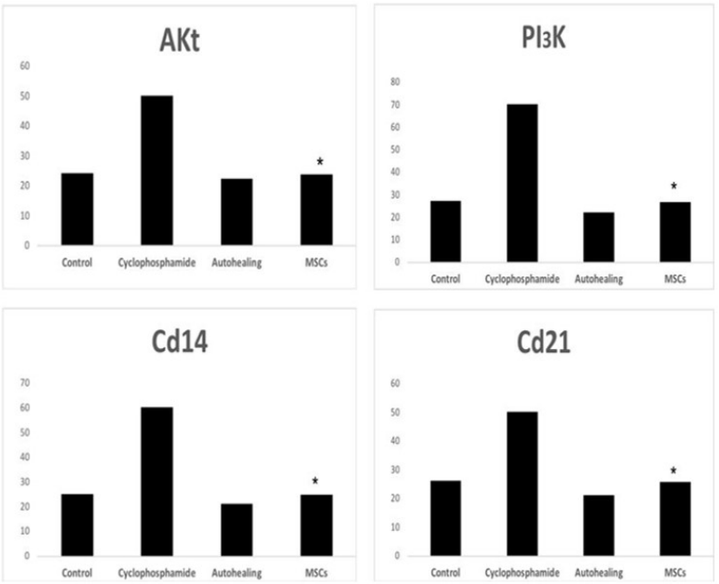

Figure 7: Mean area percentage was assessed in all groups after immunohistochemical staining of four markers as follow :
AKT- Bars represent mean ± SD. A significant difference was analyzed by one-way ANOVA test, *; p<0.05, ***; p < 0.001, compared to CP-treated group
PI3K- Bars represent mean ± SD. A significant difference was analyzed by one-way ANOVA test, **; p<0.01, ***; p < 0.001, compared to the CP-treated group.
CD14- Bars represent mean ± SD. A significant difference was analyzed by one-way ANOVA test, *; p<0.05, **; p<0.01, ***; p < 0.001, compared to the CP-treated group.

Cd21- Bars represent mean ± SD. A significant difference was analyzed by one-way ANOVA test, *; p<0.05, **; p<0.01, ***; p < 0.001, compared to the CP-treated group.

DISCUSSION

Rat lung sections were examined for apoptosis using (caspase3, BCL2 and P53) markers in pulmonary tissues, evaluation were done by using Rt pcr and immunohistochemical methods were used for detection of PI3K/Akt , Cd14 and Cd21 positive cells.

The present study showed that treatment with cyclophosphamide increased inflammatory changes, fibrosis, oxidative stress and apoptosis resulting in histopathological damage in the pulmonary architecture. These toxic effects were decreased significantly after MSCs therapy. Some authors reported the toxic effects of Cyclophosphamide on the vital organs of the experimental animals[22,23]. However, other authors showed significant improvement in these pathological hazards in various organs like the heart, liver, and kidney, when treated with Averon[24].

Cyclophosphamide is a powerful immunosuppressive agent used to treat autoimmune diseases, the AKT and PI3K signaling pathways played an essential role in cell proliferation, differentiation, and survival[25,26]. To understand the mechanism used by the Cyclophosphamide in the lung, the present study demonstrated the effects on AKT and PI3K signaling pathways. Expression of AKT and PI3K in the rat treated with Cyclophosphamide increased significantly compared to the control group and those treated with MSCs in the present study.

These results indicate that Cyclophosphamide stimulates AKT and PI3K signaling pathways. Significant upregulation of PI3K/AKT expression in lung tissue after cyclophosphamide treatment in the present study might be responsible for the observed inflammatory changes in lung tissues of these animals as; mononuclear inflammatory cell infiltration in the thickened interstitial spaces and congested blood vessels, similar observations were reported in mice, after LPS-induced sepsis in both murine and human cells through the activation of the PI3KAkt- mTOR/PFKFB3 pathway that promotes collagen synthesis, furthermore other authors supported these suggestions and mentioned that treating cells with the PI3Kinhibitor LY294002 might improve the inflammatory as well as fibrotic changes in lung tissue[25].

Moreover, significant increase in vascular endothelial growth factor (VEGF) after cyclophosphamide treatment in the present study might act in synergism with apoptotic genes especially (BCL2) thus explaining inflammatory changes observed in the lung tissue in the present study, these suggestions were supported by authors who mentioned that Proinflammatory factors, mainly are vascular endothelial growth factor (VEGF) and tumor necrosis factor might be implicated in extravasation of leukocytes and edema of the alveoli, by increasing the vascular permeability of alveolar endothelial cells, with PI3K inhibitors counteracting these effects[26].

Significant downregulation of AKT/PI3K after MSCs treatment in the present study might explain the anti-inflammatory role played by MScs in lung tissue. In accordance to these findings, authors reported the significant anti-inflammatory role that was played by Akt inhibitor in lung tissue associated with anti-fibrotic

effect due to epithelial-mesenchymal transition that further confirming that PI3K/Akt pathway is a cornerstone in the pathogenesis of lung fibrosis and might be used a new therapeutic strategy[27].

Furthermore, regulation of Cd14 positive cells after cyclophosphamide treatment in the present study raises the suggestion that macrophages are associated with PI3K/AKT pathway in the observed inflammatory changes in lung tissue, these suggestions are supported by the work of authors who used Glycyrrhetinic acid as an inhibitor for PI3K/AKT As well as macrophage Nlrp3 inflammasome, they found that this inhibition ameliorated acute lung injury as well as inflammatory changes[28].

It has been found that CD14 is expressed mainly on some inflammatory cells as monocytes, macrophages and neutrophils, minimal expression might be observed on fibroblasts, epithelial and endothelial cells, CD14 positive cells might play an important role in exaggeration of the immune and inflammatory responses in the lung tissue by being a receptor for some endogenous molecules like intercellular adhesion molecule[29]. Furthermore, cyclophosphamide might induce immunomodulation through stimulation of the expansion/activation of monocytes/dendritic cells, in a trial to make a danger perception consequent to cell death, through p53 and IFN-I-related mechanisms[30].

Meanwhile the observed significant down-regulation of CD 14 positive cells after MSCs treatment in the present study further confirm the protective anti-inflammatory, antioxidant as well as antiapoptotic roles of MSCs against cyclophosphamide- induced lung damage. Similar observations were reported by authors after the use of alogliptin in rats as an ameliorative agent against cyclophosphamide induced lung injury, they explained its role by Impact on PI3K/Akt/FoxO1 pathway and inflammatory cascades[31].

On the other hand, Dendritic cells (DCs) were considered the most dominant antigen-presenting cells that play a key role in the inflammatory immune response either initial or adaptive one. Previous studies demonstrated the association between acute lung inflammation associated with high number as well as activated dendritic cells that reflect their active role in the process of lung inflammation. It has been found that regulation of the maturation as well as the function of these cells might have great ameliorative role in cases of lung inflammations[32]. These suggestions supported our finding of upregulation of cd21 positive (dendritic cells) in lung tissue after cp treatment in a trial to counteract the associated pulmonary inflammatory changes that were observed concomitant with upregulated PI3K/Akt signaling pathway , the latter might be implicated in these inflammatory changes. Moreover other, authors supported these findings and reported The HMGB1/PI3K/Akt/mTOR signaling pathway might be responsible for the maturation and induction of antigen-presenting capability of lung DCs, as well as enhancement of their adhesion and chemotactic ability[32].

Thus upregulation of PI3K/AKT/mTOR signaling pathway might induce immunostimulatory effects particularly affecting pulmonary DCs, in accordance to these findings, it has been found that PI3K/Akt/mTOR pathway regulated a number of physiological processes such as apoptosis, cellular proliferation and autophagy[32]. The close correlation between mesenchymal stem cells treatment and downregulation of a PI3K/AKT pathway raises the suggestion of their anti-tumorigenic effect, these suggestions were supported by the work of authors who explained the anti-tumorigenic role of MScs in acute myeloid leukaemia (AML) animal model by their inhibitory effect on PI3K/AKT/mTOR pathway plays, that plays a vital role in the cell survival, proliferation, migration, cell cycle and apoptosis. Furthermore MScs might exert a potential improvement in microenvironment, cytokines in addition to improving proliferation by down-regulation of (X-linked inhibitor of apoptosis protein),thus up-regulation BCL2 and P53 in the present study after MSCs treatment further add to the potential ameliorative effect in pulmonary parenchyma[33].

CONCLUSION

Mesenchymal stem cells ameliorated CP-induced lung toxicity by decreasing the fibrotic, oxidative and inflammatory alterations. Thus, MSCs are considered as a promising potential pharmacological therapy for CP-induced lung toxicity. Furthermore, MSC's immune regulatory role might be considered a novel therapeutic strategy after lung transplantation.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Matalon, ST, Ornoy A, Lishner M. (2004): Review of the potential effects of three commonly used antineoplastic and immunosuppressive drugs (cyclophosphamide, azathioprine, doxorubicin on the embryo and placenta). Reprod Toxicol.;18 (2):219-30.

2. McDonald GB, Slattery JT, Bouvier ME, Ren S, Batchelder AL, Kalhorn TF, *et al*. (2003): Cyclophosphamide metabolism, liver toxicity, and mortality following hematopoietic stem cell transplantation. Blood.;101: 2043-2048.

3. Chen, X.G., Huang, H., Tian, Y., Guo, C.C., Liang, C.Y., Gong, *et al*. (2011): Cyclosporine, prednisone, and high-dose immunoglobulin treatment of

angioimmunoblastic T- cell lymphoma refractory to prior CHOP or CHOP-like regimen. Chin J Cancer.;30: 731-738.

4. Ozkok A, Kaymaz S, Elcioglu O, Bakan A, Odabas A. (2012): Cyclophosphamide induced early-onset interstitial lung disease. CEN Case Reports,; 1:128 -129.

5. Shokrzadeh M, Chabra A, Ahmadi A, Naghshvar F, Habibi E, Salehi F *et al*. (2015): Hepatoprotective effects of zataria multiflora ethanolic extract on liver toxicity induced by cyclophosphamide in mice. Drug Res.;65: 169-175.

6. Youssef A, Aboalola D, Han Vkm. (2017): The roles of insulin-like growth factors in mesenchymal stem cell niche. Stem Cells Int: 1-12,.

7. Kariminekoo S, Movassaghpour A, Rahimzadeh A, Talebi M, Shamsasenjan K, Akbarzadeh A (2016):Implications of mesenchymal stem cells in regenerative medicine. Artif Cells Nanomed Biotechnol. 44: 749-757,

8. Mao F, Tu Q, Wang L, Chu F, Li X, *et al*. (2017): Mesenchymal stem cells and their therapeutic applications in inflammatory bowel disease. Oncotarget. 6;8(23):38008-38021.

9. Zorzopulos J, Opal SM, Hernando Insua A (2017): Immunomodulatory oligonucleotide IMT504: Effects on mesenchymal stem cells as a first-in-class immunoprotective/immunoregenerative therapy. World J Stem Cells 9: 45-67.

10. Csobonyeiova M, Polak S, Zamborsky R (2017): iPS cell technologies and their prospect for bone regeneration and disease modeling: A mini review. J Adv Res 8: 321-327.

11. Cao Y, Gang X, Sun C (2017): Mesenchymal stem cells improve healing of diabetic foot ulcer. J Diabetes Res 9328347.

12. Corcione, A.; Benvenuto, F.; Ferretti, E.; Giunti, D.; Cappiello, V.; Cazzanti, F. *et al*. (2006): Human mesenchymal stem cells modulate B-cell functions. Blood, 107, 367–372.

13. Castro-Manrreza, M.E.; Montesinos, J.J. (2015): Immunoregulation by mesenchymal stem cells: Biological aspects and clinical applications. J. Immunol. Res., Volume 2015, Article ID 394917, 20 pages .

14. Zhang Y, Zhang Jw, Lv Gy (2012): Effects of STAT3 gene silencing and rapamycin on apoptosis in hepatocarcinoma cells. Int J Med Sci 9: 216-224.

15. Bhaskar PT, Hay N (2007): The two TORCs and Akt. Dev Cell 12: 487-502.

16. Engelman JA (2009): Targeting PI3K signalling in cancer: Opportunities, challenges and limitations. Nat Rev Cancer 9: 550-562.

17. Mangi Aa, Noiseux N, Kong, He H, Rezvani M, Ingwall Js, Dzau Vj (2003): Mesenchymal stem cells modified with Akt prevent remodeling and restore performance of infarcted hearts. Nature Med 9: 1195-120.

18. Saad El dien, H., M.; Bakhaat, G., A.; Rashwan, E., K.; Alaaeldin, R.; Fathy, M. (2022): Bone marrow-derived mesenchymal stem cells modulate apoptosis and angiogenesis in cyclophosphamide-induced spleen injury in rats. The Egyptian Journal of Histology. Available Online from 24 August Articles in Press.

19. Winter, E., *et al*., (2010) :Cell tracking using iron oxide fails to distinguish dead from living transplanted cells in the infarcted heart. Magnetic resonance in medicine, 63(3): p. 817-821.

20. Eleni Andriana Trigka, Georgia Levidou, Angelica A Saetta, Ilenia Chatziandreou, Periklis Tomos, Nikolaos Thalassinos, *et al*. (2013): A detailed immunohistochemical analysis of the PI3K/AKT/mTOR pathway in lung cancer: correlation with PIK3CA, AKT1, K-RAS or PTEN mutational status and clinicopathological features. Oncol Rep. Aug;30(2):623-36.

21. Geng-Wen Huang, Lian-Yue Yang, and Wei-Qun Lu. (2004): Effects of PI3K and p42/p44 MAPK on overexpression of vascular endothelial growth factor in hepatocellular carcinoma. World J Gastroenterol;10(6):809-812 .

22. Goodman, L. S. And Gilman, A. (2008.) Antineoplastic Agents. In Goodman, L. S. And Gilman, A. (Eds.). Goodman & Gilman's: manual of pharmacology and therapeutics. 5th ed. San Diego LaJolla, California: McGraw-Hill, p. 262-279.

23. Mythili, M.D. And Nair, Sc (2004): Effect of cyclophosphamide pretreatment on hematological indices of Indian Bonnet monkey. Indian Journal of Pharmacology, 36: 175-180.

24. Ukpo, Ge, Ehianeta, Ts., Adegoke, Ys. And Salako, Oa. (2013): Evaluation of the Haematological

and biochemical effects of Averon, an herbal formulation, against Cyclophosphamide-induced immunomodulated male rats. International Journal of Pharmaceutical Sciences and Research, 4 (9): 3556-3562.

25. Stork, C.M.; Schreffler, S.M. (2014): Cyclophosphamide. Encycl. Toxicol. 1: 1111–1113.

26. Yung, S.; Zhang, Q.; Chau, M.K.; Chan, T.M. (2015): Distinct effects of mycophenolate mofetil and cyclophosphamide on renal fibrosis in NZBWF1/J mice. Autoimmunity 2015, 48: 471–487, doi:10.3109/08916934..1054027.

27. Zhang X. L., Xing R. G., Chen L., Liu C. R., And Miao Z.G., (2016) :PI3K/Akt Signaling Is Involved In The Pathogenesis Of Bleomycin Induced Pulmonary Fibrosis Via Regulation Of Epithelial Mesenchymal Transition. Molecular medicine reports 14: 5699-5706.

28. Wang ,K., Zhang , Y., Cao Y., Shi, Z., Lin, Y., Chen, Y, *et al*., (2020): Glycyrrhetinic acid alleviates acute lung injury by PI3K/AKT suppressing macrophagic Nlrp3 inflammasome activation.Biochemical and Biophysical Research Communications 532: 555-562.

29. Van Der Poll, A., T. and De Vas, A. F. (2011): Role of CD14 in Lung Inflammation and Infection. Intensive care medicine, ,jan 31 : 129-140

30. Moschella, F., Torelli,G., F., Valentini, M., Urbani, F., Buccione,C., Teresa M. *et al*. (2013): Cyclophosphamide induces a type I interferon-associated sterile inflammatory response signature in cancer patients' blood cells: implications for cancer chemoimmunotherapy. Clin Cancer Res. Aug 1;19(15):4249-61.

31. Alsemeh, A., E., Abdullah, D., M (2022): Protective effect of alogliptin. against cyclophosphamide-induced lung toxicity in rats: Impact on PI3K/Akt/FoxO1 pathway and downstream inflammatory cascades. Cell and Tissue Research 388:417–438.

32. Li, R., Zou, X., Huang, H., Yu,Y., Zhang,H. *et al*., (2020): HMGB1/PI3K/Akt/mTOR Signaling Participates in the Pathological Process of Acute Lung Injury by Regulating the Maturation and Function of Dendritic Cells. Front. Immunol. 11:594253.

33. Ahmed, E., S., Ahmed, N., H., Medhat,A., M. Said,U., Z., Rashed, L., A. and Abdel Rahman B et., (2019): Mesenchymal stem cells targeting PI3K/AKT pathway in leukemic model. Tumor Biology : 1–11.

الملخص العربى

التأثير التحسيني المحتمل للخلايا الجذعية الوسيطة المشتقة من نخاع العظم على الرئة المصابة بالسيكلوفوسفاميد في اناث الجرذان البيضاء البالغة

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الخلفية: أظهرت الخلايا الجذعية الوسيطة وخاصة تلك المشتقة من نخاع العظم (BM-MSCs) تجديدا ذاتيا بالإضافة إلى قدرات تمايز السلالات الثلاثية. بسبب آثارها التجديدية ، تعتبر هذه الخلايا مثالا للعلاج الخلوي في العديد من الاضطرابات الطبية ، وخاصة في أنسجة الرئة . يعتبر سيكلوفوسفاميد دواء معروف مثبط للمناعة ، فهو يمارس إصابة رئوية في البشر والحيوانات. لذلك ، فإن الهدف من هذه الدراسة هو التحقق من الآثار العلاجية والمناعية للخلايا الجذعيه الوسيطة في تلف الرئة الناجم عن السيكلوفوسفاميد للجرذان.

المواد والأساليب: تم تقسيم الجرذان إلى ٤ مجموعات. كانت المجموعة الأولى هي المجموعة الضابطة (A) التي تم إعطاؤها محلول ملحي طبيعي معقم لمدة ١٠ ايام. وعولج ثلاثون فأرا بسيكلوفوسفاميد داخل التجويف البريتوني بجرعه مقدارها ٧٠ مغ/كغ من وزن الجسم/يوم لمدة ٣ أيام، ثم قسمت بالتساوي إلى ثلاث مجموعات فرعية (B, C, D): المجموعة ب (تم تشريحها بعد ثلاثة أيام). تركت المجموعة C (الشفاء التلقائي) دون علاج لمدة عشرة أيام. تم علاج المجموعة D (بالخلايا الجذعية الوسيطه) في اليومين الر ابع والعاشر بعد انتهاء السيكلوفوسفاميد بجرعة ثلاثه ملايين خلية لكل كيلو من وزن الجرذان، عن طريق الحقن داخل التجويف البريتوني. بعد عشرة أيام تم تشريح الجرذان وتم الحصول على أنسجة الرئة ومعالجتها وفحصها باستخدام المجهرالضوئي ، وتم أخذ عينات للتجميد إلى -٨٠ لاستخراج الحمض النووي الريبي. تم قياس الجينات والبروتينات بواسطة جهاز تفاعل البلمرة المتسلسل (qPCR) كما تم استخدام طرق الكيمياء النسيجية المناعية.

النتائج: اظهرت النتائج تحسين في الرئة التالفة باستخدام الخلايا الجذعيه الوسيطة. لقد أعادوا مستويات الجينات المختلفة مثل: p53 و caspase3 و bcl2 لتلك الموجودة في المجموعة الضابطة.كما تم ارتفاع جين VEGF بعد حقن الخلايا الجذعيه الوسيطة أيضا لوحظ ان استخدام الخلايا الجذعيه الوسيطة عمل علي انخفاضا كبيرا لتعبير بروتينات CD14 و CD21 و Akt و PI3K بعد زيادتها الناجم عن السيكلوفوسفاميد.

الاستنتاج: أكدت هذه الدراسة أن استخدام الخلايا الجذعيه الوسيطة ادى الى تحسين التغيرات الالتهابية والليفية الرئوية من خلال آثارها المناعية ، وبالتالي فهي تعتبر علاجا دوائيا واعدا لسمية الرئة الناجمة عن السيكلوفوسفاميد

الكلمات المفتاحية: الرئة ; سيكلوفوسفاميد. PI3K، AKT; CD21; Cd14; MSCs، كاسباز.