MEDICAL SCIENCE MONITOR

ANIMAL STUDY

e-ISSN 1643-3750
© Med Sci Monit, 2019; 25: 2623-2632
DOI: 10.12659/MSM.915804

Received: 2019.02.20
Accepted: 2019.03.26
Published: 2019.04.10

Mesenchymal Stem Cells Alleviate Acute Lung Injury and Inflammatory Responses Induced by Paraquat Poisoning

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEF Lichun Zhang
BCDE Qiuhe Li
BCDE Wei Liu
BCDF Zhenning Liu
BCDF Haitao Shen
ADFG Min Zhao

Emergency Department, Shengjing Hospital Affiliated to China Medical University, Shenyang, Liaoning, P.R. China

Corresponding Author: Min Zhao, e-mail: zhaomsy@163.com
Source of support: This work was funded in part by the National Key Clinical Specialist Fund (2012-649), China

Background: Mesenchymal stem cells (MSCs) show anti-oxidative and anti-inflammatory effects that have prompted further research into their potential applications in treating paraquat (PQ) poisoning cases in emergency rooms. We assessed the protective effects, underlying mechanisms, and secondary inflammatory responses of MSCs on PQ-induced acute lung injury.

Material/Methods: Sprague-Dawley rats were injected intraperitoneally with PQ (20 μg per gram of body weight). MSCs were injected through the caudal vein 1 h after PQ treatment. The severity of lung injury and oxidative stress and levels of inflammatory mediators were examined with and without MSC grafting. Expression levels of TLR4, NF-κB, p65, Nrf2, HO-1, and activated caspase-3 protein were determined by Western blotting.

Results: Administration of MSCs significantly decreased the levels of TNF-α, IL-1β, and IL-6 and polymorphonuclear neutrophil (PMN) count in the bronchoalveolar lavage fluid (BALF) of rats with PQ-induced ALI. In addition, MSC also effectively reduced the wet-to-dry lung weight ratio, lung injury score, and the levels of MDA and 8-OHdG. Conversely, MSC increased SOD and GSH-PX activity in the lung tissue. Moreover, MSC significantly upregulated HO-1, Nrf-2 protein expression in the lung tissue. In contrast, the levels of TLR4, NF-κB p65 and activated caspase-3 protein were decreased in MSC-treated rats ($P<0.05$).

Conclusions: Treatment with MSCs overexpressed Nrf2 gene and activated downstream antioxidant HO-1, leading to inhibit oxidative stress, cell apoptosis and inflammatory response in lung tissue, thereby significantly improving PQ-induced acute lung injury in rats.

MeSH Keywords: Acute Lung Injury • Inflammation • Mesenchymal Stromal Cells • Paraquat • Tissue Therapy, Historical

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/915804

3290 — 5 35

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Background

Paraquat dichloride (PQ, or *N*, *N*'-dimethyl-4,4'-bipyridinium dichloride) is a widely used herbicide in developing countries. It is recognized for its quick and non-selective action on green plant tissues. However, it is also extremely toxic to humans and animals, mostly due to its redox activity, which produces reactive oxygen species (ROS). After absorption, PQ concentrates in cells where redox cycling undergoes, which is a repetitive enzyme-mediated cycling between PQ and PQ radicals. A byproduct of this process is a superoxide radical, a highly reactive oxygen species, which can cause direct cellular damage or further react to form other ROS and nitrite radicals. Redox cycling consumes NADPH, one of the key players in cellular antioxidant defenses. The oxidative stress resulting from the production of free radicals and the depletion of NADPH directly causes cell damage (via lipid peroxidation, mitochondrial dysfunction, necrosis and apoptosis) and it also triggers pronounced secondary inflammatory responses [1].

PQ exposure through the digestive tract, skin and respiratory tract can cause multiple organ dysfunction syndrome (MODS), in which the lungs are among the major target organs. "Paraquat lung" is a disease showing acute lung injury or acute respiratory distress syndrome (ARDS) symptoms, and/or pulmonary interstitial fibrosis, which cause an extremely high case-fatality rate (50% to 80%) [2,3]. The lethal ingestion dose of paraquat for humans is approximately 35 mg/kg, which is equivalent to 10-15 ml of a 20% solution. Thousands of individuals succumb to PQ ingestion every year in developing countries. In the rural areas where PQ remains readily available, PQ ingestion is a common method of intentional self-poisoning. However, there is still no specific antidote for paraquat poisoning, for which to formulate a treatment regimen for protecting poisoned organs from irreversible damage is necessary.

Recently, an increasing number of reports have indicated protective effects of stem cells in acute or chronic injury in multiple organs. Mesenchymal stem cells (MSCs) are multipotent cells with the capability to differentiate into mesenchymal lineages including cartilage, bone, muscle, tendon, ligament and adipose tissue. Except for their functions in regeneration, MSCs have shown possess potent immunomodulatory [4], anti-inflammatory [5] and antifibrotic capacities [6]. For example, a 2003 study [7] showed that MSC grafts, originating from murine bone marrow, could prevent pulmonary fibrosis in mice exposed to bleomycin (BLM), a cancer medication known to have this side effect. Recently, MSCs have shown potential for the treatment of PQ-poisoned patients in multiple studies [8].

To better understand the protective effects and the potential mechanisms of MSCs in treating PQ poisoning, we established a PQ poisoning animal model through intraperitoneal injection of PQ into Sprague-Dawley rats, followed by intravenous injection of bone marrow-derived MSCs 1 h after PQ injection. The effects of MSC grafts were assessed based on their ability to reverse PQ-induced damage, as well as inflammatory indicators.

Material and Methods

MSC preparation and characterization

Rat MSCs derived from normal rat bone marrow were purchased from Cyagen Bioscience, Inc. (Guangzhou, China). They expressed CD90and CD44, and did not express CD34, CD45, and CD11b/c, as determined by flow cytometry assay. The Sprague-Dawley rat MSC basal medium (Cyagen Bioscience, Inc., Guangzhou, China) was supplemented with 10% Sprague-Dawley rat MSC qualified fetal bovine serum, 1% glutamine, and 1% penicillin-streptomycin at 37°C and 5% CO_2 saturated humidity to grow MSC cells. The growth medium was changed every day. The cells were dissociated with Trypsin-EDTA and passaged when they were approximately 80–90% confluent. Lentivirus carrying GFP (pLenO-GFP) was purchased from GenePharma (Shanghai, China). The MSCs were infected by pLenO-GFP with MOI=50 and cultured in 6-well plates (10^6 cells/well) with 5% CO_2 at 37°C. The culture medium was changed after 24 h. The infection efficiency of pLenO-GFP was identified by fluorescence microscopy 48 h after transfection (Olympus Co., Tokyo, Japan).

Animals

Male Sprague-Dawley rats weighing 200–250 g were provided by the Experimental Animal Center of China Medical University. Animals were housed with a 12: 12-hour light-dark cycle, with free access to food and water. Animal experimental procedures were approved by the Animal Ethics Committee of China Medical University. Sprague-Dawley rats were randomly divided into 3 groups: (1) Saline-treated (control group, n=18); (2) PQ-treated (PQ group, n=18); and (3) PQ + MSCs (MSC group, n=18). PQ solution (2 mg/ml in saline) or an equal amount of saline was injected into the rats intraperitoneally (20 μg per gram of body weight). MSCs (3×10^6 viable cells) re-suspended in PBS were injected through the caudal vein 1 h after PQ injection.

Bronchoalveolar lavage fluid (BALF) collection

Rats were anesthetized by intraperitoneal injection of 1mL of 2.5% Avertin in PBS 24 h after PQ injection. The BALF samples were collected from the left lung following standard procedures. The BALF samples were cleared by centrifugation at 1500g at 4°C for 10 min, and the supernatants were collected and subjected to subsequent assays.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Lung wet/dry weight ratio (W/D)

The upper lobe of the right lung was dissected from the rats. The wet weight (W) was measured after removal of the liquid on the surface of the collected lung tissue, and the dry weight (D) was measured after drying the lung tissue at 80°C for 24 h. The lung tissue W/D ratio was calculated by dividing the wet weight (W) by the dry weight (D).

Histology

Lung tissue dissected from the right upper lobe was fixed with 10% paraformaldehyde solution, and then the specimen was sectioned to tissue blocks with a thickness of about 0.5 cm. We then performed conventional gradient alcohol dehydration, paraffin-embedding, and serial sectioning. The paraffin-embedded sections were stained with hematoxylin and eosin (HE) following standard procedures. Histopathological changes of lung tissue were observed under an optical microscope. The severity of lung injury was graded based on edema, congestion, neutrophil infiltration, intrabronchial hemorrhage, and cell proliferation. These characteristics were subjectively scored on a scale from 0 to 3: 0=normal, 1=slight effect, 2=moderate effect, and 3=strong effect, calculated by the sum of the scores of 5 markers. The overall injury score is the sum of the scores from the 5 indicators [9].

Polymorphonuclear neutrophil (PMN) count

A small amount of each BALF sample was placed onto a coverslip for Giemsa staining. Polymorphonuclear neutrophils (PMN) were imaged and counted under a microscope (400×, Eclipse E200, Nikon Corporation, Japan). Five randomly selected independent fields were counted.

Oxidative stress test with lung tissue

Left lung tissue was homogenized using tissue lysis buffer (0.01 mol/L Tris-HCl, pH 7.4, 0.1 mmol/L EDTA-2Na, 0.01 mol/L sucrose, 0.8% NaCl). The homogenate was centrifuged, and the supernatant was collected to test the level of malondialdehyde (MDA) using thiobarbituric acid colorimetry (Nanjing Jiancheng Bioengineering Institute), super oxidative dismutase (SOD) using the xanthine oxidase method (Nanjing Jiancheng Bioengineering Institute), GSH-PX activity using chemical colorimetry (Nanjing Jiancheng Bioengineering Institute), and 8-OHdG content using ELISA (Biolegend, San Diego, CA)

Cytokine analysis

The BALF samples were tested to determine the concentrations of TNF-α, IL-1β, and IL-6 by ELISA (Bio-Rad, Hercules, CA) following the manufacturer's protocols. The levels of TNF-α, IL-1β, and IL-6 in the samples were calculated based on a standard curve. The detection ranges of the TNF-α, IL-1β and IL-6 ELISA assays were 56.25–3600, 7.8–500, and 62.5–4000 pg/ml, respectively. Samples with a concentration over the limit of the standard curve were measured after dilution.

Western blot analysis

Cytoplasmic and nuclear proteins were isolated from lung tissue samples following the instructions of the KGI Nuclear and Cytoplasmic Protein Extraction kit (Nanjing KGI Biotechnology). Protein concentrations were determined using the BCA method (Beyotime Institute of Biotechnology, Haimen, China). Proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and were transferred onto polyvinylidene fluoride membranes (Millipore, Bedford, MA). The membranes were incubated overnight at 4°C with rabbit polyclonal antibodies against TLR4, NF-κB p65, Nrf2, HO-1, and activated caspase-3 (Santa Cruz Biotechnology, Inc., Dallas, TX). The membranes were then incubated with horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (Beyotime Institute of Biotechnology) at 37°C for 45 min. The enhanced chemiluminescence (ECL) imaging system was to visualize protein bands. β-actin was used as the internal control.

Statistical analysis

SPSS16.0 statistical software (SPSS Inc., Chicago, IL) was used for data analysis. All data were recorded as mean ± standard deviation (SD). The difference among groups was analyzed by one-way ANOVA, and the LSD test was used for comparison between 2 groups. A p value <0.05 was considered to be statistically significant.

Results

Isolation and characterization of MSCs

MSCs arranged in order were cultured for 6 generations. Lentivirus transfection did not affect proliferation or morphology of MSCs. Fluorescence microscopy revealed that over 80% of cells were GFP-positive cells (Figure1A, 1B). Then, at 1 h after establishment of the paraquat-induced ALI animal model carrying GFP, MSCs were injected through the caudal vein. All rats in the experiment survived. Fluorescence was measured in lungs 24 h after MSCs administration using fluorescence microscopy. As shown in Figure 1C–1E, the fluorescence was distributed in the distal lungs.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

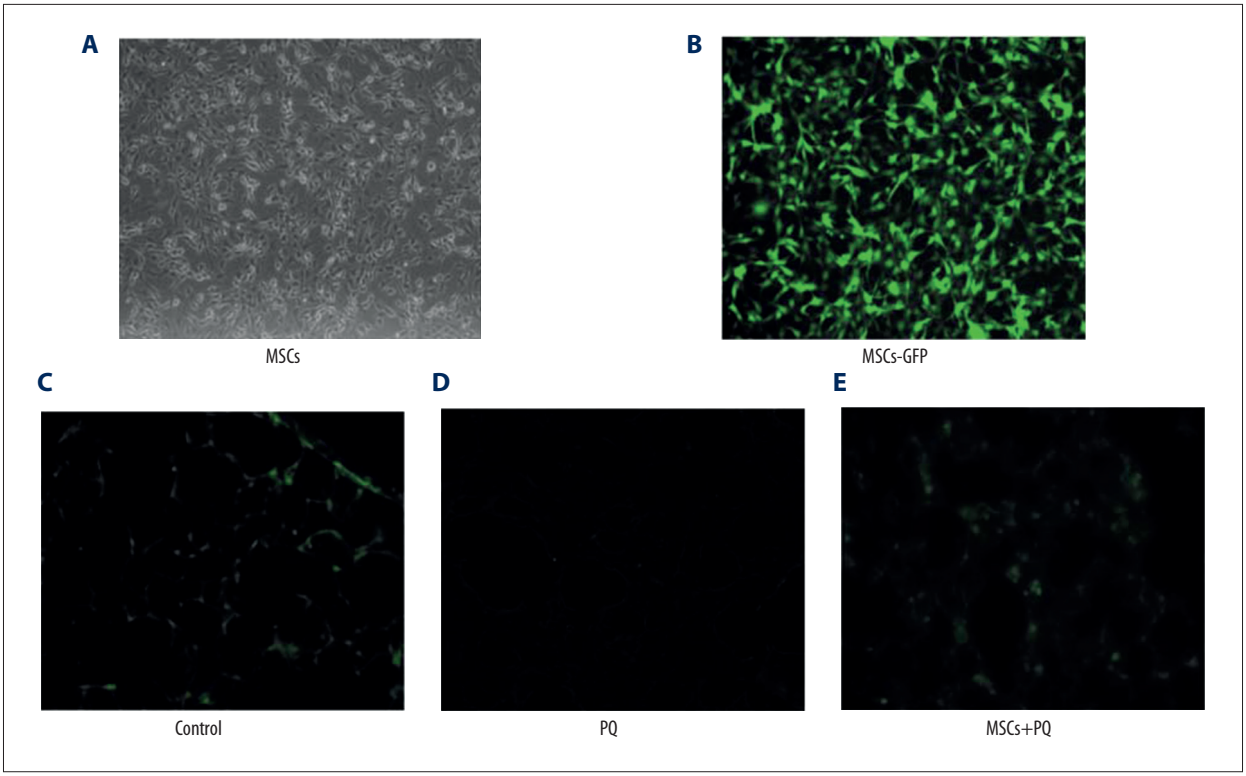

Figure 1. MSCs carrying GFP were observed via fluorescence microscopy. The transfection efficiency was over 80% (**A, B**). At 24 h after MSCs administration, MSCs were distributed throughout the distal lungs (**C–E**).

Treatment with MSCs attenuates lung necrosis, edema, and inflammation induced by PQ

To assess the effects of PQ and MSC treatment, we dissected lung tissue from the right upper lobe for wet/dry (W/D) ratio measurement and histological examination. We found a significantly higher lung W/D ratio in the PQ group, indicating severe pulmonary edema due to PQ ingestion (Figure 2A, $p<0.05$). We stained paraffin sections of lung tissues with hematoxylin and eosin (HE) (Figure 2B, 2D) for examination of the lung injury severity. Normal lung tissue showed structural integrity of alveoli, without inflammatory exudates (score: 0, Figure 2E). PQ treatment induced severe lung injury in rats, manifested by visible alveolar structural damage, lung interstitial edema, and a large number of infiltrating inflammatory cells (Figure 2E). Consistent with the HE staining results, the number of polymorphonuclear neutrophils (PMNs) in the bronchoalveolar lavage fluid (BALF) dramatically increased in PQ-treated rats (Figure 2F, $p<0.05$).

We injected MSCs (approximately 3 million viable cells) into PQ-poisoned rats 1 h after PQ treatment. The results showed that administration of MSCs significantly protected lung tissue from damage by PQ poisoning, as indicated by W/D weight ratio (Figure 2A), histological characteristics (Figure 2B–2D), overall injury severity scores (Figure 2E), and the number of PMNs (Figure 2F).

MSCs protect lung tissues from necrosis by alleviating oxidative stress

Our results show that the amounts of MDA and 8-OHdG in lung tissues of the PQ-treated rats increased, but this was reversed by MSC grafting (Figure 3A, 3B). In addition, the activity of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) was decreased in PQ-treated rats and was significantly restored in PQ-treated rats injected with MSCs (Figure 3C, 3D).

Compared with the control group, Nrf2 and HO-1 protein levels in the PQ group were slightly increased, but there was no significant difference between the 2 groups. In contrast, the expression of Nrf2 and HO protein in the MSC group increased significantly ($p<0.05$, Figure 3E–3G).

MSCs inhibit proinflammatory mediators

Consistent with the histological changes and the elevated number of PMNs shown above (Fig. 2F), the levels of TNF-α, IL-1β, and IL-6 were all detected by ELISA in the BALF samples from the PQ-treated rats at 20–70-fold increases compared with the control group. Conversely, MSC grafting remarkably decreased the levels of these cytokines by 60–70% when compared with the rats treated with PQ alone (Figure 4A–4C).

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

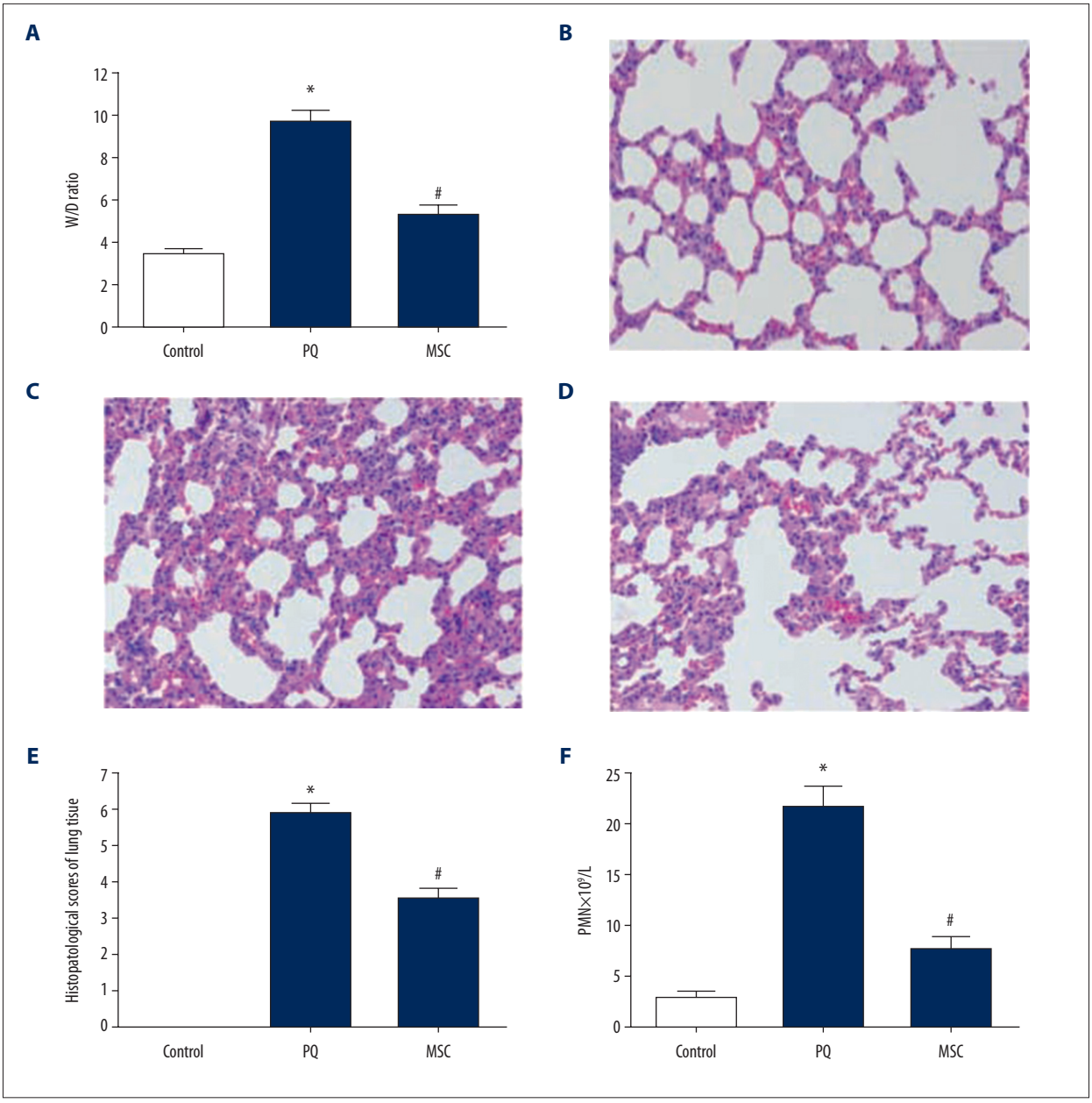

Figure 2. SD rats exposed to paraquat showed less lung injury after treatment with mesenchymal stem cells. (**A**) The lung tissue W/D ratio was calculated by dividing the wet weight by the dry weight. (**B–D**) Representative HE staining of the lung tissue from the Control, PQ-treated, and MSC-treated groups. (**E**) Injury scores of the 3 groups. (**F**) The number of PMNs from BALF samples. Data are mean ±SD. n=12/group * $p<0.05$ compared with the normal control group and # $p<0.05$ compared to the PQ group.

We observed elevated expression of TLR4 and NF-κB p65 protein synchronously in the PQ group, which decreased in the MSC group ($p<0.05$, Figure 4D–4F), suggesting that the PQ-induced inflammation is related to TLR4/NF-κB. In our experiments, PQ treatment induced Caspase-3 activity, which was downregulated by MSC grafts ($p<0.05$, Figure 5A, 5B).

Discussion

At present, comprehensive measures such as gastric lavage, catharsis, activated carbon adsorption, blood perfusion, antioxidant free radical drugs, and immunosuppressive agents are applied for paraquat poisoning treatment. Although the survival time of poisoned patients has been increased, the mortality rate has not significantly improved. The main cause of

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

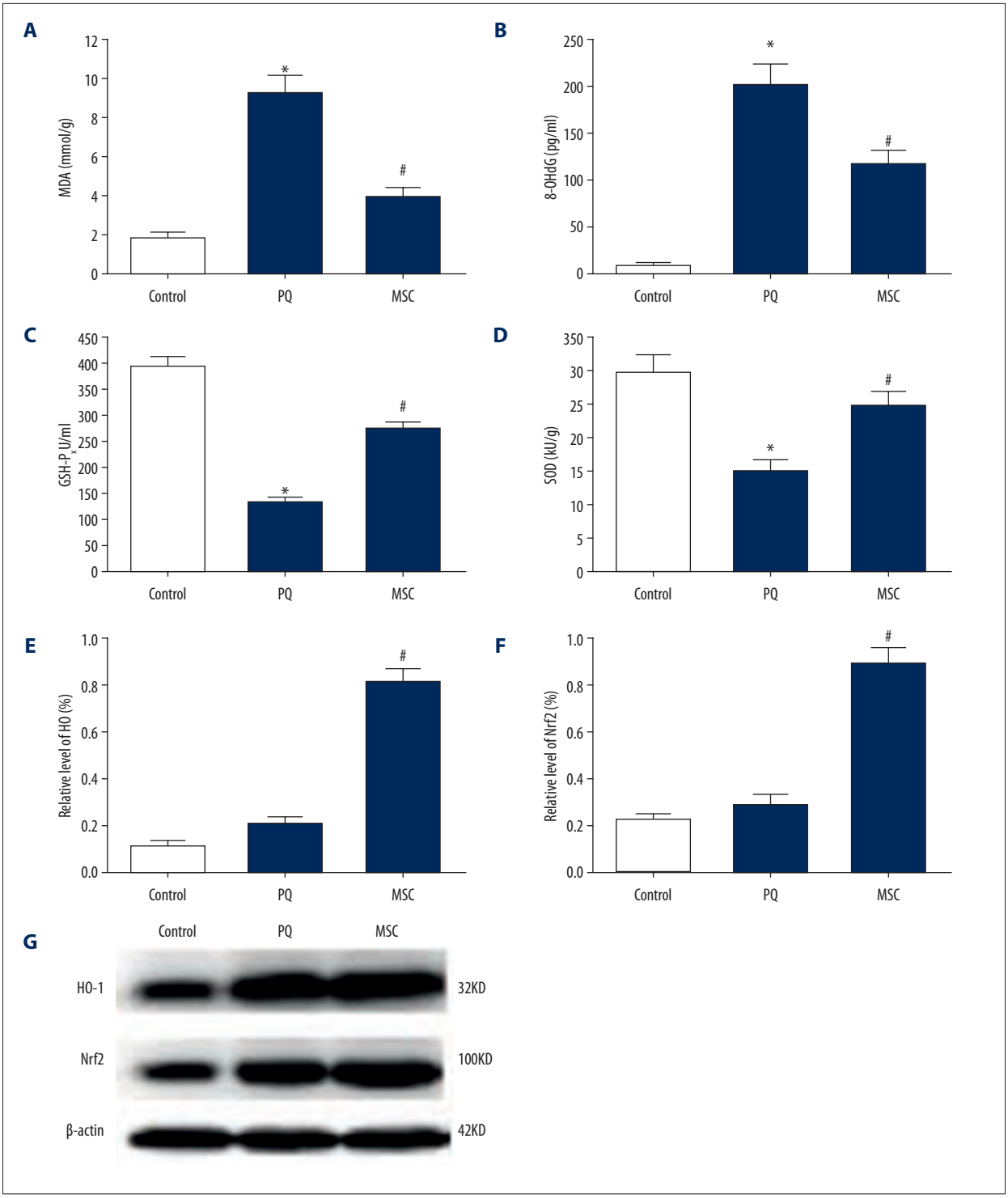

Figure 3. Mesenchymal stem cell treatment reduced oxidative stress from paraquat exposure. Oxidative stress was measured in tissue lysates from the left lung as the level of MDA (**A**), 8-OHdG (**B**), GSH-PX (**C**), and SOD (**D**) using ELISA. Expression of HO-1 (**E, G**) and Nrf2 (**F, G**) were measured by Western blot analysis and normalized to β-actin. Data are mean ±SD. n=12/group * $p<0.05$ compared to the normal control group and # $p<0.05$ compared to the PQ group.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

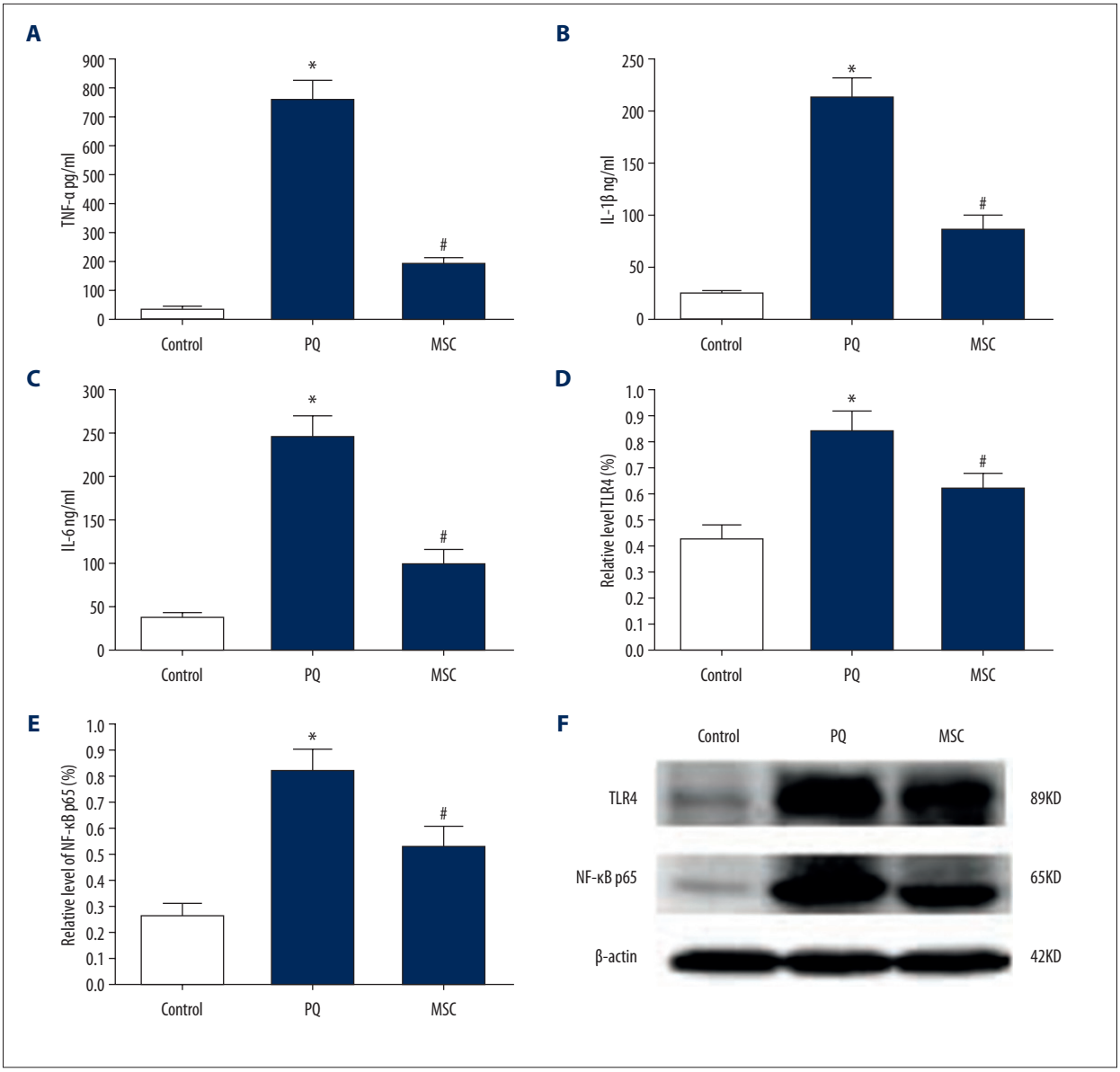

Figure 4. Proinflammatory mediators are reduced by mesenchymal stem cell treatment. Protein levels of TNFα (**A**), IL-1β (**B**), and IL-6 (**C**) in BALF samples were determined by ELISA. Protein levels of TLR4 (**D, F**) and NF-κB p65 (**E, F**) were measured by Western blot and normalized to β-actin. Data are mean ±SD. n=12/group. * $p<0.05$ compared to the normal control group and # $p<0.05$ compared to the PQ group.

death is respiratory failure caused by lung injury. Therefore, exploring new and effective therapeutic strategies for PQ poisoning lung injury has become an important research focus.

Numerous reports have shown that adult MSCs can suppress the maturation of T cells and dendritic cells, reduce the activation and proliferation of B-cells, inhibit the proliferation and cytotoxicity of NK cells, and promote the generation of regulatory T cells via soluble factors or cell–cell contact mechanisms, which eventually result in modulating the adaptive and innate immune systems [10–12]. MSCs are derived from bone marrow, fat, and other tissues, and they have been applied in the treatment of inflammatory diseases and other immune disorders in experimental animal models. Potential treatment effects have been showed on autologous, allogeneic, and even xenogeneic MSCs. MSCs have been approved and used as an immunomodulator in clinical trials because of their efficacy in GvHD, organ transplantation, diabetes, multiple sclerosis, and Crohn's disease [13]. There are more than 400 studies on treatment using MSCs registered in the clinical trial database. Some of these studies have focused on poisoning cases. For example, the effect of a combination therapy of MSC transplantation

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

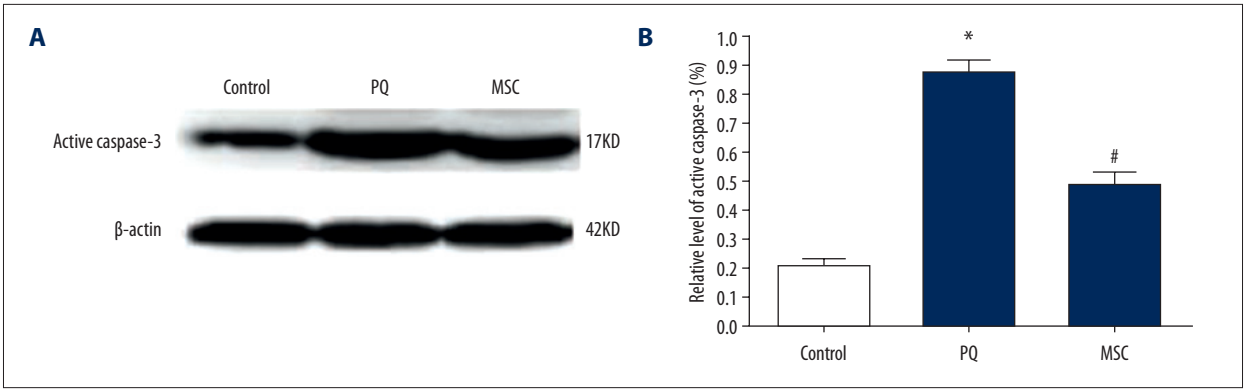

Figure 5. Apoptosis was reduced by mesenchymal stem cell treatment. Active Caspase-3 was measured by Western blot (**A**). β-actin was used to normalize the expression levels (**B**). Data are mean ±SD. n=12/group. * $p<0.05$ compared to the normal control group and # $p<0.05$ compared to the PQ group.

and butylphthalide was examined and found to be effective in the treatment of patients with delayed encephalopathy after carbon monoxide (CO) poisoning (DEACMP) [14].

Considering the unique anti-oxidative and immunomodulatory properties of MSCs, the promising results from the above studies on MSCs in the treatment of other immunological disorders suggest MSCs may be a potential treatment option in PQ poisoning. In the present study, a PQ poisoning rat model was established to explore the effects and the underlying mechanisms of MSCs in PQ-induced acute lung injury. We found that MSCs protect against PQ-induced acute lung injury in rats through alleviating oxidative stress and the inflammatory response. Most of the rats showed improvement in the overall severity of lung injury, suggesting that MSCs could be used as an alternative option or in combination with other anti-oxidative and anti-inflammatory medications.

The main mechanism of acute lung injury caused by PQ poisoning is the oxygen free radical damage theory. PQ is an electron acceptor that acts on the oxidation-reduction reaction in cells to generate a large number of reactive oxygen radicals, causing lipid peroxidation of cell membranes and mitochondrial dysfunction, and eventually resulting in oxidative damage to tissue cells [15]. Levels of SOD and GSH-Px basically reflect the body's ability to scavenge free radicals and resist lipid peroxidation damage [16]. Oxidative stress causes profound alterations of various biological structures, including cellular membranes, lipids, proteins, and nucleic acids. Malondialdehyde (MDA) is a final product from peroxidation of polyunsaturated fatty acids in cells. An increase in free radicals causes overproduction of MDA. MDA levels are commonly used as a marker of oxidative stress and the antioxidant status of patients [17]. 8-Hydroxy-2'-deoxyguanosine (8-OHdG), which is another predominant product from free radical-induced oxidative lesions, has therefore been widely used as a biomarker for oxidative stress and carcinogenesis. The results of this study showed that the content of MDA and 8-OHdG in the lung tissue of the PQ group increased, while the activity of SOD and GSH-Px decreased, suggesting that strong oxidative stress occurs in PQ poisoning, which causes an imbalance in the ratio of oxidation and anti-oxidation in cells.

Recent studies have shown that an imbalance of primary oxidation leads to the occurrence of alveolitis, and results in infiltration of neutrophils, alveolar macrophages, and lymphocytes in the body [18,19]. PQ can directly cause tissue and cell damage by lipid peroxidation, and the generation of reactive oxygen species (ROS) can also activate inflammatory cells such as neutrophil and monocyte macrophages, causing many inflammatory cytokines to be released, including tumor necrosis factor (TNF-α) and interleukin (IL-1β, IL-6). A cascade of inflammatory reactions is caused by TLR4, which triggers a systemic inflammatory response syndrome (SIRS) and aggravates lung tissue damage [20,21]. In normal cells, nuclear factor kappa B (NF-κB) binds with inhibitory proteins, and ROS can transfer NF-κB from stationary state into active state. Inhibitory proteins are rapidly phosphorylated by NF-κB inducer. Once NF-κB is activated, it moves to the nucleus and combines with promoter regions, inducting target gene transcription of inflammatory factors, cytokines, and chemokines [22]. As a result, PQ induces nuclear enrichment and DNA fragmentation by ROS production and NF-κB activation, leading to further apoptosis.

Caspase-3 is directly involved in the initiation and execution of the apoptosis process and the associated signal transductions. Given that cleaved activated caspase-3 is an important factor in apoptosis, the status of apoptosis can be inferred by the detection of activated caspase-3 [23]. The results of our study showed that the concentration of TNF-α, IL-1β, and IL-6 were increased, and large numbers of PMN were observed in BALF from PQ-treated rats, showing an obvious inflammatory response in lung tissue. The expression of TLR4 and NF-κB was upregulated, indicating that the TLR4/NF-κB pathway

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

was involved in the early stage of pathophysiological process of lung injury in PQ-treated rats. MSCs can suppress activity of the TLR4/NF-κB signaling pathway, reducing inflammation, oxidative stress, and cell apoptosis in lung tissue, thus alleviating lung injury. Recently, MSCs have been demonstrated to be highly resistant to oxidative damage and to be potent scavengers of free radicals [24].

PQ can activate the Nrf2-ARE signaling pathway in multiple ways as a strong stressor. Nuclear factor (erythroid-derived 2)-like 2, which is also called Nrf2, is one of the basic leucine zipper (bZIP) proteins. When the body experiences injury and inflammation, Nrf2 regulates the expression of antioxidant proteins involved in protection against oxidative damage [25]. Nrf-2 is located in the cytoplasm in physiological states and combines with cytoplasm joint protein (Keap-1) to form dimers. When stimulated by oxidative stress, Nrf-2 combines with the antioxidant response element (ARE) and is phosphorylated into the nucleus, inducing its target gene expressing HO-1, GSH-Px, SOD, NQ01 (NADPH: quinoneoxidoreductase1), and other antioxidant and II detoxifying enzymes [26], thus protecting against oxidative stress caused by ROS and protecting normal cells from damage [27]. The treatment of diseases caused by oxidative stress has been studied for several drugs that stimulate the Nrf2 pathway [28]. Heme oxygenase-1 (HO-1) is one of the Nrf2 target genes; it has been reported to protect against many pathologies, including sepsis, hypertension, atherosclerosis, acute lung injury, kidney injury, and pain [29]. Studies have also shown that co-culturing with MSCs can reduce the volume of PQ in type II alveolar epithelial cells and increase the expression of antioxidant genes, increasing epithelial cell survival [30]. After implantation of bone marrow MSCs into PQ-treated rats, lung injury in model animals was reduced [31,32]. The results of our study showed that the pulmonary alveolar hemorrhage, inflammatory cell infiltration, pulmonary interstitial hyperemia, and edema were significantly reduced in the MSCs group, and the expression of Nrf-2 and HO-1 in the lung tissue was significantly higher than that in the PQ group, suggesting that MSCs can reduce the lung injury caused by PQ poisoning by activating Nrf2 signaling.

Besides their immunomodulatory properties, MSCs have also demonstrated excellent self-renewal and regeneration capabilities [33]. MSCs not only selectively aggregate, increase the secretion of surfactant, and reduce inflammatory responses [34], but also differentiate, repair damaged tissue, and contribute to "scarless" wound healing to prevent the occurrence of chronic pulmonary fibrosis [35]. At 24 h after MSCs administration, fluorescence microscopy showed distribution of MSCs throughout the distal lung in PQ rats. Combining their immunomodulatory functions and regenerative capabilities, MSCs could be a unique clinical treatment option for acute poisoning cases and the wounded tissue healing processes that follow.

Conclusions

MSCs have protective effects on PQ-induced lung injury in rats. The direct mechanism is that MSC can participate in the repair by colonizing damaged distal lung tissues. The indirect mechanism is that treatment with MSCs led to overexpression of the Nrf2 gene and activated the downstream antioxidant HO-1, thus inhibiting oxidative stress, cell apoptosis, and inflammatory response in lung tissue.

Conflicts of interest

None.

References:

1. Sun B, Chen YG: Advances in the mechanism of paraquat-induced pulmonary injury. Eur Rev Med Pharmacol Sci, 2016; 20: 1597–602
2. Huang J, Xuan D, Li X et al: The value of APACHE II in predicting mortality after paraquat poisoning in Chinese and Korean population: A systematic review and meta-analysis. Medicine (Baltimore), 2017; 96: e6838
3. Shen H, Wu N, Wang Y et al: 95JNK inhibitor SP600125 attenuates paraquat-induced acute lung injury: An *in vivo* and *in vitro* study. Inflammation, 2017; 40: 1319–30
4. Yagi H, Soto-Gutierrez A, Parekkadan B et al: Mesenchymal stem cells: Mechanisms of immunomodulation and homing. Cell Transplant, 2010; 19: 667–79
5. Xiang B, Chen L, Wang X et al: Transplantation of menstrual blood-derived mesenchymal stem cells promotes the repair of LPS-induced acute lung injury. Int J Mol Sci, 2017; 18. pii: E689
6. Gazdhar A, Grad I, Tamò L et al: The secretome of induced pluripotent stem cells reduces lung fibrosis in part by hepatocyte growth factor. Stem Cell Res Ther, 2014; 5: 123
7. Ortiz LA, Gambelli F, McBride C et al: Mesenchymal stem cell engraftment in lung is enhanced in response to bleomycin exposure and ameliorates its fibrotic effects. Proc Natl Acad Sci USA, 2003; 100: 8407–11
8. Tsai HL, Chang JW, Yang HW et al: Amelioration of paraquat-induced pulmonary injury by mesenchymal stem cells. Cell Transplant, 2013; 22: 1667–81
9. Wu R, Dong W, Zhou M et al: Ghrelin attenuates sepsis-induced acute lung injury and mortality in rats. Am J Respir Crit Care Med, 2007; 176: 805–13
10. Contreras RA, Figueroa FE, Djouad F, Luz-Crawford P: Mesenchymal stem cells regulate the innate and adaptive immune responses dampening arthritis progression. Stem Cells Int, 2016; 2016: 3162743
11. Gao F, Chiu SM, Motan DA et al: Mesenchymal stem cells and immunomodulation: current status and future prospects. Cell Death Dis, 2016; 7: e2062
12. Kovach TK, Dighe AS, Lobo PI, Cui Q: Interactions between MSCs and immune cells: Implications for bone healing. J Immunol Res, 2015; 2015: 752510
13. Gao F, Fish BL, Szabo A et al: Short-term treatment with a SOD/catalase mimetic, EUK-207, mitigates pneumonitis and fibrosis after single-dose total-body or whole-thoracic irradiation. Radiat Res, 2012; 178: 468–80
14. Wang H, Li Y, Wu Q et al: Combination of butylphthalide with umbilical mesenchymal stem cells for the treatment of delayed encephalopathy after carbon monoxide poisoning. Medicine (Baltimore), 2016; 95: e5412

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

15. Blanco-Ayala T, Andérica-Romero AC, Pedraza-Chaverri J: New insights into antioxidant strategies against paraquat toxicity. Free Radic Res, 2014; 48: 623–40
16. Li X, Xiao Y, Cui Y et al: Cell membrane damage is involved in the impaired survival of bone marrow stem cells by oxidized low-density lipoprotein. J Cell Mol Med, 2014; 18: 2445–53
17. Gil HW, Seok SJ, Jeong DS et al: Plasma level of malondialdehyde in the case of acute qaraquat intoxicatin. Clin Toxicol (Phila), 2010; 48: 149–52
18. Gao F, Fish BL, Szabo A et al: Short-term treatment with a SOD/catalase mimetic, EUK-207, mitigates pneumonitis and fibrosis after single-dose total-body or whole-thoracic irradiation. Radiat Res, 2012; 178: 468–80
19. Pan J, Su Y, Hou X et al: Protective effect of recombinant protein SOD-TAT on radiation-induced lung injury in mice. Life Sci, 2012; 91: 89–93
20. Rosa SI, Rios-Santos F, Balogun SO, Martins DT: Vitexin reduces neutrophil migration to inflammatory focus by down-regulating pro-inflammatory mediators via inhibition of p38, ERK1/2 and JNK pathway. Phytomedicine, 2016; 23: 9–17
21. de Oliveira MR, de Souza IC, Fürstenau CR: Carnosic acid induces anti-inflammatory effects in paraquat-treated SH-SY5Y cells through a mechanism involving a crosstalk between the Nrf2/HO-1 axis and NF-κB. Mol Neurobiol, 2017; 12: 890–97
22. Toygar M, Aydin I, Agilli M et al: The relation between oxidative stress, inflammation, and neopterin in the paraquat-induced lung toxicity. Hum Exp Toxicol, 2015; 34: 198–204
23. Hai Y, Zhou X, Dai Q et al: Hydrogen rich saline ameliorates lung injury associated with cecal ligation and puncture induced sepsis in rats. Exp Mol Pathol, 2015; 98: 268–76
24. Calió ML, Marinho DS, Ko GM et al: Transplantation of bone marrow mesenchymal stem cells decreases oxidative stress, apoptosis, and hippocampal damage in brain of a spontaneous stroke model. Free Radic Biol Med, 2014; 70: 141–54
25. Gold R, Kappos L, Arnold DL et al., DEFINE Study Investigators: Placebo-controlled phase 3 study of oral BG-12 for relapsing multiple sclerosis. N Engl J Med, 2012; 367: 1098–107
26. Kobayashi M, Yamamoto M: Nrf2-Keapl regulation of cellular defense mechanisms against electrophiles and reactive oxygen species. Adv Enzyme Regul, 2006; 46: 113–40
27. Hayes JD, Dinkova-Kostova AT: The Nrf2 regulatory network provides an interface between redox and intermediary metabolism. Trends Biochem Sci, 2014; 39: 199–218
28. Ma Q: Role of nrf2 in oxidative stress and toxicity. Annu Rev Pharmacol Toxicol, 2013; 53: 401–26
29. Mittal M, Siddiqui MR, Tran K et al: Reactive oxygen species in inflammation and tissue injury. Antioxid Redox Signal, 2014; 20: 1126–67
30. Tsai HL, Chang JW, Yang HW et al: Amelioration of paraquat-induced pulmonary injury by mesenchymal stem cells. Cell Transplant, 2013; 22: 1667–81
31. Yang H, Wen Y, Yu HY et al: Combined treatment with bone marrow mesenchymal stem cells and methylprednisolone in paraquat-induced acute lung injury. BMC Emerg Med. 2013; 13(Suppl. 1): S5
32. Yang H, Wen Y, Bin J et al: Protection of bone marrow mesenchymal stem cells from acute lung injury induced by paraquat poisoning. Clin Toxicol, 2011; 49: 298–302
33. Giudice A, Arra C, Turco MC: Review of molecular mechanisms involved in the activation of the Nrf2-ARE signaling pathway by chemopreventive agents. Methods Mol Biol, 2010; 647: 37–74
34. Yu J, Zheng C, Ren X et al: Intravenous administration of bone marrow mesenchymal stem cells benefits experimental autoimmune myasthenia gravis mice through an immunomodulatory action. Scand J Immunol, 2010; 72: 242–49
35. Hostettler KE, Gazdhar A, Khan P et al: Multipotent mesenchymal stem cells in lung fibrosis. PLoS One, 2017; 12: e0181946

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]