hAMSC-CM Attenuates Paraquat-Induced A549 Cell Damage by Reducing Endoplasmic Reticulum Stress and Oxidative Stress

Futuan Liao

Affiliated Hospital of Zunyi Medical University

Liming Gong

Affiliated Hospital of Zunyi Medical University

Lijing Jia

301 Military Hospital: Chinese PLA General Hospital

Jianhong Wang

Affiliated Hospital of Zunyi Medical University

Tongying Liu

Affiliated Hospital of Zunyi Medical University

Tingting Huang

Affiliated Hospital of Zunyi Medical University

Jing Liang

Affiliated Hospital of Zunyi Medical University

manhong zhou (✉ manahongzhou@126.com)

Affiliated Hospital of Zunyi Medical College <https://orcid.org/0000-0002-0824-1787>

Research

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Abstract

Acute paraquat (PQ) poisoning results in severe acute lung injury and pulmonary fibrosis, and there is no specific antidote; thus, the mortality rate of PQ poisoning is extremely high. The mechanism of poisoning may be associated with endoplasmic reticulum stress, oxidative stress damage and organ/tissue inflammation. Recent studies have reported that human amnion-derived mesenchymal stem cells (hAMSCs) secrete a variety of cytokines, and that hAMSC-conditioned medium (CM) has anti-inflammatory and immunomodulatory effects. The aim of the present study was to investigate whether hAMSC-CM exerts protective effects against PQ toxicity in A549 cells. The data demonstrated that the activity of A549 cells was decreased after 24 h of PQ exposure and that the cell viability of the hAMSC-CM intervention group was higher compared with the PQ-only group. hAMSC-CM intervention decreased cell damage, apoptosis rates, oxidative stress indexes, Bax/Bcl-2 ratios and CHOP expression levels in poisoned cells by CCK-8 experiment, apoptosis detection, ROS content detection, and Western blot analysis respectively. In conclusion, hAMSC-CM may attenuate the cell damage caused by PQ by reducing endoplasmic reticulum stress and oxidative stress.

Introduction

In total, 250,000-370,000 people die from pesticide poisoning worldwide each year, particularly in less developed countries and regions (1). Paraquat (1,1-dichloro-4,4-bipyridine; PQ), which has been produced since 1962, has become one of the most commonly used herbicides in developing countries due to its low price, good herbicidal effects and availability. PQ can enter the body through the digestive tract, skin, and respiratory tract resulting in poisoning. In clinical practice, oral intake is the most common route of exposure. Previous studies have confirmed that there is a specific polyamine transporter system (PTS) on the membranes of alveolar epithelial cells (AECs)(2). After acute PQ poisoning, the PTS present on the alveolar cell membrane can specifically and actively transport PQ from the blood into alveolar cells, thus causing PQ to concentrate in the lungs and subsequently damage AECs and other cells. Therefore, the lungs represent the primary target organs after PQ poisoning (3). Acute PQ poisoning is often serious, and there is still no specific detoxification drug for the treatment of PQ poisoning; thus, the mortality rate is high, and it is essential to further explore methods to treat PQ poisoning(4). Mesenchymal stem cells (MSCs) are a class of adult stem cells with strong abilities to proliferate and differentiate (5). Previous studies have demonstrated that bone marrow and cord blood-derived MSCs selectively undergo chemotaxis, colonize lung injury sites, repair damaged alveolar cells and tissues directly or via the factor-1/chemokine receptor CXCR4 matrix signaling pathway, exert paracrine effects and produce a variety of cytokines that exert protective effects against lung injury (6,7). Human amnion-derived mesenchymal stem cells (hAMSCs) exert similar effects and have the advantages of easy and non-invasive acquisition, relatively few ethical concerns, low immunogenicity and strong amplification ability. hAMSCs can directly repair cellular and tissue damage. These beneficial therapeutic effects may originate from the hAMSCs themselves, possibly due to the various soluble factors produced by hAMSCs. hAMSCs can exert various therapeutic effects by releasing a variety of cytokines (8-13). In the present study, the aim was to

investigate whether the soluble cytokines produced by hAMSCs and present in hAMSC-conditioned medium (hAMSC-CM) exert protective effects on human alveolar type II-like epithelial cells (A549 cells) against damage caused by acute PQ poisoning, and to perform preliminary studies to elucidate the underlying mechanisms.

Materials And Methods

Isolation and extraction of hAMSCs. Human fetal placentas were obtained from the Department of Obstetrics and Gynecology at the Affiliated Hospital of Zunyi Medical University (Zunyi, China). The present study was approved by the Ethics Committee of the Affiliated Hospital of Zunyi Medical University, and all volunteers signed informed consent form before participating. The human amniotic membrane is a thin film composed of a single layer of interconnected epithelial cells that contains human amnion epithelial stem cells (hAECs) and hAMSCs (14). After amniotic membrane separation, the tissue samples were washed with PBS three times and the tissue was shredded by mechanical methods. Next, 0.25% trypsin-EDTA was added to a final concentration of 0.05 mg/ml, and the tissues were digested by shaking at 37°C for 30 min before being filtered with a 300-mesh cell strainer. Then, the tissue in the strainer was transferred to a 50 ml centrifuge tube and digested with collagenase IV at 37°C for 2 h until tissue lysis was complete. The samples were then rinsed with PBS and filtered, and the filtrate was collected and centrifuged at 790 x g (2500 rpm) for 10 min at 4°C. The supernatant was discarded, and original hAMSC medium was added. The cells were mixed and seeded in 25 cm² cell culture flasks at a density of 1×10⁶ cells/ml for later use after cell counting.

Culture of cell lines and collection of hAMSC-CM. A549 human alveolar type II-like epithelial cells from Beina Chuanglian Biotechnology Co., Ltd (Beijing, China) and hAMSCs (between passages 3 and 5) were seeded at a density of 2×10⁶ cells/ml (determined with a fully automatic multifunction microplate reader; Thermo Fisher Scientific, Inc.) in an incubator (37°C, 5% CO₂) and cultured in DMEM/low-glucose medium (HyClone; GE Healthcare Life Sciences) containing 10% fetal bovine serum (FBS; Gemini Bio Products). The medium was changed once every two days. When the cells reached 80% confluence, they were subjected to 1:2 passaging. When the hAMSCs covered 80–90% of the culture flask, they were washed with PBS twice, and the medium was replaced with 5 ml of DMEM/low- glucose medium containing 10% FBS. After additional culture for 24 h at 37°C, the culture supernatant was aspirated, filtered through a 0.22 µm filter, dispensed into an EP tube and stored at -80°C.

Experimental protocol. A549 cells were cultured in 96-well plates, treated with PQ to establish the model (Sigma-Aldrich; Merck KGaA) and immediately treated with hAMSC-CM for 24 h before Cell Counting Kit-8 (CCK-8) detection. A549 cells with appropriate PQ and hAMSC-CM concentrations were selected for intervention experiments and were divided into the following groups: i) control group; ii) PQ group (exposed to PQ only); iii) PQ + CM group (treated first with PQ and then with hAMSC-CM); and iv) CM group (the hAMSC-CM control group). A549 cells were also cultured in 6-well plates, and apoptosis was measured by flow cytometry 24 h after treatment. Other A549 cells were cultured in a 25 cm² cell culture flask. At 24 h after intervention, the morphology of the cells was observed, and the levels of ROS were

detected. The expression levels of related proteins in the cells were detected by western blot analysis. The cytokine content in the supernatant was detected by ELISA.

CCK-8 experiment. A549 cells were cultured and then exposed to different concentrations of PQ solution (0, 250, 500, 750 and 1,000 μM) for 24 h at 37°C (6 replicate wells per group). Then, 10 μl of CCK-8 reagent (Beijing Soleil Technology Co., Ltd.) was added to each well, and the cells were incubated at 37°C for 2 h. The absorbance value at a wavelength of 450 nm was measured, and cell proliferation activity was calculated according to the following formula: cell proliferation activity (%) = (experimental group OD value - zero well OD value) / (control group OD value - zero well OD value). Analyses were conducted in the same manner after hAMSC-CM was administered at concentrations of 25, 50 and 75% volume fraction (ie, medium volume/total volume). The order of addition was medium, PQ solution and hAMSC-CM, and the cells were analyzed after 24 h of culture at 37°C.

Qualitative observation of intracellular ROS. 2,7-Dichlorofluorescein diacetate (DCFH-DA) itself has no fluorescence and is hydrolyzed into DCFH by esterase after entering cells. In the presence of active oxygen, DCFH is oxidized to form a substance with strong green fluorescence, DCF, that cannot penetrate the cell membrane (15). The fluorescence of DCF has an excitation wavelength of 502 nm, the emission peaks near 530 nm and the intensity is proportional to the level of intracellular ROS. Reactive oxygen species Assay Kit was obtained from Nanjing Jiancheng Bioengineering Institute. (catalog number: E004-1-1 and was used according to the manufacturer's protocol. After 24 h of culture at 37°C, the cells in each group were washed once with PBS. Next, 40 μM DCFH-DA dye was added to each group, and the mixture was incubated for 30 min at 37°C. The development of fluorescence in the cells of each group was observed under a green light source with a fluorescence microscope (Olympus Corporation), 100 $\times$.

Observation of cell morphology. After cells were cultured for 24 h, cell morphology was observed under an inverted phase-contrast microscope (OLYMPUS, 200 $\times$). The cells in each group were washed twice with PBS, digested and centrifuged (2,200 $\times$ g; 5 min) at 25°C. The supernatant was discarded. The cells in the pellets were fixed with 1% citric acid for 24h at 4°C, dehydrated with different concentrations of ethanol and acetone, soaked, embedded, trimmed, sectioned and stained (stains: Uranyl Acetate and Lead Citrate were purchased from HEAD (BeiJing) Biotechnology Co. Ltd). A transmission electron microscope (Zeiss, Germany, bar = 2 μm) was used to observe changes in tissue structure.

Detection of oxidation index values and inflammatory factor levels in cells and their supernatants.

Samples were prepared according to the corresponding kit instructions. The activity of superoxide dismutase (SOD) in A549 cells was determined using the xanthine oxidase method (Superoxide Dismutase assay kit, Nanjing Jiancheng Bioengineering Institute). The MDA content in A549 cells was detected by the thiobarbituric acid method (Cell Malondialdehyde assay kit, Nanjing Jiancheng Bioengineering Institute). The levels of interleukin (IL)-1 β , IL-6 and IL-10 in the supernatants of A549 cells were detected by ELISA (Wuhan Sanying Biotechnology). Blank control wells and standard wells were included in the experiment. The absorbance was measured at a wavelength of 450 nm to prepare a standard curve, and the concentrations of the samples to be tested were calculated.

Apoptosis detection with Annexin V-FITC/propidium iodide (PI). Annexin V-FITC (TransDuetce Annexin V-FITC/PI Cell Apoptosis Detection Kit) was purchased from TransGen Biotech (), and it can specifically bind to phosphatidylserine, which translocates to the membrane during apoptosis, and emit green fluorescence under excitation by blue light to enable apoptotic cells and normal cells to be distinguished. PI is a nucleic acid dye that cannot penetrate intact cell membranes; however, it can penetrate damaged cell membranes (including those of late apoptotic and dead cells) and stain the nuclei red. The signals of these two labels can be compared to distinguish between early and late apoptotic cells. After 24 h of culture, cells (density 2×10^6) were digested with 0.25% trypsin without EDTA for 1 min and then collected, washed twice with cold PBS, and resuspended in binding buffer. Annexin V-FITC and PI were added to the cell suspensions, and the solutions were mixed and protected from light for 5 min. Flow cytometry (BD, FACSCanto™ II Flow Cytometry System) was performed within 1 h. BD FACSDiva Software (v8.0.2) was used for analysis. Quantification of the respective flow plots. Q1, Q2, Q3 and Q4 represent necrotic cells, late apoptotic cells, early apoptotic cells and normal cells, respectively.

Western blot detection of related protein expression. A549 cells of each group were collected, appropriate amounts of lysis buffer were added, and the mixtures were centrifuged at 4°C ($39,588 \times g$, 10 min; Beckman Coulter, Inc.). The supernatants were collected, and the protein concentrations were determined by the BCA method. Loading buffer was then added, and the proteins were denatured at 100°C for 5 min. A total of 20 µg of each protein sample was added to each lane for SDS-PAGE (separation gel concentration 12% and concentrated gel 5%). After electrophoresis, the proteins were transferred to a PVDF microporous membrane at 200 mA for 70 min and blocked with 3% BSA (Beijing Solarbio Science & Technology Co., Ltd) in TBST (5% of Tween 20) blocking solution for 2 h at room temperature. Then, the membrane was incubated with diluted primary antibodies, including a b-actin monoclonal antibody (cat. no. 66009-1-Ig; dilution 1:20,000), a Bcl-2-related X protein (Bax) polyclonal antibody (cat. no. 50599-2-Ig; dilution 1:7,000), a Bcl-2 polyclonal antibody (cat. no. 12789-1-AP, dilutions 1:3,000), and a CHOP polyclonal antibody (cat. no. 15204-1-AP, dilution 1:1,000) (Wuhan Sanying Biology Technology Co., Ltd.), overnight at 4°C and washed three times with TBST (15 min each time). The membrane was then incubated with secondary antibodies, including HRP-conjugated Affinipure goat anti-rabbit IgG (H + L) (dilution 1:5,000; Wuhan Sanying Biology Technology Co., Ltd.) and HRP-conjugated Affinipure goat anti-mouse IgG (H + L) (dilution 1:5,000; Wuhan Sanying Biology Technology Co., Ltd.) for 2 h at room temperature before being washed three times with TBST. A mixture of luminescent liquids A and B was evenly dropped onto the membrane ECL ENhanced Kit, Beijing Ding Biotechnology Co., Ltd., which was then placed in a Bio-Rad gel imaging system for imaging. The Western blot results were analyzed by grayscale scanning (Bio-Rad Laboratories, Inc.) using ImageJ software (National Institutes of Health v5.2).

Statistical analysis. Experimental and measurement data are expressed as the mean $\pm$ standard deviation ($\bar{x} \pm s$). SPSS 18.0 statistical software (SPSS, Inc.) was used for statistical analysis. One-way ANOVA was conducted among groups, the post hoc test used following one way ANOVA was Tukey's HSD, and $P < 0.05$ was considered to indicate statistical significance.

Results

hAMSC-CM alleviates the cytotoxicity induced by PQ. In order to identify the dose of PQ, A549 cells were exposed to PQ at concentrations of 250, 500, 750 and 1,000 μM for 24 h and cell proliferation activity was decreased (Fig. 1A). In addition, decreased cell viability was observed after PQ exposure for more than 24 h. The half-maximal inhibitory concentration (IC_{50}) of PQ was calculated to be 816 μM , which was closest to 750 μM ; therefore, 750 μM PQ was selected as the modeling concentration for subsequent experiments.

The hAMSC-CM group was subdivided into groups treated with volume fractions of 25, 50 and 75% hAMSC-CM. The concentration of CCK-8 reagent was detected after 24 h of exposure or intervention in order to evaluate the toxicity of PQ to A549 cells. There were no differences in cell proliferation activity between the hAMSC-CM groups (with different concentrations of hAMSC-CM) and the control group. Cell proliferation was detected after the addition of different concentrations of hAMSC-CM. The proliferation of cells in the PQ + CM group was higher than that in the PQ group at all concentrations. Among the different concentrations of hAMSC-CM, the 50% volume fraction resulted in the highest proliferation rate of A549 cells (Fig. 1B).

hAMSC-CM alleviates PQ-induced oxidative stress and increases in inflammatory mediator levels hAMSC-CM reduces the production of ROS in poisoned cells. Intracellular ROS content was measured with a DCFH-DA probe. The green fluorescence and ROS content of the control group and the CM group were lower than those of the PQ group and the PQ + CM group; however, the PQ + CM group had less fluorescence and a lower ROS content than the PQ group. At 24 h after exposure to PQ at 750 μM or intervention (with the 50% concentration of hAMSC-CM), DCFH-DA was added, and fluorescence microscopy was used to observe green fluorescence (Fig. 2A).

hAMSC-CM alleviates tissue damage in poisoned cells. The morphology of the cells was observed under an inverted microscope. The cells in the control group and the CM group were uniform under the inverted microscope, showing cobblestone-like morphology, good adherence and tight connections. After 24 h of PQ exposure, the morphology, connections and adherence of the cells were altered; the cells were not tightly connected, the number of dead cells was increased and the amount of cell debris at the bottom of the culture flask was increased. The PQ + CM group had a greater cell density, more adherent cells and fewer dead cells than the PQ group (Fig. 2B).

The ultrastructural morphology of the cells in the control group and the CM group was observed via transmission electron microscopy. The cell size was uniform, the cell membranes were intact, microvilli were observed on the cell surface, the cytoplasm was uniform, various organelles and the nuclear membrane were intact. After PQ exposure, the numbers of microvilli and protrusions on the surfaces of A549 cells were reduced, and cell membrane budding, apoptotic bodies, swelling of the endoplasmic reticulum, increased number of autophagosomes, decreased numbers of organelles (such as mitochondria), abnormal nuclear chromatin distribution, and damage to organelles (such as the

endoplasmic reticulum) were all observed in the PQ + CM group. The changes were milder in the PQ + CM group than in the PQ group (Fig. 2C).

hAMSC-CM reduces PQ-induced cellular oxidation. The MDA content in each group was detected with an MDA kit. The MDA content in the PQ group was higher than that in the control group, while the MDA content in the PQ + CM group was lower than that in the PQ group (Fig. 2D). The SOD content in each group was also detected. The SOD content in the PQ group was lower than that in the control group, while the SOD content in the PQ + CM group was significantly higher than that in the PQ group (Fig. 2E). There were no significant differences in the levels of MDA and SOD between the CM group and the control group.

hAMSC-CM reduces PQ-induced increases in inflammatory cytokine levels. The levels of certain ILs in the supernatant in each group was detected using ELISA kits. The levels of IL-1 β and IL-6 in the supernatant were significantly higher in the PQ group compared with the control group, while the levels of these cytokines in the PQ + CM group were significantly lower than those in the PQ group (Fig. 2F and G). The level of IL-10 in the supernatant of the control group was used as the baseline value. Compared with the control group, the other three groups exhibited significantly increased IL-10 levels, and the levels in the CM group were the highest. In addition, the IL-10 levels in the PQ + CM group were significantly higher compared with those in the PQ group (Fig. 2H).

hAMSC-CM reduces PQ-induced increases in apoptosis rates. Flow cytometry was used to detect apoptosis in A549 cells. After exposure (to PQ at 750 μ M) and intervention (with 50% hAMSC-CM), apoptosis in each group was detected with an Annexin V-FITC/PI apoptosis kit. The apoptotic rate of the PQ group was higher than those of the other groups. The apoptosis rates of the control, PQ, PQ + CM and CM groups were $14.44 \pm 0.86\%$, $49.39 \pm 0.81\%$, $22.65 \pm 0.95\%$ and $14.49 \pm 1.46\%$, respectively. The early + late apoptotic rate of the PQ + CM group was lower than that of the PQ group (Fig. 3A and B).

The expression levels of proteins in apoptosis-related and endoplasmic reticulum-related pathways were detected by western blot analysis. The results showed that the Bax/Bcl-2 ratio in the PQ group was significantly higher compared with in the control group, while the ratio in the PQ + CM group was significantly lower than that in the PQ group. There was no significant difference between the PQ + CM group and the control group (Fig. 3C and D).

hAMSC-CM and PQ affects the protein expression level of CHOP in cells. After exposure to PQ at 750 μ M, the protein expression of CHOP in the PQ group was increased compared with the control group, as determined by western blot analysis. The concentration of CHOP protein in the PQ group was 50% higher than that in the control group. Following hAMSC-CM treatment, the expression of CHOP protein in the PQ + CM group was significantly decreased compared with the PQ group ($P < 0.05$) (Fig. 4A and B).

Discussion

Under normal circumstances, ROS produced in the body can be quickly removed by antioxidant defense system components, such as SOD, without causing physiological damage. If ROS cannot be removed in time, they cause oxidative stress damage, resulting in cell membrane phospholipid peroxidation and inducing apoptosis. The mechanism of PQ poisoning has not yet been fully elucidated, but numerous studies have confirmed that PQ undergoes a series of redox reactions in the body to form superoxide anions, which results in the accumulation of ROS and can directly cause cell damage (16, 17).

In the present study, upon observing the distribution of ROS in A549 cells, it was revealed that PQ increased ROS production in cells, resulting in cellular damage and decreased cell viability. hAMSC-CM itself did not damage A549 cells or promote cell proliferation. However, hAMSC-CM reduced the production of ROS in PQ-treated cells and alleviated PQ-induced cell damage. In addition, detection of the intermediate metabolites MDA and SOD, combined with microscopic observation of cell structure changes, revealed that hAMSC-CM reduced MDA content, increased SOD content, enhanced antioxidant capacity and alleviated cell damage caused by superoxide anions.

MSCs have certain immunosuppressive properties that can serve key roles in inflammation-related diseases. In the current experiment, IL levels were examined in A549 cell culture supernatant to evaluate the release of inflammatory cytokines, including anti- or Pro-inflammatory, after cells were exposed to PQ. It was previously revealed that hAMSC-CM contained a certain amount of IL-10, an anti-inflammatory cytokine with strong immunomodulatory activity that can inhibit inflammatory reactions. In the present study, hAMSC-CM downregulated the levels of proinflammatory factors, such as IL-1 β and IL-6, and upregulated IL-10 levels, which may explain the reduction in inflammation associated with hAMSC-CM treatment (18, 19). It has been confirmed that Human Placental Mesenchymal Stem Cells (also termed PMSCs) can induce macrophages to switch from pro-inflammatory M1 cells (which secrete proinflammatory cytokines such as IL-1 β and IL-6) into an anti-inflammatory M2 phenotype (which secretes anti-inflammatory cytokines such as IL-10). This may explain further the fact that IL-10 was upregulated, and the other two cytokines were downregulated under hAMSC-CM conditions (20).

Apoptosis, also known as programmed cell death, is primarily responsible for maintaining homeostasis by clearing abnormal or damaged cells (21). In the present study, the apoptosis rates of A549 cells were significantly increased after PQ exposure, and endoplasmic reticulum stress (ERS) and injury were significantly enhanced, suggesting that PQ exposure caused persistent or excessive ERS in cells, which resultantly induced apoptosis. Increased ERS may have been the cause of apoptosis in the A549 cells. Abnormal accumulation of apoptotic cells increases the release of inflammatory mediators, and causes a persistent inflammatory response, which in turn further enhances apoptosis and results (22). Intravenous injection of hAMSC-CM after PQ poisoning may reduce the production of inflammatory and damaging substances in the circulation, interrupting this cycle and ultimately reducing apoptosis. In addition, the present study revealed that hAMSC-CM intervention decreased the expression levels of Bax within cells while increasing the expression of the anti-apoptotic protein Bcl-2.

CHOP is a member of the CCAAT/enhancer binding protein (C/EBP) transcription factor family, which inhibits the transcriptional activity of C/EBP and is a target for other selective enhancers (23). CHOP regulates apoptosis by activating pro-apoptotic genes and downregulating anti-apoptotic genes, such as the Bcl-2 gene (24). Detection of the expression of CHOP protein in ERS-related pathways revealed that PQ exposure increased CHOP protein expression, while hAMSC-CM treatment decreased CHOP protein expression in poisoned cells, confirming that hAMSC-CM attenuates increases in ERS within PQ-exposed A549 cells. ERS can also cause apoptosis via a cell death mechanism independent of the unfolded protein response. For example, excessive ERS induces release of calcium into the cytosol, thus causing apoptosis via Bax and Bcl-2 (25).

The present in vitro cell experiment revealed that PQ increases the production of ROS, which results in the release of inflammatory factors, induces oxidative stress and aggravates cell damage. hAMSC-CM alleviates the apoptosis induced by PQ and serves a protective role. However, the other underlying mechanisms and associated pathways still need to be further explored. hAMSC-CM has anti-inflammatory and immunomodulatory effects and can attenuate PQ-induced A549 cell injury by reducing oxidative stress levels and ERS levels.

Declarations

Acknowledgments

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

FL and LG performed the CCK-8 experiment, apoptosis detection, ROS content detection, and Western blot analysis experiments, analyzed the data and wrote the manuscript. LJ analyzed information of transmission electron microscope pictures. LW, HT and MF performed ELISA testing experiments, and analyzed the data. MZ, XL, YW and MW analyzed the data and modified of the manuscript. MZ received funding for the project, conceived the study and performed the experiments. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The experiments in this study were approved by the Experiment Ethics Committee of Zunyi Medical University (2020-1-252).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

PQ reduces the proliferation of A549 cells and hAMSC-CM attenuates this reduction. (A) Comparison of the effects of exposure to different concentrations of PQ (250, 500, 750 and 1,000 μM) and the control condition (0 μM) on cell viability using CCK-8. ** $P < 0.01$ vs. control group. (B) Cell viability after treatment with PQ and various concentrations of hAMSC-CM was determined using CCK-8. $n = 6$. * $P < 0.05$; ** $P < 0.01$ as indicated. PQ, paraquat; hAMSC, human amnion-derived mesenchymal stem cells; CM, conditioned medium; CCK-8, Cell Counting Kit-8.

Figure 2

hAMSC-CM reduces PQ-induced oxidative stress and alleviates increases in inflammatory mediator levels in A549 cells. * $P < 0.05$; ** $P < 0.01$ as indicated. PQ, paraquat; hAMSC, human amnion-derived mesenchymal stem cells; CM, conditioned medium; SOD, superoxide dismutase; Nu, nucleus; cyto, cytoplasm.

Figure 3

hAMSC-CM reduces PQ-induced apoptosis in A549 cells. * $P < 0.05$; ** $P < 0.01$ as indicated. PQ, paraquat; hAMSC, human amnion-derived mesenchymal stem cells; CM, conditioned medium.

Figure 4

Western blot analysis of intracellular CHOP protein expression. * $P < 0.05$; ** $P < 0.01$ as indicated. PQ, paraquat; hAMSC, human amnion-derived mesenchymal stem cells; CM, conditioned medium.