Effect of human umbilical cord mesenchymal stem cell exosomes on aerobic metabolism of human retinal pigment epithelial cells

Lian Liu · Chunlan Liang · Wei Fan · Jingxiang Zhong

Received: 23 November 2021 / Accepted: 11 September 2022 / Published online: 5 October 2022
© The Author(s), under exclusive licence to Springer Nature B.V. 2022

Abstract
Purpose To investigate the effect of exosomes secreted by human umbilical cord mesenchymal stem cells (HUCMSC-Exo) on aerobic metabolism of cobalt chloride (CoCl₂)-induced oxidative damage in the human retinal pigment epithelial cell line (ARPE-19), and to explore the protective mechanism of HUCMSC-Exo on oxidative damage in ARPE-19 cells.

Methods HUCMSC-Exo were extracted and identified; CCK-8 assay was used to establish the oxidative damage mode of ARPE-19 cells induced by CoCl₂; JC-1 flow cytometry was used to detect the effects of exosomes with different concentrations (0, 25, 50, or 100 μg/mL) on the mitochondrial membrane potential (MMP) of oxidatively damaged ARPE-19 cells. The effects of exosomes with different concentrations on the activity of oxidative metabolic enzymes (oxidative respiratory chain complexes I, III, IV, and V) and ATP synthesis in oxidatively damaged ARPE-19 cells were detected by spectrophotometry.

Results Under transmission electron microscope, HUCMSC-Exo were round or oval membrane vesicles with diameters of about 40–100 nm. Western blot results showed that HUCMSC-Exo expressed specific marker proteins CD63 and CD81. CCK-8 dates showed that the cell viability of ARPE-19 cells was significantly decreased with increasing CoCl₂ concentration, and the concentration of 400 μmol/L CoCl₂ was chosen to be the optimal concentration for oxidative damage. MMP was increased in exosomes intervention group (25, 50 or 100 μg/mL), and the dates were statistically different from 0 μg/mL exosome intervention group (P < 0.05). The activities of mitochondrial complexes I, IV, and V in exosomes intervention groups (100 μg/mL) were higher than those in 0 μg/mL exosome intervention group. In 50 μg/mL and 100 μg/mL exosome intervention group, ATP synthesis was significantly different from the 0 μg/mL exosome intervention group (P < 0.05).

Conclusion HUCMSC-Exo had a certain protective effect on ARPE-19 cells induced by CoCl₂ in vitro. The protective mechanism of HUCMSC-Exo on oxidative damage ARPE-19 cells might be through saving its aerobic metabolic function, restoring cell ATP synthesis, and improving the ability of cells to repair damage and deal with the hypoxic environment.
**Introduction**

Retina is one of the tissues with high oxygen demand. The balance between oxygen supply and oxygen consumption can maintain retinal homeostasis. Once this balance is destroyed, it will lead to many retinal diseases [1]. Oxidative stress produced by this large amount of reactive oxygen species (ROS) is associated with the pathogenesis of various eye diseases, such as glaucoma, diabetic retinopathy (DR), age-related macular degeneration (AMD), and the retinopathies [2]. For a variety of reasons, the retina is particularly vulnerable to oxidative damage [3]. Oxidative stress may be the key factor leading to retinal pigment epithelium (RPE) dysfunction-related retinal diseases [4]. Current cellular strategies for protection against oxidative damage include antioxidants, molecular repair (removal or repair of oxidation-modified biomolecules to counteract functional effects), and cell replacement (using stem cells or progenitor cell populations) [5].

Exosomes have attracted the attention of researchers due to their powerful biological functions, such as tissue repair, inhibition of inflammation, and regulation of immunity. Exosomes are vesicles with the lipid bilayer structure of about 40–100 nm in diameter [6]. It is secreted by a variety of cell lines and cell types, including tumor cell lines, stem cells, and neurons. Human umbilical cord mesenchymal stem cells (HUCMSCs) are a kind of multifunctional stem cells that exist in neonatal umbilical tissue. Compared with other sources of mesenchymal stem cells, such as bone marrow and adipose tissue, HUCMSCs have obvious advantages because of low cost, are easy obtained, non-invasive procedure to the donors, and representing the noncontroversial source of mesenchymal stem cells [7]. HUCMSCs have a stronger capacity of expansion than bone marrow mesenchymal stem cells (BMSCs) [8].

Because the mesenchymal stem cells (MSCs) and their exosomes (MSCs-Exo) had a similar function [9], this study used HUCMSC-Exo to explore the protection mechanism against oxidative damage in ARPE-19 cells.

**Materials and methods**

**HUCMSCs-Exo purification and identification**

Briefly, HUCMSCs (Salial, Guangzhou, China) were cultured in a 5% CO₂ incubator (Thermo Fisher, Shanghai, China) at 37 °C in DMEM/F12 (Gibco, Guangzhou, China) medium containing 10% fetal bovine serum (FBS)(Gibco, Australia) and 1% penicillin–streptomycin solution (Gibco, Guangzhou, China). 48 h before extraction of the 4–10 generation hUCMSCs with good growth, the original medium was discarded and replaced with DMEM/F12 medium containing 10% exosome-free FBS (SBI, Guangzhou, China) and 1% penicillin–streptomycin solution. After 48 h of cell culture, the culture medium was collected and put into the 15-ml centrifuge tube. The cells and cell fragments were removed by 1000 rpm centrifugation for 10 min at 4 °C. The supernatant was prepared and filtered to the ultrafilter tube through a 0.22-μm aseptic membrane. The exosome concentration was collected by centrifugation at 4000 rpm for 8–10 min at 4 °C. After adding exosome extraction reagent ExoQuick-TC (SBI, Guangzhou, China), the mixture was taken out after standing for at least 12 h and centrifuged at 10000 rpm for 30–40 min at 4 °C. The bottom precipitate was collected, resuspended with 100-500 μl phosphate buffer saline (PBS) (Gibco, Guangzhou, China), and stored in the -80°C ultra-low temperature refrigerator for later use.

**Characterization of HUCMSCs-Exo**

The exosome stored in -80°C was quickly moved into a 37 °C thermostat water bath for about 1 min, and the freezing tube was gently shaken until it melted completely. The exosome suspension was prepared by diluting the exosome samples with PBS buffer at 1:10–1:20. The copper mesh was dipped in a small amount of diluted exosome sample and was placed in a 3% phosphotungstic acid solution for negative staining for about 5 min. The excess dye solution was removed with a filter paper. The morphological characteristics of exosomes were detected with transmission electron microscopy (TEM, JEOL 2100F, Beijing, China).
Quantification and detection of exosomes protein

The exosomes were lysed with RIPA lysis buffer (Solarbio, Beijing, China) containing protease inhibitors, and A562 was determined by Microplate Reader (Bio-Tek, Guangzhou, China). The protein concentration of the exosomes was calculated according to the standard curve. Western blot was used to detect the expression of CD63 (proteintech, Wuhan, China) and CD81 (proteintech, Wuhan, China) in the exosome after quantification.

Establishment of oxidative damage model of ARPE-19 cells

ARPE-19 cells (ATCC, Beijing, China) in the logarithmic growth phase were digested and centrifuged to form a single-cell suspension and inoculated in 96-well plates. After the cells were adherent overnight, the original medium was absorbed and replaced with the medium containing different concentrations (0, 50, 100, 200, 400, 800) μmol/L of CoCl₂ (Sigma, Guangzhou, China). Each group was replicated 5 times and incubated at 37 °C in 5% CO₂ incubator for 24 h. Each well was incubated with 10 μl CCK-8 (MCE, Guangzhou, China) for 30 min at 37 °C in 5% CO₂ incubator. A450 was determined by Microplate Reader. The survival rate of ARPE-19 cells was calculated, and the concentration of 50% cell survival rate was used as the optimum concentration of (CoCl₂)-induced oxidative damage of ARPE-19 cells.

Measurement of MMP

The cells were divided into six groups: normal group; control group/ non-intervention group (CoCl₂-damaged cells were cultured for 24 h without changing the original medium); in all exosome intervention groups, the original medium was replaced after 24 h of CoCl₂-damage, then exosomes were added at the concentration of either 0, 25, 50, or 100 μg/mL, respectively, and cells were cultured for another 24 h. The adherent ARPE-19 cells were digested by 0.25% trypsin–EDTA (Gibco, Guangzhou, China) and suspended by 0.5 ml cell culture medium. 5.5',6.6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolcarbocyanine iodide (JC-1) (Solarbio, Beijing, China) solution of 0.5 ml was added and mixed, and then, cells were incubated at 37 °C for 20 min away from the light. Then, cells were treated with JC-1 staining buffer and analyzed by flow cytometry (BD, Shanghai, China) [10].

Activity detection of oxidative respiratory chains complex

The activity of I, III, IV, and V (I: NADH dehydrogenase, III: Cytochrome C reductase, IV: Cytochrome C oxidase, V: F1F0-ATP synthase) the mitochondrial respiratory chains complex was determined by spectrophotometry with reference to the method of Vyatлина et al. [11] 10~20 μg of mitochondrial protein was added into the buffer solution with a final volume of 2 mL, and distilled water was used as a blank tube to correct the absorbance to 0 point. The changes of absorbance values at wavelength of 340 nm and 550 nm for 3 min were measured, respectively. The unit of enzyme activity was nmol/min/10⁴ cell.

Detection of ATP synthesis quantity

**Cellular extraction of ATP**

First, the cells were collected into the centrifuge tube, the supernatant was excluded, and 1 ml of the acid extract was added into the 5 million cells at a ratio of 500~1000:1. The cells were crushed by ultrasonic for 1 min (ice bath, intensity 20% or 200 W, ultrasonic 2S stopped for 1 s). The cells were centrifuged at 8000 g at 4 °C for 10 min. The supernatant was integrated into another centrifuge tube, and an equal volume of alkaline extract (Solarbio, Beijing, China) was added to neutralize and mix. The supernatant was centrifuged at 8000 g at 4 °C for 10 min; then, the supernatant was taken and placed on ice to be measured.

**ATP synthesis assay**

Preheat spectrophotometer for more than 30 min; adjust the wavelength to 700 nm and distilled water zero. One blank tube and one standard tube are made, respectively, and one pair of care is set for each measuring tube. Add relevant reagents in each tubes according to the instructions (Table1). Preparation of chromogenic agent: according to the volume of proposed chromogenic agent (sample number *0.87 ml) (Solarbio, Beijing, China), reagent 4(ml) (Solarbio,
Beijing, China): reagent 5 (ml) (Solarbio, Beijing, China) = 1:5 should be prepared before use. Sample determination: The mixture was thoroughly mixed and was incubated in water bath at 37°C for 20 min. After the water bath at 37°C for 20 min, the absorbance value of each tube was measured at 700 nm. Calculation of ATP content (μmol/10^4 cell) = [S*(M-C)/(S-B)*V1]/(500*V1/V2) = 0.004*(M-C)/(S-B).

Standard liquid concentration, 1 𝝀mol/mL; V1: solution in the reaction system, 0.03 mL; V2: extraction liquid volume, 2 mL; V3: serum (slurry) volume, 0.1 ml.

**Statistical analysis**

The statistical software SPSS 19.0 (IBM, Armonk, NY, USA) and GraphPad Prism 8.0 (GraphPad Software, California, USA) was used to analyze data. The results are represented as mean ± SD. For comparison of different groups and evaluation multiple comparisons, statistical comparisons were performed by one-way ANOVA and Tukey. In these analyses, P < 0.05 was considered statistically significant.

**Results**

Characteristics of exosomes

Exosomes were extracted by ExoQuick-TC, and TEM demonstrated that HUCMScs-Exo were round or oval membranous vesicles with diameters between 40 ~ 100 nm (Fig. 1A-D), which was consistent with the basic morphology of exosomes. Western blot was used to detect the expression of CD63 and CD81 in HUCMScs-Exo (Fig. 1E).

Optimal damage concentration of CoCl₂ in ARPE-19 cell model

Cells were treated with six different concentrations (normal/ 0 μg/mL, 50 μg/mL, 100 μg/mL, 200 μg/mL, 400 μg/mL, and 800 μg/mL) of CoCl₂ for 24 h and then incubated with 10μL CCK8 for 30 min. The survival rate of ARPE-19 cells was (80.94 ± 6.87)%, (87.37 ± 2.81)%, (93.38 ± 4.28)%, (80.26 ± 4.67)%, (50.71 ± 2.33)% and (25.06 ± 3.75)%.

As shown in Fig. 2, the cell viability of ARPE-19 cells was significantly decreased with increasing CoCl₂ concentration compared with the normal group. The survival rate of ARPE-19 cells treated with 400 μg/mL and 800 μg/mL CoCl₂ concentrations was statistically significant reduced compared with the normal group (P < 0.01). When the CoCl₂ concentration is 400 μmol/L, the survival rate of ARPE-19 cells is 50%, which was chosen to be the optimal concentration for oxidative damage.

Effect of HUCMScs-Exo on the morphology of ARPE-19 cells injured by CoCl₂

On observation under an inverted microscope, normal ARPE-19 cells were fusiform or polygonal monolayer adherent cells with a clear outline. The cytoplasm might contain pigment, namely lipofuscin, which was brown and mostly located in the inner side of the cell (Fig. 3A). When 400 μg/mL CoCl₂ was added, the number of ARPE-19 cells decreased, the space widened, the arrangement was disordered, and the cells shrank and the nuclei aggregated (Fig. 3B-C). After treated with exosomes (25 μg/mL, 50 μg/mL and 100 μg/mL), respectively, ARPE-19 cells morphology was similar to the normal group, with clear cell boundaries and increased cell numbers (Fig. 3D-F). The cell morphology of the 50 μg/mL and 100 μg/mL exosome intervention groups was similar to that of the normal group, with clear cell boundaries and increased cell number.

| Table 1 ATP synthesis assay |
|-----------------------------|
| Tube | Measuring tube, M | Control tube, C | Standard tube, S | Blank tube, B |
| ATP sample(μL) | 30 | 30 | / | / |
| Standard liquid(μL) | / | / | 30 | 30 |
| Reagent 1(μL) | 60 | / | 60 | / |
| Reagent 2(μL) | 30 | 30 | 30 | 30 |
| Reagent 3(μL) | 10 | / | 10 | / |
| Distilled water(μL) | / | 70 | / | 70 |
Effect of HUCMSCs-Exo on the MMP of ARPE-19 cells injured by CoCl₂

MMP is an index to evaluate mitochondrial function, and the decrease in MMP potential is one of the early markers of apoptosis. We used flow cytometry to detect cellular MMP, and the results showed that proportion of MMP in normal group, control group, and exosomes intervention group (0, 25, 50 or 100 μg/mL) were (92.9 ± 0.56)%,(74.3 ± 0.45)%, (79.65 ± 0.31)%, (83.7 ± 0.21)%, and (79.65 ± 0.25)%. Compared with normal group (92.9 ± 0.56)%, there was statistically significant difference (\(P < 0.05\)) in 0 μg/mL exosome intervention group (74.3 ± 0.45)% (Fig. 4). These results indicate that CoCl₂ may initiate the mitochondrial apoptotic pathway in ARPE-19 cells. However, MMP was increased in exosomes intervention group (25, 50 or 100 μg/mL).
Fig. 2 Cobalt chloride (CoCl₂) reduces the survival of ARPE-19 cells. **Statistically significant difference as compared with normal group ($P < 0.01$)

Fig. 3 Effect of HUCMSCs-Exo on CoCl₂-induced ARPE-19 cell morphology ($400\times$). A: normal ARPE-19 B: 400 µg/mL CoCl₂ damaged ARPE-19 C: ARPE-19 treated with 0 µg/mL HUCMSCs-Exo D: ARPE-19 treated with 25 µg/mL HUCMSCs-Exo E: ARPE-19 treated with 50 µg/mL HUCMSCs-Exo F: ARPE-19 treated with 100 µg/mL HUCMSCs-Exo
100 μg/mL) and the dates were statistically different from 0 μg/mL exosome intervention group (P < 0.05).

Effect of HUCMSCs-Exo on the activity of oxidative respiratory chains complex in ARPE-19 cells injured by CoCl₂

Respiratory chain complex I, namely NADH-Co Q reductase or NADH dehydrogenase, is the main part of O²⁻⁻⁻ generated in the respiratory electron transport chain. Its activity reflects the state of respiratory electron transport chain and reactive oxygen species (ROS) production.

Based on statistical analysis (Fig. 5A), the enzyme activities of respiratory chain complex I in normal group, control group, and exosomes intervention group (0, 25, 50 or 100 μg/mL) were (0.0183 ± 0.0037, 0.0548 ± 0.0037, 0.0150 ± 0.0030, 0.0183 ± 0.0037, 0.0256 ± 0.0037, 0.0657 ± 0.0001) nmol/min/10^4 cell, respectively. The activities of respiratory chain complex I in 100 μg/mL exosome intervention group were (0.0657 ± 0.0001) nmol/min/10^4 cell, which had statistical difference with the normal groups and 0 μg/mL exosomes intervention group (P < 0.001).

Respiratory chain complex III, namely CoQ-cytochrome C reductase, is a common component of the main circuit and branch of mitochondrial respiratory electron transport chain. The activities of respiratory chain complex III in normal group, control group, and exosomes intervention group (0, 25, 50, or 100 μg/mL) were (0.0620 ± 0.0024, 0.0248 ± 0.0002, 0.0250 ± 0.0040, 0.0149 ± 0.0025, 0.0248 ± 0.0008, 0.0248 ± 0.0007) nmol/min/10^4 cell, respectively (Fig. 5B). Activities of respiratory chain complex III were straightly decreased in control group and 0 exosomes μg/mL intervention group, which had significant difference with the normal group (P < 0.001). However, there were no significant differences between normal group and exosomes intervention group (P > 0.05).

Respiratory chain complex IV (cytochrome C oxidase) is a common component of the main and branch pathways of the mitochondrial respiratory electron transport chain. Activities of respiratory chain complex IV in normal group, control group, and exosomes intervention group (0, 25, 50, or 100 μg/mL) were (0.0089 ± 0.0001, 0.0044 ± 0.0002, 0.0044 ± 0.0002, 0.0044 ± 0.0002, 0.0044 ± 0.0002, 0.0044 ± 0.0002, 0.0067 ± 0.0022, 0.0089 ± 0.0001) nmol/min/10^4 cell, respectively (Fig. 5C). Compared to normal group, activities of respiratory chain complex IV were slightly decreased in control group and 0 exosomes μg/mL intervention group. However, there were no significant differences between any two groups (P > 0.05).
Respiratory chain complex V, also known as F1F0-ATP synthase, is the key enzyme for mitochondrial oxidative phosphorylation to synthesize ATP. Activities of respiratory chain complex V in normal group, control group, and exosomes intervention group (0, 25, 50 or 100 μg/mL) were (0.0010 ± 0.0003, 0.0010 ± 0.0003, 0.0010 ± 0.0004, 0.0020 ± 0.0004, 0.0778 ± 0.0005) nmol/min/10^4 cell, respectively (Fig. 5D). The activities of respiratory chain complex V in 100 μg/mL exosome intervention group were (0.0778 ± 0.0005) nmol/min/10^4 cell, which had significant differences with the other 5 groups (P < 0.0001).

Effect of HUCMSCs-Exo on ATP synthesis of ARPE-19 cells injured by CoCl2

ATP is a complex high-energy compound, which widely exists in animals, plant microorganisms, and cultured cells. ATP is involved in many life processes and referred to as the "monetary molecular unit" for intracellular energy transfer. Measuring ATP content and calculating energy charge can reflect the state of energy metabolism. As shown in Fig. 6, ATP content in normal group, control group, and exosomes intervention group (0, 25, 50 or 100 μg/mL) was (0.0176 ± 0.0009, 0.0020 ± 0.0007, 0.0036 ± 0.0006, 0.0044 ± 0.0002, 0.0129 ± 0.0009, 0.0201 ± 0.0001).
μmol/10^4 cells. Compared with the normal group ((0.0176 ± 0.0009) μmol/10^4 cells, cellular ATP was significantly decreased (P < 0.05) after CoCl2 processing, as it was showed in 0 μg/mL exosome intervention group (0.0036 ± 0.0006) μmol/10^4 cells. With the intervention of exosomes, the amount of cellular ATP synthesis increased. The dates of ATP synthesis in 0 μg/mL exosome intervention group (0.0036 ± 0.0006) μmol/10^4 cells had significant differences (P < 0.05) with the 50 μg/mL and 100 μg/mL exosome intervention group, whose ATP synthesis was (0.0129 ± 0.0009, 0.0201 ± 0.0001) μmol/10^4 cells.

Discussion

In recent years, more and more evidence proved MSCs or MSCs-Exo could rescue the aerobic metabolism of damaged cells [12]. Arslan et al. [13] injected MSCs-Exo into the mouse model of myocardial infarction through the tail vein, and the results showed that the levels of ATP and NADH could be restored within 1 h. This study also showed that MSCs-Exo enhanced myocardial viability by reducing oxidative stress and increasing phosphorylated Akt and phosphorylated GSK-3β [13]. Panfoli et al. [14] suggested that the UCMSCs-Exo of full-term neonates could express functional respiratory chain complexes I, IV, and V, which consumed oxygen and produced ATP. Besides, some studies had shown that there were functional expressions of respiratory chains complexes and the tricarboxylic acid cycle (TAC cycle) enzymes in urinary exosomes; its proteomics showed that proteins were concentrated in certain specific functions, one of which was aerobic metabolism [15, 16].

The ability of exosomes to restore cellular aerobic metabolism may be due to their oxidative phosphorylation independent of mitochondria. Panfoli et al. [14] showed that ND4L, expressed by exosomes, was a complex I subunit encoded by DNA, indicating that the mechanism of oxidative phosphorylation (OXPHOS) in exosome membrane was the same as that in mitochondria. There was a supramolecular arrangement of the respiratory complex in the mitochondrial membrane, which could be transferred to endoplasmic reticulum (ER), through heterologous fusion between mitochondrial and ER, and eventually transferred to the exosomes body when sprouting in Polyneices [14]. This may be one of the explanations for the origin of the exosome OXPHOS mechanism. Additionally, Islam et al. [17] reported that there was a gap junction between mitochondrial DNA and alveolar cells in human MSCs after acute lung injury, so mitochondrial transferred and exosome transferred
might also play a role in restoring the aerobic metabolism of injured cells. If the MSCs-Exo could save the aerobic metabolism and restored the ATP synthesis of cells, it was hoped to improve the ability of cells to repair injuries and deal with the hypoxic environment, thereby promoting cell repair and regeneration and restoring their functions. This would provide a theoretical basis for the therapeutic efficacy of MSCs and MSCs-Exo in a variety of diseases.

Our results showed that the vesicles extracted by ExoQuick-TC showed a 40−100-nm round or oval bilayer membrane structure, highly expressed CD63 and CD81, and demonstrated that HUCMSC-Exo were successfully isolated. We tested ARPE-19 cells proliferation ability by CCK-8, and results showed that CoCl2 inhibited ARPE-19 cells proliferation. However, compared with the non-intervention group, ARPE-19 cell after exosomes intervention group (50 or 100 μg/mL) had significantly more surviving cells, and the cell morphology was also closer to that of normal cells.

Besides, in the results of MMP detection, the proportion of MMP roughly increased with the increasing of exosomes concentration, and in the 50 μg/mL exosomes intervention group was closest to that of the normal group. The elevated MMP may indirectly reflect mitochondrial functional recovery, which had been demonstrated in the detection of respiratory chain complex and ATP synthesis. The enzyme activities of the respiratory chain complex I and complex V in 100 μg/mL exosomes intervention group were significantly higher than those in other groups. However, there was no significant change in the enzyme activity of respiratory chain complex III and complex IV in exosomes intervention group, and it was lower than that in the normal group. Also, the amount of ATP synthesis in 50 μg/mL and 100 μg/mL exosome intervention group had significant difference with 0 μg/mL exosome intervention group, and the ATP synthesis in 100 μg/mL exosomes intervention group was the highest.

The above results suggested that the addition of exosomes at a certain concentration could restore the aerobic metabolism and ATP synthesis of ARPE-19 cells damaged by oxidation and have a protective effect on the cells. Moreover, the MMP and the activity of the respiratory chain complex I in the control/non-intervention group were higher than those in the low concentration exosomes intervention group, which might be due to the production of a certain amount of exosomes in ARPE-19 itself after oxidative damage. Due to the replacement of the original culture medium in the 0 μg/mL and 25 μg/mL exosomes concentration group, the exosomes produced by ARPE-19 itself were removed, while the exosome concentration was lower; it might not be enough to have a significant effect on cell function. In this study, hUCMSC was "starved" before exosomes were extracted; exosome extraction can be improved by removing exosome serum for hUCMSC culture. Weiss ML et al. released cells from umbilical cord matrix by enzymatic degradation of extracellular matrix, and this improved isolation method can help to produce a sufficient number of exosomes [7]. Further experiments are needed to determine whether there are other more effective pretreatment methods.

It is remarkable that the therapeutic effect of MSCs is mainly mediated by paracrine signaling of exosome [18]. And compared with MSCs, exosome is a kind of cell-free therapy, which has more advantages [19]. Our results showed that HUCMSC-Exo had a certain protective effect on ARPE-19 cells damaged by CoCl2 in vitro. The protective mechanism may be to restore cellular ATP synthesis and improve the ability of cells to repair damage and deal with hypoxic environment by saving their aerobic metabolic function.

Acknowledgements We are grateful to our colleagues for their helpful suggestions during the planning and editing of this work.

Author contributions All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by WF, LL, and CL. The first draft of this manuscript was written by CL and LL, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding This study was funded by the National Natural Science Foundation of China (grant number 81970806, 82101116) and Fundamental Research Funds for the Central Universities (grant number 21621052).

Declarations

Conflicts of Interest The authors declare that they have no competing interests, and the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.
References

1. Yu DY, Cringle SJ (2005) Retinal degeneration and local oxygen metabolism. Exp Eye Res 80(6):745–751. https://doi.org/10.1016/j.exer.2005.01.018
2. Ung L, Pattamatta U, Carnt N et al (2017) Oxidative stress and reactive oxygen species: a review of their role in ocular disease. Clin Sci (London, England: 1979) 131(24):2865–2883. https://doi.org/10.1042/cs20171246
3. Ambati J, Ambati BK, Yoo SH et al (2003) Age-related macular degeneration: etiology, pathogenesis, and therapeutic strategies. Surv Ophthalmol 48(3):257–293. https://doi.org/10.1016/s0039-6257(03)00030-4
4. Gong X, Draper CS, Allison GS et al (2017) Effects of the macular carotenoid lutein in human retinal pigment epithelial cells. Antioxidants (Basel) 6(4):100. https://doi.org/10.3390/antiox6040100
5. Jarrett SG, Boulton ME (2012) Consequences of oxidative stress in age-related macular degeneration. Mol Aspects Med 33(4):399–417. https://doi.org/10.1016/j.mam.2012.03.009
6. Lee Y, El Andaloussi S, Wood MJ (2012) Exosomes and microvesicles: extracellular vesicles for genetic information transfer and gene therapy. Hum Mol Genet 21(R1):R125–R134. https://doi.org/10.1093/hmg/dds317
7. Weiss ML, Medicetty S, Bledsoe AR et al (2006) Human umbilical cord matrix stem cells: preliminary characterization and effect of transplantation in a rodent model of Parkinson’s disease. Stem Cells 24(3):781–792. https://doi.org/10.1634/stemcells.2005-0330
8. Chen MY, Lie PC, Li ZL et al (2009) Endothelial differentiation of Wharton’s jelly-derived mesenchymal stem cells in comparison with bone marrow-derived mesenchymal stem cells. Exp Hematol 37(5):629–640. https://doi.org/10.1016/j.exphem.2009.02.003
9. He GH, Zhang W, Ma YX et al (2018) Mesenchymal stem cells-derived exosomes ameliorate blue light stimulation in retinal pigment epithelium cells and retinal laser injury by VEGF-dependent mechanism. Int J Ophthalmol 11(4):559–566. https://doi.org/10.18240/ijo.2018.04.04
10. Liu L, Sha XY, Wu YN et al (2020) Lycium barbarum polysaccharides protects retinal ganglion cells against oxidative stress injury. Neural Regen Res 15(8):1526–1531. https://doi.org/10.4103/1673-5374.274349
11. Vyatkina G, Bhatia V, Gerstner A et al (2004) Impaired mitochondrial respiratory chain and bioenergetics during chagasic cardiomyopathy development. Biochim Biophys Acta 1689(2):162–173. https://doi.org/10.1016/j.bbadis.2004.03.005
12. Lai RC, Yeo RW, Lim SK (2015) Mesenchymal stem cell exosomes. Semin Cell Dev Biol 40:82–88. https://doi.org/10.1016/j.secmdb.2015.03.001
13. Arslan F, Lai RC, Smeets MB et al (2013) Mesenchymal stem cell-derived exosomes increase ATP levels, decrease oxidative stress and activate PI3K/Akt pathway to enhance myocardial viability and prevent adverse remodeling after myocardial ischemia/reperfusion injury. Stem Cell Res 10(3):301–312. https://doi.org/10.1016/j.scr.2013.01.002
14. Panfoli I, Ravera S, Podestà M et al (2016) Exosomes from human mesenchymal stem cells conduct aerobic metabolism in term and preterm newborn infants. Faseb J 30(4):1416–1424. https://doi.org/10.1096/fj.15-279679
15. Panfoli I, Ravera S, Bruschi M et al (2011) Proteomics unravels the exportability of mitochondrial respiratory chains. Expert Rev Proteomics 8(2):231–239. https://doi.org/10.1586/epr.11.1
16. Bruschi M, Santucci L, Ravera S et al (2016) Human urinary exosome proteome unveils its aerobic respiratory ability. J Proteomics 136:25–34. https://doi.org/10.1016/j.jprot.2016.02.001
17. Islam MN, Das SR, Emin MT et al (2012) Mitochondrial transfer from bone-marrow-derived stromal cells to pulmonary alveoli protects against acute lung injury. Nat Med 18(5):759–765. https://doi.org/10.1038/nm.2736
18. Yu B, Li XR, Zhang XM (2020) Mesenchymal stem cell-derived extracellular vesicles as a new therapeutic strategy for ocular diseases. World J Stem Cells 12(3):178–187. https://doi.org/10.4252/wjssc.v12.i3.178
19. Nuzzi R, Caselgrandi P, Vercelli A (2020) Effect of mesenchymal stem cell-derived exosomes on retinal injury: a review of current findings. Stem Cells Int 2020:8883616. https://doi.org/10.1155/2020/8883616

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.