

Dexmedetomidine Attenuates Lung Ischemia-reperfusion Injury in Rats by Reducing Mitochondrial Dysfunction

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Abstract

Background: Lung ischemia-reperfusion injury (LIRI) is a significant clinical problem occurring after lung transplantation. LIRI is mediated by the overproduction of reactive oxygen species (ROS) and inflammatory activation. Previous studies have confirmed that dexmedetomidine (DEX) exerts a protective effect on LIRI, which potentially causes severe mitochondrial dysfunction. However, the specific mechanisms remain unclear. Our study was to explore whether dexmedetomidine exerts a beneficial effect on LIRI by reducing mitochondrial dysfunction.

Methods: Two different models were used in our study. For the in vivo experiment, thirty-two male Sprague-Dawley rats were randomly divided into Sham, ischemia-reperfusion (I/R), DEX+I/R and DEX+yohimbine+I/R (DY+I/R) groups. Similarly, pulmonary vascular endothelial cells (PVECs) from SD rats were divided into Control, oxygen glucose deprivation (OGD), D+OGD and DY+OGD groups.

Results: In our experiment, we confirmed severe lung damage after LIRI that was characterized by significantly pulmonary histopathology injury, a decrease in the oxygenation index ($\text{PaO}_2/\text{FiO}_2$) and an increase in the wet-to-dry weight ratio, while DEX treatment mitigated this damage. In addition, the DEX pretreatment significantly attenuated I/R-induced oxidative stress by decreasing the level of ROS in the mitochondria in vitro. Moreover, the DEX treatment enhanced mitochondrial biogenesis and autophagy by increasing the expression of peroxisome proliferator-activated receptor-gamma coactivator-1 α (PGC-1 α), mitochondrial transcription factor A (Tfam), PTEN-induced putative kinase 1 (PINK1), Parkin and dynamin 1-like protein 1 (Drp1).

Conclusions: These data suggest that DEX may alleviate LIRI by reducing mitochondrial dysfunction through the induction of mitochondrial biogenesis and autophagy.

Background

Lung ischemia-reperfusion injury (LIRI) is a well-known and important clinical problem occurring after lung transplantation that is characterized by the overexpression of many pro-inflammatory cytokines, generation of reactive oxygen species (ROS), and cellular swelling, resulting in severe pulmonary edema and primary graft dysfunction.¹⁻³

Mitochondria play a core role in circulation if death signals are triggered inside cells, including oxidative stress, regulating cell autophagy and death/apoptosis pathways.⁴ Mitochondria are related to many lung injuries and diseases, including LIRI.⁵⁻⁷ Damaged mitochondria induce autophagy through a mechanism that is mainly mediated by PINK1/Parkin-mediated mitophagy, and then the damaged mitochondria may be replaced by the progeny of more bioenergetically active mitochondria.^{8,9}

Dexmedetomidine (DEX), a specific 2-adrenoreceptor agonist, exerts many pharmacological effects, including synergistic sedation and analgesia. Many studies have verified that DEX attenuates ischemia-reperfusion injury and lipopolysaccharide and ventilator-induced lung injury.¹⁰⁻¹³ However, the specific

mechanism remains unknown. According to a recent report, α_2 -adrenergic stimulation suppresses oxidative damage and alters the mitochondrial membrane potential (MMP),¹⁴ suggesting that dexmedetomidine, a specific α_2 -adrenoreceptor agonist, probably exerted a protective effect by influencing mitochondrial function. Additionally, the mitochondrial ATP-sensitive potassium channel might participate in the protective effect of dexmedetomidine on cerebral ischemia-reperfusion (I/R) injury.¹⁵ Therefore, in the present study, we hypothesized that DEX might exert a beneficial effect on LIRI by reducing mitochondrial dysfunction and enhancing mitochondrial autophagy. The protective effect was partially blocked by yohimbine, an α_2 -adrenergic receptor antagonist.

Methods

Animals

All experimental protocols were authorized by the Animal Care and Use Committee of the Xuzhou Medical University (Xuzhou, China). Adult (aged 8–10 weeks) male Sprague-Dawley rats ($n = 32$; weight: 180–220 g) were purchased from the Experimental Animal Center at Xuzhou Medical University (Xuzhou, China). The care of all animals was provided according to the Chinese National Guidelines. All animals were housed in a temperature-controlled room (25 ± 2 °C) with a 12-h light/12-h dark cycle and 60–65% humidity and were provided free access to normal chow and tap water. All rats were allowed to acclimate to the housing conditions for 5 days prior to experiments. Rats were fasted for 8 h before surgery but were provided drinking water and allowed free access to food and water after surgery.

Rat ischemia-reperfusion (I/R) injury model

All SD rats were divided into four groups: Sham group, I/R group, DEX + I/R group and DY + I/R group ($n = 8$ per group, total $n = 32$) using a computer based random order generator. All SD rats were anesthetized with 1% sodium pentobarbital (50 mg/kg, Hengrui Medicine Co., Ltd., China). After a sufficient depth of anesthesia was established, all rats were intravenously injected with saline (10 ml/kg) or DEX (Hengrui Medicine Co., Ltd., China) at a concentration of 50 μ g/kg for 1 h prior to the induction of I/R or treatment with yohimbine (Shanghai Yuanye Bio-Technology Co., Ltd., China) at a concentration of 1.0 mg/kg, followed by the infusion of DEX, as described in previous studies.^{5,16} The tracheal tube was connected to a small animal ventilator for mechanical ventilation (the respiratory rate was 60 breaths/min, the tidal volume was 15 ml/kg, I:E = 1:2.5 and FiO_2 : 99%). The chest of each rat was opened at the fourth intercostal space, and the subcutaneous tissue was bluntly separated. The pleura was exposed and cut in the expiratory phase. The left thoracic cavity was exposed and the left hilar was carefully separated to expose the trachea. Then, the portal vein was injected with 300 U/kg of unfractionated heparin via the tail vein. After 10 min, the left hilar was clamped with a noninvasive vascular clamp to establish an ischemic model. After 30 min, the noninvasive vascular clamp was released to restore the ventilation and blood supply to the left lung for 2 h. Then the rats were euthanized with an overdose of isoflurane (5%). The left lung was removed immediately and washed with pre-cooled saline to eliminate the blood clot. The left

hilar of rats in Sham group were not clamped as a vehicle control. Four different researchers are responsible for anesthesia, administering drugs, surgery and data collection.

Histological examination.

At the end of the experiment, the lung tissues were removed. The upper parts of the lung tissues were fixed with 10% formalin, embedded in paraffin and subsequently sectioned. After deparaffinization and rehydration, the tissue sections were stained with hematoxylin and eosin. Finally, the pathological sections were evaluated in a blinded manner.

Measurement of the lung W/D weight ratio.

The wet lung tissues were weighed and then placed in an 80 °C drying oven for 48 h and were subsequently weighed when the tissues were dried. The W/D weight ratio was calculated.

Analysis of the oxygenation index (PaO₂/FiO₂).

At the end of experiment, arterial blood was acquired from the carotid artery and tested by using a blood gas analyzer (Premier3000, GEM).

In vitro cell viability test

To evaluate the cytotoxicity of DEX and yohimbine against PVECs, DEX was added to the culture medium at concentrations of 2, 4, 6, 8 and 10 nmol/L, and yohimbine was added to the culture medium at concentrations of 0.25, 0.5, 0.75 and 1.0 μmol/L. The viability of DEX/yohimbine-treated PVECs was determined by using the cell counting kit-8 (CCK-8) assay.

Cellular oxygen glucose deprivation (OGD) model.

Rat pulmonary vascular endothelial cells (PVECs) were acquired from ScienCell (San Diego, CA, USA). The PVECs were pretreated with DEX (10 nmol/L) or DEX and yohimbine (1 μmol/L followed by the DEX) for 30 min, and then PVECs were cultured in glucose-free DMEM (GIBCO, New York, USA) without FBS in a hypoxic incubator (95% N₂ and 5% CO₂) at 37 °C for 10 h. The control group was treated with DMEM. After exposure to OGD, the cells were transferred to absolute medium containing 10% FBS and cultured under normoxic conditions (95% air, 5% CO₂, and 21% FiO₂) for another 24 h.

Measurement of the mitochondrial membrane potential (MMP) in vitro.

At the end of the experiment, PVECs were stained with the JC-1 probe (2 μmol/L) (Beyotime Institute of Biotechnology) for 30 min, rinsed twice with PBS, and then imaged with a confocal microscope (original magnification, × 400). The MMP was determined by monitoring the dual emissions from the mitochondrial JC-1 monomers (green) and the aggregates (red) under a confocal microscope with excitation using a 488-nm-laser. The MMP was reported as the emission intensity ratio (the ratio of green to red fluorescence), which represents the relative arbitrary MMP level. A higher ratio indicates a lower MMP.

Measurement of ROS production in vitro.

After treatment, the PVECs were measured using MitoSOX™ Red mitochondrial superoxide indicator (Molecular Probes, Inc., Eugene, OR, USA) at 37 °C for 20 min, washed gently 3 times with warm buffer, and imaged in × 200 magnification using Leica microscope. Mitochondrial ROS generation was expressed as the mean fluorescence intensity of the red color, according to previously described.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). After treatment, total RNA was extracted from lung tissues and PVECs with Trizol reagent (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer's instructions. Total RNA (1 µg) was reverse transcribed into cDNA using the PrimeScript™ RT reagent kit (Takara Bio, Dalian, China). And then, the cDNA templates were amplified with SYBR Premix Ex Taq™ (Takara Bio) by using the following cycling conditions: 30 sec at 95 °C for 1 cycle, following 40 cycles of 5 sec at 95 °C and 30 sec at 60 °C. The following primer sequences were used: peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α) sense, 5'-CGG AGC AAT CTG AGT TAT ACG-3' and antisense, 5'-CAG TCA CAG GAG GCA TCT-3'; mitochondrial transcription factor A (Tfam) sense, 5'-ATC TCA TCC GTC GCA GTG-3' and antisense, 5'-GCA CAG TCT TGA TTC CAG TTC-3'; PINK1 sense, 5'-CAA TGC CGC TGT GTA TGA A-3' and antisense, 5'-CCG TCT CTG GAT CTT CTG TAA-3'; Parkin sense, 5'-CTC AGA CAA GGA CAC ATC AGT A-3' and antisense, 5'-GCG GTG GTT ACA TTG GAA G-3'; dynamin-related protein 1 (Drp1) sense, 5'-ACT GGC CCC CGT CCA GCT TA-3' and antisense, 5'-TGG ACC AGC TCC ACA CAG CG-3'; and GAPDH sense, 5'-GTG AAG GTC GGT GTG AAC-3' and antisense, 5'-GGT GAA GAC GCC AGT AGA-3'. The threshold cycle (Ct) values were set in the exponential phase of the PCR. Relative gene expressions were calculated by comparing the cycle times for each target PCR. The Ct values of the target PCR were normalized by subtracting the Ct value of GAPDH; and then, the relative mRNA expression of each sample was calculated by the $2^{-(\Delta Ct \text{ sample} - \Delta Ct \text{ control})}$ method.

Western blot analysis. Total cellular protein was extracted from lung tissues and PVECs using standard protocols, and protein concentration from the supernatant was determined by the BCA protein assay kit (Thermo Fisher Scientific). Equal amounts (30 µg) of purified protein were electrophoresed on 8% SDS-PAGE gels, then transferred and blotted onto polyvinylidene difluoride membranes. After blocking (5% nonfat milk, 2 h), membranes were incubated with the following primary antibodies at 4 °C overnight : anti-PGC-1α antibody (1:1,000; ab54481; Abcam), anti-Tfam antibody (1:2,000; ab131607; Abcam), anti-dynamin 1-like protein 1 (Drp1) antibody (1: 1,000; ab56788, Abcam), anti-PINK1 antibody (1:1,000; Abcam, ab23707), anti-Parkin antibody (1:1,000; Abcam, ab179812) and anti-GAPDH antibody (1:1,000; 5174P; Cell Signaling Technology, Inc, Danvers, MA, USA). After four washes, membranes were then incubated with HRP-conjugated secondary antibodies, containing the anti-rabbit antibody (1:5,000; ab6721, Abcam) and anti-mouse antibody (1:2,000; ab97023, Abcam); all steps were performed at room temperature. Finally, the signals were detected by the chemiluminescence substrate (Super Signal West Pico, Thermo Fisher Scientific, USA), the intensities of bands were calculated by Gel-Pro Analyzer software(Media Cybernetics, Rockville, MD, USA). In addition, all intensity values were normalized to GAPDH and then reported as relative expression.

Statistical analysis. All data are presented as the means values \pm SD, with the number of independent experiments indicated in the figure legends. Statistical analyses were performed with Student's t-test or one-way ANOVA, followed by Dunnett's post hoc tests. Differences are considered statistically significant at $p < 0.05$.

Results

DEX alleviated LIRI in vivo.

In our study, our results revealed a protective effect of DEX on LIRI in rats. The pathological changes of lung tissue in the I/R group were characterized by neutrophil aggregation in the alveolar space, a thickened alveolar wall and less alveolar space. These changes were significantly alleviated by DEX treatment. The protective effect of DEX was offset by yohimbine (Fig. 1A). The lung W/D weight ratio increased in the I/R group compared with the sham group. The DEX pretreatment decreased the W/D weight ratio compared with the I/R group. The yohimbine treatment increased the W/D weight ratio of the lung compared with the DEX group (Fig. 1B). Additionally, the $\text{PaO}_2/\text{FiO}_2$ was markedly decreased in the I/R group, and the DEX pretreatment increased the $\text{PaO}_2/\text{FiO}_2$, an effect that was counteracted by the yohimbine treatment (Fig. 1B and 1C). Based on these results, the DEX pretreatment alleviated LIRI by activating α_2 adrenergic receptors.

Beneficial effects of DEX on mitochondrial morphology and function in vitro.

A cell viability test was performed to verify the possible cytotoxicity of DEX and yohimbine towards PVECs. The cell viability of each group was approximately 100%, indicating that the different concentrations of DEX and yohimbine did not interfere with the growth of PVECs (Fig. 2A). These results confirmed the low toxicity and biological safety of DEX and yohimbine. Based on the results, DEX (10 nmol/L) and yohimbine (1 $\mu\text{mol/L}$) were used in subsequent experiments. Transmission electron microscopy (TEM) was used to observe the mitochondrial ultrastructural damage and examine the protective effects of DEX on OGD-induced mitochondrial damage. The mitochondrial morphology remained intact in the control group, but the mitochondria in the OGD group were swollen and displayed vacuolization and a loss of cristae. In contrast, the mitochondrial structure was significantly improved after the DEX pretreatment and was counteracted by the yohimbine treatment.

DEX prevented the LIRI-induced excessive generation of mtROS and the decrease in the MMP in vitro.

During the progression of LIRI, mtROS production might be a potential mechanism associated with mitochondrial dysfunction, and the HR-induced depolarization of the MMP has been reported to depend on the excessive generation of ROS. In our experiment, a marked increase in the generation of mtROS and a decrease in the MMP (as evidenced by an increase in the green/red fluorescence ratio) was observed in the OGD group compared with the control group. However, the pretreatment of the PVECs with DEX decreased these effects. The changes induced by DEX were neutralized by the yohimbine treatment (Figs. 3 and 4).

Beneficial effects of DEX on the expression levels of proteins associated with mitochondrial autophagy and biogenesis in vivo and in vitro.

Mitochondrial autophagy is associated with PINK1, Parkin and Drp1,^{8,17,18} while mitochondrial biogenesis is related to PGC-1 α and Tfam.¹⁹ The expression levels of the PINK1, Parkin and Drp1 mRNAs and proteins, which are essential for mitochondrial autophagy, increased in the I/R group, and the DEX pretreatment further increased the levels of these proteins (Fig. 5A). Additionally, the DEX pretreatment markedly increased the expression levels of the PGC-1 α and Tfam mRNAs and proteins compared with those of the OGD group (Fig. 5B).

Discussion

Lung ischemia-reperfusion injury is strongly correlated with the occurrence of primary graft failure following lung transplantation, which can even lead to significant morbidity and mortality.²⁰ Research has recently focused on the protective effects of DEX on IR-induced injury, while the specific mechanism remains largely unknown.^{21,22} Mitochondrial autophagy is critical to maintain the physiological status and is involved in the development of many pulmonary diseases.^{23,24} Recently, α_2 -adrenergic stimulation was reported to suppress oxidative stress-induced injury and reduce mitochondrial damage, while yohimbine, an α_2 -adrenergic receptor antagonist, reversed these changes.¹⁴ Therefore, the present study was performed and designed based on the hypothesis that DEX exerts a protective effect on lung I/R injury by reducing mitochondrial dysfunction and increasing mitochondrial autophagy.

The LIRI model was established in SD rats to determine whether DEX attenuates LIRI. Following model establishment, the lung damage was manifested as a deterioration of pathological changes, increased W/D weight ratio and decreased oxygenation index, consistent with previous reports.²⁵ The beneficial effect of DEX had been verified in many different models in other studies.^{11, 12, 14} After the DEX treatment (50 ug/kg), IR-induced lung injury was reduced, as evidenced by the improvements in the histopathological changes, lung water content and oxygenation index, which were related to the effects on the α_2 receptor.

DEX exerts a beneficial effect on a rat cerebral IR model that might be associated with the activation of the mitochondrial ATP-sensitive potassium channel.¹⁷ Notably, DEX-induced neutrophil apoptosis is related to a loss of the MMP.²⁶ The mtROS levels and MMP, which are characterized as the representative manifestations of mitochondrial injury, were detected in the present study to explore the mechanism underlying the protective effect of DEX on LIRI. In our study, the DEX pretreatment suppressed mtROS production and changes in the MMP in vitro—effects that were subsequently reversed by the yohimbine treatment.

In the present experiment, PVECs treated with OGD exhibited abnormal mitochondrial ultrastructures upon the visualization of the mitochondrial morphology, but the morphology of damaged mitochondria

was markedly attenuated in PVECs pretreated with DEX. Mitophagy is a quality control system characterized by the specific elimination of dysfunctional or damaged mitochondria, and it reduces cell apoptosis signals and prevents unwarranted cell loss after cells encounter various stimuli.²⁷ According to many researchers, mitochondria experiencing ROS-induced injury should be selectively transferred to lysosomes and then degraded during mitophagy; these mitochondria might be replaced by more biogenetically active mitochondria.²⁸ Therefore, we inferred that the DEX pretreatment would improve mitochondrial autophagy and mitochondrial biogenesis.

PTEN-induced putative kinase 1 (PINK1) is intimately involved in mitophagy by identifying impaired mitochondria and selectively removing them from the mitochondrial network through PINK1/Parkin-mediated mitophagy. While damaged mitochondria are being recognized by PINK1, PINK1 accumulates on the outer membrane of the mitochondria and recruits Parkin, an E3 ubiquitin ligase, and then the damaged mitochondria are designated for degradation by the lysosomes through the PINK1/parkin pathway.²⁹ During the process of PINK/Parkin-related mitochondrial fission, Drp1 is essential for mitochondrial autophagy.^{8,9,30} Recent studies reported an Ang II-induced increase in the ROS levels in the mitochondria, which induced mitochondrial injury and the activation of Parkin-related mitochondrial autophagy.¹⁹ In our experiment, the levels of the PINK1, Parkin and Drp1 mRNAs and proteins were increased in the OGD group and I/R group, consistent with previous studies. Interestingly, the DEX pretreatment further increased the expression of PINK1, Parkin and Drp1 further, changes that were partially reversed by the yohimbine treatment. Based on these results, DEX promotes mitochondrial autophagy. Accordingly, we postulated that these findings indicated an effect of DEX on promoting the autophagy of impaired mitochondria.

PGC-1 α is a multifunctional protein that exerts important effects on controlling the transcription of genes related to mitochondrial biogenesis and respiratory function. It also participates in the mitochondrial energy-generating process, according to the metabolic demands imposed by many diseases.³¹ The expression of Tfam is activated by PGC-1 α , and the translocation of Tfam activates the transcription and replication of mtDNA.³² In the current study, the levels of the PGC-1 α and Tfam mRNAs and proteins were increased in the OGD group and I/R group, suggesting that damaged mitochondria might be replaced by more biogenetically active mitochondria following the activation of PGC-1 α and Tfam. Additionally, the DEX pretreatment led to the overexpression of PGC-1 α and Tfam. A recent study also implied that a DEX treatment directly increased the levels of the PGC-1 α protein in the regions with traumatic brain injury.³³ Taken together, DEX increased the expression levels of PGC-1 α and Tfam to improve mitochondrial biogenesis.

Our experiments had several limitations. Although two different lung injury models were chosen in our study, the LIRI occurring in many diseases is more complex than our models. In addition, the protective effect of DEX was only partially blocked by the yohimbine treatment, suggesting the existence of other mechanisms unrelated to α_2 -receptor stimulation, and additional studies are needed to clarify the protective effect of DEX.

Conclusions

In summary, DEX significantly alleviated I/R-induced lung injury in vivo and in vitro. Notably, the possible mechanism may be associated with a reduction in mitochondrial dysfunction through the activation of mitochondrial biogenesis and autophagy. DEX potentially represents a new type of antioxidant reagent for the treatment of I/R-induced injury. However, we have not clearly determined the mechanism by which DEX induced the expression of PGC-1 α /Tfam and PINK1/Parkin/Drp1; these questions are currently being investigated in our lab.

Abbreviations

DEX Dexmedetomidine

Drp1 dynamin 1-like protein 1

DY Dexmedetomidine and Yohimbine

I/R Ischemia-reperfusion

LIRI Lung ischemia-reperfusion injury

MMP Mitochondrial membrane potential

mtROS Mitochondrial reactive oxygen species

OGD Oxygen glucose deprivation

PINK1 PTEN-induced putative kinase 1

PGC-1 α Peroxisome proliferator-activated receptor-gamma coactivator-1 α

PVECs Pulmonary vascular endothelial cells

ROS Reactive oxygen species

Tfam Mitochondrial transcription factor A

Declarations

Ethics approval and consent to participate

All experimental protocols were authorized by the Animal Care and Use Committee of the Xuzhou Medical University (Xuzhou, China).

Consent for publication

Not applicable.

Authors' contributions

YZ and YL designed the study, directed the project, processed rats samples, performed the experiments and prepared the figures and table. KW performed statistical analysis of gene expression data. MYZ and CYW participated in data analysis. LQ contributed new reagents or analytic tools. LWW participated in research design and the writing of the paper. All authors read and approved the manuscript.

Competing interests

The authors declare no conflicts of interest.

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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.

References

1. Guerra-Mora JR, Perales-Caldera E, Aguilar-Leon D, et al. Effects of Sildenafil and Tadalafil on Edema and Reactive Oxygen Species Production in an Experimental Model of Lung Ischemia-Reperfusion Injury. *Transplant Proc.* 2017;49(6):1461-6.
2. Novick RJ, Gehman KE, Ali IS, Lee J. Lung preservation: the importance of endothelial and alveolar type II cell integrity. *Ann Thorac Surg.* 1996;62(1):302-14.
3. Nakou M, Katsikas G, Sidiropoulos P, et al. Rituximab therapy reduces activated B cells in both the peripheral blood and bone marrow of patients with rheumatoid arthritis: depletion of memory B cells correlates with clinical response. *Arthritis Res Ther.* 2009;11(4):R131.
4. Shrivastav A, Sarkar G. Medical image. Hand in chronic arsenicosis. *N Z Med J.* 2013;126(1377):75-6.

5. Fu C, Dai X, Yang Y, Lin M, Cai Y, Cai S. Dexmedetomidine attenuates lipopolysaccharide-induced acute lung injury by inhibiting oxidative stress, mitochondrial dysfunction and apoptosis in rats. *Mol Med Rep.* 2017;15(1):131-8.
6. Jiang T, Liu Y, Meng Q, et al. Hydrogen sulfide attenuates lung ischemia-reperfusion injury through SIRT3-dependent regulation of mitochondrial function in type 2 diabetic rats. *Surgery.* 2019;165(5):1014-26.
7. Liu WC, Chen SB, Liu S, et al. Inhibition of mitochondrial autophagy protects donor lungs for lung transplantation against ischaemia-reperfusion injury in rats via the mTOR pathway. *J Cell Mol Med.* 2019;23(5):3190-201.
8. Narendra DP, Youle RJ. Targeting mitochondrial dysfunction: role for PINK1 and Parkin in mitochondrial quality control. *Antioxid Redox Signal.* 2011;14(10):1929-38.
9. Deng H, Dodson MW, Huang H, Guo M. The Parkinson's disease genes pink1 and parkin promote mitochondrial fission and/or inhibit fusion in *Drosophila*. *Proc Natl Acad Sci U S A.* 2008;105(38):14503-8.
10. Chen H, Sun X, Yang X, et al. Dexmedetomidine reduces ventilator-induced lung injury (VILI) by inhibiting Toll-like receptor 4 (TLR4)/nuclear factor (NF)-kappaB signaling pathway. *Bosn J Basic Med Sci.* 2018;18(2):162-9.
11. Gu J, Chen J, Xia P, Tao G, Zhao H, Ma D. Dexmedetomidine attenuates remote lung injury induced by renal ischemia-reperfusion in mice. *Acta Anaesthesiol Scand.* 2011;55(10):1272-8.
12. Cavalcanti V, Santos CL, Samary CS, et al. Effects of short-term propofol and dexmedetomidine on pulmonary morphofunction and biological markers in experimental mild acute lung injury. *Respir Physiol Neurobiol.* 2014;203:45-50.
13. Sen V, Guzel A, Sen HS, et al. Preventive effects of dexmedetomidine on the liver in a rat model of acid-induced acute lung injury. *Biomed Res Int.* 2014:621827.
14. Kelly AC, Camacho LE, Pendarvis K, et al. Adrenergic receptor stimulation suppresses oxidative metabolism in isolated rat islets and Min6 cells. *Mol Cell Endocrinol.* 2018;473:136-45.
15. Yuan F, Fu H, Sun K, Wu S, Dong T. Effect of dexmedetomidine on cerebral ischemia-reperfusion rats by activating mitochondrial ATP-sensitive potassium channel. *Metab Brain Dis.* 2017;32(2):539-46.
16. Jiang L, Li L, Shen J, Qi Z, Guo L. Effect of dexmedetomidine on lung ischemiareperfusion injury. *Mol Med Rep.* 2014;9(2):419-26.
17. Lee J, Giordano S, Zhang J. Autophagy, mitochondria and oxidative stress: cross-talk and redox signalling. *Biochem J.* 2012;441(2):523-40.
18. Eid N, Ito Y, Horibe A, Otsuki Y, Kondo Y. Ethanol-Induced Mitochondrial Damage in Sertoli Cells is Associated with Parkin Overexpression and Activation of Mitophagy. *Cells.* 2019;8(3).
19. Lu Y, Li S, Wu H, et al. Beneficial effects of astragaloside IV against angiotensin II-induced mitochondrial dysfunction in rat vascular smooth muscle cells. *Int J Mol Med.* 2015;36(5):1223-32.

20. Laubach VE, Sharma AK. Mechanisms of lung ischemia-reperfusion injury. *Curr Opin Organ Transplant*. 2016;21(3):246-52.
21. Chen Z, Qiu PY, Ma CG. Dexmedetomidine preconditioning protects against retinal ischemia/reperfusion injury and inhibits inflammation response via toll-like receptor 4 (TLR4) pathway. *Biomed Pharmacother*. 2017;93:1018-24.
22. He L, Hao S, Wang Y, et al. Dexmedetomidine preconditioning attenuates ischemia/reperfusion injury in isolated rat hearts with endothelial dysfunction. *Biomed Pharmacother*. 2019;114:108837.
23. Li T, Liu Y, Xu W, et al. Polydatin mediates Parkin-dependent mitophagy and protects against mitochondria-dependent apoptosis in acute respiratory distress syndrome. *Lab Invest*. 2019;99(6):819-29.
24. Ghanta S, Tsoyi K, Liu X, et al. Mesenchymal Stromal Cells Deficient in Autophagy Proteins Are Susceptible to Oxidative Injury and Mitochondrial Dysfunction. *Am J Respir Cell Mol Biol*. 2017;56(3):300-9.
25. Deng C, Zhai Z, Wu D, et al. Inflammatory response and pneumocyte apoptosis during lung ischemia-reperfusion injury in an experimental pulmonary thromboembolism model. *J Thromb Thrombolysis*. 2015;40(1):42-53.
26. Kishikawa H, Kobayashi K, Takemori K, Okabe T, Ito K, Sakamoto A. The effects of dexmedetomidine on human neutrophil apoptosis. *Biomed Res*. 2008;29(4):189-94.
27. Zhong Z, Umemura A, Sanchez-Lopez E, et al. NF-kappaB Restricts Inflammasome Activation via Elimination of Damaged Mitochondria. *Cell*. 2016;164(5):896-910.
28. Mammucari C, Rizzuto R. Signaling pathways in mitochondrial dysfunction and aging. *Mech Ageing Dev*. 2010;131(7-8):536-43.
29. McWilliams TG, Muqit MM. PINK1 and Parkin: emerging themes in mitochondrial homeostasis. *Curr Opin Cell Biol*. 2017;45:83-91.
30. Kageyama Y, Hoshijima M, Seo K, et al. Parkin-independent mitophagy requires Drp1 and maintains the integrity of mammalian heart and brain. *EMBO J*. 2014;33(23):2798-813.
31. Scarpulla RC, Vega RB, Kelly DP. Transcriptional integration of mitochondrial biogenesis. *Trends Endocrinol Metab*. 2012;23(9):459-66.
32. Wu Z, Puigserver P, Andersson U, et al. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell*. 1999;98(1):115-24. .
33. Li F, Wang X, Deng Z, Zhang X, Gao P, Liu H. Dexmedetomidine reduces oxidative stress and provides neuroprotection in a model of traumatic brain injury via the PGC-1alpha signaling pathway. *Neuropeptides*. 2018;72:58-64.

Figures

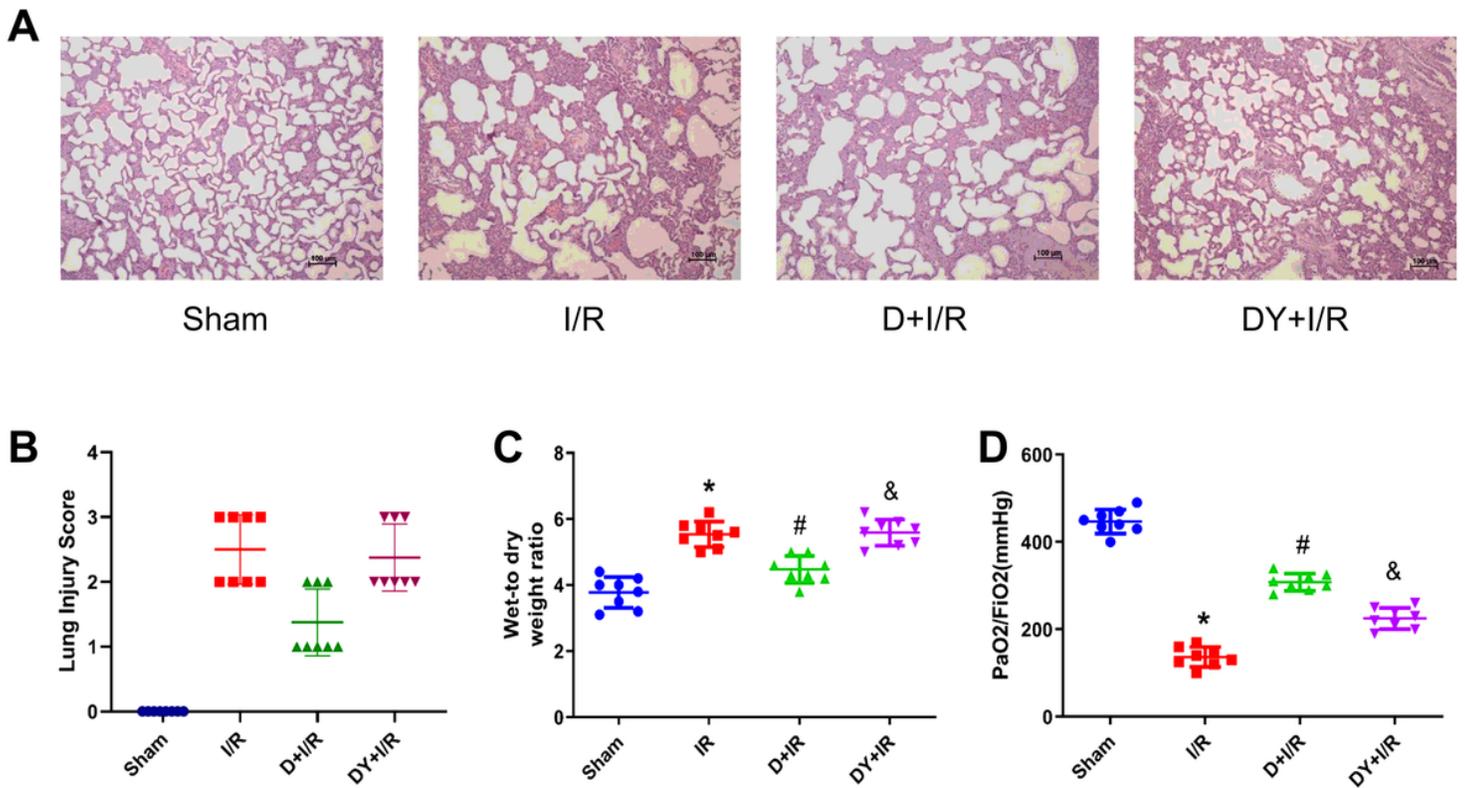


Figure 1

Dexmedetomidine (DEX) alleviated IR-induced lung injury in rats. (A) Comparison of histopathological changes in the lungs of the sham group, I/R group, D+I/R group and DY+I/R group as determined by using hematoxylin and eosin staining. The lungs of rats in the I/R group exhibited neutrophil aggregation in the alveolar space, a thickened alveolar wall and less alveolar space. DEX obviously alleviated the I/R-induced histopathological changes, an effect that was counteracted by yohimbine treatment. (B) DEX reduced lung injury score in the D+I/R group. (C) DEX distinctly alleviated I/R-induced vascular hyperpermeability in the lung. (D) DEX significantly alleviated I/R-induced lung dysfunction, which was determined by calculating the oxygenation index (PaO₂/FiO₂). The yohimbine treatment reversed the protective effect of DEX. The data are presented as means±SD (n=8). *P<0.05 compared with the sham group, #P<0.05 compared with the I/R group, and &P<0.05 compared with the D+I/R group). DEX, dexmedetomidine; I/R, ischemia-reperfusion. Scale bar, 100 nm.

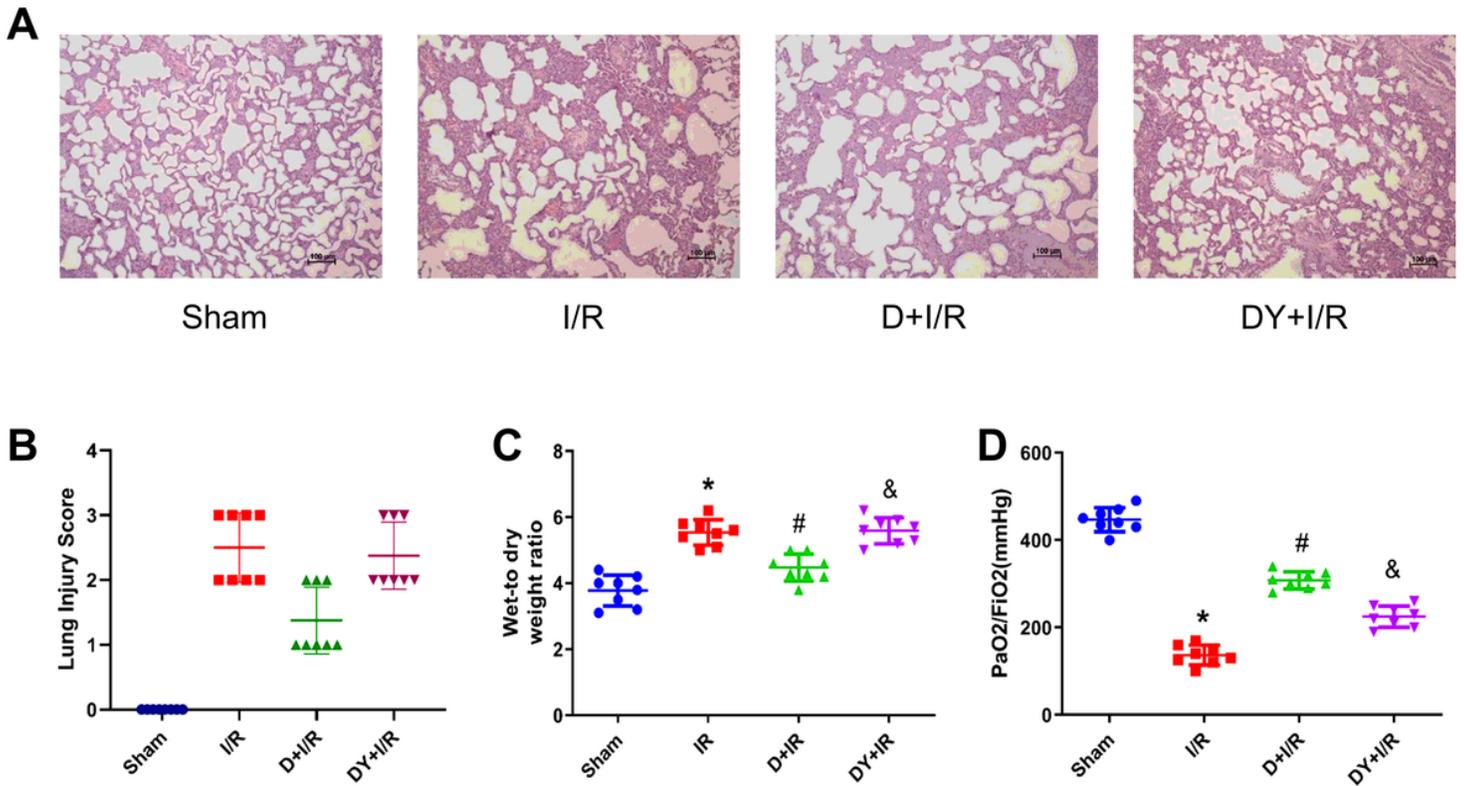


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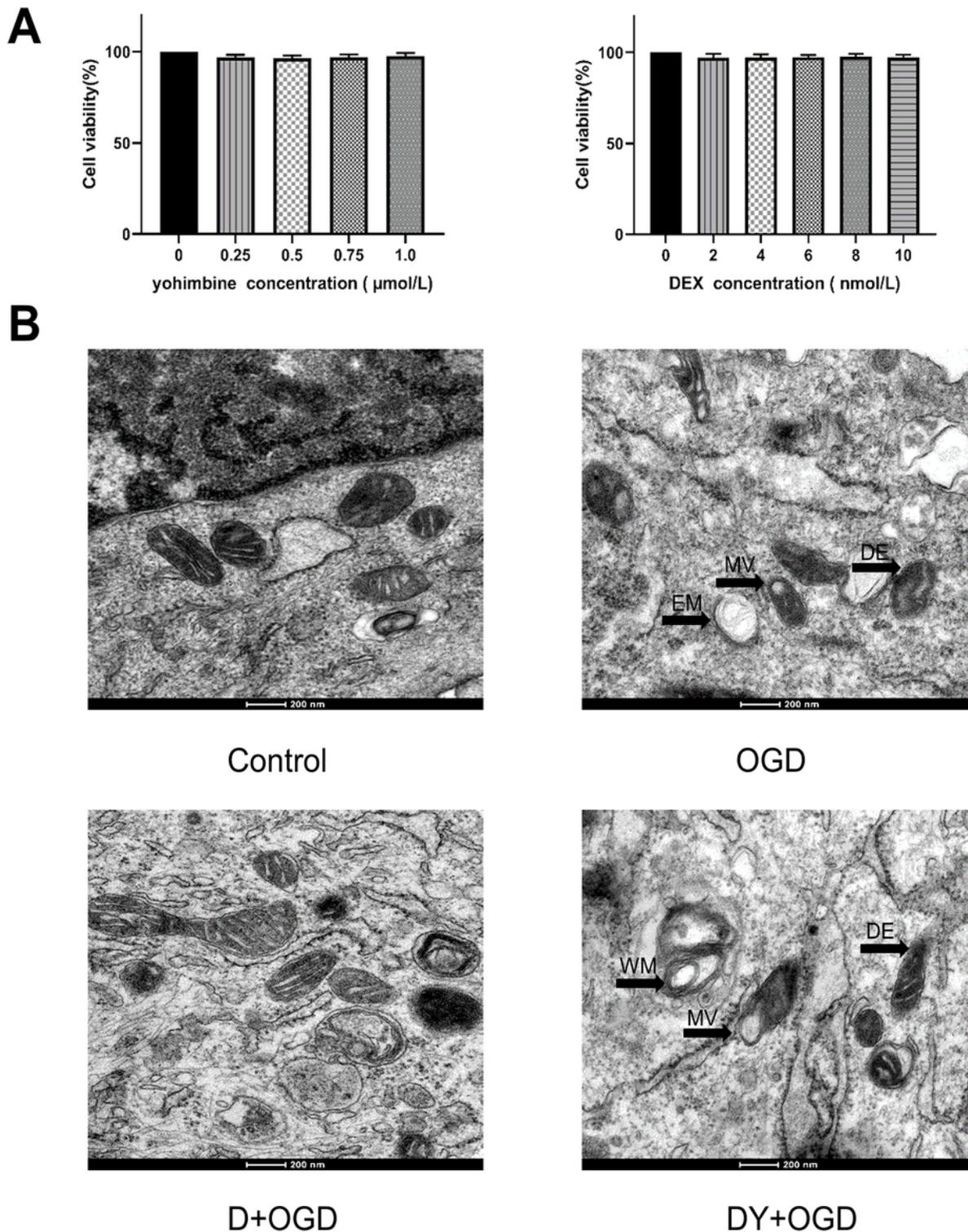


Figure 2

Beneficial effects of DEX on mitochondrial morphology in vitro. (A) Viability of pulmonary vascular endothelial cells (PVECs) incubated with DEX and yohimbine for 10 h. Data are presented as means \pm SD of 3 independent experiments. (B) Transmission electron microscopy images of the mitochondrial morphology in PVECs (original magnification, $\times 80,000$). The mitochondrial morphology generally remained normal in the control group. PVECs treated with oxygen glucose deprivation (OGD) exhibited

substantial damage. Mitochondria displayed vacuolization, electron dense deposits and whorl-like inner membrane with nearly a complete loss of cristae. The mitochondrial damage was significantly reduced by the DEX pretreatment compared with the OGD group, which was counteracted by the yohimbine treatment. MV, mitochondrial vacuolization; EM, empty mitochondria; WM, whorl-like inner membrane; DE, dense electron. Scale bar, 200 nm.

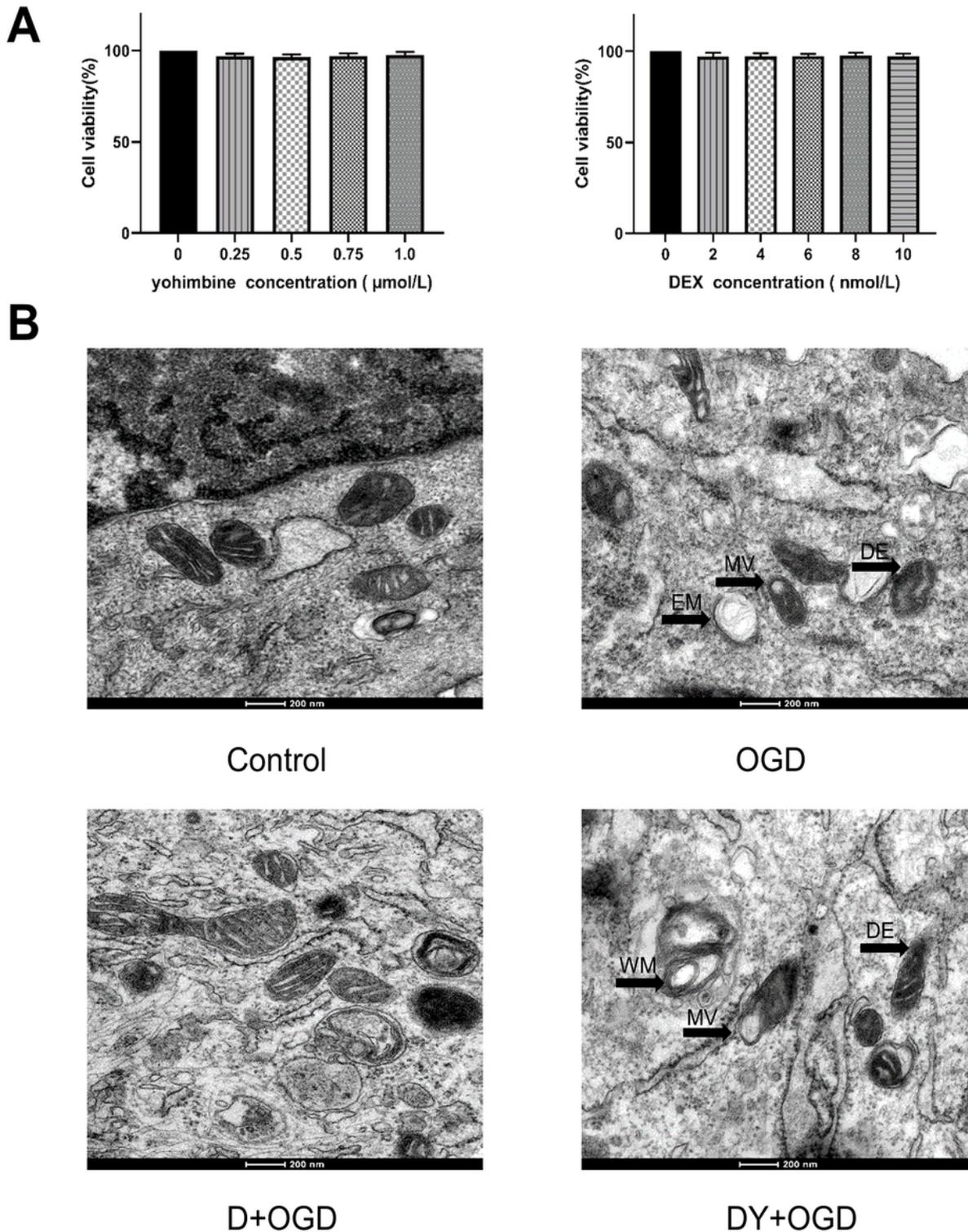


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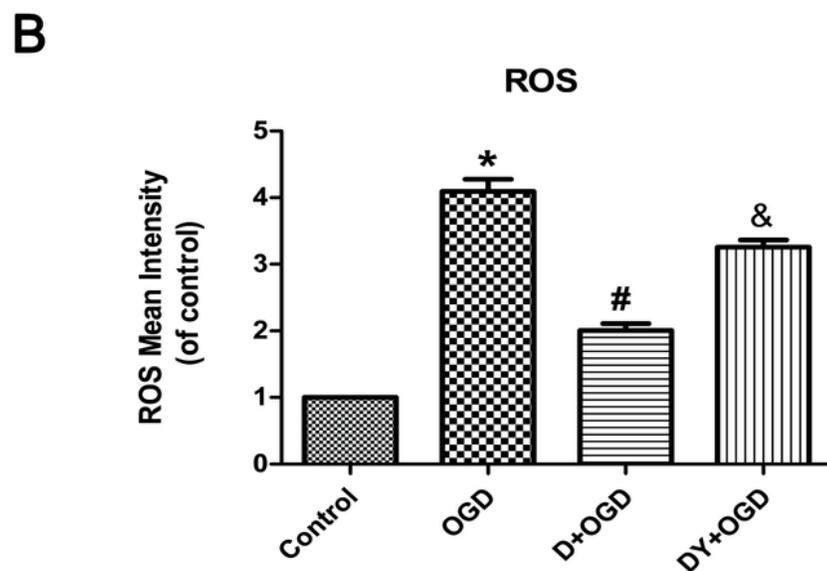
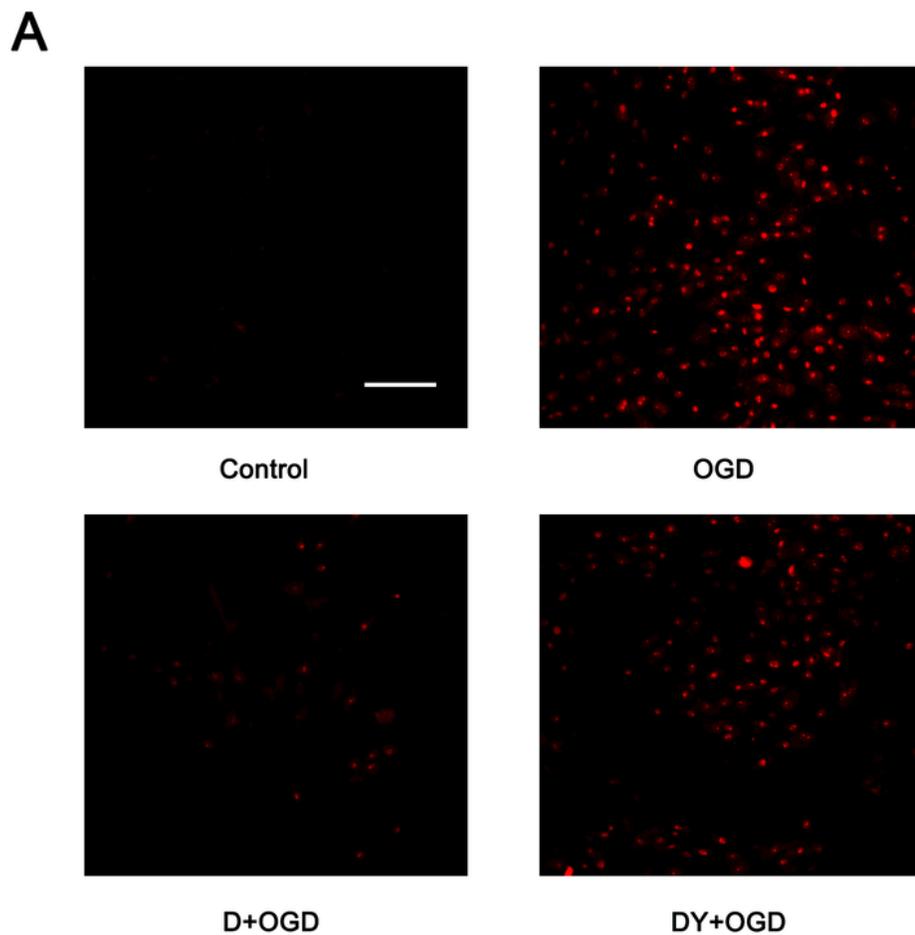
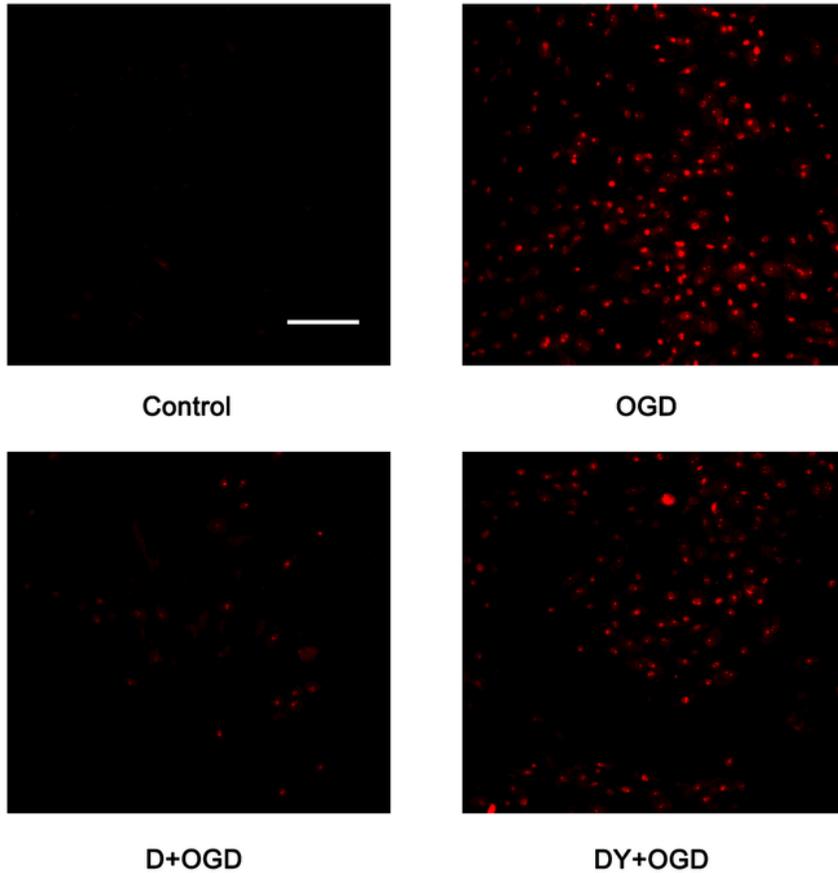


Figure 3

Effects of DEX on the overproduction of mitochondrial ROS (mtROS). (A) Confocal microscopy images of mtROS (scale bars represent 200 μ m). (B) Quantitative analysis of the effects of DEX on the mtROS level. PVECs were divided into four groups: a control group (Control), PVECs treated with OGD (OGD), PVECs pretreated with DEX followed by the OGD treatment (D+OGD), and PVECs pretreated with yohimbine and DEX followed by OGD treatment (DY+OGD). Data are presented as means \pm SD of 3 independent

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A



B

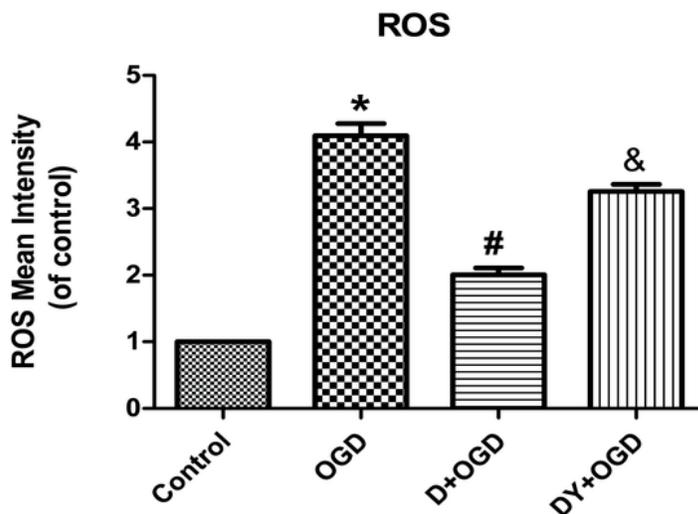


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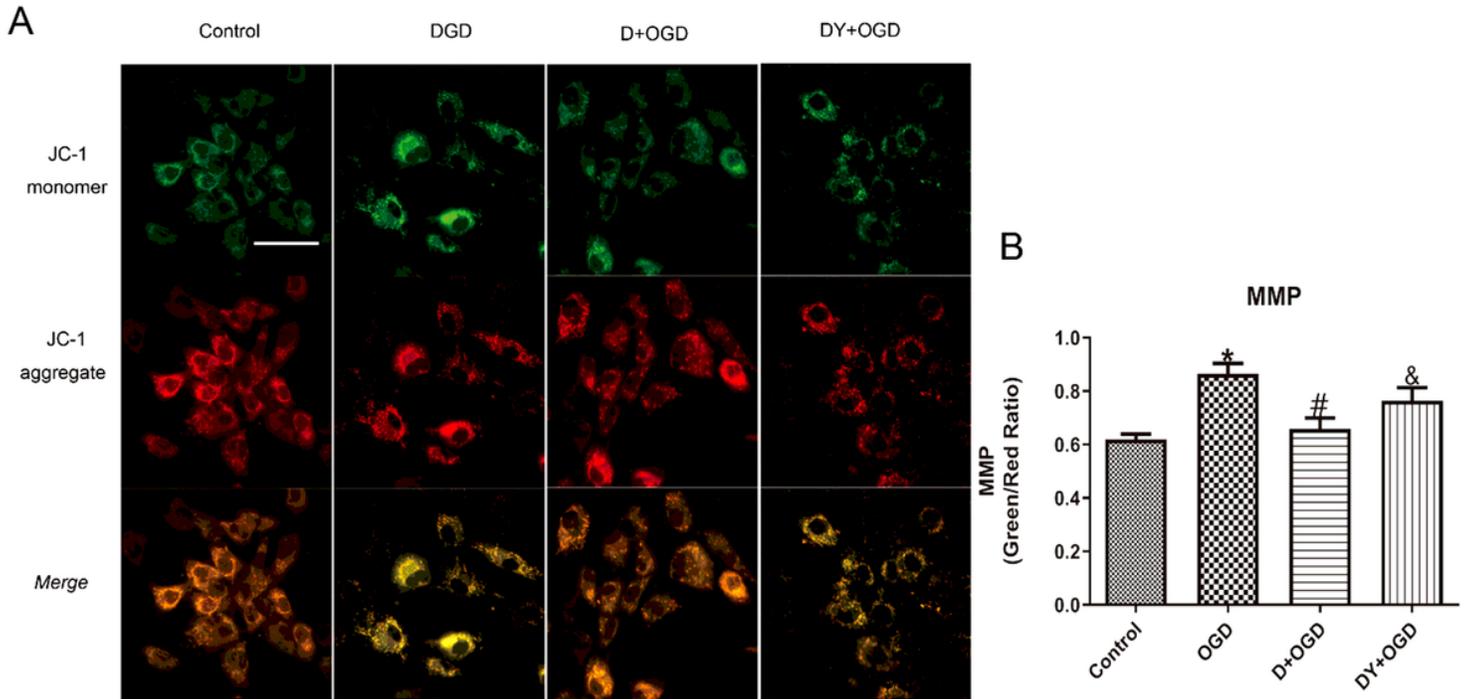


Figure 4

Effects of DEX on the mitochondrial membrane potential (MMP). (A) Confocal microscopy images of the MMP (scale bars represent 100 μ m). The orange color indicates the combination of red (JC-1 aggregate) and green (JC-1 monomer). (B) Quantitative analysis of the effects of DEX on the MMP. PVECs were divided into four groups: control group (Control), PVECs treated with OGD (OGD), PVECs pretreated with DEX followed by the OGD treatment (D+OGD), and PVECs pretreated with yohimbine and DEX followed by the OGD treatment (DY+OGD). Data are presented as means \pm SD of 3 independent experiments. * P <0.05 compared with the control group, # P <0.05 compared with the OGD group, and & P <0.05 compared with the D+OGD group.

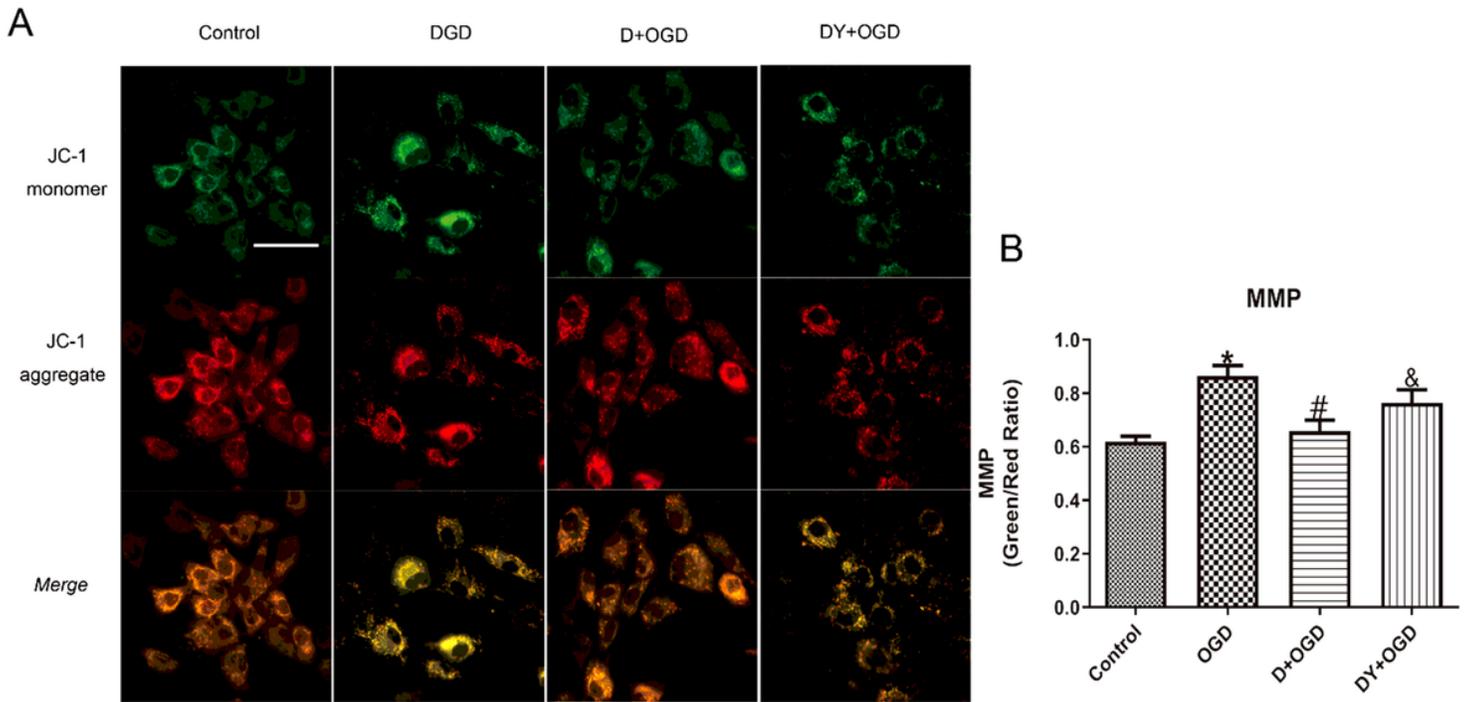


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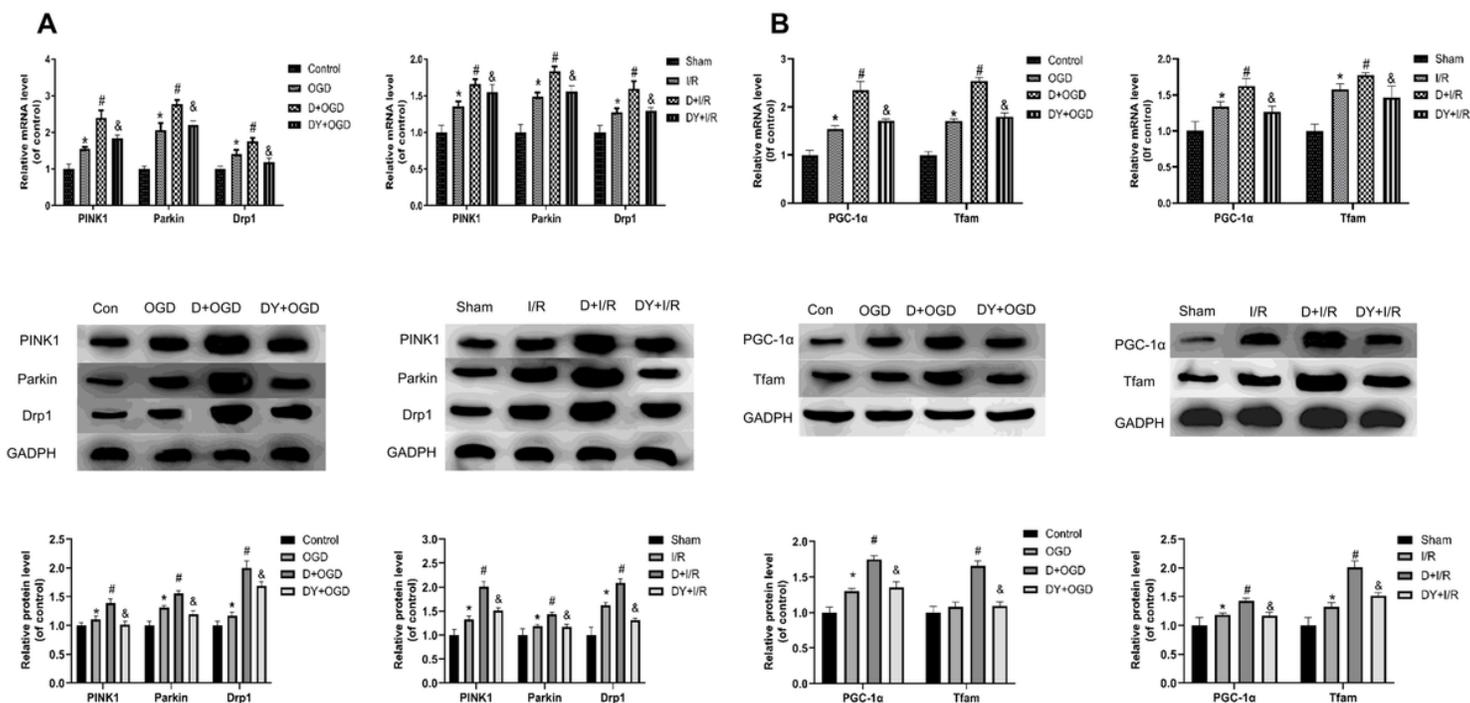


Figure 5

Effects of DEX on mRNA and protein expression in vivo and in vitro. (A) Expression levels of the PINK1, Parkin and Drp1 mRNAs and proteins. (B) Expression levels of the PGC-1 α and Tfam mRNAs and proteins. PVECs were divided into four groups: control group (Control), PVECs treated with OGD (OGD), PVECs pretreated with DEX followed by the OGD treatment (D+OGD), and PVECs pretreated with yohimbine and DEX followed by the OGD treatment (DY+OGD). Rats were divided into four groups (n=8): a sham group (Sham), rats treated with I/R (I/R), rat pretreated with DEX followed by an I/R treatment (D+I/R), and rats pretreated with yohimbine and DEX followed by an I/R treatment (DY+I/R). Data are presented as means \pm SD of 3 independent experiments. *P<0.05 compared with the control group, #P<0.05 compared with the OGD or I/R group, and &P<0.05 compared with the D+OGD or D+I/R group.

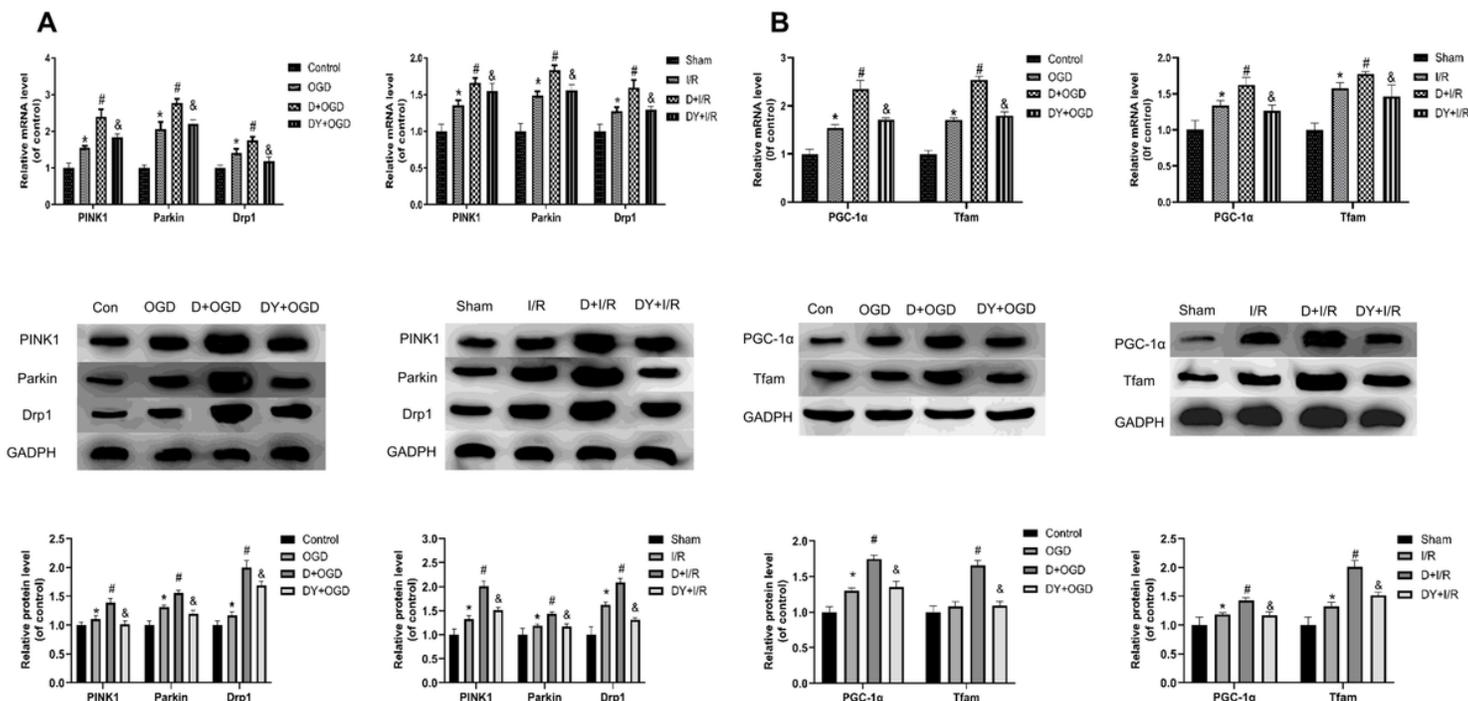


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Effects of DEX on mRNA and protein expression *in vivo* and *in vitro*. (A) Expression levels of the PINK1, Parkin and Drp1 mRNAs and proteins. (B) Expression levels of the PGC-1 α and Tfam mRNAs and proteins. PVECs were divided into four groups: control group (Control), PVECs treated with OGD (OGD), PVECs pretreated with DEX followed by the OGD treatment (D+OGD), and PVECs pretreated with yohimbine and DEX followed by the OGD treatment (DY+OGD). Rats were divided into four groups (n=8): a sham group (Sham), rats treated with I/R (I/R), rat pretreated with DEX followed by an I/R treatment (D+I/R), and rats pretreated with yohimbine and DEX followed by an I/R treatment (DY+I/R). Data are presented as means \pm SD of 3 independent experiments. *P<0.05 compared with the control group, #P<0.05 compared with the OGD or I/R group, and &P<0.05 compared with the D+OGD or D+I/R group.

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