

# Dexmedetomidine Provides Protection to Neurons Against OGD/R-Induced Oxidative Stress and Neuronal Apoptosis

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## Research article

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# Abstract

**Background:** Dexmedetomidine, a potent  $\alpha_2$ -adrenoceptor ( $\alpha_2$ -AR) agonist, is extensively used in the operating room (OR) and intensive care unit (ICU) and has applied in several diseases. However, the precise role of dexmedetomidine in oxygen and glucose deprivation/reoxygenation (OGD/R)-treated neurons, and the mechanisms underlying its effect, has yet to be elucidated.

**Methods:** OGD/R-treated neurons served as a cellular model in our study. Western blotting was used to investigate the protein levels of  $\alpha$ -adrenergic receptor ( $\alpha$ -AR) in OGD/R-treated neurons, apoptosis related proteins (Bcl-2, Bax and Cleaved Caspase 3) and a range of proteins associated with the Nrf2/ARE pathway (Nrf2, HO-1, NQO-1, SOD). The CCK-8 assay was used to determine cell survival rates while Co-IP was used to determine the interactions between  $\alpha_2$ -AR and Nrf2. The TUNEL assay was used to detect the levels of apoptosis in neurons.

**Results:** OGD/R treatment reduced the level of  $\alpha_2$ -AR protein in neurons and reduced neuronal survival in a time-dependent manner. However, treatment with dexmedetomidine led to an elevation of  $\alpha_2$ -AR protein expression in OGD/R-treated neurons and the survival rate of OGD/R-treated neurons. These results indicated that dexmedetomidine treatment promoted the viability of OGD/R-treated neurons but inhibited OGD/R-mediated oxidative stress and neuronal apoptosis. From a mechanistic point-of-view, Nrf2 can bind effectively with  $\alpha_2$ -AR. We believe that dexmedetomidine exerted effect on the Nrf2/ARE pathway in OGD/R-treated neurons. Silencing the expression of Nrf2 reversed the effects of dexmedetomidine on cell viability, oxidative stress, and neuronal apoptosis in OGD/R-treated neurons.

**Conclusion:** Collectively, our data indicate that elucidated that the activation of  $\alpha_2$ -AR by dexmedetomidine had a protective effect in neurons against OGD/R-triggered oxidative stress and neuronal apoptosis by modulating the Nrf2/ARE pathway, thus providing a novel way forward to develop clinical therapies to reduce oxidative stress induced by neuronal injury.

## Background

Ischemic stroke is a form of neurological disorder that is caused by an insufficiency in oxygen supply and blood flow restoration, frequently resulting in permanent disability [1, 2]. Following neuronal injury, post-cerebral ischemia can be a significant problem. Over recent years, there has been some progress in the treatment of ischemia and evaluating the progression of patients affected by post-cerebral ischemia. Studies have revealed that a range of mechanisms are involved in post-cerebral ischemia, including oxidative stress, neuronal apoptosis and excitatory toxicity; collectively, these processes can lead to irreversible brain damage [3]. Although strategies for reducing neuronal injury have been investigated, at least to some extent, the prognosis of patients affected by this condition is still not satisfactory. Therefore, it is vital that we investigate the pathological process underlying neuronal injury so that we may identify new treatment strategies for ischemic stroke.

Dexmedetomidine is a potent  $\alpha$ 2-adrenoceptor ( $\alpha$ 2-AR) agonist that has been extensively applied in the intensive care unit (ICU) and operating room (OR) because it is more efficient than most other sedatives or anesthetics [4, 5]. Several lines of evidence have shown that dexmedetomidine is able to play a pivotal part in the treatment of several diseases. For example, dexmedetomidine is known to inhibit inflammation in lipopolysaccharide-induced endotoxemia by regulating the cholinergic anti-inflammatory pathway [6]. Furthermore, dexmedetomidine has been shown to suppress inflammation following renal ischemia reperfusion injury [7]. Moreover, Dexmedetomidine regulates BDNF signaling to alleviate the apoptosis of neurons in kainic acid-induced excitotoxicity [8]. Other research has shown that dexmedetomidine attenuates the apoptosis of neurons following cerebral ischemia-reperfusion injury [9]. Nonetheless, the exact function of dexmedetomidine in glucose deprivation/reoxygenation (OGD/R)-treated neurons, or the mechanisms involved, have yet to be investigated.

NF-E2-related factor 2 (Nrf2) is an pivotal modulator of antioxidants and can balance oxygen free radicals and inflammation within cells [10]. Under conditions involving inflammation and oxidative stress, levels of reactive oxygen species (ROS) begin to increase. In turn, this causes increased oxidation of Keap1, thus causing the release of Nrf2. Following translocation to the nucleus, Nrf2 is capable of binding to the antioxidant response element (ARE) in order to activate the expression of antioxidant genes, including superoxide dismutase (SOD), heme oxygenase-1 (HO-1), and NAD(P)H: quinone oxidoreductase-1 (NQO-1) so as to remove excessive ROS [11, 12]. As oxidative stress can be induced by OGD/R treatment, and can therefore result in neuronal injury, studies have begun to focus on biological molecules that can reduce oxidative stress in OGD/R-treated neurons. Interestingly, numerous lines of evidence indicate that Nrf2/ARE signaling is a vital regulator of oxidative stress in a variety of diseases. For instance, the upregulation of CKIP-1 attenuates high-glucose triggered oxidative stress in human retinal endothelial cells by regulating the Nrf2/ARE signaling pathway [13]. Another study demonstrated that the activation of the Nrf2/ARE pathway suppressed cognitive deficits in a mouse model of Alzheimer's disease by modulating oxidative stress [14]. Other research has indicated that the Nrf2/ARE pathway plays a pivotal role in Parkinson's Disease and could therefore represent a promising target for the development of new therapeutic options for Parkinson's disease [15]. However, the regulatory mechanisms associated with the activation of the Nrf2/ARE pathway activation in OGD/R-treated neurons has not been investigated specifically.

In the present study, we confirmed that dexmedetomidine exerts a protective protect in neurons against OGD/R-triggered oxidative stress and neuronal apoptosis by modulating the Nrf2/ARE pathway. The findings of our study might provide novel insight with which to facilitate the development of new therapies for the reduction of oxidative stress following ischemic stroke.

## Methods

### Cell culture and transfection

Human neurons were obtained from the BeNa Culture Collection (BNCC, Beijing China) and cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, USA) containing 10% fetal bovine serum (FBS) and 1% Penicillin and Streptomycin. Cells were grown at 37°C in 95% air and 5% CO<sub>2</sub>. RNA interfering assays were carried out by transfecting neuronal cells with siRNA designed against Nrf2 (si-Nrf2). Transfection was carried out using a Lipofectamine2000 (Thermo Fisher Scientific, Waltham, USA). The primer sequences were as follows, si-Nrf2-forward-5'-GAGACUACCAUGGUUCCAA (dTdT)-3', si-Nrf2-reverse-5'-UUGGAACCAUGGUAGUCUC (dTdT)-3'; si-NC-forward-5'-CCUACGCCACCAUUUCGU-3', si-NC-reverse-5'-ACGAAAUUGGUGGCGUAGG-3'.

### **Construction of a model for OGD/R**

We constructed an OGD/R model in accordance with previous studies [16]. Neurons (5×10<sup>5</sup> cells/ml) were then incubated at 37°C in 95% air and 5% CO<sub>2</sub> for 24 h. Thereafter, neurons were maintained in glucose-free Dulbecco's Modified Eagle's Medium (DMEM) under hypoxic conditions (3% O<sub>2</sub>, 5% CO<sub>2</sub> and 92% N<sub>2</sub>) for 8 h. Thereafter, the medium was replaced with fresh medium containing glucose (20%). Neurons were then cultured for 24 h under normal conditions. An additional group of neurons were cultured in normal media and under normal conditions to form a control group.

### **The Cell Counting Kit-8 (CCK-8) assay**

Cell survival was evaluated at 24 h post-OGD/R induction by performing a CCK-8 assay. In brief, 1×10<sup>4</sup> cells were seeded into 96-well plates and cultured over night at 37°C. The following morning, 10 µl of CCK-8 solution (Beyotime Biotechnology) was added into each well and cultured at 37°C for another 2 h. Finally, cell survival was calculated by detecting the absorbance at 450 nm with a microplate reader (BMG Labtech GmbH).

### **Co-immunoprecipitation (Co-IP)**

Co-IP assays were conducted using a method that was described previously [17]. In order to perform Co-IP for α2-AR and/or Nrf2 proteins, we used anti-α2-AR and anti-Nrf2 agarose beads to pull down α2-AR and Nrf2, respectively. Mouse or rabbit purified IgGs were used as negative controls. The supernatants were transferred to new microcentrifuge tubes for western blot analysis.

### **Western blot**

For western blotting, neurons were first homogenized in Radio-Immunoprecipitation Assay (RIPA) buffer. Then, equal amounts of total protein were separated by 10% SDS-PAGE and then transferred onto a nitrocellulose membrane. Membranes were then blocked with 5% non-fat milk in Tris-buffered saline containing 0.1% Tween 20 (TBST) and probed with primary antibodies against Nrf2 (ab62352), HO-1 (ab13243), NQO-1 (ab28947), SOD (ab80946), Bax (ab32503), Bcl-2 (ab185002), Cleaved Caspase 3 (ab2302), α2-AR (ab198394) and GAPDH (ab181602). Thereafter, proteins were probed with species-specific HRP-conjugated secondary antibodies. All antibodies were obtained from Abcam company

(USA). Immunoreactive bands were visualized using enhanced chemiluminescence reagents (ECL) and the optical density of each band was quantified by mage Lab™ Software (Bio-Rad, Hercules, CA, USA)

### **The detection of MDA, SOD and GSH-PX**

Specific Activity Assay Kits (Cayman Chemical, USA) were used to determine the levels of MDA, SOD and GSH-Px in neurons. When the assays were complete, the absorbance at 450 nm was read via a spectrophotometric assay and a microplate reader (Syntron, USA).

### **TdT-mediated dUTP Nick-End Labeling (TUNEL) staining**

The extent of apoptosis in neurons was using an In Situ Cell Death Detection Kit (TUNEL fluorescence FITC kit, Roche). Neurons were grown in 24-well culture plates. The neurons were then fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100 for 5 min on ice. Next, 50 µl of the TUNEL reaction was added to the samples and cultured for 60 min at 37°C. The nuclei were then stained with DAPI. TUNEL staining was then visualized by fluorescence microscopy (Leica, Germany).

### **Statistical analysis**

All data analysis involved SPSS version 20.0 (Chicago, IL, USA) and all experiments were carried out in triplicate. Data are shown as mean ± standard deviation (SD). For comparisons between two independent groups, we used the Student's t-test. When comparing differences among three or more groups, we used one-way analysis of variance (ANOVA). Statistical significance was set to  $P < 0.05$ .

## **Results**

### **Dexmedetomidine promotes the cell viability of OGD/R-treated neurons**

Dexmedetomidine was previously identified to play an essential role in the treatment of several diseases. We attempted to ascertain whether dexmedetomidine could help protect neurons against oxidative damage. First, we investigated the expression of  $\alpha_2$ -AR in OGD/R-treated neurons at different time points. Western blotting showed that the levels of  $\alpha_2$ -AR protein decreased significantly in response to OGD/R treatment in a time-dependent manner ( $P < 0.05$  vs control; Figure 1A). CCK-8 assays further showed that cell survival also decreased significantly following OGD/R treatment ( $P < 0.05$  vs control; Figure 1B). Next, we used different concentrations of dexmedetomidine to treat the OGD/R-treated cells and found that dexmedetomidine led to a significant increase in the expression of  $\alpha_2$ -AR protein in OGD/R-treated neurons at different time points ( $P < 0.05$  vs 0 ng/ml; Figure 1C). In addition, the survival rate of OGD/R-treated cells also increased significantly following treatment with dexmedetomidine ( $P < 0.05$  vs 0 ng/ml; Figure 1D). Thus, dexmedetomidine treatment promoted the cell viability of OGD/R-treated neurons.

### **Dexmedetomidine inhibits OGD/R-induced oxidative stress and neuronal apoptosis in OGD/R-treated neurons**

Next, we investigated whether dexmedetomidine exerted an effect on oxidative stress and neuronal apoptosis in OGD/R-treated neurons. First, we determined the levels of MDA, SOD and GSH-PX and found that OGD/R-treatment significantly increased the levels of MDA but decreased the levels of SOD and GSH-PX; however, these effects were abrogated by dexmedetomidine treatment (@P<0.05 vs control; #P<0.05 vs OGD/R+saline; Figure 2A), thus indicating that dexmedetomidine inhibited OGD/R-induced oxidative stress. TUNEL assays further revealed that dexmedetomidine treatment reversed an OGD/R-mediated increase in cell apoptosis (@P<0.05 vs control; #P<0.05 vs OGD/R+saline; Figure 2B). In addition, we determined the levels of several proteins associated with apoptosis (Bcl-2, Bax and Cleaved Caspase-3) by western blotting. Analysis showed that OGD/R triggered the down-regulation of Bcl-2 and the upregulation of Bax and Cleaved Caspase-3; dexmedetomidine treatment abolished these effects (@P<0.05 vs control; #P<0.05 vs OGD/R+saline; Figure 2C). Thus, dexmedetomidine inhibited OGD/R-triggered oxidative stress and neuronal apoptosis in OGD/R-treated neurons.

### **Dexmedetomidine modulates the Nrf2/ARE pathway in OGD/R-treated neurons**

Next, we investigated the mechanisms underlying the actions of dexmedetomidine on OGD/R-treated neurons. The Nrf2/ARE pathway, a pathway shown to play a key role in the inhibition of oxidative stress, was previously implicated with numerous diseases, including OGD/R-induced neuronal injury. We attempted to ascertain whether dexmedetomidine could exert influence on the Nrf2/ARE pathway in OGD/R-induced neuronal injury. The CCK-8 assay showed that treatment with Lir (an activator of the Nrf2/ARE pathway) could reverse the poor viability of the OGD/R-treated neurons (@P<0.05 vs control; #P<0.05 vs OGD/R+DMSO; Figure 3A), implying that the Nrf2/ARE pathway might play a role in OGD/R-induced neuronal injury. Furthermore, the Co-IP assay revealed that  $\alpha$ 2-AR was able to bind with Nrf2 (Figure 3B). Next, we investigated the levels of  $\alpha$ 2-AR protein and a range of proteins associated with the Nrf2/ARE pathway by Western blotting. We found that dexmedetomidine treatment significantly increased the protein levels of  $\alpha$ 2-AR, n-Nrf2, HO-1, NQO-1 and SOD but reduced the protein level of c-Nrf2 in OGD/R-treated neurons (@P<0.05 vs control; #P<0.05 vs OGD/R+saline; Figure 3C), illustrating that dexmedetomidine treatment can activate the Nrf2/ARE pathway. Consequently, our data show that dexmedetomidine can modulate the expression of the Nrf2/ARE pathway in OGD/R-treated neurons.

### **Nrf2 silencing reverses the influence of dexmedetomidine on cell viability, oxidative stress and neuronal apoptosis in OGD/R-treated neurons**

Rescue assays were carried out to ensure that dexmedetomidine was able to modulate cell viability, oxidative stress and neuronal apoptosis in OGD/R-treated neurons by activating the Nrf2/ARE pathway. First, we knocked Nrf2 in neurons; the protein levels of Nrf2 fell significantly following the transfection of neurons with the si-Nrf2 vector (\*P<0.05 vs NC; Figure 4A). Furthermore, the protein levels of HO-1, NQO-1 and SOD increased following treatment with Lir but decreased in line with Nrf2 depletion. We also found that Nrf2 deficiency reversed the effects of Lir treatment and caused an upregulation in the protein levels of HO-1, NQO-1 and SOD (@P<0.05 vs OGD/R + DMSO + NC; #P<0.05 vs OGD/R + Liraglutide + NC; \$P<0.05 vs OGD/R + Liraglutide + si-Nrf2; Figure 4B). In addition, cell survival increased in response to Lir

treatment but decreased when Nrf2 was downregulated. The downregulation of Nrf2 was able to counteract the effects of the Lir treatment-induced enhancement of cell survival (@P<0.05 vs OGD/R + DMSO + NC; #P<0.05 vs OGD/R + Liraglutide + NC; \$P<0.05 vs OGD/R + Liraglutide + si-Nrf2; Figure 4C). We also found that the levels of MDA were lower, while SOD and GSH-PX levels were elevated, following Lir treatment, and that Lir treatment increased the levels of these proteins. Attenuation of Nrf2 was shown to abrogate the effects that Lir had on neurons with respect to the levels of MDA, SOD and GSH-PX levels (@P<0.05 vs OGD/R + DMSO + NC; #P<0.05 vs OGD/R + Liraglutide + NC; \$P<0.05 vs OGD/R + Liraglutide + si-Nrf2; Figure 4D). Finally, the TUNEL assay revealed that Lir treatment alleviated cell apoptosis while Nrf2 silencing enhanced apoptosis. The downregulation of Nrf2 abolished the -induced alleviation of apoptosis (@P<0.05 vs OGD/R + DMSO + NC; #P<0.05 vs OGD/R + Liraglutide + NC; \$P<0.05 vs OGD/R + Liraglutide + si-Nrf2; Figure 4E). Lir caused an increase in the levels of Bcl-2 protein level and a reduction in the levels of Bax and Cleaved Caspase-3 while the suppression of Nrf2 had the opposite effects. Furthermore, we found that the effects of Lir treatment on the protein levels of Bcl-2, Bax and Cleaved Caspase-3 were changed as a result of Nrf2 suppression (Figure 4F). Nrf2 silencing therefore reversed dexmedetomidine-mediated cell viability, oxidative stress and neuronal apoptosis in OGD/R-induced neurons.

## Discussion

Ischemic stroke is a terrible disease that affects millions of people across the world. A large body of evidence now indicates that the pathophysiology of neuronal injury following ischemic stroke is associated with an increased level of apoptosis [18]. Consequently, it is very important that we investigate mechanisms that may be used to inhibit apoptosis in neurons. Previous studies demonstrated that oxidative stress can be induced by OGD/R treatment and that this can subsequently result in neuronal injury [19]; consequently, a range of studies are focusing on identifying biomarkers that might be able to reduce oxidative stress in OGD/R-treated neurons. In the present study, we constructed a cell model of OGD/R to help us to investigate potential biomarkers associated with neuronal injury. We showed that cell survival decreased significantly as a result of OGD/R treatment in a time-dependent manner, thus suggesting that the OGD/R cell model had been successfully established and could be used for further studies.

Dexmedetomidine is widely used in the operating room and ICU and plays a vital role in the treatment of several diseases, including acute lung injury after kidney ischemia reperfusion injury [7], kainic acid-induced excitotoxicity [8], and cerebral ischemia-reperfusion injury [9]. However, the exact effect of dexmedetomidine on OGD/R-treated neurons, and the mechanisms involved, had not been investigated prior to the present study. In this study, we found that the levels of  $\alpha$ 2-AR protein decreased in response to OGD/R treatment in a time-dependent manner, implying that dexmedetomidine might play an essential role in neuronal injury. In addition, dexmedetomidine promoted the viability of OGD/R-treated neurons. More importantly, dexmedetomidine was confirmed to inhibit OGD/R-induced oxidative stress and apoptosis in OGD/R-treated neurons. Taken together, these data indicate that dexmedetomidine is able to protect neurons against OGD/R-triggered oxidative stress and neuronal apoptosis.

Next, we investigated the mechanisms that might be involved with these observations. Numerous previous studies have reported that the Nrf2/ARE signaling pathway is a vital regulator of oxidative stress in a range of diseases, including high-glucose triggered oxidative stress in human retinal endothelial cells [13] and Alzheimer's disease [14]. The Nrf2/ARE pathway is also known to play a pivotal role in Parkinson Disease [15]. However, the exact mechanisms underlying how dexmedetomidine and the Nrf2/ARE pathway cause effect on OGD/R-treated neurons has not been fully explored previously. In the present study, we found that Liraglutide (Lir), an activator of the Nrf2/ARE pathway, could improve cell survival after OGD/R treatment. Moreover, we showed that Nrf2 was able to bind with  $\alpha$ 2-AR. We also showed that dexmedetomidine was able to modulate the expression of proteins associated with the Nrf2/ARE pathway in OGD/R-induced neurons. Finally, we showed that Nrf2 depletion reversed dexmedetomidine-mediated cell viability, oxidative stress, and neuronal apoptosis in OGD/R-treated neurons.

## Conclusions

In summary, this study proved that the activation of  $\alpha$ 2-AR by dexmedetomidine exerted a protective effect on neurons against OGD/R-triggered oxidative stress and neuronal apoptosis by modulating the Nrf2/ARE pathway. Our findings may therefore help us to develop methods to reduce the effects of neuronal injury induced by oxidative stress.

## Abbreviations

OGD/R: oxygen and glucose deprivation/reoxygenation;  $\alpha$ 2-AR:  $\alpha$ 2-adrenoceptor; BNCC: BeNa Culture Collection; DMEM: Dulbecco's modified Eagle's medium; FBS: fetal bovine serum; CCK-8: Cell Counting Kit-8; RIPA: Radio-Immunoprecipitation Assay; TBST: Tris-buffered saline containing 0.1% Tween 20; ECL: enhanced chemiluminescence reagents; Co-IP: Co-immunoprecipitation; TUNEL: TdT-mediated dUTP Nick-End Labeling; SD: standard deviation; Nrf2: NF-E2-related factor 2; c-Nrf2: cytoplasm Nrf2; n-Nrf2: nuclear Nrf2

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Availability of data and materials

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

## Competing interests

None of the authors have any conflicts of interest to declare.

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## Authors' contributions

DMX and CBZ conceived and supervised the study; JYL designed experiments; WHC and WL performed the experiments and analyzed the data; DMX wrote the manuscript. All authors reviewed the results and approved the final version of the manuscript.

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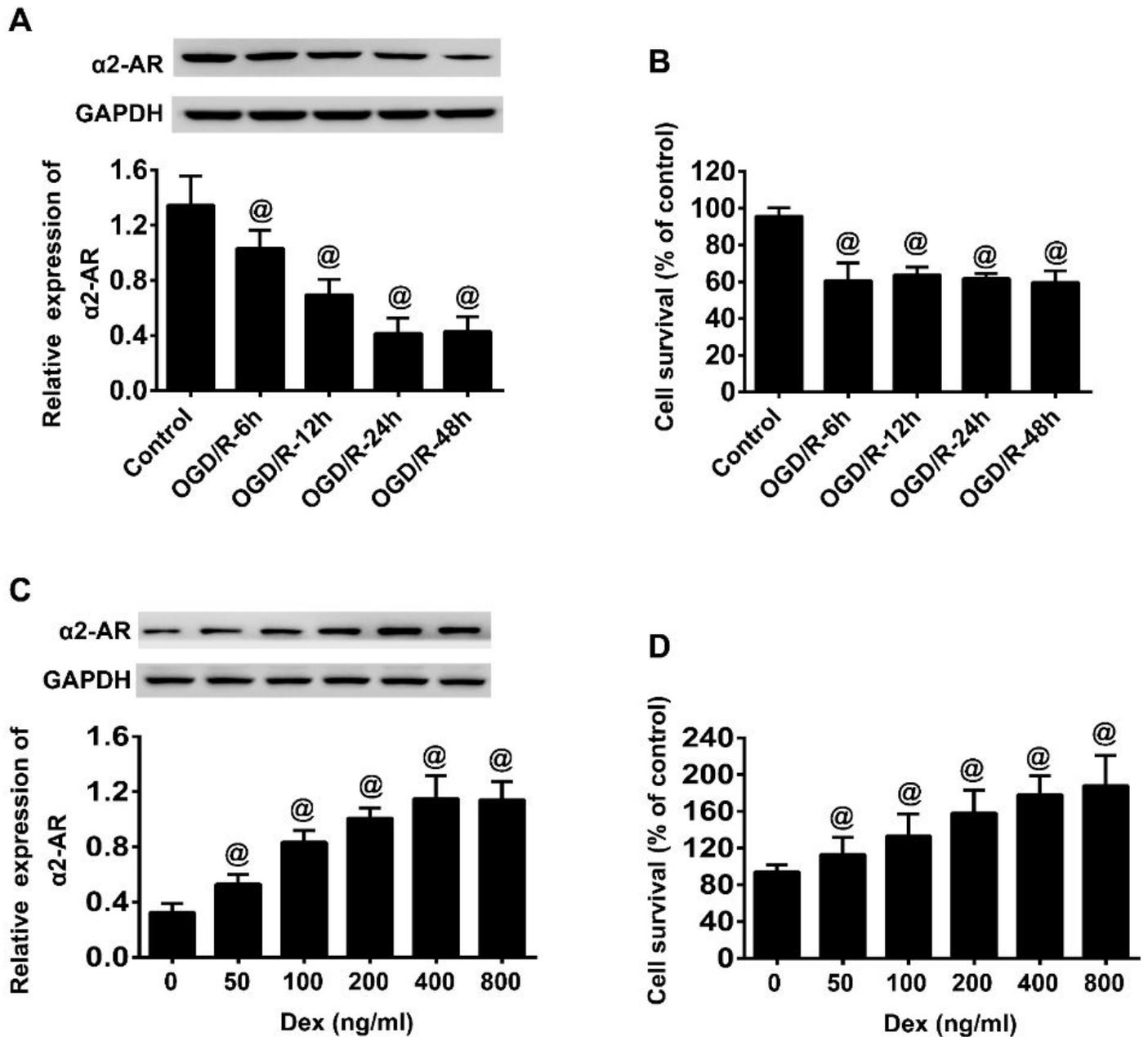
We thank all participators for their help.

## References

1. Murphy TH, Corbett D. Plasticity during stroke recovery: from synapse to behaviour. *Nat Rev Neurosci.* 2009;10:861-72.
2. Sun B, Ou H, Ren F, Huan Y, Zhong T, Gao M, et al. Propofol inhibited autophagy through Ca(2+)/CaMKKbeta/AMPK/mTOR pathway in OGD/R-induced neuron injury. *Mol Med.* 2018;24:58.
3. George PM, Steinberg GK. Novel Stroke Therapeutics: Unraveling Stroke Pathophysiology and Its Impact on Clinical Treatments. *Neuron.* 2015;87:297-309.
4. Lam SW, Alexander E. Dexmedetomidine use in critical care. *AACN Adv Crit Care.* 2008;19:113-20.
5. Gurbet A, Basagan-Mogol E, Turker G, Ugun F, Kaya FN, Ozcan B. Intraoperative infusion of dexmedetomidine reduces perioperative analgesic requirements. *Can J Anaesth.* 2006;53:646-52.
6. Xiang H, Hu B, Li Z, Li J. Dexmedetomidine controls systemic cytokine levels through the cholinergic anti-inflammatory pathway. *Inflammation.* 2014;37:1763-70.
7. 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;55:1272-8.
8. Chiu KM, Lin TY, Lee MY, Lu CW, Wang MJ, Wang SJ. Dexmedetomidine protects neurons from kainic acid-induced excitotoxicity by activating BDNF signaling. *Neurochem Int.* 2019;129:104493.
9. Zhai M, Liu C, Li Y, Zhang P, Yu Z, Zhu H, et al. Dexmedetomidine inhibits neuronal apoptosis by inducing Sigma-1 receptor signaling in cerebral ischemia-reperfusion injury. *Aging (Albany NY).* 2019;11:9556-68.
10. Sandberg M, Patil J, D'Angelo B, Weber SG, Mallard C. NRF2-regulation in brain health and disease: implication of cerebral inflammation. *Neuropharmacology.* 2014;79:298-306.

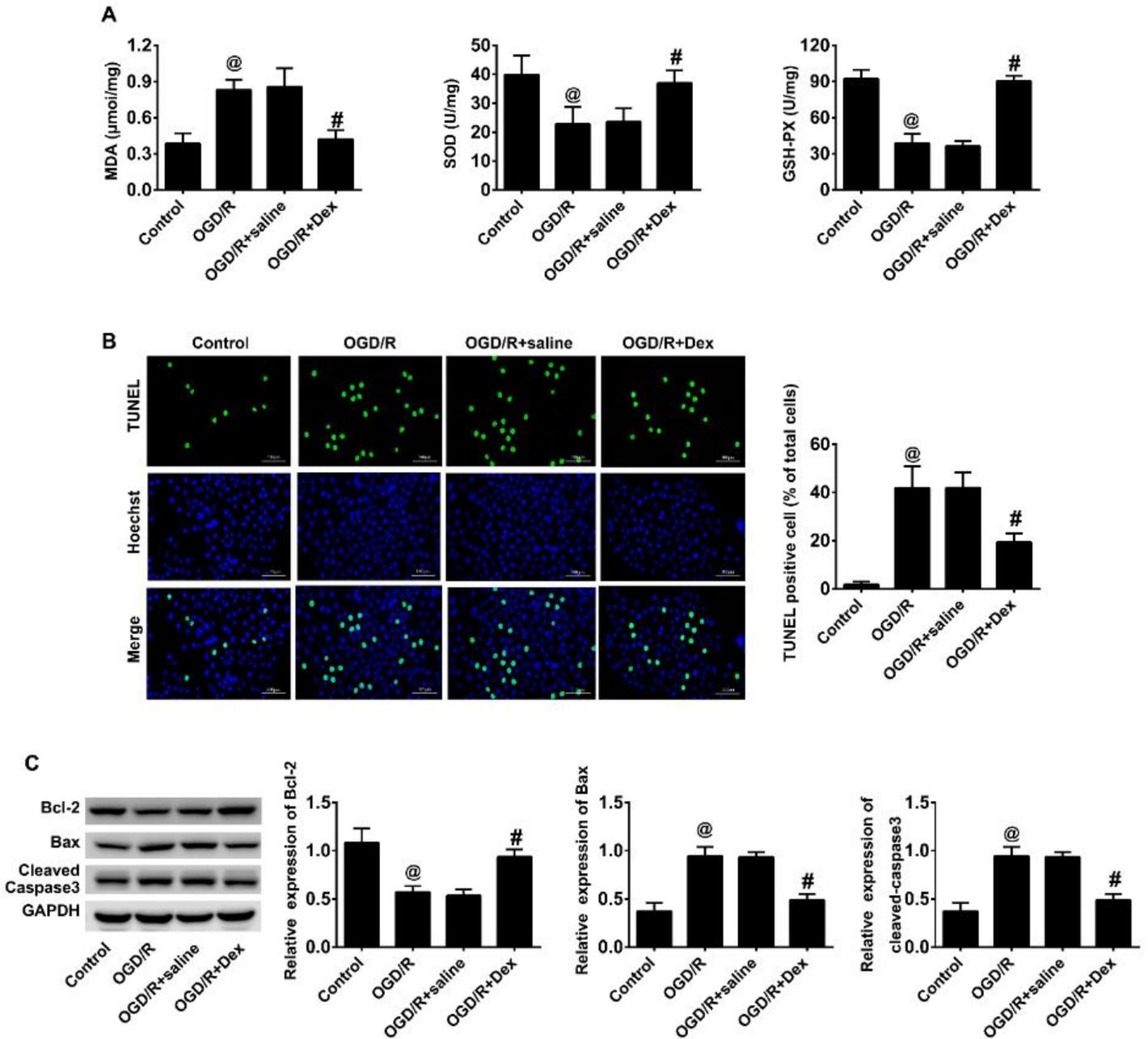
11. Wasik U, Milkiewicz M, Kempinska-Podhorodecka A, Milkiewicz P. Protection against oxidative stress mediated by the Nrf2/Keap1 axis is impaired in Primary Biliary Cholangitis. *Sci Rep.* 2017;7:44769.
12. Xue F, Huang JW, Ding PY, Zang HG, Kou ZJ, Li T, et al. Nrf2/antioxidant defense pathway is involved in the neuroprotective effects of Sirt1 against focal cerebral ischemia in rats after hyperbaric oxygen preconditioning. *Behav Brain Res.* 2016;309:1-8.
13. Zhang L, Yu J, Ye M, Zhao H. Upregulation of CKIP-1 inhibits high-glucose induced inflammation and oxidative stress in HRECs and attenuates diabetic retinopathy by modulating Nrf2/ARE signaling pathway: an in vitro study. *Cell Biosci.* 2019;9:67.
14. Tian Y, Wang W, Xu L, Li H, Wei Y, Wu Q, et al. Activation of Nrf2/ARE pathway alleviates the cognitive deficits in PS1V97L-Tg mouse model of Alzheimer's disease through modulation of oxidative stress. 2019;97:492-505.
15. Gureev AP, Popov VN. Nrf2/ARE Pathway as a Therapeutic Target for the Treatment of Parkinson Diseases. 2019;44:2273-9.
16. Agrawal M, Kumar V, Singh AK, Kashyap MP, Khanna VK, Siddiqui MA, et al. trans-Resveratrol protects ischemic PC12 Cells by inhibiting the hypoxia associated transcription factors and increasing the levels of antioxidant defense enzymes. *ACS Chem Neurosci.* 2013;4:285-94.
17. Liu J, Zhang C, Wang XL, Ly P, Belyi V, Xu-Monette ZY, et al. E3 ubiquitin ligase TRIM32 negatively regulates tumor suppressor p53 to promote tumorigenesis. *Cell Death Differ.* 2014;21:1792-804.
18. Min HM, Wang Y, Ren DY, Cheng X, Li J, Jiang XQ, et al. Protective effect of 2-deoxy-D-glucose on the brain tissue in rat cerebral ischemia-reperfusion models by inhibiting Caspase-apoptotic pathway. *Histol Histopathol.* 2017;32:57-67.
19. Liu X, Zhu X, Chen M, Ge Q, Shen Y, Pan S. Resveratrol protects PC12 cells against OGD/ R-induced apoptosis via the mitochondrial-mediated signaling pathway. *Acta Biochim Biophys Sin (Shanghai).* 2016;48:342-53.

## Figures



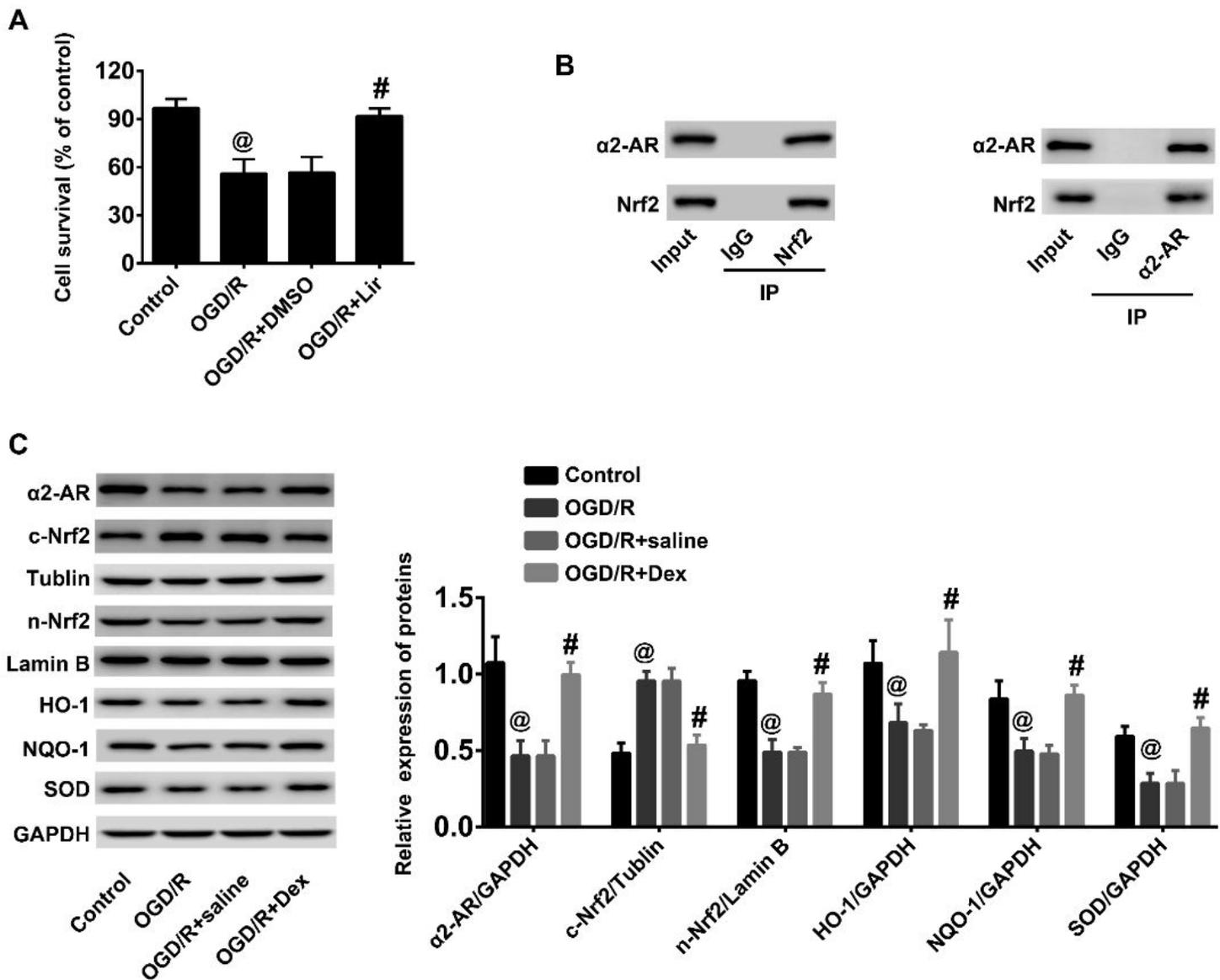
**Figure 1**

Dexmedetomidine promotes the cell viability of OGD/R-treated neurons. (A) Western blot analysis showed that OGD/R treatment led to a reduction in  $\alpha 2$ -AR protein levels in a time-dependent manner. (B) CCK-8 assays showed that cell survival was also significantly decreased as a result of OGD/R treatment. (C) Western blot analysis further revealed that dexmedetomidine treatment significantly increased the expression of  $\alpha 2$ -AR protein in OGD/R-treated neurons at different time points. (D) CCK-8 assays also showed that dexmedetomidine also elevated the survival rate of OGD/R-treated neurons. @P<0.05 vs control in A and B; @P<0.05 vs 0 ng/ml in C and D.



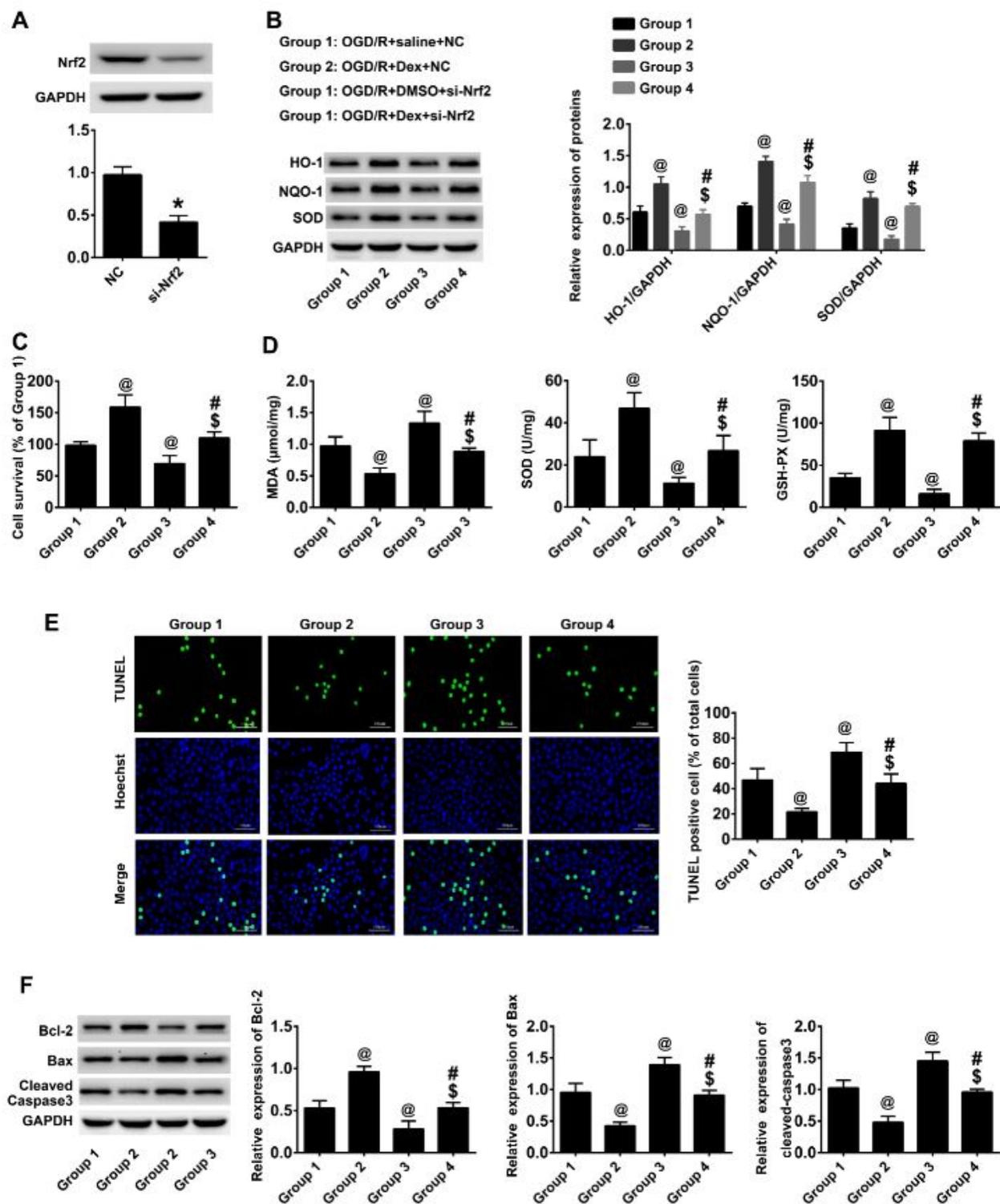
**Figure 2**

Dexmedetomidine inhibits OGD/R-triggered oxidative stress and neuronal apoptosis in OGD/R-treated neurons. (A) OGD/R treatment upregulated the levels of MDA but downregulated the levels of SOD and GSH-PX in neurons; these effects were abrogated by dexmedetomidine treatment. (B) TUNEL assays revealed that dexmedetomidine treatment reversed cellular apoptosis induced by OGD/R treatment. (C) Western blotting showed that OGD/R triggered a down-regulation in Bcl-2 protein levels but upregulated the levels of Bax and Cleaved Caspase-3. Dexmedetomidine treatment abolished these effects. @P<0.05 vs control; #P<0.05 vs OGD/R+saline.



**Figure 3**

Dexmedetomidine modulates the Nrf2/ARE pathway in OGD/R-treated neurons. (A) CCK-8 assays showed that Lir treatment could reverse the reduced cell survival in OGD/R-treated neurons. (B) Co-IP assays revealed that  $\alpha 2$ -AR could bind with Nrf2. (C) Western blotting showed that dexmedetomidine treatment elevated the protein levels of  $\alpha 2$ -AR, n-Nrf2, HO-1, NQO-1 and SOD but reduced the protein levels of c-Nrf2 in OGD/R-treated neurons. <sup>@</sup>P<0.05 vs control; <sup>#</sup>P<0.05 VS OGD/R+saline. c-Nrf2: cytoplasm Nrf2; n-Nrf2: nuclear Nrf2.



**Figure 4**

Nrf2 reverses dexmedetomidine-mediated cell viability, oxidative stress and neuronal apoptosis in OGD/R-treated neurons. (A) Western blotting showed that Nrf2 protein levels decreased following the transfection of OGD/R-treated neurons with si-Nrf2 vector. (B) Western blotting demonstrated that Li treatment caused the upregulation of HO-1, NQO-1 and SOD protein I but reduced the levels of Nrf2. Lir treatment resulted in the upregulation of HO-1, NQO-1 and SOD proteins. (C) CCK-8 assays further revealed that Lir treatment

increased cell survival but downregulated the levels of Nrf2 protein. However, the downregulation of Nrf2 prevented Lir treatment from enhancing cell survival. (D) Specific Activity Assay Kits were used to demonstrate the MDA, SOD and GSH-PX levels changes in different groups. (E) TUNEL assays further showed that Lir treatment alleviated cell apoptosis while Nrf2 silencing enhanced apoptosis. The downregulation of Nrf2 abolished the effects of Lir treatment on cellular apoptosis. (F) Western blotting revealed that the protein levels of Bcl-2 increased following Lir treatment while Bax and Cleaved Caspase-3 decreased; however, the suppression of Nrf2 had the opposite effects. However, the suppression of Nrf2 led to a decreased in Bcl-2 and an increase in the levels of Bax and Cleaved Caspase-3. @P<0.05 vs OGD/R + DMSO + NC; #P<0.05 vs OGD/R + Liraglutide + NC; \$P<0.05 VS OGD/R + Liraglutide + si-Nrf2.

## Supplementary Files

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