Dexmedetomidine preconditioning alleviates H₂O₂-induced oxidative stress and apoptosis in human umbilical vein endothelial cells via p38MAPK signaling pathway

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Abstract

Background: Ischemia-reperfusion injury (IRI) is the main cause of perioperative organ injury, and the morbidity increase constantly in population. Due to a lack of effective treatment, IRI is associated with a high mortality rate. Thus, we need to discover an effective means to alleviate IRI.

Methods: HUVECs were treated with different concentrations of H_2O_2 alone and in combination with Dex to explore the dose-effect relationship of different concentrations of H_2O_2 and Dex on HUVEC. In order to explore the relationship between Dex and p38MAPK signal pathway, HUVEC was treated with SB202190, an inhibitor of p38MAPK signal pathway. Cell viability was detected by CCK-8 method. ELISA kit was used to detect lactate dehydrogenase (LDH). Fluorescence probe DCFH-DA staining was used to detect ROS level. Flow cytometry analysis with Propidium Iodide (PI) was used to determine the rate of cell apoptosis. And the protein expression of p-p38MAPK, total p38MAPK, p-ERK1/2 and caspase9 was detected by western blot.

Results: 1. H_2O_2 could damage HUVEC and lead to the release of LDH. The higher the concentration of H_2O_2 , the more severe the injury. 2. Dex pretreatment attenuated H_2O_2 -induced oxidative stress injury and apoptosis of HUVEC. The cell activity of HUVEC increase as Dex concentration increase, the release of LDH decreased, the intracellular ROS level and apoptosis rate decreased, the protein expression of p-p38MAPK and caspase9 decreased, while the protein expression of p-ERK1/2 increased. 3. Dex had the same effect as SB202190, an inhibitor of p38MAPK signaling pathway. After the application of SB202190, the activity of HUVECs increased significantly, while LDH, intracellular ROS and apoptosis rate decreased significantly. Western blot showed that the protein expression of p-p38MAPK and caspase9 decreased significantly, while the protein levels of p-ERK1/2 increased significantly.

Conclusion: 1. Dex attenuates H_2O_2 -induced oxidative stress injury and apoptosis of HUVEC in a concentration-dependent. 2. Dex may attenuate H_2O_2 -induced oxidative stress injury and apoptosis of HUVEC by inhibiting p38MAPK signal pathway.

Introduction

Ischemia reperfusion injury (IRI) is the major type of cardiovascular and cerebrovascular diseases, usually resulting in death or disability among the middle-aged and older people[1]. As ageing population keeps increasing, the incidence of cardiovascular and cerebrovascular is also included in the rise[2]. In addition, organ transplantation[3], replantation[4, 5]and tourniquet application[6]are become more and more common. These events of IRI not only impact on the prognosis of patients, but also represent a substantial financial burden, in terms of both work productivity loss and health and social care expenditures. Simultaneously, it presents a growing challenge for clinical practices. Consequently, new approaches and targets for preventive treatments of IRI are urgently needed.

Vascular endothelial has been the hot spots for vascular-related disease research in recent years, especially for IRI[7]. A growing body of evidence has indicated that a shift in the functions of the endothelium, including the barrier function, paracrine, proangiogenic and adhesion molecules expression, plays a non-negligible role in IRI[8, 9]. The activation of endothelial cells can lead microcirculation to a dangerous prethrombotic state and their central role in immunoreactive also can exacerbate IRI[10, 11]. Studies have shown that oxidative stress is closely related to endothelial cell dysfunction, inflammation, hypertrophy, apoptosis, cell migration, fibrosis and angiogenesis[12, 13]. Therefore, it is necessary to use vascular endothelial cells as therapeutic targets in IRI, and finding new drugs and targets that can reduce oxidative stress damage of vascular endothelial cells has also become an important research hotspot.

Mitogen-activated protein kinases (MAPK) are a group of serine-threonine protein kinases that can be activated by different extracellular stimuli, such as cytokines, neurotransmitters, hormones, cell stress and cell adhesion. P38 protein is one of the subclasses of MAPKs. The function of p38MAPK is mainly through phosphorylation cascade reaction and activating important transcription factors to respond to different stimuli, and jointly regulate a variety of important cellular physiological / pathological processes, such as cell growth, differentiation, environmental stress adaptation, inflammatory response and so on[14]. Some studies have shown that the activation of p38MAPK signaling pathway can lead to vascular endothelial cells, hepatocytes dysfunction and apoptosis, but also promote cardiomyocyte fibrosis and hypertrophy, participate in the occurrence of cardiovascular diseases such as IRI and ventricular remodeling[15, 16]. When IRI occurs, it can activate NF- κ B and p38MAPK signal pathways, accelerate the production of cytokines such as interleukin (IL) and tumor necrosis factor (TNF) - α , and then promote the expression of adhesion molecules and aggravate IRI[17, 18].

Dexmedetomidine (Dex) is a highly selective α 2-adrenoceptor agonist and has the effects of sedative, anti-anxiety, hypnotic, analgesic and sympathetic. It is mainly used in surgical anesthesia sedation, auxiliary analgesia, diagnosis and human intervention[19]. With the widely application in clinic, Dex has been found to have organ protective effects, which can alleviate IRI via antioxidant stress, anti-apoptosis, anti-inflammation[20, 21]. A prospective, randomized, single-blind study of hepatectomy found Dex can decrease the concentrations of glutathione- α , transferase- S, IL-6 and TNF- α , participate in alleviating IRI after hepatic blood flow occlusion[22]. Furthermore, Fang[23] et al found that Dex treatment significantly improve the survival rate of mouse skin flap by anti-inflammatory and anti-oxidant stress.

Therefore, we constructed a cell-level oxidative stress injury model in vitro to evaluate whether Dex pretreatment protects HUVECs from oxidative stress and to further reveal if p38MAPK signaling pathway is involved in the protective effects of Dex.

Materials And Methods

Cell culture

Human umbilical vein endothelial cells (HUVECs) were recruited from the China Center for Type Culture Collection (kindly donated by the Institute of Cardiovascular Disease, Hengyang Medical College,

University of South China, Hengyang, Hunan, China). The HUVECs were cultured in DMEM (Gibco, USA) medium supplemented with 10% fetal bovine serum (FBS) (Gibco®USA) and 1% penicillin/streptomycin (Gibco®USA) in an incubator which maintain stable operating condition of 37 °C, 95% air and 5% CO₂.

CCK-8 assay

HUVECs were resuspended into single-cell suspension with medium containing 10% FBS, and 100µL cell suspension inoculated into 96-well plates with a density of about 1×10^4 / per well. After the treatment of a specified concentration of H₂O₂ (Sigma®USA), Dex (Sigma®USA) and SB202190 (Abmole®USA), 10µL of CCK-8 (Vazyme®China) was added to the plate and incubated for 4 h at 37 °C. the absorbance at 450nm was measured by enzyme labeling instrument (Molecular Devices®USA). Cell survival rate (%) = $(OD_{450} \text{ of treated group} - OD_{450} \text{ of blank group}) / (OD_{450} \text{ of control group} - OD_{450} \text{ of blank group})$.

LDH detection assay

LDH Cytotoxicity Assay Kit (Abbkine®USA) was used to detect the release of LDH after cell injury. Single-cell suspension of HUVECs (1×10^4 cells/well) were cultured in 96-well plates. Once the experiments were completed, centrifuged 96-well plate 5 min at 400× g and transferred 100µL of supernatant to the a new 96-well plate. Then added 100 µ L LDH Solution to each well and incubated for 30 min at 37 °C. Absorbance at 565 nm was measured. LDH release is proportional to the absorbance.

LDH release rate (%) = $(OD_{565} \text{ of treated group} - OD_{565} \text{ of blank group}) / (OD_{565} \text{ of control group} - OD_{565} \text{ of blank group})$.

Evaluation of endogenous ROS

CheKine™ Reactive Oxygen Species (ROS) Detection Fluorometric Assay Kit (Abbkine®USA) was used to detect the level of endogenous oxidative stress. DCFH-DA (2,7-dichlorodihydro-fluorescein diacetate) can be oxidized by intracellular ROS to produce fluorescent DCF. More fluorescent DCF led to higher level of ROS. After the experiments were completed, removed the cell culture medium and washed the cells with serum-free medium for 2 times. DCFH-DA with a final concentration of 10 µ M was added to the 96-well plate and incubated 30 min at 37 °C without light. The fluorescence images were taken under fluorescence microscope (Olympus®Japan). Image J software (V1.8.0.112) was used to measure fluorescence intensity.

Apoptosis assay

HUVECs (1×10^6 cells/well) were plated on 6-well plate. After the treatment of a specified concentration of the corresponding drugs, the treated HUVECs was stained with Annexin V-FITC/PI Apoptosis Detection Kit (Vazyme®China) and detected by flow cytometry (BD,USA), then. All the steps were carried out in accordance with the manufacturer's instructions.

Western blot assay

Cells were collected after the treatment of the corresponding drugs and lysed in a RIPA Lysis Buffer (Epizyme, China) with 1% protease inhibitor (Abmole, USA). Protein concentrations were measured using BCA assay (Vazyme, China), Separated proteins by gel electrophoresis and constant current transferred onto polyvinylidene fluoride (PVDF) membranes. Proteins were blocked with 5%BSA (Epizyme, China) for 1 h and cultured overnight at 4°C with the corresponding primary antibodies—total p38MAPK rabbit antibody (1:1000—Abclonal—USA)—p-p38MAPK rabbit antibody (1:1000—Abclonal—USA)—caspase9 rabbit antibody(1:1000—Beyotime—China)—p-ERK1/2 mouse antibody(1:1000—Beyotime—China)—β-actin mouse antibody(1:1000—Beyotime—China). And then cultured with HRP-labeled goat anti-mouse IgG(H+L) or HRP-labeled goat anti-rabbit IgG(H+L) (1:2000—Beyotime—China) for 1 h. The protein bands were visualized by ECL™ Light Chemiluminescence Kit (Epizyme, China) and ChemiDoc MP Imaging System (Bio-Rad—USA) was used as chemiluminescence reader. Image J software was used to analysis the mean grey value of western blot bands and Adobe Illustrator CC 2019 software was used to crop and make pictures.

Statistical analysis

All data are expressed as mean ±standard deviation. Statistical analyses were conducted using one-way analysis of variance (one-way ANOVA, GraphPad Prism8) and then compared by t-test. P < 0.05 was statistically significant. All the experiments were repeated 3 times.

Results

Dex preconditioning attenuates H₂O₂-induced cytotoxicity in HUVECs

To verify the effects of H₂O₂ on HUVECs, various concentrations of H₂O₂ (100-700μM) were used to treat normal HUVECs. After exposed to H₂O₂ for 12 h, the results showed H₂O₂ can induce HUVECs injure—the higher the concentration of H₂O₂, the stronger the cytotoxicity. The concentration of H₂O₂ needed for 50% inhibition (IC₅₀) was about 550μM (**Figure 1A**). Furthermore, after pretreatment for 2 h with various concentrations of Dex (0.1-20μM), the cell viability was tested using the CCK-8 method. The results suggested Dex (0.1-20μM) is nontoxic and Dex pretreatment did not have a significant effect on the cell viability of HUVECs (**Figure 1B**).

To investigate the effects of Dex on the cell viability of HUVECs under H₂O₂-treated oxidative stress, HUVECs were pretreated with varying concentrations of Dex (0.1-20μM) for 2h followed by exposure for 12 h with 550μM H₂O₂. The results showed Dex pretreatment can attenuate H₂O₂-induced cytotoxicity in HUVECs and the cell viability of HUVECs were elevated as the concentration of Dex increased (**Figure 1C**). As the results of those experiment we chose a Dex concentration of 1μM and 10μM for subsequent experiments and a H₂O₂ concentration of 550μM, respectively.

Dex preconditioning attenuates the oxidative stress injure mediated by elevated cellular ROS levels of HUVECs in a dose-dependent manner

To investigate the effects of various concentrations of Dex preconditioning on oxidative stress injury of HUVECs, HUVECs were pretreated with 1 μ M and 10 μ M Dex for 2 h and then exposed to 550 μ M H₂O₂ for 12 h. DCFH-DA staining observations with fluorescence microscopy showed that H₂O₂ treatment significantly increased ROS generation in HUVECs, whereas Dex preconditioning reduced intracellular ROS generation and oxidative stress treated with H₂O₂ (**Figure 2B, C**). LDH release assay showed H₂O₂ caused significant increases in increment in LDH release, which indicated increased cells damage. After Dex pretreated, there was striking downregulation of LDH release (**Figure 2A**). In addition, those results also showed that pretreatment with Dex attenuates the oxidative stress injury mediated by elevated cellular ROS levels of HUVECs in a concentration-dependent manner (**Figure 2**).

Dex preconditioning attenuates H₂O₂-induced apoptosis and inhibits p38MAPK phosphorylation of HUVECs in a dose-dependent manner

Next, we evaluated the effects of various concentrations of Dex preconditioning on H₂O₂-induced apoptosis in HUVECs. After the treatment of a specified concentration of the corresponding drugs, the treated HUVECs were stained with Annexin V-FITC/PI and detected by flow cytometry. The results showed H₂O₂ treatment produced an obvious increase in apoptosis. While Dex preconditioning significantly reduced apoptosis compared with the H₂O₂ group and the effect of anti-apoptosis rose with increasing the concentration of Dex (**Figure 3A, B**). Furthermore, we also explored whether Dex exerted effects on the expression of apoptosis-related proteins. As shown in **Figure 3C, D**, the expression of caspase-9 increased significantly after H₂O₂ treatment and the expression of p-ERK1/2 decreased significantly, whereas 1 μ M and 10 μ M Dex pretreatment partially reversed H₂O₂-induced the expression of apoptosis-related proteins. The higher the concentration of Dex, the stronger the effect of reversible. These experiments demonstrated Dex preconditioning attenuates H₂O₂-induced apoptosis of HUVECs in a concentration-dependent manner.

To investigate the effects of Dex preconditioning on p38MAPK signaling pathway, we detected the expression of p-p38MAPK and total p38MAPK by Western blotting, and found that the expression of total p38MAPK remained stable in all groups. The protein expression of p-p38MAPK increased significantly in H₂O₂ group, while Dex pretreatment significantly inhibited phosphorylation of p38MAPK. We also found that Dex (10 μ M) had a stronger inhibitory effect than Dex (1 μ M) (**Figure 3E**).

SB202190 pretreatment attenuates the oxidative stress injury mediated by elevated cellular ROS levels of HUVECs

To further validate potential mechanisms of the effects of Dex in attenuating oxidative stress injury, HUVECs were pretreated with Dex (10 μ M) or p38MAPK inhibitor SB202190 (5 μ M) for 2 h or 10 min, and then exposed to 550 μ M H₂O₂ for 12 h. Our results revealed that both Dex and SB202190 pretreatment significantly alleviated the cellular ROS levels (**Figure 4C, D**) and LDH release (**Figure 4B**). The cytoprotective effects of Dex and SB202190 against oxidative stress injury were also confirmed

by the CCK-8 assay (**Figure 4A**). In addition to this, we also found that Dex and SB202190 combination therapy have a stronger protective effective than alone. These results therefore suggest that SB202190 pretreatment attenuates the oxidative stress injure mediated by elevated cellular ROS levels of HUVECs.

SB202190 inhibits p38MAPK phosphorylation and attenuates H₂O₂-induced apoptosis

Next, we also evaluated the effects of SB202190 on H₂O₂-induced apoptosis and p38MAPK signaling pathway in HUVECs. After treated with SB202190, p38MAPK phosphorylation (**Figure 5E**) and H₂O₂-induced apoptosis (**Figure 5A, B**) of HUVECs significantly decreased. Apoptosis-related proteins caspase9 expression also significantly decreased (**Figure 5C**), whereas the expression of p-ERK1/2 had a significant increase (**Figure 5D**). Furthermore, comparing with the Dex or SB202190 group, p-p38MAPK expression and apoptosis rate were remarkable reduced in the Dex and SB202190 combination group. Altogether, SB202190 inhibits p38MAPK phosphorylation and attenuates H₂O₂-induced apoptosis and Dex had a similar influence as SB202190.

Discussion

Ischemia reperfusion injury (IRI) is a common complication in the perioperative period and always causes perioperative patients to organ dysfunction and poor prognosis due to the lack of effective prevention and treatment. Vascular endothelial cells are the first-line defense cells in IRI. Previous studies have shown that the barrier function, paracrine, expression of adhesion molecules and angiogenesis of vascular endothelial cells play an important role in the regulation of IRI[13, 24]. Endothelial cell dysfunction is a complex pathophysiological event, in which oxidative stress is the key component. Some studies have shown that oxidative stress plays an important role in the development of IRI through ROS-mediated damages, such as endothelial dysfunction, inflammation, hypertrophy, apoptosis, cell migration, fibrosis and angiogenesis, and vascular remodeling caused by hypertension[12, 13]. Therefore, the protective therapy based on vascular endothelial cells may become a promising direction for the prevention and treatment of perioperative IRI, and the search for new drugs and targets that can reduce oxidative stress injury of vascular endothelial cells has also become an important research hotspot.

In the process of exploring the prevention and treatment of IRI, the potential targets and preventive benefits of drugs based on narcotic drugs have become the subject of a large number of studies. So far, many narcotic drugs have been shown to have a protective effect—including Dex[21]—propofol [25]—sevoflurane [26] and sufentanil [27], etc. Among them, Dex has attracted the attention of many researchers. Dex is a highly selective α_2 -adrenoceptor agonist, which is widely used in the clinical. Currently, Dex has appeared in a variety of off-label application, such as examination of sedation (intranasal or oral administration)[28, 29] and as an analgesic adjuvant[30]. Furthermore, Dex is also widely used in medical research and has been applied in other clinical settings. It has been found that Dex can reduce the oxidative stress and apoptosis LPS-induced by improving the activity of superoxide dismutase, thereby attenuating the acute kidney injury in septicemic mice[21]. It can also alleviate the oxidative stress injury of cardiomyocytes by reducing the level of ROS[31]. Other studies have found that

cardiac surgery patients who receive Dex infusion can achieve better short-term and long-term survival and reduce the incidence of postoperative cognitive impairment[32]. However, it has not been clearly whether Dex can alleviate the oxidative stress injury of vascular endothelial cells. Consequently, the current study sought to evaluate the effect of Dex on vascular endothelial cells under oxidative stress.

In this study, the H_2O_2 -induced cell model of oxidative stress was established to mimic the IRI in vitro. Our study showed the cytotoxic effect of H_2O_2 on HUVECs in a dose-dependent manner. After treated with $550\mu M H_2O_2$ for 12 h, the cultured HUVECs showed obvious oxidative stress injury, the cell activity significantly decreased and the LDH release increased a lot. Which is also consistent with previous work, but the concentration of H_2O_2 was slightly different. The reason may be due to differences in cell type, complexity (comparison of isolated cells / tissues with the entire organism), or time of exposure to H_2O_2 . Previous studies showed human melanocytes induced with $400\mu M H_2O_2$ for 24 hours will cause membrane blistering and cell contraction, resulting in cell death[33], while cardiomyocytes require only 12h with $500\mu M H_2O_2$ [31]. In addition, we also found that the intracellular ROS level, apoptosis rate and apoptosis-related protein caspase9 expression of HUVECs significantly increased after treatment with $550\mu M H_2O_2$, but lead to a decrease in ERK1/2 phosphorylation. It is well known that oxidative stress is the result of the imbalance between ROS and antioxidant defense system. Existing studies have proved that, physiologically concentrations of H_2O_2 plays an important role in the signal transduction of oxidative stress. Higher concentration of H_2O_2 will lead to oxidative stress adaptation through NF- κ B pathway, while super-physiological concentration of H_2O_2 will cause a series of pathological processes such as lipid peroxidation, nucleic acid damage and inhibition of enzyme activity, which will change the molecular structure and function of cells, and eventually lead to growth arrest, apoptosis and necrosis. It may also cause aseptic inflammation and microvascular dysfunction[34, 35]. ERK1/2 is a survival-related kinase, which requires pre-ERK1/2 nuclear accumulation and ERK1/2-mediated gene transcription in the process of anti-apoptosis[36]. In recent studies, it has also been found that ERK1/2 signal transduction is involved in the regulation and expression of a variety of apoptotic proteins, which can control the promotion of apoptosis and anti-apoptosis[37]. Inhibiting the phosphorylation of ERK1/2 protein can effectively reduce cardiomyocyte apoptosis and treat myocardial hypertrophy and heart failure[38]. Therefore, this study and the above studies have proved that apoptosis is closely related to oxidative stress injury, and apoptosis is a certain outcome in the process of oxidative stress.

Next, we also observed that the phosphorylation of p38MAPK signal pathway in HUVECs was significantly enhanced after H_2O_2 treatment. Many studies have shown that oxidative stress is closely related to p38MAPK signaling pathway. High levels of ROS can activate p38MAPK phosphorylation and mediate cell senescence or death. Conversely, activated p38MAPK can further increase ROS[39, 40].

When we pretreated HUVECs with $1\mu M$ and $10\mu M$ Dex, the results showed that the cell activity of HUVECs in Dex pretreatment group increased, the concentration of LDH in culture medium decreased, and the intracellular ROS level and apoptosis decreased significantly, and this effect was enhanced with the increase of Dex concentration. Western blot assay also showed that the phosphorylation of p38MAPK in

HUVECs was inhibited and the phosphorylated of ERK1/2 was enhanced in Dex pretreatment group compared with H₂O₂ group. Our results are consistent with previous studies[41], which confirmed that Dex can reduce oxidative stress injury and apoptosis of HUVECs by inhibiting p38MAPK phosphorylation. In addition, we also compared Dex with p38MAPK signaling pathway inhibitor SB202190. After the application of SB202190, the activity of HUVECs increased significantly, while LDH, intracellular ROS and apoptosis decreased significantly. Western blot results showed that the expression of p-p38MAPK decreased significantly, while the expression of p-ERK1/2 increased significantly. In other studies, it has been found that Dex has a similar function to SB202190, which can alleviate hypoxia / reoxygenation injury of cardiomyocytes by inhibiting p38MAPK and downstream apoptosis signal pathway[42]. These results strongly suggest that Dex has the same effect as SB202190, and further confirm that Dex can protect HUVECs from oxidative stress by inhibiting p38MAPK pathway.

Conclusion

Altogether, our results suggest that Dex can inhibit p38MAPK signaling pathway to reduce H₂O₂-induced oxidative stress injury and apoptosis of HUVECs. Perioperative application of Dex may be an effective strategy for prevention and treatment of IRI. The identification of p38MAPK phosphorylation as the target of IRI will also inspire many studies on the targeting strategies of p38MAPK in physiological and pathological processes. However, our research also has some shortcomings, which is only limited to in vitro cell experiments, which needs to be confirmed by more animal and clinical trial.

Declarations

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

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SL and HZ have contributed equally to this work and share first authorship. SL, BW and JL conceived and designed the study, SL wrote the manuscript. SL, JL, HZ, CZ, DX, QZ, WL and NL analyzed the data. HZ revised the manuscript. BW contributed to project supervision. All authors have read and approved the manuscript.

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Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Conflict of interest statement

The author declares that they have no competing interests.

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Figures

Figure 1

Effects of different concentrations of Dex or H₂O₂ on cell viability. (A) Cell viability of different concentrations of H₂O₂-induced HUVECs. IC₅₀ is expressed as the concentration inhibiting 50% of cell viability. (B) Cell viability of different concentrations of Dex pretreatment. (C) Cell viability of different concentrations of Dex pretreatment in H₂O₂-induced HUVECs. ****P<0.0001 vs Control #P<0.05 vs H₂O₂ +P<0.05 vs Dex(1μM)

Figure 2

Effects of different concentrations of Dex pretreatment on oxidative stress injure induced by H₂O₂. (A) LDH release rate (B) Fluorescence microscopy images stained with DCFH-DA. (C) Mean fluorescence quantification stained with DCFH-DA. **P<0.01, ***P<0.001 vs Control #P<0.05, ##P<0.01 vs H₂O₂ +P<0.05 vs Dex(1μM)

Figure 3

Effects of different concentrations of Dex pretreatment on apoptosis and the protein expression levels induced by H₂O₂. (A and B) Flow cytometry analysis of apoptosis. (C) The expression levels of caspase9. (D) The expression levels of p-ERK1/2. (E) The expression levels of p-p38MAPK and total p38 MAPK. **P < 0.01, ***P < 0.001 vs Control; #P < 0.05, ##P < 0.01 vs H₂O₂; +P < 0.05, ++P < 0.01 vs Dex(1μM)

Figure 4

Effects of inhibition of p38MAPK signaling pathway on oxidative stress injure induced by H₂O₂. (A) Cell viability after pretreated with p38MAPK inhibitor. (B) LDH release rate. (C) Fluorescence microscopy images stained with DCFH-DA. (D) Mean fluorescence quantification stained with DCFH-DA. ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$ vs Control; # $P \leq 0.05$, ## $P \leq 0.01$ vs H₂O₂; + $P \leq 0.05$ vs Dex(10µM)

Figure 5

Effects of inhibition of p38MAPK signaling pathway on apoptosis and the expression levels of p-p38MAPK and total p38 MAPK induced by H_2O_2 . (A and B) Flow cytometry analysis of apoptosis. (C) The expression levels of caspase9. (D) The expression levels of p-ERK1/2. (E) The expression levels of p-p38MAPK and total p38 MAPK. ** $P \leq 0.01$, *** $P \leq 0.001$ vs Control; # $P \leq 0.05$, ## $P \leq 0.01$ vs H_2O_2 ; + $P \leq 0.05$ vs Dex(10 μ M)