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ORIGINAL ARTICLE

Dexmedetomidine alleviates hepatic injury via the inhibition of oxidative stress and activation of the Nrf2/HO-1 signaling pathway

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ABSTRACT. Background: **Dexmedetomidine (Dex), frequently used as an effective sedative, was reported to play a critical role in the protection of multiple organs. However, its underlying mechanism of a putative protective effect on ischemia/reperfusion (I/R)-induced liver injury is still unclear. Methods**: **A hepatocyte injury model was established by treating WRL-68 cells with oxygen and glucose deprivation/reoxygenation (OGD/R). Enzyme Linked Immunosorbent Assay (ELISA) kits were used to determine the level of inflammatory factors (IL-6, IL-1β, and TNF-α), and oxidative stress indicators (ROS, MDA, GSH-Px, and SOD). MTT assay and flow cytometry analysis were used to determine the influence of Dex on cell viability and cell apoptosis. Expression of nuclear factor erythroid-derived 2- like 2 (Nrf2), HO-1, and apoptosis-related proteins (Bax, Bcl-2, caspase3, and caspase9) were detected by qRT-PCR and western blotting. Results**: **Dex promoted cell viability and suppressed cell apoptosis in OGD/R-treated WRL-68 cells. Dex reduced TNF-α, IL-6, IL-1β, ROS, and MDA production, whereas it increased that of SOD and GSH-Px in OGD/R-treated WRL-68 cells. Moreover, Nrf2, HO-1, and Bcl-2 expression was upregulated, whereas, in contrast, transcripts for Bax, caspase3, and caspase9 were downregulated following Dex treatment under OGD/R. Knockdown of Nrf2 reversed the Dex effects on cell proliferation, apoptosis, and expression of TNF-α, IL-6, IL-1β, ROS, MDA, SOD, and GSH-Px. Conclusion**: **Dex protects WRL-68 cells against OGD/R-induced injury by inhibiting inflammation, oxidative stress, and cell apoptosis via the activation of Nrf2/HO-1 signaling pathway, suggesting that Dex may be a potential protector against hepatic injury.**

Key words: Dexmedetomidine, hepatic injury, inflammation, oxidative stress, Nrf2/HO-1 signaling pathway

INTRODUCTION

The liver is one of the most easily injured organs of human body in blunt or penetrating abdominal trauma and ischemia/reperfusion (I/R), due to its anterior location in the abdomen, large size in the abdominal cavity, and fragile parenchyma [1, 2]. Data have shown that hepatic injuries were accounting for more than 5% of all trauma admissions, and it significantly contributed to the mortality [3, 4]. I/R-induced injury, defined as the process in which injury exerts in initial hypoxia followed by the recovery of oxygen supply, is a common injury that may be caused by organ transplantation, resection, and other clinical manipulates [5, 6]. According to the American Association for the Surgery of Trauma Hepatic Injury Scale, minor hepatic injuries made up the majority of liver trauma with more than 80% being grade I or II [3]. However, mortality increase with the grade of hepatic injury, and hepatic injury being grade VI was frequently considered fatal [7]. Up to now, hepatic injuries were still the leading cause of death in abdominal trauma, accounting for approximately 10% to 15% mortality rate [3]. Oxidative stress, defined as an unbalance between the production and elimination of free radicals, frequently results from the accumulation of reactive oxygen species (ROS), damaged antioxidant system, and dysregulation of mitochondrion [8]. Accumulating evidences have shown that oxidative stress may pose a secondary injury to organ injuries by promoting cell

Abbreviations

Dex	dexmedetomidine
ELISA	enzyme linked immunosorbent assay
GSH-Px	glutathione peroxidase
I/R	ischemia/reperfusion
NC	negative control
Nrf2	nuclear factor erythroid-derived 2-like 2
Nrf2	nuclear factor erythroid 2-related factor 2
OGD/R	oxygen and glucose deprivation/reoxygenation
qRT-PCR	quantitative real-time PCR
SOD	superoxide dis-mutase

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apoptosis, suggesting its important roles in the therapy of organ injuries [9]. Li H et al. reported that anwulignan protected against D-galactose-induced hepatic injury by increasing the activities of superoxide dis-mutase (SOD) and glutathione peroxidase (GSH-Px) via MAPK/p38 signaling pathway [10]. Moreover, Zhang et al. demonstrated that taraxasterol protected against ethanol-induced liver injury by attenuating the inflammatory response via decreasing the production of TNF-α and IL-6 [11]. This implied that inflammatory response and ROS-mediated oxidative stress might play an important role in liver injury.

Dexmedetomidine (Dex), a highly selective α_2-adrenergic receptor agonist, is an adjuvant that frequently used anesthesia in clinical practice [12, 13]. Due to its properties in sedation, analgesia, and anti-inflammation, Dex exhibited protective functions in various human diseases, such as posttraumatic stress disorder, and acute lung injury [14-16]. In recent years, increasing *in vitro* and *in vivo* studies reported that Dex might exhibit potential protective effects on multiple organ injuries, such as brain, kidney, lung, and heart injury [17-19]. For instance, Dex showed a significant neuroprotective effect on traumatically injured hippocampal cells [18]. Previous studies also demonstrated that Dex showed protective effects on hepatic injuries [19], but its potential mechanisms remain unknown.

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a critical transcription factor that is responsible for cellular redox homeostasis. Once activated by oxidative stress agents, Nrf2 would dissociate from its endogenous suppressor Keap-1 and translocated into the cell nucleus, and finally activating the transcriptional process of defense-related targeted genes [20]. It was reported that Nrf2 could participate in the protective effects against foam cells formation by modulating the production of antioxidant proteins (HO-1 and Prxs), ATP-binding cassette efflux transporters, and scavenger receptors [21]. The triterpenoid CDDO-imidazolide was demonstrated to ameliorate liver I/R injury by activating Nrf2/HO-1 pathway [22]. Furthermore, Nrf2 pathway was reported to mediate the reducing effects of Dex on I/R injury in skeletal muscle [23]. However, the regulatory relationship between Dex and Nrf2/HO-1 pathway in liver injury is still unclear.

In this study, we demonstrated that Dex protected hepatocyte against OGD/R-induced injury by reducing inflammation and oxidative stress, thereby decreasing cell apoptosis. In mechanism, Nrf2/HO-1 signaling pathway was revealed to mediate the protective effects of Dex on OGD/R-induced hepatocyte injury. Our findings revealed a critical role of Dex in ameliorate of I/R-induced injury and it might be a potential protector in hepatic injury.

METHODS

Cell culture and transfection

The normal human hepatocytes WRL-68 were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA), and maintained in the RPMI-1640 (GIBCO, Grand Island, NY), which supplemented with 1% penicillin/streptomycin and 10% fetal bovine serum (FBS, Invitrogen, Carlsbad, USA). WRL-68 cells were transfected with sh-Nrf2 (purchased from GeneChem Corp., Shanghai, China) or negative control (NC) using Lipofectamine 2000 (Invitrogen, USA) according to the manufacturer's instruction.

Establishment of the cell model of I/R injury

WRL-68 cells were first treated with 0.01 nM or 0.1 nM Dex for 1 h, and then the I/R injury cell model was performed by oxygen and glucose deprivation/reoxygenation (OGD/R). WRL-68 cells were first washed twice with phosphate-buffered saline and then cultured with OGD medium (glucose-free and serum-free RPMI-1640) under hypoxia conditions (5% CO_2, 1% O_2, and 94% N_2) at 37 for 4 h, followed by re-oxygenation (5% CO_2, 95% O_2) and the medium was replaced with a regular medium for 8 h.

ELISA assay

The productions of TNF-α, IL-6, IL-1β, MDA, SOD, and GSH-Px in the supernatant fluid of WRL-68 cells were determined by corresponding commercial kits according to the instructions obtained from the manufacturers (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

MTT assay

MTT assay was applied to detect the cell viability of treated WRL-68 cells. In brief, cells were seeded into 96-well plates at a density of 3×10^3 cells/well, and cultured overnight at 37 with 5% CO_2 and 95% O_2. Then the cells were incubated with 30 μL of MTT reagent (5 mg/mL) and maintained for a further 4 h. After being treated with 100 μL dimethyl sulfoxide for 15 min, the 96-well plates were analyzed by a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA) at 490 nm.

Apoptosis analysis

Flow cytometry analysis was used to analyze the apoptosis of treated WRL-68 cells. Briefly, the cells were harvested by trypsinization and washed with cold PBS for three times. Subsequently, the cells were stained with Annexin V-fluorescein isothiocyanate (FITC, R&D Systems Inc, Minneapolis, USA) and propidium iodide (PI, R&D Systems Inc, USA) for 15 min in the dark. Finally, the cells were subjected to apoptosis analysis with a Becton-Dickinson FACS Calibur Flow Cytometer.

Measurement of intracellular ROS (DCFH-DA assay)

Intracellular ROS levels of treated WRL-68 cells were evaluated by the OxiSelect Intracellular ROS Assay Kit (Cell Biolabs, STA342, USA) according to the instructions provided by the manufacturers. In brief, the cells were first incubated for 5 min with a cell permeable fluorescent 2′, 7′-dichlorodihydrofluorescin diacetate (DCFH-DA), which can be degraded into

non-fluorescent 2′, 7′-DCFH. The DCFH could be oxidized by cellular ROS into fluorescent dichlorodihydrofluorescin (DCF). Thus, the fluorescent intensity is proportional to the intracellular ROS levels. And then the formation of DCF was detected by DCF fluorescence intensity recorded at 520 nm via a Spectramax M5 Microplate Reader (Molecular Devices, Pennsylvania, USA). Results were presented as DCF AFU (arbitrary fluorescence unit of DCF).

Quantitative real-time PCR (qRT-PCR)

Total RNAs of WRL-68 cells were prepared using TRIzol reagent (Takara,Japan). A 2 μg aliquot of total RNAs was applied to reverse transcribed into cDNA viaTakara RT kit following the protocols provided by the manufacturer. qRT-PCR was performed using an ABI Prism 7700 sequence detection system (PE Applied Biosystems, California, USA) with protocols shown as follows: 95 °C, 4 mins; 35 cycles of 95 °C 25 s, 62 °C 40 s, and 72 °C 90 s; 72 °C 10 mins. Sequence of primers used in the present study was shown as follows: β-actin: forward 5′-TGGCACCCAGCACAATGAAGA-3′, reverse 5′-GAAGCATTTGCGGTGGAC GAT-3′; Nrf2: forward 5′-TGAGCCCAGTATCAGCAACA-3′, reverse 5′-AGTGAAATGCCGGAGTCAGA-3′; HO-1: forward 5′-GCTCAAAAAGATTG CCCAGA-3′, reverse 5′-GCTCTGGTCCTTGGTGTCAT-3′. β-actin was used as the internal control, and the expression of each gene was calculated using the $2^{-\Delta\Delta CT}$ method.

Western blot analysis

Proteins of WRP-68 cells were extracted using RIPA buffer (0.5 M Tris, 250 Mm NaCl, 0.1% Nonidet P-40, 0.2 M Na3VO4, 0.2 M NaF) containing the protease inhibitor (Roche, Mannheim, Germany). The concentration of extracted proteins was determined by a BCA kit (Pierce, Rockford, USA). Subsequently, 30 μg of total proteins were isolated by 10% SDS-PAGE. Targeted proteins were then electrophoretic transferred into polyvinylidene difluoride (PVDF) membranes (Millipore, Bedfordshire, UK). The nonspecific binding sites were blocked by incubating membranes with 5% skimmed milk at room temperature for 2 h. Primary antibodies that against Nrf2 (Rabbit, 1:3000, ab62352, abcam, UK), HO-1 (1:2000, ab13243, abcam, UK), Bax (1:5000, ab32503, abcam, UK), Bcl-2 (1:2000, ab182858, abcam, UK), caspase3 (1:500, ab13847, abcam, UK), caspase9 (1:5000, ab32539, abcam, UK), and GAPDH (1:3000, ab62352, abcam, UK) were incubated with membranes overnight at 4. After being washed with TBST twice, membranes were incubated with horseradish peroxidase conjugated donkey-anti-rabbit secondary antibodies (IgG-HRP, ab6802, Abcam, 1:2000, UK) for 2 h. The signals were visualized using the enhanced chemiluminescent reagents (ECL, Germany).

Statistical analysis

All the experiments in this study were carried out for three times. Data were expressed as mean ± standard deviation (SD), and analyzed using Graghpad (GraphPad Prism Software, USA). Student's t test (two tailed) was utilized to compare the difference between the two groups, and one-way analysis of variance was used for multiple groups comparison, followed by the Tukey post hoc test. A P value of less than 0.05 was considered statistically significant.

RESULTS

Dex increased cell viability and inhibited apoptosis in OGD/R treated WRL-68 cells.

We first established a cell injury model by treating WRL-68 cells with OGD/R condition. In the cell viability and apoptosis analysis, OGD/R treated WRL-68 cells exhibited a significant down-regulation of cell viability (*figure 1A*), and a remarkable up-regulation of cell apoptosis (*figure 1B-C*). However, these effects could be abolished by the application of Dex, and high dose group (0.1 nM) exhibited better reversed effects than low dose group (0.01 nM) (*figure 1A-C*). These results demonstrated that Dex might prevent liver injury by increasing hepatocyte viability and inhibiting apoptosis.

Dex inhibited inflammation and oxidative stress caused by OGD/R in WRL-68 cells.

The expression of inflammatory factors (TNF-α, IL-1β, and IL-6) was significantly higher in OGD/R treated WRL-68 cells, indicating that inflammation played a critical role in I/R-induced hepatic injury (*figure 2A-C*). However, their expression levels were significantly reduced by the application of Dex under OGD/R conditions (*figure 2A-C*). In addition, the expression levels of ROS and MDA were significantly up-regulated while antioxidase SOD and GSH-Px were remarkably down-regulated in the OGD/R-induced hepatocyte injury model group compared with normal group, suggesting severe redox imbalance in I/R-mediated liver injury (*figure 2D-G*). However, the up-regulation of ROS and MDA and the down-regulation of SOD and GSH-Px were reversed by the application of low and high dose of Dex (*figure 2D-G*), illuminated that Dex might alleviate I/R-induced oxidative stress damage by elevating the antioxidase activity. These results clearly showed that Dex might attenuate I/R-induced liver injury by inhibiting the release of inflammatory factors and ROS-mediated oxidative stress.

Dex activated Nrf2/HO-1 signaling pathway in OGD/R-treated WRL-68 cells.

To investigate whether Nrf2/HO-1 signaling pathway involved in the protective effects of Dex on WRL-68 cells, we measured the relative mRNA and protein expression of Nrf2 and HO-1 by qRT-PCR and western blot assays, respectively. Results showed the relative mRNA and protein expression of Nrf2 and HO-1 were significantly down-regulated in the OGD/R-induced hepatocyte injury model group compared with normal group (*figure 3A-D*). Furthermore, this OGD/R-induced down-regulation of Nrf2 and HO-1 was reversed in the Dex-treated groups, and high dose group exhibited better reversed effects than low dose group (*figure 3A-D*). In addition, Dex could reverse the reduced expression of Bcl-2 and increased expression

of Bax, caspase3, and caspase9 induced by OGD/R in hepatocytes (*figure 3E-F*), indicating Dex could decrease OGD/R-induced cell apoptosis by inhibiting caspase signaling pathway.

Knockdown of Nrf2 attenuated the protective effects of Dex on hepatocytes against I/R injury.

Next, Nrf2 knockdown was carried out to determine the exact roles of Nrf2 in OGD/R-induced hepatocyte injury. The knockdown efficiency of Nrf2 was evaluated by qRT-PCR, results showed that Nrf2 expression was significantly down-regulated in sh-Nrf2-treated cells compared to those cells treated with sh-NC (*figure 4A*). Dex significantly inhibited OGD/R-induced apoptosis and decreased cell viability (*figure 4B-D*). However, knockdown of Nrf2 could reverse this change, suggesting that Dex could play a role in inhibiting liver injury by activating Nrf2 signaling.

Furthermore, the up-regulation of IL-6, IL-1β, and TNF-α induced by OGD/R was significantly abolished by Dex in WRL-68 cells, but knockdown of Nrf2 markedly attenuated the abolished effects of Dex (*figure 5A-C*). In addition, knockdown of Nrf2 could significantly reverse down-regulation of ROS and MDA, as well as antioxidase up-regulation caused by Dex (*figure 5D-G*). These results demonstrated that Dex could inhibit inflammatory response and oxidative stress damage in liver injury by activating Nrf2 signaling pathway.

Dex inhibited apoptosis-associated protein expression by activating Nrf2/HO-1 signaling pathway in OGD/R-treated WRL-68 cells.

As shown in *figure 3A-3D*, the down-regulation of Nrf2 and HO-1 induced by OGD/R was significantly reversed by the application of Dex; however, these reversed effects of Dex were significantly attenuated in the sh-Nrf2-treated group (*figure 6A-D*). Additionally, Dex could inhibit apoptosis-associated proteins expression of caspase3, caspase9, and Bax induced by OGD/R in WRL-68 cells, but knockdown of Nrf2 significantly reversed this condition (*figure 6E-F*). These results implied that Dex inhibited apoptosis-associated caspase pathway by activating Nrf2/HO-1 signaling pathway, thereby alleviating OGD/R-induced hepatocyte apoptosis.

DISCUSSION

Liver injuries, often occuring in the blunt and penetrating abdominal trauma as well as I/R-induced injury caused by liver transplantation or resection, are

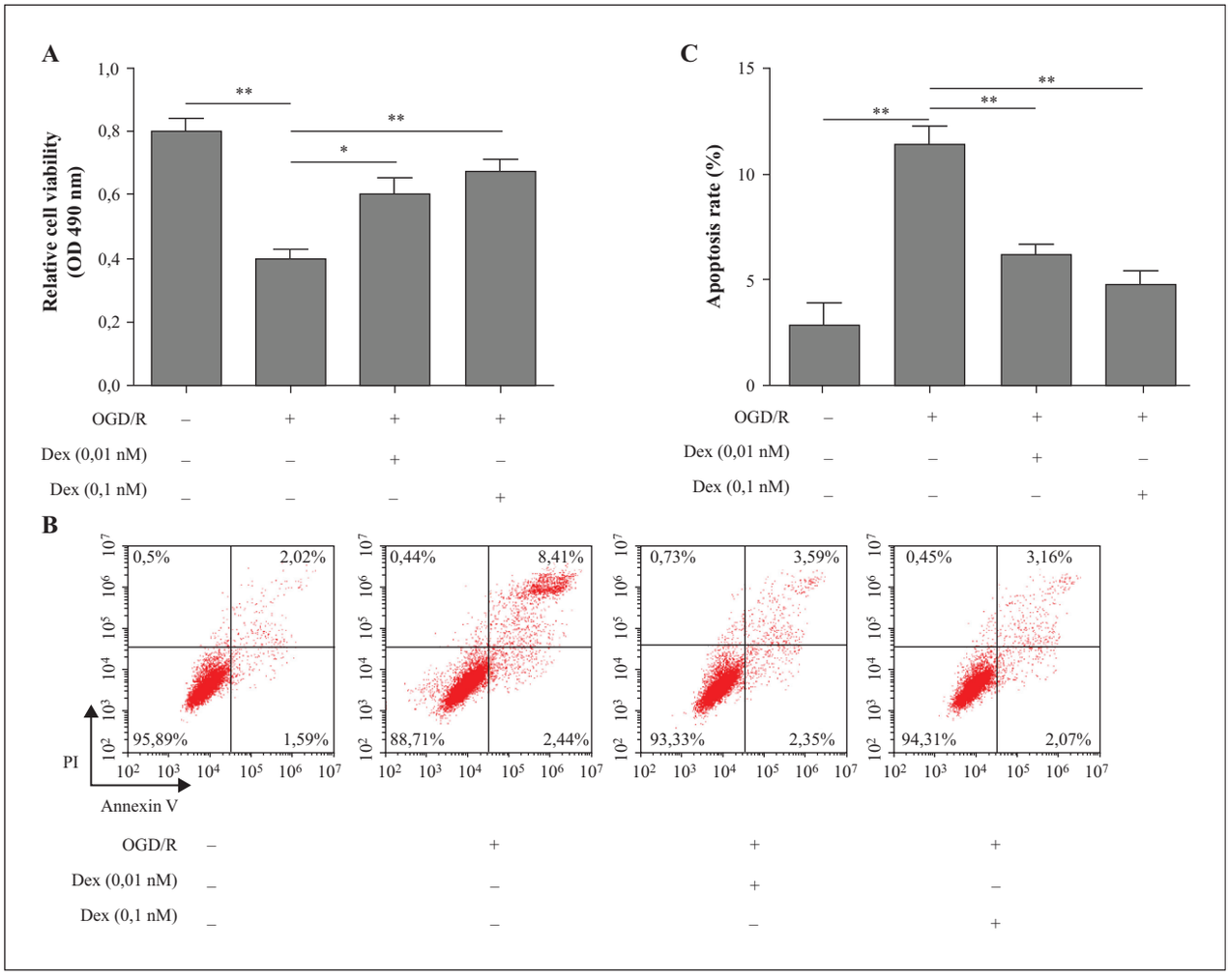

Figure 1

Dex increased cell viability and inhibited cell apoptosis under OGD/R condition. (A) MTT assay was used to examine the viability of WRL-68 cells treated with OGD/R or Dex. (B) Representative pictures of WRL-68 cell apoptosis measured by flow cytometry. (C) Apoptosis cells were quantified. All the results were shown as mean ± SD (n = 3), which were three separate experiments performed in triplicate. * $p < 0.05$ and ** $p < 0.01$.

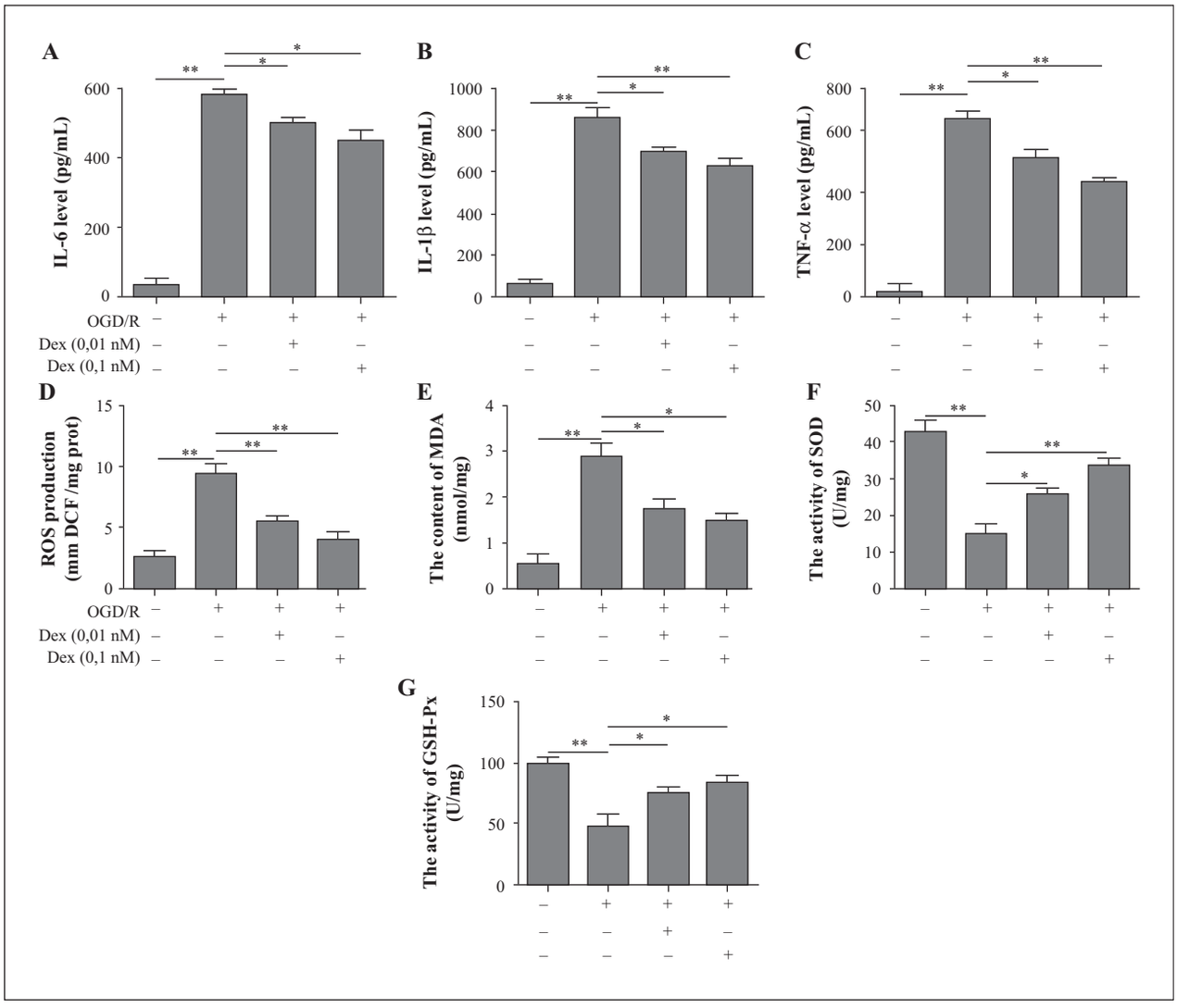

Figure 2
Dex attenuated inflammation and oxidative response in OGD/R-treated WRL-68 cells. ELISA assay was used to detect the release level of IL-6 (A), IL-1β(B), TNF-α (C), ROS (D), MDA (E), SOD(F), and GSH-Px (G) in WRL-68 cells treated with OGD/R or Dex. All the results were shown as mean ± SD (n = 3), which were three separate experiments performed in triplicate. * $p < 0.05$ and ** $p < 0.01$.

a leading cause of liver disease-related death [3]. The protective effects of Dex were well documented in a wide range of organ systems in accumulating preclinical experiments, and Dex has already exhibited promise in protecting organs against injuries in various animal models of organ injuries [19, 24, 25]. Recently, Dex was also demonstrated to show protective effects on hepatic injury that was induced by partial hepatectomy and I/R [19, 26, 27]. However, the potential protective mechanisms of Dex in liver injury remain largely undetermined. In the present study, we explored the influence of Dex on inflammatory response, oxidative stress, cell apoptosis, and proliferation in the hepatocyte injury model induced by OGD/R. The results demonstrated that Dex could inhibit inflammation, oxidative stress, and cell apoptosis by activating Nrf2/HO-1 signaling pathway, thereby protecting hepatocyte against OGD/R injury.

In the liver I/R injury, the return of blood supply may lead to the release of inflammatory cytokines and excessive ROS-mediated oxidative stress damage, which could damage the structure and function of liver, and even lead to liver failure [24]. The generated ROS could be cleared by endogenous anti-oxidant enzyme such as SOD and GSH-Px. The pathophysiology of liver injuries is extremely complex, and elucidating the underlying mechanisms contributes to the development of novel protective measures. Owing to the inhibitory effects on inflammatory response and oxidative stress, Dex was reported to exhibit protective effects on multiple organ injuries by regulating multiple cellular inflammatory cytokines and oxidative factors [19]. In our study, Dex was found to reverse the OGD/R-induced up-regulation of TNF-α, IL-1β, IL-6, ROS, and MDA, as well as the down-regulation of GSH-Px and SOD in WRL-68 cells, indicating that Dex might protect liver from I/R-induced injury by inhibiting the release of inflammatory cytokines and increasing the activity of antioxidant enzymes.

Apoptosis is an irreversible cellular process, which occurs in multiple pathophysiological contests, such as

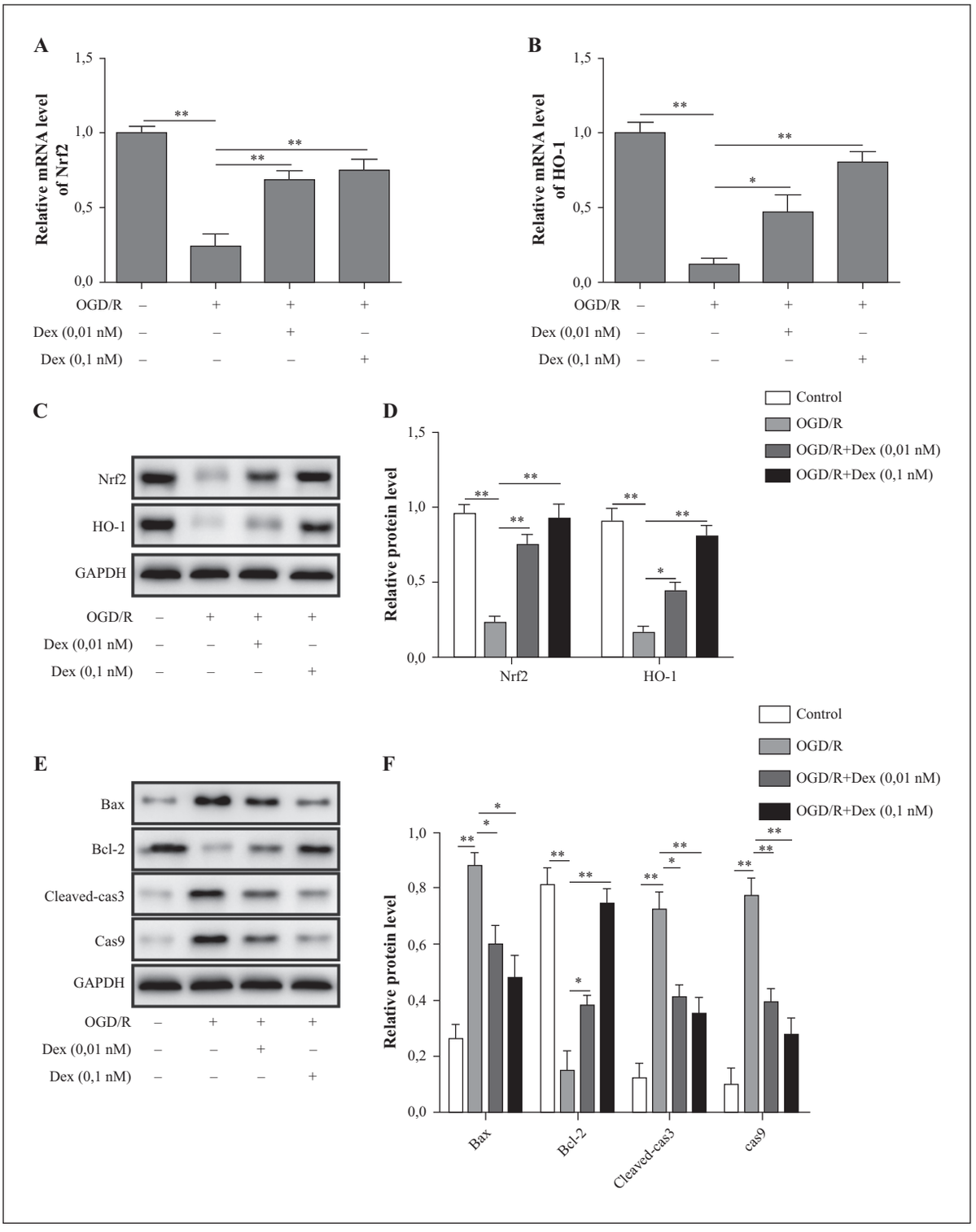

Figure 3

Dex activated Nrf2/HO-1 pathway and inhibited caspase pathway in OGD/R-treated WRL-68 cells. The relative mRNA expression levels of Nrf2 (A) and HO-1 (B) were measured by qRT-PCR in WRL-68 cells treated with OGD/R or Dex. (C) The protein level of Nrf2 and HO-1 was detected via western blot assay. (D) Quantitative analysis of protein band gray in *figure 3C*. (E) The protein levels of Bax, Bcl-2, caspase3, and caspase9 were measured by western blot assay in WRL-68 cells treated with OGD/R or Dex. (F) Quantitative analysis of protein band gray in *figure 3E*. GAPDH was used as internal control. All the results were shown as mean ± SD (n = 3), which were three separate experiments performed in triplicate. * $p < 0.05$ and ** $p < 0.01$.

hypoxia, toxicity, and trauma. Cell apoptosis that occurs in liver diseases frequently results in long-term alterations in organ structure and functions, affecting the outcomes of various hepatic diseases [26]. Evidences suggested that excessive inflammatory response and oxidative stress occurred in damaged hepatocyte could activate multiple cellular signaling pathways that lead to apoptosis, including caspase and Bcl-2 [27]. Previous studies demonstrated that Dex could reduce hepatocyte apoptosis against OGD/R-induced injury by inhibiting caspase3 signal in HL7702 cells [28]. Moreover, Dex protected against

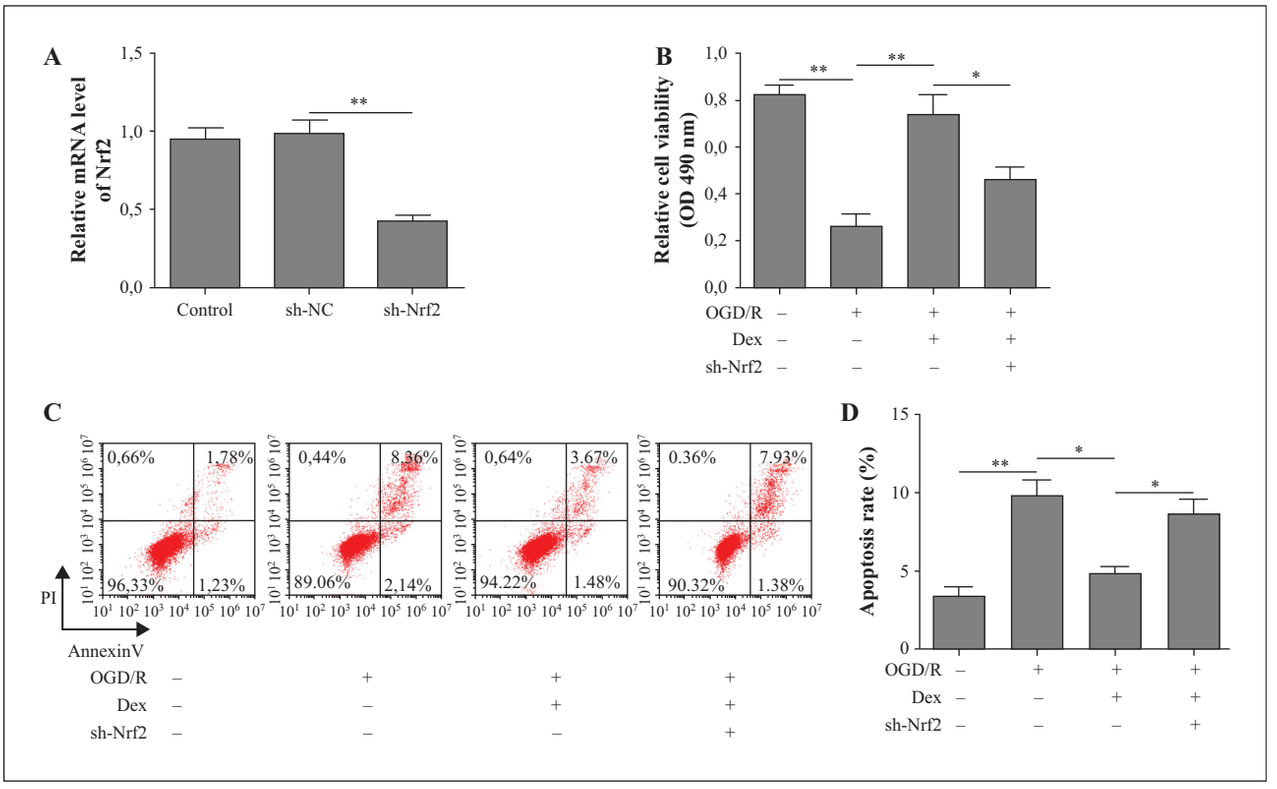

Figure 4

Nrf2 knockdown partially abolished OGD/R-induced cell viability suppression and apoptosis. (A) The relative mRNA expression of Nrf2 was evaluated by qRT-PCR in WRL-68 cells treated with sh-Nrf2. (B) MTT assay was used to examine the viability of WRL-68 cells treated with OGD/R, Dex or sh-Nrf2. (C) Representative pictures of WRL-68 cell apoptosis measured by flow cytometry. (D) Apoptosis cells were quantified. All the results were shown as mean ± SD (n = 3), which were three separate experiments performed in triplicate. * $p < 0.05$ and ** $p < 0.01$.

hepatic I/R injury in NLRC5-deficient mice through suppression of apoptosis by inhibiting inflammation and oxidative stress [15]. In the present study, we also found that Dex could alleviate OGD/R-induced apoptosis and inhibit the expression of Bax, caspase3, and caspase9 while increasing Bcl-2 expression in WRL-68 cells, indicating that Dex might exhibit its inhibitory effects on OGD/R-induced cell apoptosis

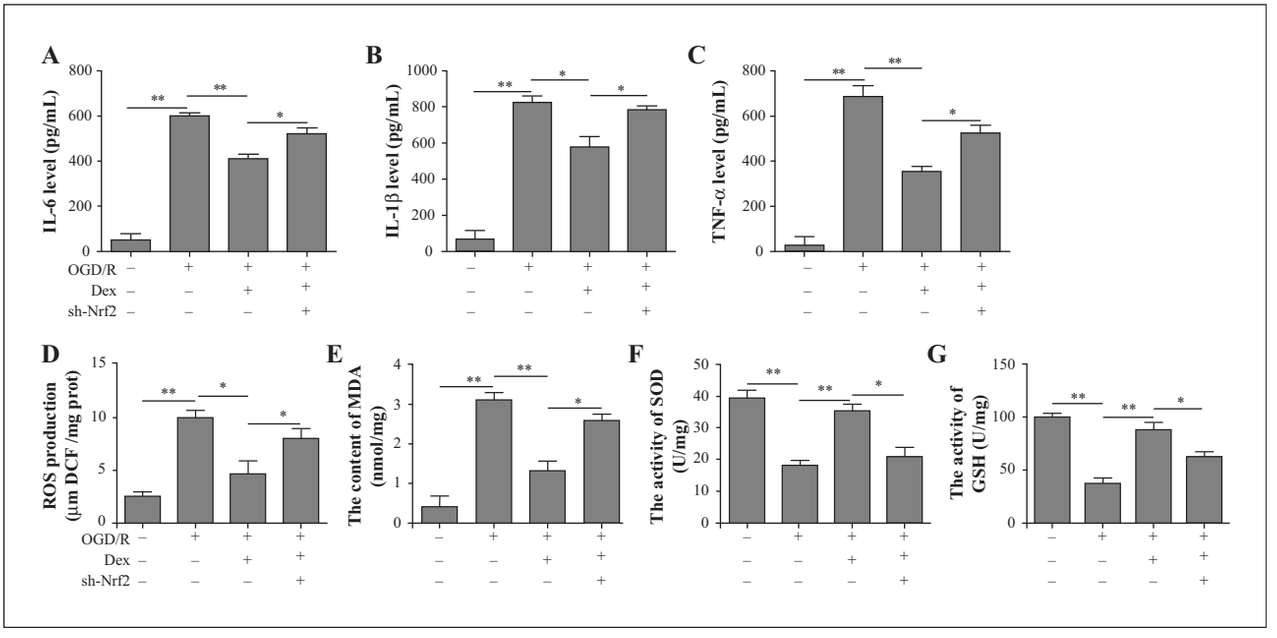

Figure 5

Nrf2 knockdown reversed the effects of Dex on inflammation and oxidative response in OGD/R-treated WRL-68 cells. ELISA assay was used to detect the release level of IL-6 (A), IL-1β (B), TNF-α (C), ROS (D), MDA (E), SOD (F), and GSH-Px (G) in WRL-68 cells treated with OGD/R, Dex, or sh-Nrf2. All the results were shown as mean ± SD (n = 3), which were three separate experiments performed in triplicate. * $p < 0.05$ and ** $p < 0.01$.

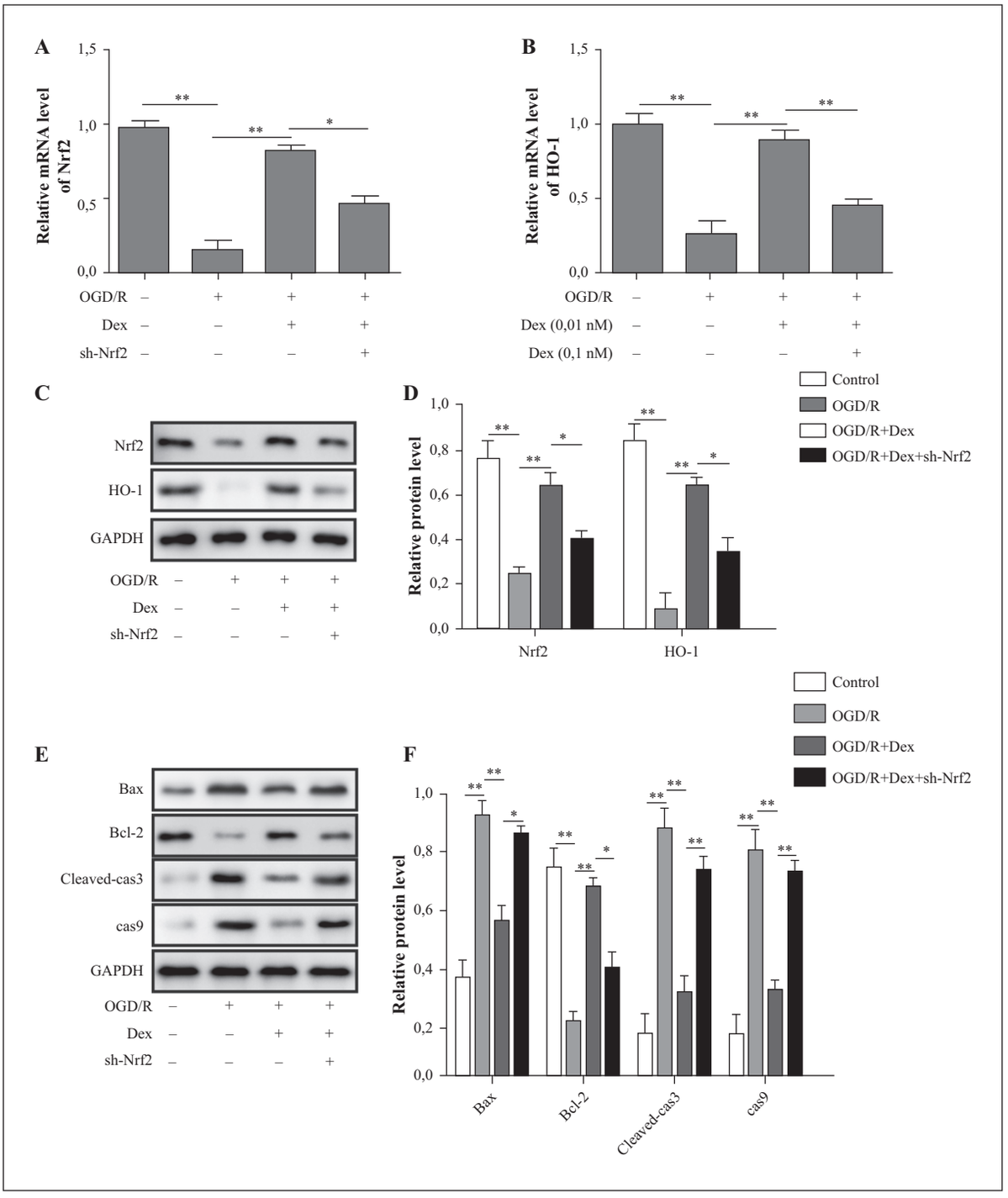

Figure 6

Nrf2 knockdown attenuated the effects of Dex on apoptosis-related proteins in OGD/R-treated WRL-68 cells. The relative mRNA expression levels of Nrf2 (A) and HO-1 (B) were measured by qRT-PCR in WRL-68 cells treated with OGD/R, Dex, or sh-Nrf2. (C) The protein level of Nrf2 and HO-1 was detected via western blot assay in WRL-68 cells treated with OGD/R, Dex, or sh-Nrf2. (D) Quantitative analysis of protein band gray in *figure 6C*. (E) The protein levels of Bax, Bcl-2, caspase3, and caspase9 were measured by western blot assay in WRL-68 cells treated with OGD/R, Dex, or sh-Nrf2. (F) Quantitative analysis of protein band gray in *figure 6E*. GAPDH was used as internal control. All the results were shown as mean ± SD (n = 3), which were three separate experiments performed in triplicate. * $p < 0.05$ and ** $p < 0.01$.

by suppressing apoptosis-related caspase signaling pathway.

Nrf2 initiated the transcription of multiple cytoprotective genes under oxidative stress [28]. It played as a primary defensive molecule against the hepatotoxicity caused by multiple chemicals [29]. For example, Ginsenoside Rg1 was demonstrated to protect against acetaminophen-induced hepatic injury by increasing Nrf2 nuclear translocation [30]. Umbelliferone was demonstrated to alleviate hepatic injury by suppressing inflammatory response and promoting Nrf2-mediated antioxidant via increasing the production of SOD in diabetic db/db mice [31]. Moreover, salvianolic acid A was revealed to abolish the upregulation of MDA, TNF-α, IL-1β, and IL-6 induced by I/R treatment by activating Nrf2/HO-1

signaling pathway [32]. In the present study, Dex could protect hepatocytes from OGD/R-induced damage by activating the Nrf2/HO-1 signaling pathway, and knockdown of Nrf2 could attenuate this protective effect of Dex on hepatocytes. These results fully suggested that Dex alleviated OGD/R-induced hepatocyte injury by activating Nrf2/HO-1 signaling pathway.

In conclusion, we show that Dex protected against I/R caused hepatocyte injury by inhibiting caspase signaling-mediated cell apoptosis. Furthermore, Dex can alleviate OGD/R-induced inflammatory response and oxidative stress by decreasing inflammatory cytokines level of TNF-α, IL-1β, and IL-6 and increasing the activity of antioxidant factors of SOD and GSH-Px. Additionally, the protective effects of Dex on hepatocytes injury were demonstrated to be Nrf2/HO-1 dependent. These findings reveal that Dex might act as a protective agent during hepatic injury by activating Nrf2/HO-1 signaling pathway to alleviate hepatic injury.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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