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ORIGINAL RESEARCH

Dexmedetomidine Attenuates Cellular Injury and Apoptosis in H9c2 Cardiomyocytes by Regulating p-38MAPK and Endoplasmic Reticulum Stress

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Background: Myocardial ischaemia-reperfusion injury (IRI) has been confirmed to induce endoplasmic reticulum stress (ERS) when myocardial cell function continues to deteriorate to a certain degree. The clinical applications of effective tested strategies are sometimes inconsistent with the applications evaluated in experiments, although reasonable mechanisms and diverse signalling pathways have been broadly explored. Dexmedetomidine (DEX) has been shown to attenuate IRI of the heart in animal studies. This study aimed to determine whether DEX can protect injured cardiomyocytes under hypoxia/reoxygenation (H/R) at the cellular level and whether the mechanism is related to ERS and the p38 MAPK pathway.

Methods: H9c2 cells were subjected to H/R or thapsigargin (TG) to build a model. DEX or 4-PBA was added to the medium either 1 h or 24 h before modelling, respectively. Model parameters were determined by assessing cell viability and injury, which were measured by assessing cell counting kit-8 (CCK8), lactate dehydrogenase (LDH) release and flow cytometry results, and the expression of GRP78, CHOP and caspase-12. In addition, the protein expression of p38MAPK and p-p38MAPK was examined, and SB202190, a negative regulator, was also preincubated in medium.

Results: Compared to that of cells in the control group, the activity of cells in the H/R and TG groups was decreased dramatically, and the LDH concentration and proportion of apoptotic cells were increased. DEX could correspondingly reverse the changes induced by H/R or TG. Additionally, DEX effectively attenuated ERS defined as increased expression of GRP78, CHOP and caspase-12. Additionally, DEX could obviously depress the P38 MAPK phosphorylation and high p-p38 MAPK expression in the TG group, indicating DEX has a function similar to that of SB202190.

Conclusion: H/R injury in H9c2 cells can lead to abnormal ERS and apoptosis, as well as activation of the p38MAPK signalling pathway. DEX can protect cardiomyocytes by intervening in ERS, regulating p38MAPK and the downstream apoptotic signalling pathway.

Keywords: dexmedetomidine, hypoxia/reoxygenation injury, endoplasmic reticulum stress signalling pathway, p38 mitogen-activated protein kinase

Introduction

Myocardial ischaemia-reperfusion injury (MIRI), which usually occurs in clinical settings in an inevitable manner, always produces severe outcomes for patients if no effective strategies to induce downstream apoptotic cascades to shift in a favourable direction are employed. Fortunately, numerous animal studies evaluating diverse protective mechanisms have confirmed the efficacy of cardioprotection in ameliorating MIRI.[1–6] The actual

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clinical application of some of these strategies, however, does not match the animal study applications, and it is difficult to predict the consequences in clinical practice from those seen in experimental research.[7–9] In this context, the unclear mechanism underlying the development of apoptotic pathways or involving key molecules in MIRI might significantly matter, especially for cell death and even associated signalling pathways during ischaemia-reperfusion, such as ERS-related apoptosis or signalling pathways; a noteworthy report by Davidson in 2019 shared the opinion that multitarget strategies must be adopted to reduce MIRI if appropriate because single approaches have a limited capacity to overcome complicated MIRI situations.[10] Based on these unknown factors, we explored the possibility that there is a clinical medication with organ-protecting properties that can be adopted to attenuate IRI and then tried to illuminate the underlying mechanism to shorten the development time needed to successfully treat IRI.

DEX, a highly selective α^2 adrenergic receptor agonist, is frequently used in clinical practice, especially to protect the heart and other organs during surgery.[11–14] At this point, the most likely function of DEX in this application could be related to the anti-inflammatory response and inhibition of ischaemia-reperfusion injury. Regarding ERS, the effects of DEX on ERS and subsequent apoptosis have not been thoroughly illustrated. Judging from existing studies, DEX performs a protective role in inhibiting IRI in the heart of diabetic mice by interfering with ERS or autophagy;[6,15] however, these results were partly attributed to the diabetes context. Furthermore, researchers have focused on studying other non-cardiac cells, such as endothelial cells, under IRI or H/R conditions[16,17] and examining several crucial ERS chaperones, proteins and apoptosis indicators produced by organs other than the heart under IRI or H/R conditions,[6,18–22] finding that DEX can effectively regulate the function of non-cardiac cells and interfere in the endoplasmic reticulum stress signalling pathway under certain circumstances. Few studies have explored the function of DEX in H9c2 cardiomyocytes under H/R conditions;[23,24] however, the exact regulatory effect of DEX on ERS remains unknown. In recent years, an increasing number of basic research studies have focused on the p38MAPK signalling pathway; p38MAPK belongs to the family of MAPK-activated protein kinases and participates in many cellular processes, such as cell proliferation, survival and apoptosis. Additionally, it has been shown that stress is the leading cause of p38 MAPK activation. Accumulating evidence

has shown that DEX has the capacity to alleviate inflammation and IRI by regulating p38 MAPK signalling.[25–27] Wang et al found that DEX could attenuate mouse Neuro 2A neuroblastoma cell apoptosis under H/R conditions by targeting p38 MAPK signalling,[28] which confirmed that DEX might positively affect cells under H/R conditions.

In this study, we hypothesized that DEX could prevent cardiac cell injury under ERS conditions, which would verify its involvement in ERS. Moreover, we assume that the cardioprotective function of DEX could be fulfilled by regulating the p38MAPK and ERS signalling pathways.

Methods
Cell Culture

H9c2 cardiomyocytes, a rat embryonic myocardial cell line, were purchased from Process Life and Technology Corporation Limited (Wuhan, China). Cells were cultured in 45 g/L glucose DMEM (Corning, USA) supplemented with 10% foetal bovine serum (FBS, Invitrogen, USA), 100 g/mL streptomycin and 100 U/mL penicillin (Solarbio, China). All the experimental cells were grown in a 95% air plus 5% CO_2 incubator. The medium was replaced as needed, according to cell growth, approximately every 1–3 days. When cells reached 70–80% confluence, the cells were used in experiments.

H/R Injury Model

According to our previous study illustrating the optimal in vitro H/R model, H9c2 cells were processed into a single-cell suspension, seeded in advance in 96-well plates at approximately 5×10^3 cells/mL, and cultivated in two plates (a control plate and an experimental plate). The control plate was incubated in a 37 °C 5% CO_2 cell incubator, and the medium was changed at the same time point for both plates. All the groups in the experimental plates underwent a three-hour anoxia period with all the cells cultured at 37 °C in a hypoxia chamber filled with 5% CO_2 and 95% N_2, and the medium was changed to serum-free DMEM when the anoxia procedure began. According to the different protocols adopted in the study (see below), the experimental groups were subjected to normoxic culture when the reoxygenation procedure started at the end of the previous step. For the second part of the study, an ERS model was induced with thapsigargin (TG) (the exact protocol is explained below). At the end of each procedure, cell viability, the injury rate, apoptosis and several ERS molecular chaperone markers, such

as GRP78, CHOP and caspase-12, were detected and examined according to corresponding procedures (see below).

Experimental Protocols

To verify that DEX was an effective protector and attenuated H/R injury in H9c2 cells, and to determine whether DEX is involved in the signalling of ERS, cultured cells in 96-well or 6-well plates were divided into a control group, an H/R group, an H/R+DEX group and an H/R+DEX +4-PBA (4-phenylbutyrate, P-21,005, Sigma, USA) group. 4-PBA, which is a classic chemical chaperone used to reduce protein misfolding and ameliorate ERS, was dissolved in a DMSO solution in a sterile, dark environment and was added to the medium 24 h prior to H/R at a dilution of 1:1000. In addition to cell viability and injury assays, flow cytometry was used to evaluate apoptosis in these groups. In addition, the mRNA and protein expression of GRP78, CHOP and caspase-12 was also detected by RT-PCR and Western blot analysis, respectively (the specific methods are described below).

To test whether p38MAPK is targeted by DEX in the experimental system to attenuate ERS, TG, which is a specific ER stress inducer, was used to induce ERS at the recommended dose of 0.5 µM 10 h before the start of procedures. The other pre-treatments were similar to the above treatment protocol. First, the p38MAPK (dilution ratio 1:1000) and P-P38MAPK (dilution ratio 1:1000) expression of cells in the control group, TG group and DEX + TG group were detected at the end of the experiment by Western blotting, and β-actin was used as the internal control for sample loading. Second, SB202190, a p38MAPK-specific inhibitor, was selected to further study the possible signalling pathway involved in this experiment and was added to the medium at a dose of 5 µM 1 h before the beginning of the experiment. The incubated cells were randomly divided into control, TG, DEX + TG, DEX + TG + 4-PBA, SB202190 + TG, DEX + TG + SB202190, and DEX + TG + 4-PBA + SB202190 groups, and each group followed the appropriate specific procedure. Every group was analysed with cell viability assays, flow cytometry, and detection of the protein and mRNA expression of GRP78, CHOP and caspase-12.

Cell Viability and Injury Assays

According to the instructions of a CCK-8 kit (Dojindo, Japan), H9c2 cells (approximately 5×10^3 cells/well) were seeded in a 96-well plate and used in the subsequent protocols. When required for viability testing, the cells in each well were covered with 10 µL of Beyotime and incubated for at least 1 h at 37 °C in the incubator. Then, the OD value at 450 nm was measured with a microplate reader (Molecular Devices, Sunnyvale, CA, USA). Cell viability (%) was then calculated. In addition, cell injury was assessed based on the amount of LDH released into the supernatant with an LDH kit (Beyotime Biotechnology, China). According to the instructions of the LDH assay kit, all the cell groups were plated in 96-well plates, with 3 replicates per group, and outcomes were measured with a microplate reader at 450 nm. The LDH concentration and cell injury rate were then calculated.

Apoptosis Assay

The apoptosis rate of the H9c2 cells in each group was examined by flow cytometry with an Annexin V-FITC/PI (propidium iodide) kit (Sigma, USA). Briefly, after digestion and centrifugation, cells were washed with PBS twice, resuspended in binding buffer, and then incubated with 10 µL of Annexin V-FITC and 5 µL of PI for 20 min. Apoptotic cells were detected using a flow cytometer (BD, San Jose, CA), and the apoptosis rate was calculated.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

H9c2 cells were prepared in advance for mRNA analysis. The primers for GRP78, caspase12, CHOP, and β-actin were synthesized by Invitrogen, and the primer sequences are shown in Table 1. Total RNA was extracted from H9c2 cells with TRIzol, and the purity of the RNA from each group of cells was determined. With the Reverse Transcription Kit (Takara, China), 500 ng of RNA from each sample was reverse transcribed into cDNA according to the manufacturer's instructions. Subsequently, polymerase chain reaction was conducted with 2 µL of cDNA and other necessary reagents according to the instructions of a Premix Ex Taq kit (Takara, China), and the final 25-µL reaction was run on the ABI Prism 7500 System (Bedford, USA). The amplification process was initiated at 95 °C for 3 min, followed by 40 cycles of amplification including denaturation at 95 °C for 30 s, annealing at 55 °C for 20 s, and elongation at 72 °C for 20 s. mRNA expression was calculated as the relative ratio of the target gene level to the β-actin level by using the $2^{-\Delta\Delta CT}$ method.

Table 1 Primers Sequences

Gene	Forward (5′-3′)	Reverse (5′-3′)
GRP78	ACTGGAATCCCTCCTGCTC	CAAACTTCTCGGCGTCAT
CHOP	TGCCTTTCGCCTTTGAGAC	GCTTTGGGAGGTGCTTGTG
caspase-12	GGGATAGCCACTGCTGATA	GCCACTCTTGCCTACCTTC
β-actin	TGAGAGGGAAATCGTGCGTG	TTGCTGATCCACATCTGCTGG

Western Blots

Following the treatments described in previous procedures, H9c2 cells were washed well with an ice-cold PBS solution, and then, we added a RIPA solution (Beyotime, China) to the wells and incubated the cells for 30 min on ice. Subsequently, the supernatants were collected after centrifugation of the lysates. The BCA method was used to measure the protein concentration. The proteins were separated by SDS-PAGE and transferred to PVDF membranes at 4 °C and 200 mA for 2 h. Then, the membranes were blocked in a TBST solution for 2 h at room temperature and incubated at 4 °C overnight with the following primary antibodies: anti-dual phospho-p38 MAPK (Thr[180] and Tyr[182]) (Sigma, 1:1000), anti-p38 MAPK (Sigma, 1:1000), anti-CHOP (Affinity, 1:1000), anti-GRP78 (Affinity. 1:1000), anti-caspase12 (Affinity, 1:1000) and mouse anti-β-actin (Affinity, 1:1000). A secondary antibody conjugated to horseradish peroxidase (Jackson, 1:2000) was added and incubated for 2 h at room temperature. Finally, the membranes were washed in TBST, and the signals were visualized with an enhanced chemiluminescence detection kit (ECL, Beyotime, China). The density of the protein bands was quantified by IQuantTL (GE Healthcare, USA). Relative protein expression was calculated with normalization to β-actin expression.

Statistical Analysis

All the data are expressed as the mean ± standard deviation (SD), and one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test was performed to examine the significance of differences among multiple groups with SPSS 19.0 software (SPSS, Chicago, USA). GraphPad Prism (San Diego, USA) was used to construct the graphics. P<0.05 was considered to indicate a statistically significant difference.

Results
DEX Protects H9c2 Cells from H/R Injury

Based on the successful H/R model established in our previous study, the cell viability and LDH release of incubated cells in randomized groups given different treatments were detected (Figure 1). Compared to the control group, the DEX pretreatment group under normal conditions and the 4-PBA pretreatment group under normal conditions showed similar and comparable changes in terms of the two abovementioned variables (P>0.05). However, it was observed that the H/R group showed dramatically decreased cell viability and increased LDH release compared with the control group (P<0.05), which corresponded well with previous results.
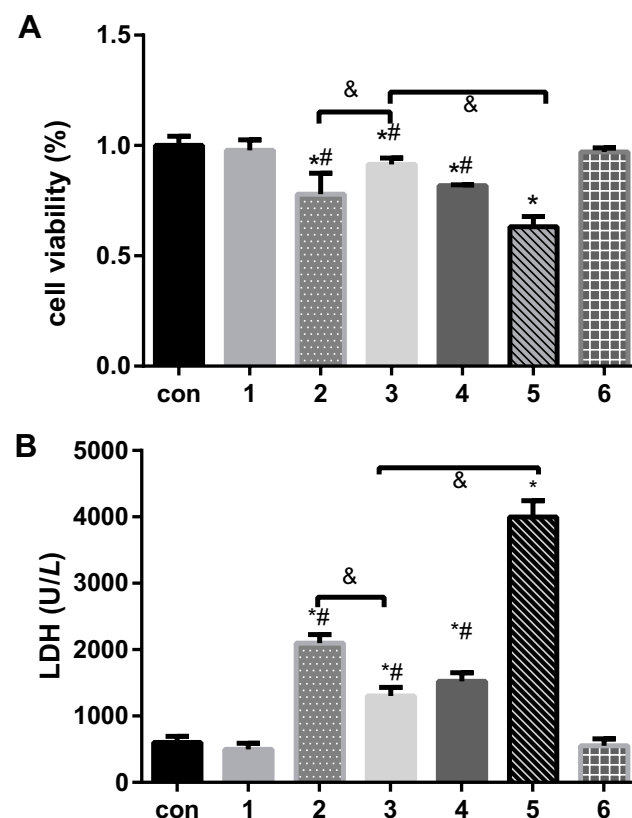
Figure 1 The effects of dexmedetomidine on the levels of myocardial cell injury due to H/R under different conditions were determined by measuring (**A**) the proportions of cell viability and (**B**) LDH release. The results are expressed as the mean ± SD. *p < 0.05 vs the control group; #P<0.05 vs the H/R group; &P<0.05 vs the DEX+H/R group. Con, control group; 1, normoxic incubation with dexmedetomidine; 2, hypoxic/reoxygenation incubation with dexmedetomidine and 4-phenyl butyric acid; 3, hypoxic/reoxygenation incubation with dexmedetomidine; 4. hypoxic/reoxygenation incubation with 4-phenyl butyric acid; 5. hypoxic/reoxygenation incubation; 6. normoxic incubation with 4-phenyl butyric acid.
Abbreviations: LDH, lactate dehydrogenase; H, hypoxia; R, reoxygenation

Compared with the H/R group, the DEX group showed reduced cellular injury and LDH release, as did the 4-PBA group. In addition, 4-PBA successfully reversed the protective effect of DEX on H/R in terms of cell viability and LDH release (Figure 1).

In a cell apoptosis assay, there was an average 10% increase in the H/R group compared with the control group (Figure 2). However, the DEX+ H/R and 4-PBA+ H/R pretreatment groups exhibited reduced cell apoptosis, and apoptosis in these 2 groups was significantly attenuated by 7% or 6%, respectively, compared with that in the H/R group (P<0.05). Additionally, 4-PBA reversed the anti-apoptotic effect of DEX and resulted in an obvious increase of approximately 4% (Figure 2).

DEX Reduced the Expression of GRP78, CHOP and Caspase12 in H9c2 Cells After H/R

With the goal of determining the role of DEX, three molecules involved in ERS and related signalling pathways were examined (Figure 3). As illustrated in Figure 3, consistent results were observed between mRNA expression and protein expression. Compared to the control group, the H/R group exhibited obvious upregulation of the indicators of ERS and apoptosis. DEX or 4-PBA pretreatment significantly alleviated the expression of GRP78, CHOP and Caspase-12 induced by H/R (P<0.05). Moreover, the alleviation of ERS and apoptosis in the DEX+H/R group were dramatically reversed by 4-PBA, as was indicated by the results for the DEX+H/R+4-PBA group.

DEX Attenuated TG-Induced H9c2 Cell Injury

Previously, ERS in H9c2 cells was shown to be induced by TG, and cell viability, LDH release and the apoptotic proportion were evaluated (Figures 4 and 5). Figure 4 shows that the cell viability in the TG group was significantly decreased compared with that in the normal group, and the LDH concentration was increased dramatically. The cell viability and LDH concentration in the DEX + TG group were increased and decreased, respectively, due to DEX intervention (P < 0.05). Like LDH release, apoptosis showed similar contrasting trends among the different groups (Figure 5). Compared with the DEX + TG group, the sb202190 + TG group had comparable outcomes in terms of cell viability, the LDH concentration and apoptosis (P > 0.05), but those indexes were reversed

by sb202190, a p-p38MAPK inhibitor, and showed opposite changes in the DEX + sb202190+TG group (P < 0.05).

DEX Inactivated the Phosphorylation of P38 MAPK

As shown in Figure 6, Western blot analysis of P38MAPK and P-P38MAPK was performed for groups with or without DEX pretreatment. In all groups, the overall expression of p38-MAPK remained stable, and no obvious difference was found, even in the TG group (P > 0.05). Compared with that of the control group, the protein expression level of P-P38MAPK in the TG group was almost 3-fold increased (P < 0.05). Compared with the TG group, however, the DEX + TG group had lower protein expression of P-P38MAPK, which is normally accompanied by stress (P < 0.05) (Figure 6). These above results indicate that ERS induced by TG can cause p38MAPK phosphorylation and activate the p38MAPK signalling pathway, which directly affects cell viability, LDH release and apoptosis. DEX has the potential to attenuate cell injury, which may be involved in the activation of p38MAPK.

Effects of P38MAPK Inactivation by DEX on the Expression of Markers of the ERS Signalling Pathway

To further verify whether p38MAPK is involved in the process by which DEX attenuates the H9c2 cell damage induced by TG and elucidate the possible mechanism linking DEX pretreatment and the ERS-apoptosis signalling pathway, the expression of GRP78, CHOP and caspase-12 was further evaluated. The results showed that there was no significant difference in GRP78, CHOP and caspase-12 gene or protein expression between the DEX + TG group and SB202190 + TG group (P > 0.05); compared with that in the TG group, the expression of GRP78, CHOP and caspase-12 in the 3 intervention groups including the DEX+ TG group, SB20219+ TG group and 4-PBA + TG group was decreased (P < 0.05). However, in contrast to that in the DEX + TG group, the expression of those indexes in groups with SB20219 and 4-PBA intervention was significantly increased (P < 0.05). Furthermore, the DEX + TG + sb20219 + 4-PBA group had the highest increase (Figure 7). In conclusion, the SB20219 group exhibited an inhibitory effect similar to that in the 4-PBA group to prevent ERS, and SB20219 could inhibit the protective function of DEX under ERS
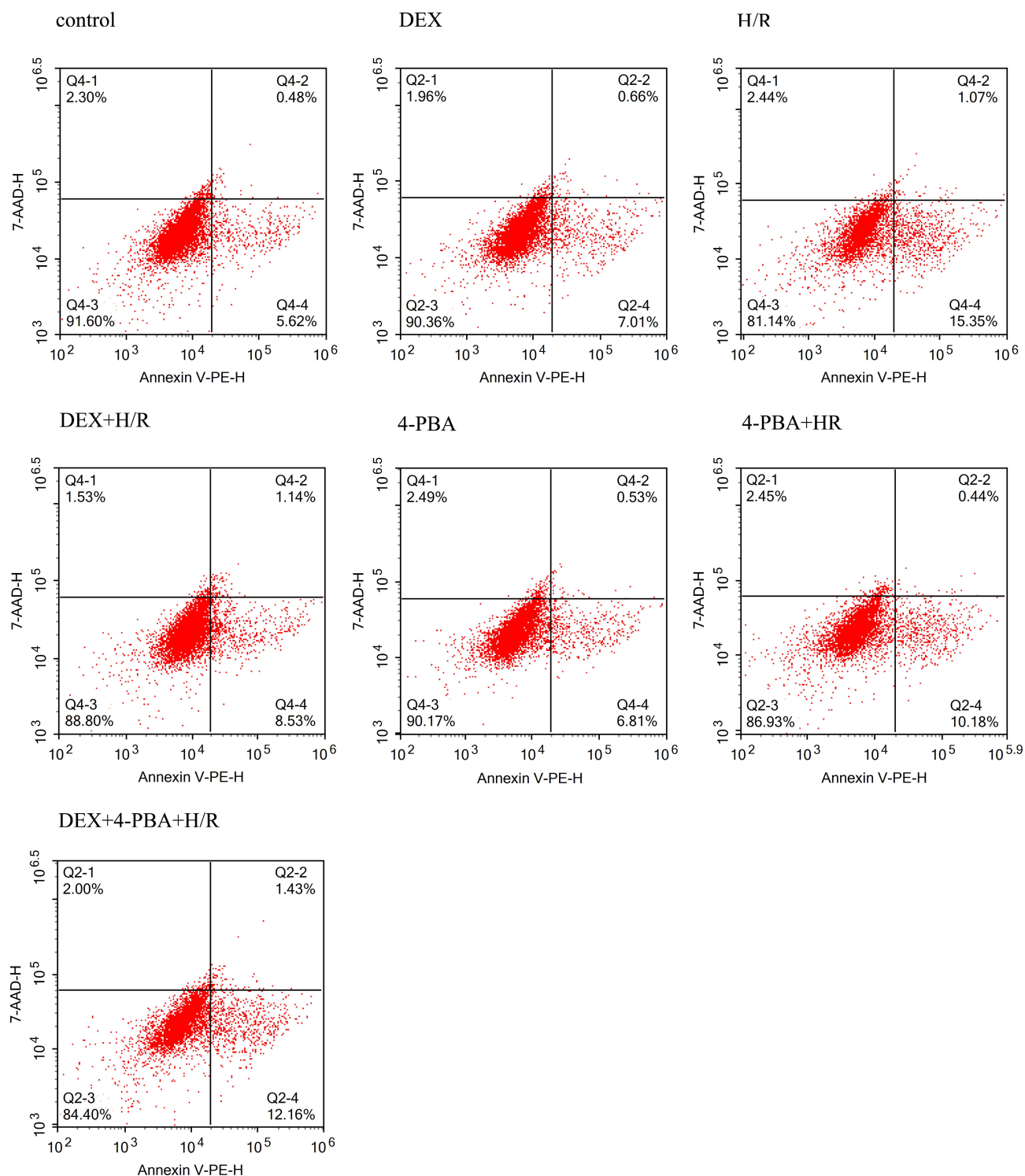
Figure 2 The percentages of apoptotic H9C2 cardiomyocytes were examined by flow cytometry under different conditions.
Abbreviations: DEX, dexmedetomidine; 4-PBA, 4-phenyl butyric acid; H/R, hypoxia/reoxygenation.

conditions, which significantly reduced the expression of GRP78, CHOP and caspase-12. These three enzymes might be downstream signalling molecules of the p38MAPK-apoptosis signalling pathway (Figure 7).

Discussion

It is well known that DEX shows a variety of protective properties in clinical use, especially in ischaemic heart disease patients, but the specific mechanism remains
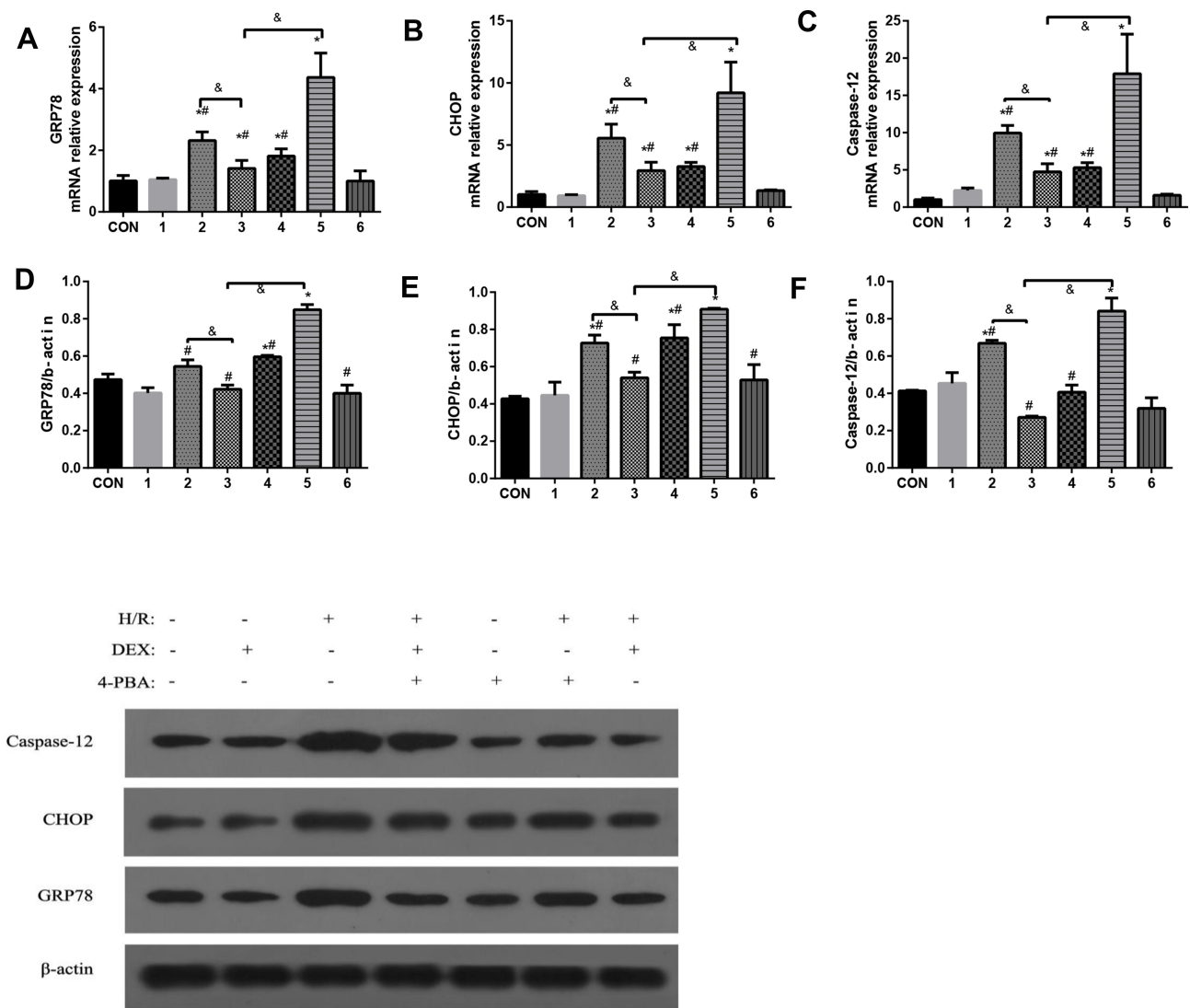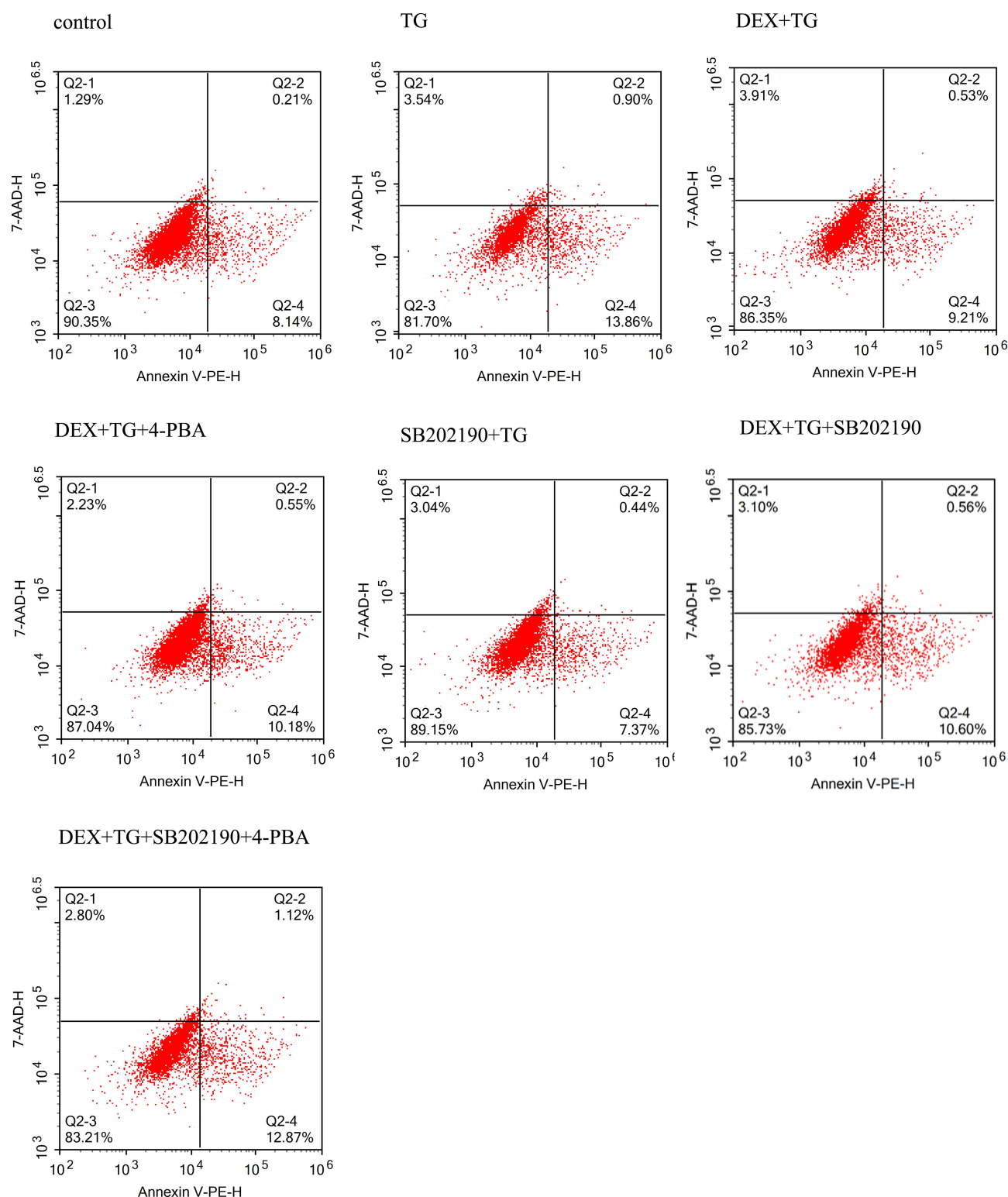
Figure 3 The effects of dexmedetomidine on the expression of GRP78, CHOP and caspase-12 in myocardial cells treated under different conditions were examined. (**A–C**) mRNA expression of GRP78, CHOP and caspase-12; (**D–F**) Protein expression of GRP78, CHOP and caspase-12. The results are expressed as the mean±SD. *$P<0.05$ vs the control group; #$P<0.05$ vs the H/R group; &$P<0.05$ vs the DEX+H/R group. Con, control group; 1, normoxic incubation with dexmedetomidine; 2, hypoxic/reoxygenation incubation with dexmedetomidine and 4-phenyl butyric acid; 3, hypoxic/reoxygenation incubation with dexmedetomidine; 4. hypoxic/reoxygenation incubation with 4-phenyl butyric acid; 5. hypoxic/reoxygenation incubation; 6. normoxic incubation with 4-phenyl butyric acid.

unknown. In recent years, a great number of basic research studies have reported that DEX has the capacity to regulate in vivo MIRI and even H/R injury via the regulation of either the inflammatory response pathway or apoptosis,[1,12,13,27,29] which are two key downstream branches of the MAPK signalling pathway.[30–33] However, few basic research studies have paid attention to the modulation of ERS by DEX in the regulation of IRI outcomes, and the exact mechanism underlying this modulation has not been elucidated. Recently, DEX was reported to alleviate IRI of the intestine or sepsis in the lungs by targeting the p38MAPK signalling pathway.[25,26] In our present study, we applied a classic H/R model and

TG-induced specific ERS model to examine the effects of DEX on H/R and stress injury based on ERS and further determined if the targeting molecule in this process is p38MAPK. This study confirmed that DEX attenuated the myocardial damage induced by either H/R or TG by attenuating ERS, which subsequently led to the downregulation of the expression of several key molecules related to ERS and apoptosis. Additionally, p38MAPK might be a potential target involved in this process.

It has been well accepted that MIRI can lead to severe ERS, which is usually detected by upregulated GRP78 expression.[34,35] If ERS increases, apoptotic cascades would be considered an underlying mechanism of MIRI,

control

TG

DEX+TG

| Q2-1 1.29% | Q2-2 0.21% |
| Q2-3 90.35% | Q2-4 8.14% |

| Q2-1 3.54% | Q2-2 0.90% |
| Q2-3 81.70% | Q2-4 13.86% |

| Q2-1 3.91% | Q2-2 0.53% |
| Q2-3 86.35% | Q2-4 9.21% |

DEX+TG+4-PBA

SB202190+TG

DEX+TG+SB202190

| Q2-1 2.23% | Q2-2 0.55% |
| Q2-3 87.04% | Q2-4 10.18% |

| Q2-1 3.04% | Q2-2 0.44% |
| Q2-3 89.15% | Q2-4 7.37% |

| Q2-1 3.10% | Q2-2 0.56% |
| Q2-3 85.73% | Q2-4 10.60% |

DEX+TG+SB202190+4-PBA

| Q2-1 2.80% | Q2-2 1.12% |
| Q2-3 83.21% | Q2-4 12.87% |

Figure 4 The percentages of apoptotic H9C2 cardiomyocytes were examined by flow cytometry under different conditions.
Abbreviations: DEX, dexmedetomidine; 4-PBA, 4-phenyl butyric acid; TG, thapsigargin; SB202190, a p38MAPK-specific inhibitor.

and the expression of some specific transcription-related molecules, such as CHOP and caspase-12, would be upregulated in an ERS-dependent manner. Most researchers have confirmed that these molecules can be used to represent the status of ERS and even the developing direction of cell survival as downstream markers of the ERS signalling
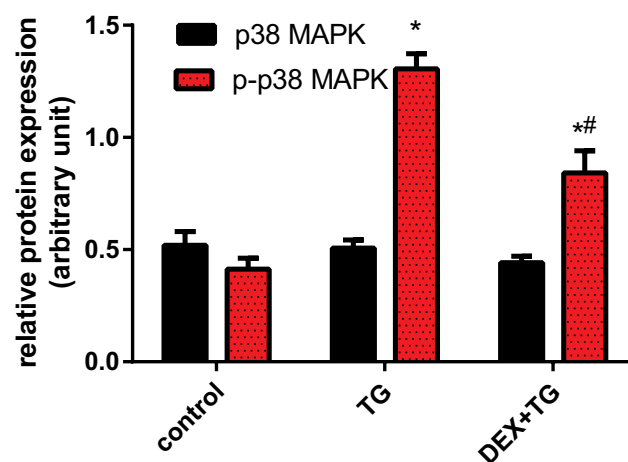
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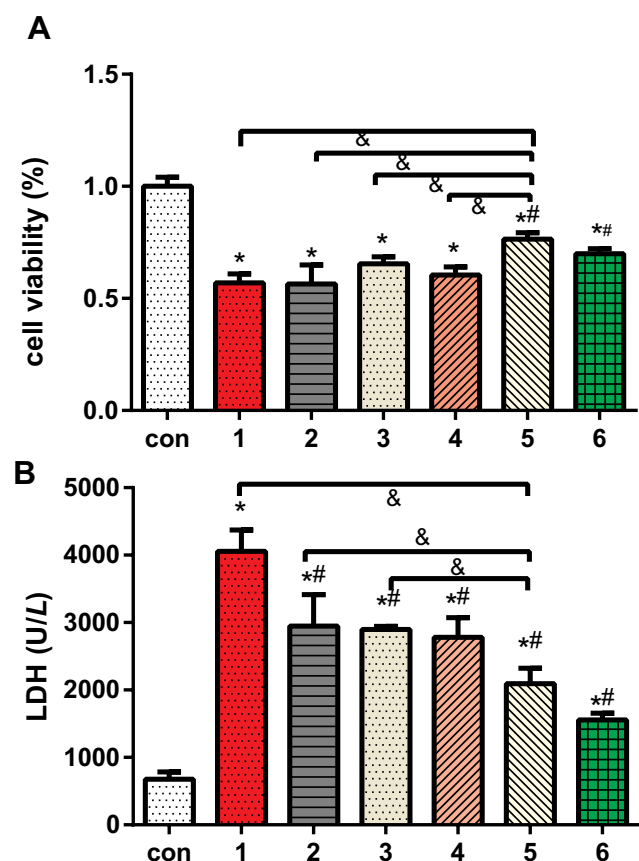
Figure 5 The effects of dexmedetomidine on the levels of myocardial cell injury induced by TG and different conditions were determined by measuring (**A**) the proportions of cell viability and (**B**) LDH release. The results are expressed as the mean ± SD. *$p < 0.05$ vs the control group; #$P<0.05$ vs the TG group; &$P<0.05$ vs the DEX+TG group. Con, control group; I, normoxic incubation with TG; 2, incubation with a combination of SB202190, 4-phenyl butyric acid and DEX on TG model; 3, incubation with DEX and 4-phenyl butyric acid on TG model; 4. incubation with SB202190 and DEX on TG model; 5. incubation with DEX on TG model; 6. incubation with a combination of TG and SB202190.

Abbreviations: LDH, lactate dehydrogenase; TG, thapsigargin; SB202190, a p38MAPK-specific inhibitor.

Figure 6 The protein expression of p38MAPK and p-p38MAPK in 3 separate groups was detected by Western blotting and is illustrated as the mean ± SD. *$p < 0.05$ vs the control group; #$P<0.05$ vs the TG group. DEX, dexmedetomidine; TG, thapsigargin; p38MAPK, p38 mitogen-activated protein kinase; p-p38MAPK, phosphorylated p38MAPK.

pathway.[36–38] Moreover, ERS in MIRI can be alleviated through the suppression of caspase-12 and CHOP.

In this preclinical study, these 3 molecules were highly expressed under H/R or TG induction but dramatically alleviated by DEX and 4-PBA. This result clearly indicated that DEX is potentially involved in ERS-associated apoptosis and has the capacity to inhibit ERS with a function similar to that of 4-PBA, as reflected by the decreased expression of CHOP, GRP78 and caspase-12 at both the mRNA and protein levels. Similar to studies by Chai and other researchers,[19,39,40] which have similar outcomes to ours, DEX could eventually attenuate the apoptotic outcomes induced by CHOP or caspase-12, even though those studies used diverse models or approaches to induce apoptosis, such as IRI, ERS and oxidative stress pathways. In regard to the mechanism at the cellular level, several studies have confirmed the cardioprotective effect of DEX in I/R injury based on H/R models.[23,24,41] The consistent conclusions advanced from these studies share similar outcomes with our conclusions, although the H/R models used varied among the studies. The major outcomes evaluated were cell viability, the apoptosis rate and LDH release, and by evaluating the expression of miR-208b,[23] intracellular calcium overload[24] and TLR4,[41] no ERS was found to be involved.

From another aspect, apoptosis in H/R mimic models of IRI can have different causes, and it is extremely difficult to specify the cause and consequence. According to Price and Chaudhari's research,[42,43] hypoxia, to some extent, is considered a beneficial factor in cells that undergo subsequent TG induction, which could affect the efficacy of TG, or hypoxia could be independent of apoptosis, which was unlike the results for the function of TG. Therefore, TG induction was adopted as a specific enhancement model to clarify the inhibitory effect of DEX on the ERS pathway in the present study.

p38 MAPK is a member of the evolutionarily conserved serine/threonine protein kinases that mediate a variety of cellular processes linking cellular signals from outside the cell to intracellular processes.[44] It is activated through the phosphorylation of Thr-X-Tyr by mitogen-activated protein kinase (MAPK). Among its family members, p38 MAPK is related to infection and inflammatory stress.[45] Then, how is p38 MAPK activated? The first answer is ERS. ERS directly leads to the upregulation of PERK, IRE1 and ATF6 expression, and if ERS is not alleviated, it may lead to an interaction
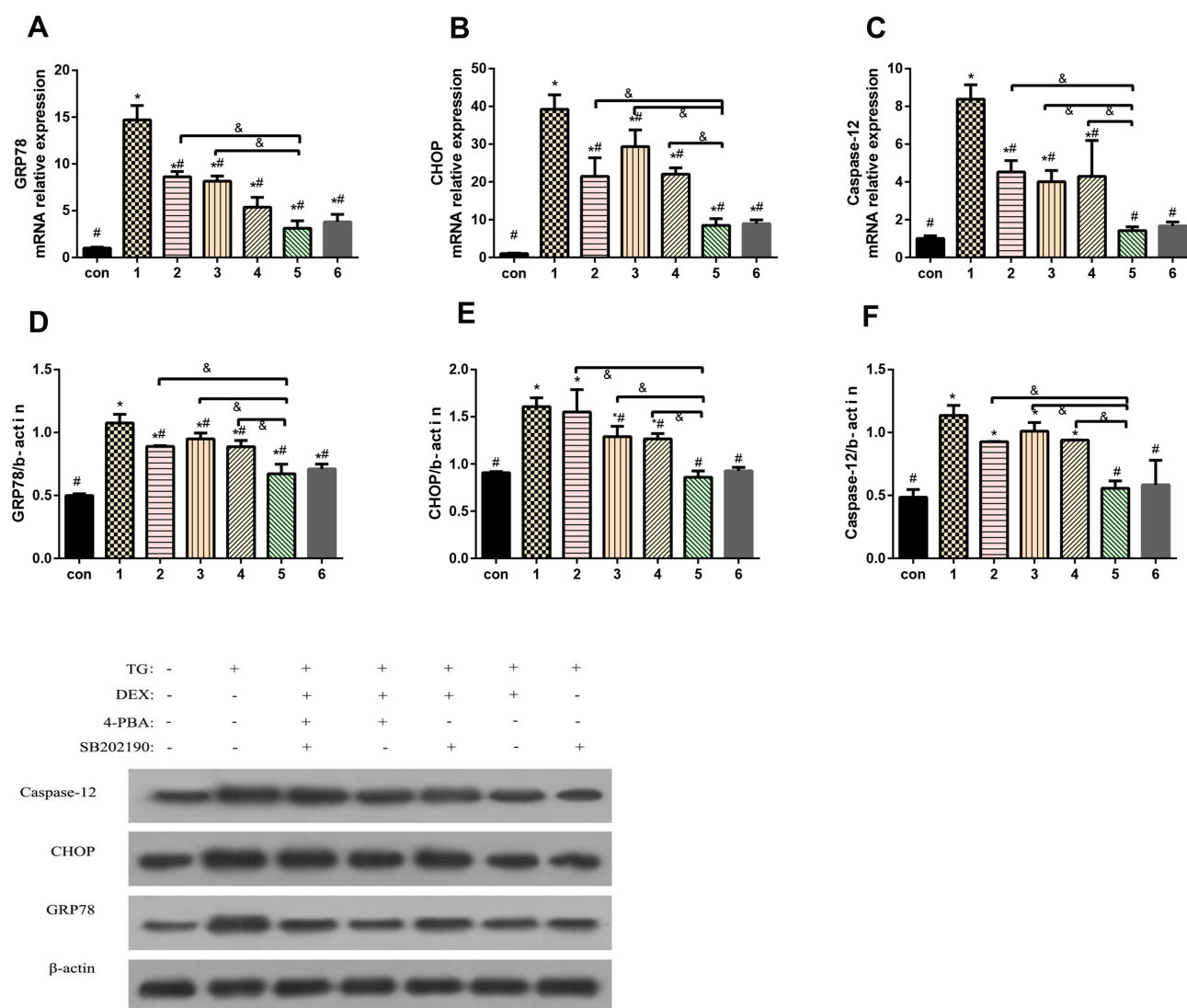
Figure 7 The effects of dexmedetomidine on the expression of GRP78, CHOP and caspase-12 in myocardial cells treated under different conditions were evaluated. A-C, mRNA expression of GRP78, CHOP and caspase-12; D-F, protein expression of GRP78, CHOP and caspase-12. The results are expressed as the mean±SD. *$P<0.05$ vs the con group; #$P<0.05$ vs the TG group; &$P<0.05$ vs the DEX+TG group. Con, control group; 1, normoxic incubation with TG; 2, incubation with a combination of SB202190, 4-phenyl butyric acid and DEX on TG model; 3, incubation with DEX and 4-phenyl butyric acid on TG model; 4. incubation with SB202190 and DEX on TG model; 5. incubation with DEX on TG model; 6. incubation with a combination of TG and SB202190.
Abbreviations: DEX, dexmedetomidine; TG, thapsigargin; SB202190, a p38MAPK-specific inhibitor; 4-PBA, 4-phenyl butyric acid.

between downstream ASK1 and TRAF2 and even the autophosphorylation of ASK1.[32] A study showed that autophosphorylation of ASK1 could activate MKK and p38 MAPK.[46] Rasheed et al also noted that ERS can activate multiple signalling pathways, such as the eIF2 α, MAPK and NF-κB pathways, and thus trigger downstream cascade reactions.[47] Finally, p38 MAPK phosphorylation is achieved by MAPK3/MAPK6 activation. In addition, infection and other factors can also induce p38 MAPK activation.[48] In the present study, consistent results were achieved with TG induction in H9c2 cells, and high expression of p-p38 MAPK was found.

Then, what kinds of downstream changes would be found in the canonical p38 MAPK signalling pathway? It has been reported that p-p38 MAPK can lead to NF-κB-mediated inflammation,[31,33] autophagy,[49,50] apoptosis[30,51] and other processes. Thus, targeting p38 MAPK for the development of novel strategies combating ERS or acute pathologies has been tested for years. For instance, Bonney EA t et al[52] reported attenuated ischaemia-reperfusion injury mediated by inhibition of the p38MAPK signalling pathway. Wang et al[28] also illustrated the effect of DEX in altering oxygen deprivation/reoxygenation-induced apoptosis via the p38MAPK signalling pathway in N2A cells. In this study,

DEX pretreatment was first used to produce a similar experimental result in H9c2 cells, including the high expression of P-P38MAPK produced by ERS and myocardial apoptosis following the activation of the p38MAPK signalling pathway.

Given the importance of p38 MAPK in the ERS-apoptosis pathway, specific p38 MAPK inhibitors are commonly used as experimental tools in pilot studies or the treatment of cancers and inflammatory diseases. SB202190 is an inhibitor that can interact with p38α and p38β via competitive binding to the binding site on p38. In the present study, SB202190 eventually decreased the increases in indexes induced by TG and reversed the decreases in parameters produced by DEX in the DEX+TG group. This conclusion indirectly indicated the roles of DEX in protecting ERS and apoptosis through p38 MAPK intervention.

Several limitations should be taken into consideration. Primarily, human cells or stem cells that more accurately reflect the clinical scenario were not investigated in the present study, so these findings should not be applied to other types of cells, especially human cells. Further in vivo animal studies or clinical trials will be warranted. Likewise, this study was only a pilot study, so no advanced techniques, such as RNAi, were used. Second, the purpose of any in vitro H/R model is to mimic IRI that occurs in a clinical setting so the condition-dependent models might vary. In other words, the outcomes derived from the present study might not apply to other models.

Conclusions

In conclusion, DEX, a promising agent in the clinical setting, has been confirmed to protect H9c2 cells from hypoxia/reoxygenation injury at the cellular level, which occurred via modulation of ERS-induced apoptosis. Furthermore, DEX pretreatment alleviated p-p38 MAPK expression under ERS conditions induced by TG, which significantly reduced the downstream expression of GRP78, CHOP and caspase-12. Additional studies are needed to identify the specific signalling pathway linking p38 MAPK and apoptosis.

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Disclosure

The authors report no conflict of interest related to this paper.

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