Dexmedetomidine mitigates oxidative stress in H9C2 cardiac myoblasts under a high-glucose environment via the PI3K/AKT signaling pathway

YAN QU, WEI XIONG, RUI ZHOU, NING SONG and JINQIAO QIAN

Department of Anesthesiology, First Affiliated Hospital of Kunming Medical University, Kunming, Yunnan 650032, P.R. China

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Abstract. Dexmedetomidine (Dex) is a selective α2-adrenergic receptor agonist used for its sedative effects in anesthesia and critical care. Although Dex exhibits cardioprotective effects, to the best of our knowledge, the mechanisms underlying these effects, particularly in a high-glucose (HG) environment, remain unclear. Research into the role of Dex in alleviating oxidative stress injury in cardiac myoblasts through the PI3K/AKT signaling pathway may reveal novel cardioprotective mechanisms, enhance the understanding of cell survival and metabolic regulation, and offer potential clinical applications in cardiac surgery and critical care. The aim of the present study was to assess the protective effect and mechanism of Dex preconditioning (DP) against hydrogen peroxide (H_2O_2)-induced H9C2 cardiac myoblast injury under HG conditions. H9C2 cardiac myoblasts were either untreated or pretreated with 10 nM Dex and the PI3K inhibitor LY294002 before exposure to H_2O_2 to induce oxidative cellular damage in the presence of HG culture medium. Cell viability assays were carried out, and apoptosis was evaluated using flow cytometry, TUNEL assays and western blotting. Additionally, the relative levels of oxidative stress indicators, including superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA), were determined. Exposure to H_2O_2 significantly decreased cell viability and increased apoptosis in H9C2 cardiac myoblasts cultured in HG conditions. Treatment with Dex significantly mitigated H_2O_2-induced apoptosis, as evidenced by reduced expression of caspase-3 and BAX, and increased levels of BCL-2. In addition, oxidative stress was elevated in the HG + H_2O_2 group, as indicated by increased levels of the oxidative stress marker MDA, and reduced levels of the antioxidant enzymes SOD and CAT compared with those in the HG group. By contrast, DP in the DP + HG + H_2O_2 group reduced MDA levels, and increased SOD and CAT levels, indicating improved oxidative stress regulation. Treatment with the PI3K/AKT inhibitor LY294002 in the LY294002 + HG + DP + H_2O_2 group prevented these effects, further increasing MDA levels, and decreasing SOD and CAT levels compared with the DP + HG + H_2O_2 group, suggesting that the protective effects of Dex were abrogated by inhibition of the PI3K/AKT pathway. The present study revealed that Dex pretreatment attenuated H9C2 cardiac myoblast injury via the PI3K/AKT signaling pathway under HG conditions. Its protective effects may be achieved by reducing oxidative stress damage to cardiac myoblasts.

Introduction

Myocardial infarction (MI) remains one of the leading causes of morbidity and mortality worldwide, with an estimated 9.4 million deaths globally attributed to ischemic heart disease in 2022 posing a burden on healthcare systems (1). The pathophysiology of MI is multifaceted, involving ischemia-induced apoptosis, inflammation and oxidative stress, which together contribute to the extensive myocardial damage observed in affected patients (2). Notably, perioperative hyperglycemia resulting from diabetes or stress responses has been linked to the occurrence of adverse cardiovascular events such as MI and impaired left ventricular function following ischemia/reperfusion (I/R) injury (3,4). Despite the known association between hyperglycemia and adverse cardiac outcomes, the precise mechanisms by which hyperglycemia contributes to increased myocardial vulnerability during I/R injury are still not fully understood.

Dexmedetomidine (Dex) is a highly selective α2-adrenergic receptor agonist. At present, it is used for the induction and maintenance of anesthesia in the operating room, as well as for sedation therapy in the intensive care unit (5). Preclinical studies in animal models and cell experiments have demonstrated that Dex exerted beneficial effects on myocardial cells, reducing injury through mechanisms such as suppression of inflammation, attenuation of calcium overload, reduction of oxidative stress and inhibition of apoptosis (6-8). These protective effects have made Dex a drug of interest in cardiac surgery and perioperative care. However, despite its well-documented benefits, limited research has been conducted on the

Correspondence to: Professor Jinqiao Qian, Department of Anesthesiology, First Affiliated Hospital of Kunming Medical University, 295 Xichang Road, Kunming, Yunnan 650032, P.R. China
E-mail: qianjinqiao@126.com

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cardioprotective efficacy of Dex in hyperglycemic environments, which are often encountered in patients with diabetes or patients experiencing stress-induced hyperglycemia.

Oxidative stress is a key contributor to myocardial damage during I/R injury, as it promotes the accumulation of free radicals, exacerbates calcium overload and activates inflammatory pathways involving neutrophils (9,10). In clinical hyperglycemic conditions, oxidative stress is further amplified, worsening myocardial injury (11). Although Dex has been revealed to mitigate oxidative stress in normoglycemic conditions, to the best of our knowledge, it remains unclear whether this cardioprotective effect extends to myocardial cells exposed to high glucose (HG) levels (12,13). This presents a considerable gap in the current understanding of the potential of Dex to reduce myocardial injury in patients with diabetes or hyperglycemia.

The present study investigated the cardioprotective effects of Dex under HG conditions, a model that reflects the metabolic environment of diabetic cardiomyopathy. Specifically, the present study aimed to determine whether the protective effects of Dex in H9C2 cardiac myoblasts exposed to a HG environment are mediated by the reduction of oxidative stress. Furthermore, the mediation of these effects through the PI3K/AKT signaling pathway was evaluated.

Materials and methods

Cell culture and treatments. H9C2 cells used in the present study were obtained from BeNa Culture Collection; Beijing Beina Chunglian Institute of Biotechnology. The cells were cultured in HG (33 mM) DMEM (Gibco; Thermo Fisher Scientific, Inc.) with 10% FBS (Biological Industries), 100 mg/ml streptomycin and 100 units/ml penicillin. The culture was maintained at 37˚C with 95% humidity and 5% CO_2.

At H_2O_2 concentrations ≥800 μM, the viability of H9C2 cells dropped to ~20%. As previously reported, when exposed to 200 μM H_2O_2 for 8 h, the range of cell viability was 40-60% (14). In preliminary experiments (data not shown), H9C2 cardiac myoblasts were treated with various concentrations of H_2O_2 (300, 600, 900 and 1,200 μM) for 6 h at 37˚C to identify the optimal dose for inducing oxidative stress. A concentration of 600 μM of H_2O_2, which resulted in ~50% cell viability, was selected for subsequent experiments. This concentration is consistent with those used in previous studies that reported similar levels of oxidative injury in H9C2 cells (15,16).

LY294002 (10 μM; MedChemExpress) was applied for 10 min at 37˚C. Subsequently, cells were pretreated at 37˚C with 10 nM Dex for 2 h and then exposed to 600 μM H_2O_2 for 6 h. The H9C2 cells were divided into four experimental groups (Fig. 1): i) HG group (H9C2 cells cultured in DMEM containing 33 mM glucose for 490 min); ii) HG + H_2O_2 group (H9C2 cells were cultured in DMEM containing 33 mM glucose for 130 min, then treated with 600 μM H_2O_2 for 360 min at 37˚C); iii) DP + HG + H_2O_2 group (DMEM supplemented with 33 mM glucose and 10 nM Dex for 120 min and then treated with 600 μM H_2O_2 for 360 min at 37˚C); and iv) LY294002 + HG + DP + H_2O_2 group (H9C2 cells were cultured in DMEM containing 33 mM glucose, treated with 10 μM LY for 10 min, then treated with 10 nM Dex for 120 min and finally treated with 600 μM H_2O_2 for 360 min at 37˚C).

Cell viability assay. Cell viability was assessed using a Cell Counting Kit-8 (CCK-8; Dojindo Laboratories, Inc.). Specifically, $8x10^3$ cells/well were seeded in a 96-well plate. After completing the experimental treatments, CCK-8 reagent was added to each well at a 1:10 ratio with the cell culture medium and cells were incubated at 37˚C for 2 h. Absorbance, which served as an indirect measure of cell viability, was read at 450 nm using a microplate reader. All measurements were carried out in triplicate by a blinded expert and the results from the treated groups were compared with those of the control group.

Apoptosis assay. The degree of apoptosis was measured using a TUNEL assay with the Servicebio Tetramethylrhodamine TUNEL Cell Apoptosis Detection Kit (Wuhan Servicebio Technology Co., Ltd.) according to the manufacturer's instructions. Briefly, cells were fixed with 4% paraformaldehyde at room temperature for 30 min, followed by rinsing with PBS. Following permeabilization with 0.1% Triton X-100 for 20 min at room temperature, the TUNEL reaction mixture was applied and incubated at 37 ˚C for 1 h in the dark. Nuclei were counterstained with DAPI, and images were captured using a fluorescence microscope. TUNEL-positive cells had nuclei that were stained red. After staining, the sections were mounted using an anti-fade fluorescence mounting medium (Servicebio, Wuhan, China) before imaging. A total of eight random fields per slide were selected for blind analysis using a fluorescence microscope (Nikon Eclipse C1, Nikon Corporation) at x200 magnification. The apoptotic index was calculated as the percentage of TUNEL-positive (red) nuclei compared with the total number of DAPI-stained (blue) nuclei. Cells were stained with DAPI (2 μg/ml) for 10 min at room temperature before imaging.

H9C2 cells were seeded in 6-well plates at a density of $2x10^6$ cells/well. Following the treatments, apoptosis rates were assessed using the Annexin V-FITC/PI Apoptosis Detection Kit (Beyotime Institute of Biotechnology) according to the manufacturer's protocol, and analyzed by flow cytometry using a BD FACSCanto™ II (Becton, Dickinson and Company) and data were analyzed with FlowJo software (version 10.6.2; BD Biosciences).

Western blotting. H9C2 cells from the various groups were collected and lysed on ice for 30 min in RIPA buffer (Beyotime Institute of Biotechnology) containing 1% PMSF. After centrifugation at ~18,000 x g for 15 min at 4˚C, the supernatant was collected as the total cell protein. The protein concentration was determined using a BCA Protein Quantitation Kit (Biomiga, Inc.). Equal amounts of protein (30 μg/lane) from the cell lysates were separated using 10-12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred to a PVDF membrane. The membranes were blocked with 5% non-fat dry milk in Tris-buffered saline with 0.1% Tween-20 (TBST) at room temperature for 1 h, then incubated overnight at 4˚C with primary antibodies against caspase-3 (1:500; cat. no. GB11767C; Wuhan Servicebio Technology Co., Ltd.), AKT (1:1,000; cat. no. GB11689; Wuhan Servicebio Technology Co.,

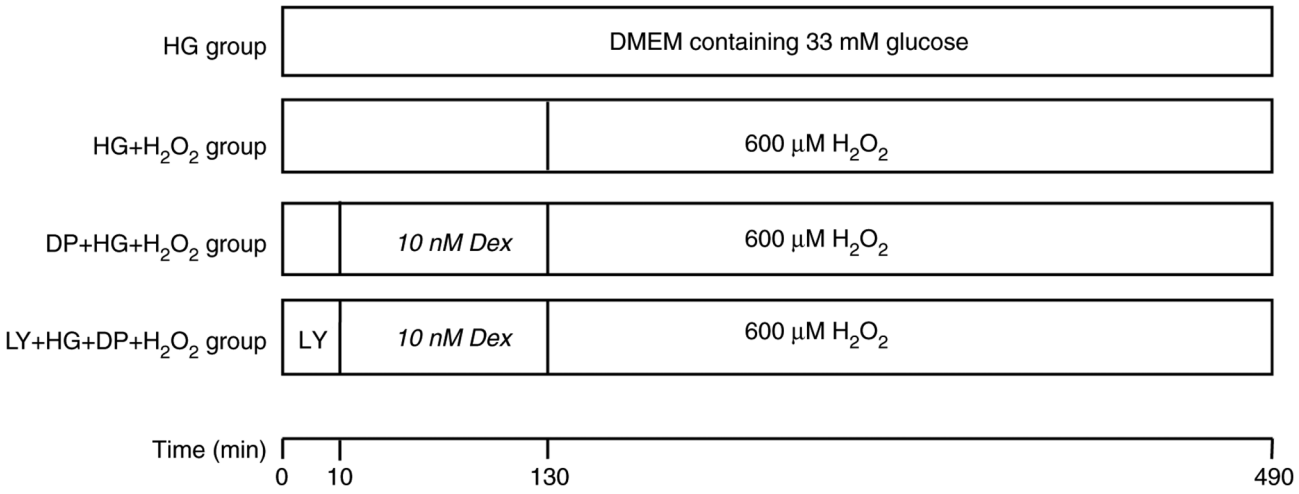

Figure 1. Cell experiment protocol. H9C2 cells were cultured overnight in plates and divided into the following four groups (n=5 wells/group): i) HG group: H9C2 cells were cultured in DMEM containing 33 mM glucose for 490 min; ii) HG + H_2O_2 group: H9C2 cells were cultured in DMEM containing 33 mM glucose for 130 min, then treated with 600 μM H_2O_2 for 360 min; iii) DP + HG + H_2O_2 group: H9C2 cells were cultured in DMEM containing 33 mM glucose, pretreated with 10 nM Dex for 120 min and then treated with 600 μM H_2O_2 for 360 min; and iv) LY + HG + DP + H_2O_2 group: H9C2 cells were cultured in DMEM containing 33 mM glucose, treated with 10 μM LY for 10 min, then treated with 10 nM Dex for 120 min and finally treated with 600 μM H_2O_2 for 360 min. HG, high-glucose; Dex, dexmedetomidine; DP, dexmedetomidine preconditioning; H_2O_2, hydrogen peroxide; LY, LY294002.

Ltd.), PI3K p85α (1:500; cat. no. AF6241; Affinity Biosciences), phosphorylated (p)-AKT (phospho-pan-AKT1/2/3 ser473; 1:500; cat. no. AF0016; Affinity Biosciences), p-PI3K (1:500; cat. no. AF3241; Affinity Biosciences), BAX (1:500; cat. no. GB11690; Wuhan Servicebio Technology Co., Ltd.), BCL-2 (1:1,000; cat. no. 26593-1-AP; Proteintech Group, Inc.) and GAPDH (1:1,000; cat. no. ab8245; Abcam). The PVDF membranes were washed three times with Tris-buffered saline containing 0.1% Tween-20. Protein bands were detected using ECL Western Blotting Detection Reagent (Thermo Fisher Scientific, Inc.) after incubating the membranes with the secondary antibody, HRP-labeled Goat Anti-Rat IgG (H + L) (1:1,000; cat. no. A0216; Beyotime Institute of Biotechnology), for 2 h at room temperature. Protein levels were normalized to GAPDH to correct for loading differences. Data are presented as a percentage change relative to the control. A ChemiScope 6000 Touch was used to read the chemiluminescence, and ImageJ (version 1.53t; National Institutes of Health) was utilized to analyze the density of the immunoreactive bands.

ELISA. Supernatants from treated H9C2 cardiac myoblasts were used to evaluate the oxidative and antioxidant status by measuring the levels of superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA). These measurements were carried out using ELISA kits from Nanjing Jiancheng Bioengineering Institute, including the SOD (cat. no. A001-3-2), CAT assay kit (cat. no. A007-2-1), and MDA assay kit (cat. no. A003-4-1).

Statistical analysis. Statistical analyses were carried out using GraphPad Prism (version 8.0; Dotmatics). Data are presented as the mean ± standard error of the mean. In the present analysis, the normality of the data was assessed using the Shapiro-Wilk test, which is well-suited for small sample sizes. For comparisons among multiple groups, when the data were normally distributed but the assumption of equal variances was violated, Welch's ANOVA was used. If the data were not normally distributed, the Kruskal-Wallis test was used as a non-parametric alternative to Welch's ANOVA, followed by Dunn's post hoc test for multiple comparisons. All experiments were repeated ≥3 times independently. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Impact of H_2O_2 treatment on H9C2 cardiac myoblasts under HG conditions. Compared to HG group, exposure to H_2O_2 in a HG environment significantly impaired H9C2 cardiac myoblasts viability (Fig. 2; $P<0.01$) and increased the proportion of TUNEL-positive cells (Fig. 3; $P<0.01$), indicating enhanced apoptosis (Fig. 4; $P<0.01$). Western blot analysis (Fig. 5A) demonstrated that H_2O_2 treatment upregulated Caspase-3 (Fig. 5B; $P<0.01$) and Bax (Fig. 5D; $P<0.01$), while downregulating Bcl-2 (Fig. 5C; $P<0.01$). Furthermore, MDA levels were increased (Fig. 6C; $P<0.01$), whereas SOD (Fig. 6A; $P<0.01$) and CAT (Fig. 6B, $P<0.01$) activities were significantly decreased in the HG + H_2O_2 group compared to the HG group.

Dex attenuates H_2O_2-induced cardiac myoblast injury in a HG environment. Dex significantly reduced H_2O_2-induced cardiac myoblast injury by increasing cell viability (Fig. 2; HG + H_2O_2 vs. DP + HG + H_2O_2; $P<0.01$). DP markedly alleviated H_2O_2-induced apoptosis, as evidenced by a reduction in the TUNEL-positive cell ratio (HG + H_2O_2 vs. DP + HG + H_2O_2; $P<0.01$; Fig. 3) and the apoptosis rate (HG + H_2O_2 vs. DP + HG + H_2O_2; $P<0.01$; Fig. 4). Furthermore, Dex treatment significantly reduced the expression levels of apoptotic proteins caspase-3 (Fig. 5B; HG + H_2O_2 vs. DP + HG + H_2O_2; $P<0.05$) and BAX (Fig. 5D; HG + H_2O_2 vs. DP + HG + H_2O_2; $P<0.01$).

Inhibition of PI3K signaling weakens Dex-mediated reduction of H_2O_2-induced oxidative stress in cardiac myoblasts under HG conditions. Although DP significantly reduced the number and

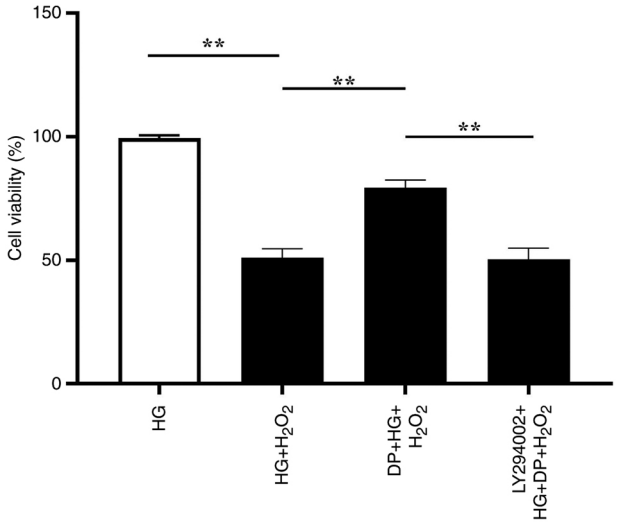

Figure 2. Cell viability was assessed using a Cell Counting Kit-8 assay. Data are presented as the mean ± SEM; n=5 per group. **P<0.01. HG, high-glucose; DP, dexmedetomidine preconditioning; H_2O_2, hydrogen peroxide.

percentage of apoptotic cells, the protective effects of Dex on cell viability were abolished by the PI3K inhibitor LY294002 (Fig. 2; HG + DP + H_2O_2 vs. LY294002 + HG + DP + H_2O_2; P<0.01). Simultaneously, the percentage of TUNEL-positive cells (Fig. 3B; HG + DP + H_2O_2 vs. LY294002 + HG + DP + H_2O_2; P<0.01) and apoptosis rate (Fig. 4E; HG + DP + H_2O_2 vs. LY294002 + HG + DP + H_2O_2; P<0.01) were significantly increased in the presence of LY294002.

Dex prevents H_2O_2-induced cardiac myoblast apoptosis via the PI3K/AKT signaling pathway under HG conditions. H_2O_2 treatment induced apoptosis. After administration of the PI3K inhibitor LY294002, the protective effects of Dex were abolished as there was a significant increase in the expression of caspase-3 (Fig. 5B; P<0.05) and BAX (Fig. 5D; P<0.01) in the LY294002 + HG + DP + H_2O_2 group compared with the HG + DP + H_2O_2 group. Furthermore, the levels of p/total-PI3K (Fig. 5E; P<0.01), p-AKT (Fig. 5F; P<0.05) and BCL-2 (Fig. 5C; P<0.01) were also reduced in the LY294002 + HG + DP + H_2O_2 group compared with the HG + DP + H_2O_2 group. The calculated fold-changes, derived from normalized densitometric values, accurately represented the protein expression changes across replicates. These findings suggested that Dex acted through the PI3K/AKT signaling pathway to reduce H9C2 cell death in acute hyperglycemic microenvironments.

Protective effects of Dex under HG conditions are achieved by reducing oxidative damage to cardiomyocytes through the PI3K/AKT signaling pathway. As shown in Fig. 6, the oxidative stress indicator MDA, and the antioxidant stress indicators SOD and CAT were examined. Compared with those in the HG group, the MDA levels in the HG + H_2O_2 group were increased (30.3±2.8 vs. 70.1±8.5; P<0.01; Fig. 6C), while the SOD (138.6±24.1 vs. 57.2±10.9; P<0.01; Fig. 6A) and CAT (11.2±1.8 vs. 6.7±1.5; P<0.01; Fig. 6B) levels were decreased. However, compared with the HG + H_2O_2 group, the DP + HG + H_2O_2 group exhibited decreased MDA levels (70.1±8.5 vs. 45.6±2.5; P<0.01; Fig. 6C), and increased SOD (57.2±10.9 vs. 115.4±24.7; P<0.01) and CAT (6.7±1.5 vs. 10.6±1.8; P<0.01; Fig. 6B) levels. Following treatment with LY294002, the LY294002 + HG + DP + H_2O_2 group exhibited increased MDA levels (45.6±2.5 vs. 112.4±11.7; P<0.01; Fig. 6C), and decreased SOD (115.4±24.7 vs. 62.4±12.2; P<0.01; Fig. 6A) and CAT (10.6±1.8 vs. 7.8±1.1; P<0.01; Fig. 6B) levels, compared with the DP + HG + H_2O_2 group.

Discussion

Perioperative cardiac protection is a key area of research (17-19), particularly due to its potential to improve outcomes in patients with preexisting cardiovascular conditions or those undergoing high-risk surgical procedures (20). Of particular concern is the impact of diabetes and elevated blood glucose levels, both of which have been demonstrated to notably impair the effectiveness of traditional cardioprotective strategies (4). High blood glucose levels, commonly observed in patients with diabetes, can diminish or nullify the protective effects of traditional interventions (21). This highlights the need for innovative strategies to safeguard the heart under HG conditions, especially during periods of I/R injury (22). Given the increasing prevalence of diabetes (23), understanding the impact of a HG environment on cardiac cells is important. The present study specifically simulated diabetic conditions, thereby addressing an underexplored clinical need.

The PI3K/AKT signaling pathway is known for its key role in cell survival, metabolism and proliferation. Under normal conditions, this pathway promotes cell survival and tissue repair by regulating key processes such as glucose metabolism, protein synthesis and anti-apoptotic signaling (24-26). However, in the context of HG, such as in diabetes, this pathway becomes dysregulated (27). Previous studies have revealed that HG levels inhibit AKT phosphorylation, leading to reduced nitric oxide production, endothelial dysfunction and impaired myocardial protection (28,29). These disruptions are considered to contribute to the increased risk of cardiovascular events in patients with diabetes, as the heart becomes more vulnerable to I/R injury (23).

The findings of the present study suggested that Dex pretreatment effectively mitigated myocardial injury in HG environments, potentially through the modulation of the PI3K/AKT signaling pathway. This is a potential advancement in understanding the mechanisms underlying cardiac protection in diabetes, where conventional protective strategies often fail (21). Specifically, analysis revealed that Dex reduced oxidative stress, a known contributor to myocardial injury, and preserved myocardial function during I/R injury. This result is in line with previous studies (30,31) demonstrating that oxidative stress, mediated by an excess of reactive oxygen species, serves a key role in driving myocardial apoptosis, fibrosis and remodeling, processes that ultimately lead to heart failure. In the present study, the ability of Dex to protect against oxidative damage suggested a mechanism through which it conferred myocardial protection under diabetic conditions.

While previous studies have demonstrated the cardioprotective effects of Dex in models such as hypoxia/reoxygenation injury (30) and septic myocardial dysfunction (31), the present study offered novel insights by examining its effects under HG conditions. This experimental model mimics the pathophysiological environment of diabetic cardiomyopathy, where

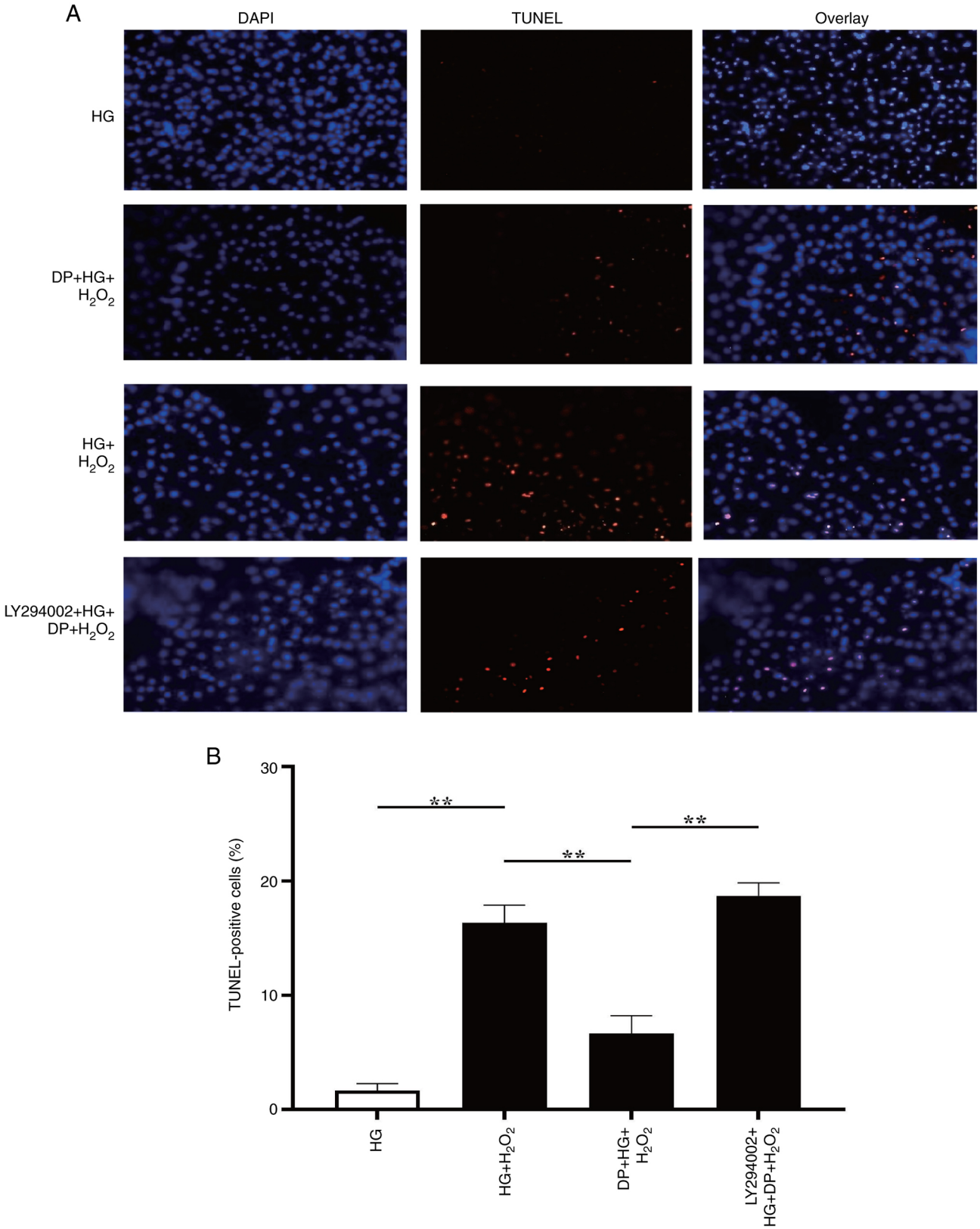

Figure 3. TUNEL staining analysis. (A) Morphological changes were detected by TUNEL staining (magnification, x40). (B) Quantification of TUNEL staining. Data are presented as the mean ± SEM; n=5 per group. **P<0.01. HG, high-glucose; DP, dexmedetomidine preconditioning; H_2O_2, hydrogen peroxide.

oxidative stress is a key contributor to cardiac injury (32). By focusing on this clinically relevant condition, the present study provided a novel perspective on the role of Dex in modulating oxidative damage. Furthermore, the present study demonstrated that the protective effects of Dex were mediated, at least in part, through activation of the PI3K/AKT signaling pathway, a mechanism that supports cardiomyocyte survival and function under metabolic stress. As nearly 589

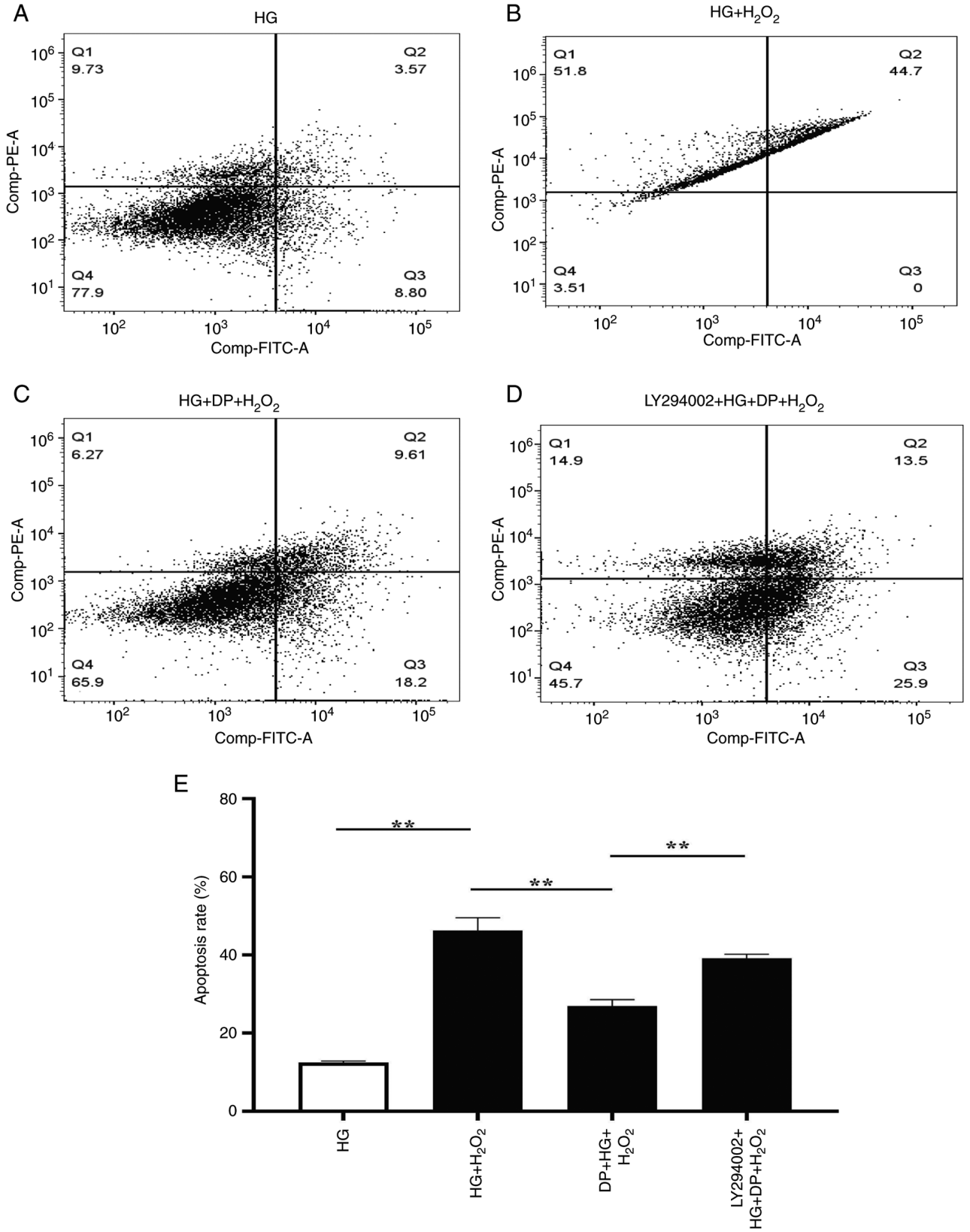

Figure 4. Flow cytometry analysis of apoptosis. (A) HG group. (B) HG + H_2O_2 group. (C) DP + HG + H_2O_2 group. (D) LY294002 + HG + DP + H_2O_2 group. (E) Quantification of H9C2 cell apoptosis. $^{**}P<0.01$. Data are presented as the mean ± SEM, n=5 per group. HG, high-glucose; DP, dexmedetomidine preconditioning; H_2O_2, hydrogen peroxide.

million adults (about one in nine) worldwide are currently living with diabetes and that high blood glucose accounts for roughly 11% of global cardiovascular deaths (33), the findings of the present study highlighted the potential of Dex as a therapeutic candidate for attenuating oxidative stress in diabetic cardiomyopathy.

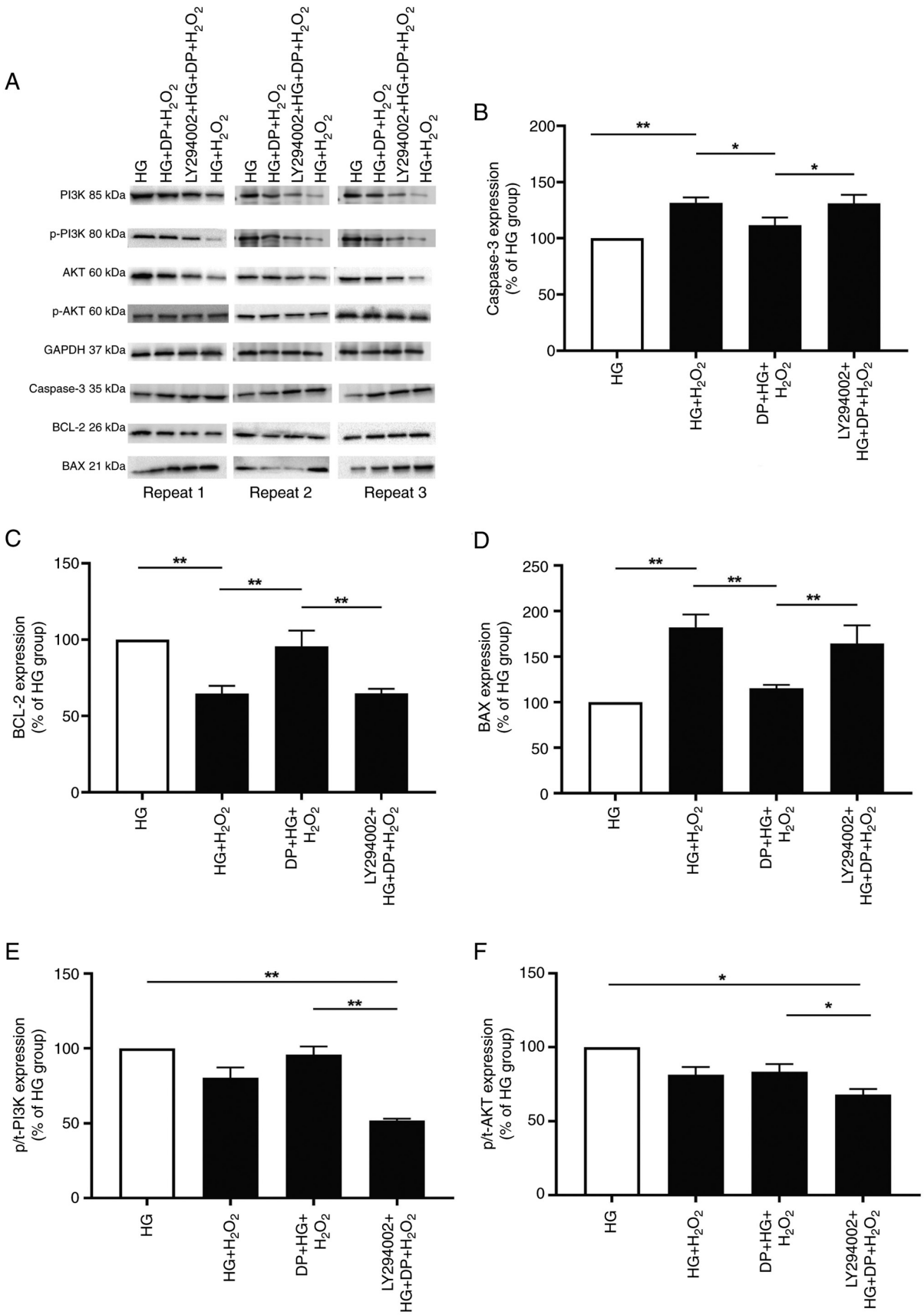

Figure 5. Detection of the levels of apoptosis-related proteins by western blotting. (A) Protein bands of apoptosis-related proteins caspase-3, PI3K, AKT, BCL-2, BAX, p-PI3K and p-AKT in H9C2 cells from the four groups assessed by western blotting. GAPDH served as an internal control for sample loading. The differing band curvatures observed ('frown' effect for p-PI3K and 'smile' effect for t-PI3K) may result from minor membrane handling variations during blotting or localized gel inconsistencies during protein migration. These factors cause uneven stretching, folding or pressure, leading to the observed discrepancies. Semi-quantification of western blotting data of apoptosis-related proteins, including (B) caspase-3, (C) BCL-2, (D) BAX, (E) p/t-PI3K and (F) p/t-AKT, in H9C2 cells from the four groups. n=3 per group. *P<0.05, **P<0.01. HG, high-glucose; Dex, dexmedetomidine; DP, dexmedetomidine preconditioning; p, phosphorylated; t, total; H_2O_2, hydrogen peroxide.

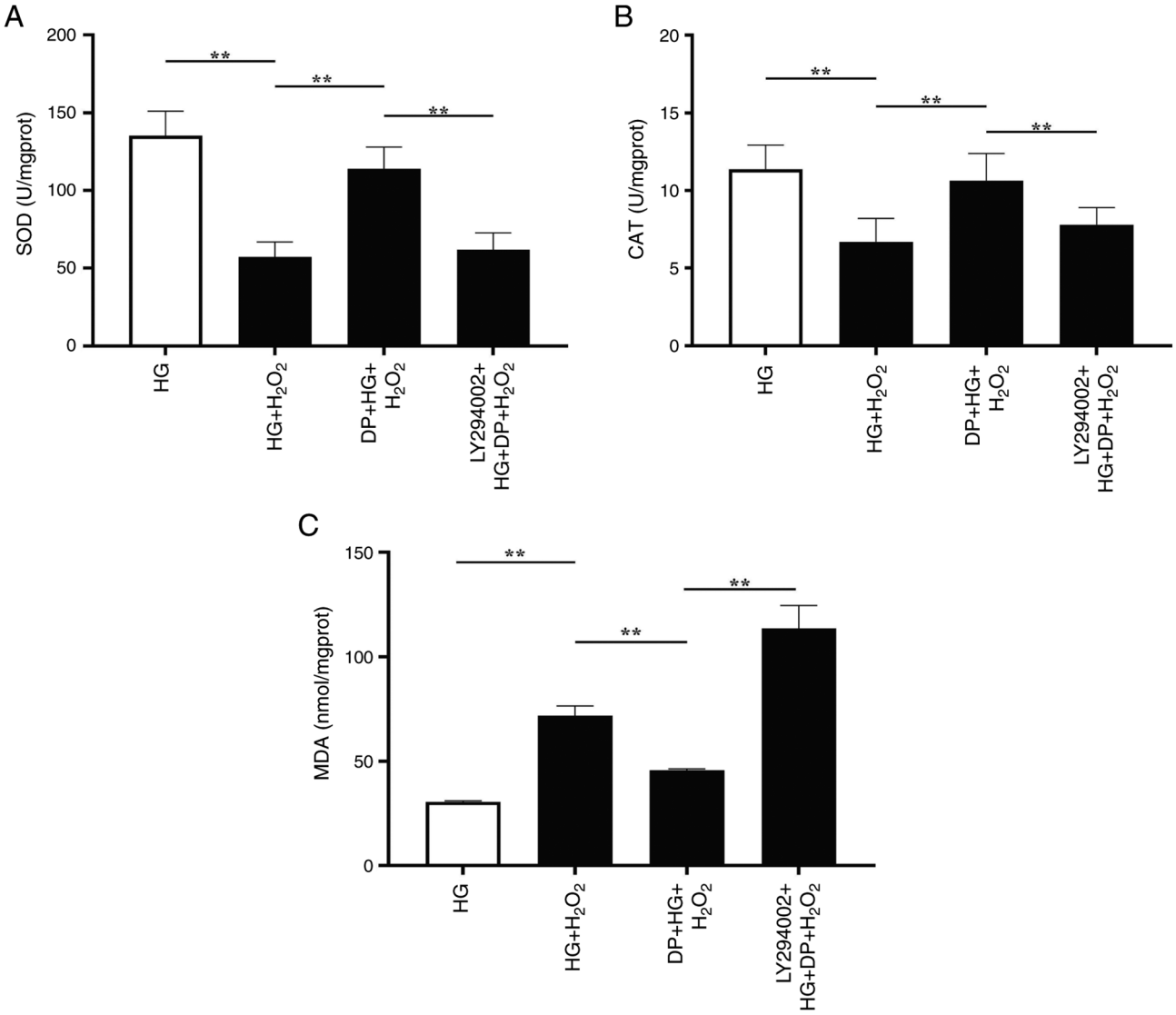

Figure 6. Analysis of oxidative stress markers in each group. (A) SOD), (B) Catalase (CAT), and (C) Malondialdehyde (MDA). Data are presented as the mean ± SEM; n=5 per group. **P<0.01, SOD, superoxide dismutase; CAT, catalase; MDA, malondialdehyde; HG, high-glucose; DP, dexmedetomidine preconditioning; H_2O_2, hydrogen peroxide; mgprot, mg protein.

The selection of 33 mM glucose was informed by review of the literature, which indicated that such a concentration effectively mimics the HG environment observed in patients with diabetes while inducing marked myocardial injury (34). The variability in glucose concentrations across different studies, particularly those investigating vascular injury or atherosclerosis, reflects the complexity of replicating human metabolic conditions in animal models (35-37). This highlights the need for consistent and context-specific model selection when studying cardiac protection under diabetic conditions.

While the results of the present study suggested that Dex offered protection through the PI3K/AKT pathway, there are certain limitations. First, all experiments were conducted using H9C2 cardiac myoblasts, which, although widely used, may not fully recapitulate the complex pathophysiology of myocardial injury in *in vivo* systems. To establish a direct causal association between the effects of Dex and PI3K/AKT signaling, small interfering RNA-mediated knockdown of PI3K and AKT in H9C2 cells will first be carried out to verify the involvement of this pathway. Subsequently, streptozotocin-induced diabetic rats will be used to measure cardiac function, infarct size and reactive oxygen species markers to validate the cardioprotective role of Dex.

LY294002 is a well-known PI3K inhibitor that exerts context-dependent effects on cardiac myoblasts. While it may reduce maladaptive PI3K/AKT pathway activation in specific scenarios such as I/R injury, its cardioprotective effects are limited and situational (38). More commonly, LY294002 inhibits key survival signaling pathways in cardiac myoblasts, which can lead to increased apoptosis and cardiac myoblast death, particularly under stress conditions such as hypoxia or ischemia (39). Given its dual role, the cardiotoxic potential of LY294002 is well-documented when the PI3K/AKT pathway is essential for survival. Without a LY294002-only group, it is challenging to definitively conclude whether the cardioprotective effects of Dex

were mediated specifically through the PI3K/AKT pathway. Therefore, future studies incorporating a LY294002-only control group will be key to clarify the role of the PI3K/AKT pathway in the cardioprotective mechanisms of Dex. Finally, a single concentration of Dex was used and its effects were assessed at only one time point. This approach limits the understanding of the dose-response relationship and the temporal dynamics of the protective effects of Dex on H9C2 cells.

In conclusion, the present study provided novel insights into the potential therapeutic role of Dex in protecting the myocardium under HG conditions. By reducing oxidative stress through modulation of the PI3K/AKT pathway, Dex may offer a novel strategy for protecting the heart in patients with diabetes, particularly during I/R injury. Future studies on Dex will investigate its protective effects in comorbidity *in vivo* models of diabetic cardiomyopathy, providing a rationale for personalized treatment approaches.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

YQ and JQ designed the study. YQ carried out the main experiments. WX, RZ and NS analyzed data. YQ and JQ confirm the authenticity of all the raw data. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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