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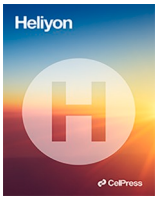

Dexmedetomidine combined with propofol attenuates myocardial ischemia/reperfusion injury by activating the AMPK signaling pathway

Ke Yang [1], Yinhong Ma [1], Chunmei Xie, Lixian He, Haoxing Zhao, Zheng Dai [*], Xiaoqi Wang [**]

Department of Anesthesiology, Fuwai Yunnan Cardiovascular Hospital, Kunming, Yunnan, 650000, China

A R T I C L E I N F O

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A B S T R A C T

Objective: Myocardial ischemia/reperfusion (MI/R) injury is a major cause of cardiac tissue damage, with high disability and death rates. Although both dexmedetomidine (Dex) and propofol (PPF) have been indicated to alleviate MI/R injury in rat models, the effects of the combined use of these two drugs remain unclear. This study aimed to investigate the combined effects of Dex and PPF against MI/R injury and related mechanisms.

Methods: A rat model of MI/R injury was established and used to explore the combined effects of Dex and PPF on MI/R injury. Hematoxylin-eosin (HE) and Masson staining were used for histopathological evaluation. 2,3,5-triphenyltetrazolium chloride (TTC), echocardiography, terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) staining were used to determine myocardial infarction size, cardiac function, and apoptosis, respectively. Enzyme-linked immunosorbent assay (ELISA) was performed to assess myocardial function and oxidative stress (OS). Autophagy was observed through transmission electron microscopy. Moreover, western blotting was conducted to detect autophagy markers and the AMPK pathway.

Results: The combination of Dex and PPF alleviated histopathological injury, reduced myocardial infarction, and rescued cardiac dysfunction in MI/R rats. Furthermore, Dex combined with PPF decreased the levels of MDA and ROS and increased the SOD level in MI/R rats. Besides, Dex combined with PPF inhibited myocardial apoptosis in MI/R rats. After combined treatment with Dex and PPF, the number of autophagosomes, expression levels of Beclin-1 and LC3II/LC3I were elevated, while the expression levels of p62 were reduced in MI/R rats. The combined use of Dex and PPF activated the AMPK pathway in MI/R rats. Compound C (an AMPK inhibitor) could abolish the combined effects of Dex and PPF on alleviating myocardial injury and enhancing autophagy in MI/R rats.

Conclusion: The combination of Dex and PPF attenuated MI/R injury in rats, which may be associated with the activation of the AMPK signaling pathway.

* Corresponding author. Department of Anesthesiology, Fuwai Yunnan Cardiovascular Hospital, No.528 Shahu North Road, Kunming, Yunnan, 650000, China.

** Corresponding author. Department of Anesthesiology, Fuwai Yunnan Cardiovascular Hospital, No.528 Shahu North Road, Kunming, Yunnan, 650000, China.

E-mail addresses: daizheng@kmmu.edu.cn (Z. Dai), wxqfwn@163.com (X. Wang).

[1] Co-first Author: These authors contributed equally to this work.

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1. Introduction

Ischemic heart disease occurs in numerous clinical pathologies and is a huge burden on humans associated with high mortality[1]. Targeting for myocardial ischemia/reperfusion (MI/R) is the most common and effective strategy for ischemic heart disease[2]. However, restoring the blood flow to the ischemic myocardium also triggered cardiac injury, which is called MI/R injury[3]. MI/R injury causes oxidative stress (OS) and inflammation, thus leading to apoptosis and necrosis of myocardial cells[4]. Moreover, it can inducereperfusion arrhythmia and even sudden cardiac death [5]. Despite different strategies for MI/R injury, more effective and practical curative approaches are desperately needed.

Dexmedetomidine (Dex) is a highly selective α-2 adrenoceptor agonist, which has sympatholytic, sedative, amnestic, and analgesic properties and is applied for sedating patients in operation rooms or intensive care units[6]. Dex has been employed to decrease intraoperative blood loss, improve intraoperative and postoperative analgesia, and sedate damaged airways without respiratory depression[7]. Notably, relevant studies have shown that Dex may attenuate MI/R injury in some rat models[8–10]. Similarly, propofol (PPF) is a potent intravenous hypnotic agent and has been used in some clinical problems, such as lung cancer tumorigenesis and cerebral ischemia reperfusion (CI/R) injury. Currently, PPF is used to avoid cardio-pulmonary injuries[11–13]. Importantly, the previous research also demonstrated that PPF can perform protective functions on MI/R injury[14–16]. Interestingly, a prior clincal study indicated that the combination therapy of Dex and PPF in open-heart surgery provides enhanced cardioprotective effects compared to propofol used alone[17]. Therefore, we hypothesized that the combined use of Dex and PPF might also exert a more beneficial effect on the treatment of MI/R injury than Dex or PPF used alone. These facts pushed us to further explore the effects of Dex and combined with PPF on MI/R and underlying mechanisms.

Autophagy is a cellular self-renewing process that relies on the degradation of the cytoplasmic proteins or organelles of lysosomes [18]. Evidence has demonstrated that autophagy was involved in the pathogenesis of MI/R injury[19–21]. The AMPK signaling pathway is an intracellular signal transduction pathway related to autophagy. Relevant studies have suggested that activating AMPK-mediated autophagy can mitigate MI/R injury in mouse models[22,23]. Nevertheless, some studies have shown that overactivation of AMPK-mediated autophagy aggravated MI/R injury[24,25]. These facts further manifest the complexity of autophagy mechanisms in MI/R injury. A study has indicated that Dex protects human cardiomyocytes from I/R injury via activating AMPK-dependent autophagy[26]. Based on these findings, we speculated that Dex and PPF may exert therapeutic effects on MI/R injury via targeting AMPK signaling.

In our study, we investigated the therapeutic effects of the combined use of Dex and PPF on MI/R injury with a focus on its effect on autophagy. Moreover, the related pathway (the AMPK signaling pathway) was explored. This study aims to explore the combined effects of Dex and PPF on the treatment of MI/R injury and related mechanisms, thereby providing novel insights into the mechanisms of MI/R injury and directions for the development of future treatment.

2. Materials and methods

2.1. Establishment of a MI/R rat model

A total of 36 male Sprague-Dawley (SD) rats (aged 8 weeks, weighing 230–270 g) were purchased from Kaixue Biotechnology company (Shanghai, China). All SD rats were housed in separate cages under a 12-h light/dark cycle at room temperature, with free access to food and water. After a week, all SD rats were randomly divided into the control (n = 6) and model groups (n = 30)[27]. A rat model of MI/R was established based on previous research[28,29]. Briefly, rats in the model group were anesthetized with 2–5% isoflurane using the gas anesthesia machine and put on a temperature-controlled heating pad to expose the left anterior descending coronary artery (LAD), and a 6-0 suture was passed through the LAD to form a slipknot around the LAD. After 30 min of ischemia, the knot was released to restore the flow of blood, followed by 2 h of reperfusion. Blood samples were collected 4 h after reperfusion and stored at −80 °C before assays. At the same time, the control group underwent myocardial exposure after thoracotomy without LAD ligation [27][29,29].

The 30 model rats were assigned to the following five groups (6 rats/group) [29][27,29]: Model, Model + Dex, Model + PPF, Model + Dex + PPF, Model + Dex + PPF + Compound C (an AMPK inhibitor). Before reperfusion after myocardial ischemia, rats in Control and Model groups were intravenously injected with 1 % dimethyl sulfoxide (DMSO) and 0.9 % normal saline. Meanwhile, rats in Model + Dex group were intravenously injected with 5 μg/kg Dex, and rats in Model + PPF group were intravenously injected with 25 mg/kg PPF alone. Rats in Model + Dex + PPF group were intravenously injected with 5 μg/kg Dex and 25 mg/kg PPF, while rats in Model + Dex + PPF + Compound C group were intravenously injected with 5 μg/kg Dex, 25 mg/kg PPF, and 0.25 mg/kg compound C. The processes of drug treatment referred to the previous study[30]. Fourteen days after model establishment, all rats were euthanized by intraperitoneal injection with 2 % pentobarbital (100 mg/kg), and the myocardial tissues of rats were collected and stored at −80 °C for later use. All animal protocols were approved by the Animal Experimental Ethics Committee of Kunming Medical University.

2.2. Hematoxylin-eosin (HE) and Masson staining

Fourteen days after model establishment, all rats were sacrificed and their myocardial tissues were harvested. The myocardial tissues from the rats in each group were fixed in 4 % paraformaldehyde, for 24 h. The tissues were then routinely dehydrated using gradient ethanol. Subsequently, the tissues were transparentized with xylene, followed by paraffin embedding. The paraffin-embedded

sections were sliced at 5 μm. The sections were dewaxed and rehydrated in xylene, and fractionated ethanol. For HE staining, the sections were stained with hematoxylin for 5 min and eosin for 2 min. For Masson staining, the sections were stained with hematoxylin for 5 min, ponceau acid fuchsin for 5 min, and fast green FCF for 1 min. The sections were observed using a fluorescence microscope (BX53, Olympus, Tokyo, Japan).

2.3. 2,3,5-Triphenyltetrazolium chloride (TTC) staining

The myocardial tissues were placed in the rat section mold, and 2 mm continuous sections were obtained with a surgical blade. Then, the sections were soaked in TTC dye solution and incubated at 37 °C for 30 min. After that, the sections were fixed in 4 % paraformaldehyde. The stained sections were photographed by a camera (SC180, Olympus, Tokyo, Japan).

2.4. Echocardiography

Echocardiography was used to assess cardiac function of rats in each group. Briefly, the rats were anesthetized with 2 % isoflurane, and cardiac function was evaluated by small animal ultrasound system (VEVO-2100, VisualSonics Inc, Toronto, Canada). The vital parameters of cardiac function, including left ventricular ejection fraction (LVEF), left ventricular fractional shortening (LVFS), left ventricular end-systolic volume (LVESV), and left ventricular end-systolic diameter (LVESD), were analyzed.

2.5. Enzyme-linked immunosorbent assay (ELISA)

The levels of AST, CK-MB, cTnl, LDH, and MDA in serum, as well as the levels of ROS and SOD in the myocardial tissues, were assessed by the ELISA kits (Solarbio, Beijing, China) according to the manufacturer's instructions. Finally, the levels of these parameters were obtained at 450 nm wavelength using a DR-3518G microplate reader (Wuxi Hiwell Diatek, China).

2.6. Terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) staining

TUNEL kits (Beyotime Biotech, Shanghai, China) were used to measure the apoptosis in the myocardial sections. Prepared paraffin-embedded sections were dewaxed and dehydrated in gradient ethanol and then incubated with proteinase K solution (50 μg/mL) at 37 °C for 30 min. Finally, the stained cells were observed with a fluorescence confocal microscope (FV3000, Olympus, Tokyo, Japan).

2.7. Immunofluorescence assay

The paraffin-embedded myocardial tissue sections were fixed in 4 % formaldehyde for 24 h. The myocardial tissue sections were sealed with 5 % skimmed milk powder (Beyotime Biotech, Shanghai, China) at 37 °C for 1 h and were then incubated overnight with LC3 antibody (Cell Signal Technology, USA) at 4 °C. After that, they were incubated in fluorescent dye-bound secondary antibodies at room temperature for 1 h and were stained with DAPI. Finally, the images were captured by a fluorescence confocal microscope (FV3000, Olympus, Tokyo, Japan). The mean fluorescence intensity (integrated intensity/area) was quantified by ImageJ software (NIH, Bethesda, MD, USA).

2.8. Western blot assay

The myocardial tissue samples were collected and lysed with RIPA lysis buffer (Beyotime Biotech, Shanghai, China) for extracting total protein. Then, the lysate was collected and detected for protein concentration by a BCA kit (Beyotime Biotech, Shanghai, China). The obtained proteins were transferred to several polyvinylidene difluoride (PVDF) membranes (Beyotime Biotech, Shanghai, China), and the membranes were blocked by 5 % skimmed milk powder (Beyotime Biotech, Shanghai, China) for 1 h. Then, the membranes were incubated with anti-Beclin-1 (1:1,000, Abcam, UK), anti-p62 (1:1,000, Cell Signal Technology, USA), anti-LC3II/I (1:1,000, Cell Signal Technology, USA), anti-AMPK (1:1,000, Abcam, UK), anti-*p*-AMPK (1:1,000, Abcam, UK), and anti-GAPDH (1:10,000, Abcam, UK) primary antibodies at 4 °C overnight. After that, the membranes were incubated with Goat anti-Rabbit IgG H&L (HRP) (1:2,000, Abcam, UK) secondary antibody for 1 h. The blots were developed using an enhanced chemiluminescence (ECL) kit (#P1000, Applygen, Beijing, China). The gray values of target proteins were quantified by ImageJ software using GAPDH as the internal reference.

2.9. Transmission electron microscopy (TEM)

The myocardial tissues were fixed with 2.5 % glutaraldehyde for 24 h, washed with PBS buffer, and fixed with 1 % osmium tetroxide for 2 h. After dehydration with ethanol, uranium ethanolacetate, and epoxypropane, the tissues were soaked and embedded in pure Eponate 12 resin (#18005, Ted Pella Inc, CA, USA). Then, the tissues were sectioned at 50–70 nm to obtain ultrathin slices using an ultramicrotome (UC-7, Leica, Germany) and stained with 1 % uranyl acetate and lead citrate. Finally, autophagy was observed under a TEM (Tecnai F30, FEI, OR, USA).

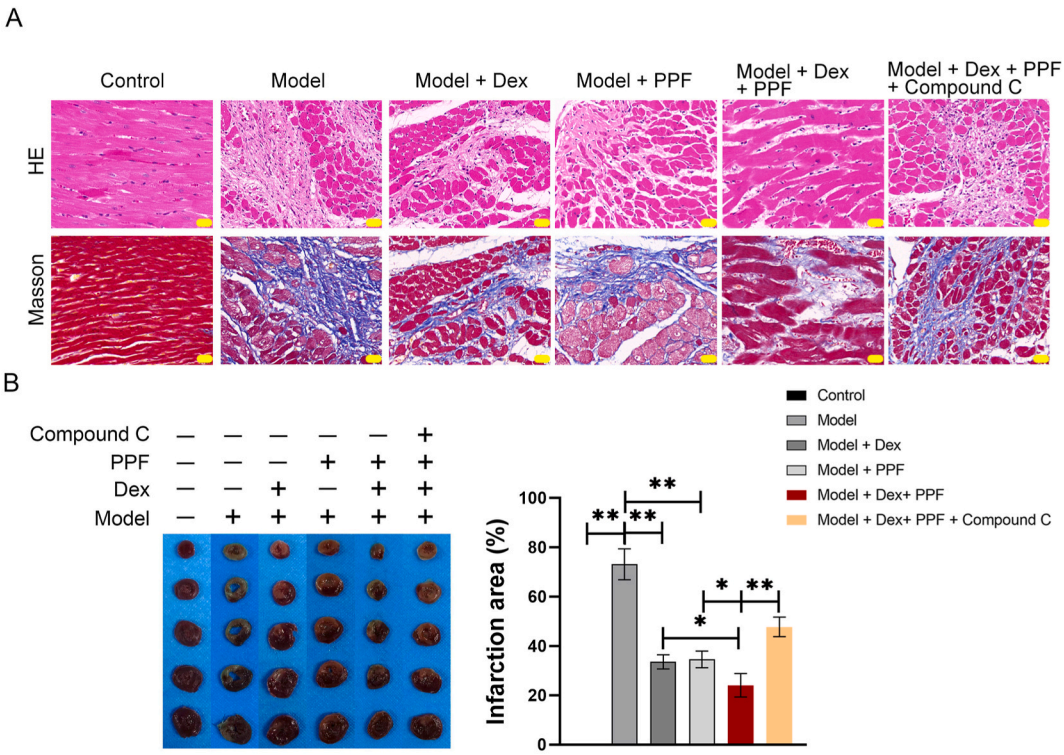

Fig. 1. The combination of Dex and PPF attenuates MI/R injury in rats. (A) Detection of histopathological injury using HE and Masson staining. Scale bar = 20 μm; magnification : 40 × . **(B)** Detection of the myocardial infarction size using TTC staining. Data were expressed as the mean ± standard deviation.*$P < 0.05$ and **$P < 0.01$ between each group. Dex, dexmedetomidine; PPF, propofol; MI/R, myocardial ischemia/reperfusion; HE, hematoxylin-eosin; TTC, 2,3,5-triphenyltetrazolium chloride.

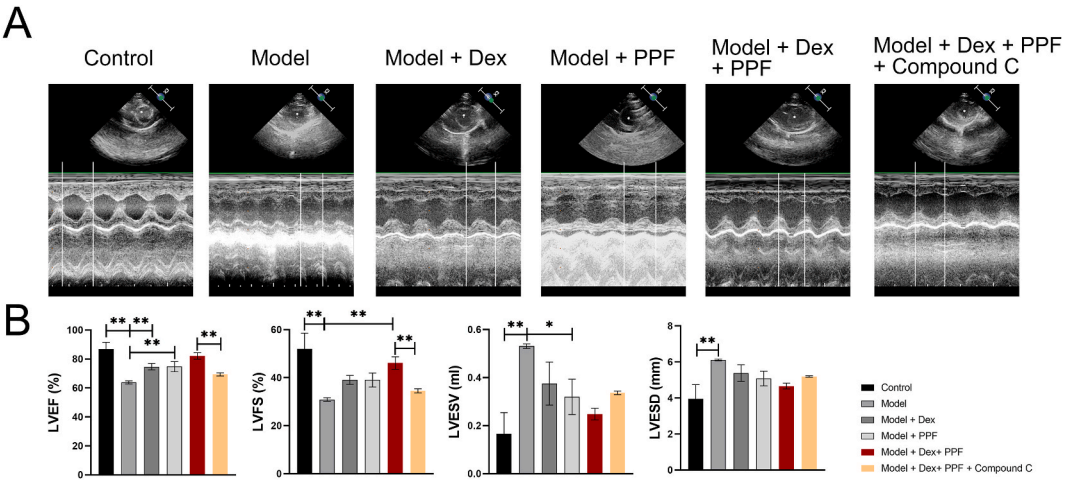

Fig. 2. The combination of Dex and PPF alleviates cardiac dysfunction in MI/R rats. Echocardiographic images (**A**) and evaluation of cardiac function indicators (**B**), LVEF, LVFS, LVESV, and LVESD. Data were expressed as the mean ± standard deviation. *$P < 0.05$ and **$P < 0.01$ between each group. Dex, dexmedetomidine; PPF, propofol; MI/R, myocardial ischemia/reperfusion; LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening; LVESV, left ventricular end-systolic volume; LVESD, left ventricular end-systolic diameter.

2.10. Statistical analysis

All data were presented as the mean ± standard deviation. GraphPad 7.0 software was applied for statistical analysis. A one-way ANOVA test was used to compare the differences between groups. $P < 0.05$ was considered statistically significant.

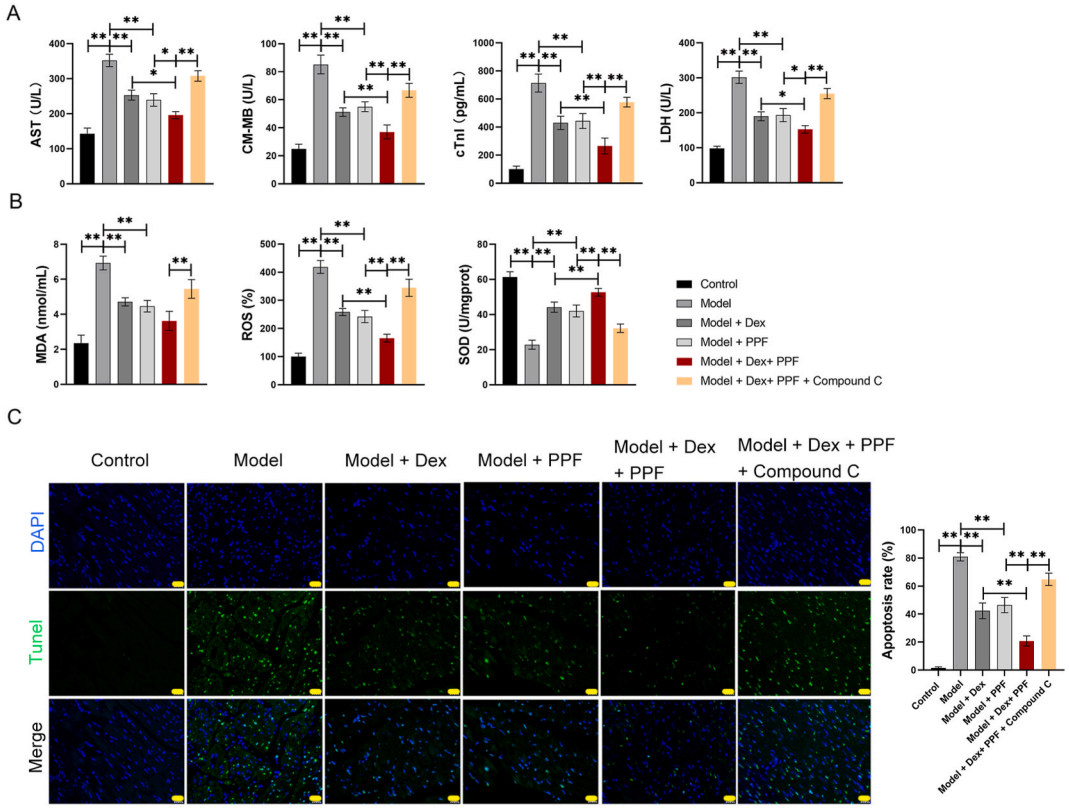

Fig. 3. The combination of Dex and PPF inhibits OS and myocardial apoptosis in MI/R rats. (A and B) The levels of AST, CK-MB, cTnI, LDH, MDA, ROS, and SOD using ELISA. **(C)** Detection of the number of myocardial cell apoptosis using TUNEL staining. Scale bar = 20 μm; magnification : 40 × . Data were expressed as the mean ± standard deviation. *$P < 0.05$ and **$P < 0.01$ between each group. Dex, dexmedetomidine; PPF, propofol; OS, oxidative stress; MI/R, myocardial ischemia/reperfusion; ELISA, enzyme-linked immunosorbent assay; TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labeling.

3. Results

3.1. Dex combined with PPF ameliorated myocardial infarction in MI/R rats

To investigate the effects of Dex combined with PPF on myocardial injury, MI/R model rats were utilized and treated with Dex, PPF, or/and compound C (an AMPK inhibitor). HE staining showed that the myocardial cells were sparse and hypertrophy, the collagen fibers thickened, and the collagen fiber spacing increased in myocardial tissues of MI/R rats compared with those in control rats. After treatment with Dex or/and PPF, the histopathological injury of MI/R rats was alleviated, in particular when treated with both Dex and PPF. AMPK activation can mitigate organ/cell damage by maintaining mitochondrial homeostasis and metabolism[31]. Our results showed that compound C weakened the combined effect of Dex and PPF on mitigating pathological damage in MI/R rats (Fig. 1A). Myocardial tissue fibrosis is a common pathological phenomenon in injured myocardial tissues. Masson staining presented that the number of smooth muscles dropped and the number of collagen fibers increased in MI/R rats compared with those in control rats. After treatment with Dex or/and PPF, the number of smooth muscles increased and the number of collagen fibers fell, especially Dex combined with PPF. The results showed that compound C abolished the effect of Dex combined with PPF on reducing myocardial tissue fibrosis (Fig. 1A). Furthermore, TTC staining showed the myocardial infarction area in MI/R rats was significantly larger than that in control rats ($P < 0.01$), which was reversed following Dex or/and PPF treatment ($P < 0.05$) (Fig. 1B). Also, compound C eliminated the effect of Dex combined with PPF on mitigating myocardial infarction ($P < 0.01$) (Fig. 1B).

3.2. Dex combined with PPF alleviated cardiac dysfunction in MI/R rats

To further determine the combined effects of Dex combined with PPF on MI/R injury, we employed echocardiography to assess cardiac function of rats in each group. Our results showed the crucial indicators of cardiac function, LVEF and LVFS, markedly declined in MI/R rats relative to control rats, while LVESV and LVESD increased ($P < 0.01$) (Fig. 2). After treatment with Dex or/and PPF, LVEF and LVFS were markedly upregulated in MI/R rats ($P < 0.01$), while LVESV and LVESD showed dcreasing trends (Fig. 2). These results indicated the cardioprotective effects of Dex or/and PPF treatments. Furthermore, the combination of Dex and PPF exerted a

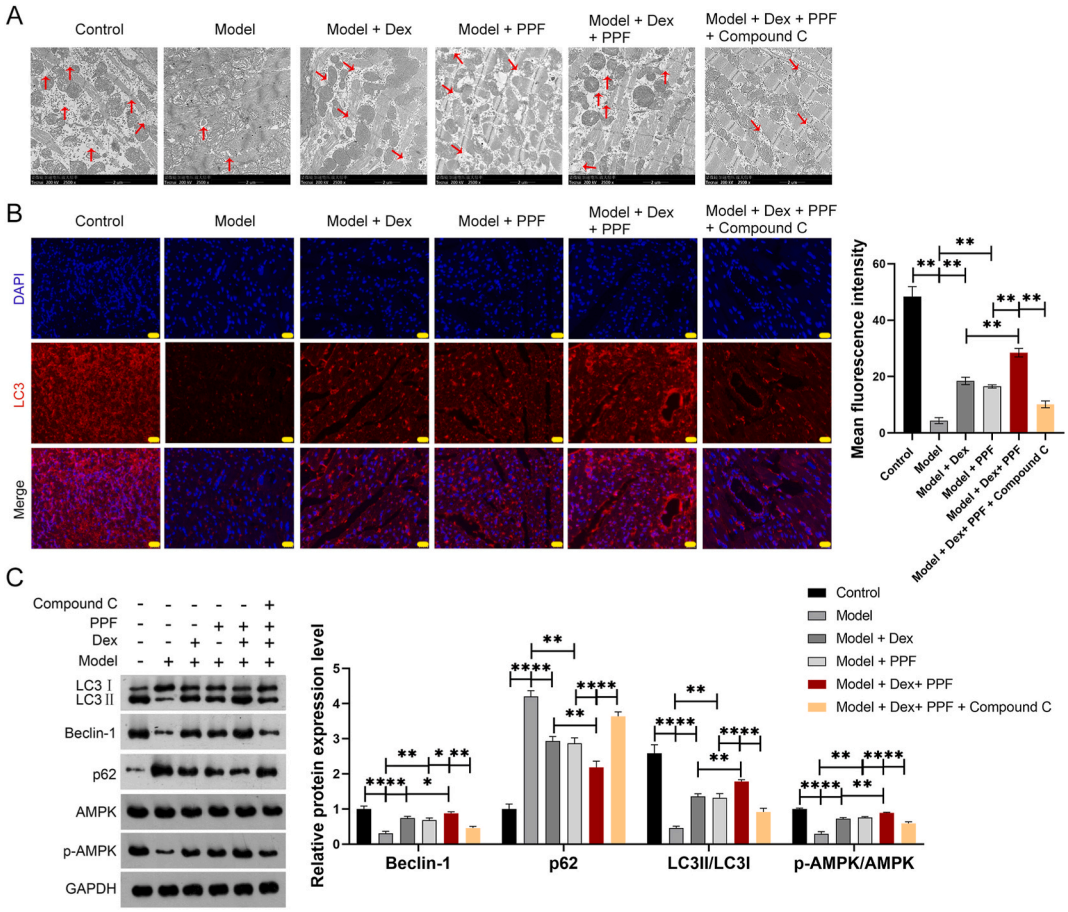

Fig. 4. The combination of Dex and PPF promotes autophagy in MI/R rats probably by activating the AMPK signaling pathway. (A) Observation of showed the state of autophagy through TEM. Scale bar = 20 μm; magnification : 2500 × . **(B)** Detection of LC3 fluorescence intensity using immunofluorescence. Scale bar = 20 μm; magnification : 40 × . **(C)** Detection of expression of Beclin-1, p62, LC3II/LC3I, *p*-AMPK/AMPK using Western blotting. Data were expressed as the mean ± standard deviation. **P* < 0.05 and ***P* < 0.01 between each group. Dex, dexmedetomidine; PPF, propofol; MI/R, myocardial ischemia/reperfusion; TEM, transmission electron microscope.

synergistic effect on alleviating cardiac dysfunction on MI/R rats, which was reversed by compound C (Fig. 2).

3.3. Dex combined with PPF alleviated OS and myocardial apoptosis in MI/R rats

We measured the levels of myocardial injury markers (AST, CK-MB, cTnl, and LDH) to further explore the effect of Dex combined with PPF on myocardial function. Here, we found that the levels of AST, CK-MB, cTnl, and LDH in MI/R rats were significantly increased compared with those in control rats ($P < 0.01$), which were reversed after Dex or/and PPF treatment ($P < 0.01$) (Fig. 3A). Compound C weakened the effect of Dex combined with PPF on promoting myocardial function ($P < 0.01$) (Fig. 3A). OS reportedly plays an important part in the pathological process of MI/R injury[32]. To determine the effects of Dex and PPF on inhibiting OS, the levels of OS markers (MDA, ROS, and SOD) were measured. The results showed that the levels of MDA and ROS increased considerably and the level of SOD decreased in MI/R rats compared with control rats ($P < 0.01$). We found decreased MDA and ROS levels and increased SOD level after administration with Dex or/and PPF ($P < 0.01$) (Fig. 3B). Compound C weakened the combined effect of Dex and PPF on inhibiting OS in MI/R rats (Fig. 3B). Apoptosis is an important form of cell death during MI/R injury. TUNEL staining showed more apoptotic myocardial cells in MI/R rats than those in control rats ($P < 0.01$), and myocardial apoptosis was inhibited following treatment with Dex or/and PPF ($P < 0.01$) (Fig. 3C). The combination of Dex and PPF exhibited a better effect on inhibiting cell apoptosis than when Dex or PPF treatment alone, while compound C abolished this effect ($P < 0.01$) (Fig. 3C).

3.4. Dex combined with PPF enhanced autophagy partly by activating the AMPK signaling pathway in MI/R rats

To explore the combined effect of Dex and PPF on autophagy, we observed autophagy through TEM and detected autophagy markers (LC3, Beclin-1, and p62) in MI/R rats. Through TEM, we observed that the number of autophagosomes decreased in MI/R rats

relative to control rats (Fig. 4A). Similarly, the LC3 fluorescence intensity in MI/R rats decreased compared with those in control rats ($P < 0.01$) (Fig. 4B). Moreover, the protein expression levels of Beclin-1 and LC3II/LC3I in MI/R rats were markedly lower than those in control rats, while the p62 level was higher ($P < 0.01$) (Fig. 4C). After Dex or/and PPF treatment, especially Dex combined with PPF, the number of autophagosomes, LC3 fluorescence intensity ($P < 0.01$), and protein expression levels of Beclin-1 and LC3II/LC3I ($P < 0.01$) were notably elevated in MI/R rats, while the p62 level was reduced ($P < 0.01$) (Fig. 4A–C). These results suggest the promotive effect of the combined treatment of Dex and PPF on autophagy in MI/R rats. The AMPK signaling pathway is a well-studied pathway related to autophagy. Thus, we also analyzed the AMPK axis to further explore the autophagy mechanism in MI/R injury. Importantly, we found that the protein expression level of *p*-AMPK/AMPK was markedly lower in the MI/R rats than that in control rats ($P < 0.01$), which was reversed after treatment with Dex or/and PPF, particularly Dex combined PPF ($P < 0.01$) (Fig. 4C). Nevertheless, compound C eliminated the promotive effects of Dex combined with PPF on autophagy and the AMPK signaling pathway ($P < 0.01$) (Fig. 4).

4. Discussion

MI/R injury, which results from the initial interruption of blood flow and the sequent restoration of blood flow, is associated with complicated mechanisms, including cardiomyocyte OS, apoptosis, inflammatory responses, and autophagy. It has been a significant clinical problem with varied cardiac reperfusion strategies after acute myocardial infarction [33–35]. It is known that Dex has a protective effect on a variety of organs, such as the heart, lungs, kidneys, and liver[36]. In addition, existing evidence also shows that PPF can protect against I/R injury[37]. The AMPK signaling pathway is a basic intracellular signaling cascade that regulates autophagy. Therefore, we proposed a hypothesis that Dex combined with PPF could alleviate MI/R injury in rats by targeting the AMPK signaling pathway.

Myocardial infarction is a serve pathological progress involved in myocardial necrosis induced by acute or persistent coronary ischemia and hypoxia. Collagen gradually replaces the original tissue owing to the loss of many cardiomyocytes, the myocardial contractile function was decreased, and myocardial fibrosis finally leads to heart failure after myocardial infarction[38]. Previous studies have demonstrated that Dex mitigated myocardial infarction by inhibiting myocardial fibrosis[39]. Besides, PPF played a protective effect on experimental myocardial infarction by reducing adverse ventricular remodeling, cardiac dysfunction, myocardial hypertrophy, and fibrosis[40]. Our results showed that after Dex or/and PPF treatment, the myocardial tissue damage and fibrosis were effectively alleviated, myocardial infarction was notably reduced, and cardiac dysfunction was rescued, especially when Dex integrated with PPF. These data suggest that Dex combined with PPF may ameliorate myocardial infarction and cardiac dysfunction in MI/R rats.

Myocardial dysfunction is often induced by MI/R, so the recovery of myocardial function is vital for alleviating MI/R. AST, CK-MB, cTnl, and LDH are common factors that can reflect myocardial function damage. Dex and PPF are known to have cardioprotective effects according to previous research[36,41]. In the present study, Dex or/and PPF down-regulated the levels of cTnl, CK-MB, AST, and LDH, especially Dex combined with PPF. Furthermore, OS is a key reaction induced by MI/R injury referring to the oxidative damage due to the imbalance in production and elimination of oxygen-free radicals in the body or cells, resulting in ROS accumulation in the body or cells. Excessive ROS can damage cardiomyocytes, thereby triggering cardiomyocyte apoptosis[42]. MDA and SOD are important OS markers in mitochondria. MDA is a remarkable factor produced by OS, while SOD is a key antioxidant enzyme. Previous studies have presented that Dex can ease OS reactions and stress responses and PPF can inhibit the production of OS-related factors[43,44]. Our results showed that the combination of Dex and PPF better inhibited OS were used alone. In addition, apoptosis is a major form of programmed cell death and one of the main characteristics of myocardial ischemia, followed by cardiomyocyte loss, myocardium remolding, and the aggravation of the inflammatory response[45]. Dex has been reported to exert an antiapoptotic effect by activating related signaling pathways, thus protecting cells from damage[36]. Furthermore, PPF can reduce myocardial apoptosis, thereby presenting a cardioprotective effect[46]. Herein, we found that the combination of Dex and PPF had a better effect on inhibiting myocardial apoptosis than the single use of Dex or PPF. That suggests that Dex combined with PPF may suppress OS and myocardial apoptosis in MI/R rats.

Recent evidence shows the crosstalk between OS and autophagy in the pathological process of MI/R injury[47]. Autophagy is a highly conserved cellular process maintaining intracellular homeostasis by fusing with lysosomes to eliminate the damaged organelles and abnormal proteins in cells[48,49]. ROS accumulation and mitochondrial damage are closely linked to decreased autophagic flux, which in turn contributes to the generation of ROS[50]. LC3II, Beclin-1, and p62 are important autophagy markers. Beclin1 is a crucial gene for autophagy, and Beclin-1-PI3KC3 signaling complexes have significant implications for the generation of autophagosomes [51]. LC3II can be converted from LC3-I when autophagy is activated (Lu, Li et al., 2018). During autophagy, p62 is bound to ATG8/LC3, followed by degradation[52]. Evidence has demonstrated that autophagy is a crucial mechanism in the development of MI/R injury. For example, restoring impaired cardiomyocyte autophagy can alleviate MI/R injury in cell and animal models[53]. The AMPK signaling pathway reportedly is a critical node in the regulation of autophagy[54]. During acute starvation, autophagy functions quickly via AMPK activation to maintain energy homeostasis and cell survival[55]. Furthermore, previous research has presented that the activation of the AMPK axis could relieve MI/R injury by regulating autophagy in cardiomyocytes[56]. Interestingly, a recent study has indicated that activation of AMPK signaling pathway can increase autophagy level to inhibit OS, thereby alleviating MI/R injury in mice[57]. We found that after Dex or/and PPF administration, the protein expression levels of LC3II/LC3I and Beclin-1 were increased, the p62 level was decreased, and the *p*-AMPK/AMPK level was elevated in MI/R rats. Compound C attenuated the combined effect of Dex and PPF on promoting autophagy. That implies that the combination of Dex and PPF may facilitate autophagy to alleviate MI/R injury in rats partly by activating the AMPK signaling pathway.

This study has some limitations. First, the therapeutic effects of Dex combined with PPF on MI/R injury were explored only at the

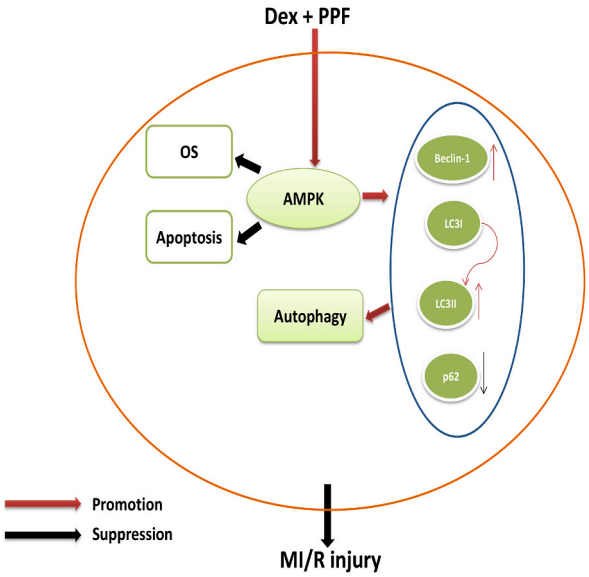

Fig. 5. The schematic diagram of the therapeutical effects of Dex combined with PPF against MI/R injury by activating the AMPK signaling pathway. Dex, dexmedetomidine; PPF, propofol; MI/R, myocardial ischemia/reperfusion; OS, oxidative stress.

rat level, and thus additional studies are required for further verification. Second, this study focused on the AMPK signaling pathway in the anti-MI/R injury mechanisms of Dex combined with PPF, which may neglect the involvement of other important pathways. Third, although we found the promotive effect of Dex combined with PPF on the AMPK signaling pathway, it would be interesting to figure out the relative effectiveness of Dex combined with PPF compared with the known AMPK activators (e.g., metformin). In our subsequent studies, we will further investigate the efficacy of Dex combined with PPF on MI/R injury by comparing it with that of metformin, and the downstream molecules of the AMPK signaling will be analyzed.

5. Conclusion

In summary, this study demonstrated that Dex combined with PPF alleviated myocardial infarction, cardiac dysfunction, OS, and cell apoptosis, as well as enhanced autophagy in MI/R rats, which may be partly related to the activation of the AMPK axis. The mechanisms by which Dex combined with PPF may alleviate MI/R injury were exhibited in Fig. 5. This study may further provide a novel therapeutic strategy for MI/R injury and a new direction for follow-up animal and clinical studies.

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Data availability

Data will be made available on request.

Ethics approval

This work was conducted according to Directive 2010/63/EU of the European Parliament and of the Council of September 22, 2010 for animal experiments. This study was approved by the Animal Experimental Ethics Committee of Kunming Medical University (kmmu20220230).

CRediT authorship contribution statement

Ke Yang: Conceptualization, Data curation, Formal analysis, Methodology, Visualization, Writing – original draft. **Yinhong Ma:** Data curation, Formal analysis, Investigation, Methodology, Validation, Software. **Chunmei Xie:** Data curation, Methodology, Resources, Validation. **Lixian He:** Data curation, Methodology, Validation, Resources. **Haoxing Zhao:** Data curation, Methodology, Resources, Validation. **Zheng Dai:** Conceptualization, Methodology, Software, Validation, Writing – review & editing. **Xiaoqi Wang:**

Conceptualization, Formal analysis, Project administration, Software, Validation.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Zheng Dai reports was provided by Yunnan Provincial Cardiovascular Disease Clinical Medical Center Project. Ke Yang reports was provided by Yunnan Provincial Science and Technology Department and Kunming Medical University Applied Basic Research Joint Project. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e22054.

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