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Article

Compound Danshen Dripping Pills pretreatment protects the heart from ischemia/reperfusion injury by enhancing autophagic flux

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Compound Danshen Dripping Pills (CDDPs) have been used in clinical treatment to protect the heart from ischemia/reperfusion (IR) injury for many years. However, the underlying mechanism implicated in the protective effects remains to be explored. Here, we determined the effects of CDDPs in Sprague–Dawley rats with the IR model. Cardiac function *in vivo* was assessed by echocardiography. Transmission electron microscopy, histological and immunohistochemical techniques, Western blotting and recombinant adeno-associated virus 9 transfection were used to illustrate the effects of CDDPs on IR and autophagy. Our results showed that pretreatment with CDDPs decreased the level of serum myocardial enzymes and infarct size in rats after IR. Apoptosis evaluation showed that CDDPs significantly ameliorated the cardiac apoptosis level after IR. Meanwhile, CDDPs pretreatment increased myocardial autophagic flux, with upregulation of LC3B, downregulation of p62, and increased autophagosomes and autolysosomes. Moreover, the autophagic flux inhibitor chloroquine could increase IR injury, while CDDPs could partially reverse the effects. Furthermore, our results showed that the activation of AMPK/mTOR was involved in the cardioprotective effect exerted by CDDPs. Herein, we suggest that CDDPs partially protect the heart from IR injury by enhancing autophagic flux through the activation of AMPK/mTOR.

KEYWORDS: ischemia/reperfusion. Compound Danshen Dripping Pills. Autophagy. Apoptosis.

INTRODUCTION

Myocardial ischemia/reperfusion (IR) injury is the major limitation for revascularization after myocardial infarction, which results in heart dysfunction (Murphy, Steenbergen, 2008). Finding prevention and treatment strategies to alleviate IR injury is of great clinical significance. An increasing amount of evidence suggests that pre- or posttreatment with pharmacological alternative methods can effectively reduce IR injury (Ling *et al*., 2016).

Traditional Chinese medicine (TCM) has been practiced for thousands of years and provides a vast source of pharmaceutical materials (Zheng *et al*., 2017). Compound Danshen Dripping Pills (CDDPs), a Chinese herb medicine that has been widely used for the prevention and treatment of coronary arteriosclerosis, angina pectoris, and hyperlipemia in China for decades, is also a dietary medicine in the worldwide field (Chu *et al*., 2011).

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In previous studies, various cardioprotective effects of CDDPs have been revealed. Strong evidence has been accumulated to illustrate that CDDPs exert significant prevention effects in the process of atherosclerosis (Zhou *et al.*, 2016). A study reported that CDDPs reduced microcirculatory disturbance and myocardial damage in rats subjected to IR surgery (Zhao *et al.*, 2010a).

In the isoproterenol-induced acute myocardial ischemia model, CDDPs were indicated to help modulate energy metabolism and ameliorate cardiac damage (Guo *et al.*, 2016). However, little is known about the mechanism of CDDPs in IR injury.

Autophagy, a regulated intracellular catabolic process, serves as the cellular quality control mechanism for the disposal of damaged organelles and proteins, and it is widely implicated in cardiovascular diseases (Ghavami *et al.*, 2014; Zheng, *et al.*, 2017). AMP-activated protein kinase (AMPK), a cellular energy homeostasis regulator, inhibits mammalian target of rapamycin (mTOR) to induce autophagy (Gatica *et al.*, 2015). Our previous publications showed that enhancing autophagic flux reduced IR or hypoxia/reoxygenation (H/R) injury by reducing ER stress and regulating the AMPK/mTOR and PI3K/AKT signaling pathways(Chen, 2016; Zhong *et al.*, 2017; Tang *et al.*, 2017). However, it is unknown whether and how CDDPs pretreatment regulates autophagy during IR.

To address this issue, we designed this study to confirm the concentration-dependent effects of CDDPs on myocardial IR injury, clarify the effects of CDDPs on autophagy, and explore the related signaling pathway.

MATERIAL AND METHODS

Animals

All animal experiments were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978), and approval was obtained from the Ethics Committee of Zhujiang Hospital Southern Medical University. Rats were housed under standard laboratory conditions with a light/dark cycle of 12 hours and had free access to sufficient food and water.

Drugs

CDDPs (batch number: z10950111) were obtained from Tasly Pharmaceutical (Tianjin, China) and prepared from the water-ethanol extract of Salvia miltiorrhiza (SM), Panax notoginseng (PN), and borneol under the guidelines of Good Manufacturing Practice and Good Laboratory Practice verified by the Chinese, Australian, and US government agencies. One pill of CDDPs contains 9 mg of SM, 1.76 mg of PN, 0.5 mg of borneol, and 13.74 mg of polyethylene glycol (Zhao *et al.*, 2010b).

Animal IR model

Male Sprague–Dawley rats (280-300 g) were anesthetized with a mixture of 5 mg/kg xylazine and 100 mg/kg ketamine by intraperitoneal injection (IP). A mini-ventilator (Taimeng, Chengdu, China) was used to maintain gas exchange in the lungs with an endotracheal tube. After the heart was exposed, the left coronary artery (LCA) was ligated for 30 min followed by 3 h of reperfusion, with no ligation for the sham group.

Drug administration

Rats were randomly divided into different groups and received the following treatments for 1 week before the IR surgery: (1) Sham group: 0.9% saline; (2) IR group: 0.9% saline; (3) IR + CDDPs group: different concentrations of CDDPs dissolved in 0.9% saline (0.1 g/kg, 0.4 g/kg, 0.8 g/kg body weight/day, TASLY PHARM, China); (4) IR + CDDPs + chloroquine (CQ) group: CDDPs (0.8 g/kg body weight/day) dissolved in 0.9% saline and CQ (50 mg/kg body weight/day, Sigma, CA, USA) by intraperitoneal injections; (5) IR + CQ group: intraperitoneal injections of CQ (50 mg/kg body weight/day). CDDPs (TASLY PHARM) were diluted in 0.9% saline at the target concentration and administered by gavage.

Echocardiography

Echocardiography was performed using a Vevo 2100 System equipped with a 30 MHz transducer

(FUJIFILM Visual Sonics, Inc., Toronto, Canada). Rats were anesthetized with 2% isoflurane. M-mode echocardiographic examinations of the short axis were obtained. The cardiac output (CO), left ventricular end-diastolic (Did) and systolic (Dis) diameter, end-diastolic (Vid), and systolic dimension (Vis) were measured. Left ventricular ejection fraction (LVEF %) and fractional shortening (LVFS%) were evaluated as follows: LVFS% = (Did - Dis)/Did × 100, LVEF% = (Vid - Vis)/Vid × 100.

Serum myocardial zymogram testing

Blood samples were collected from rats by cardiac puncture. Serum was separated from blood by centrifugation (1000 x g, 10 min). Levels of creatine kinase-MB (CK-MB) and high-sensitivity troponin T (TnT-hs) were measured according to the ROCHE test (Tang *et al.*, 2017).

Myocardial infarct size (IS) measurement

The myocardial IS was detected by 2,3,5-triphenyltetrazolium chloride (TTC, Sigma, USA) and Evans blue (Sigma, USA) costaining as previously described (Ling, *et al.*, 2016). The infarct size/area at risk (IS/AAR) was used to evaluate the infarct level. Images were obtained using a stereomicroscope (Leica S8AP0) and analyzed by ImageJ software.

Immunohistochemistry of cleaved caspase 3

Heart tissues were fixed in 4% paraformaldehyde, dehydrated in ethanol, cleared in xylene, and embedded in paraffin. Samples were then cut into 6-μm sections on a microtome and deparaffinized. Then, the sections were blocked with 1% BSA at room temperature for 30 min and incubated with a primary antibody against cleaved caspase 3 (Servicebio, Wuhan, China) at 4 °C overnight. Then, the sections were washed and incubated with secondary antibodies (Beyotime, Biotech, Beijing, China) at room temperature. After being incubated with diaminobenzidine and counterstained with hematoxylin, dehydrated, and mounted, sections were viewed under a light microscope (Leica DFC7000T).

Transmission electron microscopy (TEM)

Rat heart tissues were fixed in 2.5% glutaraldehyde overnight and immersed in 1% osmium tetroxide in 0.1 M cacodylate buffer for 1 hour followed by incubation with 2% aqueous uranyl acetate for 2 hours. Samples were dehydrated with a graded series of ethanol and sliced into small grids. Grids were examined with a Philips CM 10 electron microscope operated at 80 kV.

Autophagic flux measurement

Recombinant adeno-associated virus 9 (rAAV9) tandem RFP-GFP-LC3 adeno-associated virus (rAAV9-CMV-LC3) was obtained from Hanbio (Shanghai, China) and administered by direct injection into the left ventricular free wall (5 sites, 10 μl/site) in rats at 4 weeks of age using a syringe with a 30-gauge needle. Four weeks later, sham or IR surgery was performed. The transduction efficiency of *in vivo* gene transfer by rAAV9 was assessed by observing EGFP fluorescence (510 nm) in cryosectioned heart slices using confocal microscopy (Leica, SP8, USA). Punctuate localization of LC3 in cells had both red and green fluorescence and appeared yellow in merged images, which indicates the autophagosome. The instability of GFP in the acidic pH of the lysosome results in the loss of the green fluorescent signal in the fused autophagosome–lysosomes (autolysosomes), and the red dots that do not overlie green dots in merged images indicate autolysosome formation. The numbers of autophagosomes (yellow dots) and autolysosomes (red dots) and the ratio of autophagosomes/autolysosomes were measured.

TUNEL assay for apoptosis determination

Apoptosis levels were analyzed using a TdT-mediated dUTP nick end labeling (TUNEL, Roche, Germany, cat: 11684817910) assay according to the manufacturer's instructions. TUNEL-stained cells (%) were calculated according to the distribution of myocardial cells under microscopy (Leica DFC7000T), five views were chosen in each section, and the average percentage of apoptotic cells was determined as the apoptosis index.

Western blot

Rat hearts were lysed in RIPA buffer (Beyotime Institute of Biotechnology, China). The protein concentrations were measured by BCA protein assay kits (Thermo Scientific, USA). Protein was separated by SDS–PAGE and transferred to a polyvinylidene fluoride membrane (Millipore), followed by incubation in a 5% nonfat milk-blocking buffer for 2 hours. Membranes were then incubated with the following primary antibodies at 4 °C overnight: anti-LC3B (1:1000, CST cat# 2775S), anti-p62 (1:1000, CST cat# 5114S), anti-p-AMPK (1:1000, CST cat# 2535S), anti-AMPK (1:1000, CST cat# 2532S), anti-p-mTOR (1:1000, CST cat# 5536S), anti-mTOR (1:1000, CST cat# 2972S), and anti-GAPDH (1:10000, Boster cat# AP0063). The membranes were incubated with secondary antibodies (1:8000, Boster, Shanghai, China) and visualized with ECL kits (Engreen, Beijing, China). The levels of protein were quantified using ImageJ software and were normalized to the sham group.

Data and statistical analysis

Data were analyzed with SPSS 23.0 software. All data are expressed as the mean ± SD. Comparisons between multiple groups were evaluated using one-way ANOVA followed by Bonferroni's or Dunnett's T3 tests. Values of $p < 0.05$ were considered statistically significant.

RESULTS

CDDP pretreatment ameliorates myocardial IR injury

To investigate the effect of CDDPs on myocardial IR injury, the serum levels of CK-MB and TnT-hs were examined. Our results showed that serum TnT-hs was increased significantly in the IR group, whereas CDDPs pretreatment, especially in the 0.8 g/kg group, significantly decreased the levels of TnT-hs and CK-MB (Figure 1A). TTC-Evans blue staining was used to detect the infarct size of rat hearts after IR surgery. Representative pictures of different groups showed that CDDPs reduced the infarct area (Figure 1B, 1E). The TUNEL assay showed that myocardial apoptosis was increased in the IR group compared with the sham group, whereas CDDPs decreased apoptosis with fewer apoptotic nuclei (Figure 1C, 1F). Similarly, immunohistochemistry staining showed that the expression of cleaved caspase 3 in the IR group was significantly increased compared with that in the sham group, and pretreatment with CDDPs helped to decrease cleaved caspase 3 in the IR+CDDPs group (Figure 1D, 1G). Taken together, our findings indicated that CDDPs exert a protective effect on cardiac IR injury.

CDDPs pretreatment enhanced autophagic flux in myocardium suffering from IR injury

Western blot analysis of autophagy-associated proteins indicated that the ratio of LC3 II and LC3 I+II was decreased, but p62 was increased in the IR group (Figure 2A-D, 2E-H). Myocardium with rAAV9-CMV-LC3 injection subjected to IR was observed with fewer autophagosomes and autolysosomes (Figure 2I-K). These results suggest that autophagy was suppressed in the myocardial IR group. However, compared with the IR group, LC3II and LC3 I+II protein expression was increased, and p62 protein expression was decreased (Figure 2A-D, 2E-H). As shown in Figure 2I, more autophagosomes and autolysosomes were observed in the CDDPs-pretreated group. CQ (autolysosome fusion inhibitor) could block autophagic flux with more p62, more autophagosomes and fewer autolysosomes. However, cotreatment with CDDPs partially blunted the effects of CQ (Figure 2E-H, 2I-K). These results indicated that CDDPs pretreatment promoted the formation of autophagosomes and enhanced autophagic flux in myocardial IR injury.

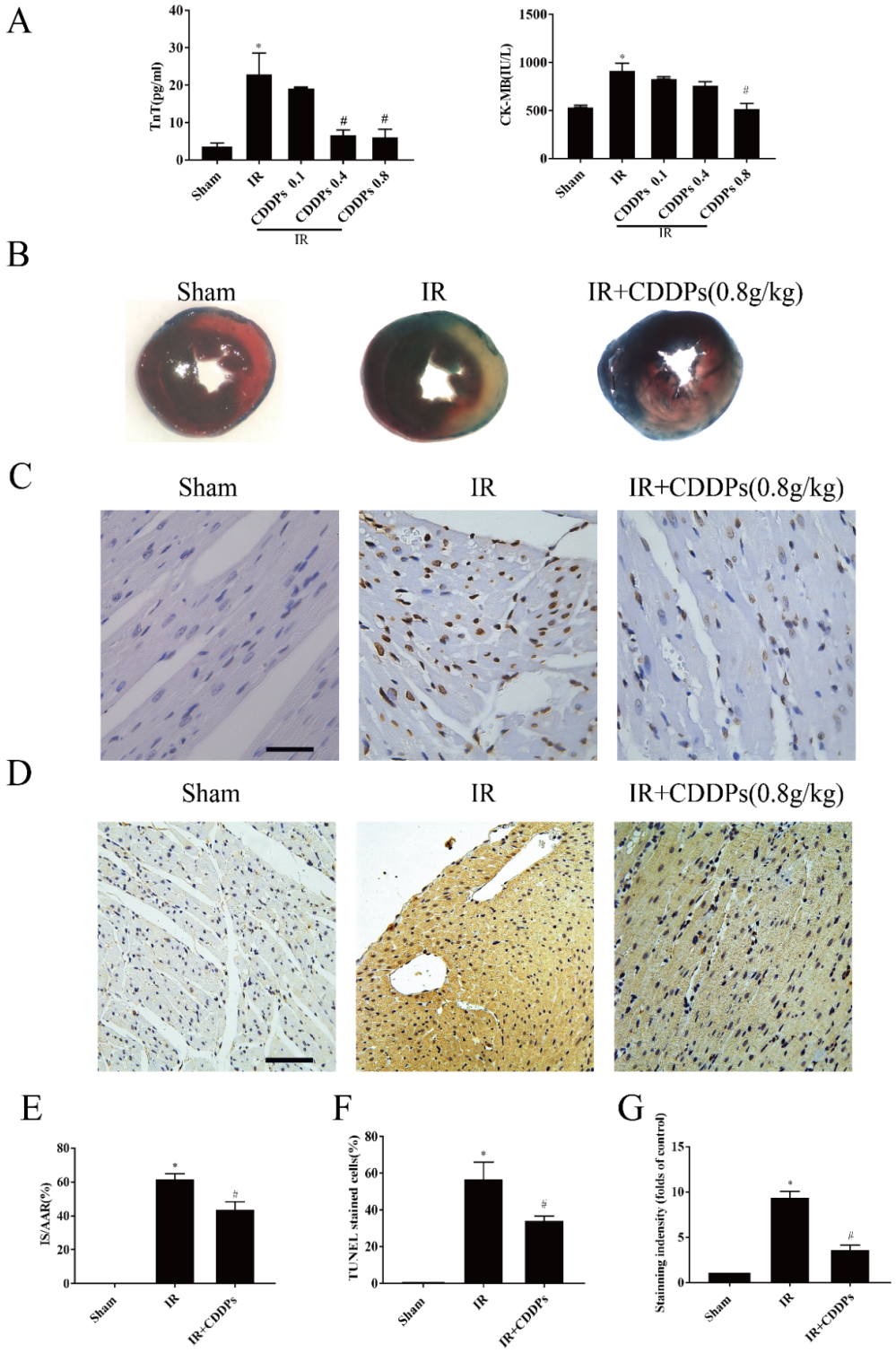


FIGURE 1 - Effects of CDDPs pre-treatment showed cardioprotective effects in rats subjected to IR injury. (A)The TnT-hs and CK/MB concentrations in different groups. (B) Representative TTC–Evans Blue stained sections of hearts from each group. The brick red-stained area represents viable myocardium, whereas the unstained (white) area represents infarcted myocardium. (C)TUNEL staining (brown nuclei) was used to evaluate the apoptotic cells in 0.8g/kg groups, scale bar=250um. (D) Immunostaining of the Cleaved-caspcase-3 in rats with different concentrations of CDDPs under IR injury, the brown area presents the positive expression of Cleaved-caspcase-3. The scale bar represents 100 μm. (E-G) The semi-quantification for panel B-D. Data are expressed with Mean±SD, **P*<0.05, significantly different from the sham group, #*P* <0.05 versus the IR group.

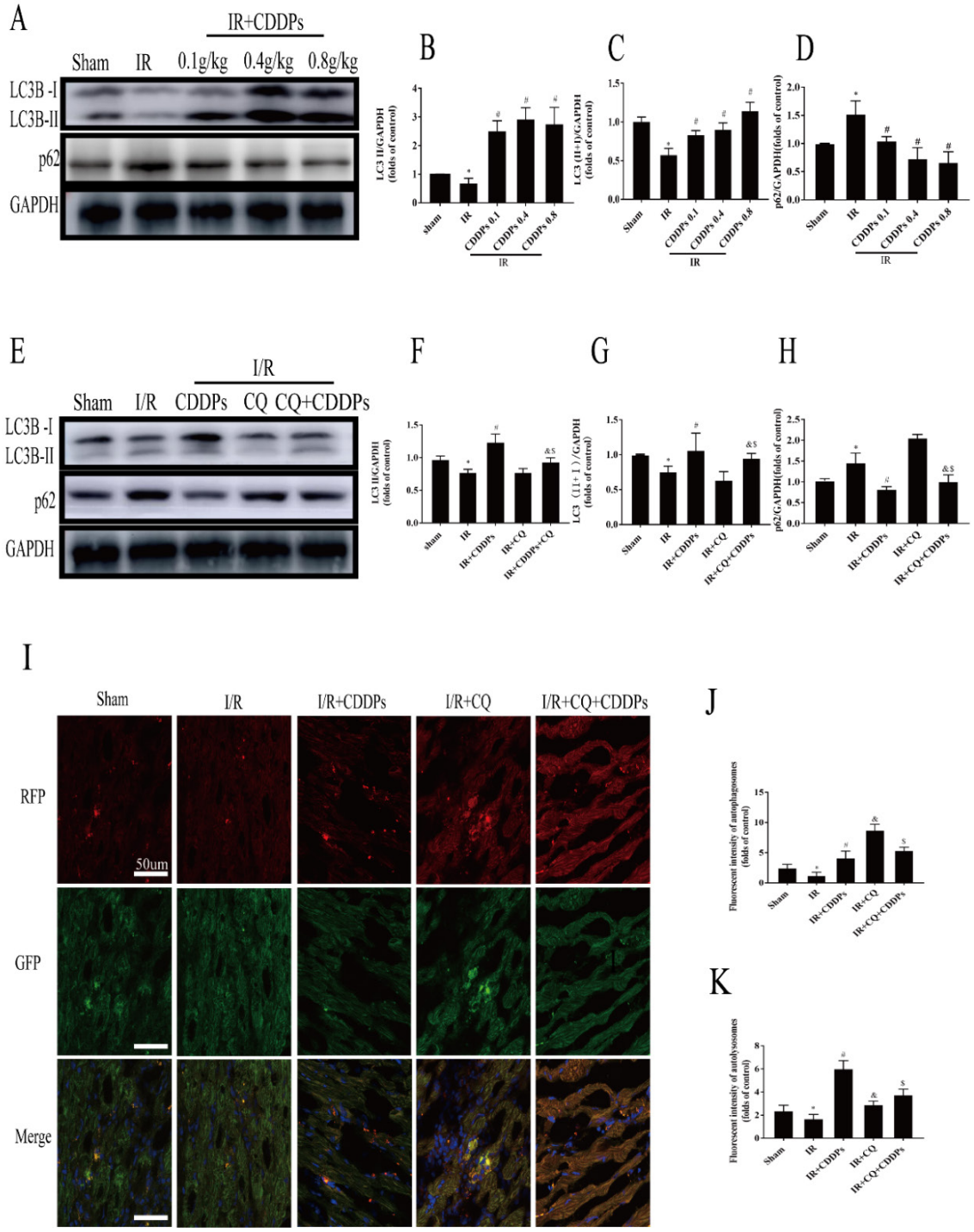


FIGURE 2 - CDDPs enhanced autophagic flux in myocardium of rats under IR injury. (A) Western blotting analysis of LC3B, p62 in rats with different concentrations of CDDPs. (B-D) Semi-quantification of LC3-I+II/GAPDH, LC3II/GAPDH, and p62/GAPDH in panel A are shown. (E) The Western blotting analysis of LC3B, and p62 in rats after cotreatment with CQ. (F-H) Semi-quantification of LC3-I+II/GAPDH, LC3II/GAPDH, p62/GAPDH in B are shown. (I) Rat hearts were transfected with rAAV9-CMV-LC3 for 4 weeks and were subjected to different treatments. Representative pictures of immunofluorescent myocardium expressing mRFP-GFP-LC3. The nuclei were labeled with DAPI (blue staining), GFP dots are green, and mRFP dots are red. (J-K) Semi-quantitative analysis of autophagosomes (AP; yellow dots in merged images) and autolysosomes (AL; red only dots in merged images), scale bar=50um. Data shown are individual values with means ± SD; n = 5. *P < 0.05, significantly different from sham group, #P <0.05, significantly different from IR group, &P<0.05, significantly different from IR+CDDPs group, SP<0.05, significantly different from IR+CQ group.

CDDPs pretreatment activated AMPK/mTOR signaling

To explore the potential molecular mechanisms of CDDPs-promoted autophagy against IR injury, the AMPK/mTOR signaling pathway was assessed by western blotting. Our results showed that the phosphorylation of AMPK (p-AMPK) was decreased and the phosphorylation of mTOR (p-mTOR) was increased in the IR group. However, CDDPs pretreatment significantly increased the level of p-AMPK and decreased p-mTOR, especially in the IR+CDDPs (0.8 g/kg) group (Figure 3A-3C). CQ decreased p-AMPK expression and increased p-mTOR expression, and the effect was reversed by cotreatment with CDDPs (Figure 3D-3F). Collectively, our results suggested that AMPK/mTOR was activated in the regulation of autophagy by CDDPs.

CDDPs relieved cardiac IR injury by enhancing autophagic flux

CQ, a kind of autophagic flux inhibitor, was used in this experiment. As shown in Figure 4A, CDDPs pretreatment significantly reduced the serum CK-MB and TnT levels. Treatment with CQ did not decrease the levels of CK-MB and TnT, while after cotreatment with CDDPs, CK/MB and cTnT were decreased. Moreover, TUNEL analysis indicated that CQ could increase the apoptosis level of the myocardium, while CDDPs could reverse the effect and reduce apoptosis (Figure 4B). Furthermore, as shown in Figure 4C, IR increased the expression of the apoptotic protein cleaved caspase-3, while CDDPs reduced cleaved caspase-3 expression. Compared with IR+CQ, IR+CQ+CDDPs significantly reduced the expression of cleaved caspase 3. Collectively, our results indicated that enhancing autophagic flux could partially ameliorate myocardial IR injury.

CDDPs pretreatment improved cardiac function and reduced myocardial damage by enhancing autophagic flux

M-mode echocardiography revealed that cardiac LVEF and LVFS were decreased in the IR group compared with the Sham group, and CDDPs could partly reverse the reduction in LVEF and LVFS in the IR injury (Figure 5A-C). Moreover, the role of CDDPs in myofibril composition was investigated by TEM. Abundant tight mitochondria, neatly arranged, and intact myofibrils could be seen in the heart tissue from the Sham group. In contrast, the IR group showed fewer mitochondria, more swollen mitochondria, and more ruptured myofibrils. However, the reduction in mitochondria and myofibrils in the IR group could be alleviated by pretreatment with CDDPs. However, CQ cotreatment with CDDPs partially reversed the effect of CDDPs (Figure 5D-F). These results demonstrated that CDDPs protected the myocardium against IR injury in structure and function partially by enhancing autophagic flux.

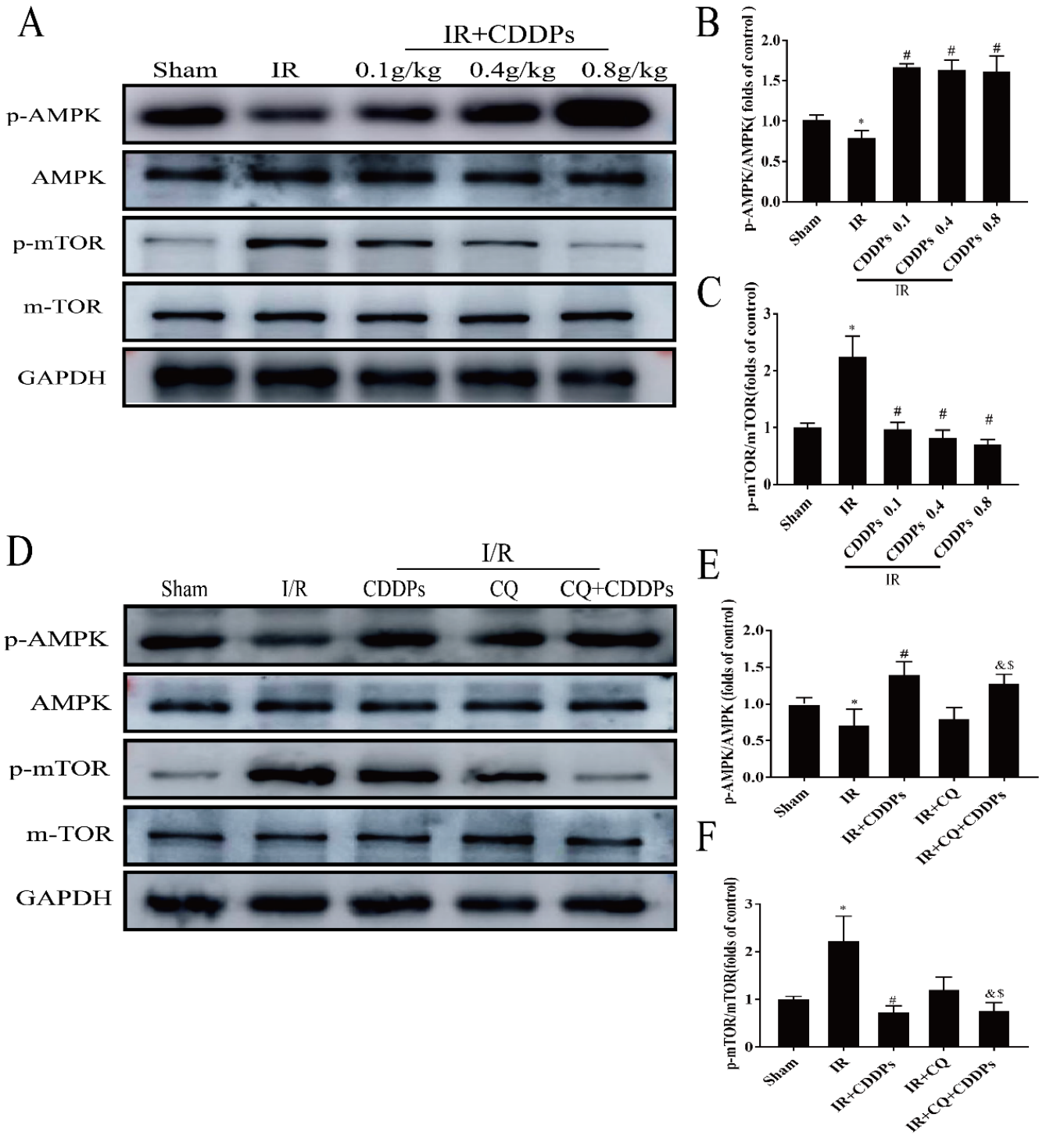


FIGURE 3 - AMPK/mTOR is involved in the cardioprotective effects of CDDPs on myocardial IR injury (A) Western blotting analysis of p-AMPK and p-mTOR in rats with different treatments. (B-C) Semi-quantification for panel A. Data shown are individual values with means ±SD; n = 5. *P <0.05, significantly different from sham group, #P < 0.05, significantly different from IR group. (D) Western blotting analysis of p-AMPK and p-mTOR in rats with different treatments. (E-F) Semi-quantification for panel D. Values are expressed with means ± SD; n =5. *P< 0.05, significantly different from sham group, #P < 0.05, significantly different from IR group, &P<0.05, significantly different from IR+CDDPs group, $$P$<0.05, significantly different from IR+CQ group.

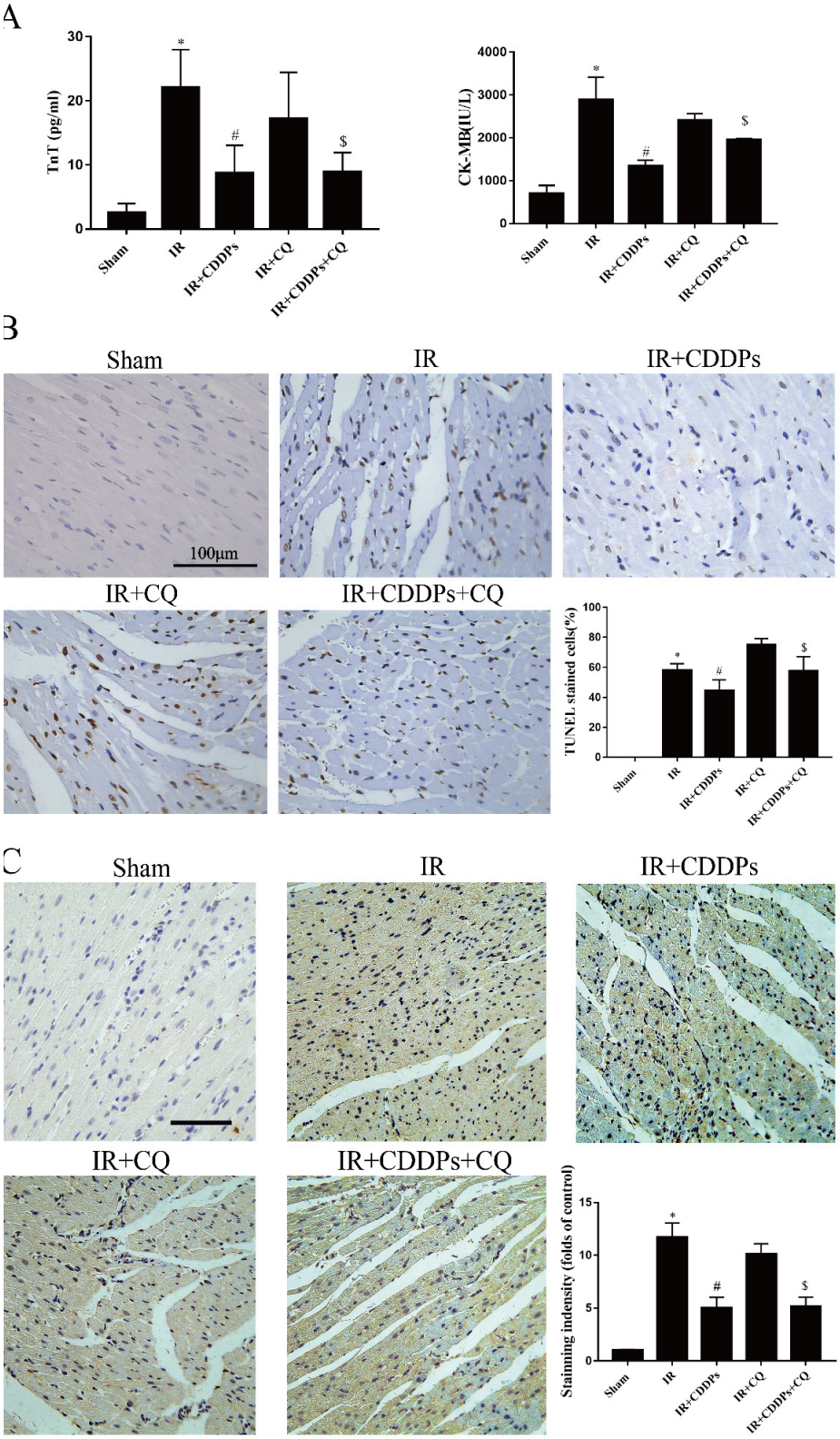


FIGURE 4 - CDDPs ameliorated IR injury via enhancing autophagy and promoting autophagic flux. (A)The serum cTnT and CK/MB concentrations in different groups. (B) TUNEL staining (brown nuclei) was used to evaluate the apoptotic cells in different groups, scale bar=100um. (C) Immunostaining of the Cleaved-caspcase 3 in rats in different groups under IR injury, the brown area presents the positive expression of Cleaved-Caspase 3, scale bar represents 100 μm. Data are expressed with Mean±SD, **P*<0.05, significantly different from the sham group, #*P* <0.05 versus the IR group, $P<0.05, significantly different from IR+CQ group.

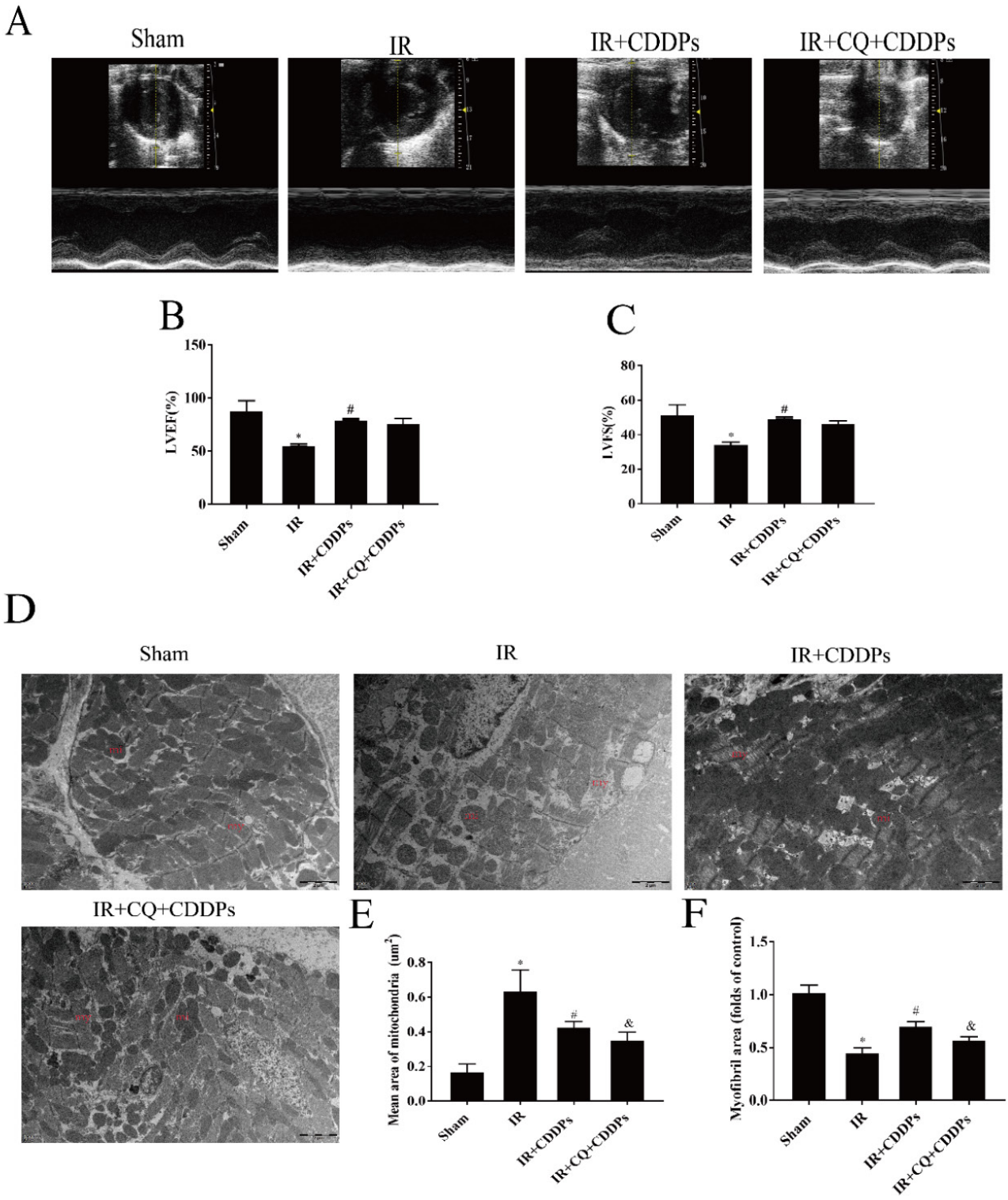
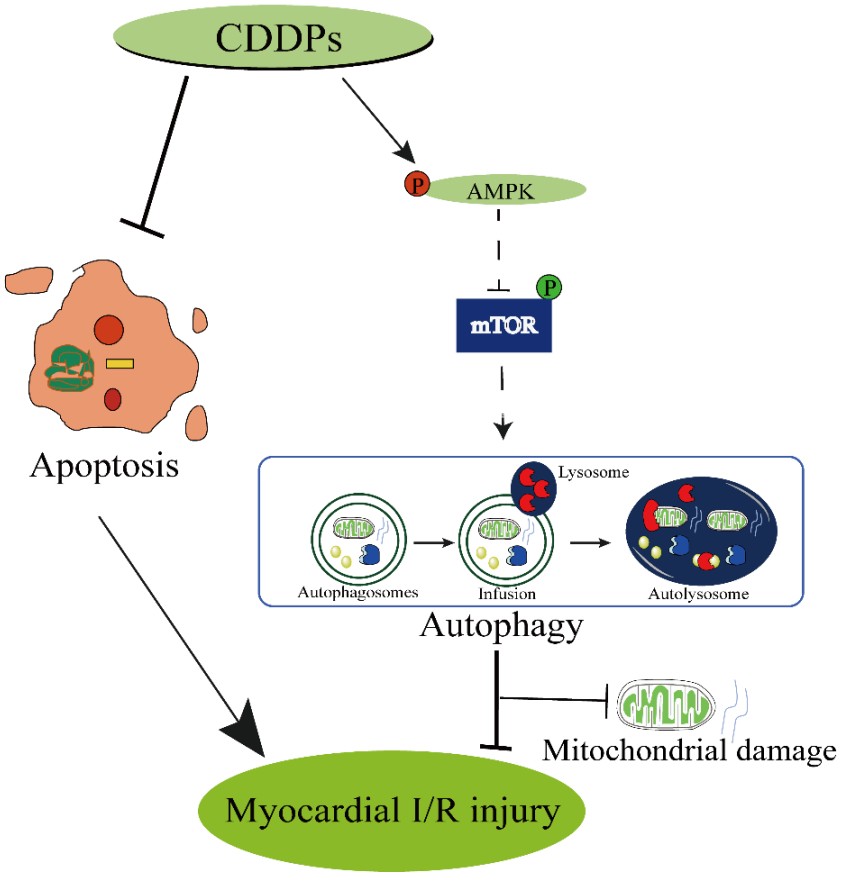


FIGURE 5 - CDDPs enhanced-autophagic flux reduced mitochondrial damage and improved cardiac function in IR. (A) Representative echocardiograms were shown, and M-mode echocardiography was used to measure LVEF(B) and LVFS(C) in different groups. (D) The ultrastructure of the myocardium in different groups is shown in TEM pictures. m, mitochondria, and the mean mitochondrial area in different groups are shown in the bar graph Mitochondria (Mi) and Myofibrils (My) were marked in red. The scale bar represents 2um. (E) The mean mitochondrial area in different groups is shown in the bar graph. (F) The mean myofibrils area in different groups is shown in the bar graph. Values are expressed with means ±SD; n = 5. *$P < 0.05$, significantly different from the sham group, #$P < 0.05$, significantly different from the IR group, \$P<0.05, significantly different from IR+CQ group.

DISCUSSION

As shown in the graphical abstract below, in this study, we demonstrated that CDDPs have a protective effect on myocardial IR injury in rats by enhancing autophagic flux via activation of the AMPK/mTOR signaling pathway. Our results revealed the mechanism underlying the cardioprotective effect of CDDPs in IR injury and provided new insight into the prevention of IR injury.



CDDPs represent protective effect on myocardial IR injury in rats with the involvement of enhancing autophagic flux and reducing apoptosis via activation of the AMPK/mTOR signaling pathway.

Myocardial IR injury resulting from reperfusion leads to a paradoxical increase in cell death and cardiac function deterioration (Huang *et al.*, 2019). Effective therapies for preventing IR injury are limited. Our study showed that CDDPs pretreatment improves myocardial function and reduces myocyte apoptosis, which is consistent with previous studies (Zhao, *et al.*, 2010a; Zhao *et al.*, 2010b; Ren-an *et al.*, 2014). In addition to improving cardiac function and reducing myocardial apoptosis, CDDPs significantly limited the infarct size and reduced the serum CK/MB and cTnT levels. This effect should also contribute to the CDDPs-affording protection against reperfusion. Our results in M-mode echocardiography parameters and myofibril damage investigated by TEM also indicated that CDJP pretreatment alleviated myocardial IR injury.

Our results showed that CDDPs pretreatment protects the heart from IR injury and is related to

autophagy regulation. Autophagy is a vital catabolic pathway involved in multiple cellular physiological or pathological processes (Giampieri *et al.*, 2018). In recent years, autophagy has been accepted as a dynamic process; similar to "a flowing river", the autophagosome is "the upstream of the river" and the autolysosome is "the lower course of the river". In this "river", decreased generation of autophagosomes means a decrease in autophagy; the excessive increase in autophagosomes but less formation of autolysosomes could cause "impaired autophagic flux", which induces further injury (Denton, Nicolson S, Kumar, 2012; Sciarretta *et al.*, 2011). However, the appropriately increased autophagosomes and well-functioning autolysosomes indicate smooth autophagic flux. Our previous publications found that the autophagic flux in C57BL/6 mice that suffered IR was impaired, while restoring autophagic flux helped to ameliorate IR injury (Tan *et al.*, 2019; Ling, *et al.*, 2016). In this study, we found that the number of autophagosomes was decreased with less LC3II and total LC3 (LC3I+II) tested by Western blot, and fewer autophagosomes were detected by tandem fluorescent mRFP-GFP-LC3 in rat heart tissue after IR, which means that autophagy activation was decreased. However, in this study, Western blotting showed increased LC3 II and LC3 I+II and decreased p62 in the CDDPs pretreatment group, and tandem fluorescent mRFP-GFP-LC3 showed that autophagosomes and autolysosomes were increased in the CDDPs pretreatment group. CDDPs not only increased autophagy but also enhanced autophagic flux. To identify the role of autophagic flux in CDDPs in IR, we used the autophagic flux inhibitor CQ, which inhibits the fusion of autophagosomes and lysosomes. We found that CQ could partially block the cardioprotective effects of CDDPs. These results indicated that CDDPs could improve cardiac function by enhancing autophagy and autophagic flux in the setting of IR injury.

AMP-activated protein kinase (AMPK) signaling is a critical regulator of cellular metabolism by inhibiting mammalian target of rapamycin (mTOR) and plays an important role in diabetes, cancer, and vascular diseases (Qi, Young, 2015). Our previous research demonstrated that enhancing autophagy via activation of the AMPK/mTOR signaling pathway in myocardial ischemia and diabetic cardiac myopathy could reduce myocyte damage (Yan *et al.*, 2019; Zhang *et al.*, 2017). Our present results showed that IR surgery decreased p-AMPK accompanied by upregulation of p-mTOR, while CDDPs could upregulate p-AMPK and decrease p-mTOR accompanied by increased LC3II and autophagosomes. Considering the role of CDDPs in promoting autophagosome formation, our results showed that CDDPs activated autophagy and promoted autophagic flux, which might be related to the AMPK/mTOR signaling pathway. Nevertheless, the upstream regulators of AMPK in the context of the autophagy response are still largely unknown.

Cardiomyocytes need a large amount of energy, and mitochondrial content is the most noticeable component in the ultrastructure. The steady state of mitochondrial structure and function is indispensable for cardiac function (Marek-Iannucci *et al*., 2019). Our TEM results showed that the mitochondria of the myocardium were swollen and the number was significantly decreased in the IR group compared with the Sham group, whereas CDDPs pretreatment could relieve the loss of mitochondria and ameliorate the swelling of mitochondria. Moreover, CDDPs pretreatment helped alleviate the loss of myofibrils, which was important for the restoration of cardiac diastolic and systolic function. Similarly, our results showed that cotreatment with CQ could partially reverse the effects, which indicates that CDDPs-enhanced autophagy and autophagic flux play an important role in the cardioprotective effect during IR injury.

CONCLUSION

We established that CDDPs exert a cardioprotective effect on myocardial IR injury by enhancing autophagy and smoothing autophagic flux, which might be related to the activation of the AMPK/mTOR signaling pathway. Our study provides new and solid evidence for traditional Chinese medicine in clinical applications.

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ABBREVIATIONS

CDDPs: Compound Danshen Dripping Pills, IR: ischemia/reperfusion, TUNEL: TdT-mediated dUTP nick end labeling, ER: endoplasmic reticulum, TCM: Traditional Chinese medicine, AMPK: AMP-activated protein kinase, mTOR: mammalian target of rapamycin, H/R: hypoxia/reoxygenation, CQ: chloroquine, LVEF: left ventricle ejection fraction, LVFS: left ventricle fractional shortening, MB/CK: creatine kinase-MB(CK-MB) to CK, TnT-hs: high-sensitivity Troponin T, IS/AAR: infarct size/area at risk, rAAV9: Recombinant adeno-associated virus 9.

DECLARATION OF INTERESTS

The authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS

Haiqiong Liu: writing-original Draft, investigation and revising manuscript. Qian Liang: conceptualization and methodology. Xiheng Mei: software, validation. Hekai Li, Jing Yan, and Man Long: resources. XiLi Yang: formal analysis. Wei Wang: visualization, WeiJie Li: data curation. Aihua Chen: Supervision. Yuanna Ling: project administration, writing-review & editing.

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