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Original Article

Effect of blood lipid on myocardial injury in rats with myocardial infarction

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Abstract: Aim: This study investigated the effect of elevated blood lipid levels on myocardial injury in rats with myocardial infarction. Materials and methods: Sprague-Dawley (SD) rats were randomly assigned to six groups (n=9 per group): normal, sham, acute myocardial infarction (AMI), AMI+30% high-fat (HF), AMI+70% HF, AMI+100% HF and AMI+100% HF+PD (atorvastatin treatment). After 3 weeks of dietary intervention or treatment, serum and heart tissue samples were collected. Serum concentrations of triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), lactate dehydrogenase (LDH), creatine kinase-MB (CK-MB) and cardiac troponin I (cTnI) were measured. Myocardial pathologic changes were examined using H&E staining. Cardiomyocyte apoptosis was evaluated using the TUNEL assay, andcaspase-3 and caspase-12 protein expression levels in myocardial tissue were determined using western blot analysis. Results: Compared to the normal group, TG, TC, LDL, LDH, CK-MB and cTnI levels were significantly increased, whereas HDL levels were significantly decreased in the AMI+30% HF, AMI+70% HF and AMI+100% HF groups. These changes were accompanied by a significant increase in cardiomyocyte apoptosis and marked pathological deterioration of myocardial tissue. By contrast, atorvastatin supplementation markedly improved lipid profiles and cardiac function and reduced cardiomyocyte apoptosis. Conclusions: Alterations in blood lipid levels are closely associated with cardiac dysfunction and myocardial injury following AMI *in vivo*.

Keywords: Blood fat, AMI, myocardial injury, cardiac function

Introduction

Acute myocardial infarction (AMI) is a prevalent clinical condition. With the widespread application of thrombolytic therapy, stent implantation and pharmacologic interventions, the prognosis and symptoms of patients with myocardial infarction have improved considerably. However, serious complications may still occur after AMI, adversely affecting the quality of life and long-term health of patients. The pathologic process of AMI involves ischaemic cell death, which triggers stress-induced repair mechanisms. Damaged myocardial tissue is replaced by fibrotic scars formed by fibroblasts and myofibroblasts. Excessive fibrosis, particularly when it extends beyond the infarct zone, can progressively impair cardiac function and ultimately lead to heart failure [1, 2]. The timely reperfusion of ischemic myocardium remains a cornerstone of AMI management because it can alleviate myocardial injury. However, reperfusion itself can cause cardiac damage, namely, myocardial ischemia-reperfusion injury (MIRI). This condition may manifest as coronary artery spasm, thromboembolism and endothelial dysfunction, further aggravating myocardial damage. The clinical incidence of MIRI is up to 60% [3]. Alterations in glucose, lipid and amino acid metabolism; oxidation-reduction reactions and various other metabolic processes maycontribute to the pathogenesis of MIRI, and

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lipid metabolism plays a critical role because of its influence on myocardial energy supply [4]. Under physiologic conditions, lipid peroxidation and oxygen-free radical activity remain in dynamic balance. During reperfusion, the excessive production of oxygen free radicals rapidly enhances lipid peroxidation, alters cell membrane fluidity and permeability and induces cardiomyocyte injury or even death [5]. Multiple studies employing the LC-MS technology have revealed considerable alterations in lipid profiles in patients with AMI during the onset of and after PCI. Lipid metabolism-related molecules, such as 16-hydroxyeicosatetraenoic acid, 20-hydroxyeicosatetraenoic acid, leukotriene B4, pentadecanoic acid and 1-oleoyl-glycerophosphocholine, have been shown to exert important pathophysiologic effects in MIRI [6, 7]. To date, the role and underlying mechanisms of blood fat in AMI-related myocardial injury remain unclear. Therefore, the present study aimed to investigate the extent of myocardial tissue damage in a rat AMI model subjected to varying degrees of high-fat (HF) dietary intervention and evaluate the protective effects of atorvastatin on myocardial injury in this model.

Materials and methods

Animal grouping and model establishment

In total, 63 male SD rats (body weight: 180-220 g; SPF grade) were purchased from Beijing Weitong Lihua Experimental Animal Technology Co., Ltd. All rats were healthy at baseline. Eighteen rats were randomly assigned to the normal (n=9) and sham groups (n=9). The remaining 45 rats were used to construct AMI models. Before modelling, the animals were fasted and deprived of water for 12 h. The body weight of each group of rats was recorded, and anaesthesia was induced through intraperitoneal injection of 4% chloral hydrate. The rats were fixed in a supine position, and tracheal intubation was performed. Then, a ventilator and electrocardiogram electrodes were connected. A skin incision was made at the level of the fourth rib, from left to right, and the pectoral muscles between the third and fourth ribs were separated to expose the thoracic cavity. The heart was gently removed, and the left anterior descending coronary artery was ligated 2-3 mm below its origin. Successful AMI induction was confirmed by visible pallor of the left ventricular myocardium, weakened systolic function and an elevation of approximately 0.2 V in the ST segment on electrocardiogram. Rats in the sham group underwent the same surgical procedures, except for LAD ligation. Following surgery, all animals received intraperitoneal penicillin injections once daily for 3 consecutive days.

After successful modelling, the 45 rats were randomly assigned to five groups: AMI, AMI+30% HF (w/w, containing 30% fat), AMI+70% HF (w/w, containing 70% fat), AMI+100% HF (w/w, containing 100% fat) and AMI+100% HF+PD. Rats in the AMI group were fed a standard diet; those in the AMI+30% HF group received a diet containing 30% HF content; those in the AMI+70% HF group received a diet containing 70% HF content, and those in the AMI+100% HF group were fed a HF diet exclusively. Rats in the AMI+100% HF+PD group were fed a HF diet in combination with atorvastatin (10 mg/kg) administered by oral gavage once daily [8]. After 3 weeks of dietary intervention, all animals were euthanised by intravenous administration of pentobarbital sodium (150 mg/kg) for tissue and blood sample collection.

Detecting blood fat in rats

Four millilitres of fasting blood were collected via cardiac puncture, and plasma was obtained via centrifugation. Serum total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) levels were determined using a direct homogeneous assay. Serum creatine kinase isoenzyme-MB (CK-MB) and lactate dehydrogenase (LDH) activities were measured using the enzyme-coupling method, and serum cardiac troponin I (cTnI) concentration was quantified using chemiluminescence immunoassay.

Heart tissue sampling

To induce cardiac arrest during ventricular diastole, 3 mL of 10% potassium chloride solution was injected into the tail vein of each rat. The heart was then immediately excised, and the atrial appendage and atrium were removed. The ventricle was sectioned parallel to the atrioventricular groove at the midpoint of the left ventricular long axis. The apex was sepa-

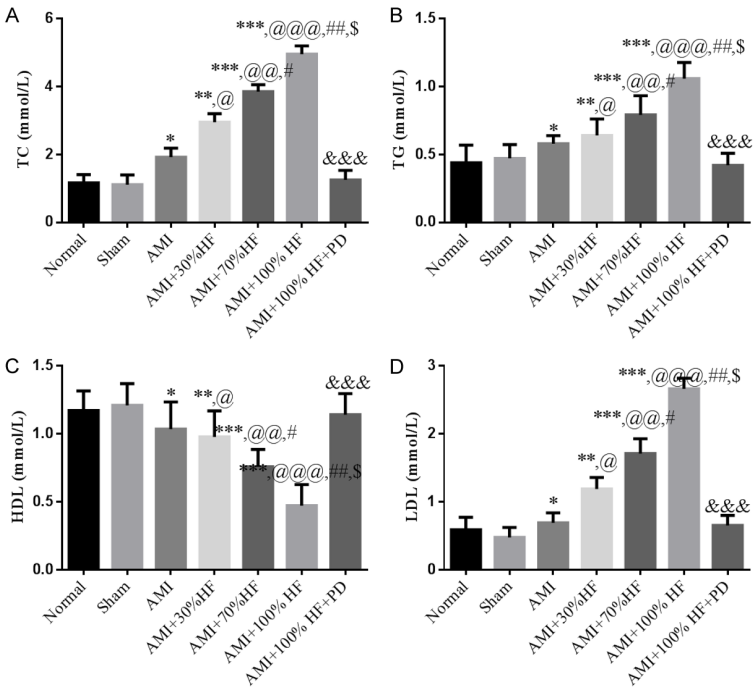

Figure 1. TC, TG, HDL and LDL concentrations levels from serum in different groups. A. TC concentrations in different rat groups. B. TG concentrations in different rat groups. C. HDL concentrations in different rat groups. D. LDL concentrations in different rat groups. *: P<0.05, **: P<0.01, **: P<0.001, compared to Normal group; @: P<0.05, @@: P<0.01, @@@: P<0.001, compared to AMI group; #: P<0.05, ##: P<0.01, compared to AMI+30% HF group; $: P<0.05, compared to AMI+70% HF group; &&&: P<0.001, compared to AMI+100% HF group.

rated from the base, snap-frozen in liquid nitrogen and stored at -80°C for subsequent western blot (WB) analysis. The base of the heart was fixed in 4% paraformaldehyde for 12 h, embedded in paraffin and serially sectioned into at least 12 sections, each with a thickness of 4 μm, for TUNEL detection of cardiomyocyte apoptosis and H&E staining.

H&E staining

Myocardial tissue fixed in formalin was excised, and a 1 mm^3 block from the left ventricular cross-section was trimmed, dehydrated, embedded in paraffin, sectioned into 4 μm slices, dried, stained with eosin, dehydrated, mounted with neutral gum and examined under a microscope.

TUNEL detection of myocardial cell apoptosis

TUNEL staining was performed strictly according to the manufacturer's instructions. Under light microscopy, apoptotic cell nuclei appeared brownish-yellow (TUNEL positive). Ten random fields were selected for each section, and the number of apoptotic cells was determined using ImageJ Version 1.8.0 (National Institutes of Health).

Western blot (WB) assay

Myocardial tissue stored at -80°C was cut into small pieces, lysed and used for protein extraction. Protein concentration was determined using the BCA method, and the samples were aliquoted and stored at -20°C. Protein solution and loading buffer were mixed in a 4:1 ratio and boiled for denaturation, and 50 μg of protein was loaded onto the electrophoresis gel. After separation, the proteins were transferred to a PVDF membrane, blocked with skim milk for 1 h and incubated with the primary antibody. Following washing, the membrane was incubated with the secondary antibody for 10 min, and bands were visualized using DAB staining. Band intensities were quantified using ImageJ Version 1.8.0 (National Institutes of Health) by measuring greyscale values. The greyscale value of the untreated control group was normalized to 1, and the relative intensities of the treated groups were calculated accordingly.

Statistical analysis

Statistical analysis was performed using SPSS Version 22.0. Comparisons between two groups were conducted using the Student's *t*-test, while comparisons among multiple groups were performed using one-way ANOVA followed by the Bonferroni *post hoc* test. Data were expressed as mean ± SD, and differences were considered significant at P<0.05.

Results

Comparison of blood fat among different groups of rats

Compared to the normal group, serum TC, TG and LDL levels in the AMI, AMI+30% HF, AMI+70% HF and AMI+100% HF groups were

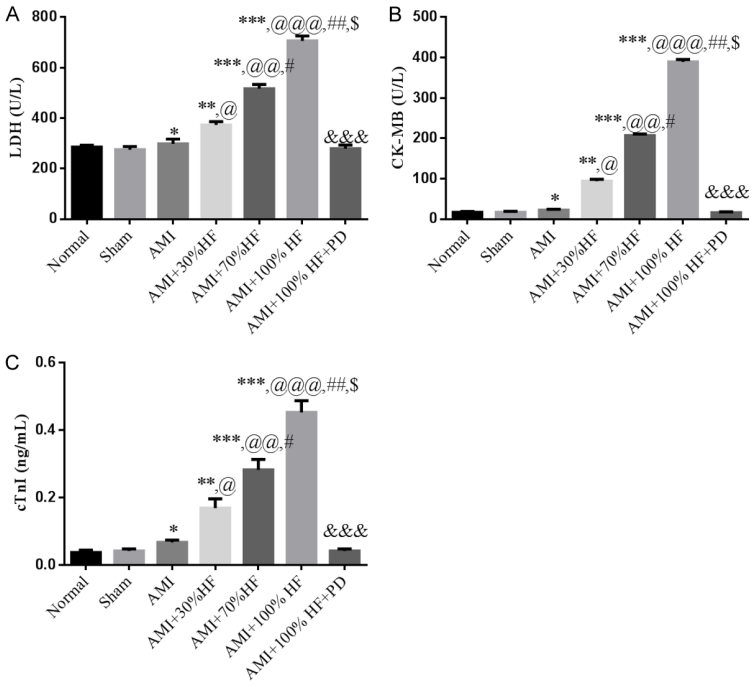

Figure 2. LDH, CK-MB and cTnI concentration levels from serum in different groups. A. LDH concentrations in different rat groups. B. CK-MB concentrations in different rat groups. C. cTnI concentrations in different rat groups. *: P<0.05, **: P<0.01, **: P<0.001, compared to Normal group; @: P<0.05, @@: P<0.01, @@@: P<0.001, compared to AMI group; #: P<0.05, ##: P<0.01, compared to AMI+30% HF group; $: P<0.05, compared to AMI+70% HF group; &&&: P<0.001, compared to AMI+100% HF group.

significantly elevated (P<0.05), whereas HDL levels were significantly reduced (P<0.05). Significant differences were also observed among the AMI+30% HF, AMI+70% HF and AMI+100% HF groups (P<0.05). Following atorvastatin intervention, the AMI+100% HF+PD group exhibited a marked reduction in TC, TG and LDL levels (P<0.001) and a significant increase in HDL levels (P<0.001) compared to the AMI+100% HF group. Data are presented in **Figure 1**.

Comparison of myocardial function among the different groups of rats

Compared to the normal group, serum LDH, CK-MB and cTnI levels were significantly elevated in the AMI, AMI+30% HF, AMI+70% HF and AMI+100% HF groups (P<0.05). Significant differences were also observed among the AMI+30% HF, AMI+70% HF and AMI+100% HF groups (P<0.05). Following atorvastatin treatment, the AMI+100% HF+PD group demonstrated markedly lower LDH, CK-MB and cTnI levels than the AMI+100% HF+PD group (P<0.001). Data are presented in **Figure 2**.

Comparison of pathologic changes in the myocardial tissue of rats in different groups

As shown in **Figure 3**, no pathologic abnormalities were observed in the myocardial tissue of rats in the normal and sham groups. In contrast, rats in the AMI, AMI+30% HF, AMI+70% HF and AMI+100% HF groups exhibited disorganised myocardial architecture, marked degeneration and necrosis of cardiomyocytes, pronounced interstitial oedema and extensive inflammatory cell infiltration. Following atorvastatin intervention, the AMI+100% HF+PD group demonstrated substantial improvement in myocardial cell morphology, mitigating degeneration and necrosis.

Comparison of the apoptosis numbers of myocardial cells in different groups of rats

TUNEL-positive staining was localized to the nuclei of cardiomyocytes, appearing brownish-yellow, whereas normal nuclei appeared pale blue (**Figure 4**). Compared to the normal group, the number of apoptotic cells was significantly increased in the AMI, AMI+30% HF, AMI+70% HF and AMI+100% HF groups (P<0.001). Significant differences were also observed among the AMI+30% HF, AMI+70% HF and AMI+100% HF groups (P<0.05). Following atorvastatin intervention, the number of apoptotic cells in the AMI+100% HF+PD group was markedly reduced compared to the AMI+100% HF group (P< 0.001). Corresponding quantitative data are presented in **Figure 4**.

Correlation analysis between the number of apoptotic cells and blood lipid in each group of rats

Correlation analysis revealed that the number of apoptotic cells was positively correlated with TC, TG, and LDL levels and negatively correlated with HDL levels, and all correlations reached significance (P<0.001; **Figure 5**).

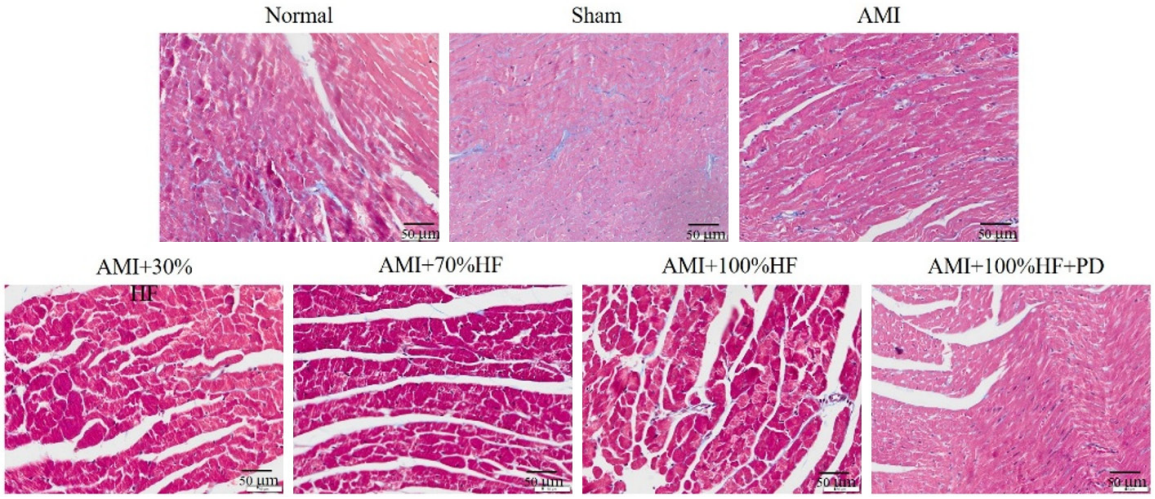

Figure 3. Pathologic changes in different groups in rats by H&E staining (200×).

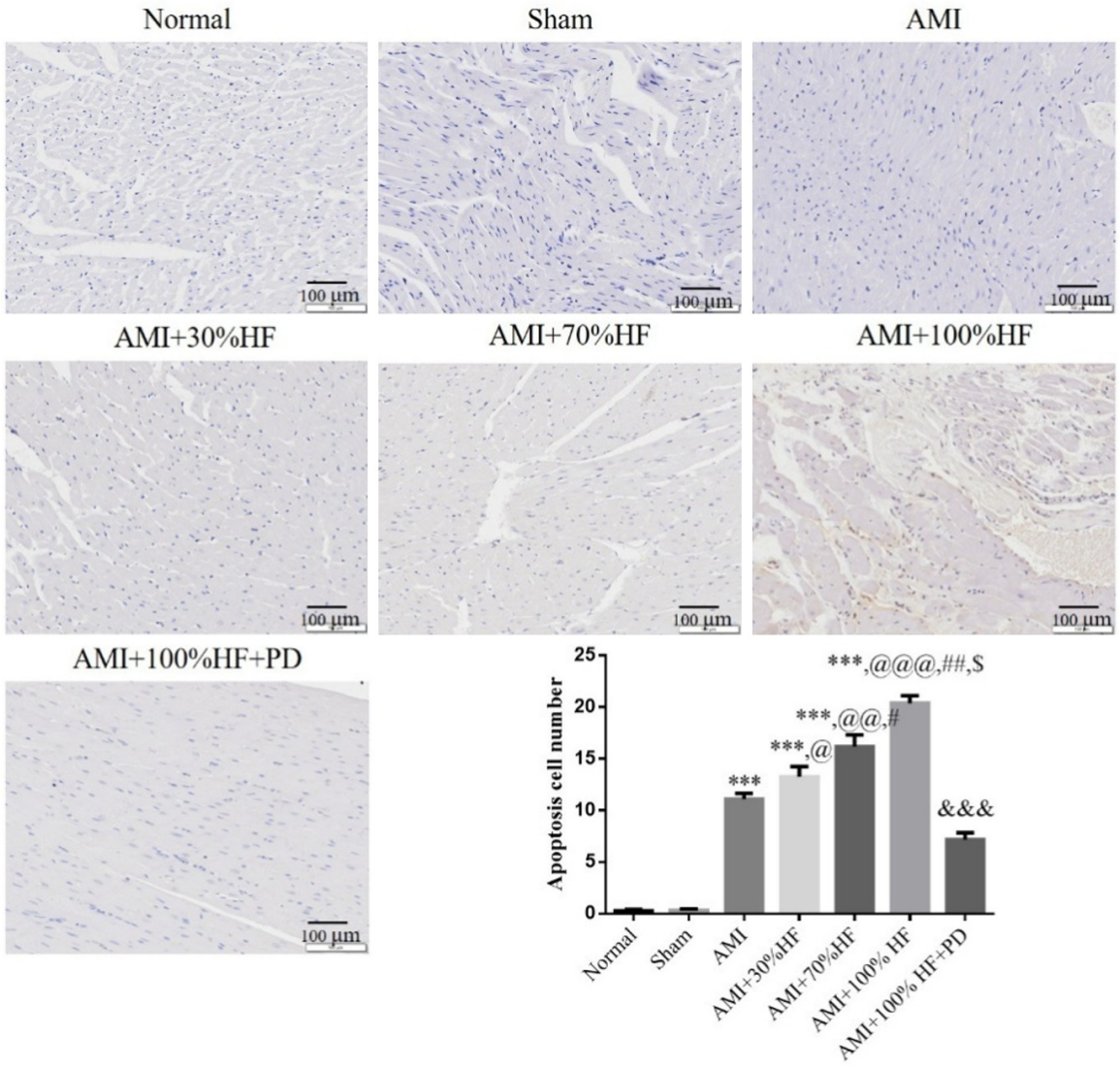

Figure 4. Apoptosis cell number of different groups by TUNEL assay (100×). *: P<0.05, **: P<0.01, **: P<0.001, compared to Normal group; @: P<0.05, @@: P<0.01, @@@: P<0.001, compared to AMI group; #: P<0.05, ##: P<0.01, compared to AMI+30% HF group; $: P<0.05, compared to AMI+70% HF group; &&&: P<0.001, compared to AMI+100% HF group.

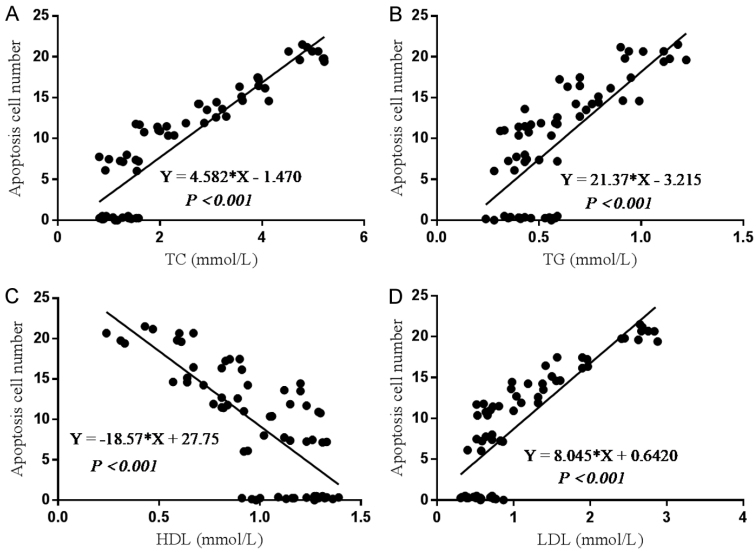

Figure 5. Correlation analysis between the number of apoptotic cells and blood lipid levels in each group of rats. A. Analysis correlation between TC and Apoptosis cell number. B. Analysis correlation between TG and Apoptosis cell number. C. Analysis correlation between HDL and Apoptosis cell number. D. Analysis correlation between LDL and Apoptosis cell number.

Comparison of the expression of apoptosis-related proteins caspase-3 and caspase-12 in myocardial tissue of rats in different groups

The WB test results showed that compared to the normal group, caspase-3 and caspase-12 protein levels were significantly elevated in the AMI, AMI+30% HF, AMI+70% HF and AMI+100% HF groups (P<0.05; **Figure 6**). Significant differences were also observed among the AMI+30% HF, AMI+70% HF and AMI+100% HF groups (P<0.05). Following atorvastatin intervention, the AMI+100% HF+PD group exhibited a marked reduction in caspase-3 and caspase-12 protein levels compared with the AMI+100% HF group (P<0.001; **Figure 6**).

Discussion

Previous studies have established that dyslipidemia is closely associated with recurrent myocardial infarction in patients with AMI and is recognized as an independent risk factor for AMI [9]. A meta-analysis reported that long-term lipid-lowering therapy, resulting in a 1-mmol/L reduction in LDL cholesterol, was associated with a 21% decrease in the risk of major cardiovascular adverse events and a 27% reduction in recurrent coronary events [10]. Elevated levels of LDL, TC and TG, together with reduced HDL, can exacerbate cardiovascular inflammation and oxidative stress, ultimately leading to the structural and functional damage of myocardial cells [11]. In the present study, the levels of TG, LDL and TC in the AMI model rats were significantly higher than those of the normal group, whereas the level of HDL was significantly reduced. HF diet interventions at different ratios considerably increased the levels of TC, TG, and LDL in the sera of AMI rats, whereas the level of HDL was significantly reduced. Along with the abnormal expression of blood fat, the number of myocardial cell apoptosis considerably increased. Correlation analysis confirmed that TC, TG and LDL concentrations were significantly positively correlated with the number of myocardial apoptotic cells, and HDL was significantly negatively correlated with the number of myocardial apoptotic cells (P<0.001). After intervention with the lipid-lowering drug atorvastatin, serum TC, TG, and LDL levels significantly decreased, HDL levels significantly increased, and the number of myocardial apoptotic cells significantly decreased relative to those in the AMI+100% HF group. This result suggests that dyslipidemia has considerable implications for myocardial injury after AMI.

Caspase activation is a pivotal event in myocardial cell apoptosis [12]. Caspase-3 is an essential component of the apoptotic protease cascade, serving as a key executioner enzyme in mammalian cell apoptosis. By contrast, caspase-12 is a central mediator of endoplasmic reticulum stress-induced apoptosis [13]. It is

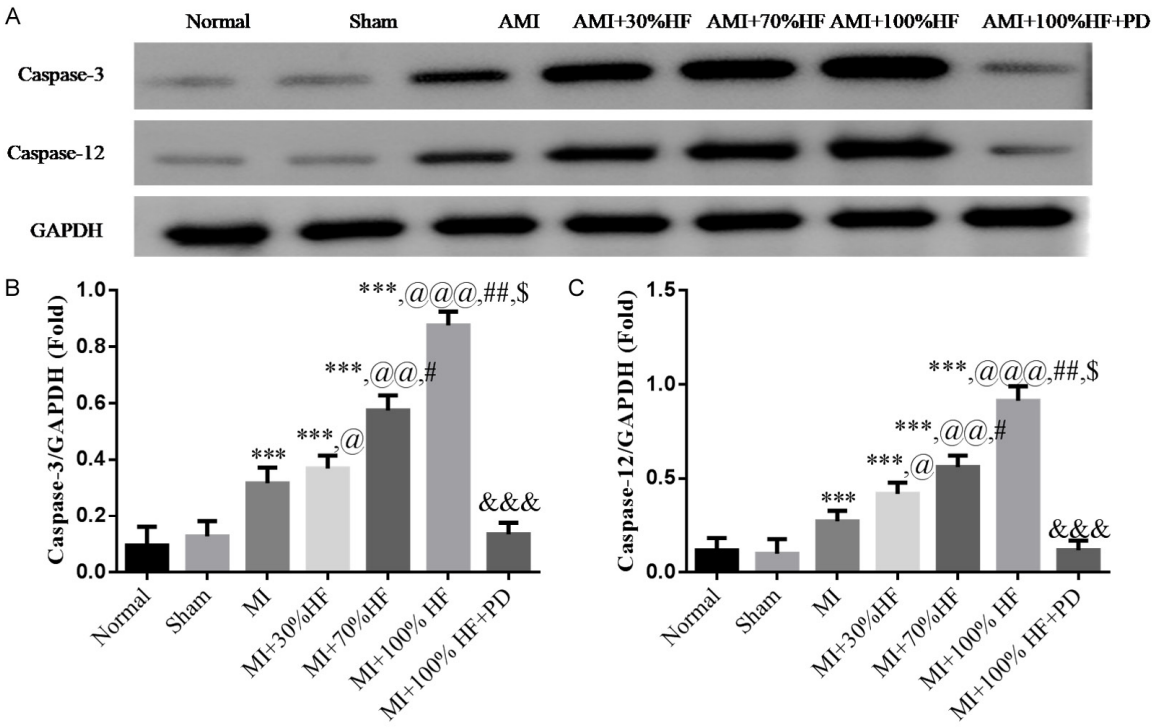

Figure 6. Caspase-3 and Caspase-12 protein expressions in difference groups in rats by WB assay. A. Caspase-3 and Caspase-12 proteins expressions by WB assay. B. Caspase-3/GAPDH ratio in different rat groups. C. Caspase-12/GAPDH ratio in different rat groups. *: P<0.05, **: P<0.01, **: P<0.001, compared with Normal group; @: P<0.05, @@: P<0.01, @@@: P<0.001, compared to AMI group; #: P<0.05, ##: P<0.01, compared to AMI+30% HF group; $: P<0.05, compared to AMI+70% HF group; &&&: P<0.001, compared to AMI+100% HF group.

localized exclusively to the cytoplasmic surface of the endoplasmic reticulum membrane in its inactive zymogen form. Moderate endoplasmic reticulum stress can restore calcium and protein homeostasis and enhance cellular stress tolerance. Sustained and severe endoplasmic reticulum stress triggers endoplasmic reticulum-mediated apoptosis, leading to cell injury. Under prolonged strong stress, procaspase-12 is cleaved into active caspase-12, which subsequently activates downstream caspase-3, initiating the apoptotic process [14]. The caspase-12-mediated endoplasmic reticulum apoptosis pathway is markedly activated and contributes to myocardial injury after AMI [15]. The present study found that dyslipidemia following AMI may promote the overexpression of caspase-3 and caspase-12, thereby increasing apoptosis in myocardial tissue. Atorvastatin treatment effectively inhibited the activation of the endoplasmic reticulum apoptosis pathway, as evidenced by suppression of caspase-12 activation and reduced expression of activated caspase-3. These results suggest that correcting dyslipidemia can attenuate myocardial cell apoptosis and improve cardiac function after AMI by blocking the caspase-12-mediated endoplasmic reticulum stress-induced apoptotic pathway.

In conclusion, dyslipidemia plays a critical role in myocardial dysfunction and injury following AMI. The correction of abnormal lipid levels after AMI can inhibit the activation of the key apoptotic protein caspase-12 in the endoplasmic reticulum stress-mediated apoptosis pathway, preventing the progression of myocardial cell apoptosis induced by dyslipidemia in rats.

Disclosure of conflict of interest

None.

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