Tohoku J. Exp. Med., 2025, **265**, 151-159

Activation of the AMPK-mTOR Pathway by Astaxanthin Against Cold Ischemia-Reperfusion in Rat Liver

Shujun Lu,[1] Yajing Zhang[2] and Wenli Yu[1]

[1]Department of Anesthesiology, Tianjin First Central Hospital, Tianjin, China
[2]First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, National Clinical Research Center for Chinese Medicine Acupuncture and Moxibustion, Tianjin, China

To investigate the protective effect and mechanism of astaxanthin on myocardial injury through activation of AMPK-mTOR pathway. In this study, 32 SPF adult male Wistar rats aged 8 to 10 weeks, weighing 250-300 g were divided into 4 groups (n = 8): sham surgery group (S group), autologous orthotopic liver transplantation group (T group), astaxanthin pretreatment surgery group (Group A) and compound C + astaxanthin pretreatment surgery group (Group C). Group A was given astaxanthin 500 mg/kg, group C received compound C 50 mg/kg + astaxanthin 500 mg/kg once a day for 2 weeks, group S and T received same volume of 0.9% saline. 8 h after portal vein opening, blood samples were collected to determine serum concentrations of TNF-α, IL-6 and HMGB 1 and myocardial injury markers. Myocardial tissue was collected to determine the MDA content, SOD activity and activation of AMPK-mTOR pathway. Compared with the S group, higher serum concentrations of TNF-α, IL-6, HMGB 1, CK-MB, cTnI, and H-FABP in groups T, A, and C, increased MDA content and decreased SOD activity, higher expression of activated Caspase-3 was observed; Compared with the T group, in group A, the serum concentrations of TNF-α, IL-6, and HMGB 1, CB-MB, cTnI, and H-FABP were significantly decreased, with decreased MDA content, increased SOD activity, the reduced expression of activated Caspase-3, elevated P-AMPK/AMPK, and decreased P-mTOR/mTOR. In Conclusion, Astaxanthin protects against liver ischemia-induced myocardial injury in rats mediating by the activation of the AMPK-mTOR pathway.

Key words: AMPK-mTOR; astaxanthin; liver transplantation; myocardial injury
Tohoku J. Exp. Med., 2025 March, **265** (3), 151-159.
doi: 10.1620/tjem.2024.J103

Introduction

Cold ischemia and reperfusion of the liver is an inevitable pathophysiological process in liver transplantation surgery, which not only leads to liver injury, but also causes damage to distant organs such as heart and lung. Myocardial troponin I (cTnI) was greater than 0.1 μg/L as the standard of myocardial injury. The incidence of myocardial injury in liver transplantation patients reached 40.4%, and the 30-day mortality of patients with myocardial damage was as high as 11.4%, which was one of the main causes of postoperative death in patients (Huang et al. 2016).

The myocardial injury of varying severity can occur during the perioperative period of liver transplant recipients. Researchers have found that serum cTnI and creatine kinase isoenzyme (CK-MB) were significantly higher at the end of surgery than at the preoperative level (Hei et al. 2006). Therefore, myocardial damage does occur during the perioperative period of liver transplantation, but the specific mechanism is not clear. It is believed that hepatic cold ischemia-reperfusion can stimulate the massive production of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), and the produced proinflammatory cytokines can aggravate liver ischemia-reperfusion injury, forming a malignant circle (Nong et al. 2016). Excessive release of inflammatory factors is closely related to the perioperative myocardial injury in liver transplantation. Marfella et al. (2009) showed that TNF-α activated the overexpression of nitric oxide synthase (iNOS) in cardiomyocytes and vascular endothelial cells, released a large amount of nitric oxide (NO), and damaged the lipid peroxidation of the cell membrane, thus damaging tissue cells. The time point of increasing serum TNF-α and IL-6 concentrations in liver

Received April 10, 2024; revised and accepted September 17, 2024; J-STAGE Advance online publication September 26, 2024
Correspondence: Wenli Yu, 24 Fukang Road, Nankai District, Tianjin 300192, China.
e-mail: yuwenli8363@163.com

transplantation was slightly earlier than the time point of increasing myocardial injury markers, and the peak time point was basically consistent, suggesting that the excessive release of numerous inflammatory cytokines may be an important factor in inducing myocardial injury (Weng et al. 2015). Some clinical trials have also confirmed that the inhibition of serum TNF-α and IL-6 release amount can improve the left ventricular structure and left heart function in patients with heart failure (Lianza et al. 2014). At present, no effective treatment has been found for perioperative myocardial injury of liver transplantation recipients. In the liver transplantation, using vasoactive drugs such as dopamine, and adrenaline to maintain circulation stability, and using nitroglycerin and cyclic adenosine for myocardial protection, still found new liver stage cTnI is greater than 0.2 μg/L, indicating that the patient has obvious myocardial injury. Therefore, it is urgent to reveal the pathogenesis of myocardial damage in liver transplantation and to find safe and effective protection measures.

Astaxanthin (AST) is a carotenoid widely found in microorganisms and marine organisms. AST has a α-hydroxyketone structure with strong antioxidant activity by capturing singlet oxygen and reacts with free radicals (Ambati et al. 2014). The antioxidant activity of AST was 500-fold and 10-fold higher than vitamin E and β-carotene, respectively (Kishimoto et al. 2016). In recent years, AST has been reported to prevent cardiovascular disease. In rats with isoproterenol-induced MI, AST treatment reduced heart weight, inflammatory cell infiltration, and myocardial fibrosis by improving antioxidant enzyme activity (Ghosh et al. 2020). In addition, AST attenuated cardiac infarction (myocardial infarction, MI) -induced cardiac dysfunction and fibrosis (Shi et al. 2018). AST can enhance myocardial contraction index and cardiomyocyte mitochondrial membrane potential to protect damaged myocardia by fighting oxidative stress and inhibiting inflammatory response (Alam et al. 2018). In ochratoxin A induced myocardial injury model, AST pretreatment by raising cardiomyocyte Nrf2 level, increase the antioxidant enzyme SOD, catalase (CAT), glutathione (GSH) content, maintain mitochondrial structural integrity and normal function, improve the heart rate, myocardial enzyme level, play the role of myocardial protection (Cui et al. 2020).

Adenylate-activated protein kinase (AMPK) is an important enzymatic energy balance and regulated signaling pathway, which acts as an intracellular energy sensor (Bouma et al. 2010). Studies have found that AMPK has anti-inflammatory effects after reactivation. Activation of AMPK suppresses the postischemic adhesion between leukocytes and endothelial cells and reduces the local aggregation of inflammatory cells, thus exerting an anti-inflammatory effect. Since AMPK has anti-apoptotic and anti-inflammatory effects after activation, it is a hot topic and an innovative point to exert myocardial protective effect by intervening in AMPK signaling pathway (Gao et al. 2015). In this study, a rat model of myocardial injury induced by hepatic cold ischemia and reperfusion was established to explore the protective effect of AST in regulating AMPK-mTOR pathway on myocardial injury under oxidative stress in rat autologous liver transplantation.

Materials and Methods

Laboratory animals

32 SPF adult male Wistar rats, aged 8 to 10 weeks of age and weighing 250-300 g, were purchased from Oriental Biological Company (Gyeonggi Province, Korea). All rats were housed in a constant temperature environment (23°C) with 2 rats per cage on a 12-h light / dark cycle with ad libitum access to food and water. The animal treatment protocol was approved by the Institutional Animal Care and Use Committee of Nankai University (Tianjin, China) (IACUC approval number: 2010-0113A).

Experimental grouping and establishment of an autologous liver transplantation model

The rats were divided into 4 groups (n = 8): sham group (S group), orthotopic liver transplantation group (T group), AST pretreatment surgery group (Group A) and compound C + AST pretreatment surgery group (Group C). Rats in Group A and C completed pretreatment with AST before the start of the experiment: Group A was administered AST 500 mg/kg, Group C received compound C 50 mg/kg + AST 500 mg/kg, dissolved in 100 g/L of hydroxypropyl-β-cyclodextrin saline 0.2 ml once a day for 2 weeks, Group S and T were given the same volume of 0.9% saline. All patients were fasted for about 12 h before surgery. In group S, only free liver and peripheral vessels were open without vascular blockade and liver perfusion. A rat orthotopic liver transplantation model was prepared in groups T, A and C according to reference. 5% chloral hydrate 0.5 ml/100 g of anesthesia, skin disinfection, and median abdominal incision into the abdomen. The rat severed the perihepatic ligament and flipped the liver to the left to reveal the right retroperitoneum, separate and expose inferior hepatic vena cava (IHVC), subhepatic inferior vena cava (SHVC), portal vein (PV) and the hepatic native arteries. The sequential interruption of the above hepatic vessels with the onset of the anhepatic phase.1-2mm was cut above the IHVC clip at a 6-8 ml/min perfusion rate and a pressure of 10 kPa (1 kPa = 7.5 mmHg). The liver surface was covered with fine ice chips to maintain low temperature and cold perfusion time for 30 min. The liver changes from bright red to earthy yellow, and the touch is cold, and the perfusion is completed. At the end of perfusion, the IHVC outflow tract was closed and the vessel clips was removed to restore blood flow from SHVC, IHVC, PV and native hepatic arteries. After the reflow of the above vessels, the liver changed from khaki to bright red, and the abdominal cavity was washed with warm saline. During the operation, pay attention to maintain the body temperature, avoid hypothermia, and eat normally after surgery.

Blood, Serum, and Tissue Sampling

At the end of the liver cold ischemia and reperfusion period (8 h), 3.5 mL of blood was collected through the inferior vena cava. Set in 4°C refrigerator for 30 min, centrifuged at 3,000 rpm centrifuge for 10 min, the supernatant was removed and stored at 80°C until use. Take 0.2-l g of heart tissue, rinse thoroughly with normal saline. Quickly cut the tested myocardial tissue with small ophthalmic scissors, put it into a homogenate tube, fully grind the myocardial tissue, and make the cardiac muscle ground into tissue homogenate for about 10 min. The liquid in the homogenizer was moved into the centrifuge tube, set the centrifugation speed of 15,000 rpm, temperature of 4°C, centrifugation time of 10 min, remove the supernatant and obtained the concentration of 10% tissue homogenate 80°C.

Hematoxylin-Eosin (HE) Staining

The myocardial tissue was fixed in 4% paraformaldehyde solution, and after 12-24 h of fixation, the proteins of the tissue to be examined were solidified and degenerated, so as to prevent the destruction and deformation of the cell morphology and structure. Cardiac tissue was dehydrated with gradient alcohol, embedded in wax, and then sliced into conventional sections (4 μm thick). Sections were dehydrated in 100%, 95%, 90%, 80% and 75% ethanol for 10 min, rinsed in distilled water, stained with hematoxylin for 1 min, differentiated with 1% hydrochloric acid, stained with 1% eosin for 2 min, rinsed with tap water, and histologically by optical microscopy. Staining results: the nucleus is blue; the cytoplasm and other tissues are pink.

Determination of TNF-α, IL-6 and HMGB1 content

TNF-α concentration was determined by double antibody sandwich ELISA. First, the microplate was prepared for use in advance, and the coated anti-rat TNF-α monoclonal antibody was placed in it. When the TNF-α in the standard and tested products were fully combined with the monoclonal antibody, the free unbound part was removed, and then the horseradish peroxidase-labeled phosphine and biotinylated anti-rat TNF-α antibody were placed. After this, biotin binds to phosphine, and the rat TNF-α coated on the monoclonal antibody binds to the anti-rat TNF-α antibody through the principle of antigen and antibody binding. After this binding, it forms an immune complex, and the excess is removed by washing. The chromogen was placed, and when TNF-α was present in the reaction well, the chromogen was turned blue under horseradish peroxidase catalysis, and the termination solution was placed to turn it yellow. The absorbance (OD) value was measured at 450 nm, and the sample concentration was calculated by correlation. The principle and procedure for detecting serum IL-6 and HMGB1 concentration were the same as for detecting TNF-α.

Measurement of malondialdehyde levels and superoxide dismutase activity in the heart

Fat peroxidation is estimated by measuring the content of malondialdehyde (MDA), a product of lipid oxidation. The heart tissue was homogenized with RIPA buffer and a 10% homogenate was obtained using a homogenizer (50 mg of tissue in 0.5 ml cold buffer). MDA levels were measured using a kit purchased from the Cayman Islands (MI, USA). This assay relies on the MDA-thiobarbituric acid (TBA) adduct, which is formed by MDA reaction with TBA at high temperatures (90-100°C) and acidic conditions and can be calorimetrically measured at 532 nm. Superoxide dismutase (SOD) was evaluated using a kit purchased from Cayman. This assay uses tetrazolium salts to detect superoxide radicals produced by xanthine oxidase and hypoxanthine. This kit mainly detects Mn-SOD in mitochondria, but also includes cytosolic Cu / Zn SOD and extracellular SOD.

Determination of CK-MB, cTnI and H-FABP content

The levels of myocardial injury markers in hepatic cold ischemia-reperfusion rats were measured by ELISA. The coated microwells were coated with CK-MB, cTnI and H-FABP antibody and ready for use. Serum samples, standards and horseradish peroxidase-labeled antibodies were placed in turn, and then warmed, then washed, and set aside for the next operation. TMB is applied to color the substrate. After peroxidase and a chemical reaction, TMB becomes blue and further turns yellow when catalyzed by the acid. In the sample, the concentration of the sample was used to measure the absorbance value at 450 nm, and the sample concentration was calculated by the correlation.

Western Blotting

Rat heart tissue was cut into small pieces, weighed, and radioimmunoprecipitation (RIPA) lysis buffer (Beyotime, Tianjin, China) (100 mg: 1 mL) was added and homogenized. Subsequently, proteins were extracted using lysis buffer and quantified using the bicinchonini acid (BCA) Protein Assay Kit (Santa Cruz, United States). Then, the samples were loaded for electrophoresis and transferred to a polyvinylidene difluoride (PVDF) membrane. Then, the membranes were blocked, incubated overnight with primary antibody, and incubated with secondary antibody for 1h. Band exposure was performed using enhanced chemiluminescence (ECL) and analyzed using an Odyssey film scanner. Protein levels were normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Western blot bands were quantified using the Image Lab software (Bio-Rad, United States).

Statistical analysis

Statistical analysis was performed using SPSS 22.0 statistical software, measurement data were expressed as mean ± standard deviation ($\bar{x}$ + s), and comparisons between groups using one-way analysis of variance, P <

0.05 was considered as statistically significant.

Results

HE staining

Under light microscope, no obvious pathological changes were seen in myocardial tissue of S group, and pathological damage occurred in groups T, A and C: including cardiomyocyte contraction, scattered pyknotic cells, and neutrophil infiltration. The degree of pathological damage in group A was significantly reduced compared with the T group, while the pathological changes in group C were similar to those in the T group (Fig. 1).

Changes in levels of serum inflammatory factors

Serum concentrations of TNF-α, IL-6 and HMGB1 increased 8 h after reperfusion in the T, A and C rats, compared with the S group ($P < 0.05$). Compared with group T, serum concentrations of TNF-α, IL-6 and HMGB1 decreased in group A ($P < 0.05$). In group C, serum concentrations of TNF-α, IL-6 and HMGB1 were increased with group A ($P < 0.05$) (Fig. 2).

Changes in MDA content and SOD activity in myocardial tissue

Compared with S group, MDA content increased and SOD activity decreased in groups T, A and C ($P < 0.05$). Compared with the group A, MDA decreased and SOD activity increased in group A ($P < 0.05$); compared with group A, MDA increased and SOD activity decreased ($P < 0.05$) (Fig. 3).

Changes in levels of myocardial injury markers

Serum levels of CK-MB, cTnI and H-FABP were increased in groups T, A and C ($P < 0.05$). Compared with group T, the serum CK-MB, cTnI, and H-FABP levels were all decreased in group A ($P < 0.05$). Compared with group A, serum CK-MB, cTnI, and H-FABP levels were increased in group C ($P < 0.05$) (Fig. 4).

Activated caspase-3 protein and changes in levels of P-AMPK / AMPK and P-mTOR / mTOR in rats

In myocardial tissue, Caspase-3 expression was increased in T, A and C groups compared with S group ($P < 0.05$). Compared with the T group, the Caspase-3 expression level decreased in group A ($P < 0.05$). Compared with group A, the Caspase-3 expression level was increased in group C ($P < 0.05$) (Fig. 5). Compared with the S and T groups, P-AMPK / AMPK and P-mTOR / mTOR ($P < 0.05$). Compared with group A, P-AMPK / AMPK decreased and P-mTOR / mTOR increased in Group C ($P < 0.05$) (Fig. 6).

Discussion

The establishment of the rat liver ischemia-reperfusion

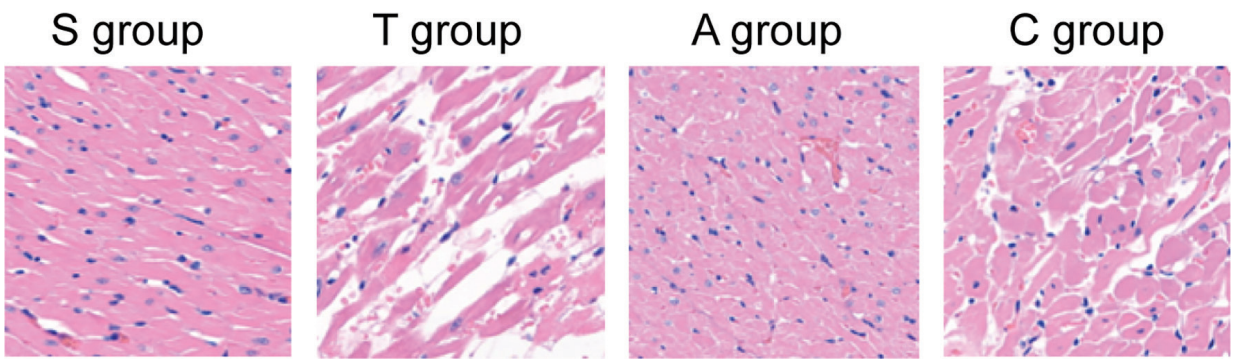

Fig. 1. Myocardial histomorphological changes in rats in each group.

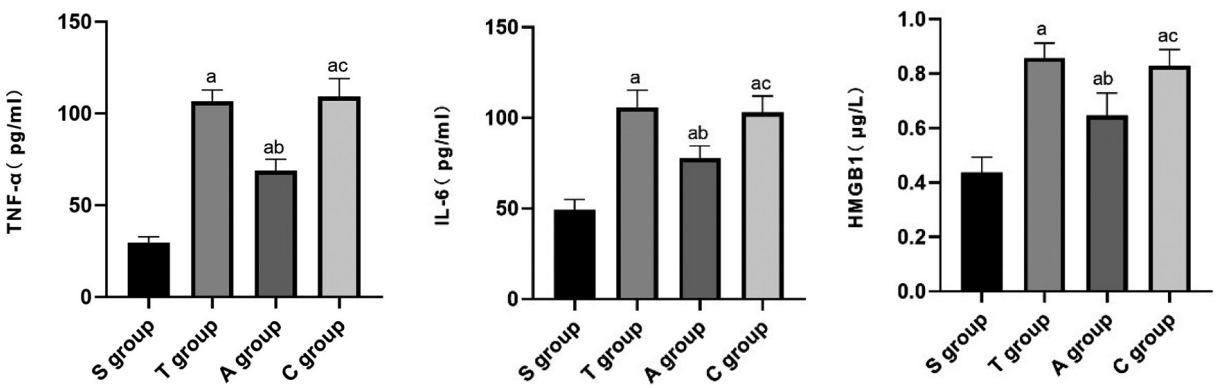

Fig. 2. Comparison of serum TNF-α, IL-6, and HMGB1 level in each rat group.
Compared with S group, $^{a}P < 0.05$; compared with T group, $^{b}P < 0.05$; compared with group A, $^{c}P < 0.05$.

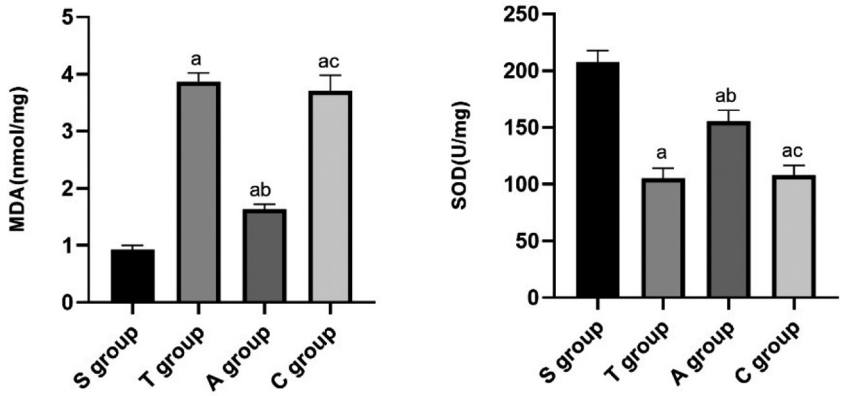

Fig. 3. Comparison of MDA content and SOD activity in myocardial tissues of rats.
Compared with S group, $^{a}P < 0.05$; compared with T group, $^{b}P < 0.05$; compared with group A, $^{c}P < 0.05$.

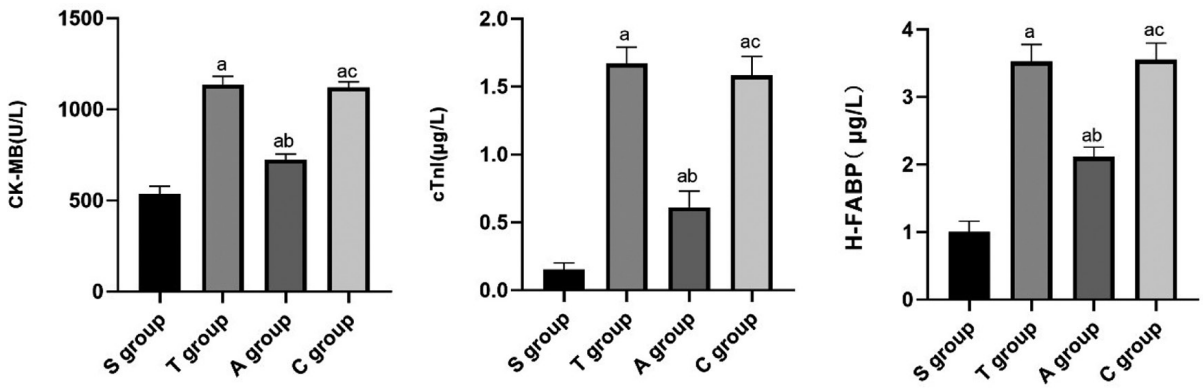

Fig. 4. Comparison of serum CK-MB, cTnI, and H-FABP levels in each group of rats.
Compared with S group, $^{a}P < 0.05$; compared with T group, $^{b}P < 0.05$; compared with group A, $^{c}P < 0.05$.

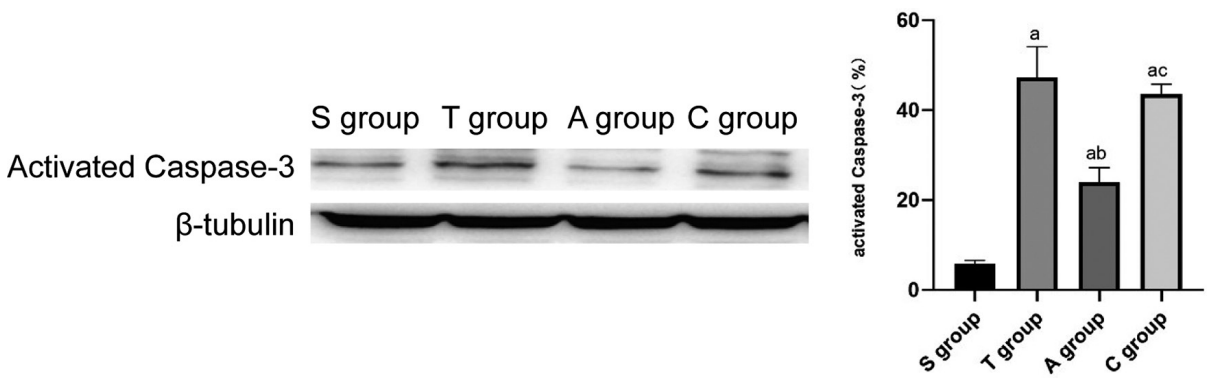

Fig. 5. Comparison of activated Caspase-3 expression in each tissue of rats.
Compared with S group, $^{a}P < 0.05$; compared with T group, $^{b}P < 0.05$; compared with group A, $^{c}P<0.05$.

model is an important way to study the liver ischemia-reperfusion injury and its mechanism, among which the Kamada liver transplantation model is the most typical (Kamada et al. 1979). This model includes both the hot and cold ischemic injury processes of the liver, but the model relies on higher surgical skills and has large surgical trauma, and different manipulation techniques may bring different experimental results. The hepatic thermal ischemia and reperfusion model was established by clamping the rat hepatic artery, portal vein, superior hepatic vein, and inferior vena cava, which is also one of the animal models commonly used in the experimental study (Serafín et al. 2004). Although the operation of this model is easy to master, it cannot mimic the surgical procedure of clinical liver transplantation. Liver cold ischemia reperfusion during clinical course is an important factor leading to hepatic and distant organ damage (Liu et al. 2013). Therefore, the application of hepatic thermal ischemia reperfusion model is not effective to analyze the mechanism of distal organ injury in liver transplantation. Hu et al. (2001) established

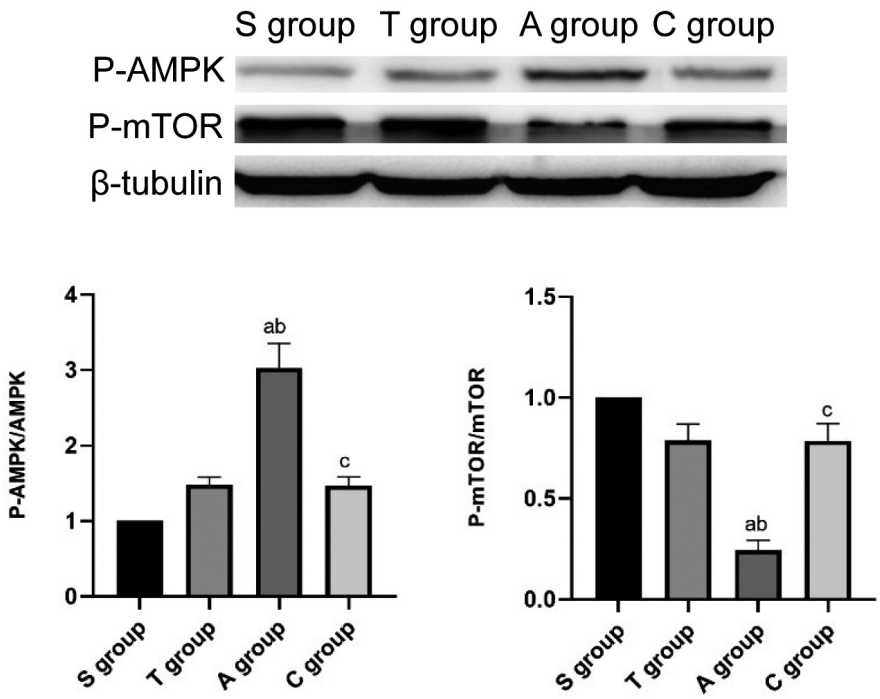

Fig. 6. Western blots of P-AMPK / AMPK and P-mTOR / mTOR levels in myocardial groups of rats.
Compared with S group, $^{a}P < 0.05$; compared with T group, $^{b}P < 0.05$; compared with group A, $^{c}P < 0.05$.

the in situ hepatic cryogenic perfusion and reflux model in rats, including the liver ischemia of the inferior hepatic inferior vena cava, portal vein and hepatic native artery, and then infused 0°C of homemade perfusion fluid through the splenic vein for 20 min, and the right adrenal vein flowed the perfusion fluid. This model simulates the process of cold ischemia reperfusion in rat liver and can be used to analyze cold ischemia reperfusion injury in the liver. However, in this model, using the right adrenal vein of the fine rat as the outflow channel of the perfusion fluid easily tends to increase the outflow resistance of the perfusion fluid and cause edema of liver tissue. In this study, we used a modified model of rat hepatic cold ischemia reperfusion with convenient operation, less trauma and high survival rate, significantly improved the accuracy of the experimental results (Nozato et al. 1998). This model effectively simulates the surgical procedure of liver transplantation, laying an experimental foundation for the analysis of myocardial injury caused by hepatic cold ischemia and reperfusion.

AST is a carotenoid extracted from Marine algae, yeast and aquatic animals. It has strong anti-oxidative stress function and has clear anti-inflammatory and anti-tumor effects on the body without serious side effects. AST can play a protective role in a variety of cardiovascular diseases, and prevent exercise, myocardial injury caused by ethanol and fat, and cardiac dysfunction caused by coronary microembolism. Some clinical studies have found that the prior administration of AST for three months can improve the cardiac of left ventricular systolic dysfunction in patients with heart failure (Kato et al. 2020). We have shown that AST administration reduces cardiac dysfunction and myocardial fibrosis in mice (Zhang et al. 2017). Gross et al. (2006) found that continuous administration of AST could significantly reduce the area of myocardial infarction in cardiac ischemia and reperfusion in rats, and could improve the myocardial survival rate. AST can also increase glutathione peroxidase and superoxide dismutase viability in rat serum by upregulating Nrf 2 expression, thus enhancing its antioxidant capacity and reducing the production of oxidation products, and protecting cardiomyocyte (Deng et al. 2016). However, the effect of AST on myocardial injury caused by ischemia-reperfusion injury in vital organs remains unknown. Our results showed that AST could significantly reduce serum CK-MB, cTnI and H-FABP levels and reduce the activated Caspase-3 expression level in myocardial tissue, so using AST could improve the myocardial injury caused by hepatic cold ischemia and reperfusion injury.

The biological activity of TNF-α is multifaceted, and through its immune conditioning, it strengthens the damage effect of other inflammatory factors, leading to organ and tissue damage. TNF-α expression is not detected in normal heart muscle, but increase when heart failure occurs (Heberto Herrera Garza et al. 2002). TNF-α can be released after isolated myocardial tissue ischemia, which decreases in coronary blood flow and weakened myocardial ejection capacity, and leads to increased ventricular pressure in the end-diastolic phase. Clinical studies have shown that various cytokines are closely related to patient heart failure after myocardial infarction, and TNFR1 can be used as an independent factor affecting patient prognosis (Valgimigli et al. 2005). Excessive release of TNF-α can lead to nitric

oxide and rising enzyme levels, causing an increase of NO content in cardiomyocytes and inhibiting myofilament contraction. NO may be an important mediator of the negative allotropic effect of TNF-α myocardium (Marfella et al. 2009). Cardiomyocytes will undergo apoptosis under certain conditions. TNF-α leads to tissue damage, apoptosis may be mediated through the death receptor pathway or mitochondrial pathway. After the binding of TNF-α to its receptor, TNFR1 may enable the adaptor molecule to aggregate the TRADD, cause the trimerization of TNFR1. The secondary adaptor protein FADD was recruited. Further agglomeration of the Caspase-8 precursors to allow the final activation of Caspase-3, causing cell apoptosis. The mitochondrial pathway is distinct from the death receptor pathway. A large number of stimuli, such as intracellular signaling caused by oxidative stress, inflammatory cytokines, ischemia/reperfusion. These factors may cause alterations in mitochondrial membrane permeability and the release of cytochrome C from cytochrome into the cytoplasm, and form apoptotic bodies with Caspase-9 precursor, apoptotic protease activator factor 1 and ATP, eventually causing the Caspase to be activated, resulting in cell apoptosis.

Ischia-reperfusion of organ tissue can cause increased production of IL-6, intercellular adhesion molecule-1 (ICAM-1) can be produced by cardiomyocytes due to the induction of IL-6, and ICAM-1 promotes the enhancement of neutrophil adhesion, leading to microcirculatory embolism. Thus, we concluded that cardiomyocyte ischemia-reperfusion injury is closely related to the mediation of IL-6. In addition, ICAM-1 induced by IL-6 can increase the adhesion of leukocytes to cardiomyocytes, which is one of the important factors leading to the cytotoxic effect of leukocytes during cardiomyocyte reperfusion. For patients with coronary atherosclerosis, plaque instability may have adverse consequences. And inflammatory cytokines can increase the expression of metalloproteinases, leading to instability and even rupture of atherosclerotic plaques. Serum IL-6 levels are associated with poor clinical outcomes in patients with angina pectoris. Thus, IL-6 levels are associated with not only disease progression but also as an important inflammatory factor predictive of adverse complications. Plenz et al. (2002) compared different functional state of donors in heart transplantation, and the results showed that IL-6 level significantly affected the ventricular function. The increased myocardial level of IL-6 expression will cause obvious cardiac insufficiency. So, in clinical practice, reducing the heart transplant recipient IL-6 level may improve heart function, reduce postoperative cardiac complications, and improve the success rate of surgery. TNF-α causes isolated cardiomyocyte injury, and IL-10 can combat its injury effect, thus avoiding the imbalance between anti-inflammatory factors and inflammatory factors, and alleviating myocardial injury (Kaur et al. 2006). Some clinical trials have also confirmed that increasing TNF-α and IL-6 concentrations can significantly improve the LV structure and function in HF patients (Lianza et al. 2014).

HMGB1 can significantly reduce myofibrils contractility in cardiomyocytes and has negative inotropic effects on the heart (Hagiwara et al. 2008; Tzeng et al. 2008). In addition, the combination of HMGB1 and its receptor can affect the outward potassium current and L-type calcium current in cardiomyocytes, causing action potential lengthening, indicating that HMGB1 can aggravate its damage through myocardial electrical remodeling (Liu et al. 2010). HMGB1 could induce overexpression of iNOS, leading to increased NO levels, lipid peroxidation of cell membrane, disrupted membrane integrity, and further caused tissue cell damage (Ren et al. 2006). In this study, the relative levels of TNF-α, IL-6, and HMGB 1 were increased in the T group, suggesting an increased inflammatory response after hepatic cold ischemia-reperfusion injury. AST pretreatment in rats significantly reduced this level, suggesting an anti-inflammatory effect of AST after ischemia-reperfusion.

Cold ischemia and reperfusion of the liver reduces the production of oxygen free radicals, disrupting the balance between oxidants and antioxidants, leading to impaired myocardial oxygen metabolism and functional regulation, and oxidative stress plays an important role in myocardial injury. Organ ischemia-reperfusion injury induced oxidative stress, increased myocardial lipid peroxidation and protein oxidation, mitochondrial membrane destruction, and Cytochrome C release and Caspase 3 lysis activation, which leads to cardiomyocyte apoptosis (Shen et al. 2018). In this study, compared with S group, the MDA content and SOD activity decreased significantly in T group, and the oxidative stress response decreased and SOD activity increased, indicating that AST had obvious antioxidant effect on oxidative stress of cold ischemia and reperfusion in the liver.

AMPK is a key sensor of the cellular energy state found in almost all eukaryotes. It is a heterotrimer with the catalytic α subunit and the regulatory β and γ subunits, and in the heart, AMPKα2 highly expresses in cardiomyocytes (Taghiyar et al. 2023). Cellular metabolism is an important mechanism for cardioprotection, and AMPK is an important hub for restoring ATP production after myocardial injury. As the main cellular energy sensor, AMPK activation can be involved in the regulation of glucose and lipid metabolism during organ ischemia and reperfusion injury, and downregulates the level of proinflammatory factors, inhibit myocardial injury, and enhance the antioxidant capacity of myocardial tissue (Casin and Calvert 2021). During body organ ischemia, AMPK is rapidly activated and stimulates glucose uptake and glycolysis to promote the production of anaerobic ATP. This adaptive mechanism can restore cellular energy status and have protective and limiting effects on cardiac injury caused by liver ischemia and reperfusion during the acute phase of ischemia.

Liver cold ischemia reperfusion, AMPK stimulate myocardial fatty acid oxidation through phosphorylation to restore energy homeostasis, this process caused by inflam-

matory reaction, characterized by massive infiltration of immune cells (such as neutrophils and macrophages), inflammatory cells is the source of cytokines, oxidants and growth factors, they support fibroblast proliferation and angiogenesis to repair the damaged myocardial (Marino et al. 2021). However, during the liver cold ischemia reperfusion, the massive accumulation of inflammatory cells in the ischemic area is intensified, leading to further increase of oxidative substances and involvement in myocardial injury, remodeling, and heart failure (Fei et al. 2020). Activated AMPK attenuates the production of oxidative stress, and we can speculate that it protects the heart from injury by blocking the excessive oxidative stress produced by immune cells during the initial phase of reperfusion. In accordance with this hypothesis, this study shows that AST can alleviate the production of oxidative stress in inflammatory cells through pharmacological activation of AMPK and phosphorylation of AMPK, thereby preventing myocardial injury induced by reperfusion. Furthermore, we examined phosphorylated mTOR, a member of the key downstream signaling pathway of AMPK, and mTOR is negatively regulated by AMPK. Our results were consistent with another study that found the activation of AMPK-mTOR signaling during myocardial ischemia (Wang et al. 2019). The results of this study showed that AMPK phosphorylation further increased and reduced the level of p-mTOR in autologous liver transplantation rats, and accompanied the decrease of myocardial injury index, indicating that AST had myocardial protective effect, and the anti-inflammatory antioxidant action of AMPK inhibitor compound C was inhibited, and the expression of myocardial injury index increased, inhibiting the myocardial protective effect of AST, suggesting that the protective effect of AST on the mechanism of liver ischemia and reperfusion myocardial injury in autologous liver transplantation rats was related to the activation of AMPK-mTOR pathway.

Conclusions

AST can reduce the inflammatory response and oxidative stress by activating the AMPK-mTOR pathway. This study provides a rationale for the prevention and treatment of myocardial injury induced by hepatic cold ischemia and reperfusion.

Funding

This study was supported by Tianjin Key Medical Discipline (Specialty) Construction Project (TJYXZDXK-045A) and Natural Science Foundation of China (82072219).

Conflict of Interest

The authors declare no conflict of interest.

References

Alam, M.N., Hossain, M.M., Rahman, M.M., Subhan, N., Mamun, M.A.A., Ulla, A., Reza, H.M. & Alam, M.A. (2018) Astaxanthin Prevented Oxidative Stress in Heart and Kidneys of Isoproterenol-Administered Aged Rats. *J. Diet. Suppl.*, **15**, 42-54.

Ambati, R.R., Phang, S.M., Ravi, S. & Aswathanarayana, R.G. (2014) Astaxanthin: sources, extraction, stability, biological activities and its commercial applications–a review. *Mar. Drugs*, **12**, 128-152.

Bouma, H.R., Ketelaar, M.E., Yard, B.A., Ploeg, R.J. & Henning, R.H. (2010) AMP-activated protein kinase as a target for preconditioning in transplantation medicine. *Transplantation*, **90**, 353-358.

Casin, K.M. & Calvert, J.W. (2021) Dynamic Regulation of Cysteine Oxidation and Phosphorylation in Myocardial Ischemia-Reperfusion Injury. *Cells*, **10**, 2388.

Cui, G., Li, L., Xu, W., Wang, M., Jiao, D., Yao, B., Xu, K., Chen, Y., Yang, S., Long, M., Li, P. & Guo, Y. (2020) Astaxanthin Protects Ochratoxin A-Induced Oxidative Stress and Apoptosis in the Heart via the Nrf2 Pathway. *Oxid. Med. Cell Longev.*, **2020**, 7639109.

Deng, Z. (2016) The protective effect of astaxanthin on the Beagle dog myocardial infarction model and its reduction of oxidative stress mechanism. *Clinical Pharmacology and therapeutics in China*, **21**, 509-513.

Fei, Q., Ma, H., Zou, J., Wang, W., Zhu, L., Deng, H., Meng, M., Tan, S., Zhang, H., Xiao, X., Wang, N. & Wang, K. (2020) Metformin protects against ischaemic myocardial injury by alleviating autophagy-ROS-NLRP3-mediated inflammatory response in macrophages. *J. Mol. Cell Cardiol.*, **145**, 1-13.

Gao, Y., Hao, J. & Zhao, G. (2015) Activation of AMPKα by paeoniflorin pretreatment treatment against myocardial ischemia-reperfusion injury in somatic rats. *Hainan Med. J. Aug.*, **26**, 2185-2189.

Ghosh, R., Vinod, V., Symons, J.D. & Boudina, S. (2020) Protein and Mitochondria Quality Control Mechanisms and Cardiac Aging. *Cells*, **9**, 933.

Gross, G.J., Hazen, S.L. & Lockwood, S.F. (2006) Seven day oral supplementation with Cardax (disodium disuccinate astaxanthin) provides significant cardioprotection and reduces oxidative stress in rats. *Mol. Cell Biochem.*, **283**, 23-30.

Hagiwara, S., Iwasaka, H., Uchino, T. & Noguchi, T. (2008) High mobility group box 1 induces a negative inotropic effect on the left ventricle in an isolated rat heart model of septic shock: a pilot study. *Circ. J.*, **72**, 1012-1017.

Heberto Herrera Garza, E., Herrera Garza, J.L., Rodriguez Gonzalez, H., Trevino Trevino, A., Ibarra Flores, M. & Torre Amione, G. (2002) Importance of tumor necrosis factor-alpha in the pathogenesis of heart failure. *Rev. Esp. Cardiol.*, **55**, 61-66.

Hei, Z.Q., Liu, D.Z., Luo, C.F., Li, S.R., Ma, W.H. & Luo, G.J. (2006) Perioperative changes of ventricular function and three indicators of myocardial injury during orthotopic liver transplantation. *Chin. Med. J.* (Engl), **119**, 939-943.

Hu, J., Qian, J., Wang, X., Lu, S., Sun, B. & Zhang, H. (2001) Establishment and significance of cryogenic perfusion and reflow in rats. *The Chinese Journal of Experimental Surgery*, **18**, 366-367.

Huang, S., Apinyachon, W., Agopian, V.G., Wray, C.L., Busuttil, R.W., Steadman, R.H. & Xia, V.W. (2016) Myocardial injury in patients with hemodynamic derangements during and/or after liver transplantation. *Clin. Transplant.*, **30**, 1552-1557.

Kamada, N. & Calne, R.Y. (1979) Orthotopic liver transplantation in the rat. Technique using cuff for portal vein anastomosis and biliary drainage. *Transplantation*, **28**, 47-50.

Kato, T., Kasai, T., Sato, A., Ishiwata, S., Yatsu, S., Matsumoto, H., Shitara, J., Murata, A., Shimizu, M., Suda, S., Hiki, M., Naito, R. & Daida, H. (2020) Effects of 3-Month Astaxanthin Supplementation on Cardiac Function in Heart Failure Patients with Left Ventricular Systolic Dysfunction-A Pilot Study. *Nutrients*, **12**, 1896.

Kaur, K., Sharma, A.K., Dhingra, S. & Singal, P.K. (2006) Interplay of TNF-alpha and IL-10 in regulating oxidative stress in isolated adult cardiac myocytes. *J. Mol. Cell Cardiol.*, **41**, 1023-1030.

Kishimoto, Y., Yoshida, H. & Kondo, K. (2016) Potential Anti-Atherosclerotic Properties of Astaxanthin. *Mar. Drugs*, **14**, 35.

Lianza, A.C., Aikawa, N.E., Moraes, J.C., Leal, G.N., Morhy, S.S., Andrade, J.L., Bonfa, E. & Silva, C.A. (2014) Long-term evaluation of cardiac function in juvenile idiopathic arthritis under anti-TNF therapy. *Clin. Exp. Rheumatol.*, **32**, 754-759.

Liu, W., Deng, J., Xu, J., Wang, H., Yuan, M., Liu, N., Jiang, Y. & Liu, J. (2010) High-mobility group box 1 (HMGB1) down-regulates cardiac transient outward potassium current (Ito) through downregulation of Kv4.2 and Kv4.3 channel transcripts and proteins. *J. Mol. Cell Cardiol.*, **49**, 438-448.

Liu, X., Wang, B., Yu, L., Liu, C., Xiang, J., Tian, M. & Lv, Y. (2013) A single-center clinical study of classical orthotopic liver transplantation in a cardiac deceased donor. *J. Organ Transplant*., **4**, 23-27.

Marfella, R., Di Filippo, C., Portoghese, M., Ferraraccio, F., Rizzo, M.R., Siniscalchi, M., Musacchio, E., D'Amico, M., Rossi, F. & Paolisso, G. (2009) Tight glycemic control reduces heart inflammation and remodeling during acute myocardial infarction in hyperglycemic patients. *J. Am. Coll. Cardiol.*, **53**, 1425-1436.

Marino, A., Hausenloy, D.J., Andreadou, I., Horman, S., Bertrand, L. & Beauloye, C. (2021) AMP-activated protein kinase: A remarkable contributor to preserve a healthy heart against ROS injury. *Free Radic. Biol. Med.*, **166**, 238-254.

Nong, K., Wang, W., Niu, X., Hu, B., Ma, C., Bai, Y., Wu, B., Wang, Y. & Ai, K. (2016) Hepatoprotective effect of exosomes from human-induced pluripotent stem cell-derived mesenchymal stromal cells against hepatic ischemia-reperfusion injury in rats. *Cytotherapy*, **18**, 1548-1559.

Nozato, E., Shiraishi, M., Miyaguni, T., Hiroyasu, S., Okuhama, Y., Tomori, H.H., Higa, T., Nagahama, M., Taira, K. & Muto, Y. (1998) In situ perfusion of the liver under portosystemic shunt in rats: a stable model of cold ischemia for orthotopic liver transplantation. *Transplant. Proc.*, **30**, 3718-3720.

Plenz, G., Eschert, H., Erren, M., Wichter, T., Bohm, M., Flesch, M., Scheld, H.H. & Deng, M.C. (2002) The interleukin-6/interleukin-6-receptor system is activated in donor hearts. *J. Am. Coll. Cardiol.*, **39**, 1508-1512.

Ren, D., Sun, R. & Wang, S. (2006) Role of inducible nitric oxide synthase expressed by alveolar macrophages in high mobility group box 1--induced acute lung injury. *Inflamm. Res.*, **55**, 207-215.

Serafín, A., Rosello-Catafau, J., Prats, N., Gelpi, E., Rodes, J. & Peralta, C. (2004) Ischemic preconditioning affects interleukin release in fatty livers of rats undergoing ischemia/reperfusion. *Hepatology*, **39**, 688-698.

Shen, C., Wang, L., Kang, W. & Wang, X. (2018) Effect of astaxanthin on cardiomyocyte apoptosis and caspase-3 expression in rats with acute myocardial infarction. *Journal of Shanxi Medical University*, **49,** 749-753.

Shi, Y., Lin, P., Wang, X., Zou, G. & Li, K. (2018) Sphingomyelin phosphodiesterase 1 (SMPD1) mediates the attenuation of myocardial infarction-induced cardiac fibrosis by astaxanthin. *Biochem. Biophys. Res. Commun.*, **503**, 637-643.

Taghiyar, S., Pourrajab, F. & Aarabi, M.H. (2023) Astaxanthin improves fatty acid dysregulation in diabetes by controlling the AMPK-SIRT1 pathway. *EXCLI J.*, **22**, 502-515.

Tzeng, H.P., Fan, J., Vallejo, J.G., Dong, J.W., Chen, X., Houser, S.R. & Mann, D.L. (2008) Negative inotropic effects of high-mobility group box 1 protein in isolated contracting cardiac myocytes. *Am. J. Physiol. Heart Circ. Physiol.*, **294**, H1490-1496.

Valgimigli, M., Ceconi, C., Malagutti, P., Merli, E., Soukhomovskaia, O., Francolini, G., Cicchitelli, G., Olivares, A., Parrinello, G., Percoco, G., Guardigli, G., Mele, D., Pirani, R. & Ferrari, R. (2005) Tumor necrosis factor-alpha receptor 1 is a major predictor of mortality and new-onset heart failure in patients with acute myocardial infarction: the Cytokine-Activation and Long-Term Prognosis in Myocardial Infarction (C-ALPHA) study. *Circulation*, **111**, 863-870.

Wang, L., Yuan, D., Zheng, J., Wu, X., Wang, J., Liu, X., He, Y., Zhang, C., Liu, C., Wang, T. & Zhou, Z. (2019) Chikusetsu saponin IVa attenuates isoprenaline-induced myocardial fibrosis in mice through activation autophagy mediated by AMPK/mTOR/ULK1 signaling. *Phytomedicine*, **58**, 152764.

Weng, Y., Yu, W., Du, H., Lv, D., Wang, G., Wang, Y., Sheng, M. & Yu, Y. (2015) Preliminary study of the myocardial protection mechanism of ulinastatin during perioperative liver transplantation. *The Chinese Journal of Hepatobiliary Surgery*, **21**, 849-851.

Zhang, J., Wang, Q.Z., Zhao, S.H., Ji, X., Qiu, J., Wang, J., Zhou, Y., Cai, Q., Zhang, J. & Gao, H.Q. (2017) Astaxanthin attenuated pressure overload-induced cardiac dysfunction and myocardial fibrosis: Partially by activating SIRT1. *Biochim. Biophys. Acta Gen. Subj.*, **1861**, 1715-1728.