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Metformin protects septic rats from myocardial injury

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Abstract

This study assessed metformin's effect on myocardial injury in sepsis rats. Forty male Wistar rats were randomly allocated into sham group, sham/M group (metformin, 100mg/kg), CLP group (cecal ligation and puncture) and CLP/M group (metformin treatment before operation) followed by analysis of ALT、AST、CRE, and plasma TNF-α, IL-1β and cTnI levels by ELISA at 12 and 24 hours after operation, cardiomyocytes apoptosis by TUNEL staining and caspase-3 level by western blot. The levels of ALT, AST, CRE, TNF-α, IL-1β and cTnI showed no differences between sham group and sham/M group after operation. However, they were significantly increased in CLP group and CLP/M group and decreased in CLP/M group compared to CLP group. Myocardial apoptosis indexes in CLP group and CLP/M group were significantly increased than sham group with a lower apoptosis index in CLP/M group than CLP group. At 24 hours after operation, caspase-3 level was significantly higher in CLP group and CLP/M group compared to sham group with a lower level in CLP/M group than CLP group. In conclusion, metformin exerts protective effect on CLP model rats with myocardial injury possibly through reducing myocardial cell apoptosis and inhibiting TNF-α secretion.

Keywords: Metformin; Sepsis; Myocardial injury; Apoptosis

Introduction

Sepsis refers to the uncontrolled response of the host to infection and life-threatening organ dysfunction[1]. Sepsis with myocardial injury aggravates the disease progression, leading to multiple organ failure and even death. Some studies reported that the mortality of sepsis patients with myocardial injury can reach 70-90%[1]. At present, it is believed that myocardial injury in sepsis is caused by the interaction of gene, molecule, metabolism, structure, autonomic nerve and hemodynamic changes[3]. The theory of "cytokine storm" in sepsis is gradually verified, which proposes that a large number of cytokines, chemokines and colony-stimulating factors are produced and provided the basis for the occurrence and development of sepsis[4].

Metformin is a widely used first-line hypoglycemic drug. It can delay glucose uptake in gastrointestinal tract, improve insulin sensitivity and inhibit gluconeogenesis, and reduce respiratory chain complex activity in mitochondria as well as reduce ATP production to activate AMP-activated kinase (AMPK)[5]. Recent studies found that metformin may reduce myocardial mitochondrial membrane potential by activating AMPK signaling, thus reducing mitochondrial damage[6, 7]. However, whether metformin affects myocardial injury in sepsis remains unclear. Therefore, this study examined the effect of metformin on myocardial injury.

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Materials and Methods

Animals and Reagents

Male Wistar rats (180-200g, 4 weeks) from Hubei Provincial Center for Disease Control and Prevention were randomly assigned to different experimental groups. Metformin was purchased from Shanghai Shiguibao Pharmaceutical Co. Ltd. TUNEL kit was from Roche Applied Science (Product No. 11684817910), and ELISA kits for TNF-α, IL-1β, cTnI were purchased from Wuhan YILAITE Biotechnology Co. Ltd. (Product No. E-EL-R0019c、E-EL-R0012c、E-EL-R1253c).

Grouping of Experimental Animals

40 rats were randomly assigned into sham group, sham/M group, CLP group and CLP/M group. In sham group, the cecum was turned over only

after laparotomy and then returned to abdominal cavity without cecal ligation and puncture (CLP). Metformin (100mg/kg) was given to sham/M group rats by gavage 1.5 hours before operation and other procedures were the same as sham group. CLP group rats were treated with CPL for constructing sepsis model. CLP/M group was treated with metformin (100mg/kg) 1.5 hours before operation and CLP.

Preparation of Sepsis Model in Rats

Sepsis model is caused by cecal ligation and puncture. Rats were anesthetized using 1.5% of isoflurane in air and abdomen was shaved. Then rats were subjected to a longitudinal median laparotomy. Ligation was performed with No.4 silk thread 1 cm away from blind end. Then, No.16 needle penetrated cecum twice at distal end of ligation site. After ligation and puncture, cecum was returned to abdominal cavity, which was then sutured. After operation, saline 2ml/100g was injected subcutaneously to supplement the fluid loss during operation.

Relevant measurements

The rats were sacrificed and blood samples and tissue were collected. ALT, AST and CRE values were measured using biochemical methods. Plasma levels of TNF-α, IL-1β and cTnI were measured by ELISA. Western blot detected Caspase-3 expression in cardiomyocytes. The apoptosis of cardiomyocytes was detected by TUNEL method.

Statistical analysis

SPSS21.0 software analyzed data which were shown as mean ± standard deviation and assessed by one-way ANOVA. $P < 0.05$ were considered significant.

Results

Effects of Metformin on Plasma ALT, AST and CRE values

The plasma levels of ALT, AST and CRE were increased after operation without differences between sham group and sham/M group. The values of ALT, AST and CRE in CLP group and CLP/M group were significantly higher than sham group after operation ($P<0.05$). However, CLP/M group had significantly lower values of ALT, AST and CRE than CLP group ($P < 0.05$). (Figure 1)

Effects of metformin on TNF-α, IL-1β and cTnI levels

There were no differences in TNF-α, IL-1β and cTnI levels between sham group and sham/M group at 12h and 24h after operation. However, their values in CLP group and CLP/M group were significantly increased ($P<0.05$). But they were significantly decreased in after metformin treatment ($P<0.01$). (Figure 2)

Metformin inhibits apoptosis of myocardial cells

TUNEL staining showed no difference in myocardial apoptosis index (AI) between sham group and sham/M group, but it was higher in CLP group and CLP/M group than sham group ($P <0.05$). AI of myocardial cells was decreased after metformin administration ($P < 0.05$). (Figure 3)

Metformin decreases caspase-3 expression

At 24 hours after operation, no significant differences were found in caspase-3 expression in cardiomyocytes between sham group and sham/M group. Compared with sham group, caspase-3 expression in CLP group and CLP/M group were significantly elevated ($P<0.05$). However, it was significantly reduced by metformin ($P<0.05$) (Figure 4).

Discussion

Metformin is a widely used first-line hypoglycemic drug, which can significantly relieve the complications of type 2 diabetes and effectively improve the prognosis of diabetic patients[5]. Recently, several studies suggest that metformin not only has a hypoglycemic effect but also has remarkable anti-inflammatory, antioxidant, anti-tumor, anti-aging and other pharmacological effects, which might have extensive clinical application potential [5].

Studies have shown that metformin can inhibit the activity of mitochondrial respiratory chain complex, reduce intracellular ATP production and increase the ratio of AMP/ATP and ADP/ATP[8], which may be the key cellular basis for metformin to exert metabolic regulatory effects on hypoglycemia[9]. The protective effect of metformin on acute myocardial infarction induced by isoproterenol was validated in animal experiments, which was mainly manifested as the reduction of myocardial fiber structure disorder, inhibition of inflammatory cell infiltration, and reduction of myocardial cell apoptosis and necrosis[10]. Therefore, metformin can play a protective role in diabetic cardiovascular complications, myocardial infarction and other pathological conditions. Our study found that cTnI concentration in plasma was increased significantly in rat sepsis model caused by CLP which can be inhibited by metformin. In addition, apoptosis indexes of myocardial cells

were significantly reduced with a short-term treatment of metformin in CLP/M group compared to CLP group. The results were consistent with the previous findings and indicated that metformin exerts protective effect on myocardial injury in sepsis.

Our results found that CLP induced myocardial injury was accompanied with the increased inflammatory cytokines such as TNF-α and IL-1β. Related studies also demonstrated that TNF-α and other inflammatory cytokines participate in sepsis-induced myocardial injury. TNF-α can directly inhibit myocardial contractility and lead to reversible ventricular enlargement[11]. This mechanism may be related to the activation of sphingomyelinase, proteolytic enzyme by TNF-α and inhibition of calcium influx. Our study found that plasma TNF-α level in CLP group was significantly increased. It further demonstrated that TNF-α involves in sepsis-induced myocardial injury. Researchers found that activation of AMPK can inhibit endotoxin-induced TNF-α production in macrophages, while interference with AMPK expression can promote endotoxin-induced TNF-α expression[12]. It was also found that metformin significantly inhibited the plasma transaminase, caspase activation and hepatocyte apoptosis, leading to a higher survival rate in mice with fulminant hepatitis induced by endotoxin[13]. These effects may be related to inhibition of the production of pro-inflammatory and pro-apoptotic cytokines TNF-α by metformin [13]. Labuzek found that metformin can prevent myocardial cell death by activating AMPK, thus providing myocardial protection in sepsis[14]. The mechanism of action includes vasodilation, inhibition of oxidative stress and apoptosis proteins. Caspase-3 participates in apoptosis cascade and involves in the regulation of cardiomyocyte apoptosis. According to Kewal Ramani's study, AMPK can inhibit caspase-3 activity, thus reducing cardiomyocytes apoptosis induced by TNF-α[15].

In this study, we found that apoptosis index of myocardial cells and caspase-3 expression in CLP group were significantly upregulated. However, apoptosis index of myocardial cells and caspase-3 expression could be significantly reduced after administration of metformin. These results indicated that as sepsis-induced cardiomyocyte apoptosis leads to myocardial injury, metformin could effectively reduce myocardial cell apoptosis and alleviate myocardial injury in sepsis. The possible mechanism is that the production of TNF-α was inhibited by activating AMPK, meanwhile caspase-3 was also inhibited in the apoptotic cascade. It can further reduce the apoptosis of myocardial cells and protect the myocardium. However, the exact mechanism by how metformin affects TNF-α secretion and caspase-3 expression was not assessed in our study which is the main study limitation. In the future study, we plan to investigate the molecular mechanism by how metformin regulates TNF-α secretion and caspas-3 activation in sepsis.

In conclusion, metformin can prevent myocardial injury in sepsis through inhibiting TNF-α production and the activity of caspase-3, indicating that metformin might be a novel agent for the treatment of myocardial injury in sepsis.

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Disclosure of conflict of interest

None.

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Figure legends

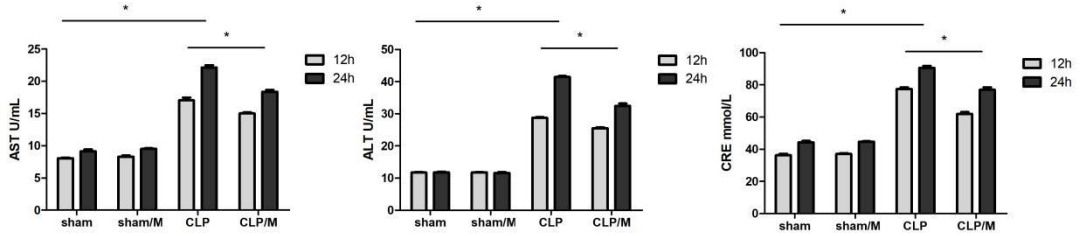

Figure 1. **Effect of metformin on plasma ALT, AST and CRE values**

The plasma values of ALT, AST and CRE were detected by biochemical methods. The results showed no significant changes between sham group and sham/M group at 12 hours and 24 hours after the operation. However, the values of ALT, AST and CRE in CLP group and CLP/M group were significantly increased compared to sham group at both 12 hours and 24 hours after the operation. Meanwhile, CLP/M group had significantly reduced values of ALT, AST and CRE than CLP group. (* P<0.05)

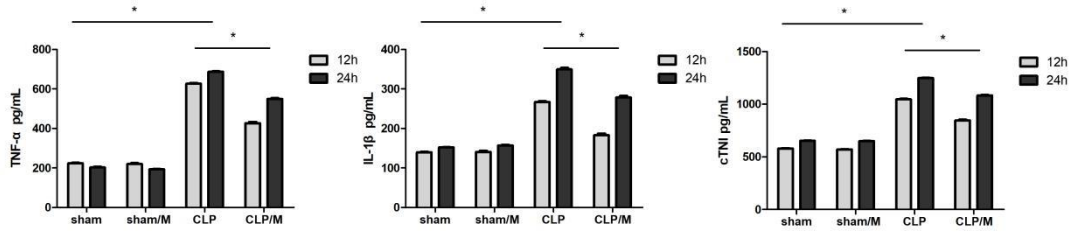

Figure 2. **Effect of metformin on plasma TNF-α, IL-1β and cTnI concentrations**

The concentrations of TNF-α, IL-1β and cTnI in plasma were measured by ELISA. There were no significant changes between sham group and sham/M group at 12 hours and 24 hours after the operation. But, the values of TNF-α, IL-1β and cTnI in CLP group and CLP/M group were significantly higher than that in sham group at both 12 hours and 24 hours after the operation. Meanwhile, CLP/M group had significantly decreased values of TNF-α, IL-1β and cTnI compared to CLP group. (* P<0.05)

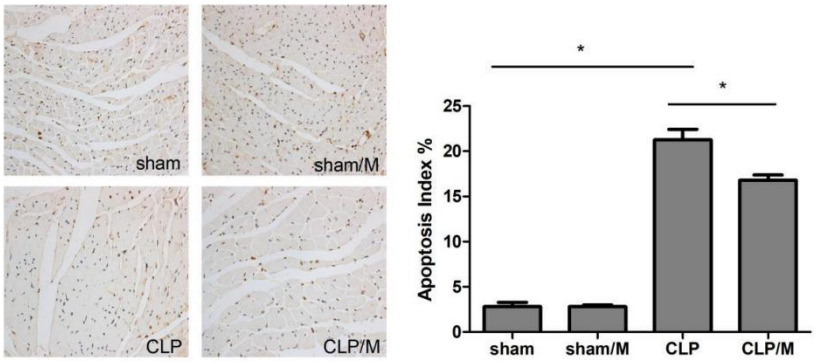

Figure 3. **Effect of metformin on apoptosis of myocardial Cells**

Cardiomyocyte apoptosis was detected by TUNEL staining. The apoptosis indexes of myocardial cells in CLP group and CLP/M group was significantly increased compared to sham group. However, the AI of myocardial cells in CLP/M group was significantly reduced than that in CLP group. (* P<0.05)

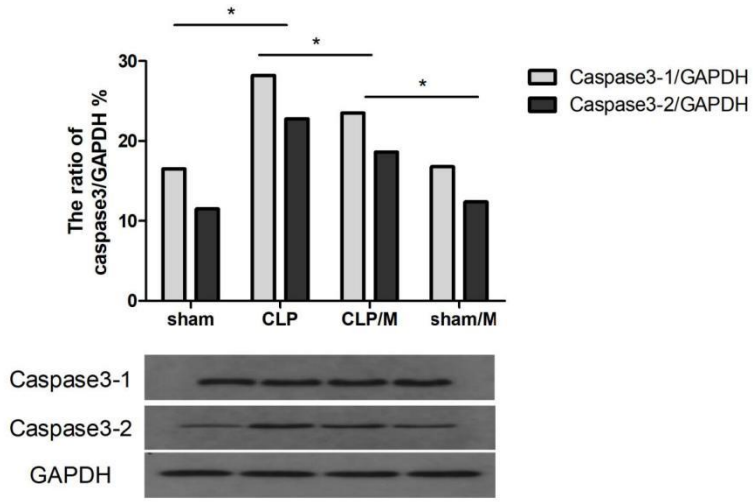

Figure 4. **Effect of metformin on expression of Caspase-3 Protein**

Western blot was used to measure caspase-3 expression in cardiomyocytes. There was no significant differences between sham group and sham/M group. However, caspase-3 expression in CLP group and CLP/M group were significantly increased than that in sham group. And the protein expression level of caspase-3 in CLP/M group was significantly decreased compared to CLP group. (* P<0.05).