Protective effects of rutin on lipopolysaccharide-induced heart injury in mice

Liu Xianchu, Zheng Lan, Liu Ming and Mo Yanzhi

Key Laboratory of Physical Fitness and Exercise Rehabilitation of Hunan Province, Hunan Normal University, Changsha, China

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ABSTRACT — Rutin has a wide range of beneficial health properties in the amelioration of multi-organ injury owing to its various biological effects. The aim of this study was to investigate the effects of rutin on lipopolysaccharide (LPS)-induced heart injury and clarify its potential cardioprotective mechanism. The mouse model of heart injury was intraperitoneal infection with LPS, and rutin was orally administered for 8 consecutive days. One day after LPS injection, heart histopathology, cardiac marker enzymes and cardiac fibrosis related genes were determined to evaluate the cardioprotective effects of rutin. In addition, oxidative parameters and inflammatory cytokines were tested to explore its possible underlying mechanism. The presented results showed that rutin significantly improved morphological changes of myocardium and relieved cardiac marker enzymes (creatine kinase (CK) and lactate dehydrogenase (LDH)) level to protect heart in LPS-induced sepsis. And more, rutin observably mitigated fibrosis related genes (matrix metalloproteinase 2 (MMP-2) and matrix metalloproteinase 9 (MMP-9)) expression in the heart to prevent against LPS-induced cardiac fibrosis. In addition, rutin markedly increased antioxidant enzymes (superoxide dismutase (SOD) and catalase (CAT)) activity, and improved oxidative production (malondialdehyde (MDA) and H2O2) level to balance the oxidation and anti-oxidation systems in the heart. Lastly, rutin dramatically ameliorated (tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6)) activity to restrain inflammatory responses in the heart. In conclusion, rutin possessed anti-oxidant and anti-inflammatory properties to improve LPS-induced heart injury, which suggested rutin could be used as a potential cardioprotective medicine in sepsis.

Key words: Rutin, Lipopolysaccharide, Heart, Anti-oxidant, Anti-inflammatory

INTRODUCTION

Sepsis is one of major pathological features in ICU patients, and can cause failure of multiple organs and even death (Antonucci et al., 2014; Polat et al., 2017; Dellinger, 2013). The heart is one of the sensitive organs in sepsis (Rudiger and Singer, 2013; Zhang and Chen, 2016), which is characterized by ventricular dilation, reduced ventricular ejection fraction, and abnormal cardiac diastolic and contractility (Fernandes and de Assuncao, 2012; Niederbichler et al., 2006; Vieillard-Baron et al., 2008). Furthermore, heart disease is the main cause of a high mortality rate in sepsis (Court et al., 2002; Parrillo, 1989). However, many critically ill patients frequently died interrelated with heart injury because of the lack of effective drug treatments for sepsis (Eichacker et al., 2002; Vincent et al., 2002). Therefore, it is necessary to clarify the relationship between sepsis and heart injury, and further find an effective therapeutic approach to ameliorate sepsis-associated heart injury in basic and clinical research.

Lipopolysaccharide (LPS) form gram-negative bacteria endotoxin is widely used for the induction of sepsis (Lee et al., 2012b). In sepsis-induced heart injury, excessive oxidative stress may be closely related to imbalance between the oxidation and anti-oxidation systems, which is attributed to reduced antioxidant enzymes and increased oxidative indicators (Sebai et al., 2011; Li et al., 2016). What is more, LPS can trigger inflammatory response by stimulating overproduction of TNF-α, IL-1β and IL-6 (Zanotti-Cavazzoni and Hollenberg, 2009). Therefore, drugs which possess the function of inhibiting oxidative stress and inflammatory response may bring beneficial health effects against LPS-induced heart injury.
Rutin, a natural flavonol glycoside, extensively exists in flowers and fruits. Rutin is extensively reported to have a wide range of beneficial health properties in multiple organs owing to its various pharmacological effects, including antioxidant and anti-inflammatory (Habtemariam, 2016). Previous research has showed that treatment with rutin possesses the cardioprotective function of antioxidant effect and alteration of TNF-α level in diabetic rats (Saklani et al., 2016). In LPS-induced sepsis, treatment with rutin can improve acute lung injury via inhibition of oxidative stress and inflammatory response, which is characterized by increasing the activity of SOD and CAT, and inhibiting the level of TNF-α and IL-1β (Yeh et al., 2014; Chen et al., 2014; Feng et al., 2014). Thus, we assumed that rutin had a cardioprotective function against sepsis because of its antioxidant and anti-inflammatory properties.

**MATERIALS AND METHODS**

**Animals**

Male BALB/c mice (weighing 23 g ± 2 g, 8-10 weeks old) from Hunan SJA Laboratory Animal Co., Ltd. (Changsha, China) were kept in a specific pathogen free laboratory at the Animal Center of Hunan Normal University. All animals were raised with standard water and food. All animal experiments were authorized by the Animal Care Committee of Hunan Normal University in this study.

**Experimental protocols**

In the present study, lipopolysaccharide (LPS) was purchased from Sigma (Shanghai, China), and intraperitoneally injected at a dose of 10 mg/kg on day 8 to establish the mouse model of heart injury. Rutin (purity: > 95%) was purchased from Shanghai Sangon Biotech Engineering Co., Ltd. (Shanghai, China) and orally treated at a dose of 100 mg/kg per day for eight consecutive days (Fig. 1). Mice were randomly divided into three groups: (1) Control group, which was given 0.1 mL physiological saline, (2) LPS group, which was intraperitoneally challenged with LPS, (3) LPS+Rutin group, which was treated with rutin and LPS. One day after LPS challenge, mice were killed by cervical dislocation. Blood samples and heart tissues were harvested for other studies.

**Heart Histopathological evaluations**

One day after intraperitoneal infection with LPS, heart tissues were collected and fixed with 4% paraformaldehyde. The heart tissues were embedded in paraffin and cut into slices. Then, the sections were stained with hematoxylin & eosin (HE) to evaluate morphological change, which was observed by light microscope (200 X).

**Cardiac marker enzyme determinations**

Blood was collected for the serum biochemical measurements at 1 day after LPS challenge. The cardiac marker enzymes [Catalog number: A032 for creatine kinase (CK), A020-1 for lactate dehydrogenase (LDH), Nanjing Jiancheng Biotechnology Institute, Nanjing, China], which absorb light at 440 nm and 412 nm respectively as directed by the manufacturer’s instructions, were determined to assess heart injury. The activity of CK and LDH was represented as units per liter.

**Oxidative stress measurements**

The supernatant from heart tissues were harvested for oxidative stress analysis as represented by antioxidant enzymes activity and oxidative indicators level. The release of oxidative stress parameters [Catalog number: A001-1 for superoxide dismutase (SOD), A007-1 for catalase (CAT), A003-1 for malondialdehyde (MDA), A064 for H2O2, Nanjing Jiancheng Biotechnology Institute] was measured with the corresponding detection kit as directed.

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![Fig. 1.](image-url) The research programme in the present study.
by the manufacturer’s instructions.

**ELISA analyses**

The level of cardiac fibrosis related genes and inflammatory cytokines (Catalog number: EK0460 for matrix metalloproteinase 2 (MMP-2), EK0466 for matrix metalloproteinase 9 (MMP-9), EK0527 for tumor necrosis factor α (TNF-α), EK0411 for interleukin 6 (IL-6), BOSTER Biological Technology, Wuhan, China) in the heart tissues was analyzed by ELISA. The absorbance value was measured at 450 nm to analyze cardiac fibrosis and inflammatory responses according to manufacturer’s instructions.

**RNA extraction and RT-PCR analysis**

At 1 day after LPS challenge, the heart tissues were collected and stored at -80°C for RNA research. RNA from heart tissues was extracted by using the trizol method, and reversed into cDNA. RT-PCR was used to determine the mRNA expression of cardiac fibrosis related genes (MMP-2 and MMP-9) and inflammatory cytokines (TNF-α and IL-6). Primer sequences for RT-PCR were synthesized by TSINGKE Biological Technology Co., Ltd., (Beijing, China) and Biosune Biological Technology Co. Ltd. (Shanghai, China) (Table 1). The results were expressed as a ratio by normalizing to GAPDH mRNA levels.

**Statistics**

All results are shown as mean ± S.D. All statistical data were analyzed with SPSS 16.0 software. p < 0.05 was considered statistically significant.

**RESULTS**

**Rutin alleviated LPS-induced heart injury in mice**

To investigate the effect of rutin on LPS-induced heart injury, hematoxylin and eosin-stained sections were used to measure heart histopathology. In comparison with the control group, cardiac lesions characterized by prominent cardiac inflammation and inflammation area were apparently observed after LPS challenge, while pre-treatment with rutin statistically reduced morphological changes of myocardium (Fig. 2A). Cardiac marker enzymes are widely used to detect heart diseases in the clinic. Throughout our experiment, the release of serum CK and LDH was elevated in LPS-induced heart injury. In contrast, pre-treatment with rutin significantly inhibited CK and LDH levels in serum (Fig. 2B and C). These results indicated that rutin was involved in protective properties in LPS-induced heart injury.

**Effects of rutin on cardiac fibrosis in the heart of LPS-treated mice**

To evaluate the effect of rutin on LPS-induced cardiac fibrosis, the fibrosis genes, including MMP-2 and MMP-9, in the heart were detected in this study. The expression and release of MMP-2 and MMP-9 were markedly increased in the heart of LPS-induced mice when compared with those of the control group, while pre-treatment with rutin dramatically alleviated LPS-induced increase of MMP-2 and MMP-9 levels in the heart (Fig. 3). These results suggested that rutin significantly prevented cardiac fibrosis in LPS-treated mice.

**Effects of rutin on oxidative stress in the heart of LPS-treated mice**

To examine the effect of rutin pre-treatment on oxidative stress in LPS-treated mice, we evaluated the antioxidant enzyme activity and oxidative indicator levels in the heart. The antioxidant enzyme activity of SOD and CAT was used to evaluate anti-oxidative functions. Compared with the control group, the activity of SOD and CAT in the heart was significantly reduced in the heart of LPS-treated mice. In contrast, pre-treatment with rutin had significantly enhanced SOD and CAT activity (Fig. 4A and B), which indicated that rutin had cardioprotective properties by promoting antioxidant ability in sepsis.

MDA and H₂O₂ were used as indicators to assess oxidative effects. In this study, pre-treatment with rutin observably ameliorated LPS-induced alteration of MDA and H₂O₂ levels (Fig. 5A and B). These results suggested that pre-treatment with rutin had the ability of resisting oxidative effect in heart tissues of LPS-treated mice.

**Effects of rutin on inflammation response in the heart of LPS-treated mice**

To uncover the effects of rutin on inflammation responses in the heart of LPS-treated mice, we analyzed the levels of proinflammatory cytokines, including

**Table 1.** Primer sequences used in RT-PCR.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequences (5’-3’)</th>
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<tbody>
<tr>
<td>GAPDH</td>
<td>Forward: AACTTTGGCAATTGGAAGG</td>
</tr>
<tr>
<td></td>
<td>Reverse: ACACATTGGGGGTAGGAACA</td>
</tr>
<tr>
<td>MMP-2</td>
<td>Forward: GTGCCCTTAAAACAGACAA</td>
</tr>
<tr>
<td></td>
<td>Reverse: GGTCTCGATGGGTGTCTTGGT</td>
</tr>
<tr>
<td>MMP-9</td>
<td>Forward: CGTCGATCCCCACATTACT</td>
</tr>
<tr>
<td></td>
<td>Reverse: AACACAGGGTTTGCCTTC</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Forward: ACCCTACACTCACAAACCA</td>
</tr>
<tr>
<td></td>
<td>Reverse: GGCAGAGAGGGGTTGACTT</td>
</tr>
<tr>
<td>IL-6</td>
<td>Forward: CCACCAAGAAGGATAGTCAA</td>
</tr>
<tr>
<td></td>
<td>Reverse: TTTCCACGATTCCAGA</td>
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TNF-α and IL-6. It was noted that the TNF-α and IL-6 was remarkably increased in the heart of LPS-treated mice. In contrast, pre-treatment with rutin differentially suppressed the expression and release of TNF-α and IL-6 (Fig. 6). These results indicated that the protective effects of rutin on LPS-induced heart injury were attributable to its inhibition of inflammatory response.

DISCUSSION

In this study, the mouse model of LPS-induced sepsis was established to evaluate the protective functions of rutin in heart injury. Our results are as follows. Firstly, pretreatment with rutin markedly improved morphological changes of myocardium and ameliorated CK and LDH activity in serum induced by LPS challenge, which suggested that rutin had the protective functions in heart injury. Secondly, pretreatment with rutin significantly alleviated the LPS-induced increase of MMP-2 and MMP-9 expression in the heart to inhibit fibrosis. Thirdly, pretreatment with rutin enhanced the level of antioxidant enzymes of SOD and CAT, and suppressed the release of oxidative productions of MDA and H₂O₂ against LPS-induced oxidative stress to balance the anti-oxidation and oxidation systems in the heart. Fourth, the overexpression of TNF-α and IL-6 in the heart induced by LPS challenge was noticeably alleviated by rutin pretreatment, which suggested that rutin has the protective functions by inhibiting inflammation responses in the heart.

Rutin, also named rutoside or quercetin-3-O-rutinoside, is a polyphenol flavonoid with various pharmacological effects, such as anti-inflammatory and anti-oxidant. Increasing studies have showed that rutin plays a pivotal role in the amelioration of multi-organ injury in various experimental conditions. Rutin can also alleviate serum activity of CK and LDH to reduce cardiac risk in animals fed hypercaloric diet and receiving ethanol (Chuffa et al., 2014). In LPS-induced acute lung injury, rutin pro-
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**Fig. 3.** Effects of rutin on cardiac fibrosis in the heart of LPS-treated mice. (A, B) Expression of fibrosis related genes MMP-2 and MMP-9 was measured by RT-PCR. The level of MMP-2 (C) and MMP-9 (D) were measured by ELISA. ##p < 0.01: LPS group compared with CON group; *p < 0.05, **p < 0.01: LPS+Rutin group compared with LPS group.

**Fig. 4.** Effects of rutin on antioxidant enzymes in the heart of LPS-treated mice. SOD (A) and CAT (B) activity was measured to evaluate anti-oxidative functions in the heart. ##p < 0.01: LPS group compared with CON group; *p < 0.05: LPS+Rutin group compared with LPS group.
Fig. 5. Effects of rutin on oxidative indicators in the heart of LPS-treated mice. MDA (A) and $H_2O_2$ (B) level was measured to assess oxidative effects in the heart. *$p < 0.01$, *$p < 0.01$: LPS group compared with CON group; **$p < 0.01$, *$p < 0.05$: LPS+Rutin group compared with LPS group.

Fig. 6. Effects of rutin on inflammatory cytokines in the heart of LPS-treated mice. (A, B) Expression of TNF-$\alpha$ and IL-6 was measured by RT-PCR. The level of TNF-$\alpha$ (C) and IL-6 (D) were measured by ELISA. *$p < 0.01$, *$p < 0.05$: LPS group compared with CON group; **$p < 0.01$, *$p < 0.05$: LPS+Rutin group compared with LPS group.
vides potential protective effects by partially inhibiting LPS-induced expression of MMP-9 (Chen et al., 2014). In streptozotocin (STZ)-induced diabetic rats, rutin attenuates cardiomyopathy by regulating oxidative stress and inflammatory response (Wang et al., 2015). Therefore, rutin was hypothesized to possess potential protective effects in LPS-induced sepsis. In this study, our results showed that rutin could improve heart injury, as well as represented by reducing level of cardiac marker enzymes and cardiac fibrosis relevant genes, due to enhancing the activity of antioxidant enzymes and improving the elevation of oxidative stress markers and inflammatory factors in the heart of the sepsis mice.

Sepsis is involved in the development of heart injury, which leads to a high mortality rate in intensive care units. Patients with heart injury have increased levels of cardiac marker enzymes in serum, which are often measured as indicators of heart disease in the clinic. CK and LDH are cardiac marker enzymes, which play a pivotal role in energy metabolism and membrane permeability in the heart (He et al., 2015). Previous studies have indicated that LPS can induce heart injury, which is characterized by the elevation of CK and LDH in serum. What is more, rutin has been reported to have a cardioprotective effect in animals fed hypercaloric diet and receiving ethanol by restoring CK and LDH activity (Chuffa et al., 2014). However, there is no report on the cardioprotective effect of rutin in LPS-sepsis. In this study, our results showed the elevation of CK and LDH in serum after administrating LPS to mice, while pretreatment with rutin can observably reverse the excessive release of CK and LDH to protect the heart tissues in sepsis.

The matrix metalloproteinase gene family, such as MMP-2 and MMP-9, is involved in myocardial remodeling (DeLeon-Pennell et al., 2017). Therefore, cardiac fibrosis can result in the elevation of MMP-2 and MMP-9 in the heart (Lew et al., 2013). Rutin displays its protective effects in mice by down-regulation of MMP-9 activation on LPS-induced acute lung injury (Chen et al., 2014). In agreement with previous studies, LPS increased the expression of MMP-2 and MMP-9 in the heart, while pretreatment with rutin could markedly improve the elevation of MMP-2 and MMP-9 to restraint cardiac fibrosis in sepsis.

Oxidative stress is involved in various heart diseases, and occurs as a result of imbalance between the oxidation and anti-oxidation systems. Oxidative indicators are used to assess the effect of oxidative stimulation, while antioxidant enzymes are an important contributor to the antioxidative defense system (Elias et al., 2008; Vassort and Turan, 2010; Niemann et al., 2017). SOD and CAT are major antioxidant enzymes, which are involved in protective effects in organ injury. In ischemia/reperfusion-associated hemodynamic alteration, rutin improves left ventricular end diastolic pressure by enhancing the antioxidant enzyme SOD activity (Bhandary et al., 2012). In addition, rutin has protective effects in amylin-induced neurocytotoxicity by increasing the activity of CAT, which suggests that rutin can be used as a feasible agent to protect the aging brain (Yu et al., 2015). What is more, previous study has shown that the activity of anti-oxidant enzymes, as represented by SOD and CAT, is reduced on LPS-induced heart injury (Sebai et al., 2011; Li et al., 2016). In our experimental model, our results were consistent with a previous report showing that rutin had antioxidant effects by increasing SOD and CAT activity, which suggested that rutin protected the heart from oxidative stimulation on LPS-induced sepsis.

In LPS-induced sepsis, treatment with rutin can improve acute lung injury via inhibition of oxidative stress and inflammatory response, which is characterized by increasing the activity of SOD and CAT, and inhibiting the level of TNF-α and IL-1β (Yeh et al., 2014; Chen et al., 2014; Feng et al., 2014). MDA and H2O2 have been recognized as an important target in the oxidative process, and previous studies have shown that the elevation of oxidative stress indicators is related to heart injury. In LPS-induced sepsis, pretreatment with butyrate improves heart depression through ameliorating MDA content (Wang et al., 2017). Meanwhile, administration of alpha-lipoic acid relieves LPS-induced increase of H2O2 content in the heart (Goraca et al., 2009). What is more, rutin can also decrease oxidative stress indicators during the pathogenesis of heart injury. In diabetic rats, treatment with rutin mitigates the MDA level in the heart tissue to improve myocardial dysfunction (Wang et al., 2015). In this study, our results showed that rutin was involved in inhibiting the overproduction of MDA and H2O2 in the heart, which suggested that its protective effects were also associated with anti-oxidation actions.

Excessive inflammation plays a pivotal role in the pathogenesis of organ injury, especially in the heart. LPS is widely used to induce inflammation response, as evaluated by increase of pro-inflammatory cytokines (Lee et al., 2012a). What is more, MMPs, which is increased upon exposure to LPS, can provoke inflammatory signaling in remodeling process (DeLeon-Pennell et al., 2017). TNF-α is one of pro-inflammatory cytokines which is closely associated with inflammation response (Beutler, 1995). In addition, the increased level of TNF-α is one of cardiac responses in LPS-sepsis (Balija and Lowry,
Previous study revealed that LPS could increase the expression of IL-6 in the heart, which is involved in myocardial fibrosis via overproduction of collagen (Lee et al., 2012b; Meléndez et al., 2010). On LPS-induced lung injury, pre-treatment with rutin has potential protective effects by inhibition of iNOS and VCAM-1 (Huang et al., 2016). Meanwhile, rutin also attenuates TNF-α activity to protect heart in STZ-induced diabetic rats (Saklani et al., 2016). In this study, the level of TNF-α and IL-6 was increased in the heart after LPS treatment, as previously described. Rutin was shown to have an inhibitory effect on the activity of TNF-α and IL-6 against inflammatory response in LPS-induced heart injury.

In conclusion, this is the first evidence that rutin attenuates LPS-induced heart injury with its anti-oxidative and anti-inflammatory effects. According to our results, rutin may be a promising therapeutic agent to improve heart injury in sepsis.

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Conflict of interest——The authors declare that there is no conflict of interest.

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