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Original Article

Protective effects of Lycium barbarum polysaccharide (LBP) on rats with renal ischemia-reperfusion injury (IRI)

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Abstract: Renal ischemia-reperfusion injury (RIRI) is a common issue in clinics. Previous studies showed multiple pharmaceutical roles of Lycium barbarum polysaccharides (LBP); however very few studies focused on its preventive function on RIRI. This study thus investigated the effect of LBP on renal functions in rat RIRI models and the protective mechanisms. SD rats were randomly assigned into three groups: sham group, ischemia-reperfusion (IR) group and IR + LBP group. Serum urea and blood creatine levels were analyzed, along with superoxide dismutase (SOD) and malondialdehyde (MDA) quantification. Real-time PCR and Western blot were employed to determine mRNA and protein expression of rat Bcl-2 and Bax. Enzyme linked immunosorbent assay (ELISA) was used to measure the expression of tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) in rat serum. IR rats had significantly elevated serum urea and creatine levels, along with decreased SOD or MDA contents. IR rats also showed decreased mRNA and protein expression of Bcl-2 yet increased Bax expression, plus enhanced secretion of TNF-α and IL-1β ($P < 0.05$ compared to sham group). LBP treatment decreased serum urea or creatine levels in IR rat, enhanced SOD activity, decreased MDA content, increased mRNA or protein level of Bcl-2 but decreased Bax expression, and suppressed the secretion of TNF-α and IL-1β ($P < 0.05$ compared to IR group). In summary, LBP can exert protective effects on RIRI via improving oxidative stress (OS), suppressing apoptosis, and decreasing secretion and inflammatory cytokines.

Keywords: Renal ischemia reperfusion injury, Lycium barbarum polysaccharides, oxidative stress, apoptosis, inflammatory cytokine

Introduction

Ischemia reperfusion injury (IRI) refers to the tissue damage caused by ischemia followed by blood reperfusion, leading to the production of large amounts of free radicals [1]. Kidney is one prominent organ with hyper-perfusion and is thus sensitive to hypoxia and ischemia, leading to high incidence of renal ischemia reperfusion ischemia (RIRI), which can occur during traumatic shock, surgical operation and organ transplantation [2, 3]. During RIRI progression, it is commonly observed in clinics that aggressiveness damage of renal structure and function can cause multi-organ failure, acute vascular dysfunction, and in some cases, death [4, 5]. RIRI significantly increases serum creatine level, indicating severe renal dysfunction. Pathological examination reveals prominent swelling of mitochondria, deformation, decreased ridges with disrupted regulation, or even mitochondrial collapse and vacuole formation, all of which mostly attack renal tubules and may underlie acute renal failure [6, 7]. Therefore, RIRI has become the critical factor that potentially could cause acute renal failure, and further impact on acute and chronic rejection phase of kidney transplantation and the long-term survival of the disease [8, 9]. Consequentially, the alleviation of RIRI-induced renal damage and facilitation of kidney function recovery are both crucial in clinical treatment [10]. As enhanced oxygen free radical products by IR can eventually lead to cell apoptosis and necrosis, increased oxidative stress (OS) and weakened anti-oxidation balance are important regulatory mechanism of RIRI [11, 12].

Lycium barbarum polysaccharides (LBP) is a major active ingredient in wolfberry and is one group of polysaccharides composed of six monosaccharides including glucose and galactose [13]. Previous studies have confirmed that LBP could protect neurons post hypoxic damage. More studies have demonstrated multiple pharmaceutical roles of LBP [14, 15]. However, whether it can prevent or treat RIRI has not been clearly illustrated yet. This study thus aimed to investigate the effect of LBP on renal functions in rat model of RIRI and underlying protective mechanisms.

Materials and methods

Experimental animals

A total of 45 healthy Wistar rats (2 months old, SPF grade, body weight 250 ± 20 g) were purchased from laboratory animal center of Hebei Medical University and were kept in an SPF grade animal facility. All animal experiments followed approved protocol and were performed by experienced technicians to minimize animal pain. The ethical committee of Affiliated Hospital of Hebei Engineering University has approved this study.

Major reagent and equipment

LBP was purchased from Kangpu Biotech (China). Rabbit anti-mouse Bcl-2 and Bax monoclonal antibody and goat anti-rabbit horseradish peroxidase (HRP) conjugated IgG secondary antibody were purchased from CST (US). ELISA kit for TNF-α and IL-1β was purchased from R&D (US). RNA extraction kit and reverse transcription kit were purchased from Axygen (US). SOD assay kit and MDA quantification kit were purchased from Jiancheng Bio (China). Other common reagents were purchased from Sangon (China). Model 7700 Fast fluorescent quantitative PCR cycler was purchased from ABI (US). Labsystem Version 1.3.1 microplate reader was purchased from Bio-rad (US). Surgical instrument was purchased from Suzhou Medicial Instrument (China). Cold centrifuge was purchased from Sigma (Germany). JY92-IIDN ultrasonic rupture was purchased from Fanqi Instrument (China).

Grouping and processing of rats

Those Wistar rats purchased for the study were randomly assigned into three groups: sham group, i.e., received surgery to expose renal tissues without RIRI treatment; IR group, i.e., received bilateral ischemia treatment of renal tissues for 45 min followed by 24 h reperfusion; and IR + LBP group, i.e., received LBP (10 mg/kg via gavage for 7 days) on RIRI model [16].

Rat RIRI model preparation

Based on previous literatures [17], rats were anesthetized by 1% pentobarbital, and were fixed on the operation table with a supine position. A midline incision was made to open the abdominal cavity for renal tissues exposure. Bilateral renal arteries were freed on proximal end and were clapped by non-invasive arterial clap. After clapping, the change of renal artery was observed for the purpose of recording arterial pulsation and tissue color. The weakened pulsation of artery and whitening of renal tissues indicated successful generation of RI model. 45 min after arterial clapping and detection of RI, the renal tissue was returned to the abdominal cavity before suturing the incision. After 45 min maintenance of RI, the midline incision was re-opened to expose the renal cavity. The arterial clap was relieved for preparing reperfusion model. Post re-opening, the reddish color change of renal tissues from gray white indicated successful reperfusion.

Sample collection and processing

A cardiac puncture was performed to collect blood samples using a negative pressurized tube. Blood samples were centrifuged at 3600 rpm for 10 min at 4°C. The supernatant was saved at -80°C storage. Renal tissues were repeatedly rinsed by sterilized saline to remove residual blood and were kept in liquid nitrogen.

Kidney function analysis

Rat serum urea and blood creatine levels were quantified as previously described [17].

Real-time PCR for renal expression of Bcl-2 and Bax mRNA

Under sterile conditions, RNA was extracted from renal tissues using Trizol reagent. Primers were designed and synthesized by Gimma Gene (China). cDNA was synthesized based on relevant primers (**Table 1**). Quantitative analysis was performed by $2^{-\Delta Ct}$ approach.

Western blot for Bcl-2 and Bax protein expression in renal kidney tissues

Total proteins were extracted from renal tissues on ice. Primary antibody (1:1000 diluted

Table 1. Primer synthesis sequence

Gene	Forward primer 5'-3'	Reverse primer 5'-3'
GADPH	AGTGCCAGCCTCGTCTCATAG	CGTTGAACTTGCCGTGGGTAG
Bcl-2	ACCATCAGCCTCAACAC	TCTCCCCACTTCCGTGGG
Bax	AGGCTACCATAACACTGATGG	GGTTCCCCTCACATGCGTA

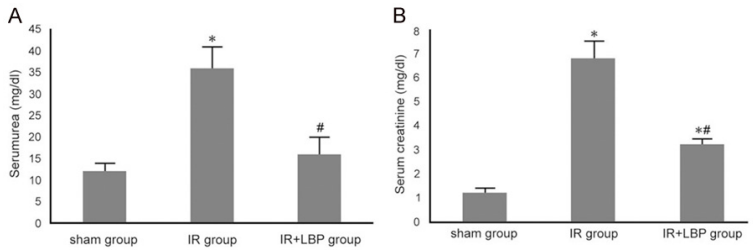

Figure 1. Protective effects of LBP on RIRI rats. A. Serum urea level; B. Serum creatine level. *, P < 0.05 compared to sham group; #, P < 0.05 compared to IR group.

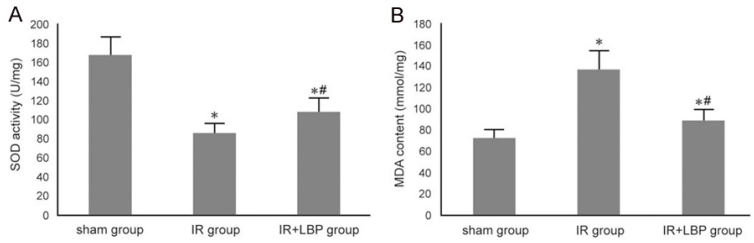

Figure 2. Effects of LBP on oxidative stress indexes of RIRI rats. A. SOD activity; B. MDA content. *, P < 0.05 compared to sham group; #, P < 0.05 compared to IR group.

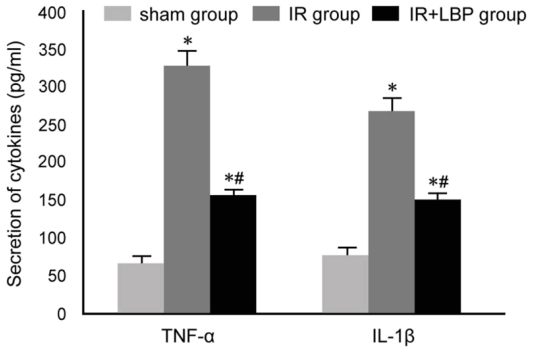

Figure 3. Effects of LBP on serum inflammatory cytokines in RIRI rats. *, P < 0.05 compared to sham group; #, P < 0.05 compared to IR group.

Bcl-2 and 1:1500 diluted Bax) was added for incubation, followed by incubation with 1:2000 goat anti-rabbit secondary antibody. Images were analyzed for band density.

ELISA for rat serum inflammatory factor expression

All samples were measured for serum levels of inflammatory factor TNF-α and IL-1β, using ELISA kit as per the manual instruction of test kit.

Assay for oxidative stress index

According to the instruction of test kit, SOD content assay kit, GSH quantification kit and MDA assay kit were used for measuring SOD activity, GSH content and MDA concentration, respectively.

Statistical processing

SPSS 16.0 statistical software was used for data analysis. Measurement data were presented as mean ± standard deviation (SD). The comparison of means among multiple groups was employed using one-way analysis of variance (ANOVA). A statistical significance was defined when $P < 0.05$.

Results

Protective effects of LBP on RI rats

The study firstly focused on analyzing the protective effect of LBP on RIRI rats. Results showed that RI rat model had significantly enhanced serum urea and creatine levels (P < 0.05 compared to sham rats). LBP treatment significantly decreased serum urea or creatine levels in RI rats (P < 0.05 compared to model group, **Figure 1**).

Effects of LBP on oxidative stress indexes of RIRI rats

We further analyzed the effect of LBP on oxidative stress indexes of RIRI rats. Results showed that I/R rats had decreased SOD activity and enhanced MDA content (P < 0.05 compared to sham group). LBP treatment effectively increased SOD activity and decreased MDA con-

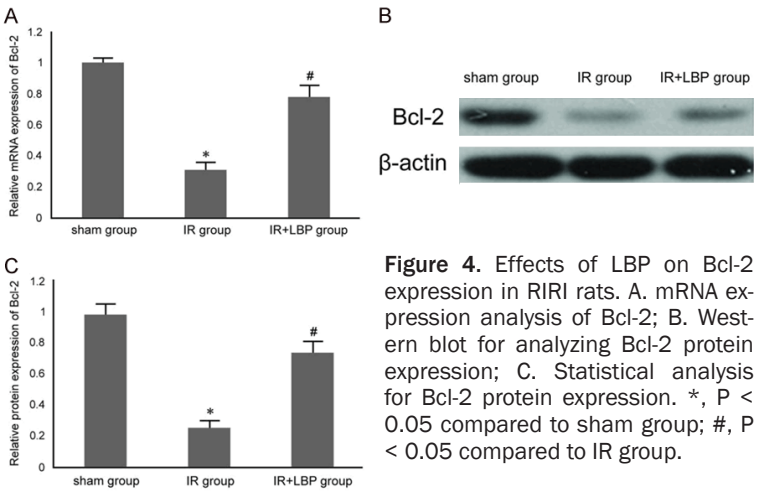

Figure 4. Effects of LBP on Bcl-2 expression in RIRI rats. A. mRNA expression analysis of Bcl-2; B. Western blot for analyzing Bcl-2 protein expression; C. Statistical analysis for Bcl-2 protein expression. *, P < 0.05 compared to sham group; #, P < 0.05 compared to IR group.

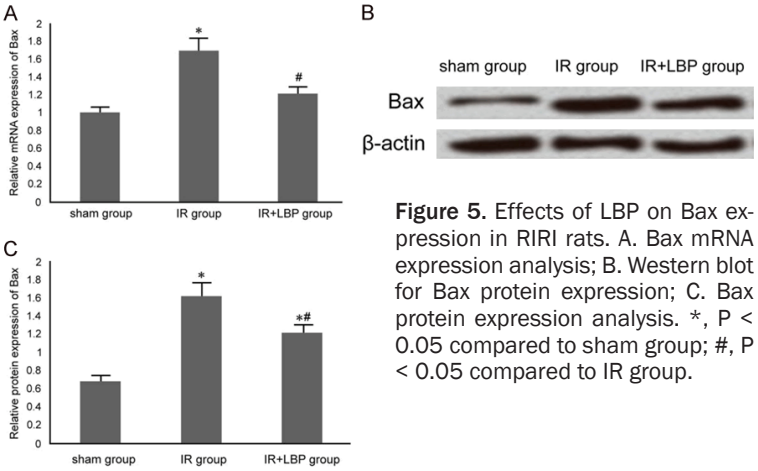

Figure 5. Effects of LBP on Bax expression in RIRI rats. A. Bax mRNA expression analysis; B. Western blot for Bax protein expression; C. Bax protein expression analysis. *, P < 0.05 compared to sham group; #, P < 0.05 compared to IR group.

tent in RIRI rats (P < 0.05 compared to IR group, **Figure 2**).

Effects of LBP on serum inflammatory cytokines in RIRI rats

ELISA was employed to analyze the effect of LBP on serum inflammatory factors of RIRI rats. Results showed that I/R rats had enhanced secretion of serum inflammatory cytokines TNF-α and IL-1β (P < 0.05 compared to sham group). LBP treatment remarkably inhibited secretion of serum inflammatory cytokines TNF-α and IL-1β (P < 0.05 compared to IR group, **Figure 3**).

Effects of LBP on Bcl-2 or RIRI rats

Real-time PCR and Western blot were used to analyze the effect of LBP on mRNA and protein expression of Bcl-2 in RIRI rats. Results showed that I/R model rats had significantly decreased mRNA or protein levels of Bcl-2 in renal tissues (P < 0.05 compared to sham group). LBP treatment significantly enhanced renal expression of Bcl-2 mRNA or protein expression (P < 0.05 compared to IR group, **Figure 4**).

Effects of LBP on Bax expression in RIRI rats

We used real-time PCR and Western blot to analyze the effect of LBP on Bax mRNA or protein expression in RIRI rats. Results showed that I/R rats had significantly enhanced Bax mRNA or protein expression (P < 0.05 compared to sham group). LBP treatment remarkably decreased mRNA or protein expression of Bax in renal tissues (P < 0.05 compared to IR group, **Figure 5**).

Discussion

Serum urea and creatine levels are important indexes reflecting kidney functions [18]. This study thus prepared rat RIRI model accordingly and measured serum urea and creatine levels. Results showed significantly elevated levels of urea and creatine in RIRI model. Natural extract compounds LBP has fruitful bioactivities yet minimal teratogenic or carcinogenic effects. LBP can also facilitate glucose oxidation in lymphocytes, accelerate tricarboxylic acid cycle and protect DNA damage, thus has been widely used in clinics [19]. LBP has been demonstrated to have satisfactory efficiency in treating tumor, diabetes, protecting neurons and preventing cell apoptosis [20, 21]. This study utilized LBP to study its effect on rat RIRI, and results showed that LBP significantly decreased serum urea and creatine levels, indicating protective role of LBP on RIRI rats.

Pathological process of RIRI involves multiple factors e.g. polymorphonuclear leukocyte, inflammatory cytokines, reactive oxygen species

(ROS), nitric oxide (NO), and complement activation. Nonetheless, imbalance of oxidation-reduction process at cellular level is the primary cause for RIRI pathology [22]. This study thus further investigated protective role and mechanism of LBP on RIRI rats. Results showed that LBP can exert protective roles via enhancing SOD activity, decreasing MDA content, and regulating oxidation-reduction balance. Enhanced SOD in endogenous anti-oxidation system and decreased MDA levels can benefit the clearance of ROS and help to maintain balance of body oxidation-reduction system [23]. The increase of free radicals such as ROS can lead to enhanced secretion of inflammatory cytokines, imbalance of cell apoptosis/anti-apoptosis, and potentiated cell apoptosis [24]. Bcl-2/Bax is the critical anti-apoptotic/apoptotic protein modulating cell apoptosis pathway [25]. This study demonstrated decreased Bcl-2 mRNA/protein expression in RIRI model rats, which also had increased Bax mRNA/protein expression, plus potentiated secretion of TNF-α and IL-1β. LBP treatment enhanced Bcl-2 mRNA and protein expression, decreased Bax mRNA or protein expression, and suppressed TNF-α and IL-1β secretion. These studies suggested that LBP can retard IR progression via modulating oxidative stress homeostasis during RIRI process, further leading to decreased TNF-α and IL-1β secretion, modulating anti-apoptosis/apoptosis balance. Further studies can be performed to investigate the protective effect of LBP on human RIRI, as well as related mechanisms, in order to provide relevant evidence for clinical application.

Conclusion

LBP can exert protective effects against RIRI in rats by improving oxidative stress, inhibiting apoptosis and decreasing secretion of inflammatory cytokines.

Disclosure of conflict of interest

None.

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