Life Sciences 157 (2016) 82–90

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Lycium barbarum polysaccharides ameliorates renal injury and inflammatory reaction in alloxan-induced diabetic nephropathy rabbits

Qihan Zhao, Jingjing Li, Jun Yan, Shuai Liu, Yulin Guo, Dajie Chen, Qiong Luo *

Department of Nutrition and Food Hygiene, School of Public Health, Wuhan University, Wuhan, 430071, Hubei, PR China

ARTICLE INFO

Article history:
Received 18 October 2015
Received in revised form 26 May 2016
Accepted 31 May 2016
Available online 1 June 2016

Keywords:
Lycium barbarum polysaccharides
Diabetic nephropathy
Renal function
Inflammatory reaction

ABSTRACT

Aims: This study was aimed to investigate the effect of *Lycium barbarum* polysaccharides (LBP) on renal function and inflammatory reaction in rabbits with diabetic nephropathy.
Main methods: Diabetes was induced by injecting alloxan (ALX). Japanese male white rabbits were randomly assigned into 5 groups: normal control group, diabetic nephropathy (DN) model group, LBP prevention group, positive control group and LBP treatment group. LBP (10 mg/kg) was given to the LBP prevention group after diabetes mellitus (DM) model succeeded for 12 weeks and to the LBP treatment group after DN model succeeded for 4 weeks. Telmisartan (3.7 mg/kg) was given to the positive group after DN model succeeded for 4 weeks, and the same volume of balanced saline was given to the normal group and DN group for 12 weeks. Urea nitrogen (BUN), creatinine (SCr), and C-reaction protein (CRP) in serum were detected at the end of the 12th week. The expression of MCP-1 mRNA and ICAM-1 mRNA extracted from cortex were detected by RT-PCR. Western blot analysis was carried out to examine NF-κB p65 protein expression.
Key findings: LBP improves the renal function and alleviates the inflammatory reaction in the kidneys of diabetic rabbits. In addition, the prevention effect of LBP is better than the treatment effect of LBP.
Significance: LBP has obvious protective effect on the diabetic nephropathy rabbits' renal function and postpones the appearance and development of DN. The mechanisms may be related to the reduction the expression of MCP-1mRNA and ICAM-1mRNA by restraining the expression of NF-κB and AngII.

1. Introduction

Diabetes mellitus (DM) is a serious and complex chronic condition, characterized by chronic hyperglycemia. According to a prediction by the International Diabetes Federation, the worldwide prevalence of diabetes is likely to reach 552 million by year 2030 [1]. Without intervention, approximately 80% of patients with type I diabetes would develop overt nephropathy in 10–15 years. Diabetic nephropathy (DN) is the most serious complication of diabetes and the main cause of end-stage renal disease, which leads to high morbidity and mortality rates in diabetic patients.

The incidence of DN is presently considered to be influenced by many aspects, including the hemodynamic disorders, genetic predisposition, and disorder of biochemical metabolism [2]. Inflammation and immunity theories have also attracted much attention in recent years. Some studies have shown that chronic inflammation plays critical roles in exacerbating the development of DN [3–5]. MCP-1 and ICAM-1 are the key inflammatory factors mediating kidney inflammation in DN. Some studies indicated that the expression of MCP-1 and ICAM-1 were significantly increased in DN rats, and kidney damage could be alleviated by the inhibition of the expression of MCP-1 and ICAM-1 [6–8].

Lycium barbarum, a solanaceous deciduous shrubbery, has been used as a traditional Chinese herbal medicine for thousands of years [9]. Its name was assigned by a Swedish botanist, Carolus Linnaeus in 1753 [10]. It was listed as Taxonomic Serial no. 503599 in the taxonomy and nomenclature (with taxonomic hierarchy) set by United States Department of Agriculture (USDA) [11]. The traditional medicinal part is the fruit, which has a history of use as an ingredient in various soft and alcoholic drinks for the benefits of anti-aging and protecting the kidney and liver [12–14]. The bioactive components of *Lycium barbarum* fruit have been mainly attributed to LBP. LBP consist of a complex mixture of highly branched and only partly characterized polysaccharides and proteoglycans. The glycosidic part accounts, in most cases, for about 90%–95% of the mass and consists of arabinose, glucose, galactose, mannose, rhamnose, xylose, and galacturonic acid [15]. The β-glycan structural aspect of a polysaccharide is related to the biological function [16].

Research indicated that LBP has a large variety of bioactivities, such as antioxidant, anti-aging, antitumor and immunomodulation. Our research since 1995 has found the biological activities and functions of crude or pure polysaccharides from *Lycium barbarum* fruits, including

* Corresponding author.
E-mail address: drluoqiong@sina.com (Q. Luo).

http://dx.doi.org/10.1016/j.lfs.2016.05.045
0024-3205/

hypoglycemic, hypolipidemic, and antifatigue effects, anti-oxidant and immune activity, protective effects against heat-induced damage of rat testes, H_2O_2-induced DNA damage in mouse testicular cells, beneficial effects on sexual behavior and reproductive function of hemicastrated rats, and the formation of extracellular matrix of human mesangial cells in high glucose [17–22]. Some recent studies have demonstrated that LBP had anti-inflammatory properties, namely, capable of inhibiting chronic inflammation-related injury [23–24]. Sun et al. reported that serum Advanced Glycation End Products (AGEs) and plasma Glycosylated Hemoglobin (HbAlc) levels and IL-8 secretion were reduced in diabetic rats after they had been given LBP and their kidney lesion was ameliorated [25]. Huang et al. also reported that LBP had obvious hypoglycemic and hypolipidemic effects and reduced serum levels of inflammatory factor [26]. Yang et al. confirmed that treatment of LBP significantly reduced expression of inflammatory genes, such as MCP-1, IL-6, and TNF-α [24]. Furthermore, a previous study revealed that LBP attenuated hepatic inflammation via a down-regulation of pro-inflammatory mediators and chemokines, partly through the down-regulation of NF-κB [27]. In addition, our previous study showed that LBP could reduce blood glucose and protect against high-glucose damages to the glomerular mesangial cells through reducing the expression of transforming growth factor-b (TGF-b) and fibronectin (FN) [22,28]. Therefore, the effect of LBP on the development of DN in alloxan (ALX) diabetes rabbits was explored in this study. Telmisartan was selected for positive drug in this experiment [29–30]. The diabetic rabbit model, induced by a single intravenous injection of ALX, is well documented to produce hyperglycemia and insulitis similar to human counterparts [31–33]. Urea nitrogen, creatinine, and urine protein are the most common indicators of kidney functions [34].

The objectives of this study included three aspects: First, to evaluate effects of LBP that delay the occurrence of proteinuria in diabetic nephropathy rabbits; Second, to assess protective effects of LBP against inflammation of kidney tissue reaction in diabetic nephropathy rabbits; and Third, to evaluate its efficacy to protect the renal function in diabetic nephropathy rabbits.

2. Materials and methods

2.1. Preparation of LBP

LBP was extracted as described in our previous study [20–21]. In brief, *Lycium barbarum* fruits, produced in Ningxia Autonomous Region of China, were dried at 60 °C and ground to a fine powder which were further double refluxed to remove lipids with chloroform: methanol solvent (2:1, in vol) first and then refluxed with 80% ethanol solvent at 80 °C to remove oligosaccharides. After filtering, the residues were extracted and concentrated by a rotary evaporator (R-210, Buchi, Switzerland) at 60 °C, and then precipitated with 95% ethanol, 100% ethanol and acetone, respectively. After filtering and centrifuging, the precipitate was collected and vacuum-dried. The precipitate was collected and vacuum-dried to obtain 11.3 g LBP with an extraction ratio of 4.52%.

2.2. Experimental animals and reagents

A total of 25 healthy Japanese male white rabbits, weighed approximately 2.4 ± 0.2 kg, were purchased from the Animal Center of Hubei Provincial CDC (Wuhan, China) (Certificate No.: 4200694919). They were caged individually and allowed free access to food and water. All animal experiments were performed in accordance with the guidelines for care and use of laboratory animals established by Wuhan University (Wuhan, China). Alloxan (ALX) was obtained from Sigma (St Louis, USA). Telmisartan tablets were purchased from Boehringer-Ingelheim Co. (Shanghai, China). Urea nitrogen (BUN), creatinine (Cr) and C-reaction protein (CRP) kits were purchased from Jiancheng Bioengineering Institute (Nanjing, China).

2.3. Modeling of DM and DN rabbits

Experimental diabetes of the rabbits was induced by a single intravenous injection of ALX (100 mg/kg body weight) freshly dissolved in 0.9% balanced saline after a 12-hour overnight fasting. In addition, the rabbits were treated with 10% glucose solution orally to combat the early phase of drug-induced hypoglycemia. Rabbits of the normal control group received equivalent volume of balanced saline intravenously. Induction of the diabetes was confirmed by measuring the blood glucose level on the 3rd day after ALX administration. The rabbits with fasting blood glucose higher than 16.7 mmol/L were classified as successful diabetes model. The diabetic rabbits were fed high fat (4% lard + 1% cholesterol + 95% standard diet) feed for 2 consecutive weeks to develop of DN after the DM model success. The fasting blood glucose concentration > 16.7 mmol/L and the appearance of proteinuria over two weeks were used as the DN subjects for further experiment at the end of the 8th week of this experiment.

2.4. Experimental design

The ALX-induced diabetic rabbits were randomly assigned into four groups (5 rabbits per group), and normal rabbits were used as the control group. All studies were carried out one week after ALX had been injected. The five groups are described below:

Group I: Normal control group. The rabbits were treated with same volume of balanced saline by gavage for 12 weeks.
Group II: DN model control group. The rabbits were treated with same volume of balanced saline after the success of DM model by gavage for 12 weeks.
Group III: LBP prevention group. The rabbits were treated with 10 mg/kg/day of LBP (3 mL) after the success of DM model by gavage for 12 weeks.
Group IV: Positive control group. The rabbits were treated with 3.7 mg/kg/day of Telmisartan (3 mL) after the success of DN model (at the end of the 8th week) by gavage for 4 weeks.
Group V (n = 5): LBP treatment group. The rabbits were treated with 10 mg/kg/day of LBP (3 mL) after the success of DN model (at the end of the 8th week) by gavage for 4 weeks.

Body weight and fasting blood glucose (FBG) were monitored every 2 weeks throughout the study. FBG was measured on ear-vein blood samples using an Accu-CHEK® Active II glucometer (Roche, Switzerland). At the end of the 12th week, overnight-fasted rabbits were anaesthetized with intravenous injection of sodium pentobarbital (30 mg/kg body weight). Bilateral kidneys were taken out, rinsed with cold saline solution and weighted. The kidney weight index (KWI) was estimated by the ratio of both kidneys weight to their body. The left kidney was fixed in 10% neutral formaldehyde and paraffin-embedded for histological examination. The right kidney was flash frozen in liquid nitrogen and then stored in −80 degree Celsius (°C) freezer until being assayed.

2.5. Twenty-four hours urinary protein determination

Twenty-four hours urinary samples after ALX injection were collected respectively at the end of the 4th, 6th, 8th and 12th week. The urine sample volume was recorded and 5 mL of urine were centrifuged at 2000 runs per minute (rpm) for 10 minutes (min). The supernatant was collected and detected by spectrophotometry with urinary protein kit according to the manufacturer's instructions (Nanjing Jiancheng Institute of Bioengineering, Nanjing, China).