

Original Article

Protective effects of berberine on high fat-induced kidney damage by increasing serum adiponectin and promoting insulin sensitivity

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Abstract: Berberine (BBR) has been reported in several studies in cell and animal models. However, the mechanism of actions is not fully understood. The present study was therefore aimed to explore the effects of berberine on insulin sensitivity and kidney damage in a high fat diet rat model. Impaired glucose tolerance rats induced by injection of berberine while fed with high fat laboratory chow. After rats were treated for 4 weeks, OGTT and IPITT were determined. Mass and PAS were used to study the kidney tissue. ELISA was used to detect the protein concentration of CRP and TNF- α . Western blot was used to detect the proteins adiponectin, adipoR1, adipoR2 and p-AMPK expression level. These encouraging findings suggest that berberine has excellent pharmacological potential to prevent kidney damage.

Keywords: Berberine, adiponectin, p-AMPK, adipoR1, adipoR2

Introduction

It has been hypothesized that chronic progressive kidney disease often associated with abnormalities of lipid metabolism [1]. Numerous experiments and clinical studies confirmed that abnormal lipid metabolism may be an independent risk factor in the kidney damage [2]. The metabolic sensor AMP-activated protein kinase is stimulated upon exercise and may augment lipid and glucose metabolism in muscle [3, 4]. Numerous diseases are associated with obesity including cardiovascular disease, diabetes mellitus, hypertension and chronic kidney disease [5]. In obesity, reduced adiponectin levels are associated with insulin resistance [6]. The mechanism of action of adiponectin in the kidney appears to be related to AMPK activation. Further studies are needed to elucidate this pathway and investigate the role of potential targets of adiponectin AMPK pathway.

Rhizoma coptidis has been used to treat diseases for more than one thousand years in the history of Chinese medicinal remedy. Berberine, one of the main constituents of Rhizoma coptidis, is a kind of isoquinoline alkaloid. BBR has

been demonstrated as having potential as a treatment for diarrhea, cancer, diabetes and obesity [7-9]. Studies on the anti-obesity effects of berberine have been reported by several groups showing. In addition, recent studies have indicated that berberine prevents obesity in vivo by inducing glycolysis and activating AMP-activated protein kinase [10, 11]. However, the effect of berberine on kidney damage in a high fat diet induced obesity mouse model has not been reported.

This study was to explore BBR and the relationship between the serum adiponectin and kidney damage. Our results indicate that berberine inhibits kidney damage in high fat (HF) mice accompanied with up-regulated AMPK expression without any obvious toxicity in HF and normal mice.

Materials and methods

Materials

Basal diet (AIN-93G diet) and the high-carbohydrate/high-fat diet (66% basal diet; 15% lard; 10% plantation white sugar; 6% casein and 3%

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yolk powder) were produced by Shanghai SLAC Laboratory Animal Co. Ltd. (Shanghai; China). Insulin and TNF- α ELISA kit were bought from Wuhan Gene Biotech Co. Ltd (Wuhan, China). Anti-GAPDH (#5174) antibody was obtained from Cell Signaling Technology (Beverly, MA). The antibodies for adiponectin (sc.7940), AMPK and p-AMPK were obtained from Santa Cruz Biotechnology (California, USA). The antibodies for adipoR1 (ab126611) and adipoR2 (ab77-612) were obtained from Abcam (Cambridge, MA). Trichrome Stain (Masson) Kit (KGMST-8003) and Periodic Acid-Schiff (PAS) Kit (LEA-GENEDG0007) were bought from Nanjing Key-GEN Biotech (Nanjing, China). Blood Glucose Kit (F006) was obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Berberine, insulin and D-glucose were obtained from Sigma.

Rat high-fat-diet model

40 male Wistar rats were obtained from Shanghai SLAC Laboratory Animal Co. Ltd. All rats used in the experiments were 8 week old, and housed 5 per cage with 12 h light-12 h dark cycles under controlled humidity ($60 \pm 5\%$) and temperature ($25 \pm 1^\circ\text{C}$). Experiments were approved by the local (Shanghai) Animal Care and Use Committee (ACUC) and conducted according to the National Research Council publication Guide for Care and Use of Laboratory Animal.

Rats were acclimatized to new environment for 1 week, and were then randomly divided into 4 groups. Group 1 (n=10, named Normal Group) and group 2 (n=10, named Normal+BBR Group) were fed with basal diet while Group 3 (n=10, named HF Group) and Group 4 (n=10, named HF+BBR Group) were fed with the high-fat diet for 12 weeks. Every day of the last 8 weeks, rats of group 2 and group 4 were given a gavage of 380 mg BBR/kg body weight while rats of group 1 and group 3 were given placebo. After BBR intervention was finished, rats were fasted for 12 hours and OGTTs and IPITTs were then performed on these rats. After made a full recovery, rats were fasted for 12 hours then sacrificed; blood samples and kidneys were collected. The kidneys were frozen for Western blot or fixed in 4% paraformaldehyde for histological analysis.

OGTT and IPITT

OGTT and IPITT were performed at the end of the 12 weeks after induction of diabetes. Being fasted (rats were food restricted and were given only water to drink) for 12 h, for OGTT, the rats were given a gavage of D-glucose (20% solution; 2 g/kg BW); for IPITT, the rats were given insulin (1 unit/kg body weight in approximate 0.1 ml 0.9% NaCl) by intraperitoneal injection. Blood samples for plasma glucose were then collected from the tail vein at 0, 30, 60, 90 and 120 min.

Histopathology

Kidneys tissues were fixed in 10% formalin for 48 h at room temperature. Fixed tissues were embedded in paraffin and sectioned into 8 μm (for Masson's trichrome (MT) staining), 4 μm (for Periodic acid-Schiff (PAS) staining) thick slices. For Masson's trichrome staining, kidney sections were deparaffinized and hydrated to water, then treated with Bouin's solution overnight. After being washed with running tap water to remove yellow color, sections were stained by Weigert's iron hematoxylin and Biebrich Scarlet-acid Fuchsin solution for 5 min separately. These sections were reacted with phosphomolybdic-phosphotungstic acid solution for 5-10 min and then stained with aniline blue. Twenty randomly chosen fields of cortex and medulla were analyzed (n=10 animals per group) under 200 \times magnification. For PAS staining, after being deparaffinized and hydrated to water, sections were then oxidized for 15 min with 1% periodic acid, washed 3 times with deionized water, and stained with Schiff's reagent for 30 min, washed 3 times with PBS for 2 to 3 min.

ELISA

The concentrations of insulin and TNF- α in rats' serum were determined by ELISA according to the manufacturer's protocol. Briefly, 50 μl of each sample was added to EIA/RIA plate and washed. After blocking with 2% bovine serum albumin, the plate was incubated with anti insulin or TNF- α monoclonal antibody and washed. After adding stop solution, the absorbance was measured at 450 nm by using a spectrophotometer.

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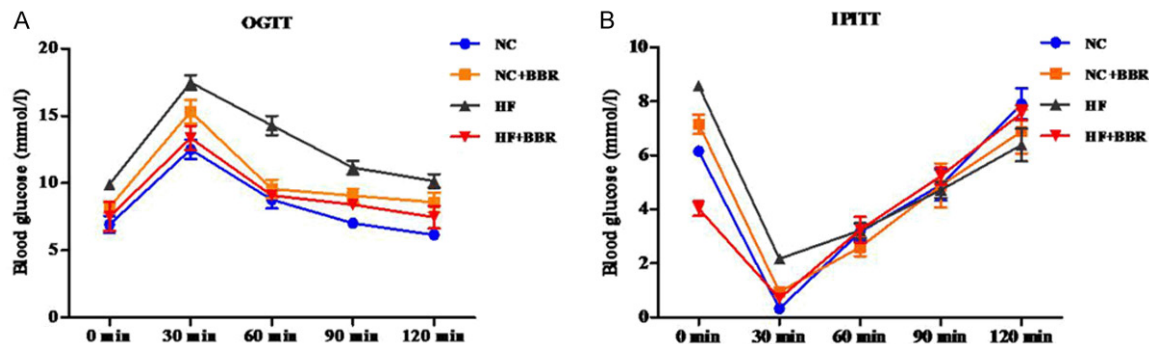


Figure 1. Effects of berberine on oral glucose tolerance test and insulin sensitivity test of high fat diet rats. A: The result of OGTT in the test rats. B: The result of IPITT in the test rats. Data are means \pm SD, $n=3$.

Western blotting

The protein extraction and western blot procedures were conducted as described previously. Briefly, kidneys isolated from rats were put in a glass homogenizer for homogenizing, added RIPA lysis buffer and PMSF, standing on ice for 30 min, collected supernatant after centrifuge. Protein concentrations were determined using Pierce BCA protein assay reagent (Pierce Biotechnology). Protein samples were separated by SDS-PAGE and blotted onto PVDF membranes by electrophoretic transfer. After blocking nonspecific binding sites, proteins were probed using antibody dilutions (anti Adiponectin, AMPK, p-AMPK, adipoR1, adipoR2), followed by incubation with secondary antibody. Specific bands were visualized with chemiluminescence and exposed to X-ray film. GAPDH was used as an internal control.

Statistical analysis

Numerical data were presented as mean \pm SD. Statistical analyses were performed using prism 5.0 software. The significance of difference between groups was assessed by Student's two-tailed t-test. $P<0.05$ was considered statistically significant.

Results

Berberine improves glucose tolerance and promotes insulin sensitivity

To determine if intermittent administration of BBR can affect glucose metabolism in rats, we performed glucose tolerance tests and insulin tolerance tests. As shown in **Figure 1**, in rats fed with high fat diets, glucose tolerance and

insulin sensitivity of BBR-treated rats significantly improved, compared with the placebo-treated rats. These results suggest that daily injection of BBR is likely to improve glucose and lipid metabolism, all of which are the risk factors of kidney disease.

BBR protected high-fat-induced kidney damage

To determine the effect of BBR on high-fat-induced kidney damage, the kidney sections were observed by light microscope with Masson Trichrome staining and PAS staining. Histomorphological assessment of Masson Trichrome-stained renal sections revealed that basement membrane was thinned and blue positive matter deposition was decreased in BBR-intervened HF group compared with that of HF group (**Figure 2A**). Similarly, basement membrane thickening and mesangial matrix proliferation have been rectified in BBR-intervened HF group compared with that of HF group in PAS-stained sections (**Figure 2B**). C-reactive protein (CRP) is an acute-phase protein of hepatic origin that increases following interleukin-6 secretion from macrophages and T cells. It was reported that CRP is not only a biomarker, but also a mediator in diabetic kidney disease (DKD) [12]. On the other hand, it was also reported that TNF- α plays a pathogenic role in nephropathy [13]. For these reasons, CRP and TNF- α levels in rats fasting serum were detected by ELISA. As is shown in **Figure 2C, 2D**, the CRP and TNF- α levels in rat's serum of BBR-intervened HF group are lower than that of HF group. These results suggested that the renal lesion of the BBR-intervened HF group was less than that of the HF group.

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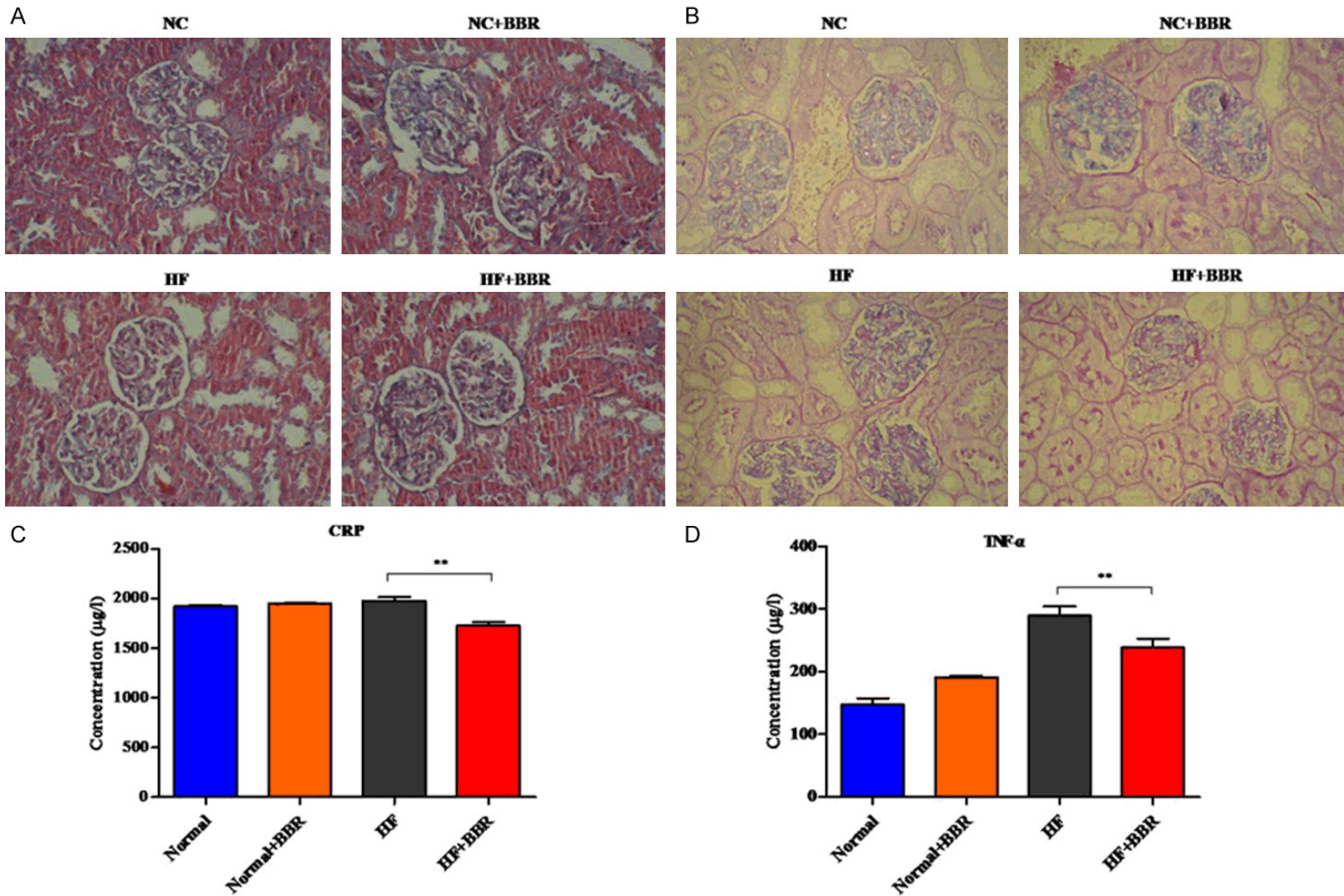


Figure 2. Effects of berberine on high-fat-induced kidney damage. A: Representative magnified image of Masson's trichrome stain for BBR in HF group. B: The PAS staining of kidney tissue in each rats group. C, D: The concentration of CRP and TNF- α in kidney tissue was detected by ELISA. The values are expressed as means \pm SD, n=3, **P<0.01.

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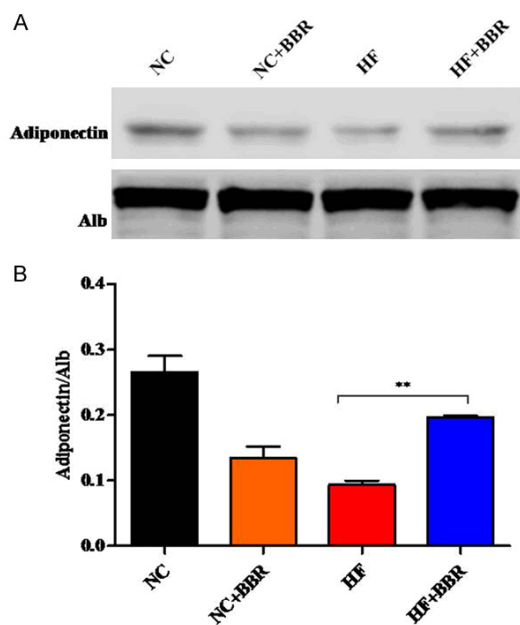


Figure 3. Effects of berberine on protein expression of adiponectin. A: A representative data. B: The adiponectin level was significantly increased in BBR treated high fat diet group mice.

BBR increase serum adiponectin level

Adiponectin is an insulin-sensitizing adipokine that modulates glucose and lipid metabolism. Levels of adiponectin in the blood are decreased under conditions of obesity, insulin resistance and type 2 diabetes. Administration of adiponectin causes glucose-lowering effects and ameliorates insulin resistance in mice [14]. Adiponectin is secreted primarily by adipose tissue and plays a key role in kidney disease [15]. Several clinical studies have confirmed an inverse association between circulating adiponectin and renal function in both in Africans and Caucasians. New research now shows that the drop in adiponectin is also associated with an inflammatory-driven decline in kidney function, causing protein to leak into the urine (albuminuria). To determine the effects of BBR on serum adiponectin level, all groups fasting blood sample were test by Western. As shown in **Figure 3**, compared with HF group, serum level of adiponectin significantly increased in BBR-intervened HF group.

BBR promoted AdipoR1 and AdipoR2 expression and AMPK activation in kidney of high-fat-diet rats

Two seven-transmembrane Adiponectin receptors have been identified: AdipoR1 and Adipo-

R2. AdipoR1 are expressed ubiquitously, with the most abundant expression in skeletal muscle, whereas AdipoR2 is expressed predominantly in the liver. The mechanism of action of adiponectin in the kidney appears to be related to AMP-activated Protein Kinase (AMPK) activation [15]. The ultrasensitive energy sensor AMPK orchestrates the regulation of energy-generating and energy-consuming pathways. AMPK is highly expressed in the kidney where it is reported to be involved in a variety of physiological and pathological processes [16]. The phosphorylated AMPK (p-AMPK) is an indicator of the activation of AMPK pathway. Many studies demonstrate that the kidney is an early responder to the challenge of high fat feeding, and the energy sensor AMPK mediates the early renal effects of a high-fat diet [17]. To determine the effects of BBR on AdipoR1 and AdipoR2 expression in kidney of high-fat-diet rats and whether by activating AMPK signal pathway BBR protected high-fat-induced kidney damage, the expression level of AdipoR1, AdipoR2 and pAMPK in kidney of all group were test by Western. As shown in **Figure 4**, both the expressions of AdipoR1/R2 and pAMPK were up-regulated in BBR intervened HF group compared to HF group, while the AMPK and GADPH were used as a loading control.

Discussion

Berberine an herbal compound traditionally used in Chinese medicine as an anti-microbial or anti-obesity [18, 19]. In this study, we used a high fat diet mice model to not only verify the inhibitory effects of berberine on adiponectin but to also show strong experimental data that berberine had an effect on other important factors known to be involved in the kidney damage. Following berberine treatment in this mouse model, we observed that BBR improved glucose tolerance and promoted insulin sensitivity. In addition CRP and TNF- α levels in high fat diet induced obesity mice were all lowered, showing that berberine to be a potential natural compound for the treatment of obesity.

An interesting finding in this study is that berberine caused a increase in adiponectin. Thus, raising the question of the mechanism by which berberine affects. Increased body mass index and high fat intake have been associated with the progression of renal disease [20, 21]. Unlike other organs, such as the heart, liver, and brain, the kidney is covered by abundant perirenal fat

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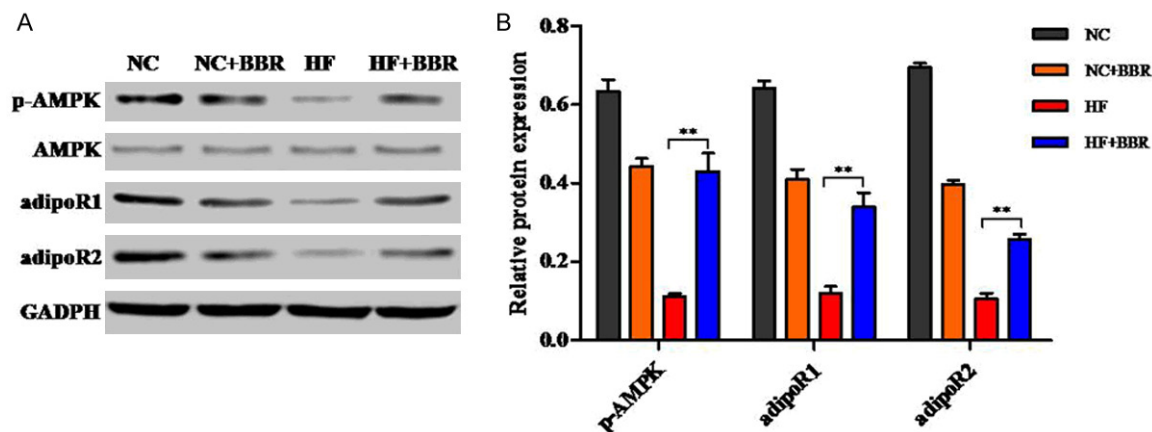


Figure 4. Effects of berberine on protein expression of p-AMPK, adipoR1 and adipoR2. A: A representative data of western blot result. B: The expression level of p-AMPK, adipoR1 and adipoR2 were significantly increased when treated with BBR in HF group. Data are means \pm SD, $n=3$, $**P<0.01$.

tissue containing adipocytes [22]. This may be related to the effect of adipokines during renal homeostasis and injury. The kidneys of high fat diet fed rats, a model for diabetes, resulted in a marked increase in fibrosis with suppression of AMPK activity.

Researchers with VA and the University of California, San Diego, have found that inducing AMPK activation can reverse damage in the early stages of kidney disease in rodents. While early kidney damage leads to reduced amounts of AMPK in rodents, when the research team upped the activity of AMPK, kidney function improved and kidney damage lessened. AMPK is an ubiquitous heterotrimeric enzyme that is considered to be the master energy sensor in all eukaryotic cells. AMPK has been reported to be reduced in various organs, including the kidney after exposure to a high fat diet [17]. BBR treatment resulted in increased levels of AMPK, adipoR1 and adipoR2, suggesting that BBR increased adiponectin level and enhanced insulin sensitivity that may reduce the risk for kidney damage, at least through the activation of AMPK signaling pathway.

From this study, we believe that berberine had excellent potential as an effective agent to protect the kidney damage from the effects of high fat feeding.

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Disclosure of conflict of interest

None.

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