Effect of Berberine on Expression of Hepatocyte Nuclear Factor-4α in Rats with Fructose-induced Insulin Resistance*  

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Summary: The effects of berberine on the expression of hepatocyte nuclear factor-4α (HNF-4α) in liver of rats with fructose-induced insulin resistance and the molecular mechanism of berberine preventing insulin resistance were investigated. The experimental animals were divided into two groups of 16 animals each. The control group received a control routine diet containing 60% carbohydrate, and the study group a high-fructose diet containing 60% fructose as the sole source of carbohydrate. At the end of 6 weeks these were each subdivided into two groups. One was administered with berberine [187.5 mg/(kg·d) in 5 g/L carboxymethyl cellulose] by intragastric intubation and the other group was treated with a vehicle (5 g/L carboxymethyl cellulose). The rats were fed on the same dietary regimen for the next 4 weeks. After the experimental period of 10 weeks, plasma glucose, insulin and triglyceride levels were measured. HOMA insulin resistance index (HOMA-IR) was assayed. Immunohistochemistry, semiquantitative RT-PCR and western blot were used to detect the expression of HNF-4α in liver. Compared with control diet, fructose feeding induced hyperinsulinemia, HOMA-IR and increased triglyceride (all P < 0.01). Berberine prevented the rise in plasma insulin (P < 0.01), HOMA-IR (P < 0.01) and triglyceride (P < 0.05) in the fructose-fed rats. No change in plasma glucose was seen among these groups. The mRNA and protein expression of HNF-4α was decreased in the fructose-fed rats, but berberine could promote its expression. It was concluded that berberine could prevent fructose-induced insulin resistance in rats possibly by promoting the expression HNF-4α in liver.  

Key words: insulin resistance; berberine; liver; HNF-4α; high fructose diet

Insulin resistance (IR) is a state in which higher than normal concentrations of insulin are required for normal response and plays a role in the pathophysiology of the most common human diseases including type 2 diabetes mellitus (T2DM), hypertension, obesity, dyslipidemia and coronary heart disease, but there is no very effective method for the treatment of IR at present. Berberine (BBR) is an isoquinoline derivative alkaloid isolated from many medicinal herbs, such as Hydrastis Canadensis (goldenseal), Cortex phellodendri (Huangbai) and Rhizoma coptidis (Huanglian). Studies have shown that BBR, the major component of these herbs, has many pharmacological effects including inhibition of adipocyte differentiation, anti-cancer effects, anti-microbial effects, lipid-lowering effects and anti-inflammatory potential.  

Recently, it has been reported that BBR had antidiabetic properties such as lipid-lowering properties and improving IR[1]. BBR could even down-regulate the expression of genes involved in lipogenesis and up-regulate those involved in energy expenditure in adipose tissue and muscle[2], but the role and mechanism of BBR in inhibition of IR have not yet been clarified.  

Hepatocyte nuclear factor-4α (HNF-4α), a zinc finger protein, is the most abundant transcription factor in the liver. HNF-4α gene is associated with T2DM in some populations, and mutations in the HNF-4α gene cause the disorder maturity onset diabetes of the young (MODY1). It has been suggested that HNF-4α is a central regulator of glucose and lipid metabolism and insulin secretion. In the liver, HNF-4α plays an important role in regulating various genes involved in glucose, fatty acid, amino acid, and cholesterol metabolism, as well as blood coagulation and hepatic development and differentiation[3]. This study investigated whether BBR could prevent fructose-induced insulin resistance and the effect of BBR on the expression of hepatatic HNF-4α levels in rats.  

1 MATERIALS AND METHODS

1.1 Animal Modeling, Grouping and Treatment

Six-week-old male Sprague-Dawley rats (170−190 g, purchased from Experimental Animal Center of Tongji Medical College, HUST, China) were used in the experiments. The rats were housed in plastic cages in an animal room with a constant temperature of 23±2°C and a fixed 12 h light-dark cycle and were given water and chow ad libitum. After one week of acclimatization, the rats were randomly assigned to two groups and fed separa-
rately either a regular chow diet or a 60% fructose-enriched diet for 6 weeks. The high fructose diet was composed of 60% fructose (ADM, USA), 20.7% protein, 5% fat, 8% cellulose, w/w, and standard vitamins and mineral mix[5]. Each group of rats was further divided randomly into two subgroups: with BBR (National Institute for the Control of Pharmaceuticals and Biology, China) treatment (187.5 mg/kg body weight every day by gastric intubation) or without BBR treatment (a vehicle by gastric intubation). The rats were fed on the same dietary regimen for the next 4 weeks. Then 4 groups were assigned (n=8 per group): control (C), control BBR-treated (B), fructose-fed (F), and fructose-fed -BBR treated (FB). At termination, all the rats were killed using pentobarbital sodium anesthesia. Blood samples were taken from the abdominal aorta under anesthesia, and the liver was removed, immediately frozen in liquid nitrogen and stored at −80°C, except for a portion cut out for histology.

1.2 Biochemical Analyses

Plasma insulin levels were assayed using a radioimmunoassay (RIA) kit (Northern Bioengineering Institute, China). Fasting blood glucose was determined by the glucose-oxidase method and plasma triglyceride (TG) by enzyme end-point method (Nanjing Jiancheng Bioengineering Institute, China).

The homeostasis model assessment for insulin resistance (HOMA-IR; [Fasting insulin (μIU/mL)×Fasting glucose (mmol/L)]/22.5) was used to estimate insulin sensitivity[6].

1.3 Immunohistochemical Detection of HNF-4α

All immunohistochemical studies using the streptavidin peroxidase technique were performed on 5-μm thick formaldehyde-fixed and paraffin-embedded liver tissue sections mounted on APES-coated slides. Slides were deparaffinized in xylene and rehydrated in graded ethanol. Endogenous peroxidase activity was quenched with a 3% hydrogen peroxide solution in methanol at room temperature for 30 min, followed by rinsing in pH 6.0 phosphate buffered saline (PBS). After antigen retrieval in a water bath set in a 10 mmol/L citrate buffer (pH 6.0) at 94°C for 8 and 10 min, respectively, the slides were immediately cooled for 20 min at room temperature. Non-specific binding sites were blocked by serum at 37°C for 30 min. The sections were then incubated overnight at 4°C with a goat polyclonal antibody directed against HNF-4α (Goat anti-HNF-4α, sc-6556, Santa Cruz, USA) at a dilution of 1:100. The positive stains were shown as brown color with peroxidase substrate solution DAB, and samples were lightly counterstained with hematoxylin. Specimens were examined under a light microscope.

1.4 RNA Extraction and RT-PCR Assay

The expression of HNF-4α mRNA was detected by RT-PCR. Total RNA was extracted from livers using TRIzol reagent according to the manufacturer’s instructions, then reversed by transcribing it into cDNA. Total RNA (5 μg) and random primer (2 μg) in DEPC water were denatured at −80°C for 5 min, then 5 μL 5× reverse transcriptase buffer, 2 μL 20 mmol/L dNTPs, 1 μL M-MLV reverse transcriptase (200 U) and DEPC water were added to the total volume of 15 μL. The reaction was performed at 37°C for 1.5 h and at 100°C for 5 min to inactivate the reverse transcriptase. PCR was performed in a 25 μL reaction mixture containing 1 μL reactant, 2.5 U Taq DNA polymerase and 20 pmol primers. HNF-4α genes were amplified with specific primers, and the gene for GAPDH was used as an internal control. Primer sequences were as follows: HNF-4α, forward 5'-GGTGCCAACTCCAACCTCA-3' and reverse 5'-AGGCTGCTCTCAGTAAAG-3', fragment length 314 bp; GAPDH, forward 5'-GACTCCCGCTAATCATCAAT-3' and reverse 5'-ATCAAAGTGAGAATGGG-3', fragment length 648 bp. Each PCR cycle included an initial denaturation at 94°C for 5 min and a final extension at 72°C for 10 min. The cycling conditions were as follows (30 cycles for HNF-4α and 28 cycles for GAPDH, respectively): denaturation at 94°C for 1 min, annealing for 50 s (at 57°C for HNF-4α), or for 1 min (at 54°C for GAPDH) and extension at 72°C for 50 s (HNF-4α) or 1 min (GAPDH) on a Mastercycler gradient thermal cycler (Eppendorf, Germany). The amplified products were electrophoresed in 2 g/L agarose gels containing 0.5 g/L ethidium bromide and visualized under UV light. The absorbance (A) for each sample was analyzed by using computerized image analysis software (Peiqing Technology, China). The ratio of HNF-4α/GAPDH was regarded as the expression levels of HNF-4α.

1.5 Protein Isolation and Western Blotting Analysis

The rat liver HNF-4α protein level was determined by Western blotting. Liver samples were homogenized in lysis buffer with protease inhibitors at 4°C. The supernatant was collected and used as the source of protein for Western blots, as described elsewhere[6]. Briefly, for each sample, 100 μg protein was resolved by electrophoresis on a 12% SDS-polyacrylamide gel and transferred to a polyvinylidene difluoride membrane (Pierce Company, USA). Membranes were blocked for 2 h in a 7% milk buffer before incubation with an antibody raised against rat HNF-4α (Goat anti-HNF-4α, sc-6556, Santa Cruz, USA), 1:500 dilution in Tris-buffered saline, pH 7.4 at room temperature. Non-specific binding sites were blocked by normal goat serum at 37°C for 1.5 h and at 100°C for 1 min, then 5 μL 5× reverse transcriptase buffer, 2 μL 20 mmol/L dNTPs, 1 μL M-MLV reverse transcriptase (200 U) and DEPC water were added to the total volume of 15 μL. The reaction was performed at 37°C for 1.5 h and at 100°C for 5 min to inactivate the reverse transcriptase. PCR was performed in a 25 μL reaction mixture containing 1 μL reactant, 2.5 U Taq DNA polymerase and 20 pmol primers. HNF-4α genes were amplified with specific primers, and the gene for GAPDH was used as an internal control. Primer sequences were as follows: HNF-4α, forward 5'-GGTGCCAACTCCAACCTCA-3' and reverse 5'-AGGCTGCTCTCAGTAAAG-3', fragment length 314 bp; GAPDH, forward 5'-GACTCCCGCTAATCATCAAT-3' and reverse 5'-ATCAAAGTGAGAATGGG-3', fragment length 648 bp. Each PCR cycle included an initial denaturation at 94°C for 5 min and a final extension at 72°C for 10 min. The cycling conditions were as follows (30 cycles for HNF-4α and 28 cycles for GAPDH, respectively): denaturation at 94°C for 1 min, annealing for 50 s (at 57°C for HNF-4α), or for 1 min (at 54°C for GAPDH) and extension at 72°C for 50 s (HNF-4α) or 1 min (GAPDH) on a Mastercycler gradient thermal cycler (Eppendorf, Germany). The amplified products were electrophoresed in 2 g/L agarose gels containing 0.5 g/L ethidium bromide and visualized under UV light. The absorbance (A) for each sample was analyzed by using computerized image analysis software (Peiqing Technology, China). The ratio of HNF-4α/GAPDH was regarded as the expression levels of HNF-4α.

1.6 Statistical Analysis

Results were expressed as t±s. Significant difference was determined by one-way ANOVA followed by LSD test (Student’s t-test) between different groups. Statistical analyses were performed using SPSS 11.0 software. A P value less than 0.05 was considered to be statistically significant and a P value less than 0.01 was regarded as extremely significant.

2 RESULTS

2.1 Plasma Glucose, Insulin, Triglyceride and HOMAIR

The plasma fasting glucose, insulin and triglyceride