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Protective effect of berberine on antioxidant enzymes and positive transcription elongation factor b expression in diabetic rat liver

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ABSTRACT

The protective effect of berberine against antioxidant, antilipid peroxidation in serum and liver tissue, and positive transcription elongation factor b (P-TEFb) expression in liver tissue of type 2 diabetic rats was investigated. Overnight fasted rats were intraperitoneally injected 35 mg/kg streptozotocin. Diabetic rats were admitted after 2 weeks and given a high-carbohydrate/high-fat diet to induce hyperlipidemias. From week 16, diabetic rats were treated with 75, 150, 300 mg/kg berberine, 100 mg/kg fenofibrate or 4 mg/kg rosiglitazone for another 16 weeks. P-TEFb (composed of cyclin-dependent kinase 9 and cyclin T1) mRNA and protein expression in liver tissue were detected by real time PCR and immunohistochemistry, respectively. Berberine significantly up-regulated the declined cyclin-dependent kinase 9, cyclin T1 mRNA and protein expression in diabetic rat liver. Berberine obviously decreased malondialdehyde level and increased catalase, superoxide dismutase, glutathione peroxidase, and glutathione activities in liver tissue and serum of diabetic rats. These results suggest that the effects of berberine on up-regulation of P-TEFb expression, antioxidant and antilipid peroxidation may be related to its protective potential on diabetes.

1. Introduction

Diabetes is a chronic disorder of metabolism caused by an absolute or relative lack of insulin as old as mankind and its incidence is considered to be about 5% all over the world. The number of people in the world with diabetes has increased dramatically over recent years and is expected to reach 300 million by the year 2025 [1]. Hyperglycemia and several other symptoms (i.e., hyperlipidemia) are involved in development of diabetic complications which are the major causes of morbidity and death [2]. These traits are hypothesized to damage cell membranes and to elevate production of reactive oxygen species. The combination of excessive generation of reactive oxygen species and decreased antioxidant defenses is the major pathogenetic mechanism of diabetes [3,4].

Anti-oxidants protect against living organism damage caused by uncontrolled production of reactive oxygen species concomitant lipid peroxidation, protein damage, and DNA strand breaking. As a traditional Chinese medicine, *Rhizoma coptidis* is often used to treat several diseases (i.e., diabetes) in which reactive oxygen species and free radicals play a major role [5]. Lipid peroxidation occurs when free radicals attack the unsaturated fatty acids in the cell membranes. Malondialdehyde is one of the end products of oxidation of polyunsaturated fatty acids and an index of lipid peroxidation. Some cyclin-dependent kinases (CDKs) appear to have a direct role in transcription [6]. Positive transcription elongation factor b (P-TEFb) is composed of cyclin T1 (CycT1) and CDK9. CycT1 is a regulatory subunit of CDK9. P-TEFb is required as a basic transcription elongation factor and is recruited by some transcription factors to activate transcriptional elongation from specific promoters. CycT1 and CDK9 all highly expressed in differentiated tissues, which indicate the participation of P-TEFb in the activation or maintenance of specific differentiation processes.

Plants remain a major source for drug discovery notwithstanding the quick advance of synthetic techniques. Traditional plant extract is used to treat various diseases widespread in the

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world [7–9]. Berberine (Fig. 1) is one of the principal antidiabetic components of *Rhizoma coptidis*, which has been used to treat diabetes for more than 1400 years in China. As several studies report [10–13], we also find that berberine has hypoglycemic, hypolipidemic effects and β cell protection on diabetic rats [14,15]. Some clinical trials show that berberine is a potential and safe oral hypoglycemic drug to treat type 2 diabetic patients with dyslipidemia [16,17].

Since diabetes is known to induce oxidative stress, in this study, we determined the effects of berberine on P-TEFb mRNA and protein expression, malondialdehyde level, various antioxidant enzymes (catalase, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and glutathione activities) in liver tissue of type 2 diabetic rats induced by a low-dose streptozotocin and a high-carbohydrate/high-fat diet.

2. Materials and methods

2.1. Animals

Male Wistar rats (180–220 g, Grade II) were obtained from Third Military Medical University, Chongqing, China and housed in a temperature and humidity-controlled environment with a 12-h light/dark cycle. All animals were maintained in individual cages and allowed free access to food and water. The animal experiment was approved by Third Military Medical University according to the guidelines of the Institutional Animal Care and Use Committee.

2.2. Diabetes induction and treatment

Overnight fasted rats were intraperitoneally injected with 35 mg/kg streptozotocin (Sigma, Saint Louis, USA) (freshly prepared in 0.1 mM citrate-phosphate buffer, pH 4.5). Control rats were injected with citrate-phosphate buffer alone. After injection, all animals continued on the standard diet for two weeks. The animals with fasting blood glucose level of above 16.7 mM were considered to be diabetic and only uniformly diabetic rats were used [18]. Diabetic rats were given the high-carbohydrate/high-fat diet (70% standard diet, 12% lard, 9% yolk powder, and 9% plantation white sugar) to induce diabetic dyslipidemias. Control rats were fed the standard diet throughout the experiment. The animals were divided into 7 groups (10 per group) at week 16: control rats; diabetic rats; diabetic rats treated with 75, 150 or 300 mg/kg berberine (Northeast General Pharmaceutical Factory, China); diabetic rats treated with 100 mg/kg fenofibrate (Kaifeng Pharmaceutical Group Co, Ltd, China) or 4 mg/kg rosiglitazone (Beijing Comens Chemical Co, Ltd, China) every day (both served as positive control). Berberine, fenofibrate or rosiglitazone was mixed daily with a vehicle consisting of the standard diet for 16 weeks. The standard diet or the high-carbohydrate/high-fat diet was given only after the vehicle was completely ingested by the animals.

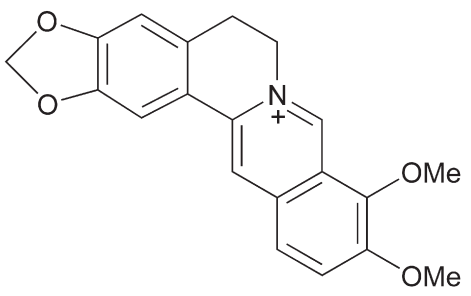

Fig. 1. Structural formula of berberine.

2.3. Tissue preparation

After a 32-week induction of diabetes, the overnight fasted rats were anesthetized with 120 mg/kg sodium pentobarbital and blood samples were collected from the heart. Half of each group rats were perfused with 200 mL physiological saline through the left ventricle via the ascending aorta at 25 °C, followed by 500 mL 4 °C 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 1 h. The livers were excised and fragments of the right lobe liver were postfixed in 4% paraformaldehyde overnight. The other animals were directly sacrificed after blood collection. The livers were rapidly excised and most of the right lobe liver was cut into slices, frozen in liquid nitrogen and stored at −70 °C for biochemical assay and mRNA measurement. Another part of the right lobe liver was postfixed in 4% paraformaldehyde overnight. All the tissues were dehydrated through graded ethanol series, made transparent with xylene, embedded with paraffin, cut into slices (7 μm thickness), and mounted onto 3-aminopropyltriethoxysilane-coated glass slides for immunohistochemical staining.

2.4. Biochemical assays

The frozen right lobe liver tissue was homogenized with ten times of their weights in volume of the ice-cold 0.9% sodium chloride using homogenizer to obtain a 10% solution. The samples were immediately centrifuged at 10,000 rpm for 5 min at 4 °C to obtain the tissue homogenate. The protein content of tissue homogenates was measured by the Bradford method as described in the Bio-Rad protein assay kit (Nanjing Jiancheng Bioengineering Institute, China). The tissue homogenate and serum were used for the determination of the scavenging enzymes SOD, catalase, glutathione, GSH-Px activities, and lipid peroxidation using malondialdehyde level by commercial kit as per the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, China), respectively.

2.5. Real time PCR

Total RNA was isolated using the RNAout kit (Tianze Technologies, Chengdu, China) according to the manufacturer's guidelines. First-strand cDNAs were generated through oligo (dT) priming with a cDNA synthesis kit (Tianze Technologies, Chengdu, China), following the manufacturer's instructions. Real time PCR was performed with Real time PCR Master Mix containing SYBR® Green I and hotstart Taq DNA polymerase. Real time detection of the emission intensity of