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Original article

Effects of Berberine on Hepatic Sirtuin 1-uncoupling Protein 2 Pathway in Non-alcoholic Fatty Liver Disease Rats Induced by High-fat Diet

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ABSTRACT

Objective To investigate the involvement of sirtuin 1 (SIRT1)-uncoupling protein 2 (UCP2) pathway in the development of non-alcoholic fatty liver disease and whether berberine exerts its effects by regulating this pathway. **Methods** Male SD rats were divided into three groups: normal control group, high-fat diet group, and berberine supplement group. The rats in the normal control group were given normal diet while the rats in the other two groups were fed with high-fat diet. Rats in the berberine supplement group were concurrently given berberine (100 mg/kg body weight) once daily. After 16 weeks, the levels of serum, liver lipids, and serum aminotransferase were measured using an automatic biochemical analyzer. Superoxide dismutase (SOD) activity and malondialdehyde (MDA) content in the liver were measured using commercial kits. Histopathological changes of liver tissues were observed by hematoxylin and eosin (HE) staining and Oil Red O staining. The hepatic mRNA and protein levels of SIRT1 and UCP2 were assayed by reverse transcription polymerase chain reaction (RT-PCR) or Western blotting. **Results** Berberine supplement could significantly decrease the serum and liver lipid contents in rats fed with high-fat diet. Meanwhile, SOD level was significantly elevated, but MDA level was reduced in the liver. The results of HE and Oil Red O staining showed that the hepatic steatosis was alleviated in berberine supplement group. Furthermore, berberine induced an increase in SIRT1 expression but a decrease in UCP2 expression. **Conclusion** The regulation of hepatic SIRT1-UCP2 pathway may be an important mechanism by which berberine exerts the beneficial effects in NAFLD rats.

Key words

berberine; non-alcoholic fatty liver disease; oxidative stress; sirtuin 1; uncoupling protein 2

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1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is recognized as a hepatic manifestation of metabolic syndrome characterized by predominant macrovesicular steatosis of the liver without alcohol consumption (Loomba and Sanyal, 2013). It comprises a wide spectrum of disease that includes simple steatosis, non-alcoholic steatohepatitis (NASH), fibrosis, and cirrhosis (Cohen et al, 2011; de Alwis and Day, 2008). NAFLD has become the most common cause of chronic liver disease in Western countries, affecting up to 30% of the general population (Loomba and Sanyal, 2013). In Asia, the prevalence of NAFLD ranges from 15% to 45%, with the epidemic of obesity and type 2 diabetes (Farrell et al, 2013). Although it has been proposed that NAFLD is closely associated with obesity and insulin resistance, the pathogenesis of NAFLD remains ill-defined (Cohen et al, 2011). As a result, the treatment of NAFLD remains controversial and novel therapeutic strategies are needed for the prevention and treatment of NAFLD.

Berberine, also known as umbellatine, is a kind of isoquinoline alkaloid isolated from Chinese medicinal herb *Coptidis Rhizoma*, which has been used in traditional Chinese medicine (TCM) for centuries. It is well known that berberine has many pharmacological properties concerning metabolic diseases, such as obesity and type 2 diabetes (Vuddanda et al, 2010; Wan et al, 2015). Recently, berberine has been reported to have beneficial roles in preventing or treating NAFLD *in vivo* and *in vitro*, suggesting that berberine may be a potential drug for NAFLD (Liu et al, 2013). Sirtuins are the mammalian homologues of silent information regulator-2 (Sir2), a group of class III histone deacetylases, which are NAD^+-dependent protein deacetylases. Sirtuin 1 (SIRT1), first identified among sirtuins, has been demonstrated to regulate the cellular protection against oxidative stress in many diseases including metabolic disorders (Chong et al, 2012; Colak et al, 2011). Since oxidative stress is strongly implicated in the pathogenesis of NAFLD (Videla et al, 2006, Rolo et al, 2012), SIRT1 has been proposed to be a potential therapeutic target in the treatment of NAFLD (Colak et al, 2011). Recently, berberine has been demonstrated to activate SIRT1 *in vivo* and *in vitro* (Chi et al, 2014; Gomes et al, 2012). Uncoupling protein 2 (UCP2) is a member of the super family of anion carrier proteins located in the inner membrane of mitochondria (Fisler and Warden, 2006). Our previous study demonstrated that berberine could improve NAFLD in rats along with a decrease in UCP2 expression, but the underlying mechanisms have not been elucidated (Yang et al, 2011). A previous study has demonstrated that SIRT1 represses mitochondrial UCP2 transcription by binding directly to the UCP2 promoter (Bordone et al, 2006).

Based on these data, it is tempting to speculate that berberine could lead to SIRT1 activation which in turn repressed UCP2 in liver tissues, thereby protect against NAFLD. In the present study, we used an NAFLD rat model to further investigate whether SIRT1-UCP2 pathway and oxidative stress were involved in the effects of berberine in protecting against NAFLD.

2. Materials and methods

2.1 Animals

Thirty specific pathogen-free male Sprague-Dawley (SD) rats aged 6−7 weeks (200 ± 20) g were purchased from the Laboratory Animal Research Center of Guangzhou University of Traditional Chinese Medicine, China (Approval No. SYXK (Yue) 2013−0034). The rats were kept in separate cages under conditions of controlled temperature (24 ± 2) ^oC on a regular 12-h light/dark cycle (lights on from 8:00 am to 8:00 pm), with free access to diet and water.

2.2 Grouping and modeling

After one week adaptive breeding, the rats were randomly distributed into three groups, 10 rats in each group: normal control (NC) group, high-fat diet (HFD) group, and high-fat diet supplemented with berberine (HFB) group. Rat models were duplicated according to the method as described previously with minor modifications (Yang et al, 2011). Rats in NC group got free access to normal chow diet supplied by Experimental Animal Centre of Jinan University, while rats in HFD and HFB groups were fed with a high-fat diet (composed of 88% regular chow, 10% axungiaporci, 1.5% cholesterol, and 0.5% bile salt) which were purchased from Experimental Animal Centre of Guangdong Province. Rats in HFB group were ig administered with berberine (Mysun Pharma, China) by 100 mg/kg, once daily (Gomes et al, 2012). Rats in NC and HFD groups were given distilled water daily. The intervention lasted for 16 weeks. After the last administration, all rats were made to fast for 12 h and then anesthetized by ip injecting 3% pentobarbital (1 mL/kg). Blood samples were collected from abdominal aorta and then centrifuged at 1500 × *g* for 10 min at 4 °C. The clear supernatants were collected for assays. The livers were immediately removed. All rats were treated in compliance with the *Guiding Principles for Animal Experiments* and the protocols were approved by the Animal Experimental Ethnics Committee of Jinan University, Guangzhou, China.

2.3 Biochemical analysis

The concentration of total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) in serum were measured using automatically biochemical analyzer (Hitachi Company, Japan). Liver tissues were homogenized using a Tissue Lyser-II Homogenizer (Qiagen, Germany) and centrifuged at 3000 × *g* for 10 min at 4 °C. Then the clear supernatants were collected to determine liver levels of TC and TG using an automatic biochemical analyzer.