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Original article

Mangiferin ameliorates fatty liver via modulation of autophagy and inflammation in high-fat-diet induced mice

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease globally. The progression of NAFLD is complex and associated with inflammation, oxidative stress, autophagy, endoplasmic reticulum stress, and insulin resistance. Mangiferin, a natural C-glucosyl xanthone, has been reported to show multiple biological activities. The aim of this study was to investigate the therapeutic effect of mangiferin on NAFLD and the underlying molecular mechanism. We established a mouse model of NAFLD using a high-fat diet (HFD), and injected the mice with different doses of mangiferin (15, 30, and 60 mg/kg, intraperitoneal) for 12 weeks. Liver tissue was assessed to evaluate changes in inflammatory responses, autophagy, and glycolipid metabolism. We found that mangiferin decreased body weight, as well as the levels of triglycerides and total cholesterol in plasma and the liver. It also increased glucose tolerance in HFD-fed mice. In addition, mangiferin decreased inflammatory responses by inhibiting the activities of nuclear factor kappa B and c-Jun N-terminal kinase, regulated autophagy via the AMP-activated protein kinase/mechanistic target of rapamycin signaling pathway, and improved glycolipid metabolism via modulation of the insulin receptor substrate/phosphoinositide 3-kinase/protein kinase B signaling pathway. This study demonstrated that mangiferin significantly ameliorates NAFLD development in HFD-fed mice by inhibiting inflammatory responses, activating autophagy, and improving glycolipid metabolism.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is one of the most common liver diseases globally. It encompasses a wide spectrum of disorders ranging from simple steatosis to non-alcoholic steatohepatitis, which can progress to fibrosis and even liver cirrhosis [1]. The pathogenesis of NAFLD is complex, owing to interactions between the host genetic background and environmental factors. Although numerous studies have been conducted in recent decades to develop strategies for treating NAFLD, the therapeutic outcome remains unsatisfactory. Therefore, more effective therapeutic approaches with fewer side effects are required.

The pathological process of NAFLD is strongly associated with inflammatory responses, autophagy, and glycolipid metabolism. Inflammatory pathways involving nuclear factor kappa B (NF-κB) and c-Jun N-terminal protein kinase (JNK) lead to hepatic inflammation and a decrease in insulin sensitivity [2,3]. The AMP-activated protein kinase (AMPK)/mechanistic target of rapamycin (mTOR) signaling pathway is the most typical autophagy pathway that controls autophagy and lipid metabolism [4].

The NF-κB signaling pathway is important in signal transduction since transcription factors in the NF-κB family bind to specific DNA sequences and control transcriptional activity. NF-κB participates in the pathogeneses of inflammatory injuries and fibrogenesis of the liver [5]. It can also control the expression of tumor necrosis factor-α, which is involved in liver fibrosis. The JNK pathway is implicated in hepatic inflammation and fatty degeneration [6], which can impair oxidative metabolism and contribute to insulin resistance and hepatosteatosis [7]. Autophagy is a crucial intracellular process involved in the degradation of defective organelles. It can decrease the number of lipid droplets through lipophagy. The AMPK/mTOR signaling pathway is critical for autophagy activation and regulation of lipid accumulation in NAFLD.

Mangiferin is a natural C-glucosyl xanthone and polyhydroxy

Abbreviations: NAFLD, non-alcoholic fatty liver disease; HFD, high-fat diet; SREBP-1, sterol regulatory element-binding protein-1; TG, triglyceride; TC, total cholesterol; ipGTT, intraperitoneal glucose tolerance test; IRS, insulin receptor substrate

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polyphenol that can be isolated from mango stem bark extract. It has been reported that mangiferin has antioxidant [8], antidiabetic [9], antitumor [10], anti-inflammatory [11], and immunomodulatory effects [12]. Thus, it has the potential to treat NAFLD. It has been demonstrated that mangiferin inhibits activation of NF-κB in multiple myeloma cell lines [13]. In addition, it has a prophylactic effect against myocardial ischemia-reperfusion injury [14] because it inhibits JNK activation. These findings indicate that mangiferin may be used to treat NAFLD because it suppresses inflammation. Autophagy also plays a major role in lipid metabolism in the liver and is regulated via the AMPK/mTOR pathway [15]. Activation of AMPK results in the inhibition of mTOR activity and the upregulation of autophagy.

It was recently shown that mangiferin can ameliorate lipid accumulation in the liver [16,17], with upregulation of proteins for mitochondrial biogenesis and downregulation of proteins for lipogenesis. However, the underlying mechanism is not completely understood and still needs to be investigated. Therefore, in the present study, we evaluated the effects of three different concentrations of mangiferin on NAFLD in HFD-fed mice. We investigated whether mangiferin could affect the development of NAFLD and improve inflammatory responses, autophagy, and glycolipid metabolism in the livers of the mice to provide greater mechanistic insight.

2. Materials and methods

2.1. Animals and experimental design

Male Kunming mice [18] (6 weeks old) were purchased from the Center for Experimental Animals, Xuzhou Medical University, and were acclimated for 1 week before treatments were administered. The animals were housed under humidity- (50 ± 10%) and temperature-controlled (23 ± 2 °C) standard conditions, and an alternating 12-h light/dark cycle. The mice had free access to water and rodent chow. Body weights were recorded twice per week. All animal care and handling protocols were approved by the Animal Ethics Committee of Xuzhou Medical University. All experiments were carried out in conformity with the recommendations of the Guidelines for Ethical Conduct in the Care and Use of Animals.

After 1 week of acclimation, the mice were randomly divided into five groups (n = 8 per group). In the normal control (Cont) group, the mice received vehicle (normal saline) for 12 weeks but no HFD or mangiferin. In the HFD group, the mice received HFD, containing 74% basal feed, 10% lard, 7% sucrose, 5% casein, 2% fishmeal, 2% maltose, and 0.1% methionine (Slaccas, Shanghai, China), for 12 weeks. In the mangiferin-treated HFD groups, the mice were administered mangiferin in normal saline (intraperitoneal injection) at the following doses for 12 weeks: low, 15 mg/kg body weight/day (HFD + L); middle, 30 mg/kg body weight/day (HFD + Mid); and high, 60 mg/kg body weight/day (HFD + H) [19,20].

2.2. Antibodies and chemicals

Mangiferin was purchased from Fengshanjian Medicinal Research Co., Ltd. (purity > 97%, Kunming, China). β-Actin antibody was purchased from Bioworld Technology, Inc. (St. Louis Park, MN, USA). Sterol regulatory element-binding protein-1 (SREBP-1) antibody was purchased from Santa Cruz Biotechnology (Dallas, TX, USA). Microtubule-associated protein 1A/1 B light chain 3 (LC3), mTOR, phosphorylated mTOR (p-mTOR), 70-kDa ribosomal protein S6 kinase (p70S6 K), phosphorylated p70S6 K (p-p70S6 K), AKT, p-AKT, AMPK, and p-AMPK antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA), whereas NF-κB, JNK, p-JNK, and nucleoporin p62 antibodies were purchased from Abcam (Cambridge, UK). Secondary antibodies were purchased from Beijing Emarbio Science & Technology Co., Ltd. (Beijing, China).

2.3. Histological examination

For histological assessments, liver tissues were fixed in 10% buffered formalin for 24 h, embedded in paraffin, and cut into 5-μm sections before being stained with hematoxylin and eosin (H & E) for assessment by light microscopy.

2.4. Determination of blood and liver triglyceride (TG) and total cholesterol (TC) levels

Mice were fasted overnight and ocular blood was collected. The blood samples were centrifuged at 3000 rpm for 15 min to obtain serum. Total liver lipids were extracted by the Folch method. Hepatic tissue (0.5 g) was homogenized with 10 mL of chloroform-methanol (2:1), agitated for 15–20 min in an orbital shaker at room temperature, and centrifuged at 2500 rpm for 5 min at 4 °C. The supernatant was collected, placed in a 125-mL separatory funnel, washed with 2 mL of 0.9% NaCl, shaken for 2 min, and allowed to stand for the components to separate into layers. Next, the chloroform-methanol solution was discharged in a 100-mL round-bottom flask and evaporated on a water bath at 70 °C. Finally, the residue was dissolved in 4 mL of chloroform. TG and TC levels in the serum and tissue homogenate samples were analyzed using a fully automated biochemical analyzer (Beckman Coulter Inc., Brea, CA, USA).

2.5. Intraperitoneal glucose tolerance test

Glucose tolerance was determined by the intraperitoneal glucose tolerance test (ipGTT). All the mice were fasted in fresh cages, with water supply provided, for 16 h on the day before the experiment and were injected intraperitoneally with 10% glucose in phosphate-buffered saline at a dose of 2 mg/g body weight [21]. Blood samples were collected from the tail vein at 0, 30, 60, 90, and 120 min, and glucose levels were measured using a glucometer (Johnson & Johnson Medical (China), Ltd., Shanghai, China).

2.6. Western blotting analysis

An amount of preserved liver was homogenized in lysis buffer using a glass homogenizer in an ice water bath. The homogenate was placed on ice for at least 30 min and centrifuged at 12,000g for 10 min, after which the supernatant was analyzed by western blotting. Protein concentrations in the extracts were determined using a bicinchoninic acid protein assay kit (Beyotime Institute of Biotechnology, Nanjing, China). The protein samples were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred onto Biotrace™ nitrocellulose membranes. The membranes were blocked with 3% blocking buffer for 60 min and incubated overnight at 4 °C with primary antibodies diluted at 1:1000. Next, the membranes were incubated with secondary antibodies (diluted at 1:1000) at room temperature for 60 min, followed by analysis by electrogenerated chemiluminescence. Image J software (National Institutes of Health, Bethesda, MD, USA) was used to quantify signal intensities. The signals were normalized to those of β-actin, which was used as the internal control.

2.7. Statistical analysis

All results are presented as means ± standard errors (SE). Intergroup variations were analyzed by one-way analysis of variance using SPSS 13.0 software (IBM Corporation, Armonk, NY, USA), followed by Tukey's test. P values < 0.05 were considered statistically significant.

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