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Original article

Natural products berberine and curcumin exhibited better ameliorative effects on rats with non-alcohol fatty liver disease than lovastatin

Wu-wen Feng[a,b,1], Shuang-yu Kuang[c,1], Can Tu[a], Zhi-jie Ma[a], Jing-yao Pang[a], Yan-hui Wang[a], Qing-ce Zang[a], Ta-si Liu[d], Yan-ling Zhao[a], Xiao-he Xiao[a], Jia-bo Wang[a,*]

[a] Integrative Medical Center for Liver Diseases, Beijing 302 Hospital, Beijing, PR China
[b] College of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu, PR China
[c] Department of Clinical Pharmacy, The Second Affiliated Hospital, University of South China, Hengyang, PR China
[d] College of Pharmacy, Changsha University of Traditional Chinese Medicine, Changsha, PR China

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ABSTRACT

Studies have shown that satins and herbal products have potential to treat non-alcohol fatty liver disease (NAFLD) in clinic. However, no study has compared their effects, and their mechanisms remain unresolved. Here, we choose lovastatin and two herbal products including berberine and curcumin to compare their effects in treating NAFLD. NAFLD model was established by high fat food, and rats were administrated with lovastatin, berberine, curcumin, berberine + curcumin at the dosage of 100, 100, 100, 50 + 50 mg/kg bw, respectively. The body weight, visceral fat gain, histological inspection and serum parameters were studied to exam the curative effects. In addition, mediators including SREBP-1c, caveolin-1, pERK, NF-κB, TNF-α, and pJNK were studied. Results showed that berberine + curcumin group exhibited lower body and fat weigh compared with lovastatin group. Biochemical assays showed that LDL-c, ALT, AST, ALP, MDA, LSP level were lower in berberine + curcumin group compared with lovastatin group. Lower expression of SREBP-1c, pERK, TNF-α, and pJNK were also observed in berberine + curcumin group. We conclude that combination of curcumin and berberine exhibited better ameliorative effects in treating NAFLD than lovastatin, and this enhanced effect is associated with oxidative stress, hepatic inflammation and lipid metabolism.

1. Introduction

With the transition of life style and changes in diet structure, non-alcohol fatty liver disease (NAFLD) is emerging as one of the most common type of chronic liver diseases in clinic with 10–24 percent of the general population in many countries being affected [1]. It encompasses a broad spectrum of hepatic pathology such as steatohepatitis, liver fibrosis and cirrhosis which may even progress to hepatocellular carcinoma [2,3]. As it severely impairing human life quality and life span and for its high incidence, NAFLD is gaining increasing appreciation around the world. It is recommended that first line treatment should consist of lifestyle change with weight loss and exercise to improve insulin sensitivity. However, because of long term compliance difficulties, pharmaceutical agents aimed at reducing insulin resistance or protecting the liver from additional insults are needed [4]. For medical treatment of NAFLD, several medicines for it have been studied, such as ursodeoxycholic acid and vitamin E, yet, those drugs used for treating on NAFLD are sometimes insufficient and can have serious side effects [5,6]. Therefore, searching for medicines of low toxicity and high effect is of great significance. The cynosures of those medicines are satins and herbal compounds.

Of a variety of statins, lovastatin is the first statin that was approved in 1987 for clinic use as cholesterol-lowering agent. In the following years, it was proved to be safe in NAFLD patients [7,8]. With its safety demonstrated, experts encourage administration of it to patients of NAFLD [9]. Even so, animal and large scale randomized controlled trials are needed to conform the efficacy of them [9]. Except for statins, many herbal compounds are demonstrated to be safe in treating NAFLD. The typical herbal compounds for treating NAFLD are berberine and curcumin. Berberine is one major active constituent extracted from *Coptis chinensis* Franch. It has long been used to treat diarrhea, inflammatory diseases, and metabolic disorders including obesity and diabetes [10,11]. Curcumin is a natural yellow polyphenol isolated from *Curcuma longa* L. It has played important role in culinary and medicinal use in the traditional medicine of several countries for its antioxidant, anti-inflammatory, anti-mutagenic, antimicrobial, and

* Corresponding author.
E-mail address: pharm_sci@126.com (J.-b. Wang).
[1] These authors contributed equally to this work.

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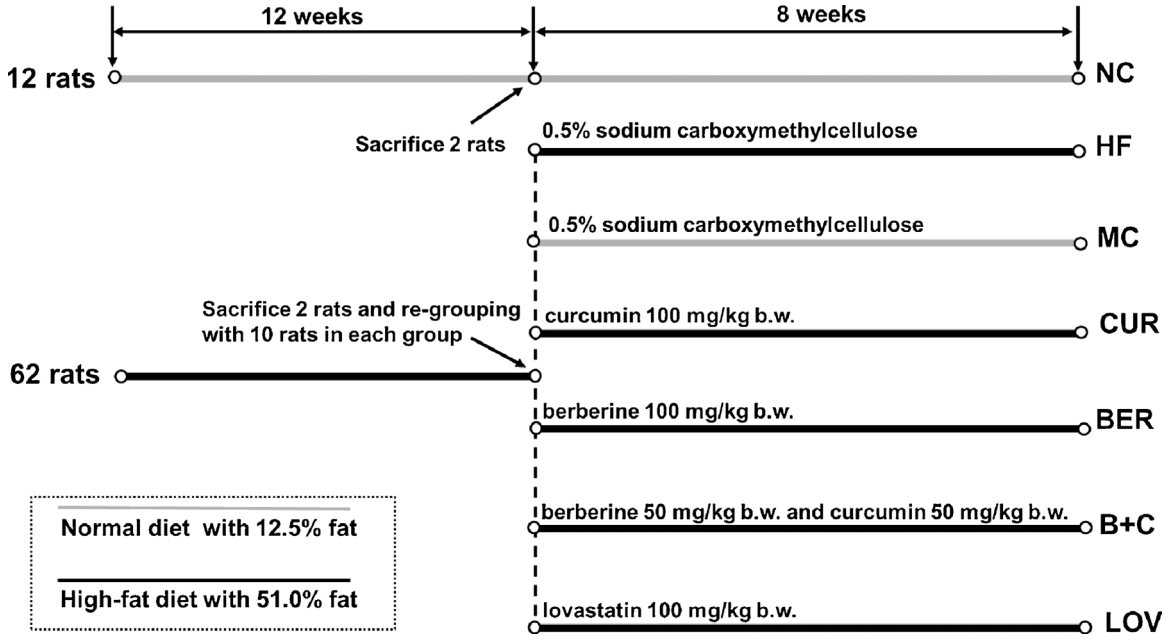

Fig. 1. The schematic diagram of the animal experimental design.

anticancer properties [12]. Recently, a lot of studies have shown that both constituents exhibit ameliorative effects on rats and patients with NAFLD [13–19]. Although the safety of lovastatin, berberine and curcumin has been confirmed, the efficacy of them in clinic has not been confirmed in large clinical study [9,20]. Therefore, determination of which of them is of better efficacy can be important for clinical selection of medicines for treating NAFLD.

Studies have showed that combination of different drugs can have stronger curative effects in curing some diseases such as hypertension and cancer [21,22]. And the strategy of combining herbals is commonly used in treating of diseases, such as traditional Chinese medicine. Therefore, the present investigation was designed not only to determine which one of them (lovastatin, berberine or curcumin) is the best candidate for treating NAFLD, but also to confirm whether combination of berberine and curcumin could exhibit enhanced curative effects. In addition, the possible mechanisms responsible for the enhanced effects of combination of herbal medicines were discussed.

2. Materials and methods

2.1. Reagents and equipment

Berberine and curcumin of purity > 98% were purchased from Sichuan Weikeqi Biological Technology Co., Ltd. (Chengdu, Sichuan, China) and Zhengzhou Stars Shine Technology Co., Ltd. (Zhengzhou, Henan, China) respectively. Detection kits for low-density lipoprotein cholesterol (LDL-c), low-density lipoprotein cholesterol (HDL-c), alanine aminotransferase (ALT), asparate aminotransferase (AST), alkaline phosphatase (ALP), total cholesterol (TC) and triglyceride (TG) were provided by Shanghai Kehua Bio-engineering Co., Ltd. (Shanghai, China). Free fatty acids (FFA) detection kits were obtained from Sekisui Medical Co., Ltd. (Tokyo, Japan). Detection kits for glutathione peroxidase (GSH-Px), Malondialdehyde (MDA), Glucose detection kits were obtained from NanJing JianCheng Bioengineering Institute (Nanjing, Jiangsu, China) to detect the fasting serum glucose (FSG). Insulin detection kits were got from Beijing North Biotechnology Institute (Beijing, China) to detect the fasting serum insulin (FSI). Lipopolysaccharide (LPS) were determined using a professional tool kit (Shanghai Kunkeng biochemical engineering Co., Ltd., Shanghai, China). TIANScript RT kit was supplied by Tiangen Biotech Co., Ltd (Beijing, China). SYBR FAST qPCR Kit Master Mix (2×) Universal was obtained from Kapa Biosystems (Woburn, MA, USA). Mouse monoclonal antibodies against caveolin-1, pJNK, TNF-α and SREBP-1c (Abcam, USA) and NF-κB (Abgent, USA) were used for western blot analysis.

2.2. Animals and treatment

A total of 74 male Sprague-Dawley rats aged 6 weeks (160–180 g) were obtained from Laboratory Animal Center of the Academy of Mititary Medical Sciences (Certification number SCXK-JUN 2007-004). The rats were allowed with free access to water and food for 1 week for acclimation. The room temperature was maintained at 20 ± 2 °C with 60%–70% humidity with 12-h light/dark cycle. The experimental protocol was approved by the Institutional Animal Care and Use Committee of 302 hospital of PLA and was in conformity with the US Public Health Service Policy on Humane Care and Use of Laboratory Animals.

After acclimation for 1 week, rats were allocated to normal control group (NC) fed with normal diet (62.3% carbohydrate, 12.5% fat and 24.3% protein in total calories) or a high-fat diet group (32.6% carbohydrate, 51.0% fat and 16.4% protein). The high-fat diet was made by mixing normal diet with cholesterol, lard, yolk powder, and bile salts. In the 2nd, 4th, 5th week, blood samples were drawn from orbit to detect the TG, TC, LDL-c, and HDL-c. In the 12th week, 2 rats in each group were sacrificed to inspect the effect of high fat diet on the liver, besides, the high-fat diet group were further randomized into six subgroups: the high fat group (HF, intragastrically treated with 0.5% sodium carboxymethylcellulose), model control group (MC, intragastrically treated with 0.5% sodium carboxymethylcellulose), berberine group (BER, intragastrically treated with berberine 100 mg/kg bw,), curcumin group (CUR, intragastrically treated with curcumin 100 mg/kg bw,), berberine + curcumin group (B + C, intragastrically treated with berberine 50 mg/kg bw and curcumin 50 mg/kg bw), and lovastatin group (LOV, intragastrically treated with lovastatin 100 mg/kg bw). The treatment was continued for 8 weeks. Except for MC and NC group, all the groups were treated with high-fat diet in the last 8 weeks. Fig. 1 shows the detailed grouping and treating of rats. Body weight was recorded every week in the whole course of study