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Liraglutide protects non-alcoholic fatty liver disease via inhibiting NLRP3 inflammasome activation in a mouse model induced by high-fat diet

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ABSTRACT

Liraglutide, a glucagon-like peptide-1 (GLP-1) analogue that has recently become the first-line treatment for type 2 diabetes mellitus (T2DM), has also been reported to decrease fatty degeneration of the liver. The purpose of this study is to explore whether liraglutide improves high-fat diet-induced non-alcoholic fatty liver disease (NAFLD) in mice through inhibiting the NLRP3 inflammasome in the liver. After daily intraperitoneal injection of liraglutide (0.6 mg/kg body weight) for four weeks, the liver, liver/body weight, serum levels of ALT, AST, total cholesterol, triglycerides and LDL were significantly decreased in a high-fat diet-induced NAFLD mouse model. The hepatic steatosis among sections of H&E and Oil Red O staining was also markedly reduced after treatment with liraglutide. The expressions of NLRP3 inflammasome components (including NLRP3, ASC, and caspase-1) in the liver of mice after treatment with liraglutide were decreased substantially. In vitro studies found that the mitochondrial dysfunction in Kupffer cells induced by palmitic acid was attenuated, and the protein levels of NLRP3, ASC and caspase-1 were also decrease markedly. These results demonstrate that liraglutide was able to alleviate high-fat diet-induced hepatic steatosis via inhibiting NLRP3 inflammasome activation, suggesting that liraglutide is a potent drug that can reverse the pathological hallmarks of NAFLD.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD), characterized by excessive accumulation of fat in the liver tissue, is a condition that develops in the absence of excessive alcohol intake [1]. It has become a broad-spectrum chronic liver disease in the world [2]. A large proportion of NAFLD cases may progress from simple non-alcoholic steatosis to non-alcoholic steatohepatitis (NASH), and eventually fibrosis, cirrhosis and hepatocellular carcinoma (HCC) [3]. Increasingly, hypertension, hyperlipidemia, central obesity and type 2 diabetes mellitus (T2DM) are the risk factors for NAFLD and NASH [4]. NAFLD is closely related to insulin resistance and is associated with hepatocytes of metabolic syndrome [5,6]. The two hits theory is widely accepted as explaining the pathogenesis of NAFLD. The first hit is the accumulation of free fatty acids in hepatocytes, which results in the second hit in the pathogenesis of NAFLD – an excess of inflammatory mediators and oxidative stress that ultimately results in liver injury, inflammation and fibrosis [7,8]. There are currently no specific therapeutic strategies for NAFLD management. Previous studies have indicated that improving insulin sensitivity with lifestyle interventions and reducing weight may contribute to NAFLD therapy [9,10]. However, a pharmacologic treatment for NAFLD is urgently needed [11].

The NOD-like receptor family pyrin containing 3 (NLRP3) inflammasome has been proven to play a key role in NAFLD and NASH [6,12]. It is well known that IL-1β contributes to the progression of atherosclerosis and fibrosis [13]. Previous studies suggested that IL-1β secretion results from saturated fatty acids via activation of NLRP3, resulting in the aggravation of liver injury [14,15]. When danger signaling molecules are released, the

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inflammatory cells gather in the liver to repair [16,17]. The critical role of the NLRP3 inflammasome in the pathogenesis of NAFLD makes it an attractive target for the development of pharmaceutical therapies to treat NAFLD.

We tried to find a pharmacological inhibitor that targets the NLRP3 inflammasome in order to improve or halt the progression of NAFLD. Liraglutide is a human glucagon-like peptide-1(GLP-1) analogue that has become the first-line treatment for T2DM [18,19]. Recent studies demonstrated that GLP-1 receptor agonist improves insulin resistance and reduces high-fat diet-induced lipid accumulation and hepatic steatosis [20]. Additionally, GLP-1 receptor agonist is able to improve beta-cell growth, thus enhancing insulin secretion and reducing appetite and body weight [20]. Recent advances showed that liraglutide also has beneficial effects on NASH during the treatment of T2DM [21,22]. In this study, we investigate whether liraglutide improves high-fat diet-induced NAFLD in a mouse model via inhibiting the activation of the NLRP3 inflammasome in the liver.

2. Materials and methods

2.1. Animals and diets

Forty-five 6-week-old C57BL/6 mice (male) were purchased and fed in the laboratory animal research center of Chongqing Medical University (Chongqing China). Mice were randomly divided into three groups (15 mice in each group): normal diet (ND), high-fat diet (HFD), and high-fat diet with daily intraperitoneal injection of liraglutide (HFD + liraglutide). After feeding for 32 weeks, the ND and HFD groups were given daily intraperitoneal injection of saline (0.6 mg/kg body weight, intraperitonially) for four weeks, and the HFD + liraglutide group was given a daily intraperitoneal injection of liraglutide (0.6 mg/kg body weight, intraperitoneally) for four weeks. Subsequently, mice were euthanized. Serum and liver tissue were stored at low temperatures for further experiments. All animals were fed under IVC conditions and were kept in a 12-h light/12-h dark cycle (20 ± 2 °C, lights on 7:00 a.m. to 19:00 p.m.) and given human care.

2.2. Biochemical analysis

Serum levels of liver function (ALT, AST, LDL) and blood fat (TC, TG) were examined in the clinical laboratory of the Children's hospital of Chongqing Medical University. Serum or supernatant IL-1β and TNF-α levels were measured with enzyme-linked immunosorbent assay (ELISA) kits. After fasting for 12 h, the glucose tolerance tests (GPTTs) and the insulin tolerance tests (IPGTTs) were measured using Roche blood sugar test paper (Roche Diagnostics Limited Company of Germany) at 0, 15, 30, 60, and 120 min after the glucose challenge.

2.3. Histological analysis

A part of the liver tissues was fixed in 10% formalin for 48 h, embedded in paraffin, and sectioned at a thickness of 5 μm. Some tissue was stained with hematoxylin and eosin (H&E) to detail the fatty degeneration in the liver. Another portion of the tissue was prepared for immunohistochemical analysis. Another part of liver tissues was embedded in oct medium and sectioned at a thickness of 10 μm. These sections were stained with Oil Red O to detail lipid droplets. The images of the H&E sections and immunohistochemical sections and Oil Red O sections were captured via transmission electron microscopy (TEM, Hitachi, Japan). The ultrastructures of the cell mitochondria were observed using a transmission electron microscope in the center of Chongqing Medical University.

2.4. Western blotting

Approximately 0.2 g liver tissue was crushed, and the raw or macrophage cells were extracted using a RIPA lysate and then centrifuged at 12,000×*g* for 15 min. The supernatant contained total protein, which was extracted into a loading buffer. The protein

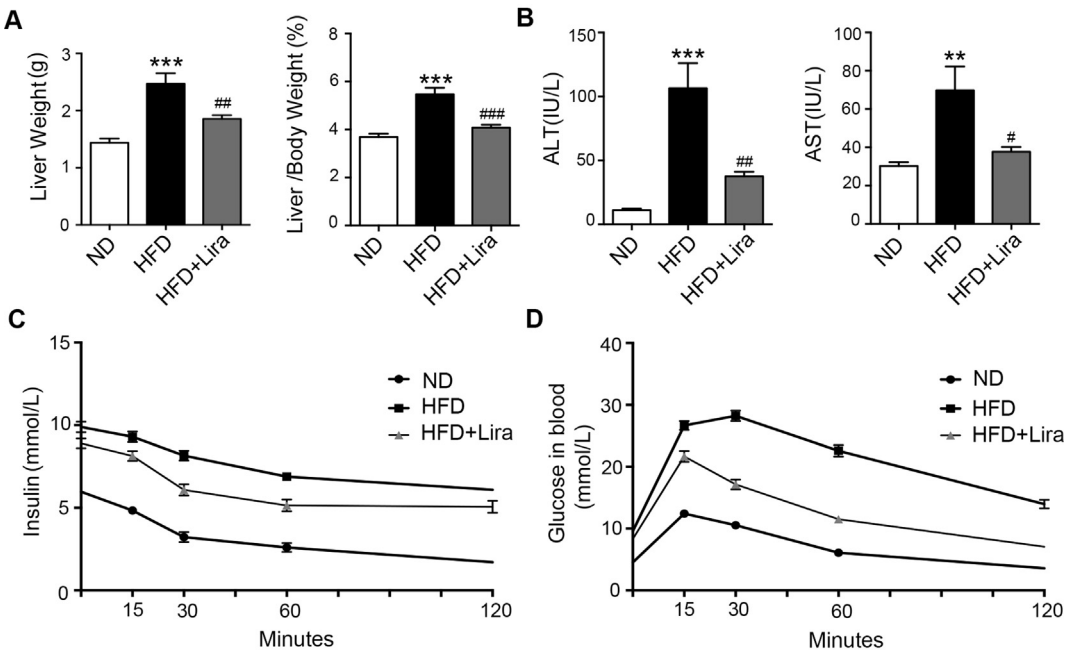

Fig. 1. Liraglutide improves hepatic function, glucose tolerance and insulin tolerance in a high-fat diet-induced NAFLD mouse model. A. Liver weight and liver/body weight were decreased in the HFD-Lira group. B. The serum levels of ALT and AST were significantly decreased in mice after treatment with liraglutide. C. The insulin tolerance was lower in the HFD-Lira group than that in the HFD group. (D) The glucose tolerance was lower in the HFD-Lira group than that in the HFD group. N = 12 for each group. Values are presented as the mean ± SEM, one-way analysis of variance. *Significantly different from ND. #Significantly different from HFD. * or $^{\#}P < 0.05$, ** or $^{\#\#}P < 0.01$, *** or $^{\#\#\#}P < 0.001$.

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