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Sitagliptin attenuates methionine/choline-deficient diet-induced steatohepatitis

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ABSTRACT

Aims: Accumulating evidence suggests that inhibitors of dipeptidyl peptidase-4 (DPP-4), such as sitagliptin, may play an important role in the prevention of non-alcoholic steatohepatitis (NASH). This study was conducted to elucidate whether sitagliptin could prevent steatohepatitis by inhibiting pathways involved in hepatic steatosis, inflammation, and fibrosis.

Methods: C57BL/6 mice were fed a methionine/choline-deficient (MCD) diet with or without supplement with sitagliptin for 5 weeks. Liver and adipose tissue from mice were examined histologically and immunohistochemically to estimate the effect of sitagliptin on the development of NASH.

Results: Supplementation with sitagliptin resulted in significant improvement of MCD diet-induced fat accumulation in the liver. In addition, sitagliptin treatment lowered fatty acid uptake, expression of VLDL receptor and hepatic triglyceride content. Sitagliptin also effectively attenuated MCD diet-induced hepatic inflammation, endoplasmic reticulum (ER) stress, and liver injury, as evidenced by reduced proinflammatory cytokine levels, ER stress markers, and TUNEL staining. Expression of CYP2E1 and 4NHE were strongly increased by the MCD diet, but this effect was successfully prevented by sitagliptin treatment. Furthermore, sitagliptin significantly decreased levels of MCD diet-induced fibrosis-associated proteins such as fibronectin and α-SMA in the liver. Inflammatory and atrophic changes of adipose tissue by MCD diet were restored by sitagliptin treatment.

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Conclusions: Sitagliptin attenuated MCD diet-induced hepatic steatosis, inflammation, and fibrosis in mice through amelioration of mechanisms responsible for the development of NASH, including CD36 expression, NF-κB activation, ER stress, CYP2E1 expression, and lipid peroxidation. Treatment with sitagliptin may represent an effective approach for the prevention and treatment of NASH.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) has become an important worldwide health problem due to its high prevalence and close association with various metabolic diseases, including type 2 diabetes, metabolic syndrome, and cardiovascular disease [1]. NAFLD is a clinicopathological term that encompasses a full spectrum ranging from simple steatosis and non-alcoholic steatohepatitis (NASH) to cirrhosis and hepatocellular carcinoma [2,3]. NASH is characterized by an increase in intrahepatic triglyceride content with inflammation and fibrosis [3]. Numerous clinical trials showed that NASH is closely associated with insulin resistance and type 2 diabetes [4–7]. However, evidence for the use of insulin sensitizers or anti-diabetic agents in NASH is limited. Although glitazones are known to improve steatohepatitis and delay the progression of fibrosis, there is now evidence that prolonged treatment with these agents may offer no additional histological benefit and that metabolic improvement does not necessarily parallel histological improvement [8].

Dipeptidyl peptidase 4 (DPP-4) inhibitors are a new leading class of oral hypoglycemia drugs that avoid many of the safety and tolerability issues associated with conventional agents. Glucagon-like peptide-1 (GLP-1), an incretin that stimulates glucose-dependent insulin secretion, is rapidly degraded by DPP-4. Thus, DPP-4 inhibition resulted in elevated bioactive incretin levels and improved glucose tolerance and reduced glucagon levels. DPP-4 is also expressed on the surface of most cell types and has a multiplicity of biological functions [9]. For these reasons, recent studies have investigated the metabolic benefits of DPP-4 inhibitors on various organs and diseases such as adipose tissue inflammation, diabetic nephropathy, and cardiovascular disease [10–12]. In addition to anti-diabetic effects, several lines of evidence suggest that DPP-4 inhibitors have considerable therapeutic potential for NAFLD. Several recent clinical studies showed that NASH patients had significantly increased levels of serum DPP-4 compared with control groups [13] and that the histological liver steatosis grade was associated with DPP-4 staining in the liver [14]. Moreover, DPP-4 may also be involved hepatic fibrosis by controlling interactions between DPP-4-expressing hepatocytes [15,16]. Sitagliptin, a DPP-4 inhibitor, showed promising activity against NASH in humans and diet-induced animal models [17,18]. However, experience with sitagliptin use for the treatment of NASH is still very limited.

The mechanisms responsible for the development and progression of NASH in humans have not been fully elucidated. Several lines of evidence suggest that increased fatty acid uptake to the liver and subsequent cytochrome P450 2E1 (CYP2E1) activation, endoplasmic reticulum (ER) stress, and activation of redox-sensitive inflammatory signaling pathways such as NF-κB are implicated in human NASH as well as methionine/choline-deficient (MCD) diet-induced steatohepatitis [19,20]. Because an MCD diet causes a steatohepatitis in rodents that is histologically similar to human NASH, it has been frequently used as an animal dietary model of NASH. MCD-fed mice show hepatic histological changes characterized by steatosis, focal inflammation, and fibrosis [21]. The mechanisms responsible for the development of steatohepatitis in the MCD diet model have been established [22]. Similar to human NASH, the MCD diet considerably increases fatty acid uptake and reduces very low-density lipoprotein (VLDL) secretion, which causes activation of inflammatory signaling, induction of ER stress, activation of CYP2E1, and lipid peroxidation [22].

The purpose of the current study was to determine the protective effect of sitagliptin on the development of experimental steatohepatitis. Using the MCD diet-induced NASH model, we delineated the effects of sitagliptin on the signaling pathways and histological changes involved in NASH progression.

2. Materials and methods

2.1. Materials

Sitagliptin was provided by Merck Shap & Dohme (Kenilworth, NJ, USA). The anti-CD36 antibody, anti-F4/80 antigen antibody, and anti-cleaved ATF6 antibody were purchased from Abcam (Cambridge, UK). The anti-CYP2E1 antibody was purchased from ENZO Life Sciences (Farmingdale, NY, USA). The anti-4HNE antibody was purchased from Alpha Diagnostic (San Antonio, TX, USA), the anti-fibronectin antibody and anti-PAI-1 antibody were purchased from BD Biosciences (San Jose, CA, USA), the anti-α-SMA antibody was purchased from Sigma (St. Louis, MO, USA), and the anti-phospho-NFκB antibody, anti-CHOP antibody, anti-Phospho-eIF2α antibody, and anti-GAPDH antibody were purchased from Cell Signaling Technology (Beverly, MA, USA).

2.2. Animals and diets

All animal procedures were carried out in accordance with institutional guidelines for animal research. Experiments were conducted using male 8-week-old C57BL/6 mice (Hanasangsa, Pusan, Korea). Mice were divided into three groups and fed a methionine/choline-sufficient (MCS) diet (Dyets, PA, USA), an MCD diet (Dyets, PA, USA), or an MCD diet supplemented with sitagliptin (20 mg/kg) (Dyets, PA, USA).

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