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Lipid-lowering agents inhibit hepatic steatosis in a non-alcoholic steatohepatitis-derived hepatocellular carcinoma mouse model

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is associated with various metabolic disorders, and the therapeutic strategies for treating NAFLD and non-alcoholic steatohepatitis (NASH) have not been fully established. In the present study, we examined whether lipid-lowering agents inhibited the progression of NAFLD and tumorigenesis in a non-alcoholic steatohepatitis-derived hepatocellular carcinoma model mouse (STAM mice) generated by streptozotocin injection and a high-fat diet.

Seven-week-old STAM mice were divided into groups fed a high-fat diet (Ctl) or a high-fat diet supplemented with ezetimibe (Ez), fenofibrate (Ff), rosuvastatin (Rs), ezetimibe plus fenofibrate (EF), or ezetimibe plus rosuvastatin (ER) for 4 weeks. At the end of the experiments, an oral glucose tolerance test, an insulin tolerance test, biochemical analyses using serum and liver, and a histological analysis of liver were performed in 11-week-old STAM mice.

The lipid-lowering agents did not affect the body weight or the casual blood glucose levels in any of the groups. The serum triglyceride level was significantly decreased by Ff, Rs, and EF. Glucose tolerance was improved by Ez and Ff, but none of these agents improved insulin sensitivity. A histochemical analysis revealed that the lipid-lowering agents, with the exception of Rs, significantly inhibited the progression of hepatic steatosis. Nonetheless, no significant changes in the incidence of hepatic tumors were observed in any of the groups.

Lipid-lowering agents inhibited the progression of hepatic steatosis without suppressing tumorigenesis in STAM mice. Our data has implications for the mechanism underlying steatosis-independent hepatic tumorigenesis in mice.

1. Introduction

The prevalence of non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) is increasing because of the dramatic increase in obesity and diabetes worldwide. NAFLD and NASH have become serious issues that can cause liver cirrhosis and subsequently lead to hepatocellular carcinoma. Insulin resistance observed in obese individuals and patients with type 2 diabetes (T2D) is one of the causes of NAFLD (Chitturi et al., 2002). The prevalence of ultrasonographic NAFLD in individuals with T2D has been reported to be 69.4% (Leite et al., 2009), and the prevalence of NAFLD has been shown to increase in a linear manner with body mass index, triglycerides, and low-density lipoprotein cholesterol (Eguchi et al., 2012). To date, activators of peroxisome proliferator-activated receptor, such as thiazolidinediones, have been reported to attenuate insulin resistance and to prevent the progression of NAFLD (Belfort et al., 2006). However, a strategy for the treatment of NAFLD has not yet been established.

Dyslipidemia is also known to be a risk factor for NAFLD as well as insulin resistance (Chalasani et al., 2012). Several studies have reported that lipid-lowering agents may improve liver biochemistry and histology in humans (Foster et al., 2011; Rallidis et al., 2004). A number of studies in mice have also shown the inhibitory effect of lipid-lowering agents on the progression of NAFLD. Pitavastatin suppressed diethylnitrosamine-induced liver pre-neoplasms and improved steatosis in C57BL/KsJ-db/db mice (Shimizu et al., 2011). Furthermore, a peroxisome proliferator-activated receptor α (PPARα) agonist, fenofibrate reduced hepatic steatosis in fatty liver Shionogi mice (Harano et al., 2006). Ezetimibe improved

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high-fat and cholesterol diet-induced NAFLD in C57BL/6J mice (Zheng et al., 2008). In these previous studies, the favorable effects of these lipid-lowering agents on the progression of steatohepatitis were assessed using NASH models, which rarely develop hepatocellular carcinoma (HCC), and the suppressive effects of these agents on the progression of HCC remain obscure in NASH-derived HCC models.

Rodent NASH models, including leptin or leptin receptor-mutation models, dietary methionine choline-deficient (MCD) models, and long-term high-fat diet (HFD)-fed models, rarely develop typical tumorigenesis in the liver, as observed in human HCC (Horie et al., 2004; Muraoka et al., 2011; Sahai et al., 2004; Weltman et al., 1996). Recently, Fujii et al. (2013) generated a NASH-derived HCC mouse model that they named "STAM mice". STAM mice are generated by subcutaneous streptozotocin (STZ) injection and feeding with a HFD and develop hyperglycemia, hypoinsulinemia, and elevated transaminase and liver triglyceride levels. This model is thought to replicate the clinicopathological course from fatty liver, steatohepatitis, and fibrosis to hepatocellular carcinoma (Fujii et al., 2013). L-carnitine, which is an essential nutrient for the conversion of fat into energy in mitochondria, prevented the progression of non-alcoholic steatohepatitis by upregulating mitochondrial β-oxidation in the STAM mouse model (Ishikawa et al., 2014). STAM mice are thought to be a useful model for investigating the pathogenic mechanism of NAFLD-derived hepatocellular carcinoma. These findings inspired us to investigate whether lipid-lowering agents prevented the progression of NAFLD and hepatic tumorigenesis in STAM mice.

2. Materials and methods

2.1. Animal model

Six-week-old male STAM mice, a NASH-cirrhosis-hepatocellular carcinoma model, were purchased from Stelic Institute & Co., Inc. (Tokyo, Japan). The mice were given free access to food and water and were kept in a room with a constant room temperature (25 °C) and a 12-h light/dark cycle. After 1 week of HFD feeding, the seven-week-old male STAM mice were divided into six groups and were fed for another 4 weeks as follows: (1) HFD (Ctl); (2) HFD supplemented with 0.005% wt/wt ezetimibe (Ez); (3) HFD supplemented with 0.1% wt/wt fenofibrate (Ff); (4) HFD supplemented with 0.01% wt/wt rosuvastatin (Rs); (5) HFD supplemented with combination of 0.005% wt/wt ezetimibe and 0.1% fenofibrate (EF); and (6) HFD supplemented with combination of 0.005% wt/wt ezetimibe and 0.01% wt/wt rosuvastatin (ER). After 4 weeks of feeding, the 11-week-old mice were killed (Fig. 1A). A

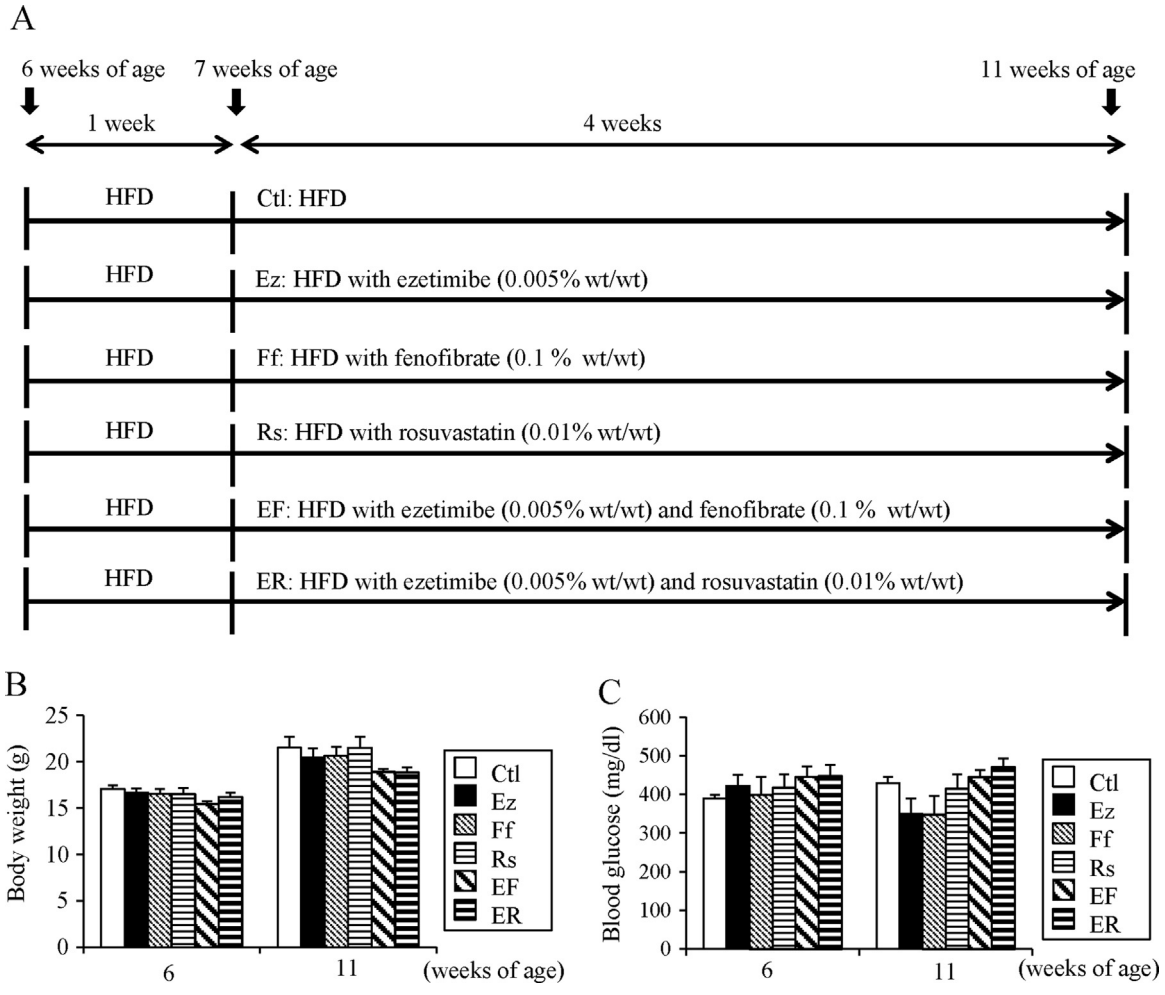

Fig. 1. *Comparison of body weight and casual blood glucose level at 6 and 11 weeks of age in STAM mice.* (A) Experimental protocol. Six-week-old STAM mice were fed HFD until 7 weeks of age. The mice were then fed HFD supplemented with lipid-lowering agents until 11 weeks of age. (B) Body weight was determined at both 6 and 11 weeks of age in groups of mice fed either HFD or HFD supplemented with lipid-lowering agents. Mice were fed HFD (Ctl) (open bar) or HFD supplemented with the following agents: ezetimibe (Ez) (solid bar), fenofibrate (Ff) (light hatched bar), rosuvastatin (Rs) (light striped bar), combination of ezetimibe with fenofibrate (EF) (dark hatched bar), or combination of ezetimibe with rosuvastatin (ER) (dark striped bar) (each group, $n=5–6$). (C) Casual blood glucose levels were determined at 6 and 11 weeks of age in each group of mice (each group, $n=5–6$).