REGULATION OF HEPATIC LIPID METABOLISM IN LEPTIN DEFICIENT (OB/OB) MICE BY 6,7-DIMETHYLESCEULTIN

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Background and Aim: Nonalcoholic fatty liver disease (NAFLD) has become increasingly recognized as the most common cause of chronic liver disease. 6,7-Dimethylesculetin activates constitutive androstane receptor in primary hepatocytes and accelerates bilirubin clearance in vivo. The present study was to test the efficacy of 6,7-Dimethylesculetin, for it’s ability to modulate pathways implicated in obese, ob/ob mice that develop hyperlipidemia and fatty livers.

Methods: To evaluate this possibility, leptin deficient ob/ob mice with fatty livers were treated with 6,7-Dimethylesculetin (100 mg/kg, po), an agent that possessed lipid lowering and anti-inflammation properties. At the end of the experiment, serum biochemical parameters, liver histology and lipid profile were analyzed. In the liver, the SREBP-1 and PPAR gamma protein levels were determined by Western blot and messenger RNA levels of some enzymes involved in lipogenesis were examined by real-time polymerase chain reaction. The effects of 6,7-Dimethylesculetin on hepatic CB1 and total GSH/GSSG were also examined.

Results: Following a 4 week protocol, 6,7-Dimethylesculetin suppression of hepatomegaly and steatosis was reflected by a 18% lower liver average triglyceride content as compared with obese controls. In addition, 6,7-Dimethylesculetin simultaneously decreased hepatic fatty acid synthesis (SREBP-1c, FAS) and increased beta-oxidation (CPT-1, CPT-2) genes expression of ob/ob mice. Reduction of SREBP-1 protein level in livers of ob/ob mice by 6,7-Dimethylesculetin treatment modestly improves fat drop deposition, and completely normalizes the increased expression of CB1-mediated effects. 6,7-Dimethylesculetin also suppressed systemic inflammation by reducing the serum level of tumor necrosis factor alpha. Moreover, ob/ob mice display decreased senescence marker protein-30 in liver that is normalized by 6,7-Dimethylesculetin, this in turn, helps to restore GSH concentrations and viability.

Conclusions: These data suggest that obese subjects in the persistent inflammatory states, such as elevated TNF-alpha, may have up-regulated CB1-mediated signaling by increasing SREBP-1 proteins, leading to up-regulation of fatty acid synthesis expression and increased oxidative stress in liver. Thus, 6,7-Dimethylesculetin may play an modulate role in pathogenesis of the hepatic steatosis by it’s biological effects of antioxidant and anti-inflammation properties.
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Abstract

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Figure 1. Regression of body weight in ob/ob mice (leptin -/-) (A). Leptin (+/+) mice display the characteristic signs of fatty liver (B, upper) and fat mass (B, lower) typically exhibited at 20 weeks.

Figure 2. Light microscopic analysis of liver sections of ob/ob mice (leptin -/-) and mice treated with scapornone. Histopathology of liver. Paraffin embedded sections were stained with H&E (A, B), and liver sections were incubated with Oil-Red O (D, E). Original magnification × 200.

Figure 4. Hepatic free fatty acid (FFA) (A), triglyceride (B), plasma FFA (C) and triglyceride (D) in ob/ob mice (leptin -/-) and scapornone treatment groups. Each bar represents the mean value of experiments performed in triplicate assay±SEM.

Figure 5. Detection of TNF-α expression from serum (A) and hepatic tissue (B) in ob/ob mice (leptin -/-) and scapornone treatment groups. Cytokine array pattern were scanned, and the intensities of signals were quantitated by densitometry. Amplification of hepatic TNF-α mRNA by RT-PCR from livers of mice. GAPDH was used as an internal control.

Figure 6. Scapornone attenuated leptin deficiency (–/–)– induce oxidative stress in the liver. (A) The levels of glutathione (GSH) in the liver were analyzed by HPLC. Scapornone reversed the down-regulated of total GSH in the liver of ob/ob mice. (B) SMP30 levels indicated the antioxidant status in the liver. Scapornone attenuated ob/ob mice induced suppression of SMP30 levels. Each bar represents the mean value of experiments performed in triplicate assay±SEM.

Figure 7. Western blot analysis of nuclear SREBP-1 in ob/ob mice after scapornone treatment. Histone was used as an internal control (upper). Amplification of hepatic SREBP-1c, FAS, SCD-1, and CPT-1 mRNA by RT-PCR from livers of ob/ob mice (lower). GAPDH was used as an internal control.

Figure 8. Inhibitory effects on C1 levels of scapornone in leptin (–/–) obese mice. Leptin (+/+) mice were treated with 100 mg/kg scapornone for 4 weeks. The amounts of C1 were determined by western blot (A) and immunohistochemistry analysis (B). The relative value of C1 protein is expressed as the intensity of β-actin.

Conclusion

Scapornone prevents lipid accumulation and thereby blocks the oxidative stress in the liver. Scapornone could also prevent the C1-lactivities, therefore, attenuates the sequential recruitment of lipogenesis factors, results in diminishes leptin deficiency-associated fatty liver diseases.

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