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Osthol regulates hepatic PPARα-mediated lipogenic gene expression in alcoholic fatty liver murine

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ABSTRACT

Our previous studies found that osthol, an active constituent isolated from *Cnidium monnieri* (L.) Cusson (Apiaceae), could ameliorate the accumulation of lipids and decrease the lipid levels in serum and hepatic tissue in alcohol-induced fatty liver mice and rats. The objective of this study was to investigate its possible mechanism of the lipid-lowering effect. A mouse model with alcoholic fatty liver was induced by orally feeding 52% erguotou wine by gavage when they were simultaneously treated with osthol 10, 20, 40 mg/kg for 4 weeks. The BRL cells (rat hepatocyte line) were cultured and treated with osthol at 25, 50, 100, 200 μg/ml for 24 h. The mRNA expressions of peroxisome proliferator-activated receptor (PPAR) α, diacylglycerol acyltransferase (DGAT), 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase and cholesterol 7α-hydroxylase (CYP7A) in mouse hepatic tissue or cultured hepatocytes were determined by reverse transcription polymerase chain reaction (RT-PCR). After treatment with osthol, the PPARα mRNA expression in mouse liver and cultured hepatocytes was increased in dose dependent manner, while its related target genes for mRNA expression, e.g., DGAT and HMG-CoA reductase, were decreased, the CYP7A was inversely increased. And osthol-regulated mRNA expressions of DGAT, HMG-CoA reductase and CYP7A in the cultured hepatocytes were abrogated after pretreatment with specific inhibitor of PPARα, MK886. It was concluded that osthol might regulate the gene expressions of DGAT, HMG-CoA reductase and CYP7A via increasing the PPARα mRNA expression.

Introduction

Alcohol remains one of the most common causes of chronic liver diseases in the world (Diehl 2002). Fatty liver, characterized by an accumulation of triglycerides (TG) and cholesterol in the liver, is the most common and earliest response of the liver to alcohol in heavy alcohol users. Although the pathogenesis of fatty liver by ethanol is complex and multifactorial, the lipogenesis has long been proposed as an important biochemical mechanism underlying the development of alcoholic fatty liver. It has been reported that impaired mitochondrial oxidation of long-chain fatty acids might result in the "fatty acid overload" and these accumulated fatty acids are diverted into esterification (TG synthesis) (Kaikaus et al. 1993; Ockner et al. 1993). In a study with human subjects, it was shown that the hepatic lipogenesis was activated after an ethanol consumption of 24 g per day (Siler et al. 1999). So, it is now accepted that long-term ethanol consumption can increase the rate of lipid synthesis in the liver of human being and animals by inducing the key lipogenic gene expression.

Peroxisome proliferator-activated receptor (PPAR) α, one of the PPAR receptors, is predominantly expressed in the liver (Braissant et al. 1996) and involved in the clearance of circulating and cellular lipids by controlling the expression of lipogenic genes including diacylglycerol acyltransferase (DGAT), 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase and cholesterol 7α-hydroxylase (CYP7A) (Berger and Moller 2002; Waterman and Zammit 2002; Konig et al. 2007).

DGAT is a microsomal enzyme expressed in the liver and rate-limited enzyme in the process of TG synthesis, catalyzes the final step in TG synthesis by converting diacylglycerol and fatty acyl-coenzyme A into TG (Farese et al. 2000). Some research data have shown that DGAT mRNA expression was close to PPARα (Waterman and Zammit 2002; Duval et al. 2007). And it is now accepted that PPARα activation may increase the uptake and β-oxidation of hepatic fatty acids, which decrease the availability of fatty acids for TG synthesis (Schoonjans et al. 1996). HMG-CoA reductase is the rate-limiting enzyme of cholesterol synthesis. In wild-type mice, an antiparallel relationship exists between the gene expression of PPARα and that of HMG-CoA reductase. It was also reported that PPARα activation could inhibit the expression

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of HMG-CoA reductase by reduction of nuclear sterol regulatory element binding protein-2 (Konig et al. 2007). Thus, these data strongly suggested that PPARα activation might reduce cholesterol synthesis by diminishing expression of its target gene HMG-CoA reductase. CYP7A is a liver-specific enzyme that catalyzes the transformation of cholesterol into bile acids. Deletion of the CYP7A gene in mice had a dramatic effect on bile acid development, while over-expression of the gene in hamsters could greatly reduce plasma cholesterol level. Since secretion of cholesterol and bile acids in the bile is the major route for the removal of cholesterol from the body, CYP7A plays an important role in cholesterol homoeostasis (Noshiro et al. 2007). It had been shown that PPARα was apt to induce the hepatic expression of liver X receptor, which subsequently up-regulates the gene expression of CYP7A (Chiang 2002).

Osthol (7-methoxy-8-isopentenoxycoumarin) (Fig. 1) is an active constituent isolated from the fruit of *Cnidium monnieri* (L.) Cusson, a Chinese herbal medicine. In the clinic, the fruit has been used in the treatment of skin disease and gynecopathy for many years (Lian 2003). Modern pharmacological studies have shown that osthol has anti-inflammation (Liu et al. 2005), anti-oxidation (Wang et al. 2005), anti-osteoporosis (Zhang et al. 2007), anti-apoptosis (Okamoto et al. 2003), anti-tumor (Zhou et al. 2002) and estrogen-like effects (Kuo et al. 2005). Our previous studies found that osthol could ameliorate the accumulation of lipids and decrease the lipid levels in serum and hepatic tissue in alcohol-induced fatty liver mice and rats, (Song et al. 2006; Sun et al. 2009), but its mechanism was not completely understood. In this study, the effects of osthol on PPARα and PPARα-mediated genes such as DGAT, HMG-CoA reductase, and CYP7A were investigated *in vivo* and *in vitro*.

Materials and methods

Reagents

Osthol was prepared and provided by Dr. Jia Zhou of Green Spring Natural Product Co., Ltd. (Xi'an, China), the purity was > 98% as determined by HPLC. Erguotou wine was a product of Bejing Red Star Co., Ltd. (Bejing, China), the composition of the wine is alcohol and water (52:48, v-v). Trizol was a product of Invitrogen (Carlsbad, CA, USA). Taq DNA polymerase and reverse transcriptase were products of Sangon Gene Company (Shanghai, China) and Fermentas (Vilnius, Lithuania), respectively. The primers used for amplification by reverse transcription polymerase chain reaction (RT-PCR) were synthesized by Sangon Gene Company (Shanghai, China). MK886 (purity > 99%), an inhibitor of PPARα, was obtained from Cayman Chemical Company. All other reagents used in this study were of analytical grade.

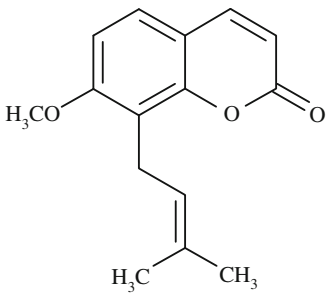

Fig. 1. Structure of osthol.

Mouse model of alcoholic fatty liver (Zhao et al. 2005; Guo et al. 2006)

Kunming mice (male, 7 weeks of age), weighing 23-27 g, were obtained from Animal Breeding Center of Soochow University (Suzhou, China), and were housed in regular cages in a room controlled temperature and humidity, and allowed free access to food and water. The animals were allowed to acclimatize to the laboratory environment for 7 days prior to the study. All animal studies were approved by the University Ethic Committee and conducted according to the regulations for the use and care of experimental animals at Soochow University (number of Ethic Commitee: 2008-84). These mice were randomly divided into 5 groups (n=6): control group, fatty liver model group, osthol 10, 20 and 40 mg/kg groups. The treated groups were orally given osthol 0.1 ml/10 g body weight per day by gavage based on different dose in the morning for 4 weeks, and control and model animals were orally given an equivalent volume of 0.5% sodium carboxymethyl cellulose solution. In order to induce the development of alcoholic fatty liver, all mice except the control group were simultaneously given 52% erguotou wine by gavage, which was administered in the afternoon for 4 weeks. The amount of the wine administered to the animals was gradually increased from 0.1 ml/10 g to 0.4 ml/10 g (full dose) body weight per day within one week according to animal tolerance. The mice were then killed by stunning and cervical dislocation after oral administration for 4 weeks, partial hepatic tissues were quickly taken and frozen in liquid nitrogen and stored at -80 °C for RT-PCR.

RT-PCR

RT-PCR was used to measure the mRNA expressions of PPARα, DGAT, HMG-CoA reductase, CYP-7A and GAPDH in mouse hepatic tissue. Hepatic tissue samples were immediately placed into Trizol reagent, total RNA was extracted according to the manufacturer's instructions. The final RNA pellet was resolved by 0.1% diethyl pyrocarbonate-treated water, the concentration and purity of the RNA were determined spectrophotometrically by the absorbance ratio 260:280 nm. Total RNA (2 μg) was used as the RT reaction following the manufacturer's introduction. After RT, 22 μl of a PCR master mix, including all PCR components and the primers (Table 1), was added to tubes containing 3 μl of cDNA. After the samples were overlaid with mineral oil, the tubes were placed in the DNA thermal cycler. The PCR conditions were as follows: 33 cycles of denaturation at 94 °C for 30 s, annealing (the temperature was seen in Table 1) for 45 s, and extension at 72 °C for 45 s after an initial step of 94 °C for 5 min. A final extension was 72 °C for 10 min. PCR products were conducted with 1.5% agarose gel electrophoresis and ethidium bromide staining, and quantitated by densitometry using the Image Master VDS system and associated software (Pharmacia, USA). Data were expressed as a ratio of the signals of interest band to that of GAPDH band, the latter acted as the internal control in the experiment.

BRL cell culture

The rat hepatocyte line (BRL cells) under passage 30 (Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Shanghai, China) was cultured in RPMI 1640 supplemented with L-glutamine and 10% calf serum, and was maintained at 37 °C in a humidified atmosphere with 5% CO_2.