Licochalcone A regulates hepatic lipid metabolism through activation of AMP-activated protein kinase

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ABSTRACT

Licochalcone A (LA) is a major phenolic ingredient of *Glycyrrhiza* plant. Although multiple pharmacological activities of LA have been reported, effect on hepatic lipid metabolism is unknown yet. The present study showed LA to suppress the hepatic triglyceride accumulation in HepG2 cells and ICR mice fed on a high fat diet (HFD). LA inhibited lipogenesis via suppression of sterol regulatory element-binding protein 1c (SREBP1c) and its target enzymes (stearoyl-CoA desaturase 1, fatty acid synthase and glycerol-3-phosphate acyltransferase) transcription. In addition, LA up-regulated gene expression of proteins such as peroxisome proliferator-activated receptor α (PPARα) and fatty acid transporter (FAT/CD36), which are responsible for lipolysis and fatty acid transport, respectively. These effects were mediated through activation of AMP-activated protein kinase (AMPK), and were abrogated when HepG2 cells were treated with an AMPK inhibitor, compound C. To explore how LA activates AMPK, oxygen consumption rate and ATP levels were measured in HepG2 cells. LA significantly inhibited the mitochondrial respiration and ATP levels, suggesting that LA activated AMPK indirectly. In animal study, LA (5 and 10 mg/kg) was orally administered to six-week-old mice once a day for 3 weeks. In vitro results were likely to hold true in vivo experiment, as LA markedly lowered the triglyceride levels and activated AMPK signaling pathway in the liver of ICR mice fed on a HFD. In conclusion, the current study suggests that LA suppressed hepatic triglyceride accumulation through modulation of AMPK-SREBP signaling pathway and thus LA may be a potential therapeutic agent for treating fatty liver disease.

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1. Introduction

Hepatic metabolism plays a key role in the regulation of whole-body energy status since the liver is the major site for storage and release of carbohydrates and for fatty acid synthesis. According to the American Association for the Study of Liver Diseases, non-alcoholic fatty liver disease (NAFLD) is defined as the accumulation of fat in the liver that exceeds 5 to 10% of its weight without significant ethanol consumption [1]. In the various stages of NAFLD, hepatic lipid accumulation, primarily triglyceride (TG), has become a significant public health concern, because it can lead to more harmful hepatitis and cirrhosis [2,3]. Moreover, NAFLD is strongly associated with obesity and insulin resistance in the liver [4,5]. Therefore, preventing and treating NAFLD are relevant to health promotion.

AMP-activated protein kinase (AMPK) is a phylogenetically conserved serine/threonine kinase which acts as a 'metabolic master switch' mediating the cellular adaptation to environmental or nutritional stress factors [6]. AMPK acutely controls lipid metabolism through phosphorylation of acetyl-CoA carboxylase (ACC), while mediating long-term adaptive effects through phosphorylation of sterol regulatory element-binding protein 1 (SREBP1) and suppression of expression of lipogenic enzymes. Once activated, AMPK leads to increases in fatty acid oxidation and simultaneously inhibition of hepatic lipogenesis,

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cholesterol synthesis, and glucose production. Consequently, AMPK cascades have emerged as novel targets in the treatment of fatty liver [7].

Recent studies on fatty liver in food science have focused on identifying functional food ingredients or herbal extracts that can suppress hepatic lipid accumulation. Licochalcone A (LA, Fig. 1A) is a major phenolic constituent of *Glycyrrhiza* plant and is also found in *Brassica rapa* L. LA has been documented to have osteogenic [8], anti-inflammatory [9,10], anti-parasitic [11], anti-fungal [12], anti-cancer [13,14] and anti-hyperglycemic [15] activities. However, the hypolipidemic effect and potential mechanism of LA in the liver are unknown. In this study, we investigated whether LA has an inhibitory effect on intracellular lipid accumulation in insulin resistant HepG2 cells. Rodents fed a high fat diet (HFD) demonstrate visceral adiposity, hyperglycemia, dyslipidemia, hyperinsulinemia and hepatic steatosis, which are similar to human NAFLD [16]. In order to simulate the real life situation, the effects of LA on liver fat metabolism in ICR mice fed on a HFD were also investigated.

2. Materials and methods

2.1. Materials

LA was isolated from *B. rapa* L. and kindly supplied by Prof. N. I. Baek of Kyung Hee University (Yongin, Korea). Antibodies against AMPK, phospho-AMPK, ACC and phospho-
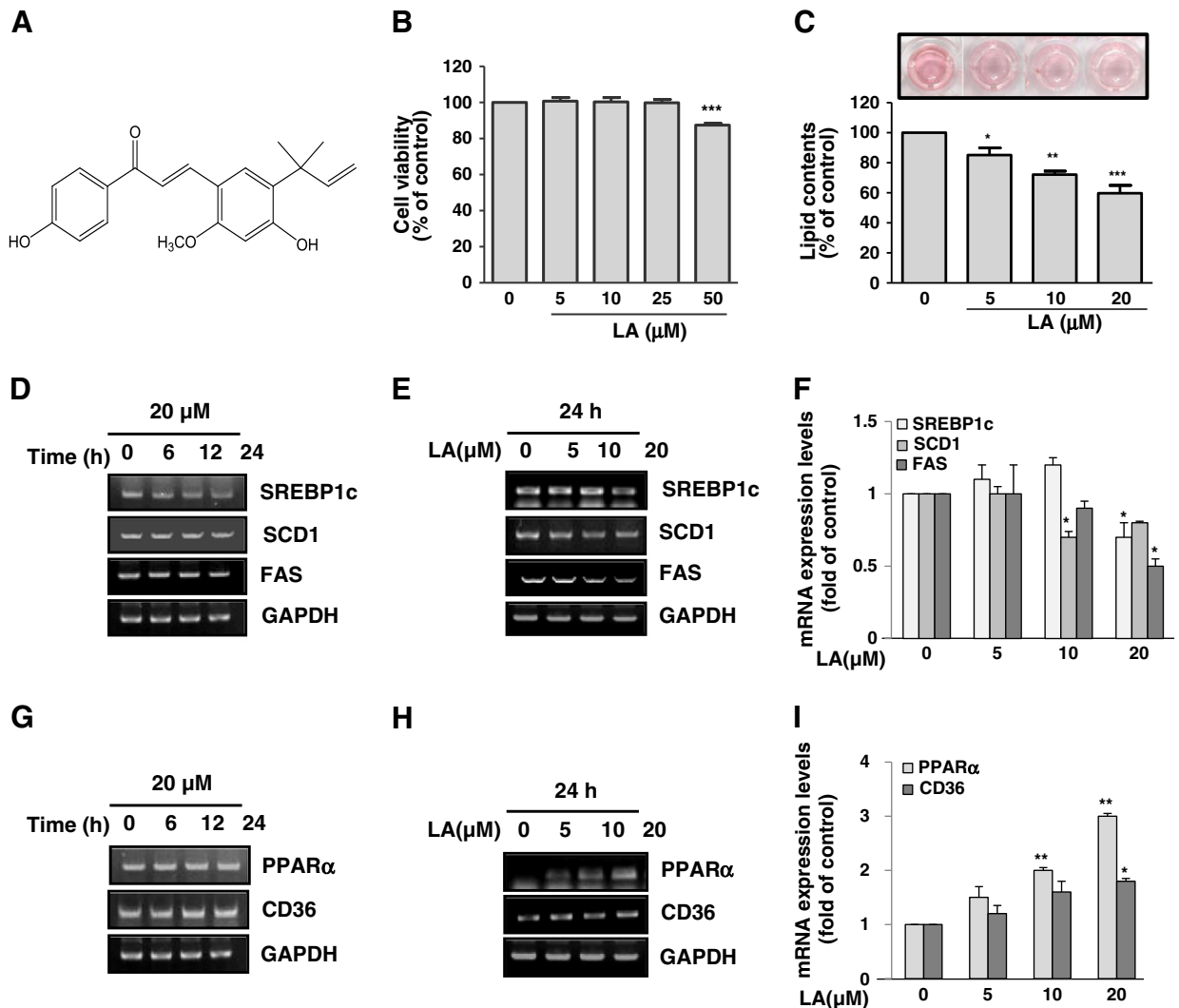

Fig. 1. Effects of LA on lipid accumulation and lipogenic- and lipolytic-related gene expression in HepG2 cells. Chemical structure of licochalcone A (A). Cells were incubated with various concentrations of LA (0–50 μM) for 24 h, and cell viability was measured by MTS assay (B). HepG2 cells treated with LA at indicated concentrations were stained with Oil Red O and lipid contents were quantified (C). Effects of 20 μM of LA on lipogenic and lipolytic gene expressions in HepG2 cells were examined in time-dependent manner with LA up to 24 h (D, G) or concentration-dependent manner with LA (5–20 μM) for 24 h (E, F, H, I). F and I were experimental data obtained from real-time PCR. Each bar represents the mean ± SEM of three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to the control.