Inhibitory effect of schisandrin B on free fatty acid-induced steatosis in L-02 cells

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AIM: To investigate the effects of schisandrin B (Sch B) on free fatty acid (FFA)-induced steatosis in L-02 cells.

METHODS: Cellular steatosis was induced by incubating L-02 cells with a FFA mixture (oleate and palmitate at the ratio of 2:1) for 24 h. Cytotoxicity and apoptosis were evaluated by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide assay and Annexin V/propidium iodide staining, respectively. Cellular total lipid was determined using a photocolorimetric method after Nile red staining, and triglyceride content was measured using an enzymatic kit. To study the effects of Sch B on steatosis, L-02 cells were treated with Sch B (1-100 μmol/L) in the absence or presence of 1 mmol/L FFA for 24 h, and cellular total lipid and triglyceride levels were measured. To explore the mechanisms of action of Sch B in the steatotic L-02 cells, mRNA levels of several regulators of hepatic lipid metabolism including adipose differentiation related protein (ADRP), sterol regulatory element binding protein 1 (SREBP-1), peroxisome proliferator-activated receptor (PPAR)-α and PPAR-γ were measured by quantitative real-time polymerase chain reaction (PCR), and protein levels of ADRP and SREBP-1 were measured by immunoblotting.

RESULTS: Treatment with 1 mmol/L FFA for 24 h induced intracellular lipid accumulation in L-02 cells comparable to that in human steatotic livers without causing apparent apoptosis and cytotoxicity. Sch B mitigated cellular total lipid and triglyceride accumulations in the steatotic L-02 cells in a dose-dependent manner. Quantitative real-time PCR and Western blot analyses revealed that treatment of L-02 cells with 100 μmol/L Sch B reverted the FFA-stimulated up-regulation of ADRP and SREBP-1.

CONCLUSION: Sch B inhibits FFA-induced steatosis in L-02 cells by, at least in part, reversing the up-regulation of ADRP and SREBP-1.

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Key words: Free fatty acid; Hepatic lipid metabolism; Hepatocellular steatosis; L-02 cells; Schisandrin B

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) has emerged...
as a serious and widespread metabolic disease, which entails a wide spectrum of liver disorders and damages ranging from simple steatosis through steatohepatitis and fibrosis to end stage liver diseases including cirrhosis and hepatocellular carcinoma[1]. The clinical significance of NAFLD is largely due to its high prevalence of around 20% in general populations and up to 80% in obese and diabetic individuals worldwide[2]. Although the pathogenesis of NAFLD has not been clearly defined, hepatic steatosis characterized by uncontrolled triglyceride accumulation in hepatocytes and oxidative stress are believed to play a crucial role[3]. Therefore, agents that are capable of lowering hepatic lipid levels and alleviating oxidative stress may be beneficial to the control of NAFLD.

Schisandrin B (Sch B) (Figure 1) is the most abundant and active dibenzocyclooctadiene derivative isolated from the fruits of *Schisandra chinensis*, a traditional Chinese medicinal herb commonly used in treatment of viral and chemical hepatitis. A growing body of evidence has shown that Sch B can protect liver from damage caused by oxidative stress. Sch B may inhibit oxygen free-radical lipoperoxidative damage to plasma membrane of rat hepatocytes in vitro[4]. Sch B pretreatment protects mouse livers against tumor necrosis factor α-induced apoptosis in a dose-dependent manner[5]. In addition, Sch B can protect mice against carbon tetrachloride-induced hepatic toxicity by inhibiting lipid peroxidation[6]. Recently, we have reported that Sch B has hepatic lipid lowering effects in mice fed a high-fat diet[7]. These lines of evidence underscore both hepatic lipid-lowering and antioxidant effects of Sch B, making it a promising candidate for the treatment of NAFLD. Although the antioxidant role of Sch B has been well investigated, the mechanism underlying its hepatic lipid-lowering action remains unknown. This study was designed to investigate the anti-hepatosteatotic effects and mechanisms of Sch B using cultured steatotic cells.

NAFLD patients exhibit an elevated lipolysis and high circulating free fatty acid (FFA) levels[8]. High circulating FFA levels can trigger a series of biological changes in hepatic lipid metabolism, thus ultimately leading to hepatic steatosis[9]. Therefore, cellular FFA loading is commonly utilized to develop in vitro models of steatosis. These models can reliably reproduce the key features of hepatic steatosis in human beings[10,11], rendering them useful for the identification of potential therapeutic targets and effective intervention approaches against NAFLD. Human hepatocytes in primary culture represent the model closest to human liver tissues. Nevertheless, their use is often greatly hampered due to scarcity of liver samples[12]. HepG2 and Huh-7, two human hepatoma cell lines, are frequently used in establishing in vitro steatosis models. However, the validity of cancer cell-based models is concerned because metabolic regulation is often altered in cancer cells. For example, it has been highlighted that cancer cells may carry out an increased fatty acid *de novo* synthesis irrespective of the extracellular lipid levels[13]. Therefore, in this study, we first established FFA-induced steatotic cells using an immortalized normal human hepatocytes-derived cell line L-02[14,15]. Then, we investigated the in vitro effects of Sch B on hepatosteatosis in the steatotic L-02 cells, and explored the underlying mechanisms.

**MATERIALS AND METHODS**

**Cell culture and treatment**

L-02 (Institute of Biochemistry and Cell Biology, Shanghai Institute for Biological Sciences, Shanghai, China) and HepG2 (ATCC) cells were grown in Dulbecco’s modified Eagle’s medium (DMEM, Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, USA) and 1% penicillin/streptomycin (P/S, Gibco, USA) at 37°C in an atmosphere containing 5% CO₂. When FFA mixture (sodium salts of oleate and palmitate, Sigma, Malaysia) was added, bovine serum albumin (BSA) was supplemented to a final concentration of 1% in the culture medium. Cell cultures were used in experiments when they reached 75% confluence.

Sch B was purchased from Ningli Technology Co. Ltd. (Kunming, China) with a purity of 98% as determined by HPLC. A stock solution of Sch B (100 mmol/L) was prepared in dimethylsulfoxide (DMSO). The concentration of vehicle DMSO was 0.1% in treated cell cultures.

**Cell viability assay**

Cytotoxicity of FFA to L-02 cells was assessed by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay. L-02 cells in 96-well plates were treated with FFA. After incubated for 24 h, 20 μL of MTT solution (5 mg/mL, USB, Austria) was added to each well and the plates were further incubated at 37°C for 4 h. After medium removal, 100 μL of DMSO was added to each well of the plates which were then gently shaken for 5 min. Optical absorbance was determined at 570 nm with a microplate spectrophotometer (BD Bioscience, USA). Each treatment was performed in triplicate.

**Quantification of apoptosis**

Early and late phase apoptotic cells were assessed using the Annexin V-fluorescein isothiocyanate (FITC) apoptosis detection kit I (BD Bioscience, USA) following the manufacturer’s instructions. After treatment with FFA, L-02 cells were harvested and rinsed twice with cold PBS, resuspended in the binding buffer, and incubated with Annexin V-FITC and propidium iodide (PI) staining solution. Samples of 10000 stained cells were analyzed using a flow cytometer (BD Bioscience, USA).

**Nile red staining**

L-02 cells in F96 microwell black plates (Nunc, Denmark) were treated with FFA in the presence or absence of Sch B for 24 h. Photocolorimetric measurement of intracellular lipid contents in Nile red stained cells was performed as previously described[16]. Each treatment was performed in triplicate.
Phase-contrast and fluorescence microscope imaging

L-02 cells in 6-well plates were treated with FFA for 24 h, washed with PBS, stained with 1 μmol/L Nile red in HBSS for 15 min and then examined under phase-contrast (Leica, Germany) and fluorescence (Nikon, Japan) microscopes.

Measurement of intracellular triglyceride content

Cellular triglyceride content was measured using an enzymatic kit (Zhongsheng Beikong Biotechnology and Science Inc, China) following the manufacturer's instructions. Triglyceride content was expressed in microgram of triglycerides per microgram protein. Protein concentration was measured by Bio-Rad protein assay (Bio-Rad, USA). Each treatment was performed in triplicate.

Semi-quantitative and real-time quantitative polymerase chain reaction analyses

Total RNA was extracted with Trizol reagent (Invitrogen, USA), and 2 μg of RNA was reverse-transcribed with oligo-dT using the M-MLV reverse transcriptase (Promega, USA) according to the manufacturer’s instructions. For semi-quantitative polymerase chain reaction (PCR), the resultant cDNA was subjected to 25-30 cycles of PCR amplification (denaturing at 95°C for 30 s,annealing at 55-60°C for 30 s, extension at 72°C for 60 s). The PCR products were separated by electrophoresis on a 2% agarose gel and visualized with ethidium bromide staining. Quantitative real-time PCR was performed using SYBR green reaction mixture in the ABI 7500 fast real-time PCR system (Applied Biosystems). The PCR conditions were one cycle at 55°C for 2 min and at 95°C for 10 min, followed by 40 cycles of amplification at 95°C for 15 s and at 60°C for 1 min. The fluorescent signals were detected using the ABI Prism 7500HT sequence detection system (Applied Biosystems). The gene expression data were normalized to the endogenous control β-actin. The relative expression levels of genes were measured according to the formula 2^ΔCt, where ΔCt is the difference in threshold cycle values between the targets and β-actin. All samples were analyzed in triplicate. The specific primer pairs used for detecting messenger RNA are listed in Table 1.

Western blot analysis

L-02 cells were harvested and lysed on ice with the RIPA buffer consisting of 50 mmol/L Tris-Cl, 1% NP-40, 0.35% sodium-deoxycholate, 150 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L EGTA, pH 7.4, 1 mmol/L phenylmethysulfonil fluoride, 1 mmol/L NaF, 1 mmol/L Na3VO4 and 10 μg/mL each of aprotinin, leupetin and pepstatin A. Protein concentration in each sample was measured by the Bio-Rad protein assay. Protein samples (each 15 μg) were separated by SDS-PAGE and then electro-transferred onto nitrocellulose membranes (Amersham Biosciences, USA), which were blocked for 30 min with 5% skim milk in the TBST buffer containing 50 mmol/L Tris (pH 7.6), 150 mmol/L NaCl and 0.1% Tween-20 and incubated with specific antibodies against adipose differentiation related protein (ADRP) (Abcam), sterol regulatory element binding protein 1 (SREBP-1) (Santa Cruz) or β-actin (Santa Cruz) overnight at 4°C. The membranes were then incubated with HRP-conjugated secondary antibodies, and immunoreactive bands were visualized using the ECL detection kit (Amersham Biosciences, USA) following the manufacturer’s instructions.

Statistical analysis

All results were expressed as mean ± SE. The difference between two groups was analyzed using the Student’s t test.

RESULTS

Cytotoxic effect of FFA treatment on L-02 cells

L-02 cells were treated with 0.5-2 mmol/L FFA mixture (oleate and palmitate at the ratio of 2:1) for 24 h and the cytotoxicity of FFA to L-02 cells was detected by MTT

Table 1  Primers used for polymerase chain reaction amplification of mRNA

| Gene       | Forward primer                      | Reverse primer                  |
|------------|------------------------------------|----------------------------------|
| SREBP-1    | ACGGCCGCCCCGGTTTAAGCCACTTGCA       | TGCCAAGATGGTTCGCCACTCAGG         |
| ADRP       | GGAGCTCCCTGTACCAAGG               | AGATGGCTGGTCCCACTAGG             |
| PPAR-α     | CCGATATTACAGGACCTTCTGT            | CGTGTGTGACATCCGACAG             |
| PPAR-γ     | GTGACTCTATATGAGCGCCGAA            | GCGAACAGCGTGTGAGACTCAG           |
| β-actin    | GACTACCTCATGAAGATC                | GATCCACATCTGCTGGA               |

SREBP-1: Sterol regulatory element binding protein 1; ADRP: Adipose differentiation related protein; PPAR: Peroxisome proliferator-activated receptor.

Figure 1  Chemical structure of schisandrin B.

Table 1  Primers used for polymerase chain reaction amplification of mRNA
We next examined the cellular lipid accumulation in L-02 cells. FFA treatment induced lipid accumulation in L-02 cells, as the percentage of triglycerides increased from 0.8% ± 0.2% in control cells to 8.9% ± 0.5% in FFA-treated cells (Figure 2A), which is consistent with previous reports showing that FFA causes about 2.5-fold higher in human steatotic livers than in non-steatotic livers. We also measured the intracellular triglyceride levels in L-02 cells treated with FFA mixture at the concentration of 1 mmol/L. The triglyceride content was increased by about 2.5-fold from 0.108 ± 0.027 μg/μg protein in control cells to 0.241 ± 0.030 μg/μg protein in FFA-treated cells (Figure 3C), which is similar to the results obtained from human liver samples. The triglyceride content is about 2.7-fold higher in human steatotic livers than in non-steatosis livers.

The above data indicate that steatotic cells can be prepared by incubating L-02 cells with a FFA mixture (oleate and palmitate at the ratio of 2:1) at the concentration of 1 mmol/L for 24 h, in which lipid accumulation can reach a level similar to that in human steatotic livers in the absence of apoptosis.

Apoptotic effect of FFA treatment on L-02 cells
To evaluate the apoptotic effect of FFA treatment on L-02 cells, we treated L-02 cells with 1 mmol/L FFA (oleate and palmitate at the ratio of 2:1) for 24 h, and then stained with Annexin V/PI. Apoptosis of L-02 cells was monitored by flow cytometry. For comparison, apoptosis of HepG2 cells induced by FFA treatment was also analyzed. FFA treatment did not trigger early- or late-stage apoptosis of L-02 cells but significantly induced early-stage apoptosis of HepG2 cells (Figure 2B and C). The percentage of apoptotic HepG2 cells was increased from 1.500% ± 0.473% in control cells to 9.267% ± 0.203% in FFA-treated cells (Figure 2C), which is consistent with previous reports showing that FFA causes apoptosis of HepG2 cells under the same conditions. These results suggest that L-02 and HepG2 cell lines do have different responses to FFA treatment.

FFA treatment induced lipid accumulation in L-02 cells
We next examined the cellular lipid accumulation in L-02 cells treated with the FFA mixture for 24 h at the concentration of 0.5 mmol/L or 1 mmol/L using Nile red staining. The results showed that FFA induced lipid accumulation (Figure 3A) in L-02 cells in a dose-dependent manner, which was confirmed by fluorescent microscopy (Figure 3B). When the L-02 cells were treated with FFA mixture at the concentration of 1 mmol/L, the intracellular lipid content was increased by 5.34 ± 0.65-fold in L-02 cells compared to that in FFA-untreated controls (Figure 3A). The cellular lipid accumulation level in L-02 cells treated with FFA at the concentration of 1 mmol/L was comparable to that in human steatotic livers, which is 5.5-fold over non-steatotic livers.

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Sch B treatment alleviated FFA-induced lipid accumulation in L-02 cells

To investigate the anti-steatotic effect of Sch B in L-02 cells, L-02 cells were exposed to various concentrations of Sch B (1-100 μmol/L) in the absence or presence of FFA mixture at the concentration of 1 mmol/L for 24 h. The intracellular total lipid levels in L-02 cells were measured after Nile red staining and the triglyceride contents were assayed using an enzymatic kit. Nile red staining assay showed that Sch B at all concentrations had no significant effect on the cellular lipid content in L-02 cells in the absence of FFA, but substantially ameliorated the lipid accumulation induced by FFA in a dose-dependent manner (Figure 4A). The lipid-lowering effect of Sch B at the concentration of 100 μmol/L was further confirmed by microscopic examination of the fluorescence of Nile red-stained L-02 cells (Figure 4B). The intracellular triglyceride measurements showed that Sch B inhibited the fat accumulation in a dose-dependent manner, and exerted a significant inhibitory effect in L-02 cells treated with FFA at the concentration of 1 mmol/L (Figure 4C). It was noteworthy that Sch B at each tested concentration did not elicit apparent cytotoxicity or apoptosis in L-02 cells in the presence or absence of 1 mmol/L FFA at 24 h (data not shown).

Sch B decreased mRNA and protein expression levels of ADRP and SREBP-1 in FFA-induced steatotic L-02 cells

To explore the mechanisms underlying Sch B-mediated lipid-lowering action in L-02 cells, the mRNA expression lev-
els of ADRP, SREBP-1, peroxisome proliferator-activated receptor (PPAR-α) and PPAR-γ, four important regulators of hepatic lipid metabolism, were first measured by semi-quantitative PCR and quantitative real-time PCR, respectively. Sch B attenuated the FFA-induced fat accumulation most effectively at the concentration of 100 μmol/L, thus this dosage of Sch B was used in this experiment. The mRNA expression level of PPAR-α remained unchanged in FFA-treated L-02 cells, while the mRNA expression levels of the other three genes were up-regulated (Figure 5A and B). Concurrent treatment with Sch B at the concentration of 100 μmol/L for 24 h restored the FFA-upregulated ADRP and SREBP-1 expression to normal levels, but did not affect FFA-stimulated PPAR-γ mRNA expression. In addition, Sch B treatment did not obviously influence the expression of PPAR-α in L-02 cells in the presence of FFA. 

Immunoblot analysis showed that the protein levels of ADRP and SREBP-1 were dramatically elevated in L-02 cells in response to FFA treatment, but returned to normal levels after concurrent treatment with Sch B (Figure 5C).

DISCUSSION
FFA-induced hepatocellular steatosis models have been widely applied in studies on NAFLD pathogenesis and anti-NAFLD drugs[13,17]. Although human hepatocytes in primary culture represent the most stringent model of the human liver, they are tedious to prepare and the reproducibility of experimental results is often a big problem[18,19]. On the other hand, the use of liver cancer cell lines including HepG2 and Huh-7 is often questioned about their acquired genetic and epigenetic alterations which may endow them with numerous properties including metabolism regulation distinct from normal hepatocytes[20]. In the present study, we successfully prepared FFA-induced steatotic cells using a normal human hepatocytes-derived cell line L-02. The steatotic L-02 cells behave similarly to human steatotic livers[13]. These aforementioned features make FFA-induced steatotic L-02 cells suitable for the investigations of NAFLD pathogenesis and anti-NAFLD agents. High circulating FFA concentration may aggravate hepatic fat accumulation by disrupting lipid metabolism in NAFLD patients, and thus studying how FFA overload influences metabolic regulators will further improve our understanding of NAFLD.
understanding about the pathogenesis of NAFLD. Given that palmitic and oleic acids are the most abundant fatty acids in liver triglycerides in both normal subjects and NAFLD patients\(^\text{[21]}\), clarification of their effect on hepatocytes is of great importance. In the present study, treatment of L-02 cells with a FFA mixture (oleate and palmitate at the ratio of 2:1) did not significantly affect PPAR-\(\alpha\) mRNA expression level but increased the mRNA expression levels of ADRP, SREBP-1 and PPAR-\(\gamma\). The unchanged PPAR-\(\alpha\) mRNA expression in L-02 cells treated with FFA suggests that PPAR-\(\alpha\)-mediated mitochondria fatty acid \(\beta\)-oxidation may not been affected by FFA in our experimental conditions. The increased SREBP-1 and PPAR-\(\gamma\) mRNA expression levels in response to FFA treatment are in good accord with a recent report assuming that the up-regulations of SREBP-1 and PPAR-\(\gamma\) are linked to the steatogenic property of oleic acid\(^\text{[17]}\). Moreover, the ADRP mRNA expression was elevated in L-02 cells challenged with FFA, which is in agreement with the reported observations in other cell lines treated with FFA\(^\text{[22,23]}\). ADRP is a lipid storage droplet-associated protein found in most cells and tissues, and has been suggested to be a marker of lipid accumulation, because the cellular level of ADRP is proportional to the total mass of neutral lipids within the cells\(^\text{[24]}\). Fatty acids have been implicated as ligands for PPAR family members including PPAR-\(\alpha\) and PPAR-\(\gamma\), it is thus believed that the stimulation of ADRP gene by FFA is at least in part due to PPAR activation. It has been shown that the activated PPAR can complex with retinoid X receptor and bind to the PPAR response element in the promoter of ADRP gene\(^\text{[25,26]}\). As both PPAR-\(\alpha\) and PPAR-\(\gamma\) were detectable in L-02 cells, further studies are needed to ascertain whether one or both of them are required for the up-regulation of ADRP gene expression induced by FFA treatment. These findings suggest that exposure to exogenous FFA may interfere with lipid metabolism through the modulation of metabolic regulators in L-02 cell line derived from normal human hepatocytes.

![Figure 5](image_url)

Figure 5 Effect of schisandrin B on mRNA and protein expression levels of several lipid metabolism-related molecules in free fatty acid-treated L-02 cells. A, B: Semi-quantitative polymerase chain reaction (PCR) and real-time quantitative PCR showing mRNA levels of adipose differentiation related protein (ADRP), sterol regulatory element binding protein 1 (SREBP-1), peroxisome proliferator-activated receptor (PPAR)-\(\alpha\) and PPAR-\(\gamma\). Results shown are the representative of three independent experiments. *\(P < 0.05\), **\(P < 0.01\) vs control group; &\(P < 0.05\) vs free fatty acid-treated groups; C: Immunoblotting showing expression levels of ADRP and SREBP-1 proteins. The representative blots out of three experiments are shown. FFA: Circulating free fatty acid; Sch B: Schisandrin B.

We demonstrated that Sch B exerted a drastic inhibitory effect on FFA-induced steatosis in L-02 cells. This finding and the hepatic lipid-lowering action of Sch B observed in high-fat diet-fed mice\(^\text{[7]}\) strongly highlight the anti-steatosis potential of Sch B. Since FFA overloading contributed to hepatic fat accumulation through modulation of lipid metabolism-related genes in our established steatotic L-02 cells, it is conceivable that Sch B may attenuate fat accumulation by countering or reversing the unfavorable changes in expression of genes evoked by FFA. In this study, Sch B treatment restored the FFA-induced up-regulation of both mRNA and protein levels of ADRP and SREBP-1 to normal levels, indicating that ADRP and SREBP-1 are the potential targets of Sch B in relation to its lipid-lowering property.
ADRP expression is closely associated with intracellular lipid droplets and up-regulated in hepatic steatosis in human and mouse models\(^{27,28}\). It has been reported that ADRP overexpression may promote lipid accumulation in fibroblasts and macrophages without changing the expression of adipogenic genes and genes involved in lipid efflux\(^{29,30}\). Intriguingly, ADRP overexpression may facilitate the uptake of long chain FFA in COS-7 cells\(^{15}\). Moreover, Edvardsson et al\(^{31}\) have recently proposed that ADRP may enhance cellular triglyceride accumulation in hepatocytes by increasing fatty acid uptake, driving fatty acids to triglyceride formation as well as preventing the use of triglyceride in VLDL assembly. In this connection, the down-regulation of ADRP may contribute to the anti-hepatosteatotic effect of Sch B by inhibiting the uptake of exogenous long chain FFA, decreasing the incorporation of FFA into triglyceride and increasing the availability of triglyceride for VLDL assembly. It has been demonstrated that ADRP-deficient mice produced by either knock-out or anti-sense oligonucleotide technology do not acquire diet-induced hepatic steatosis\(^{30,33,34}\), raising the possibility that ADRP may become a putative molecular target for the prevention of NAFLD. Thus, screening for compounds that can repress hepatic ADRP expression may provide a new direction for the identification of potential therapeutic agents against NAFLD. It has been recently demonstrated that pycnogenol, a French maritime pine bark extract, can reduce oleic acid-induced lipid droplet formation in mouse liver epithelial cells MMuLi by inhibiting ADRP expression, and interestingly, the suppression of ADRP expression is mediated in part by facilitating mRNA degradation\(^{53}\). How Sch B impairs ADRP expression in steatotic L-02 cells remains to be evaluated.

SREBP-1 is the most important transcription factor regulating de novo lipogenesis in the liver. There is compelling evidence that supports the involvement of SREBP-1 in NAFLD development. It has been reported that SREBP-1 expression is significantly elevated in livers from NAFLD and obesity patients, and from insulin-resistant and hyperinsulinemic \(ob/ob\) mice\(^{27,30,34}\). Overexpression of SREBP-1 in cultured hepatocytes or mouse livers can increase hepatic triglyceride deposition and mRNA expression of genes involved in lipogenesis\(^{37,39}\). Moreover, in Lep\(^{28,30}\) mice deficient in SREBP-1, hepatic steatosis is markedly attenuated, which is accompanied by decreased mRNA levels of lipogenic enzymes\(^{36}\). These lines of evidence strongly suggest that SREBP-1 plays a pivotal role in the regulation of hepatic lipid metabolism, thus pharmacological manipulation of SREBP-1 may be beneficial to the management of NAFLD. In this study, Sch B could reverse FFA-induced up-regulation of SREBP-1. Therefore it is plausible to infer that the down-regulation of SREBP-1 may partly contribute to the lipid-lowering activity of Sch B by inhibiting de novo lipogenesis. Since SREBP-1 may transcriptionally activate a variety of genes required for lipogenesis in the liver\(^{41}\), it is of interest to investigate which SREBP-1 target genes are regulated by Sch B. Another question is how Sch B regulates the expression of SREBP-1. A most recent study showed that resveratrol inhibits palmitate-induced lipid accumulation in HepG2 cells by reducing the up-regulation of SREBP-1 via the Sirt1-FOXO1 pathway\(^{42}\). Whether the Sirt1-FOXO1 pathway is involved in Sch B-mediated down-regulation of SREBP-1 remains to be clarified.

In summary, Sch B has an inhibitory effect on FFA-induced steatosis in L-02 cells, and the decreased expression of ADRP and SREBP-1 may account for the inhibitory effect of Sch B by reducing FFA uptake, incorporation of FFA into triglycerides and de novo fatty acid synthesis, as well as by increasing VLDL assembly. Changes in ADRP and SREBP-1 expression may also provide mechanistic explanations for the hepatic lipid-lowering effect of Sch B in mice fed a high-fat diet as reported previously by us. The results of this study provide the molecular evidence for developing Sch B as a therapeutic agent against NAFLD.

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