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(5R)-5-hydroxytriptolide ameliorates liver lipid accumulation by suppressing lipid synthesis and promoting lipid oxidation in mice

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A R T I C L E I N F O

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A B S T R A C T

Aims: (5R)-5-hydroxytriptolide (LLDT-8) is a triptolide analog with excellent capability against cancers, cerebral ischemic injury and rheumatoid arthritis. Here, we discovered its hepatoprotective effects in a mouse model of non-alcoholic fatty liver disease (NAFLD) by ameliorating liver lipid accumulation.
Main methods: Male C57BL/6J mice were fed with a high-fat/high-fructose (HFHFr) diet for 29 weeks to induce the pathological phenomena of NAFLD. Then the mice were treated with LLDT-8 (0.5mg/kg and 1mg/kg) or Vehicle for 8 weeks. Finally, the serum biochemical indexes, liver histological features, fatty acids (FAs) profile and related gene expression in liver were detected to investigate the effect of LLDT-8 on lipid accumulation and its possible mechanism.
Key findings: LLDT-8 treatment significantly inhibited hepatic injury featured by the decrease of serum alanine aminotransferase (ALT) and aspartate transaminase (AST), the lessening of hepatic ballooning and macrovesicular steatosis. Moreover, LLDT-8 could downregulate the expression of stearoyl-CoA desaturase 1 (SCD1), which further led to the lower ratios of C16:1/C16:0 and C18:1/C18:0 and thus inhibited lipid synthesis. LLDT-8 treatment also could upregulate liver peroxisome proliferator-activated receptor α (PPARα), carnitine palmitoyltransferase 1a (Cpt1a), peroxisomal acyl-CoA oxidase 1 (Acox1), long-chain acyl-CoA dehydrogenase (Acadl) and medium-chain acyl-CoA dehydrogenase (Acadm) expression levels involved in fatty acids oxidation (FAO) and markedly promoted lipolysis.
Significance: Our results provide a novel application of LLDT-8 in improving NAFLD.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is a chronic epidemic of liver disease and is involved a wide spectrum of pathological condition that ranges from simple steatosis to nonalcoholic steatohepatitis (NASH), cirrhosis and hepatocellular carcinoma [1]. In this process of gradual deterioration, hepatic steatosis is the initial stage and can be reversed [2,3]. Therefore, inhibiting excessive fat deposition in liver is considered as an effective therapeutic strategy in preventing it from developing to a more severe stage [4].

Excessive lipids accumulation in hepatocytes is one of characteristics of NAFLD, which is a result of imbalance between the lipid synthesis, uptake of serum free fatty acids (FAs), export of very low density lipoprotein (VLDL) and oxidation of FAs in liver [5,6]. It also has been well established that increase of hepatic de novo lipogenesis (DNL) and uptake of peripheral FAs are major contributors to lipid accumulation in liver of NAFLD [7,8]. After serum FAs were taken up by hepatocytes, it metabolized either by fatty acid oxidation (FAO) in mitochondria and peroxisome or esterification in endoplasmic reticulum to produce triglycerides (TG) [9]. However, mitochondrial and peroxisomal β-oxidation are impaired in NAFLD, which also contribute to the occurrence of hepatic steatosis [10,11]. That is to say, inhibiting DNL or promoting β-oxidation facilitates to less liver injury by decreasing intracellular lipids accumulation. For example, statins were

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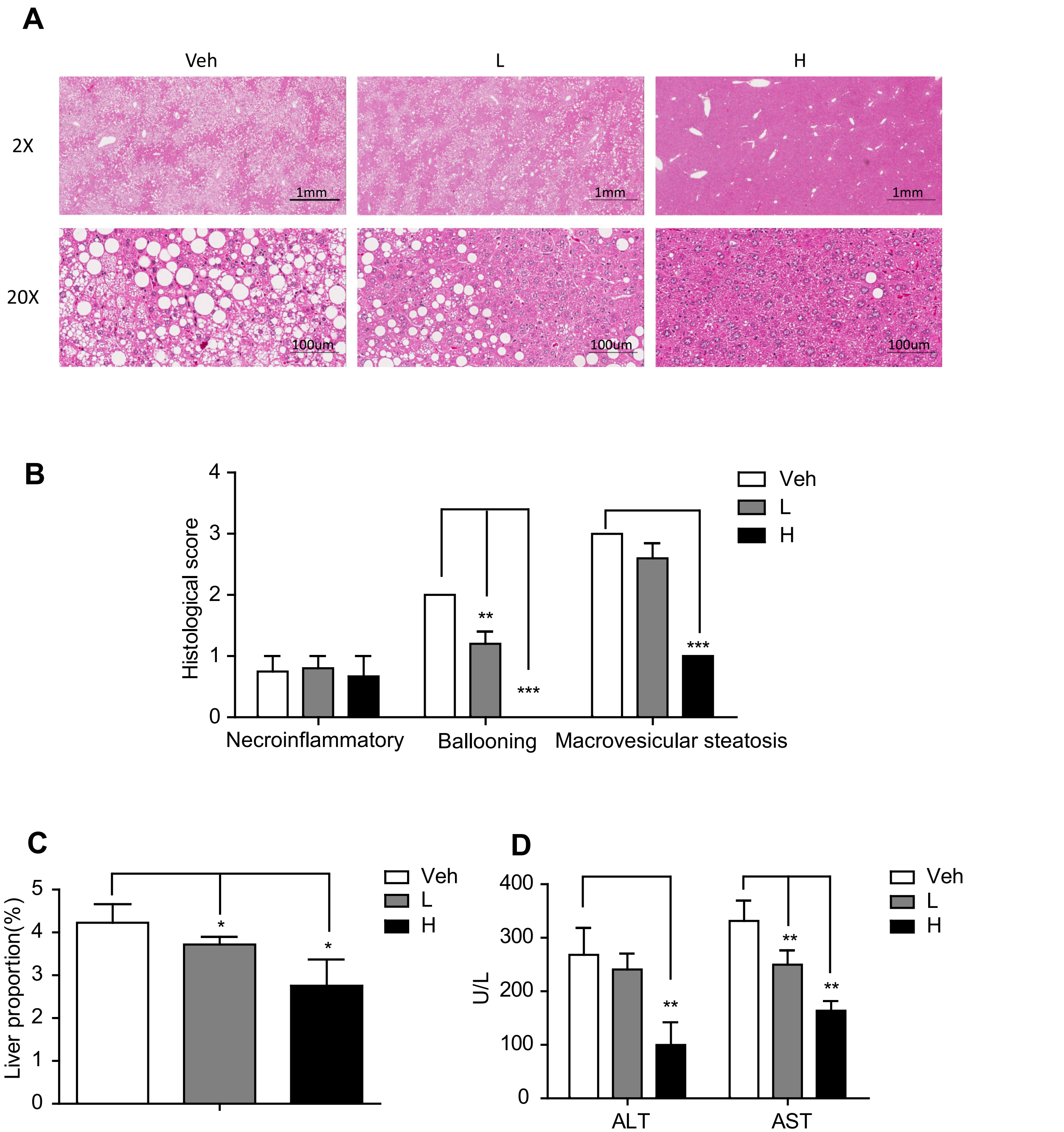

Fig. 1. Effects of LLDT-8 on HFHFr-diet induced liver injury.
(A) Tissue sections were stained with hematoxylin and eosin. (B) The histological NAS scores of necroinflammatory, ballooning and macrovesicular steatosis were determined by a certified pathologist. (C) Liver proportion was calculated as follow: Liver weight / body weight × 100. (D) ALT and AST in serum were determined by the enzymatic kinetic method using an automatic biochemical analyzer. Data were expressed as the mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs Veh group, n = 3–5 in each group.

reported to prevent NASH by increasing mitochondrial and peroxisomal FAO [11], while miR-27a can regulate hepatic lipid metabolism to alleviates NAFLD via repressing fatty acid synthase (FAS) and stearoyl-CoA desaturase 1 (SCD1) [12].

Triptolide, isolated from a Chinese traditional herb named Tripterygium wilfordii Hook. f. (TWHF), has shown strong immunosuppressive activity in vivo and in vitro [13,14]. However, its serious toxicity and poor water solubility limit its clinical application [15]. (5*R*)-5-hydroxytriptolide (LLDT-8) as a immunosuppressant for the treatment of rheumatoid arthritis in phase II clinical trials is a new derivative modified from the structure of triptolide [16], which exhibits lower toxicity and higher immunosuppressive activity compared with triptolide [17]. Current research believes that LLDT-8 can inhibit the production of Th1 type cytokines (IFN-γ, IL-2), inflammatory cytokines (TNF-α, IL-6), nitric oxide (NO) and inducible nitric oxide synthase (iNOS) expression, which is the mainly reason to play its immunosuppressive role [18]. Furthermore, LLDT-8 also has great potential for antitumor activity by inhibition of gene transcription [19,20]. However, the relationship between LLDT-8 and NAFLD has not been touched.

In current work, the mouse model of NAFLD induced by high-fat/high-fructose (HFHFr) diet was used to investigate the effects of LLDT-8

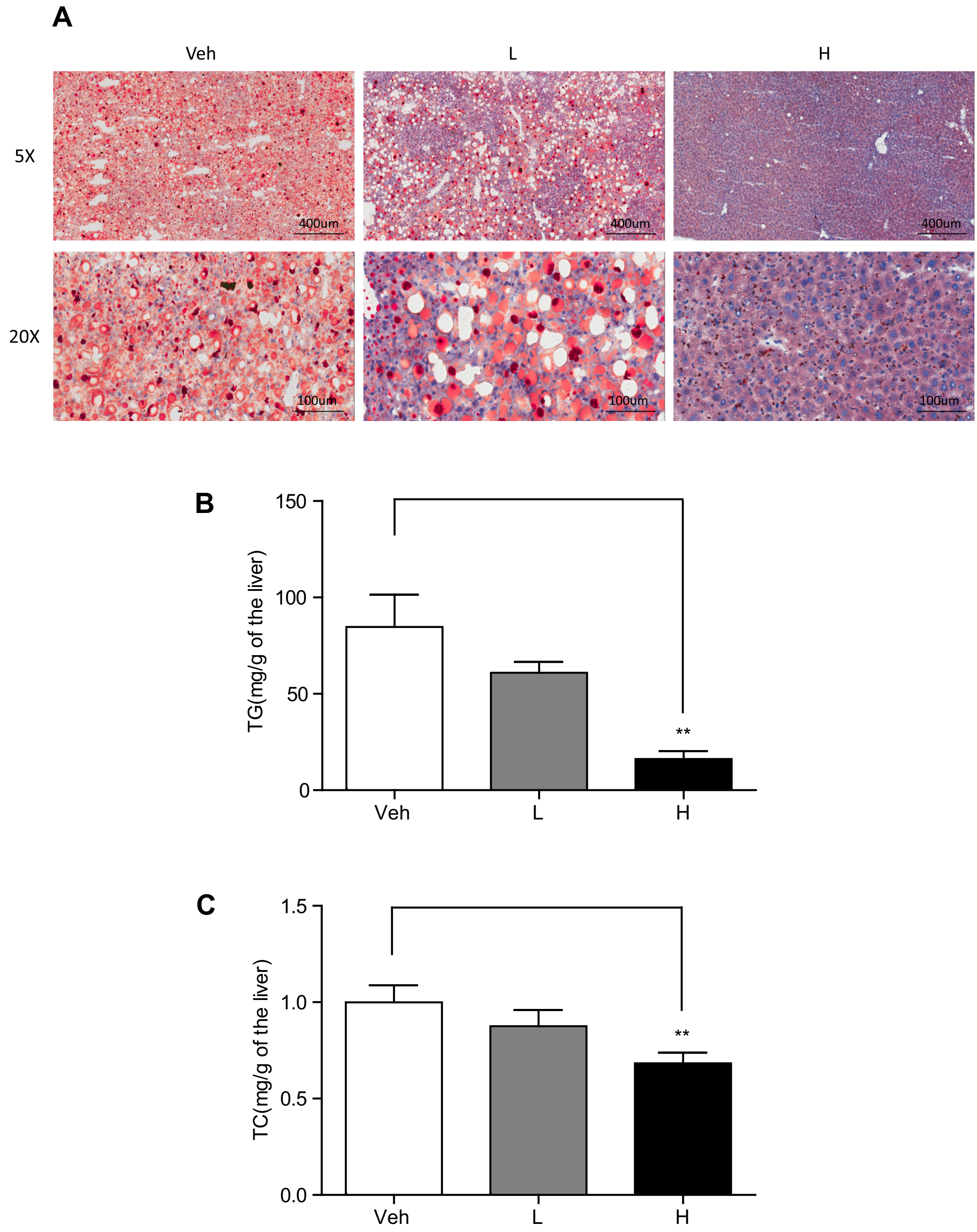

Fig. 2. Effects of LLDT-8 on HFHFr diet-induced hepatic lipid accumulation.
(A) Tissue sections were stained with Oil red O. (B) TG and (C) TC in livers were quantified using enzymatic reagent kits. Data were expressed as the mean ± SEM. **p < 0.01 vs Veh group, n = 3–5 in each group.

on NAFLD, and the likely mechanism for its action was further explored.

2. Materials and methods

2.1. Animal

Male C57BL/6J mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China) and housed in specific pathogen free laboratory (23 ± 1 °C, 12 h light/12 h dark cycles, 50% relative humidity). All animal treatments were approved by the Institutional Animal Care and Use Committee of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences (2018-01-RJ-160). After 1 week of acclimation, all mice were fed HFHFr diet (60 kcal % fat: ResearchDiets, USA, #D12492 high-fructose corn syrup: 77.3% fructose (34 g/L) and 22.7% glucose (10 g/L)). After 29 weeks feeding, the mice were divided into Vehicle (0.1% DMSO, n = 5), LLDT-8-L (0.5 mg/kg, n = 5) and LLDT-8-H (1.0 mg/kg, n = 5) according to the

Table 1
Liver fatty acids composition and enzyme activities based on fatty acid ratios.

Fatty acids	Vehicle	LLDT-8-L	LLDT-8-H
Palmitic acid (16:0)	92.31 ± 13.94 μmol/g	64.62 ± 11.11 μmol/g	89.26 ± 39.74 μmol/g
Stearic acid (18:0)	43.69 ± 4.40 μmol/g	36.73 ± 1.37 μmol/g	73.32 ± 43.56 μmol/g
Palmitoleic acid (16:1 n7)	4.40 ± 1.19 μmol/g	2.32 ± 0.74 μmol/g	1.14 ± 0.19 μmol/g
trans-9-Elaidic and *cis*-9-oleic acid (C18:1 n9)	72.72 ± 22.74 μmol/g	41.76 ± 11.16 μmol/g	50.34 ± 28.80 μmol/g
cis-11-Eicosenoic acid (20:1)	1.07 ± 0.39 μmol/g	0.67 ± 0.20 μmol/g	1.78 ± 1.80 μmol/g
cis-15-Tetracosenoic acid (24:1)	4.43 ± 0.58 μmol/g	1.53 ± 0.53 μmol/g*	0.53 ± 0.30 μmol/g*
Linolelaidic and linoleic acid (18:2 n6)	43.16 ± 9.79 μmol/g	29.41 ± 2.76 μmol/g	53.34 ± 23.84 μmol/g
cis-8,11,14-Eicosatrienoic acid (20:3 n6)	2.88 ± 0.51 μmol/g	2.30 ± 0.04 μmol/g	2.16 ± 1.03 μmol/g
Arachidonic acid (20:4 n6)	39.66 ± 3.00 μmol/g	35.73 ± 2.16 μmol/g	53.73 ± 33.93 μmol/g
cis-5,8,11,14,17-Eicosapentaenoic acid (20:5 n3)	1.07 ± 0.02 μmol/g	0.76 ± 0.01 μmol/g**	0.81 ± 0.15 μmol/g
cis-4,7,10,13,16,19-Docosahexaenoic acid (22:6 n3)	24.15 ± 1.12 μmol/g	19.55 ± 0.36 μmol/g*	35.59 ± 19.93 μmol/g
Total fatty acids	329.53 ± 42.90 μmol/g	235.37 ± 29.38 μmol/g	362.00 ± 193.27 μmol/g
SFA%	41.43 ± 2.50	43.06 ± 0.07	45.21 ± 1.13
MUFA%	24.79 ± 4.33	19.50 ± 2.48	14.65 ± 0.76
PUFA%	33.78 ± 1.83	37.43 ± 2.41	40.13 ± 0.36*
PUFA/MUFA	1.39 ± 0.32	1.94 ± 0.37	2.74 ± 0.12*
Estimations of enzyme activities			
Stearoyl-CoA desaturase 1 (16:1/16:0)	0.05 ± 0.01	0.04 ± 0.01	0.01 ± 0.00*
Stearoyl-CoA desaturase 1 (18:1/18:0)	1.70 ± 0.69	1.13 ± 0.26	0.69 ± 0.02
Delta-5 desaturase (20:4 n-6/20:3 n-6)	14.07 ± 3.53	15.50 ± 0.69	23.85 ± 4.34
Elongase (18:0/16:0)	0.48 ± 0.12	0.58 ± 0.08	0.79 ± 0.14
Elongase (18:1/16:1)	16.42 ± 0.71	18.16 ± 1.00	42.55 ± 18.01
De novo lipogenesis (16:0/18:2 n6)	2.16 ± 0.17	2.19 ± 0.17	1.67 ± 0.00

Results expressed as mean ± standard deviation, *$p < 0.05$, **$p < 0.01$ vs Vehicle. Abbreviations: SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

mean of body weight and serum low-density lipoprotein cholesterol (LDL-C) level. LLDT-8 was synthesized from triptolide that was isolated from TWHF. All of the administrations by gavage were executed daily for 8 weeks. At the end of the experiment, all mice were anesthetized and killed after a 12–16 h fast. Blood was immediately acquired and serum was isolated by centrifugation at 2000*g* for 10 min at 4 °C. The liver sections fixed with 4% paraformaldehyde were prepared for histological staining and the remained hepatic tissues were stored at −80 °C.

2.2. Serological analysis and liver lipid analysis

Serum alanine aminotransferase (ALT), aspartate transaminase (AST) and LDL-C were assayed with an automatic biochemistry analyzer from Roche (Cobas C501). Serum β-hydroxybutyrate level was measured by a commercially available kit (© Beijing Wantai Biological Pharmacy Enterprise Co. Ltd.) according to the instructions of the manufacturers.

Hepatic TG levels of mice were assayed using enzymatic reagent kit (Nanjing Jiancheng Bioengineering institute, Nanjing, China) and total cholesterol (TC) were detected using the Amplex® Red Cholesterol Assay Kit (Thermo Fisher Scientific, USA) according to the manufacturer's protocol.

2.3. Histologic analysis

For evaluation of hepatic steatosis, the tissue specimens were fixed in 4% paraformaldehyde, and then paraffin-embedded sections were subjected to standard hematoxylin-eosin (H&E) staining. Oil red O (Sigma, St Louis, USA) staining is used to identify the tissue lipid deposits and fat accumulation [21]. The tissue specimens were embedded in an optimal cutting temperature (OCT) compound (Tissue-Tek, Torrance, CA, USA). Frozen sections (10 μm) were washed with 60% isopropanol, and stained with Oil red O solution (in 60% isopropanol) for 5 min, then washed repeatedly with PBS and stained with hematoxylin for 10 s. In the end, tissue sections were sealed with glycerin gelatin. H&E as well as Oil Red O-stained liver specimens were evaluated by light microscopy.

Histological features and liver lesion including degree of steatosis, inflammation, and ballooning were assessed using the NAFLD activity scoring (NAS) scoring system: steatosis (0–3), lobular inflammation (0–3), hepatocellular ballooning (0–2) [22,23].

2.4. Liver tissue FAs measurement by GC–MS

About 10 mg of liver tissue sample were homogenized (20 Hz, 90 s) three times by a tissue grinder (QIAGEN, Germany) in 500 μL of methanol and 100 μL of homogenate were transferred into 5 mL glass centrifuge tube. Then 1 mL of methanol:hexane (4:1) and 20 μL of internal standard solution including methyl heptadecanoate (1 mg/mL), methyl tricosanate (0.5 mg/mL) and butylated hydroxytoluene (BHT) were added. After cooling with liquid nitrogen for 15 min, 100 μL of acetyl chloride was added carefully and the reaction was continued in liquid nitrogen for 10 min and then at room temperature (25 °C) for 24 h in the dark. After that, 2.5 mL of 6% K_2CO_3 was added to end the reaction. Finally, fatty acid methyl ester was extracted using 200 μL hexane, mixed and centrifuged at 800*g* for 10 min g at room temperature. Supernatant was collected and the residue was repeatedly extracted twice with hexane. Merger of these supernatants was evaporated to dryness and re-dissolved in 100 μL of hexane prior to analysis.

FAs of liver tissue samples were identified and quantified by gas chromatography coupled to mass spectrometry (7890B GC-FID-5977A MSD, Agilent, USA) (GC–MS). A split injector (the split ratio being 1:30) at 230 °C was used to add the sample (1.0 μL) onto a Agilent DB 225 (10 m × 0.1 mm I.D., 0.1 μm film thickness) capillary column and fatty acid methyl esters were separated at constant flow. The ion trap mass spectrometer was operated under electron bomb ionisation (EI) mode. Mass spectra of m/z 45–450 were collected using full scan mode. Source temperature was 230 °C with the electron energy at 70 eV.

2.5. Quantitative real-time PCR

Total RNA was isolated from the liver tissues by RNAiso Plus reagent (Takara Biomedical Technology Co. Ltd., Beijing, China) and reverse transcribed to cDNA with PrimeScript™ RT Master Mix (Takara, Japan). Quantitative real-time polymerase chain reaction (qRT-PCR) analysis was performed using SYBR Premix Ex Taq™ (Takara, Japan). The qPCR amplification program consisted of polymerase activation at

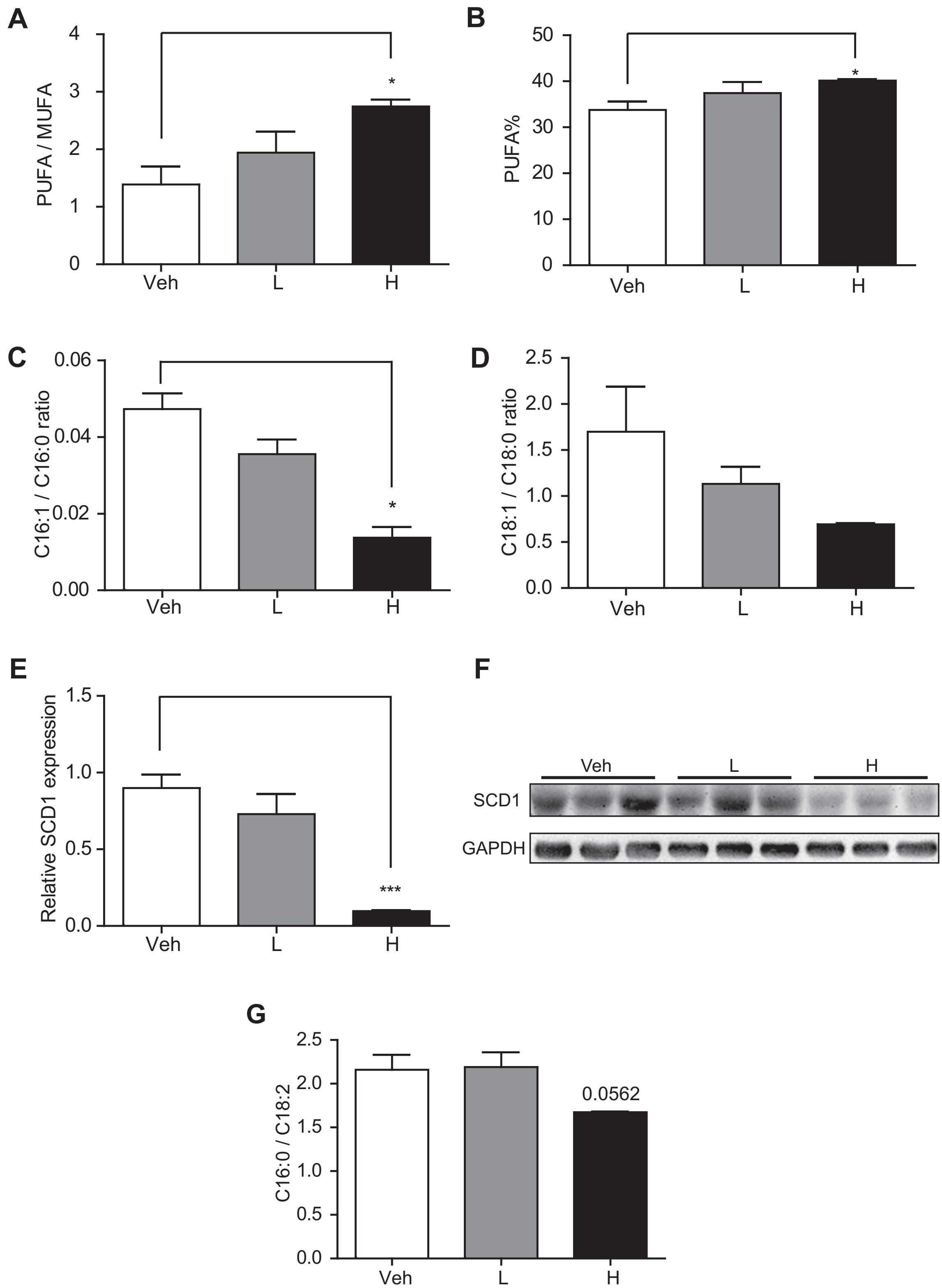

Fig. 3. Effects of LLDT-8 on the inhibition of desaturation of fatty acids.

The ratio of monounsaturated fatty acid (MUFA) to polyunsaturated fatty acid (PUFA) (A), PUFA to total FAs (B), palmitoleic acid (C16:1) to palmitic acid (C16:0) (C), oleic acid (C18:1) to stearic acid (C18:0) (D) and palmitic acid (C16:0) to linoleic acid (C18:2) (F) were calculated from GC–MS data. n = 2 in each group. (E) Liver mRNA and (F) protein levels of SCD1 were detected qRT-PCR and western blotting analyses. Data were expressed as the mean ± SEM. $^{*}p < 0.05$, $^{***}p < 0.001$ vs Veh group, n = 3–5 in each group.

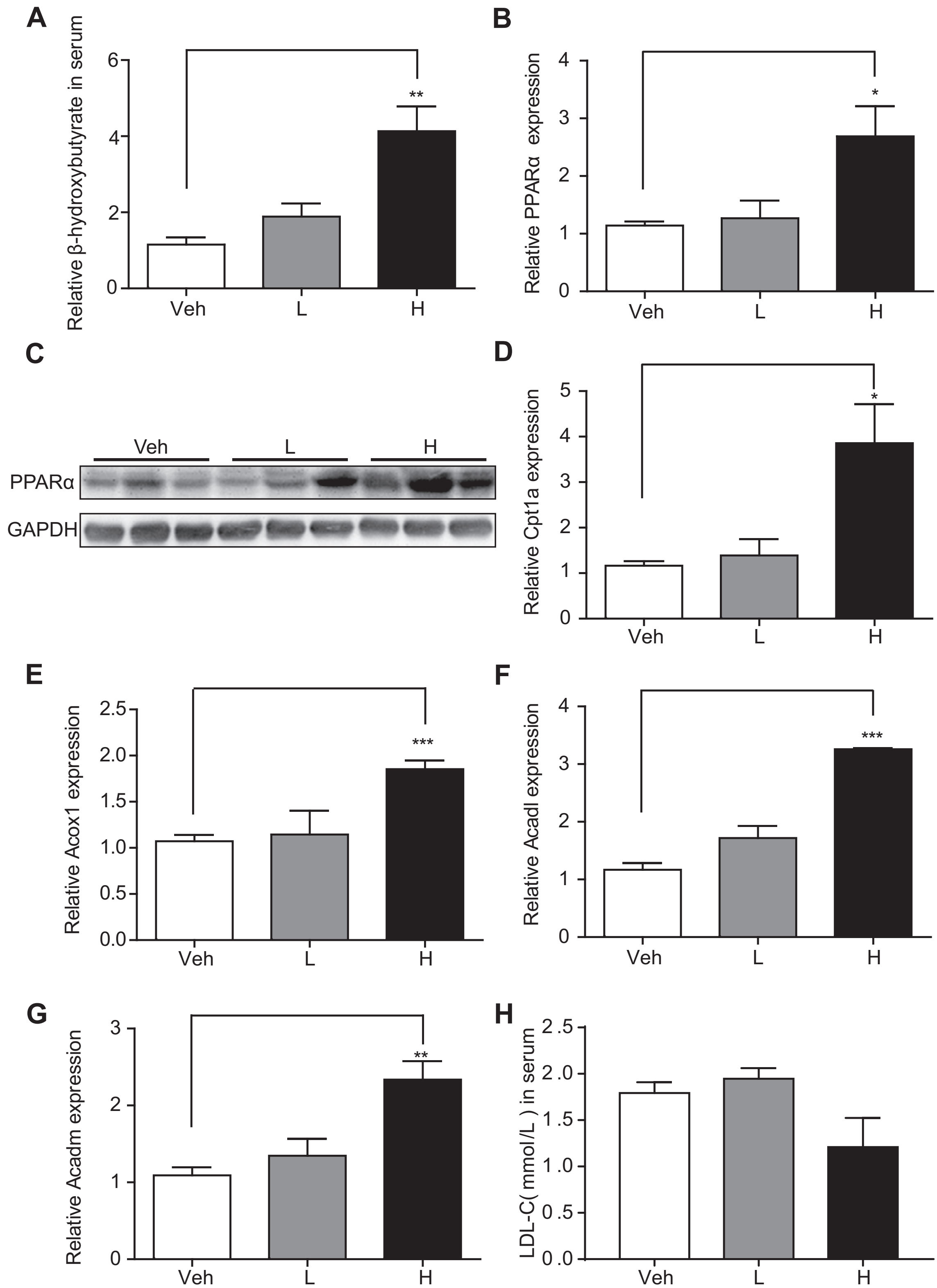

Fig. 4. Effects of LLDT-8 on β-oxidation of fatty acids.

(A) Serum β-hydroxybutyrate level was measured by a commercially available kit. Hepatic mRNA (B) and protein (C) levels of PPARα were detected by qRT-PCR and western blotting analyses. The mRNAs of (D) Cpt1a, (E) Acox1, (F) Acadl and (G) Acadm were detected by qRT-PCR analyses. (H) Serum LDL-C levels were assayed by automatic biochemistry analyzer. Data were expressed as the mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 vs Veh group, n = 3–5 in each group.

50 °C for the 2 min, 95 °C for the 20 s and 40 cycles of denaturation at 95 °C for 5 s, annealing, and extension at 60 °C for 30 s. The sequences of the primers used for quantitative PCR are shown as following: SCD1 forward primer is 5′-TTCTTGCGATACACTCTGGTGC-3′, reverse primer is 5′-CGGGATTGAATGTTCTTGTCGT-3′; peroxisome proliferator-activated receptor α (PPARα) forward primer is 5′-AGAGCCCCATCTGTC CTCTC-3′, reverse primer is 5′-ACTGGTAGTCTGCAAAACCAAA-3′; carnitine palmitoyltransferase 1a (Cpt1a) forward primer is 5′-CTCCG CCTGAGCCATGAAG-3′, reverse primer is 5′-CACCAGTGATGATGCCA TTCT-3′; peroxidase acyl-CoA oxidase 1 (Acox1) forward primer is 5′-TAACTTCCTCTCACTCGAAGCCA-3′, reverse primer is 5′-AGTTCCA TGACCCATCTCTGTC-3′; long-chain acyl-CoA dehydrogenase (Acadl) forward primer is 5′-TCTTTTCCTCGGAGCATGACA-3′, reverse primer is 5′-GACCTCTCTACTCACTTCTCCAG-3′; medium-chain acyl-CoA dehydrogenase (Acadm) forward primer is 5′-AGGGTTTAGTTTTGAGTTGA CGG-3′, reverse primer is 5′-CCCCGCTTTTGTCATATTCCG-3′; GAPDH forward primer is 5′-AGGTCGGTGTGAACGGATTTG-3′, reverse primer is 5′-GGGGTCGTTGATGGCAACA-3′. The CT values for the samples were normalized to the corresponding GAPDH CT values.

2.6. Western blotting analyses

Mouse liver tissues were lysed in RIPA lysis buffer (Sangon, Shanghai, China) with phenylmethanesulfonyl fluoride (PMSF). Proteins were separated on 10% SDS-polyacrylamidegels (SDS-PAGE) and electro-transferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). After blocking in 5% non-fat milk, proteins on the membrane were incubated with specific antibodies for SCD1 (ab236868, Abcam, Cambridge, UK), PPARα (ab97609, Abcam, Cambridge, UK) and GAPDH (2118S, Cell Signaling Technology, Danvers, USA) overnight at 4 °C. Protein bands were visualized using an enhanced chemiluminescence (ECL) kit (Millipore, Billerica, MA, USA). GAPDH was selected as the internal control.

2.7. Statistical analysis

Statistical significance in this study was assessed by two-tailed Student's *t*-test and $P < 0.05$ was considered a statistically significant difference. All data were given as the mean ± S.E.M.

3. Results

3.1. LLDT-8 ameliorated HFHFr diet-induced liver injury

C57BL/6J mice induced by HFHFr diet is a classic model, which presented the similar clinical features, biochemical changes to NAFLD in human [24]. After 36 weeks of HFHFr diet feeding, liver sections displayed significant ballooning and macrovesicular steatosis as confirmed by H&E staining. However, high-dose LLDT-8 treatment significantly attenuated HFHFr diet-induced liver injury (Fig. 1A). NAS of ballooning and macrovesicular steatosis was reduced consistent with the result of H&E staining (Fig. 1B). In this study, the ultimate liver weight was determined. We found that LLDT-8 treatment made the weight proportion of liver to body lowered in a dose-dependent manner compared with the vehicle-treated mice (Fig. 1C). At the same time, the levels of serum ALT and AST were markedly decreased by LLDT-8 treatment (Fig. 1D), which were biochemical diagnostic markers for the presence of hepatocellular damage. These results clearly suggested that LLDT-8 may protect against the hepatic dysfunction induced by HFHFr diet in mice.

3.2. LLDT-8 reduced HFHFr diet-induced hepatic steatosis and TG accumulation

To investigate the effect of LLDT-8 on hepatic steatosis, liver sections were stained with Oil Red O, a histological marker of hepatocytes TG accumulation [21]. As shown in Fig. 2A, the extent of TG accumulation was reduced obviously in LLDT-8-treated mice compared with the vehicle group. The levels of liver TG and TC were also reduced in the high-dose LLDT-8 treated group (Fig. 2B, C). The above results showed that LLDT-8 has an improved effect on fat accumulation and steatosis in the liver.

3.3. LLDT-8 prevented fat deposition through regulating the composition of free FAs

We further investigate the underlying differences in endogenous FAs metabolism between LLDT-8 treatment and vehicle group in liver to explain the LLDT-8 possible mechanism of inhibiting lipid accumulation. As shown in Table 1, there was no obvious change in total FAs of liver, whereas the ratio of polyunsaturated fatty acids (PUFA) to monounsaturated fatty acids (MUFA) and PUFA to total FAs significantly increased (Fig. 3A, B). As reported, the decrease of PUFA/MUFA ratio characterizes the excessive lipid peroxidation and oxidative stress in high-fat diet (HFD)-fed mice [25] and down of PUFA/total FAs ratio plays a role in the development from simple steatosis to NASH [26], which indicated the possible protective effect of LLDT-8 for NAFLD.

Additionally, a significant decrease was observed in C16:1 to C16:0 and C18:1 to C18:0 ratios (Fig. 3C, D), which were usually used to estimate the activity of SCD1 and were correlated with steatosis, inflammation or ballooning and reported higher in those individuals with steatosis or NASH compared to those with normal liver [5,27–29]. Then, we detected the mRNA and protein level of SCD1 and discovered that high-dose LLDT-8 could obviously lower SCD1 expression (Fig. 3E, F). Moreover, the ratio of C16:0 to C18:2 also has a decreased trend (Fig. 3G), which is one of markers of hepatic DNL [5] and suggested that LLDT-8 has an ability to inhibit hepatic lipogenesis.

3.4. LLDT-8 inhibited hepatic steatosis through increasing lipolysis

In order to assess others molecular mechanisms by which LLDT-8 reversed hepatic steatosis, we performed serological analyses to discover that high-dose LLDT-8 treatment significantly increased β-hydroxybutyrate level in serum (Fig. 4A) which is one of FAO products [30,31]. Since the activation of PPARα can promote FAO, the effects of LLDT-8 on PPARα mRNA and protein in liver were then detected. As shown in Fig. 4B–G, PPARα mRNA and protein were obviously elevated in high-dose LLDT-8-treated group, while the mRNA of its downstream genes, Cpt1a, Acox1, Acadl and Acadm coded for related enzymes involved in FAO pathways, were also increased accordingly [32,33]. In addition, the level of serum LDL-C was examined to exclude the possible risk of LLDT-8 in cardiovascular disease for its effect on increasing FAO. As shown in Fig. 4H, there is no obviously difference in LDL-C after LLDT-8 treatment. Therefore, LLDT-8 could regulate beta-oxidation of FAs via affecting related genes expression without inducing the change in LDL-C.

4. Discussion

Emerging evidence suggests that excessive hepatic TG and TC accumulation in the liver is a main risk factor in the development of NASH [34]. Therefore, it is an urgent need to find effective strategies to reduce fat accumulation involved in the progression of NAFLD.

Mice fed the HFHFr diet had increased levels of liver TG and TC, and exhibited histological features of NAFLD, including macrovesicular steatosis, ballooning and lobular inflammation [35]. The results of the current study were showed LLDT-8 obviously attenuated liver Oil staining and decreased hepatic TG and TC. What's more, our study exhibited a significant decrease in serum ALT and AST levels when LLDT-8 was fed along with the HFHFr diet, thus improved liver function.

There are four main pathways of TG accumulation in the liver, including lipid synthesis, FFA uptake, VLDL export and FAO [6]. Firstly, SCD1 is a rate-limiting enzyme in the biosynthesis of monounsaturated FAs. Enhanced the activity of SCD-1 promotes the hepatic DNL and exacerbates the progress of NAFLD [36]. The present study stated that LLDT-8 treatment downregulated the expression of SCD1 to against hepatic lipid accumulation. Secondarily, PPARα is a key nuclear receptor that regulates the transcription of genes involved in FAO, and HFD was reported to down-regulate PPARα expression in C57BL/6J mice [37]. Cpt1a is responsible for transporting FAs from the cytosol to the mitochondria before beta-oxidation, and is also remarkably decreased in NAFLD patients and HFD-induced obesity mice [38,39]. Acadl and Acadm is the first step in catalyzing the oxidation of FAs, while Acox1 is critical for FAs oxidation in peroxisome [40]. In our study, LLDT-8 effectively promoted FAO by increasing the expression of those genes and gradually reduced the hepatic TG level. Finally, several genes involved in hepatic FAs uptake and VLDL export (data not shown) were no obvious changes in mice after LLDT-8 supplement, indicated LLDT-8 may have no effect on FAs uptake and VLDL export to reduce TG accumulation. In summary, LLDT-8 might inhibit lipogenesis via SCD1 pathway and increase FAO via PPARα pathway to ameliorates liver lipid accumulation.

Compared to triptolide, LLDT-8 has lower toxicity and overcome the problem of poor water solubility and narrow therapeutic window [41]. However, LLDT-8 also targets spermatocytes in the testes to cause testicular injury in a dose and time-dependent manner [16,42], which is the main adverse effect in rodents. So, we need to consider developing a liver-targeted drug delivery system to avoid this side effect in the future. In addition, we will modify its structure to further reduce toxicity. The dose of LLDT-8 in phase I clinical trial was from 0.25 mg/d to 4 mg/d [17], which was set from pharmacokinetic and pharmacodynamic results in experimental animals where the dose used in rodents was from 0.125 mg/kg to 2 mg/kg. In our work, 1 mg/kg of LLDT-8 can obviously ameliorate liver lipid accumulation in C57BL/6J mice, indicating the result of LLDT-8 in phase I clinical trial might provide useful information to LLDT-8 in the treatment of NAFLD.

5. Conclusions

In conclusion, we have demonstrated that LLDT-8 effectively attenuated HFHFr diet-induced hepatic steatosis and lipid accumulation. LLDT-8 may protect against the deleterious effects via down-regulation of SCD1, a key regulator of lipid synthesis, as well as up-regulation of several genes involved in oxidation of FA, including PPARα, Cpt1a, Acox1, Acadl and Acadm. These results suggest that LLDT-8 could be a promising agent for preventing the progression of NAFLD.

Author contributions

Yunxia Dong, Qiang Li and Henglei Lu performed the experiments; Yunxia Dong wrote the manuscript; Yunxia Dong, Henglei Lu, Xinming Qi and Jing Chen contributed experiment design; Yuanchao Li contributed to provide compound. Jin Ren, Zean Zhang and Jing Chen revised the manuscript. We confirm that the manuscript has been read and approved by all named authors. We also confirm that the order of authors listed in the manuscript has been approved by all of us.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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