Introduction

Recently, the prevalence of obesity has increased dramatically worldwide along with obesity-related metabolic disorders, such as hyperlipidemia, hepatic steatosis and type 2 diabetes[1, 2]. The primary defect in obesity is the excessive accumulation of triglycerides in the blood, white adipose tissue (WAT), liver, and other tissues[3]. Inhibition of triglyceride synthesis may be a feasible strategy for the treatment of obesity and its related medical consequences[4, 5].

The final and only committed step in mammalian triglyceride synthesis is catalyzed by acyl CoA:diacylglycerol acyltransferase (DGAT) enzymes, which convert diacylglycerol and fatty acyl-CoA into triglycerides[6]. Mammals have two DGAT enzymes (DGAT1 and DGAT2), which share no sequence homology[7, 8]. Both enzymes are ubiquitously expressed in mammalian tissues and are highly expressed in adipose tissue, liver and intestine, where triglyceride synthesis is most active[9]. DGAT2-deficient (Dgat2−/−) mice die early in the postnatal period due to severe triglyceride depletion, implying that DGAT2 function is crucial for survival[10]. DGAT1-deficient (Dgat1−/−) mice are viable, have significantly reduced tissue triglycerides, and are resistant to high-fat diet-induced body weight gain through increased energy expenditure rather than decreased food intake[11-13]. Dgat1−/− mice are also protected from insulin resistance caused by a high-fat diet or by genetic crosses onto the Agouti yellow (AY/a) background[12-14]. In addition, knockdown of DGAT1 expression with antisense oligonucleotides protects against hepatic steatosis induced by a high-fat diet[15]. Thus, DGAT1 may represent a novel therapeutic target for obesity, diabetes, and hepatic steatosis[4, 16, 17].

Pharmacological inhibition of diacylglycerol acyltransferase 1 reduces body weight gain, hyperlipidemia, and hepatic steatosis in db/db mice

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Aim: To test whether pharmacological inhibition of Diacylglycerol acyltransferase 1 (DGAT1) by a small-molecule inhibitor H128 can improve metabolism disorders in leptin receptor-deficient db/db mice.

Methods: To investigate the effect of H128 on intestinal fat absorption, db/db mice were acutely given a bolus of corn oil by gavage. The mice were further orally administered H128 (3 and 10 mg/kg) for 5 weeks. Blood glucose, lipids, insulin, ALT, and AST as well as hepatic triglycerides were measured. The insulin tolerance test was performed to evaluate insulin sensitivity. The expression of genes involved in fatty acid oxidation was detected by RT-PCR.

Results: Oral administration of H128 (10 mg/kg) acutely inhibited intestinal fat absorption following a lipid challenge in db/db mice. Chronic treatment with H128 significantly inhibited body weight gain, decreased food intake, and induced a pronounced reduction of serum triglycerides. In addition, H128 treatment markedly ameliorated hepatic steatosis, characterized by decreased liver weight, lipid droplets, and triglyceride content as well as serum ALT and AST levels. Furthermore, H128 treatment increased the expression of the CPT1α and PPARα genes in liver, suggesting that H128 enhanced fatty acid oxidation in db/db mice. However, neither blood glucose nor insulin tolerance was affected by H128 treatment throughout the 5-week experimental period.

Conclusion: DGAT1 may be an effective therapeutic target for the treatment of obesity, hyperlipidemia and hepatic steatosis.

Keywords: diabetes; hepatic steatosis; obesity; hyperlipidemia; small-molecule inhibitor; H128; diacylglycerol acyltransferase 1; db/db mice; CPT1 gene; PPARα gene
Recent reports show that pharmacologic inhibition of DGAT1 by small molecules suppresses high-fat diet-induced body weight gain in diet-induced obese (DIO) mice\[18, 19]. However, to the best of our knowledge, no studies have examined the effects of small-molecule DGAT1 inhibitors on glucose metabolism in animal models. H128 (Figure 1A) is a potent DGAT1 inhibitor with an IC_{50} value of 98 nmol/L against human DGAT1\[19]. In this study, we sought to determine whether H128 could improve glucose metabolism in leptin receptor-deficient db/db mice, which spontaneously develop obesity and diabetes. We also evaluated the effects of H128 on body weight, blood lipids, and hepatic steatosis in db/db mice.

**Materials and methods**

**Compound**

H128 was synthesized in the Shanghai Institute of Materia Medica, Chinese Academy of Sciences. H128 was prepared in 0.5% Tween-80 solution in water for in vivo studies.

**Animals and experimental protocols**

Male C57BL/Ks-J-Lep \( ^{db} \) (db/db) and their lean littermates, obtained from Jackson Laboratories (Bar Harbor, Maine, USA), were maintained in a 12:12 light–dark cycle with ad libitum access to water and a normal chow diet. The db/db mice at 10 weeks of age were divided into three groups (n=5−6/group) on the basis of fasting blood glucose level and body weight. The db/db mice were gavaged once daily with vehicle (0.5% Tween-80) or H128 (3 mg/kg and 10 mg/kg) for 5 weeks. The lean mice were also treated with vehicle in an identical manner. Blood glucose was monitored in tail vein blood using a glucometer (One-Touch Ultra, Lifescan, Milpitas, USA) every 30, 60, 90, and 120 min after insulin administration.

**Acute lipid challenge test**

Male db/db mice at 8 weeks of age, previously maintained on a chow diet, were fasted for 16 h. Animals were given H128 or vehicle by oral gavage and, 1 h later, given a bolus of corn oil (5 mL/kg). After an additional 1 h, blood samples were collected from the ophthalmic venous plexus. Serum triglyceride level was determined using a commercially available colorimetric kit (Rongsheng, Shanghai, China).

**Insulin tolerance test**

After 4 weeks receiving vehicle or H128, mice were fasted for 6 h, followed by intraperitoneal administration of insulin (1 unit/kg). Blood glucose was measured from the tail vein using the same glucometer at 0 (prior to insulin administration), 30, 60, 90, and 120 min after insulin administration.

**Biochemical measurements**

Serum triglyceride and cholesterol levels were measured using commercial enzyme assay kits (Rongsheng, Shanghai, China). Serum insulin was assayed with an insulin ELISA kit (Linco Research, MO, USA). The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in serum were determined by commercial kits (Rongsheng, Shanghai, China)

To measure liver triglyceride content, liver triglycerides were extracted with ethanol by a procedure modified from Cool et al\[20]. Briefly, liver samples were homogenized in 30 volumes of ethanol, then vortexed and centrifuged at 15000×g for 10 min at room temperature. The supernatant was used to measure liver triglyceride content as described above.

**Histological analysis**

Liver samples were fixed in 10% buffered formalin and embedded in paraffin wax. After dehydration, the sections were stained with hematoxylin & eosin. To visualize neutral lipids, liver samples were frozen in Tissue-Tek OCT compound (Sakura Finetek USA Inc, CA, USA) and sections were stained with Oil-Red O. Images of the histological sections were analyzed for hepatic steatosis.

**Gene expression analysis**

Total RNA was extracted from frozen liver samples (50 mg) using a RNeasy Lipid Tissue Mini Kit (Qiagen, Tokyo, Japan). For gene expression analysis, the cDNA was synthesized from total RNA using M-MLV reverse transcriptase (Invitrogen Life Technologies, California, USA). Semi-quantitative real-time PCR was performed using SYBR® Premix Ex Taq™ II (Takara, Dalian, China) on the 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, USA). All of the primers used are listed in Table 1. The mRNA levels were normalized relative to the amount of β-actin mRNA and are presented as arbitrary units.

**Statistical analysis**

All data are expressed as the mean±SEM. The comparison of different groups was assessed by two-tailed unpaired Student’s t test. Differences were considered statistically significant at P<0.05.

**Results**

**H128 acutely inhibits intestinal triglyceride absorption in db/db mice**

To investigate the effect of inhibition of DGAT1 on intestinal triglyceride absorption in db/db mice, H128 was evaluated in an acute lipid challenge model by measuring serum triglyceride level following a bolus of corn oil. One hour after a lipid challenge, serum triglycerides were increased by 54% (0.94±0.07 vs 1.44±0.05, P<0.01) in db/db mice (Figure 1B). However, when mice were pretreated with 10 mg/kg of H128 1 h followed by a lipid challenge, serum triglycerides remained close to the original level (Figure 1B). Thus, H128 can acutely inhibit intestinal triglyceride absorption following
a lipid challenge in db/db mice.

**Effects of H128 on body weight gain and food intake in db/db mice**

Next, we investigated the chronic effects of H128 on body weight gain and food intake in db/db mice. H128 treatment at 10 mg/kg for 5 weeks markedly suppressed body weight gain by about 50% (11.32±1.39 vs 5.74±0.53, P<0.05) relative to the vehicle-treated group (Figure 2B); this reduction began at the fourth week of H128 treatment (Figure 2A). Additionally, treatment with 3 mg/kg H128 elicited a slight decrease (about 30%) in body weight gain at the end of the experiment (Figure 2B). At both doses of 3 mg/kg and 10 mg/kg, H128 treatment modestly reduced food intake as compared with the vehicle-treated mice throughout the 5-week experimental period (Figure 2C). Food intake in H128-treated mice was decreased by 7.8% (5.85±0.06 vs 5.39±0.09, P<0.05) by 3 mg/kg and by 6.8% (5.85±0.06 vs 5.45±0.11, P<0.05) by 10 mg/kg H128 (Figure 2D).

**Effects of H128 on blood lipids and glucose metabolism in db/db mice**

db/db mice usually serve as a genetic animal model of metabolic disorders with significant obesity, hyperlipidemia, and fasting hyperglycemia. As shown in Figure 3, db/db mice displayed higher levels of triglycerides, cholesterol, glucose, and insulin in the blood compared with lean mice. Five weeks of H128 treatment at doses of 3 and 10 mg/kg produced pronounced reductions of serum triglyceride levels in db/db mice (Figure 3A). Although no significant difference was found in serum cholesterol, there was a trend towards decreased cholesterol in db/db mice treated by H128 (Figure 3B).

To evaluate whether H128 treatment also improves glucose homeostasis in db/db mice, fasting blood glucose level was measured during the experimental period. H128 did not affect blood glucose level after 1, 2, or 4 weeks of treatment (Figure 3D). We further conducted an intraperitoneal insulin tolerance test to evaluate the effect of H128 treatment on insulin sensitivity. The glucose levels at different times were similar between vehicle- and H128-treated db/db mice (Figure 3E). Although H128 treatment significantly decreased serum insulin level (Figure 3C), neither glucose homeostasis nor insulin resistance in db/db mice was improved by H128 treatment.

**H128 improved hepatic steatosis in db/db mice**

Because DGAT1 catalyzes the final step in triglyceride synthesis, decreasing DGAT1 activity is assumed to reduce hepatic steatosis[15]. Compared with the lean mice, the db/db mice (vehicle-treated) exhibited severe hepatic steatosis characterized by numerous “foamy” cells (hematoxylin & eosin staining) and lipid droplets (Oil-Red O staining) (Figure 4A). After 5 weeks of H128 treatment at the dose of 10 mg/kg, the “foamy” cells and lipid droplets were decreased (Figure 4A). Biochemical analysis of triglyceride content in liver further confirmed the histological changes (Figure 4B). H128 treatment at 10 mg/kg decreased hepatic triglyceride content by 25% (197.08±12.94 vs 148.22±7.51, P<0.05). In addition, a corresponding decrease in liver weight was observed in H128-treated db/db mice compared with the vehicle group (2.55±0.12 vs 2.04±0.02, P<0.05) (Figure 4C). Liver enzymes ALT and AST were also measured in serum as an indication of liver

### Table 1. Primers used in gene expression analysis.

| Probe     | Forward                  | Reverse                  |
|-----------|--------------------------|--------------------------|
| ACC1      | CTTCTTGACAAACGAGTCTGGG  | CTGCCGAAACATCTCGGGA      |
| CPT1a     | CTTCATGACTCGGCTCTTC     | AGCCTGAAACTGCTCCTGCTG   |
| PPARα     | TTGCTGGGAGATCGGCTCTCT   | GGATGGTGGTGCTGAGGGT     |
| PPARγ     | TTTTCAAGGCGAGGCTCTTTC   | ATACGTGACCCCTCTGAGAT    |
| SCD-1     | CAGGCTGAGATGCTGCTGGA    | CAGAGGCTGCTGTAGTAGT     |
| SREBP1c   | TAGGACATGCACAAAACAGC    | TGGTAGACACAAGCGCCTATC   |
| β-actin   | CACGATGGAGGGCGCACTCATC  | CTAAGACCTCTATGGCAACACAGT|

**Figure 1.** Structure of H128 and serum triglyceride level after an acute lipid challenge in db/db mice. (A) Structure of H128. (B) Serum triglyceride (TG) level. The acute lipid challenge test was performed as described in Materials and Methods. The data are expressed as the mean±SEM. n=5. *P<0.01 vs vehicle group.

**Figure 2.** Structure of H128 and serum triglyceride level after an acute lipid challenge in db/db mice. (A) Structure of H128. (B) Serum triglyceride (TG) level. The acute lipid challenge test was performed as described in Materials and Methods. The data are expressed as the mean±SEM. n=5. *P<0.01 vs vehicle group.
damage. H128 treatment decreased the serum levels of ALT and AST, indicating a reversal of liver pathology (Figure 4D and 4E). These results indicate that H128 treatment protects against hepatic steatosis in db/db mice.

Effects of H128 on the expression of genes related to hepatic lipid metabolism
To elucidate the molecular mechanisms by which inhibiting DGAT1 leads to protection against obesity and hepatic stea-
atosis, we further examined the expression of genes involved in hepatic lipid metabolism in *db/db* mice. Compared with the vehicle group, H128 treatment at 10 mg/kg significantly increased the levels of CPT1 and PPARα mRNAs (Figure 5E and 5F), both of which are key regulators of fatty acid oxidation in rodents [21]. However, no significant differences were detected in the levels of mRNAs encoding proteins related to fatty acid synthesis [22], such as SCD1, ACC1, SREBP1c, and PPARγ (Figure 5A−5D), implying that H128 treatment did not affect fatty acid synthesis in the liver of *db/db* mice.

**Discussion**

In this study, we demonstrated for the first time that oral administration of H128, a small-molecule inhibitor of DGAT1, can reduce body weight gain, hyperlipidemia, and hepatic steatosis but does not improve glucose metabolism in *db/db* mice. Compared with the vehicle group, H128 treatment at 10 mg/kg significantly increased the levels of CPT1 and PPARα mRNAs (Figure 5E and 5F), both of which are key regulators of fatty acid oxidation in rodents [21]. However, no significant differences were detected in the levels of mRNAs encoding proteins related to fatty acid synthesis [22], such as SCD1, ACC1, SREBP1c, and PPARγ (Figure 5A−5D), implying that H128 treatment did not affect fatty acid synthesis in the liver of *db/db* mice.

The highest levels of DGAT1 expression are found in the small intestine, suggesting an important role for DGAT1 in intestinal triglyceride synthesis and dietary fat absorption [7, 9]. In support of this notion, *Dgat1*−/− mice exhibit a delay in circulating postprandial hypertriglyceridemia, suggesting a reduction of intestinal triglyceride absorption [23]. The importance of DGAT1 in dietary fat absorption has been further confirmed by potent DGAT1 inhibitors. For example, the DGAT1 inhibitor XP620 reduces apolipoprotein B secretion in Caco-2 cells, triglyceride synthesis in primary enterocytes and dietary fat absorption in normal Sprague-Dawley rats [24]. Moreover, new, potent DGAT1 inhibitors reduce dietary fat absorption following a lipid challenge and decrease body weight in DIO mice [18, 19], recapitulating the major phenotype of *Dgat1*−/− mice. Our results show that the DGAT1 inhibitor H128 inhibited dietary fat absorption in *db/db* mice, which may be a major reason for the beneficial effects of H128 treatment in *db/db* mice.

Increased energy expenditure is believed to be one of the major reasons why *Dgat1*−/− mice are protected from diet-induced obesity [11, 12, 25]. In the present study, H128 treatment significantly increased hepatic levels of CPT1 and PPARα mRNAs in *db/db* mice, indicating that H128 enhanced fatty acid oxidation. Actually, fatty acid oxidation in *Dgat1*−/− mice is also up-regulated [12]. Thus, our results are consistent with the increased energy expenditure in *Dgat1*−/− mice because enhanced fatty acid oxidation is assumed to increase energy expenditure [26, 27]. The reason why inhibition of DGAT1 would enhance fatty acid oxidation remains unknown. Due to the increased leptin sensitivity in *Dgat1*−/− mice [12] and the positive effect of leptin on fatty acid oxidation [28, 29], DGAT1 may
regulate fatty acid oxidation via the leptin signaling pathway. However, the DGAT1 inhibitor H128 enhanced fatty acid oxidation in the leptin receptor-deficient db/db mice, implying that leptin may be not necessary for DGAT1-regulated fatty acid oxidation.

DGAT1 is expressed highly in human liver\textsuperscript{[30]}, and increased mRNA levels of DGAT1 have been found in humans with non-alcoholic fatty liver disease\textsuperscript{[30]}, underscoring the importance of DGAT1 in hepatic steatosis. Global or liver-specific knockout of DGAT1 in mice protects them from diet-induced hepatic steatosis\textsuperscript{[13, 15]}. Likewise, pharmacological inhibition of DGAT1 decreases liver triglyceride content in DIO mice\textsuperscript{[15]}. Nevertheless, liver-specific knockdown of DGAT1 in ob/ob or db/db mice does not protect against hepatic steatosis\textsuperscript{[15]}. Indeed, liver-specific knockdown of DGAT1 by intraperitoneal injection with antisense oligonucleotides is unable to diminish the effects of DGAT1 in extrahepatic tissues\textsuperscript{[19]}. In the present study, H128 was administered by oral gavage, and it inhibited intestinal fat absorption and increased fatty acid oxidation, both of which may underlie the protective effect of H128 against hepatic steatosis in db/db mice.

Dgat1\textsuperscript{−/−} mice have increased sensitivity to insulin, and homozygous deletion of Dgat1 in A\textsuperscript{+/−} mice with insulin resistance and obesity significantly improves insulin sensitivity\textsuperscript{[12]}. Therefore, it is appealing to speculate that pharmacological inhibition of DGAT1 by small-molecule inhibitors will improve glucose metabolism. However, H128 treatment for 5 weeks did not lower blood glucose or improve insulin tolerance in db/db mice. Hepatic steatosis is strongly associated with insulin resistance in animals and humans\textsuperscript{[32–34]}. Although H128 treatment significantly improved hepatic steatosis, it did not improve glucose metabolism in db/db mice. Of note, overexpressing DGAT1 in the liver of mice induces hepatic lipid accumulation and hepatic steatosis but does not impair glucose or insulin tolerance\textsuperscript{[30]}. Also, it is possible that increased fatty acid oxidation by inhibition of DGAT1 may cause oxidation stress, leading to insulin resistance\textsuperscript{[36]}, which neutralizes the effect of reduced triglycerides in liver. Thus, the increased insulin sensitivity in Dgat1\textsuperscript{−/−} mice might be not attributable to decreased liver triglyceride content. A more likely explanation is that the effects of DGAT1 deficiency on glucose metabolism require an intact leptin pathway, because homozygous deletion of Dgat1 in db/db or leptin-deficient ob/ob mice does not improve glucose metabolism\textsuperscript{[12]}. On the other hand, the positive effects of H128 in db/db models (such as reduced lipid absorption, reduced body weight gain, amelioration of hyperlipidemia and hepatic steatosis, and enhanced fatty acid oxidation) may be mediated in leptin-independent manners. Whether pharmacological inhibition of DGAT1 by small-molecule inhibitors can improve glucose metabolism needs further study in other animal models.

In conclusion, treatment with the DGAT1 inhibitor H128 reduced body weight gain, lowered blood lipids and improved hepatic steatosis in db/db mice. Although H128 did not improve glucose metabolism in db/db mice, we will further evaluate the effects of H128 on glucose metabolism in other

![Figure 5. Effects of H128 on the expression of genes related to fatty acid regulation in liver. At the end of the experiment, total RNA was extracted from the liver, and semi-quantitative real-time PCR was performed to assess the mRNA levels of the indicated genes involved in fatty acid regulation. (A) ACC1, acyl-CoA carboxylase 1. (B) PPAR\textsubscript{γ}, peroxisome proliferator-activated receptor gamma. (C) SCD1, stearoyl-CoA desaturase 1. (D) SREBP1c, sterol regulatory element binding protein 1c. (E) CPT1a, carnitine palmitoyltransferase 1a. (F) PPAR\textsubscript{α}, peroxisome proliferator-activated receptor alpha. All data are expressed as the mean±SEM. n=3. \(b\)\(P<0.05\), \(c\)\(P<0.01\) vs vehicle group.](https://www.chinaphar.com/zh/ZhangXD)
animal models, such as DIO mice. Our findings suggest that inhibition of DGAT1 is an effective strategy to treat obesity, hyperlipidemia and hepatic steatosis.

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Author contribution
He-yao WANG and You-hong HU designed the research; Xiao-dong ZHANG, Jian-wei YAN, Gui-ru Yi, Xiao-yun SUN, and Jun LI performed the experiments; Xiao-dong ZHANG analyzed the data; Xiao-dong ZHANG wrote the paper. Yi-ming LI was actively involved throughout the entire study.

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