A Novel Inhibitor of Stearoyl-CoA Desaturase-1 Attenuates Hepatic Lipid Accumulation, Liver Injury and Inflammation in Model of Nonalcoholic Steatohepatitis

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Stearoyl-CoA desaturase-1 (SCD-1) catalyzes the biosynthesis of monounsaturated fatty acids, and their abnormality is possibly responsible for obesity, insulin resistance, hepatic steatosis and nonalcoholic steatohepatitis (NASH). A novel SCD-1 inhibitor, N-(2-hydroxy-2-phenylethyl)-6-[4-(2-methylbenzoyl)piperidin-1-yl]pyridazine-3-carboxamide, has been obtained. The compound inhibited liver SCD-1 activity and increased liver triglyceride accumulation in mice fed with non-fat, high-sucrose diets. In order to evaluate the effects of the SCD-1 inhibitor on NASH development, rats were fed with lipogenic methionine and choline-deficient (MCD) diets for 8 weeks. The SCD-1 inhibitor was administered once-daily at a dose of 30 or 100 mg/kg/d by oral gavage. Administration of a high dose of the SCD-1 inhibitor decreased triglyceride accumulation in the liver of NASH rats by 80%. Administration of a high dose of the SCD-1 inhibitor attenuated the increase of aspartate aminotransferase (AST) and alanine transaminase (ALT) by 86% and 78%, respectively. Hepatic steatosis, hepatocellular degeneration and inflammatory cell infiltration were histologically observed in the liver of NASH rats, and administration of the SCD-1 inhibitor ameliorated these crucial observations in NASH. In summary, an SCD-1 inhibitor ameliorated hepatic triglyceride accumulation, liver injury, hepatocellular degeneration and inflammation in experimental NASH models. These results suggest that SCD-1 maybe a promising target for the treatment of NASH.

Key words stearoyl-CoA desaturase-1; steatohepatitis; methionine choline-deficient diet

Nonalcoholic fatty liver disease (NAFLD), which is identified with excess lipid accumulation in the liver, is strongly associated with obesity, insulin resistance, hypertension, dyslipidemia and metabolic syndrome. Most NAFLD are associated with obesity, insulin resistance, hypertension, steatohepatitis with excess lipid accumulation in the liver, is strongly associated with obesity, insulin resistance, hypertension, dyslipidemia and metabolic syndrome. The SCD-1 inhibitor on development of NASH.

Methionine and choline-deficient (MCD) diet model is one of the most useful NASH models. We previously reported that rat α1 type I collagen promoter-luciferase transgenic (Col1a1-Luc Tg) rats were an appropriate model for sensitively evaluating the fibrosis of kidney and various organs in a short-term period. In the present study, Col1a1-Luc Tg rats received MCD diets for 8 weeks to evaluate the pro-fibrogenic response in NASH development in a short-term period; however, collagen 1α1 promoter activity failed to increase,
although slight or mild fibrosis was histologically observed. On the other hand, other crucial phenotypes of NASH including hepatic steatosis, liver injury, hepatocellular degeneration and inflammation were induced in the liver of these rats fed with MCD diets for 8 weeks. Therefore, we evaluated the effects of an SCD-1 inhibitor on these crucial observations in NASH.

MATERIALS AND METHODS

Synthesis of SCD-1 Inhibitor N-(2-Hydroxy-2-phenylethyl)-6-[4-(2-methylbenzoyl)piperidin-1-yl]pyridazine-3-carboxamide (Fig. 1), a potent and orally available SCD-1 inhibitor, was discovered by Daiichi Sankyo, Co., Ltd. The detailed synthetic procedures, physicochemical properties and pharmacokinetic profile of the SCD-1 inhibitors with this structural motif have been previously reported. 21)

Animals All experimental procedures were performed in accordance with the in-house guideline of the International Animal Care and Use Committee of Daiichi Sankyo Co., Ltd. Collal1-Luc Tg rats were generated by a previously reported method, and bred in Japan SLC Inc. (Shizuoka, Japan). 23) C57BL/6J mice were purchased from Charles River Laboratories Japan, Inc. (Yokohama, Japan). They were maintained in a room under a temperature controlled at 23±2°C and a 12-h light–dark lighting cycle. The animals were allowed standard pellet chow before the experiment and water ad libitum.

Mice Fed with Non-fat, High Sucrose Diets For evaluating liver SCD-1 activity, 9 week old male C57BL/6J mice received non-fat, high sucrose (NFHS) diets (Research Diets, Inc., New Brunswick, NJ, U.S.A.) for 7 d prior to administration of the SCD-1 inhibitor. NFHS diets were composed of 22 kcal% protein and 78 kcal% carbohydrate as sucrose. The SCD-1 inhibitor was administered once-daily at a dose of 10, 30 and 100 mg/kg (n=2) to mice by oral gavage in 4:1 mixture of polypylene glycol and Tween80 as vehicle. Rats were sacrificed 8 weeks after starting the administration. Blood was collected from the aorta, and plasma was separated by centrifugation. The livers were washed with saline and weighed. The specimens of liver were immediately snap-frozen and stored at −80°C for TaqMan polymerase chain reaction (PCR) analysis, measurement of liver triglyceride content, and luciferase activity. Portions of the liver lobes were also fixed in 10% buffered formalin (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and embedded in paraffin for histopathological analysis.

Liver Luciferase Activity The liver tissues were homogenized by Polytron homogenizer with the addition of 1 mL phosphate-buffered saline per 1 g of liver. After centrifugation at 3000 rpm for 20 min, the luminescence in supernatant was measured by a PicaGene kit (Toyo Ink MFG Co., Ltd., Tokyo, Japan). The concentration of luciferase was quantified using the standard curve of luciferase protein (Toyo Ink MFG Co.). The protein concentration was measured by protein assay reagent (Bio-Rad Laboratories, Inc., Tokyo, Japan), and luciferase activity was indicated as pg/mg protein.

Liver Triglyceride Content A piece of liver was homogenized by Polytron homogenizer with the addition of phosphate buffered saline. The homogenates were mixed with the mixture of CHCl₃ and MeOH (2:1). After 5 min mixing by vortex mixer, the mixture was centrifuged at 14000 rpm for 3 min. The lower layer was collected and evaporated. After being dissolved in isopropanol containing 10% Triton, triglyceride content was measured by Triglyceride E test-Wako (Wako Pure Chemical Industries, Ltd.).

Plasma Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) AST and ALT levels in the plasma were measured using an Autoanalyzer (Hitachi 7250).

Quantitative Reverse Transcription (RT)-PCR Analysis The liver tissues were homogenized by Polytron homogenizer with the addition of Trizol reagent (Invitrogen, Carlsbad, CA, U.S.A.). After centrifugation at 10000 rpm at 25°C, 0.2 mL chloroform was added and mixed by a vortex mixer. After 10 min incubation at room temperature, these were centrifuged at 10000 rpm at 25°C, and the water layer was collected. RNA was purified by an Rneasy Mini Kit (Qiagen, Valencia, CA,
U.S.A.) and RNA concentration was measured by Gene Spec III (Hitachi, Tokyo, Japan). cDNA was synthesized by a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, U.S.A.). TaqMan PCR was carried out by ABI PRISM 7900 (PerkinElmer Life Sciences, Boston, MA, U.S.A.). The mixture containing 5 µL cDNA, 25 µL TaqMan 2×PCR Master Mix (Applied Biosystems), 0.5 µL forward primer, 0.5 µL reverse primer and 0.5 µL TaqMan probe, were reacted together. The thermal cycler conditions were 2 min at 50°C, 10 min at 95°C, and 40 cycles of 15 s at 95°C followed by 1 min at 60°C. The sequence of primers and probe of rat monocyte chemotactic protein-1 (MCP-1) were indicated as follows: Forward primer: 5′-CTTCCACGTTGCTGCTGTAGAC-3′; Reverse primer: 5′-AGTGAAATGTA GCCAGCAGCTGAG-3′; Probe: 5′-FAM-AGT GAA TGA GTA GCCAGCAGCTGAG-TAMRA-3′. The sequence of primers and probe of Liver Δ9 desaturase activity in human microsomal and cellular Δ9 desaturase activity in vitro were 15 and 51 nM, respectively, as previously reported. To confirm in vivo inhibitory activity on liver SCD-1, the compound was administered to mice fed with NFHS diets, which the animals indicated an increase of SCD-1 activity and mRNA expression in livers (data not shown). Single dosing of the compound inhibited liver SCD-1 activity in a dose dependent manner, evaluated by the ratio of C18:1 (oleate) to C18:0 fatty acids (stearate) (Fig. 2A). The IC50 was 17.2 mg/kg. Triglyceride content in liver of mice fed with NFHS diets for 7 d was twice as much as standard chow diets. Repeated dosing of the SCD-1 inhibitor at 30 and 100 mg/kg/d for 7 d decreased NFHS diets-induced triglyceride accumulation in liver in a dose dependent manner (Fig. 2B).

**SCD-1 Inhibitor Reduced Triglyceride Accumulation of Liver in NASH Rats** In order to evaluate the effects of the SCD-1 inhibitor on NASH development, rats were fed with MCD diets for 8 weeks. The doses of the SCD-1 inhibitor in the study were 30 and 100 mg/kg/d based on the results of the study shown in Fig. 2 and other preliminary studies. Feeding of MCD diets to rats induced body weight loss by 30% (Table 1). Repeated administration of a high dose of the SCD-1 inhibitor significantly decreased final body weight, although the effects on body weight gain were not significant. The SCD-1 inhibitor did not affect the food intake (data not shown). The SCD-1 inhibitor did not also change the liver weight. Triglyceride content in the liver was measured for the purpose of evaluating the effects on steatosis in NASH rats. Feeding of MCD diets increased triglyceride content in the liver of rats by 12-fold (Fig. 3A), indicating lipid accumulation in the liver. Administration of a high dose of the SCD-1 inhibitor reduced triglyceride accumulation in the liver of NASH rats by 80%.

**SCD-1 Inhibitor Attenuated Liver Injury in NASH Rats** Plasma AST and ALT levels were measured for the purpose of evaluating the effects on liver injury. Feeding of MCD diets to rats increased plasma AST and ALT levels by 3.3 and 5.5-fold, respectively (Figs. 3B,C). Administration of a high dose of the SCD-1 inhibitor significantly reduced the increase of plasma AST and ALT levels by 86% and 78%, respectively, indicating that the SCD-1 inhibitor ameliorated liver injury in NASH rats.
SCD-1 Inhibitor Attenuated Hepatic Steatosis, Hepatocellular Degeneration and Inflammation in NASH Rats

In histopathological analysis, feeding of MCD diets indicated severe steatosis in the liver of rats, but not in the control group (Figs. 4A, B). Administration of the SCD-1 inhibitor attenuated development of steatosis, indicated by reduction of lipid droplets in the liver, and it was remarkable in a high dose of the SCD-1 inhibitor-administered rats (Fig. 4C, D). Hepatocellular degeneration and necrosis was observed in the liver of rats fed with MCD diets. Administration of the SCD-1 inhibitor attenuated the severity (Fig. 5A). Inflammatory cell infiltration was also observed in the liver of rats fed with MCD diets, and it was rarely observed in a high dose of the SCD-1 inhibitor-administered rats (Figs. 4E, F, 5B). These results indicate that the SCD-1 inhibitor attenuated crucial histopathological changes including hepatic steatosis, hepatocellular degeneration and inflammatory cell infiltration in experimental NASH liver.

The mRNA expression of MCP-1, which is a pro-inflammatory cytokine concerned with NASH development, was increased in the liver of NASH rats by 36.8-fold (Fig. 6). Administration of a high dose of the SCD-1 inhibitor reduced the mRNA expression by 79.4%.

Slight or mild fibrosis was observed in the liver of rats fed with MCD diets (Fig. 7A). Administration of the SCD-1 inhibitor attenuated the development of early fibrogenic change (Fig. 7B), indicated by the scores of fibrosis (Fig. 7C). Coll1a1-Luc Tg rats were used in this study for the purpose of evaluating the pro-fibrogenic response in the short-term experiments. However, the luciferase activity, indicating collagen 1α1-promoter activity, was not significantly increased in the liver of rats fed with MCD diets (Fig. 8). Therefore, to fully evaluate the effects of the SCD-1 inhibitor on liver fibrosis, it is necessary to study for longer periods.
Fig. 4. Hematoxylin–Eosin Staining of Liver in SCD-1 Inhibitor-Treated NASH Rats

Rats were treated as shown in Materials and Methods. (A) Control, Vehicle-treated rats fed with control diets; (B) and (E) MCD, Vehicle-treated rats fed with MCD diets; (C) SCD-I 30, SCD-1 inhibitor (30 mg/kg/d) was daily administered to rats fed with MCD diets; (D) and (F) SCD-I 100, SCD-1 inhibitor (100 mg/kg/d) was daily administered to rats fed with MCD diets. Liver sections were prepared 8 weeks after the first treatment, and performed by hematoxylin–eosin staining. Representative sections of rats in each group are shown. Arrows indicate inflammatory cell infiltration. Bars indicate 0.4 mm for (A)–(D) and 0.1 mm for (E) and (F).

Fig. 5. The Scores of Hepatocellular Degeneration and Inflammation of Liver in SCD-1 Inhibitor-Treated NASH Rats

The scores of hepatocellular degeneration (A), and inflammation (B) are shown. Rats were treated as shown in Materials and Methods. Control, Vehicle-treated rats fed with control diets (n=4); MCD, Vehicle-treated rats fed with MCD diets (n=8); SCD-I 30, SCD-1 inhibitor (30 mg/kg/d) was daily administered to rats fed with MCD diets (n=5); SCD-I 100, SCD-1 inhibitor (100 mg/kg/d) was daily administered to rats fed with MCD diets (n=4). Liver sections were prepared 8 weeks after the first treatment, and performed by hematoxylin–eosin staining. The degree of hepatocellular degeneration and inflammation were scored as shown in Materials and Methods. Values are mean±S.E. *p<0.05, **p<0.01 vs. control, *p<0.05, **p<0.01 vs. MCD.
DISCUSSION

We have recently discovered novel SCD-1 inhibitors including spiropiperidine, benzoylpiperidine and thiazole-based structures. A benzoylpiperidine-based SCD-1 inhibitor with good ADME and pharmacokinetic profiles was used to evaluate the pharmacological effects in the present study. It was reported that feeding of high-carbohydrate or high-fructose diets increased expression of SCD-1 and other lipogenic genes and subsequently increased hepatic...

![Fig. 6. Effects of SCD-1 Inhibitor on MCP-1 mRNA Expressions in Liver of NASH Rats](image)

Rats were treated as shown in Materials and Methods. Control, Vehicle-treated rats fed with control diets (n=4); MCD, Vehicle-treated rats fed with MCD diets (n=8); SCD-I 30, SCD-1 inhibitor (30mg/kg/d) was daily administered to rats fed with MCD diets (n=5); SCD-I 100, SCD-1 inhibitor (100mg/kg/d) was daily administered to rats fed with MCD diets (n=4). Liver samples were prepared 8 weeks after the first treatment. Values are mean±S.E. **p<0.01 vs. control, #p<0.05 vs. MCD.

![Fig. 7. Masson-Trichrome Staining of Liver in SCD-1 Inhibitor-Treated NASH Rats](image)

Rats were treated as shown in Materials and Methods. Vehicle-treated rats fed with control diets (n=4); MCD, Vehicle-treated rats fed with MCD diets (n=8); SCD-I 30, SCD-1 inhibitor (30mg/kg/d) was daily administered to rats fed with MCD diets (n=5); SCD-I 100, SCD-1 inhibitor (100mg/kg/d) was daily administered to rats fed with MCD diets (n=4). Liver sections were prepared 8 weeks after the first treatment, and performed by Masson-trichrome staining. Representative sections of rats in MCD (A) and SCD-I 100 (B) are shown. Fibrotic area is stained as blue. Bar indicates 0.4mm. The scores of fibrosis were shown in (C). Values are mean±S.E. **p<0.01 vs. control, #p<0.05, ##p<0.01 vs. MCD.

![Fig. 8. Effects of SCD-1 Inhibitor on Luciferase Activity in Liver of NASH Rats](image)

Rats were treated as shown in Materials and Methods. Control, Vehicle-treated rats fed with control diets (n=4); MCD, Vehicle-treated rats fed with MCD diets (n=8); SCD-I 30, SCD-1 inhibitor (30mg/kg/d) was daily administered to rats fed with MCD diets (n=5); SCD-I 100, SCD-1 inhibitor (100mg/kg/d) was daily administered to rats fed with MCD diets (n=4). Liver samples were prepared 8 weeks after the first treatment. Values are mean±S.E.
triglyceride synthesis.\textsuperscript{26}\) SCD-1 deficient mice were protected from the increased hepatic lipogenesis, indicating an essential role of SCD-1 for hepatic triglyceride synthesis.\textsuperscript{27} In the present study, feeding of NFHS diets increased hepatic SCD-1 activity consistent with SCD-1 mRNA expression and hepatic de novo lipogenesis (data not shown), as previously reported. A single administration of the SCD-1 inhibitor inhibited the increased hepatic SCD-1 activity in mice fed with NFHS diets. Liver triglyceride content was also 2-fold increased in liver of mice fed with NFHS diets compared with standard chow diets. Repeated administration of the SCD-1 inhibitor completely inhibited the NFHS diets-induced triglyceride accumulation in the livers. These results also indicate that SCD-1 has a key role in hepatic triglyceride synthesis after feeding of high-carbohydrate diets. Some mechanisms for dietary carbohydrate-induced SCD-1 and lipogenic genes in liver were proposed. Increased insulin secretion by dietary carbohydrate induce lipogenic transcription factors, sterol regulatory element binding protein 1c (SREBP-1c) and liver X receptor (LXR), which can activate the transcription of SCD-1 and other lipogenic genes.\textsuperscript{28} Increased flux of glucose or fructose to liver can also induce SCD-1 and other lipogenic genes by SREBP-1c-dependent and -independent mechanisms.\textsuperscript{26,27} Therefore, excess intake of dietary carbohydrate and some kind of fatty acids, or food condition that increases insulin secretion possibly cause increased hepatic SCD-1 activity and triglyceride accumulation, and subsequently develop NAFLD/ NASH.

A “two-hit” theory has been proposed for the mechanism of NASH progression.\textsuperscript{29} The “first hit” is the lipid accumulation in liver caused by obesity, insulin resistance, dyslipidemia and other metabolic disorders. The lipid accumulation in liver leads to increased sensitivity to the “second hit” stimuli including oxidative stress, endotoxin, inflammatory cytokines, which then causes hepatoacellular injury, inflammation and fibrosis. In order to evaluate effects of the SCD-1 inhibitor on hepatoacellular injury and inflammation, rats were fed with lipogenic MCD diets, which highly contain carbohydrates. Lipogenic MCD diets induce liver triglyceride accumulation by mainly decreased triglyceride secretion as very low density lipoprotein (VLDL) and increased fatty acid uptake.\textsuperscript{30} In mice fed with MCD diets, hepatic lipogenesis was decreased, which was related to down-regulation of SCD-1.\textsuperscript{31} In the present study, SCD-1 mRNA in liver of rats fed with MCD diets was decreased (data not shown). Interestingly, the SCD-1 inhibitor effectively inhibited liver triglyceride accumulation in rats fed with MCD diets in spite of decreased expression of SCD-1. Levels of fatty acids in liver are regulated by fatty acid uptake, de novo lipogenesis, VLDL secretion and fatty acid oxidation. We speculated that the SCD-1 inhibitor decreased liver triglyceride accumulation in rats fed with MCD diets resulting from change of balance in these fatty acid metabolisms. In NAFLD patients, increased lipogenesis is a major contributor to hepatic triglyceride accumulation; therefore, reduction of hepatic triglyceride synthesis may be a major therapeutic target.\textsuperscript{32}

Obesity and insulin resistance are also key mediators for NASH pathology in addition to effects on liver triglyceride accumulation. In the present study, feeding of MCD diets caused remarkable body weight loss and decrease of plasma triglyceride, cholesterol and glucose (data not shown); therefore, the effects of the SCD-1 inhibitor on plasma lipid and glucose were not confirmed in these animals. It was reported that genetic deletion or pharmacological inhibition of SCD-1 prevented some metabolic disorders including obesity, insulin resistance and dyslipidemia.\textsuperscript{11–14,16–18,33–35} The SCD-1 inhibitor may have an advantage for NASH/NAFLD other than direct effects on hepatic triglyceride synthesis.

Feeding of MCDs diets histologically induced hepatocellular degeneration, necrosis and inflammatory cell infiltration in the liver of rats. Importantly, administration of the SCD-1 inhibitor ameliorated these notable observations. Administration of the SCD-1 inhibitor also attenuated the increase of plasma AST and ALT in rats fed with MCD diets. Hepatic stellate cells (HSCs) have an important role in fibrogenesis in NASH and other chronic liver diseases. HSCs normally function as vitamin A-stored cells. In a disease state, HSCs undergo morphological transdifferentiation to myofibroblast-like cells and produce collagen and other ECM components by some stimuli such as TGF-β, platelet-derived growth factor (PDGF), angiotensin II, connective tissue growth factor (CTGF) and reactive oxygen species.\textsuperscript{36} Activated HSCs also induce chemotaxis and activation of inflammatory cells such as monocytes/macrophages and T lymphocytes by production of inflammatory cytokines such as MCP-1.\textsuperscript{37,38} HSCs can also migrate towards these cytokine chemotractions.\textsuperscript{39} It was reported that feeding of MCDs diets to mice increased the mRNA expression of MCP-1 in livers.\textsuperscript{40} Our results also indicated that the mRNA expression of MCP-1 was increased in the liver of rats fed with MCD diets. Administration of the SCD-1 inhibitor attenuated the MCD diets-induced MCP-1 expression in liver of rats. These results indicate that the SCD-1 inhibitor ameliorates the NASH pathology including steatosis, liver injury, hepatocellular degeneration and inflammation. We expect that the direct target of the SCD-1 inhibitor is the process of triglyceride synthesis in livers. Interestingly, the SCD-1 inhibitor attenuated liver injury, hepatocellular degeneration and inflammation, although probably these processes are not a direct target. These results indicate that attenuation of the triglyceride accumulation in liver results in prevention of crucial phenotypes of NASH including liver injury, hepatocellular degeneration, inflammation and possibly fibrosis.

Fatty acids are key mediators for hepatocyte lipotoxicity, which play a role in hepatocellular death, oxidative and endoplasmic reticulum stress, inflammation and fibrosis.\textsuperscript{41} MUFAAs are generally less toxic than saturated fatty acids, and it was reported that MUFAAs protected saturated fatty acids-induced apoptosis in cells.\textsuperscript{42} Unbalanced accumulation of saturated fatty acids were reported in livers of mice fed with MCD diets concerned with decrease of SCD-1, suggesting that depletion of MUFAAs contribute to liver injury.\textsuperscript{43} Li et al. reported that feeding of MCD diets to SCD-1 deficient mice increased hepatocellular apoptosis, liver injury and fibrosis although hepatic steatosis was ameliorated.\textsuperscript{33} They suggested that hepatic SCD-1 plays a key role in prevention of steatohepatitis by partitioning excess lipids into MUFA that can be safely stored. In contrast, our results indicate that SCD-1 inhibition is protective to inflammation and liver injury in rats fed with MCD diets. Larter et al. reported that feeding of MUFA-rich diets did not protect MCD diet-induced liver injury in spite of the increased ratio of MUFA in liver fatty acids.\textsuperscript{44} They suggested that accumulation of fatty acids itself may be important
in liver injury, and it was not dependent on nature of fatty acid source. It was also reported that saturated fatty acids and MUFAs have similar effects on sensitization to TRAIL-induced apoptosis in cells.\textsuperscript{50} In human NASH, both saturated fatty acids and MUFA are accumulated in liver.\textsuperscript{56} These reports and our results suggested that MUFA and/or PUFA also play a role in liver injury in NASH.

In the present study, steatosis, hepatocellular degeneration, necrosis and inflammatory cell infiltration were histologically detected in the liver of rats fed with MCD diets for 8 weeks. On the other hand, there was only slight or mild fibrosis in the liver of the rats, although the SCD-1 inhibitor prevented the onset of the early fibrosis. It was previously reported that Colla1-Luc T\textsubscript{g} rats were useful as fibrosis models of the kidney and other organs, by which organ fibrosis was evaluated easily and in a short-term period.\textsuperscript{25} In the present study, Colla1-Luc T\textsubscript{g} rats were used to measure collagen 1a1 promoter activity as a pro-fibrogenic response in a short-term period. However, the luciferase activity failed to increase in rats fed with MCD diets for 8 weeks, although showing tendency to increase. Therefore, it is necessary to fully evaluate fibrosis by mRNA or protein levels of collagen in longer periods of experiments. It was also reported that feeding of cholesterol-containing atherogenic diets to mice induced liver injury, inflammation and fibrosis, which was concerned with increased hepatic lipogenesis.\textsuperscript{57} In hamsters, feeding of high-cholesterol diets also increased SCD-1 expression and subsequently induced insulin resistance and hepatic steatosis.\textsuperscript{58} In these models, induction of SCD-1 and lipogenesis in liver are proposed to be mediated by LXR activation by dietary cholesterol. We also expect beneficial effects of SCD-1 inhibitors on hepatic steatosis, liver injury, inflammation and fibrosis in such lipogenic models.

In conclusion, we demonstrated that a novel benzoylpylperidine-based SCD-1 inhibitor attenuated NASH development by reducing hepatic steatosis, liver injury, hepatocellular degeneration, and inflammation. SCD-1 is also associated with not only the development of NASH/NAFLD but also insulin resistance, obesity and metabolic syndrome. Therefore, SCD-1 may be a promising target for treatment of NASH/NAFLD and other metabolic syndrome-related diseases.

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