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The GPR40 full agonist SCO-267 improves liver parameters in a mouse model of nonalcoholic fatty liver disease without affecting glucose or body weight

Mitsugi Ookawara, Keisuke Matsuda, Masanori Watanabe, Yusuke Moritoh

SCOHIA PHARMA Inc., Kanagawa, Japan

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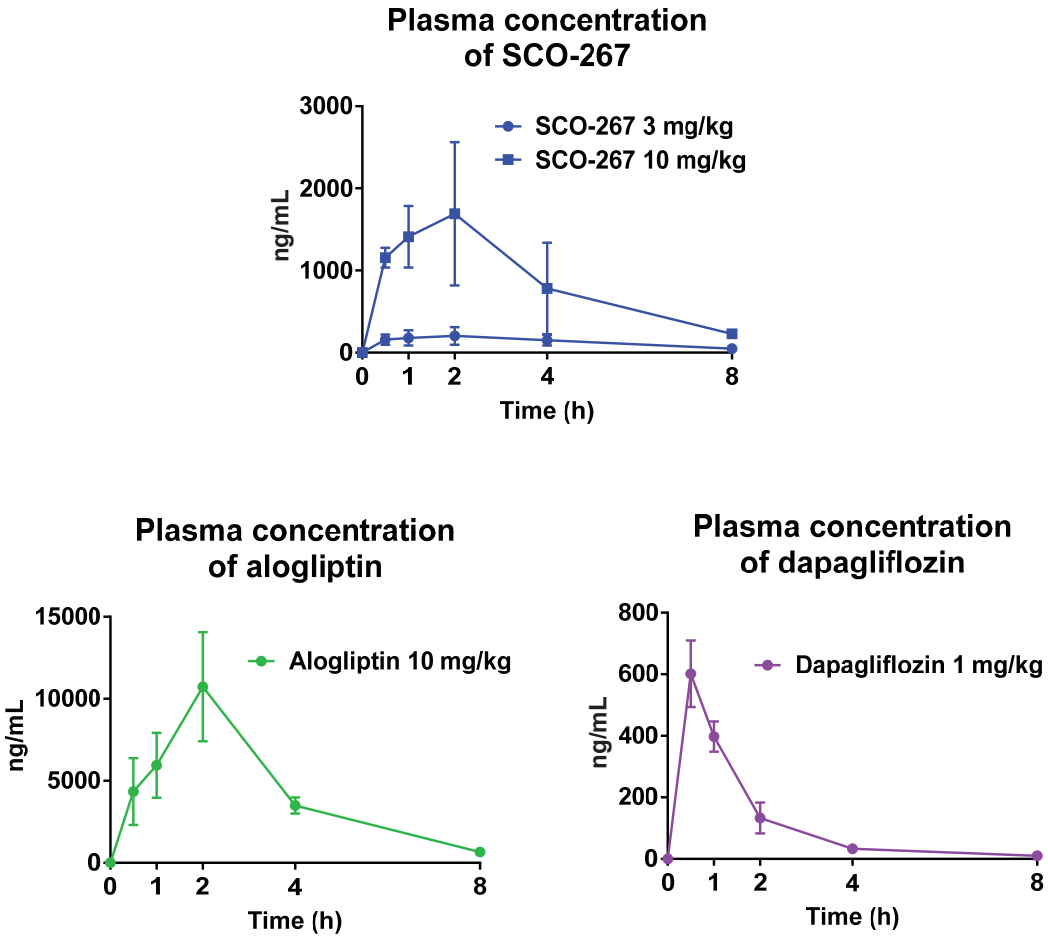
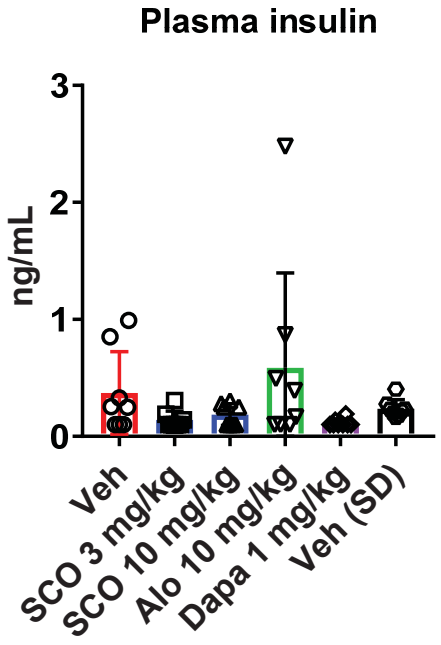
Supplementary information



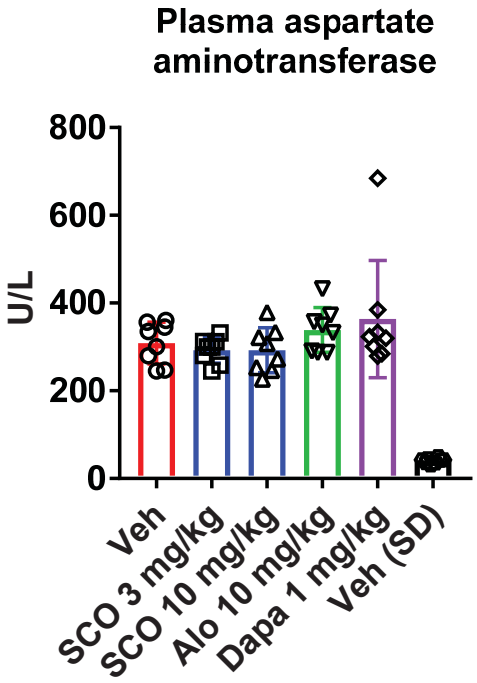
Supplementary Figure 1. Pharmacokinetic analysis of CDAHFD-fed mice

Each compound was dosed orally, and plasma concentration was determined. Values are presented as mean ± S.D. (n = 4). CDAHFD, choline-deficient, L-amino acid-defined, high-fat diet.



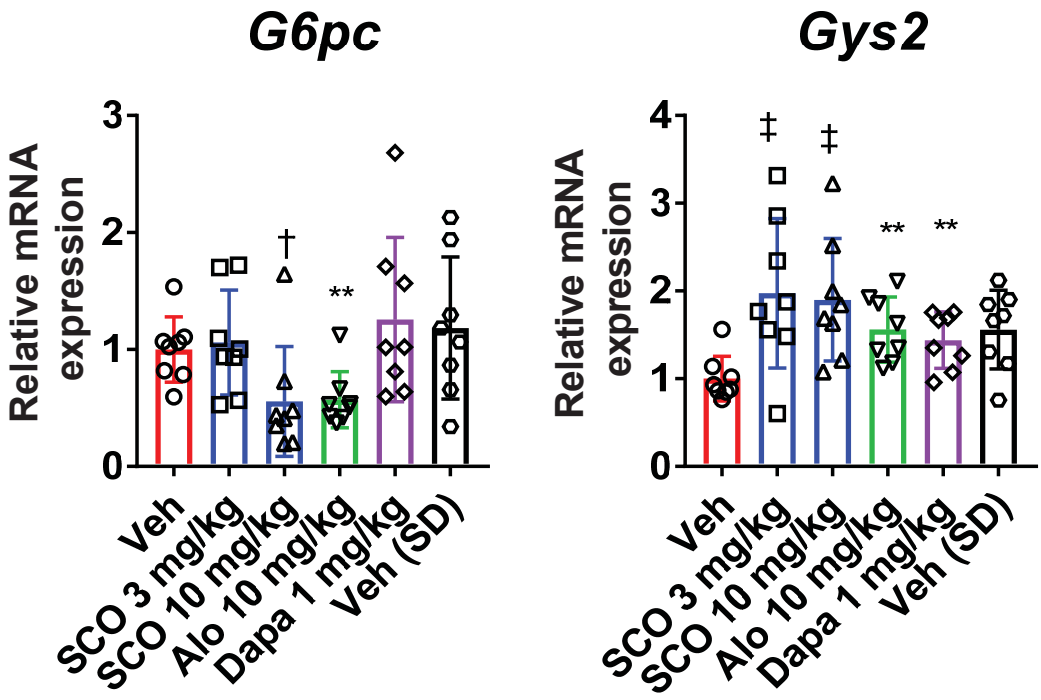
Supplemental Figure 2. Plasma insulin levels in CDAHFD-fed mice

SCO-267 did not cause any significant effect on plasma insulin levels in the 4-week study of CDAHFD-fed mice. Values are presented as mean ± S.D. (n = 8). CDAHFD, choline-deficient, L-amino acid-defined, high-fat diet; Veh, vehicle; SCO, SCO-267; Alo, alogliptin benzoate; Dapa, dapagliflozin; SD, standard diet.



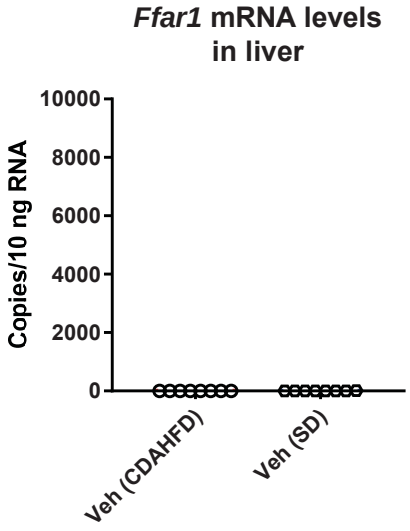
Supplemental Figure 3. Plasma aspartate aminotransferase levels in CDAHFD-fed mice

SCO-267 did not cause any significant effect on plasma aspartate aminotransferase levels in the 4-week study of CDAHFD-fed mice. Values are presented as mean ± S.D. (n = 8). CDAHFD, choline-deficient, L-amino acid-defined, high-fat diet; Veh, vehicle; SCO, SCO-267; Alo, alogliptin benzoate; Dapa, dapagliflozin; SD, standard diet.



Supplemental Figure 4. Liver mRNA levels of *G6pc* and *Gys2* in CDAHFD-fed mice

Values are presented as mean ± S.D. (n = 8). CDAHFD, choline-deficient, L-amino acid-defined, high-fat diet; Veh, vehicle; SCO, SCO-267; Alo, alogliptin benzoate; Dapa, dapagliflozin; SD, standard diet; *G6pc*, glucose-6-phosphatase, catalytic subunit; *Gys2*, glycogen synthase 2. † and ‡ $P < 0.025$ vs. vehicle by one-tailed Williams' test and Shirley–Williams test, respectively. ** $P < 0.01$ vs. vehicle by Student's *t*-test.



Supplemental Figure 5. Liver mRNA levels of *Ffar1*

Liver mRNA levels of GPR40 (*Ffar1*) in vehicle-treated mice fed with either CDAHFD or standard diet (n = 8). *Ffar1* mRNA was undetectable in liver of both groups. CDAHFD, choline-deficient, L-amino acid-defined, high-fat diet; Veh, vehicle; SD, standard diet.

Supplementary Table 1. Taqman Gene Expression Assays used for qPCR

Gene name	Code number	Uniprot Entry
Acta2	Mm00725412_s1	P62737
Ccl2	Mm99999056_m1	P10148
Cd36	Mm01135198_m1	Q08857
Col1a1	Mm00801666_g1	P11087
G6pc	Mm00839363_m1	P35576
Gapdh	Mm99999915_g1	P16858
Il6	Mm99999064_m1	P08505
Srebf1	Mm00550338_m1	Q9WTN3
Tgfb1	Mm01178820_m1	P04202
Tnf	Mm00443258_m1	P06804

Supplementary Table 2. Primer sequences for SYBR green qPCR

Gene name		Sequences (5′→3′)	Uniprot Entry
Acadl	Forward	TCAACAGCAGTTACTTGG	P51174
	Reverse	GACAATATCTGAGTGGAG	
Cybb	Forward	ACTCCTTGGGTCAGCACTGG	Q61093
	Reverse	GTTCCTGTCCAGTTGTCTTCG	
Gys2	Forward	CCTTGGGGTGTTTCCATCGTAC	Q8VCB3
	Reverse	CGGAGAGGTTTGTAGTCACACTGG	
Ncf1	Forward	ACATCACAGGCCCCATCATCCTTC	Q09014
	Reverse	ATGGATTGTCCTTTGTGCC	
Ppara	Forward	CGTACGGCAATGGCTTTATC	P23204
	Reverse	AACGGCTTCCTCAGGTTCTT	
Tfam	Forward	GGAATGTGGAGCGTGCTAAAA	P40630
	Reverse	TGCTGGAAAAACACTTCGGAATA	

Gene expression analysis for mouse *Ffar1*

To determine the absolute copy numbers of the mouse GPR40 mRNA (*Ffar1*), a standard curve was generated by amplifying known concentrations of synthetic oligonucleotides and the target gene copy number was calculated using this curve. All oligonucleotide primers and dual-labelled (FAM-TAMRA) oligonucleotide probes synthesized by Sigma-Aldrich (Supplementary Table 3). Amplification was performed under the following conditions: initial denaturation at 95 °C for 1 min, followed by 40 cycles of denaturation at 95 °C for 15 s and annealing and extension at 60 °C for 45 s on the ABI PRISM 7900HT Sequence Detector (Thermo Fisher Scientific) using the EXPRESS qPCR supermix (Thermo Fisher Scientific).

Supplementary Table 3. Primers, probe, and standard sequence for mouse *Ffar1*

Sequence Name	Sequences (5′→3′)
Mm_*Ffar1*-F	TCAGGGCAGCTTGGGTGG
Mm_*Ffar1*-R	AGCCACATTGGAGGCATTATAGG
Mm_*Ffar1*-P	CTCTCCTCACACTCCTGCTCTGCCTGG
Mm_*Ffar1*-std	GAAGCTCAGGGCAGCTTGGGTGGCCGGAGGCGCTCTCCTCACACTC CTGCTCTGCCTGGGGCCCTATAATGCCTCCAATGTGGCTAGTTT

Mm, mouse; F, forward primer; R, reverse primer; P, probe; std, standard oligonucleotide.