Acetyl-CoA carboxylase 1 and 2 inhibition ameliorates hepatic fibrosis and steatosis in a murine model of nonalcoholic steatohepatitis

Mitsuharu Matsumoto[1], Hiroaki Yashiro[2], Hitomi Ogino[1], Kazunobu Aoyama[1], Tadahiro Nambu[1], Mayumi Nishida[1], Xiaolun Wang[2], Derek Erion[2], Manami Kaneko[1]

[1]Axcelead Drug Discovery Partners, Inc., [2]Takeda Pharmaceuticals

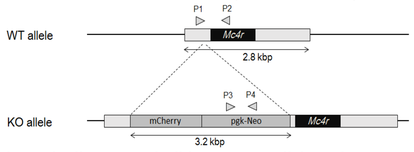
Purpose

De novo lipogenesis is increased in livers of patients with nonalcoholic steatohepatitis (NASH). Acetyl-CoA carboxylase (ACC) catalyzes the rate-limiting step in this process. Although an inhibitor of ACC reduced hepatic steatosis and fibrosis marker, serum TIMP1 in patients with NASH in a randomized-controlled trial, the influence on fibrosis is not fully elucidated. We evaluated effects of inhibition of ACC on fibrosis in addition to hepatic steatosis using melanocortin 4 receptor-deficient (MC4R KO) mice fed western diet (WD) that progressively developed hepatic steatosis and fibrosis.

Methods

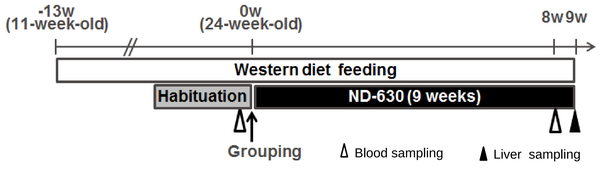
Animals :Male MC4R KO mice (produced in Takeda)
Male C57BL / 6J mice (Charles River Laboratoires Japan)

Diet : Western diet D12079B (Research Diet)



Schematic diagrams of wild-type Mc4r allele and Mc4r KO allele

Protocol



ND-630: ACC1/2 dual inhibitor (Proc Natl Acad Sci U S A. 2016 ;113:E1796-805) Low and high doses (2 and 8 mg/kg, b.i.d.) were orally administered for 9 weeks from 24 weeks of age.

Results

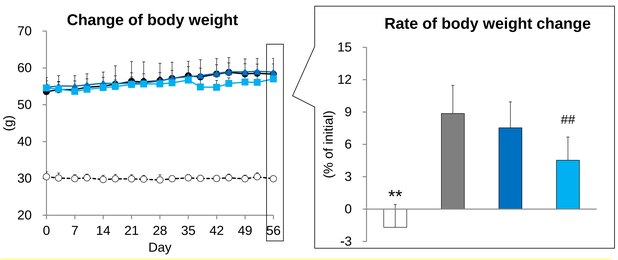


Fig 1: Body weight.
WD-fed MC4R KO mice developed obesity. ND-630 at 16 mg/kg/day significantly suppressed the weight gain. ND-630 did not affect food intake.

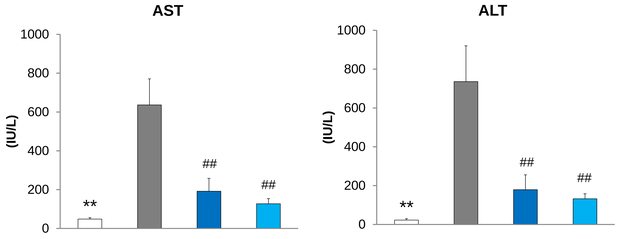
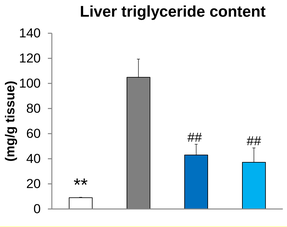


Fig 2: Plasma liver enzymes.
Plasma ALT and AST were increased in WD-fed MC4R KO mice. ND-630 robustly lowered them, suggesting that ACC inhibition improved hepatic injury.



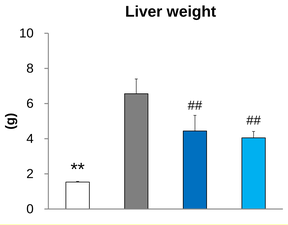
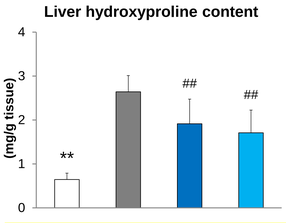


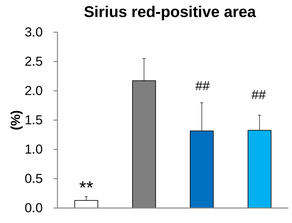
Fig 3: Hepatic steatosis.
WD-fed MC4R KO mice developed steatosis. ND-630 significantly lowered triglyceride content and liver weight. ACC inhibition improved steatosis in this model as well as in NASH patients.



C57BL/6J + Vehicle
MC4R KO + Vehicle
MC4R KO + ND-630 4 mg/kg/day
MC4R KO + ND-630 16 mg/kg/day

Mean ± SD, n= 5 - 8,
***p*<0.01 vs. MC4R KO + vehicle by *t*-test.
#*p*<0.05, ##*p*<0.01 vs. MC4R KO + vehicle by Williams' test.

Fig 4: Hydroxyproline content (collagen content) .
Fibrosis progressed in WD-fed MC4R KO mice. ND-630 significantly lowered hydroxyproline content. This result suggests that ACC1/2 dual inhibition suppressed the progress of fibrosis in addition to steatosis in WD-fed MC4R KO mice.



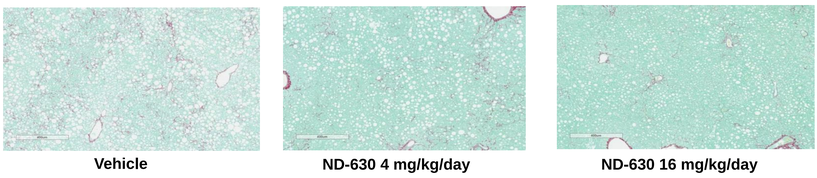
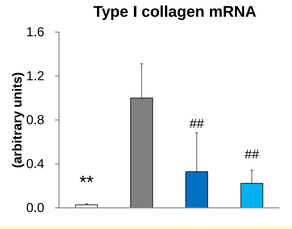
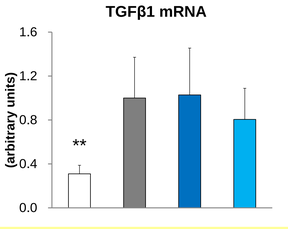
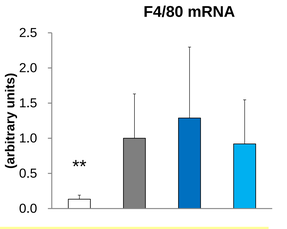


Fig 5: Sirius red-positive area in histopathological images.
Fibrosis area which is shown by Sirius red-positive staining was decreased in ND-630 treated MC4R KO mice.







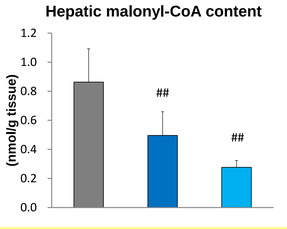


Fig 6: Hepatic mRNA expression.
Fibrosis marker genes (type I collagen and transforming growth factor β1 (TGFβ1)) and macrophage marker gene (F4/80) were upregulated in WD-fed MC4R KO mice. ND-630 significantly suppressed mRNA levels of type I collagen, but not TGFβ1 and F4/80.

Fig 7: Hepatic malonyl-CoA content.
ND-630 dose-dependently lowered hepatic malonyl-CoA content, indicating it inhibited ACC in the liver of WD-fed MC4R KO mice.

Mice	Drug	Total cholesterol (mg/dL)	Triglyceride (mg/dL)	Glucose (mg/dL)	Insulin (ng/mL)
C57BL/6J	Vehicle	85 ± 6**	86 ± 26	149 ± 9	0.7 ± 0.1**
MC4R KO	Vehicle	389 ± 39	90 ± 35	143 ± 16	9.4 ± 3.9
	ND-630 4 mg/kg/day	264 ± 37##	170 ± 47##	163 ± 15#	59.9 ± 39.8##
	ND-630 16 mg/kg/day	249 ± 28##	152 ± 54##	173 ± 18##	79.3 ± 22.7##

Table: Plasma glucose, triglyceride, total cholesterol and insulin.
WD-fed MC4R KO mice developed hypercholesterolemia and hyperinsulinemia. Although ND-630 significantly lowered plasma total cholesterol, triglyceride, glucose, and insulin were significantly increased. These data revealed that ACC1/2 dual inhibition improved cholesterol metabolism, whereas it has potentiality of deteriorating glucose and triglyceride metabolism and accelerating hyperinsulinemia.

Conclusion

ACC1/2 dual inhibition by ND-630 suppressed fibrosis progression and improved hepatic steatosis in the liver of WD-fed MC4R KO mice. Furthermore, ACC1/2 dual inhibition robustly decreased plasma liver enzymes, suggesting improvement of liver injury. These data show ACC1/2 inhibition could be a new option to suppress fibrosis progression as well as improve hepatic steatosis in NASH patients with fibrosis.

COI disclosure information :We have no financial relationship to disclose for our presentation contents.