Identification of differences in therapeutic mechanisms between Resmetirom and Semaglutide on metabolic dysfunction-associated steatohepatitis treatment in western diet-fed melanocortin 4 receptor knockout mice

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Introduction

MASLD is strongly associated with metabolic abnormalities such as obesity and insulin resistance, and MASLD develops into MASH. Resmetirom, a thyroid hormone receptor (THR)-beta agonist, is the first approved therapeutic drug for MASH, and Gulcagon-like peptide-1 (GLP-1) analogue, Semaglutide, also has ameliorative effects on MASH in clinical trials. There are some reports of Resmetirom using diet-induced obese (DIO) mice. However, DIO mice models do not fully reflect human MASH pathology. Melanocortin 4 receptor (MC4R) regulates appetite, and MC4R KO mice gain appetite and body weight. Western diet (WD)-fed MC4R KO mice show multiple aspects of pathophysiology of MASH patients such as liver injury, steatosis, and fibrosis[1]. Therefore WD-fed MC4R KO mice are ranked as having a closer resemblance to MASLD patients than other animal models[2].

Aim

In this study, we confirmed and compared anti-MASH effects of Resmetirom and Semaglutide respectively using WD-fed MC4R KO mice .

Method

MASH model mice were established by feeding MC4R KO mice with WD (D12079B, Research diets) for 6 weeks. Resmetirom (5 mg/kg) was orally administered and Semaglutide (0.1 mg/kg) was subcutaneously injected daily for 7 weeks. On the 7 weeks of treatment, body composition was measured using Magnetic Resonance Imaging. Energy expenditure was analyzed using Oxymax system. Plasma and liver were harvested from each group. For histopathological analysis, tissue samples of liver were formalin-fixed, paraffin-embedded, sectioned at 3-μm thickness, and stained with Hematoxylin and Eosin (HE). NAFLD Activity Score (NAS) was evaluated using HE section by the pathologists. Sirius Red staining was also performed, and Sirius Red positive areas were quantified.
The results of the statistical analysis are shown below.
# p<0.05, ## p<0.01, vs Vehicle (Dunnett test followed by Bonferroni correction)
* p<0.05, ** p<0.01, vs Control (Student's t-test)
$ p<0.05, $$ p<0.01, vs Vehicle (Dunnett test)

Results

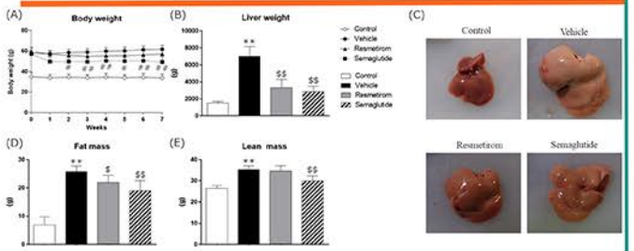


Figure 1 Effects of Resmetirom and Semaglutide on body weight and liver

(A) Body weight change for 7 weeks. (B) Liver weight. (C) Representative pictures of liver after treatment. (D) Fat mass and (E) lean mass determined by EchoMRI at week 7.
Data are represented as the mean ± SD.

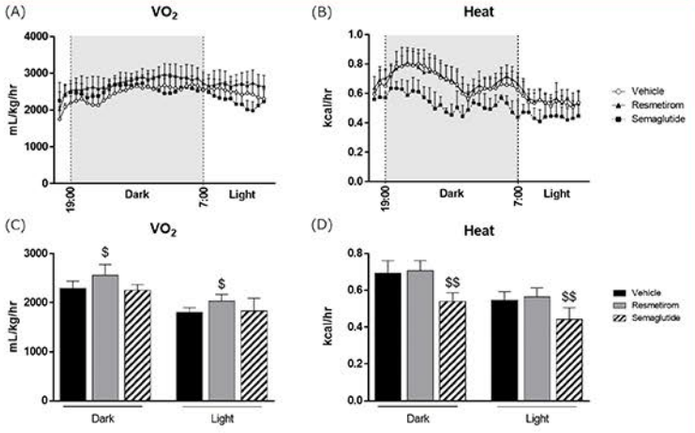


Figure 2. Comparison of oxygen consumption and energy expenditure between Resmetirom and Semaglutide

Time course of the (A) oxygen consumption, (B) energy expenditure monitored continuously for 19 hours by using an Oxymax system. Shaded regions represent the dark phase of 12-hour light-dark cycle. The average of (C) oxygen consumption, (D) energy expenditure per light and dark period.
Data are represented as the mean ± SD.

Figure 3. Effect on plasma parameters and hepatic hydroxyproline.

(A) Time course of plasma ALT for 7 weeks. (B) The change of level of plasma LDL concentration from the values before treatment. (C) Plasma TIMP-1 concentration. (D) The hydroxyproline contents in liver.
Data are represented as the mean ± SD.

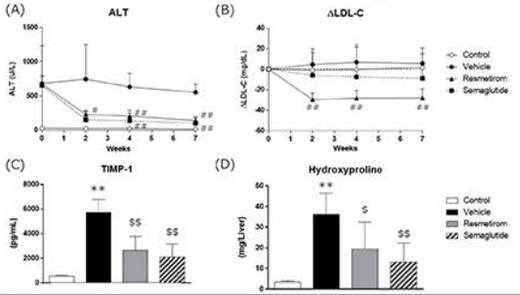
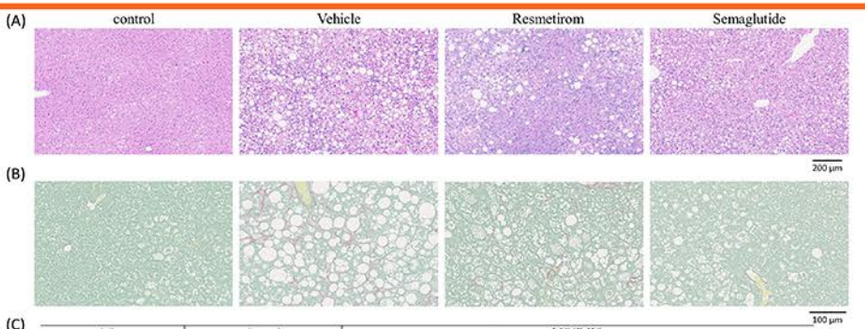


Figure 4. Effect on gene expression in liver.

Fold-changes in the mRNA expression of inflammation-, fibrosis-, and lipid metabolism-related genes in the liver were normalized to those in the control group. Glyceraldehyde-3-phosphate dehydrogenase (Gapdh) was used as the endogenous control. Data are represented as the mean ± SD.

Mice	Control	MC4R KO		
Treatment	Vehicle	Vehicle	Resmetirom	Semaglutide
	(n = 4)	(n = 8)	(n = 8)	(n = 4)
Inflammation-related gene expression				
Tnf	1.00 ± 0.14	7.45 ± 3.89**	5.96 ± 3.91	5.91 ± 3.99
Il1b	1.00 ± 0.24	4.43 ± 1.75**	2.43 ± 0.88	3.66 ± 3.16
Il6	1.00 ± 0.40	3.78 ± 2.74	2.00 ± 1.51	2.45 ± 1.19
Ccl2	1.00 ± 0.18	16.54 ± 6.39**	7.54 ± 3.81$$	11.12 ± 5.34
Fibrosis-related gene expression				
Acta2	1.00 ± 0.13	2.66 ± 0.74**	2.33 ± 0.66	2.73 ± 1.31
Col1a1	1.00 ± 0.48	22.06 ± 12.86**	11.44 ± 10.15	11.14 ± 8.14
Col3a1	1.00 ± 0.31	9.58 ± 5.26**	5.38 ± 4.92	4.99 ± 2.84
Spp1	1.00 ± 0.26	10.84 ± 5.16**	5.45 ± 4.71	5.03 ± 3.35
Lipid metabolism-related gene expression				
Scd1	1.00 ± 0.41	2.13 ± 0.43**	1.73 ± 0.31	1.99 ± 0.74
Fabp4	1.00 ± 0.16	3.74 ± 1.16**	2.03 ± 0.79	4.43 ± 4.93
Mogat2	1.00 ± 0.23	2.65 ± 0.57**	0.85 ± 0.32$$	1.35 ± 0.73$$



(C)

Mice	Control	MC4R KO		
Treatment	Vehicle	Vehicle	Resmetirom	Semaglutide
	(n = 4)	(n = 8)	(n = 8)	(n = 4)
Histopathology				
Steatosis	0.0 ± 0.0	3.0 ± 0.0	1.8 ± 0.7	2.3 ± 1.0
Lobular inflammation	0.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	0.8 ± 0.5
NAFLD activity score	0.0 ± 0.0	4.0 ± 0.0	2.8 ± 0.7	3.0 ± 1.4
Fibrosis analysis				
Sirius red-positive area (%)	0.24 ± 0.04	2.07 ± 0.91	2.29 ± 1.37	1.20 ± 0.90

Figure 5. HE staining and Sirius Red staining

Representative images of liver sections stained with (A) HE, and (B) Sirius Red.
(C) Individual scores for steatosis (0–3) and lobular inflammation (0–2) were provided and were added up to determine the NAFLD activity score as a semiquantitative measure of disease severity. The percentage of Sirius Red-positive areas were quantified using Halo® and Halo AI ®.

Conclusions

WD-fed MC4R KO mice model revealed that both of Resmetirom and Semaglutide improved MASH pathology. On the other hand, some parameters differed from each other. Resmetirom lowered fat mass and raised oxygen consumption. By contrast, Semaglutide reduced body weight accompanied with decrease in both of fat and lean mass, and lowered energy expenditure. The lean mass reduction is similar to clinical evidence. These results indicated that the anti-MASH effects between Resmetirom and Semaglutide may be due to different mechanisms. Although improvement of steatosis was clearly, improvement of fibrosis was obscure in both of Resmetirom and Semaglutide. Therefore, further development of MASH treatment drugs with more potent and direct antifibrotic effects is necessary.

References

1) Itoh M et al. Melanocortin 4 receptor-deficient mice as a novel mouse model of nonalcoholic steatohepatitis. *Am J Pathol. 2011 Nov;179(5):2454-63*
2) Vacca, M et al. An unbiased ranking of murine dietary models based on their proximity to human metabolic dysfunction-associated steatotic liver disease (MASLD). *Nat Metab. 2024 Jun;6(6):1178-1196*

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Please contact us if you are interested in drug discovery research for Liver diseases!

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