Potent Cholesterol Lowering Effect by HM12525A, A Novel Long-acting GLP-1/Glucagon Dual Receptor Agonist

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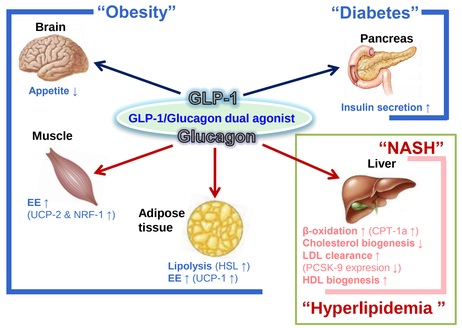
ABSTRACT

HM12525A is a novel long-acting GLP-1/glucagon receptor dual agonist for once-weekly administration. Previous studies have demonstrated that HM12525A induced greater weight loss than GLP-1 receptor agonists. In the present study, we evaluated the anti-hyperlipidemic effects and anti-NASH properties of HM12525A, and investigated underlying mechanisms of action (MoA). First, we investigated the cholesterol lowering efficacy in a pair feeding study using fructose-fed hamsters as a hyperlipidemic model. HM12525A lowered serum cholesterol levels, especially LDL, independent of weight loss and beyond what was seen with liraglutide. In addition to lowering LDL, HM12525A increased HDL levels. To investigate the responsible MoA, we evaluated the effect of HM12525A at the molecular level. In the cultured hepatocytes, HM12525A induced the expression of PPARα and CPT-1 in hepatocytes, suggesting inhibition of VLDL production by an increase of β-oxidation in the liver. Chronic HM12525A treatment increased hepatic LDL receptor expression while decreasing serum levels of PCSK9 (Proprotein convertase subtilisin/kexin type 9), a known negative regulator of the LDL receptor and inhibiting the action of hepatic HMG-CoA reductase, a rate-limiting enzyme for cholesterol bio-synthesis in fructose-fed hamsters. In addition, HM12525A increased the hepatic expression of apolipoprotein A1 (ApoA1), a main component of HDL. These results suggested that HM12525A exerted its potent cholesterol lowering effect via 1) inhibition of VLDL bio-synthesis, 2) promoting the hepatic LDLR-mediated lipoprotein clearance, 3) inhibition of hepatic cholesterol bio-synthesis and 4) HDL biogenesis. To investigate the effect on nonalcoholic steatohepatitis (NASH), we utilized ALIOS- (American lifestyle induced obesity syndrome) and methionine-choline deficient (MCD) diet-mice as NASH animal models, HM12525A resulted in greater effects on reduction of hepatic TG and the NAFLD score compared to liraglutide. Our results suggest that the novel long acting GLP-1/glucagon dual agonist HM12525A may have a multi-therapeutic potential and benefits in hyperlipidemia and NASH.

BACKGROUND

- **GLP-1/GCG dual agonist has a multi-therapeutic potential in hyperlipidemia and NASH as well as diabetes and obesity**



Properties of HM12525A compared to liraglutide

Type	Test material	Anti-Diabetes	Anti-Obesity	Lipid profile	
		HbA1c (db/db mice, 4w)	Body Weight (DIO mice, 2w)	Cholesterol (DIO mice, 2w)	LDL (DIO mice, 2w)
GLP-1 Receptor agonist	Liraglutide [100 nmol/kg/day, 3 mg/day in human]	1.2%	- 17 %,	-12%	-2%
GLP-1/GCG dual receptor agonist	HM12525A [5 nmol/kg/week, 2 mg/week in human]	-1.4%	- 31%	-68%	-48%
Reference		ADA 2014 [116-LB], ADA 2015 [1118-P]			

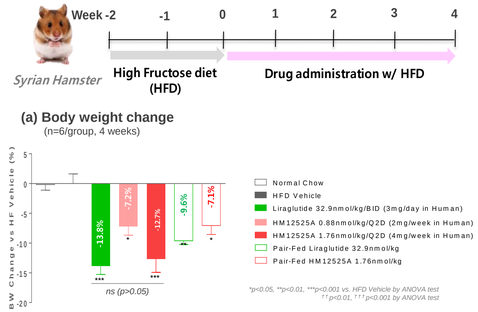
METHODS

- Fructose fed hamsters were treated (s.c.) with HM12525A Q2D and liraglutide BID for 4 weeks, respectively. Pair-fed controls were given a daily food allotment equal to that consumed by a drug-treated group. To confirm the cholesterol lowering efficacy, lipid parameters (cholesterol, HDL, LDL) were determined.
- Normal mice were treated (s.c.) with HM12525A Q2D and liraglutide BID for 7 days, respectively. Liver LDLR expression level, serum cholesterol level and serum PCSK9 level were measured.
- ALIOS- and MCD-diet C57BL/6 mice were treated (s.c.) with liraglutide BID and HM12525A (Q2D) for 4 weeks, respectively. After 4 weeks treatment, the hepatic TG level and NAFLD scores were measured after treatment for 4 weeks.
- Rat primary hepatocytes were treated with HM12525A (1μM) and glucagon (0.3μM). After 24 hours, PPARα and CPT-1 mRNA expression level were evaluated. HepG2 cells were treated with HM12525A (1μM) and glucagon (0.3μM). After 96 hours, ketone body level was determined.

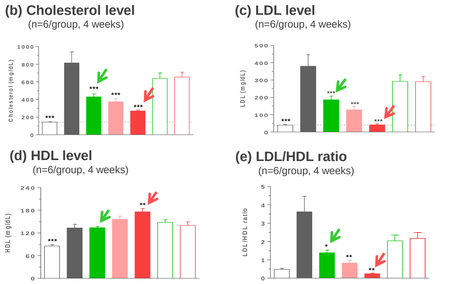
RESULTS

Cholesterol lowering efficacy beyond GLP-1RA

Figure 1. Cholesterol lowering efficacy in fructose-fed hamster

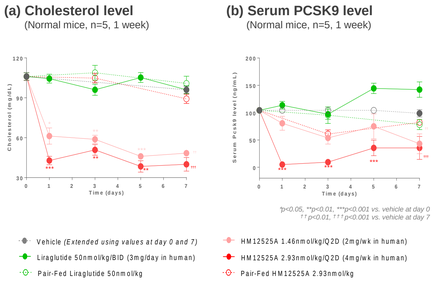


*p<0.05, **p<0.01, ***p<0.001 vs. HFD Vehicle by ANOVA test
††p<0.01, †††p<0.001 by ANOVA test



➢ HM12525A showed superior cholesterol lowering efficacy compared with liraglutide and BWL-independent improvement on lipid profile.

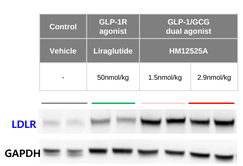
Figure 2. Decrease of serum PCSK9 level and increase of hepatic LDL-R level

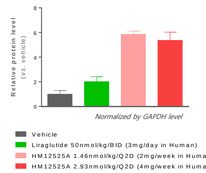


*p<0.05, **p<0.01, ***p<0.001 vs. vehicle at day 0
††p<0.01, †††p<0.001 vs. vehicle at day 7

(c) LDLR level in liver
(Normal mice, n=2, day 7)

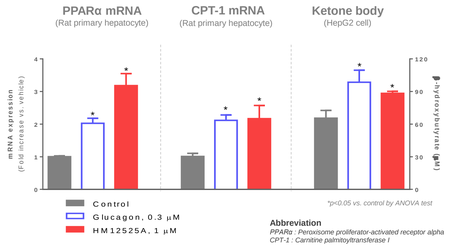
Control	GLP-1R agonist	GLP-1/GCG dual agonist
Vehicle	Liraglutide	HM12525A
-	50nmol/kg	1.5nmol/kg	2.9nmol/kg





➢ HM12525A lowered cholesterol level by decreasing of serum PCSK9 level and increasing hepatic LDLR level

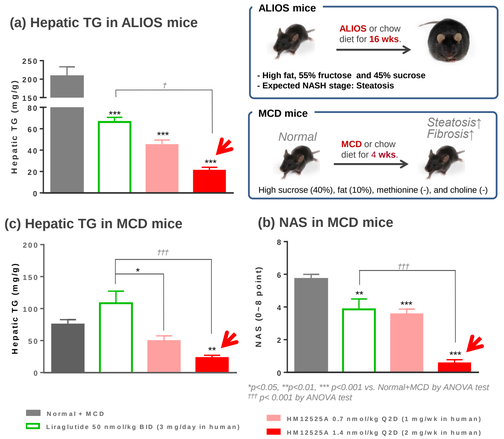
Figure 3. Increase of hepatic β-oxidation



➢ HM12525A induced the expression of PPARα and CPT-1 in hepatocytes, suggesting inhibition of VLDL production by increase of β-oxidation.

Anti-NASH efficacy beyond GLP-1RA

Figure 4. Therapeutic potential in NASH models (n=7, 4 weeks)



*p<0.05, **p<0.01, *** p<0.001 vs. Normal+MCD by ANOVA test
††† p< 0.001 by ANOVA test

➢ HM12525A decreases hepatic TG and NAFLD activity score indicating reduction of disease progression in NASH animal models

CONCLUSIONS

- In previous studies, HM12525A showed superior body weight loss compared with liraglutide maintaining glycemic lowering efficacy due to increase of energy expenditure
- In present studies, HM12525A lowered serum cholesterol levels, especially LDL and decreased LDL/HDL ratio, independent of weight loss and beyond liraglutide in a fructose fed hamster hyperlidemia animal model. From MoA studies, we confirmed that HM12525A exerts its potent cholesterol lowering effect via, (1) increase of hepatic β-oxidation, (2) decrease the hepatic LDL receptor clearance by decrease of PCSK9 expression (3) inhibition of hepatic cholesterol bio-synthesis (HMG CoA reductase inactivation: data not shown) and increasing HDL cholesterol HM12525A (Apo AI mRNA expression: data not shown).
- In NASH animal models, HM12525A reduces hepatic TG and histological scores.
- Our results suggest that the novel long acting GLP-1/glucagon dual agonist HM12525A may have multi-therapeutic potential in hyperlipidemia and NASH.

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