Bone protective effect of a novel long-acting GLP-1/GIP/Glucagon triple agonist (HM15211) in the osteoboseoporotic rodent model

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ABSTRACT

Severe weight loss is often associated with reduction in bone mineral density (BMD) and an imbalance between formation and resorption in obese people. As a consequence, there can be an increased risk of bone fractures with body weight loss. Several studies have proposed that the gut hormones, gastric inhibitory polypeptide (GIP), glucagon-like peptide-1 (GLP-1), and glucagon (GCG), might be modulators of bone growth and remodeling. HM15211 is a novel long-acting GLP-1/GIP/Glucagon that is being developed for the treatment of obesity. In this study, we investigated whether treatment with HM15211 prevented bone loss under a severe weight loss condition. To investigate bone protection efficacy of HM15211 and elucidate in osteoboseoporotic bone related mechanisms, we made use of the 60% kcal fat diet-induced obese Sprague-Dawley rat model. The subcutaneous treatment of HM15211 showed lower serum levels of decalcified collagen (Gluc-OC) and higher serum levels of osteoprotegerin (OPG) and proclL1 homologue protein-1 (proCL1) compared with those of vehicle and liraglutide treated groups. Consequently, HM15211 showed comparable BMD of femurs with vehicle group while it showed greater weight loss compared to liraglutide. For elucidating the underlying molecular mechanisms, related marker gene expression was investigated using the MC3T3-E1 cell (preosteoblastic cell). HM15211 led to significant increase in type-1 collagen and carboxylated collagen (Glu-OC expression), which were blunted by inhibition of GPR119-mediated signaling. These results suggest that HM15211 might provide potent weight loss without the deleterious imbalance bone loss.

RESULTS

Bone homoeostasis effects of GCG, GLP-1 and GIP

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CONCLUSIONS

• Lower serum level of Gluc-OC and higher serum levels of OPG and P1NP were observed compared with those of vehicle and liraglutide treated groups in obese-osteoporotic rats model.

• HM15211 showed comparable BMD of femurs with vehicle while it showed greater weight loss compared to liraglutide in obese-osteoporotic rats model.

• HM15211 led to significant increase in collagen and Gluc-OC expression, which were blunted by inhibition of GPR119-mediated signaling in osteoblastic cell.

• These results suggest that HM15211 might provide potent weight loss without bone loss.

REFERENCES

Effect of a novel long-acting GLP-1/GIP/Glucagon triple agonist (HM15211) in a NASH and fibrosis animal model

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ABSTRACT
NASH (Non-alcoholic Steatohepatitis), a potential consequence of NAFLD, may lead to end-stage liver disease including cirrhosis and hepatocellular carcinoma. Despite its severity and prevalence, NAFLD currently lacks an effective treatment. HM15211, a long-acting GLP-1/GIP/Glucagon triple agonist, HM15211. Previously, we showed that HM15211 uniquely improves liver steatosis, inflammation, and fibrosis in isolated liver (implanted) in DIO mice, and showed a liver preferential distribution suggesting HM15211 as a potential treatment option for NASH. Here, we evaluated the effect of HM15211 in NASH and fibrosis by using DIO mice and MCD-dietic mice.

In DIO mice, HM15211 treatment normalized liver steatosis and improved hepatic lipid metabolism as indicated by decrease in triglyceride related gene expression and increase in fatty acid β-oxidation related gene expression (PGC-1α and CPT-1). In addition HM15211 improved NAFLD fatty spots and hepatic TG in AMLN mice (NAFLD model) and improved hepatic lipid metabolism as indicated by decreased in liver triglyceride (TG), and increased hepatic free fatty acid (FFA) and fatty acid β-oxidation gene expression (PGC-1α and CPT-1). In addition, HM15211 reduced liver inflammation and fibrosis in MCD-dietic mice.

CONCLUSIONS
HM15211 reduced liver steatosis and improved hepatic lipid metabolism via direct hepatic activity, and may have potential therapeutic effect on NASH and fibrosis.

METHODS
To investigate the effects of HM15211 on hepatic lipid metabolism related gene expression, liver tissue samples were prepared after 4 weeks treatments of HM15211 in DIO mice. Then, the QPCR was performed on extracted liver total RNA and indicated gene expression (de novo lipogenesis, SREBP-1c, ACC1, ACC2, FAS and SCD-1) and fatty acid β-oxidation (PGC-1α, CPT-1) using qPCR primers. NAFLD adipose tissue levels and hepatic TG level were determined by the endpoint analysis at the end of study after 4 weeks treatments of HM15211 in MCD-dietic mice.

RESULTS
Liver preferential distribution of HM15211

Figure 1. Time-dependent tissue distribution of HM15211 in SD rats (n=5).

Impact of HM15211 on hepatic lipid metabolism in DIO mice

Figure 2. Effect of HM15211 on hepatic lipid metabolism related gene and lipid profile in DIO mice (n=7).

Liver preferential distribution

Figure 3. Effect of HM15211 on NASH prognosis markers in MCD-dietic mice (n=7).

Figure 6. Therapeutic effect of HM15211 on NASH and fibrosis in MCD-dietic mice (n=7).

Figure 4. Effect of HM15211 on NASH prognosis markers in MCD-dietic mice (n=7).

NASH and fibrosis improvement in animal models

Figure 5. Effect of HM15211 on hepatic NASH/fibrosis marker gene expression in MCD-dietic mice (n=7).

Figure 7. Therapeutic effect of HM15211 on NASH and fibrosis in MCD-dietic mice (n=7).

CONCLUSIONS
HM15211, a novel long-acting GLP-1/GIP/Glucagon triple agonist, improved hepatic lipid metabolism related gene expression in DIO mice and reduced NASH progression markers in MCD-dietic mice.

HM15211 reduced NASH prognosis related markers including hepatic lipid contents, oxidative stress, blood ALT, and bilirubin in MCD-dietic mice.

HM15211 reduced the expression of genes responsible for hepatic inflammation, HSC activation, and fibrosis in MCD-dietic mice.

The therapeutic effects of HM15211 on NASH is further demonstrated as reduction of NASH and hepatic fibrogenicity contents.

REFERENCES

**American Diabetes Association’s (ADA) T&O Scientific Sessions, Orlando, FL, USA; June 22-26, 2018**
Neuroprotective effects of HM15211, a novel long-acting GLP-1/GIP/Glucagon triple agonist in the neurodegenerative disease models

ABSTRACT
HM15211 is a novel long-acting GLP-1/GIP/Glucagon triple agonist that is being developed for the treatment of obesity and non-alcoholic fatty liver disease (NAFLD). Accumulated evidences have shown that obesity, type 2 diabetes, and NASH increase the risk of developing progressive neurodegenerative diseases such as Parkinson’s disease (PD) and Alzheimer’s disease (AD). Long-acting GLP-1 agonists, which mimic incretin effects following once-daily administration, have been shown to have beneficial effects in both PD and AD. In this study, we investigated the in vivo neuroprotective effects of HM15211 in several brain disorders including PD, AD and NASH. Previously, we demonstrated that HM15211 restored neuroprotective effects in MPTP-induced dopamine motor function impairment in a model of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in combination with peripheral inflammatory injection. In this study, we investigated the neuroprotective effects of HM15211 in the development of Alzheimer’s disease (AD), in the treatment of Parkinson’s disease (PD), and in the treatment of Non-Alcoholic Steato-Hepatitis (NASH). We found that HM15211 improved TH+ cell number in the striatum, reduced the release of IFN-γ in the peripheral blood, and restored the impaired motor function using Traction test and Pole test. In the NASH model, HM15211 reduced the area covered by microles (a, b) and reversed the induction of IFN-γ (c) and the reduction of AGE (d) levels in serum. In conclusion, HM15211 is a novel long-acting GLP-1/GIP/Glucagon triple agonist that has potential therapeutic effects against PD, AD, and NASH.

METHODS
Chronic Parkinson’s disease mouse model was induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), in combination with peripheral inflammation. Thirteen weeks after MPTP administration, HM15211 was intravenously administered twice a week for 5 weeks. HM15211 was administered once a week for 5 weeks. To determine effects of peripheral inflammation, HM15211 was administered twice a week (50 mg/kg, ip, twice a week), which was derived from anti-inflammatory effect by HM15211. Also, HM15211 decreased TH+ cell number in striatum of chronic mPTP model. Together with these efficacies, HM15211 significantly improved the motor function impairment in both parkinsonian and inflammation models. The α-synuclein model was induced by subcutaneous administration of 15211, which was derived from anti-inflammatory effect by HM15211. Also, HM15211 decreased α-synuclein in striatum of chronic mPTP model. Both of these efficacies, HM15211 significantly improved the motor function impairment in both parkinsonian and inflammation models. The α-synuclein model was induced by subcutaneous administration of 15211, which was derived from anti-inflammatory effect by HM15211. Also, HM15211 decreased α-synuclein in striatum of chronic mPTP model. Both of these efficacies, HM15211 significantly improved the motor function impairment in both parkinsonian and inflammation models. We investigated the in vivo neuroprotective effects of HM15211 in several brain disorders including PD, AD and NASH. In this study, we investigated the neuroprotective effects of HM15211 in the development of Alzheimer’s disease (AD), in the treatment of Parkinson’s disease (PD), and in the treatment of Non-Alcoholic Steato-Hepatitis (NASH). We found that HM15211 improved TH+ cell number in the striatum, reduced the release of IFN-γ in the peripheral blood, and restored the impaired motor function using Traction test and Pole test. In the NASH model, HM15211 reduced the area covered by microles (a, b) and reversed the induction of IFN-γ (c) and the reduction of AGE (d) levels in serum. In conclusion, HM15211 is a novel long-acting GLP-1/GIP/Glucagon triple agonist that has potential therapeutic effects against PD, AD, and NASH.

RESULTS
Neuroprotective effects of HM15211 in the neurodegenerative disease models

Neuroprotection in chronic PD mice

Figure 1. Motor function restoring effects of HM15211
(a) Traction test (b) Pole test (T-Total) (c) Relaxed test

Figure 2. Dopaminergic neuroprotection by HM15211
(a) Dopaminergic neuron staining (TH; tyrosine hydroxylase)

Mechanisms of neuroprotection in chronic PD mice

Figure 3. Anti-inflammatory effects of HM15211
(a) Microglia staining (ibai)

Figure 4. Inhibited accumulation of Aβ42 and AGE by HM15211
(a) Aβ42
(b) AGE

Figure 5. Reduced inflammation by HM15211
(a) IFN-γ
(b) IL-1β

CONCLUSIONS
In MPTP/Parkinson’s induced chronic Parkinson’s disease model, HM15211 inhibited the increase of α-synuclein, which is related to the development of neuroinflammation and progressive neurodegenerative biomarker of Parkinson’s disease. In aged db/db mice, pathological characters of Alzheimer’s disease such as Aβ42 and AGE accumulations were shown. These were reversed by HM15211 treatment. These neuroprotective effects of HM15211 are derived from anti-inflammation effect through the altered cytokine expression and reduced lipid peroxidation (data not shown). Based on these results, the novel long-acting GLP-1 / GIP / Glucagon triple agonist, HM15211 might have therapeutic potential for neurodegenerative diseases.

REFERENCES
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