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

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ABSTRACT

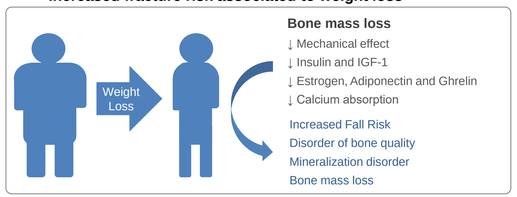
Severe weight loss is often associated with reduction in bone mineral density (BMD) and an imbalance between bone formation and resorption in obese people. As a consequence, there can be an increased risk of bone fractures with body weight loss. Several studies have proposed that the gut hormones, gastric 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 agonist that is being developed for the treatment of obesity. In this study, we investigated whether treatment with HM15211 prevents bone loss under a severe weight loss condition, and the underlying mechanism of action.

To investigate bone protection efficacy of HM15211 and liraglutide in obese-osteoporosis rats model for chronic treatment. After 4 weeks subcutaneous treatment of HM15211 showed lower serum level of decarboxylated osteocalcin (Glu-OC) and higher serum levels of osteoprotegerin (OPG) and procollagen type I pro-peptide (P1NP) 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.

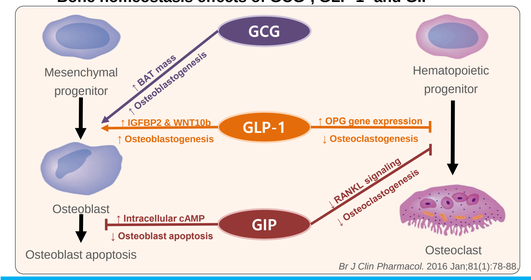
For elucidating the underlying molecular mechanism, related marker gene expression was investigated using the MC3T3-E1 cell (mouse osteoblast cell). HM15211 led to significant increase in type1 collagen-α1 and carboxylated osteocalcin (Gla-OC) expression, which were blunted by inhibition of GIPR-mediated signaling.

These results suggest that HM15211 might provide potent weight loss without the otherwise inevitable bone loss.

- **Increased fracture risk associated to weight loss[1]**



- **Bone homeostasis effects of GCG[2], GLP-1[3] and GIP[4]**



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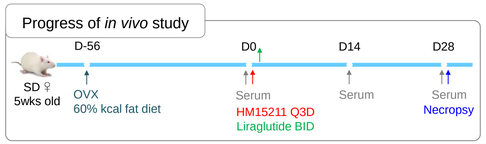
METHODS

- To investigate MoA for bone protection of HM15211, MC3T3-E1 cells were treated with HM15211. Osteoblast differentiation related markers (RUNX2, OCN, ALP and Col1α) were analyzed using real-time PCR. Additionally, collagen protein expression change and anti-apoptotic effect were evaluated using commercial kit.
- Diet induced obesity (DIO) osteoporosis rat model was induced by surgical oophorectomy (OVX) and fed 60% kcal fat diet to immatured 5 weeks old female sprague dawley (SD) rats for 8 weeks. Serum levels of bone biochemical markers (Glu-OC, OPG and P1NP) were measured by commercial ELISA kits. BMD of femurs were monitored using a high resolution *in vivo* μ-CT system (n = 7 /group).

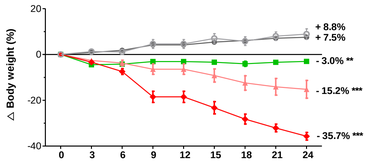


RESULTS

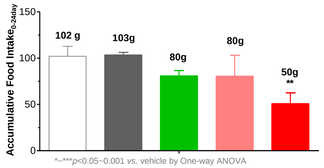
Reduction of body weight and food intake

Figure 1. Body weight change and food intake

(a) Body weight change



(b) Accumulative food intake$_{0\text{-}24day}$



*~***p<0.05~0.001 *vs.* vehicle by One-way ANOVA

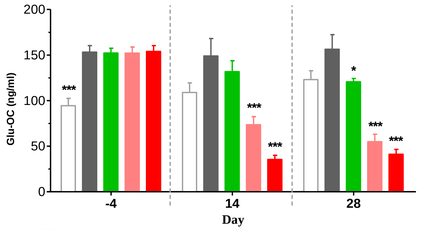
➢ HM15211 administration significantly decreased body weight and food intake, respectively.

- Shame vehicle, Q2D
- OVX vehicle, Q2D
- OVX Liraglutide 25 nmol/kg, BID (3 mg/day in human)
- OVX HM15211 2.2 nmol/kg, Q2D (4 mg/week in human)
- OVX HM15211 4.4 nmol/kg, Q2D (8 mg/week in human)

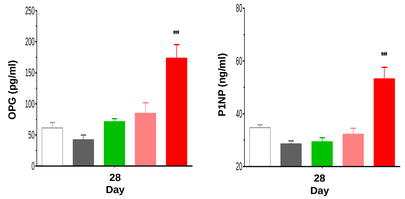
Improvement of bone biochemical markers

Figure 2. Serum levels of Glu-OC, OPG and P1NP

(a) Glu-OC (bone resorption marker)



(b) OPG (osteoclastogenesis inhibition marker) & P1NP (bone formation marker)



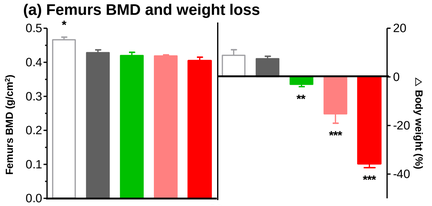
*~***p<0.05~0.001 *vs.* vehicle by One-way ANOVA

➢ Bone bio chemical markers (Glu-OC, OPG and P1NP) were dose dependently improved on HM15211 dosing group, respectively.

Prevention of BMD loss following weight loss

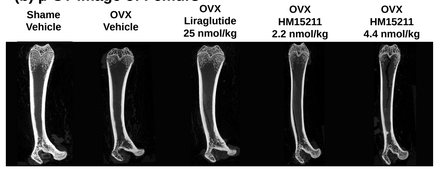
Figure 3. BMD and μ-CT image of femurs

(a) Femurs BMD and weight loss



*~***p<0.05~0.001 *vs.* vehicle by One-way ANVAO

(b) μ-CT image of Femurs

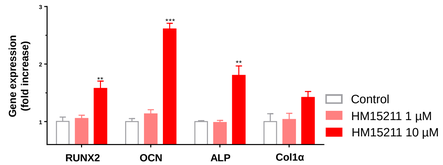


➢ Even severe weight loss condition, HM15211 prevented BMD loss of femurs

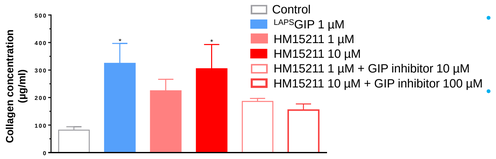
MoA studies for bone protection

Figure 4. Bone protection mechanism in MC3T3-E1 cell

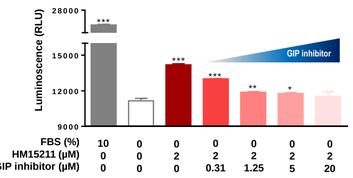
(a) Osteoblast differentiation marker genes



(b) Collagen expression in conditioned media

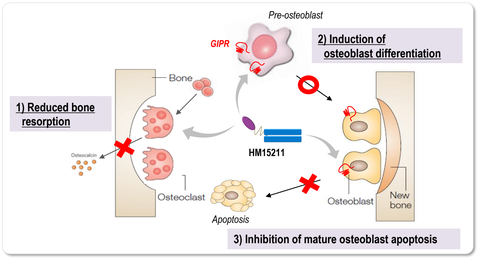


(c) Inhibition of osteoblast apoptosis



➢ HM15211 improved osteoblast differentiation and showed anti-apoptotic effect. Additionally, GIP antagonist reversed the beneficial effect of HM15211 on bone protection.

Figure 5. Schematic summary for bone protective effect



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CONCLUSIONS

- **Lower serum level of Glu-OC and higher serum levels of OPG and P1NP were observed compared with those of vehicle and liraglutide treated groups in obese-osteoporosis rats model.**
- **HM15211 showed comparable BMD of femurs with vehicle while it showed greater weight loss compared to liraglutide in obese-osteoporosis rats model.**
- **HM15211 led to significant increase in collagen and Gla-OC expression, which were blunted by inhibition of GIPR-mediated signaling in osteoblast cell.**
- **These results suggest that HM15211 might provide potent weight loss without bone loss**

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