Sustained glucagon effect on blood glucose and improvement of insulin resistance mediated by a novel long-acting glucagon analog, HM15136, in animal models

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ABSTRACT

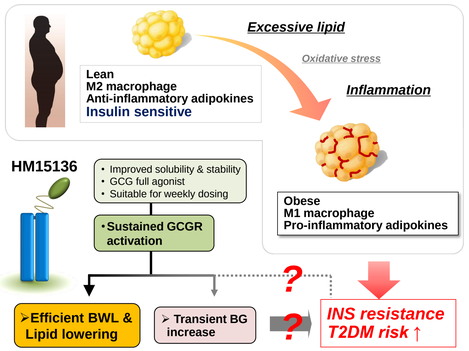
HM15136 is a novel long-acting glucagon (GCG) analog conjugated with human IgG FC fragment via a short PEG linker. Previously, HM15136 treatment led to efficient body weight loss (BWL) in obese animal models while showing transient effect on blood glucose (BG) elevation. Insulin resistance (IR) can lead to imbalances in BG homeostasis in obesity, representing a key risk factor for T2DM. Considering the potential benefit of BWL in IR improvement, HM15136 could be a novel therapeutic option for obesity treatment with substantially compromised BG elevation. To assess this novel concept, the present study evaluated the effect of HM15136 on glucose hemostasis as well as BW, and investigated the plausible mechanisms leading to IR improvement and long-term normal BG maintenance *in vitro* and *in vivo*

In DIO mice, 4 weeks treatment of HM15136 led to remarkable BWL (-39.9% *vs.* Veh). BG monitoring showed that initial BG elevation was rapidly normalized and maintained normal thereafter with no HbA1c increase in DIO mice (4.1 and 4.3% for Veh and HM15136). Interestingly, BWL effect was correlated with the reduction of blood lipids including free fatty acid (FFA) and HOMA-IR. *In vitro* studies in 3T3-L1 adipocytes further demonstrated the deteriorating effect of FFA on Akt phosphorylation by insulin (INS). These results suggested that improvements in the lipotoxic milieu explains the IR improvement seen with HM15136. To explore the additional mechanisms, inflammatory markers and adipokines affecting insulin signaling were evaluated. Interestingly, HM15136 not only inhibited F4/80 expression, but also increased FGF21 and adiponectin expression in white adipose tissue (WAT) and 3T3-L1 adipocytes. Furthermore, we unexpectedly observed that HM15136 itself also enhanced insulin signaling in 3T3-L1 adipocytes and primary hepatocytes, thereby leading to enhanced BG lowering by insulin in mice

Our results demonstrated that BWL under by HM15136 therapy is accompanied by the reduction of [1)] lipotoxicity, [2)] inflammatory biomarkers, and the expression of [3)] insulin-sensitizing adipokines, eventually leading to IR reversal and normal BG maintenance. Additionally, HM15136 might also directly affect, at least in part, the improvement of insulin action. Human studies are needed to confirm whether these findings will translate into humans

BACKGROUND

In obese condition, excessive lipid influx increases adiposity and subsequent inflammation, leading to insulin resistance and T2DM



Despite its efficient BWL, potential effects of HM15136 on glucose homeostasis & insulin resistance were not closely investigated

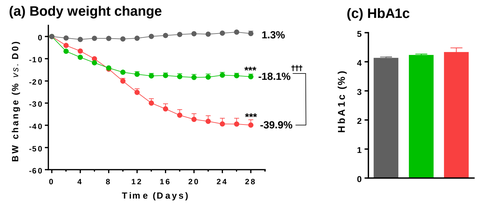
METHODS

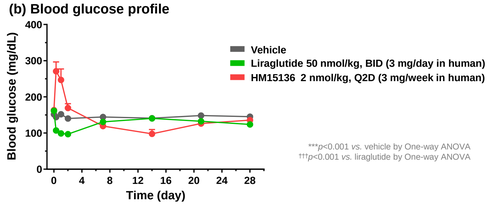
- To evaluate the therapeutic potential of HM15136 in obesity, DIO mice were chronically administered with HM15136, and BW and BG were monitored. At the end of treatment, blood lipid profiles, HOMA-IR, and HbA1c were determined. Potential impact of HM15136 in WAT inflammation was evaluated by measuring F4/80 expression in WAT
- For *in vitro* mechanism studies, insulin resistant 3T3-L1 adipocytes induced by FFA incubation and rat primary hepatocytes were utilized. After HM15136 treatment, expression and secretion of pro-inflammatory cytokines (TNF-α and IL-6) and adipokines (FGF21 and adiponectin) were determined by qPCR and ELISA, respectively. To monitor insulin-mediated signaling, Akt phosphorylation was measured

RESULTS

In vivo efficacy in obesity animal models

Figure 1. HM15136 effect on BW, BG and HbA1c in DIO mice (n=7)



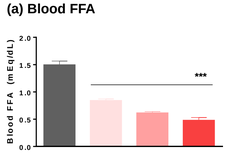


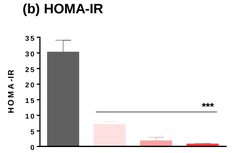
***p<0.001 *vs.* vehicle by One-way ANOVA
†††p<0.001 *vs.* liraglutide by One-way ANOVA

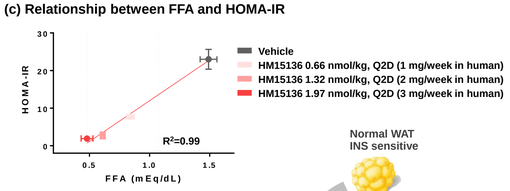
➢In DIO mice, HM15136 showed more BWL than liraglutide. Transient BG elevation is rapidly normalized. No increased HbA1c is observed

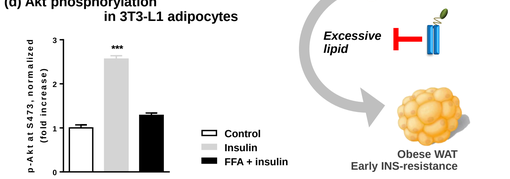
Improved lipotoxicity and insulin (INS) resistance

Figure 2. Effect of HM15136 on blood free-fatty acid (FFA) and HOMA-IR in DIO mice (n=7)







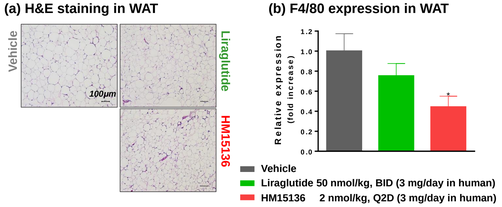


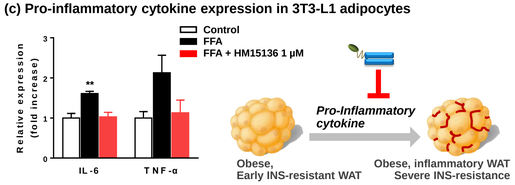
* ~ ***p<0.05 ~ 0.001 *vs.* vehicle or control by One-way ANOVA

➢HM15136 reduced blood lipids and HOMA-IR in DIO mice. FFA incubation impairs insulin signaling in 3T3-L1 adipocytes, indicating the induction of insulin resistance by lipotoxicity

Improvement of Inflammation in adipose tissue

Figure 3. Effect of HM15136 on inflammation in adipocytes



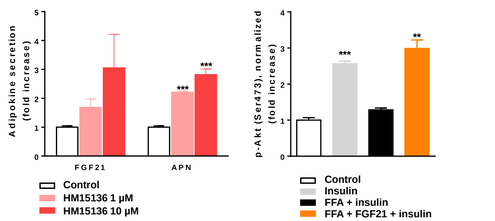


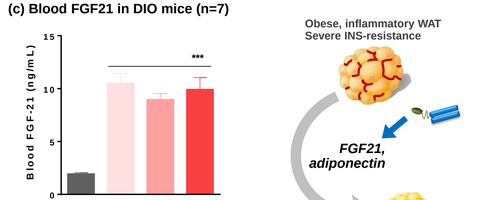
* ~ **p<0.05 ~ 0.01 *vs.* control by One-way ANOVA

➢HM15136 reduced lipid droplet size and macrophage activation in WAT of DIO mice. In addition, FFA-induced pro-inflammatory cytokine expression was reduced by HM15136 in 3T3-L1 adipocytes

Induction of INS sensitizing adipokines

Figure 4. Effect of HM15136 on FGF21 and adiponectin (APN), and its impact on INS signaling in 3T3-L1 adipocytes



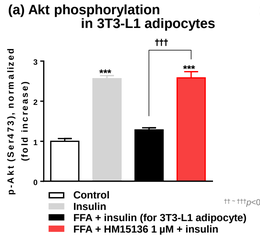


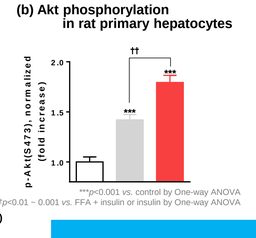
** ~ ***p<0.01 ~ 0.001 *vs.* vehicle or control by One-way ANOVA

➢HM15136 increased the secretion of FGF21 and APN, and FGF21 reversed FFA-induced insulin resistance in 3T3-L1 adipocytes. Blood FGF21 was also increased by HM15136 treatment in DIO mice

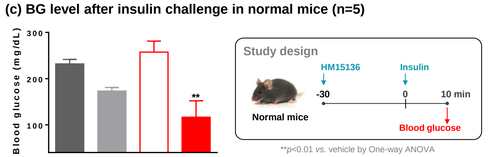
Direct involvement of HM15136 in INS signaling

Figure 5. Direct HM15136 effect on INS signaling





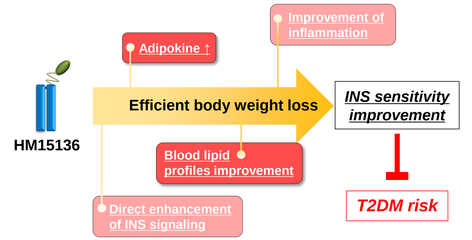
***p<0.001 *vs.* control by One-way ANOVA
†† ~ †††p<0.01 ~ 0.001 *vs.* FFA + insulin or insulin by One-way ANOVA



**p<0.01 *vs.* vehicle by One-way ANOVA

➢HM15136 reversed insulin resistance and enhanced insulin signaling in 3T3-L1 adipocytes and rat primary hepatocytes, respectively. Accordingly, pretreatment of HM15136 further enhanced BG lowering

Proposed model for INS resistance improvement and normal BG maintenance by HM15136



CONCLUSIONS

- **HM15136 is a novel long-acting glucagon analog, developed for the treatment of obesity**
- **HM15136 provides greater BWL than liraglutide. Transient BG elevation is rapidly normalized, and no HbA1 increase is observed in DIO mice**
- **HM15136 reduces blood lipids and HOMA-IR, indicating the improvement of lipotoxicity and insulin resistance**
- **HM15136 also reduces the expression of F4/80 and pro-inflammatory cytokines in WAT of DIO mice and 3T3-L1 adipocytes**
- **HM15136 increases FGF21 and APN secretion, which, in turn, enhances insulin-mediated Akt phosphorylation**
- **Unexpectedly, HM15136 also directly improves insulin action**
- **In conclusion, sustained GCG action by HM15136 could lead to insulin resistance improvement by which BG could be maintained normal, supporting a therapeutic potential of HM15136 in obesity**

REFERENCES

- Campbell JE and Drucker DJ., *Nat Rev Endocrinol.* **11**, 329-38 (2015)
- Muller TD *et al.*, *Physiol Rev.* **97**, 721-66 (2017)