A novel long-acting glucagon analog (HM15136) offers favorable stability, PK/PD, and therapeutic potentials in CHI (congenital hyperinsulinism) animal model

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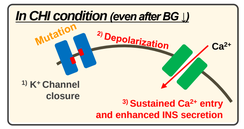
BACKGROUND

Long-acting glucagon could be one of the most favorable therapeutic strategies for CHI, in terms of efficacy and convenience

- **Congenital hyperinsulinism (CHI)**
  - Mutation in potassium channel
  - Inappropriate insulin secretion lead to hypoglycemia
  - Incidence : 1/25,000 ~ 1/50,000 (Orphan disease)



- **To date, no drug available for CHI**

- **Treatment scheme**

Diazoxide → Octreotide ✚
- Glucose loading
- Glucagon : Not appropriate as a long-term therapy due to unfavorable chemical stability ✓
- Pancreatectomy (Diabetic risk ↑)

(Several month ~ years)

- **Orphan drug status for CHI (FDA)**

	Exendin 9-39	Glucagon	Glucagon infusion (G-Pump™)	hIgG$_2$ against IR (XOMA 358)
Orphan Designation	O (Jun. 2011)	O (Dec. 2012)	O (Sep. 2014)	O (Jun. 2015)
Orphan Approval	X	X	X	X
Drug approval	X	O (for hypoglycemia)	X, Ph2	X, Ph2

"Long-acting drug"

AIMS

- **We have developed a novel long-acting glucagon analog, HM15136, which consists of a glucagon analog conjugated to the human aglycosylated Fc fragment via a short PEG linker.**
- **To investigate the therapeutic potential of HM15136 by evaluating its 1) solubility and stability, 2) in vitro biological functions, and 3) PK/PD in rodent models.**

METHODS

- To measure intracellular cyclic AMP level, CHO cells stably expressing either mouse or human GlucagonR was treated with HM15136 for 15 minutes. The native glucagon was used as a reference control. Accumulated intracellular cAMP was measured using the LANCE™ cAMP assay Kit (Perkinelmer)
- To investigate glucose producing ability of HM15136, rat primary hepatocytes were prepared, and subjected to glucose production assay. Briefly, hepatocytes were incubated with insulin to enrich glycogen production, which then were treated with HM15136 for 30 minutes to induce glycogenolysis. For gluconeogenesis, serum starved hepatocytes were treated with HM15136 for 6 hrs in the presence of pyruvate & lactate. After treatment, glucose level in collected medium was determined via GOPOD assay.
- Pharmacokinetics of HM15136 was investigated in ICR mice after single subcutaneous or intravenous administration of HM15136. The blood samples were collected at indicated time points, and the blood HM15136 concentration was determined by using in-house developed ELISA method
- Therapeutic potential of HM15136 in acute hypoglycemia was evaluated in SD rats. Briefly, after fasting for 4 hrs, SD rats were challenged with 0.65 U/kg human insulin to induce acute hypoglycemia. 45 min after insulin challenge, either glucagon or HM15136 was administered, and the blood glucose was monitored up to 180 min.
- To evaluate therapeutic potential of HM15136 in CHI, human-mimetic CHI rat model was established by implanting osmotic pump filled with human insulin. Filled insulin dose (60 nmol/kg/day) was previously determined to effectively induce chronic hypoglycemia. HM15136 was multiply administered, and daily BG was monitored for 2 weeks. To rule out sudden food intake effect on BG, the rats were fasted for 2 hrs before measuring BG
- Statistical analysis was performed using GraphPad Prism by one-way ANOVA, followed by Dunnett *post-hoc* analysis. A value of $p < 0.05$ was considered as statistically significant.

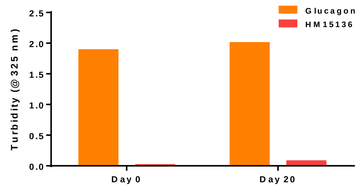
RESULTS

Improved solubility and physical stability of HM15136

Table 1. Solubility of HM15136

Test article	Solubility at pH 7.0
Glucagon	0.03 mg/mL
HM15136	≥ 150 mg/mL

Figure 1. Physical stability of HM15136 in PBS (pH 7.0) at 25°C



➢ HM15136 shows improved solubility and physical stability at physiological pH compared to the native glucagon.

In vitro properties of HM15136

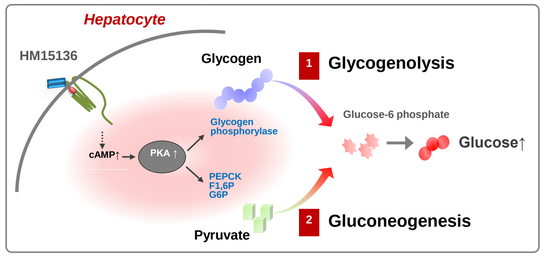
Table 2. cAMP accumulation by glucagon receptor (GCGR)

	Test articles	% Activity *vs.* native GCG
Human GCGR	Glucagon	100%
	HM15136	11.83 ± 4.42%
Mouse GCGR	Glucagon	100%
	HM15136	20.67 ± 8.22%

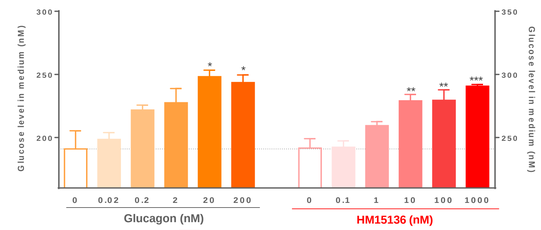
➢ Despite LAPS-conjugation, HM15136 shows intact glucagon-like action with full-agonistic nature

Figure 3. Glucose production by HM15136 in rat primary hepatocytes

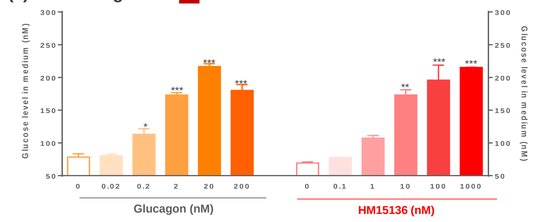
(a) Glucose production by HM15136



(b) Glycogenolysis 1



(c) Gluconeogenesis 2

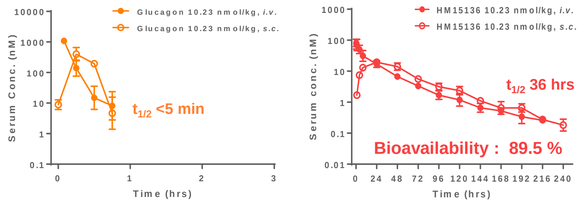


➢ Similar to the native glucagon, HM15136 is able to produce glucose *via* both glycogenolysis and gluconeogenesis in rat primary hepatocytes.

PK and short-term PD (reversal of acute hypoglycemia)

Figure 4. PK in normal mice (n=3/time point)

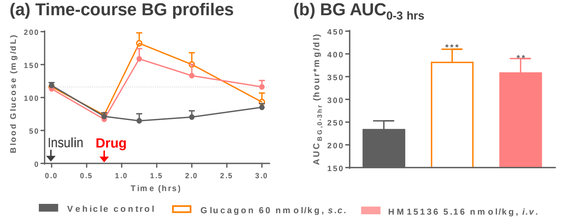
(a) For glucagon (Glucagen®) (b) For HM15136



➢ Compared to commercial glucagon, HM15136 shows substantially extended half-life as well as improved bioavailability, suggesting its weekly dosing potential

Figure 5. Reversal of acute hypoglycemia in normal rats (n=5)

(a) Time-course BG profiles (b) BG AUC$_{0\text{-}3\ hrs}$

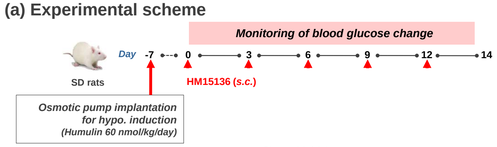


➢ Intravenous administration of HM15136 could effectively reverse acute hypoglycemia induced by insulin challenge (0.65 U/kg) in SD rats

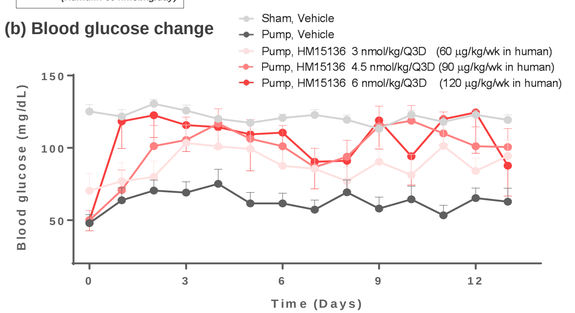
Sustained BG increasing efficacy in hyperinsulinemia-induced chronic hypoglycemic rats

Figure 6. Blood glucose after chronic administration of HM15136 in CHI model rats (n=9)

(a) Experimental scheme



(b) Blood glucose change



➢ When chronically administered, HM15136 sustainably increases BG in CHI mimetic rats, demonstrating its therapeutic potential in CHI

CONCLUSIONS

- **HM15136 is a long-acting glucagon receptor agonist developed for the treatment of CHI**
- **HM15136 induces GCGR activation with full agonistic nature**
- **HM1536 not only induce glycogenolysis, but also gluconeogenesis in rat primary hepatocytes, indicating its glucose producing potential**
- **PK results demonstrate its prolonged half-life and improved BA, indicating weekly and self injection potential**
- **Intravenous administration of HM15136 could reverse acute hypoglycemia-induced by insulin challenge**
- **When chronically administered, HM15136 sustainably increases BG in CHI mimetic rats, demonstrating its therapeutic potential in CHI**
- **In conclusion, HM15136 shows prolonged glucagon-like action with improved physicochemical features which may allow the development of a novel therapeutic option for CHI for easy weekly use**

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