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

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ABSTRACT

Congenital hyperinsulinism (CHI) is a rare genetic disorder characterized by unregulated insulin secretion leading to persistent and severe hypoglycemia especially during fasting condition. Although glucagon is considered one of the most potent therapeutic options, its utilization is limited due to poor solubility, limited stability at physiological pH, and short duration of action. To overcome these limitations, we have developed a novel long-acting glucagon analog, HM15136. HM15136 consists of a glucagon analog conjugated to the human aglycosylated Fc fragment via a short PEG linker. This study investigated the therapeutic potential of HM15136 in CHI by evaluating its [1] solubility and stability, [2] *in vitro* biological functions, and [3] PK/PD in rodent models.

First, we demonstrated that HM15136 shows improved solubility at pH 7.0, compared to the solubility of the native glucagon (≥150 *vs.* 0.03 mg/mL). Regarding *in vitro* biological functions, HM15136 showed selective glucagon receptor (GCG-R) activation. In addition, HM15136 induced glycogenolysis and gluconeogenesis in rat primary hepatocytes in a dose dependent manner, demonstrating its glucagon-like nature.

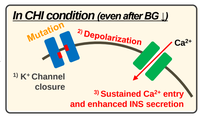
Finally, PK/PD of HM15136 was measured. HM15136 showed longer half-life (36 hr *vs.* < 5 min), and better bioavailability (90%) than that of the native glucagon after single subcutaneous administration in mice, suggesting potential for once-weekly use in human. As to therapeutic potential, HM15136 could effectively reverse acute hypoglycemia induced by insulin challenge. Furthermore, multiple administrations of HM15136 could sustainably increase blood glucose in CHI rats induced by osmotic insulin pump.

In conclusion, HM15136 with improved physicochemical features, shows prolonged glucagon-like action allowing the development of a novel once a weekly therapeutic option for CHI.

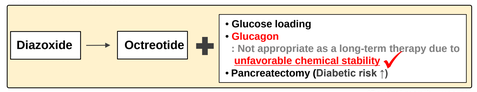
BACKGROUND

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**



- **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"

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.

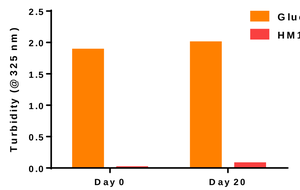
RESULTS

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

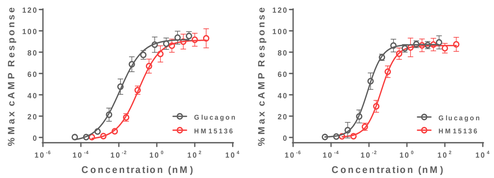


➢HM15136 shows improved solubility and physical stability at physiological pH compared to the native glucagon.

In vitro properties of HM15136

Figure 2. Intracellular cAMP accumulation by HM15136

(a) Human GCGR/CHO cells (b) Mouse GCGR/CHO cells

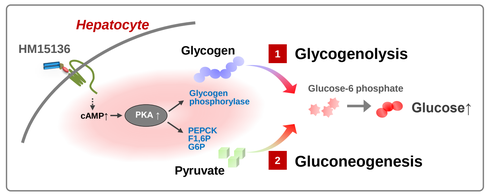


	Test articles	EC_{50} (nM)	% Activity *vs.* native GCG
Human GCGR	**Glucagon**	0.010 ± 0.001	100%
	HM15136	0.092 ± 0.029	**11.83 ± 4.42%**
Mouse GCGR	**Glucagon**	0.009 ± 0.003	100%
	HM15136	0.044 ± 0.007	**20.67 ± 8.22%**

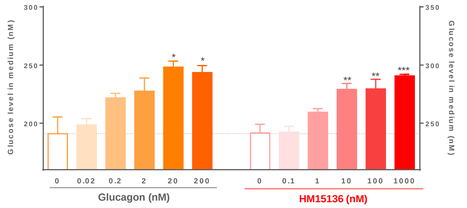
➢Despite LAPS-conjugation, HM15136 shows intact glucagon-like action with full-agonistic nature

Figure 3. Glucose production by HM15136 in rat primary hepatocytes

(a) Glucose production by HM15136

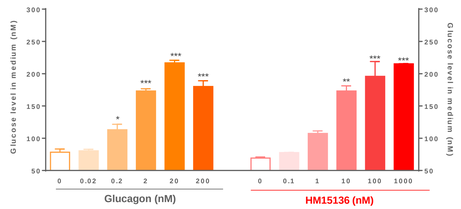


(b) Glycogenolysis



*p < 0.05, **p < 0.01, *** p < 0.001 vs. 0 nM by one-way ANOVA

(c) Gluconeogenesis

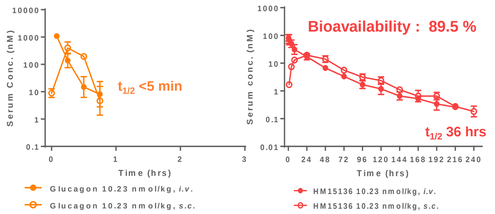


➢Similar to the native glucagon, HM15136 is able to produce glucose *via* both glycogenolysis and gluconeogenesis in rat primary hepatocytes.

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

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

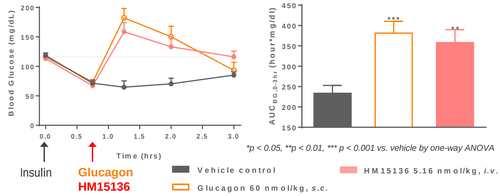
(a) For glucagon (Glucagen®) (b) For HM15136



➢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 SD rats (n=5)

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



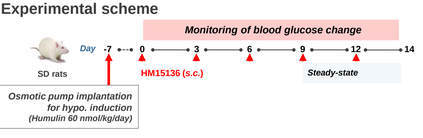
*p < 0.05, **p < 0.01, *** p < 0.001 vs. vehicle by one-way ANOVA

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

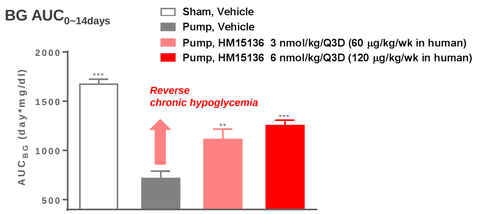
Sustained BG increasing efficacy in hyperinsulinemia-induced chronic hypoglycemic rats

Figure 6. Blood glucose after chronic administration of HM15136 in CHI rats (n=5)

(a) Experimental scheme



(b) BG $AUC_{0\sim14days}$



➢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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