Underlying Mechanisms for Long-Acting Properties of the Novel Weekly Insulin, **LAPS**Insulin 115 (HM2470)

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**ABSTRACT**

The ultra-long-acting basal insulin HM2470 has been developed by conjugating an in vivo 48-fold insulin fragment in CHO cells under a human immunoglobulin G (IgG) recombinant human epidermal growth factor receptor-2 (HER-2) construct. Although the LAPS-Insulin 115 (115 = 1150 nM) in rats has shown superior PK and PD properties compared to LAPS-12470 in rats, HM2470 has been developed for clinical testing in humans. However, the mechanism of prolonged properties remained to be fully elucidated. This study investigated the underlying mechanisms of the long-lasting properties of insulin 115 and HM2470 in comparison with insulin-12470 in human HepG2 cells and rat adipocytes. We demonstrated that HM2470, as compared to LAPS-12470, may have stronger receptor binding affinity to human insulin receptor than insulin and HM2470-2A. Insulin was found to be internalized via receptor-mediated endocytosis, with cellular stability in HepG2 cells. The residual activity of insulin 115 (60%) and insulin 12470 (25%) was measured using a radioactivity assay. It was measured using the **Figure 1**. Cellular stability of insulin 115 and LAPS-Insulin 115 in a cell environment.

**METHODS**

- **Receptor binding affinity**: was measured by SPA (scintillation proximity assay), insulin and HM2470 were incubated in the presence of insulin receptor antibody, and radioactivity was measured using a scintillation counter. Binding affinity (Ki) was calculated using GraphPad Prism 7.
- **Receptor internalization**: was determined by measuring the remaining amount of insulin in 32P-labeled HepG2 cells. Cells were incubated with 115 nM insulin for 24 h. Cell surface-bound insulin was removed using acid wash (PBS 1% and 30%), and residual activity was measured using a radioactivity assay.
- **Cellular stability**: was measured in HepG2 cells. Cells were treated with 500 nM of insulin 115 or 12470 for 48 h, and 115 nM insulin 115 or 12470 for 48 h. Cells conditioned media were harvested and residual insulin concentrations were measured using a radioactivity assay.
- **Serum stability**: was determined in rat and human serum. Insulin was spiked into rat serum (1000 nM) or human serum (100 nM), and residual radioactivity was measured using a radioactivity assay. For the PK/PD of Insulin 115, 65.1 nM insulin was subcutaneously injected to rats. Residual radioactivity of insulin in the cell was determined using a radioactivity assay at 0 h and 3, 6, 12 h after injection. Insulin in serum was measured using a radioactivity assay at 0 h and 3, 6, 12 h after injection. For the PK/PD of Insulin 115, 65.1 nM insulin was subcutaneously injected to rats. Residual radioactivity of insulin in the cell was determined using a radioactivity assay at 0 h and 3, 6, 12 h after injection. Insulin in serum was measured using a radioactivity assay at 0 h and 3, 6, 12 h after injection.

**RESULTS**

In vitro properties of Insulin 115 and LAPS-Insulin 115

<table>
<thead>
<tr>
<th>Test material</th>
<th>Insulin</th>
<th>LAPS-Insulin 115</th>
<th>LAPS-Insulin 115</th>
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<th>LAPS-Insulin 115</th>
<th>LAPS-Insulin 115</th>
<th>LAPS-Insulin 115</th>
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<tbody>
<tr>
<td>Receptor binding affinity (Ki, nM)</td>
<td>100</td>
<td>66</td>
<td>2.6</td>
<td>1.7</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Receptor internalization</td>
<td>100</td>
<td>53</td>
<td>5.6</td>
<td>2.0</td>
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**Stability of insulin 115 and LAPS-Insulin 115 in a cell environment**

**CONCLUSIONS**

- **Insulin 115 and LAPS-Insulin 115 have lower receptor binding affinity to receptor rather than insulin and LAPS-Insulin 115. Lower receptor binding affinity of insulin 115 and LAPS-Insulin 115 may trigger less receptor phosphorylation and internalization compared to insulin.**
- **Insulin 115 and LAPS-Insulin 115 shows higher cellular stability. The improved cellular stability of insulin 115 and LAPS-Insulin 115 may be derived from weaker receptor binding affinity as well as improved serum stability.**
- **Taken together, the long-lasting properties of insulin 115 and LAPS-Insulin 115 may be associated with the combined effects of both reduced in vitro activity and improved stability in the cellular/serum compartments.**

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Cells chronically exposed to the novel weekly insulin HM12470 are protected against desensitization of the insulin signaling cascade

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Abstract

The novel basal insulin HM12470 consists of an insulin analog (Insulin 115) conjugated to the human aglycosylated Fc fragment via a small PEG linker and is developed for once-weekly administration. In the present study, we mimic chronic exposure to HM12470 in vitro to analyze the insulin-induced desensitization of target cells. To establish an in vitro model of chronic insulin stimulation, human coronary artery smooth muscle cells (HCASM) were used in light of their high insulin sensitivity and a good tolerance towards long-term treatment. Cells were exposed for 5 days to 500 nM regular insulin (reg. Ins) or HM12470 and allowed to recover for up to 48 h. After 5 days of chronic exposure, insulin receptor (IR) protein abundance was significantly decreased in both HM12470 and reg. Ins treated cells by 42 and 78%, respectively. However, in the HM12470 exposed cells, IR levels recovered completely within 48 h in contrast to reg. Ins. Interestingly, under these conditions IR mRNA levels were not altered, indicating a more efficient translational and/or post-translational recovery of IR in HM12470 treated cells. The most prominent effect was observed at the signaling level, where the insulin stimulated Akt phosphorylation dropped tremendously in both HM12470 (to 28%) and reg. Ins (to 12%) treated cells. However, total Akt levels were not altered. Insulin signaling in the HM12470 treated cells was almost completely restored after 48 h recovery (72%), whereas the reg. insulin treated cells showed no significant improvement under these conditions. In conclusion, cells chronically exposed to the basal insulin HM12470 showed an improved recovery flexibility of the insulin signaling cascade probably due to changes in IR translation or processing. Thus, HM12470 does not irreversibly desensitize target cells under chronic exposure, and therefore represents an excellent candidate for a weekly insulin.

Summary & conclusion

- HM12470 displays...
  - Substantially prolonged pharmacokinetic and pharmacodynamic profile
  - No increased mitogenic potency compared to regular insulin
  - Similar metabolic potency compared to regular insulin after long-term stimulation
  - Decreased insulin receptor downregulation
  - Improved insulin signaling under chronic conditions

HM12470 exhibits a unique action profile and thereby represents an excellent candidate for once-weekly administration.

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