A NOVEL GLUCAGON ANALogue, ZP-GA-1, DISPLAYS INCREASED CHEMICAL AND PHYSICAL STABILITY IN LIQUID FORMULATION

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Background

Glucagon is a peptide hormone produced in response to low blood sugar levels. Pharmacologically, glucagon is used for emergency treatment of severe hypoglycemia in diabetes patients treated with insulin. Glucagon possesses poor solubility in aqueous buffers at or near physiological pH, and is also resistant to exhibiting poor chemical and physical stability.

We have present data for ZP-GA-1, a novel glucagon analogue, which displays superior solubility and stability at physiological pH, while retaining high potency in the glucagon receptor and a comparable pharmacodynamic action profile.

Superior solubility and physical stability

The solubility of ZP-GA-1 was greatly enhanced at or near physiological pH when compared to native glucagon, which had limited solubility at pH 5.2.

Table 1: Solubility of native glucagon and ZP-GA-1 in various buffers at different pH (mean values, n=3).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Buffer</th>
<th>pH 4.0</th>
<th>pH 5.2</th>
<th>pH 5.6</th>
<th>pH 7.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucagon</td>
<td>0.04 μg/ml</td>
<td>0.04 μg/ml</td>
<td>0.01 μg/ml</td>
<td>0.005 μg/ml</td>
<td>0.005 μg/ml</td>
</tr>
<tr>
<td>ZP-GA-1</td>
<td>1 μg/ml</td>
<td>1 μg/ml</td>
<td>1 μg/ml</td>
<td>1 μg/ml</td>
<td>1 μg/ml</td>
</tr>
</tbody>
</table>

ZP-GA-1 displayed superior physical stability compared to native glucagon; no turbidity was detected for ZP-GA-1 over 10 days in a Titertek 1 assay at physiological pH. Moreover, absorbance data did not show increased turbidity over time, indicating that no amorphous aggregates have been formed. Contrary, native glucagon turbid within 24 hours and increased turbidity was observed.

Excellent chemical stability

The chemical stability of ZP-GA-1 was measured over times near physiological pH and was found to be drastically improved compared to native glucagon which showed 50% degradation after 7 days at pH 5.2. The degradation of ZP-GA-1 after 30 days was only 0.1% and 0.7% when measured at 4°C and 25°C, respectively.

Potent glucagon receptor activation

The glucagon receptor activation of ZP-GA-1 was found to be very potent and comparable to native glucagon with a EC50 of 0.032 μM vs. 0.013 μM for glucagon.

Conclusions

We have successfully developed a glucagon analogue, ZP-GA-1, which demonstrates superior pharmacochromical properties over native glucagon along with a comparable pharmacodynamic profile. These studies suggest ZP-GA-1 as a potential candidate for the treatment and/or prevention of severe hypoglycemia in the form of a ready-to-use rescue kit or as an artificial pancreas device.

Comparative pharmacokinetics

Pharmacokinetic evaluation of ZP-GA-1 and native glucagon was performed in Sprague-Dawley rats. The two compounds showed comparable pharmacokinetic profiles, with a similar Cmax and Tmax, and a comparable concentration at a 1 mg/kg dose.

Retained pharmacodynamic profile

The effect of ZP-GA-1 on acute glucose release in male Sprague-Dawley rats was investigated and compared with native glucagon.

Our data shown that injection of ZP-GA-1 induced a blood glucose releasing effect that was comparable to glucagon with respect to time to reach maximum and area under the curve.

Table 2: Pharmacokinetic data at ZP-GA-1 and native glucagon in Sprague-Dawley rats.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value (μM)</th>
<th>Value (μM)</th>
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<tbody>
<tr>
<td>Glucagon</td>
<td>0.013</td>
<td>0.013</td>
</tr>
<tr>
<td>ZP-GA-1</td>
<td>0.013</td>
<td>0.013</td>
</tr>
</tbody>
</table>

Figure 1: Glucagon receptor activation of ZP-GA-1 and native glucagon at pH 5.2 using a cAMP assay with the glucagon receptor using a cAMP assay.

Figure 2: The solubility of the samples was measured at 100% and 0% and 100% values.

Figure 3: Chemical stability of ZP-GA-1 was measured by HPLC in a simple buffer solution at 4°C, and at 25°C for comparison. ZP-GA-1 in water buffer at pH 5.2 showed no change in concentration of ZP-GA-1 at 4°C for 10 days.

Figure 4: Turbidity assay at physiological pH and at 4°C measured by Titertek 1 assay with native glucagon and ZP-GA-1.

Figure 5: Pharmacokinetic data at ZP-GA-1 and native glucagon in Sprague-Dawley rats.

Figure 6: Plasma concentration of glucagon and ZP-GA-1 in sedated rats after intramuscular administration.

Figure 7: The effect of ZP-GA-1 on acute glucose release in male Sprague-Dawley rats.

Figure 8: Time to a 250-fold increase in blood glucose level and time for ZP-GA-1 and native glucagon in Sprague-Dawley rats.
<table>
<thead>
<tr>
<th>Compound</th>
<th>pH 2.5</th>
<th>pH 4</th>
<th>pH 5</th>
<th>pH 5</th>
<th>pH 6</th>
<th>pH 7</th>
<th>pH 7.5</th>
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<tbody>
<tr>
<td>Glucagon</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>ZP-Ga 1</td>
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