The Role of Glucagon in Closed Loop Treatment for T1D

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Disclosures:
• Patent holder iSense/Bayer: Continuous Glucose Monitoring
• Patent holder Legacy, OHSU Artificial Pancreas algorithms
• Patent applicant, OHSU glucagon chemical stabilization
• Current employee, Pacific Diabetes Technologies
• Scientific Advisory Board, Xeris Pharmaceuticals

Treatments discussed here are not FDA-approved
BACKGROUND:
Bihormonal automated glycemic control in T1D: **INSULIN and GLUCAGON**

Are there risks of using glucagon in bihormonal systems?
- Can repeated low dose glucagon cause glycogen depletion from the liver?
- Can high circulating insulin levels prevent the liver from responding to glucagon?

(Note: Even with glucagon, occasional hypoglycemia still occurs)

Can glucagon be biochemically stabilized?
DISCUSSION POINTS:

• To avoid glucagon failure, what are optimal insulin levels? *(Non-radioactive tracer experiments in TID)*:

• Is there depletion of carbohydrate stores after repeated doses of low dose SC glucagon? *Non-invasive measurement of liver glycogen*:

• Glucagon is biochemically unstable— Can excipients be added to make it stable and pumpable?

• **Clinical use of a fully-automated bihormonal AP system**
  - What are the difficulties?
  - Sensor accuracy in AP studies- metrics including frequency of egregious errors
The rationale for glucagon delivery--

--Hypoglycemia is the greatest fear of many persons with diabetes (coma, seizure, auto accidents, social embarrassment--

”Is this person under the influence?”
Effect of glucagon in the liver:

Large number of end points at which glycogen phosphorylase can act to release glucose 1-phosphate.

(muscle glycogen – cannot raise BG)
Why can’t you just stop the insulin?
**Rationale for the use of glucagon in automated control systems:**

*PK & PD are FAST!*

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**GLUCAGON LEVELS BY DOSE - change from baseline**

- MEAN 25 mcg
- MEAN 75 mcg
- MEAN 125 mcg
- MEAN 175 mcg

**GLUCAGON LEVELS (pg/mL)**

**TIME AFTER DOSE (min)**

**MEAN ± SEM**

*n = 25*
In Closed Loop Setting, SC Glucagon is ineffective in some (10-20%) of cases (Oregon and Boston groups).

Question 1:
At what circulating concentrations of insulin does SC glucagon lose its effectiveness in people with TID?

• Study Design:
  – carry our studies where SINGLE doses of glucagon are given at 3 DIFFERENT insulin levels (continuous IV insulin infusions).
  – Clamp glucose at euglycemia
  – Dideuterated glucose to measure glucagon effect (glucose output)
  – Octreotide infusion
INFUSIONS:
Insulin/Octreotide/Dextrose (if needed)

Study Start
Glucagon Dose 1
Glucagon Dose 2
Glucagon Dose 3
Glucagon Dose 4

Study End
Table 1: Glucagon pseudo-randomization table, showing 4x4-block method.

<table>
<thead>
<tr>
<th>Block</th>
<th>Dose 1</th>
<th>Dose 2</th>
<th>Dose 3</th>
<th>Dose 4</th>
<th>Subjects</th>
</tr>
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<tbody>
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<td>Block 4</td>
<td>175</td>
<td>25</td>
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<td>125</td>
<td>2</td>
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</tbody>
</table>
• 10 subjects with type 1 DM underwent three 10-hour euglycemic clamps in hospital CRC setting:

• On each day, **4 different SC glucagon doses** were given, separated by 2 hours.
ENDOGENOUS GLUCOSE PRODUCTION VS. GLUCAGON DOSE AND INSULIN INFUSION RATE

Bar height = glucagon effect

ENDOGENOUS GLUCOSE PRODUCTION AUC OVER 60 MINUTES (MG/KG)

GLUCAGON DOSE (MCG)

INSULIN INFUSION RATE
To achieve a brisk glucagon effect (up to 175 µg), what are the ideal plasma insulin concentrations? *

- **0-35 µU/ml** Usually safe
- **35-40 µU/ml** Risky
- **>40 µU/ml** Likely to fail

* In fasted patients of normal or mildly elevated body weight
** Probably safe to exceed this level in the hyperglycemic state soon after meals
A custom-built $^{13}$C/$^1$H transmit and receive surface coil. $^{13}$C coil is 10 cm and located in the interior.

Extensive software development to refine technique developed at Yale.

High resolution Siemens MAGNETOM 7 Tesla whole-body instrument at OHSU Advanced Imaging Research Center.

High resolution, non-invasive measurement of liver glycogen using $^{13}$C, 7T magnet.

Jessica R. Castle MD, Mark Woods PhD, Joseph El Youssef MD, Yu Cai PhD, T. Barbara PhD, W. K. Ward MD

Question 2: Do repeated doses of SC glucagon lead to depletion of liver glycogen?
**Not MR Imaging!**
The spectrum showing the glycogen signal -- naturally-occurring C13

1% of our carbon is $^{13}$C!

- **Hepatic Glycogen Signal**
- Fed (before glucagon)
- Fasted (before glucagon)

**Relative Signal Intensity (AU)**

**Parts Per Million (ppm)**
High resolution, non-invasive measurement of liver glycogen with a 7 Tesla magnet

IV insulin infusion and euglycemic glucose clamp

SC GLUCAGON, 2 µg/kg

fed, pre glucagon

fasting, pre glucagon

fed, post glucagon

fasting, post glucagon
• The position of the liver within the torso can vary significantly on both an inter-and intra-subject basis.

• These differences can give rise to erroneous liver glycogen level determinations, either too high or low: it is therefore critical to account for the position of the liver during data analysis.

• By developing a method for accounting for the position of the liver, and therefore the amount of liver tissue interrogated by the $^{13}$C coil, we found that it was possible to correct the signal intensity data to give a more accurate determination of liver glycogen concentrations \textit{in vivo}.

(ms submitted)
Fasting
Fed

[Glucagon] g/L

No Glucagon
Fed
Fasting
Glucagon

Fed
Fasting
Using multivariate analysis, if one accounts for the insulin infusion rate, the glycemic response to later doses of glucagon (potential for glycogen depletion) was maintained.

**Summary of Magnetic Resonance Spectroscopy:**

Using a high-res magnet, we found that:

In the absence of prolonged fasting, depletion of liver glycogen will be unlikely even with repeated low/moderate glucagon dosing.

--imaging of liver glycogen
--rise in blood glucose in response to glucagon
Glucagon usually works in the setting of an artificial endocrine pancreas........ BUT

It is highly unstable and cannot be pumped!

• protein aggregation---
Question 3: How to biochemically stabilize glucagon?

1. By raising the pH to 9-10 reduces amyloid fibrillation-aggregation

2. By avoiding water in formulation (Prestrelski et al, Xeris Pharmaceuticals)

3. By creating analogues of glucagon (DiMarchi et al, IU and Roche)

4. Formulation at neutral pH (Biodel, Latitude)

5. By adding the Indian spice, curcumin, or compounds related to curcumin (OHSU).
Protein aggregation (fibrillation) contributes to pathogenesis of:
- Alzheimer’s Disease
- Parkinson’s Disease
- Prion disease
The other problem with glucagon: DEGRADATION

Spontaneous Degradation of Glucagon in Aqueous Solution:

- Deamidation
- Oxidation
- Chain scission, others
- Specific mechanisms of degradation have unpredictable effects on bioactivity

GLUCAGON ALONE (7 DAYS)
Dense fibrils

GLUCAGON + CURCUMIN + HSA (7 DAYS): No fibrils

Key pharmacodynamics results were very similar between fresh and aged formulation of curcumin-stabilized glucagon.
Ferulic acid does not spontaneously degrade
A: injection peak;
B: ferulic acid;
C: a peak that has been identified as glucagon oxidation at Met27 through analysis of an oxidized Met27 glucagon
Charles Roberts, Jr. PhD

- n = 7
- No shift in bioactivity after aging at 37°C

Graph showing percent of maximal fluorescence versus glucagon (pg/ml). Two curves are compared: one for unaged FAFG and another for 7 day aged FAFG.
Rise in blood glucose from the baseline of fresh, no-excipient synthetic glucagon (black), fresh FAFG (blue) and 7-day aged FAFG (red) at 37º C.
CRC-based study of T1DM subjects, 21 studies, single-blinded:

**CROSSOVER PORTION:**

No glucagon: 40 min/day <70
With glucagon: 15 min/day <70

Mean BG: 138.0 ± 17.5 (with glucagon) vs. 130.6 ± 16.9 mg/dl (with placebo), p = NS

Recent Studies: Bihormonal AP System

Concept: to provide “rescue” delivery of SC glucagon to minimize hypoglycemia.

– When given, the goal is to keep the dose as low as possible to avoid rebound hyperglycemia

Study Summary and Preliminary Findings

• n = 29 subjects with C-peptide negative T1DM
• Fully-automated control system
• 30 hour experiments, n=11 in directly supervised clinic setting, n=18 in hotel setting with physician monitoring via cloud server
• Meals announced and estimated to nearest 20 grams with insulin bolus
A Complex System:

The subject side

• 1 smart phone AP controller (Motorola)
• 2 CGM’s (Dexcom G4) with receivers
• 2 pumps (OmniPod); one for insulin, one for glucagon
• 1 pump controller
• Batteries
• Cables, USB hub
• Glucagon (Novo) changed every 8 hrs

Physician monitoring side (Peter Jacobs PhD)

• Cloud server communicating either via wireless hotspots or cell towers
STUDY MANAGEMENT: Jessica Castle MD; Parkash Bakhtiani, MD; Deborah Branigan
ENGINEERING: Peter Jacobs, PhD; Nicholas Prieser; Joseph El Youssef, MBBS

Master controller: Motorola ES400
GUI: home screen

entering BG values
estimating carbohydrate intake
remote monitoring
mean glucose: 147 mg/dl

**Hypoglycemia (sensor glucose)** based on 720.5 hours
- Average min/day below 70: **13.2 min**
- Average min/day below 60: **3.8 min**

**Hypoglycemia (sensor glucose)** *Gen4 ONLY* based on 498 hours
- Average min/day below 70: **7.5 min**
- Average min/day below 60: **0.5 min**

Mean glucagon amount:

280 micrograms per day
Performance of Dexcom Gen4 CGM in Oregon AP studies 2013

- n=20 subjects
- 268 data pairs

**Mean + SEM**

- **Bias:** 0.35 mg/dl
- **RD:** 0.9%
- **ARD:** 9.3%

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1. Calibration by the research subjects using One Touch II meter
2. Data are mean of two sensors
3. Calibration every 6 hours

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**Note:** Data shown are based on average of dual sensors.
Performance of Dexcom Gen4 CGM in Oregon AP studies 2013
Automated Bihormonal Control of Type 1 Diabetes

Conclusions

• In the fasting state, to maximize glucagon effectiveness, insulin levels should ideally be <40 μU/ml.

• Repeated dosing of low dose SC glucagon is unlikely to cause depletion of liver glycogen in people with T1D who are eating normally.

• The addition of glucagon to AP systems reduces frequency of hypoglycemia further than a comparable insulin only method.

• SC interstitial CGM devices are increasingly accurate and egregious errors are very rare.
  – This improvement in sensor accuracy contributes to lower rates of hypoglycemia.

Room for Improvement

– Still need a “pumpable” glucagon formulation.

– The connectivity issue: Need consolidations of devices!
  • Need to minimize the number of on-the-skin devices.
  • Need to minimize the number of devices carried in pockets.
  • Need to develop hardware and software with ultra high reliability.
CURRENT STATE-of-the-ART (2014)

A need to have 2 through-the-skin devices to properly manage one’s diabetes

a sensor...............................and ........................ an insulin catheter

combined into a sensing catheter (for research only)

--SPIDIMAN – Graz, Austria
--Pacific Diabetes Technologies, Inc --- pacificdt.com
Clinical Studies of Artificial Pancreas

- **Jessica R. Castle MD**: Director of Clinical Studies
- **Joseph El Youssef MD**: Algorithm and Mathematics Specialist
- **Peter Jacobs PhD, Nick Preiser**: Software Engineers
- **Deborah Branigan**: Lead Research Technician
- **Matthew Breen**: Research Associate

Glucagon Chemistry Project:

- **Nick Caputo MS, Melanie Jackson, Colin Bergstrom**: biochemistry
- **Parkash Bakhtiani**: microscopy
- **Charles Roberts PhD**: assays

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