Biochaperone Glucagon Exenatide (BC Glu Exe), A Stable Combination Of Glucagon (Glu) And Exenatide (Exe) Achieved Larger Body Weight (BW) Loss Than Exe Alone In DIO Mice

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Abstract

Over the last decades, obesity and its associated consequences have become a major health issue worldwide. GLP-1 Receptor Agonists (GLP-1 RA) are one of the few treatment options currently available, but achieve limited weight loss.

A combination of human glucagon (Glu) and GLP-1 RA is expected to provide a superior body weight (BW) reduction than GLP-1 RA alone. GLP-1 RA acts in the brain to induce satiety while Glu has a lipolytic and thermogenic effect in liver and adipose tissues. BioChaperone (BC) proprietary technology allows the solubilization and stabilization of poorly soluble and/or unstable peptides, such as Glu, at neutral pH and thus the combination of Glu and GLP-1 RA.

A combination formulation of glucagon and exenatide (BC Glu Exe) was developed. Such combination is physically and chemically stable for at least 4 weeks at 37°C in cartridges. It was tested *in vivo* against reference exenatide and vehicle in diet induced obese (DIO) male mice. Mice were dosed with a subcutaneous continuous infusion of vehicle, Exe or BC Glu Exe via osmotic minipumps.

After 14 days, Exe and BC Glu Exe induced a BW loss of 10% and 25% respectively, compared to the vehicle. The BW loss was significantly more pronounced with BC Glu Exe than with Exe. In conclusion, these results support the testing of BC Glu Exe in clinics as a treatment for obesity.

Introduction & Background

- Over a relatively short period, obesity and its associated consequences have become a large medical and economic burden. Today, medical treatment options comprise on the one hand bariatric surgery, a highly invasive technique with irreversible secondary effects, that can lead to weight loss of 20 to 30% and on the other hand pharmacological treatments that result in weight loss reduction ranging from 3 to 7% (1). Among those treatments, GLP-1 RAs have received a lot of attention for their efficacy in treating both Type II diabetes and obesity, in particular due to their effect on food intake reduction.

- One GLP-1 RA, liraglutide is currently approved for weight loss in obesity and a second generation is in late stage development. However, the initial weight loss induced by GLP-1 RA is associated with a reduction in energy expenditure which limits the efficacy of the treatment in the long term.

- Oxyntomodulin, another gut hormone, has been shown to have an effect in rodents on both food intake via the GLP-1 receptor and energy expenditure via the glucagon receptor (2) leading to the development of dual agonist combining both GLP-1 R and glucagon receptor agonist activities for T2D patients. However, as glucose control is the primary target in T2D, the respective GLP-1 and glucagon activities are balanced towards the GLP-1 activity and may not be fully suited for non diabetic obese patients. Having a formulation that enables the establishment of the optimal balance between GLP-1 RA and glucagon effects in those patients over the course of its development could lead to an improved weight loss benefit.

- BioChaperone® (BC) technology relies on compounds that form reversible interactions with therapeutic hormones. BC technology solubilizes glucagon at neutral pH, enabling its co-formulation with exenatide, a GLP-1 RA to deliver both molecules at the required dose through continuous subcutaneous infusion.

1 Srivastava et al. Nat rev Endocrinology 2017
2 Scott et al. Peptides 2018

Aim

- Develop a fully-aqueous formulation of glucagon and exenatide that is physically and chemically stable at physiological pH, can be used in the treatment of obesity and validate the formulation efficacy in an animal model for obesity.

Methods

- BC Glucagon Exenatide formulations
  - Formulations for both *in vivo* studies in mice and human clinical trials were designed using PK modeling to ensure a similar molar ratio of GLP-1 RA vs glucagon at steady state in both species.

- Assessment of stability of the BC Glucagon Exenatide formulations
  - Vial inspection and vial photographs were performed under a vertical grazing white light to reveal visible objects.
  - Chemical stability was assessed by RP-HPLC

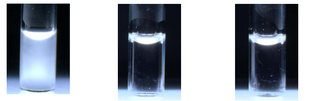
- Animal Study
  - 26 weeks HFD induced C57Bl/6J male mice (n=10) were continuously infused (s.c.) using Alzet mini pump with Exenatide alone, a combination formulation of Biochaperone, glucagon and exenatide, or Saline, for 2 weeks. Animals were kept in collective cages with two cages of five animals per group.
  - The body weight was monitored by individual weighting every day and the food intake was monitored by measuring food consumption in each cage.

  - An extra arm consisting of BioChaperone and exenatide without glucagon was tested in the study and showed identical results to the exenatide alone, demonstrating that the Biochaperone itself had no effect.

BC technology enables glucagon exenatide co-formulation

Glucagon poses a challenge for formulation due to very low solubility at physiologic pH and low stability over time resulting from rapid fibrillation.

Figure 1. Pictures of solutions containing glucagon (2 mg/mL), glucagon (2 mg/mL) + BC and the combination glucagon (2 mg/mL), exenatide (57 µg/ml) and BC at neutral pH.



- Glucagon is insoluble at neutral pH but the addition of BC allows to solubilize glucagon producing a clear and colorless solution.
- The addition of exenatide in the formulation did not impact glucagon solubility.

Stability of BC Glu Exe formulation

Human formulation: BC Glu Exe in vials

→ Visual inspection of the formulation at 4°C and 37°C.

	BC glucagon Exe	
Temperature	37°C	4°C
Stablility	>12W	>13M

- BC Glu Exe coformulation is a clear and colorless solution, essentially free of particle, after storage in vials at 4°C for at least 13 months and at 37°C for at least 12 weeks.

→ Chemical stability after 4 weeks storage at 37°C in vials.
- Both glucagon and exenatide recoveries are around 90 %
- Glucagon and exenatide impurity contents are of 5.4 and 7.6 % respectively (Figure 2)

Mice formulation: BC Glu Exe in cartridges

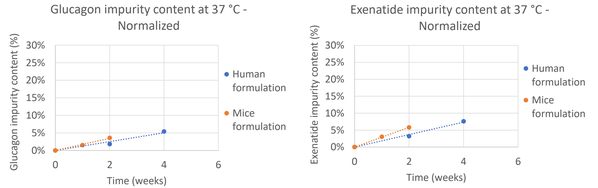
→ Visual inspection of the formulation at 4°C and 37°C.

	BC Glucagon Exe	
Temperature	37°C	4°C
Stablility	>12W	>12M

- BC Glu Exe coformulation is a clear and colorless solution, essentially free of particle, after storage in cartridges at 4°C for at least 13 months and at 37°C for at least 12 weeks

→ Chemical stability after 2 weeks storage at 37°C in cartridges
- Glucagon and exenatide impurity contents are of 3.6 and 5.8 % respectively (Figure 2)

Figure 2. Glucagon and exenatide impurity content in BC Glu Exe coformulations designed for mice in vivo studies or human clinical trials.



Animal Study

The treatments were well tolerated in all groups without any clinical finding reported during the study.

Exenatide alone, used as a refence and infused for 14 days led to a significant 10% decrease in BW compared to vehicle (Saline) (Figure 3). The total food intake in this group was significantly decreased when compared to the vehicle (Figure 4).

Figure 3 Relative body weight (n=10)

Values expressed as mean of n = 10 + SEM
Dunnett's test one-factor linear model
*: P < 0.05 compared to Saline
***: P < 0.001 compared to Saline
###: P < 0.001 compared to Exenatide

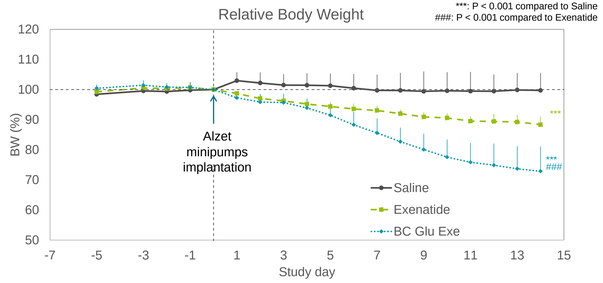
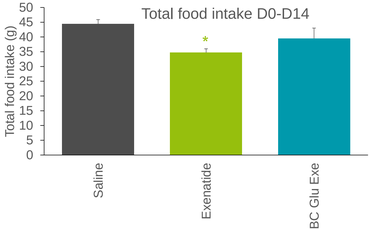


Figure 4 Cumulated food consumption (n=10)



- The coformulation BC Glu Exe induced a significant BW loss compared to vehicle (25%) and to exenatide alone (15%) (Figure 3). In this group the total food intake was intermediate between vehicle and exenatide without statistical significance (Figure 4) although the BW loss was superior to both groups. This confirms the glucagon effect on weight loss in the combination. This effect can be attributed to an increase in energy expenditure.

Conclusions

- ✓ BioChaperone technology enables the combination of glucagon with exenatide, a GLP1-RA.
- ✓ BC Glu Exe coformulation is clear and essentially free of aggregate after storage in cartridges for at least 12 weeks at 37°C and at least 13 months at 2-8°C, as well as chemically stable for at least 4 weeks at 37°C.
- ✓ *In vivo* the combination of glucagon with exenatide has a significantly stronger effect on BW loss than exenatide alone in DIO mice treated for 14 days.

These results would warrant further development of BC Glu Exe formulations as potential treatment for non diabetic obese people.

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