SOLUBLE NON-AQUEOUS GLUCAGON FORMULATIONS FOR THE TREATMENT OF SEVERE HYPOGLYCEMIA.

Steven J. Prestrelski, John Kinzell. Xeris Pharmaceuticals, Inc., Austin, TX

ABSTRACT

Severe hypoglycemia remains a significant unmet medical need. Recent studies estimate that 6% to 10% of deaths of patients with type 1 diabetes are attributable to hypoglycemia. Administration of glucagon is effective in reversing severe hypoglycemia. However, development of a simple, ready-to-use glucagon product has been hampered by the property of glucagon to spontaneously assemble into fibrils in aqueous solution. Thus, currently approved products (Lilly, Glucagon for Injection; Novo Glucagen®) are based on lyophilized formulations. The need for reconstitution has made these products difficult to administer in emergency situations, and thus, they are infrequently used. We have developed a soluble glucagon formulation based on biocompatible, non-aqueous solvents. These formulations effectively suppress the fibrillation of glucagon observed in aqueous solutions, even at high concentrations and temperatures. Further, the chemical stability of glucagon in these formulations is similar to that of dry powders. Non-aqueous solutions of glucagon (5 mg/ml) have been demonstrated to be free of fibrillation after incubation at 40°C for two months (compared to just hours for aqueous solutions). Additionally, minimal chemical degradation of glucagon is observed in non-aqueous solutions (apparent degradation rate at room temperature is ~0.25%/month). Comparative pharmacology studies in a rodent model show the non-aqueous solutions of glucagon to have equivalent pharmacokinetics and pharmacodynamics to aqueous formulations. Similar to aqueous solutions, injection of non-aqueous formulations of glucagon show rapid absorption (Tmax ~ 5 min) and elevation of glucose levels (within 15 minutes). These data support the development of a ready-to-use rescue pen for severe hypoglycemia as well as a glucagon formulation suitable for a bi-hormonal (insulin-glucagon) infusion pump.

INTRODUCTION

One of the main complications with insulin therapy is hypoglycemia. Type 1 diabetics suffer ~two symptomatic hypoglycemic events per week – and an event of such severity that is temporarily disabling approximately once a year (Diabetes Control and Complications Research Group, 1993; Reichard. P. et al., 1991; McLeod et al, 1993). Insulin-using diabetics typically have several hypoglycemic episodes in a given year, 1-2 of these being severe episodes. There are currently approximately 1.4 million Type 1 and 3.8 million insulin-using Type 2 diabetics in the US alone. On average this patient population experiences a total of 3 million severe hypoglycemic events per year.

- **Two to ten percent of deaths in Type 1 diabetics have been attributed to hypoglycemia** (JDRF, 2012).
- **The current standard of care for counteracting severe hypoglycemia is using a Glucagon Emergency Kit (GEK).** ADA recommends all insulin- and sulfonylurea-using diabetics carry two GEKs and use glucagon as first line therapy in the event of severe hypoglycemia. Schools and paramedics also keep GEKs on hand. However a recent survey indicates only about 30% of the insulin-using diabetics carries GEK's (CloseConcerns, 2010).
- **The limitation of currently available Glucagon Emergency Kits (GEKs) is the complex assembly and reconstitution procedure** required during an emergency situation. Lilly's GEK, includes a vial containing 1 mg of glucagon (the standard adult dose). Glucagon in the vial is a lyophilized cake, which must be reconstituted with 1 ml of diluent included in the kit in a pre-filled syringe. Prior to injection, the person administering the treatment must insure complete dissolution of the drug and examine for the absence of particulates. **In total there are nine (9) steps that must be followed carefully**. This is a complex procedure for an untrained person in an emergency situation.
- **There is a compelling need for an improved delivery system for glucagon administration.** One that simplifies administration during an emergency medical procedure increasing patient compliance and improving the experience for both patient and caregiver.

OBJECTIVE

- **Xeris is developing a formulation and delivery system that allows subcutaneous injection of a ready-to-use, ultra-low formulation of glucagon via an auto-injector pen.** Xeris' technology relies on a unique non-aqueous, concentrated drug formulation that can be injected subcutaneously without reconstitution. Further, the small volume of the dose allows rapid auto-injection. **The current nine-step process could be reduced to two-simple steps.**

MATERIALS AND METHODS

Analytical Methods - Turbidity – Turbidity was measured via light scattering using UV absorbance at 630 or 650 nm. Reverse phase HPLC - A gradient method was developed based on 0.1% trifloroacetic acid (TFA) and acetonitrile and a Thermo Scientific BioBasic-8, 250 mm x 4 mm, 5 µm, 300 Å column. Detection was at 280 nm; flow Rate was 1.0 mL/min; column Temperature was 37°C; injection quantity was 30 µg of glucagon. Size exclusion HPLC – A size exclusion method was develop using a Mobile Phase consisting of 3.2 mM HCl, 100 mM NaCl, pH 2.5 and a Tosoh G3000SWXL (7.8 x 300 mm) column. Detection was at 280 nm; flow Rate wass 1.0 mL/min; column Temperature of 35°C. **Solubility Studies** - Glucagon (Bachem, Bubendorf, Switzerland) was prepared at 1.0 mg/mL via dissolution into 2 mM glycine in aqueous solution. Solutions were filetered through 0.45 µm filters and aliquoted into sterile, 2 cc glass vials, each at 1 mL fill volume. These solutions were lyophilized to dryness and stoppered under vacuum. Lyophilized glucagon formulations were reconstituted with appropriate amounts of various solvent systems. Solubility samples were analyzed via visual analysis, turbidity (A_{630}), RP- and SE-HPLC. **Stability Studies** - Glucagon (Bachem) was prepared at 1.0 mg/mL via dissolution into 2 mM glycine in aqueous solution. Solutions were filtered through 0.45 µm filters and aliquoted into sterile, 2 cc glass vials, each at 1 mL fill volume. These solutions were lyophilized to dryness and stoppered under vacuum. Lyophilized glucagon formulations were reconstituted to 5 mg/ml glucagon, 10 mM glycine in various solvent-excipient systems. Samples for stability were stored at 5, 25 and 40°C in stability chambers (ambient humidity). Stability samples were analyzed via visual analysis, turbidity (A_{630} or A_{650}), RP- and SE-HPLC. **Pre-Clinical Studies** - Jugular vein-canulated, Sprague-Dawley rats (~250 g) were all dosed subcutaneously with 10 µg glucagon. The non-aqueous glucagon solutions were given as 2 µl injections of 5 mg/ml. Aqueous glucagon was injected as 10 ml injections of 1 mg/mL glucagon, pH 3.0. Pharmacokinetic parameters were analyzed for the three treatment groups plus the aqueous control. Plasma glucagon was assayed via an RIA at Intertek (San Diego, CA). Blood glucose measurements were performed with a handheld glucometer. A non-compartmental PK analysis was performed for each rat. C_{max} and T_{max} were computed from observed data. Area-under-the-curve (AUC) estimates were computed without extrapolation. Data were analyzed using a four group ANOVA to compare PK parameters across groups.

CONCLUSIONS

- **Glucagon has significantly enhanced solubility and stability in polar aprotic solvents when compared to aqueous solutions.**
- **Fibrillation of glucagon prevalent in aqueous solutions is effectively suppressed in polar aprotic solvents, even at high concentrations and temperatures**
- **Polar aprotic solvents stabilize soluble, monomeric glucagon**
- **Glucagon is highly stable in polar aprotic solvents, with stability comparable to that of freeze-dried powders**
- **The pharmacokinetics and pharmacodynamics of glucagon in polar aprotic solvents are essentially equivalent to low pH aqueous solutions when delivered as a subcutaneous injection**

REFERENCES AND ACKNOWLEDGEMENTS

REFERENCES

JDRF, 2012 (http://www.jdrftalk.org/2011/11/07/percentage-people-type1-diabetes-die-low-blood-sugar-hypoglycemia/)

Cryer, PE, Management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement from the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care, January 2007, Vol, 30 no. 1: 1190-192

Diabetes Therapeutics and Diagnostics: A Strategic Market Analysis. Frontline Strategic Consulting, Inc. Report, (2001).

Diabetes Control and Complications Trial Research Group "The effect of intensive therapy of diabetes on the development and progression of long-term complications in glucagon-dependent diabetes mellitus" *New England Journal of Medicine*, 329 pp 977-986 (1993).

McLeod, K.M., Hepburn, D.A. and B.M. Frier, 1993. Frequency and morbidity of severe hypoglycaemia in insulin-treated diabetic patients. Diabet. Med. 10:238-245.

Reichard, P., Bergland, B., Britz, A. Cars, I., Nilsson, BY and U. Rosenqvist, 1991. Intensified conventional insulin treatment retards the microvesicular complications of insulin dependent diabetes mellitius (IDDM): the Stockholm Diabetes Intervention Study (SDIS) after 5 years. J. Intern. Med. 230:101-108.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the support from the National Institutes of Health, National Institute for Diabetes and Digestive and Kidney Diseases, Small Business Innovation Research grants (2R44DK085809-02).

RESULTS

Solubility Studies – Glucagon is highly soluble in biocompatible, polar aprotic solvents and co-solvents. It is also surprisingly soluble in aqueous buffer, pH 2.5, though gelation was typically evident within a day of reconstitution.

Solubility Results for Glucagon in Non-aqueous Solvents and Co-Solvents.

Concentration (mg/ml)	Aqueous, pH 2.5		DMSO		DMSO/NMP (50/50)		NMP	
	Recon	Aged	Recon	Aged	Recon	Aged	Recon	Aged
1	Clear	***Gelled***	Clear	Clear	Clear	Clear	Clear	Clear
10	Clear	***Gelled***	Clear	Clear	Clear	Clear	Clear	Clear
30	Clear	***Gelled***	Clear	Clear	Clear	Clear	Clear	Clear

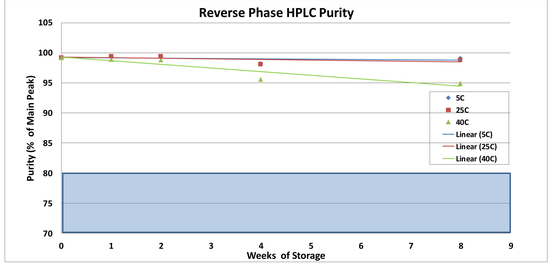
Stability Studies—Physical Stability—Non-aqueous formulations of glucagon at 5 mg/ml in polar aprotic solvents are very stable. Samples remained visually clear and free flowing over an 8 week period stored at 40°C. No significant increase in turbidity was apparent over this 8 week period (data not shown). Similarly, no new higher molecular weight peaks were apparent of SEC chromatograms for samples stored at 40°C.

SEC Purity of Glucagon Stored at 40°C.

Time (weeks)	0	1	2	4	8
SEC Purity (%Main)	100	100	100	100	99*

*Impurity is a lower molecular weight species, likely due to hydrolysis.

Stability Studies—Chemical Stability – Non-aqueous formulations of glucagon demonstrated a high degree of chemical stability after 8 weeks of storage. Figure 1 shows the Reverse phase –HPLC purity of glucagon over 8 weeks of storage at various temperatures. Very little degradation is observed at 25°C (the expected storage temperature) and 5°C. Some degradation is observed at 40°C. The apparent degradation rates are ~0.2%/month at 5°C, ~0.3%/month at 25°C and 2.5%/month at 40°C.

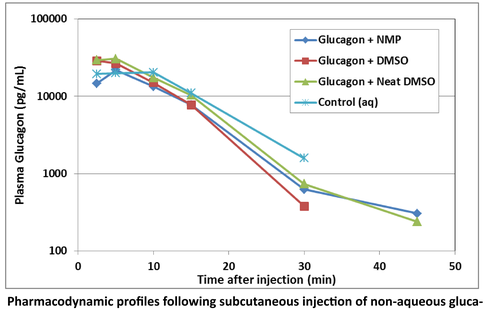


Comparative Pharmacology Studies – Following subcutaneous injection in a rat model, non-aqueous glucagon formulations demonstrate essentially equivalent pharmacokinetics and pharmacodynamics as low pH aqueous formulations. Statistical analysis (4-way ANOVO) indicates no significant differences in AUC, T_{max} and C_{max} among the four groups.

Formulations Used in Rat Pharmacology Study

Formulation #	Formulation Components
1	Glucagon + NMP + glycine + trehalose
2	Glucagon + DMSO + glycine + trehalose
3	Glucagon + Neat DMSO
4	Glucagon + H_2O (control)

Pharmacokinetic profiles following subcutaneous injection of non-aqueous glucagon and low pH aqueous control.



Pharmacodynamic profiles following subcutaneous injection of non-aqueous glucagon and low pH aqueous control.