Nonaqueous, Mini-Dose Glucagon for Treatment of Mild Hypoglycemia in Adults With Type 1 Diabetes: A Dose-Seeking Study

DOI: 10.2337/dc15-2124

OBJECTIVE
To evaluate mini-dose glucagon in adults with type 1 diabetes using a stable, liquid, ready-to-use preparation.

RESEARCH DESIGN AND METHODS
Twelve adults with type 1 diabetes receiving treatment with insulin pumps received subcutaneous doses of 75, 150, and 300 μg of nonaqueous glucagon. Plasma glucose, glucagon, and insulin concentrations were measured. At 180 min, subjects received insulin followed in ~60 min by a second identical dose of glucagon.

RESULTS
Mean (±SE) fasting glucose concentrations (mg/dL) were 110 ± 7, 110 ± 10, and 109 ± 9 for the 75-, 150-, and 300-μg doses, respectively, increasing maximally at 60 min by 33, 64, and 95 mg/dL (all P < 0.001). The post–insulin administration glucose concentrations were 70 ± 2, 74 ± 5, and 70 ± 2 mg/dL, respectively, with maximal increases of 19, 24, and 43 mg/dL post–glucagon administration (P < 0.02) at 45–60 min.

CONCLUSIONS
Subcutaneous, nonaqueous, ready-to-use G-Pen Mini glucagon may provide an alternative to oral carbohydrates for the management of anticipated, impending, or mild hypoglycemia in adults with type 1 diabetes.

Near-normal glycemic control is known to prevent or delay the microvascular complications of type 1 diabetes but increases the risk of hypoglycemia (1–3). Our previous findings of the utility of mini-dose glucagon in treating mild-to-moderate hypoglycemia in children and adolescents (4) has been confirmed at a diabetes summer camp and in the hospital setting, and is now widely recommended (5). Because rehydrated glucagon is unstable (6), Xeris Pharmaceuticals, Inc., has developed a ready-to-use, stable, nonaqueous liquid form of glucagon. In this study, we evaluated the glycemic responses and the safety, tolerability, and pharmacokinetic profile of the Xeris G-Pen Mini glucagon.

RESEARCH DESIGN AND METHODS
Twelve adults (26 ± 3 years of age, 8 females, BMI 25.0 ± 1.3 kg/m²) with type 1 diabetes for 12 ± 2 years (HbA₁c 7.7 ± 0.1%) using insulin pumps were studied on...
three occasions separated by 2–21 days. A unique dose of glucagon (75, 150, or 300 μg) was used on each study day, and the order of doses was randomized across study days. The night before the study, subjects maintained their normal routine. At ~4:00 to 5:00 A.M., the subjects reported their blood glucose concentration to one of the investigators. If their blood glucose concentration was >140 mg/dL, subjects were instructed to administer a small dose of insulin in a bolus, and, if the concentration was <70 mg/dL, to treat with a small amount of oral glucose. Subjects arrived at the study unit at ~7:00 A.M.

At ~9:00 A.M., the subjects received the assigned dose of glucagon using an insulin syringe. Each injection site was graded at +30 and +120 min using the scale of Draize et al. (7), a subjective ranking (0 to 4+) of skin erythema and edema. Blood pressure, heart rate, and temperature measurements were performed at 30, 60, and 120 min after each dose. At 180 min, the subjects received a subcutaneous insulin bolus using an insulin syringe, equivalent to cover 30 g of glucose to induce mild hypoglycemia, and blood glucose concentration was monitored until it decreased to <70 mg/dL or for 2 h, whichever occurred first. At ~60 min, the second glucagon dose was administered (same as the previous dose). Serial blood samples (~4 mL) were obtained prior to and after both doses of glucagon. At the end of the study, subjects were fed, and when their glucose concentration was stable, they were discharged.

Standard methodologies were used to measure plasma concentrations of glucose (Yellow Springs Instruments, Yellow Springs, OH), glucagon (Glucagon RIA Kit, catalog #GL32K; Millipore, Billerica, MA), and insulin (Mercodia Iso-Insulin Kit, catalog #10–1128–01; Mercodia Inc., Winston-Salem, NC). All statistical testing was two sided. SAS version 9.3 for Windows (SAS Institute, Cary, NC) was used throughout. All data are presented as the mean ± SE. The derivations for glucagon (PK) and glucose (PD) efficacy variables included the following: maximal glucagon concentration (C\text{max}) between 0 and 180 min, the time to maximal concentration (T\text{max}), the area under the curve from 0 to 120 min using the trapezoidal rule (AUC\text{0–120}), and baseline adjusted AUC\text{0–120} with negative values set at 0. The glucose, insulin, and glucagon concentrations were compared using ordinary Sidak analyses (8).

Pairwise comparison of the least squares arithmetic mean of each dose was performed by applying a mixed model (dose, sequence, dose period, and subject). The PK/PD parameters were analyzed using the Schuirmann (9) two one-sided tests for equivalence. The two one-sided tests from the SAS t test procedure were applied to test the bioequivalence.

RESULTS
Dose-dependent increases in glucose were observed after an overnight fast and mild hypoglycemia, but were more robust after an overnight fast (Fig. 1). Plasma glucose concentrations increased (P < 0.05) by 20 min with all doses of glucagon. Increases in glucose, C\text{max}, and adjusted AUC were dose dependent (P < 0.05). The fasting C\text{max} values for glucose were 155, 186, and 213 mg/dL for the 75-, 150-, and 300-μg doses, respectively, and the fasting mean adjusted AUC estimates for glucose were 4,872, 8,565, and 12,420 mg/dL/min for the 75-, 150-, and 300-μg doses, respectively (P < 0.05 for all pairwise comparisons).

After insulin-induced mild hypoglycemia, glucagon administration increased (P < 0.05) blood glucose concentrations at 20 min, although changes were not as robust as in the morning fasted state. The change in blood glucose concentrations from baseline to 20 min was 4.6, 1.8, and 6.9 mg/dL for the 75-, 150-, and 300-μg doses, respectively.

Increases in glucagon C\text{max} and AUC\text{0–120} were dose dependent and statistically significant (P < 0.05) (Fig. 1). Glucagon C\text{max} estimates were 233, 381, and 664 pg/mL for the 75-, 150-, and 300-μg doses, respectively, and the fasting AUC\text{0–120} values for glucagon were 265, 390, and 735 pg/mL/h for the 75-, 150-, and 300-μg doses, respectively. The glucagon C\text{max}, AUC\text{0–120}, and T\text{max} were similar between the fasted and post–insulin administration states. After subcutaneous insulin administration, plasma insulin concentrations increased from ~22 to ~33 μU/mL and were unaffected by the dose of glucagon or the study condition (Fig. 1).

Heart rate, blood pressure, and temperature were neither clinically nor significantly changed. The most common adverse event (AE) reported was a burning sensation at the injection site, and it was similar across doses. The majority of these AEs were a mild (68%) or moderate (30%) burning sensation. The median duration was similar among the doses (~3.0 min). One subject reported burning for 4 h after one dose (but not after other doses) that was administered at the infusion site from which she had just removed her insulin pump; this event was excluded as an outlier from the analysis of duration. Four subjects reported transient nausea without vomiting on a single occasion after the 300-μg dose (17% of the total), but none after the 75- or 150-μg dose. All other AEs were reported in two or fewer subjects and had a similar incidence across doses, and the vast majority were mild.

CONCLUSIONS
Based on the suboptimal response with the 75-μg dose of glucagon and the occasional mild nausea with the 300-μg dose, the 150-μg dose of glucagon appears to be optimal. This is similar to the dose we have used in older adolescents using aqueous glucagon (4,5). The glycemic response after an overnight fast was greater than that after mild hypoglycemia, perhaps because of the known effects of insulin on glycogen metabolism (10,11). Because most hypoglycemia occurs some hours after the last insulin bolus, we anticipate that the glycemic response to mini-dose glucagon would be similar to that observed after the overnight fast in this study. However, hypoglycemia occurring immediately after an insulin bolus could dramatically blunt the response to glucagon; therefore, rechecking blood glucose concentrations will always be recommended, and repeating the dose may be necessary (4,5).

Two areas of concern are mild transient nausea, which appears to be dose related, and injection site discomfort after some injections. This may be related to the DMSO used in the Xeris glucagon formulation. However, the discomfort was inconsistent in each individual and unrelated to the site or dose.

Mini-dose glucagon in a ready-to-use form would provide an alternative to the current management of mild-to-moderate hypoglycemia in patients...
with insulin-requiring diabetes of all ages. Fear of progression to severe hypoglycemia and a general sense of not “feeling good” cause many to avoid even mild hypoglycemia (12–14). The current American Diabetes Association recommendations (15) are to treat mild hypoglycemia with 15 g of oral carbohydrate. Patients frequently repeat the recommended treatment several times, resulting in dramatic hyperglycemia (16). Treatment with mini-dose glucagon may provide a more precise therapeutic option for mild-to-moderate hypoglycemia, avoiding the consumption of additional calories and the resulting hyperglycemia. In addition, the liquid, stable, and ready-to-use glucagon circumvents the complexities and the potential failure to reconstitute and administer lyophilized glucagon correctly that interfere with timely treatment. We believe that mini-dose glucagon administration using stable, ready-to-use G-Pen Mini glucagon (Xeris) will be a clinically attractive and cost-effective tool that patients with type 1 diabetes can use to help manage their disease.

Acknowledgments. The authors thank the volunteers and their families who participated in the studies and those of other investigators, and the research nurse and staff at the Metabolic Research Unit, Children’s Nutrition Research Center, Baylor College of Medicine, Houston, TX, and other institutions. They also thank the study coordinators Rebecca Balhoff and Allison Rounds for the outstanding job they did in the execution of this study. In addition, they thank S. Sharma, Department of Pediatrics, Baylor College of Medicine, for her dedication and technical support of the studies undertaken by our group, and Dr. Wong for help in overseeing the security and validation of the supply of the Xeris glucagon. Finally, the authors thank Leon Shi, PhD, and Poul Strange, MD, Integrated Medical Development, Princeton Junction, NJ, and O’Brian Smith, PhD, Baylor College of Medicine, for statistical analysis and interpretation of the study data.

Funding. The studies reported in this article were supported by U.S. Department of Agriculture grant CRIS 6250-51000 to M.W.H., and by National Institutes of Health grant NIDDK SBIR1R44-DK-096715 to M.W.H. and J.K., coprincipal investigators, 2/06/2014–2/05/16, and the Helmsley Charitable Trust to S.P.

Duality of Interest. This work is a publication of the U.S. Department of Agriculture/Agricultural Research Service Children’s Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine (Houston, TX). M.J.C. is Vice President of Drug Development at Xeris Pharmaceuticals. B.N. is Senior Director of Glucagon Products at Xeris Pharmaceuticals. J.K. is Executive Vice President of Xeris Pharmaceuticals. S.P. is Chief Scientific Officer of Xeris Pharmaceuticals. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. M.W.H. performed subject screening and physical examinations, oversaw all of the subjects on their days of study, spoke to virtually all of the subjects during the night preceding their study days, adjusted insulin and/or oral glucose concentrations to permit subjects to come to the Metabolic Research Unit with a reasonable blood glucose concentration, reviewed the data, and wrote initial drafts of the article. M.J.R. and S.M. performed subject screening and physical examinations, oversaw all of the subjects on their days of study, reviewed the data, and wrote initial drafts of the article. M.J.C. was responsible for monitoring the study and maintaining the electronic database, and oversaw analysis of the data and preparation of the clinical study report. B.N. acted as the study coordinator for Xeris Pharmaceuticals, and oversaw analysis of the data and preparation of the clinical study report. J.K. was coinvestigator on the Small Business Innovation Research (SBIR) grant listed in the Funding section. S.P. contributed to the study design. All of the authors contributed to the review of the data and discussion of

Figure 1—Plasma glucose (A), glucagon (B), and insulin (C) responses in 12 individuals using doses of 75, 150, and 300 μg of G-Pen Mini glucagon (Xeris) administered in random order. The initial injection of glucagon (Dose 1) was administered after an overnight fast. The second injection of glucagon (Dose 2) occurred between 10 and 30 min after a subcutaneous injection of insulin equivalent to what each subject would use to cover 30 g of carbohydrate using their insulin/carbohydrate ratio.
the results; reviewed and collectively edited the content of this article; and gave final approval of the version of the article to be published. M.W.H. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Prior Presentation. This study was presented at the 75th Scientific Sessions of the American Diabetes Association, Boston, MA, 5–9 June 2015.

References
1. Imura H, Nakao K, Shimatsu A, et al. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993;329:683–689
2. Cryer PE. Hypoglycaemia: the limiting factor in the glycaemic management of Type I and Type II diabetes. Diabetologia 2002;45:937–948
3. Frier BM. The incidence and impact of hypoglycaemia in type 1 and type 2 diabetes. International Diabetes Monitor 2009;21:210–218
4. Haymond MW, Schreiner B. Mini-dose glucagon rescue for hypoglycemia in children with type 1 diabetes. Diabetes Care 2001;24:643–645
5. Chung ST, Haymond MW. Minimizing morbidity of hypoglycemia in diabetes: a review of mini-dose glucagon. J Diabetes Sci Technol 2015;9:44–51
6. Onoue S, Iwasa S, Kojima T, et al. Structural transition of glucagon in the concentrated solution observed by electrophoretic and spectroscopic techniques. J Chromatogr A 2006;1109:167–173
7. Draize JH, Woodward G, Calvery HO. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharmacol Exp Ther 1944;83:377–390
8. Westfall PH, Wolfinger RD. Multiple tests with discrete distributions. Am Stat 1997;51:3–8
9. Schuirmann DJ. A comparison of the two one-sided tests procedure and the power approach for assessing the equivalence of average bioavailability. J Pharmacokin Biopharm 1987;15:657–680
10. Tse TF, Clutter WE, Shah SD, Cryer PE. Mechanisms of postprandial glucose counterregulation in man. Physiologic roles of glucagon and epinephrine vis-a-vis insulin in the prevention of hypoglycemia late after glucose ingestion. J Clin Invest 1983;72:278–286
11. Ahren B, Nohin A, Schersten B. Insulin and C-peptide secretory responses to glucagon in man: studies on the dose-response relationships. Acta Med Scand 1987;221:185–190
12. Martyn-Nemeth P, SchwarzFarabi S, Mihailescu D, Nemeth J, Quinn L. Fear of hypoglycemia in adults with type 1 diabetes: impact of therapeutic advances and strategies for preventiona review. J Diabetes Complications. 7 September 2015 [Epub ahead of print]. DOI: 10.1016/j.jdiacomp.2015.09.003
13. Gonder-Frederick LA, Vajda KA, Schmidt KM, et al. Examining the Behaviour subscale of the Hypoglycaemia Fear Survey: an international study. Diabet Med 2013;30:603–609
14. Böhme P, Bertin E, Cosson E, Chevalier N; GEODE group. Fear of hypoglycaemia in patients with type 1 diabetes: do patients and diabetologists feel the same way? Diabetes Metab 2013;39:63–70
15. American Diabetes Association. 6. Glycemic Targets. Diabetes Care 2015;38(Suppl. 1):S33–S40
16. Nakamura K, Walker T, Leach J, Bohnett L, Valdes J, Baio A. Incidence of hypoglycemia overtreatment in the SHARE Real Life Use Population. Late-breaking abstract presented at the 75th Annual Meeting of the American Diabetes Association, 5–9 June 2015, at the Boston Convention and Exhibition Center, Boston MA