Pharmacokinetic and Pharmacodynamic Characteristics of Dasiglucagon, a Novel Soluble and Stable Glucagon Analog

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OBJECTIVE

Treatment of severe hypoglycemia outside of the hospital setting is limited to glucagon formulations requiring reconstitution before use, which may lead to erroneous or delayed glucagon administration. We compared the pharmacokinetic (PK) and pharmacodynamic (PD) characteristics and safety and tolerability of different doses of dasiglucagon, a novel soluble glucagon analog, with approved pediatric and full doses of GlucaGen in insulin-induced hypoglycemia in patients with type 1 diabetes.

RESEARCH DESIGN AND METHODS

In this single-center, randomized, double-blind trial, 58 patients with type 1 diabetes received single subcutaneous injections of 0.1, 0.3, 0.6, or 1.0 mg dasiglucagon or 0.5 or 1.0 mg GlucaGen in a state of hypoglycemia (blood glucose target 55 mg/dL) induced by an intravenous insulin infusion.

RESULTS

Dasiglucagon demonstrated a dose-dependent and rapid increase in plasma concentrations, reaching a maximum at ∼35 min with a half-life of ∼0.5 h. Dasiglucagon rapidly increased plasma glucose (PG) by ≥20 mg/dL (9–14 min) to PG ≥70 mg/dL (within 6–10 min), similar to GlucaGen, but with a longer-lasting and greater effect on PG. All patients on both treatments reached these end points within 30 min (predefined success criteria). Both treatments were well tolerated. Nausea was the most frequent adverse event, occurring at a similar rate (44–56%).

CONCLUSIONS

Dasiglucagon was well tolerated and showed an early PD response similar to that of GlucaGen at corresponding doses, suggesting comparable clinical effects of the two glucagon formulations. Dasiglucagon has the potential to become an effective and reliable rescue treatment for severe hypoglycemia in a ready-to-use pen.

Currently available glucagon formulations for rescue treatment of severe hypoglycemia require reconstitution of dry powder in aqueous solution immediately prior to each use. The process of reconstitution and delivery is complex and requires adequate education of families and caregivers, which is not ideal for an emergency drug. Despite training, the reconstitution process could still lead to erroneous or delayed administration of glucagon, at least when used by medical nonprofessionals in stressful emergency situations (1,2).

Native glucagon is a highly unstable peptide prone to spontaneous polymerization and formation of amyloid-like fibrils, resulting in the product becoming unusable within

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1 day of reconstitution (3). Instructions for commercially available glucagon allow only immediate usage after reconstitution (4,5). Stable liquid formulations of a glucagon analog in a ready-to-use injection device would offer major clinical advantages, such as speed and ease of use for rescue treatment in patients experiencing severe hypoglycemia. Furthermore, a simplified glucagon application might reduce the fear of hypoglycemic events, which is sometimes the underlying source of suboptimal glycemic control in patients with diabetes, resulting in an increased risk for complications (6).

Bihormonal artificial pancreas systems might be another promising option for a stable glucagon analog. Maintaining euglycemia in artificial pancreas settings is challenging because of the slow onset and the relative long duration of action of subcutaneous (s.c.) prandial insulins, so a more aggressive insulin titration could easily lead to hypoglycemia. Hypoglycemia could be avoided with an s.c. glucagon formulation in the bihormonal artificial pancreas systems, but currently available glucagon formulations are only stable for 24 h. Undoubtedly, a glucagon analog with longer stability at body temperature would substantially increase the feasibility of bihormonal pump delivery devices (7–14).

Dasiglucagon is a novel stable peptide analog of human glucagon in an aqueous solution at neutral pH, consisting of 29 amino acids with 7 amino acid substitutions relative to native glucagon. (Dasiglucagon is the proposed international nonproprietary name.) These amino acid substitutions result in improved physical and chemical stability compared with currently available glucagon formulations (Supplementary Fig. 1).

Dasiglucagon can be dissolved to at least 20 mg/mL at pH 7.0 in the presence and absence of preservatives (*m*-cresol). Ongoing stability studies show stability at 40°C under shaking conditions with absence of fibrillation in a Thioflavin T fluorescence assay (samples containing 40 μmol/L Thioflavin T, excitation 450 nm, and emission 485 nm) for at least 7 days, while native glucagon in the same assay fibrillated within 3 h, enabling the use of dasiglucagon in a ready-to-use rescue device and potential use in pump delivery devices.

The objectives of the current study were to compare the pharmacokinetic (PK) and pharmacodynamic (PD) properties as well as safety and tolerability of dasiglucagon with those of GlucaGen different dose ranges in insulin-induced hypoglycemia in patients with type 1 diabetes.

RESEARCH DESIGN AND METHODS

Trial Design

This was a single-center (Profil, Neuss, Germany), randomized, double-blind trial in patients with type 1 diabetes. The trial included four groups of patients, with the first eight patients (group 1) randomly allocated (3:1) to either a mini-dose of dasiglucagon (0.1 mg) (6 patients) or a full dose (1.0 mg) of GlucaGen (2 patients). Subsequent patients were randomly allocated to one of the three other treatment groups (groups 2–4, with 16 patients in each group) and received a single dose of 0.3 mg (group 2), 0.6 mg (group 3), or 1.0 mg (group 4) dasiglucagon and, in a cross-over fashion, a single pediatric dose of GlucaGen of 0.5 mg (group 2) or full dose of 1.0 mg (groups 3 and 4) (Supplementary Fig. 2). (Pediatric dose of GlucaGen has been approved for children <25 kg or younger than 6–8 years of age.)

The trial protocol was reviewed and approved by the local health authority (Bundesinstitut für Arzneimittel und Medizinprodukte) and by an independent ethics committee (Ärztekammer Nordrhein). The trial was performed in accordance with the Declaration of Helsinki and the International Conference on Harmonization and Good Clinical Practice. Written informed consent was obtained before initiation of any trial-related activities. The trial was registered at ClinicalTrials.gov (trial identifier: NCT02660008).

Participants

Eligible adults were aged between 18 and 50 years, both inclusive, had been diagnosed with type 1 diabetes per American Diabetes Association criteria, and had been treated with insulin for ≥12 months (15). Participants were required to have a glycosylated hemoglobin (HbA_{1c}) <8.5% (69.4 mmol/mol) and body weight between 60 and 90 kg (both inclusive). Patients were excluded if they had clinically significant concomitant diseases, had clinically significant abnormal values in clinical laboratory screening tests, were habitual smokers, or had any other condition conflicting with trial participation or evaluation of study results.

Procedures

The trial consisted of an informed consent visit obtained at least 1 day prior to screening visit, a screening visit (3–30 days before the first dosing visit), one dosing visit for group 1 and two dosing visits for groups 2–4 separated by 7 ± 3 days washout, and a follow-up visit (21 ± 3 days after the last dosing visit). In group 1, patients received a single s.c. dose of 0.1 mg dasiglucagon (1 mg/mL, liquid formulation in prefilled syringes; Zealand Pharma, Copenhagen, Denmark) or a single s.c. dose of lyophilized glucagon (1 mg for reconstitution, GlucaGen; Novo Nordisk, Copenhagen, Denmark). In groups 2–4, patients were administered three different single s.c. doses for dasiglucagon and two different single doses for GlucaGen. Both treatments were received in a randomized sequence.

For maintenance of double blinding, the appropriate dose/volume was transferred from the prefilled syringes (dasiglucagon) or from the vial filled with freshly reconstituted solution (GlucaGen) into 1-mL disposable syringes with attached 27 G needles (Becton Dickinson) by staff not otherwise involved in trial procedures. Both trial products were administered by s.c. injection into a lifted skinfold of the abdominal wall around the umbilicus. Basal insulin was continued as usual during the dosing day, whereas short-acting insulin was replaced by insulin glulisine (Apidra; Sanofi Deutschland GmbH, Frankfurt, Germany) from 12 h prior to each dosing onwards. Patients using continuous s.c. insulin infusion continued their basal insulin rate during the experiment.

Patients attended the clinical site in the morning after an overnight fast and participated in a manual hypoglycemic clamp procedure that started with a variable infusion of intravenous (i.v.) insulin glulisine (15 units Apidra dissolved in 49 mL saline and 1 mL of the patient's own blood to prevent insulin adherence to tubing material), targeting a blood glucose level of 55 mg/dL ± 10%, corresponding to a plasma glucose (PG) level of 62 mg/dL ± 10% (3.4 mmol/L) prior to dose administration. If PG levels decreased <56 mg/dL (3.1 mmol/L) prior to dosing, i.v. glucose was infused and the run-in period extended until the target range was established for at least 10 min before dose administration. After dose administration, subjects with hypoglycemic PG concentrations <56 mg/dL were immediately treated with i.v. glucose until a PG value of >70 mg/dL (3.9 mmol/L) was established.

The PK and PD effects of the study drugs were assessed over 360 min postdosing with plasma samples for the determination of dasiglucagon/glucagon and glucose being taken predose and then every 5 min from dosing until 40 min postdose followed by at 50, 60, 75, 100, 150, 200, 260, 300, and 360 min postdose.

Assessments

Determination of dasiglucagon in human plasma was done by use of a validated analytical method using off-line and on-line solid phase extraction and liquid chromatography with tandem mass spectrometric detection, which had a lower limit of quantification (LLOQ) of 10.0 pmol/L. Glucagon was determined using a validated radioimmunoassay (Euro Diagnostica AB, Malmö, Sweden) performed on a 1470 Wizard Automatic Gamma Counter (PerkinElmer) with an LLOQ of 4.7 pmol/L.

PG was determined with a validated colorimetric assay (hexokinase/glucose-6-phosphate dehydrogenase test kit for glucose; Roche Diagnostics, Mannheim, Germany), performed on a MODULAR EVO/P-Module (Roche Diagnostics) with an LLOQ of 2.9 mg/dL (0.16 mmol/L). During insulin-induced hypoglycemia, blood glucose levels were monitored closely on-site using a laboratory glucose analyzer (Super GL glucose analyzer; Dr. Müller Gerätebau GmbH, Freital, Germany). A validated ELISA method was used for the detection of IgG- and IgM-ZP4207/glucagon antibodies in human serum (YBS, York, U.K.). The sensitivity of the assays was 13.6 and 11.8 ng/mL for the anti-ZP4207 and anti-glucagon methods, respectively.

Safety assessments included adverse events, hypoglycemic episodes (defined as PG $<$56 mg/dL), local tolerability at the injection site (assessed predose and 30, 120, and 360 min postdose), laboratory safety parameters, vital signs and electrocardiogram (assessed predose and 30 and 360 min postdose), physical examination, and anti-drug antibody measurements (samples taken prior to start of each insulin-induced hypoglycemic procedure and at follow-up).

End Points

For evaluation of early PK and PD effects, partial areas under the curve (AUCs) of plasma dasiglucagon and glucagon concentrations ($AUC_{0–30min}$) and PG concentrations (area under the effect curve $[AUE]_{0–30min}$) were analyzed as well as PG excursions 30 min postdose (CE_{30min}). For correction for endogenous glucagon concentrations, glucagon concentrations were baseline adjusted (BL). Other PK measures comprised the total ($AUC_{0–360min\ and}$ $AUC_{0–inf}$) ($AUC_{0–inf}$ is defined as area under the plasma dasiglucagon concentration vs. time curve from 0 to infinity, whereas it is calculated from baseline-adjusted and truncated for GlucaGen profiles with a cutoff at 2.5 h) and maximum (C_{max}) plasma dasiglucagon and $glucagon_{[BL]}$ concentrations, time to maximum (t_{max}), terminal elimination rate constant (λ_z), terminal plasma elimination half-life ($t_{1/2}$), total body clearance, volume of distribution, and mean residence time.

PD effects were analyzed with use of total PG area under effect curves ($AUE_{0–last}$), maximum PG concentration effect above baseline (CE), and t_{max} excursion. Key secondary end points evaluated success criteria for glucose rescue such as the time to achieve a PG $\geq$70 mg/dL (3.9 mmol/L) and the time to an increase of $\geq$20 mg/dL (1.1 mmol/L), as well as the proportion of patients reaching these goals within 30 min after dosing.

Statistical Analyses

No formal sample size calculation was performed for this study, which was aimed at providing a first insight on the PK/PD properties of dasiglucagon in a new, optimized formulation.

All statistical analyses were performed using SAS System for Windows, version 9.4 (SAS Institute, Cary, NC). PK parameters were calculated with WinNonlin, version 6.4 (Pharsight Corporation, Mountain View, CA).

The primary PK and PD end points ($AUC_{0–30min}$, $AUC_{0–360min,}$ $C_{max,}$ and t_{max} and $AUE_{0–30min}$, AUE, CE_{30min}, CE, and t_{max}) for group 1 were analyzed descriptively. For groups 3 and 4, data for 1.0 mg GlucaGen were pooled across groups for summary statistics. PK/PD end points were log transformed and compared between treatments with a linear model ANOVA with treatment, period, sequence, and patient within sequence as fixed effects. Least squares (LS)-mean values of dasiglucagon and GlucaGen as well as the differences of the means and 90% CIs were estimated and backtransformed (exponentially transformed) in order to find the estimated ratios and CIs of responses. As t_{max} was dependent on sampling intervals, it showed neither normal nor log-normal distribution and was therefore analyzed using the Wilcoxon signed rank test for paired observations within each group. In addition, point estimates for median differences between treatments and corresponding 90% CIs were determined using Hodges and Lehmann procedure.

As λ_z and related parameters ($t_{1/2}$, total body clearance, volume of distribution, and mean residence time) showed markedly skewed distributions with $glucagon_{[BL]}$ concentrations, these end points were calculated from baseline-adjusted glucagon profiles truncated at 2.5 h in a post hoc analysis. No statistical comparisons were done with these parameters.

Dose proportionality of C_{max} and AUCs of dasiglucagon were analyzed using a regression analysis with the log-transformed end points as response and log dose as fixed effect. If 1 was included in the 95% CIs of the estimated slope of the regression line, dose proportionality was assumed.

RESULTS

Subject Disposition and Characteristics

A total of 76 patients were screened and 58 subjects were randomized and treated with trial products. Two patients withdrew consent after the first dosing visit (from group 2, GlucaGen 0.5 mg, and group 3, dasiglucagon 0.6 mg). Fifty-six patients completed the trial. One patient (group 1) was excluded from both PK and PD analyses owing to a postdose hypoglycemic event treated with i.v. glucose infusion. Therefore, 57 exposed patients were included in the PK and PD analysis (full analysis set) and 58 exposed patients were included in the safety analysis set. One PK and PD data set from one visit (group 4) was excluded from analysis, as PD measurements were missing between 10 and 50 min. All groups were comparable with regard to age, weight, height, and BMI (Supplementary Table 1).

PK Results

The PK profile of dasiglucagon was characterized by a dose-dependent and rapid increase in plasma levels. Dasiglucagon reached maximum plasma concentrations later than GlucaGen (35 vs. 20 min, respectively, based on medians over all dasiglucagon and all GlucaGen doses). The half-life of dasiglucagon was ~0.5 h (Table 1 and Fig. 1). Total exposure

Table 1—PK data

	Dasiglucagon dose				GlucaGen dose	
	0.1 mg	0.3 mg	0.6 mg	1.0 mg	0.5 mg	1.0 mg
N	5	16	16	16	17	33
$AUC_{0–30min}$ (pmol * h/L)	99.4 (32.0)	302 (78.9)	444 (163)	884 (307)	375 (104)	600 (180)
$AUC_{0–360min}$ (pmol * h/L)	451 (123)	1,360 (166)	2,630 (368)	4,800 (697)		
$AUC_{0–360minBL}$ (pmol * h/L)					939 (177)	1,660 (315)
$AUC_{0–inf}$ (pmol * h/L)	451 (123)	1,360 (166)	2,640 (365)	4,810 (696)		
$AUC_{0–inf,BL,Trunc}$ (pmol * h/L)					895 (169)	1,630 (311)
C_{max} (pmol/L)	334 (113)	976 (208)	1,570 (445)	2,800 (767)		
$C_{max,BL}$ (pmol/L)					1,100 (307)	1,720 (526)
t_{max} (h)	0.50 (0.5–0.6)	0.63 (0.3–0.8)	0.58 (0.5–1.7)	0.63 (0.3–0.8)	0.25 (0.2–0.6)	0.37(0.2–0.8)
$t_{1/2}$ (h)	0.43 (0.09)	0.43 (0.07)	0.49 (0.14)	0.54 (0.17)		
$t_{1/2,BL,Trunc}$ (h)					0.37 (0.07)	0.42 (0.13)

Data are mean ± SD unless otherwise indicated; t_{max} shows median (range). GlucaGen $AUC_{0–30min}$, $AUC_{0–360min}$, and C_{max} are shown calculated from baseline-adjusted data, whereas $AUC_{0–inf}$ and $t_{1/2}$ are displayed as calculated from baseline-adjusted and truncated ($_{Trunc}$) GlucaGen profiles with a cutoff at 2.5 h.

($AUC_{0–inf}$) at compared dose levels was higher for dasiglucagon compared with GlucaGen, while C_{max} values were comparable. Treatment ratios for $AUC_{0–inf,BL}$ for 0.3 mg and 0.6 mg dasiglucagon versus 0.5 and 1.0 mg GlucaGen administration were 1.46 (90% CI 1.213; 1.752) and 1.59 (1.299; 1.950), respectively, whereas treatment ratios for $C_{max,BL}$ for 0.3 and 0.6 mg dasiglucagon versus 0.5 and 1.0 mg GlucaGen administration were 0.91 (90% CI 0.817; 1.007) and 1.03 (0.885; 1.199), respectively. Dasiglucagon met the dose-proportionality criteria for $AUC_{0–30}$, $AUC_{0–360}$, and $AUC_{0–inf}$, so dose proportionality can be assumed even though for C_{max} the upper limit of the 95% CI was slightly <1 (Supplementary Table 2).

PD Results

Despite later PK t_{max}, the early PD response ($AUE_{0–30min}$ and CE_{30min}) of 0.3 mg dasiglucagon was comparable with that of 0.5 mg GlucaGen, as were the early PD responses of 0.6 mg dasiglucagon and 1.0 mg GlucaGen. Treatment ratios for dasiglucagon versus GlucaGen are shown for PD end points in Table 3.

The overall effect in terms of AUE was higher with 0.3 mg dasiglucagon versus 0.5 mg GlucaGen ($P < 0.0001$) and for both 0.6 mg ($P = 0.0043$) and 1.0 mg ($P < 0.0001$) dasiglucagon versus 1.0 mg GlucaGen. Time to maximum PG increased with increasing dose levels, as observed with both treatments. While median t_{max} values trended to be higher for dasiglucagon versus GlucaGen (Table 2 and Fig. 2), there were no differences in the median time to achieve PG ≥70 mg/dL: 6 min for dasiglucagon doses of ≥0.3 mg and 6–7 min for both GlucaGen doses (10 min for 0.1 mg dasiglucagon). All patients achieved PG ≥70 mg/dL within 30 min after dosing across all treatments and doses. Median time to reach a PG increase of 20 mg/dL was 9–10 min for dasiglucagon doses of ≥0.3 mg, similar to the 10 min observed with both GlucaGen doses (14 min for 0.1 mg dasiglucagon) (Table 2).

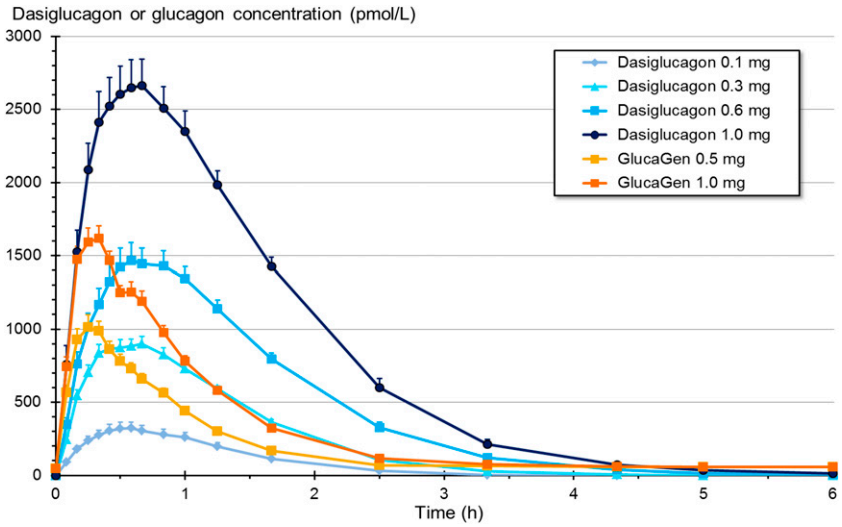

Figure 1—PK profiles. Mean plasma concentration profiles and SEM after single s.c. doses of dasiglucagon and GlucaGen.

Safety

All doses of dasiglucagon were safe and well tolerated. Gastrointestinal side effects occurred with a similar frequency after dasiglucagon and GlucaGen treatments, and the most frequent treatment-emergent adverse event (TEAE) was nausea, accounting for 53 of the total of 143 TEAEs observed. A considerable proportion of these patients also experienced vomiting (22) (in most cases 2–3 h postdose). The second most frequent TEAE was headache, accounting for 30 of 143 TEAEs in 27 patients. While there were numerically more headaches with dasiglucagon (40 vs. 15%), there were no indications of dose dependency of adverse events for either dasiglucagon or GlucaGen (Supplementary Table 3). Headache occurred most often (50%) in the dasiglucagon 0.1 mg group, whereas the incidence in the 1-mg dose groups was relatively small (dasiglucagon 31% and GlucaGen 21%). Postdose hypoglycemic events occurred infrequently with either treatment (5 events in 4 patients

Table 2—PD data

	Dasiglucagon dose				GlucaGen dose	
	0.1 mg (*N* = 5)	0.3 mg (*N* = 16)	0.6 mg (*N* =17)	1.0 mg (*N* =16)	0.5 mg (*N* = 17)	1.0 mg (*N* = 33)
$AUE_{0–30min}$ (mg * h/dL)	12.9 (5.21)	20.9 (6.13)	21.1 (6.10)	24.1 (5.18)	22.1 (5.48)	21.9 (5.74)
AUE (mg * h/dL)	344 (149)	666 (247)	788 (165)	895 (213)	462 (273)	566 (232)
CE_{30min} (mg/dL)	66.1 (23.8)	93.4 (23.7)	98.2 (25.0)	100 (20.3)	93.5 (21.4)	96.5 (21.9)
CE (mg/dL)	334 (113)	174 (44.6)	190 (32.2)	209 (40.2)	142 (42.6)	166 (42.5)
t_{max} (h)	1.25 (0.8–1.7)	1.67 (1.0–2.5)	1.67 (1.7–4.3)	2.50 (1.7–2.5)	1.0 (0.7–5.0)	1.25 (0.8–6.1)
	Dasiglucagon dose				GlucaGen	
	0.1 mg (*N* = 5)	0.3 mg (*N* = 16)	0.6 mg (*N* = 17)	1.0 mg *N* = 16)	0.5 mg (*N* = 15)	1.0 mg (*N* = 31)
Time to reach PG levels ≥70 mg/dL (min)	10.0 (2.0–17.0)	6.0 (0–13.0)	6.0 (0–9.0)	6.0 (0–9.0)	6.0 (0–9.0)	7.0 (0–10.0)
Time to increase in PG levels ≥20 mg/dL (min)	14.0 (11.0–27.0)	10.0 (7.0–20.0)	9.0 (6.0–16.0)	9.0 (7.0–15.0)	10.0 (6.0–13.0)	10.0 (5.0–15.0)

Data are mean ± SD or median (minimum–maximum), except for t_{max}, which shows median (range).

with dasiglucagon and 9 events in 8 patients with GlucaGen). Four events occurred within 2 h postdosing (2 events each with 0.1 mg dasiglucagon and 1.0 mg GlucaGen). These events might be mostly attributed to a protracted blood glucose decline after the induction of hypoglycemia with i.v. insulin. Seven additional events were observed between 4 and 6 h postdosing with GlucaGen, whereas the other three hypoglycemic events with dasiglucagon occurred >100 h postdosing.

No serious adverse event occurred, and all adverse events were either of mild (113 events) or moderate (30 events) intensity.

Local tolerability findings were rare (7 findings in 5 patients with dasiglucagon and 5 findings in 4 patients with GlucaGen) and mild, and all disappeared within 30 min postdosing. No anti-drug antibodies were detected.

All doses of dasiglucagon were consequently considered safe and well tolerated.

CONCLUSIONS

In this study, dasiglucagon at all dose levels consistently and quickly reestablished euglycemia after insulin-induced hypoglycemia in adults with type 1 diabetes. Most importantly, the time to reach a PG increase of 20 mg/dL and time to reach PG ≥70 mg/dL were similar for dasiglucagon and GlucaGen across the tested doses, as were $AUE_{0–30min}$ and CE_{30min} (Table 2).

This indicates that dasiglucagon and GlucaGen have similar early PD properties to treat insulin-induced hypoglycemia. It is worth pointing out that the overall glycemic response over the 6-h observation period, as represented by AUE and CE (Table 2), was higher with dasiglucagon for all three comparisons with GlucaGen, mostly due to higher PG concentrations observed subsequent to the 1-h time point after dosing (Table 2). The higher total and longer-lasting glucose response with dasiglucagon suggests not only a higher biopotency of dasiglucagon versus GlucaGen but also that the longer-lasting effect of dasiglucagon could potentially reduce the risk of recurrent hypoglycemia after administration as rescue medication. This speculative view may be supported by the observation that no recurrent hypoglycemic events occurred in the time period of 4–6 h after dasiglucagon in contrast to seven events observed after GlucaGen administration. However, clinical investigations are needed to show whether these PD differences between dasiglucagon and GlucaGen are of any clinical relevance, in particular in settings that are more typical for daily clinical life than the i.v. induction of hypoglycemia with insulin, e.g., after strenuous physical activity or after s.c. injection of a high insulin dose.

Stability of dasiglucagon has been demonstrated in an extensive evaluation program. Publication of these results is underway. In general, substitution of 7 out of 29 amino acids could potentially result in changed specificity in biological action and in immunogenic reactions. However, anti-drug antibodies related to dasiglucagon treatment have not been observed in phase 1 or 2 clinical trials, and there were not any indications of neutralizing antibodies in the preclinical or clinical development program. Further long-term data are needed to further assess immunogenic reactions. The benefits of a stable, easy-to-use glucagon formulation are undisputed, and other approaches are in development to achieve this goal (10–14). One of the first developments reported by Chabenne and colleagues uses a pH adjustment and backbone stabilization to achieve aqueous solubility and stability (12,13). Another development is an intranasal glucagon releasing 3 mg glucagon powder into the nose after pushing of a small plunger on the bottom of the device. While reconstitution is not required before use, the formulation contains phospholipid dodecylphosphocholine as absorption enhancer and β-cyclodextrine as bulking agent. The nasal administration was reported to have a higher rate of head/facial discomfort (25% vs. 9% with intramuscularly administered (i.m.) GlucaGen (16,17). Published data on this intranasal approach are still scarce, but a noninferiority

Table 3—Treatment comparisons

Treatment comparisons	*N*	Treatment ratios	90% CI
$AUE_{0–30min}$			
Dasiglucagon 0.3 mg vs. GlucaGen 0.5 mg	16/17	0.934	(0.8042; 1.0858)
Dasiglucagon 0.6 mg vs. GlucaGen 1.0 mg	17/16	0.965	(0.8613; 1.0822)
CE_{30min}			
Dasiglucagon 0.3 mg vs. GlucaGen 0.5 mg	16/17	1.01	(0.8933; 1.1353)
Dasiglucagon 0.6 mg vs. GlucaGen 1.0 mg	17/16	1.01	(0.9255; 1.1020)

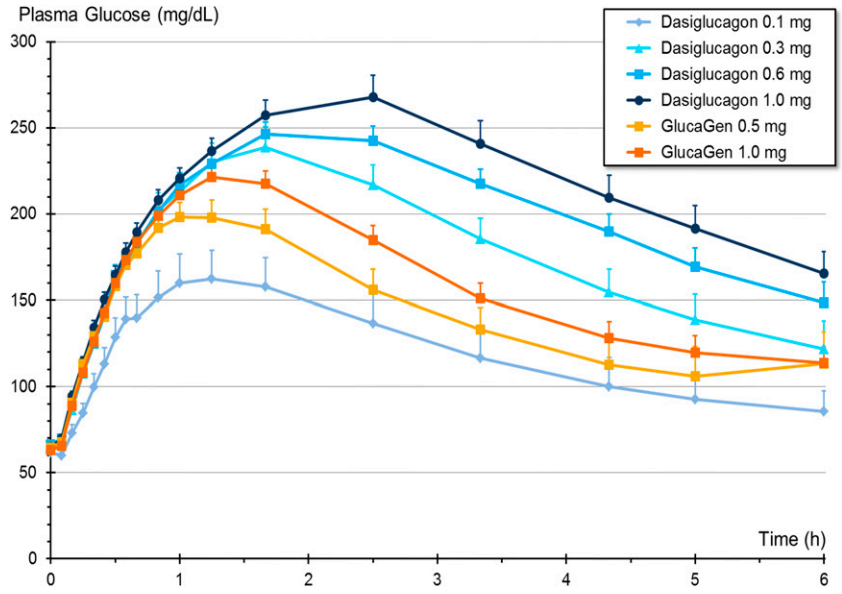

Figure 2—PD profiles. Mean PG concentration profiles and SEM after single s.c. doses of dasiglucagon and GlucaGen.

trial indicated a slightly (~3–5 min) slower rise in PG with nasal glucagon compared with i.m. GlucaGen (18). Our study used s.c. GlucaGen and did not include an i.m. comparator. However, the mean time to a PG increase of 20 mg/dL in our study (9–10 min with dasiglucagon) compares favorably with the reported data for both nasal glucagon (16 min) and i.m. GlucaGen (13 min). Nevertheless, across-study comparisons are always difficult, and head-to-head comparisons would be needed to assess a potential faster rise in PG concentration with dasiglucagon versus intranasal glucagon application. The main difficulty with currently marketed glucagon products is the need for reconstitution and handling issues, in particular for untrained medical nonprofessionals (2), again highlighting the importance of an easy-to-use glucagon formulation. Indeed, the rate of successful glucagon rescue injections was substantially higher in both experienced and training-naïve (to glucagon injections) caregivers in a study performed with the use of an auto-injector (G-Pen) compared with using the currently available glucagon kits (19). The auto-injector solution was preferred by all participants in this human factors study.

Furthermore, a stable, liquid glucagon formulation would also enable the development of bihormonal artificial pancreas systems. Safety and efficacy of such systems have been demonstrated in adults, adolescents, and children (20,21). In comparison with glucagon used as hypoglycemia rescue therapy, glucagon doses in the artificial pancreas setting are much smaller and have been reported to be effective in the management of mild or expected forthcoming hypoglycemia in a range of 20–150 μg in children and adolescents with type 1 diabetes (22,23). Likewise, low-dose glucagon boluses of 100–300 μg were demonstrated to effectively increase PG levels of 54 mg/dL after insulin overdosing in pump users (24). In line with these findings, another study demonstrated clinically relevant rises in blood glucose levels with glucagon doses of 0.11–1.00 mg administered in euglycemic or hypoglycemic baseline conditions (25). These data and the clear need for lower glucagon doses in an artificial pancreas setting made us investigate low doses of dasiglucagon and a pediatric dose of GlucaGen in this study. Our results confirm that even these low doses of dasiglucagon (100 or 300 μg) efficiently raise PG concentrations from hypoglycemia, making it potentially usable in a bihormonal artificial pancreas setting and also a viable treatment option for mild hypoglycemia.

Strengths of the current trial comprise the inclusion of patients with type 1 diabetes, who are the most sensitive and relevant target population for hypoglycemia rescue therapy. Furthermore, a study in people with type 1 diabetes avoids the confounding influence of endogenous insulin and minimizes the influence of counterregulatory hormonal responses to insulin-induced hypoglycemia that would have been more pronounced in healthy subjects who are rarely or never exposed to hypoglycemia (26–28). Hypoglycemia was induced with an i.v. insulin infusion under tightly controlled conditions, which is a well-established and ethical way to induce hypoglycemia in clinical trials. A controlled setting is also needed to establish comparable baseline conditions across treatments—a prerequisite for valid comparisons in a study like ours with a limited sample size. This is in particular important for prevailing insulin levels, as high insulin concentrations have been shown to partially prevent glucagon from increasing endogenous glucose production and thereby affecting glucagon efficacy (29). We therefore used a variable i.v. insulin infusion titrated to inducing (but not running below) the desired hypoglycemic PG target concentration, and we succeeded in establishing similar insulin concentrations across treatments in our study. As advantageous as this design was for creating similar baseline conditions, this setup is different from the usual clinical causes of severe hypoglycemia, which most often comprise of insulin dosing errors, exercise, and alcohol consumption (30). A constraint of the current study is that the use of different analytic methods for GlucaGen (radioimmunoassay) and dasiglucagon (liquid chromatography–mass spectrometry) might limit a direct comparison of PK values. However, the observed PK differences were consistent with the observed PD effects, and the use of a specific assay for dasiglucagon could also be regarded as an advantage, as any interference with endogenous glucagon could be avoided.

In addition to the controlled, but artificial, experimental design, the small sample size is a limitation of this study. While larger clinical trials are needed, the chosen sample size in combination with the crossover design (thereby excluding interindividual confounders [29]) was sufficient to demonstrate small differences in, for example, time to maximum concentrations and total exposure. The clinical relevance of these differences needs to be explored in future trials.

In conclusion, our study shows that the novel glucagon analog dasiglucagon in a stable liquid formulation quickly and effectively reestablished euglycemia after insulin-induced hypoglycemia at the tested doses from 0.1 to 1.0 mg in adults with

type 1 diabetes. In comparison with GlucaGen, dasiglucagon needed similar time to increase PG by 20 mg/dL or to reach PG ≥70 mg/dL, and the overall rise in PG levels was slightly longer lasting and higher. With these characteristics, dasiglucagon is a promising candidate for hypoglycemia rescue therapy. Further clinical trials confirming this potential are underway.

Duality of Interest. This study was funded by Zealand Pharma, A/S, Denmark. B.V.B., U.M., and F.M. are employees of Zealand Pharma A/S, and D.V.M. was an employee of Zealand Pharma A/S during trial conduct. T.H. is a shareholder of Profil, which received research funds from Adocia, Biocon, Dance Pharmaceuticals, Eli Lilly, Johnson&Johnson, Julphar, Medimmune, Mylan, Nordic Bioscience, Novo Nordisk, Poxel, Roche Diagnostics, Saniona, Sanofi, Senseonics, SkyePharma, and Zealand Pharma. In addition, T.H. is a member of advisory panels for Novo Nordisk and received speaker honoraria and travel grants from Eli Lilly, Novo Nordisk, and Sanofi. No other potential conflicts of interest relevant to this article were reported.

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