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Original Research

Dasiglucagon Effects on QTc in Healthy Volunteers: A Randomized, Placebo-Controlled, Dose-Escalation, Double-Blind Study

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ABSTRACT

Background: Dasiglucagon is a novel glucagon analog that is stable in aqueous formulation and approved for use in severe hypoglycemia. Concentration QTc analyses are critical for assessing risk of drug-induced QTc prolongation and potential for fatal cardiac arrhythmias such as torsades de pointes.
Objective: The aim of this study was to determine whether dasiglucagon treatment resulted in any clinically relevant effect on cardiac repolarization in healthy volunteers.
Methods: This double-blind, placebo-controlled, dose-escalation Phase I trial was conducted at a single center in Germany between November 2018 and June 2019. Sixty healthy volunteers aged 18 to 45 years were randomized within dose cohorts to receive intravenous dasiglucagon, intravenous placebo, or subcutaneous dasiglucagon. In the intravenous administration cohorts, doses ranged from 0.03 mg to 1.5 mg. The subcutaneous administration cohort received the approved 0.6 mg dose. In the intravenous administration cohorts, serial electrocardiograms were extracted from continuous Holter monitors at prespecified time points beginning the day before dosing and through 24 hours postdose. Heart rate, PR interval, and QRS duration were evaluated. Concentration-QT analyses corrected by Fridericia's formula (QTcF) were performed using both a linear mixed-effects and a maximum estimated effect (E_{max}) model.
Results: At the doses studied, dasiglucagon did not have any clinically relevant effect on heart rate, PR interval, or QRS duration. A minor prolongation of the QTcF interval was observed without any clear dose or concentration dependency. Both the linear and E_{max} models predicted mean and 90% CIs of placebo-corrected change in QTcF remained below 10 ms (the threshold of regulatory concern), although the linear model did not fit the data well at low dasiglucagon plasma concentrations. In the E_{max} model, the E_{max} of dasiglucagon was 3.6 ms (90% CI, 1.23–5.95 ms), and the amount to produce half the effect of E_{max}) was 426.0 pmol/L (90% CI, −48.8 to 900.71 pmol/L). The treatment effect-specific intercept was −0.44 ms (90% CI, −2.37 to 1.49 ms). The most frequently observed treatment-emergent adverse events reported in the trial were gastrointestinal disorders such as nausea and vomiting.
Conclusions: Dasiglucagon does not cause clinically relevant QTc prolongation in concentrations up to ≈30,000 pmol/L, a level 5-fold higher than the highest observed plasma concentrations in clinical trials investigating use of the approved 0.6 mg SC dose. ClinicalTrials.gov Identifier: NCT03735225; EudraCT identifier: 2018-002025-32. (*Curr Ther Res Clin Exp*. 2022; 83:XXX–XXX)

Introduction

The classic physiologic role of glucagon involves stimulating hepatic glucose production to maintain euglycemia in concert with insulin.[1] Both glucagon and insulin play central, essential, and interdependent roles in the regulation of plasma glucose, along with extended effects on fat and protein metabolism and energy balance. Glucagon acts to counterregulate the blood glucose-lowering effects of insulin by stimulating glycogenolysis and gluconeogenesis in the liver.[1] As a result, pharmacologic preparations of glucagon are often used to treat severe hypoglycemia induced by glucose-lowering therapies. However, the physiologic effects of glucagon are not constrained to a strictly glucoregulatory role.

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Preclinical studies and noncontrolled trials suggest that intravenous administration of supraphysiologic concentrations of glucagon can increase heart rate, blood pressure, and heart contractility.[2–4] These positive chronotropic and inotropic effects form the basis for clinical use of glucagon to treat overdoses induced by cardioinhibitory drugs such as beta blockers and calcium channel blockers.[4] Elevated levels of glucagon may also be associated with chronic tachycardia, which, in addition to recurrent hypoglycemia, is a risk factor for cardiovascular morbidity.[5–7] Therefore, it is important to understand any potential chronotropic effects of novel pharmacologic agents that regulate glucose metabolism and blood glucose levels.

Dasiglucagon is a novel glucagon analog that is stable in aqueous solutions and is approved as a rescue treatment for severe hypoglycemia.[8,9] Median time to plasma glucose recovery following insulin-induced hypoglycemia with dasiglucagon is comparable to that achieved with reconstituted lyophilized glucagon.[9] Given that dasiglucagon retains selectivity and potency at the glucagon receptor, it is important to understand any potential chronotropic consequences of dasiglucagon treatment.

In clinical development programs, it is critical to determine via nonclinical and clinical studies whether a drug may exert harmful effects on cardiac repolarization (eg, due to drug-induced ion channel blockade) potentially resulting in fatal arrythmias such as torsades de pointes. Nonclinical studies with dasiglucagon have not raised any cardiac safety concerns: dasiglucagon had no effect on 8 different human cardiac ion channels (including human ether-a-go-go related gene [hERG]) in vitro and showed no potential for QTc prolongation in a cardiovascular telemetry study in dogs at plasma concentrations up to ≈200 times higher than what is observed in human beings at a rescue dose of 0.6 mg.

For clinical studies, International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines recommend a concentration QTc modeling approach that employs a prespecified linear mixed-effects model.[10] Notably, physiologic changes over a normal range of blood glucose levels in relation to activities such as meal ingestion can give rise to minor fluctuations in the corrected QT interval.[11–15] This phenomenon is likely a result of a complex interplay among glucose, insulin, C-peptide, and the activation of the autonomic nervous system.[11,14,15] In the present study, we sought to determine whether there is any clinically relevant effect of dasiglucagon on cardiac repolarization in healthy volunteers. This article presents an interpretation of clinical manifestations and ECG findings based on concentration QT analyses corrected by Fridericia's formula (QTcF) to assess the potential effect of dasiglucagon on the QT interval.

Methods

Study design

In this randomized, double-blind, placebo-controlled, dose-escalation Phase I trial (NCT 03735225; EudraCT 2018-002025-32), healthy adult volunteers received either intravenously administered dasiglucagon, intravenously administered placebo, or subcutaneously administered dasiglucagon. The primary objective of the trial was to evaluate the safety and tolerability of ascending doses (0.03 mg to 1.5 mg) of dasiglucagon via intravenous administration. An open-label, noncontrolled subcutaneous administration cohort was included to assess the bioavailability of dasiglucagon following subcutaneous compared with intravenous administration. In terms of ECG findings, the primary objective was to evaluate any potential effect by dasiglucagon on cardiac repolarization (ie, QTc interval), whereas the secondary ECG objective included assessing the effect of dasiglucagon on other quantitative ECG parameters, such as heart rate (HR), PR interval, and QRS duration.

The trial was conducted at a single site in Germany between November 2018 and June 2019. Within the intravenous administration cohorts, the administered doses of dasiglucagon (4 mg/mL) were 0.03 mg, 0.1 mg, 0.3 mg, 0.6 mg, and 1.5 mg. Six additional study participants were included in the 0.6 mg dose cohort due to possible improper intravenous dose administration. Participants were assigned a random number on the day of dosing after eligibility was confirmed according to the dosing day exclusion criteria. Dilution of dasiglucagon was performed with vehicle by unblinded personnel of a contracted vendor, who prepared a syringe with the dose determined by randomization for each participant on the day of dosing. Treatments were formulated in identical aqueous solutions, and all doses were thereafter administered by blinded trial personnel. Dasiglucagon was provided in a prefilled syringe for subcutaneous administration and in vials for intravenous administration.

The trial protocol and other information provided to study volunteers were approved by an independent ethics committee and the responsible competent authority (Bundesinstitut für Arzneimittel und Medizinprodukte). This study was conducted in accordance with ethical principles according to the Declaration of Helsinki and ICH guidelines with written informed consent obtained from participants before trial enrollment. Participants were informed that they were free to withdraw from the trial at any time at their own discretion without necessarily giving reasons.

Participants

Participants included healthy male and female adult volunteers aged 18 to 45 years weighing between 60 and 90 kg. Volunteers were eligible to participate if systolic blood pressure was ≥90 mm Hg and ≤140 mm Hg, diastolic blood pressure was ≤90 mm Hg, and pulse rate was ≥50 and ≤90 bpm after at least 5 minutes' rest in a supine position. Furthermore, if 12-lead ECG with QTcF was <450 ms, PR interval was <220 ms, and QRS duration was <110 ms, volunteers were eligible to participate in the study.

Intravenous dosing

Healthy volunteers were screened between 21 and 3 days before dosing with the trial drug. Eligible participants attended a single dosing visit involving an overnight stay and Holter ECG monitoring 24 hours pre- and postdose. All ECG data were collected using a Global Instrumentation (Manlius, New York) M12R ECG continuous 12-lead digital recorder.[16]

The intravenous route of administration was chosen to increase the time between ECG extractions and nausea and vomiting expected to occur 2 to 3 hours after administration of dasiglucagon, a time frame that did not coincide with the expected t_{max} of dasiglucagon. Participants in the intravenous administration cohort fasted for at least 8 hours before drug administration and were given the option of a 25 g granola bar snack (112 kcal: fat = 4.3 g, carbohydrates = 16.4 g, sugar = 6.1 g, and protein = 1.6 g) at 1.5 hours postinjection and standard meals at 4 hours, 8 hours, and 12 hours postinjection. The time points at which study volunteers were permitted to eat did not coincide with ECG time points. Doses of dasiglucagon were administered in a sentinel manner and ranged from 0.03 mg to 1.5 mg. After intravenous administration, blood was collected for pharmacokinetic (PK) measurements at predefined time points over a period of 4 hours. Blood sampling for PK measurements was paired with ECG data extractions from continuous Holter ECG monitoring. Blood samples were collected predose and at 5, 10, 15, 20, 25, 35, 50, and 60 minutes after trial drug administration for plasma glucose determination.

Subcutaneous dosing

In the subcutaneous administration cohort, 0.6 mg IV dasiglucagon was administered in the abdomen via a prefilled syringe. Blood was subsequently collected for PK measurements at predefined time points over a period of 5 hours.

Continuous 12-lead ECG assessment

A Holter device was applied to all participants on the day before dosing, and participants were resting in a supine position for at least 15 minutes before each ECG extraction. Replicate 12-lead ECGs were extracted at a central ECG laboratory (ERT, Rochester, New York) at 45, 30, and 15 minutes predose and paired with PK samples at 5, 10, 15, 20, 25, 40, 60, 80, 100, 120, 140, 180, and 240 minutes and 24 hours postdose. ECGs were performed at corresponding time points on day −1 but were not relevant due to lack of an HR effect (mean placebo-corrected change from baseline [ΔΔ] HR was <10 bpm). This informed the decision to correct the QT interval using Fridericia's formula rather than using other correction methods such as optimized HR-corrected QT interval or individualized HR-corrected QT interval. The TQT Plus method was used to identify periods of stable HR in the 5-minute extraction window to ultimately generate up to 10 nonoverlapping 10-second ECG replicates within each nominal prespecified time point. TQT Plus is an advanced computer-assisted and statistical process utilized to extract ECGs from continuous 24-hour recordings.[17] The primary analysis lead was lead II, followed sequentially by lead V2 and lead V5 if the primary lead was nonanalyzable. Categorical T-wave morphology analysis and measurement of PR interval and QRS duration were analyzed by a cardiologist in 3 of the 10 ECG replicates at each time point.

Other safety assessments

In addition to 12-lead ECG, safety was monitored throughout the study by means of adverse event (AE) recording, vital sign measurements, clinical laboratory evaluations, physical examinations, and monitoring for injection site reactions. Antibody formation was assessed at the dosing visit (before dosing) and at up (28 days after dose) using a multitiered assessment approach.[18] An antidasiglucagon antibody-positive participant was defined as a someone with at least 1 treatment-induced or treatment-boosted (titer increase above 5-fold) antidasiglucagon antibody-positive sample at any time during the treatment or follow-up observation period.

Statistical methods

In general, data for placebo were pooled across cohorts, whereas data for dasiglucagon were analyzed by dose for intravenous administration cohorts or by route of administration (subcutaneous vs intravenous) for the 0.6 mg dose cohort specifically. All planned statistical analyses for PK and pharmacodynamic (PD) end points were exploratory, and a confirmatory statistical analysis was not intended. As an exploratory analysis, dose proportionality of C_{max} and AUCs of dasiglucagon administered intravenously were analyzed using a power model by means of a regression analysis with the log-transformed end point as a response and log-dose as a fixed effect. The estimated slope of the regression was estimated. Dose proportionality was supported if the 90% CI was within the critical interval for slope. Absolute bioavailability was estimated using a linear model (SAS software [SAS Institute, Cary, North Carolina], procedure = Mixed) for the total exposure (AUC) of 0.6 mg SC formulation in comparison to the 0.6 mg IV formulation of dasiglucagon. No inference statistical analyses were performed on PD end points.

HR correction of the QT interval was calculated based on Fridericia's formula ($QTcF = QT/(RR(sec))^{1/3}$).[19] By-time point analyses were based on a linear mixed-effects model with change from baseline (Δ) QTcF as the dependent variable; time point, treatment, and time-by-treatment interaction as fixed effects; and baseline QTcF as a covariate. For HR, PR interval, and QRS duration, a similar model was used as described for ΔQTcF and was based on change from baseline postdosing. By-time point analyses of HR and QTcF were performed, including data from participants with typical intravenous PK profiles to allow for a proper evaluation of a potential dose-dependent effect. The relationship between plasma concentrations of dasiglucagon and ΔQTcF was also quantified using a linear mixed-effects modeling approach evaluating all available intravenous administration data. The model included ΔQTcF as the dependent variable, time-matched concentrations of dasiglucagon as a continuous covariate, centered baseline QTcF as an additional covariate, treatment and time point as categorical factors, and a random intercept and slope per participant. The Kenward-Roger method was used to estimate the degrees of freedom. The slope of dasiglucagon (the regression parameter for the concentration), and the treatment effect-specific intercept (defined as the difference between dasiglucagon-treated and placebo) were estimated together with the 2-sided 90% CI. In line with regulatory guidelines, the primary hypothesis for the analysis was that dasiglucagon does not cause a clinically relevant QTc effect by demonstrating that the upper bound of the 2-sided 90% CI for placebo-corrected ΔQTcF (ΔΔQTcF) was <10 ms at the geometric mean C_{max} for the highest dose level in the trial (1.5 mg dasiglucagon).[10] An upper bound of 10 ms represents the threshold of regulatory concern and is chosen to provide reasonable assurance that the mean effect of the study on the QTc interval is not >5 ms (drugs that prolong the mean QTc interval by around 5 ms or less do not appear to cause torsades de pointes).[10]

Additional exploratory analyses included assessment of a possible delayed effect (hysteresis) and fitting a model with the original term and a quadratic term for dasiglucagon concentration and testing the quadratic term at the 2-sided 5% level. The quadratic term was found to be statistically significant at the .05 level ($P = 0.0313$) and thus indicated that a linear model may not be appropriate. Therefore, a maximum estimated effect (E_{max}) model was also applied to the data to further explore the concentration-QTc relationship for dasiglucagon.

All statistical analyses were performed using the SAS System for Windows, version 9.3 or higher (SAS Institute Inc). PK characteristics were calculated using Phoenix WinNonlin version 8.0 (Certara, Princeton, NJ). No formal sample size calculation was performed for this study.

Results

Participant disposition and demographic characteristics

In all, 141 volunteers were screened; of those, 60 were randomized and received a single dose of the trial drug (n = 6 dasiglucagon × 7 dose cohorts; n = 3 placebo × 6 dose cohorts) (see Supplemental Figure 1. The majority of participants were White (97%) and 52% were women. Mean age was 30 years and mean body weight was 72.7 kg (Table 1). Baseline ECG parameters were similar among all participants. Mean HR across dose groups ranged from 55.7 to 62.9 bpm. Mean QTcF varied between 388.1 and 402.9 ms. Mean PR interval varied between 134.7 and 157.3 ms, and mean QRS duration varied between 102.3 and 107.2 ms.

Table 1
Demographic and baseline characteristics of the full analysis population (N = 60).*

Characteristic	Dasiglucagon						Placebo (n = 18)	Full analysis population (n = 60)
Dose, mg	0.03 IV n = 6	0.1 IV n = 6	0.3 IV n = 6	0.6 IV n = 12	1.5 IV n = 6	0.6 SC n = 6		
Age, y	35.0 (25-45)	30.8 (21-45)	23.3 (18-30)	32.4 (25-42)	33.3 (23-40)	27.8 (23-41)	28.3 (18-43)	30 (18-45)
Body weight, kg	76.05 (65.7-84.1)	70.30 (60.0-80.7)	71.48 (61.9-79.7)	73.24 (63.8-85.6)	74.87 (63.3-89.8)	72.67 (62.8-83.6)	71.67 (60.1-89.9)	72.7 (60.0-89.9)

IV = intravenous; SC = subcutaneous.
* Values are presented as mean (range).

PK and PD

The PK properties of dasiglucagon over a range of 0.03 mg to 1.5 mg were predictable in healthy volunteers. AUC and C_{max} were dose-proportional across the intravenous administration dose cohorts. AUC during the first 240 minutes (AUC_{0-240}) for intravenous dasiglucagon ranged from 251 to 13,656 h*pmol/L across dose groups, whereas AUC_{0-300} for SC 0.6 mg dasiglucagon was 2357 h*pmol/L. $AUC_{0-\infty}$ for the subcutaneous cohort was approximately half of that for the 0.6 mg IV dose cohort (2362 vs 4633 h*pmol/L). Therefore, the absolute bioavailability of dasiglucagon following subcutaneous administration in the abdomen was ≈51%. With the exception of the 0.03 mg dose cohort, the dasiglucagon C_{max} was higher following intravenous administration than following subcutaneous administration (Figure 1A). However, within 30 to 35 minutes, dasiglucagon concentrations in the intravenous administration cohorts had declined to near 0. By 35 minutes, plasma dasiglucagon concentrations in the subcutaneous administration cohort had peaked at 1534 pmol/L. Dasiglucagon was rapidly eliminated following both routes of intravenous and subcutaneous administration with a terminal $t_{½}$ of approximately 18 and 30 minutes, respectively.

Plasma glucose levels increased after dasiglucagon administration in all dose groups. The observed mean maximum glucose concentrations ($C_{max,\ glucose}$) after IV administration ranged from 135 mg/dL (0.03 mg) to 160 mg/dL (1.5 mg). Median time to $C_{max,\ glucose}$ ranged from 17.5 minutes (0.03 mg) to 42.5 minutes (1.5 mg) and increased with ascending doses of intravenous dasiglucagon (Figure 1B). Subcutaneous administration dosing was associated with higher plasma glucose levels (172 mg/dL) than intravenous dosing.

HR and cardiac conduction

At the studied doses, dasiglucagon did not exert a substantial HR effect (mean ΔΔHR was <10 bpm) (Figure 2). Mean ΔΔHR was small across all dose groups, ranging from –7.0 bpm at 5 minutes postdose in the 0.03 mg dose group to 7.5 bpm at 24 hours postdose in the 0.6 mg dose group. At the doses investigated, dasiglucagon did not have a clinically relevant effect on either PR interval or QRS duration.

Cardiac repolarization: By-time point analysis

Because dasiglucagon did not exert a substantial effect on HR, Fridericia's formula was chosen as the QT correction method. The greatest change in placebo-corrected ΔQTcF (ΔΔQTcF) was observed within the first 40 minutes postdose, in which plasma dasiglucagon concentrations rapidly declined to near 0, and again 180 minutes to 24 hours postdose, in which plasma dasiglucagon concentrations were negligible (Figure 3). Within the first 40 minutes postdose, the largest mean ΔΔQTcF were observed in the 4 highest dose groups but without a clear dose- or concentration-dependent relationship. Mean ΔΔQTcF ranged from −3.4 ms (90% CI, −8.96 to 2.23) at 40 minutes postdose in the 0.03-mg dose group to 6.2 ms (90% CI, 1.07 to 11.23) at 25 minutes postdose in the 0.1-mg dose group. Between 60 and 120 minutes postdose, mean ΔΔQTcF was largely negative across all dose groups. ΔΔQTcF exceeded 10 ms at 180 minutes postdose in the 1.5-mg (13.4 ms [90% CI, 8.55 to 18.16]) and 0.1-mg (12.3 ms [90% CI, 7.19 to 17.34]) dose cohorts. This effect was mainly driven by a negative placebo response at this time point as shown in Supplemental Figure 2 (ΔQTcF over time). Dasiglucagon plasma concentrations were negligible at this time point, and there was again no clear dose or concentration dependency.

Cardiac repolarization: Concentration-QTc analysis

The initial concentration-QTc analysis utilizing a linear regression model with a random slope and intercept assigned per participant did not fit the data well at low dasiglucagon plasma concentration levels. In a goodness-of-fit plot, the majority of dasiglucagon concentration observations clustered around 0, and ΔΔQTcF varied without relation to concentration. However, the linear model did predict that the mean and 90% CI of ΔΔQTcF remained below 10 ms across all observations. The estimated population slope of the concentration-QTc relationship was −0.00002 ms per pmol/L (90% CI, −0.000080 to 0.000049) with a treatment effect-specific intercept of 0.8 ms (90% CI, −1.05 to 2.56). Neither slope nor the treatment effect-specific intercept was statistically significant at the 0.1 level.

An E_{max} model was subsequently fitted as per the protocol and statistical analysis plan. The E_{max} model similarly predicted that both mean and 90% CI of ΔΔQTcF remained below 10 ms across all observations (Figure 4 and Table 2). The E_{max} of dasiglucagon was 3.6 ms (90% CI, 1.23 to 5.95; $P = 0.0137$), and the amount that produces half the effect of E_{max} was 426.0 pmol/L (90% CI, −48.8 to 900.71). The treatment effect–specific intercept was −0.44 ms (90% CI, −2.37 to 1.49). Both the amount that produces half the effect of E_{max} and the treatment effect-specific intercept were not statistically significant at the 0.1 level.

As shown in Figure 4, the vast majority of data points were below a dasiglucagon plasma concentration of 30,000 pmol/L, with only 6 observations above 30,000 pmol/L. There was no indication of a clinically relevant QTc prolongation in the concentration range from 30,000 to 80,000 pmol/L (ie, 90% CI of ΔΔQTcF remained below 10 ms for all observations), but due to the limited number of observations, data in this high concentration range should be interpreted with caution.

Safety

A total of 33.3% (14 out of 42) and 11.1% (2 out of 18) of participants reported treatment-emergent AEs (TEAEs) after administration of dasiglucagon and placebo, respectively. All TEAEs were

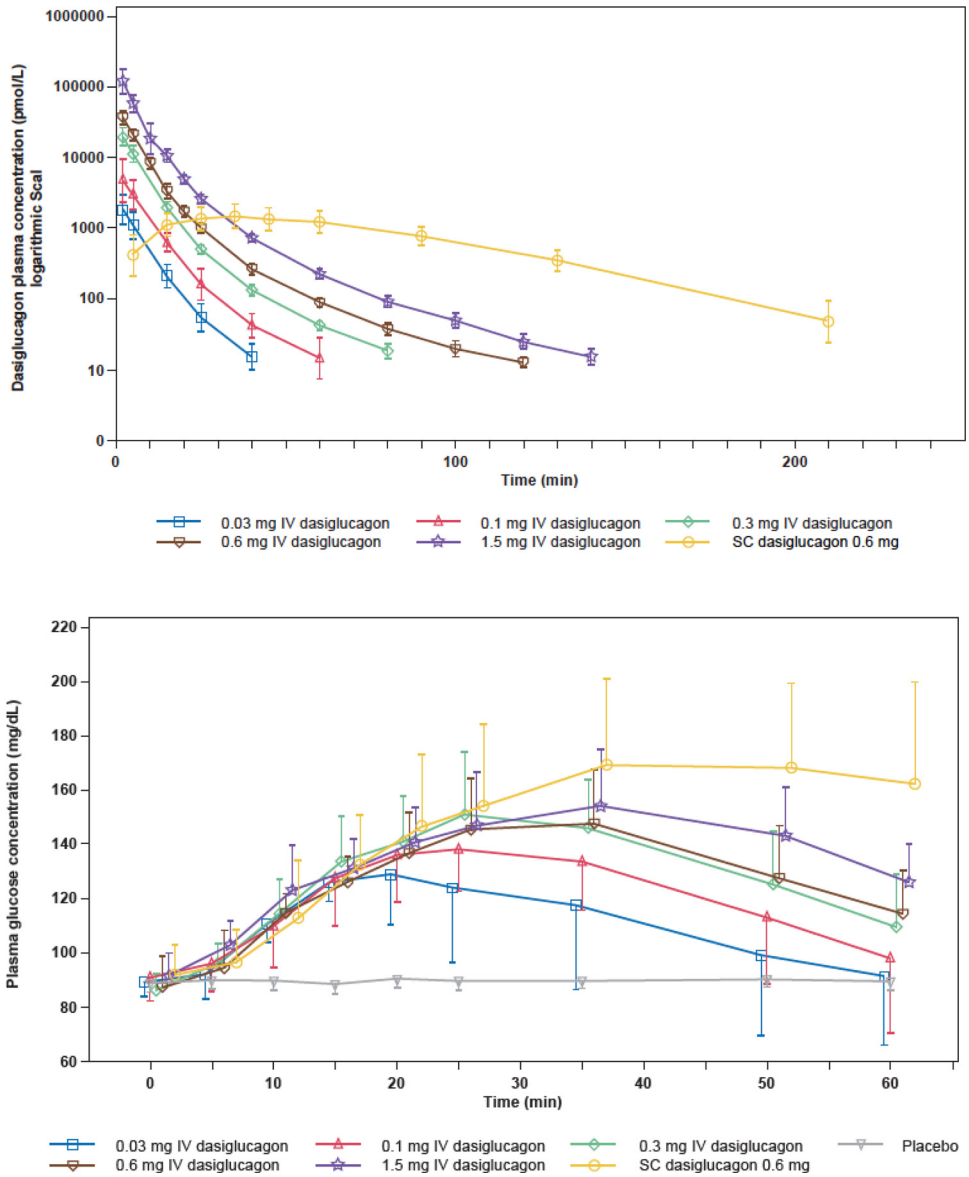

Figure 1. Pharmacokinetic (PK) and pharmacodynamic response to intravenous (IV) and subcutaneous (SC) dasiglucagon. (A) Log-transformed dasiglucagon plasma concentrations over time in healthy volunteers (n = 34) with typical PK profiles receiving dasiglucagon SC 0.6 mg or IV 0.03 to 1.5 mg. Data are presented as geometric mean and 95% CI. (B) Plasma glucose concentrations over time in healthy volunteers with typical PK profiles (n = 52) receiving placebo or dasiglucagon SC 0.6 mg or IV 0.03 to 1.5 mg. Data are presented as mean and 95% CI.

mild to moderate in intensity, and all TEAEs were resolved. The most frequently observed TEAEs reported in both the subcutaneous and intravenous administration dasiglucagon cohorts were nausea (9 participants), vomiting (4 participants), headache (2 participants), extravasation (2 participants), and dizziness (2 participants). Nausea and vomiting occurred more frequently at the higher dose levels. These events had an onset of 1.5 to 2.5 hours after dasiglucagon administration and resolved within 3 hours. In the 0.6 mg SC cohort, which is the approved route of administration, two-thirds of participants (4 out of 6) experienced nausea and half of participants (3 out of 6) vomited. Notably, no ECG-related TEAEs or TEAEs in the system organ class "cardiac disorders" occurred. No effect on vital signs or safety laboratory parameters were observed. Two injection site reactions were reported: a case of spontaneous pain (0.1 mg IV group) and a hematoma (0.6 mg SC group). Both cases were mild and considered possibly related to investigational product by the investigator.

One volunteer had positive test results for antidasiglucagon antibodies at predose and follow-up visits. However, because the increase in titer at follow-up compared with predose was <5-fold, the response was not classified as treatment-boosted. There was no cross-reactivity to endogenous glucagon.

Discussion

This study investigated the potential effect of dasiglucagon exposure on the QT interval and other ECG parameters in 54 healthy adult volunteers. Dasiglucagon had no clinically relevant effect on any ECG parameters studied at any of the investigated doses ranging from 0.03 mg to 1.5 mg IV. Dasiglucagon did not exert a substantial chronotropic effect (mean ΔΔHR was <10 bpm). In line with ICH E14 and US Food and Drug Administration recommendations, a concentration-QTc analysis was performed using a mixed-effects linear model.

The upper bound of the 2-sided 90% CI for ΔΔQTc was <10 ms (ie, below the threshold of regulatory concern) at the geometric mean of C_{max} for the highest dose levels studied. Based on this model, a clinically relevant effect on QTcF was excluded in the

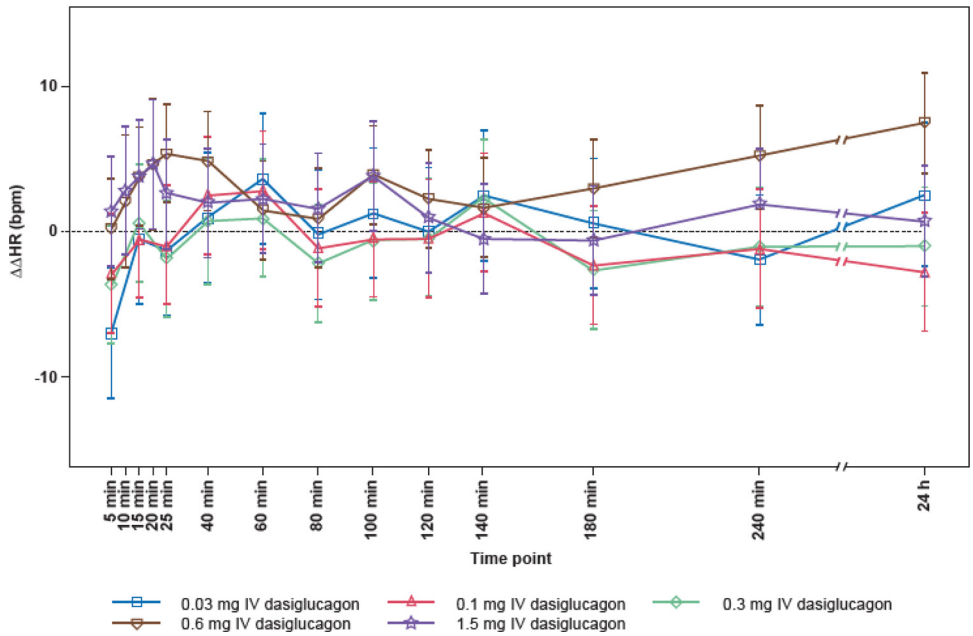

Figure 2. Placebo-corrected change from baseline in heart rate (ΔΔHR) over time. ΔΔHR across prespecified ECG time points in healthy volunteers with typical pharmacokinetic (PK) profiles receiving either placebo or dasiglucagon IV 0.03 to 1.5 mg (n = 46) is shown. Least squares mean and 90% CI based on a linear mixed-effects model: ΔHR = time + treatment + time * treatment + baseline HR is reported. A compound symmetry covariance structure was used to specify the repeated measures (time within participant).

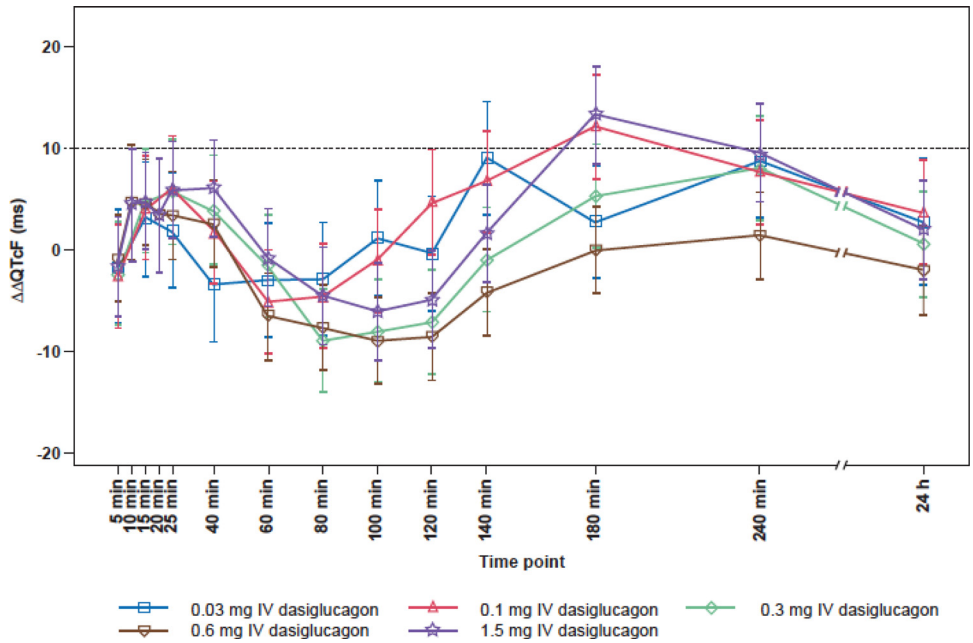

Figure 3. Placebo-corrected change from baseline in QTcF (ΔΔQTcF) over time. ΔΔQTcF across prespecified ECG time points in healthy volunteers with typical pharmacokinetic (PK) profiles receiving either placebo or dasiglucagon IV 0.03 to 1.5 mg (n = 46) is shown. Least squares mean and 90% CI based on a linear mixed-effects model: ΔQTcF = time + treatment + time × treatment + baseline QTcF is reported. A compound symmetry covariance structure was used to specify the repeated measures (time within participant).

concentration range up to ≈30,000 pmol/L, below which the vast majority of observations were made. However, the mixed-effects linear model provided a poor fit to the data at low concentrations of dasiglucagon and an E_{max} model was therefore explored, as prespecified in the protocol and statistical analysis plan. In the E_{max} model, an effect on ΔΔQTcF exceeding 10 ms could also be excluded in the concentration range up to ≈30,000 pmol/L.

In the analysis evaluating QTcF over time, a small delay was observed between dasiglucagon peak concentrations and the small increases in ΔΔQTcF observed during the first 40 minutes post-dose for dasiglucagon doses of ≥0.1 mg. During this time period, dasiglucagon plasma concentrations rapidly declined to near zero. It is therefore possible that this delay represents a delayed effect of dasiglucagon on the QTc interval (ie, hysteresis) or a PD effect mediated by an increase in plasma glucose.

In the initial 60 minutes after intravenous dosing, the increase in ΔΔQTcF coincided temporally with increases in plasma glucose (see Supplemental Figure 3), indicating that the PD effect of dasiglucagon (ie, increased hepatic glucose output) could be at play. An association between increases in plasma glucose levels

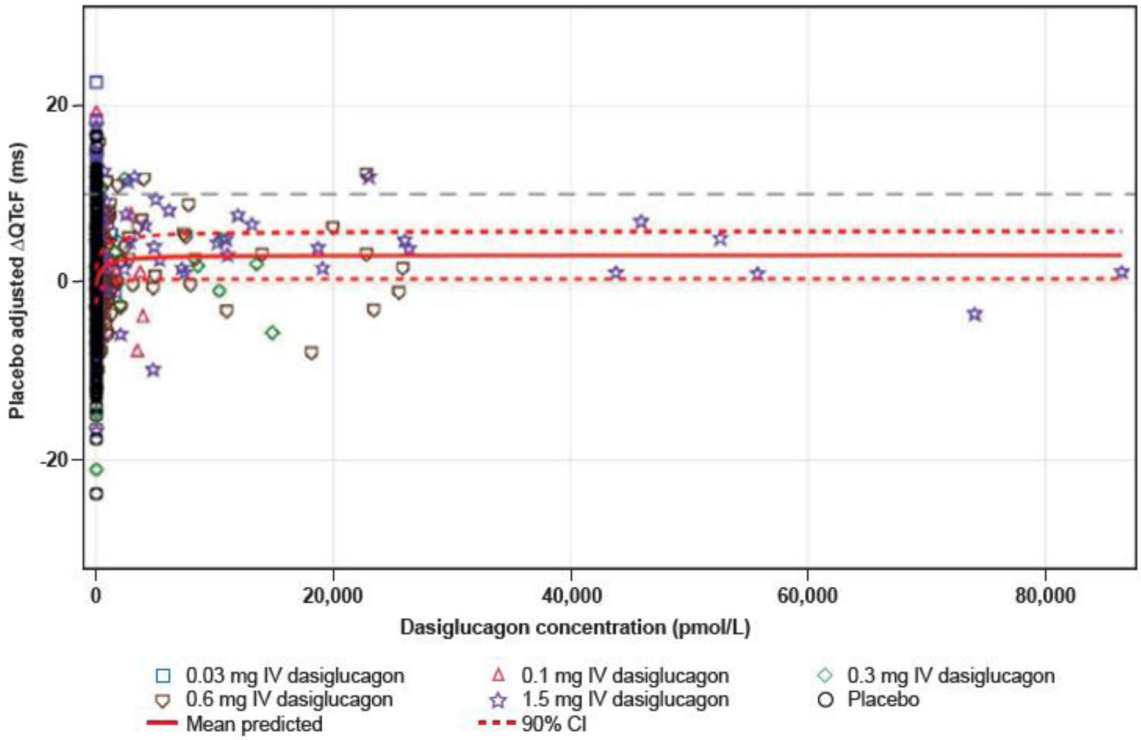

Figure 4. Scatterplot of observed dasiglucagon plasma concentrations and placebo-adjusted change from baseline in QTc by Fridericia's formula (ΔQTcF) from the estimated maximum effect (E_{max}) model. The relationship between the individually observed dasiglucagon plasma concentrations and estimated placebo-adjusted ΔQTcF is shown. Data are from all participants receiving either placebo or dasiglucagon IV 0.03-1.5 mg (N = 54). The solid red line with dashed red lines denotes the model-predicted mean ΔΔQTcF with 90% CI. The blue squares, red triangles, green diamonds, brown homedowns, purple stars, and black circles denote the pairs of observed dasiglucagon plasma concentrations and estimated placebo-adjusted ΔQTcF by participants for the 0.03 mg IV dasiglucagon, 0.1 mg IV dasiglucagon, 0.3 mg IV dasiglucagon, 0.6 mg IV dasiglucagon, 1.5 mg IV dasiglucagon, and placebo treatment groups, respectively. The individually estimated placebo-adjusted $\Delta QTcF_{i,j}$ equals the individual $\Delta QTcF_{i,j}$ for participant$_i$ administered with dasiglucagon at time point$_j$ minus the estimation of time effect at time point$_j$.

Table 2
Placebo-corrected change from baseline in predicted concentration-QT analyses corrected by Fridericia's formula (ΔΔQTcF) in all intravenous (IV) administration cohort participants at geometric mean peak concentration of dasiglucagon, from the maximum estimated effect model (n = 36).*

Dasiglucagon IV dose, mg	Geometric mean C_{max} of dasiglucagon, pmol/L	ΔΔQTcF estimate, ms
0.03	568.1 (223.32 to 1445.32)	1.61 (−0.45 to 3.68)
0.1	2391.3 (1425.88 to 4010.42)	2.61 (0.26 to 4.97)
0.3	6456.6 (2053.26 to 20303.05)	2.93 (0.40 to 5.47)
0.6	10217.9 (5481.17 to 19047.88)	3.01 (0.42 to 5.60)
1.5	57895.6 (46386.06 to 72260.93)	3.13 (0.45 to 5.80)

* Values geometic mean C_{max} and ΔΔQTcF are presented as mean (90% CI).

and QTc interval prolongation has been previously reported, with another study indicating that C-peptide, released as part of the counterregulatory response to elevated glucose levels, has the opposite effect.[11,13] Thus, the observed first peak in ΔΔQTcF in our study is likely to be a physiologic response to the PD effect of dasiglucagon resulting from a complex interplay between glucose and counterregulatory responses (eg, increases in insulin and C-peptide levels). Because neither insulin nor C-peptide were measured in the initial 60 minutes after intravenous dosing, further research would be required to fully understand this interplay.

The second peak of ΔΔQTcF, when dasiglucagon plasma concentrations were near 0, was largely driven by a negative placebo response at 180 minutes postdose. It cannot be ruled out that the consumption of a snack at 90 minutes may have also played a role, because food intake is known to influence the QT interval.[12,14,20,21] However, because plasma glucose levels were not measured beyond 60 minutes postdosing, it is not possible to associate this second peak in ΔΔQTcF with elevated plasma glucose levels. Another potentially confounding variable is the onset of nausea and vomiting 1.5 to 2.5 hours postdose. However, our data do not support a causal relationship between AEs of nausea and/or vomiting and QTc prolongation because only 4 of the 28 volunteers receiving an intravenous injection of dasiglucagon and contributing to the by-time point analysis experienced AEs of nausea and/or vomiting.

It is important to note that even at high dose levels up to 1.5 mg, dasiglucagon did not exert a significant effect on HR. This is in contrast to evidence from preclinical studies suggesting that pharmacologic levels of glucagon in the microgram to milligram range are associated with positive cardiac chronotropic effects.[3] Clinical studies regarding cardiac effects of glucagon performed in humans are few and limited to small, specific populations of patients with heart failure. These studies also lacked control groups and were performed without randomization and blinding, and interindividual differences typically exceed 50%.[3] Taken together, these factors make results from available clinical studies difficult to interpret. Generally, the stimulating effects of supraphysiologic doses of glucagon (>1 mg) in humans do not exceed 20 minutes and increase HR to varying degrees.[3] The lack of any observed chronotropic effect with high doses of a novel hypoglycemia rescue agent is particularly reassuring for a patient population at increased risk for cardiovascular morbidity and mortality.

This study assessed the potential effect of dasiglucagon on the QT interval in healthy volunteers. The intravenous route of administration was chosen to increase the time between ECG extractions and the onset of nausea and vomiting expected to occur 2 to 3 hours after drug administration. An effect of dasiglucagon on ΔΔQTcF exceeding 10 ms was excluded in the observed intravenous administration concentration range up to

≈30,000 pmol/L. It is important to note that there were a limited number of observations (n = 6) in the concentration range of >30,000 to 80,000 pmol/L dasiglucagon. Taken together with the observed minor QTc prolongation, it may be necessary to interpret data in this high concentration range with caution. However, such high concentrations far exceed the maximum concentrations (5000–6000 pmol/L) observed in any clinical trial with 0.6 mg dasiglucagon administered as the approved single subcutaneous dose. This would also be the case should, as specified in the prescribing information, an additional dose be required 15 minutes after the first dose due to the absence of a response.[22] Other limitations in this study include a small number of participants enrolled in the subcutaneous administration cohort to determine absolute bioavailability and lack of a positive control. Although ICH E14 revision R3 allows for concentration-response analyses to be performed on data from trials conducted without a positive control,[10] this study could have been strengthened by including a direct measure of assay sensitivity or an active comparator arm to enhance the confidence with which clinically relevant differences in QT prolongation were detected. This therapeutic agent was also assessed in a highly controlled investigational inpatient setting, which may not fully reflect real-world settings and outcomes.

The strengths of this study include multiple trial design elements to deal with potential bias, including randomization, blinding, and use of a concurrent placebo control group. ECG data were analyzed at a central ECG laboratory in a blinded manner and included a methods bias sensitivity analysis.[17] The purpose of this analysis was to determine whether the expert precision QT method used to evaluate QTc introduced a negative bias that could have underestimated QT prolongation. Concentration-QTc analysis was based on a robust modeling approach, and ECG data were derived from continuous, replicate ECGs captured at multiple time points postdosing. An important problem to address in QT interval measurement is intrinsic variability, which can result from several factors, including posture changes and food intake. Intrinsic variability was minimized in this study by collecting multiple ECGs at both baseline and several time points postdosing while volunteers were in a supine position and by regulating ingestion of food.

Conclusions

Dasiglucagon was generally safe in healthy volunteers, even at doses up to 1.5 mg IV. The most frequently reported TEAEs were nausea and vomiting, which are known side effects that occur with glucagon receptor agonism. No effect on vital signs, safety laboratory parameters, or antidasiglucagon-induced or -boosted antibody formation was observed in study volunteers. PK properties of intravenous dasiglucagon were predictable across a wide dose range (0.03–1.5 mg), and dasiglucagon had no clinically relevant effects on studied ECG parameters. Therefore, an effect of dasiglucagon on ΔΔQTcF exceeding 10 ms was excluded in the observed concentration range up to ≈30,000 pmol/L, a level 5-fold higher than the highest observed plasma concentrations in clinical trials investigating use of the approved 0.6 mg SC dose.

Conflicts of Interest

This study was funded by Zealand Pharma (Søborg, Denmark). R. Tehranchi, S. J. Maarbjerg, J. Pettersson, A. E. Melgaard, and A. Valeur are employees of Zealand Pharma. As authors, employees of Zealand Pharma were involved in the study design; collection, analysis, and interpretation of data; the writing of the manuscript; and the decision to submit the manuscript for publication. The authors have indicated that they have no other conflicts of interest regarding the content of this article.

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R. Tehranchi was the medical project director responsible for the trial and contributed to study design and data interpretation. J. Pettersson was consulted for data analysis and interpretation of results. F. Seitz was the principal trial investigator and contributed to data collection and interpretation. A. E. Melgaard was the statistician responsible for final protocol and statistical analysis. A. Valeur was responsible for PK sampling design, bioanalysis, and blinded interim PK analysis to guide dose escalation between cohorts. S. J. Maarbjerg was the clinical pharmacologist responsible for final protocol, trial conduct, and data interpretation. All authors were involved in drafting and critically revising the manuscript, approved the final version to be published, and take full responsibility for the accuracy and integrity of the content reported herein.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.curtheres.2022.100668.

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