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ORIGINAL ARTICLE

Efficacy and pharmacokinetics of subcutaneous exendin (9-39) in patients with post-bariatric hypoglycaemia

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Aim: To evaluate the efficacy, pharmacokinetic (PK) profile and tolerability of subcutaneous (s.c.). exendin 9-39 (Ex-9) injection in patients with post-bariatric hypoglycaemia (PBH).

Methods: Nine women who had recurrent symptomatic hypoglycaemia after undergoing Roux-en-Y gastric bypass were enrolled in this 2-part, single-blind, single-ascending-dose study. In Part 1, a single participant underwent equimolar low-dose intravenous (i.v.) vs s.c. Ex-9 administration; in Part 2, 8 participants were administered single ascending doses of s.c. Ex-9 during an oral glucose tolerance test (OGTT). Glycaemic, hormonal, PK and symptomatic responses were compared with those obtained during the baseline OGTT.

Results: Although an exposure–response relationship was observed, all doses effectively prevented hyperinsulinaemic hypoglycaemia and improved associated symptoms. On average, the postprandial glucose nadir was increased by 66%, peak insulin was reduced by 57%, and neuroglycopenic symptoms were reduced by 80%. All doses were well tolerated with no treatment-emergent adverse events observed.

Conclusions: Injection s.c. of Ex-9 appears to represent a safe, effective and targeted therapeutic approach for treatment of PBH. Further investigation involving multiple doses with chronic dosing is warranted.

KEYWORDS

bariatric surgery, GLP-1, hypoglycaemia, incretins, pharmacodynamics, pharmacokinetics

1 | INTRODUCTION

Post-bariatric hypoglycaemia (PBH) is an increasingly recognized complication of bariatric surgery, occurring in 0.2% to 11.6% of individuals undergoing Roux-en-Y gastric bypass (RYGB),[1–3] that is characterized by repeated episodes of symptomatic, postprandial hypoglycaemia. Affected patients report a high degree of functional disability; many cannot work, drive, care for others, or be left alone, and are at increased risk of severe neuroglycopenic outcomes, such as loss of consciousness, seizure, motor vehicle accidents, or death.[4] To date, no pharmacotherapies have been developed for treatment of PBH. As the use of bariatric surgery for metabolic gain continues to rise, and perhaps faster than before with the recent addition of metabolic surgery to the treatment algorithm for Type 2 diabetes,[5] the demand for a safe and effective treatment for PBH will continue to grow.

While the precise mechanisms underlying the development of PBH have yet to be determined, a primary role for the incretin hormone glucagon-like peptide-1 (GLP-1) has been described.[6,7] It is well known that postprandial concentrations of GLP-1 are several-fold higher after RYGB,[8,9] including in asymptomatic patients. This hormone, secreted by enteroendocrine cells in response to oral nutrients in the lumen of the hindgut, enhances insulin secretion and, in the case of PBH, contributes to postprandial hypoglycaemia. This is demonstrated clearly by the observation that in patients with PBH, gastrostomy tube feeding into the remnant stomach (from which nutrients transit via the foregut) normalizes postprandial GLP-1, insulin and glucose concentrations.[10,11] Patients with PBH, as compared with asymptomatic RYGB controls, exhibit even higher postprandial GLP-1 concentrations with increased insulin/glucose ratios,[12] insulinogenic index[13] and β-cell glucose sensitivity,[7] indicating that an exaggerated incretin effect may be responsible. While the

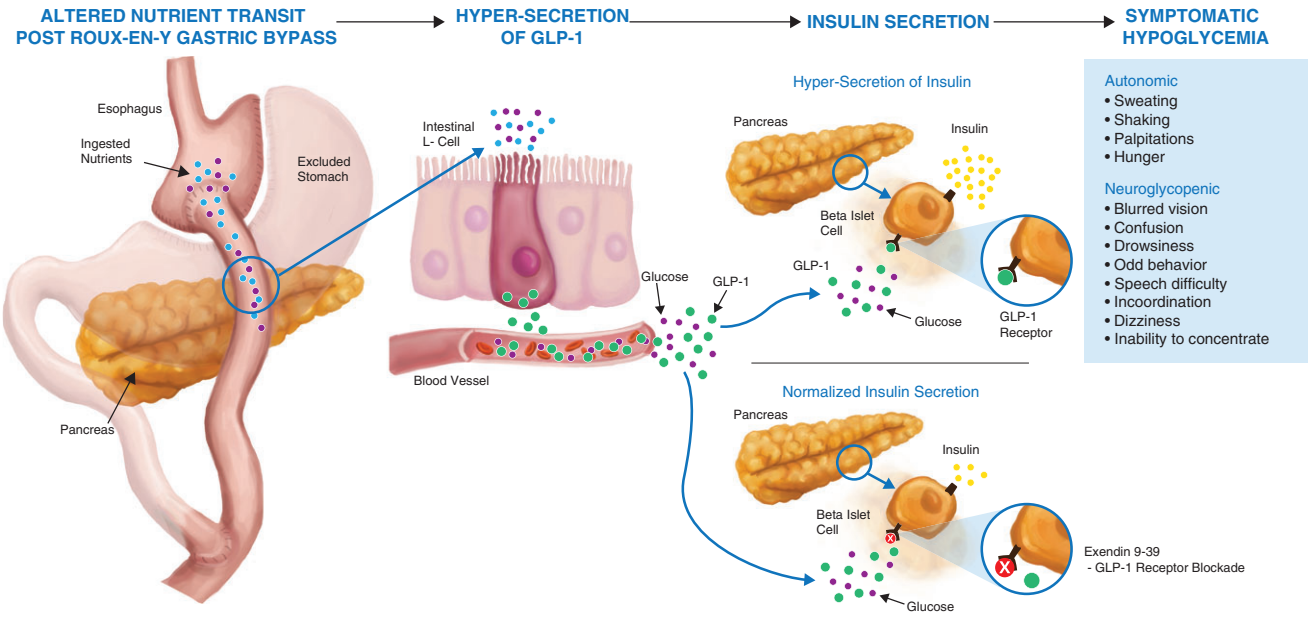


FIGURE 1 Illustration of the proposed mechanism of GLP-1-mediated hyperinsulinemic hypoglycaemia in patients with post-bariatric hypoglycaemia (PBH) and its reversal with exendin 9-39. In patients with PBH, altered nutrient transit triggers hyper-secretion of GLP-1 from intestinal L-cells, causing an exaggerated incretin effect with dysregulated secretion of insulin from pancreatic beta cells, resulting in symptomatic hypoglycaemia. Exendin 9-39 competes with endogenous GLP-1 for the GLP-1 receptor, thereby normalizing insulin secretion and reducing symptomatic hypoglycaemia.

insulinotropic action of GLP-1 is widely thought to be glucose-dependent and dose-dependent, a potentiation of the otherwise linear dose–response relationship between plasma glucose and insulin secretion rate (ISR) has been observed at supraphysiological concentrations of exogenous[14] and endogenous[6] GLP-1. Together, these data suggest that GLP-1 plays a critical role in the development of PBH after RYGB. Indeed, placebo-controlled studies evaluating GLP-1 receptor blockade with the GLP-1 antagonist, exendin 9-39 (Ex-9) administered by continuous intravenous (i.v.) infusion have demonstrated: complete reversal of hyperinsulinaemic hypoglycaemia[6,7]; significant reduction in neuroglycopenic symptoms[6]; and normalization of the glucose-ISR dose–response curve[6] in patients with PBH. Figure 1 depicts the proposed mechanism of GLP-1-mediated hyperinsulinemic hypoglycemia in patients with PBH and its reversal with exendin 9-39.

In light of this physiology, development of Ex-9 for treatment of PBH would represent a uniquely targeted therapeutic approach. This study was designed to evaluate the efficacy, pharmacokinetic (PK) profile, safety and tolerability of single ascending doses of subcutaneously administered Ex-9 in patients with PBH.

2 | MATERIALS AND METHODS

2.1 | Study design

This randomized, single-blind, single-ascending-dose study was conducted in two parts. The first part (Part 1) was aimed at comparing the bioavailability of a single dose of Ex-9 administered by i.v. bolus vs by subcutaneous (s.c.) injection in 1 patient with documented PBH. The second part (Part 2) was aimed at evaluating the efficacy, PK profile, safety and tolerability of single ascending doses of s.c. Ex-9 in 8 patients with PBH during an oral glucose tolerance test (OGTT). The study was conducted at Stanford University School of Medicine in accordance with Good Clinical Practice Guidelines, and with Stanford University Institutional Review Board approval. The protocol was registered with clinicaltrials.gov (NCT02996812), and conducted after US Food and Drug Administration review as an investigational new drug (IND 126123). All participants provided written informed consent before taking part in the study.

2.2 | Participants

Eligible participants were men or women, aged 18 to 65 years, who had undergone RYGB surgery at least 12 months previously, with a documented history of Whipple's triad, with inappropriately elevated insulin concentrations (>21 pmol/L) at the time of hypoglycaemia (≤3.1 mmol/L),[15] and a minimum of 1 symptomatic episode per month by patient report. The sample size was not determined based on a power calculation, as the current phase I study was conducted as an exploratory pilot study.

2.3 | Experimental procedures

2.3.1 | Part 1

In Part 1, an i.v. bolus dose of 0.025 mg/kg Ex-9, prepared as previously described,[6] was administered over 1 minute. On a separate day and in the same participant, an s.c. injection was administered, consisting of 0.025 mg/kg of lyophilized Ex-9 (solubilized and diluted in 0.7 mL of 0.9% normal saline). Post-administration plasma samples were obtained, and PK responses to each route of administration were compared.

2.3.2 | Part 2

In Part 2, single ascending doses of Ex-9 (0.13, 0.25, or 0.38 mg/kg) were administered by s.c. injection to 8 participants with PBH before OGTT provocation. For all participants, timing (−150 minutes relative to OGTT) and volume (0.7 mL) of Ex-9 injections were held constant as doses were escalated, and either the concentration of the injectate was increased with increasing dose (participants 2-5) or the concentration was maintained ≤15 mg/mL (participants 6-9), as shown in Table 2. Dose escalation was based on interim review of PK, pharmacodynamic (PD), and safety data.

A baseline, fasting OGTT was performed, wherein metabolic and symptomatic responses were assessed. On a separate day, a repeat OGTT was conducted after administration of a single s.c. dose of Ex-9: At −150 minutes, participants were injected with s.c. Ex-9 in the anterolateral aspect of the upper arm with the dose to which they were randomized and blinded. Between 0 and 20 minutes, participants consumed a 75-g glucola drink, divided into quarters, with each quarter consumed evenly over 5 minutes; plasma samples were collected at −151 minutes and every 15 minutes from 0 to 180 minutes. The rate of sampling doubled if the plasma glucose fell below 5.55 mmol/L. At 2.78 mmol/L the test was stopped and investigators intervened as needed with i.v. dextrose. Samples for PK evaluation were obtained from 0 to 480 minutes and at 1440 minutes.

2.3.3 | Peptide and assays

Good manufacturing practices-grade Ex-9 acetate was acquired as a sterile, lyophilized powder (Clinalfa, Läufelfingen, Switzerland) and stored at −20°C in the Stanford Investigational Drug Pharmacy. For participants 1 to 5, each 10 mg vial of peptide was reconstituted in the following volumes of 0.9% normal saline for each of the following dose levels: 2 mL for 0.025 mg/kg, 0.5 mL for 0.13 mg/kg, 0.25 mL for 0.25 mg/kg, and 0.15 mL for 0.38 mg/kg. The reconstituted volume (0.7 mL for participant 1 or the full reconstituted volume for participants 2-5) was then drawn up in a 1-mL syringe, and further diluted with normal saline to a total volume of 0.7 mL. For participants 6 to 9, the injection volume was maintained at 0.7 mL and the injectate concentration was maintained at ≤15 mg/mL, with each 10-mg vial reconstituted in 0.5 mg normal saline and further diluted to a total volume of 0.7 mL, with more than one injection administered to achieve the full dose, as necessary. Analytical characterization of the reconstituted stock concentrations for participants 1 to 5 was performed on an interim basis prior to the dosing of participants 6 to 9.

Glucose concentrations were determined by a glucose oxidase method (Analyzer 2; Beckman, Brea, California). Insulin and C-peptide concentrations were measured by radioimmunoassay according to the manufacturer's specifications (Millipore, St Charles, Missouri). Analytical characterization of the reconstituted Ex-9 solutions prepared for participants 1 to 5 and analysis of the concentrations of Ex-9 in all plasma samples were conducted using liquid chromatography–tandem mass spectrometry (File S1).

2.3.4 | Patient-reported symptom questionnaire

A 6-point severity gradation (0 = none; 5 = severe), imposed on the Edinburgh Hypoglycaemia Symptom Scale (EHSS),[16,17] was used to assess the temporal presence and severity of autonomic symptoms (sweating, shaking, palpitations, hunger), neuroglycopenic symptoms (blurred vision, confusion, drowsiness, odd behaviour, speech difficulty, incoordination, dizziness, inability to concentrate), and symptoms of malaise (nausea, headache) at baseline and every 30 minutes during each OGTT. Severity-ranked scores were recorded by time point, and a composite score for "all time points" generated. Results were then partitioned (1) temporally into the "glucose rise" period (from 0 minute to the individual participant's glucose peak) and the "glucose fall" period (from glucose peak to nadir), and (2) by symptom type (autonomic, neuroglycopenic or malaise).

2.3.5 | Calculations and statistical methods

Metabolic variables

Homeostatic model assessment of insulin resistance (HOMA-IR)[18] was used as a surrogate measure of insulin sensitivity, given the known discordance between fasting and postprandial insulin concentrations in patients with PBH caused by an exaggerated incretin effect, rendering postprandial insulin secretion invalid in assessing insulin resistance. The ISR was calculated through deconvolution of peripheral C-peptide concentrations using a 2-compartment model of C-peptide kinetics[19] and

TABLE 1 Participant baseline clinical characteristics

Characteristic	n = 8
Age, years	45 ± 3.8
Sex: men/women	0/8
Pre-surgical BMI	49 ± 2.3
Postsurgical BMI, kg/m^2	29 ± 1.3
Systolic BP, mm Hg	122 ± 3.9
Diastolic BP, mm Hg	76.2 ± 2.4
Post-surgical time to hypoglycaemia, years	2.3 ± 0.87
Post-surgical time with hypoglycaemia, years	4.6 ± 1.5
History of T2D: yes/no	1/7
HOMA-IR, U	0.9 ± 0.2
Frequency postprandial blood glucose <2.7 mmol/L, %	
≥ Daily	37.5
≥ Weekly	50
≥ Monthly	12.5
Frequency neuroglycopenic symptoms, %	
≥ Daily	37.5
≥ Weekly	25
≥ Monthly	37.5
Frequency neuroglycopenic outcomes, %	
Seizure	
≥ Monthly	50
Never	50
Loss of consciousness	
≥ Weekly	37.5
≥ Monthly	25
Never	37.5
Functional status, %	
Able to work	50
Able to drive	25

Abbreviations: BP, blood pressure; T2D, type 2 diabetes.

Values are mean ± s.e.m., unless otherwise indicated.

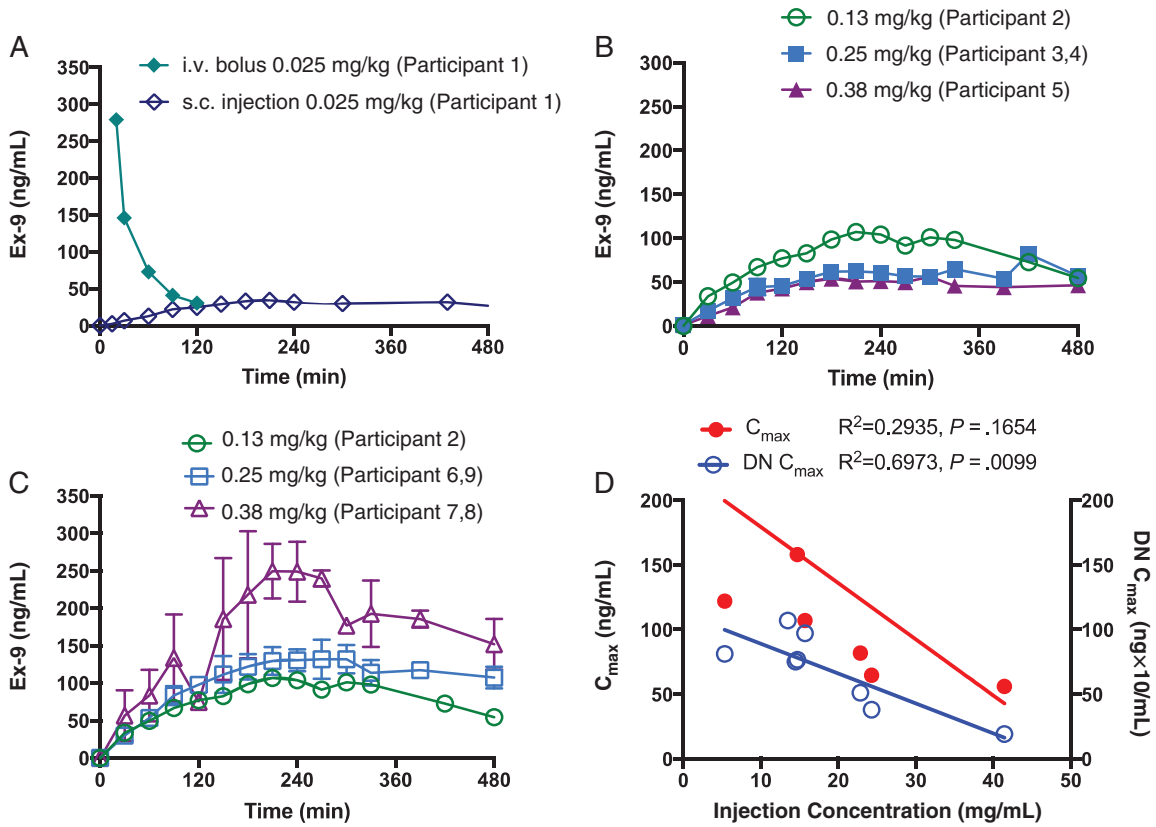


FIGURE 2 Effect of route of administration, dose level, and injectate concentration on PK profiles of patients with PBH after a single s.c. dose of Ex-9. Plasma concentration vs time for A, participant 1 (i.v. vs. s.c. injection); B, participants 2 to 5 (s.c. injection, increasing dose, increasing injectate concentration); and C, participants 2 and 6 to 9 (s.c. injection, increasing dose, consistently low injectate concentration). Individual participant C_{max} and DN C_{max} as a function of injectate concentration for participants 2 to 9 (D), demonstrating a strong inverse relationship between injectate concentration and exposure.

population-based C-peptide kinetics with adjustment for age, sex and body mass index (BMI).[20] β-cell function was assessed by calculation of ISR adjusted for glucose stimulus (ISR2h/G2h), which was derived by taking the ratio of the integral of insulin secretion to the integral of glucose concentration over the first 120 minutes.[21] The rate of glucose decline was calculated as ($glucose_{peak}$ − $glucose_{peak+30min}$)/30 min. The trapezoidal rule was used to calculate the area under the curve (AUC), which was also partitioned into 0 to 60 and 90 to 180 minutes to address the potential cancelling effect of the early rise and late fall in plasma glucose and insulin over 180 minutes. In instances of hypoglycaemia (ie, during baseline assessments) when the test was stopped early because of investigator intervention with i.v. dextrose, the last data point was carried forward. This practice introduces bias toward the null hypothesis of "no effect," but was used as the best model for comparison of AUC values with and without treatment.

Pharmacokinetic variables

Values of AUC extrapolated to infinity (AUC_{inf}) were calculated as AUC + C_{LQC}, where C_{LQC} was the last quantifiable concentration. The apparent elimination rate constant (K_{el}) was estimated by linear regression of the terminal linear portion of the log concentration vs time curve. Terminal elimination half-life ($T_{1/2}$) was calculated as ln (2)/K_{el}. PK analyses were conducted using R 3.4.0. software.

Data are presented as mean ± standard error of the mean (s.e.m.). Two-tailed paired Student's *t*-tests were used for mean intra-group comparisons for baseline (no Ex-9) vs Ex-9. *P* values <.05 were taken to indicate statistical significance. Data were graphed using PRISM software (GraphPad, La Jolla, California).

3 | RESULTS

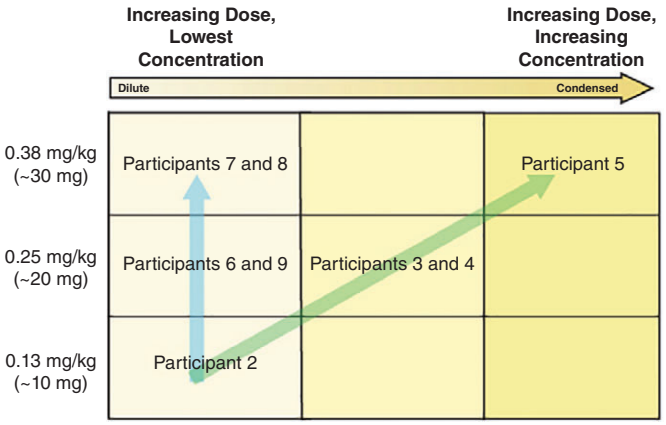
3.1 | Participants and demographics

Nine people with recurrent symptomatic hypoglycaemia post-RYGB were enrolled. Because this study was the first human study to investigate Ex-9 by the s.c. route, the first participant underwent very-low-dose i.v. bolus vs s.c. injection PK comparison (Part 1) with no metabolic testing. The subsequent 8 participants underwent single-ascending-dose s.c. injection PK evaluation with concomitant metabolic testing (Part 2). All 8 single-ascending-dose study participants were women, with a mean age of 45 years and BMI of 29 kg/m^2. On average, a first documented episode of symptomatic hypoglycaemia occurred 2 years postoperatively, followed by 5 years of symptomatic postprandial episodes. All participants experienced hypoglycaemic episodes with capillary glucose <2.78 mmol/L at least monthly, with 50% reporting at least weekly and 38% reporting at least daily episodes. Half reported seizure at least monthly, and 63% reported loss of consciousness at least monthly. Impaired functional status was reported by many participants: 75% were not able to drive and 50% were not able to work (Table 1).

TABLE 2 PK responses to OGTT after s.c. Ex-9 by dose and injectate concentration

	Dose level, mg/kg					
	Increasing dose with increasing concentration[a,b]			Increasing dose with low concentration[a,c,d]		
Variable	0.13 (n = 1)	0.25 (n = 2)	0.38 (n = 1)	0.13 (n = 1)	0.25 (n = 2)	0.38 (n = 2)
Patient number	2	3, 4	5	2[c]	6[c], 9[d]	7[c], 8[c]
Injectate characteristics						
Injectate concentration, mg/mL	15.7	23.6 ± 0.1	41.4	15.7	10.0 ± 4.7	14.0 ± 0.5
Total dose administered, mg	11	16.5 ± 0.5	29	11	17.8 ± 2.8	29.4 ± 1.1
Weight-based dose, mg/kg	0.13	0.25	0.38	0.13	0.25	0.38
PK responses						
C_{max}, ng/mL	107	73.4 ± 8.6	56	107	140 ± 18	266 ± 37
C_{max}DN, ng/mL mg	9.7	4.5 ± 0.7	1.9	9.7	7.9 ± 0.2	9.1 ± 1.6
T_{max}, h	3.5	6.3 ± 0.8	5.0	3.5	6.3 ± 1.8	3.8 ± 0.8
AUC_{last}, h ng/mL	1081	901 ± 93	720	1081	1693 ± 36	2604 ± 591
AUC_{last} DN, h ng/mL/ mg	98.3	54.8 ± 7.3	24.8	98.3	97.9 ± 17.4	89.6 ± 23.3
AUC_{inf}, h ng/mL	1102	923 ± 98	740	1102	1711 ± 36	2621 ± 591
AUC_{inf} DN, h ng/mL/mg	100	56.2 ± 7.6	25.5	100	98.9 ± 17.6	90.1 ± 23.4
$T_{1/2}$, h	3.6	3.8 ± 0.7	3.6	3.6	2.6 ± 0.0	2.4 ± 0.1

Dosing schematic for single-ascending-dose study (Part 2) shown right: participants 2 to 5 received increasing doses (0.13, 0.25, 0.38 mg/kg) with increasing concentrations (15, 24, 43 mg/mL). Participants 6 to 9 received increasing doses with low concentrations (≤15 mg/mL).



Values are mean ± s.e.m., unless otherwise indicated.

[a] Constant volume per injection of 0.7 mL.

[b] Concentration of the injection solution increased incrementally with dose escalation.

[c] Concentration of the injection solution maintained at approximately 15 mg/mL.

[d] Concentration of the injection solution reduced to 5 mg/mL.

3.2 | Pharmacokinetic responses

3.2.1 | Part 1

The PK profile of a single 0.025 mg/kg dose of Ex-9 was assessed in participant 1 after i.v. bolus vs s.c. injection. The maximum plasma concentration (C_{max}) was reduced >8-fold after s.c. injection as compared with after i.v. bolus (34 ng/mL vs 279 ng/mL), the timing of which (T_{max}) occurred at 3.5 hours after s.c. injection (Figure 2A). Exposure was more prolonged after s.c. injection than anticipated, consequently bioavailability could not be determined because of a lack of sampling time points after 550 minutes.

3.2.2 | Part 2

Dose escalation for the subsequent 4 participants (participants 2-5) resulted in surprisingly paradoxical reductions in the PK profile as the concentration of the injectate increased with dose escalation (Table 2 and Figure 2B). C_{max} incrementally declined ~1.4-fold as the dose escalated from 0.13 mg/kg to 0.25 mg/kg to 0.38 mg/kg, and injectate concentration increased from 15 mg/mL to 24 mg/mL to 41 mg/mL. The AUC_{last} also decreased incrementally with dose escalation. Investigating the non-linear and inverse relationship to dose that was observed, dose normalization (DN) was performed, consisting of dividing the PK variables (C_{max} or AUC) by the dose (in mg) administered. This revealed an inverse linear relationship between (1) C_{max} and injectate concentration and (2) AUC and injectate concentration, such that as injectate concentration increased, DN C_{max} (R^2 = 0.6973) and DN AUC (R^2 = 0.6919) decreased (Table 2, Figure 2D). While T_{max} ranged from 3.5 to 7 hours, a predilection for 2 peaks to occur was observed, and in cases where the second peak was higher than the first, T_{max} was delayed, offering a possible explanation for the range observed in T_{max}. $T_{1/2}$ was on average 3.7 hours. Interim analytical characterization of the reconstituted

TABLE 3 Metabolic and clinical responses to OGTT after s.c. Ex-9 by dose and injectate concentration

Variable	Dose level					
	Increasing dose with increasing concentration[a,b]			Increasing dose with consistently low concentration[a,c,d]		
	0.13 mg/kg (n = 1)	0.25 mg/kg (n = 2)	0.38 mg/kg (n = 1)	0.13 mg/kg (n = 1)	0.25 mg/kg (n = 2)	0.38 mg/kg (n = 2)
Patient number	2	3, 4	5	2	6[c], 9[d]	7[c], 8[c]
Injectate characteristics						
Injectate concentration, mg/mL	15.7	23.6 ± 0.1	41.4	15.7	10.0 ± 4.7	14.0 ± 0.5
Total dose, mg	11	16.5 ± 0.5	29	11	17.8 ± 2.8	29.4 ± 1.1
Weight-based dose, mg/kg	0.13	0.25	0.38	0.13	0.25	0.38
Metabolic responses (% change from baseline)						
Glucose, mmol/L						
Fasting	93 (5)	102 ± 10 (5)	93 (0)	93 (5)	95 ± 3 (4)	93 ± 2 (6)
Peak	244 (−14)	227 ± 40 (−1)	311 (21)	244 (−14)	277 ± 90 (6)	228 ± 50 (14)
Nadir	88 (87)	71 ± 8 (64)	58 (36)	88 (87)	82 ± 7 (73)	85 ± 3 (65)
$AUC_{(90,180)}$	13 508 (82)	9634 ± 116 (55)	6668 (18)	13 508 (82)	10 328 ± 2873 (95)	10 459 ± 1834 (87)
Insulin, pmol/L						
Fasting	5.9 (71)	6.2 ± 3 (71)	7.8 (−22)	5.9 (71)	4.4 ± 1 (45)	5.6 ± 1 (10)
Peak	93 (−28)	72 ± 16 (−66)	48 (−83)	93.4 (−28)	85 ± 6 (−67)	108 ± 1 (−40)
$AUC_{(0,60)}$	2669 (−24)	2805 ± 710 (−38)	2268 (−73)	2669 (−24)	3128 ± 103 (−56)	3139 ± 620 (−49)
ISR_{2h}/G_{2h}	0.52 (−43)	0.6 ± 0.2 (−35)	1.13 (−36)	0.52 (−43)	0.43 ± 0.01 (−71)	0.36 ± 0.1 (−61)
C-peptide, pmol/L						
Fasting	212 (−44)	477 ± 251 (22)	265 (10)	212 (−44)	225 ± 80 (−56)	358 ± 132 (−12)
Peak	2225 (−87)	2126 ± 920 (−89)	6569 (−13)	2225 (−87)	3218 ± 238 (−120)	2437 ± 66 (−111)
$AUC_{(0,60)}$	70 324 (−64)	65 357 ± 14 501 (−55)	234 811 (22)	70 324 (−64)	105 089 ± 10 131 (−83)	102 010 ± 7847 (−69)
Clinical responses[e] (% change from baseline)						
Overall symptom score						
All time points	25 (−32)	22 ± 7 (−38)	2 (−750)	25 (−32)	24 ± 5 (−6)	0 (NA)
Glucose rise	22 (−14)	16 ± 1 (−11)	2 (−50)	22 (−14)	22 ± 7 (45)	0 (NA)
Glucose fall	12 (−83)	4 ± 2 (−760)	0 (NA)	12 (−83)	7 ± 2 (−198)	0 (NA)
Autonomic symptoms						
All time points	4 (−100)	7 ± 3 (−63)	0 (NA)	4 (−100)	4 ± 2 (−20)	0 (NA)
Glucose rise	3 (67)	5 ± 1 (75)	0 (NA)	3 (67)	4 ± 1 (13)	0 (NA)
Glucose fall	2 (−300)	3 ± 2 (−440)	0 (NA)	2 (−300)	6 ± 2 (−13)	0 (NA)
Neuroglycopenic symptoms						
All time points	15 (−67)	8 ± 1 (−157)	0 (NA)	15 (−67)	12 ± 5 (−20)	0 (NA)
Glucose rise	13 (−69)	10 ± 3 (79)	0 (NA)	13 (−69)	11 ± 6 (94)	0 (NA)
Glucose fall	9 (−56)	2 ± 1 (−850)	0 (NA)	9 (−56)	3 ± 2 (−875)	0 (NA)

Data are presented as mean ± s.e.m.

[a] Constant volume per injection of 0.7 mL.

[b] Concentration of the injection solution increased incrementally with dose escalation.

[c] Concentration of the injection solution maintained at or below 15 mg/mL.

[d] Concentration of the injection solution reduced to 5 mg/mL.

[e] Symptoms of hypoglycaemia graded on 5-point Likert scale (0 = none; 5 = severe) imposed on the EHSS: autonomic (sweating, shaking, palpitations, hunger); neuroglycopenic (blurred vision, confusion, drowsiness, odd behaviour, speech difficulty, incoordination, dizziness, inability to concentrate); malaise (nausea, headache). Composite scores for "all time points"; subscores to isolate symptoms temporally associated with the "glucose rise" period (from T = 0 to glucose peak) and the "glucose fall" period (from glucose peak to nadir); and scores by symptom category (autonomic or neuroglycopenic).

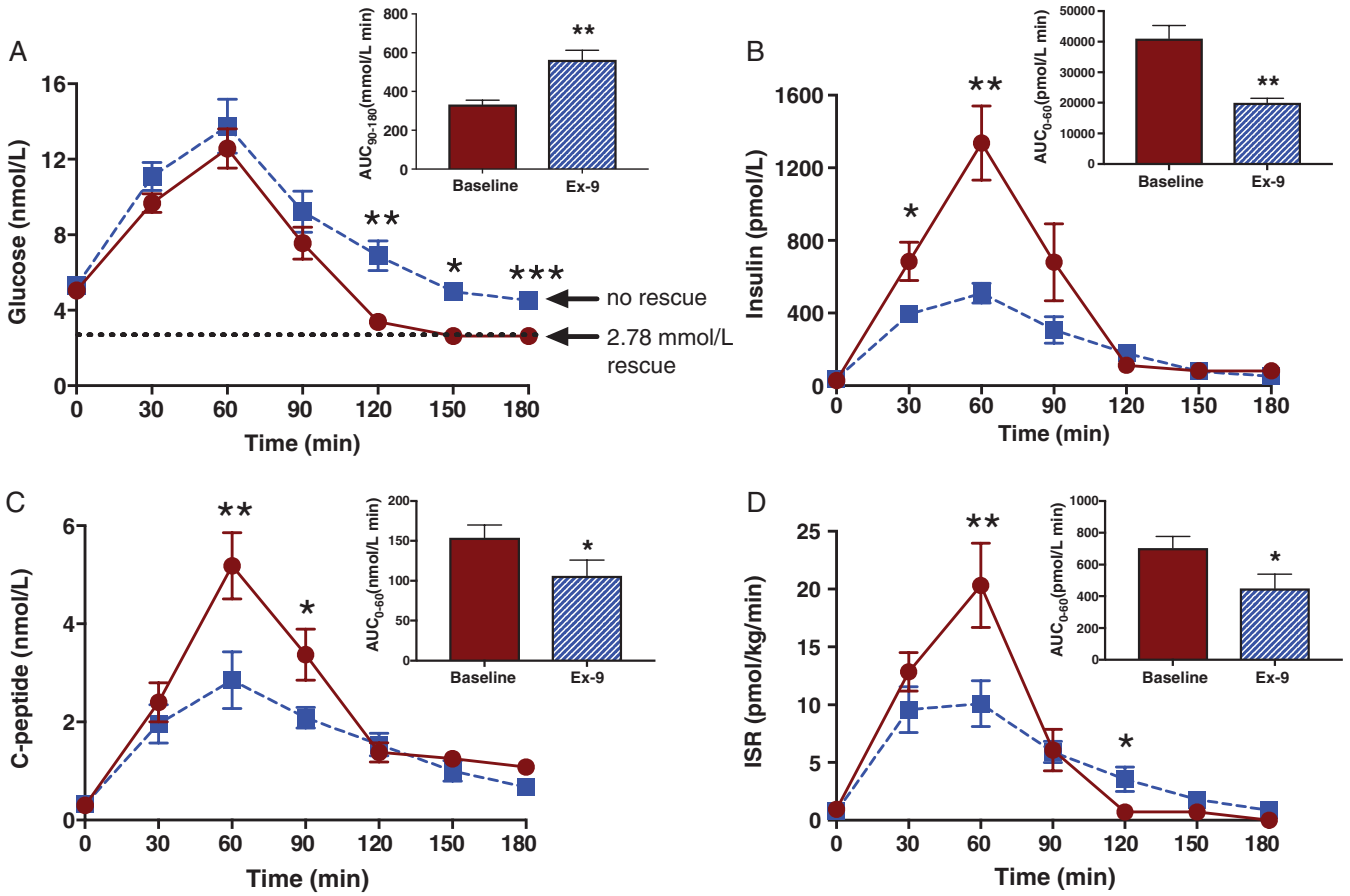


FIGURE 3 Mean metabolic responses to OGTT at baseline and after a single s.c. dose of Ex-9. Plasma concentration (mean ± s.e.m.) vs time and shown inset AUC levels (mean ± s.e.m.) for A, glucose; B, insulin; C, C-peptide; and D, ISR. Baseline: solid red line, circle symbols. Ex-9: dashed blue line, square symbols. *P* values: * ≤.05; ** ≤.01; *** ≤.001

solutions prepared for participants 1 to 5 demonstrated a detected concentration within 20% of the nominal concentration of the test solutions and did not reveal any bias toward reduced recovery with increasing nominal concentration (File S1).

The injection concentration was held at the relatively dilute concentration of ~15 mg/mL for participants 6 to 8 to control for the apparent role of injectate concentration in absorption kinetics. In contrast to participants 2 to 5, in whom dose escalation resulted in unexpected decreases in exposure, dose escalation in participants 6 to 8 yielded linear increases in DN C_{max} and DN AUC. T_{max} ranged from 3 to 4.5 hours (Table 2 and Figure 2C). For participant 9 the effect of an even more dilute injection concentration was investigated through administration of a 5 mg/mL solution. Notably, this resulted in a considerably slowed absorption rate, as demonstrated by a relatively flat PK profile up to T_{max} at 8 hours where C_{max} occurred. Individual PK results for all 9 participants are shown in File S1.

3.3 | Pharmacodynamic responses

3.3.1 | Metabolic responses

A PK–PD relationship was observed at each dose level and injectate concentration administered. In concert with the paradoxically reduced PK responses to dose escalation occurring in participants 2 to 5, PD responses were also inversely correlated with dose level and injectate concentration. For these participants, the percent increase in postprandial glucose nadir and $AUC_{glucose}$ was incrementally diminished rather than enhanced as the dose of Ex-9 was escalated from 0.13 mg/kg to 0.25 mg/kg to 0.38 mg/kg (Table 3). Similarly, the percent reduction in peak and AUC insulin was reduced as doses increased. After adjustment to control for injectate concentration on the basis of interim analyses from participants 2 to 5, a direct dose–response relationship was observed for participants 6 to 9 as doses were escalated. After DN, a linear relationship between percent increase in glucose nadir and Ex-9 exposure (DN C_{max} [R^2 = 0.6831] and DN AUC [R^2 = 0.5123]) was revealed across dose levels (Table 3 and File S1).

Hypoglycaemia was prevented in all 8 participants (Table 3, Figure 3A). Early postprandial glycaemic rises (fasting, peak, time to peak, and $AUC_{[0,60]}$) were not worsened by Ex-9, and late glycaemic variables were improved: the rate of glucose decline was diminished by 27% (P = .05), while glucose $AUC_{(90,180)}$ and glucose nadir were increased by 72% (P < .01) and 66% (P < .001), respectively. The postprandial insulin spike was substantially diminished with Ex-9 injection: peak and AUC insulin were reduced by 57% (P < .01) and 48% (P < .01), and peak and AUC C-peptide concentrations were reduced by 44% (P = .001) and 31% (P < .05), respectively (Figure 3B,C). Peak ISR was reduced by 51% (P < .001), and by 49% (P < .01) when adjusted for incremental unit of plasma glucose (ISR_{2h}/G_{2h}; Figure 3D).

3.3.2 | Clinical responses

Administration of s.c. Ex-9 reduced overall symptom scores at all dose levels. There was no apparent relationship between PK exposure and the degree of symptomatic improvement observed. On average, the composite score accounting for both the degree and occurrence of any symptom reported from the EHSS questionnaire was reduced by ~80% ($P < .01$) during the glucose fall period, and this was primarily attributable to the substantial reduction in the presence and severity of neuroglycopenic symptoms, which were also decreased by ~80% ($P < .01$) during this timeframe. Autonomic symptoms during the glucose fall period were reduced by 39% ($P < .05$; Table 3).

3.4 | Safety and tolerability

Ex-9 was well tolerated at all dose levels with no injection site reactions observed. No serious adverse events or treatment-emergent adverse events occurred, and no participants were discontinued from the study because of an adverse event.

4 | DISCUSSION

This is the first-in-human study involving the s.c. administration of the GLP-1 receptor antagonist Ex-9. The results of this study show that s.c. doses of Ex-9 ranging from 0.18 to 0.38 mg/kg effectively prevented postprandial hyperinsulinaemic hypoglycaemia and mitigated associated symptoms in patients with PBH. On average, the postprandial glucose nadir was increased by 66%, peak insulin was reduced by 57%, and neuroglycopenic symptoms were reduced by 80%, results that mirror those observed under the same experimental conditions involving continuous i.v. infusion of Ex-9.[6] S.c. Ex-9 was well tolerated, with no drug-related adverse events or serious adverse events observed.

Historically, Ex-9 has been administered by continuous i.v. infusion in many clinical investigations involving primarily healthy humans or participants with diabetes in doses up to 900 pmol/kg/min (0.003 mg/kg/min), as shown in File S1. In aggregate, > 300 people have received Ex-9 by i.v. infusion to date, with no adverse effects reported. In the first human trial, Edwards et al.[22] examined the physiological role of GLP-1 in the postprandial state in humans, demonstrating in healthy fasted volunteers that continuous i.v. infusion of Ex-9 could abolish the insulinotropic effect of exogenous GLP-1 administered at physiological doses, fully reversing the glucose-lowering effect. More recently, placebo-controlled trials involving equivalent or higher i.v. doses of Ex-9 in patients with PBH have demonstrated an ability to abolish the insulinotropic effect of endogenous GLP-1 and normalize postprandial glucose, even at the supraphysiological postprandial concentrations observed in patients with PBH.[6,7,13]

To date, s.c. injection as a route of administration, representing a more practical method for daily use, has not been examined. The bioavailability of the related 39-amino acid GLP-1 agonist, exenatide (of which Ex-9 is a 31-amino-acid fragment), is substantially reduced when injected into the s.c. compartment as compared with when infused intravenously, such that several-fold higher doses are required to achieve equal concentrations after s.c. administration. In addition, equivalent plasma concentrations of exenatide (or equally of native GLP-1) have demonstrated reduced PD "efficiency" after s.c. vs i.v. administration.[23] Indeed, in Part 1 of the present investigation, bioavailability, while not formally assessed, was clearly markedly lower after s.c. vs i.v. administration, similar to the pattern observed with exenatide; however, in Part 2, an exposure–response correlation relating s.c. exposure to Ex-9 (both C_{max} and AUC) with glycaemic improvements (% increase in glucose nadir) was observed. Ultimately, efficacy, defined as prevention of postprandial hypoglycaemia and improvement in symptoms, was observed at *all* dose levels. These results, while surprising, bode extremely well for the development of Ex-9 as an s.c. injectable for treatment of PBH.

From a PK standpoint, an intriguing paradoxical relationship between injectate concentration, plasma concentration, and glycaemic response was observed, such that as injectate concentrations increased, PK and glycaemic responses decreased. This was overcome by maintaining a relatively dilute injection solution. Further dilution resulted in a more protracted rise to C_{max} with longer duration of coverage. There may be many possible explanations for these PK phenomena.

Characterization of the pharmaceutical formulation conducted on an interim basis on stock solutions ranging in concentration from 5 to 67 mg/mL yielded detected concentrations that fell within a reasonable margin of the nominal concentrations of the test solutions, suggesting that the inverse dose–linear relationship was probably not attributable to a dosing or bioanalytical method error. In addition, recovery was not reliably reduced with increasing concentration of the test solution, indicating that under the same temperature-controlled conditions in which the dosing solutions were maintained, pre-dosing effects probably did not contribute to the observed decrements in exposure. Rather, these data were more suggestive of post-dosing effects in the s.c. compartment, where exposure to local conditions may have induced aggregation and precipitation, potentially resulting in reduced exposure at higher dosing solution concentrations.

The observed PK tendency for 2 peaks to occur may provide evidence to suggest the presence of a distribution phase, which, among other possibilities, could be the outcome of target-mediated drug disposition. A further consideration is that, while s.c. injection site (abdomen vs thigh vs arm) for the related peptide exenatide has demonstrated similar bioavailability,[24] s.c. administration of Ex-9 into the abdominal tissue in this population, which after bariatric surgery-induced weight loss may be composed disproportionately more of redundant skin than adipose tissue, may differ from bioavailability after arm or thigh administration. Consequently, in the present study, all injections were administered into the anterolateral aspect of the arm.

The present study has some limitations. This investigator-initiated study was conducted in a small cohort of participants at a single centre involving a single s.c. injection of Ex-9. The clinical applicability of these findings must be confirmed by multicentre studies involving multiple doses. Timing and frequency of administration and any relationship to the timing of meals should be explored, as should the durability of response over time in light of the potential for (1) compensatory increases in GLP-1 secretion or receptor upregulation in the face of chronic GLP-1 receptor blockade and

(2) development of antidrug antibodies, although in the instance of exenatide, low-titre antibodies are commonly observed with no apparent effect on efficacy.[25] Another consideration is the use of OGTT as a provocative metabolic test. While for patient safety, provocation must occur in the clinical setting under direct observation, mixed-meal tolerance testing more closely resembles the mixed macronutrient composition of at-home meals, has demonstrated good reproducibility for glucose, insulin and C-peptide measures,[26] and is recommended for diagnosis of PBH by the American Society for Metabolic and Bariatric Surgery (ASMBS).[27]

In conclusion, the results of this first-in-human study involving s.c. injection of the GLP-1 receptor antagonist Ex-9 demonstrate that a single s.c. administration can effectively prevent postprandial hypoglycaemia and improve symptoms in patients with PBH. In light of the efficacy demonstrated at every dose level evaluated in the present investigation, s.c. Ex-9 appears to represent a promising, practical and targeted therapeutic approach for the treatment of PBH. The PK properties observed may present opportunities for modulation of absorption kinetics via formulation and optimization of dosing regimen. Multicentre, multi-dose trials are warranted with a view toward durability of effect, safety and tolerability with chronic dosing.

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Conflict of interest

C. M. C., T. L. M., L. L., T. N. and C. A. P. were affiliates of Stanford University with no duality of interest at the time of study conduct and analysis. C. M. C. and T. L. M. were consultants affiliated with Eiger BioPharmaceuticals at the time of manuscript submission. J. B. was an independent contractor with a consulting relationship to RRD International at the time of manuscript submission.

Author contributions

All authors meet the criteria for authorship as recommended by the International Committee of Medical Journal Editors (ICMJE). C. M. C. and T. L. M. conceived of and designed the study, carried out the study and the analysis and interpretation of data, drafted the manuscript, and contributed to critical revisions. L. L., C. A. P., T. N. and E. C. were involved in the acquisition of the data. J. B. was involved in the analysis of PK data. All authors were involved with the drafting, critical review or revision of the manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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