Pharmacokinetic and Glucodynamic Comparisons of Recombinant and Animal-Source Glucagon after IV, IM, and SC Injection in Healthy Volunteers

CASEY J. GRAF, JAMES R. WOODWORTH,* MARY E. SEGER, JOHN H. HOLCOMBE, RONALD R. BOWSHER, AND RENEE LYNCH

Contribution from *Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, Indiana.*

Received March 9, 1999. Final revised manuscript received July 14, 1999.
Accepted for publication July 23, 1999.

Abstract □ The structure of the hormone glucagon is identical among humans and several species of other mammals. Equivalence of recombinant glucagon (rG) to animal-source glucagon (aG) was assessed in this two-part, open-label, randomized study. Part I was a four-way crossover intravenous dose-ranging study of rG (pH 2.8) involving 12 subjects. Part II was a six-way crossover study of 29 subjects comparing rG (diluent pH 2.0 and 2.8) with aG administered subcutaneously (sc) and intramuscularly (im). Maximum glucagon plasma concentrations (C_{max}) and area under the glucagon concentration curve (AUC) were calculated. Additionally, maximum blood glucose concentrations (BG_{max}), maximum absolute BG excursion (MAE), and area under the glucose concentration curve from time of dosing to return to baseline (AUC_{rtb}) were calculated. The primary focus was equivalence of the formulation intended for marketing (rG pH 2.0) to aG. Administration of rG pH 2.0 through the im route demonstrated equivalence to aG for all pharmacokinetic and glucodynamic comparisons. Subcutaneous administration of rG pH 2.0 demonstrated standard bioequivalence for AUC (5.87 versus 6.63 ng·h/mL; NS) and near equivalence for C_{max} (7.94 versus 9.12 ng/mL; $p < 0.05$). rG pH 2.0 showed glucodynamic equivalence to aG (BG_{max}, 136 versus 133 mg/dL; MAE, 50.0 versus 47.4 mg/dL, respectively) and statistically greater AUC_{rtb} values (151 versus 126 mg·h/dL, $p < 0.05$). rG and aG were equally safe and well tolerated. In conclusion, rG provides equivalent safety and efficacy to aG.

Introduction

Glucagon is a naturally occurring protein hormone secreted from the α cells of the pancreas. The primary sequence of glucagon is highly conserved in mammals and is identical in man, cattle, pigs, dogs, and rats. The principal function of glucagon is to maintain glucose production, through both glycogenolysis and gluconeogenesis, at a rate sufficient to meet glucose requirements. In man, approximately 75% of net glucose production is mediated through glucagon.[1]

Glucagon is used therapeutically to treat severe hypoglycemia, particularly in patients with diabetes when intravenous glucose is unavailable.[2,3] Glucagon is also used intravenously to relax the intestinal tract to facilitate radiographic examination of the upper and lower gastrointestinal tract.[3,4] Although glucagon is used to treat diabetic hypoglycemia, it may induce hyperglycemia in patients with diabetes when used for radiologic purposes if the patients are in good metabolic control. However, glucagon can induce gut immobilization at lower doses than 1 mg. Lower doses of glucagon may provide necessary gut immobilization while inducing lesser amounts of hyperglycemia and therefore achieving greater safety in patients with diabetes.

Historically in the United States, commercial glucagon has been produced through an extraction process of beef and pork pancreas glands, followed by a high degree of purification. With a trend away from the manufacturing of beef and pork insulins, the availability of quality animal pancreas glands has diminished. Recent advances in recombinant deoxyribonucleic acid (DNA) technology have provided a reliable and efficient source of purified glucagon, recombinant glucagon (rG), with an amino acid sequence identical to that of animal glucagon. The purpose of this study was to compare the pharmacokinetic and pharmacodynamic parameters of rG and animal-source glucagon (aG).

Methods

Patient Population—Forty-one healthy volunteers (24 males, 18 females) between the ages of 23 and 60 years and who were within 15% of normal body weight for their height and frame size (Metropolitan Life Insurance standards) were enrolled in Part I ($n = 12$) or in Part II ($n = 29$) of this study. Subjects participated in only one part of the study. Each subject had a complete medical history, physical examination, complete blood count, urinalysis, a fasting chemistry panel, and chest X-ray prior to enrollment.

For this study, the primary study drug was rG, and the comparator study drug was aG. Each was supplied by Eli Lilly and Company (Indianapolis, IN). The study drug was supplied as a lyophilized powder and reconstituted at the time of injection to a concentration of 1 mg/mL. The diluting solutions were pH 2.0 and 2.8 for rG and pH 2.8 for aG. The Institutional Review Board of the participating institution approved the study, and each subject gave written informed consent for the study.

Study Design—Part I was a randomized, open-label, four-way crossover study that assessed the pharmacokinetics, glucodynamics, dose proportionality, and safety of rG after intravenous (iv) administration. Each healthy volunteer received four doses (0.25, 0.5, 1.0, and 2.0 mg) of rG pH 2.8 as an iv bolus injection with a 7−10 day interval between each dose.

Part II was a randomized, open-label, six-way crossover study that assessed the bioequivalence of rG pH 2.0 and pH 2.8 with aG pH 2.8 after intramuscular (im) and subcutaneous (sc) administration. Two separate pH values were used, since the pH used currently (2.8, for aG) occasionally results in a gel formation. Reduction of the pH to 2.0 reduces the occurrence of this phenomenon. Each subject was scheduled to receive all six possible dose combinations: (1) aG sc, (2) rG pH 2.8 sc, (3) rG pH 2.0 sc, (4) aG im, (5) rG pH 2.8 im, and (6) rG pH 2.0 im. Each dose (sc or im, aG or rG) was 1 mg and was separated by a 7−10 day interval. Administrations were given sc in the lower abdomen and im in the upper deltoid muscle.

* Corresponding author. Telephone: (317) 276-1304. Fax (317) 276-5495. e-mail: WOODWORTH_JAMES_R@lilly.com.

10.1021/js990077p CCC: $18.00
Published on Web 08/27/1999

Table 1—Pharmacokinetic and Glucodynamic Parameters, Intravenous Administrations[a]

	C_{max}, ng/mL	$AUC_{(0-t)}$, ng·h/mL	$AUC_{(0-inf)}$, ng·h/mL	CL, L/h	V_{ext}, L	V_{ss}, L	$t_{1/2}$, h	BG_{max}, mg/dL	TBG_{max}, h	$AUC_{(0-rtb)}$, mg·h/dL
recombinant 0.25 mg (A)	37.4 ± 9.24	4.07 ± 0.631	4.08 ± 0.632	62.5 ± 9.0	11.9 ± 6.6	4.2 ± 2.0	0.13 ± 0.06	131 ± 17.5	0.34 ± 0.1	137 ± 96.2
recombinant 0.5 mg (B)	77.6 ± 20.7	8.47 ± 1.83	8.48 ± 1.84	61.1 ± 11.3	12.7 ± 6.9	4.6 ± 2.2	0.15 ± 0.09	138 ± 16.8	0.35 ± 0.1	129 ± 61.7
recombinant 1.0 mg (C)	171 ± 67.3	17.9 ± 4.04	17.9 ± 4.04	58.4 ± 13.3	18.5 ± 10.7	3.8 ± 2.0	0.22 ± 0.12	132 ± 21.0	0.36 ± 0.2	101 ± 52.9
recombinant 2.0 mg (D)	368 ± 117	37.7 ± 6.98	37.7 ± 6.97	54.6 ± 10.1	23.8 ± 9.2	3.5 ± 2.1	0.30 ± 0.09	129 ± 23.1	0.35 ± 0.2	123 ± 103
p-value[b]	NS	NS	NS	nc	nc	nc	<0.001	NS	NS	NS

[a] All data are reported as mean (±SD). NS = not significant ($p > 0.05$). nc = not compared. [b] From the ANOVA comparing treatment means.

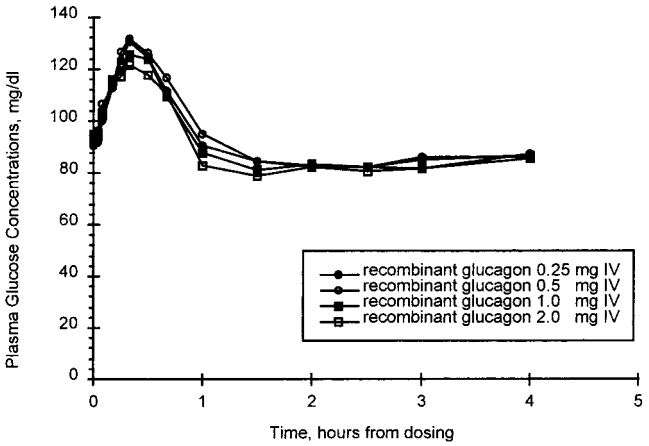

Figure 1—Mean blood glucose concentration versus time curves, all intravenous treatments (part I). $n = 10$. ● = 0.25 mg, ○ = 0.5 mg, ■ = 1.0 mg, □ = 2.0 mg.

For Part I and II, patients received doses of glucagon after an overnight fast, and they remained fasting during the test. Serum samples were collected over a 4-h period after each injection for measurement of glucose and glucagon concentrations. For Part I, samples were collected at 0, 2, 5, 10, 15, 20, 30, 40, 60, 90, 120, 150, 180, and 240 min after administration. For Part II, samples were collected at 0, 5, 10, 15, 20, 25, 40, 60, 90, 120, 150, 180, and 240 min after administration. Serum glucagon concentrations were measured at a central laboratory (Pharmaco International, Richmond, VA) using radioimmunoassay (RIA) techniques. A modified commercial glucacon RIA kit (LINCO, St. Louis, MO) was used for measurement of rG. The modification consisted of replacing the kit calibration standards with standards prepared with rG in the kit's ligand-free matrix. Test samples, standards, and QC samples were analyzed after extracting all samples with four volumes of methanol, evaporation of the extracts, and reconstitution in assay buffer. Although the primary sequence of aG is highly conserved in mammals (the 29-amino acid sequence is identical for man, cattle, pigs, dogs, and rats), aG was cross-validated within this assay, showing equivalent potency, parallel dilution, and nearly identical recovery. The lowest quantifiable concentration for the assay was 40 pg/mL.

Additionally, blood glucose concentrations were determined using a glucose hexokinase method (Clinical Laboratory of MDS Harris, Lincoln, NE).

Pharmacokinetic Measurements—Pharmacokinetic parameters were estimated by noncompartmental pharmacokinetic methods. Maximum glucagon concentration (C_{max}), the time at which C_{max} was observed relative to drug administration (t_{max}), area under the glucagon concentration versus time curve from time 0 to infinity ($AUC_{(0-inf)}$), and apparent terminal elimination phase half-life ($t_{1/2}$) for glucagon plasma concentrations derived from Parts I and II were calculated. Additional parameters, including total systemic clearance (CL), the volume of distribution at steady-state (V_{ss}), and the extrapolated volume of distribution (V_{ext}) were calculated from the iv bolus data (Part I).

Glucodynamic Measurements—Several glucodynamic measurements were derived from the blood glucose concentrations, including the following: maximum blood glucose concentration (BG_{max}), time to BG_{max} (TBG_{max}), area under the glucose versus time curve from time 0 to the time of return to baseline ($AUC_{(0-rtb)}$), area under the glucose excursion versus time curve from 0 to return to baseline (AUC_{ex}), maximum absolute BG excursion (MAE), and earliest recorded time of the MAE (TBG_{ex}). "Baseline" is defined as the blood glucose concentration reported just prior to injection. "Return to baseline" is the achievement of that baseline concentration after blood glucose peaked. If necessary, $AUC_{(0-rtb)}$ and AUC_{ex} values were interpolated. The excursion values (AUC_{ex}, MAE, TBG_{ex}) reflect a subtraction of the baseline value from all measured concentrations and calculations in a fashion similar to the nonadjusted values. All AUC measurements were calculated using the trapezoidal rule.

Statistical Methods—For Part I, a parametric (normal theory) general linear model was applied to the pharmacokinetic and glucodynamic parameters using the SAS GLM procedure. The analysis of variance (ANOVA) model included sequence, period, and treatment as fixed effects and subject within sequence as a random effect. Differences between the treatments with respect to any given parameter were further assessed using pairwise multiple *t*-tests (Bonferroni method). Dose linearity was assessed by linear regression of the dose-normalized parameters with respect to dose. Dose linearity with respect to a parameter was concluded if the slope was not significantly different from 0 ($\alpha = 0.05$). For Part II, a similar ANOVA was performed comparing the pharmacokinetic and glucodynamic parameters and the log-transformed values of C_{max}, $AUC_{(0-t)}$, $AUC_{(0-inf)}$, BG_{max}, $AUC_{(0-rtb)}$, AUC_{ex}, and MAE. The two one-sided hypotheses (Schuirmann two one-sided tests procedure) were tested at the 5% level for the parameters by constructing 90% confidence intervals for the ratio of the test and reference means. The 90% confidence intervals were obtained from the antilogs of the lower and upper bounds of the 90% confidence intervals for the difference in the means of the log-transformed data. Bioequivalence was concluded if the 90% confidence intervals for the variables C_{max}, $AUC_{(0-t)}$, $AUC_{(0-inf)}$, BG_{max}, $AUC_{(0-rtb)}$, AUC_{ex}, and MAE were contained within the range of 80% to 125%.

Results

Part I—For Part I, 10 out of the 12 subjects successfully completed the study. Two subjects withdrew from the study for reasons unrelated to the study. All adverse events in Part I occurred within 24 h following dosing and were mild in severity. The most common adverse events were dizziness and nausea.

Pharmacokinetics—The pharmacokinetic parameters are shown in Table 1. rG pH 2.8 exhibited dose proportionality for C_{max} ($p = 0.186$ for the test of 0 slope), $AUC_{(0-t)}$ ($p = 0.099$), and $AUC_{(0-inf)}$ ($p = 0.104$) when administered intravenously over the 0.25 to 2.0 mg dose range. Mean maximal plasma glucagon concentrations ranging from 37 to 368 ng/mL occurred within 0.05 h following the iv bolus dose. Glucagon was rapidly eliminated, with mean half-lives ranging from 0.13 to 0.30 h. Half-life appeared to increase with increasing dose. This could be an effect of the appearance of a second compartment that only becomes apparent with higher glucagon doses. The mean clearance was similar between the treatments (≈59 L/h). The volume of distribution (V_{ext}), which is affected by changes in the rate of elimination, increased with increasing doses.

Glucodynamics—Glucodynamic parameters are shown in Table 1. Mean maximal blood glucose concentrations were similar for each treatment (129 to 136 mg/dL) and occurred within 0.36 h after the iv bolus dose of glucagon. This finding indicates that even at the lowest glucagon dose, a

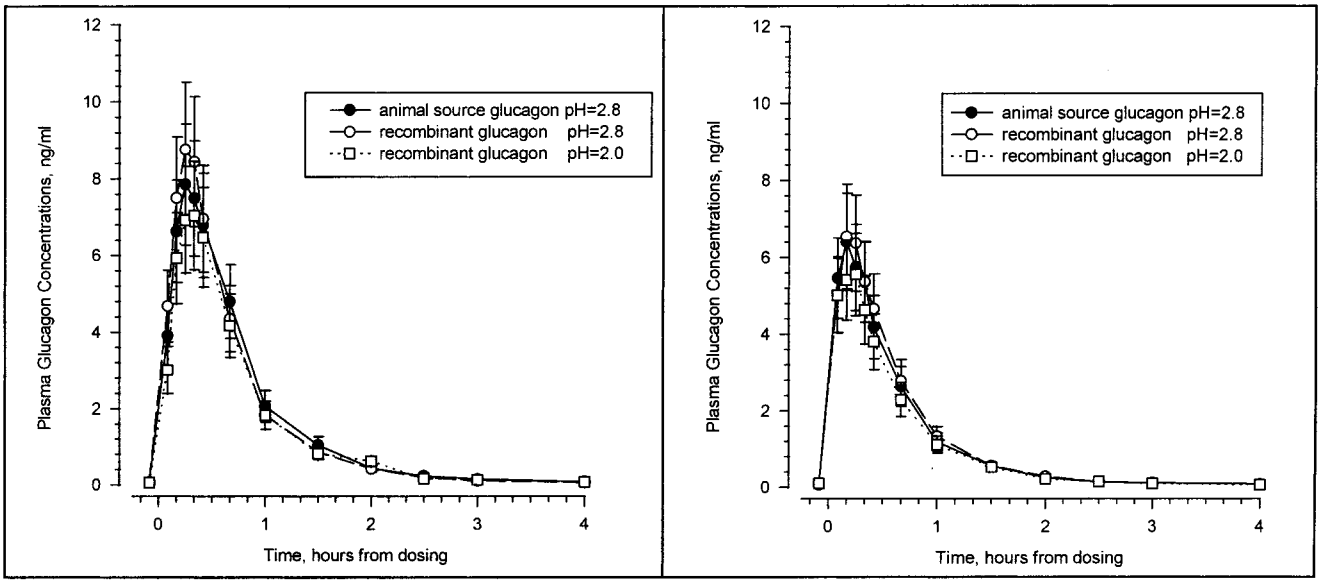

Figure 2—Mean plasma glucagon concentration versus time curves, all treatments (part II). All glucagon doses were 1.0 mg. Left panel shows subcutaneous (sc) administrations; right panel shows intramuscular (im) administrations. Bars indicate standard errors. $n = 25$. ● = animal-source glucagon pH 2.8, ○ = recombinant glucagon pH 2.8, □ = recombinant glucagon pH 2.0.

Table 2—Pharmacokinetic Parameters and Bioequivalence Assessments, Subcutaneous Administrations[a]

	$t_{1/2}$, h	C_{max}, ng/mL	t_{max}, h	$AUC_{(0-t)}$, ng·h/mL	$AUC_{(0-inf)}$, ng·h/mL
animal-source pH = 2.8 (A)	0.488 ± 0.166	9.12 ± 5.11	0.33 ± 0.10	6.57 ± 2.29	6.63 ± 2.30
recombinant pH = 2.8 (B)	0.451 ± 0.146	10.0 ± 3.65	0.27 ± 0.11	6.43 ± 2.15	6.47 ± 2.15
recombinant pH = 2.0 (C)	0.461 ± 0.166	7.94 ± 3.83	0.35 ± 0.098	5.82 ± 1.61	5.87 ± 1.62
		90% CI		90% CI	90% CI
B vs A[b]		102–126		91.4–109	91.2–109
C vs A[b]		79.1–97.8		82.4–98.2	82.4–98.1

[a] All data are reported as mean (±SD). All glucagon doses were 1.0 mg. [b] Comparisons reflect bioequivalence assessments based on log-transformed parameters. The specified range for any given parameter is the 90% confidence interval (90% CI) of the comparative ratios. If the interval falls between a range of 80% to 125%, it meets the standard bioequivalence criteria.

Table 3—Pharmacokinetic Parameters and Bioequivalence Assessments, Intramuscular Administrations[a]

	$t_{1/2}$, h	C_{max}, ng/mL	t_{max}, h	$AUC_{(0-t)}$, ng·h/mL	$AUC_{(0-inf)}$, ng·h/mL
animal-source pH = 2.8 (D)	0.414 ± 0.147	7.36 ± 2.51	0.22 ± 0.10	4.31 ± 1.36	4.36 ± 1.38
recombinant pH = 2.8 (E)	0.382 ± 0.100	7.81 ± 3.57	0.21 ± 0.11	4.62 ± 1.86	4.67 ± 1.87
recombinant pH = 2.0 (F)	0.364 ± 0.141	6.90 ± 2.64	0.22 ± 0.095	3.92 ± 1.48	3.97 ± 1.49
		90% CI		90% CI	90% CI
D vs E[b]		93.6–115		97.1–115	97.4–116
D vs F[b]		85.2–105		83.6–99.5	83.8–99.6

[a] All data are reported as mean (± SD). All glucagon doses were 1.0 mg. [b] Comparisons reflect bioequivalence assessments based on log-transformed parameters. The specified range for any given parameter is the 90% confidence interval (90% CI) of the comparative ratios. If the interval falls between a range of 80% to 125%, it meets the standard bioequivalence criteria.

maximum glucodynamic effect was present. Blood glucose returned to baseline value by 1 h in most subjects (Figure 1). There were no statistically significant differences among the four glucagon doses with respect to any glucodynamic parameters.

Part II—For Part II, 25 out of the 29 subjects successfully completed the study. Three subjects withdrew from the study for reasons unrelated to the study, and one patient withdrew due to difficult venipuncture. One subject was removed from the study due to nausea, dizziness, and pallor after a 1 mg dose of aG. rG and aG appeared to be equally safe and well tolerated. The most common adverse events reported were nausea, dizziness, and headache, occurring throughout treatment with both aG and rG.

Pharmacokinetics—Visual inspection of the mean glucagon concentration−time plots suggests that the profiles have similar absorption and elimination when comparing the glucagon formulations (aG, rG pH 2.8, and rG pH 2.0) for either route of administration (Figure 2). Slight differences in glucagon concentrations were noted between the injection routes with higher plasma concentrations occurring after sc administration. The absorption was rapid by either route, with maximum concentrations attained approximately 0.21 to 0.35 h after dosing.

A summary of the pharmacokinetic parameters is given in Table 2 and Table 3 for the sc and im routes of administration, respectively. rG pH 2.0 and pH 2.8 administered sc met the standard criteria for bioequivalence to aG with respect to $AUC_{(0-t)}$ and $AUC_{(0-inf)}$ and was nearly equivalent with respect to C_{max} comparisons. Following im administration, both rG formulations met standard bioequiv-

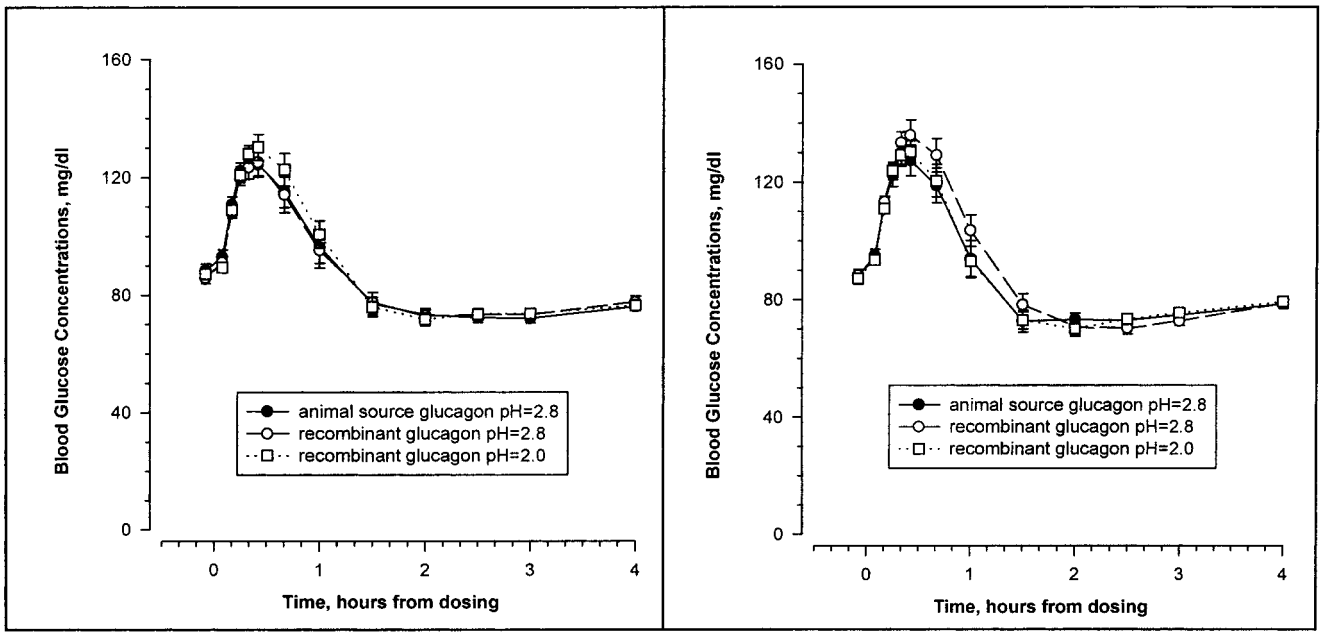

Figure 3—Mean blood glucose concentration versus time curves, all treatments (part II). All glucagon doses were 1.0 mg. Left panel shows subcutaneous (sc) administrations; right panel shows intramuscular (im) administrations. Bars indicate standard errors. $n = 25$. ● = animal-source glucagon pH 2.8, ○ = recombinant glucagon pH 2.8, □ = recombinant glucagon pH 2.0.

Table 4—Glucodynamic Parameters and Glucodynamic Equivalence Assessments, Subcutaneous Administrations[a]

	BG_{max}, mg/dL	TBG_{max}, h	$AUC_{(0-rtb)}$, mg·h/dL	MAE, mg/dL	TBG_{ex}, h	AUC_{ex}, mg·h/dL
animal-source pH = 2.8 (A)	133 ± 20.6	0.49 ± 0.46	126 ± 65.3	47.4 ± 19.2	0.60 ± 0.55	29.0 ± 25.7
recombinant pH = 2.8 (B)	132 ± 19.0	0.43 ± 0.18	130 ± 68.1	48.0 ± 13.6	0.60 ± 0.45	30.5 ± 21.4
recombinant pH = 2.0 (C)	136 ± 19.8	0.52 ± 0.54	151 ± 61.5	50.0 ± 18.5	0.59 ± 0.57	35.0 ± 20.5
	90% CI		90% CI	90% CI		90% CI
B vs A[b]	96.2–103		86.5–109	95.8–113		87.0–125
C vs A[b]	99.2–106		111–139	98.1–116		119–171

[a] All data are reported as mean (±SD). All glucagon doses were 1.0 mg. [b] Comparisons reflect bioequivalence assessments based on log-transformed parameters. The specified range for any given parameter is the 90% confidence interval (90% CI) of the comparative ratios. If the interval falls between a range of 80% to 125%, it meets the standard bioequivalence criteria.

Table 5—Glucodynamic Parameters and Glucodynamic Equivalence Assessments, Intramuscular Administrations[a]

	BG_{max}, mg/dL	TBG_{max}, h	$AUC_{(0-rtb)}$, mg·h/dL	MAE, mg/dL	TBG_{ex}, h	AUC_{ex}, mg·h/dL
animal-source pH = 2.8 (D)	137 ± 22.3	0.37 ± 0.14	136 ± 77.7	51.7 ± 17.4	0.59 ± 0.52	32.2 ± 25.9
recombinant pH = 2.8 (E)	143 ± 20.6	0.43 ± 0.15	147 ± 72.7	56.4 ± 16.8	0.61 ± 0.57	39.7 ± 29.4
recombinant pH = 2.0 (F)	138 ± 16.5	0.45 ± 0.16	129 ± 60.4	50.6 ± 16.3	0.67 ± 0.62	32.1 ± 22.8
	90% CI		90% CI	90% CI		90% CI
E vs D[b]	101–108		98.9–124	101–119		99.6–143
F vs D[b]	96.7–103		85.7–108	88.1–104		87.0–125

[a] All data are reported as mean (±SD). All glucagon doses were 1.0 mg. [b] Comparisons reflect bioequivalence assessments based on log-transformed parameters. The specified range for any given parameter is the 90% confidence interval (90% CI) of the comparative ratios. If the interval falls between a range of 80% to 125%, it meets the standard bioequivalence criteria.

alence criteria to aG with respect to all pharmacokinetic parameters.

The mean absolute bioavailability for the subcutaneous administrations of aG, rG pH 2.0, and rG pH 2.8 were 0.39, 0.35, and 0.38, respectively, using mean $AUC_{(0-inf)}$ measurements from the intravenous administrations as an index. Similar absolute bioavailability calculations for im administrations showed values of 0.25, 0.23, and 0.27 for of aG, rG pH 2.0, and rG pH 2.8, respectively.

Glucodynamics—All glucagon formulations produced nearly identical glucose response curves after sc or im administration (Figure 3). A summary of the glucodynamic parameters is given in Table 4 and Table 5 for the sc and im administrations, respectively. Comparison of glucodynamic response following sc administration showed that both rG formulations had equivalent BG_{max} and MAE values when compared to aG. Furthermore, aG and rG pH 2.8 were equivalent with respect to $AUC_{(0-rtb)}$ and glucose excursion AUC_{ex}. Compared with aG, rG pH 2.0 had a statistically greater $AUC_{(0-rtb)}$ and AUC_{ex}. All im administrations had statistically equivalent $AUC_{(0-rtb)}$ values. The rG pH 2.8 formulation showed greater BG_{max} and MAE values. However, since maximum activity appears to be achieved with low intravenous doses, this "greater" activity is likely a type I statistical error. Additionally, the AUC_{ex} of rG pH 2.0 was glucodynamically equivalent to that of aG.

Discussion

Historically, commercial glucagon has been produced through an extraction of beef and pork glands. However, recombinant DNA technology has led to an efficient process for producing pure glucagon. Moreover, the Food and Drug Administration has recently approved for marketing glucagon manufactured by that technology. The present study was designed to compare the pharmacokinetic and pharmacodynamic parameters of recombinant glucagon and animal-source glucagon. Since the sequence of animal-source glucagon is identical to that of recombinant glucagon, no difference in biological effect was expected when comparing the two formulations.

Part I of the study provided a dose-ranging assessment of rG given intravenously. Our analysis showed that rG exhibits linear disposition, with AUC and C_{max} increasing in a dose-proportional fashion. The half-life was short, with relatively high clearance (approximately 1 L/h) and small volume of distribution. The small volume of distribution is typical with proteins with their large molecular weight and polarity.

No dose−response was found with rG between the various intravenous doses. A maximum response was achieved by even the lowest dose tested, suggesting that the use of even small doses may induce hyperglycemia in patients with diabetes who use rG for radiologic procedures.

Part II of the present study demonstrated that both rG formulations administered sc were glucodynamically equivalent with respect to BG_{max} and MAE values when compared to aG. Furthermore, rG pH 2.8 was equivalent with respect to $AUC_{(0-rtb)}$ and glucose excursion AUC_{ex} when compared to aG. However, rG pH 2.0 induced higher glucose-related areas than aG following sc administration. When calculating $AUC_{(0-rtb)}$ and AUC_{ex}, it is necessary to assume that baseline glucose concentrations are stable within subject. In reality, fluctuations can occur in a subject's baseline, which can cause high intersubject variability in $AUC_{(0-rtb)}$ (41% to 53%) and AUC_{ex} (29% to 89%) and broad confidence intervals. For these reasons, the glucose-related areas may not be the most robust pharmacoynamic parameters for comparing the glucagon formulation.

TBG_{max} was observed to be greater than t_{max} after administration of glucagon by any route, although the discrepancy is smallest for sc and largest for iv. The size of this hysteresis and relative delay appears to be inversely related to the rate of appearance in the bloodstream (rate of absorption for sc and im routes) and is typical for a compound which exerts an effect in a tissue distant to where it is being measured. Nonetheless, the onset can still be considered rapid with the peak effects from the slowest absorption (sc) averaging approximately 30 min after dosing.

A review of the literature yielded a single study that investigated the pharmacokinetics and glucodynamics of a recombinant glucagon. Urae and colleagues[5] measured the pharmacokinetics and glucodynamics of a 1 mg dose of recombinant glucagon administered iv and sc (upper arm). Unlike the present study, recombinant glucagon was not compared to animal-source glucagon to determine bioequivalence. Minor differences in pharmacokinetic and glucodynamic parameters for rG pH 2.0 were noted compared to those determined by Urae et al. The glucodynamic parameter of $AUC_{(0-rtb)}$ for both iv and sc administrations was lower than those reported by Urae et al.[5] One possible explanation for this difference is the duration of blood sampling. In the Urae study, blood samples were collected over 720 min,[5] while in the present study blood samples were collected for only 240 min. Although we cannot verify the calculations of the Urae study, it appears that calculations were performed to the end of collection rather than the return to baseline. Both studies show rapid absorption of glucagon following sc administration (TBG_{max}, 0.34 ± 0.083 h and 0.52 ± 0.54 h, respectively). However, the mean BG_{max} was slightly lower in the present study when compared to the values determined by Urae et al.[5] (136 ± 19.8 mg/dL versus 160.4 ± 26.5 mg/dL, respectively). In general, mean values of pharmacokinetic parameters in the current study tended to be higher than those determined by Urae et al.[5] The minor discrepancies that were noted may be related to differences in assays, injection sites, and methods of injection.

In conclusion, we have demonstrated that glucagon produced through recombinant DNA technology demonstrates equivalent activity to the currently marketed animal-source glucagon following im and sc administration. Additionally, we have provided evidence of the pharmacokinetic equivalence between rG and aG when given by the im and sc routes. We have also shown the pharmacokinetic dose-proportionality of glucagon and that the glucose response appears to be saturated with even low doses of glucagon. Nonetheless, it is best to ensure the achievement of a maximum glucose response in emergency situations, with a clinical dose of 1 mg recommended.

References and Notes

1. Unger R. H.; Orci L. Glucagon. In *Joslin's Diabetes Mellitus*; Kahn C. R., Weir G. C., Eds.; Lea & Febiger: Philadelphia, 1994; p 170.
2. Marks V. Glucagon in the diagnosis and treatment of hypoglycemia. In *Glucagon II*; Lefebvre P. J., Ed.; Springer-Verlag: New York, 1983; pp 646−649.
3. Khan C. R.; Shechter Y. Insulin, oral hypoglycemic agents. In *The Pharmacological Basis of Therapeutics*, 8th ed.; Gilman A. G., Rall T. W., Nies A. S., Taylor P., Eds.; Pergamon Press: New York, 1990; pp 1488−1489.
4. Diamant B.; Picazo J. Spasmolytic action and clinical use of glucagon. In *Glucagon II*; Lefebvre P. J., Ed.; Springer-Verlag: New York, 1983; pp 611−634.
5. Urae A.; Irie S.; Makino K.; Amamoto T.; Morise H.; Sakata Y.; Urae R. Phase I study of glucagon (GL-G) produced by genetic engineering technology. *Jpn. J. Clin. Pharmacol. Ther.* **1991**, *22*, 451−458.

Acknowledgments

This work was sponsored by Eli Lilly and Company (Indianapolis, IN) and has been presented in part at the American Diabetes Association (ADA) 58th Scientific Sessions in Chicago, IL, 1998.

JS990077P