Comparison of the Pharmacokinetics and Pharmacodynamics of LY2963016 Insulin Glargine and EU- and US-Approved Versions of Lantus Insulin Glargine in Healthy Subjects: Three Randomized Euglycemic Clamp Studies

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OBJECTIVE

LY2963016 (LY IGlar) and Lantus (IGlar) are insulin glargine products manufactured by distinct processes but with identical amino acid sequences. Three studies evaluated the pharmacokinetic (PK) and pharmacodynamic (PD) similarity of LY IGlar and the European Union– and US-approved versions of IGlar.

RESEARCH DESIGN AND METHODS

These were three single-site, randomized, double-blind, two-treatment, four-period, crossover, euglycemic clamp studies. In each study, fasted healthy subjects received 0.5 units/kg s.c. doses of two different insulin glargine products on two occasions each, following a randomized sequence. A ≥7-day washout period separated the doses. Blood samples were collected predose and up to 24 h postdose to assess PK; PD was assessed by a euglycemic clamp lasting up to 24 h.

RESULTS

A total of 211 subjects participated in the three studies. The PK (area under the curve [AUC]; maximum observed concentration [C_{max}]) and PD (maximum glucose infusion rate [R_{max}]; total glucose infusion during the clamp [G_{tot}]) were similar between LY IGlar and IGlar, with the ratios of geometric means ranging from 0.90 to 0.95 for PK parameters and from 0.91 to 0.99 for PD parameters across studies. In all cases, the 90% CIs for the ratios of geometric means were completely contained in the prespecified acceptance limits of 0.80–1.25. Adverse events were similar between treatments.

CONCLUSIONS

These studies demonstrated that the PK and PD properties of LY IGlar and IGlar were similar after single 0.5 units/kg s.c. doses in healthy subjects, contributing to the totality of evidence supporting similarity of these products.

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Insulin glargine (Lantus; a registered trademark of Sanofi, hereafter referred to as "IGlar"), a long-acting basal insulin, is manufactured using recombinant DNA technology and is based on the human insulin amino acid sequence. IGlar is indicated for the treatment of patients with type 1 or 2 diabetes for the control of hyperglycemia (1).

LY2963016 (LY IGlar) is an insulin glargine product with an amino acid sequence identical to that of IGlar. A therapeutic protein molecule ("biologic") that is highly similar to a previously marketed product, with no clinically meaningful difference in safety or efficacy, is commonly referred to as a "biosimilar" (2). "Biosimilar" is a regulatory designation; LY IGlar may be designated as a biosimilar in some geographic regions (e.g., the European Union [EU]) and not in others (e.g., the US, where insulins are not classified as biologics) (3,4).

LY IGlar has been compared with IGlar in a comprehensive development program, including preclinical, phase 1, and phase 3 studies. The extensive preclinical comparison program demonstrated similarity between LY IGlar and IGlar in terms of chemical (structural and physicochemical properties, impurity profiles) and in vivo (toxicokinetics, repeat-dose toxicity profiles, carcinogenicity, and local tolerance testing) characteristics (5). In addition, in vitro data from binding affinity, functional, metabolic potency, and rat hepatoma mitogenesis assays showed that LY IGlar is similar to IGlar (5). Clinically, a series of studies assessed the pharmacokinetic (PK) and pharmacodynamic (PD) similarity of LY IGlar to IGlar in healthy subjects and subjects with type 1 diabetes. The safety and efficacy of LY IGlar have also been assessed in two randomized, controlled clinical trials in patients with type 1 and 2 diabetes. These phase 3 trials demonstrated equivalent efficacy, as measured by change in HbA_{1c} from baseline, of LY IGlar and IGlar, with the insulin glargine products demonstrating similar safety profiles, including immunological profiles, and effects on blood glucose levels and weight (6,7).

This article presents the results of three PK/PD studies conducted in healthy subjects as part of the LY IGlar development program. These studies were designed to collectively evaluate the similarity in the PK and PD of LY IGlar, the EU-approved version of IGlar, and the US-approved version of IGlar, to establish a scientific bridge between the three products and support comparisons made in global trials between LY IGlar and IGlar.

RESEARCH DESIGN AND METHODS

Study Design

These were three phase 1, single-site, randomized, double-blind, two-treatment, four-period, crossover, replicate euglycemic clamp studies, each evaluating the similarity in PK and PD of two insulin glargine products in healthy subjects (LY IGlar vs. EU-approved IGlar [clinical trial reg. no. NCT01476345], LY IGlar vs. US-approved IGlar [clinical trial reg. no. NCT01688635], and EU-approved IGlar vs. US-approved IGlar, respectively). In each study, subjects were randomly allocated to one of two treatment sequences, in which they received 0.5 units/kg s.c. doses of two different insulin glargine products (as listed above) on two occasions each. The study design is illustrated in Fig. 1.

Subjects were admitted to the clinical research unit on day −1 of each treatment period. On day 1, subjects received a single dose of LY IGlar or IGlar after an overnight fast of at least 8 h, and a euglycemic clamp procedure that lasted up to 24 h postdose was performed. Subjects were discharged on day 2 of each treatment period. A minimum of 7 days' washout separated each dose.

Study Drugs

All US-approved IGlar was purchased from a wholesaler in the US, and EU-approved IGlar was purchased in the EU from a wholesaler or directly from a pharmacy.

Study Subjects

The inclusion criteria varied slightly between studies, dependent on the geographical location at which the study was conducted. For the two studies in which US-approved IGlar was administered (both of which were conducted in Singapore), subjects were required to be overtly healthy men or women, aged 21–65 years, with a BMI between 18.5 and 29.9 kg/m^2 and a fasting plasma glucose value $<$108 mg/dL (6.0 mmol/L). For the study comparing LY IGlar and EU-approved IGlar (conducted in South Africa), subjects were required to be overtly healthy men or women, aged 18–60 years, with a BMI between 18.5 and 32.0 kg/m^2, HbA_{1c} levels $\leq$6.4% (46 mmol/mol), fasting plasma glucose levels $\leq$99 mg/dL (5.5 mmol/L), and a normal glucose tolerance.

The study protocols were approved by an institutional review board (National Healthcare Group Domain Specific Review Board, Singapore, or Ethics

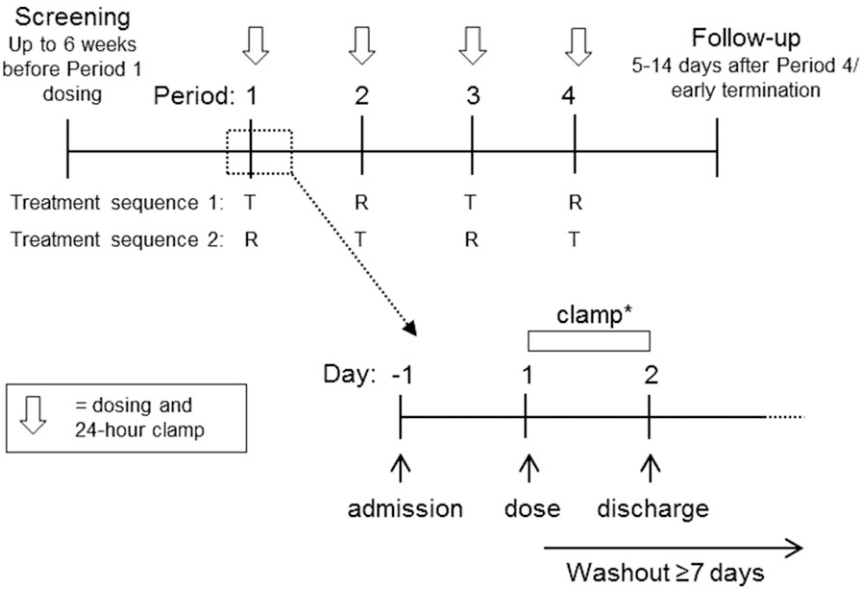

Figure 1—Study design. Schematic of study design for all three studies. The test and reference treatments for each study were LY IGlar vs. EU-approved IGlar, LY IGlar vs. US-approved IGlar, and EU-approved IGlar vs. US-approved IGlar, respectively. *Including collection of serial blood samples for pharmacokinetic and pharmacodynamic assessments. R, reference treatment; T, test treatment.

Committee of the Faculty of Health Sciences of the University of the Free State, South Africa) and were conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. All subjects provided written informed consent prior to participating.

Pharmacokinetic Data Collection

For the PK analysis, blood samples were collected prior to dosing (−0.5 h), at the time of dosing (0 h), and at 0.5, 2, 4, 6, 9, 12, 15, 18, 21, and 24 h postdose after the LY IGlar or IGlar dose on day 1 of each treatment period. Additional samples were taken at −1 h and at 3 h postdose in the study comparing LY IGlar with EU-approved IGlar.

Euglycemic Clamp Procedures

The euglycemic clamp procedure aimed to maintain blood glucose at a target level (equivalent to predose fasting glucose in 1 of the studies [LY IGlar vs. EU-approved IGlar] or 5 mg/dL [0.3 mmol/L] below the mean predose fasting glucose in the other 2 studies) for ∼24 h after administration of LY IGlar or IGlar by infusing intravenous glucose. During the clamp, the glucose infusion rate (GIR) was varied as necessary to maintain the blood glucose concentration for each subject within approximately ±5% of the target value to the extent possible. In this way, blood glucose concentrations were kept constant while the GIR varied, allowing the profile of GIR over time to be used as a direct measure of insulin action.

On the morning of day 1 in each treatment period, a forearm vein was catheterized for infusion of glucose, with another catheter placed in the contralateral arm for blood sampling. This area was heated to ∼55–60°C to "arterialize" the venous blood by opening arteriovenous anastomoses (8). This method helps minimize any differences in blood glucose concentrations between venous and arterial blood, an effect that may be particularly noticeable in the insulin-sensitive population used in these studies (9).

Blood samples were obtained at the bedside for immediate determination of whole blood glucose concentrations using an automated glucose oxidase technique (YSI2300; Stat Plus glucose analyzer, YSI Inc., Yellow Springs, OH). These blood samples were taken predose (−30, −20, −10, and 0 min; baseline was defined as the mean of the glucose measurements at these 4 time points), then at least every 10 min from 0 to 480 min (first 8 h), every 20 min until 900 min (8 to 15 h), and every 30 min until the end of the clamp (remaining 10 h), where 0 min was defined as the time of LY IGlar or IGlar dosing.

The glucose was infused as a 20% dextrose or glucose solution. Blood glucose concentrations and the GIR required to maintain euglycemia were documented throughout the procedure. If the GIR fell to zero for at least 30 min after the clamp had been underway for at least 8 h (or at least 4 h for the study comparing LY IGlar with EU-approved IGlar), the clamp was discontinued.

Subjects were fasted (apart from water) throughout the clamp procedure and were provided a meal at the end of the clamp. If the clamp was completed earlier than 24 h postdose, the meal was to be delayed until the last PK sample had been taken, unless the investigator deemed it necessary to administer the meal for safety reasons, to avoid confounding the bioanalytical assay by stimulating secretion of endogenous insulin.

A medical assessment was conducted prior to discharge from the clinical research unit to ensure subject safety.

Bioanalytical Methods

Serum samples were analyzed for immunoreactive insulin glargine (i.e., uncorrected [observed] serum insulin) using a validated, competitive radioimmunoassay [RIA] method at Covance Laboratories, Inc. (Chantilly, VA).

This RIA measured "free" immunoreactive insulin glargine (i.e., insulin and insulin analogs not bound to endogenous anti-insulin antibodies) in human serum. After dosing of either LY IGlar or IGlar, the RIA detected in human serum a combined response to insulin glargine, the active metabolites M1 (21A-Gly-insulin) and M2 (21A-Gly-des-30B-Thr-insulin), and endogenous insulin. The lower and upper limits of quantification were 50 and 2,000 pmol/L, respectively. Both precision and accuracy, as expressed, respectively, by the interassay coefficient of variation (CV%) and the interassay relative error, were ≤16.0% for the measurement of immunoreactive insulin glargine in human serum. The long-term stability of immunoreactive insulin glargine in human serum was demonstrated up to 12 months when stored at −15 to −30°C and −60 to −80°C. Freeze/thaw stability was demonstrated for immunoreactive insulin glargine in human serum through five cycles. Room-temperature stability was demonstrated for up to 24 h.

Pharmacokinetic Analyses

Because the studies were conducted in healthy subjects, it was necessary to correct serum immunoreactive insulin glargine (hereafter called "serum insulin") concentrations for endogenous insulin. To this end, each subject had blood samples taken for the measurement of serum C-peptide concentrations at the same time points as the PK samples. Correction for endogenous insulin concentrations using C-peptide concentration data was performed based on Owens' method (10) using the following equation:

$$[\text{LY IGlar or IGlar}] = [\text{serum insulin}] - F^{*}[\text{C-peptide}]$$

where F is the average of the ratios of serum insulin to C-peptide at baseline (where baseline was −30 and 0 min for the studies in which US-approved IGlar was administered and −60, −30, and 0 min for the study comparing LY IGlar with EU-approved IGlar). Values of insulin or C-peptide below the lower limit of quantification were set to half the lower limit of quantification. If an insulin value below zero was obtained after C-peptide correction, the value was excluded from the analyses.

Pharmacokinetic parameter estimates for LY IGlar and IGlar were calculated using standard noncompartmental methods with WinNonlin (version 6.3). The primary parameters for analysis were area under the concentration-time curve from zero to 24 h (AUC[0–24]) and maximum observed drug concentration (C_{max}). Additional PK parameters, such as apparent clearance (CL/F), apparent volume of distribution (V_z/F), and half-life ($t_{1/2}$), were also calculated.

Pharmacodynamic Analysis

A locally weighted scatterplot smoothing (LOESS) function was applied to all individual GIR-time profiles using TIBCO Spotfire S+ 8.2 for Windows. The fitted data for each subject were used to calculate the primary PD parameters, maximum GIR (R_{max}) and total glucose infusion over the clamp duration (G_{tot}), and a secondary PD parameter, the time of R_{max} (TR_{max}). Raw (that is, observed)

GIR values from each clamp procedure were used to calculate the other secondary PD parameters, such as the time of first change of GIR postdose (T_{onset}), the time of last measurable GIR (T_{last}), time to 50% maximal GIR before TR_{max} (early $TR_{max50\%}$), time to 50% maximal GIR after TR_{max} (late $TR_{max50\%}$), and the value of the last measurable GIR (GIR_{last}).

Safety Assessments

Safety assessments included physical examinations, clinical laboratory evaluations, vital signs, electrocardiograms, and adverse events (AEs).

Pharmacokinetic and Pharmacodynamic Statistical Analysis

Log-transformed AUC[0–24] and C_{max} were evaluated with a linear mixed-effects model including subject as a random effect with period, sequence, and treatment as fixed effects. For each parameter, the difference in least squares (LS) means along with the 90% CIs was backtransformed to produce the ratio of geometric means and the CI comparing treatments. Pharmacokinetic similarity was to be concluded if the 90% CIs for both AUC[0–24] and C_{max} were completely contained within the interval 0.80–1.25 (2,3).

A similar analysis was performed for R_{max} and G_{tot}. Sample sizes were planned to provide at least 90% power to demonstrate that the 90% CI of the ratios of key PK or PD parameters between treatments would be contained within 0.80–1.25.

A nonparametric approach based on the Hodges-Lehmann method was taken to evaluate time of C_{max} (t_{max}).

RESULTS

Demographics and Disposition

A total of 211 subjects participated in the three studies. Subject demographics are presented in Table 1.

Ninety-one subjects (85 males and 6 females) aged 22–62 years participated in the study comparing LY IGlar with US-approved IGlar, of whom 82 subjects completed the study. Three subjects were withdrawn owing to subject decision, two subjects were withdrawn owing to physician decision (inadequate venous access and noncompliance with study procedures, respectively), three subjects were withdrawn owing to dosing or glucose infusion errors, and one subject was withdrawn owing to an AE of lethargy not considered by the investigator to be related to study treatment.

Eighty subjects (56 males and 24 females) aged 18–60 years participated in the study comparing LY IGlar with EU-approved IGlar, of whom 78 subjects completed the study. Two subjects were withdrawn owing to subject decision.

Forty subjects (33 males and 7 females) aged 21–60 years participated in the study comparing US-approved IGlar with EU-approved IGlar, of whom 34 subjects completed the study. Two subjects were withdrawn owing to subject decision. Four subjects were withdrawn owing to physician decision; each of these subjects had one of their doses delayed owing to illness, and when they were well enough to be dosed the physician decided not to expose them to further study drug.

Pharmacokinetics

The mean C-peptide–corrected serum insulin concentration profiles were similar after administration of 0.5 units/kg s.c. LY IGlar, EU-approved IGlar, and US-approved IGlar (Fig. 2). Lucidi et al. (11) have reported that after injection, insulin glargine is rapidly and almost completely metabolized to M1 (21A-Gly-insulin) and that serum insulin concentrations measured by RIA closely correspond to circulating M1 concentrations. In all profiles, maximum concentrations of serum insulin were reached at 12 h (median), with serum insulin concentrations falling approximately twofold by 24 h postinjection.

Similar mean serum C-peptide profiles were observed for LY IGlar, E.U.-approved IGlar, and US-approved IGlar, indicating similar levels of endogenous insulin after administration of each of the three insulin glargine products (Supplementary Fig. 1).

Pharmacokinetic parameter summaries and analysis results are presented in Table 2. The PK parameters were similar between treatments; 90% CIs for the ratios of LS geometric means for AUC[0–24] and C_{max} were completely contained within the interval 0.80–1.25 for all three studies. There were no statistically significant differences in t_{max} between treatments, with the 95% CIs for the median differences in t_{max} between treatments containing zero. Overall, intrasubject variability for AUC[0–24] and C_{max} (as assessed by CV%) was similar between treatments within the respective studies and ranged from 24.1 to 30.3%. The secondary PK parameters are presented in Supplementary Table 1.

Pharmacodynamics

The mean GIR profiles during the euglycemic clamp procedure were similar after administration of 0.5 units/kg s.c. LY IGlar, EU-approved IGlar, and US-approved IGlar (Fig. 2). Each profile demonstrated sustained glucose-lowering activity over 24 h, with no pronounced peak. The time of R_{max} was similar between treatments, occurring between ~11 and 14 h postdose in all three studies.

Pharmacodynamic parameter summaries and analysis results are presented in Table 2. The PD parameters

Table 1—Subject demographics

	Study		
Demographics	LY IGlar vs. EU-approved IGlar (*N* = 80)	LY IGlar vs. US-approved IGlar (*N* = 91)	EU-approved IGlar vs. US-approved IGlar (*N* = 40)
Male/female (%)	70.0/30.0	93.4/6.6	82.5/17.5
Race (%)	80.0 white, 12.5 black, 7.5 multiple	98.9 Asian, 1.1 white	95.0 Asian, 5.0 white
Age (years)	32.0 ± 10.9	32.7 ± 9.0	31.9 ± 9.9
Weight (kg)	74.8 ± 12.5	70.2 ± 10.3	67.8 ± 9.4
BMI (kg/m^2)	24.9 ± 3.2	24.1 ± 2.8	23.8 ± 2.4

Data are means ± SD unless otherwise indicated. *N*, number of subjects.

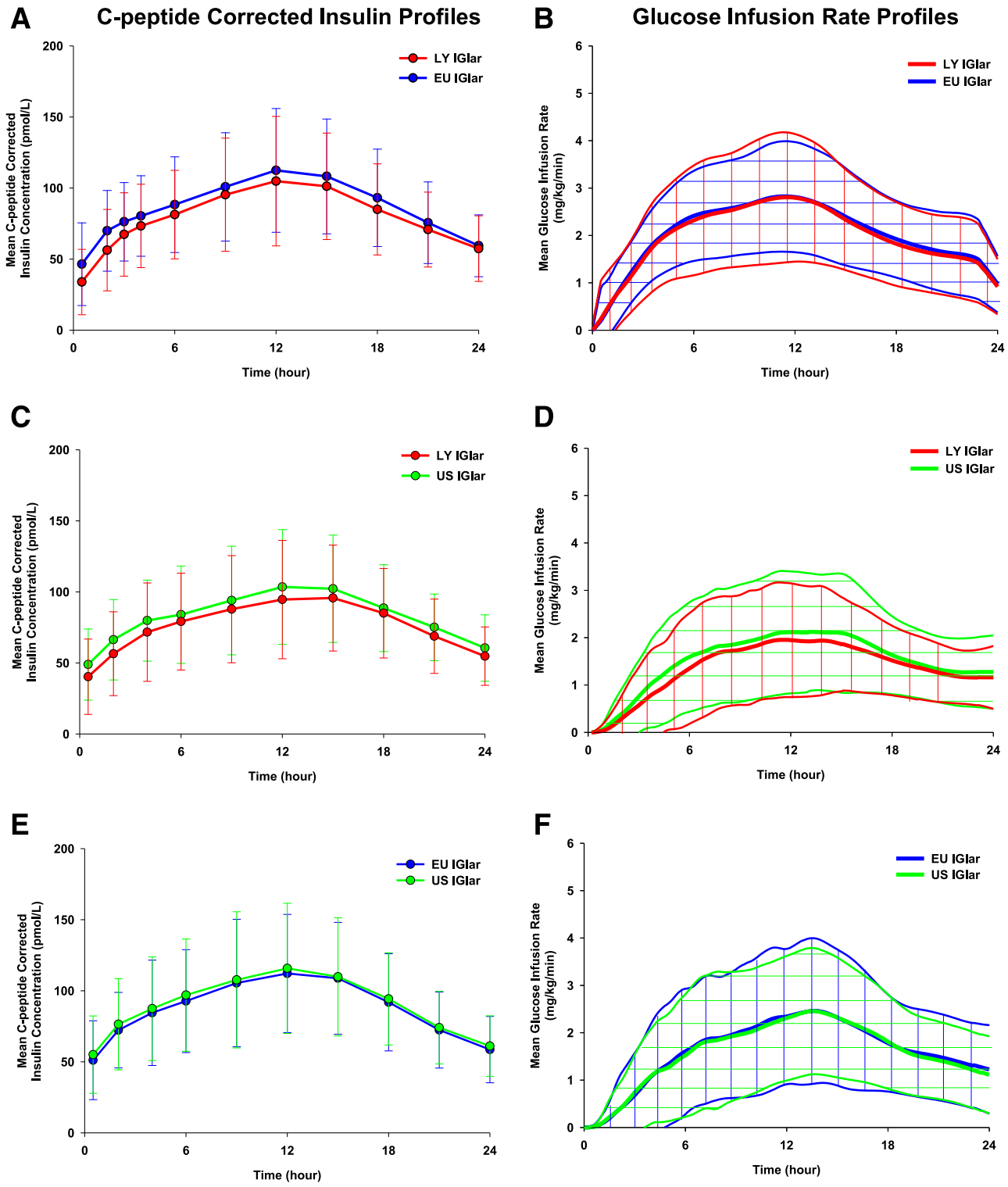

Figure 2—Mean ± SD C-peptide–corrected serum insulin concentrations and GIRs after administration of 0.5 units/kg s.c. LY IGlar, EU-approved IGlar, or US-approved IGlar in three 2-treatment, 4-period, crossover design studies. *A*, *C*, and *E*: Plots of mean C-peptide–corrected insulin (LY IGlar or IGlar [Lantus]) concentration in the 24 h after 0.5 units/kg s.c. administration of three insulin glargine products. (*A*, *C*, and *E* are from 3 separate studies conducted in separate groups of subjects.) *B*, *D*, and *F*: Plots of mean glucose infusion rate, a measure of insulin action, in the 24 h after 0.5 units/kg s.c. administration of 3 insulin glargine products. (*B*, *D*, and *F* are from 3 separate studies conducted in separate groups of subjects.)

were similar between treatments. The 90% CIs for the ratios of LS geometric means for G_{tot} and R_{max} were completely contained within the interval 0.80–1.25 for all three studies. Overall, intrasubject variability for G_{tot} and R_{max} (as assessed by CV%) was similar between treatments within the respective studies and ranged from 24.3 to 47.7%. The secondary PD parameters are presented in Supplementary Table 2.

Table 2—Comparison of the primary pharmacokinetic and pharmacodynamic parameters of LY IGlar, EU-approved IGlar, and US-approved IGlar in three 2-treatment, four-period, crossover design studies

Treatment (0.5 units/kg)	*N* (*n*)	Geometric mean (CV%)*	Ratio of LS geometric means (test†/reference) (90% CI)‡
Statistical analysis of pharmacokinetic parameters			
AUC[0–24] (pmol · h/L)			
LY IGlar†	87 (165)	1,720 (42)	0.90 (0.86, 0.94)
US IGlar	89 (167)	1,900 (35)	
LY IGlar†	79 (156)	1,810 (40)	0.91 (0.87, 0.96)
EU IGlar	80 (157)	1,980 (36)	
EU IGlar†	40 (75)	2,000 (35)	0.98 (0.91, 1.05)
US IGlar	40 (76)	2,060 (39)	
C_{max} (pmol/L)			
LY IGlar†	88 (167)	103 (41)	0.92 (0.87, 0.96)
US IGlar	89 (169)	111 (34)	
LY IGlar†	80 (158)	112 (39)	0.95 (0.90, 1.00)
EU IGlar	80 (158)	119 (34)	
EU IGlar†	40 (76)	120 (33)	0.99 (0.92, 1.06)
US IGlar	40 (77)	122 (37)	
t_{max} (h)§			
LY IGlar†	88	12.00	0.50 (−0.76, 1.25)
US IGlar	89	12.00	
LY IGlar†	80	12.00	0.00 (−0.75, 0.75)
EU IGlar	80	13.50	
EU IGlar†	40	12.00	−0.75 (−1.50, 0.50)
US IGlar	40	12.00	
Statistical analysis of pharmacodynamic parameters			
G_{tot} (mg/kg)			
LY IGlar†	88 (171)	1,670 (60)	0.91 (0.85, 0.98)
US IGlar	88 (170)	1,820 (74)	
LY IGlar†	80 (158)	2,580 (45)	0.95 (0.91, 1.00)
EU IGlar	80 (158)	2,710 (40)	
EU IGlar†	40 (76)	1,870 (84)	1.00 (0.89, 1.13)
US IGlar	40 (77)	1,880 (77)	
R_{max} (mg/kg/min)			
LY IGlar†	88 (171)	2.12 (54)	0.93 (0.88, 0.98)
US IGlar	88 (170)	2.27 (58)	
LY IGlar†	80 (158)	2.85 (46)	0.99 (0.94, 1.04)
EU IGlar	80 (158)	2.88 (41)	
EU IGlar†	40 (76)	2.35 (67)	0.97 (0.88, 1.07)
US IGlar	40 (77)	2.44 (63)	

n, number of observations; *N* = number of subjects. *Summary statistics of pharmacokinetic and pharmacodynamic parameters; does not reflect results of the statistical analysis. †The test treatment in each comparison. ‡Statistical model: log(parameter) = period + sequence + treatment + error, subject (random), period sequence treatment (categorical). §Median or median difference (95% CI) are presented for t_{max}. t_{max} was analyzed using a nonparametric approach based on the Hodges-Lehmann method. Analysis was based on subject's t_{max} values averaged across the 2 occasions where the same treatment was administered, if applicable.

Safety and Tolerability

No notable differences were observed in the AE profiles of LY IGlar and IGlar in healthy subjects, and no safety concerns were noted in the clinical laboratory evaluations, vital signs, or electrocardiogram data, in any of the three studies.

Six hypoglycemic events (blood glucose <54 mg/dL [3.0 mmol/L]) occurred during the glucose clamp procedure in a single study; the episodes were transient and quickly resolved with intravenous glucose infusion. The events were evenly divided between subjects receiving LY IGlar and those receiving IGlar. Four of the six events occurred within 15 min postdose, and the other two events occurred ~12 and 15 h postdose, respectively. Some of the hypoglycemic events, especially those that occurred immediately after injection, were associated with unusually high serum insulin concentrations, suggesting that hypoglycemia may have been caused by injection of study drugs into or near a blood vessel.

CONCLUSIONS

These three studies collectively provided an assessment of the PK and PD similarity of LY IGlar, EU-approved IGlar, and US-approved IGlar after single 0.5 units/kg s.c. doses in healthy subjects and established a scientific bridge between the three insulin glargine products as part of a series of clinical trials in healthy subjects and patients with diabetes. The findings of these studies contributed evidence to support the approval of the first biosimilar insulin analog in the EU (September 2014) (12).

The results demonstrate similarity in the systemic exposure (as measured by AUC[0–24] and C_{max}), absorption kinetics (t_{max}), and PD parameters (G_{tot} and R_{max}) of LY IGlar, EU-approved IGlar, and US-approved IGlar. Although PK and PD parameters were slightly lower for LY IGlar compared with IGlar (with LS geometric mean ratios ranging from

0.90 to 0.95 for AUC[0–24] and C_{max} and from 0.91 to 0.99 for G_{tot} and R_{max} across studies), the numerical differences are within the range of variability for these parameters. For further understanding of whether these differences may be of clinical relevance, a mechanistic model-based approach was applied to translate the GIR-time profiles of LY IGlar and IGlar during the clamp procedures into end points of more clinical relevance, namely, 24-h blood glucose profiles and HbA_{1c}. Simulations were performed using a physiology-based model of plasma glucose metabolism, which includes equations representing glucose absorption, hepatic glucose production, muscle glucose uptake, brain and splanchnic glucose uptake, and urinary glucose excretion as well as regulation by plasma insulin and glucagon (13). The result suggested that as much as a 20% difference in G_{tot} does not lead to meaningful differences in 24-h glucose profiles or HbA_{1c} after single- or multiple-dose administration of LY IGlar and IGlar (data on file). The lack of clinically relevant differences is further supported by the results of a phase 3 study in patients with type 2 diabetes, which showed that the 7-point self-monitored blood glucose levels were not higher with LY IGlar compared with IGlar (7).

In accordance with regulatory guidance, the statistical assessment of PK/PD similarity is based on the 90% CI for the ratio of the test and reference products being contained within the predefined acceptance limits 0.8–1.25 (2,3); this criterion was met for all primary PK and PD parameters in all studies. The conclusion of PK/PD similarity is supplemented by the results of phase 3 studies in which equivalent efficacy was shown between LY IGlar and IGlar, as measured by change in HbA_{1c} from baseline at end point, with similar overall rates/incidence of hypoglycemia and mean change in insulin dose from baseline (6,7).

The PK and PD profiles and parameters of IGlar observed in these studies are consistent with those previously reported after a single IGlar dose (11,14–17). No safety concerns were noted in the present studies; furthermore, similar safety profiles were demonstrated in phase 3 studies between LY IGlar and IGlar in patients with type 1 and 2 diabetes for up to 52 weeks (6,7).

It is conceivable that differences may exist in drug products approved in different regions, and such differences could potentially affect their relative safety, efficacy, PK, and/or PD profiles. As there are no published data demonstrating the similarity between EU- and US-approved IGlar, it was important from both a regulatory and a scientific perspective to establish a scientific bridge between the three products. A three-study approach was selected for this purpose; a single study comparing all three products would not have been feasible, as it would have exceeded the clamp capacity for a single clinical site and required unfeasibly large blood sampling volumes. It may also have introduced unacceptable clamp technique variability owing to the need for multiple sites.

Each study used a replicate design to reduce the number of subjects exposed to study drug and euglycemic clamp procedures, while maintaining the necessary statistical power to demonstrate similarity between treatments. The study conditions were strictly standardized, and the use of a crossover design allowed each subject to act as his or her own control, reducing variability in the measured parameters. The double-blinding method avoided a potential source of bias in the results, ensuring that subjects and clinical site staff remained blind to the treatment allocations for individual subjects.

A single-dose regimen was considered appropriate because there is no evidence that the PK of IGlar is nonlinear in time; therefore, single-dose PK of IGlar should be a good predictor of PK at steady state. The dose of 0.5 units/kg LY IGlar or IGlar was selected, as this dose yields measurable insulin serum concentrations, is a clinically relevant dose, and is within the range of doses used in phase 3 trials (mean dose at study end points of 0.36–0.50 units/kg/day) (6,7). Insulin glargine has been administered at 0.3–0.6 units/kg to healthy subjects in similar euglycemic clamp trials (18,19).

The use of a hyperinsulinemic-euglycemic clamp technique allowed direct assessment of the glucodynamic properties of LY IGlar and IGlar. The euglycemic clamp is a long-standing, widely used technique to evaluate insulin action and is considered the reference standard for determining insulin sensitivity in human subjects (9). The technique is recommended by the European Medicines Agency for use in clinical pharmacology studies aiming to demonstrate biosimilarity between two insulins (3). The quality of the clamp performance is critical for the interpretation of data; the three clamp studies were well executed, as evidenced by mean blood glucose concentrations of 78.3–81.4 mg/dL (4.3–4.5 mmol/L) and intrasubject CV% of 4.8–5.7%, for each treatment across studies during the 24-h procedure. Consistent with the currently approved once-daily dosing indication for IGlar (1), a clamp period of 24 h was chosen to capture a clinically meaningful dosing interval while minimizing the length of time for which subjects were required to fast and be subjected to intensive clamp procedures. It would be of interest to evaluate the duration of action exceeding 24 h; such an assessment should ideally be performed in patients with type 1 diabetes owing to the lack of interfering endogenous insulin. A study that compared the duration of action of LY IGlar and IGlar for up to 42 h in patients with type 1 diabetes demonstrated similar duration of action of the two insulin glargine products; the PD characteristics remained comparable between treatments during this period (20).

The studies were performed in healthy subjects; this population is homogenous and insulin sensitive and was therefore the most sensitive population in which to detect any treatment-related differences. Regulatory guidance recommends using healthy subjects in bioequivalence studies (21,22), and biosimilar guidance recommends the use of either healthy subjects or patients with type 1 diabetes in studies comparing similar insulin products, with the caveat that action may be required to adjust for or suppress endogenous insulin if healthy subjects are included (3). In the present studies, a correction was applied to the assayed insulin concentrations using the serum C-peptide concentration, based on Owens' method (10). This method is well established and has been applied in studies of insulin glargine in healthy subjects for the estimation of endogenous insulin (15,23). Therefore, the estimation of LY IGlar and IGlar concentrations by means of C-peptide correction was considered reasonable and reliable. Further, immunoreactive LY IGlar and immunoreactive

IGlar concentrations (uncorrected for endogenous insulin) (Supplementary Fig. 2) and C-peptide concentrations (Supplementary Fig. 1) were similar between treatments, consistent with the C-peptide–corrected insulin data.

In summary, these studies demonstrated that the PK (AUC[0–24] and C_{max}) and PD (R_{max} and G_{tot}) properties of LY IGlar, EU-approved IGlar, and US-approved IGlar were similar after single 0.5 units/kg s.c. doses in healthy subjects, contributing to the totality of evidence supporting similarity of these products.

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