Basal Insulin Peglispro Demonstrates Preferential Hepatic Versus Peripheral Action Relative to Insulin Glargine in Healthy Subjects

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OBJECTIVE
We evaluate the endogenous glucose production (EGP) and glucose disposal rate (GDR) over a range of doses of basal insulin peglispro (BIL) and insulin glargine in healthy subjects.

RESEARCH DESIGN AND METHODS
This was a single-center, randomized, open-label, four-period, incomplete-block, crossover study conducted in eight healthy male subjects. Subjects had 8-h euglycemic clamps performed with primed, continuous infusions of BIL (5.1 to 74.1 mU/min) in three dosing periods and insulin glargine (20 or 30 mU/m²/min) in a fourth period, targeted to achieve 50–100% suppression of EGP. D-[3-3H] glucose was infused to assess rates of glucose appearance and disappearance.

RESULTS
Mean BIL and insulin glargine concentrations (targeted to reflect the differences in intrinsic affinities of the two basal insulins) ranged from 824 to 11,400 and 212 to 290 pmol/L, respectively, and increased accordingly with increases in dose. Suppression of EGP and stimulation of GDR were observed with increasing concentrations of both insulins. At insulin concentrations where EGP was significantly suppressed, insulin glargine resulted in increased GDR. In contrast, at comparable suppression of EGP, BIL had minimal effect on GDR at lower doses and had substantially less effect on GDR than insulin glargine at higher doses.

CONCLUSIONS
The novel basal insulin analog BIL has relative hepatopreferential action and decreased peripheral action, compared with insulin glargine, in healthy subjects.

In vivo, insulin is secreted from pancreatic β-cells and enters the circulation via the portal vein, where on first pass the liver extracts ~40–80% (1–7). As a result, systemic circulating insulin levels are reduced compared with those in the portal vein, and subsequent insulin action in the peripheral target tissues is also reduced compared with the liver. Consequently, the relative ratio of hepatic action to peripheral action ranges between 2:1 and 4:1 (1,2,4,8–10). In contrast, when exogenous insulin is administered peripherally, insulin is distributed equally across the liver and peripheral tissues (11,12) and thus does not mimic normal physiology.

1Center for Metabolic Research, VA San Diego Healthcare System, San Diego, CA
2University of California, San Diego, La Jolla, CA
3Eli Lilly and Company, Indianapolis, IN
4Eli Lilly and Company, Singapore, Singapore
Corresponding author: Helle Linnebjerg, linnebjerg_helle@lilly.com.
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V.P.S. is currently affiliated with the Office of Clinical Pharmacology, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Silver Spring, MD.
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Intraperitoneal administration of insulin via implanted infusion pumps has been pursued experimentally (13,14) to attempt to restore this physiologic balance and provide the potential benefits compared with existing exogenous insulin therapy (15).

Basal insulin peglispro (BIL; LY2605541), a novel, polyethylene glycolylated insulin analog currently in phase III, has a hepatic preferential action and decreased peripheral action in an insulinopenic dog model (16). The half-life of BIL is ~2 to 3 days (17) and therefore requires ~7 to 10 days to reach steady state when administered subcutaneously. However, under experimental conditions for short-term studies, more rapid attainment of steady state can be achieved by intravenous infusion. BIL has reduced affinity for the insulin receptor compared with insulin lispro (18) and a volume of distribution that is only slightly larger than the circulating blood volume (19), thus lower than that reported for insulin lispro and regular human insulin (20). The higher systemic circulating BIL levels (like insulin detemir [15]) reflect the differences in vitro insulin receptor–binding affinity (Ki) and volume of distribution. In phase II trials in type 1 and type 2 diabetes, in which BIL showed similar glycemic-lowering to insulin glargine (21,22), the steady-state BIL concentrations were estimated to be ~3,200 and 4,300 pmol/L, respectively.

Additionally, these phase II studies (21,22) demonstrated that BIL compared with insulin glargine was associated with lower rates of nocturnal hypoglycemia and weight loss and a lack of suppression of serum triglycerides. In patients with type 1 diabetes (21), these findings were also associated with a modest increase in LDL cholesterol, a modest decrease in HDL cholesterol, and a higher rate of total hypoglycemia, which were noted with both a lower hemoglobin A1c (GHb) and lower dose of prandial insulin. All these findings led to the speculation that BIL may have lower peripheral action, which may result in a relative hepatopreference compared with insulin glargine. This hypothesis was first confirmed in an acute dog model of insulinopenic diabetes (somatostatin and portal glucagon-infused conscious, fasted dogs), where intravenous infusions of BIL and human insulin were compared (16). The results suggested that BIL may restore the physiologic ratio of hepatic to peripheral insulin action.

The aim of this present study was to determine in humans a dose response of intravenous infusions of BIL compared with an intravenous infusion of insulin glargine with regard to suppression of endogenous glucose production (EGP), whole body glucose disposal rate (GDR), and glucose infusion rate (GIR).

**RESEARCH DESIGN AND METHODS**

**Study Subjects**

This study was conducted in accordance with the International Conference on Harmonization Guidelines for Good Clinical Practice and the Declaration of Helsinki (23). All procedures were approved by the Institutional Review Board at the University of California, San Diego, Human Research Protections Program, and all subjects provided written, informed consent.

Healthy male subjects between the ages of 23 and 27 years, inclusive, were screened and enrolled in this randomized, open-label, four-period, incomplete-block crossover study. Subjects were required to be overtly healthy (as determined by medical history and physical examination), have a BMI of 20.0 to 29.9 kg/m², and have a fasting blood glucose <108 mg/dL (6.0 mmol/L). Subjects were excluded if their GHb level was <12.0 g/dL or if they had used over-the-counter or prescription medication within 7 and 14 days, respectively, prior to dosing.

**Study Protocol**

Healthy subjects who met the study enrollment criteria were admitted to the clinical research unit in the afternoon/evening prior to dosing. Subjects fasted overnight for ~8 h and then received a continuous infusion of D-[3,3H] glucose for ~4 h to label the glucose pool prior to determination of the rate of EGP. Plasma glucose–specific activity was measured at ~30, ~20, and ~10 min and immediately prior to BIL or insulin glargine dosing. Measurement of serum glucose, serum immunoreactive insulin, and C-peptide was also obtained during the basal period. Following basal measurements, the continuous infusion of D-[3,3H] glucose was continued for the duration of the glucose clamp study. Subjects received an intravenous infusion of BIL or insulin glargine given in a primed continuous fashion, as described in Table 1. Euglycemia was maintained at 90 mg/dL (5.0 mmol/L) using a simultaneous variable intravenous infusion of 20% dextrose containing D-[3,3H] glucose, as described previously (24). Blood samples were collected at selected time points to measure the plasma glucose, plasma glucose–specific activity, and C-peptide levels. Blood samples were collected for the determination of drug concentrations at 20, 40, and 60 min and 2, 3, 4, 6, and 8 h postdose (BIL and insulin glargine) and also at 9 and 10 h postdose (BIL only). There was a minimum of 6 days of washout between doses. Safety assessments included monitoring of adverse events (AEs), physical examinations, clinical laboratory evaluations, vital signs, and electrocardiogram parameters.

**Study Treatments**

Subjects were randomized to either cohort A or B and received intravenous BIL in the first three dosing periods and intravenous insulin glargine in the fourth period (Table 1). Based on BIL pharmacokinetics after intravenous administration (19), infusion rates were selected to attain steady-state BIL concentrations of 2,000, 4,000, 6,000, 8,000, 10,000, and 12,000 pmol/L, respectively.

**Table 1—Treatment sequences**

<table>
<thead>
<tr>
<th>Sequence/cohort</th>
<th>Period 1 BIL</th>
<th>Period 2 BIL</th>
<th>Period 3 BIL</th>
<th>Period 4 insulin glargine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cohort A (n = 4)</td>
<td>Priming dose</td>
<td>660 mU</td>
<td>1,320 mU</td>
<td>2,000 mU</td>
</tr>
<tr>
<td></td>
<td>Constant infusion</td>
<td>5.1 mU/min</td>
<td>10.2 mU/min</td>
<td>15.3 mU/min</td>
</tr>
<tr>
<td>Cohort B (n = 4)</td>
<td>Priming dose</td>
<td>2,000 mU</td>
<td>4,000 mU</td>
<td>8,000 mU</td>
</tr>
<tr>
<td></td>
<td>Constant infusion</td>
<td>15.3 mU/min</td>
<td>37.0 mU/min</td>
<td>74.1 mU/min</td>
</tr>
</tbody>
</table>

*The priming dose was given over ~10 min. b The constant infusion for both BIL and insulin glargine was given over ~8 h.
8,000, and 10,000 pmol/L. Insulin glargine infusion rates were selected based on a previous publication (24), the dose of 20 mU/m²/min glargine was selected to approximate the ED₃₀ to achieve half-maximal suppression of EGP, and the dose of 30 mU/m²/min glargine was chosen to achieve near-maximal suppression of EGP.

**Analysis**

Serum concentrations of BIL were analyzed at Charles River in Senneville, Quebec (Canada) using a validated ELISA specific for BIL with no cross-reactivity with human insulin. The lower limit of quantification was 20 pmol/L, and the upper limit of quantification was 500 pmol/L. The interassay accuracy (percentage relative error) during validation ranged from −1.4 to 3.5%. The interassay precision (percentage relative SD) during validation ranged from 5.2 to 8.5%.

Glargine concentrations were analyzed at Covance Laboratories Inc., Chantilly, VA, using a validated radioimmunoassay. The lower limit of quantification was 50 pmol/L, and the upper limit of quantification was 2,000 pmol/L. The radioimmunoassay used to measure insulin glargine was nonspecific, and concentrations represent insulin glargine, its two main metabolites (M1 and M2), and endogenous and exogenous human insulin. The interassay accuracy (percentage relative error) during validation ranged from 0.9 to 15.0%. The interassay precision (percentage relative SD) during validation ranged from 7.1 to 16%. The insulin glargine concentrations were corrected for endogenous insulin based on C-peptide measurements.

C-peptide concentrations were analyzed by Covance Central Laboratory Services, Indianapolis, IN, using ADVIA Centaur C-Peptide assay. The assay is a two-site sandwich immunoassay using direct chemiluminescent technology.

The primary pharmacodynamic measurement in this study was the evaluation of the glucodynamic response to the study insulins during the euglycemic clamp, including the assessment of EGP and glucose turnover. Plasma glucose was analyzed using a YSI 2300 STAT Plus glucose analyzer (YSI Inc., Yellow Springs, OH). Glucose turnover was calculated using modified Steele equations for non-steady-state conditions (24–26), where Rₑ is EGP and R₉ is the GDR. In the basal state, Rₑ equals EGP. During the insulin infusion, the rate of EGP is calculated as the difference between the Rₑ and the GIR. EGP values less than 0 were imputed to 0 to indicate full suppression of EGP, as, conceptually, EGP less than 0 is implausible. Averages of preclamp measurements were used to establish basal rates. The average BIL concentration, C-peptide–corrected glargine concentrations, EGP, and GDR were calculated using the last 2 h of the clamp when serum concentrations of study insulins, EGP, and GDR had stabilized.

Safety parameters were listed and summarized using standard descriptive statistics.

**RESULTS**

**Subject Characteristics**

A total of eight male subjects completed the study. Subjects had a mean ± SD age of 26.1 ± 1.5 years, mean BMI of 24.4 ± 1.8 kg/m², and mean fasting blood glucose 89.25 ± 4.76 mg/dL (5.26 ± 0.28 mmol/L) prior to dosing.

**Pharmacokinetic and Glucodynamic Results**

Figure 1 presents a panel of time-course profiles for the serum concentrations (pmol/L) and GIRs (mg/kg/min) in healthy subjects following the intravenous infusion of different doses of BIL or insulin glargine.

As shown in Fig. 1A and C, following the 10-min priming infusion, serum concentrations of BIL and insulin glargine remained stable during the 8-h constant infusion. Average concentrations tended to increase with increased doses of BIL and insulin glargine. Average concentrations ranged between 824 and 11,400 pmol/L for BIL (Fig. 1A), and the average C-peptide–corrected insulin glargine concentrations were 212 and 290 pmol/L, respectively (Fig. 1C).

GIR versus time profiles are shown in Fig. 1B and D for BIL and insulin glargine, respectively. At the lowest dose of BIL, GIR rapidly (∼1 h) stabilized; intermediate doses required 3 to 4 h for the response to stabilize; while at the highest BIL dose, there was a tendency for GIR to increase for at least 6 to 7 h. For insulin glargine, GIR increased over time until reaching stable values at ∼3 h.

Figure 2 shows the time-course profiles for EGP and GDR and relative responses (percentage EGP suppression, fold change from baseline in GDR stimulation) during infusion of BIL or insulin glargine. BIL suppressed EGP in a time- and dose-dependent manner. Except for the lowest doses of BIL, essentially complete suppression of EGP was reached within 1 to 2 h of exposure to BIL or insulin glargine. Differences between the effects of BIL and insulin glargine were observed with regard to stimulation of GDR. The lower BIL doses had essentially no effect on GDR, while the highest BIL dose showed a gradual increase in GDR that continued to rise up to 8 h of exposure. Both dose levels of insulin glargine resulted in substantial increases in GDR with values up to 15 mg/kg/min, considerably higher than the largest BIL response (6 mg/kg/min) (Supplementary Fig. 1B).

These differences between BIL and insulin glargine action are accentuated by comparing concentration–response curves for percentage EGP suppression (Fig. 3A) and fold GDR stimulation (Fig. 3B). The x-axis of the figures present the concentrations of BIL or insulin glargine measured in the circulation at steady state for each separate infusion, while the y-axis is the activity measured in the same procedure. With regard to EGP, both BIL and insulin glargine were able to attain 100% suppression, though the curve for BIL was right shifted by ∼15-fold compared with insulin glargine. The insulin glargine–concentration response curve for stimulation of GDR (Fig. 3B) was similar to that for suppression of EGP (Fig. 3A), with maximal effects of greater than sixfold increase above basal rate. Two differences are apparent for BIL action on GDR when compared with insulin glargine. The first is that the maximal effect observed, predominately an approximately twofold increase, is much lower than that attained with insulin glargine, even at serum concentrations where suppression of EGP is similar. The second is that the BIL concentration–response curve for stimulation of GDR is shifted to the right of that for insulin glargine. The BIL concentration–response relationship for GDR was observed to be nonlinear when analyzed using a linear regression model (with random effects for subjects) fitted to the log-transformed
GDR values and log-transformed BIL concentrations (slope of 0.372; \( P < 0.001 \)).

Figure 3C and D show similar concentration-response curves to Fig. 3A and B when using the equivalent human insulin concentration to compensate for the difference in receptor binding between BIL, insulin glargine, and human insulin (19). Equivalent human insulin concentrations were calculated by dividing each study insulin concentration by its Ki and multiplying by the Ki of human insulin. As a result, the right shift observed in Figs. 3A and B was corrected, with the relative slopes of these relationships being maintained. Therefore, both BIL and insulin glargine were able to attain 100% suppression of EGP, while the simultaneously observed GDR was substantially less for BIL than for insulin glargine.

There were four subjects (50%) who experienced at least one AE during the study. Three subjects experienced AEs related to study procedures; no drug-related AEs were reported. All except one of the AEs were mild in severity and resolved by the end of the study. No subjects discontinued due to an AE and there were no serious AEs or deaths reported during the study. The most frequently reported AE was headache (nervous system disorders). No clinical laboratory parameter, physical examination, vital sign, or electrocardiogram findings were considered to be clinically significant by the investigator.

CONCLUSIONS

The primary aim of the current study was to evaluate suppression of EGP and stimulation of GDR over a dose range of BIL and insulin glargine in healthy subjects. At circulating concentrations of BIL similar to those observed in the phase II studies (19,27), comparable suppression of EGP was achieved by BIL and insulin glargine. Importantly, for similar EGP suppression, the effects on GDR between BIL and insulin glargine were substantially different. Similar relationships between equivalent human insulin concentrations of BIL and insulin glargine were demonstrated with suppression of EGP, but the same human insulin equivalent BIL concentration provided much less effect on GDR than did insulin glargine in healthy subjects.
These findings demonstrate that BIL has a relative hepatopreferential action with reduced peripheral action in healthy subjects compared with insulin glargine. In contrast, insulin glargine delivered intravenously has been shown to have similar hepatic and peripheral effects to intravenous human insulin (24).

These observations in healthy human subjects support those noted in an acute dog model of insulinopenic diabetes, where GIRs were matched between peripheral intravenous infusions of BIL and human insulin (16). Under these conditions, BIL was found to produce a more rapid suppressive effect on hepatic glucose production accompanied by a lesser, more delayed effect on peripheral action. These findings in the dog were corroborated by two methodologies: the same radioisotopic method used in this present study and direct organ balance (liver and hindlimb) obtained through invasive catheterization. The results obtained in this dog experiment suggested that BIL may restore the physiologic ratio of hepatic to peripheral insulin action.

Some insulin analogs have been demonstrated to have a hepatopreferential effect based on the hypothesis that molecular size or binding to other proteins may inhibit permeability into the periphery. For example, dimerization of the insulin molecule and a thyroxyl-insulin analog, which binds to thyroid hormone–binding proteins, has shown relative hepatopreferential activity, but these insulins apparently have not been further developed for use in humans (28–30). The binding to albumin by insulin detemir has also been used to explain a mild hepatopreferential action of that insulin analog under hypoglycemic conditions (31). Additionally, the insulin precursor, proinsulin, has demonstrated a greater effect on EGP than GDR (32,33).

Figure 2—Time courses of suppression of EGP and stimulation of GDR by varying infusions of BIL and insulin glargine. Results are presented as absolute values (EGP and GDR), percentage reduction in EGP from the baseline (percentage EGP suppression), or increase over basal GDR (fold change in GDR), mean ± SD. A: EGP absolute values over the infusion period. B: GDR absolute values over the infusion period. C: Percentage EGP suppression over the infusion period. D: Fold increase in GDR over the infusion period.
predetermined doses of BIL to compare with known EGP effects of insulin glargine doses, and therefore a wider spectrum of BIL dosing was used. Because of the rigors of the euglycemic clamp technique in combination with radioisotopic tracer infusions, subjects could not receive all doses, and an incomplete-block design was used in this study. Only male subjects were allowed to participate to limit the impact of variations of insulin sensitivity over the menstrual cycle on the course of the study. The radioisotopic techniques along with the Steele calculations used in this study lack the precision of the organ balance techniques used in the dog study (16), which are extremely invasive procedures and inappropriate for human studies. Lastly, these studies were conducted in healthy subjects, thus these findings require confirmation in patients with both type 1 and type 2 diabetes. At the highest doses of BIL, stable values of GIR may not have been achieved during the 8-h clamp.

In conclusion, this study in healthy subjects supports the findings of studies performed in an acute, insulinopenic dog model, which demonstrated the hepatopreferential action of BIL. Importantly, the hepatopreferential action of BIL results from a lesser peripheral action compared with human insulin and insulin glargine and not an accentuated or enhanced effect on the liver itself. A hepatopreferential insulin analog could offer several advantages over other insulins (15), such as the weight benefit, lower rate of nocturnal hypoglycemia, and decreased prandial insulin requirements that were observed in the BIL phase II trials (21,22).

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Figure 3—Relationship between percentage EGP suppression, fold GDR stimulation, and drug concentration (pmol/L), with equivalent human insulin concentration (pmol/L) for individual subjects. Each data point represents individual subject results for each infusion rate. The means of insulin concentrations, percentage EGP suppression, and fold GDR increase for each dose are connected by lines for each study insulin. Equivalent human insulin concentrations were calculated by dividing each study insulin concentration by its Ki and multiplying by the Ki of human insulin. A: Percentage EGP suppression versus serum concentration. B: Fold increase in GDR versus serum concentration. C: Percentage EGP suppression versus equivalent human insulin concentration. D: Fold increase in GDR versus equivalent human insulin concentration.
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