ORIGINAL ARTICLE doi: 10.1111/j.1463-1326.2008.01013.x

Dose–response relationship of insulin glulisine in subjects with type 1 diabetes

R. H. A. Becker,[1] A. D. Frick,[1] L. Teichert,[1] L. Nosek,[2] L. Heinemann[2] and K. Rave[2]

[1]sanofi-aventis, Frankfurt/Main, Germany
[2]Profil Institute for Metabolic Research, Neuss, Germany

Aim: Little is known about the dose–response relationships of rapid-acting insulin analogues in subjects with diabetes. This study compared the dose–exposure and dose–response relationships of insulin glulisine and regular human insulin (RHI) in subjects with type 1 diabetes.
Methods: Eighteen male subjects with type 1 diabetes (mean glycosylated haemoglobin, HbA_{1c}, 7.7%; body mass index 24.5 kg/m^2) received subcutaneous injections of insulin glulisine followed by RHI (both at doses of 0.075, 0.15 and 0.3 U/kg) in the same three-way crossover, randomized order in a euglycaemic glucose-clamp study.
Results: Insulin glulisine and RHI showed dose-proportional increases in exposure (INS-AUC_{total}) and maximum serum concentration (INS-C_{max}) in the dose ranges 0.075, 0.15 and 0.3 U/kg. At all doses, within 2 h after injection, about twice as much insulin glulisine was absorbed as RHI (INS-$AUC_{0–2h}$: 3855, 6832 and 13237 vs. 2356, 3630 and 6231 μU.min/mL; $p < 0.05$) and INS-C_{max} was reached in about half the time (INS-T_{max}: 47, 57 and 72 vs. 82, 104 and 119 min; $p < 0.05$). Corresponding glucose disposition was twice as large with insulin glulisine as with RHI (GIR-$AUC_{0–2h}$: 314, 491 and 536 vs. 127, 219 and 294 mg/kg; $p < 0.05$), but was similar in extent upon completion (GIR-AUC_{total}: 499, 1090 and 1476 vs. 416, 1076 and 1555 mg/kg; not significant). With escalating doses, a steady increase in insulin exposure was noticed for both insulins across the entire dose range, whereas glucose disposition increased in a dose-proportional manner only for the dose range 0.075–0.15 U/kg with insulin glulisine only. For both insulins, the end of euglycaemia occurred at insulin concentrations <10 μU/mL, with a subsequent rise in plasma glucose taking 80–90 min to reach ≥8.3 mmol/L (≥150 mg/dL) and a difference in time of ~120 min between the insulins at any dose.
Conclusions: Insulin glulisine presents rapid, dose-proportional absorption, resulting in saturable glucodynamic activity in subjects with type 1 diabetes.

Keywords: dose–response proportionality, euglycaemic glucose-clamp, insulin analogues, insulin glulisine, type 1 diabetes
Received 31 October 2008; accepted 31 October 2008

Introduction

Rapidly absorbed and rapid-acting insulin analogues are increasingly being used to improve postprandial metabolic control [1,2]. Reduced postprandial glucose excursions, in turn, may help in reducing cardiovascular-related and all-cause mortality in patients who already have reasonably good metabolic control (glycosylated haemoglobin, HbA_{1c}, < 8%) [3–5].

Correspondence:
Reinhard Becker, MD, Clinical & Exploratory Pharmacology, sanofi-aventis, Building H831, Room C 0441, Frankfurt 65926, Germany.
E-mail:
reinhard.becker@sanofi-aventis.com
Conflicts of interest:
R. H. A. Becker, A. D. Frick and L. Teichert are employees of sanofi-aventis, and K. Rave and L. Nosek are employees of Profil, of which L. Heinemann is the CEO.

Insulin glulisine is an insulin analogue [6] that differs from regular human insulin (RHI) by the replacement of lysine with glutamic acid at position 29 and asparagine with lysine at position 3 on the B-chain of the human insulin molecule. This substitution reduces self-association of the insulin glulisine molecules, but still allows inherently more stable dimers to exist at pharmaceutical concentrations, unlike other insulin analogues where proline at B28 [7,8] is exchanged and which exist as pure monomers. This also allows the glulisine drug product to be formulated without added zinc to achieve sufficient physical shelf life and, thereby, avoiding hexamer formation that may impede rapid absorption [9–11].

Pharmacokinetic, pharmacodynamic and safety studies of insulin glulisine in a sizeable number of healthy volunteers and patients have shown that subcutaneous injection of insulin glulisine more aptly mimics physiological postprandial insulin action than RHI [12–14]. Hence, improved metabolic control was achieved with rapid-acting insulin analogues like insulin glulisine or insulin aspart as compared with RHI in subjects with type 1 diabetes on effectively titrated basal insulin regimens [15,16].

Despite the increasing use of rapid-acting insulin analogues, surprisingly little is known about dose escalation on systemic insulin levels and metabolic activity in subjects with diabetes, whereas there is some information from healthy volunteers [17–21]. Therefore, this study was conducted to investigate the dose–exposure and dose–response relationships of insulin glulisine compared with RHI in subjects with type 1 diabetes.

Methods

Study Design

This study was a single-centre, randomized, euglycaemic, glucose-clamp trial, with two sequential three-way crossover treatments. It comprised eight trial periods: a screening visit (trial period 0), three glucose-clamp visits with insulin glulisine (trial periods 1, 2 and 3), three glucose-clamp visits with RHI (trial periods 4, 5 and 6) and a follow-up visit (trial period 7). Two to 21 days passed between trial visits. The study followed the principles of the Good Clinical Practice guidelines of the European Union and the Declaration of Helsinki, and was reviewed and approved by the local Ethics Committee. Subjects were provided with information about the study and a consent form with which to obtain written informed consent prior to the start of the study.

Study Population and Baseline Characteristics

Eighteen of 24 screened male subjects with non-progressed type 1 diabetes (e.g. without progressive diabetic retinopathy) who met the inclusion criteria participated in the study. Participants were 35 years of age (21–50 years) [mean (range)] with a body mass index (BMI) of 24.5 kg/m^2 (19.0–28.4 kg/m^2). HbA_{1c} levels at screening were 7.7% (7.0–9.0%) at a stable insulin regimen with less than 1 U/kg/day for at least 2 months.

Study Protocol

On the evening prior to the trial days, subjects were admitted to the research unit and had dinner, after which they were prepared with in-dwelling lines and connected to a Biostator (glucose-controlled insulin infusion system; MTB-Medizintechnik, Ulm, Germany). From a hand vein kept in a warming box heated to 55 °C, arterialized venous blood was continuously sampled and analysed for blood glucose concentrations. Using intravenous RHI infusion (Insuman Rapid U100; Sanofi-Aventis, Frankfurt, Germany), blood glucose concentrations were manually maintained at 4.4–6.7 mmol/L (80–120 mg/dL) from 22:00 hours until 1 h before dosing the following day when blood glucose was adjusted to an euglycaemic clamp level of 5.5 mmol/L (100 mg/dL) and maintained by the Biostator with an algorithm-based automated infusion of 20% glucose solution until the end of the glucose-clamp (up to 10 h). Intravenous insulin infusion was discontinued immediately before injecting either insulin subcutaneously. At time point 0 of each glucose-clamp day, subjects received, in a sequence of doses (0.075, 0.15 or 0.3 U/kg body weight) according to the randomization plan, insulin glulisine followed by RHI via a subcutaneous injection 5 cm lateral to the umbilicus (Microfine IV syringe; Becton Dickinson, Heidelberg, Germany).

Blood glucose and glucose infusion rates (GIR) were recorded throughout the glucose-clamp periods on a minute-to-minute basis by the Biostator. The glucose-clamp was stopped when blood glucose levels reached $\geq$10 mmol/L ($\geq$180 mg/dL) for 30 min in the absence of an intravenous glucose infusion (end-of-dose phenomenon) or after 10 h, whichever came first.

Data Analysis

Pharmacokinetic and Pharmacodynamic Assessments

Serum concentrations of insulin glulisine were measured using a radioimmunoassay (RIA; Linco Research Inc., St Charles, MO, USA). A specific RIA for insulin glulisine

was used [lower limit of quantification (LLOQ) 5.0 μU/mL], while serum concentrations of human insulin were determined using a RIA for human insulin (LLOQ 4.3 μU/mL). Areas under the curve (AUCs) of insulin concentrations were calculated by the trapezoidal rule and AUCs of GIR as sum of rectangles. The AUCs of insulin concentration–time profiles were characterized between 0 and 2 h (INS-$AUC_{0–2h}$) and to the end of the glucose-clamp (INS-AUC_{total}); the AUCs of GIR–time profiles were similarly determined (GIR-$AUC_{0–2h}$ and GIR-AUC_{total}, respectively). Times to 10 and 90% of INS-AUC_{total} and GIR-AUC_{total} (INS-$T_{10\%}$ and INS-$T_{90\%}$ or GIR-$T_{10\%}$ and GIR-$T_{90\%}$, respectively) were derived from the ratios of AUCs per time point ($AUC_{0–t}/AUC_{total}$). Time to the end of euglycaemic clamp level (BG-T_{EU}) and to the first occurrence of a spontaneous rise in blood glucose concentrations ≥7.2, ≥8.3 and 10.0 mmol/L (≥130, ≥150 and ≥180 mg/dL; BG-T_{130}, BG-T_{150} and BG-T_{180}) during the glucose-clamps were derived from blood glucose readings, which were confined to the duration of the clamp (10 h) and were, therefore, right censored. Maximum serum insulin concentration (INS-C_{max}) and corresponding time to INS-C_{max} (INS-T_{max}) values were derived from predicted data, while maximum GIR (GIR-max) and time to GIRmax (GIR-T_{max}) were obtained from curves smoothed with a weighted regression technique (procedure LOESS, factor 0.15, SAS version 8.2; SAS Institute Inc., Cary, NC, USA).

Statistics

ANOVA models, allowing the estimation of least square (LS) means with corresponding 95% confidence limits, were applied on untransformed GIR-AUC and GIR_{max} and on natural logarithm-transformed INS-AUC and INS-C_{max} data. For INS-data, the 95% confidence intervals for the differences between LS means were calculated and retransformed to derive the respective confidence limits for the mean ratios of the pairwise treatment comparisons. For GIR data, the 95% confidence limits for the mean ratios of the pairwise treatment comparisons were calculated according to Fieller's theorem.

Insulin exposure and metabolic response were individually investigated for strict monotonic increases with dose. Dose proportionality was assessed by pairwise dose comparisons of the LS means for: INS-$AUC_{0–2h}$, INS-AUC_{total}, INS-C_{max}, GIR-$AUC_{0–2h}$, GIR-AUC_{total} and GIR_{max}, applying bioequivalence (BE) criteria for dose-normalized results, with half the 'investigational' dose as the reference dose (BE; 80–125%). Dose proportionality within the BE criteria was confirmed when the 95% confidence interval for a treatment ratio was within 1.6–2.5.

Time parameters based on INS or GIR (INS-$T_{10\%}$, INS-$T_{90\%}$, GIR-$T_{10\%}$, GIR-$T_{90\%}$, INS-T_{max} and GIR-T_{max}) were subjected to non-parametric analysis for pairwise comparisons between treatments. The 95% non-parametric confidence intervals for the respective median difference in treatment were calculated using ranks, based on the method of Steinijans and Diletti [22].

Estimation of treatment differences in BG-derived time parameters (BG-T_{EU}, BG-T_{130}, BG-T_{150}, BG-T_{180} and related differences), based on observations that were right censored by the duration of the clamp (10 h), were analysed by parametric failure time modelling (procedure LIFEREG). Estimations are not given for parameters with greater than 50% right-censored data.

Results

Pharmacokinetics

The time–concentration profiles after subcutaneous injection of 0.075, 0.15 and 0.3 U/kg of insulin glulisine and RHI are displayed in figure 1a–c. The same total insulin exposure (INS-AUC_{total}) was observed with insulin glulisine and RHI for each corresponding dose (table 1); INS-$AUC_{0–2h}$ and INS-C_{max}, by contrast, were twice as large with insulin glulisine compared with any corresponding dose of RHI.

At any dose, insulin glulisine was about twice as rapidly absorbed ($p < 0.05$) as RHI (INS-$T_{10\%}$, INS-T_{max}, but also INS-$AUC_{0–2h}$; table 1) and completion of absorption (INS-$T_{90\%}$) occurred approximately 2.5 h earlier for insulin glulisine than for RHI, at any dose. Notably, doubling the dose increased INS-$T_{90\%}$ by roughly 1 h for either insulin. Onset of absorption (INS-$T_{10\%}$) took about 25 min with 0.075 U/kg insulin glulisine and about 20 min more with RHI. The overall increment with increasing doses in INS-$T_{10\%}$ was, although significantly later, only 5–10 min for both insulins and at either dose step. In contrast, INS-T_{max} increased, from about 50 min after 0.075 U/kg insulin glulisine, by 10 min for each dose increment ($p < 0.05$), whereas it took almost 20 min more for each increment, from about 80 min, with RHI and with considerable overlap between steps.

Dose-proportional increases were observed in INS-AUC_{total}, INS-$AUC_{0–2h}$ and INS-C_{max} for both insulin glulisine and RHI (table 1, figure 2a–c). Moreover, dose separation was observed for any subject with both insulin glulisine and RHI, with regard to INS-AUC_{total}, INS-$AUC_{0–2h}$ and INS-C_{max}, as depicted by no overlapping mid-ranges of values, but not for INS-T_{max} (figure 2d).

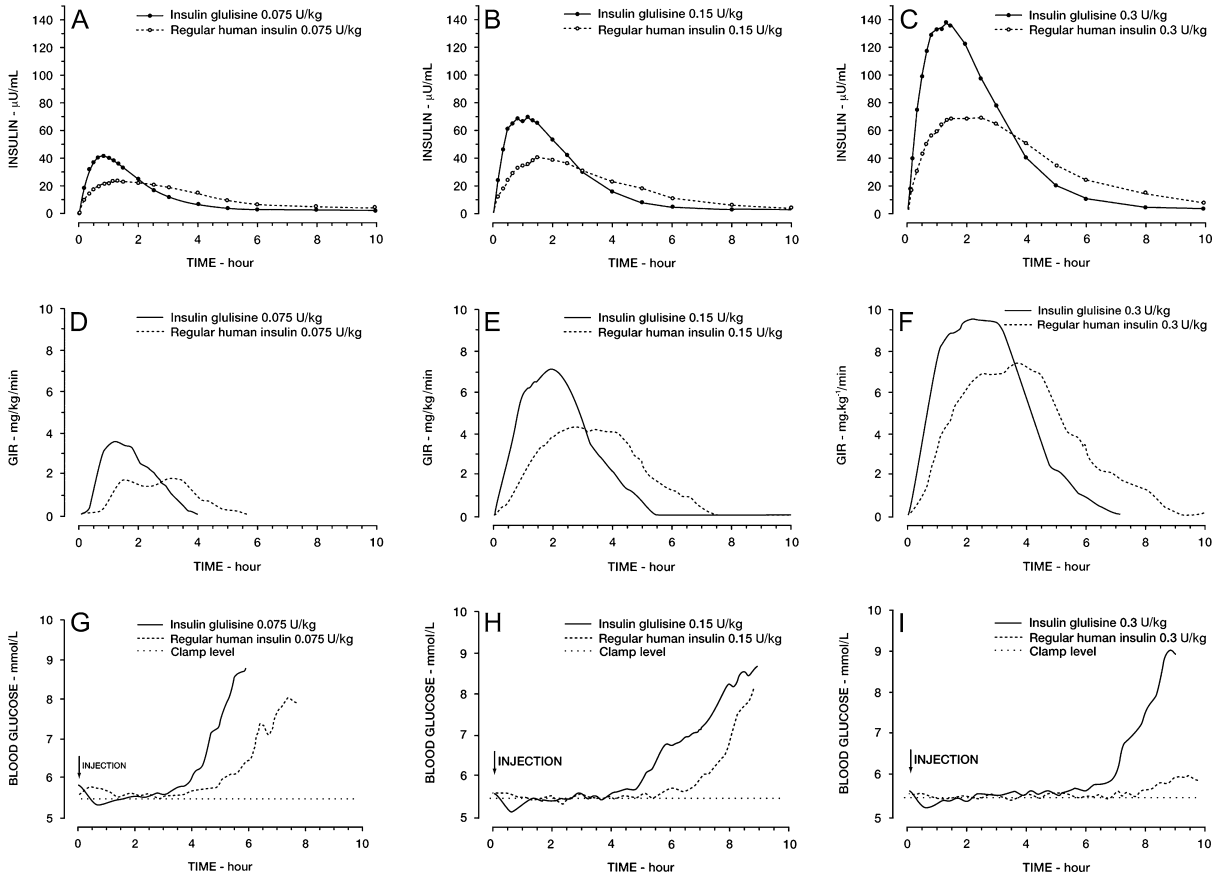

Fig. 1 Time–concentration (upper panel a–c), time–action (middle panel, d–f) and corresponding blood glucose concentration profiles (lower panel, h–i) after subcutaneous injection of 0.075, 0.15 and 0.3 U/kg of insulin glulisine (closed circle, solid lines) and regular human insulin (open circles, dotted lines) in subjects with type 1 diabetes. GIR, glucose infusion rate.

Pharmacodynamics

The time–action profiles after subcutaneous injection of 0.075, 0.15 and 0.3 U/kg of insulin glulisine and RHI are shown in figure 1d–f. Insulin glulisine and RHI displayed similar GIR-AUC_{total} per dose (table 1), but insulin glulisine took metabolic effect more rapidly at any dose and with an onset in activity approximately twice as strong and fast as RHI ($p < 0.05$; GIR-$AUC_{0–2h}$, GIR-$T_{10\%}$; figure 2e–f, table 1). It also reached greater maximal effect (GIR_{max}) more rapidly than RHI ($p < 0.05$, GIR-T_{max}; figure 2h, table 1). For all doses, GIR-T_{max} was between 1.5 and 2 h for insulin glulisine and approximately 3 h for RHI (figure 2h, table 1). Completion of metabolic activity was earlier with insulin glulisine by about 1.5–2.5 h at any dose ($p < 0.05$; GIR-$T_{90\%}$; table 1). In contrast, GIR_{max} increased significantly with increasing dose with either insulin, except for the final step to 0.3 U/kg for insulin glulisine (figure 2g).

A monotonically increasing dose–response relationship in GIR-AUC_{total} (total glucose disposal) was observed in 16 of 18 subjects for either insulin, but dose proportionality was only shown for insulin glulisine between 0.075 and 0.15 U/kg doses (figure 2f, table 1). In contrast, only 5–6 subjects displayed individual dose separation for early glucose disposal (GIR-$AUC_{0–2h}$) with each step and insulin.

Times to end of euglycaemia and times to $\geq$7.2 and $\geq$8.3 mmol/L ($\geq$130 and $\geq$150 mg/dL) (BG-T_{EU}, BG-T_{130} and BG-T_{150}) increased with increasing dose for either insulin (table 2). In line with shorter GIR-$T_{90\%}$, end-of-dose phenomena (i.e. increase of blood glucose $\geq$10.0 mmol/L ($\geq$180 mg/dL) within 10 h during glucose-clamps) occurred more frequently with insulin glulisine (in 15, 11 and 12 of 18 subjects for 0.075, 0.15 and 0.3 U/kg, respectively) than with RHI (in 10, 4 and 3 of 18 subjects for corresponding doses of RHI). Similarly, the median time to end-of-dose phenomenon was 6.3,

Table 1 Pharmacokinetics and pharmacodynamics in subjects with type 1 diabetes after subcutaneous injection of insulin glulisine and regular human insulin at three different doses, and between- and within-treatment comparisons

	Insulin glulisine			Regular human insulin			Insulin glulisine PE (95% CI)		Regular human insulin PE (95% CI)		Insulin glulisine/regular human insulin PE (95% CI)		
A: Concentration/effect parameters*													
Dose (U/kg)	0.075	0.15	0.3	0.075	0.15	0.3	0.15/0.075	0.3/0.15	0.15/0.075	0.3/0.15	0.075	0.15	0.3
INS-C_{max} (μU/mL)	43	73	142	23	40	72	1.7 (1.6; 1.9)†	2.0 (1.8; 2.1)†	1.7 (1.6; 1.9)†	1.8 (1.6; 2.0)†	1.9 (1.7; 2.1)‡	1.9 (1.7; 2.1)‡	2.0 (1.8; 2.3)‡
INS-$AUC_{0–2h}$ (μU.min/mL)	3855	6832	13237	2356	3630	6231	1.8 (1.6; 1.9)†	1.9 (1.8; 2.1)†	1.6 (1.4; 1.8)†	1.7 (1.5; 1.9)†	1.7 (1.5; 2.0)‡	1.9 (1.7; 2.2)‡	2.2 (1.9; 2.6)‡
INS-AUC_{total} (μU.min/mL)	5372	11284	25076	6242	10,932	21,700	2.1 (2.0; 2.2)†	2.2 (2.1; 2.3)†	1.8 (1.6; 2.0)†	2.0 (1.8; 2.2)†	0.9 (0.8; 1.1)	1.1 (0.9; 1.2)	1.2 (1.0; 1.3)
GIR_{max} (mg/kg/min)	4.1	6.4	6.7	2.5	4.6	5.4	1.6 (1.3; 1.9)	1.1 (0.9; 1.2)	1.9 (1.5; 2.4)†	1.2 (1.1; 1.3)	1.7 (1.4; 2.0)‡	1.4 (1.2; 1.6)‡	1.2 (1.1; 1.4)‡
GIR-$AUC_{0–2h}$ (mg/kg)	314	491	536	127	219	294	1.6 (1.2; 2.1)	1.1 (0.9; 1.3)	1.7 (1.2; 2.6)	1.3 (1.1; 1.7)	2.5 (1.9; 3.5)‡	2.2 (1.7; 3.1)‡	1.8 (1.5; 2.3)‡
GIR-AUC_{total} (mg/kg)	499	1090	1476	416	1076	1555	2.2 (1.7; 2.9)†	1.4 (1.2; 1.5)	2.6 (2.0; 3.6)	1.4 (1.3; 1.7)	1.2 (1.0; 1.5)	1.0 (0.9; 1.2)	0.9 (0.8; 1.1)
B: Time parameters§													
Dose (U/kg)	0.075	0.15	0.3	0.075	0.15	0.3	0.15–0.075	0.3–0.15	0.15–0.075	0.3–0.15	0.075	0.15	0.3
INS-T_{max} (min)	47	57	72	82	104	119	10.8 (4.5; 17.8)	10.5 (4.7; 15.6)	27.4 (10.2; 38.7)	9.0 (−3.3; 21.3)	−35 (−45; −26)	−48 (−64; −36)	−49 (−64; −34)
INS-$T_{10\%}$ (min)	26	31	39	44	53	59	5.4 (3.0; 8.5)	5.9 (2.6; 9.7)	9.3 (5.1; 15.8)	7.6 (2.5; 13.8)	−16 (−21; −12)	−20 (−27; −15)	−23 (−29; −17)
INS-$T_{90\%}$ (min)	149	205	242	313	348	410	39.0 (28.1; 53.4)	31.8 (13.1; 50.4)	20.9 (–40.1; 71.5)	53.2 (4.4; 89.7)	−160 (−227; −107)	−141 (−186; −101)	−159 (−187; −131)
GIR-T_{max} (min)	87	114	121	178	169	207	11.0 (–10.0; 33.5)	14.0 (–12.5; 43.5)	28.5 (–24.0; 49.5)	−3.5 (−52.5; 37.5)	−79 (−115; −45)	−86 (−118; −51)	−64 (−107; −29)
GIR-$T_{10\%}$ (min)	45	45	52	75	88	83	0.0 (–10.5; 9.0)	7.5 (–2.0; 16.0)	1.0 (–41.5; 20.0)	−3.5 (−20.5; 8.0)	−42 (−79; −19)	−39 (−63; −25)	−30 (−49; −20)
GIR-$T_{90\%}$ (min)	180	238	275	278	330	422	65.0 (39.5; 91.5)	42.5 (19.0; 74.5)	72.0 (41.0; 117.0)	49.0 (18.5; 79.0)	−101 (−137; −65)	−115 (−157; −70)	−116 (−149; −88)

GIR_{max}, maximum glucose infusion; GIR-$AUC_{0–2h}$ and GIR-AUC_{total}, area under the glucose infusion rate (GIR)–time curve between 0 and 2 h; and to the end of clamp; GIR-T_{max}; GIR-$T_{10\%}$ and GIR-$T_{90\%}$, time to the maximum; to 10% and to 90% of GIR-AUC_{total}; INS-AUC_{total} and INS-$AUC_{0–2h}$, area under the serum insulin concentration curve at the end of the study and after 2 h; INS-C_{max}, maximum insulin concentration; INS-T_{max}; INS-$T_{10\%}$ and INS-$T_{90\%}$, time to the maximum; to 10 and 90% of INS-AUC_{total}.

*Data are arithmetic means or ratios of least square means.

†Dose-proportional [point estimate (PE) and upper and/or lower limit are within bioequivalence limits based on 95% CI].

‡Significantly different to regular human insulin ($p < 0.05$).

§Data are medians or differences of medians.

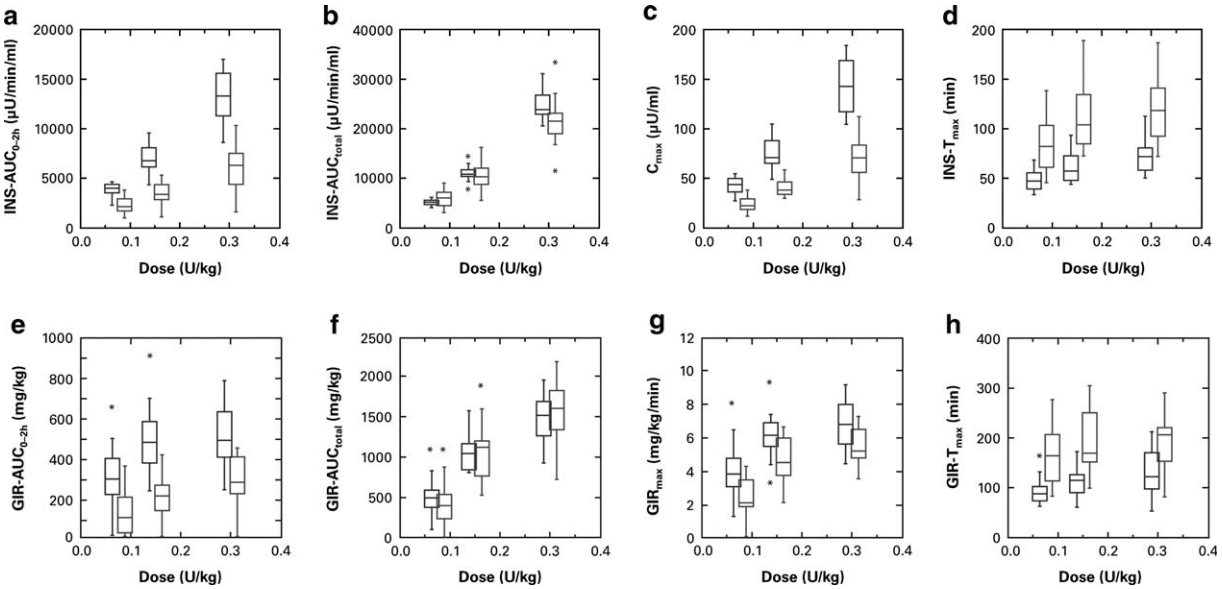

Fig. 2 Insulin exposure (upper panel, a–d) and glucose disposal (lower panel, e–h) for insulin glulisine (blue) and regular human insulin (red); Box plots represent distribution of data with percentiles at 25% (lower box hinge), 50% (median) and 75% (upper box hinge). Lower and upper whiskers indicate observed minimum and maximum value within plus or minus 1.5 times the H-spread (the distance between the hinges) from the hinges. *Values that are outside 1.5 times but less than 3 times the H-spread from the hinge.

8.5 and 9.1 h for insulin glulisine and 8.8, 9.6 and >10.0 h for RHI.

The rise in plasma glucose after the end of euglycaemia was similar with either insulin. It took about 30–40 min, regardless of dose, to reach ≥7.2 mmol/L (≥130 mg/dL) and 80–90 min to reach ≥8.3 mmol/L (≥150 mg/dL), while the difference in time to 7.2 and 8.3 mmol/L (130 and 150 mg/dL) between the insulins for the corresponding doses was about 120 min, also at any dose.

Of note, euglycaemia ceased when insulin concentrations fell below approximately 10 µU/mL with either insulin, reflecting the absence of a long-acting insulin in this study setting, which would otherwise cover basal insulin requirements.

Safety

All subjects completed the eight trial visits without clinically relevant adverse events. Three instances of headaches occurred with the highest dose of insulin glulisine.

Discussion

This euglycaemic glucose-clamp study in subjects with type 1 diabetes showed dose-proportional exposure in serum insulin in clinically relevant doses (0.075, 0.15 and 0.3 U/kg – corresponding to 6, 12 and 24 U for an 80-kg subject) of a rapidly absorbed and rapid-acting insulin analogue, insulin glulisine, and RHI. This is accompanied by dose proportionality in total metabolic response between 0.075 and 0.15 U/kg for insulin glulisine only, and a less-than-proportional increment was observed with the large dose (0.3 U/kg) for either insulin. The data confirm that insulin glulisine at any dose is absorbed approximately twice as fast and takes effect twice as rapidly compared with RHI, but disposes the same quantity of glucose as RHI at any dose.

The monotonically increasing dose–exposure relationship in early (INS-$AUC_{0–2h}$) and total insulin exposure (INS-AUC_{total}) and INS-C_{max}, observed in each subject and displaying strict individual dose separation, should translate into individually predictable dose–exposure adjustments with either insulin. However, this individual dose separation in exposure was not associated with corresponding individual dose separation in glucodynamic responses in any case, and less so with RHI. Furthermore, there was even less association seen between individual 2 h insulin exposure (INS-$AUC_{0–2h}$) and glucose disposal (GIR-$AUC_{0–2h}$). Taken together, dose proportionality was seen only in total effect (GIR-AUC_{total}) and for only the lower doses of insulin glulisine, while the total metabolic effect from 0.15 U/kg to the top dose increased less than proportionally with either insulin.

Table 2 Times to specified blood glucose concentrations after cessation of insulin activity in subjects with type 1 diabetes after subcutaneous injection of insulin glulisine and regular human insulin at three different doses, and between- and within-treatment comparisons

	Insulin glulisine median*			**Regular human insulin median***			**Insulin glulisine PE (95% CI)†**		**Regular human insulin PE (95% CI)†**		**Insulin glulisine – Regular human insulin PE (95% CI)†**		
Dose (U/kg)	0.075	0.15	0.3	0.075	0.15	0.3	0.15–0.075	0.3–0.15	0.15–0.075	0.3–0.15	0.075	0.15	0.3
BG-T_{EU} (min)	230	316	416	370	410	*>600*	100 (73; 127)	74 (43; 105)	88 (55; 121)	‡	126 (160; 92)	100 (149; 50)	‡
BG-T_{130} (min)	273	356	446	387	465	*>600*	93 (61; 125)	71 (46; 96)	94 (64; 125)	‡	116 (163; 69)	103 (148; 58)	‡
BG-T_{150} (min)	312	415	485	424	505	*>600*	84 (58; 111)	90 (68; 113)	75 (44; 105)	‡	119 (160; 79)	91 (138; 44)	‡
BG-T_{180} (min)	378	510	547	527	573	*>600*	124 (82; 166)	79 (32; 125)	53 (1; 106)	‡	141 (173; 109)	77 (145; 10)	‡
BG-T_{130} − BG-T_{EU} (min)	44	33	32	35	40	‡	−14 (−28; 1)	−1.0 (−11; 10)	9 (−91; 9)	‡	−9 (−28; 11)	10 (−32; 2)	‡
BG-T_{150} − BG-T_{EU} (min)	81	70	84	89	77	‡	−16 (−32; −1)	17 (3; 31)	−1 (−22; 24)	‡	1 (−17; 19)	−10 (−25; 4)	‡
BG-T_{180} − BG-T_{EU} (min)	138	134	123	157	124	‡	8 (14; 30)	−8 (−38; 22)	−7 (−50; 36)	‡	15 (−11; 40)	−1 (−37; 38)	‡

*Data are medians: BG-T_{EU} is time of euglycaemia/clamp level; BG-T_{130}, BG-T_{150} and BG-T_{180}, time to 7.2, 8.3 and 10.0 mmol/L (130, 150 and 180 mg/dL). Censored data [i.e. affected by the end of the observation period (maximum 600 min)] are italicized.
†Data are point estimates for treatment differences, with 95% CI estimated by the LIFEREG procedure (SAS).
‡Not estimable as a result of frequency of censored values.

It is noteworthy that, irrespective of the activity profile of either insulin, doubling the dose from 0.15 to 0.3 U/kg produced only a 50% increase in total metabolic effect. This observation points towards saturation of efficiency for both insulins, even well within the range of therapeutically applied doses. Conversely, this less-than-proportional increase in metabolic efficiency explains the substantially more-than-proportional increase in insulin dose necessary to achieve a doubling of the metabolic effect with high doses, as commonly experienced by patients with diabetes on insulin therapy.

Comparable observations in dose–response relationships and absence of dose sensitivity in INS-T_{max} and GIR-T_{max} have been reported for similar doses of both rapid-acting insulin analogues and RHI in healthy Caucasian volunteers [17,18,21], and at lower doses in healthy Japanese volunteers [20]. Although this allows generalizing dose–response information for all rapidly absorbed insulin analogues and RHI in subjects with type 1 diabetes, additional studies are needed for subjects with type 2 diabetes. Larger subcutaneous and visceral fat layers typically associated with patients with type 2 diabetes require higher insulin doses than in patients with type 1 diabetes to overcome both impaired absorption and increased insulin resistance [23]. A recent study obtained in obese to severely obese healthy subjects confirms consistently markedly reduced non-dose–proportional metabolic activity with increasing weight (BMI) for 0.2 and 0.4 U/kg insulin glulisine and insulin lispro, despite maintained dose-proportional exposure [24].

For reliable dosing, there should be no substantial shift in the absorption and action profile with increasing doses. Indeed, there were no relevant changes in onset of activity, GIR-$T_{10\%}$, with increasing doses, and no consistent rise in time to maximum absorption and time to maximum activity (INS-T_{max} and GIR-T_{max}, respectively) for either insulin. Therefore, these data indicate that the initial time–exposure and time–action profiles of these insulins are barely time sensitive to dose increments. However, the difference in GIR-$T_{10\%}$ between insulin glulisine and RHI at any dose ranged between 30 and 40 min (table 1), which reflects the difference in the recommended injection–meal interval [25,26].

In contrast to GIR-$T_{10\%}$, duration of action (GIR-$T_{90\%}$) did increase by roughly 1 h per dose step with either insulin. However, GIR-$T_{90\%}$ for insulin glulisine did not extend beyond 5 h, even at the highest dose; this is in agreement with results for insulin aspart in healthy volunteers [21]. Therefore, an increase in dose from 0.15 to 0.3 U/kg does not result in a higher early glucose

disposal, but predominantly contributes to late metabolic activity. This is more pronounced with RHI, which shows substantial metabolic effect beyond 4 h at any dose. For daily clinical practice, this means that high late metabolic activity might translate into late postprandial hypoglycaemic events with RHI, which reflects the longer duration of action (GIR-$T_{90\%}$) of up to a maximum of 7 h at the highest dose (0.3 U/kg). Conversely, the low late metabolic activity beyond the fourth hour with insulin glulisine may very well translate into a lower risk for late hypoglycaemic events when using the rapid-acting analogue and when basal insulin requirements are covered.

However, the times to end of euglycaemia and the subsequent spontaneous rise in blood glucose to concentrations >8.3 or >10.0 mmol/L (>150 or >180 mg/dL) during the glucose-clamp were longer for RHI compared with insulin glulisine, with the difference amounting to about 2 h at any dose, including the delay in onset of activity. However, also during routine treatment with rapid-acting insulin analogues, interprandial insulin requirements are not always adequately covered by basal insulin so that blood glucose control with RHI may at times be advantageous. For example, although absorption of carbohydrates following an extensive meal with high fat or high fibre content may also be covered with rapid-acting insulin analogues given up to 20 min after onset of the meal, it may be socially more convenient to administer RHI immediately prior to the meal to take advantage of its extended metabolic activity. Hence, patients may benefit from employing prandial insulins of different profiles, with the choice dependent on injection time, and size and composition of the meal [27–29].

Some methodological features differentiate the present study from others: a specific assay was used to measure serum insulin glulisine levels; the lack of a basal intravenous insulin infusion during the glucose-clamps, which allowed systemic availability of either insulin to be quantified without correction; and the predominant use of untransformed data for characterization of activity (such as GIR-AUC_{0-t} and GIR-$T_{10\%}$), which reduced methodological bias incurred with smoothing [30].

In conclusion, clearly differentiated time–concentration and time–action profiles for both insulin glulisine and RHI showed dose-proportional increases in insulin exposure (INS-AUC_{total}) and maximum serum insulin concentration (INS-C_{max}) in the dose ranges 0.075, 0.15 and 0.3 U/kg. By contrast, dose proportionality in corresponding metabolic activity was limited to insulin glulisine and to the dose range 0.075–0.15 U/kg, indicating a saturation of metabolic effect with either insulin.

Acknowledgements

This study was sponsored by sanofi-aventis. Data from this study have been previously presented at the ADA meeting in 2005. In addition, a short report containing part of the data presented here was previously published in *Diabetes Care* (Becker RH, Frick AD, Nosek L, Heinemann L, Rave K. Dose–response relationship of insulin glulisine in subjects with type 1 diabetes. *Diabetes Care* 2007; **30**: 2506–2507). The ClinicalTrials.gov identifier number of the study was NCT00368394.

References

1 Bolli GB, Di Marchi RD, Park GD, Pramming S, Koivisto VA. Insulin analogues and their potential in the management of diabetes mellitus. Diabetologia 1999; **42:** 1151–1167.
2 Hirsch IB, Insulin analogues. N Engl J Med 2005; **352:** 174–183.
3 Leiter LA, Ceriello A, Davidson JA *et al.* Postprandial glucose regulation: new data and new implications. Clin Ther 2005; **27:** S42–S56.
4 Home P. Contributions of basal and post-prandial hyperglycaemia to micro- and macrovascular complications in people with type 2 diabetes. Curr Med Res Opin 2005; **21:** 989–998.
5 Monnier L, Colette C. Contributions of fasting and postprandial glucose to hemoglobin A1c. Endocr Pract 2006; **12:** 42–46.
6 Hennige AM, Kellerer M, Strack V *et al.* New human insulin analogs: characteristics of insulin signaling in comparison to AspB10 and regular insulin. Diabetologia 1999; **42:** A178.
7 Richards JP, Stickelmeyer MP, Flora DB *et al.* Self-association properties of monomeric insulin analogs under formulation conditions. Pharm Res 1998; **15:** 1434–1441.
8 Zoete V, Meuwly M, Karplus M. Study of the insulin dimerization: binding free energy calculations and per-residue free energy decomposition. Proteins 2005; **61:** 79–93.
9 Howey DC, Bowsher RR, Brunelle RL, Woodworth JR. [Lys(B28), Pro(B29)]-human insulin. A rapidly absorbed analogue of human insulin. Diabetes 1994; **43:** 396–402.
10 Bakaysa DL, Radziuk J, Havel HA *et al.* Physicochemical basis for the rapid time–action of LysB28ProB29-insulin: dissociation of a protein–ligand complex. Protein Sci 1996; **5:** 2521–2531.
11 Aspart I 2000. http://www.fda.gov/cder/foi/label/2000/20986lbl.pdf. Accessed September 2008.
12 Becker R, Frick A, Wessels D, Scholtz H. Evaluation of the pharmacodynamic and pharmacokinetic profiles of insulin glulisine – a novel, rapid-acting, human insulin analogue. Diabetologia 2003; **46:** A775.

13 Becker R, Frick A, Wessels D, Scholtz H. Pharmacodynamics and pharmacokinetics of a new, rapidly acting insulin analogue, insulin glulisine. Diabetes 2003; **52:** A471.
14 Frick A, Becker RH, Wessels DH, Scholtz HE. Pharmacokinetic and glucodynamic profiles of insulin glulisine: an evaluation following subcutaneous administration at various injection sites. Diabetologia 2003; **46:** A776.
15 Garg SK, Rosenstock J, Ways K. Optimized basal–bolus insulin regimens in type 1 diabetes: insulin glulisine versus regular human insulin in combination with basal insulin glargine. Endocr Pract 2005; **11:** 11–17.
16 Hermansen K, Fontaine P, Kukolja KK *et al.* Insulin analogues (insulin detemir and insulin aspart) versus traditional human insulins (NPH insulin and regular human insulin) in basal–bolus therapy for patients with type 1 diabetes. Diabetologia 2004; **47:** 622–629.
17 Woodworth J, Howey D, Bowsher R *et al.* [Lys (B28), Pro (829)] human insulin(K): dose-ranging vs. humulin R (H). Diabetes 1993; **42:** 54A.
18 Heinemann L, Woodworth JR. Pharmacokinetics and glucodynamics of insulin lispro. Drugs Today 1998; **34:** 23–36.
19 Lindholm A, Sasaki T, Edwards A, Hedge A. Dose dependency in absorption of insulin aspart in healthy Caucasians and Japanese. Diabetes 2001; **50:** A441.
20 Kaku K, Matsuda M, Urae A, Irie S. Pharmacokinetics and pharmacodynamics of insulin aspart, a rapid-acting analog of human insulin, in healthy Japanese volunteers. Diabetes Res Clin Pract 2000; **49:** 119–126.
21 Nosek L, Heinemann L, Kaiser M, Arnolds S, Heise T. No increase in the duration of action with rising doses of insulin aspart. Diabetes 2003; **52:** A128.
22 Hauschke D, Steinijans VW, Diletti E. A distribution-free procedure for the statistical analysis of bioequivalence studies. Int J Clin Pharmacol Ther Toxicol 1990; **28:** 72–78.
23 Rave K, Nosek L, Heinemann L *et al.* Dependency of the metabolic effect of sc-injected human regular insulin on intra-abdominal fat in patients with type 2 diabetes. Horm Metab Res 2004; **36:** 307–311.
24 Heise T, Nosek L, Spitzer H *et al.* Insulin glulisine: a faster onset of action compared with insulin lispro. Diabetes Obes Metab 2007; **9:** 746–753.
25 ADA. Insulin administration. Diabetes Care 2003; **26:** S121–S124.
26 Glulisine I, Apidra prescribing information. 2006. http://products.sanofi-aventis.us/apidra/apidra.html.
27 Burge MR, Castillo KR, Schade DS. Meal composition is a determinant of lispro-induced hypoglycemia in IDDM. Diabetes Care 1997; **20:** 152–155.
28 Strachan MW, Frier BM. Optimal time of administration of insulin lispro. Importance of meal composition. Diabetes Care 1998; **21:** 26–31.
29 Howey DC, Bowsher RR, Brunelle RL *et al.* [Lys(B28), Pro(B29)]-human insulin: effect of injection time on postprandial glycemia. Clin Pharmacol Ther 1995; **58:** 459–469.
30 Frohnauer MK, Woodworth JR, Anderson JH. Graphical human insulin time–activity profiles using standardized definitions. Diabetes Technol Ther 2001; **3:** 419–429.