These results are supplied for informational purposes only. *Prescribing decisions should be made based on the approved package insert in the country of prescription*			
Sponsor/company:	sanofi-aventis	**ClinialTrials.gov Identifier:**	NCT00310297
		Study Code:	HMR1964A_1505
Generic drug name:	insulin glulisine	**Date:**	5 November 2007

Title

A randomised, open label, two-arm, cross-over design study to compare the pharmacodynamics and pharmacokinetics of insulin glulisine and insulin lispro in obese patients with type 2 diabetes.

Investigator, study site

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Study duration and dates	Enrollment/treatment period 6 weeks: 16th November 2004 to 23rd December 2004	**Phase**	I

Objectives

Primary objective: The primary objective of the study was to compare the pharmacodynamics of insulin glulisine and insulin lispro injected subcutaneously before three 500 kcal standard meals during a 12 hour day, in obese subjects with type 2 diabetes.

Secondary objectives: The secondary objectives of the study were to compare the pharmacokinetics of insulin glulisine and insulin lispro in obese subjects with type 2 diabetes, injected subcutaneously before three standard meals during a 12-hour day. In addition, the safety of insulin glulisine, the relationship of the pharmacodynamics and pharmacokinetics with skin thickness and C-peptide, non-esterified fatty acid, triglyceride and β-hydroxybutyrate levels in these subjects were also to be assessed.

Study design

The study consisted of an open, randomized, single-centre, two-arm crossover design in obese patients with type 2 diabetes. During a 12-hour day (trial period 1), subjects received three doses of

insulin (0.15 IU/kg) of either insulin glulisine or insulin lispro immediately prior to three standard test meals (breakfast, lunch and dinner). Subjects received the alternative insulin during the second, 12-hour treatment day.

- Treatment A: Insulin glulisine injected subcutaneously immediately prior (within 2 minutes) to a standard test meal
- Treatment B: Insulin lispro injected subcutaneously immediately prior (within 2 minutes) to a standard test meal

The study consisted of four trial periods – trial period 0 (screening), trial periods 1 and 2 (treatment visits) and trial period 3 (follow-up visit). Subjects were randomized to receive treatments A and B in the order AB or BA during trial periods 1 and 2.

Number of subjects planned

18

Inclusion criteria

Subjects fulfilling the following criteria were enrolled: Patients with type 2 diabetes, of either gender, with a body mass index (BMI) between 35 and 40 kg/m^2, aged between 18–75 years of age, with HbA_{1c} ≤10% and plasma C-peptide levels ≥0.1 nmol/L. Female subjects had to be postmenopausal, surgically sterilized, or not pregnant and using approved methods of contraception.

Treatments

Insulin glulisine was injected as a single dose (0.15 IU/kg) subcutaneously in the periumbilical abdominal area immediately prior (within 2 minutes) to each of three standard meals (breakfast, lunch and dinner); thus the total daily dose was 0.45 IU/kg.

Insulin lispro was injected as a single dose (0.15 IU/kg) subcutaneously in the periumbilical abdominal area immediately prior (within 2 minutes) to each of three standard meals (breakfast, lunch and dinner); thus the total daily dose was 0.45 IU/kg.

Pharmacodynamic data

The primary analysis variables were:

- Maximum plasma glucose concentration (GLU_{max}, mmol/L)
- Maximum plasma glucose excursion (baseline subtracted glucose concentration, ΔGLU_{max}, mmol/L)
- Time to GLU_{max} (T_{max}, min)

Further variables included:

- Area under the baseline subtracted plasma glucose concentration curve (calculated using the trapezoidal rule) between 0 h and 1 h ($AUC_{0–1h}$), 0 h and 1.5 h ($AUC_{0–1.5h}$), 0 h and 2 h ($AUC_{0–2h}$) and 0 h and 4 h ($AUC_{0–4h}$) after injection (mmol.h/L)
- Minimum plasma glucose concentration (GLU_{min}, mmol/L)
- Time to GLU_{min}, after GLU_{max} (T_{min}, min)

Pharmacokinetic data

The secondary pharmacokinetic analysis variables were:

- Area under the insulin concentration–time curve between 0 h and 1 h ($AUC_{0–1h}$), 0 h and 1.5 h ($AUC_{0–1.5h}$), 0 h and 2 h ($AUC_{0–2h}$) and 0 h and 4 h ($AUC_{0–4h}$) after injection (µIU.min/mL)
- Maximum concentration (C_{max}, µIU/mL)
- Time to maximum concentration (T_{max}, min)

Additional post-hoc analysis: a comparison of insulin absorption rates in the first 30 minutes after each meal (at times 0, 10, 20 and 30 minutes).

Efficacy data

The relationship between the following variables and skin thickness and leptin concentrations was investigated:

- Glucose $AUC_{0–2h}$
- Insulin $AUC_{0–2h}$

Secondary efficacy variables included:

- Area under the non-esterified fatty acid (NEFA) time curve
- Area under the triglyceride time curve
- Area under the β-hydroxybutyrate time curve
- Area under the C-peptide time curve, C_{max} (C-peptide), T_{max}, (C-peptide)

Safety data

Safety variables included:

- Adverse events (AEs)
- Hypoglycaemic episodes
- Haematology
- Clinical chemistry

- Urinalysis
- Electrocardiogram readings
- Vital signs (blood pressure, pulse rate and temperature)
- Physical examination
- Inspection of injection site

Statistical procedures

Pharmacodynamics:

Mixed models analysis of variance (ANOVA) was performed on the baseline-subtracted maximum (GLU_{max}), the $AUC_{0–1h}$, $AUC_{0–1.5h}$, $AUC_{0–2h}$, $AUC_{0–4h}$, and minimum (GLU_{min}) plasma glucose concentration data. Since the four AUC measures, denoted by AUC_{time}, within each meal were likely to be highly correlated with each other, the analysis considered all of them in the same model. Various patterns of covariance structure for the repeated observations were considered, allowing for constant correlations, correlations decreasing with increased time separation (i.e. allowing for a higher correlation between $AUC_{0–1h}$ and $AUC_{0–1.5h}$ than between $AUC_{0–1h}$ and $AUC_{0–4h}$), and varying correlations between treatments. Likelihood ratio statistics were used to help determine the most appropriate structure to use. The term for time within study period (i.e. meal) was included, as well as the interaction between treatment and time; if the interactions were not significant, they were dropped from the final model. Results were expressed as the ratio of geometric means accompanied by 90% confidence intervals (CIs).

Treatment differences for maximum baseline-subtracted plasma glucose concentrations were derived from arithmetic means, with CIs of the ratio of the mean of glulisine to lispro derived using Fieller's theorem.

The time to GLU_{max} and T_{min} were analysed using ANOVA if the assumption of normality appeared to hold for the data derived from the three observations for each subject in each period.

The maximum baseline-subtracted (ΔGLU_{max}) plasma glucose concentrations were not ln-transformed, as several of the values were negative.

All data were summarized in tables and individual subject data were listed. Graphs of the glucose-versus-time curves were produced, both for individual subjects, and for the mean data.

Pharmacokinetics:

Mixed models ANOVA was performed on ln-transformed $AUC_{0–1h}$, $AUC_{0–1.5h}$, $AUC_{0–2h}$, $AUC_{0–4h}$ and C_{max} of insulin concentration data as above. As there were three observations in each study period corresponding to the three meals, additional terms for time and the interaction between time and treatment were also fitted. If there was no evidence of a significant interaction between treatment and time ($p < 0.1$) then this term was to be dropped from the final model.

The residuals were plotted against fitted values and normal scores to check the assumptions of normality and stable variance. Since there were some missing values, and since the covariance structure of the data was fairly complex, random effects models were used for all analyses.

The methods described for AUC and C_{max} above were also applied to T_{max}. However, the data was not log-transformed, so the estimated treatment difference was in the form of a difference between arithmetic means rather than a ratio. The assumption of normality was investigated by plotting the residuals. If the assumption of normality was doubtful, then the three observations were combined into one estimate of T_{max}, using the mean of the three, and a Wilcoxon rank-sum test was applied to the resulting data. The differences between the data observed for the two insulins were compared between sequence groups to derive the overall significance of the difference between the insulins. Ninety percent CIs were calculated for the difference between the medians for the two treatments.

For all variables other than t_{max}, if the two-sided 90% CIs for the ratios of the means were within the conventional equivalence range (80%; 125%), bioequivalence was concluded.

All data were summarized in tables, and individual subject data were listed. Graphs of the concentration-versus-time curves were produced, both for individual subjects and for the mean data.

For the post-hoc comparison of the insulin absorption rates, the rates were estimated using linear regression for each subject, and these were then compared between the treatments using mixed models ANOVA with terms for subject within sequence (random), sequence, period, meal and treatment. The treatment difference is presented as the difference between least squares mean with its 90% CI.

Efficacy analyses

The relationship between the skin thickness and leptin concentration and the pharmacokinetic and pharmacodynamic variables was investigated graphically. The relative bioavailability for each subject, estimated as the back-transformed value of the mean difference for the $AUC_{0–2h}$ (insulin) and $AUC_{0–2h}$ (glucose) between the three observations (i.e. one for each meal) on insulin glulisine and the three for insulin lispro was plotted against skin thickness and leptin concentration.

The analysis of the additional variables – C-peptide, NEFA, triglyceride and β-hydroxybutyrate – were analysed using methods similar to those used to analyse the primary pharmacodynamic variables.

Safety analyses

The period for observing AEs was divided into three segments: pre-treatment, period one treatment and period two treatment. All AEs were coded using MedDRA version 7.1, coded to lower level terms.

Laboratory safety data were listed, and the data at the end of the study was plotted against the screening data, to see if there was any pattern.

Vital signs were listed but no formal statistical analysis was performed.

Electrocardiogram outcomes (normal or abnormal) were to be cross-tabulated at the beginning and end of the study.

Interim analysis

No interim analysis was performed

Results - Study subjects and conduct

Twenty-two subjects with type 2 diabetes of either gender were enrolled in this trial. Four of the subjects enrolled were withdrawn due to screen failure. One subject (Subject 12) was treated but was withdrawn prematurely from the trial after trial period 1 due to a non-treatment-related AE (lower lobe consolidation), following treatment with insulin glulisine. This subject was replaced with another subject (Subject 23); thus, the safety population consisted of 19 subjects who were exposed to the study medication. Eighteen subjects completed the study according to the protocol. There were no major protocol deviations.

Results - Pharmacokinetics and pharmacodynamics

There were no statistically significant differences between the pharmacodynamic profiles of insulin glulisine and insulin lispro, for the primary variables of GLU_{max} (p=0.27) and T_{max} (p=0.85). However, there were overall between-treatment differences in ΔGLU_{max}, with a 12% reduction in ΔGLU_{max} seen with insulin glulisine treatment, compared to ΔGLU_{max} observed with insulin lispro (p=0.007). The largest between-treatment differences in ΔGLU_{max} were demonstrated during the post-lunch period, where the estimated reduction of ΔGLU_{max} with insulin glulisine was ~25% compared with insulin lispro.

The estimated mean baseline-subtracted glucose AUCs were as follows:

- Insulin glulisine: 218.94 mmol/L.min
- Insulin lispro: 224.32 mmol/L.min

The difference between insulin glulisine and insulin lispro was –5.38 (90% CI: –39.32 to 28.56; p=0.79).

There were no statistically significant differences between the two insulin treatments for the overall baseline-subtracted glucose AUC (p=0.79), or at each AUC timepoint ($AUC_{0–1}$, p=0.64; $AUC_{0–1.5}$, p=0.54; $AUC_{0–2}$, p=0.76; $AUC_{0–4}$, p=0.97). The overall difference was expressed as a ratio of the means (0.98), with a 90% CI of 0.84 to 1.14, which is within the range defining bioequivalence.

Although there were no between-treatment differences for the baseline-subtracted AUC variables, there were, however, highly significant differences between these values for the three meals (p <0.0001), with larger baseline-subtracted AUCs occurring during the post-dinner period.

Pharmacodynamics

Variable	**Glulisine**	**Lispro**	**Ratio* (Glulisine:Lispro)**	**90% Confidence Interval**	**p-value**
GLU_{max} (mmol/L)	10.00	10.25	0.98	(0.94, 1.01)	0.27
GLU $AUC_{overall}$ (mmol/L.min)	218.94	224.32	–5.38	(–39.32, 28.56)	0.79
ΔGLU_{max} (mmol/L)					
Breakfast	3.39	3.72	0.91	(0.79, 1.04)	0.26
Lunch	2.58	3.44	0.75	(0.65, 0.86)	<0.01
Dinner	5.11	5.20	0.98	(0.86, 1.13)	0.83
Overall	3.55	4.06	0.88	(0.81, 0.95)	<0.01
GLU_{min} (mmol/L)	4.61	4.53	1.02	(0.96, 1.07)	0.60
T_{max} (mins)	56.26	56.87	–0.60	(–5.85, 4.64)	0.85
*Difference for areas under the curve and T_{max}					

Pharmacokinetics

Insulin levels achieved after subcutaneous injection of insulin glulisine immediately before a meal were significantly higher (~20%) than insulin levels seen after subcutaneous injection of insulin lispro in the same time frame. This difference was particularly marked during the post-lunch and post-dinner periods (p <0.01 for both).

The maximal level of insulin was achieved ~19 minutes later in patients treated with insulin glulisine, than in patients treated with insulin lispro (p=0.004). Although the times to the maximal insulin values were higher with insulin glulisine than with insulin lispro, the combined estimated values with maximal values and times to those values give similar absorption rates. In addition, the time action profiles for insulin levels (with both insulins used) mirrored the time-action profiles for mean glucose levels.

The overall mean endogenous insulin levels were slightly higher after insulin lispro, compared to those observed with insulin glulisine, although the mean total insulin data show that levels were

higher with insulin glulisine, than insulin lispro. However, there were some negative values from the individual data which may reflect variability in the assay methods.

			Geometric means				
Variable	**Meal**	**Time**	**Glulisine**	**Lispro**	**Ratio* (Glulisine:Lispro)**	**90% Confidence Interval**	**p-value**
INS–AUC (µIU.min/mL)	Breakfast	0–1 hour	2307.37	1904.27	1.21	(1.02, 1.44)	0.07
		0–1.5 hours	4307.24	3794.30	1.14	(0.95, 1.36)	0.23
		0–2 hours	6653.25	5931.07	1.12	(0.97, 1.29)	0.18
		0–4 hours	14836.81	12487.97	1.19	(1.09, 1.30)	<0.01
	Lunch	0–1 hour	5473.18	3772.08	1.45	(1.34, 1.57)	<0.01
		0–1.5 hours	9034.56	6537.81	1.38	(1.30, 1.47)	<0.01
		0–2 hours	12731.39	9176.47	1.39	(1.30, 1.48)	<0.01
		0–4 hours	23849.87	17292.60	1.38	(1.30, 1.47)	<0.01
	Dinner	0–1 hour	5908.03	4356.89	1.36	(1.23, 1.50)	<0.01
		0–1.5 hours	9784.40	7487.47	1.31	(1.20, 1.42)	<0.01
		0–2 hours	13678.32	10510.95	1.30	(1.21, 1.40)	<0.01
		0–4 hours	25767.48	18858.18	1.37	(1.30, 1.44)	<0.01
INS_{max} (µIU/mL)	Breakfast	NA	83.86	77.35	1.08	(0.99, 1.18)	0.13
	Lunch	NA	129.01	99.92	1.29	(1.18, 1.41)	<0.01
	Dinner	NA	141.51	115.11	1.23	(1.13, 1.34)	<0.01
	Overall	NA	115.25	96.18	1.20	(1.14, 1.26)	<0.01
$INS–T_{max}$ (mins)	NA	NA	108.87	89.52	19.35	(8.45, 30.25)	<0.01

*Difference for **$INS–T_{max}$**

Results – Efficacy

There were no statistically significant differences between the two insulins in the AUCs, C_{max} or T_{max} for the C-peptide curves (p=0.35, 0.53 and 0.48, respectively). However, there were significant differences between the AUC, C_{max} and T_{max} values related to the meals (p <0.0001 for all), where the values recorded during the post-breakfast period were larger than those recorded during the post-lunch and post-dinner periods.

There were no significant differences between the insulin treatments in the NEFA AUC (p=0.84; estimated ratio=1.025, 90% CI: 0.83 to 1.27 (which lie mainly within the range defining bioequivalence). However, there were highly significant differences between the NEFA AUC values

for the three meals (p <0.0001), with higher peaks in the post-breakfast period and the post-dinner period, compared with the levels seen after lunch.

The results from the triglyceride analysis reflect the findings from the NEFA analysis, such that there were no significant differences between-treatment differences in triglyceride levels (p=0.89; estimated ratio=1.01, 90% CI: 0.93 to 1.09), but there were highly significant differences between the triglyceride AUC values for the three meals (p <0.0001), with higher levels noted during the post-lunch period, compared with the levels noted after breakfast and dinner. There were no significant interactions between treatments and meals (p=0.41).

Similarly to the results obtained with the NEFA and triglyceride data, there were significant differences between the β-hydroxybutyrate AUC values for the three meals (p <0.0001), the highest level being seen during the post-breakfast period, compared with the levels seen after lunch and dinner. As before, there were no significant interactions between treatments and meals (p=0.34), nor between treatments.

Results – Safety

The results of the safety analysis show that none of the 19 randomized patients experienced any treatment-related AEs with either insulin glulisine or insulin lispro. Two subjects experienced one AE each; a serious AE (lower lobe consolidation) which resulted in the withdrawal of that subject, and non-serious AE (mild, intermittent chest pain). Neither of these AEs was attributed to study medication.

One subject demonstrated two hypoglycaemic episodes during this study during an insulin lispro treatment day, but did not require assistance on either occasion.

Laboratory data and vital signs remained consistent throughout the study; any decreases in haemoglobin, haematocrit or erythrocyte levels were attributed to blood withdrawal for sampling. In addition, subjects did not experience any pain at the injection sites. The safety results, therefore, indicate that both insulin glulisine and insulin lispro were well-tolerated in this study.

Report Date

26 January 2006