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ORIGINAL ARTICLE

Bioequivalence between two human insulin analogs in Chinese population: Glulisine and Lispro

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Abstract Intensive insulin therapy for diabetic patients has been demonstrated as an appropriate treatment. Regular fast-acting insulin can hardly mimic the efficiency of endogenous meal-activated insulin secretion. Glulisine is a new rapid-acting insulin analog for mealtime insulin supplementation. We compared the pharmacokinetics and pharmacodynamics end points between the two rapid-acting insulin analogs Glulisine and Lispro. Twenty healthy adult males age ranging from 22 to 32 years were included in a randomized, open-label, cross contrast research. Two long duration hyperinsulinemic euglycemic clamp tests, one with Glulisine and the other with Lispro, were conducted on two separate days for all the participants. The two rapid-acting insulin analogs were administrated randomly to each participant. Glucose infusion rate (GIR) began to increase 20 min after injection in both Glulisine and Lispro groups. GIR increased sharply during the first 150 min and reached a peak at 6.23 ± 1.35 mg/(kg min) in the Glulisine group and 6.02 ± 1.27 mg/(kg min) in the Lispro group. It returned to the initial level at hour 5. The Area Under Curve ($AUC_{0\text{-clamp end}}$) in Glulisine and Lispro groups were 1455.04 ± 381.88 mg/kg and 1356.25 ± 287.30 mg/kg ($P > 0.05$), respectively. However, $AUC_{0–1h}$ between the two groups showed significant difference, with Glulisine showed greater $AUC_{0–1h}$ in the first hour after injection. Other parameters showed no significant difference between the two groups. Insulin analogs Glulisine and Lispro were proved to have equivalent pharmacokinetic and pharmacodynamic parameters when administered to healthy Chinese adults, but with Glulisine showing greater $AUC_{0–1h}$ after injection.

Keywords Euglycemic hyperinsulinemic clamp test · Insulin Glulisine · Insulin Lispro · Pharmacokinetic and pharmacodynamic parameters

Introduction

To prevent the onset and aggravation of micro- and macrovascular complications, blood glucose level is required to be maintained within normal range [1, 2]. Intensive insulin therapy has been used on diabetic patients and demonstrated as an appropriate treatment, especially for type 1 diabetic patient [3, 4]. Endogenous insulin secretion has been illustrated with a basal-bolus and three meal-related peaks [5]. The regular fast-acting insulin such as Novolin R and Humilin R can hardly mimic the endogenous meal-activated insulin secretion. To get physiological insulin secretion model and achieve optimal metabolic control, a new rapid-acting insulin analog has been synthesized by DNA-recombinant technique, which shows more rapid glucose-lowering effect than regular human insulin and reaches more optimal postprandial glucose levels and hemoglobin A1c (Hb_{A1c}) [6].

Insulin Glulisine is a new rapid-acting insulin analog intended for mealtime insulin supplementation via subcutaneous injection or external infusion pump. Compared with regular human insulin, the amino acids asparagines and lysine in insulin Glulisine at positions B3 and B29 are

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replaced by lysine and glutamic acid (3B-Lys-29B-Glu-insulin) [7], respectively. Other rapid-acting insulin analogs such as Lispro and Aspart have been on the market for many years, and in this study, we evaluated insulin analogs Glulisine and Lispro on their bioequivalence by euglycemic hyperinsulinemic clamp technique and reviewed the safety issues in subjects administrated with Glulisine.

Research design and methods

Subjects

Twenty healthy adult males were included in the study ranging from 22–32 years of age (mean age: 26.2 ± 2.7 years), they were all in similar physiques with a body mass indexes (BMIs) of 19.6–24.9 kg/m^2 (mean BMI: 22.3 ± 1.6 kg/m^2; Table 1). All subjects are Chinese living in Shanghai and had given informed consent. The Institutional Review Board of the Ruijin Hospital had approved the study protocol. All participants were non-smokers and without family history of diabetes mellitus, and their plasma glucose concentrations were within normal range (fasting glucose level < 6.1 mmol/l, OGTT 2 h < 7.8 mmol/l and Hb_{A1c} < 6.4%). Volunteers were excluded if they enrolled in any other clinical trails within the 3 months before this study or with diseases which could influence the metabolism of the drugs being tested.

Research designs

To investigate the bioequivalence in human insulin analogs Glulisine and Lispro, a randomized, open-label, two-period crossover study was performed. The study protocol was reviewed and approved by the local ethics committee and was performed according to GCP/ICH guidelines and the Declaration of Helsinki. During the 2-week trial period, volunteers received initial screening including medical history collection, physical examination, and biochemistry tests (renal and hepatic function, blood/urine regular test, electrolyte analysis, Hb_{A1c} and a 72.5 g glucose-tolerant test) in the clinical research center of Shanghai Ruijin Hospital. Next, subjects went through two long duration euglycemic hyperinsulinemic clamp tests. The two rapid-acting insulin analogs were separately and randomly administrated to each participant on separate days in two different weeks, and there was a week's break period between the two clamp tests to prevent the interaction of the two insulin analogs. Subjects were requested to come back to the clinical research center for follow-up visit within 2 weeks after clamp tests. Physical examination and biochemistry tests were repeated to evaluate the safety of these two insulin analogs.

Table 1 General characteristics of participants ($N = 20$, male subjects)

Item	
Age (years)	26.2 ± 2.7
Body weight (kg)	67.7 ± 8.1
BMI (kg/m^2)	22.3 ± 1.6
FBG (mmol/l)	4.93 ± 0.44
PBG (mmol/l)	4.91 ± 0.97
Hb_{A1c} (%)	5.20 ± 0.33
INS0' (iu/ml)	5.27 ± 2.17

Materials

Glulisine and Lispro were provided by Aventis Pharma Deutschland GmbH (LOT: 40N097) and Lily France S.A.S. (LOT: A343910), respectively. All the drugs were stored at 2–8°C (36–46°F). Biochemical measurements of Hb_{A1c} and insulin were performed in the same laboratory (Shanghai Institute of Endocrinology and Metabolism, Shanghai, China). Plasma glucose concentration was measured using an enzymatic method (Beckman CX-7 Biochemical Auto-analyser, Brea, CA, USA). Serum insulin was measured using a double antibody radioimmunoassay (DSL, Webster, Texas, USA). Hb_{A1c} was measured by high performance liquid chromatography (HPLC) using the BioRad Variant Hemoglobin Hb_{A1c} assay (Hercules, CA, USA).

Euglycemic hyperinsulinemic clamp

Participants came to the research center in the morning after an overnight fasting. Throughout the clamp test, the subjects remained in fasting and in a supine position. A polyethylene cannula was inserted into an antecubital vein for blood drawing, and a second catheter was inserted retrogradely into a wrist vein on the dorsum of the hand for infusion of test substances. The hand for blood drawing was kept in a heated box at 65°C. In the first hour of the test, a variable intravenous infusion of human regular insulin was administrated in order to suppress the endogenous insulin secretion, and a variable infusion of 20% glucose solution was implemented to adjust the negative feedback principle to maintain the plasma glucose concentration at about basal level. When glucose concentration had been stablized (about an hour from the start), Glulisine or Lispro (0.2 IU/kg) was injected subcutaneously through a lifted skin fold on the abdomen, and due to the rapid glucose-lowering effect of the insulin analogs, 20% glucose solution was added immediately to prevent a decline

in blood glucose. To keep blood glucose level constant, the plasma glucose concentration was recorded with a glucose analyzer (Biosen 5130, Neckar Healthcare. Co. Ltd., Magdeburg, Germany) every 5 min in the first 6 h and every 10 min in the next 3 h. The euglycemic glucose clamp test would be stopped earlier if glucose infusion rate (GIR) dropped down to initial level for at least 30 min. Insulin and c-peptide were drawn at 10-min intervals in the first 3-h and at the specific time points in the next 5 h: 3, 3.5, 4, 4.5, 5, 6, 7, and 8 h after injection.

Statistical analysis

A comparison of Glulisine and Lispro was carried out using a t test with $P < 0.05$. Statistical analysis was performed by the SPSS 13.0 system, and data were presented as mean ± SD. All end points derived were analyzed by analysis of variance (ANCOVA) for crossover design with sequence (subjects and treatment included in the model) and by a Wilcoxon rank-sum test. A sample size of 20 subjects was considered sufficient to detect differences between the study products.

Results

Treatment period

Preinjection period (0–1 h)

Plasma glucose concentration, rate of intravenous insulin infusion, plasma insulin concentration, and plasma c-peptide concentration were observed to be similar before the injection either Glulisine or Lispro (NS, data not shown).

Serum insulin and c-peptide concentrations postinjection period (1–9 h)

After subcutaneous injection of Glulisine or Lispro, c-peptide concentration was measured and this was used to reflect the endogenous insulin secretion during the clamp test. Throughout the clamp test, the concentration of c-peptide was constant at near 1.2 ng/ml, and the concentration difference between the two groups had no significance (Fig. 1). These results showed that the secretion of endogenous insulin was completely suppressed during the test.

Analysis of GIR and other related pharmacodynamics parameters

After 20 min either Glulisine or Lispro was injected, GIR began to increase, and it increased sharply and reached a peak at 150 min; it then decreased to the initial level by hour 5. The GIR curves in both insulin analog groups were highly similar, except for some differences among individuals (Fig. 2). The area under curves ($AUC_{0\text{-clamp end}}$) in Glulisine and Lispro groups were 1455.04 ± 381.88 mg/kg and 1356.25 ± 287.30 mg/kg, respectively, showing no statistical difference between the two groups ($P = 0.1264$) (Table 2). However, the AUC_{0-1h} was 69.22 ± 38.59 mg/kg in Glulisine group and 45.95 ± 28.84 mg/kg in Lispro group ($P < 0.05$). The maximal GIR were 6.23 ± 1.35 mg/(kg min) in Glulisine group and 6.02 ± 1.27 mg/(kg min) in Lispro group ($P = 0.1908$). The times of GIR getting up to half ($T_{0-1/2GIR}$), at the peak (T_{max}), and back to half ($T_{max-1/2GIR}$) showed no significant differences between the two groups. It could be concluded that no significant differences were detected in all pharmacodynamic parameters between the two groups (Table 3).

Analysis of plasma glucose

With the two study products, plasma glucose concentration was maintained at the target of 4.5–5 mmol/l until 8 or 9 h, and no significant difference was detected between the two groups.

Safety events

After the subcutaneous injection of the two insulin analogs, no sever side effect was observed in both groups.

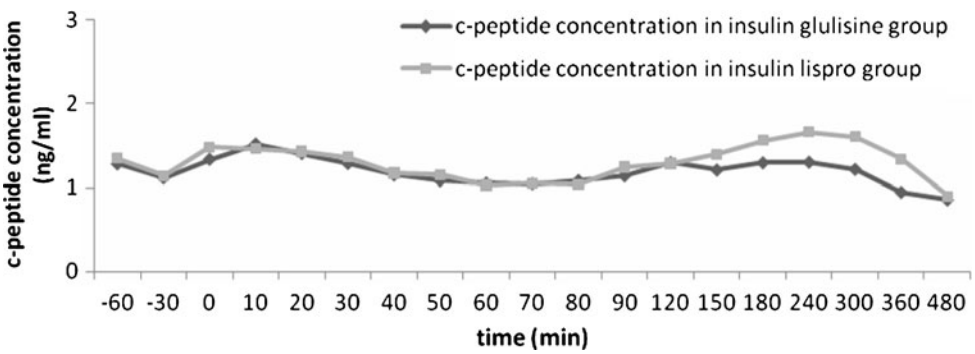

Fig. 1 Comparison of c-peptide concentration during clamp test between the two groups. The mean c-peptide concentration in Glulisine group is 1.19 ng/ml, and the mean c-peptide concentration in Lispro group is 1.29 ng/ml ($P > 0.05$)

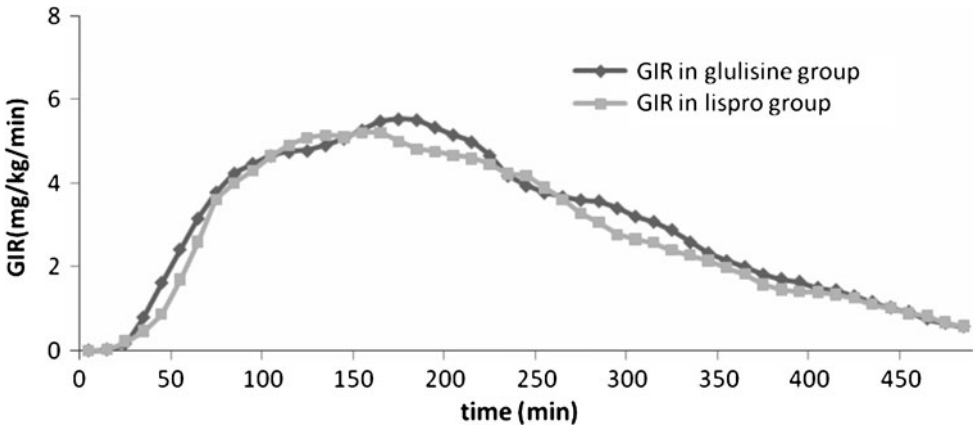

Fig. 2 Comparison of GIR during clamp test between the two groups

Table 2 Comparison of AUC0-clamp end between the two groups

Group	AUC (mg/kg)	*P*	Glulisine/Lispro 90% confidence interval
Glulisine	1455.04 ± 381.88	0.1264	107.67%
Lispro	1356.25 ± 287.30		100.69–114.64

$P > 0.05$ means AUC showed no significant difference between the two groups

Table 3 Comparison of AUC_{0-1h}, GIR_{max}, T_{max}, $T_{0-1/2GIR}$, $T_{GIR\text{-}1/2GIR}$ between the two groups

	Glulisine	Lispro	*P*
AUC_{0-1h} (mg/kg)	69.22 ± 38.59	45.95 ± 28.84	0.0237
GIR_{max} (mg/kg)	6.23 ± 1.35	6.02 ± 1.27	0.1908
T_{max} (min)	156.00 ± 44.30	161.00 ± 39.86	0.8071
$T_{0-1/2GIR}$ (min)	62.82 ± 16.38	63.87 ± 22.80	0.7972
$T_{GIR\text{-}1/2GIR}$ (min)	150.99 ± 42.94	140.63 ± 61.44	0.4249

$P > 0.05$ means there was no significant difference between the two groups. $P < 0.05$ means there was significant difference between the two groups

Discussion

This study was undertaken to compare the bioequivalence between a new rapid-acting human insulin analog Glulisine and another on the market rapid-acting insulin analog Lispro, the latter is most commonly used in intensive treatment to supplement meal-time insulin.

Insulin resistance and insulin deficiency are two main leading causes in the mechanism of diabetes mellitus. Reports have concluded that it is important to control the blood glucose within normal range by insulin replacement in patients with diabetes [8]. Currently, the most popular insulin product used around the world is regular human insulin produced by DNA recombination technique. In the second half of 1990, researchers had worked on human insulin analog by using gene recombination to modify amino acids sequence. Insulin analog can also bind to insulin receptor and shows great glucose-lowering effect; and as compared to human insulin, it can mimic physiological insulin secretion better. There are two kinds of insulin analogs on the market: long-acting and rapid-acting analogs. Long-acting insulin analog is similar with basal insulin secretion because of its stable absorption, gentle effect and long duration without a peak in blood concentration. It has been proved that long-acting insulin analog is more effective and safer than neutral protamine Hagedorn's globin insulin when administrated at bed time [9, 10]. Rapid-acting insulin analog has a fast-onset action and reaches a peak at 30–60 min after injection; it mimics the physiological mealtime insulin secretion and can better control postprandial glucose level. As the duration of rapid-acting insulin analog is about 3 h, few patients suffer from hypoglycemic events; according to its characteristics, this kind of insulin analog has been widely used in continuous subcutaneous insulin infusion. At the moment, there are two rapid-acting insulin analogs on the market: Insulin Lispro, in it the penultimate lysine and proline residues on the C-terminal end of the B-chain are reversed [2, 11, 12], and Insulin Aspart, with its amino acid proline at position B28 replaced by aspartic acid [11]. Intensive insulin treatment incorporating insulin analogs of rapid-acting with long-acting has been proved more effective and safer than traditional model.

Glulisine, a product from Sanofi-Aventis, is a human insulin analog under evaluation in this study. It is necessary to get creditable evidences of the pharmacokinetic and pharmacodynamic parameters about this insulin analog before its clinical application in China. Glulisine is synthesized by *Escherichia coli* K12 I35 expressing plasmid pINT 366 and by replacing the amino acids asparagines and lysine at positions B3 and B29 of the human insulin by lysine and glutamic acid, respectively, with empirical formula as C258H384N64O78S6 and a molecular weight of 5823 [13]. The euglycemic hyperinsulinemic clamp

technique was used in this study to suppress endogenous secretion of insulin and hepatic glucose production. Twenty healthy Chinese adults undertook two long duration euglycemic hyperinsulinemic clamp tests to compare the pharmacokinetic and pharmacodynamic parameters between the study product and insulin Lispro. To make the results more comparable, participants received each clamp test on separate day in two different weeks; and the levels of plasma insulin levels and glucose at baseline and during clamp tests were similar in the two groups. Both insulin analogs began to show the glucose-lowering effect 20 min after injection. Becker et al. [14] proved that Glulisine and Lispro demonstrated substantially more rapid time-acting profiles than regular human insulin in obese non-diabetic subjects, which prevailed with insulin Glulisine irrespective of BMI or subcutaneous fat thickness. Heise et al. [15] reported insulin Glulisine showed a faster onset of action than Lispro in the first hour, independent of BMI and dose. The AUC 0-clamp end of GIR–time curve was similar in Glulisine and Lispro groups, and the 90% confidence interval of AUC0-clamp end in Glulisine group was located in the range of 100.69–114.64 in Lispro group. This showed the two insulin products had similar biochemical equivalent. But AUC_{0-1h} showed significant difference between the two groups, with Glulisine having a greater effect at the first hour after injection, and this result was in accord with previous studies. Other pharmacokinetics and pharmacodynamics parameters had no statistical difference between the two groups. Throughout the research period, no side effect was reported in either groups. Previous studies on Glulisine were conducted on Caucasian, but there were no data about this new insulin analog in Chinese. This study provided data about the effectiveness of this new insulin analog, allowing its application in diabetics in China.

Accordingly, by using long duration euglycemic hyperinsulinemic clamp test, insulin analogs Glulisine and Lispro were proved to have similar GIR–time curves and equivalent pharmacokinetic and pharmacodynamic parameters in healthy Chinese adults. However at the first hour after injection, Glulisine showed a greater AUC_{0-1h} than that of Lispro, yet both were with satisfactory tolerance. In conclusion, Glulisine, same as Lispro, is an effective and safe rapid insulin analog, which mimics the physiological endogenous insulin secretion stimulated by food.

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