Studies of pharmacokinetic, pharmacodynamic properties and bioequivalence of recombinant insulin glargine injection in healthy man

CHENG Shi-wu,LU Jun-ming,PANG Chang-yum (Department of Endocrinology, Chinese PLA General Hospital, Beijing 100853, China.)

Abstract

Objective: To study the pharmacokinetic and pharmacodynamic properties of the subcutaneously injected long-acting insulin analog-recombinant insulin glargine injections in comparison with those of reference preparation-NPH insulin injection (Novolin® N) in healthy volunteers, and to evaluate the bioequivalence of domestic (Basalin®) and imported (Lantus®) recombinant insulin glargine injection preparation.

Methods: This single center, randomized, single-blind, three-period, crossover design study was carried out by an euglycemic glucose clamp test. 16 healthy male volunteers received single subcutaneous injection of 0.4 U/kg body weight of Basalin administration.

Results: The two injections of insulin glargine did not induce the pronounced peak in metabolic activity (P < 0.01) and neous injection of Basalin®, Lantus® or Novolin® N. Plasma insulin concentrations (P <0.05) showed a constant metabolic activity over 24-hour study period, which were different from NPH insulin. The statistic analysis of variance, two one-sided tests and 90% confidence interval were calculated. There were no significant differences in INS-AUC0~24h, INS-Cmax and INS-Tmax between the two glargine insulin preparations (P >0.05). The 90% confidence interval of BasalinTM -Ln(AUC0~24h) was 0.82 to 1.05 in the range of 0.8 to 1.25, the 90% confidence interval of Basalin®- Ln (Cmax) was 0.789 to 1.077 in the range of 0.7 to 1.43, which were in accordance with those of Lantus®.

Conclusions: The subcutaneously injected long-acting insulin analog-recombinant insulin glargine injections (Basalin® and Lantus®) induce a smoother metabolic effect and more stable plasma insulin concentrations than the NPH insulin injection (Novolin®N). The results of pharmacokinetic and pharmacodynamic properties demonstrate that the domestic (Basalin®) and imported (Lantus®) insulin glargine injection preparations were bioequivalent.

Keywords: Insulin glargine injections, Pharmacodynamics, Pharmacokinetics, Bioequivalence.

Insulin glargine is a kind of human insulin analog manufactured by using recombinant DNA technology and can smoothly and stably reduce the level of blood glucose. The recombinant insulin glargine injection (Basalin) manufactured by Gan & Lee Pharmaceutical has same active ingredients as the imported products of same kind. In order to further investigate the pharmacokinetic and pharmacodynamic properties of the product, the imported insulin glargine injection (Lantus) and NPH insulin injection (Novolin N) were used as reference preparations in this trial.

Objects and Methods

I: Objects

In accordance with the requirements of Guiding Principle for Bioavailability and Bioequivalence of Chemical Preparation in the Human Body (March 2005) and relevant foreign literatures, 16 healthy male volunteers were included in this trial according the following criteria: (1) aged 18~30; (2) body weight >50kg, BMI: 19.0~24.0kg/m2; (3) healthy without indulgence in smoking or drinking; without medical history with heart, liver, kidney and abnormal metabolism; without medical history of hypertension, diabetes mellitus or relevant family history; (4) normal in heart rate, breathing, body

temperature, blood pressure, hepatic and renal functions and blood routine upon examination, negative insulin antibody, normal serum lipid profile and normal blood glucose level according to 75g OGTT test (WHO criteria of 1999); (5) no taking other drugs in two weeks before and during the period of trial, no smoking, drinking or drinking other beverage containing caffeine; (6) voluntary to participate in this trial sign the informed consent before the trial.

II: Methods

1. Study Design and Flow

This trial adopts a single center, randomized, single-blind and crossover design.

Basalin and Lantus were administrated in all 16 objects, and Novolin N was administered to only 6 of them. All objects were randomized into 2 groups, firstly administered with Basalin and Lantus, respectively. The trial lasted for 5~8 weeks for each object, including the physical examination in the 1st week. The first kind of insulin was administered in one day in the 2nd week, the second kind of insulin 2 weeks later; Novolin N was administered to 6 of them another 2 weeks later.

All objects kept fasting in the night before the trial. The necessary glucose infusion rates (GIR) was used to conduct venous puncture in the forearms at both sides with needle tube reserved, one for infusion of insulin and 20% glucose (respectively connected to Injectomat C-IS and INCA-ST positive-displacement pump manufactured by Fresenius; both were connected to CLAMP intelligent glucose clamp system manufactured by EKF); the other side for blood collection in the trial (with forearm kept in 60°C constant temperature cabinet to maintain artery of venous blood), infused with normal saline when blood was not collected. Prompt insulin (Novolin R, 40 U/ml, produced by NovoNordisk) was continuously infused at a constant rate (0.15mU/kg/min) to keep blood glucose concentrations constant at 5.0 mmol/L. Venous blood glucose was measured once every 30 min (BIOSEN 5030 automatic blood glucose/lactic acid analyzer, glucose oxidase electrode, manufactured by EKF). GIR was adjusted according to the blood glucose level to reach stable glucose state. And 2 hours later, 0.4U/kg insulin (Basalin, Lantus or Novolin N) was subcutaneously injected at the position 5cm away from navel, then venous blood was collected respectively every 30min to measure the blood glucose level and every 60min to measure insulin and C-peptide concentration. GIR was adjusted with the save method to keep blood glucose concentrations constant at 5.0 mmol/L for 24h. The trail lasted 26h, all objects kept lying with water but not food given.

2. Candidate drug

Basalin was provided by Gan & Lee Pharmaceutical, the reference drug and other candidate drugs were supplied by PLA General Hospital's drug section. All candidate drugs were kept in 2~8°C refrigerator.

3. Collection of samples and data

The serums of all objects were separated after the examination and stored in -80°C refrigerator for central test.

Blood glucose measurement was carried out with glucose oxidase electrode method by using BIOSEN 5030 automatic blood glucose/lactic acid analyzer manufactured by EKF, CV <1.5%.

Insulin measurement was carried out with chemiluminescent method by using Access Immunoassay System manufactured by BECKMAN Coulter, the kits (lot No.: 714362) were purchased from BECKMAN Coulter in the range of 0.03~300mU/L. The recovery rates of two standard insulin products of different concentration (36.75 mU/L, 104.90 mU/L) were 90.37% and 94.54%, respectively. Cross reaction rates were measured for the standard products of two kinds with three different concentrations before the measurement of sample due to the insulin measured in the trail was insulin analog, and the results were 88.10% (Basalin) and 86.87% (Lantus), respectively. Intra-assay and inter-assay coefficients of variation (CV) of insulin-quality control serum with three different concentration (manufactured by Bio-Rad, lot No.: 40190) were measured, the results were intra-assay CV < 5.0% and inter-assay < 10.0%. C-peptide was measured with chemiluminescent method.

III: Statistical Analysis of Trial Data and Bioequivalence Determination Criteria

SPSS 10.0 statistical software was used for statistical analysis.

Statistical description was done by mean value ± standard deviation of measurement data of different injections. The area below the curve was calculated with trapezoidal method. The inter-assay deviation was compared with analysis of variance (ANOVA).

Main pharmacokinetic parameters Cmax and Tmax were taken as the measured value, and significance of Ln(Cmax) and (AUC0gt) was measured with ANOVA. The inter-drug bioequivalence was evaluated with the statistic analysis two one-sided tests and 90% CI calculation. If the candidate preparation- Ln (AUC0gt) was in the range of 80% to 125% of the reference preparation and candidate preparation -Ln(Cmax) was in the range of 70% to 143% of reference preparation, it could be determined the candidate drug was bioequivalent to the reference preparation. If there were no significant differences upon the rank test of Tmax, it could be determined the candidate preparation was bioequivalent to the reference preparation.

Results

I: General Information of Objects

Total 16 volunteers were aged 24.4±3.8 and their BMI was 21.06±1.65kg/m2. All objects completed the trial according to the schedule without adverse drug reaction and other adverse events till the end of the trial.

II: Pharmacokinetic and Pharmacodynamic Parameters

Table-1: Comparison of pharmacokinetic and pharmacodynamic parameters among subcutaneous injection of Basalin, Lantus and Novolin N ($\bar{x}\pm S$).

Item	Basalin (G1) (n = 16)	Lantus (G2) (n = 16)	Novolin N (N) (n = 6)	P value G1 vs G2	G1 vs N	G2 vs N
Pharmacodynamic parameter						
GIR						
Basal GIR (mg·kg-1·min-1)	2.96±0.88	3.03±0.55	3.21±0.45	>0.05	>0.05	>0.05
GIRmax (mg·kg-1·min-1)	6.99±1.07	7.30±1.06	11.80±0.98	>0.05	<0.01	<0.01
Tmax(h)	7.38±3.29	7.25±2.78	4.58±1.16	>0.05	<0.05	<0.05
AUC0~4h(g/kg)	1.03±0.20	1.05±0.15	1.25±0.20	>0.05	<0.05	<0.05
AUC0~8h(g/kg)	2.53±0.44	2.60±0.33	3.61±0.34	>0.05	<0.01	<0.01
AUC0~16h(g/kg)	5.33±0.74	5.50±0.66	6.78±0.63	>0.05	<0.01	<0.01
AUC0~24h(g/kg)	7.82±1.05	8.01±0.86	9.15±0.99	>0.05	<0.05	<0.05
Hharmacokinetic parameter						
Ins conc.						
Basal (mU/L)	14.83±5.60	15.34±6.80	12.90±5.37	>0.05	>0.05	>0.05
Cmax (mU/L)	39.87±8.79	43.50±10.68	54.79±8.59	>0.05	<0.05	<0.05
Tmax(h)	12.19±5.78	12.56±5.53	5.67±1.63	>0.05	<0.05	<0.05
AUC0~24h [(mU/L)·h]	4.88±1.00	5.28±1.16	3.97±0.75	>0.05	<0.05	<0.05
C-peptide conc.						
Basal (ng/ml)	1.83±0.68	1.75±0.32	1.67±0.31	>0.05	>0.05	>0.05
Cmin(ng/ml)	0.72±0.22	0.58±0.12	0.61±0.11	>0.05	>0.05	>0.05
Tmin(h)	19.63±4.03	18.38±4.93	14.67±5.77	>0.05	>0.05	>0.05
AUC0~24h [(ng/ml)·h]	32.89±11.22	29.49±5.14	34.08±7.19	>0.05	>0.05	>0.05

See Table 1 for the fasting blood glucose (FBG) and basal glucose (after 2h treatment) in normal glucose clamp test and average blood glucose levels after subcutaneous injection of Basalin, Lantus and Novolin N (after 2~26h treatment) among 3 groups of objects. There were no significant differences in FBG, basal glucose and blood glucose among 3 groups in the trial period (P > 0.05). In the trial period, the levels of blood glucose in 3 groups were kept 5.0mmol/L and the CVs were maintained within 10%.

After Basalin or Lantus was subcutaneously injected, in vivo insulin activity represented by GIR

reached a higher plateau within 4 hours and then kept stable over 24h study period without pronounced peak in metabolic activity. After Novolin N was subcutaneously injected, GIR reached pronounced peak at 4~8 h treatment and then slowly decreased till basal value at the end of 24h study, lower than that Basalin or Lantus group, but the difference between 2 groups had no statistical significance (P > 0.05) (Fig. 1).
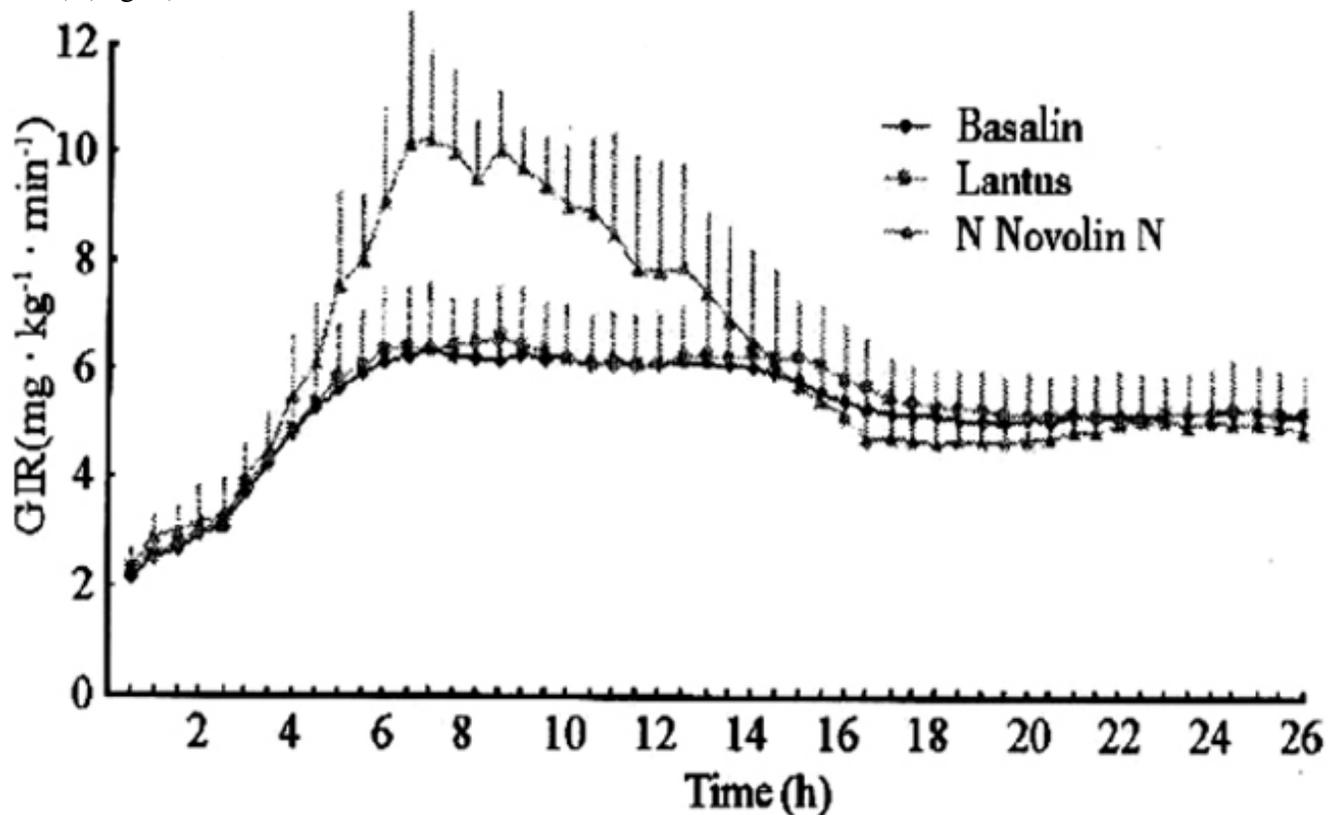

Figure-1: GIR Curve after subcutaneous injection of Basalin®, Lantus® or Novolin® N.

The maximum glucose infusion rate (GIRmax) of Basalin or Lantus group was significantly lower than that of Novolin N group (P < 0.05), the time to reach GIRmax (Tmax) was also significantly behind Novolin N group (P < 0.05) (Table 1).

The areas under GIR curves of Basalin or Lantus group or Novolin N group were compared by time, it could be found that AUC0~4h was significantly higher than Basalin or Lantus group from the very beginning (P < 0.05), similarly AUC0~8h, AUC0~16h and AUC0~24h (P < 0.05). AUC over all study period had no significant difference between Basalin group and Lantus group.

Objects' plasma insulin concentration reached high level 2 hours after subcutaneous injection of Basalin or Lantus, then always kept high level in the subsequent 18 hours, just a little decreased within the last 4 hours but still above baseline. There was no significant difference between Basalin group and Lantus group in the maximum plasma insulin value (Cmax) and the time to reach the peak (Tmax) (P > 0.05). Novolin group objects' plasma insulin concentration reached the peak at 4~8h treatment, then slowly decreased to a little above baseline at 18~24h treatment (Fig. 2, Table 1).
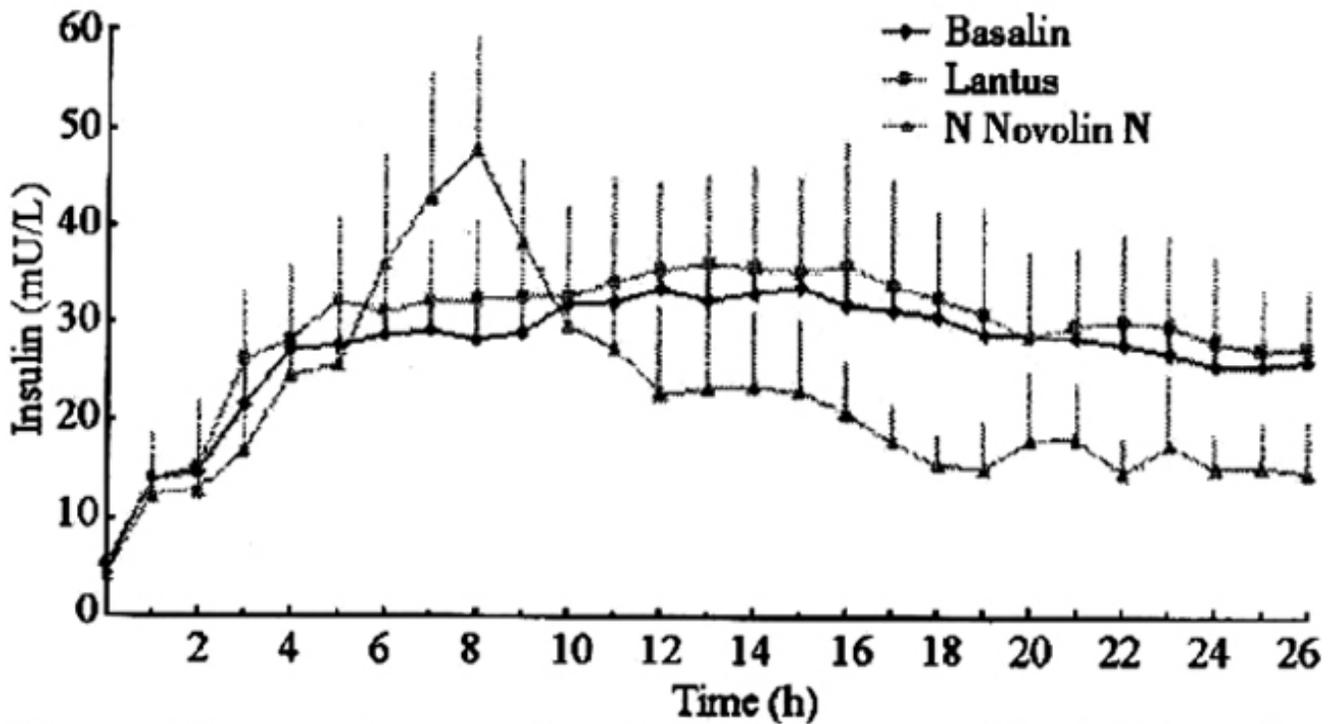
Figure-2: Plasma insulin curve after subcutaneous injection of Basalin®, Lantus® or Novolin® N.

According to the measurement results of objects who were subcutaneously injected with Basalin, Lantus or Novolin N, C peptide concentration were decreased in 3 groups, without significant difference in the minimum C peptide concentration (Cmin) and the time to reach Cmin (Tmin) (Fig. 3, Table 1).
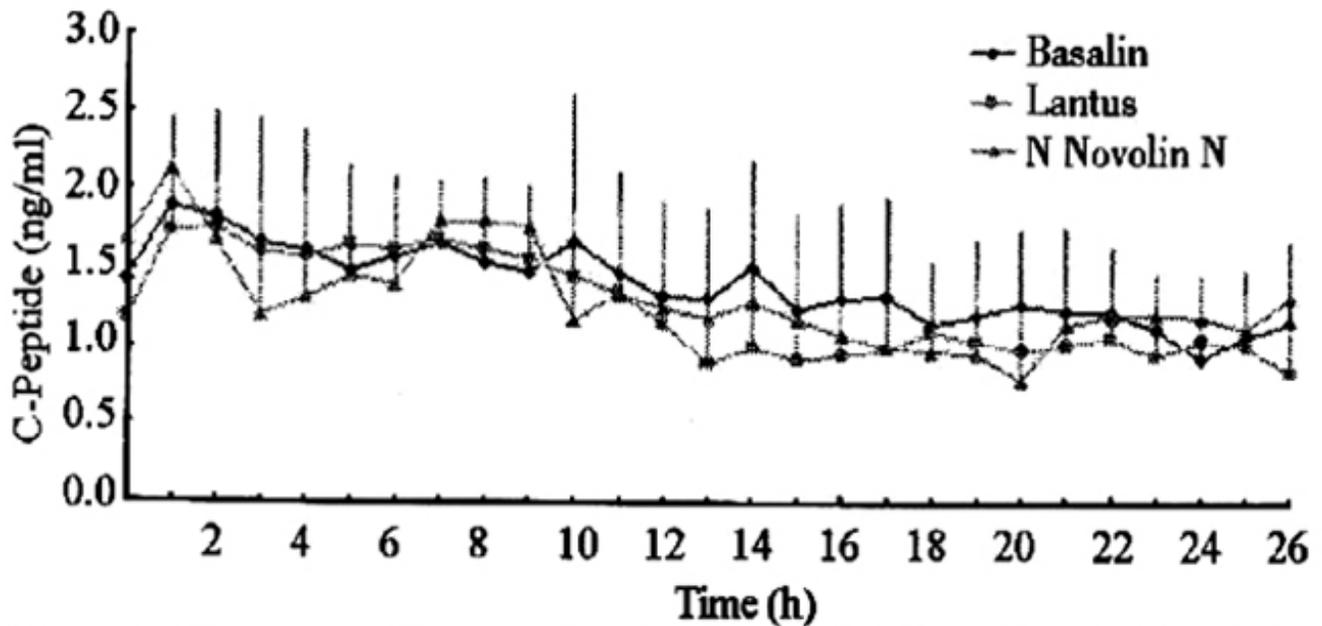

Figure-3: Plasma C peptide curve after subcutaneous injection of Basalin®, Lantus® or Novolin® N.

IIII: Bioequivalent Evaluation of Basalin and Lantus

Ln(AUC0~24h) and Ln(Cmax) were calculated according to the measured plasma insulin of 16 objects who were subcutaneously injected with Basalin and Lantus. The difference between these Basalin group and Lantus group had no statistical significance upon ANOVA of crossover study design (P > 0.05). According to two one-sided tests and 90% CI calculation, 90% CI of Ln(AUC0~24h) was in the range of 80%~125%, 90% CI of Ln(Cmax) was in the range of 70%~143% (Table 2).

Table-2: The bioequivalence of Basalin and Lantus.

Parameter	tL	tH	t1~0.05, 14	90%CI
Ln(AUC0~24h)	2.10	4.20	1.76	82.0%~105.2%
Ln(Cmax)	3.11	4.97	1.76	78.9%~107.7%

Basalin vs Lantus, *P > 0.05.

Upon the rank test of Tmax, the difference had no statistical significance (P > 0.05). According to the above results, the candidate Basalin and the reference Lantus were bioequivalent.

Discussion

The subcutaneously-injected insulin glargine had low dissolvability which made it form micro-sediment and slowly dissolve into monomer in blood. Its 24h insulin level was similar to basal insulin secreted by healthy human body. Therefore, it could stably and continuously decrease the level of blood glucose. Basalin had same active ingredients with the imported Lantus, and bioequivalent test was made in order to know well the characteristics of Basalin.

It is hard to study the pharmacokinetic property of insulin as a special drug due to the intervention of endogenous insulin in human body. The currently-used methods include using growth inhibitor inhibiting the secretion of endogenous insulin, or creating high exogenous insulin concentration to inhibit the secretion of endogenous insulin, or using T1DM patients lack of insulin as objects. All these methods have their limitations, such as growth inhibit may inhibit the secretion of hyperglycemia factor and affect subcutaneous blood circulation and insulin clearance; high exogenous insulin concentration created in trial may inhibit the secretion of endogenous insulin within a short term and it cannot do so over 26h trial; choosing T1DM patients lack of insulin as objects is easy to cause the glucose variance and affect their normal therapy. In some studies healthy volunteers are included as objects, and placebo group is added to eliminate the influence produced by the secretion of endogenous insulin. This extends the trial period. However, the result shows the pharmacokinetic property and trial result are not changed after the influence of control group is excluded. Therefore, no placebo group was set in this trial.

BECKMAN Coulter's Access Immunoassay System used in this trial had a high cross reaction rate in measuring insulin analog. The measured cross reaction rates standard insulin products of three different concentration were close to 90%, consistent with the trial requirement and similar to the reports of literatures. The measured insulin concentration might be used to calculate the pharmacokinetic parameter.

Considering the variance of drug absorption and clearance among different individuals and inter-individual CV greater than intro-individual CV, this trial was designed according to self-cross control study. Sixteen health volunteers were included as objects and the normal glucose clamp technique was used in this trial. GIR to maintain objects' stable glucose after the subcutaneous injection of insulin was used as the index judging the metabolic activity of insulin, i.e., pharmacodynamic index with which different insulin preparations were compared for the bioequivalence. Self-cross study design and strictly-controlled trial conditions might reduce the influence of endogenous excretion of insulin to the most extent. In the trial process, the influence of endogenous insulin was observed through monitoring

the change of plasma C-peptide concentration of objects. The result showed with the progress of trial, C-peptide concentration was decreased gradually by more than 50% of baseline (Table-1). This meant the endogenous excretion of insulin was inhibited and exogenous insulin mainly played the role of glucose metabolism.

It was found in this trial (Fig. 1) that GIR was still higher than baseline 24 hours after subcutaneous injection of Basalin, Lantus and Novolin N. It was reported in foreign literatures that GIR didn't decrease to baseline after 30 hours under same trial conditions. Such result was also observed in the similar trial of T1DM patients, meaning more than 24h action of Basalin or Lantus. Clinical practice showed that the metabolism almost disappeared 12~18 hours after subcutaneous injection of Novolin N. In this trial, GIR in Novolin N group was a little lower than that of Basalin or Lantus group over 24 hours, but higher than baseline. Insulin measurement result also showed plasma insulin concentration was a little higher than baseline after 24 hours, similar to foreign reports. This was possibly related to high dosage injection of insulin (0.4U/kg).

All in all, this trial showed Basalin and Lantus were bioequivalent in both pharmacokinetic and pharmacodynamic properties. Compared with Novolin N, Basalin or Lantus could induce a smoother metabolic effect and more stable plasma insulin concentrations, without peak in metabolic activity. As basal insulin supplementation, Basalin and Lantus have better pharmacokinetic property than Novolin N.

References

1. Subject group of Guiding Principle for Bioavailability and Bioequivalence of Chemical Preparation in the Human Body, Guiding Principle for Bioavailability and Bioequivalence of Chemical Preparation in the Human Body, March 2005, [H] GCL2-1, P4-21.
2. DeFronzo RA, Tobin JD, Andres R. Glucose camp technique: a method for quantifying insulin secretion and resistance. Am J Physiol, 1979, 237: E214-E223.
3. CHENG Shi-wu, LU Ju-ming, YANG Guo-qing, et al. Establishment of hyperinsulinemic-euglycemic clamp technique, Journal of the Fourth Military Medical University, 2007, 28:2082-2084
4. Ipp E, Siani Y, Bar-Oz B, Somatostatin impairs clearance of exogenous insulin in humans. Diabetes, 1987, 36:637-677.
5. Heinemann L, Linkeschova R, Rave K, et al. Time - action profile of the long acting insulin analog insulin glargine (HOE901) in comparison with those of NPH insulin and placebo. Diabetes Care, 2000: 644-649.
6. Lepore M, Kurzhals R, Pampanelli S, et al. Pharmacokinetics and dynamics of s.c. injection of the long acting insulin glargine (HOE901)
7. Nicole L, Timothy HR, Rossa C, et al. cross-reactivity of three recombinant insulin analogs with five commercial insulin immunoassays. Clinical Chemistry, 2004. 50: 257-259.