CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 21-081

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW
Division of Pharmaceutical Evaluation II

NDA:	**21-081**
Generic	**Insulin Glargine Injection**
(Brand®)	**Lantus™**
Submission Date:	**April 9, 1999**
Sponsor:	**Hoechst Marion Roussel**
Type of Submission:	**Original NDA (NME)**
Reviewers:	**Sam Haidar**
	Michael J. Fossler

Synopsis

NDA 21-081 for insulin glargine injection (Lantus™) was submitted by Hoechst Marion Roussel on April 9, 1999. The proposed indication for Lantus™ is the control of hypoglycemia in patients with diabetes mellitus who require basal insulin. Lantus™ is administered subcutaneously once daily.

In support of NDA 21-081, the sponsor submitted a number of pivotal and supportive clinical pharmacology and biopharmaceutical studies, which evaluated the action of insulin glargine in healthy subjects as well as type 1 and type 2 diabetic patients.

Results of the above studies are summarized below, including answers to questions (basis for Question-based Review) relevant for this class of drugs:

Formulation:
Unlike other long acting insulins (which are suspensions), Lantus® (HOE 901) is a clear solution in the vial. According to the sponsor, Lantus® precipitates at physiological pH. resulting in a "sustained release" type of action.

Assay:
A commercially available radioimmunoassay (RIA) was modified slightly and used to measure serum insulin. Assay validation for measuring total insulin is acceptable; however, it should be noted that the assay has only about 50% specificity to HOE 901 and its major metabolites and it has crossreactivity to endogenous insulin.

Bioequivalence:
The formulation used in the clinical trials is the same as the to-be- marketed formulation; therefore no bioequivalence studies were required.

Drug Metabolism:
HOE 901 is metabolized into two major active metabolites, 21^{A}-Gly-insulin (M1) and 21^{A}-Gly-des-30^{B}-Thr-insulin (M2). A mixture of the two metabolites were recovered in similar proportions (~50% each) from the injection site (over 24 hours).

Effect of Injection Site:
The abdomen, arm and leg were compared as injection sites. No clinically significant differences in the pharmacokinetics or pharmacodynamics of HOE 901 were found.

Special Populations:

Normal Subjects, Type 1 and Type 2 Diabetics- Lantus PK profiles were similar in normal, Type 1 and Type 2 diabetics. In all cases, the profile was relatively flat, with no distinct peak. NPH insulin, on the other hand, had a distinct peak at about – hours, and its duration of action was lower relative to Lantus®.

Age-No formal studies were performed to evaluate the effect of age on the PK or PD of Lantus.

Renal Function- No formal studies were done to evaluate the effect of renal function on the PK or PD of Lantus; however, a general statement regarding the need for glucose monitoring and dose adjustment is provided in the labeling.

Drug-Drug Interactions-
No drug-drug interaction studies were performed. The labeling provides a list of drugs, which may alter the glucose-lowering effect of insulin, requiring close glucose monitoring and possible insulin dose adjustment.

Question-based Review:

- *What is the potency of insulin glargine (HOE 901) relative to human insulin?*

  HOE 901 appears to be equipotent to NPH human insulin.

- *What effect does the site of injection have on the pharmacokinetics of insulin glargine?*

  Injection site (leg, abdomen and arm) does not show a clinically significant effect on the pharmacokinetics of HOE 901.

- *Can insulin glargine be mixed with shorter-acting insulins in the same syringe for patient convenience?*

  HOE 901 should not be mixed with any insulin since a change in pH could cause precipitation in the syringe and altered pharmacokinetics.

- *What is the optimum time of injection for insulin glargine?*

  HOE 901 is to be injected at bedtime.

- *Does the pharmacokinetics of insulin glargine differ between healthy volunteers and patients with diabetes?*

  Similar PK profiles for HOE 901 are observed in healthy subjects as well as patients with Type 1 or Type 2 diabetes.

- *Are dosing adjustments needed in special populations such as the elderly, children, or patients with renal or hepatic impairment?*

  Dosing in special populations has not been specifically addressed in the PK studies.

- *How does the PK/PD of insulin glargine compare with other long-acting insulins, such as NPH and crystalline zinc preparations?*

  HOE 901 appears to have a more flat PK profile with no distinct peaks in addition to a longer duration of action; NPH insulin has a distinct peak at about – hours.

Recommendations

The Office of Clinical Pharmacology and Biopharmaceutics/Division of Pharmaceutical Evaluation II (OCPB/DPEII) has reviewed NDA 21-081, submitted on April 9, 1999. Based on the review of the pharmacokinetic and biopharmaceutics studies submitted, OCPB/DPEII finds this NDA acceptable. However, the following labeling comments should be conveyed to the sponsor as appropriate:

Labeling Comments

Under **Pharmacodynamics**, the last sentence, which reads:

[ ] should be deleted.

The rationale for this change:

- RIA assay used in the study is not sufficiently specific to HOE 901 and major metabolites; additionally, it has high crossreactivity to endogenous insulin. Therefore, comparison of the pharmacokinetic parameters is not appropriate.

- Claim of lower [ ] is not supported by comparison of PD parameters.

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Table of Contents

Topic	*Page*
Synopsis	1
Recommendation	3
Background	5
Assay Method	6
Bioavailability and Bioequivalence	6
Absolute and Relative Bioavailability	6
Bioequivalence	9
Effect of Mixing	10
Effect of Injection Site	10
Metabolism	
In vivo	12
Pharmacokinetics In Special Populations	
Normal Volunteers	12
Type 1 Diabetics	13
Type 2 Diabetics	15
Hepatic	16
Renal	16
Age	16
Gender	16
Pediatric	
Pharmacokinetic/Pharmacodynamic Relationships	17
Drug Interactions	26
Dosage and Administration	26
Formulation	26
Labeling Comments	27
Signatures	28
Attachment A (Proposed Labeling)	29
Attachment B (Study Synopsis)	44

List of Pivotal PK-PD Studies

Study Number	Title of Study
1016	Determination of Metabolic Degradation Products of HOE 901 after Subcutaneous injection of HOE 901 in healthy subjects
1017	Comparison of the subcutaneous absorption of HOE 901 and NPH Insulin in Type 2 diabetics
1010	Comparison of the insulin absorption rate of ^{125}I-labelled HOE 901 following subcutaneous injection into the abdominal, leg and arm regions
1015	Comparison of the time-action profiles of HOE 901 and human NPH Insulin in type 1 diabetic patients using the euglycemic clamp technique
1012	Assessment of the variability in glucose lowering effect of HOE 901 compared NPH and Ultralong® (Human Ultralente) human insulins after subcutaneous doses of 0.4 IU/kg using the euglycemic clamp technique in healthy volunteers
1013	Characterization of glucose turnover of HOE 901 in comparison with human insulin in healthy male subjects

I. Background

Hoechst Marion Roussel has submitted NDA 21-081 for insulin glargine (LANTUS®), an analog of human insulin. Insulin glargine (also referred to as HOE 901) is a long acting insulin analog produced by recombinant DNA technology. It differs from human insulin in two major respects. The substitution is on the A-chain at position 21, where the native asparagine is replaced by glycine. Additionally, two arginine residues have been added to the C-terminus of the B-chain. These structural changes result in a shift in the isoelectric point as compared with human insulin. As a result, at pH 4 (in the injection solution) Lantus is completely soluble. Upon subcutaneous injection, the resulting shift to physiological pH causes the insulin analog to precipitate at the injection site. According to the sponsor, this results in slow, sustained release of insulin from the injection site, and allows once-daily administration.

Insulin glargine (21^{A}-Gly-30^{B}a-L-Arg-30^{B}b-L-Arg-human insulin) is a peptide hormone produced by recombinant DNA techniques. It has a molecular weight of 6063 and an isoelectric point of 6.7 (native human insulin's pI is 5.6). It is insoluble in water and organic solvents, but is soluble in acidic conditions.

The review of this NDA will answer the following questions:

- What is the potency of insulin glargine relative to human insulin?
- What effect does the site of injection have on the pharmacokinetics of insulin glargine?
- Can insulin glargine be mixed with shorter-acting insulins in the same syringe for patient convenience?
- What is the optimum time of injection for insulin glargine?

- Does the pharmacokinetics of insulin glargine differ between healthy volunteers and patients with diabetes?

- Are dosing adjustments needed in special populations such as the elderly, children, or patients with renal or hepatic impairment?

- How does the PK/PD of insulin glargine compare with other long-acting insulins, such as NPH and crystalline zinc preparations?

II. Assay Method and Validation

A commercial radio-immunoassay ——————————————— for quantitative determination of immunoreactive insulin (endogenous human insulin as well as HOE 901 and its metabolites), was modified slightly with regard to assay procedure and was validated by Hoechst Marion Roussel. The assay had an upper limit of quantitation of — µIU/mL. Specificity was — for HOE 901, — for the M1 metabolite, — for the M2 metabolite, and — for 21^{A}-Gly-30^{B}a-L-Arg-insulin.

Assay validation results are listed in Table I below.

Table I. Insulin assay validation (n = 10).

	Nominal Insulin Concentrations (µIU/mL)		
	2.5	10	50
Mean	2.28	9.04	47.7
Accuracy (%)	—	—	—
Intra-assay Precision (%CV)	4.5	4.2	7.4
Inter-assay Precision (%CV)	4.5	4.1	8.0

The lower limit of quantitation was — µIU/mL).

III. Bioavailability and Bioequivalence

Bioavailability

What is the bioavailability of HOE 901 relative to NPH insulin?

The bioavailability of HOE 901 relative to NPH insulin was evaluated in Study 1017. Fourteen type 2 diabetics received single subcutaneous injections of ^{125}I-HOE 901 and ^{125}I-NPH insulin (0.3 IU/kg) in a randomized, double-blind, two-way crossover study. The pharmacokinetics of the two drugs were evaluated by measuring radioactivity at the injection site, and serum concentrations of insulin. Exogenous insulin was estimated from insulin and C-peptide concentrations, using the formula listed below:

$$Insulin_{Exog} = Insulin_{OBS} - F \times C\text{-}peptide_{OBS}$$

Where

$Insulin_{Exog}$ = Estimate of exogenous insulin concentration
$Insulin_{OBS}$ = Observed serum insulin concentrations
F = Mean of the "serum insulin/C-peptide concentration" ratio at -30 min, -15 min, and -5 min (baseline)

The results are shown below.

Pharmacokinetics:

Table II. Mean pharmacokinetic parameters for serum insulin and exogenous serum insulin in Type 2 diabetics (n = 14) following single subcutaneous injections of ^{125}I-HOE 901 and ^{125}I-NPH insulin (0.3 IU/kg); Study 1017

Parameter	Mean (CV%)	
	HOE 901	**NPH Insulin**
Total Serum Insulin		
Fasting Conc. (μIU/mL)	11.5 (27)	11.5 (31)
AUC_{0-24} (μIU•h/mL)	290 (20)	391 (24)
C_{max} (μIU/mL)	16.1 (22)	29.1 (29)
t_{max} (h)	4.0[a]	4.0[a]
Exogenous Serum Insulin		
AUC_{0-24} (μIU•h/mL)	111 (50)	235 (23)
C_{max} (μIU/mL)	7.4 (60)	20.7 (32)
t_{max} (h)	12.0[a]	4.0[a]

a Median value

b Minimum and maximum values

Given that endogenous insulin was not totally suppressed, and the assay is not sufficiently specific for HOE 901, a precise determination of relative bioavailability is not possible.

Pharmacodynamics:

Table III. Mean (CV%) plasma glucose parameters in Type 2 diabetics (n = 14) following single subcutaneous injections of ^{125}I-HOE 901 and ^{125}I-NPH insulin (0.3 IU/kg); Study 1017

Parameter	Mean (CV%)	
	HOE 901	NPH Insulin
Fasting Conc. (mg/dL)	189 (27)	186 (32)
AUC_{0-24} (mg•h/dL)	2601 (31)	2328 (33)
C_{min} (mg/dL)	81.8 (34)	74.3 (38)
Maximum decrease (mg/dL)	107 (31)	111 (43)
t_{min} (h)	16.0[a]	8.5[a]

a Median value

b Minimum and maximum values

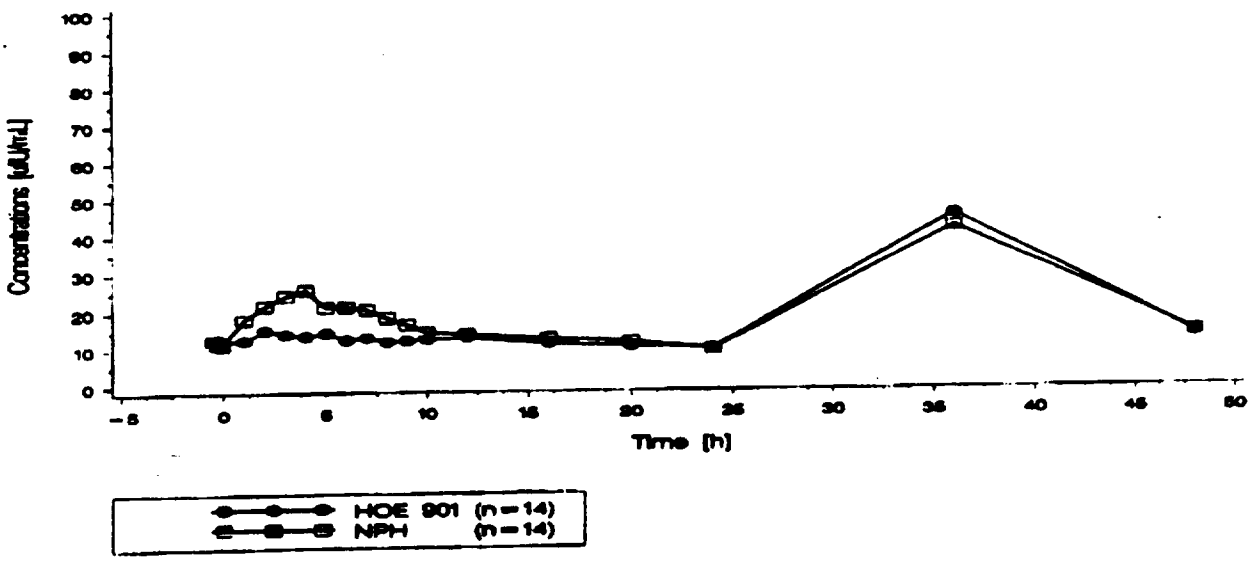


Figure 1. Serum insulin concentrations (median values) in Type 2 diabetics (n = 14) following single subcutaneous injections of ^{125}I-HOE 901 and ^{125}I-NPH insulin (0.3 IU/kg).

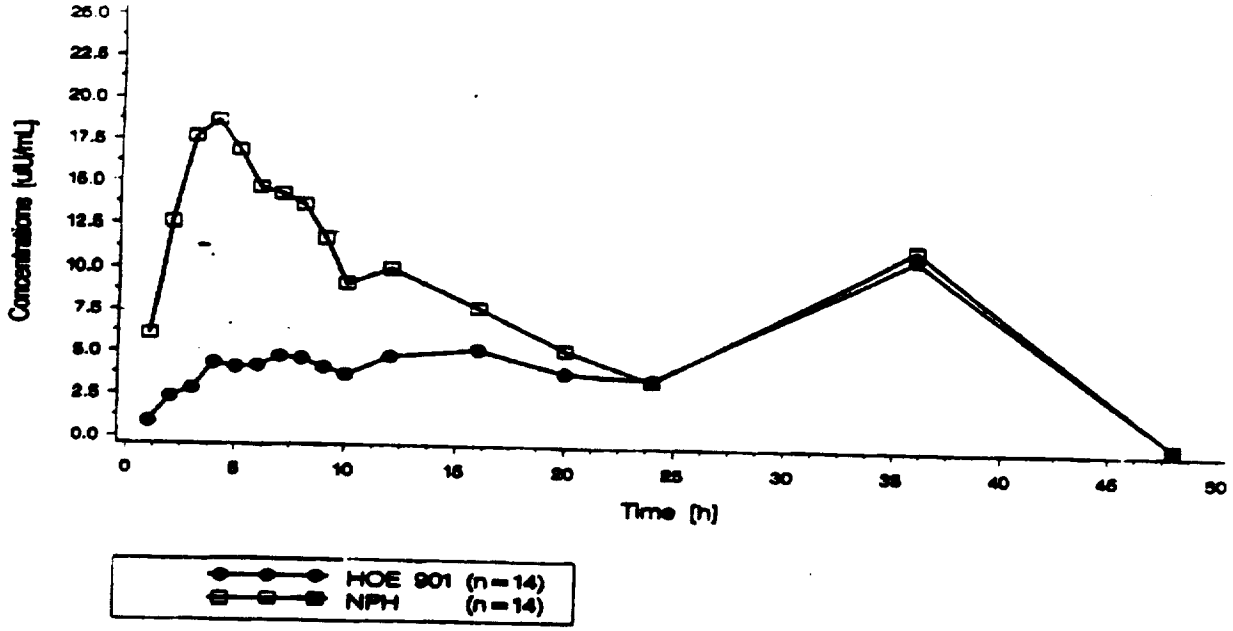


Figure 2. Serum exogenous insulin levels (median values) in Type 2 diabetics (n = 14) following single subcutaneous injections of ^{125}I-HOE 901 and ^{125}I-NPH insulin (0.3 IU/kg); Study 1017.

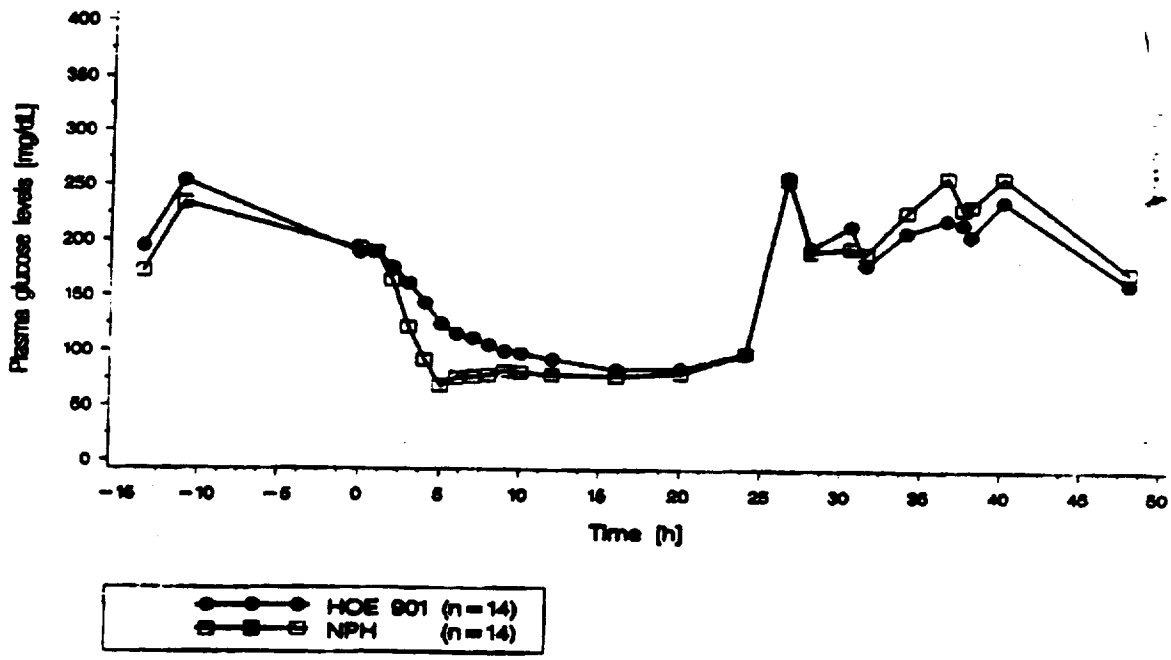


Figure 3. Serum glucose levels (median values) in Type 2 diabetics (n = 14) following single subcutaneous injections of ^{125}I-HOE 901 and ^{125}I-NPH insulin (0.3 IU/kg); Study 1017.

Bioequivalence-

Is the to-be-marketed formulation the same as the clinically tested formulation? If not, were they shown to be bioequivalent?

The to-be-marketed formulation is the same as the clinically tested formulation; therefore, no bioequivalence studies were needed.

Effect of Mixing-

Can insulin glargine be mixed with shorter-acting insulins in the same syringe for patient convenience?

No studies were performed looking at the effect of mixing insulin glargine with short-acting insulins or insulin analogs. Because of the pH-dependent solubility of HOE 901, it is likely that mixing with conventional short-acting soluble insulins or analogs (which have a pH of 7-7.8) would cause precipitation of insulin glargine in the syringe. Additionally, the soluble human insulin may also precipitate, depending on the pH of the resulting mix. In the package insert and the patient insert, it is stated that "LANTUS must not be mixed with any other insulin."

Effect of Injection Site-

What effect does the site of injection have on the PK/PD of insulin glargine?

HOE 901/1010

The effect of injection site on the absorption and pharmacodynamics of HOE 901 were evaluated in Study 1010. Twelve healthy male subjects were injected subcutaneously with single doses of ^{125}I-HOE901 (0.2 IU/kg) in the leg, arm and abdomen, in a randomized three-way crossover comparison. Absorption rate was assessed by measuring the mean time of disappearance of radioactivity. A secondary endpoint was blood glucose concentrations over the course of the study. The results suggest a small but insignificant difference in the absorption rate between the abdomen and the arm as injection sites. No significant differences were seen in response as determined by glucose plasma levels. Table IV lists glucose values following the subcutaneous injection of HOE 901 to the arm, leg, and abdomen. Figure 4 demonstrates residual radioactivity over time at the injection site. Figure 5 shows insulin levels over time for the different injection sites.

Table IV. Mean (SD) pharmacokinetic parameters for blood glucose and serum insulin following the subcutaneous injection of ^{125}I-HOE901 to the arm, leg and abdomen in healthy volunteers (N = 12); Study 1010.

Parameter	Injection Site		
	Arm	Leg	Abdomen
Blood Glucose			
AUC_{0-24} (mg•h/dL)	74.5 (3.2)	75.4 (3.1)	75.9 (3.2)
Max. Conc. Decrease (mg/dL)	23.5 (4.6)	22.6 (4.4)	20.8 (4.5)
Serum Insulin			
AUC_{0-24} (µU•h/mL)	10.9 (1.47)	10.7 (1.43)	10.2 (1.49)
C_{max} (µU/mL)	19.2 (6.86)	17.5 (6.70)	15.5 (6.96)

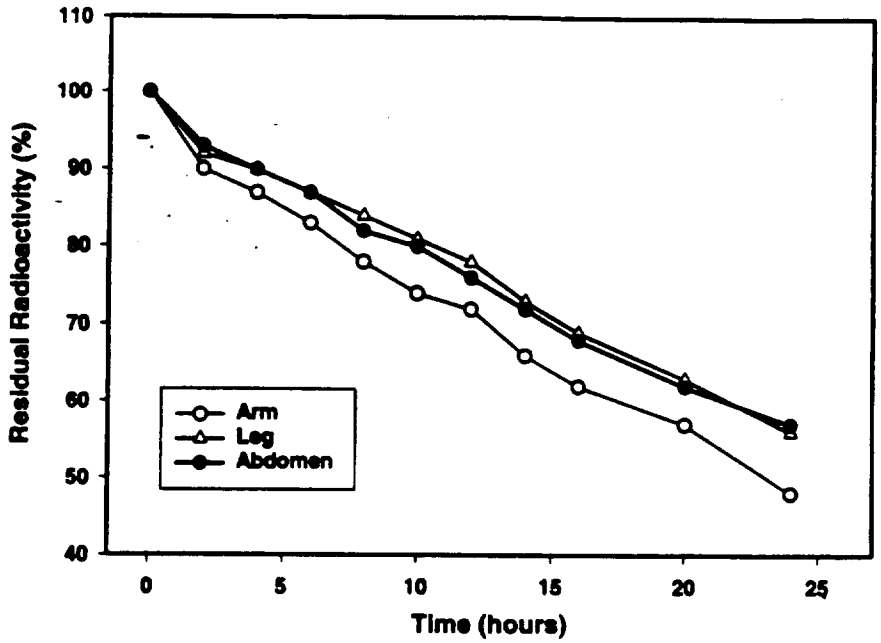


Figure 4. Residual Radioactivity over time following the subcutaneous injection of ^{125}I-HOE 901 (0.2 IU/kg) to three body sites in healthy subjects (n = 12); Study 1010.

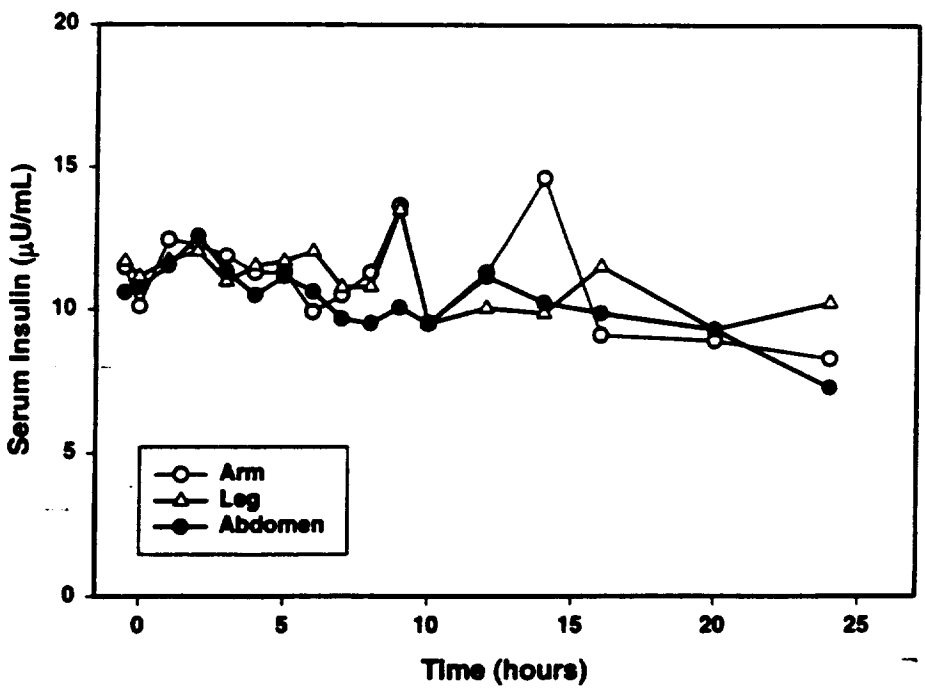
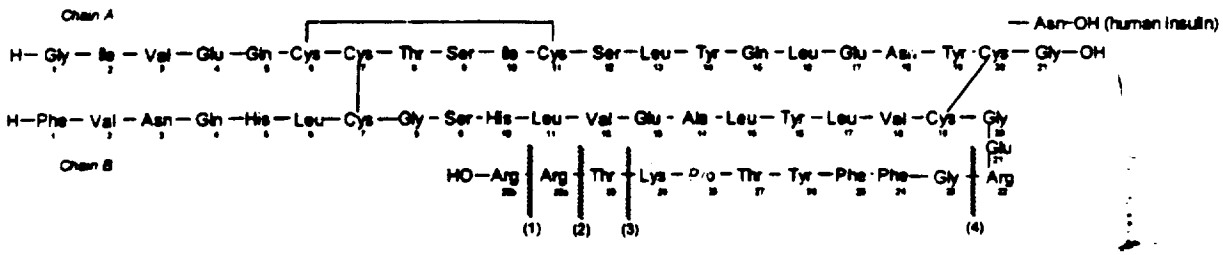


Figure 5. Serum insulin levels following the subcutaneous injection of ^{125}I-HOE 901 to the arm, leg and abdomen in healthy subjects (n = 12); Study 1010.

IV. Metabolism

The *in vivo* metabolism of HOE 901 was evaluated in Study 1016. Four healthy male subjects received a single subcutaneous dose of HOE 901 at 0.6 IU/kg. An additional subject served as control. Subcutaneous tissue at the injection site was sampled by liposuction at different time intervals. HOE 901 levels and its degradation products in the plasma and tissue at the site of injection were determined by a ———————— assay. The tissue sample obtained from the control subject was used to determine baseline insulin level at the predefined injection site. The analytical results showed that both parent drug and degradation products were present in the circulation. 21^{A}-Gly-insulin (M1) and/or 21^{A}-Gly-des-30^{B}-Thr-insulin (M2) were identified as degradation products. HOE 901 levels and its degradation products were in the same order of magnitude. Degradation took place by the loss of both arginines at the carboxy terminus of the B chain to form M1. Additional loss of the next amino acid threonine, generated the metabolite M2. The structure of HOE 901 and degradation products are illustrated in Figure 6.



HOE 901	M0	21^{A}-Gly-30^{B}a-L-Arg-30^{B}b-L-Arg-insulin	HOE 901
1	-	21^{A}-Gly-30^{B}a-L-Arg -insulin	Mono-Arg-Gly-insulin
2	M1	21^{A}-Gly-insulin	Gly-insulin
3	M2	h-insulin (21^{A}-Asn)	
	(3)	21^{A}-Gly-des-30^{B}-Thr-insulin	Des-Thr-Gly-insulin

Figure 6. HOE 901 and degradation products.

V. Pharmacokinetics in Special Populations

Does the pharmacokinetics of insulin glargine differ between healthy volunteers and patients with diabetes?

Normal Volunteers

Study 1018 was a single-dose randomized crossover trial in 15 healthy volunteers given (0.4 IU/kg) HOE 901, placebo or NPH under euglycemic clamp conditions. The PK results are shown in Table V. As compared with NPH, HOE 901 has a much more prolonged rate of absorption (median tmax =16 hrs) as compared with NPH (median tmax = 3 hrs). As shown in

Figure 7, the NPH arm shows a distinct peak at about [redacted] hours post-dose. In contrast, the HOE 901 arm shows a smooth, constant profile, similar to a constant infusion.

Table V: Mean Pharmacokinetics of HOE 901, NPH, or placebo (endogenous insulin) in 15 healthy volunteers (Study 1018)

Parameter	HOE 901	NPH	Placebo
AUC(0-30 hrs) (μIU•hr/mL)	508 ± 80.2 (16%)	590 ± 102 (17%)	320 ± 67.4 (21%)
Point estimate (95% CI) HOE901/NPH	[redacted] 86.3 (78.2, 95.2)		
Cmax (μIU/mL)	23.8 ± 4.4 (18%)	32.8 ± 6.0 (18%)	18.3 ± 4.3 (23%)
Point estimate (95% CI) HOE901/NPH	[redacted] 72.4 (63.3, 83.0)		
Tmax (hrs)	16	3.0	9.0

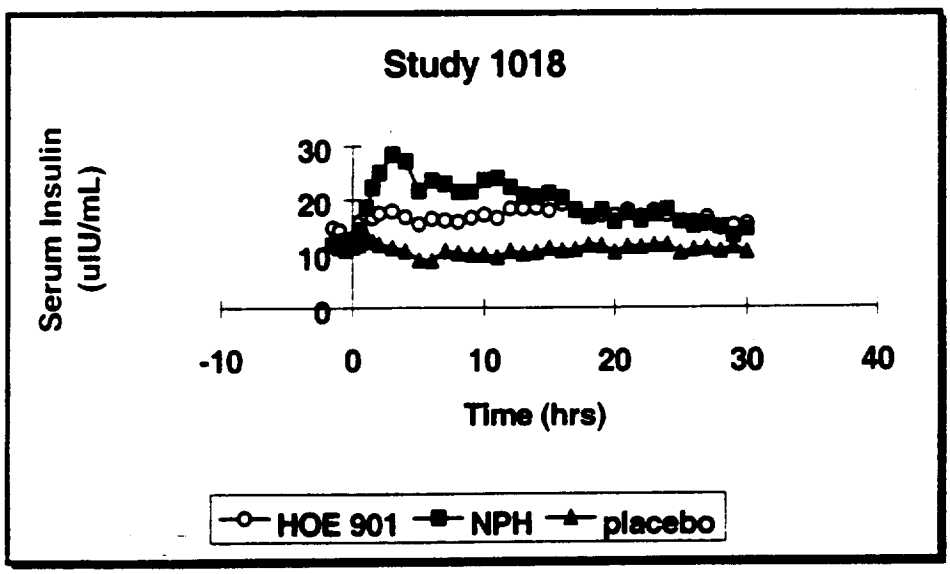


Figure 7: Mean serum insulin levels over time in 15 healthy subjects (Study 1018).

Since endogenous levels are high and the assay cannot separately measure HOE 901 and metabolites, plasma levels should be interpreted with caution.

Type 1 Diabetics

The pharmacokinetics and pharmacodynamics of HOE 901 in Type 1 diabetics were evaluated in Study 1015. The primary objective of this study was to characterize the time-action profiles of

HOE 901 in comparison with human NPH insulin in Type 1 diabetic patients. Twenty subjects received a single subcutaneous injection of HOE 901 and human NPH insulin (0.2 IU/kg) in a randomized, two-way crossover study (under euglycemic clamp conditions). The results are shown in Table VI and Figure 8 below. The sponsor's main conclusion was that HOE 901 had a longer duration of action (median of 9 hours longer than NPH insulin), and it produced a more flat glucose infusion rate (GIR) profile, relative to NPH insulin.

Table VI. Mean (CV%) pharmacokinetic parameters for serum free insulin following the subcutaneous injection of HOE 901 and human NPH insulin to subjects with Type 1 diabetes mellitus (N = 20).

Parameter	HOE 901	NPH Insulin
Baseline Conc. (μIU /mL)	16.0 (63)	14.3(78)
$AUC_{0\text{-}12\ hrs}$(μIU•hr/mL)	140 (21)	218(31)
$AUC_{0\text{-}end}$(μIU•hr/mL)	224(34)	228(39)
C_{max} (μIU/mL)	19.3(39)	27.4(51)
t_{max}(hrs)	3.0[a]	4.5[a]

[a] Median (min, max)

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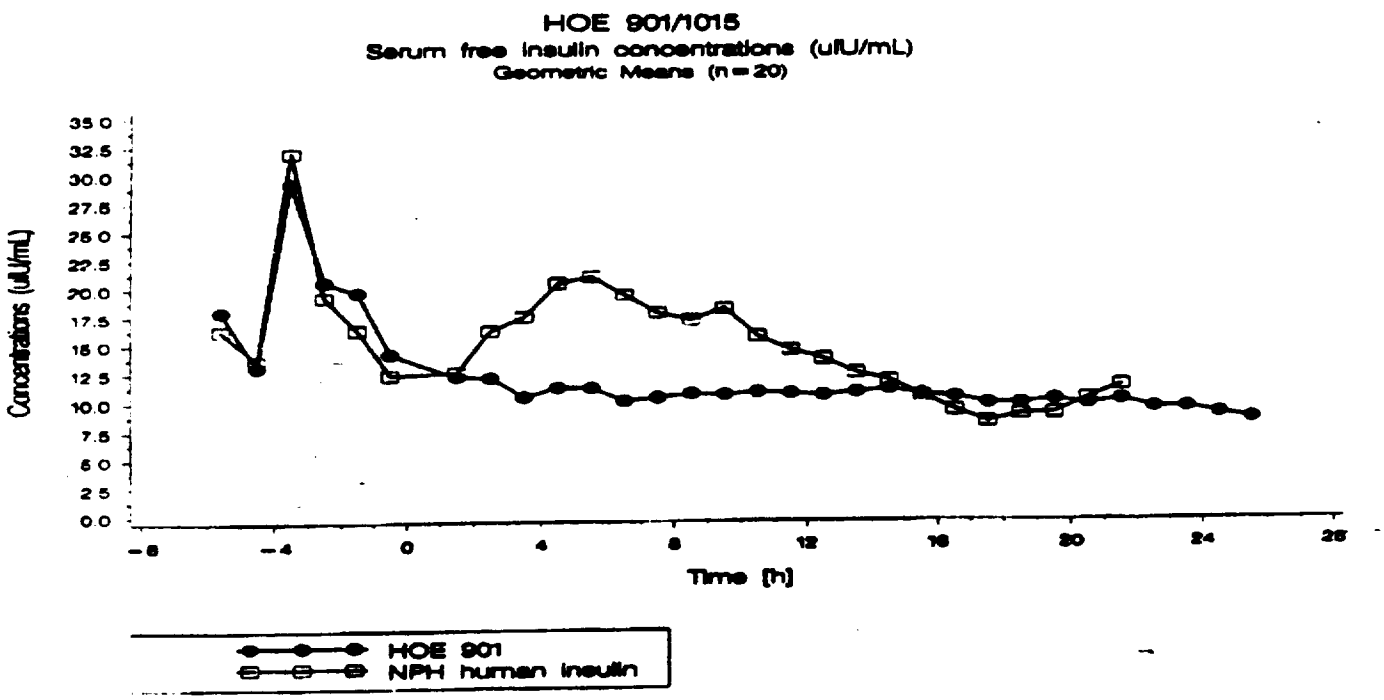

Figure 8. Free serum insulin levels over time following the subcutaneous injection of HOE 901 and human NPH insulin to subjects with Type 1 diabetes mellitus (N = 20), under euglycemic clamp conditions; Study 1015.

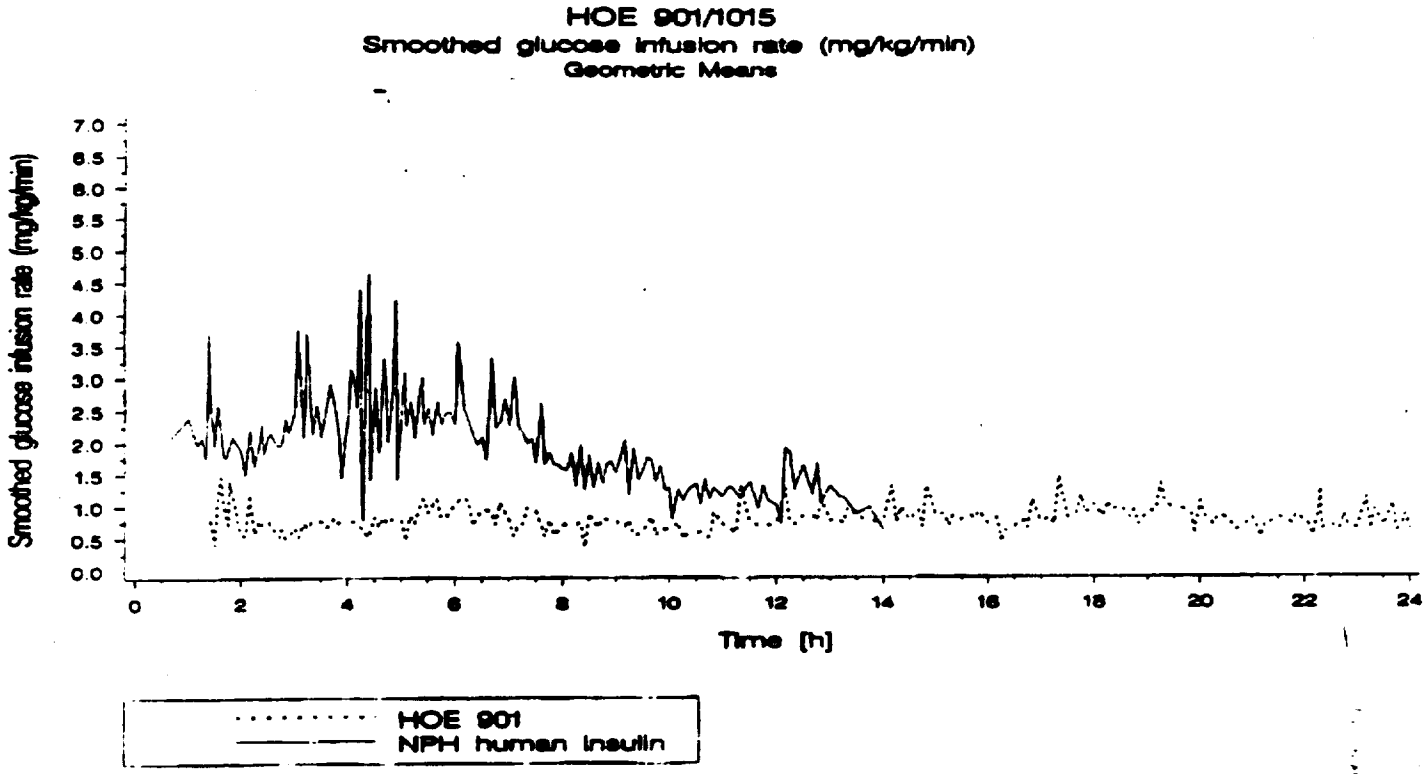

Figure 9. Glucose infusion rate following the subcutaneous injection of HOE 901 and human NPH insulin to subjects with Type 1 diabetes mellitus (N = 20), under euglycemic clamp conditions; Study 1015.

Type 2 Diabetics

Study 1017 used ^{125}I-labeled insulin (NPH) or HOE 901 to compare the absorption profiles of the two products in patients with type 2 diabetes. Each subject was randomized to receive 0.3 U/kg radio-labeled NPH or HOE 901 in a randomized fashion. Radioactivity at the site of injection was followed for 48 hours post-dose using [redacted] Serum samples for insulin measurement were also collected up to 24 hours post-dose. The primary endpoint was the time needed to reach 75%, 50%, or 25% of radioactivity at the site of injection. These results are shown in Table VII, and clearly demonstrate that insulin glargine is absorbed more slowly than NPH in Type 2 patients.

Table VII: Median times (range) to reach 75%, 50%, and 25% of radioactivity from the injection site in 14 Type 2 patients (Study 1017)

Variable	HOE 901	NPH
T75% (hrs)	15.0	6.5
T50% (hrs)	26.3	13.4
T25% (hrs)	42.4	26.6

Figure 10 depicts the mean % radioactivity vs. time plots for both NPH and HOE 901 along with fitted lines from a first-order model (NPH) or a zero-order model (HOE 901). This plot suggests

that HOE 901 is absorbed in a concentration-independent manner, unlike NPH, which shows a classic first-order absorption profile. The clinical significance of this is not clear.

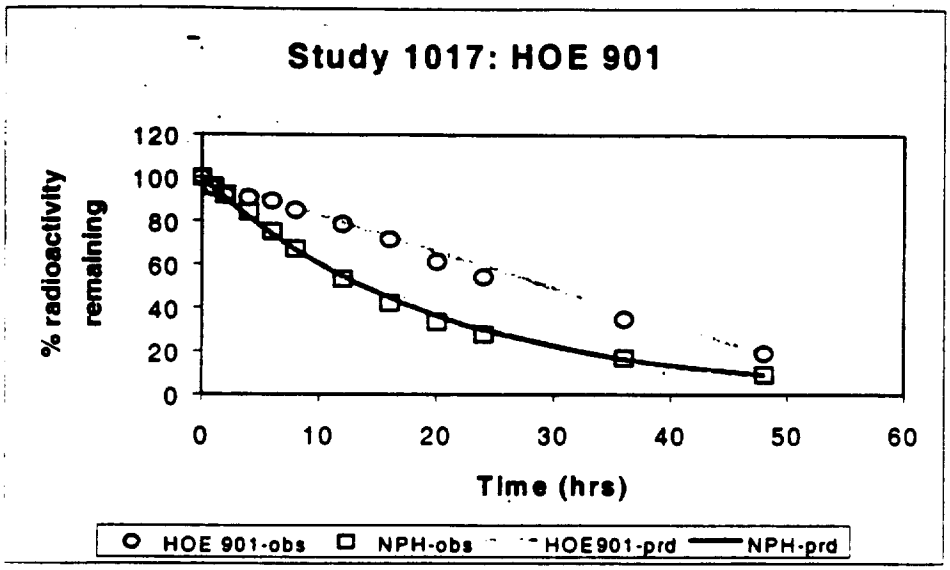

Figure 10: Mean % radioactivity vs. time profiles for HOE 901 and NPH insulin in 14 Type 2 diabetics.

Hepatic, Renal, Age, Gender, Pediatric

No formal studies were performed to evaluate the effect of these factors on the pharmacokinetics of HOE 901.

VI. Pharmacokinetic/Pharmacodynamic Relationships

How does the PK/PD of insulin glargine compare with other long-acting insulins, such as NPH and crystalline zinc preparations?

Potency: Comparison with Human Insulin

In Study 1013, 12 healthy males were given either HOE 901 or regular insulin under constant infusion conditions (40 mU/m^2/min). Prior to initiating insulin infusions, the subjects received (after fasting overnight) a continuous infusion of D-[3-^{3}H]-glucose for about 4 hours to label the glucose pool. After basal measurements were made, the insulin infusions were started. Simultaneously, a 20% dextrose solution containing D-[3-^{3}H]-glucose (hot-GINF) was infused at a variable rate to sustain euglycemia at 90 mg/dL. Blood samples were collected at selected time points to measure the blood glucose, plasma glucose specific activity, C-peptide levels, and serum free fatty acids. The clamp study lasted about 7 hours.

The glucose turnover rate was assessed during the basal state as well as during the activation and deactivation phases. Glucose turnover rate was calculated using the modified Steele equations for non-steady state conditions[1]:

$$Ra\ (t) = \bar{I}/SA_p(t) - pVG(t)[dSA_p(t)/dt]/SA_p(t) + [SA_g/SA_p(t)]GINF\ (t) - GINF\ (t)$$
$$Rd\ (t) = I/SA_p(t) - pVG(t)[dSA_p(t)/dt]/SA_p(t) + [SA_g/SA_p(t)]GINF\ (t) - pVdG(t)/dt$$

Where Ra (t) is the endogenous glucose production at time (t), Rd (t) is the glucose disposal rate at time (t), I is the concentration of tracer infusion rate ($\mu Ci/min^{-1}/kg^{-1}$), $SA_p(t)$ is the specific activity of glucose in plasma ($\mu Ci/mg$), p is the pool fraction, V is the distribution volume of glucose (dL/kg), G(t) is the plasma glucose concentration (mg/dL), $dSA_p(t)/dt$ is the rate of change of specific activity in the plasma ($\mu Ci/mg^{-1}/kg^{-1}$), GINF(t) is the exogenous glucose infusion rate ($mg/min^{-1}/kg^{-1}$), SA_g is the specific activity of the glucose infusate ($\mu Ci/mg$), and dG(t)/dt is the rate of change of the plasma glucose concentration ($mg/dL^{-1}/min^{-1}$). The equations for estimation of Ra and Rd are derived from the mass balances for glucose and tracer.

Measurement of hepatic glucose output (HGO)

According to the sponsor, "D-[3-^{3}H]-glucose is a suitable tracer substance to measure rates of glucose appearance (Ra) and disappearance (Rd) *in vivo* under both steady-state and non steady-state conditions. In the basal state, Ra equals HGO[2]. During the insulin infusion and subsequent deactivation phase, the rate of HGO is calculated as the difference between the Ra and the infusion rate of exogenous glucose."

Results of the study (Table VIII; Figures 11-13) suggest that both insulins suppressed hepatic glucose production to a similar extent. Both insulins also stimulated peripheral glucose utilization to a similar extent (Table VIII). Based on these data, it appears that HOE 901 is equipotent to human insulin.

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[1] Finegood *et al.*, ***Diabetes*** 1987; 36: 914.
[2] Prager *et al.*, ***J Clin Invest*** 1986; 78:472.

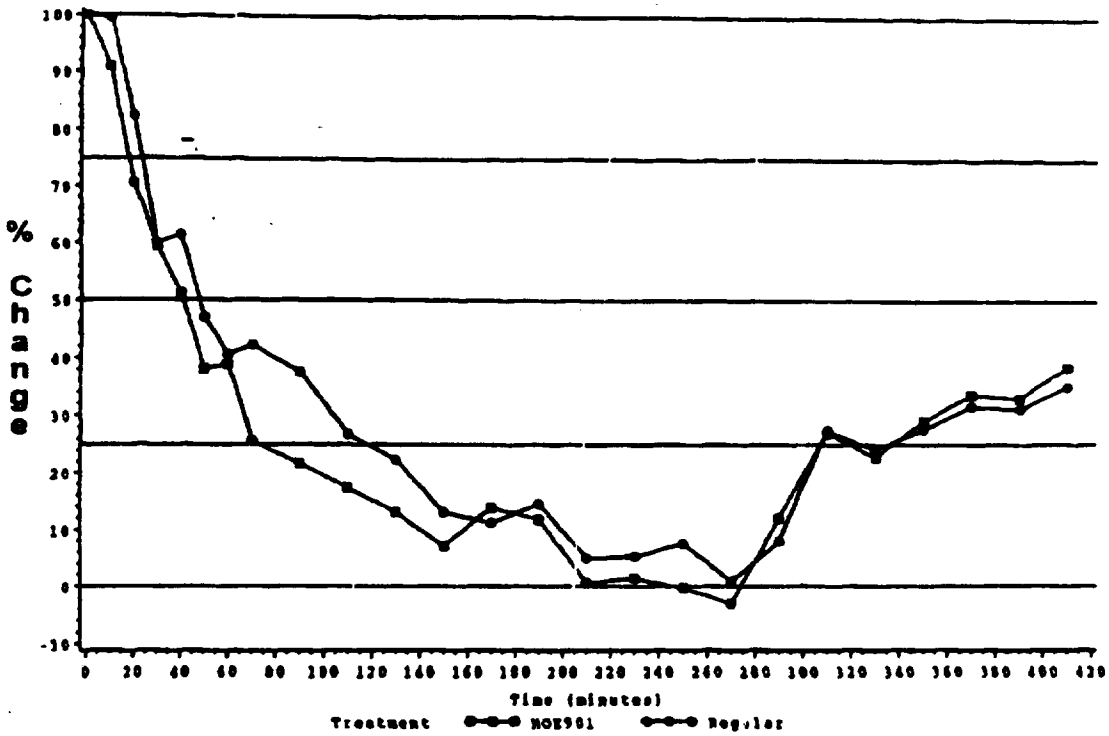

Figure 11. Mean suppression (%) of hepatic glucose output (HGO) following iv administration of HOE 901 or regular insulin under constant infusion conditions (40 mU/m^2/min) for 4 hours in healthy male subjects (n = 12); Study 1013.

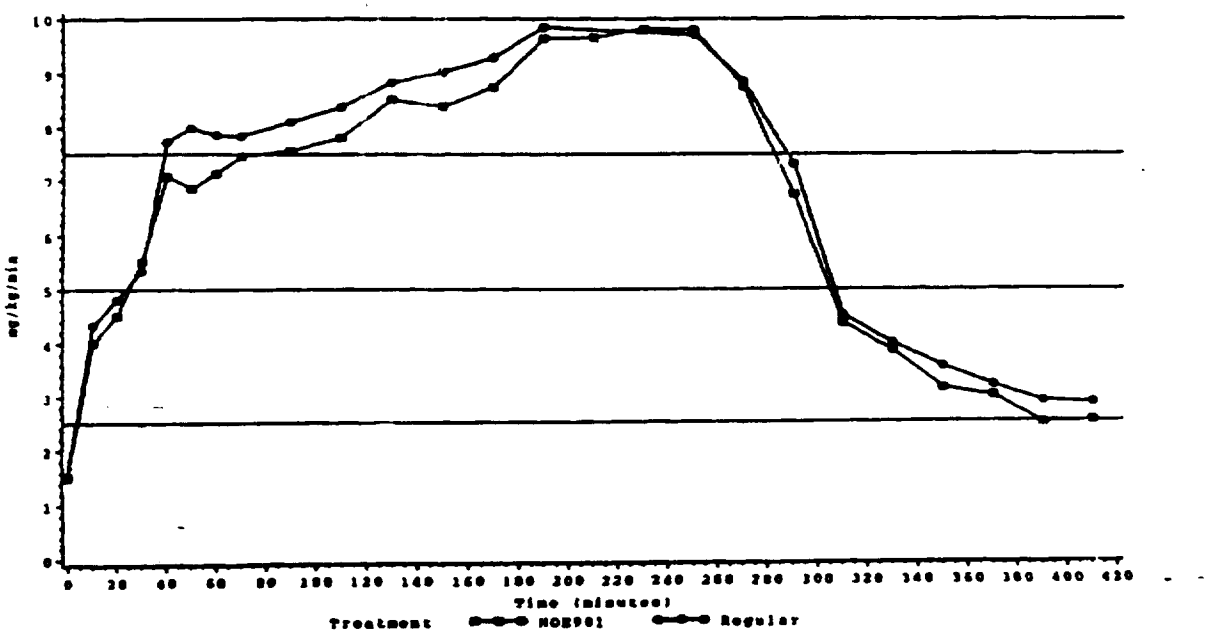

Figure 12. Mean moving average of rate of glucose disposal (Rd) following iv administration of HOE 901 or regular insulin under constant infusion conditions (40 mU/m^2/min) for 4 hours in healthy male subjects (n = 12); Study 1013.

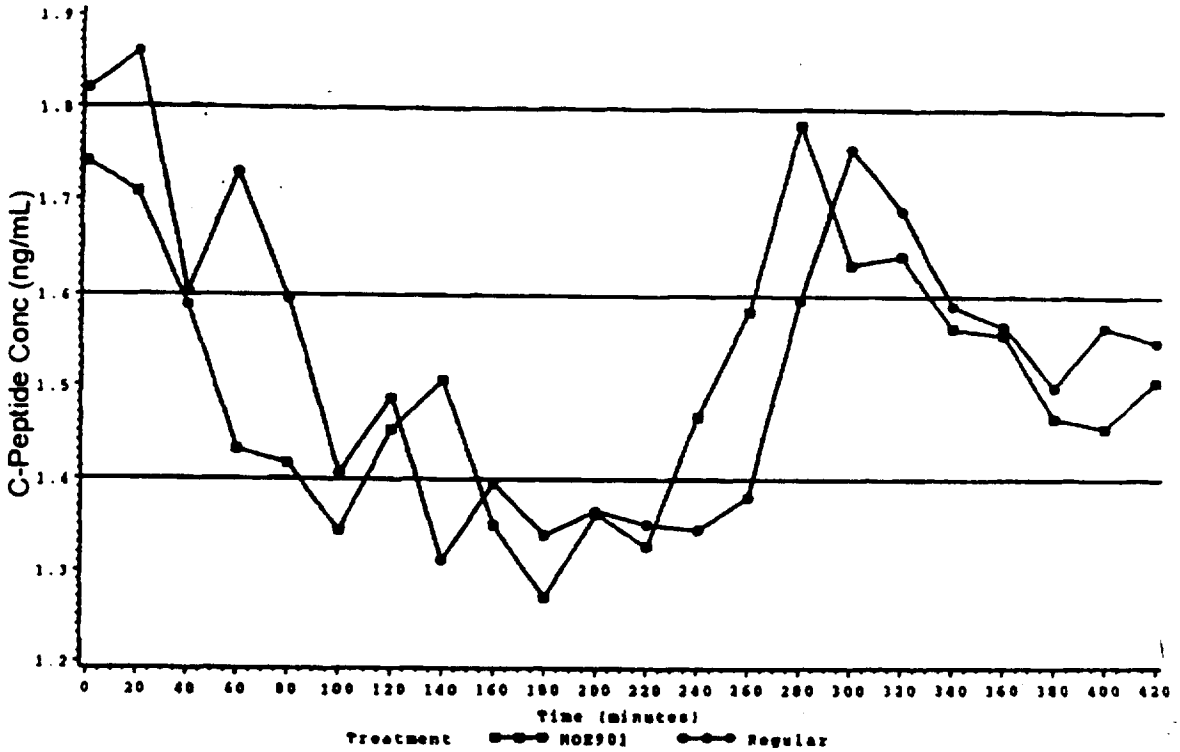

Figure 13. C-Peptide suppression following iv administration of HOE 901 or regular insulin under constant infusion conditions (40 mU/m^2/min) for 4 hours in healthy male subjects (n = 12); Study 1013.

Table VIII: mean suppression of hepatic glucose production and stimulation of peripheral glucose utilization in 12 healthy volunteers (Study 1013). The numbers in the table are adjusted means.

Parameter	HOE 901	Human Insulin
†A_{50} HGO (min)	59.47	77.32
Treatment Differences (95% CI)	-17.9 (36.5 – 0.78)	
††A_{50} IGDR (min)	41.99	31.56
Treatment Differences (95% CI)	10.43 (-12.1 – 32.93)	

†time required for 50% suppression of hepatic glucose output
††time required for half-maximal stimulation of incremental glucose disposal rate (IGDR)

Comparison with other basal insulins

Human NPH insulin

The pharmacokinetic differences between HOE 901 and basal insulin such as NPH demonstrated above are also reflected in the pharmacodynamics. Figure 14 depicts the glucose infusion rate vs. time profiles for HOE 901, NPH, and placebo in normal volunteers. Like the serum insulin

levels, the GIR resulting after HOE 901 administration is a smooth curve without an obvious peak. In fact, as depicted in Figure 14, except for slightly higher levels, the HOE 901 curve looks very similar to placebo. By contrast, the GIR profile resulting after NPH administration shows a pronounced peak ~hours post-dose, followed by declining levels.

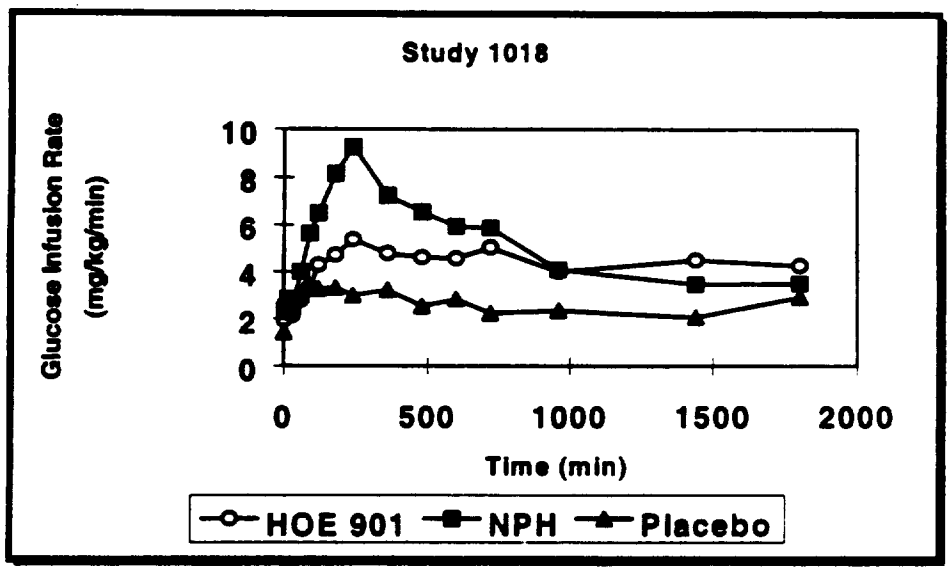

Figure 14: Median glucose infusion rates after administration of NPH, placebo, or HOE 901. (Study 1018)

Ultralong® insulin

The pharmacokinetics and pharmacodynamics of HOE 901 were compared to those of NPH insulin and the long acting insulin Ultralong® (Ultra lente) in Study 1012. According to the sponsor, the main objective of this study was to investigate the intra-subject variability in glucose lowering effect of HOE 901 compared to NPH and Ultralong® human insulins following a single subcutaneous dose of 0.4 IU/kg body weight in healthy subjects using the euglycemic technique. Study 1012 was a single-dose, double-blind, randomized parallel group (3 groups of 12 subjects per group), replicate design study. Each subject was injected twice (on separate visits) with the same formulation. The treatments were separated by a washout period of at least 7 days. The graphs below illustrate glucose levels, C-peptide levels, and GIR for HOE 901, human NPH and human Ultralong® insulin. The sponsor claims that HOE 901 had significantly lower intra subject variability relative to Ultralong® insulin in measured serum exogenous insulin. ——————————— The intra subject coefficient of variation (AUC_{0-24}, exogenous insulin) for the three formulations were 14%, 16% and 70% for HOE 901, NPH and Ultralong, respectively. The inter-subject coefficients of variation (CV) were 25%, 20%, and 46% for HOE 901, NPH and Ultralong, respectively. The sponsor does not explain why intra-subject variability for Ultralong insulin is much higher than inter-subject variability, while it is lower for HOE 901 and NPH insulin (the expected trend). Variability in C-peptide suppression, which reflects suppression of endogenous insulin, was similar across the three formulations. Additionally, variability in GIR, which is the primary endpoint in this study, was lowest for NPH insulin, and similar between HOE 901 and Ultralong insulin. Intra-subject CV for GIR AUC_{0-24}, for HOE 901, NPH insulin and Ultralong insulin were 32%, 19%, and 38%,

respectively. Therefore, the sponsor's conclusion that HOE 901 exhibits lower intra subject variability is not supported by the pharmacodynamic parameters. Pharmacokinetic parameters derived using exogenous insulin have limited value due to lack of precision. Exogenous insulin is estimated indirectly (see equation in Section III, Absolute and Relative Bioavailability), and the RIA used has only about 50% cross reactivity for HOE 901. Furthermore, the study was not powered to show the difference claimed by the sponsor.

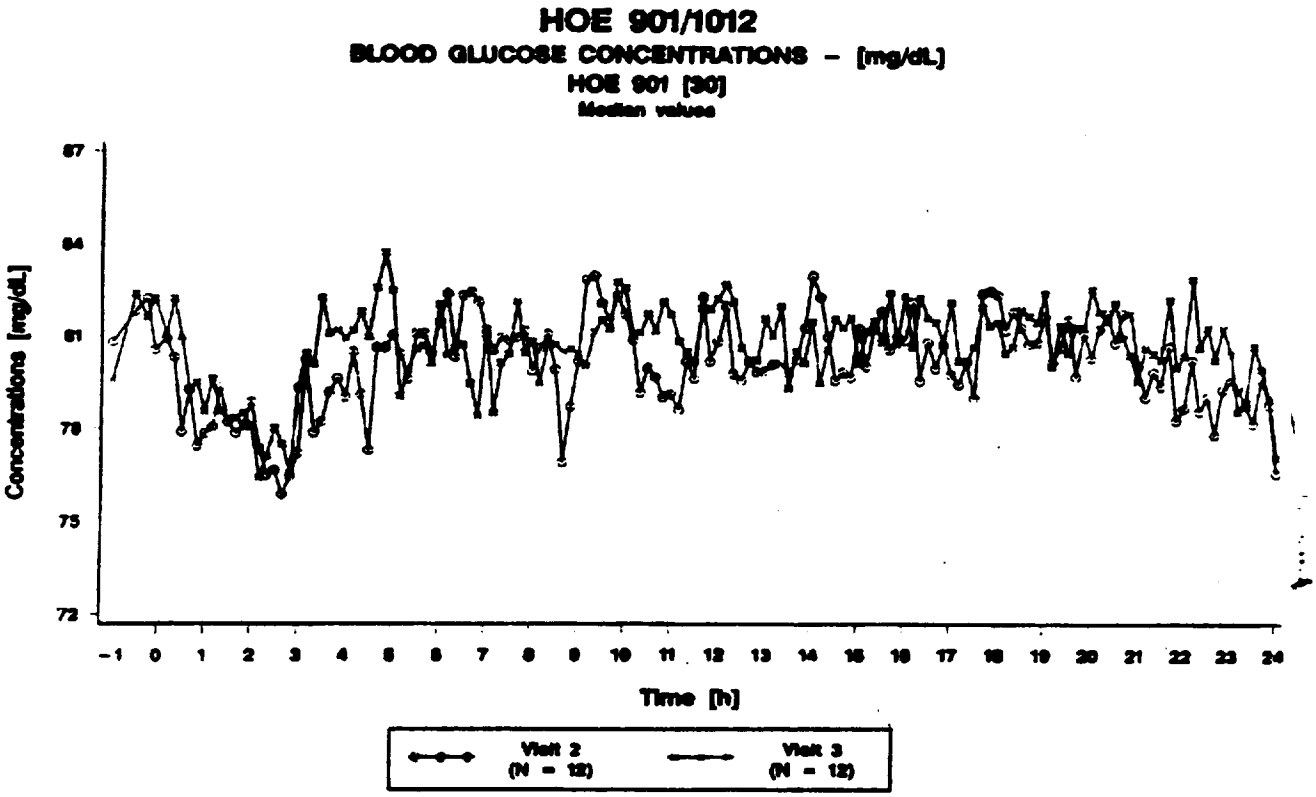

Figure 15. Blood glucose levels following two consecutive subcutaneous injections of HOE 901 (0.4 IU/kg) to healthy male subjects (n = 12); Study 1012.

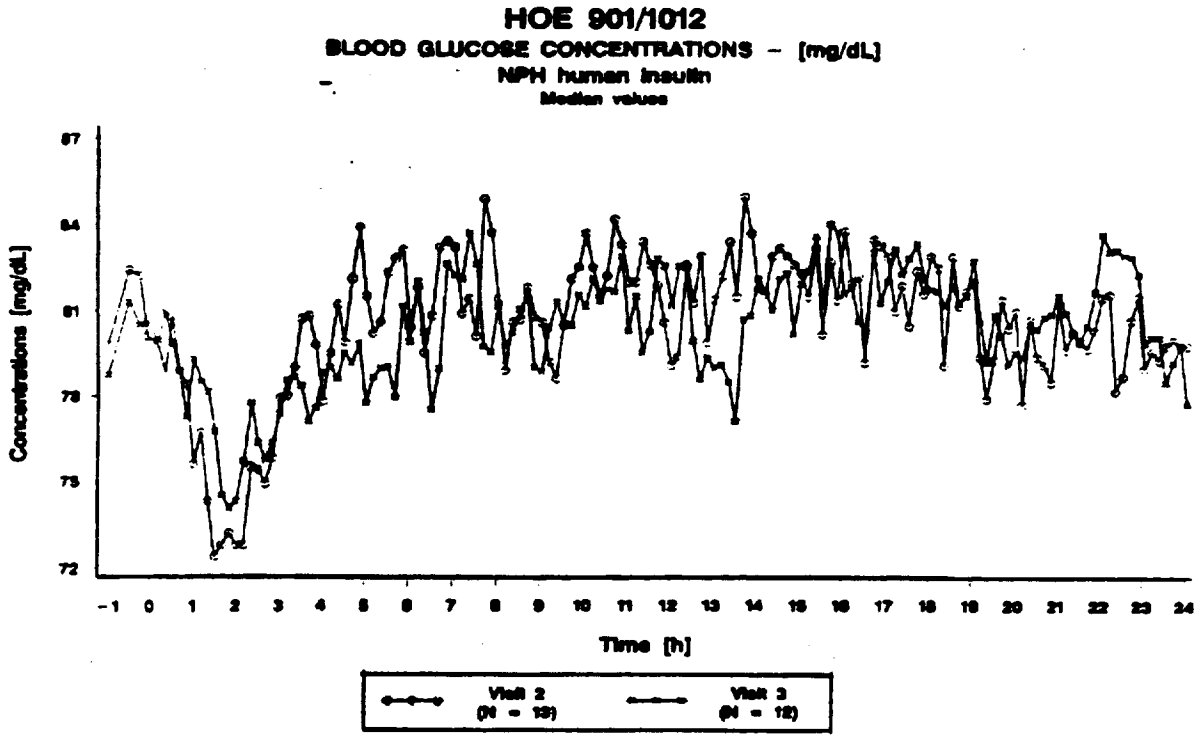

Figure 16. Blood glucose levels following two consecutive subcutaneous injections of NPH insulin (0.4 IU/kg) to healthy male subjects (n = 12); Study 1012.

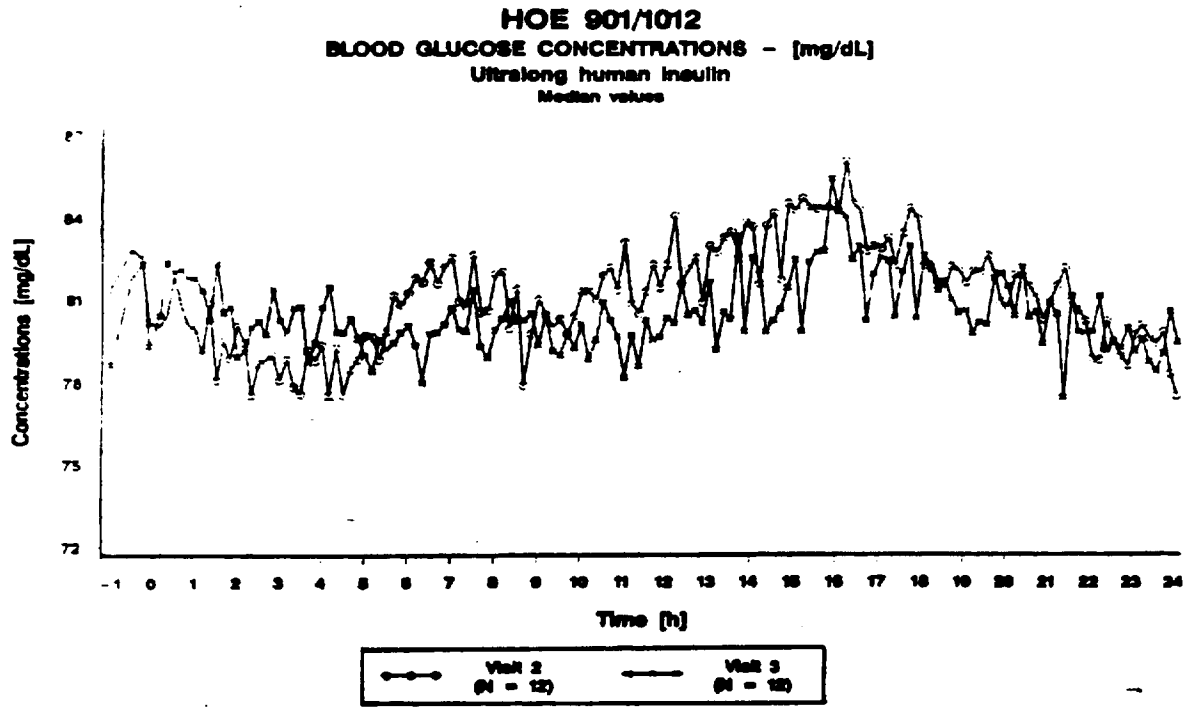

Figure 17. Blood glucose levels following two consecutive subcutaneous injections of Ultralong insulin (0.4 IU/kg) to healthy male subjects (n = 12); Study 1012.

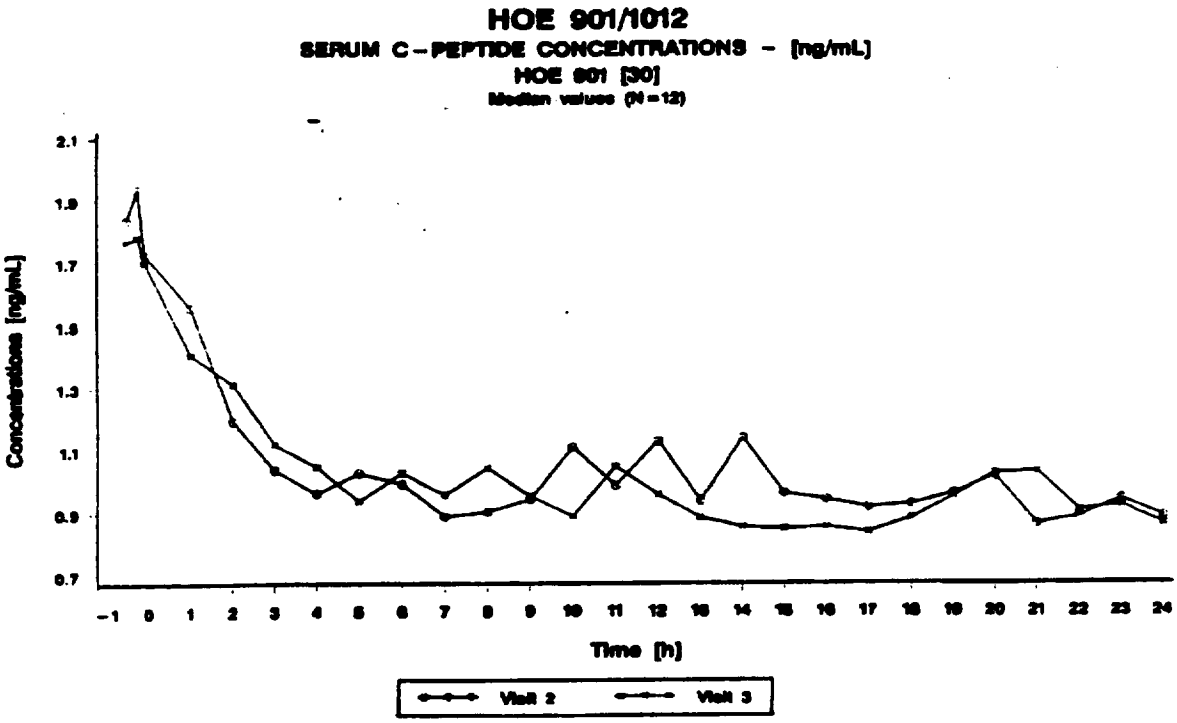

Figure 18. C-Peptide levels following two consecutive subcutaneous injections of HOE 901 insulin (0.4 IU/kg) to healthy male subjects (n = 12); Study 1012.

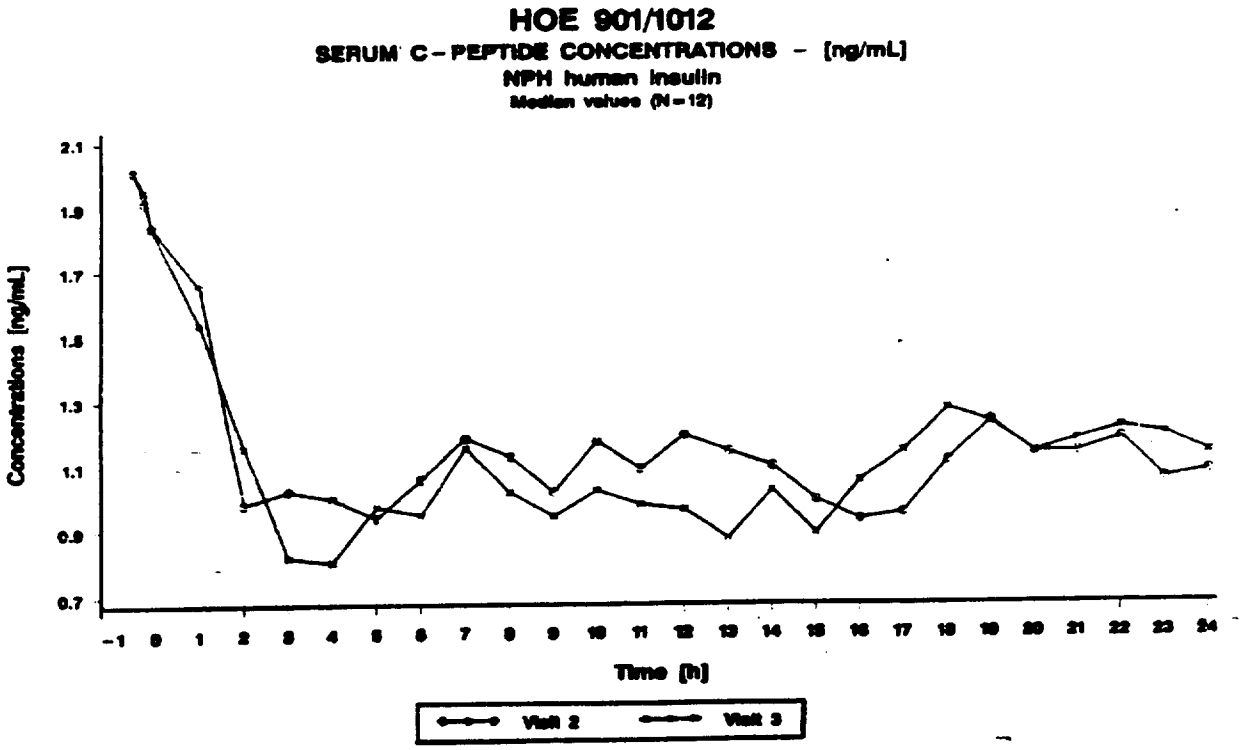

Figure 19. C-Peptide levels following two consecutive subcutaneous injections of NPH insulin (0.4 IU/kg) to healthy male subjects (n = 12); Study 1012.

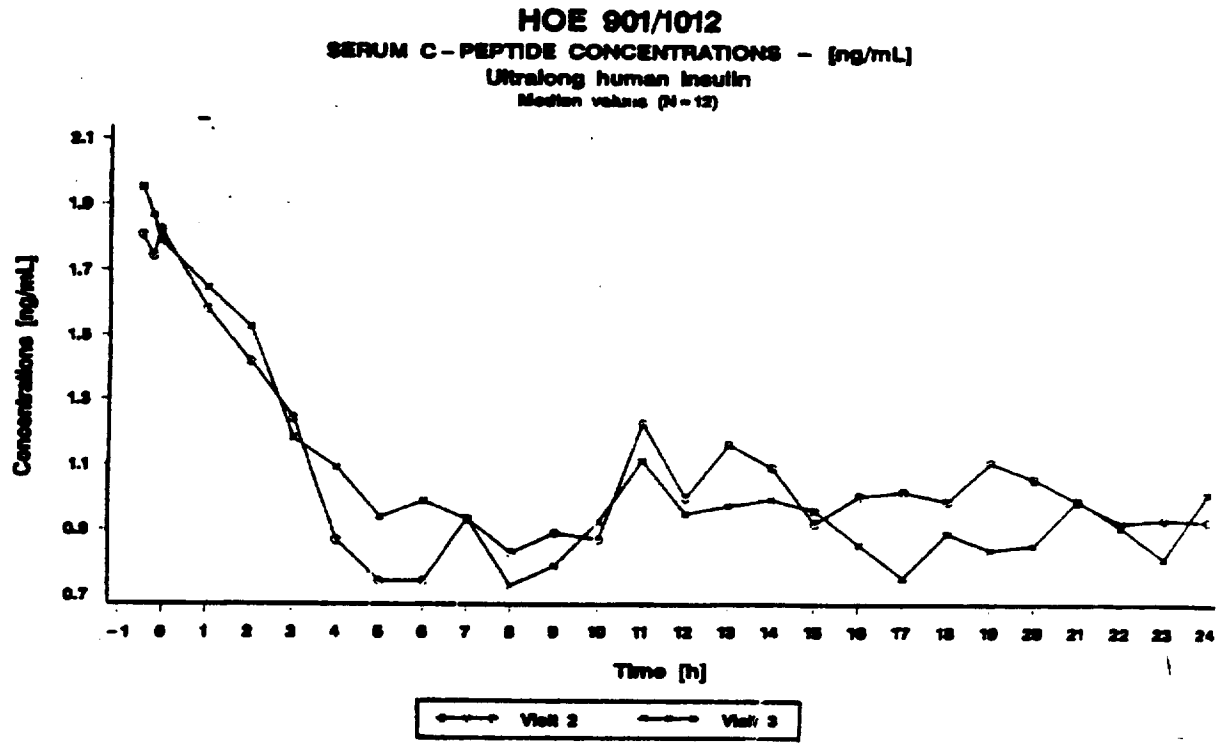

Figure 20. C-Peptide levels following two consecutive subcutaneous injections of Ultralong insulin (0.4 IU/kg) to healthy male subjects (n = 12); Study 1012.

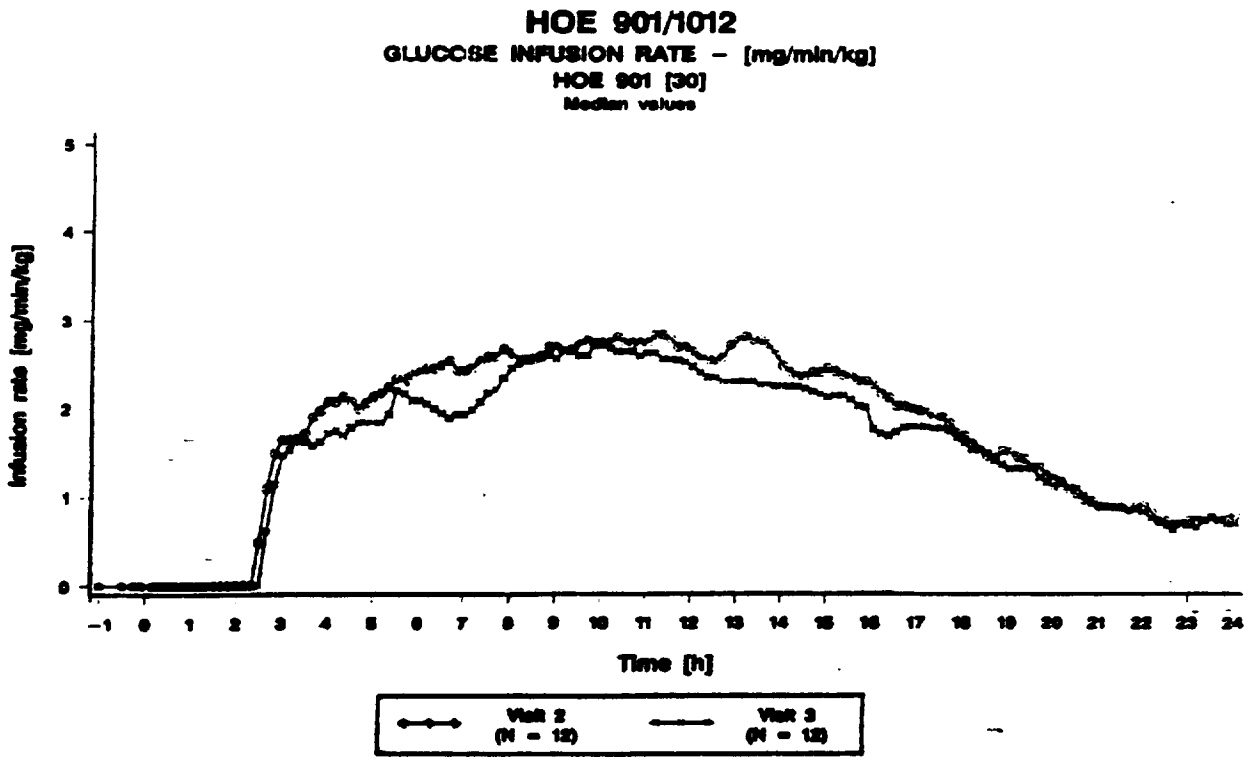

Figure 21. Glucose infusion rate following two consecutive subcutaneous injections of HOE 901 (0.4 IU/kg) to healthy male subjects (n = 12); Study 1012.

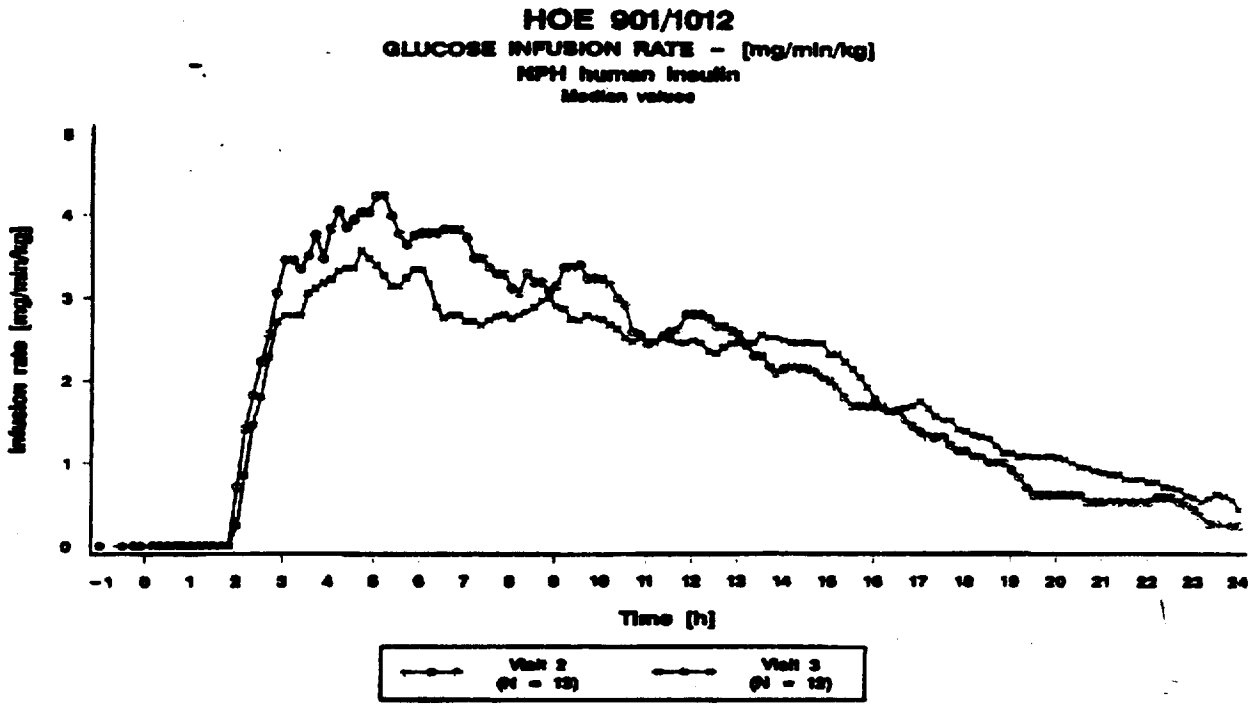

Figure 22. Glucose infusion rate following two consecutive subcutaneous injections of NPH insulin (0.4 IU/kg) to healthy male subjects (n = 12); Study 1012.

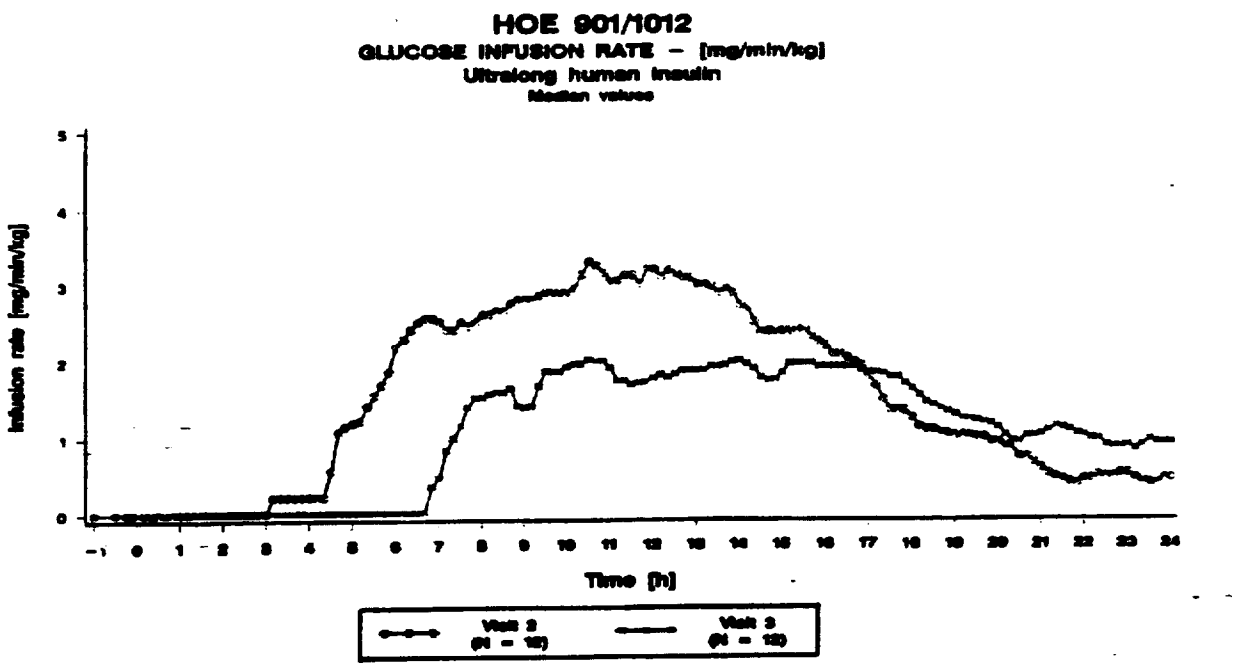

Figure 23. Glucose infusion rate following two consecutive subcutaneous injections of Ultralong insulin (0.4 IU/kg) to healthy male subjects (n = 12); Study 1012.

VII. Drug Interactions

No drug interaction studies were performed.

VIII. Dosage and Administration

The dose of all insulin products must be individualized for each patient. Because of its slow release from the site of injection, insulin glargine may be administered once daily.

IX. Formulation

Table IX contains formulation information for insulin glargine. Unlike most other long-acting insulin preparations (which are suspensions), HOE901 is a solution in the vial and only becomes a precipitate once exposed to physiological pH.

Table IX: Formulation of to-be-marketed product.

Ingredient	Amount (mg/ml)
Insulin glargine	3.6378 (100 units)
m-cresol	2.70
Zinc	[redacted]
Glycerol (85%)	20.00
NaOH	[redacted]
HCL	[redacted]
Water for injection	[redacted]

[1] nominal amount needed for pH adjustment

Question Based Review:

- *What is the potency of insulin glargine (HOE 901) relative to human insulin?*

  HOE 901 appears to be equipotent to NPH human insulin.

- *What effect does the site of injection have on the pharmacokinetics of insulin glargine?*

  Injection site (leg, abdomen and arm) does not show a clinically significant effect on the pharmacokinetics of HOE 901.

- *Can insulin glargine be mixed with shorter-acting insulins in the same syringe for patient convenience?*

  HOE 901 should not be mixed with any insulin since a change in pH could cause precipitation in the syringe and altered pharmacokinetics.

- *What is the optimum time of injection for insulin glargine?*

  HOE 901 is to be injected at bedtime.

- *Does the pharmacokinetics of insulin glargine differ between healthy volunteers and patients with diabetes?*

  Similar PK profiles for HOE 901 are observed in healthy subjects as well as patients with Type 1 or Type 2 diabetes.

- *Are dosing adjustments needed in special populations such as the elderly, children, or patients with renal or hepatic impairment?*

  Dosing in special populations has not been specifically addressed in the PK studies.

- *How does the PK/PD of insulin glargine compare with other long-acting insulins, such as NPH and crystalline zinc preparations?*

  HOE 901 appears to have a more flat PK profile with no distinct peaks in addition to a longer duration of action; NPH insulin has a distinct peak at about – hours.

X. Labeling Comments

Under **Pharmacodynamics**, the last sentence, which reads:

[ ]

[ ] should be deleted.

The rationale for this change:

- The study was not powered to allow appropriate comparison of the different formulations
- RIA assay used is not sufficiently specific to HOE 901 and major metabolites; additionally, it has high crossreactivity to endogenous insulin. Therefore, comparison of the pharmacokinetic parameters is not appropriate
- Claim of ———— is not supported by comparison of PD parameters.

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XII. Signatures

/S/

Sam H. Haidar, R.Ph., Ph.D.

Division of Pharmaceutical Evaluation II
Office of Clinical Pharmacology and Biopharmaceutics

CPB Briefing: February 2, 2000; Attendees: Malozowski S, Misbin R, Metz W, Selen A, Huang S, Hunt J, Lee P, Ahn H, Madani S, Johnson S

FT Signed by Hae-Young Ahn, Ph.D., Team Leader /S/ 2/3/00

CC: NDA 21-081 (orig., 1 copy), HFD-510 (R. Misbin, J. Rhee), HFD-850 (Lesko), HFD-870 (S. Huang, S. Haidar, H. Ahn), HFD-340 (Vish), Central Document Room (Barbara Murphy)
Code : AP/S/
2/03/00

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Attachment A

NDA 21-081

Proposed Labeling

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Labeling

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Attachment B

NDA 21-081

Study Synopsis

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STUDY SYNOPSIS

HOE 901/1015

Title
Comparison of the time-action profiles of HOE 901 and human NPH insulin in type 1 diabetic patients using the euglycaemic clamp technique.

Investigators, study site
Investigator: [redacted]
Subinvestigators: [redacted]

Phase I

Indication
Not applicable

Objective
To characterise the time-action profiles of HOE 901 in comparison with human NPH insulin in type 1 diabetic subjects.

Design
Single-centre, single-dose, double-blind, randomised, two-way crossover study on type 1 diabetic subjects.

Population
Twenty male and/or female subjects with type 1 diabetes mellitus for more than three years, aged between 18 and 50 years and with a body mass index (BMI) of between 18 and 26 kg/m^2.

Treatments
Single subcutaneous injections of 0.3 IU/kg HOE 901 or 0.3 IU/kg NPH insulin in the inner thigh region. The study medication was supplied by Hoechst Marion Roussel. The batch numbers of the medication used were – (HOE 901) and — (NPH insulin).

Pharmacodynamic data
The duration of insulin action (HOE 901 or NPH insulin) was defined as the time period between the onset of action (the time when the regular insulin infusion rate decreased to 50% of the average insulin infusion rate recorded before administration of the study medication) and the end of action. The end of action was defined as the time when the plasma glucose concentration was ≥ 150 mg/dL after discontinuation of glucose infusion. However if this value was not reached by 24 h after treatment, the clamp procedure was to be terminated and this time point was regarded as the end of action. Blood samples were taken during the euglycaemic clamp procedure, and plasma was analysed in the investigator's laboratory for glucose concentrations using [redacted] glucose analyser. The

infusion rates of glucose (GIR) and regular insulin were recorded during the euglycaemic clamp procedure from the instruments used to conduct the procedure.

Pharmacokinetic data

Blood samples were taken during the euglycaemic clamp procedure and serum was analysed for free insulin at Hoechst Marion Roussel, Milton Keynes, UK, using a radio-immunoassay. The assay range was — µIU/mL using Hoechst Marion Roussel's bioanalytical method ——— . The assay was 100% specific for human insulin, and had a cross-reactivity of about 50% with HOE 901 and its metabolites.

Safety data

- Haematology, clinical chemistry, urinalysis
- Physical examination, vital signs, 12-lead electrocardiogram
- Adverse events.

Study duration and dates

The study took place between 17 February 1998 and 2 July 1998.

Statistical procedures

Descriptive summary statistics were calculated for the duration of action (primary variable), onset of action, GIR (baseline, $AUC_{0\text{-}12\ h}$, $AUC_{0\text{-end of glucose infusion}}$, GIR_{max}, time to GIR_{max}, sums of time intervals where GIR > 75%, 50% and 25% of GIR_{max}), plasma glucose concentrations (fasting, $AUC_{0\text{-}12\ h}$, $AUC_{0\text{-end of glucose infusion}}$, C_{min}, maximum decrease, t_{min}), and infusion rates of regular insulin (total amount infused). The duration of action, onset of action, time of GIR_{max}, and sums of time intervals where GIR > 75%, 50% and 25% of GIR_{max} were analysed non-parametrically and 95% confidence intervals were calculated for the median treatment differences. 95% confidence intervals were calculated for the mean treatment ratios of $AUC_{0\text{-}12\ h}$ and $AUC_{0\text{-end of glucose infusion}}$ for GIR, and GIR_{max} according to Fieller's Theorem based on untransformed data.

The distribution of plasma glucose concentrations was investigated by constructing a frequency table reflecting the numbers and percentages of plasma glucose concentrations.

Descriptive summary statistics were calculated for serum free insulin concentrations (baseline, $AUC_{0\text{-}12\ h}$, $AUC_{0\text{-end of glucose infusion}}$, C_{max}, t_{max}).

Interim analysis

Interim data were presented by the investigator at four symposia while the study was still blinded.

Results – Study subjects and conduct

Twenty subjects with type 1 diabetes mellitus were enrolled in the study, randomised, treated with HOE 901 and NPH insulin according to the randomisation plan, and completed all examinations according to the study protocol. All 20 subjects were biometrically evaluable for the pharmacodynamic and pharmacokinetic analyses, and were included in the analysis of safety.

The mean age of the 20 study subjects was 32.4 years, ranging between 20 and 52 years. The mean height was 170.6 cm (range 150 to 186 cm) and the mean weight was 64.8 kg (range 48 to 84 kg).

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Results – Pharmacodynamics and pharmacokinetics

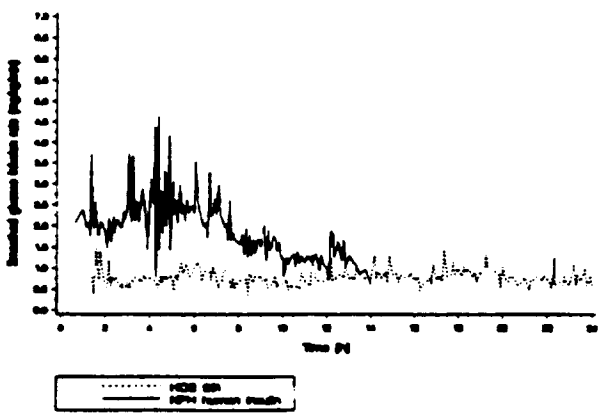
Duration of action, onset of action, and GIR pharmacodynamic variables

Variable	Statistic	HOE 901	NPH insulin	Intra-individual CV (%)[a]	PE (%)	95% Confidence interval
Duration of action (h)	Median (min;max)	22.8[b]	13.8	-	7.78[c]	5.28 ; 8.70[c]
Onset of action (h)	Median (min;max)	1.11	0.71	-	0.40[c]	-0.06 ; 0.69[c]
GIR: $AUC_{0\text{-end}}$ (mg/kg)	Mean (CV)	976 (84%)	1527 (78%)	35.3	63.9[d]	51.5 ; 77.6[d]
GIR_{max} (mg/kg/min)[e]	Mean CV (%)	3.69 (89%)	10.0 (70%)	60.2	36.9[d]	22.1 ; 55.1[d]
GIR: t_{max} (h)[e]	Median (min;max)	2.22[f]	1.40	-	1.87[c]	0.34 ; 7.08[c]

[a] Intra-individual CV was calculated only from the ANOVA with treatment, subject and period effect, based on untransformed data.
[b] The median duration of action for HOE 901 has to be regarded as a minimum estimate because in most cases the study was discontinued before the end of action had been reached.
[c] Nonparametric analysis of the median differences "HOE 901 - NPH insulin", based on the method of Hauschke et al. 1990.
[d] Point estimates and 95% confidence intervals for the ratios of treatment means ("HOE 901 / NPH insulin") according to Fieller's Theorem, based on untransformed data.
[e] Determined from "smoothed" GIR profiles.
[f] n = 19, subject 103 had no GIR values during the HOE 901 treatment period (visit 3).

The median duration of action for HOE 901 was significantly longer than that of NPH insulin. However, because the study was terminated after a maximum of 24 hours after treatment, which in 14 cases was before the predefined end of insulin action had been reached after HOE 901 treatment, the durations of action calculated for HOE 901 have to be regarded as minimum estimates.

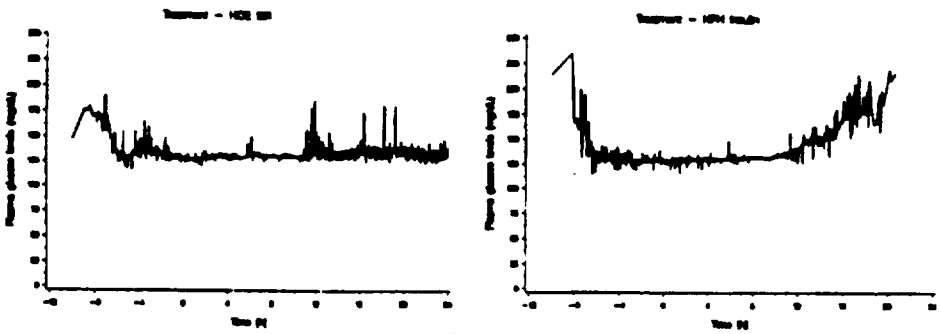
Smoothed glucose infusion rate – geometric mean values



The greater variability in individual values for t_{max} in the HOE 901 treatment group reflects the smoother curve (i.e., the lack of a distinct peak) after HOE 901 treatment, compared with after NPH

treatment. The low AUC values and the flat profile after HOE 901 treatment indicate that the action of HOE 901 was well matched to the liver glucose output over an extended period of time, i.e. HOE 901 was more successful than NPH insulin at fulfilling the subjects' basal insulin requirements over an extended period of time. In one extreme case, no glucose infusion at all was required after treatment with HOE 901.

Mean plasma glucose concentrations



As intended, plasma glucose concentrations were maintained at around 130 mg/dL during the clamp period for as long as the administered HOE 901 or NPH insulin was active.

Serum free insulin pharmacokinetic variables

Variable	Statistic	HOE 901	NPH Insulin
Baseline conc. (µIU/mL)	Mean (CV)	16.0 (63%)	14.3 (78%)
AUC_{0-end} (µIU/mL·h)	Mean (CV)	224 (34%)	228 (39%)
C_{max} (µIU/mL)	Mean (CV)	19.3 (49%)	27.4 (51%)
t_{max} (h)	Median (min;max)	3.0	4.5

The comparison of AUC values for serum free insulin concentrations was influenced by the fact that the profile period was longer for the HOE 901 group. The differences in the AUC and C_{max} values between the treatment groups reflect the fact that the radio-immunoassay used for quantifying insulin has a cross-reactivity of only about 50% to HOE 901 and its metabolites, resulting in underestimation of these components. The concentration-time profiles showed a distinct peak in serum free insulin concentrations at about five to six hours after NPH treatment, whereas serum free insulin concentrations remained relatively constant throughout the clamp period after treatment with HOE 901. The median t_{max} after treatment with HOE 901 was slightly earlier (3.0 hours) than after treatment with NPH insulin (4.5 hours). The greater variability in individual values for t_{max} in the HOE 901 treatment group reflects the smoother curve (i.e., the lack of a distinct peak) after HOE 901 treatment, compared with after NPH treatment.

Results – Safety

There were no adverse events during this study. There were no clinically relevant abnormalities in the laboratory safety data. Both insulins were safe and well-tolerated.

Conclusions

The time-action profiles in type 1 diabetic subjects, as indicated by duration of action and GIR, confirmed the long-acting profile of HOE 901 in comparison to NPH insulin. The median duration of action for HOE 901 was at least nine hours longer than that of NPH insulin. The flat, consistent GIR-time profile obtained after HOE 901 treatment, in contrast to the early peak obtained after NPH treatment, indicated that HOE 901 was more successful than NPH insulin at fulfilling the subjects' basal insulin requirements over an extended period of time. Both types of insulin were safe and well-tolerated.

STUDY SYNOPSIS

HOE 901/ 1013

Title
Characterization of glucose turnover of HOE 901 in comparison with regular human insulin in healthy male subjects

Investigator(s), study site(s)

Phase I

Indication
Not applicable.

Objectives
To investigate the equimolar potency of HOE 901 compared to regular human insulin when given intravenously (i.v.) on suppression of hepatic glucose production and stimulation of peripheral glucose utilization in healthy male subjects.

Design
This was a single-dose, double-blind, randomized, two-way crossover trial. The study consisted of 4 visits - screening visit (visit 1), treatment visits (visits 2 and 3), and follow-up visit (visit 4). Visit 2 was performed within 14 days of visit 1. The washout period between visits 2 and 3 was at least 7 days. Visit 4 was conducted within 7 days after visit 3.

Population
Healthy male subjects, aged 18 to 50 years with body mass index between 18 kg/m^2 and 26 kg/m^2.

Treatments
Crossover study with 4 hours of continuous intravenous infusion of HOE 901 or regular human insulin at a rate of 40 $mU/m^2/min$.

Medication: HOE 901 (zinc content of 30 µg/mL, batch number: HOE901/29), Regular human insulin (batch number: 1ML16M).

Pharmacokinetic data
Profiles of serum immunoreactive insulin, summarized by AUC (area under the curve), C_{max} (maximum insulin concentration), and t_{max} (time to C_{max}).

Pharmacodynamic data
At visits 2 and 3, subjects underwent 2 study periods:

Basal study: After overnight fasting, subjects received a continuous infusion of D-[3-^{3}H]-glucose for approximately 4 hours to label the glucose pool.

Glucose clamp study: After the basal measurements were obtained, subjects received a continuous i.v. infusion of HOE 901 or regular human insulin at a rate of 40 mU/m^2/min. A simultaneous infusion of 20% dextrose containing D-[3-^{3}H]-glucose (hot-GINF) was infused at a variable rate to sustain euglycemia at 90 mg/dL. Blood samples were collected at selected time points to measure the blood glucose, plasma glucose specific activity, C-peptide levels, and serum free fatty acids (FFA). The clamp study lasted about 7 hours.

- A50 IGDR: Time to half maximal stimulation of incremental glucose disposal rate (IGDR is defined as the difference between the initial basal glucose disposal rate (Rd) and the Rd values during and after cessation of the insulin infusion.)
- D50 IGDR: Time to half maximal deactivation of IGDR
- A50 HGO: Time to half maximal suppression of hepatic glucose output (HGO)
- D50 HGO: Time to half maximal deactivation of HGO
- Rd_{max}: The maximal glucose disposal rate
- Profiles of blood glucose concentration, FFA, and C-peptide

Safety data

Hematology, clinical chemistry, urinalysis, 12-lead electrocardiogram (ECG), physical examination, vital signs, and adverse events

Study duration and dates

The study took place between 30 September 1997 and 12 March 1998.

Statistical procedures

Pharmacodynamics and pharmacokinetics

An analysis of variance (ANOVA) of pharmacodynamic and pharmacokinetic parameters was used to determine if the outcomes were significantly different between the 2 insulins. The ANOVA included terms for sequence, subject within sequence, period, and treatment; 95% confidence intervals for treatment differences were calculated.

The serum immunoreactive insulin concentrations were summarized descriptively at each time point.

Safety

Safety outcomes were presented descriptively.

Interim analysis

No interim analysis was performed for this study.

Results - Study subjects and conduct

Seventeen male subjects were enrolled in the study. Subjects were between 18 and 50 years of age (mean: 33 years old). Thirteen subjects were white, 2 were black, and 2 were Asian. Fifteen subjects were randomized and completed the study. Two subjects withdrew from the study prior to randomization: one subject withdrew consent prior to randomization, and the other failed to return for visit 2 within the 2-week time period and was excluded from further participation. Only the

12 subjects who completed the study after Protocol Amendment 1 were evaluated in the pharmacokinetic and pharmacodynamic analyses. All 17 subjects who were enrolled in the study were evaluated in the safety analysis.

There were no major protocol deviations in this study.

Results - Pharmacokinetics and pharmacodynamics

Pharmacokinetics

AUC and C_{max} for regular human insulin and HOE 901 from time 0 to 7 hours were statistically different for both serum immunoreactive insulin and serum immunoreactive exogenous insulin. However, these differences in serum insulin profiles could be attributed to the radioimmunoassay (RIA) used in this study. Serum immunoreactive insulin has been measured using an antibody to regular human insulin with approximately 50% crossreactivity to HOE 901 and its active metabolites. Therefore, the concentration of HOE 901 in serum would appear to be lower than its actual concentration. There was no significant difference in t_{max} between HOE 901 and human regular insulin.

Pharmacodynamics

The suppression of HGO by HOE 901 tended to be faster than regular human insulin (p=0.0585). The mean values for HOE 901 and regular human insulin were 56.6 and 73.0 minutes, respectively. D50 HGO could not be determined for the majority of the subjects because the deactivation of HGO suppression did not reach the 50% level by 180 minutes after stopping the insulin infusion (i.e., the end of the clamp).

There was no significant difference in maximum rate of glucose disposal between regular human insulin and HOE 901. The mean values for HOE 901 and regular human insulin were 9.9 and 10.1 mg/kg/min, respectively. A comparison of AUC (mean glucose disposal rate by time) showed comparable values for HOE 901 (2740 mg/kg) and regular human insulin (2887 mg/kg).

There were no significant differences in A50 IGDR or D50 IGDR between regular human insulin and HOE 901. The A50 IGDR mean values for HOE 901 and regular human insulin were 42.1 and 32.4 minutes, respectively. The D50 IGDR mean value for HOE 901 was 57.3 minutes and for regular human insulin was 62.8 minutes.

There were no significant differences in FFA levels between regular human insulin and HOE 901. The mean values for HOE 901 were 0.43, 0.09, and 0.37 mmol/L for the basal, end and recovery periods, respectively. The mean values for regular human insulin were 0.45, 0.09, and 0.33 mmol/L for the basal, end and recovery periods, respectively.

Results - Safety

There were no serious adverse events reported in this study. All 4 nonserious adverse events reported (infection [2], arthritis, and ecchymosis) were considered not related to study medication.

HOE 901 appears to have no clinically significant effects on hematology, blood chemistry, urinalysis values, vitals signs, and ECGs.

Conclusions

HOE 901 and regular human insulin were well tolerated by all study subjects.

There was no significant difference in the kinetics of activation of HGO, nor in absolute glucose disposal rate between regular human insulin and HOE 901 when administered intravenously. D50 HGO could not be determined for the majority of the subjects because the deactivation of HGO suppression did not reach the 50% level by 180 minutes after stopping the insulin infusion. Therefore, HOE 901 and regular human insulin have similar end-organ metabolic effects when given as i.v. infusion in the same doses.

STUDY SYNOPSIS

HOE901/1016

Title
Determination of Metabolic Degradation Products of HOE 901 after subcutaneous injection of HOE 901 in healthy subjects.

Investigators, study site

[ ]

Phase I

Indication
Not applicable.

Objectives
To elucidate the metabolic profile at the site of injection and in the systemic circulation over 24 hours after subcutaneous injection of HOE 901, a HOE 901 formulation with zinc content of 30 µg/mL.

Design
Single centre, open study, single-dose HOE 901 administered to 4 subjects. One additional subject, who did not receive the s.c. injection of HOE 901, was enrolled in the study as a control subject. A subcutaneous tissue sample of the defined injection site for this study was collected from this control subject for determination of the baseline endogenous insulin level at the site of injection of the study medication.

Population
Healthy male and female, aged between 18 and 50 years, with body mass index between 18 and 26 kg/m^2.

Treatments
HOE 901, 0.6 IU/kg body weight, batch number —(HMR); single dose, subcutaneous injection.

Methods of evaluation

Pharmacokinetic data

Plasma samples for metabolic profile taken sequentially up to 24 hours after injection of HOE 901. Tissue samples from the injection site were taken by liposuction at 0, 2, 6, 12 and 24 hours, one sample per subject.

Blood samples for determination of serum immunoreactive insulin were taken hourly until the end of the clamp period. Individual profiles were plotted, and characterised by the following variables:

- C_{max}: maximum concentration.
- C_{min}: minimum concentration.
- t_{max}: time to maximum concentration.

Pharmacodynamic data

Glucose infusion rate (GIR) was determined every 5 minutes up to 6h, and every 10 minutes thereafter, up to the end of the clamp, i.e. when the GIR decreased to less than 10% of the maximum GIR over a period of 30 minutes. Individual profiles were plotted, and were characterised by the variables:

- Maximum GIR.
- Time of maximum GIR.

Safety data

Spontaneously reported and recorded adverse events
Standard laboratory haematology, clinical chemistry and qualitative urinalysis (screening and visit 2)
Physical examination (screening and visit 2)
Vital signs (all visits)

Performance of the clamp

Blood glucose was measured every 5 minutes up to 6h, and every 10 minutes thereafter up to the end of the clamp period. Individual profiles were plotted and characterised descriptively. The distribution of blood glucose levels was described by constructing a frequency table.

Study duration and dates
The study took place during October 1996.

Statistical procedures
Due to the nature of this study, and small sample size, all evaluations were descriptive only.

Interim analysis
No interim analysis was performed.

Results - Study subjects and conduct

Five Caucasian men were enrolled into the study. All completed the study and were evaluated for safety, pharmacodynamics and pharmacokinetics. Their demographic details were: age 18.9-27.7 years (mean: 22.7 years); weight 70.9-93.0 kg (mean: 83.1 kg); body mass index 23.2-25.8 kg/m^2 (mean: 24.9 kg/m^2). No major protocol deviations occurred. All clamp periods lasted between 8.50 and 23.3h.

Results - Pharmacokinetics and pharmacodynamics

Low plasma levels and significant variability in recoveries during the plasma processing procedure were observed. However, the analytical method used gave rise to a qualitative degradation pattern of

the drug in humans with respect to immunoreactive degradation products. Metabolic profiling showed that both the parent compound and degradation products (Gly-insulin (M1) and Des-Thr-Gly-insulin (M2)) were present in the circulation.

Analysis of these samples showed two immunoreactive peaks which were isolated and subjected to ——— results showed one peak to be HOE 901 and the other a mixture of M1 and M2. Other significant non-immunoreactive peaks observed in the tissue samples were identified as fragments of haemoglobin A by ——— and not to be drug related.

The blood glucose levels were "clamped" at each individual's fasting blood glucose concentration, which ranged between ——— mg/dl (mean: 85.6 mg/dL). The individual clamp periods lasted 23.3, 8.50, 22.2 and 22.8h for the four subjects respectively. The variability in blood glucose levels within each subject's clamp period ranged between ——— % (mean: 4.20%).

Maximum GIR-values of between ——— mg/min/kg (mean: 1.75 mg/min/kg) were observed, occurring between ——— h after insulin injection (median: 11.4h).

Suppression of serum C-peptide levels, indicative of suppression of endogenous insulin, was apparent in all cases. Individual maximum serum immunoreactive insulin concentrations of ——— µIU/mL (mean: 18.1 µIU/mL) were observed for the 4 subjects, between 4 and 15h (median: 12h) after injection.

Results - Safety

Adverse events: There were 2 adverse events in two subjects. Both were reports of a burning sensation at the liposuction site. These events occurred about 8 and 25 hours after the injection of HOE 901, and lasted 12 and 9 hours respectively. Both events were mild in intensity, required no treatment and resolved spontaneously. These events were most probably related to the liposuction procedure.

Laboratory investigations: Small, insignificant decreases in haemoglobin, albumin and total protein concentrations were seen in all 5 subjects. These clinically irrelevant changes were not drug related and could probably be ascribed to the haemodilution occurring with clamp procedures.

Clinical variables: The physical examination and vital signs revealed no abnormalities at screening. There were no relevant emergent changes in clinical variables.

Conclusions

Results from this study demonstrated that after subcutaneous administration, both unchanged drug and degradation products are liberated from the subcutaneous depot.

At the site of injection, HOE 901 was already degraded. On average, the parent compound and its immunoreactive degradation products were of the same order of magnitude. Degradation took place by the loss of both arginines at the carboxy terminus of the B chain to form M1. Additional loss of the next amino acid threonine, generating M2, occurred as well.

In the systemic circulation, the parent compound as well as M1 and/or M2 were detected. The mono-arginine intermediate was possibly present as well in at least one sample.

The administration of HOE 901 was found to be safe and well tolerated.

STUDY SYNOPSIS
HOE 71GT/2/GB/104/--

(HOE 71GT = HOE 901)

TITLE	Comparison of the insulin absorption rate of ^{125}I-labelled preparations of HOE 901 [30] (30 µg zinc) following subcutaneous injection into the abdominal, leg and arm regions
INVESTIGATOR, STUDY SITE	[ ]
STUDY DATES	September to November, 1995
REPORT TYPE	Clinical/biometric, final
REPORT ORIGIN	Clinical Development, Hoechst Marion Roussel GmbH, Frankfurt, Germany
DATE OF ISSUE	This document dated 25 January 1999 replaces the document dated 23 April 1998.
PHASE	I
INDICATION	Not applicable
STUDY OBJECTIVES	*Primary objective:* To measure the insulin absorption rate of a ^{125}I-labelled preparation of HOE 901 [30] by [redacted] following subcutaneous (s.c.) injection in the abdominal, leg and arm regions. *Secondary objectives:* To compare the pharmacokinetics and pharmacodynamics of HOE 901 [30] in relation to the injection sites
STUDY MEDICATION AND DOSAGE	Single s.c. 0.2 IU/kg body weight injection of HOE 901 [30] (human insulin analogue containing 30 µg/mL zinc, batch no. 6/95a & 7/95a) into either the arm, leg or abdominal regions. The study medication was radioactively labelled with ^{125}I-iodine (50 kBq per dose). A single dose of 120 mg potassium iodide was taken before each treatment day.
STUDY DESIGN	Single-center, open, randomized, three-way crossover comparison. The study comprised 5 visits: screening visit (visit 0) 1 to 28 days before visit 1, visits 1 to 3 at which study medication was given, each followed by washout phases of 7 to14 days, and a final safety visit (visit 4) 1 to 28 days after the last dose. Visits 1 to 3 were preceded by overnight fasting.
STUDY POPULATION	12 healthy male volunteers, aged between 18 and 50 years, and with a body mass index between 18 and 30 kg/m^2
STUDY VARIABLES	
Pharmacodynamics/ Pharmacokinetics	*Primary variables:* • absorption rate of radioactivity: mean time of disappearance of 25% radioactivity (T75%) and mean residual radioactivity in % *Secondary variables:* • blood glucose concentration: area under the concentration-curve divided by time for the period 0–24 h after injection ($AUC_{0-24\ h}$) and maximum decrease during 0–24 h post injection, time at which the blood glucose concentration returned to its fasting level after the minimum • serum insulin: $AUC_{0-24\ h}$, maximum concentration and time of maximum concentration within 0–24 h post injection
Clinical study manager: **Biometrician:** **Authors:**	[ ]

- serum C-peptide: $AUC_{0-24\ h}$, minimum increase and time of minimum concentration within 0–24 h post injection
- exogenous plasma insulin concentration: $AUC_{0-24\ h}$, maximum concentration and time of maximum concentration within 0–24 h post injection
- non-esterified free fatty acids (NEFA): $AUC_{0-24\ h}$

Safety

All spontaneously reported and recorded adverse events (all visits). Physical examination, Standard laboratory hematology and clinical chemistry (visits 0 and 4). Vital signs (all visits). Inspection of injection sites.

STATISTICAL METHODS

Analysis of variance (T75% and mean residual radioactivity), analysis of covariance (remaining variables). Treatments were compared using following tests: Friedman test (time of blood glucose and C-peptide minimum, time of exogenous plasma insulin maximum).

RESULTS

Study sample

12 subjects, aged between 23 and 44 years (median: 32.5 years), with BMI between 21 and 28 kg/m^2 (median 24.4 kg/m^2), completed the study according to the protocol. No anti-insulin antibodies were detected and all HbA_{1c} values were in the non-diabetic range.

Study regimen

One subject (No. 2) withdrew due to a single episode of hematemesis and associated symptoms (recorded as five serious adverse events unrelated to the study medication) and was replaced with subject 16. There were no major protocol deviations.

Pharmacodynamics/ Pharmacokinetics

Primary variables

Based on visual examination of the median radioactivity profiles, there were only limited injection site related differences in the radioactivity absorption rates. With one exception, this interpretation was confirmed by the ANOVA model of this parameter as tested using the variables T75% and mean residual radioactivity after 24 h. The arm-leg comparison of mean residual radioactivity suggested a significant difference ($p < 0.05$).

Radioactivity absorption parameters

Injection site	T75% in hours		Resid. radioactivity at injection site after 24 h [%]	
	Mean [a]	SD	Mean [a]	SD
Arm	11.9	4.66	47.7	10.12
Leg	15.3	4.66	56.3	10.12
Abdomen	13.2	4.66	57.2	10.12

[a] According to the analysis of ANOVA model

In exploratory analyses of the data, the 90% confidence intervals of the point estimates for the T75% for all sites and mean residual radioactivity after 24 h comparisons fell partly outside the conventional equivalence range suggesting that none of the injection sites were equivalent for this parameter. In contrast, the 90% confidence intervals for the leg-abdomen comparison fell within the equivalence range suggesting equivalence in the amount of radioactivity at these sites after 24 h.

Secondary variables

Based on visual examination and statistical analysis of the exogenous serum insulin, blood glucose, serum C-peptide, NEFA, and serum insulin profiles, there were no significant differences between the three injection site treatments.

On the basis of 90% confidence intervals point estimates, the exogenous serum insulin variables $AUC_{0-24\ h}$ and the maximum concentrations were not equivalent in any of the three treatment comparisons.

Exogenous serum insulin parameters

Injection site	$AUC_{0-24\ h}$ [µU/mL]		Max. conc. [µU/mL]	
	Mean [a]	SD	Mean [a]	SD
Arm	4.2	1.85	11.8	6.29
Leg	4.3	1.84	11.0	6.26
Abdomen	3.6	1.84	8.0	6.26

[a] According to the analysis of ANCOVA model

Safety

A single episode of hematemesis associated with diarrhea, gastrointestinal pain, dizziness, and sore throat with loss of voice occurred in one subject (No 2) three days after visit 2 (gastrointestinal pain and sore throat two days after visit 2). This episode was reported as 5 serious adverse events, which were not related to the study medication. This subject was withdrawn from the study. Subcutaneous HOE 901 administration in the arm, leg and abdomen was well tolerated, and there were no injection site reactions. In total 11 subjects reported 36 adverse events during this study. All adverse events were of mild to moderate intensity. Six subjects reported 10 adverse events which were considered to be possibly related to the study treatment; amongst these events, headache (3 subjects / 4 mentions) and abdominal pain (2 subjects / 3 mentions) were the most common.

COMMENTS/ CONCLUSIONS

Based on the primary variables investigated in this study (reflecting radioactivity absorption rate, there were only limited, if any, injection site-related differences in the insulin analogue HOE 901 absorption rates.

Although, exploratory analyses of the data did not suggest the equivalency of these variables for the three injection sites, this is likely to reflect the inherently high intra- and inter-subject variability in insulin absorption and the limitations of the study design. High intra- and inter-subject variability in insulin absorption rates is well documented in the literature.

As reflected by the variable exogenous serum insulin, there were no significant differences in insulin bioavailability after administration in the arm, leg, and abdomen. On the basis of 90% confidence intervals for the point estimates, the variables $AUC_{0-24\ h}$ and the maximum concentrations were not equivalent in any of the three treatment comparisons.

In summary, there were only limited injection site related differences in either the insulin analogue HOE 901 absorption rates or bioavailability. Subcutaneous HOE 901 administration in the arm, leg and abdomen was well tolerated. These data, together with published reports that NPH-insulin absorption rates are not significantly influenced by the injection site, suggest that in contrast to short acting insulins that there are only minor, if any, injection site effects on the longer acting insulin.

STUDY SYNOPSIS
HOE 71GT/2/GB/101/-

HOE 901 = HOE 71GT

INVESTIGATOR, STUDY SITE	[ ]
STUDY DATES	10 March 1993 to 15 July 1993
REPORT TYPE	Clinical/biometric, revised final
REPORT ORIGIN	HMR Clinical Development, Hoechst AG, Frankfurt / Main, Germany
DATE OF ISSUE	This document, issued 31 March 1998, replaces the document dated 15 April 1997. This document was re-issued due to an labeling error.
PHASE	I
INDICATION	Not applicable
STUDY OBJECTIVES	*Primary:* To measure the insulin absorption rate of ^{125}I-labelled preparations of HOE 71GT[15], HOE 71GT[80], HOE 36H and placebo following bolus subcutaneous injection into the abdominal wall by ——— To characterize the biological activity by comparing plasma glucose, immunoreactive insulin and C-peptide profiles in healthy subjects receiving HOE 71GT[15], HOE 71GT[80], HOE 36H and placebo. *Secondary:* To assess and compare the frequency and severity of adverse events between the different formulations.
STUDY MEDICATION AND DOSAGE	HOE 36H (semisynthetic NPH human insulin): single dose 0.15 U/kg HOE 71GT[15] (human insulin analogue): single dose 0.15 U/kg HOE 71GT[80] (human insulin analogue): single dose 0.15 U/kg (HOE 71GT[15] and HOE 71GT[80] differ only in their zinc content of 15 and 80 µg/ml respectively) Placebo (HOE 31H dilution buffer) Single dose of 120 mg potassium iodide before each study day
STUDY DESIGN	Single-center, placebo-controlled, randomized, single-blind cross-over comparison with 4×4 Latin Square. The study comprised 6 visits: screening visit (visit 0) <21 days before visit 1, visits 1–4 at which study medication was given, each with washout phases between of >7 days, and visit 5 (final) one month after the last dose. Visits 1–4 were preceded by overnight fasting.
STUDY POPULATION	12 healthy male volunteers with an age range of 18–45 years and body weight ± 20% of the ideal body weight were to be enrolled.
STUDY VARIABLES	
Pharmacodynamics/ Pharmacokinetics	*Primary variables* • Absorption rate of radioactivity: mean time of disappearance of 25% radioactivity (T75%) and mean residual radioactivity in % • Blood glucose: weighted average concentration 0–6 h after injection, maximum decrease and time of minimum between 0–6 h • Exogenous plasma insulin (immunoreactive insulin): weighted average concentration 0–6 h after injection, maximum concentration and time of maximum 0–4 h after injection. Insulin concentrations were calculated
Clinical study manager: **Biometrician:** **Authors:** **Medical Writing**	[ ]

according to Owens DR. Human Insulin. Clinical Pharmacological Study in Normal Man. Lancaster, UK: MTP Press Ltd. 1986: p46-236.

Secondary variables

- Blood glucose: weighted average concentration 0–24 and 6–24 h after injection, initial decrease, time of return to fasting level for first time after minimum
- Exogenous plasma insulin: weighted average concentration 0–24 and 6–24 h after injection, initial increase
- Plasma C-peptide: weighted average concentration 0–6, 0–24 and 6–24 h after injection, minimum concentration and time of minimum 0–14 h after injection, initial decrease
- Non-esterified free fatty acids (NEFA): weighted average concentration 0–6, 0–24 and 6–24 h after injection

Safety

Spontaneously reported and recorded adverse events. Standard clinical chemistry and haematology.

STATISTICAL METHODS

The treatments were compared using following tests: Friedman test (time of blood glucose and C-peptide minimum, time of exogenous plasma insulin maximum), analysis of variance (T75% and mean residual radioactivity), analysis of covariance (remaining variables).

RESULTS

Study sample

12 healthy male volunteers, age range 18–39 years (median: 24 years), 53.7–93.4 kg (median 72.05 kg), completed the study and were evaluable.

Study regimen

There were no major deviations. Measurements were performed according to the protocol with deviations of +/- <15 min. All subjects attended visit 0 4 to 11 days (median: 7 days) prior to visit 1. Washout phases between visits 1, 2, 3 and 4 were >7 days. The washout phase before the final visit in all subjects was between 28 and 35 days (median: 30 days).

Pharmacodynamics/ Pharmacokinetics

Primary variables

ABSORPTION OF RADIOACTIVITY	T75% (h)	Residual radioactivity (%)
Mean (and SD)		
HOE 36H	3.21 (2.375)	21.90 (9.404)
HOE 71GT[15]	8.75 (2.375)	43.84 (9.404)
HOE 71GT[80]	11.01 (2.375)	52.17 (9.404)
95% Confidence interval		
HOE 36H - HOE 71GT[15]	-7.68 ; -3.41 *	-30.39 ; -13.49 *
HOE 36H - HOE 71GT[80]	-9.93 ; -5.67 *	-38.72 ; -21.82 *
HOE 71GT[15] - HOE 71GT[80]	-4.39 ; -0.12 *	-15.85 ; -0.81 *

* $p = < 0.05$

The mean time of disappearance of 25% radioactivity (T75%) for the HOE 36H insulin indicated a significantly faster absorption rate when compared to the HOE 71GT-formulations ($p < 0.001$). This result is confirmed by the mean residual radioactivity of HOE 36H, which was significantly lower at 24 h for the same comparison ($p < 0.001$). HOE 71GT[15] indicated a faster absorption rate at T75% ($p < 0.05$) and it also yielded in less residual radioactivity at 24 h in comparison to HOE 71GT[80] ($p < 0.05$).

The weighted average blood glucose concentration 0–6 h was significantly different for HOE 36H when compared to all other treatments ($p < 0.01$). There was no difference to be seen for the comparison HOE 71GT[15] and HOE 71GT[80] ($p > 0.05$). The same significant differences between treatments applied for the variable maximum decrease.

BLOOD GLUCOSE	Weighted av. concentration 0–6 h (mmol/l)	Maximum decrease (mmol/l)
Adjusted mean[a] (and SD)		
HOE 36H	4.84 (0.237)	1.21 (0.357)
Placebo	5.34 (0.237)	0.52 (0.357)
HOE 71GT[15]	5.19 (0.242)	0.73 (0.365)
HOE 71GT[80]	5.26 (0.242)	0.63 (0.365)
95% CI for difference between adjusted means		
HOE 36H - Placebo	-0.71 ; -0.29 *	0.37 ; 1.02 *
HOE 36H - HOE 71GT[15]	-0.57 ; -0.14 *	0.15 ; 0.81 *
HOE 36H - HOE 71GT[80]	-0.64 ; -0.21 *	0.26 ; 0.91 *
Placebo - HOE 71GT[15]	-0.07 ; 0.36	-0.54 ; 0.11
Placebo - HOE 71GT[80]	-0.14 ; 0.29	-0.44 ; 0.21
HOE 71GT[15] - HOE 71GT[80]	-0.30 ; 0.15	-0.23 ; 0.44

[a] Adjusted means from the analysis of covariance model with baseline as the covariate

* p = < 0.05

EXOGENOUS PLASMA INSULIN	Weighted av. concentration 0–6 h (mU/l)	Maximum concentration (mU/l)
Adjusted mean[a] (and SD)		
HOE 36H	6.90 (2.728)	10.82 (5.989)
Placebo	0.64 (2.665)	1.85 (5.851)
HOE 71GT[15]	3.05 (2.673)	5.19 (5.870)
HOE 71GT[80]	2.78 (2.706)	5.75 (5.942)
95% CI for difference between adjusted means		
HOE 36H - Placebo	3.85 ; 8.67 *	3.69 ; 14.26 *
HOE 36H - HOE 71GT[15]	1.40 ; 6.31 *	0.25 ; 11.02 *
HOE 36H - HOE 71GT[80]	1.64 ; 6.62 *	-0.40 ; 10.54
Placebo - HOE 71GT[15]	-4.81 ; 0.00 *	-8.62 ; 1.94
Placebo - HOE 71GT[80]	-4.56 ; 0.29	-9.23 ; 1.42
HOE 71GT[15] - HOE 71GT[80]	-2.12 ; 2.67	-5.83 ; 4.69

[a] Adjusted means from the analysis of covariance model with baseline as the covariate

* p = < 0.05

The weighted average concentration of exogenous plasma insulin after HOE 36H was significantly higher than those after the two HOE 71GT formulations, which were similar. With HOE 36H the predominant peak for exogenous insulin occurred in the first 6 h after injection followed by a decline in exogenous insulin concentration. The profile of exogenous plasma insulin was smoother for the two HOE 71GT formulations than for HOE 36H.

Safety

Four subjects reported 11 nonserious adverse events: none under HOE 71GT[15], 3 under HOE 71GT[80], 4 under HOE 36H, 3 under placebo and 1 between treatment days. Headache accounted for 8 reports. Only one case of mild hypoglycaemia was reported (following HOE 71GT[80]). Changes in laboratory values were unremarkable. A mild irritation at the injection site was reported twice, in both cases the irritation subsided without treatment.

COMMENTS/ CONCLUSIONS

For almost all investigated primary variables there was a statistical difference between HOE 36H and the HOE 71GT formulations. HOE 71GT[15] and HOE 71GT[80] revealed similar results and presented the characteristics of long-acting basal insulins with a smooth, peakless plasma insulin profile. This data suggest that the absorption of both HOE 71GT formulations from the injection site is delayed. Both HOE 71GT formulations were well tolerated. The most frequent adverse event reported was headache.

STUDY SYNOPSIS

HOE 901/1018

Title
Assessment of the time-action profiles of HOE 901 compared to NPH human insulin after subcutaneous doses of 0.4 IU/kg using the euglycaemic clamp technique in healthy volunteers in a double-blind, placebo-controlled, three-way crossover study.

Investigator, study site

Phase I

Indication
Not applicable

Objective
The objective of the study was to investigate time-action profiles of HOE 901[30] (30 µg/mL zinc) and human NPH insulin in comparison to placebo following a single subcutaneous dose of 0.4 IU/kg body weight in healthy male subjects using the euglycaemic clamp technique.

Design
This was a single-dose, randomised, double-blind, placebo-controlled, three-way crossover study.

Population
Healthy men aged 18 to 45 years, with a body mass index of between 18 and 26 kg/m^2.

Treatments
Single subcutaneous injections of 0.4 IU/kg HOE 901, 0.4 IU/kg NPH insulin, or placebo in the anterior abdominal wall. The two insulin study medications and the regular insulin used in the euglycaemic clamp procedure were supplied by Hoechst Marion Roussel. The batch numbers of the medication used were – (HOE 901), — (NPH insulin), and — (regular insulin).

Pharmacodynamic data
The glucose infusion rate during the euglycaemic clamp procedure was measured by a —— Blood samples were taken during the euglycaemic clamp procedure and analysed for glucose concentrations by the —— and in the investigator's laboratory using an —— analyser. Serum C-peptide concentrations were determined by a radio-immunoassay at ——— ———. The assay range for C-peptide was —— µg/mL using Hoechst Marion Roussel's bioanalytical method ——

Pharmacokinetic data
Blood samples were taken during the euglycaemic clamp procedure and analysed for serum insulin with a radio-immunoassay at [redacted]. The assay range was [redacted] µIU/mL using Hoechst Marion Roussel's bioanalytical method [redacted]. The assay was 100% specific for human insulin, and had a cross-reactivity of about 50% with HOE 901 and its metabolites. Serum exogenous insulin was calculated from the serum insulin and C-peptide concentrations using the Owens formula.

Safety data
- Haematology, clinical chemistry, urinalysis
- Physical examination, vital signs, 12-lead electrocardiogram
- Adverse events.

Study duration and dates
The study took place between 22 April 1998 and 8 July 1998.

Statistical procedures
GIR: $AUC_{0\text{-}30\ h}$ (primary variable), baseline, $AUC_{0\text{-}4\ h}$, $AUC_{0\text{-}16\ h}$, $AUC_{0\text{-}24\ h}$, and GIR_{max} were subjected to an analysis of variance (ANOVA) with subject, treatment and period as main effects. Ninety-five percent (95%) confidence intervals were calculated for the mean treatment ratios of each of these variables according to Fieller's Theorem, based on untransformed data. To adjust for apparent baseline differences in GIR, the above variables were also analysed by an analysis of covariance (using the same model as above) with baseline GIR as covariate, after a log-transformation on the data, and 95% confidence intervals were then calculated for the respective mean treatment ratios. Time to GIR_{max} and the times to early and late half and three-quarters maximum GIR (early/late $t_{50\%}/t_{75\%}$) were analysed non-parametrically; 95% confidence intervals were calculated for median treatment differences.

Serum C-peptide concentrations: descriptive statistics (baseline, $AUC_{0\text{-}30\ h}$, C_{min}, t_{min}).

Blood glucose concentrations: descriptive statistics (fasting, $AUC_{0\text{-}30\ h}$, C_{min}, max. decrease, t_{min}).

Serum insulin and serum exogenous insulin: descriptive statistics ($AUC_{0\text{-}30\ h}$, C_{max}, t_{max}). $AUC_{0\text{-}30\ h}$ and C_{max} were analysed by analysis of variance (ln-transformed data) and 95% confidence intervals were calculated for treatment ratios. t_{max} was analysed non-parametrically and 95% confidence intervals were calculated for median treatment differences.

Interim analysis
No interim analysis was performed.

Results - Study subjects and conduct
Fifteen subjects were initially enrolled in the study. One subject (No. 107) withdrew from the study after developing a fever during visit 2 (treatment with HOE 901), and was replaced by subject 1007. Hence 15 subjects completed the study after treatment with HOE 901, NPH insulin and placebo, and were biometrically evaluable for the pharmacodynamic and pharmacokinetic analyses. Data from all 16 subjects enrolled in the study were included in the analysis of safety.

For the 15 men who completed the study the mean age was 26.7 years, ranging between 19 and 39 years; the mean height was 181.6 cm (range 170 to 190 cm) and the mean weight 73.5 kg (range 58.5 to 87.5 kg).

Results - Pharmacokinetics and pharmacodynamics

Blood glucose concentrations were maintained at approximately 90 mg/dL throughout the clamp period, thereby providing a valid basis for interpreting the pharmacodynamic and pharmacokinetic data. The C-peptide profiles indicated that the background infusion of regular insulin had successfully suppressed endogenous insulin throughout the clamp procedure during the placebo treatment as well as during each of the two insulin treatments.

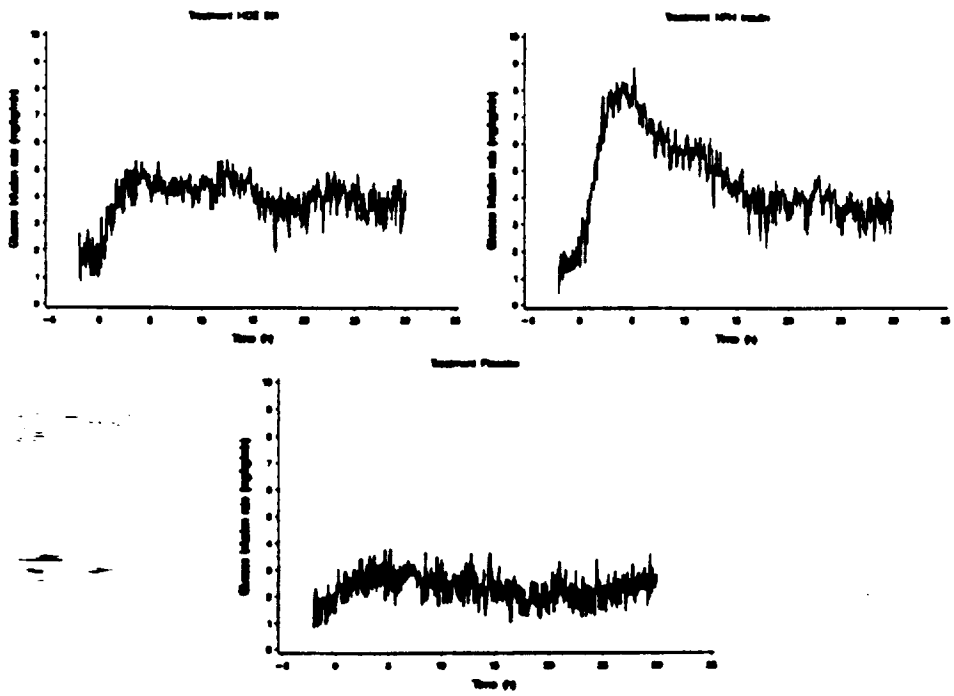
Glucose infusion rate

Variable	Mean pharmacodynamic value (CV)		
	HOE 901	NPH insulin	Placebo
$AUC_{0\text{-}30h}$ (mg/kg)	7925 (23%)	9238 (14%)	4890 (35%)
Baseline (mg/kg/min)	1.74 (69%)	2.15 (53%)	1.91 (56%)
GIR_{max} (mg/kg/min)	9.60 (17%)	11.9 (16%)	8.22 (34%)
t_{max} (h)	14.7 [illegible]	5.2 [illegible]	15.7 [illegible]

[a] Median value (min.; max.)

The mean $AUC_{0\text{-}30\,h}$ for GIR after HOE 901 treatment was about 14% lower than after NPH insulin treatment, but this difference was not statistically significant. After adjustment for baseline differences in GIR by an analysis of covariance, the "HOE 901 / NPH insulin" mean ratio for the $AUC_{0\text{-}30\,h}$ for GIR was 91.8%, a difference of about 8%, which was also not statistically significant. After an initial increase, the mean GIR-time profile was relatively constant after HOE 901 treatment, whereas there was a distinct peak between two and six hours after NPH treatment.

Glucose infusion rate – geometric mean values (n = 15)



The similarity in GIR between the two insulin treatment groups towards the end of the clamp period might have had different causes. While evidence from this study, and other studies, has shown that HOE 901 is a long-acting basal insulin, NPH insulin is known to have a shorter duration of action. Thus the elevated GIR towards the end of the clamp period after NPH insulin treatment may have reflected incomplete suppression of endogenous insulin rather than residual activity from the NPH insulin. The greater variability in individual values for t_{max} in the HOE 901 treatment group reflects the smoother curve (i.e., the lack of a distinct peak) obtained after HOE 901 treatment, compared with after NPH insulin treatment. The median late $t_{75\%}$ for GIR was significantly later for the HOE 901 treatment group than for the NPH insulin treatment group, which is consistent with the long-acting characteristics of HOE 901.

Serum insulin pharmacokinetic variables

Variable	Mean pharmacokinetic value (CV)		
	HOE 901	NPH Insulin	Placebo
Serum insulin			
Baseline conc. (µIU/mL)	12.9 (28%)	12.4 (35%)	11.4 (31%)
$AUC_{0\text{-}30h}$ (µIU·h/mL)	508.2 (16%)	590.0 (17%)	320.6 (21%)
C_{max} (µIU/mL)	23.8 (18%)	32.8 (18%)	18.3 (23%)
t_{max} (h)	16 [a]	3 [a]	9 [a]
Serum exogenous insulin			
$AUC_{0\text{-}30h}$ (µIU·h/mL)	230.8 (25%)	324.0 (16%)	66.2 (55%)
C_{max} (µIU/mL)	14.6 (20%)	22.5 (19%)	7.9 (62%)
t_{max} (h)	17 [a]	4 [a]	21 [a]

[a] Median value (min.; max.)

After an initial increase, serum insulin concentrations remained relatively constant throughout the clamp period after HOE 901 treatment, whereas there was a distinct peak within five hours of NPH treatment. A similar trend was seen in the concentration-time profiles for serum exogenous insulin. The lower values for AUC and C_{max} of serum insulin and serum exogenous insulin reflect the fact that the radio-immunoassay used for quantifying insulin has a cross-reactivity of only about 50% to HOE 901 and its metabolites, resulting in underestimation of the contribution of these components. The $AUC_{0\text{-}30\,h}$ of 66.2 µIU·h/mL and C_{max} of 7.9 µIU/mL calculated for serum exogenous insulin after placebo treatment reflect the uncertainties involved in quantifying serum exogenous insulin using the Owens formula.

Results - Safety

Both types of insulin and the placebo were safe and well-tolerated. Only one subject reported an adverse event (moderate fever), which was classified as being unrelated to the study medication (HOE 901). There were no clinically relevant abnormalities in the laboratory safety data.

Conclusions

In conclusion, the time-action profiles, as indicated by GIR, showed a smooth, peakless profile after HOE 901 treatment, compared with a distinct early peak after NPH insulin treatment. These trends were confirmed by the concentration-time profiles for serum immunoreactive insulin. Both types of insulin and the placebo were safe and well-tolerated.

STUDY SYNOPSIS

HOE 901/1012

Title
Assessment of the variability in glucose lowering effect of HOE 901 compared to NPH and Ultralong® (Human Ultralente) human insulins after subcutaneous doses of 0.4 IU/kg using the euglycemic clamp technique in healthy volunteers.

Investigators, study site
[ ]

Phase I

Indication
Not applicable.

Objectives
To investigate intra-subject variability in glucose lowering effect of HOE 901 [30] compared to NPH and Ultralong® human insulins following a single subcutaneous dose of 0.4 IU/kg body weight in healthy subjects using the euglycemic clamp technique.

Design
Single-dose, double-blind, randomized, parallel group (3 groups of 12 subjects per group), replicate design study with a wash-out period of at least 7 days between Visits 2 and 3. (Each subject received two consecutive injections of one of the study treatments).

Population
Thirty six healthy male subjects, aged between 18 and 45 years, with body mass index between 18 and 26 kg/m^2.

Treatments
Two consecutive subcutaneous injections of either HOE 901, NPH human insulin or Ultralong® at a dose of 0.4 IU/kg body weight.

Pharmacokinetic data
Serum total immunoreactive insulin and serum C-peptide were measured every 60 minutes up to 24 hours after administration of study medication. From these, profiles of serum exogenous immunoreactive insulin were derived. Secondary variables: area under the exogenous immunoreactive insulin concentration-time curve up to 24h ($AUC_{(0-24h)}$), maximum concentration (C_{max}), time to maximum concentration (t_{max}).

Serum total immunoreactive insulins and C-peptide were determined at [redacted] using radioimmunoassay methods (LLOQ: insulin [redacted] µIU/mL; C-peptide [redacted] ng/mL).

Pharmacodynamic data
Glucose infusion rate (GIR) and blood glucose concentrations were measured every 10 minutes up to 24 hours after administration of study medication. Primary variable: area under the GIR-time curve up to clamp end ($AUC_{(0-24h)}$); secondary variables: AUC of GIR for fractional periods 0-4h, 0-8h, 0-12h, 0-16h, 4-8h, 8-12h, 12-16h, 16-24h, 8-24h and 12-24h, maximum GIR, time of maximum GIR, time to early half-maximum GIR (Early $t_{50\%}$), time to late half-maximum GIR (Late $t_{50\%}$).

Safety data
Adverse events reported by the subject or noted by the investigator.
Hematology, clinical chemistry, human insulin antibodies, urinalysis and 12-lead ECG.
Physical examination and vital signs.

Study duration and dates
The study took place between February 27 and June 10, 1998.

Statistical procedures
Pharmacodynamics
Analysis of variance (ANOVA) on $AUC_{(0-24h)}$ of GIR, all fractional AUCs and maximum GIR (untransformed data). Intra-subject variability was assessed by calculating the intra-subject CV from the mean square error (MSE) from each ANOVA-table. 90% confidence intervals were calculated for the mean ratio "Visit 3/Visit 2" per product, and for the respective ratios of treatment means (secondary analysis), based on Fieller's Theorem. Non-parametric analysis was performed and 90% confidence intervals calculated for time of maximum GIR, early $t_{50\%}$ and late $t_{50\%}$.

Pharmacokinetics
Analysis of variance (ANOVA) on $AUC_{(0-24h)}$ and C_{max} of exogenous immunoreactive insulin (ln-transformed data). Intra-subject variability was assessed by calculating the intra-subject CV from the MSE from each ANOVA-table. 90% confidence intervals were calculated for the mean ratio "Visit 3/Visit 2" per product, and for the respective ratios of treatment means (secondary analysis). Non-parametric analysis was performed and 90% confidence intervals calculated for t_{max}.

Interim analysis
No interim analysis was performed.

Results - Study subjects and conduct
Thirty six healthy white male subjects were enrolled and randomized. One drop-out (no. 21; due to personal reasons), who received one administration of NPH human insulin (Visit 2), was replaced (by no. 51). The demographic details were as follows: age 18.3 to 32.5 years (mean: 23.1 years); weight 65.6 to 99.8 kg (mean: 79.6 kg); BMI 20.0 to 26.0 kg/m^2 (mean: 23.5 kg/m^2). There were no major protocol deviations. All 36 subjects who completed the trial were included in the pharmacodynamic and pharmacokinetic analyses; all 37 subjects were evaluated for safety.

Results - Pharmacokinetics and pharmacodynamics
The blood glucose levels were "clamped" at each individual's fasting blood glucose concentration; the mean values per insulin product were 81.0 mg/dL for HOE 901, 80.2 mg/dL for NPH human insulin and 81.3 mg/dL for Ultralong® human insulin. The variability in blood glucose levels within each subject's clamp period was below 7.50% in all cases (average: 4.0% (HOE 901); 4.6% (NPH); 4.1% (Ultralong®)). The decrease of serum C-peptide appeared similar for the three insulin products,

indicative of similar suppression of endogenous insulin secretion.

	Pharmacodynamic variables – GIR (n=12 per group)							
	AUC (0-24h)	AUC (0-8h)	AUC (8-12h)	AUC (12-16h)	AUC (16-24h)	AUC (0-12h)	AUC (12-24h)	Maximum GIR
Intra-subject CV[a]								
HOE 901	32%	82%	52%	22%	36%	66%	23%	45%
NPH	19%	21%	24%	27%	37%	20%	29%	17%
Ultralong®	38%	41%	42%	39%	83%	37%	55%	31%

[a]CV calculated from mean square error (MSE) in ANOVA-table.

	Pharmacokinetic variables - Exogenous insulin (n=12 per group)	
	$AUC_{(0-24h)}$	C_{max}
Intra-subject CV[a]		
HOE 901	14%	28%
NPH	16%	15%
Ultralong®	70%	67%

[a]CV calculated from mean square error (MSE) in ANOVA-table.

NPH human insulin showed lower intra-subject variability than HOE 901 and Ultralong® during the first 12 hours, due to less variable onset of action. For the later part of the profiles, i.e. during the period where all three insulins demonstrated pharmacological activity, variability was comparable for HOE 901 and NPH insulin, whereas that of Ultralong® was much higher.

Results - Safety

Adverse events:

There were no serious adverse events.

Thirty two adverse events occurred in 19 subjects. Headache was the most common adverse event (19 reports in 13 subjects). The intensity was mild to moderate and treatment (paracetamol) was administered in all 19 cases.

Iron deficiency anemia was the second most common adverse event occurring in 6 subjects. This was caused by the considerable blood loss associated with extended clamp procedures. Treatment (iron polymaltose) was administered in all cases.

Both headache and anemia are frequently seen with clamp procedures and could be ascribed to the clamp-procedure and not to the administration of the study drugs.

One subject (no. 9) developed a mild skin rash (fine, reddish appearance) on the upper body after each administration of the study drug (HOE 901). This rash was not associated with the injection-area, and subsided spontaneously. A possible causal relationship to the study drug cannot be excluded.

Other adverse events that occurred were dizziness (mild), gastro-enteritis (mild) and abdominal pain (moderate).

There was no relevant difference in frequency of reporting of adverse events between the 3 treatment groups. (HOE 901: 7 reports; NPH: 16 reports; Ultralong®: 9 reports).

Laboratory safety data:

One subject (no. 6) in the NPH-group, had significantly elevated levels of liver enzymes (SGPT, SGOT) at Visit 4 which returned to normal levels one week later. One possible explanation for this phenomenon is the sudden change in caloric intake associated with clamp procedures.

No other clinically relevant laboratory abnormalities (besides anemia in 6 subjects as described above) were observed.

Clinical variables:

No clinically relevant changes were seen in the various clinical variables.

Conclusions

HOE 901 showed lower intra-subject variability in its pharmacodynamic effect during the latter part of the time-action profile (from 12 hours after injection onwards). This is the part of the profile where all 3 drugs showed metabolic activity. NPH showed lower intra-subject variability than HOE 901 and Ultralong® during the initial 12 hours after injection. The intra-subject variability of the pharmacodynamics (GIR) during the complete 24-hour period after injection was the lowest with NPH, followed by HOE 901 and Ultralong®. However, the first 12 hours after injection were characterized by very high variability with regards to onset of action for Ultralong® and HOE 901. For this reason, inclusion of this time interval in the comparison of variability of glucose infusion rates may be misleading. Furthermore, since Ultralong® had the longest latent period by far (i.e. a GIR rate of zero for long periods during both visits), the lower intra-subject variability during the first 12 hours after injection for this drug does not reflect true variability. Regarding the pharmacokinetic variables (serum exogenous immunoreactive insulin), HOE 901 and NPH showed lower intra-subject variability than Ultralong®.

The administration of HOE 901, NPH insulin and Ultralong® human insulin was found to be safe and well tolerated.

PROTOCOL OUTLINE

HOE 901/1017

Title

Comparison of the Subcutaneous Absorption of HOE 901 and NPH Human Insulin in type 2 Diabetic Subjects

Investigator(s), Study site(s)

Investigator(s)

[redacted]

Co-investigators

[redacted]

Phase

1

Indication

Diabetes Mellitus

Objectives

To compare subcutaneous absorption of HOE 901[30] with that of NPH human insulin in type 2 diabetic subjects.

Design

This is a single dose, double blind, randomized, two-way crossover study in 14 type 2 diabetic subjects. Subjects will be randomized into one of the two sequence groups with 7 subjects each (^{125}I-HOE 901 - ^{125}I-NPH insulin or ^{125}I-NPH insulin - ^{125}I-HOE 901). The study consists of 4 study Visits, screening - Visit 1, treatment - Visits 2 and 3 and follow-up - Visit 4. Visit 1 will be performed within 14 days prior to Visit 2. The washout period between Visits 2 and 3 will be at least 7 days. Visit 4 will be conducted within 7 days after Visit 3.

During Visits 2 and 3, ^{125}I-labeled HOE 901 or ^{125}I-labeled NPH insulin will be administered subcutaneously in a dose of 0.3 IU/kg in the anterior abdominal wall. The disappearance of the radiolabeled insulins from the injection site will be measured using [redacted] at

PROTOCOL OUTLINE

HOE 901/1017

predefined time points over 48 hours. Serum immunoreactive insulins and C-peptide will be measured at selected time points.

Population

Type 2 diabetic subjects, aged between 40 and 70 years

Sample size

Fourteen type 2 diabetic subjects

Treatments

Two-way crossover study with subcutaneous injection of 0.3 IU/kg ^{125}I-HOE 901 or 0.3 IU/kg ^{125}I-NPH insulin in the anterior abdominal wall

Pharmacokinetic data

Primary variable:

- T_{75}, that is defined as the time taken for 25% of the administered radioactivity to disappear from the injection site of the anterior abdominal wall.

Secondary variable:

- T_{50} and T_{25}, that are defined as the time taken for 50% and 75% of the administered radioactivity to disappear from the injection site, respectively.

- Mean residual radioactivity after 24 , 36 and 48 hours or determined at the last measurement which is $\geq$ 5% of the initial activity that is determined immediately after the injection.

- Profiles of serum total immunoreactive insulins and serum exogenous insulin, which is derived from serum total insulin and C-peptide according to the method proposed by D.R. Owens. Serum exogenous insulin profile will be characterized by several derived variables:

  1. AUC_{0-24h}: area under the concentration-time curve from the time of drug administration to 24 hours after drug administration (calculated according to the linear trapezoidal rule)

  2. C_{max}: observed maximum concentration

  3. T_{max}: time to maximum concentration

Pharmacodynamic data

- Profile of plasma glucose levels

PROTOCOL OUTLINE

HOE 901/1017

- Profile of serum C-peptide levels.

Safety data

Hematology, clinical chemistry, urinalysis, 12-lead electrocardiogram (ECG), physical examination, vital signs, and adverse events

Statistical procedures

Demographic data will be analyzed descriptively.

Pharmacokinetics:

T_{75}, T_{50}, T_{25} and T_{max} will be subjected to non-parametric analysis. Ninety five percent (95%) confidence intervals will be calculated for the median differences "HOE 901 - Human NPH insulin" for each of these variables, based on the method of Hauschke et al, for a two-period cross-over design.

Mean residual radioactivities will be subjected to analysis of variance (ANOVA) with subject, treatment and period effects. The analysis will be performed on untransformed data. Ninety five percent (95%) confidence intervals will be calculated for the mean differences "HOE 901 - Human NPH insulin" for each of these variables.

The variables C_{max} and AUC_{0-24} of exogenous serum insulin will be analyzed by ANOVA with subject, treatment and period effects, after logarithmic (ln) transformation of data. From these, 95% confidence intervals will be calculated for the ratio "test/reference" (Steinijans V.W. et al, 1983).

Pharmacodynamics:

For serum C-peptide, individual, mean and median profiles will be plotted and characterized descriptively. For plasma glucose levels, all individual values will be listed, standard descriptive statistics calculated and individual, mean and median profiles plotted.

Safety:

Safety outcomes will be presented descriptively.

Study duration and dates

The duration of this study is expected to be four weeks, with patient recruitment proposed to start in April, 1998 and end in May, 1998. The actual overall study duration or subject recruitment period may vary.