DM

No evidence for accumulation of insulin glargine (LANTUS®): a multiple injection study in patients with Type 1 diabetes

T. Heise, S. Bott, K. Rave, A. Dressler*, R. Rosskamp† and L. Heinemann

Profil Institute for Metabolic Research, Neuss and *Aventis Pharma Deutschland GmbH, Bad Soden, Germany, and †Aventis Pharma Inc., Bridgewater, NJ, USA

Accepted 20 December 2001

Abstract

Aims Insulin glargine is a long-acting insulin analogue that is metabolically active for at least 24 h. We investigated the multiple-dose pharmacokinetic properties of insulin glargine to determine whether daily injections lead to the accumulation of circulating insulin levels and a corresponding decrease in blood glucose levels in patients with Type 1 diabetes.

Methods Fifteen patients using preprandial insulin lispro (mean age 36 ± 9 years, body mass index 24.6 ± 2.2 kg/m^2) completed the study. Each patient's optimal insulin glargine dose was determined during a dose-finding phase. After a washout period, patients were treated over 12 days with a constant daily dose of insulin glargine injected in the abdominal subcutaneous adipose tissue at 22:00 h, and with preprandial insulin lispro. Free serum insulin (FSI) and blood glucose concentrations were assessed hourly after the first, fourth, and eleventh injection, after which patients fasted for 24 h and did not use any other insulin preparation.

Results There were no changes in daily insulin doses during the dose-finding phase (insulin glargine: initial dose 24 ± 6 IU, mean change 0 ± 3 IU; insulin lispro: 18 ± 9 IU, 0 ± 7 IU). The time course of FSI was comparable on the three pharmacokinetic study days. Notably, the trough FSI at the end of the sampling periods was almost identical (day 1, 79 ± 56 pmol/l, day 4, 77 ± 56 pmol/l, day 11, 86 ± 60 pmol/l). No changes occurred in any of the pharmacokinetic parameters studied.

Conclusions There is no evidence that insulin glargine accumulates after multiple injections over 12 days. These results indicate that the predetermined dose of insulin glargine will not need to be reduced after commencing treatment because of a risk of accumulation.

Diabet. Med. 19, 490–495 (2002)

Keywords insulin glargine, long-acting insulin analogue, pharmacokinetics, accumulation, Type 1 diabetes, insulin therapy

Abbreviations BMI, body mass index; FSI, free serum insulin; IV, intravenous; CSII, continuous subcutaneous insulin infusion

Introduction

Insulin glargine (LANTUS®) is a novel, long-acting insulin analogue that has recently been approved for use in patients with diabetes. It contains two modifications compared with human insulin: the addition of two arginine molecules in positions 31 and 32 of the B-chain and the substitution of asparagine with glycine at position 21 of the A-chain. These modifications shift the isoelectric point of insulin glargine from a pH of 5.4 to 6.7, making it more soluble at a slightly acidic pH and less soluble at the physiological pH of the subcutaneous tissue.

Correspondence to: Tim Heise, Profil Institute for Metabolic Research, Stresemannallee 641460, Neuss, Germany. E-mail: tim.heise@profil-research.de

After subcutaneous injection, insulin glargine precipitates in the subcutaneous tissue, leading to delayed absorption and a subsequent prolonged duration of action [1,2].

The time–action profile of insulin glargine has been studied in healthy volunteers [3] and in patients with Type 1 diabetes [4]. In these single-dose, glucose clamp studies, insulin glargine showed a peakless metabolic action in contrast to NPH insulin, which exhibited a peak metabolic effect within 2–6 h of injection. Since a peakless time–action profile is regarded as ideal for a basal insulin regimen [1], insulin glargine may have advantages over NPH insulin for the supplementation of basal insulin needs [5].

The duration of action of insulin glargine was longer than 24 h in the majority of both healthy volunteers [3] and patients with Type 1 diabetes, as demonstrated in glucose clamp studies [4]. There is a concern that insulin preparations with a duration of action of > 24 h may lead to an accumulation of circulating insulin levels and increased metabolic effect within the first days of therapy, which could cause hypoglycaemia or at least might lead to difficulties in finding the optimal dose for each individual [6]. Pharmacokinetic absorption studies seem to support this concern with insulin glargine use because > 50% of residual radioactivity was still present at the injection site 24 h after injection [7]. The aim of the present study therefore was to investigate the pharmacokinetic and blood glucose lowering properties of multiple doses of insulin glargine in patients with Type 1 diabetes.

Patients and methods

The protocol of this open, uncontrolled study was approved by the local ethical committee, and the study was carried out according to the Declaration of Helsinki and the principles of Good Clinical Practice. After being informed of study procedures and giving their written informed consent, patients with Type 1 diabetes of a duration of > 1 year participated in this study. Of the 16 patients enrolled in the study, one patient withdrew consent after 2 days of the run-in period and withdrew from the study. Of the remaining 15 patients, 11 were male and four female, with a mean age (± SD) of 36 ± 9 years, a mean body mass index (BMI) of 24.6 ± 2.2 kg/m^2, and a mean duration of diabetes of 17 ± 8 years.

The diagnosis of Type 1 diabetes was confirmed in all patients by negative C-peptide levels after stimulation with 1 mg of intravenous (IV) glucagon. Only patients on intensified insulin therapy (NPH insulin twice daily or continuous subcutaneous insulin infusion (CSII) and injections of regular insulin before mealtime) with glycosylated haemoglobin (HbA_{1c}) levels < 8.5% and a maximum total insulin dose of 1 IU/kg per day were included. Patients with insulin antibody levels > 47 IU/ml (as determined by ELISA test), a history of hypoglycaemia unawareness, more than one episode of severe hypoglycaemia with seizure or coma during the past year, treatment with β-blockers or systemic steroids, or impaired renal or hepatic function were not permitted to enter the study.

The study consisted of four periods: screening, run-in, washout, and dosing. At the screening visit, clinically relevant concomitant diseases were excluded by means of medical history, physical examination, ECG, and laboratory parameters. Eligible patients entered the run-in period, which lasted 7–21 days and was aimed at establishing the most suitable individual dose of insulin glargine, i.e. a dose that prevented metabolic decompensation but not necessarily a dose that provided optimal blood glucose control in the long term. The first dose of insulin glargine was injected on day 1 of the run-in period at 20:00 h by the investigator. Based on (yet unpublished) experience gained in phase III clinical studies, the initial dose of insulin glargine was 20% lower than the patients' usual daily dose of NPH insulin. This dose reduction was not made in patients using CSII (n = 4), in whom the first dose of insulin glargine was equivalent to the usual daily units of basal subcutaneous insulin infusion. Throughout the rest of the run-in period, the patients injected themselves once daily at 22:00 h with insulin glargine using an Optipen® (Aventis Pharma Inc., Bridgewater, NJ, USA), and with insulin lispro (Humalog®; Eli Lilly, Bad Homburg, Germany), also administered by pen (BD-pen; Becton Dickinson, Franklin Lakes, NJ, USA), for prandial insulin substitution. Patients monitored their blood glucose five times daily (fasting in the morning, before lunch, before supper, at bedtime, and at 03:00 h). Patients contacted the investigator by telephone each day to discuss necessary adjustments of the dose of insulin glargine. During the entire study, patients kept a record of their blood glucose values, insulin doses, meal times, and carbohydrate units consumed.

The run-in period was followed by a washout period of 7–21 days, during which patients resumed their usual insulin therapy. After washout, patients entered the dosing period during which insulin lispro and insulin glargine were used for insulin supplementation. Insulin glargine was injected subcutaneously once daily into the abdomen at bedtime for 11 consecutive days at the dose established in the run-in period. On day 1 of the dosing period, the patients injected their last dose of NPH insulin in the morning or switched off their CSII at 12:00 h. The final dose of insulin lispro was injected before dinner, and no later than 17:00 h. Patients then began fasting. Patients arrived at the investigative site at about 21:00 h, and blood samples were collected using an IV cannula. At 21:30 h, blood and urine samples were taken for safety analyses (including blood glucose concentrations). At 21:55 h, the first blood sample was taken for insulin analysis (free immunoreactive serum insulin and free serum insulin lispro). At 22:00 h, the patients received the first of 11 daily injections of insulin glargine (day 1). All subsequent insulin glargine injections were given at the dose determined individually during the run-in period and at the same time on each of the following 10 days. Days on which pharmacokinetic data were measured are defined as the day the injection was given and encompass the next 24 h, i.e. day 1, day 4, and day 11. During the 24 h after the first dose of insulin glargine, blood samples were taken at hourly intervals for the determination of free immunoreactive serum insulin and blood glucose concentrations. Additional blood samples for the determination of free serum insulin lispro concentrations were taken at 23:00 h and at midnight. At 22:00 h on day 2, patients received their second injection of insulin glargine immediately after the 24-h blood samples were taken. The cannula was then removed and patients were served a snack before leaving the clinic.

Patients in the fasting state also had blood samples drawn hourly for 24 h after the administration of the fourth and

eleventh injections of insulin glargine. Additional blood samples for pharmacokinetic analyses were taken at trough level at 24 h on the days of the second (day 2) and sixth (day 6) injections of insulin glargine. On these days, patients came to the investigative site after injection of their last daily dose of insulin lispro no later than 17:00 h. A blood sample was taken for blood glucose and insulin analyses immediately before the injection of insulin glargine at 22:00 h, after which the patients left the clinic. During the dosing period, insulin glargine was administered by the investigator except on days 6, 8, and 10, when the patients injected themselves with insulin glargine at home.

During the dosing days, mild cases of hypoglycaemia (blood glucose measurements < 2.8 mmol/l and not requiring second-party assistance) were treated by the clinic staff or by the patients themselves by ingesting 10 g (1 U) of carbohydrate (100 ml apple juice or other rapidly absorbed carbohydrate). No strenuous activity was allowed within 4 days of insulin glargine administration in the run-in and dosing periods. Consumption of alcoholic beverages was not permitted 24 h before or during the run-in and dosing periods.

Laboratory assessments

Laboratory assessments for safety were made at screening and 24 h after the last injection of insulin glargine, according to standard laboratory procedures. Blood glucose was measured by the glucose oxidase method (Super GL Ambulance glucose analyser; Ruhrtal Labortechnik, Delecke-Möhnesee, Germany). Blood glucose concentrations measured outside the clinic by the patients themselves were determined using their usual method. Serum samples were analysed for free immunoreactive insulin using a modification of the Biochem Immunosystems GmbH radioimmunoassay (Freiburg, Germany). Samples were frozen at –20°C and remained frozen until analysed. The samples were thawed and homogenized immediately before use. Samples were incubated with ^{125}I-insulin tracer, and antibody-bound and free radiolabelled ligand were separated using polyethylene glycol (20%). Since the assay is sensitive to all insulin preparations, the radioimmunoassay was calibrated using insulin glargine, giving a cross-reactivity of 100% and 180% with insulin lispro and human insulin, respectively. The lower limit of quantification was 31 pmol/l and the measuring range was 31–7800 pmol/l. Free insulin lispro concentrations were measured using a commercially available radioimmunoassay (Linco Research Inc., St Charles, MO, USA). The lower limit of quantification was 15 pmol/l and the measuring range was 15–1500 pmol/l. All insulin determinations were performed by the laboratory of Professor H. K. L. Hundt (Farmovs Research Centre, Bloemfontein, Republic of South Africa).

Based upon stimulation analyses in which single-dose data from studies with 12, 14, and 15 patients using insulin glargine were used to predict steady-state concentrations using multiple doses, it was decided to enroll 16 patients in the study. Data from at least 12 patients were required for the pharmacokinetic analysis.

Statistical analysis

Data are presented as mean ± SD, unless otherwise stated. Statistical analyses were performed by Quintiles ClinData (Bloemfontein, South Africa) using SAS^{TM} version 6.12 (SAS Institute, Cary, NC, USA). The pharmacokinetic parameters of insulin were determined model-independently for each patient based on the actual sampling times using WinNonlin software. Based on the insulin concentrations for the pharmacokinetic study days 1, 4, and 11, pharmacokinetic parameters were calculated for each patient together with the actual sampling times using non-compartmental methods for all three profile days. If the time of the last observed quantifiable concentration (value above the lower limit of quantification (LOQ)) was < 24 h, all LOQ values up to 24 h were replaced by half the LOQ value. The relative total clearance (CL_{tot}/f) was calculated as CL_{tot}/f = dose/area under the curve from 0 to 24 h ($AUC_{0-24\,h}$). To assess the achievement of steady state, both mean and individual free immunoreactive serum insulin trough levels were plotted over time. Insulin lispro levels were measured to evaluate the extent to which any residual insulin lispro might still have been present at the time of insulin glargine administration.

Results

Run-in period

The median duration of the run-in period was 9 days. Insulin doses did not change significantly during this period, either for insulin glargine (initial daily dose 24 ± 6 IU, mean change 0 ± 3 IU) or insulin lispro (initial daily dose 18 ± 9 IU, mean change 0 ± 7 IU). Thus, the mean dose of insulin glargine applied during the dosing period was identical to the initial dose.

Time course of free serum insulin concentrations

Starting from slightly, but not significantly, different baseline levels (Table 1; day 1, 218 ± 299 pmol/l; day 4, 161 ± 150 pmol/l; day 11, 237 ± 210 pmol/l), free serum insulin (FSI) concentrations quickly declined to comparable levels 3 h after the administration of insulin glargine (Fig. 1a). No significant differences were observed at any time during the 24-h serum insulin profiles.

FSI concentrations measured at the start of the 24-h period were influenced by insulin lispro concentrations, ranging from 51 to 101 pmol/l (Table 1). The levels of insulin lispro declined in the first 3 h of the study (8 h after the last insulin lispro

Table 1 Free serum insulin concentrations after 24-h pharmacokinetic days

	Free serum lispro (pmol/l, mean ± SD)			Free serum insulin (pmol/l, mean ± SD)	
n = 15	0 h*	1 h	2 h	0 h*	24 h
Day 1	52 ± 87	33 ± 32	22 ± 17	218 ± 299	79 ± 56
Day 4	51 ± 45	34 ± 24	23 ± 13	161 ± 150	77 ± 56
Day 11	101 ± 99	68 ± 82	50 ± 78	238 ± 210	86 ± 60

*Free serum insulin lispro and free serum insulin at 0 h were measured 5 min before injection of insulin glargine.

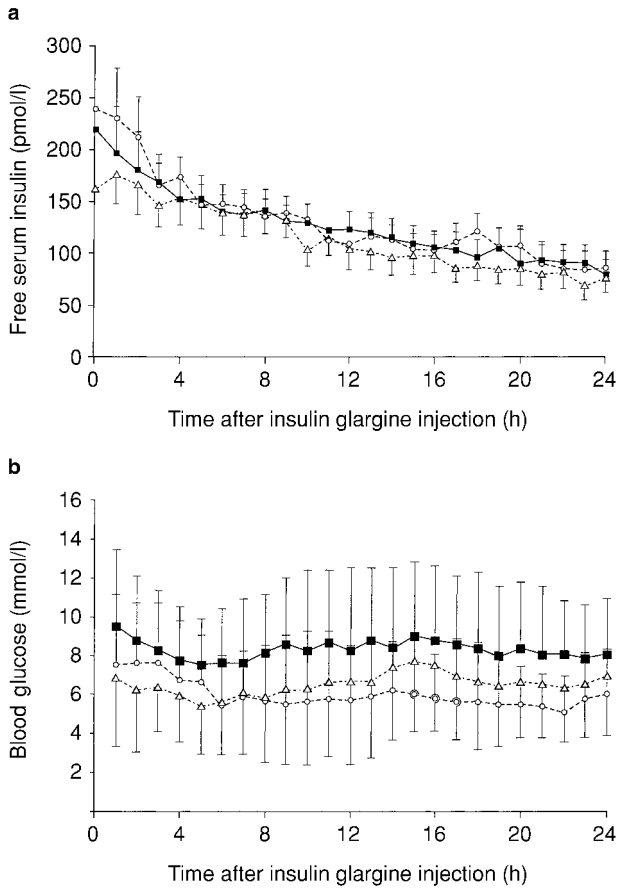

Figure 1 Time course of free serum insulin (a) and blood glucose (b) concentrations during the three pharmacokinetic study days of the dosing period, i.e. after the first, fourth, and eleventh injection of insulin glargine (applied at time point 0). Patients remained fasting and did not inject any other insulin preparation during the 24-h sampling periods. ■, Day 1; Δ, day 4; ○, day 11.

Table 2 Pharmacokinetic summary measures derived from free serum insulin concentrations on the three pharmacokinetic study days during the dosing period (mean ± SD (range))

	Day 1	Day 4	Day 11
C_{max} (pmol/l)	214 ± 167 (108–789)	192 ± 106 (77–526)	250 ± 173 (93–728)
C_{max}*	—	93.9 (79.2; 111.3)*	115.1 (97.1; 136.4)*
t_{max} (h)	4.1 ± 4.1 (1.0–13.0)	2.8 ± 2.7 (1.0–9.0)	3.1 ± 2.2 (1.0–8.0)
$AUC_{(0-24\ h)}$ (pmol × h/l)	3020 ± 1901 (1316–9257)	2746 ± 1736 (690–8173)	3137 ± 1603 (1765–8375)
$AUC_{(0-24\ h)}$	—	89.0 (77.8; 102.0)*	107.8 (94.2; 123.4)*
$Cl_{tot/f}$ (l/min)	1.0 ± 0.5 (0.4–2.3)	1.7 ± 0.9 (0.5–2.7)	1.3 ± 0.4 (0.4–1.4)

*Point estimate ratios (% and 90% confidence intervals) for day 4/day 1 and day 11/day 1.

Table 3 Distribution of mild hypoglycaemic events (defined as blood glucose measurements < 2.8 mmol/l without requiring second-party assistance)

Hypoglycaemic events (n = 17)	Number of patients (number of mentions)	
	Diary data	Clinical blood glucose data
Run-in period	9 (22)	5 (6)
Washout period	8 (24)	0
Dosing period	9 (21)	0
Profile days:		
Day 1	0	4 (7)
Day 4	0	4 (5)
Day 11	0	7 (17)
Single measurements:		
Day 2	—	1 (1)
Day 6	—	1 (1)

injection). The FSI concentrations did not show any trend to increase over the dosing period. In particular, the trough insulin concentrations after the 24-h pharmacokinetic days (days 1, 4, and 11), which definitely were not influenced by insulin lispro, were almost identical (Table 1; day 1, 79 ± 56 pmol/l; day 4, 77 ± 56 pmol/l; day 11, 86 ± 60 pmol/l). The high mean concentration and SD of FSI lispro on day 11 was due to the high dose of insulin lispro being taken by two subjects at the end of the study.

The pharmacokinetic summary measures calculated showed no differences between the three 24-h pharmacokinetic sampling days 1, 4, and 11 (Table 2). In particular, there was no indication of an increase in C_{max} or $AUC_{0-24\ h}$ over the dosing period. Point estimates and 90% confidence intervals (close to 100%) showed that there was no statistically significant difference in C_{max} and $AUC_{0-24\ h}$ between either day 4 and day 1, or day 11 and day 1. One subject had FSI concentrations that were about three times higher than the average concentration of other subjects, which may have been related to the higher doses of insulin glargine and insulin lispro that this subject received during the dosing period. These values led to increased variability in the pharmacokinetic variables. Recalculation of pharmacokinetic variables with the exclusion of this subject did not affect the conclusions of the study.

Dosing period—blood glucose

Initial blood glucose concentrations were not significantly different among the three pharmacokinetic study days (day 1, 9.3 ± 4.1 mmol/l; day 4, 6.9 ± 3.5 mmol/l; day 11, 7.5 ± 3.6 mmol/l; Fig. 1b). Mean changes in blood glucose over the 24-h observational period were comparable (day 1, −1.0 ± 4.6 mmol/l; day 4, −0.1 ± 4.2 mmol/l; day 11, −0.9 ± 4.7 mmol/l).

Hypoglycaemic episodes

There was no relevant increase in the number of hypoglycaemic events reported between the run-in and dosing periods (Table 3). No serious hypoglycaemic event (requiring second-party

assistance) occurred. The number of patients requiring carbohydrate intake for hypoglycaemia was 5, 7, and 9 on study days 1, 4, and 11, respectively. Carbohydrate administration did not affect the mean changes in blood glucose across the study period.

Adverse events

No serious adverse events occurred during the study. The only adverse event (apart from hypoglycaemia) that was considered possibly related to the study medication was injection site pain experienced by one patient in the run-in period, and by two patients in the dosing period.

Discussion

The primary goal of this study was to investigate the pharmacokinetic properties of multiple doses of insulin glargine during 11 days of daily dosing in patients with Type 1 diabetes. The comparable time course of circulating insulin concentrations after the first, fourth, and eleventh injection of insulin glargine and the nearly identical trough levels 24 h after these injections indicate that a steady-state concentration is achieved as early as day 2.

At first glance two findings seem to argue against this conclusion: first, the trend to higher baseline fasting serum insulin concentrations, and second, the trend to a higher incidence of hypoglycaemic episodes on day 11 compared with the other experimental days. However, both findings are probably due to limitations of the study design rather than to an accumulation in the metabolic effect of insulin glargine. Patients were supposed to have the last injection of insulin lispro (the short-acting insulin used during the dosing period) at least 5 h before the blood sampling for trough levels. It is possible that some of the patients used insulin lispro even after the 'deadline' of 17:00 h to correct for high blood glucose concentrations. In particular, this may have been the case at day 11 when baseline insulin lispro concentrations were almost twice as high than at the previous study days. In particular, one patient showed baseline insulin lispro concentrations about three times higher than in the other subjects. Patients may have injected extra insulin lispro at a later time-point as a result of their dissatisfaction with rather high blood glucose concentrations at days 1 and 4. As the study design did not allow any insulin injections other than insulin glargine in a predetermined dose during the study days, good blood glucose concentrations were only achievable with good baseline levels. On the other hand, good baseline blood glucose concentrations were clearly associated with a higher risk of hypoglycaemia during the study days. Thus, the assumption that some patients injected more insulin lispro at a later time-point at day 11 compared with the other study days would explain the observed higher baseline FSI concentrations, the higher baseline insulin lispro concentrations and the higher incidence of hypoglycaemia (which mostly occurred in the first hours of day 11).

Despite these limitiations, the data indicate that there was no accumulation of insulin glargine. The course and mean FSI concentrations (which were the major efficacy parameter of this study) were almost identical between the study days from 5 h onwards. This corresponds to the time when insulin lispro concentrations must have declined to zero even when injected just before the start of the experimental procedures. Previous studies have shown that FSI levels reach a plateau 6–8 h after insulin lispro administration [8]. Thus, in the present study they were not expected to influence serum insulin concentrations 5 h after insulin glargine administration. Most importantly, the almost identical trough FSI concentrations at the end of the 24-h pharmacokinetic sampling periods, 29 h after the last injection of insulin lispro, clearly indicate that no accumulation of insulin glargine occurred.

There is only one way of overcoming the limitations of the study in terms of interference from insulin lispro on FSI concentrations; that is, to conduct another study where insulin lispro is withdrawn at an earlier stage, under controlled, supervised conditions. However, in view of the efficacy parameters in our study clearly indicating that there is no accumulation of insulin glargine, a study with an even closer patient supervision is probably unnecessary.

The risk of accumulation of insulin glargine had been proposed, following glucose clamp studies, which showed a duration of action of more than 24 h in healthy volunteers and the majority of patients with Type 1 diabetes investigated [3,4,9]. Therefore, it seems surprising that no accumulation was observed in our study. However, in previous studies large insulin doses (0.4 U/kg) were administered. This resulted in a long duration of action for NPH insulin, which was estimated to be > 18 h [3,9–11]. With clinically relevant doses the metabolic effect of NPH insulin in patients with diabetes does not usually last longer than 12–15 h. It is unknown whether the duration of action of insulin glargine increases with higher doses. The results obtained in this study with a mean dose of 24 U (approximately 0.3 U/kg) do not indicate a duration of action that is significantly longer than 24 h.

Insulin glargine is not the first insulin preparation for which accumulation was considered to be a potential problem. Previously, accumulation had been demonstrated with bovine ultralente, which was recommended for use with a 'loading dose' of up to four-fold the expected basal requirement because of its long half-life of 36 h [12]. Following nocturnal hypoglycaemia, ultralente should be discontinued for an entire day. These disadvantages were overcome by human ultralente preparations that had a much shorter duration of action [13]. Human ultralente (like bovine ultralente), however, has the major disadvantage of showing a high variability of action with peak insulin concentrations occurring between 6 and 12 h [14,15]. This disadvantage led to the demand for the reintroduction of long-action bovine insulin preparations, since constant basal insulin supplementation was (and still is) regarded as a prerequisite of the basal–bolus insulin therapy concept [15].

Insulin glargine demonstrates all the advantages of ultralente insulin preparations, such as a long-acting, peakless time–action profile, without their drawbacks. The variability of the metabolic action of insulin glargine is in the range of that of NPH insulin [16] and, as the present study shows, insulin glargine does not accumulate after multiple injections. These results indicate that it is not necessary to use a 'loading dose' or dose reduction after several injections of insulin glargine.

Acknowledgements

We would like to thank our team of Research Associates, in particular Till Schröer and André Feldmann, for their excellent technical assistance, and Martina Treudler for her help during the conduct of this study. We would also like to thank Dr Bernd Rosenkranz for his major contribution to the protocol and conduct of the study. We thank Dr Catherine Hanrahan for her help during the preparation of the manuscript. The financial support of Aventis, Bridgewater, USA, is gratefully acknowledged.

References

1 Bolli GB, Di Marchi RD, Park GD, Pramming S, Koivisto VA. Insulin analogues and their potential in the management of diabetes mellitus. *Diabetologia* 1999; **42:** 1151–1167.
2 Rosskamp RH, Park G. Long-acting insulin analogs. *Diabetes Care* 1999; **22:** B109–B113.
3 Heinemann L, Linkeschova R, Rave K, Hompesch B, Sedlak M, Heise T. Time-action profile of the long-acting insulin analog insulin glargine (HOE901) in comparison with those of NPH insulin and placebo. *Diabetes Care* 2000; **23:** 644–649.
4 Lepore M, Pampanelli S, Fanelli C, Porcellati F, Bartocci L, Di Vincenzo A *et al.* Pharmacokinetics and pharmacodynamics of subcutaneous injection of long-acting human insulin analog glargine, NPH insulin, and ultralente human insulin and continuous subcutaneous infusion of insulin lispro. *Diabetes* 2000; **49:** 2142–2148.
5 Rosenstock J, Park G, Zimmerman J. Basal insulin glargine (HOE 901) versus NPH insulin in patients with type 1 diabetes on multiple daily insulin regimens. U.S. Insulin Glargine (HOE 901) Type 1 Diabetes Investigator Group. *Diabetes Care* 2000; **23:** 1137–1142.
6 Galloway JA, Chance RE. Approaches to insulin analogues. In Marshall SM, Home PD eds. *The Diabetes Annual*, 8th edn. New York: Elsevier, 1994: 277–297.
7 Luzio SD, Owens D, Evans M, Ogunku A, Beck P, Kurzhals R. Comparison of the s.c. absorption of HOE901 and NPH human insulin in type 2 diabetic patients (Abstract). *Diabetes* 1999; **48:** A111.
8 Howey DC, Bowsher RR, Brunelle RL, Rowe HM, Santa PF, Downing-Shelton J *et al.* [Lys (B28), Pro(B29)]-human insulin: effect of injection time on postprandial glycemia. *Clin Pharmacol Ther* 1995; **58:** 459–469.
9 Rave K, Heise T, Heinemann L. Time-action profile of insulin glargine (HOE901) in Japanese volunteers (Abstract). *Diabetes* 2000; **49:** A363.
10 Starke AA, Heinemann L, Hohmann A, Berger M. Wirkungsprofil von humanem Ultralente-Insulin im Vergleich mit humanem NPH-Insulin. *Dtsch Med Wochenschr* 1989; **114:** 618–622.
11 Seigler DE, Olsson GM, Agramonte RF, Lohman VL, Ashby MH, Reeves ML. Pharmacokinetics of long-acting (ultralente) insulin preparations. *Diab Nutr Metab* 1991; **4:** 267–273.
12 Turner RC, Phillips MA, Ward EA. Ultralente based insulin regimens—clinical applications, advantages and disadvantages. *Acta Med Scand Suppl* 1983; **671:** 75–86.
13 Hildebrandt P, Berger A, Volund A, Kuhl C. The subcutaneous absorption of human and bovine ultralente insulin formulations. *Diabet Med* 1985; **2:** 355–359.
14 Freeman SL, O'Brien PC, Rizza RA. Use of human ultralente as the basal insulin component in treatment of patients with IDDM. *Diabetes Res Clin Pract* 1991; **12:** 187–192.
15 Tattersall R. Bovine insulin (Letter). *BMJ* 1992; **305:** 831.
16 Scholtz HE, van Niekerk M, Meyer BH, Rosenkranz B. An assessment of the variability in the pharmacodynamics (glucose lowering effect) of HOE901 compared to NPH and ultralente human insulins using the euglycaemic clamp technique (Abstract). *Diabetologia* 1999; **42:** A235.