Time-Action Profiles of Novel Premixed Preparations of Insulin Lispro and NPL Insulin

TIM HEISE, MD
CHRISTIAN WEYER, MD
ANDREAS SERWAS, MD
SEBASTIAN HEINRICHS, MD
JOHANNES OSINGA, MD

PARIS ROACH, MD
JIM WOODWORTH, PHD
UWE GUDAT, MD
LUTZ HEINEMANN, PHD

OBJECTIVE — To study the pharmacodynamic properties of three premixed formulations of the rapid-acting insulin analog insulin lispro and its protamine-retarded preparation, neutral protamine lispro (NPL) insulin.

RESEARCH DESIGN AND METHODS — In this open, single-center, euglycemic glucose clamp study, 30 healthy volunteers (12 women, 18 men) aged 27 ± 2 years (mean ± SD), whose BMI was 23.0 ± 2.3 kg/m^2, received subcutaneous injections of 0.3 U/kg body wt of insulin mixture (high-mixture 75/25, mid-mixture 50/50, or low-mixture 25/75 insulin lispro/NPL insulin), insulin lispro, or NPL insulin on one of the five study days in randomized order. Glucose infusion rates were determined over a period of 24 h after administration.

RESULTS — Maximal metabolic activity decreased after subcutaneous injection of the mixtures with lower insulin lispro content; however, the time point of maximal and of early half-maximal metabolic activity was comparable among the three mixtures. Higher proportions of insulin lispro resulted in higher values for area under the curve within the first 360 min after injection and a more rapid decline to late half-maximal activity. Serum insulin concentrations showed a similar pattern.

CONCLUSIONS — This study shows that the pharmacodynamic and pharmacokinetic properties of insulin lispro are preserved in stable mixtures with NPL insulin.

W hen trying to achieve optimal postprandial glycemic control, timing of prandial insulin supplementation is as important as selecting the appropriate insulin dose (1). By virtue of their rapid and short action, insulin analogs such as insulin lispro allow for timely insulin substitution (2–4). Customary insulin therapies combine the use of short-acting (mealtime) insulin with an intermediate-acting insulin for basal insulin replacement.

Premixed formulations of regular and intermediate-acting insulins offer added convenience of administration and are the most often prescribed insulin formulations in the treatment of type 2 diabetic patients. Use of rapid-acting insulin analogs in such formulations may offer more appropriate prandial insulin substitution.

When insulin lispro and NPH insulin remain in prolonged contact (for weeks to months) within a mixture, an exchange

between soluble insulin lispro and protamine-bound human insulin takes place, resulting in a mixture of both soluble and protamine-bound insulin lispro and human insulin. To avoid this problem, neutral protamine lispro (NPL) insulin, an insulin lispro formulation, was developed; it is an analog of the human insulin–protamine complex (NPH insulin) (5). NPL insulin has been chosen as the intermediate-acting component of manufactured mixtures of insulin lispro and intermediate-acting insulin. Three stable mixtures of insulin lispro and its novel protamine-retarded counterpart have been formulated: low-mixture 25/75, mid-mixture 50/50, and high-mixture 75/25. This study investigates the pharmacodynamic properties of these three premixed formulations and compares them with the corresponding time-action profiles of insulin lispro and NPL insulin.
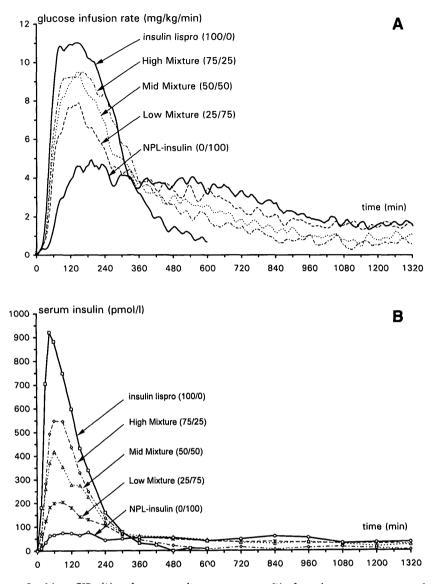
RESEARCH DESIGN AND METHODS — This study was an open-label, balanced, single-center, randomized, five-way crossover comparative trial involving 30 healthy subjects. The volunteers (12 women, 18 men; aged 27 ± 2 years; BMI 23.0 ± 2.3 kg/m^2) participated in this study after receiving detailed oral and written explanation of study objectives and possible risks. Written informed consent was obtained. The subjects were instructed to keep their body weight constant, i.e., within ±2 kg of the initial visit. Sexually active women used oral contraceptives or intrauterine devices (pregnancies were excluded by appropriate tests at the first and last visits). The protocol was approved by the local ethical committee, and the study was carried out according to the Declaration of Helsinki.

On all five study days, volunteers arrived at the study site at 8:00 A.M., after an overnight fast. Subjects were connected to a Biostator (Life Science Instruments, Elkhart, IN), and a euglycemic glucose clamp was established (clamp modus 9:1; intravenous infusion of 0.15 mU · kg^{-1} · min^{-1} regular human insulin) (3). After a baseline period of 2 h, the subjects received a subcutaneous injection of 0.3 U/kg body wt (21.3 ± 3.3 U)

of one of the five insulin preparations (U100): high-mixture 75/25, mid-mixture 50/50, or low-mixture 25/75 insulin lispro/NPL insulin; insulin lispro; or NPL insulin (Lilly, Indianapolis, IN). All subjects received all insulin preparations in a treatment sequence determined by a single Latin square crossover scheme and randomization schedule. The subjects remained fasted and supine during the course of the treatment. The minimal and maximal intervals between study days was 5 and 30 days (mean interval, 14 ± 8).

Insulin was administered into a paraumbilical skinfold by means of a syringe (Low-Dose Micro-Fine IV, Becton Dickinson, Heidelberg, Germany). Glucose infusion rates (GIRs) required to keep blood glucose levels constant at 5.0 mmol/l were monitored during the subsequent 22 h (10 h for insulin lispro). Blood samples were drawn at regular intervals (-120, -90, -60, -30, 0, 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, 360, 480, 600, 720, 840, 960, 1,080, 1,200, and 1,320 min on all study days with NPL insulin or mixtures; on the day with insulin lispro, samples were drawn after 360 min at 420, 480, 540, and 600 min) for estimation of serum insulin and serum C-peptide concentrations in a central laboratory (SciCor, Indianapolis, IN) by a commercial radioimmunoassay (RIA) kit. The displacement of iodinated insulin from the antibody used in the insulin RIA was identical for both insulin and insulin lispro. Insulin lispro was used as the assay standard. Plasma glucose concentrations were estimated by use of the glucose oxidase method (Glucose Analyzer II, Beckman, Fullerton, CA). Before the first clamp and at the final visit, blood samples were drawn to determine whether insulin administration induces an increase in insulin lispro–specific antibodies, human insulin–specific antibodies, and antibodies cross-reactive with insulin lispro and human insulin (6). No significant increases in any of the antibodies were observed. Also, no side or adverse effects were observed during this study.

Results are given as means ± SD throughout the text and as means in the figures. Data in the table are prepared by obtaining the parameter from each individual's data after each treatment to produce a group mean. The figures were prepared by averaging the data at the sampling times across all subjects for each treatment and are treatment means. An exponential function was fitted to each of the individual GIR profiles after subtraction of baseline GIR

Figure 1—*Mean GIRs (A) and serum insulin concentrations (B) after subcutaneous injection of 0.3 U/kg body wt of insulin lispro, NPL insulin, and three stable mixtures formulated with NPL insulin and the rapid-acting insulin analog insulin lispro in 30 healthy volunteers.*

(7), allowing calculation of the following pharmacodynamic summary measures: maximal GIR (GIR_{max}), time to GIR_{max} (t_{max}), time to early and late half-maximal GIR values (early $t_{50\%}$ and late $t_{50\%}$), and the area under the GIR profiles (area under the curve [AUC]). An analysis of variance for randomized block design was used for statistical comparison of the summary measures, followed by multiple testing (least-squares differences) if significant differences occurred. The pharmacokinetic summary measures were evaluated by fitting a polynomial function to the individual serum insulin concentration profiles with subsequent graphical estimation of the following parameters: maximal serum insulin con-

centration (C_{max}) and time to C_{max} (t_{max}). The trapezoidal rule was used to calculate AUCs for different time periods under the individual serum insulin profiles.

RESULTS — Independent of the proportion of soluble insulin lispro in the three different mixtures, maximal metabolic activity was seen after 2 h, and this finding was reproducible (Fig. 1A and Table 1). Comparable values for early $t_{50\%}$ as well as t_{max} were registered for the three mixtures and insulin lispro. With a greater proportion of insulin lispro in the subcutaneously injected preparation, a progressive increase in maximal effect was seen. However, the increase was not arithmetically propor-

Table 1—*Pharmacodynamic and pharmacokinetic summary measures of three premixed formulations of insulin lispro and NPL insulin registered in 30 healthy volunteers during euglycemic glucose clamps*

	IL	HM	MM	LM	NPL insulin	Differences	P value
Pharmacodynamic summary measures							
GIR_{max} (mg · kg^{-1} · min^{-1})	13 ± 3	10 ± 3	9 ± 3	7 ± 3	5 ± 2	IL HM MM LM NPL	<0.0001
t_{max} (min)	107 ± 21	120 ± 25	121 ± 22	141 ± 36	252 ± 64	IL HM MM LM NPL	<0.0001
early $t_{50\%}$ (min)	44 ± 12	47 ± 13	40 ± 12	44 ± 12	70 ± 30	IL HM MM LM NPL	<0.0001
late $t_{50\%}$ (min)	266 ± 57	339 ± 76	384 ± 110	557 ± 205	941 ± 269	IL HM MM LM NPL	<0.0001
AUC_{0-360} (g/kg × 360 min)	2.7 ± 0.6	2.5 ± 0.5	2.2 ± 0.6	1.9 ± 0.6	1.2 ± 0.7	IL HM MM LM NPL	<0.0001
$AUC_{360-1,320}$ (g/kg × 960 min)	(0.3 ± 0.3)	1.3 ± 0.6	1.6 ± 0.8	2.2 ± 1.1	2.5 ± 1.4	HM MM LM NPL	<0.0001
$AUC_{0-1,320}$ (g/kg × 1,320 min)	(3.1 ± 0.7)	3.7 ± 0.9	3.8 ± 1.2	4.0 ± 1.5	3.8 ± 2.0	HM MM LM NPL	0.62
AUC_{0-360} insulin lispro = 100%	100	92 ± 20	82 ± 23	69 ± 22	45 ± 23	IL HM MM LM NPL	<0.0001
$AUC_{360-1,320}$ NPL insulin = 100%	(17 ± 17)	57 ± 29	67 ± 29	83 ± 30	100	HM MM LM NPL	<0.0001
Pharmacokinetic summary measures							
Serum insulin							
Basal level (pmol/l)	84 ± 16	79 ± 19	79 ± 22	81 ± 17	77 ± 18	IL HM MM LM NPL	0.38
C_{max} (pmol/l)	883 ± 210	548 ± 99	401 ± 93	206 ± 79	128 ± 72	IL HM MM LM NPL	<0.0001
t_{max} (min)	71 ± 17	82 ± 20	81 ± 23	94 ± 45	200 ± 162	IL HM MM LM NPL	<0.0001
AUC_{0-90} (nmol/l × 90 min)	58 ± 15	33 ± 8	25 ± 8	13 ± 5	4 ± 5	IL HM MM LM NPL	<0.0001
AUC_{0-360} (nmol/l × 360 min)	131 ± 23	90 ± 17	71 ± 17	43 ± 15	20 ± 12	IL HM MM LM NPL	<0.0001
$AUC_{360-1,320}$ (nmol/l × 960 min)	(3 ± 6)	13 ± 16	34 ± 36	35 ± 18	43 ± 20	HM MM LM NPL	<0.0001
$AUC_{0-1,320}$ (nmol/l × 1,320 min)	(134 ± 22)	103 ± 26	105 ± 42	78 ± 23	63 ± 28	HM MM LM NPL	<0.0001
Serum C-peptide							
Basal level (ng/ml)	1.5 ± 0.7	1.4 ± 0.5	1.5 ± 0.8	1.5 ± 0.4	1.3 ± 0.4	IL HM MM LM NPL	0.39
Mean level after injection (ng/ml)	0.8 ± 0.2	0.7 ± 0.3	0.7 ± 0.2	0.7 ± 0.2	0.7 ± 0.2	IL HM MM LM NPL	0.91
% Suppression until injection	68 ± 23	66 ± 18	56 ± 15	63 ± 26	70 ± 24	IL HM MM LM NPL	0.10
Mean % suppression after injection	56 ± 20	55 ± 14	50 ± 14	52 ± 18	58 ± 19	IL HM MM LM NPL	0.23

Data are means ± SD. AUC of insulin lispro can be calculated for the study duration of 600 min only; these results were not included in some statistical comparisons (results in parentheses). *P* values are the results of the analysis of variance. Underlined insulin preparations in the differences column indicate comparable results. "% Suppression until injection" was calculated as the ratio of the serum C-peptide levels at the time of injection to the basal values. IL, insulin lispro; HM, high-mixture 75/25; LM, low-mixture 25/75; MM, mid-mixture 50/50.

tional to the percentage of soluble insulin; that is, with a 25% increase in soluble insulin lispro, the maximal metabolic effect did not increase by 25%. Higher proportions of insulin lispro resulted in a more rapid decline to late $t_{50\%}$ and in higher AUCs within the first 360 min after injection, which is the time period mainly influenced by the insulin lispro proportion.

The glucose requirements after 360 min, which were induced primarily by higher proportions of NPL insulin, were different; they increased with higher proportions of NPL insulin in the mixtures. However, the differences were smaller than those observed in the first 360 min. After 22 h, GIRs had not completely declined back to baseline values; depending on the proportion of NPL insulin, the glucose requirements were lower. The overall metabolic effect of all insulin preparations—excluding insulin lispro because of the shorter study duration—did not differ, as shown by comparable overall AUCs (Table 1).

Changes in serum insulin concentrations paralleled the glucodynamic response.

Higher proportions of soluble insulin lispro resulted in higher peak serum insulin levels in a linear dose-related manner (Fig. 1*B* and Table 1). The time points of C_{max} were comparable (with the exception of NPL insulin). The AUCs under the serum-insulin concentration profiles differed among all preparations in the first 6 h after injection, but they were comparable among most of the preparations in the time thereafter and overall. Basal serum C-peptide levels were similar on all study days (Table 1). Also, the degree of suppression seen during the treatment periods were comparable.

CONCLUSIONS — This pharmacodynamic study, which collects data from a large number of both female and male participants, shows that the pharmacodynamic and pharmacokinetic properties of insulin lispro are preserved in stable mixtures with NPL insulin. Subcutaneous injection of the individual mixtures was followed by a rapid initial insulin response and a similar t_{max}, independent of the proportion of insulin lispro in the formulation. Maximal

glucodynamic effect, in contrast, increased with the fraction of insulin lispro in the fixed mixture. Significant differences were observed between the adjacent formulations for GIR_{max}, C_{max}, and the AUCs in the first 6 h after injection.

In contrast to the serum insulin concentration, the induced metabolic effect did not increase in a linear or proportional manner with increasing proportions of soluble insulin lispro. These results are in accordance with those of a previous manual clamp study that used a balanced incomplete block study design to investigate the pharmacodynamic properties of extemporaneously prepared mixtures of insulin lispro and NPL insulin (identical mixtures and dose) in 10 healthy male subjects (8). This agreement between study results suggests that absorption and metabolic effects of premixed stable insulin mixtures are not different from those of extemporaneously prepared mixtures. The observed course of serum insulin profiles and time-action profiles is also similar to that seen by Woodworth et al. in a study of

the dose dependence of insulin lispro in comparison with regular insulin (9).

The pharmacodynamic properties of insulin lispro itself as registered in this study are in accordance with data previously published (2). Similarly, the intermediate insulin time-action profile of NPL insulin is comparable to that reported in a preliminary clamp study with eight volunteers given 0.4 U/kg NPL insulin (8). As NPH insulin itself was not included in our investigation, it remains to be studied whether the observed tendency to a more rapid onset of action with NPL insulin in comparison with NPH insulin is reproducible (8).

The total amount of insulin lispro administered (as either soluble insulin lispro or NPL insulin) does result in a comparable total metabolic effect. This suggests that nearly identical amounts of insulin lispro were absorbed across all the NPL/insulin lispro combinations, i.e., no differences in bioavailability and bioeffectiveness were observed. Analysis of the results separated by sex showed no significant differences.

The results of this study indicate that stable mixtures of a rapid-acting insulin analog and its intermediate-acting formulation combine the advantages of a fixed mixture with the benefits of timely insulin action. Thus, it can be hypothesized that subcutaneous injection of such mixtures may result in a better postprandial metabolic control than use of mixtures formulated with the more slowly absorbed human insulin. To meet basal insulin requirements between meals, the addition of a small proportion of long-acting insulin to a rapid-acting insulin analog may be beneficial (10). Accordingly, a higher proportion of long-acting insulin may be more suitable when the focus is on basal insulin substitution. Further clinical trials are needed to elucidate the possible clinical applications for these mixtures.

References
1. Bruce DG, Chisholm DJ, Storlien LH, Kraegen EW: Physiological importance of deficiency in early prandial insulin secretion in non-insulin-dependent diabetes. *Diabetes* 37:736–744, 1988
2. Howey DC, Bowsher RR, Brunelle RL, Woodworth JR: [Lys(B28), Pro(B29)]-human insulin: a rapidly absorbed analogue of human insulin. *Diabetes* 43:396–402, 1994
3. Heinemann L, Heise T, Jorgensen LN, Starke AAR: Action profile of the rapid acting insulin analogue B28Asp. *Diabet Med* 10:535–539, 1993
4. Heinemann L, Kapitza C, Starke AAR, Heise T: Time-action profile of the insulin analogue B28Asp. *Diabet Med* 13:683–684, 1996
5. DeFelippis MR, Bakaysa DL, Youngman KM, Radziuk J, Frank BH: Preparation and characterization of neutral protamine lispro (NPL) suspension (Abstract). *Diabetes* 45 (Suppl. 2):74A, 1996
6. Fineberg NS, Fineberg SE, Anderson JH, Birkett MA, Gibson RG, Hufferd S: Immunologic effects of insulin lispro [Lys(B28),Pro(B29) human insulin] in IDDM and NIDDM patients previously treated with insulin. *Diabetes* 45:1750–1754, 1996
7. Bender R, Heinemann L: Fitting nonlinear regression models with correlated errors to individual pharmacodynamic data using SAS software. *J Pharmacokinet Biopharm* 23:87–100, 1995
8. Radziuk J, Bradley BJ, Welsh L, De Fellipis MR, Roach P: Neutral protamine lispro: activity profile of s.c. administration with and without admixture of soluble lispro (Abstract). *Diabetologia* 39 (Suppl. 1):A224, 1996
9. Woodworth JR, Howey DC, Bowsher RR, Lutz S, Santa PF, Brady P: [Lys(B28), Pro(B29)] human insulin (K): dose-ranging vs. Humulin R (H) (Abstract). *Diabetes* 42 (Suppl. 1):54A, 1993
10. Torlone E, Pampanelli S, Lalli C, Del Sindaco P, Di Vincenzo A, Rambotti AM, Modarelli F, Epifano L, Kassi G, Perriello G, Brunetti P, Bolli GB: Effects of the short-acting insulin analog [Lys(B28),Pro(Pro)] on postprandial blood glucose control in IDDM. *Diabetes Care* 19:945–951, 1996