Immunologic Effects of Insulin Lispro [Lys (B28), Pro (B29) Human Insulin] in IDDM and NIDDM Patients Previously Treated With Insulin

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Insulin lispro [Lys (B28), Pro (B29) human insulin] is a rapidly absorbed analog that has diminished tendency to self-associate. In four open-label, 1-year-long international randomized trials, we contrasted the immunogenicity of insulin lispro versus regular human insulin (RHI) in patients previously treated with insulin who had IDDM or NIDDM. Using a self-blank subtraction assay, we assessed sera for the presence of insulin-specific antibodies (ISA), insulin lispro-specific antibodies (LSA), and cross-reactive antibodies (CRA). Basal insulin needs were provided either with human ultralente (UL) or NPH insulins. After 2 to 4 weeks of therapy with RHI plus UL or RHI plus NPH, 50% of patients were randomly assigned to begin insulin lispro or continue on RHI. At baseline, few pretreated patients had LSA (0–4%) and approximately 10% had ISA, whereas 41–45% of patients with IDDM and 23–27% of patients with NIDDM had CRA (IDDM vs. NIDDM, $P < 0.001$). Within studies, no significant differences were noted over time in ISA, LSA, or CRA attributable to the type of short-acting insulin. When data were pooled, inconsistent changes were noted in ISA and LSA (LSA were greater in NIDDM vs. IDDM at baseline, $P = 0.001$, and ISA were greater in IDDM vs. NIDDM at 6 months, $P = 0.007$). Significant levels of CRA were more common in IDDM at all times ($P < 0.001$, $P = 0.022$, and $P = 0.002$ at baseline, 6 months, and 12 months, respectively). For patients receiving insulin lispro, no significant changes occurred in antibody status among IDDM and NIDDM patients throughout the study (became positive, remained positive, became negative, or remained negative). IDDM patients were more likely to develop or maintain CRA levels ($P = 0.008$ vs. NIDDM), whereas antibody levels were comparable among positive individuals. No evidence was noted that insulin lispro differs in immunogenicity from RHI in previously treated IDDM and NIDDM patients. *Diabetes* 45:1750–1754, 1996

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ANOVA, analysis of variance; CRA, cross-reactive antibodies; ISA, human insulin-specific antibodies; LSA, insulin lispro-specific antibodies; PEG, polyethylene glycol; RHI, regular human insulin; UL, human ultralente insulin.

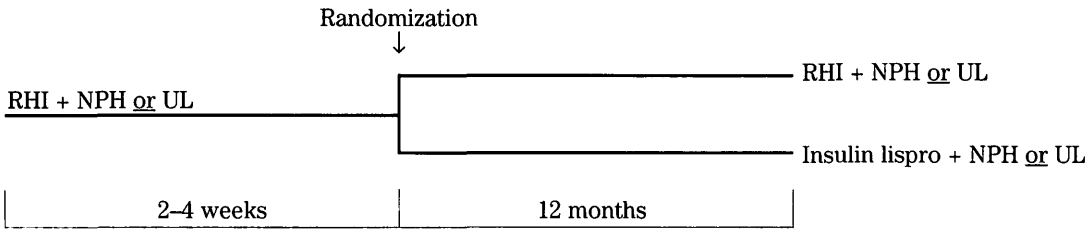
Subcutaneous insulin therapy is associated with substantial intra- and interpatient variability with regard to bioavailability and insulin action (1). To provide the most effective meal-associated bolus of insulin, soluble insulin should be administered 30 to 60 min preprandially (4–6). Despite such dosing, peak insulin levels often are delayed relative to the peak of glycemia, and the duration of absorption and activity may last up to 12 h (4,7). Studies have shown that the duration of insulin absorption is inversely proportional to insulin's tendency to self-associate (2,3,7). Others have shown that variability of action is related to prolongation of absorption (8). Monomeric and weakly dimeric insulin analogs have the potential advantages of increased rate of absorption, the convenience of injection coincident with a meal, more rapid appearance and disappearance rates, and possibly reduced intra- and interpatient variability (9–13). However, if such analogs proved to be highly immunogenic, then antibody-insulin interactions might result in a delayed peak, prolonged duration of activity (14), increased potential for hypoglycemia, and varying bioavailability.

Insulin lispro [Lys (B28), Pro (B29) human insulin] has been shown to be a rapidly absorbed analog of human insulin with a greatly diminished tendency to self-associate (15–17). In 1-year-long, international, multicenter, phase II trials of insulin lispro versus regular human insulin (RHI) in patients currently being treated with human insulin, we asked the following questions. *1*) Would insulin lispro have enhanced or diminished immunogenicity in previously treated patients with IDDM and NIDDM? *2*) Would newly acquired insulin antibodies develop more often in IDDM versus NIDDM patients and would these antibodies be specific for insulin lispro? *3*) Would alteration in antibody status (development, retention, or loss) occur more often in patients treated with insulin lispro? Specifically, we examined sera for the presence of human insulin-specific (ISA), insulin lispro-specific (LSA), and cross-reactive antibodies (CRA).

RESEARCH DESIGN AND METHODS

Four randomized, concurrent open-label studies were conducted in IDDM and NIDDM patients who had been receiving subcutaneous human insulin for at least 2 months. Approvals for these studies were obtained from the

TABLE 1
Study design



IDDM	Baseline	Therapy groups
Study 1	UL + RHI	UL + RHI or UL + insulin lispro
Study 2	NPH + RHI	NPH + RHI or UL + insulin lispro
NIDDM	**Baseline**	**Therapy groups**
Study 3	UL + RHI	UL + RHI or UL + insulin lispro
Study 4	NPH + RHI	NPH + RHI or UL + insulin lispro

human studies review boards at each institution. Informed consent was obtained from participants or their guardians prior to participation. The patients were classified as IDDM and NIDDM according to the World Health Organization criteria. Exclusion criteria for IDDM patients included life-threatening cardiovascular or renal disease, organ transplantation, liver disease, drug abuse, allergy to insulin preparations, women of child-bearing potential without the use of contraception, drug noncompliance, insulin infusion therapy, use of drugs affecting insulin action, insulin dose >2 U/kg, BMI >35 kg/m^2, and a history of hypoglycemic unawareness. Exclusion criteria for NIDDM patients were similar to IDDM but also excluded individuals who were treated with oral hypoglycemic agents within 30 days before entry into the study.

Studies consisted of a 2- to 4-week lead-in period during which patients were treated with either Humulin Ultralente (UL) plus RHI or Humulin N (NPH) plus RHI. At their second visit, they were randomized by site using computer-generated tables to either continue on their first regimen or substitute UL plus insulin lispro or NPH plus insulin lispro. Measurement of antibody levels were made at baseline (randomization) and at 2, 4, 6, 9, and 12 months. Separate trials were conducted for patients with IDDM or NIDDM (Table 1). Demographic data are given in Table 2.

UL and NPH were administered once or twice daily as deemed appropriate by the investigators. Doses of study insulins (insulin lispro or RHI) were taken before any meal that contained >20% of daily calories. Glycemic goals for the studies were overnight fasting serum glucose levels of <7.8 mmol/l without induction of nocturnal hypoglycemia and 2-h postprandial serum glucose of <10 mmol/l. Insulin doses were adjusted by the investigators to meet individual patient needs. Because of the difference in the time-action profiles for insulin lispro and RHI (15), the study insulins could not be blinded. It was recommended that insulin lispro should be taken within 15 min before meals, whereas RHI should be taken 30 to 45 min before meals.

Efficacy and safety measures were assessed and have been presented in abstract form. Sera for antibody determinations were collected and frozen at each site, shipped to a central collection site, and then transshipped to our endocrine research-Diabetes Research and Training Center (DRTC) immunoassay core laboratories. Specimens were held at –20°C until analyzed for antibody levels. We have found that antibody levels measured in sera stored in this manner are stable in excess of 2 years (unpublished data).

The methods used for liquid-phase assessment of ISA, LSA, and CRA have been modified from our previously published procedures (18). The sequence of these procedures is summarized in Fig. 1. The self-blank assay detects the percentage of binding as a qualitative indicator of antibody binding at a fixed serum dilution. Labeled and unlabeled insulin lispro and human insulin (HI) were provided by Lilly Research Laboratories (Indianapolis, IN). Parallel 120-µl aliquots of charcoal extracted sera were preincubated at 37°C for 2 h with buffer or cross-reacting antigens (0.33 nmol/l in 0.1 ml) before the addition of ^{125}I-labeled insulin lispro or ^{125}I-labeled HI (6.67 fmol in 100 µl) and incubated an additional 16 h. The final dilution of serum is 1:10.7. With the use of ^{125}I insulin lispro as a tracer, the difference in the percentage of binding between HI excess tubes and blank binding (preincubation with excess HI and insulin lispro) is attributable to LSA. With the use of ^{125}I HI as a tracer, the difference in percentage of binding between insulin lispro excess tubes and blank binding is attributable to ISA. The percentage of CRA binding is the binding that is inhibitable by insulin lipro or HI. The difference between blank and buffer preincubated binding with ^{125}I HI as a tracer is attributable to CRA. Similar results were obtained when ^{125}I insulin lispro was used as tracer to calculate CRA. The 99th percentile of percentage of binding in sera from 17 healthy, nondiabetic individuals was 1.1% for ISA and LSA and 4.3% for CRA. Therefore, >4.3% was chosen as the cut-off value to distinguish antibody positivity and negativity for CRA and >1.1% for ISA and LSA.

Statistics. Demographic data are given as mean ± SD or as the number in a particular category. Comparisons between groups at baseline were made using analysis of variance (ANOVA) for continuous data (19). Discrete data were analyzed with Fisher's exact test using StatXact (20). Because the distribution of the percentage of binding measurements was skewed to the

TABLE 2
Population description

Variable	IDDM		NIDDM	
	Ultralente	NPH	Ultralente	NPH
Sex (M/F)	79/85	82/71	74/68	76/73
Age (years)	31 ± 11	34 ± 11	56 ± 8	55 ± 9
BMI (kg/m^2)	24.4 ± 3.0	24.2 ± 2.7	28.5 ± 3.9	28.4 ± 3.9
Duration of diabetes (years)	12.9 ± 8.8	11.7 ± 9.2	11.6 ± 7.3	12.8 ± 8.3
Insulin therapy (years)	12.5 ± 8.8	11.3 ± 9.2	5.3 ± 5.0	5.9 ± 6.7
No. injections (1/>1) of basal insulin/day	102/62	68/85	120/22	45/104

Data are means ± SD or *n*.

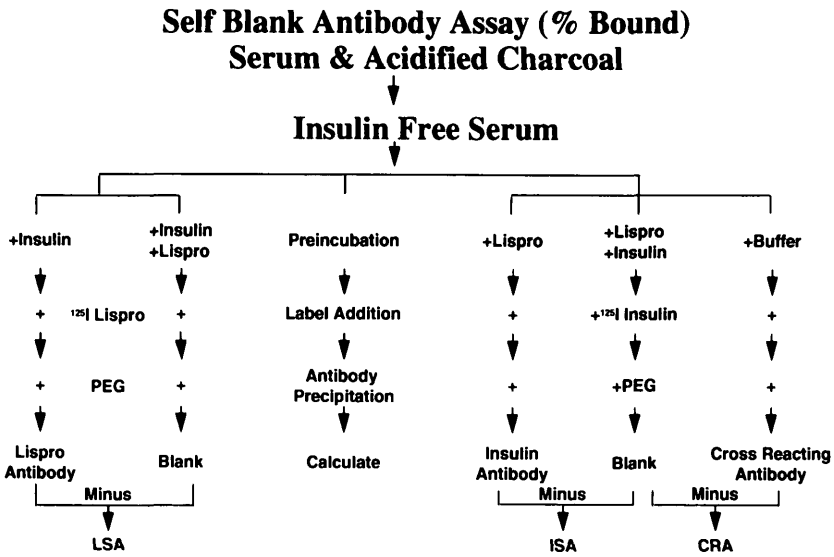


FIG. 1. Schematic diagram for liquid phase assay of insulin-specific, insulin lispro-specific, and cross-reacting antibodies.

right, these data are presented as median (interquartile range). A square root transformation was used to normalize the percentage of binding data before analysis. Antibody levels measured over time were analyzed with the use of repeated measures ANOVA. Type of basal and short-acting insulins were used as grouping factors while levels over time were treated as a repeated measure. The Huyndt Feldt adjustment to degrees of freedom was used (21).

RESULTS

No significant demographic differences were noted between the patients assigned to insulin lispro or RHI within the four studies. Population descriptions for the four studies are given in Table 2. Patients with IDDM were younger and thinner than patients with NIDDM. They also had been on insulin therapy longer. No differences were noted in the proportion of patients on more than one injection of basal insulin per day (data not shown).

Few of these pretreated patients had preexistent antibodies specific for insulin lispro (0–4%) and 8–11% had antibodies specific for RHI. However, cross-reacting antibodies were present in 41 and 45% of patients with IDDM and 23 and 27% of patients with NIDDM ($P < 0.001$ IDDM vs. NIDDM). At any point in the study, no significant differences occurred within studies between patients randomly assigned to insulin lispro or RHI in the percentage of participants with significant binding of any antigen. Therefore, we pooled the data across assignment to insulin lispro and RHI to assess the effect of type of diabetes on the presence of antibodies.

At baseline, LSA levels were higher in NIDDM [LSA: 0.1% (0–0.3%) IDDM and 0.3% (001–0.6%) NIDDM, $P = 0.001$] At 6 months, ISA levels were higher in IDDM [ISA: 0.2% (0–0.9%) IDDM and 0.1% (0–0.4%) NIDDM, $P = 0.007$]. Cross-reacting antibodies (binding inhibitable by insulin or lispro) were more common in patients with IDDM at all points ($P < 0.001$, $P = 0.022$, and $P = 0.002$ at baseline, 6 months, and 12 months, respectively). Because of the low prevalence and levels of LSA and ISA, we present additional analyses of the percentage of binding only for CRA.

Baseline values of cross-reacting antibody levels were slightly higher in the UL studies (IDDM, $P = 0.013$; NIDDM, $P = 0.025$). Within studies, no significant differences were noted over time in the levels of the percentage of binding attributable to type of short-acting insulin (Tables 3 and 4).

CRA status was stable from baseline to 1 year for most patients in the pooled study groups of IDDM (80%) and NIDDM (83%) patients. The distribution of the patients over the four possibilities (lose binding, remain positive or negative, and become positive) is not significantly different for patients receiving insulin lispro or RHI within type of diabetes. The proportion of patients with positive binding at 12 months (remaining or becoming positive) is greater in patients with IDDM (45%) than in those with NIDDM (30%) ($P = 0.008$). We also asked whether antibody levels would differ between IDDM and NIDDM indi-

TABLE 3
Cross-reacting antibodies over time: IDDM

	Ultralente		NPH	
Time	Insulin lispro	RHI	Insulin lispro	RHI
Base	4.6 (1.5–10.0)	6.0 (1.3–14.5)	2.7 (0.8–9.2)	2.2 (1.1–4.8)
2	4.8 (1.6–9.4)	5.4 (1.3–11.6)	1.9 (0.5–8.5)	2.3 (0.9–5.5)
4	4.6 (2.0– 10.5)	4.5 (1.1–12.8)	2.0 (1.1–8.2)	2.7 (1.1–6.2)
6	5.0 (2.4–11.2)	3.1 (1.0–11.5)	2.1 (0.6–8.6)	1.6 (0.7–4.7)
9	4.9 (1.6–15.9)	3.8 (1.0–11.2)	3.3 (0.9–7.8)	1.7 (1.1–4.7)
12	4.8 (2.5–10.8)	3.9 (1.2–10.6)	2.6 (1.0–7.1)	2.1 (1.3–5.6)

Data are medians (interquartile range) of percent binding.

TABLE 4
Cross-reacting antibodies over time: NIDDM

Time	Ultralente		NPH	
	Insulin lispro	RHI	Insulin lispro	RHI
Base	2.0 (0.5–25.4)	2.3 (0.4–5.1)	1.1 (0.5–2.5)	1.5 (0.4–4.2)
2	1.3 (0.6–18.0)	2.1 (0.7–4.6)	1.5 (0.7–3.4)	1.6 (0.7–3.7)
4	2.2 (0.9–26.3)	2.6 (1.0–4.4)	1.6 (0.5–3.6)	1.7 (0.6–8.7)
6	2.6 (1.4–14.2)	1.5 (0.2–7.1)	1.9 (0.7–4.5)	1.5 (0.6–6.8)
9	2.2 (0.7–18.7)	1.2 (0.7–4.0)	1.6 (0.5–5.6)	0.8 (0.3–5.0)
12	2.9 (1.3–28.3)	1.8 (0.3–3.8)	1.5 (0.8–8.0)	2.3 (0.9–5.8)

Data are medians (interquartile range) of percent binding.

viduals who had significant antibody levels at baseline (Table 5). No significant differences were noted in the levels of the percentage of binding attributable to type of diabetes or type of short-acting insulin in these patients. A small significant decrease in binding occurred as the study progressed ($P = 0.019$).

DISCUSSION

A primary consideration in the development of a rapidly absorbed subcutaneous insulin is its immunologic safety. Although highly purified animal and human insulins reduce immunogenicity in humans (22), alteration of the tertiary structure of the insulin molecule outside the α-chain loop region (amino acids 8–10) and alteration in absorption profiles could affect antigenicity. Despite a difference of only one amino acid, porcine insulin is more immunogenic than human insulin in patients with IDDM (22–24). Furthermore, treatment with repository insulin results in higher levels of antibody than treatment with soluble insulin (25). Therefore, a reduction in the self-association characteristics of insulin might have been expected to reduce immunogenicity, whereas the change in amino acid order might increase the immunogenicity of insulin (26).

No individuals were positive only for LSA. Sera of only a few individuals had LSA at baseline. This small number of positive sera may represent a minor failure to entirely block CRA because only individuals who were positive for ISA and CRA also were positive for LSA. The frequency of positive antibody binding for ISA was lower than in our previous de novo studies with combined repository and soluble human insulin (65%) (24). We have shown that after 2 years of therapy, antibody levels decrease significantly (24). Thus the duration of therapy may, in part, explain the low prevalence of ISA. When patients are treated with native or human insulin analog, the majority of antibodies are cross-reactive rather than specific. When transgenic rodents bearing human as well as rodent insulin genes are challenged with other insulin analogs having amino acid alterations outside the α-loop region, antibody formation was undetectable or minimal. When challenged with insulin analogs modified in the terminal portions of the β-chain or with native human insulin antibodies formed were cross-reactive rather than analog-specific (27). Thus analogs having amino acid differences outside the α-loop region would be predicted to produce cross-reactive antibodies in relatively low titers.

Because of the differences in CRA frequencies between IDDM and NIDDM patients, we looked at differences within studies for CRA. Within studies, we could not demonstrate any significant increase over time in CRA between individuals randomized to insulin lispro or RHI. Thus, insulin lispro did not differ in demonstrated immunogenicity from RHI for either type of diabetes.

If insulin lispro were more immunogenic than RHI, the antibody status should have differed in pooled IDDM and NIDDM studies. These differences, however, were not statistically significant. Interestingly, it is the tendency to develop antibodies that differs between patients with IDDM and NIDDM rather than the level of antibody binding. In patients who have preformed antibodies, the levels are similar in IDDM and NIDDM patients, and we observed no evidence for an anamnestic antibody response. We could not separate possible causative associations with age versus type of diabetes. Thus within type of diabetes, insulin lispro affects neither antibody level nor antibody status.

A review of the safety data available from the studies does not reveal an excess incidence of insulin allergy differing from previous experience with highly purified

TABLE 5
Cross-reacting antibodies: patients with significant binding at baseline

Time	IDDM		NIDDM	
	Insulin lispro	RHI	Insulin lispro	RHI
Base	11.2 (6.7–13.1)	13.0 (6.9–20.1)	13.5 (6.4–31.5)	8.0 (5.1–23.6)
6	9.4 (4.2–16.8)	11.2 (3.4–18.9)	12.0 (6.5–20.3)	10.6 (5.1–22.5)
12	8.3 (4.1–18.2)	8.8 (5.7–19.1)	13.4 (4.9–28.3)	9.2 (3.3–15.6)

Data are medians (interquartile range) of percent binding.

porcine or human insulins (2–3%) (28), and an immunologic insulin resistance was not observed. We conclude that insulin lispro does not differ immunogenically from RHI in patients pretreated with human insulin and that CRA are the most prevalent insulin antibodies, and we confirm that patients with IDDM are inherently more disposed to form insulin antibodies than are patients with NIDDM.

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