

Lispro Analog for Treatment of Generalized Allergy to Human Insulin

DINESH KUMAR, MD

OBJECTIVE — To evaluate the clinical usefulness of the human insulin analog lispro in a patient with generalized allergy to human insulin.

RESEARCH DESIGN AND METHODS — A 34-year-old obese female patient developed systemic allergic reactions to human insulin but tolerated insulin lispro. Sequential analyses of the anti-insulin IgE and IgG immunoglobulins were performed.

RESULTS — On intradermal test, the lispro insulin produced a 50% less intense wheal-flare response, compared with human insulin, which was presumed to be due to lispro's molecular form. The intradermal reactivity to both human and lispro insulins decreased with time and disappeared by week 25. ¹²⁵I-labeled lispro and human insulin binding antibody titers of both the IgE and IgG immunoglobulins were high initially, but decreased progressively, becoming very low by the end of 1 year. In *in vitro* immunoassay, the lispro and human insulins show a complete cross-reactivity.

CONCLUSIONS — Lispro may be useful for the management of allergy to human insulin. This analog of human insulin appears to have a reduced immunogenic potency.

With introduction of recombinant human insulin, allergic reactions to insulin preparations have become rare (1). However, whenever such a reaction occurs, it is difficult to treat. Lispro, a new human insulin analog, has been developed by transposing two amino acid residues at positions B-28 and B-29. Since this chemical modification involves the dimerization site of the insulin molecule, self-association and dimerization are markedly reduced (2). Moreover, dimers dissociate readily to their monomeric forms. These subtle differences can have immunological implications (3,4), and lispro may be beneficial for treating insulin-induced immune problems.

We present a case of systemic allergy to human insulin, which was treated with insulin lispro. The clinical response demonstrates the usefulness of this insulin for treating insulin allergy. Sequential analysis of circulating insulin antibodies suggests its reduced immunogenic potency.

RESEARCH DESIGN AND METHODS — A 34-year-old obese (BMI, 39.8 kg/m²) Latin-American woman was admitted to the University of Southern California Los Angeles County Medical Center on 25 November 1994 for a human insulin-induced generalized allergic reaction. She had had type 2 diabetes for 3.5 years. She was initially treated with human insulin (Novolin) for ~6 months, followed by glyburide for the next 1.5 years. The insulin therapy was then resumed because of poor metabolic control. She had taken human insulin (regular plus NPH, 100–120 U/day) for the past 1.5 years. Her metabolic control had been poor (random blood glucose levels ranged from 279 to 508 mg/dl and her glycohemoglobin was 14%).

Four days before admission to the hospital, she noticed erythema, swelling, and itching at the insulin injection site. Two days later, the local symptoms progressed to a generalized body rash, itching, and burning that lasted several hours. A similar

episode occurred on the day of admission. She developed acute anterior chest pressure and difficulty breathing at about midnight and came to the emergency medical department. Physical examination revealed stable vital signs and normal chest and heart findings. An acute coronary event was ruled out. She had generalized macular rash.

The patient developed systemic urticaria and angioedema involving face and both hands within an hour after the injection of human regular plus lente insulin. To evaluate the insulin allergic episodes, the intradermal tests were performed with pork regular (Squibb-Novov), human regular (Humulin R), and NPH (Humulin NPH) insulins (0.2 U in 0.02 ml/injection site). Intense immediate wheal-flare responses (WFR) were observed with all insulins, but none with diluent, indicating an insulin allergic state. A modified insulin desensitization was initiated with Humulin R, an initial dose of 0.2 U, followed with incremental doses of 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 U at 1-h intervals, administered subcutaneously under antihistamine cover (Benadryl 50 mg every 6 h). When the insulin dose was advanced to 1.6 U, the patient developed profound angioedema and shortness of breath. No further insulin injections were given that day.

The desensitization process with a revised protocol was recommenced the next day. Regular human insulin (0.2 U, Humulin R) was given subcutaneously every 2 h for 24 h, followed with 0.4 U every 2 h for 24 h and then 0.8 U every 2 h for the next 24 h. After patient had received the first 0.8-U dose of human regular insulin, we obtained approval from our Institutional Review Board to use human insulin lispro (an investigational drug at that time). Patient consented for the dermal tests and for treatment with this insulin. The intradermal tests were performed with 0.2 U of pork regular, human regular, human NPH, and lispro insulins (Eli Lilly) and 0.02 ml of diluent. There were immediate hypersensitivity reactions to all insulins. The WFR was the most intense with pork regular insulin and the least with insulin lispro. The lispro-induced WFR area was ~40–60% that of human regular insulin (Table 1). Based on these

From the Department of Medicine, Division of Endocrinology, Diabetes, and Hypertension, University of Southern California School of Medicine, Los Angeles County Medical Center, Los Angeles, California.

Address correspondence and reprint requests to Dinesh Kumar, MD, Box 393, Diabetes Section, LAC-USC Medical Center, Los Angeles, CA 90033.

Received for publication 31 January 1997 and accepted in revised form 23 May 1997.

Abbreviations: WFR, wheal-flare response.

Table 1—Intradermal test responses to lispro and human insulins and reaginic antibody profiles

Weeks of therapy	Ratio of lispro/human insulin-induced areas		Serum IgE (IU/ml)	Insulin-specific IgE*
	Wheal	Flare		
0	0.4†	0.6	3,090	High
2	0.2	0.5	—	—
4	0.2	0.3	1,530	—
16	0.5	0.4	356	—
25	None	0.6‡	189	Low
45	None	None	136	Very low

*Assayed by an insulin-specific paper radioimmunosorbent technique (5); †human insulin-induced area (wheal 80 mm², flare 900 mm²); ‡<25 mm² flare with insulin lispro.

observations, the lispro insulin was considered the best therapeutic choice. Patient received the first dose (4 U) of lispro subcutaneously. Within 15 min, there was erythema at the injection site measuring 0.5 cm in diameter but no itching. The doses were rapidly advanced to 8 U and then to 16 U at 4-h intervals, since the patient had no more local reactions. On the third day of insulin lispro therapy, the patient was tolerating 40 U before breakfast, 40 U at lunch, and 30 U at dinner.

During the past 18 months, the patient has tolerated the insulin lispro (three pre-meal injections; total daily dose, 60–80 U; fasting blood glucose range, 112–218 mg/dl; glycohemoglobin, 9.3%) with no adverse manifestations. Intradermal tests were repeated with lispro, human, and pork insulins; the ratios of WFR areas produced by lispro and human insulin on each visit are given in Table 1. The reactivity to intradermal insulin injections decreased with time. Small flare responses but no wheal formation to lispro or human insulin were seen at week 25 of therapy. No flare responses were seen to any insulin preparations at week 45. Serum samples were collected and stored at –20°C for the laboratory analysis.

RESULTS

Anti-insulin IgE and IgG antibodies

Insulin binding studies were performed using previously published radioimmunoassay techniques (5–7). Mono-iodo ¹²⁵I-human (lot A42-IYE-32D, specific activity 374 µCi/µg) and ¹²⁵I-lispro human (lot A42-IYE-34D, specific activity 374 µCi/µg) and unlabeled insulin preparations (human lot A52-7US-128, pork lot A52-L1K-021, and lispro human lot A52-AWK-087) were a gift of Drs. Bruce Frank and

Ronald Chance, Eli Lilly, Indianapolis, IN. Pools of normal human sera and insulin-treated diabetic patients' sera were used for negative and positive controls. Serum collected before desensitization was de-insulinized and analyzed for the cross-reactivity with human insulin and insulin lispro. The serum samples collected during follow-up visits were assayed for the anti-insulin IgE and IgG antibody titers.

Bindings of ¹²⁵I-labeled lispro and human insulins with both the IgE and IgG immunoglobulins were high. The concentrations of unlabeled insulin lispro required to produce 50% reduction in ¹²⁵I-lispro insulin binding were 54 nmol/l (0.32 µg/ml)

for IgE antibodies and 2,160 nmol/l (13 µg/ml) for IgG antibodies. In the cross-reactivity assays, the human and lispro insulins produced virtually identical displacement of tracers (¹²⁵I-lispro and -human insulins) from the anti-insulin IgE (Fig. 1) and IgG (data not shown) antibodies, indicating a complete in vitro cross-reactivity.

Sequential changes in bindings of ¹²⁵I-lispro and ¹²⁵I-human insulins with the anti-insulin IgE and IgG antibodies are shown in Fig. 2. There were progressive reductions in both the IgE and IgG antibodies, and their levels became very low by the end of 1 year. The binding curves for two tracer insulins (¹²⁵I-lispro and -human) were almost identical.

CONCLUSIONS — We have presented a rare patient who developed a generalized allergic reaction to human insulin (1). The intradermal tests with various insulins point to an allergen common to all of the preparations tested. Insulin products and aggregates, zinc suspensions, buffers, contaminants, and insulin itself have all been known to cause allergy in humans (1,8). Nevertheless, a quantitative difference in the WFR intensities was suggestive of the insulin or insulin product as the most likely allergen. Further support for this possibility come from the high concentration of insulin-specific reaginic IgE

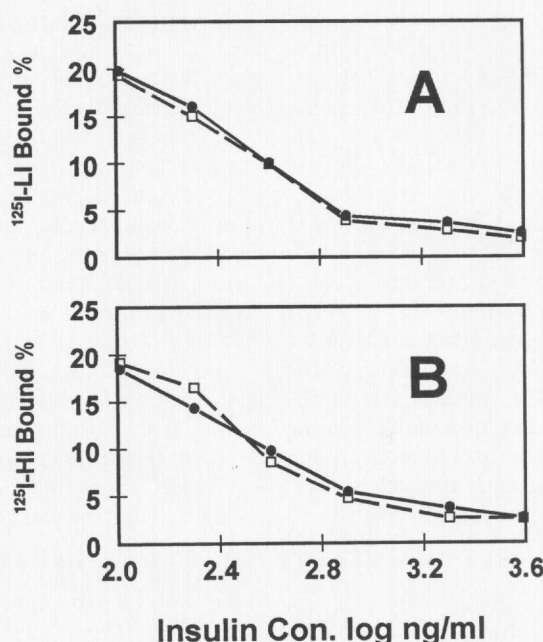


Figure 1—Effects of unlabeled lispro (□) and human (●) insulins on the binding of ¹²⁵I-lispro (A) and ¹²⁵I-human insulin (B) with anti-insulin IgE antibodies.

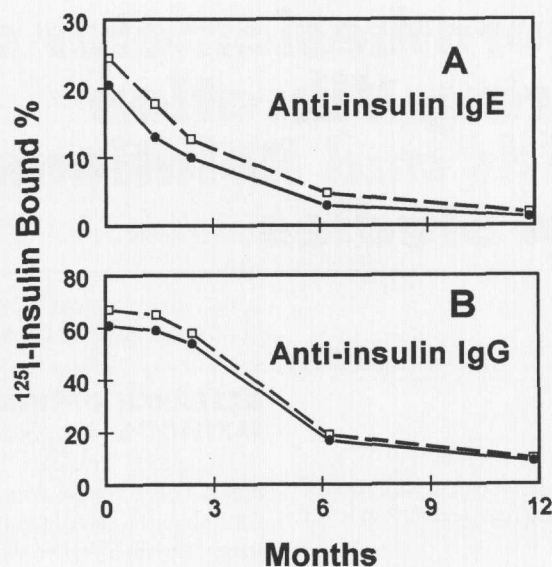


Figure 2—Changes in the ^{125}I -lispro ($\square$) and ^{125}I -human ($\bullet$) insulin binding anti-insulin IgE (A) and IgG (B) antibodies during 12 months of insulin lispro therapy.

antibodies in a patient's blood (5,9).

We observed that insulin lispro produced ~50% weaker WFR responses, compared with human regular insulin (Table 1), and this difference continued for several months until the intradermal tests became negative to all insulins (lispro, human, and pork). In vivo, the type 1 immediate hypersensitivity reaction, as seen in our patient, occurs when a specific allergen reacts with the mast cell-bound IgE, causing the release of pharmacological mediators such as histamine. This adverse reaction has specific quantitative and qualitative requirements, namely an appropriate allergen concentration and a minimum of two antibody-binding epitopes. The latter is crucial so that an allergen molecule cross-links two adjacent mast cell-bound IgEs and causes degranulation (10). Insulin molecule has well-identified immunogenic epitopes, which map at amino acid positions A-1, A-6, A-chain loop (A8-A11), A-19-20-21, B-3, and B-30 (4,6,7). Since all of those amino acids remain unchanged in the lispro analog, it is unlikely that there are real differences in the binding epitopes between lispro and human insulin monomers. Our in vitro characterization of the circulating anti-insulin IgE and IgG antibodies (cross-reactivity data) clearly supports this fact. If we assume that our patient's anti-insulin IgE antibodies were directed to a single epitope of the human insulin molecule, such a molecular form

could induce histamine release in vivo only when it existed as a dimer or higher aggregate. Indeed, human insulin makes dimer, hexamer, and larger aggregates (2,8). Although the lispro analog may also assume multimeric form, it readily dissociates to monomers (2). Thus, we propose that the observed reduced WFR to lispro insulin (i.e., reduced histamine release in vivo) was related to this property.

On lispro insulin therapy, the circulating anti-insulin IgE and IgG antibodies decreased progressively, which suggests that this insulin had a reduced antigenicity in our patient. In two immune-mediated insulin-resistant cases, the lispro insulin caused a reduction in daily dosages and concomitant decline in the insulin binding antibodies (11,12). The reduced immunogenicity may be related to lispro's molecular form. It must be appreciated that no other insulin preparation (regular, NPH, lente, or PZI) was used in our insulin-allergic patient or in two reported insulin-resistant cases (11,12). Insulin antibody levels had not changed in patients where the lispro analog was administered along with other insulins (13). Taken together, it appears that lispro has a reduced immunogenicity when used alone. This insulin may be useful for the management of insulin allergy.

Acknowledgments—I thank Drs. Ronald Chance, Bruce Frank, and James Anderson (Eli

Lilly & Company, Indianapolis, Indiana) for the insulin preparations and Drs. Chee Loo and Daniel Balmaceda for assistance in patient care.

References

1. Schemthaner G: Immunogenicity and allergenic potential of animal and human insulins. *Diabetes Care* 16 (Suppl. 3):155–185, 1993
2. Brems DN, Alter LA, Beckage MJ, Chance RE, DiMarchi RD, Green LK, Long HB, Pekar AH, Shields JE, Frank BH: Altering the association properties of insulin by amino acid replacement. *Protein Eng* 5:527–533, 1992
3. Brange J, Owens DR, Kang S, Vøland A: Monomeric insulins and their experimental and clinical implications. *Diabetes Care* 13: 923–954, 1990
4. Ottesen JL, Nilsson P, Jami J, Weilguny D, Duhrkop M, Bucchini D, Havelund S, Fogh JM: The potential immunogenicity of human insulin and insulin analogues evaluated in a transgenic mouse model. *Diabetologia* 37:1174–1185, 1994
5. Kumar D: Anti-insulin IgE in diabetics. *J Clin Endocrinol Metab* 45:1159–1164, 1977
6. Kumar D: Immunoreactivity of insulin antibodies in insulin-treated diabetics, significance of the beta-chain carboxyl-terminal amino acid (B-30) of insulin. *Diabetes* 28:994–1000, 1979
7. Kumar D: Insulin allergy; differences in the binding of porcine, bovine and human insulins with anti-insulin IgE. *Diabetes Care* 4:104–107, 1981
8. Ratner RE, Phillips TM, Steiner M: Persistent cutaneous insulin allergy resulting from high-molecular-weight insulin aggregates. *Diabetes* 39:728–733, 1990
9. deShazo RD, Galloway JA: Insulin allergy. *Ann Allergy Asthma Immunol* 76:217–218, 1996
10. Roitt IM, Brostoff J, Male DK: Hypersensitivity-Type 1. In *Immunology*. Roitt IM, Brostoff J, Male DK, Eds. St. Louis, MO, Mosby, 1985, p. 19.1–19.18
11. Lahtela JT, Knip M, Paul R, Anttonen J, Salmi J: Severe antibody-mediated human insulin resistance: successful treatment with the insulin analog lispro. *Diabetes Care* 20:71–73, 1997
12. Braimon J, O'Brien M, Kaulbach H, Moses A: The insulin analog lispro effectively treats anti-insulin antibody mediated insulin resistance (Abstract). *Diabetes* 45 (Suppl. 2):185A, 1996
13. 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