

Cameo

A case of human insulin allergy induced by short-acting and intermediate-acting insulin but not by long-acting insulin

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A 66-year-old woman suffering from pollinosis developed generalized urticaria after injection of intermediate-acting insulin for diabetes mellitus. She had human insulin-specific IgE, and in skin tests was positive for human recombinant insulin and negative for additives. Uniquely, she reacted to fast-acting and slow-acting insulin but not to long-acting insulin. We further confirmed that human insulin preparations could stimulate her peripheral basophils to release a significant amount of histamine. Genetically generated human insulin analogs, aspart and lispro, induced positive skin tests and histamine release from basophils. She was recommended to use a long-acting insulin preparation and was free from symptoms thereafter.

Introduction

Insulin is a front-line medicine in the treatment of diabetes mellitus. Although the prevalence of immediate-type allergy to insulin seems to have decreased since animal insulin preparations were replaced by human insulin,¹ insulin allergy is still a problem in the treatment of diabetes mellitus. A few recent reports have shown that long-acting human insulin does not induce immediate-type insulin allergy in certain individuals.^{2,3} In this report, we present an additional patient who had an immediate-type allergy to human insulin, showing responsiveness to short-acting and intermediate-acting insulin but not to long-acting insulin. A possible mechanism of unresponsiveness to long-acting insulin is discussed from the viewpoint of crystallization of the molecule and antigenicity.

Case Report

A 66-year-old housewife with pollinosis had been treated with Penfill N (Novo Nordisk A/S, Bagsvaerd, Denmark) for her diabetes mellitus since November 2000. She had experienced an itchy sensation and noticed the appearance of wheals at the site of insulin injection on her abdomen since March 2002. On 8 May 2002, she developed generalized urticaria and dyspnea 10 min after the injection of Penfill N and was referred to our dermatology clinic for investigation of the cause of her symptoms.

Laboratory tests revealed normal serum IgE levels (142 U/mL, normal < 300 U/mL). Her liver function and blood cell counts were normal. IgE radioallergosorbent tests showed that her serum contained specific IgE against Japanese Cedar (41.3 UA/mL; normal < 0.34 UA/mL) and human insulin (2.00 UA/mL) (CAP-FEIA, Pharmacia, Freiburg, Germany). High levels of IgG class antibody to human insulin were also detected (83 % normal < 7 %).

Materials and Methods

Skin tests

Intradermal tests were performed with 0.02 mL of 10 and 1 U/mL Penfill N (intermediate-acting) on the volar forearm. Physiological saline and histamine (10 mg/mL, Sigma, Tokyo, Japan) served as controls. An intradermal test reaction was regarded as weakly positive (2+) when it was half that of the histamine control (at 20 min), and clearly positive (3+) when it was the same as that of the histamine control. Intradermal tests were also performed for other insulin preparations including Penfill R (short-acting; Novo Nordisk A/S), Penfill 30R (biphasic-acting; Novo Nordisk A/S), Humulin N (intermediate-acting; Eli Lilly, Indianapolis, IN), Humulin U (long-acting; Eli Lilly) and two insulin analogs: Humalog (lispro: ultra-short-acting; Eli Lilly) and NovoRapid (aspart: ultra-short-acting; Novo Nordisk A/S).

Intradermal tests for various ingredients contained in Penfill N and other insulin preparations were also performed;

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human recombinant insulin (5 IU/mL), protamine sulfate (0.35 mg/mL), zinc chloride (0.05 mg/mL), methylparaben (1 mg/mL), phenol (2 mg/mL), metachlesol (3 mg/mL) and pork insulin (5 IU/mL).

Histamine release test

The amounts of histamine released from peripheral blood basophils were assessed using HRT Shionogi (Shionogi Co., Ltd, Osaka, Japan) as previously described.⁴ Briefly, the patient's peripheral blood was collected and basophils were purified using antibasophil monoclonal antibody-coated magnetic beads. The basophils were then stimulated with human recombinant insulin at concentrations of 5, 1 and 0.5 IU/mL. The amounts of released histamine were measured by an enzyme-linked immunosorbent assay. The total amounts of histamine in basophils were assessed by the destruction of the cells with digitonin, and data are presented as percentage histamine release.

Results

Intradermal test

Intradermal tests showed a positive reaction (3+) to human recombinant insulin (5 IU/mL), pork insulin (5 IU/mL) and various insulin preparations, including 1 and 10 U/mL Penfill N, Penfill R (short-acting), Penfill 30R (mixed-acting), Humulin N (intermediate-acting) and the two insulin analogs Humalog (lispro: ultra-short-acting) and NovoRapid (aspart: ultra-short-acting). Only Humulin U (long-acting) gave a negative skin test. Additives in insulin preparations, including protamine sulfate (0.35 mg/mL), zinc chloride (0.05 mg/mL), methylparaben (1 mg/mL), phenol (2 mg/mL) and metachlesol (3 mg/mL), did not provoke any skin response in intradermal tests.

Histamine release test

Peripheral blood was collected from the patient, and purified basophils were stimulated with human insulin. A concentration of 5 IU/mL of human recombinant insulin induced basophils to release a significant amount of histamine, while it did not affect a normal healthy control subject (Table 1). Humalog and NovoRapid stimulated basophils to release a significant amount of histamine; however, the same amount of Humulin U scarcely produced any induction of histamine release (Table 2). The patient has since been treated with Humulin U for her diabetes mellitus without any allergic symptoms.

Discussion

Insulin is an indispensable drug for patients with diabetes mellitus. In the old days, when pork and beef insulin was used, a number of patients allergic to insulin were described. This high incidence of sensitization could be attributed to

Table 1 Human recombinant insulin stimulated the release of histamine from peripheral basophils

Stimulant	% histamine release ^a	
	Patient	Normal healthy control
Human recombinant insulin (IU/mL)		
5	64	0
1.5	2.8	0
0.5	1.7	0
0.05	0	ND
Anti-IgE antibody	100	100

^aThe amount of histamine released from basophils was assessed and presented as percentage of total histamine content, which was determined by the addition of digitonin.
ND, not done.

Table 2 Humalog and NovoRapid induced higher amounts of histamine release than Humulin U

Stimulant	% histamine release ^a
Humulin U (2 IU/mL)	3.7
Humalog (2 IU/mL)	31.1
NovoRapid (2 IU/mL)	30.7
Anti-IgE antibody	100

^aThe amount of released histamine from basophils was assessed and presented as percentage of total histamine content, which was determined by the addition of digitonin.

differences in the amino acid arrangements of pork and beef insulin compared with human insulin.¹ Since animal insulin was replaced by human recombinant products, the number of patients allergic to insulin has significantly decreased. Immediate-type allergy to human insulin appeared to be caused by both insulin itself and the additives in insulin preparations, for example zinc⁵ and protamine.⁶ Our patient showed a positive intradermal test to human insulin (5 IU/mL) but not to any of the additives. Her serum contained human insulin-specific IgE antibodies. We further confirmed that various insulin preparations could induce histamine release from the peripheral basophils of the patient. These results indicate that her symptoms were provoked by type-I allergy to human insulin peptides.

The present case showed that, among several insulin preparations, short-acting, intermediate-acting, and biphasic-acting insulin caused her urticarial reactions, while long-acting insulin did not. Similar patients have been described in the literature.^{2,3} These findings suggest that one of the subtypes of human insulin allergy is caused by short-acting, intermediate-acting, and biphasic-acting insulin but not by long-acting

insulin. It is of interest to consider why long-acting insulin did not provoke the wheal-flare response in these patients. Long-acting insulin has a crystallized form, with a large aggregate of the enzyme. Decrystallized long-acting insulin was found to induce allergic symptoms in one such patient.² Thus, one may hypothesize that the crystallized structure in the three-dimensional conformation may mask the antigenicity of the insulin molecule.^{2,7}

Another hypothesis is that covalent dimers of insulin contain unique epitopes which are distinct from the parent molecule.⁸ Insulin aggregates rather than native monomers can cause cutaneous allergy, and therefore the antigenicity of commercial insulin preparations correlates with the relative concentration of aggregates.⁹ In short- and intermediate-acting insulin preparations, insulin molecules exist as hexamers. After subcutaneous injection, considerable amounts of dimers are released from the hexamers, and the dimers split into monomers, an active form of insulin.¹⁰ These dimers may be responsible for type-I allergy to insulin. In contrast, long-acting crystallized insulin may rather slowly release insulin monomers, which may have lower antigenicity. Therefore, it is possible that the reason our patient did not respond to long-acting insulin is that there was inadequate release of dimers or hexamers from crystallized insulin.

Certain insulin allergy patients can use insulin analogs safely. Lispro has been developed by transposing the amino acid residues B-28 and B-29,¹¹ and aspart is produced by replacing the proline at position 28 on the β chain of insulin.¹² Because these chemical modifications involve the dimerization site of the insulin molecule, self-association and dimerization are markedly reduced. In fact, these human insulin analogs have been safely used for patients with allergies to human insulin.^{11,12} Although these insulin analogs may be less immunogenic, our patient showed positive intradermal tests and a significant amount of histamine release for these insulin analogs. We could not test whether our patient was able to safely use Glargine (Aventis Pharma, Frankfurt, Germany), a synthetic human insulin analog, because this insulin preparation is not yet available in Japan.¹³ On the basis of these findings, we assume that there are at least two subtypes of allergy to human insulin peptides, i.e. a type showing unresponsiveness to insulin analogs, including aspart and lispro, and another type responding to these insulin analogs but not to long-acting insulin.

The present patient had a high titer of IgG antibody to human insulin. A similar patient with an allergy to human insulin and a high titer of IgG antibody to insulin has been described.¹⁴ It is therefore possible that IgG antibody also plays an important role in the aggravation of the allergic symptoms.

In conclusion, we report a case of human insulin allergy. The patient showed a specific unresponsiveness to crystallized insulin, and her diabetes mellitus was safely treated with this type of insulin.

References

- 1 Lamkin N, Lieberman P, Hashimoto K, *et al.* Allergic reactions to insulin. *J Allergy Clin Immunol* 1976; **58**: 213–223.
- 2 Takatsuki H, Ishii H, Yamauchi T, *et al.* A case of insulin allergy: the crystalline human insulin may mask its antigenicity. *Diabetes Res Clin Prac* 1991; **12**: 137–139.
- 3 Itoh M, Uchimura K, Makino M, *et al.* Hypersensitivity to regular and intermediate, but not to crystallized, insulin as an aggravation factor for underlying bulimia nervosa in a patient with type 1 diabetes. *Diabetes Care* 1999; **22**: 1221–1224.
- 4 Nishii H, Nishimura S, Higashiura M, *et al.* A new method for histamine release from purified peripheral blood basophils using monoclonal antibody-coated magnetic beads. *J Immunol Method* 2000; **240**: 39–46.
- 5 Feinglos MN, Jegasothy BV. Insulin allergy due to zinc. *Lancet* 1979; **22**: 22–24.
- 6 Dykewicz MS, Kim HW, Orfan N, *et al.* Immunologic analysis of anaphylaxis to protamine component in neutral protamine Hagedorn human insulin. *J Allergy Clin Immunol* 1994; **93**: 117–125.
- 7 Bentley G, Dodson E, Dodson G, *et al.* Structure of insulin in 4-zinc insulin. *Nature* 1976; **261**: 166–168.
- 8 Robbins DC, Cooper SM, Fineberg SE, *et al.* Antibodies to covalent aggregates of insulin in blood of insulin-using diabetic patients. *Diabetes* 1987; **36**: 838–841.
- 9 Ratner RE, Phillips TM, Steiner M. Persistent cutaneous insulin allergy resulting from high-molecular-weight insulin aggregates. *Diabetes* 1990; **39**: 728–733.
- 10 Kang S, Brange J, Burch A, *et al.* Subcutaneous insulin absorption explained by insulin's physicochemical properties. Evidence from absorption studies of soluble human insulin and insulin analogues in humans. *Diabetes Care* 1991; **14**: 942–948.
- 11 Kumar D. Lispro analog for treatment of generalized allergy to human insulin. *Diabetes Care* 1997; **20**: 1357–1359.
- 12 Airaghi L, Lorini M, Tedeschi A. The insulin analog aspart: a safe alternative insulin allergy. *Diabetes Care* 2001; **24**: 2000.
- 13 Moriyama H, Nagata M, Fujihira K, *et al.* Treatment with human analog (Gly^{A21}, Arg^{B31}, Arg^{B32}) insulin glargine (HOE901) resolves a generalized allergy to human insulin in type 1 diabetes. *Diabetes Care* 2001; **24**: 411–412.
- 14 Grammer LC, Roberto M, Buchanan TA, *et al.* Specificity of immunoglobulin E and immunoglobulin G against human (recombinant DNA) insulin in human insulin allergy and resistance. *J Lat Clin Med* 1987; **109**: 141–146.