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Insulin allergy; desensitization with crystalline zinc-insulin and steroid tapering

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Abstract

The insulin analogues, aspart and lispro, have been considered safe alternatives for patients with insulin allergy, because of their decreased immunogenicity. However, recent several reports showed that neither of them was completely free from allergic reactions. We also experienced a patient with insulin allergy not only to human regular insulin but also to both of the insulin analogues. Interestingly, the insulin analogues, which readily dissociate from polymer to monomer, induced the most severe allergic reaction among several types of human insulin reagents in the present case. Allergy to crystalline zinc-insulin, the three-dimensional structure of which results in delayed dissociation and absorption, was negative on intradermal tests. However, its large subcutaneous injection caused local allergic reaction. These results suggested that the allergic reaction might depend on the rapidity of insulin monomerization and absorption, and thus that the immunogenic residue of insulin is concealed when insulin is polymerized. Based on the intradermal tests, we speculated that the antigenic epitope might be B30-Thr in the present case. We also report here the modified method of insulin desensitization using crystalline zinc-insulin with prednisolone tapering. This might be a simple and useful treatment for insulin allergy.

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1. Introduction

Although the human recombinant insulin significantly lowers the frequency of insulin allergy

compared with other species' insulin used formerly [1,2], it also causes the insulin allergy. The human insulin analogues, insulin lispro and aspart, appear to be tolerated and alternated in cases of insulin hypersensitivity [3,4], because of their decreased immunogenicity. The human insulin analogues lispro and aspart are produced by interchange of the positions of the amino acids B28-Pro and B29-Lys, and replacing B28-Pro with Asp, respectively.

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These recombinations cause rapid dissociation of hexamers into monomers spontaneously, resulting in rapid absorption, reaction, and degradation [5,6]. These structural changes and pharmacological kinetics were reported to result in decreased immunogenicity [3,7,8]. Despite of these advantages, however, neither of them seems eliminate the trouble of insulin allergy. In fact, there are some reports of allergy induced by lispro [9,10]. Surprisingly, Lindholm, et al. reported that aspart caused allergic reactions as frequently as regular human insulin, although there was no consistent relationship between antibody formation and adverse events [11].

We report here a case of a type 2 diabetic patient suffering from allergy to all kinds of human insulin agents including insulin analogues lispro and aspart. Based on the reproducible results of intradermal tests, we speculated that B30-Thr might be an antigenic epitope in the present case. We also showed the modified desensitization therapy using crystalline zinc-insulin with prednisolone and subsequent prednisolone tapering. This case may present one of clues to clarify mechanisms of insulin allergy, and a novel therapeutic strategy.

2. Method

2.1. Intradermal tests

Intradermal tests were performed with eight insulin agents; human insulin analogues, aspart (NovoRapid[®], Novo Nordisk, Bagsværd, Denmark) and lispro (Humalog[®], Eli Lilly, Indianapolis, IN), human regular insulin (Penfil[®] R, Novo Nordisk), NPH (Penfil[®] N, Novo Nordisk), regular plus NPH (Penfil[®] 20R and 30R; Novo Nordisk), crystalline zinc-insulin (Monotard[®] and Novolin[®] U, Novo Nordisk) human recombinant insulin-like growth factor (IGF)-1 (Somazon[®], Fujisawa, Osaka, Japan), and concomitant materials and preservatives (protamine sulfate, paraben, phenol, *m*-cresol, isophane, and zinc from Insulin Allergy Kit[®], Novo Nordisk). A 100 µl of diluted insulin solution (0.01, 1.0 or 10 U/ml) was sequentially injected intradermally into alternate

upper arms every hour. Flare size were measured 15 min after injection. When a solution of lower concentration caused any allergic reaction, the result is documented and use of a higher concentration was omitted. Intradermal saline injection distant from the testing site in the same arm was performed as a negative control for each test. Intradermal tests were performed at least twice for each agent with the patient's agreement. Soluble prednisolone was prepared at every test against generalized allergic reactions.

3. Case report

A 64-year-old man was referred to our hospital for the management of uncontrolled diabetes and insulin allergy. He had been diagnosed as type 2 diabetes 15 years before admission and treated with oral hypoglycemic agents. However, he was lost to follow-up for the next 11 years, and stayed off all medicine. At 1998, his glycemic control was found to be very poor and his glycohemoglobin (HbA1c) was 13.5% at that time. He had already developed bilateral proliferative diabetic retinopathy, resulting in blindness. Treatment with premixed regular and NPH insulin (Penfil[®] 20R or 30R) improved his glycemic control. Fourteen months after initiation of insulin treatments he started to notice local itchy wheal-flare reactions at the injection site within 5 min of injection, accompanied by sweating and slight dyspnea lasting for 1 h. These anaphylactoid symptoms were getting severer and harder to be improved by any antihistaminic agent. His physician changed insulin treatment to the oral hypoglycemic agents glibenclamide (Daonil[®]) and metformin (Melbin[®]) under the diagnosis of insulin allergy. Radioallergosorbent test (RAST) revealed elevated levels of insulin-specific IgE antibody. Since liver dysfunction occurred in idiosyncratic fashion following treatment with the oral agents and his metabolic state was not well controlled, he was referred and admitted to our hospital in December 2001.

Physical examination revealed a blood pressure of 142/92 mmHg, with other vital signs normal. Neither lipoatrophy nor any other skin abnorm-

ality was observed. Examination revealed blindness caused by diabetic retinopathy, and peripheral and autonomic neuropathy. The relevant laboratory data on admission were as follows; white blood cell count, 5900 per μl with 3.2% eosinophils; hemoglobin, 13.1 g/dl; platelet count, 21.1×10^4 per μl ; total protein, 66 g/l; albumin, 38 g/l; total bilirubin, 0.6 mg/dl; aspartate aminotransferase, 54 IU/l; alanine aminotransferase, 69 IU/l; alkaline phosphatase, 136 IU/l; sodium, 138 mEq/l; potassium, 4.4 mEq/l; fasting plasma glucose, 8.4 mmol/l; HbA_{1c}, 7.1%; BUN, 14 mg/dl; creatinine, 79.6 $\mu\text{mol/l}$; IRI, 215 pmol/l; CPR, 0.73 nmol/l. Serum total IgE was 387 IU/ml (normal, 12–310). The insulin-specific IgE antibodies in the serum, measured by RAST, were 1.67 UA/ml (class II positive, UniCAP). The insulin autoantibody binding ratio was 65%. An HLA typing test revealed DR4 positivity, a known risk factor for induction of anti-insulin antibody [12]. Drug-induced lymphocyte stimulating tests for Penfil® 30R were negative.

Intradermal tests were performed with several insulin agents and additives. Representative results are shown in Table 1. All of the insulin agents,

except crystalline zinc-insulin, induced allergic reaction in this test. Among the concomitant materials and preservatives, protamine sulfate induced a strong reaction.

Because of the liver injury by glibenclamide and metformin, and the difficulty in controlling his plasma glucose level with any other oral hypoglycemic agents, insulin treatment using crystalline zinc-insulin, which was negative on intradermal tests, was planned. Although Monotard® is classified as a crystalline zinc-insulin, it is also known to include small part of monomeric insulin. On the other hand, Novolin® U contains only crystalline zinc-insulin, resulting in longer action than Monotard®. Because high dose of Monotard® was supposed to bring considerable monomeric insulin like a regular insulin, we chose Novolin® U among the two crystalline zinc-insulins. Insulin treatment was restarted with the single injection before breakfast at a dose of 12 U of Novolin® U, which dosage was decided on the basis of the last injection of Penfil® 30R. Despite the results of intradermal tests, the first subcutaneous injection of Novolin U caused local allergic reactions with itchy wheal-flare within 10 min lasting for 1 h. For

Table 1
Results of intradermal test for insulin preparations and additives

Preparations	0.001 U/0.1 ml	0.1 U/0.1 ml	1 U/0.1 ml
NovoRapid® (aspart)	42 × 30	–	–
Humalog® (lispro)	Negative	30 × 27	
Penfil® R (regular insulin)	Negative	50 × 38	
Penfil® 20R (regular/NPH)	Negative	45 × 38	
Penfil® 30R (regular/NPH)	Negative	45 × 39	
Penfil® N (NPH)	Negative	44 × 42	
Monotard® (crystalline zinc-insulin)	Negative	Negative	Negative
Novolin® U (crystalline zinc-insulin)	Negative	Negative	Negative
Somazon® (IGF-1)	Negative	Negative	24 × 20
Additives			
Protamine sulfate	60 × 60		
Paraben	Negative		
Phenol	Negative		
m-Cresol	Negative		
Isophane	Negative		
Zinc	Negative		

Flare size (mm) was measured 15 min after each intradermal injection. Saline injection, which was performed every time as a negative control, did not develop any flares. Intradermal test for each agent was performed at least two times, and representative data are shown here.

insulin desensitization, soluble prednisolone was injected together with Novolin® U at a dose of 0.07 mg per 1 U of insulin, referring to a previous report of successful insulin desensitization using CSII with prednisolone [13]. In this case, this mixture did not cause any allergic reactions. The dosage of Novolin U was increased every other day. When the dose of Novolin® U reached 34 U a day, his glycemic control was still insufficient, with his fasting and postprandial glucose level around 10 and 18 mmol/l, respectively. Therefore, an oral hypoglycemic agent, 6 mg of glimepiride, was additionally administered to reduce exogenous insulin dosage, as previously reported [14]. Glimepiride did not induce liver dysfunction and any other adverse effects. After his glycemic control was improved with 30 U of Novolin® U, 2 mg of prednisolone, and 6 mg of glimepiride, concomitant prednisolone was tapered carefully by 0.2 mg a day of weekdays. Two weeks after the complete termination of using prednisolone, he was discharged without any allergic reactions. Six months after, he remained free from any adverse effect with insulin therapy, and his glycemic control was improved (Fig. 1). No significant difference was

found in the serum levels of total IgE or insulin-specific IgE (RAST) before to after desensitization.

4. Discussion

Hexameric insulin, injected subcutaneously, dissociates and is absorbed in its dimeric or monomeric form. The immunogenicity of subcutaneously injected human insulin seems to be due to chemical alterations of the molecule that develop during storage or after injection, especially when aggregation occurs [7]. Crystalline zinc-insulin features altered hexamer formation with a structural change of the first eight amino acids of the B-chain, resulting in delayed dissociation and absorption [15,16]. Therefore, crystalline zinc-insulin is generally believed to have more immunogenicity than non-crystallized preparations [17]. On the other hand, the insulin analogue lispro and aspart are expected to be safe alternates in the treatment for patients with insulin allergy because of their decreased immunogenicity due principally to their rapid monomerization and absorption [3–

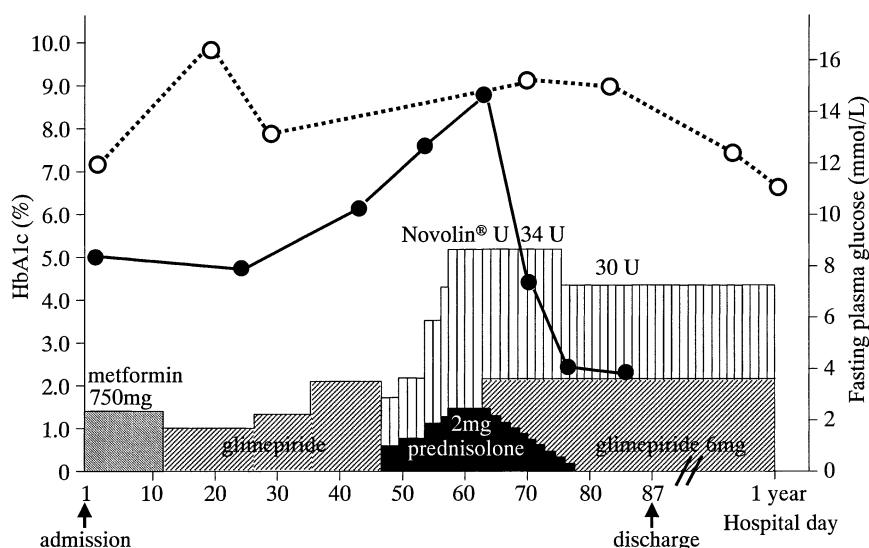


Fig. 1. Clinical course of treatments and glycemic controls. Open circles indicate HbA1c, and closed circles indicate FPG. Each square shows the administered medicine.

6]. However, in the present case, the patient exhibited marked allergy to the insulin analogues lispro and aspart, while crystalline zinc-insulin induced weaker allergy than regular and analogue insulins.

Intradermal tests revealed reproducible and remarkable results different from those noted in previous reports (Table 1): (1) insulin reagents which exhibit faster dissociation to monomers tended to induce stronger allergic reaction; (2) crystalline zinc-insulin, which features the slowest absorption, was negative on testing; and (3) IGF-1 also caused allergic reaction, although it was weak. In the present case, injected insulin may not have exposed its antigenic site until its monomeric form is released. In fact, a report showed that the three-dimensional conformation of crystallized insulin hexamer masks its antigenicity [18]. Although crystalline zinc-insulin (Novolin® U) was negative on intradermal testing, its subcutaneous injection (12 U) caused local allergic reaction. This finding may be explained by increased release of monomeric insulin from a large amount of crystalline zinc-insulin. Based on intradermal tests, we attempted to delineate a candidate antigenic epitope for insulin allergy in the present case (Fig. 2). Insulin aspart caused stronger reaction than insulin lispro. Thus, amino acids alteration of B28-Lys and B29-Pro in lispro may reduce the anti-

genicity, as compared with that of B28-Asp in aspart. Structural modification of lispro may affect the electric charges or three-dimensional structure of the C-terminal region of the B-chain, suggesting that the antigenic epitope of insulin in the present case is near the C-terminus of the B-chain. The amino acids of B22, B23, B24, and B30 of insulin are identical with those of IGF-1, which also exhibited weak reaction (Fig. 2). The three-dimensional structure of B23–B25, the receptor interaction site, seems similar between the insulin analogues, because lispro and aspart have the same affinity for insulin receptors [19]. Taken together, these findings suggest B30-Thr may be a candidate of antigenic epitope in the present case. This speculation is in good agreement with the finding that B30 is one of the immunogenic epitopes in human insulin [20]. The putative antigenic epitope of IGF-1, corresponding to B30-Thr of human insulin, might be affected by B28-Lys and B29-Pro, and C-terminal flanking sequences, resulting in less immunogenicity than that of regular insulin (Table 1).

Insulin desensitization is usually performed with frequent injection of diluted regular insulin [21]. Although the mechanism of desensitization has not yet been elucidated, depletion of allergic mediators from mast cells or induction of blocking IgG antibodies has been suggested [22,23]. Several groups reported successful desensitization using CSII by continuous microdose infusion [9,24,25]. If microdose exposure has advantages for desensitization, a lower dosage of crystalline zinc-insulin may be a beneficial alternative for desensitization because it releases monomeric insulin much more slowly than regular insulin. Furthermore, one report demonstrated successful desensitization using CSII combined with prednisolone infusion [13]. Therefore, we adopted injection of crystalline zinc-insulin with concomitant prednisolone, followed by tapering of the prednisolone. This modified desensitization method was even more convenient than that using CSII and did not require frequent injections. This method might be an option in cases where monomeric insulin appears to be immunogenic.

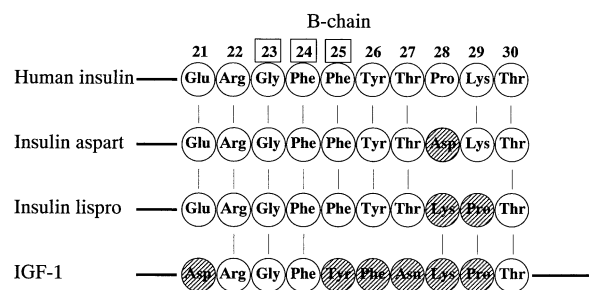


Fig. 2. Primary structures of human insulin, insulin analogues and insulin-like growth factor (IGF)-1. The C-terminal regions of the B-chains of human insulin and insulin analogues and the corresponding region of IGF-1 are shown. Open squares indicate putative sites of interaction with insulin receptor. Closed circles indicate residues different from the primary structure of human insulin.

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