

CLINICAL REPORT

Insulin-induced Drug Eruptions and Reliability of Skin Tests

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Allergic reaction to insulin preparations seemed to have decreased since the introduction of contaminant-free, human preparations. The role of protamine sulfate in decreasing the prevalence of allergy is unclear. This study examines the causative components of insulin allergy along with the value of skin tests for diagnosis. Eleven patients with insulin allergy and 53 patients receiving insulin but without an insulin allergy were included as controls. Intradermal skin tests were conducted using preparations containing various concentrations of insulin [Neutral protamine Hagedorn (NPH) insulin, regular insulin (RI)] and protamine sulfate. Of the 11 patients studied, 3 had anaphylaxis and 8 displayed localized reactions. All of the patients reacted positively during skin testing. Five patients showed positive intradermal skin test reactions to protamine sulfate, and 4 reacted to insulin. Two patients that were not tested with protamine sulfate reacted positively to NPH insulin. In the case of protamine sulfate, 4 patients with localized symptoms displayed positive reactions at concentrations of 10 µg/ml, 3 µg/ml or 0.3 µg/ml. One patient with anaphylaxis reacted positively to a concentration as low as 0.03 ng/ml. In the case of insulin protein, 3 patients reacted positively to a 100-fold dilution (1 UI/ml). Eight of the 53 controls experienced pruritus and/or skin lesions. However, none of the controls reacted at a concentration of NPH insulin of less than 10 U/ml or to protamine sulfate at less than 30 µg/ml. Allergic reactions to protamine sulfate are common and should not be ignored. This study shows a good correlation between clinical manifestations and skin test reactions for insulin allergy. **Key words:** *anaphylaxis; insulin; localized wheal; protamine sulfate; pruritus; skin tests; subcutaneous nodule.*

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Allergic reactions to insulin preparations are induced by the insulin molecule, the altered tertiary structure of insulin, the presence of non-insulin protein contaminants, or pharmaceutical additives, such as protamine sulfate or zinc (1). Highly purified and biosynthetic

insulin preparations are virtually free of protein contaminants, which has undoubtedly contributed to the decreasing prevalence of insulin allergies caused by insulin-related protein and contaminants (2–4). However, purified preparations still contain additives, and insulin allergies caused by the additives, especially protamine sulfate, still occur.

Allergic reactions to insulin preparations are frequently local, and although IgE-mediated anaphylaxis is uncommon, it is the most serious problem. Granulomatous (5) and nodular (6) reactions have also been rarely documented.

We experienced 3 cases of anaphylaxis and 8 of localized adverse reactions to insulin preparations. Although no accurate methods have been described for diagnosis in medical publications, we conducted skin tests for this purpose. Fifty-three controls were tested to evaluate the value of the skin tests. The clinical manifestations, the results of skin tests, and the causes of the insulin allergies are described.

MATERIAL AND METHODS

Subjects

Eleven patients (6 males and 5 females; aged 32–72 years, average 53 years) with allergy-suspected symptoms after the injection of insulin preparations were studied (Table I). The mean duration of diabetes mellitus (DM) was 11 years. The patients had been treated with insulin preparations (continuously or intermittently), with durations ranging from 2 months to 7 years (average 19 months). Three patients experienced generalized wheals with systemic symptoms more than once. This symptom appeared 3 years after receiving insulin injections in patient no. 1, 3 months later in patient no. 2, and one year later in patient no. 3. Insulin was needed to control blood sugar level despite an insulin allergy, and patients 1 and 2 were referred to our department for desensitization. Seven patients (nos. 4, 5, 6, 8, 9, 10, and 11) complained of localized itching and/or wheals. Some considered that their symptoms were related to the insulin, and occasionally discontinued their injections. Patient no. 7 had a severe psoriasis besides DM, and this was not controlled with oral hypoglycemic agents after the treatment of the skin lesions with oral and topical steroids. Four months after insulin injections, pruritic subcutaneous nodules developed at the injection sites. Histopathologic examination revealed dense inflammatory infiltrates consisting predominantly of eosinophils around the septa of panniculus.

Fifty-three controls (13 males and 40 females; aged 45–73 years, average 61 years) were included in the study. These controls have had DM and controlled it with insulin preparations and/or oral hypoglycemics. Seven of them experienced pruritus, but they remembered that the pruritus occurred

Table I. The characteristics of patients with results of skin tests and management

No.	Sex/Age	Clinical findings	Prick tests	Intradermal test results			Diagnosis	Management
				RI (UI/ml)	NPH (UI/ml)	Protamine sulphate		
1	F/36	Generalized wheals, systemic symptoms	+	NT	0.001	NT	Anaphylaxis	Desensitization with NPH
2	M/55	Generalized wheals, systemic symptoms	+	NT	0.001	NT	Anaphylaxis	Desensitization with NPH
3	M/62	Generalized wheals, systemic symptoms	+	–	0.00001	0.03 ng/ml	Anaphylaxis to protamine	
4	F/54	Localized itching	–	1	1	–	Immediate allergy to insulin	Change to oral hypoglycemics
5	M/44	Localized itching	+	1	1	–	Immediate allergy to insulin	Change to oral hypoglycemics
6	M/60	Localized wheals	+	1	1	–	Immediate allergy to insulin	Change to oral hypoglycemics
7	M/72	Subcutaneous nodules	–	100 Day 2	100 Day 2	–	Delayed hypersensitivity to insulin	Change to oral hypoglycemics
8	M/32	Localized wheals and papules	–	–	1	10 µg/ml	Immediate allergy to protamine	Change to oral hypoglycemics
9	F/50	Localized itching	+	–	0.1	0.3 µg/ml	Immediate allergy to protamine	Change to oral hypoglycemics
10	F/58	Localized wheals	–	–	1	3 µg/ml	Immediate allergy to protamine	Change to oral hypoglycemics
11	F/62	Wheals	–	–	1	10 µg/ml	Immediate allergy to protamine	Desensitization with NPH

RI: regular insulin; NT: not tested; NPH: neutral protamine Hagedorn.

regardless of the insulin injection. The mean duration of DM was 12 years. The duration of insulin treatment ranged from 1 to 21 years with an average duration of 11 years.

Methods

Prick and intradermal (ID) tests were conducted on the patients and controls. Neutral protamine Hagedorn (NPH) human recombinant-DNA (r-DNA) insulin (Insulatard® HM: 100 UI/ml), regular insulin (RI) (Velosulin® HM: 100 UI/ml), and injections of protamine sulfate (10 mg/ml) were used for the tests. Undiluted NPHs (human or porcine) contained 350 µg/ml protamine sulfate. The concentration of protamine sulfate injected was approximately 30-fold higher than that contained in the NPHs. A 30-fold dilution (i.e. 333 µg/ml) was usually used, instead of an undiluted injection, to make the concentration of the NPHs (which contained 350 µg/ml protamine sulfate) and the protamine injection similar, though this was not done in 3 patients (patients 7, 8, and 11). Patients 1 and 2 were given skin tests with human NPH and highly purified NPH porcine insulin (Protaphane® MC: 100 UI/ml), but not with protamine sulfate.

Undiluted insulin preparations and 30-fold diluted protamine sulfate were used for prick tests along with histamine as a positive control. If the prick tests displayed negative results within 15 min, ID tests were conducted with 0.02 ml of 10-fold dilutions of the prick testing materials. In cases where wheals caused by the testing materials were larger than those with the histamine, i.e., showing positive results, 100-fold dilutions were used for the first ID test. If anaphylaxis was suspected, the initial concentration used for the ID test was as low as a 100,000-fold dilution. Whenever ID test results were positive, the testing concentration was decreased 10-fold. If ID tests were negative, the concentration was increased 10-fold. Results were read at 15 min and 2 days after the test.

RESULTS

Prick tests displayed positive reactions in all patients with anaphylaxis (patients 1, 2, and 3) and in 3 patients with localized reactions (patients 5, 6, and 9). Patient 7 did not display any immediate reactions in either prick or ID testing. Two patients (patients 5 and 6) developed wheals with RI and NPH, as did 2 (patients 3 and 9) with NPH and protamine sulfate. None of the controls displayed positive reactions to prick tests.

Patients nos. 1 and 2 provoked wheals within 15 min at a dilution of 100,000-fold (0.001 UI/ml) or more on ID tests with serially diluted NPH preparations (human and porcine). Passive transfer tests of patients' sera to the patient's husband (patient no. 1) and to the patient's son (patient no. 2) were positive. Experimental animals were not available at the time to enable anaphylaxis diagnosis. Passive transfer tests were allowed by family members and conducted after confirming the absence of hepatitis, syphilis, and AIDS in the two patients. Patient no. 3 reacted within 15 min to protamine sulfate and NPH at 0.03 ng/ml and at 0.00001 UI/ml, respectively. Patients 4, 5 and 6 displayed a positive reaction within 15 min at 1 UI/ml of RI and NPH. Patients 8, 9, 10, and 11 reacted to protamine sulfate and NPH in 15 min at concentrations of 10 µg/ml and 1 UI/ml, 0.3 µg/ml and 0.1 UI/ml, 3 µg/ml and 1 UI/ml, 10 µg/ml and 1 UI/ml, respectively. Patient 7 did not display any immediate responses, but indurated papules developed 2 days after the ID tests with undiluted RI and NPH.

Three of the 53 controls reacted to 10 UI/ml NPH preparations and 9 displayed a positive reaction to 30 µg/ml protamine sulfate at 15 min. Two reacted to both NPH and protamine sulfate. None of the controls revealed a positive reaction to RI or delayed reactions.

Two patients (patients 1 and 2) with anaphylaxis and one with a localized reaction (patient no. 11) were desensitized successfully with NPH. Patient no. 2 still had a small number of anaphylaxis attacks after discontinuing a daily insulin injection. Seven patients with localized allergies had their insulin preparations changed to oral hypoglycemics in combination with stricter diet control.

DISCUSSION

NPHs (human and porcine) contain protamine sulfate with no zinc as a pharmacological additive. Therefore, allergic reaction to zinc in insulin preparations (7) could be discounted in all patients. The concentration of protamine sulfate in NPHs was 350 µg/ml. In contrast, RI is short acting and protamine free.

In 3 patients, anaphylaxis seemed to be frequent, although they had been treated at our department for 4 years. The number of patients who receive insulin preparations at our hospital is roughly 3,000 persons/year, and the incidence of anaphylaxis is approximately 0.025%. Localized allergic reactions occurred in 8 patients. Pruritus and/or wheals were common, as has been reported (8). Subcutaneous nodules were observed in one patient and interpreted as a delayed allergic response, which is a rare occurrence (6). The true frequency of localized insulin allergy would undoubtedly increase, if patients did not wait until the symptoms became severe. However, endocrinologists at our hospital feel that the number of insulin allergy patients has decreased now that we have adopted purified biosynthetic insulin preparations. These insulin preparations contributed to a decrease in the prevalence of insulin allergies associated with contaminating species, not from protamine sulfate. Adverse reactions to protamine were infrequent with an overall incidence of 0.13% (9). Allergy to protamine sulfate in this study was as frequent as that to insulin protein.

Prick tests with undiluted insulin preparations (100 UI/ml) and 300 µg/ml protamine sulfate displayed specificity to insulin allergy in this study. Six out of 10 patients who had immediate insulin allergy displayed positive reactions to prick tests, but none of the 53 controls displayed any reaction. However, prick testing was found to have low sensitivity and false-negative reactions in 4 out of 10 patients. Though prick testing is less painful and safer than ID tests, it seems to be insufficient to correctly diagnose insulin allergy.

A definitive skin test method for insulin allergy has yet to be agreed on. If skin test results were correlatable with clinical manifestations, the test method would be more reliable. None of the 53 controls displayed any positive reactions to ID tests with 10-fold dilutions (10 UI/ml) of RI. Patients with an immediate RI allergy reacted at 100-fold dilutions (1 UI/ml). Therefore, in the case of RI, ID tests with 10-fold dilutions could be valuable for the diagnosis of immediate allergy. Moreover, a patient with a delayed allergy reacted to undiluted RI. We did not conduct ID tests with undiluted insulin preparations on the controls, and it is uncertain whether ID tests with undiluted insulin could be meaningful for diagnosing delayed allergy. No difference was found in the ID test results of RI and NPH.

The optimal concentration of protamine sulfate in a skin test is controversial (10, 11), but ID tests with a concentration of less than 100 µg/ml are considered to be meaningful (12). Nine of the 53 controls developed immediate wheals at a protamine concentration of 30 µg/ml. This result suggests that ID tests with 30 µg/ml or more protamine could be non-specific, although a reported case of protamine allergy displayed a positive reaction at 1,000 µg/ml (9).

Five patients reacted to protamine sulfate at a concentration ranging from 10 µg/ml to 0.03 ng/ml. The reacted protamine concentrations were much lower in anaphylaxis (0.03 ng/ml in patient 3) than in localized reactions (10 µg/ml in patients 8 and 11, 3 µg/ml in patient 10, and 0.3 µg/ml in patient 9). The protamine concentration in patient 3 (0.03 ng/ml) was even lower than the lowest previously reported concentration in the case of protamine anaphylaxis (0.03–0.05 µg/ml) (13). Patients who reacted to protamine sulfate also displayed positive reactions to NPH. However, they did not react to RI as expected. Positive reactions to 10-fold diluted NPH in 3 controls probably reflected non-specific reactions to protamine sulfate.

The patients with localized insulin allergy, regardless of whether this was due to RI or protamine sulfate, were managed by discontinuing insulin preparations. Allergy to protamine sulfate can also be managed by switching NPH insulin preparations to RI or *Lente*. *Lente* insulin preparations provide prolonged pharmacologic effects, but they are not available in South Korea. RI is short acting and is difficult to use without an insulin pump, and most patients prefer not to use the pump. Some patients were able to control their DM with oral hypoglycemics, but others were not. Desensitization would be necessary for these patients and for anaphylaxis patients. Our 2 anaphylaxis patients were successfully desensitized with NPH insulin. Desensitization of the single patient with localized reactions was much easier and faster than desensitizing the 2 anaphylaxis patients, who successfully responded to a slow desensitization schedule (1).

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