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Skin Testing in Patients with High Risk of Anaphylactic Reactions to Penicillin

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SUMMARY **Sequential skin testing including immediate patch test (IPT), skin prick test (SPT), and intradermal test (IT) with sodium benzylpenicillin G (Pen G), and SPT with benzylpenicilloyl human serum albumin (BPO-HSA) was done in 58 subjects with a history of probable anaphylactic reaction or shock of unknown cause. Based on positive skin tests, the diagnosis of penicillin anphylaxis was confirmed in 30 patients. The average age of onset of penicillin allergy was 42 years ranging from 20-70 years. The sex ratio was 2:28 with marked female predominance. Anaphylactic shock, wheezing and urticaria occurred in 21, 20, 19 patients, respectively. Most symptoms were induced by skin tests and inhalation. The results of skin tests in these patients showed that IPT with 500 U/ml of Pen G was not only reliable but also safe. It is suggested that patients suspected of penicillin anaphylaxis should received IPT with 500 U/ml of Pen G as the initial diagnostic step; if a negative reaction occurred, then SPT and IT should be applied with the same concentration of Pen G, until a positive reaction developed or all the skin testing showed negative results. SPT to BPO-HSA was safe, but its positive rate was only 47.8% in our study; it seems to be less important than skin test to Pen G. As a whole, the skin testing procedure we recommend is relatively reliable, safe and practical even in individuals extremely sensitive to penicillin. In addition, once the patient develops a positive IPT, Pen G residue on the testing site should be wiped away rapidly and washed out with cool water thoroughly to disrupt further violent reaction. The positive rate of the BPO specific IgE measured with ELISA was rather low (43.5%).**

Since the discovery and clinical application of penicillin, it quickly became one of the most commonly prescribed drugs and the most common cause of anaphylactic shock in the world. Although all the four types of immunopathologic reactions classified by Gell and Coombs could be observed in penicillin allergy, the IgE-mediated reaction was the most common and serious. The diagnosis of this type of allergy is based on positive skin test, but routine allergy skin testing may cause severe even fatal allergic reactions.[1,2] The *in vitro* tests such as RAST and ELISA, and passive transfer test have been studied in China and abroad; up to now people considered they are not as sensitive as skin test yet. Skin test is still the valuable diagnostic method for anaphylaxis to penicillin.[34] This study recommends a safe, simple and reliable skin testing procedure for patients who had a history of high risk of penicillin anaphylaxis or anaphylactic shock of unknown cause.

MATERIALS AND METHODS

Patients

Over a period of 5 years, 58 subjects with symptoms resembled anaphylaxis to penicillin were encountered in our clinic. They were evaluated with various skin tests to sodium benzylpenicillin G (Pen G) and benzylpenicilloyl human serum albumin (BPO-HSA). The patient with positive skin test to any penicillin reagent was considered to have penicillin anaphylaxis and selected as a case in this study. Negative skin test should receive single blind with normal saline as placebo control by inhalation or skin testing or a dose of 50,000 U penicillin to exclude penicillin anaphylaxis.

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Skin testing reagents

Pen G was diluted to 500 U/ml and standardized weekly by the pharmacy laboratory of Peking Union Medical College Hospital, and stored at 4°C for use. Occasionally, Pen G was diluted to 200 U/ml, 1U/ml, 0.5 U/ml or 0.1U/ml prior to use, or pencillin with 400,000 U/ml, was used, to observe the different reactions.

BPO-HSA was prepared and kindly provided by Department of Immunology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and stored at -10°C for use, the protein content was 11.5 mg/ml. The solvent and histamine hydrochloride (1 mg/ml) were used for skin testing serving as negative and positive control, respectively.

Procedures for skin testing

Short-acting antihistamines should be withdrawn at least 48 hours, ketotifen 5 days, astemizole 3 weeks prior to skin testing. The methods of skin tests included immediate patch test (IPT), skin prick test (SPT) and intradermal test (IT). The procedures are briefly described below.

The patients received IPT by placing a few drops of Pen G on volar surface of the forearm. The reaction was read after 25 to 30 minutes. Once itching, erythema/wheal developed, a positive reaction was considered regardless of its size and intensity, then residual solution of Pen G on the skin was wiped away immediately and washed out with cool water thoroughly for disrupting further violent reaction.

SPT was performed using Pen G, BPO-HSA, histamine hydrochloride (1 mg/ml), and the solvent. One drop of each of them was placed on the volar surface of the patient's forearm in above order at an interval of about 3 cm. A hypodermic needle (26 gauge) was passed through the drop and penetrated into the epidermis, the needle tip was gently lifted; this method introduces about 3.3×10^{-6} ml of test solution into the skin.[4] The result was read after 15-20 minutes. In case the MWD (mean wheal diameter) of Pen G being 3 mm larger than negative control, or the MWD of BPO-HSA showing 1 HEP (histamine equivalent prick test) or more was considered a positive reaction.[5]

IT to Pen G was done as described elsewhere. According to the stipulation of routine skin testing in China, a MWD of Pen G (0.1 ml of 500 U/ml) 10 mm larger than the negative control was regarded as a positive reaction.[6]

The skin tests were arranged in the following order : IPT, SPT, IT. If any one of the above steps showed a positive reaction or IT (0.1 ml of 500 U/ml of Pen G) was negative, skin testing was discontinued or proceeded under the physician's supervision.

The measurement of BPO specific IgE

BPO specific IgE was measured with enzyme-linked immunosorbent assay (ELISA) by the Department of Immunology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences.

RESULTS

Out of 58 subjects who came to our clinic for high risk of penicillin allergy or shock of unknown cause, 30 were identified as having severe anaphylactic reactions to penicillin. The diagnosis based on the history and positive IPT and/or SPT. Twenty-nine patients showed immediate reactions only one manifested an accelerated reaction, whose positive IPT, SPT, and IT appeared in 4 hours, 4 hours, and 45 minutes respectively, and disappeared in 24 hours. Twenty-eight of 58 patients had negative routine IT. Eighteen of them who had symptoms following penicillin received normal saline as a placebo control by inhalation or skin test, symptoms similar to those after use of penicillin occurred, They were told that they were not hypersensitive to penicillin, later, no symptoms occurred after exposure to penicillin. These cases were considered to have pseudoanaphylaxis to penicillin. Six patients including those who had shock of unknown cause were given intramuscular challenge with penicillin starting with 1,000 U, increasing doses of 5,000 U, 10,000 U, and 50,000 U at 20 minutes intervals, no reaction was induced. The diagnosis of penicillin anaphylaxis was excluded. Another 4 patients refused to receive any furhter test, they were regarded

Table 1 Clinical manifestations of the patients

Clinical manifestations	No. of patients	%
Hypotension, syncope, convulsion	21	70.0
Wheezing	20	66.7
Generalized urticaria and/or angioedema*	19	63.3
Nasal itching, sneezing, watery running nose	15	50.0
Conjunctival itching, congestion	9	30.0
Ear/pharyngeal itching	5	16.7
Laryngeal edema	2	6.7
Vomiting and/or diarrhea, abdominal pain	2	6.7

* All accompanied with other symptoms.

Table 2 The results of positive skin tests to Pen G and BPO-HSA

	IPT		SPT			IT			SPT
Pt	Conc of PG (U/ml)	T of onset of Rea (min)	Conc of PG (U/ml)	T obs (min)	MWD (mm)	Am of PG (U)	MWD (mm)	MFD (mm)	BPO -HSA
1			200	1	13.0				
2			500	1	+ *				
3			500	10	15.5				
4A			0.1	15	17.5				
4B			1	15	19.0				
5	500	5							
6A	400,000	5							
6B	500	5	1	15	14.5				—
7A	500	20	5	15	3.5	0.5	18.0	53.0	—
7B			500	1	+ *				
8A	5	15	0.5	15	5.0				+
8B	500	15							
9A	500	—	5	15	—	0.5	20.0	54.0	—
9B			500	15	8.5				
10	500	15	5	15	12.0				—
11	500	5	5	15	13.5				+
12	500	1	5	15	12.0				+
13A	500	5	5	15	9.5				+
13B			500	15	15.0				
14**	500	15	500	15	5.5	50	11.5	—	—
15A	5	15	5	15	10.5				+
15B	500	4							
16A	500	15	5	15	5.5	0.25	14.0	32.0	—
16B			500	15	8.5				
17A	500	15	5	15	—	50	12.5	30.0	+
17B			500	15	4.5				
18A	500	5	500	5	3.0	0.05	9.0	13.5	—
18B						50	10.0	18.5	
19	500	10	5	15	12.5				+
20A	500	15	5	15	5.5	0.1	16.5	ND	—
20B			500	15	15.0				
21	500	5							+
22	500	10							—
23	500	10							+
24	500	15	5	15	12.5				+
25A	500	16	5	15	5.5				—
25B	400,000	3	500	15	16.0				
26	500	7	5	15	12.0				—
27	500		500			50			—
28A	500	22	5	5	11.5				
28B	400,000	5							
29	500	17							+
30	500	10							

Pt = patient; Conc = concentration; T = time; PG = Pen G; Rea = reaction; min = minute; obs = observed; Am = amount; MFD = mean flare diameter. ND = not done.

* Since postive reaction developed so quickly that emergency treatment must be given, there is no time to measure the MWD.

** Taking antihistamine during 24 hours.

Table 3 The occurence of adverse reactions of three kinds of skin tests

Pt	Methods of ST to Pen G	Adverse reactions
1	SPT	Nasal itching, sneezing, watery running nose*
2	SPT	The same reaction as the patient 1*
3	SPT	Local reaction developed seriously and promptly*
5	IPT	Wheal lasted 20 hours, conjunctival itching, congestion developed by contamination**
6A	IPT	Wheal lasted 2 days**
7B	SPT	The same reaction as the patient 3*
9	IT	Generalized urticaria, wheezing, a decreases (19%) was found in PEFR*
16	IT	The same reaction as the patient 1*
17	IT	Generalized urticaria
18B	IT	Generalized pruritus, sweating, sneezing, watery running nose, wheezing, a marked decrease (48%) was found in PEFR*

Pt = patient number; ST = skin test;
* Aqueous epinephrine (1:1,000) 0.3 ml was injected subcutaneously immediately.
** Pen G residue on the skin was not removed.

Talbe 4 Positive rate and incidence of adverse reaction in IPT, SPT and IT

	IPT	SPT_1	SPT_2	IT
	(500 U/ml)	(5 U/ml)	(200 U/ml, 500 U/ml)	(0.05-50 U/ml)
Positive rate	96.2% (25/26)	86.7% (13/15)	100% (14/14)	100% (8/8)
Incidence of adv*	4% (1/25)	0% (0/13)	28.5% (4/14)	50% (4/8)

* adv, adverse reactions.

as negative in this study. The clinical manifestations of the 30 patients were shown in Table 1. The average age of onset of penicillin allergy was 42 years, ranging from 20-70 years. The sex ratio was 2:28 with marked female predominance. The mean duration of penicillin allergy was 6 years (from 20 days to 25 years) in 26 patients, severe allergic reactions of 4 other patients occurred within the penicillin treatment courses. First anaphylactic attacks induced by routine skin tests occurred in 18 cases, by inhalation in 5, by intramuscular injection, skin contact, intravenous infusions, oral route in 3, 2, 1 and 1 cases respectively. Twenty-five patients gave no history of a prior penicillin reaction, only 5 patients had histories of strong local reactions.

The results of positive skin tests to Pen G are shown in Table 2. IPT were done in 26 patients. SPT and IT were performed under close supervision. Positive IPT were shown in 25 patients. One patient with negative IPT presented positive SPT and IT which caused asthmatic attacks and generalized urticaria. The patient had respiratory symptoms in a penicillin environment 2 years before , indicating that the result of skin tests was correlated with her clinical manifestations.

All the adverse reactions in detail in IPT, SPT, and IT are listed in Table 3. SPT_2 and IT had serious adverse reactions. In 2 patients (Nos 5 and 6A) Pen G residue on the skin were not removed, the wheal and flare reaction in test sites remained 6 hours and 2 days, respectively.

The positive rate and incidence of adverse reactions in IPT, SPT and IT with Pen G are presented in Table 4. All the patients who received SPT_2 and IT showed positive reac-

tions, but serious adverse reactions were apt to be excited. The positive rate and incidence of adverse reactions in IPT were compared with those in SPT_1, there was no significant difference between them assessed by Chi-square test.

Out of 30 patients SPT to BPO-HSA were done in 23 patients, with a positive rate of 47.8%. The measurement of serum BPO specific IgE was accomplished in 23 patients. The positive rate was 43.5%.

DISCUSSION

IgE-mediated penicillin reactions have been classified into two groups, immediate or anaphylactic reaction, and accelerated reaction. In our study, the former was 29 and the latter was only one. MDM (minor determinant mixture) and a major determinant (penicilloyl group) were responsible for them, respectively. Fresh solution of Pen G is the appropriate substitute for MDM for skin testing. Since PPL (benzylpenicilloyl poly-lysine) is not available in China, BPO-HSA was used as a substitute of PPL for SPT in most cases. Although SPT or BPO-HSA was safe, the positive rate was relatively low, moreover, no case who had negative reaction in skin tests to Pen G had a positive SPT to BPO-HSA. Fortunately, most cases of penicillin anaphylaxis were induced by MDM, only on rare occasions, they could be induced by PPL.[2]

At the initial stage of our study, SPT was applied as the first step in various skin tests. In 1981, Sullivan and his colleagues recommended using PPL, Pen G and pencilloyl acid with different concentrations for skin testing at 15-20 minutes intervals, first by SPT and then by IT, until a positive reaction to any of the above reagents appeared or all of them were negative.[7] About this suggestion, Dellen emphasized that extra caution should be paid when the patient gives a history of a severe reaction, and more dilutions of the reagents they used would increase the expense and time required for testing.[8] For these reasons, we attempted to find out a more appropriate skin test procedure for the patients with symptoms resembling anaphylaxis to penicillin. IPT was developed as the first step and compared with SPT and IT in positive rate and incidence of adverse reactions. It indicated that SPT with 200 U/ml and 500 U/ml of Pen G and IT at a dose of 0.05-50 U Pen G can not be used in the first step because of their serious reactions, even though both of them had the highest positive rates. IPT with 500 U/ml of Pen G were compared with SPT with 5 U/ml of Pen G, there was no significance of the difference either in positive rate or in incidence of adverse reactions. Since little is known of the level at which safety can be assured, and Pen G on the skin for IPT can be wiped away immediately and thoroughly, thus, IPT used as the first step to these patients is reasonable.

The purpose of IPT is that as Pen G penetrates the epidermis and interacts with penicillin-specific IgE antibodies that are bound to mast cells, the mediators released from the cells result in immunologic contact urticaria, and the diagnosis of penicillin anaphylaxis is confirmed.

However, positive IPT reaction should be differentiated from contact dermatitis, since contact dermatitis belongs to type IV allergy.

In our observation, all the itching, erythema and/or wheal formation developed within 1-22 minutes after IPT, as shown in Fig. 1. Adverse reactions of IPT were rarely seen in

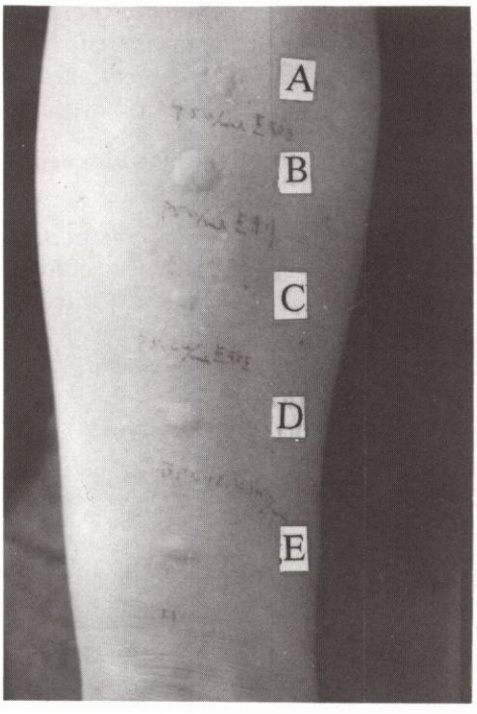

Fig. 1 The skin testing of patient 15. A = IPT to Pen G (5U/ml), Pen G residue on the skin removed at 15 min. B = SPT to Pen G (5U/ml). C = IPT to Pen G (500U/ml), Pen G residue removed at 5 min. D = SPT to BPO-HSA. E = SPT to histamine hydrochloride.

our studied patients, since there was no skin wound created in IPT, and whenever a wheal appears, Pen G residue could be wiped away promptly and followed by washing out with tap water, therefore, the occurrence of contact urticaria syndrome[9] was greatly reduced. Since hot water may cause capillary dilatation and promote Pen G absorption, its use should be prohibited. Pen G at a high concentration may provoke a risk of respiratory symptoms by inhalation, so its used for IPT is also restricted.

Therefore, we suggest that IPT with 500 U/ml of Pen G is carried out as an initial step, then SPT and IT (0.01 ml, 0.1 ml) with the same concentration of Pen G are done in order. SPT to BPO-HSA were accomplished uneventfully. If a positive reaction develops in any step, or all of the reactions is negative, skin tests should be discontinued. This procedure is relatively safe, simple, and reliable even in individuals extremely sensitive to penicillin. When PPL and/or other reagents are available, the same procedure could be adopted.

Since most anaphylactic reactions occurred in patients who have not experienced allergic reactions during previous penicillin exposure in this study and other reports,[11] and since most cases of first anaphylaxis to penicillin occurred in routine IT with Pen G (50 U), if these patients received penicillin (800,000-1,000,000 U)directly without skin testing, they might cause a fatal anaphylaxis. Thus, it seems to be reasonable that every patient even without a history of allergic reaction to penicillin should have a skin test before its use.

The reasons for skin testing for the patients with probable high sensitivity to penicillin are (1) to differentiate anaphylaxis from pseudoanaphylaxis to penicillin, (2) to rule out penicillin allergy in cases of shock with unknown cause, (3) to predict the present status of penicillin allergy in patients who had a history of penicillin reaction. All skin testing preceed under physician's supervision, and all the equipment for rescue an anaphylactic reaction should be at hand for emergency use.

However, skin testing is of no value in predicting the occurrence of non-IgE-mediated reactions such as exfoliative dermatitis, Steven-Johnson syndrome, delayed exanthema, drug fever, contact dermatitis, hemolytic anemia, or interstitial nephritis etc.[10] Therefore, the above-mentioned skin tests are unsuitable for diagnosis of these diseases.

The measurement of BPO specific IgE

BPO specific IgE measured by ELISA gave a lower positive rate in detecting penicillin anaphylaxis as compared with the skin tests mentioned above.

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