

REVIEW ARTICLE

Hypersensitivity reactions to penicillins: studies in a group of patients with negative benzylpenicillin G skin test

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SUMMARY

Background: Although skin tests are usually employed to evaluate current penicillin allergy status, a negative result does not exclude hypersensitivity. There is a need for accurate *in vitro* tests to exclude hypersensitivity. A radioallergosorbent test (RAST) is a potentially good supplementary approach, but there is little information on the suitability of this method to diagnose penicillin hypersensitivity in subjects with a negative skin test to benzylpenicillin.

Methods: A total of 133 patients with a negative skin test to benzylpenicillin G (PG) and all of whom developed allergic reactions to PG were studied. RAST was used to detect eight kinds of specific IgE antibodies to penicillins in serum, which included four kinds of major and minor antigenic determinants to four penicillin drugs. The combination sites for the specific IgE antibodies were studied by RAST inhibition test.

Results: The rate of positive reactions for the specific IgE antibodies was 59.40% (79/133). Of the eight kinds of antigenic determinants, the positive rates for specific IgE against the major and minor determinants were 39.10% (52) and 42.86% (57) respectively. Of the four drugs, positive cases only to PG were 10 (7.5%), were significantly fewer than the cross-reacting positive cases (36) to PG ($P < 0.01$). In the RAST inhibition studies all drugs exhibited good inhibitory potencies, and in some instances the side-chain of the penicillins could induce specific

responses with a variable degree of cross-reactivity among the different penicillins.

Conclusion: Radioallergosorbent test is a good complementary test in persons who are skin-test negative with PG, and the sensitivity of RAST increases with increasing specificity of IgE antibodies to be detected. 6-APA and the groups, making part of the different side-chains on penicillins, all contributed to the cross-reactivity.

Keywords: allergy, cross-reactivity, penicillins, side-chain identity, skin test

INTRODUCTION

Penicillins are the antibiotics that most frequently induce allergic reactions by specific immunological mechanisms. There are multiple manifestations in penicillin allergy, ranging from a slight urticaria to serious anaphylactic shock and death (1, 2). Penicillin skin testing is usually done to assess penicillin allergy status (3). Materials used for penicillin skin testing are typically generated from benzylpenicillin: benzyl penicilloyl (BPO) and minor determinant mixture (MDM) (4). Although there is general agreement that MDM should be used in penicillin skin testing, there are no commercial sources for these reagents in China and the quality criteria have not been standardized (5). Penicillin skin testing using only benzylpenicillin cannot accurately diagnose all potentially true-positive cases of penicillin allergy. Negative results do not exclude hypersensitivity. Diagnosis of penicillin allergy in these subjects is difficult as *in vivo* testing may involve significant risks. There is a need for alternative *in vitro* tests, and previous investigations (6) have shown that the radioallergosorbent test (RAST) a specific IgE antibody assay might be a good alternative.

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However, little information is available on the usefulness of RAST for diagnosing penicillin allergy in subjects who are negative in skin tests.

In this study, we detected eight kinds of specific IgE antibodies in sera of patients who were skin test negative with benzylpenicillin G (PG), but who nonetheless developed an allergic response after exposure to PG. Additionally, to evaluate the molecular mechanism of cross-recognition of various penicillins at the humoral level, we explored specific IgE antibodies combining sites by RAST inhibition testing.

MATERIALS AND METHODS

Subjects

A total of 133 patients (64 male and 69 female; mean age 23.03 ± 10.83), were recruited mainly from the first affiliated hospital of Zhengzhou University. Patient selection was based on a negative skin test (diameter <5 mm) and clinical symptoms after PG injection (such as dyspnea, urticaria, pruritus, fever and anaphylactic shock). A questionnaire was used which included a full history related to allergic reaction and disease. Informed consent was obtained from all participants in this study.

Skin testing was performed with PG on the inner side of the forearms by intradermal injection (7). Briefly, the PG sodium was dissolved in saline solution. Approximately 0.1 mL (20 U) of reagent was injected for intradermal testing. After 20 min, the diameter of any wheal was measured. The skin test was considered negative when the diameter was <5 mm with a surrounding area of erythema.

Radioallergosorbent test was used to detect eight kinds of specific IgE to penicillins [benzylpenicilloyl-polylysine (BPO-PLL), benzylpenicillanyle-polylysine (BPA-PLL), phenoxomethylpenicilloyl-polylysine (PVO-PLL), phenoxomethylpenicillanyl-polylysine (PVA-PLL), amoxicilloyl-polylysine (AXO-PLL), amoxicillanyl-polylysine (AXA-PLL), ampicilloyl-polylysine (APO-PLL), ampicillanyl-polylysine (APA-PLL)] in serum, which included four kinds of major and minor antigenic determinants to four penicillins [penicillin G (PG), penicillin V (PV), ampicillin (AMP) and amoxycillin (AX)] respectively. Cyanogen-bromide-activated cellulose discs were coupled to PLL and then

treated with the penicillins (PG, PV, AMP and AX). Antibodies were detected as previously described (8). The criterion used for a positive response for each of the eight kinds of specific IgE is described in our previous report (9). Briefly, the sera of 101 control subjects with no allergic reactions (including drug, food, pollen and so on) and 30 fetal cord blood samples were selected to establish the RAST cut-off point. The RAST results reflected the radiolabeled substance uptake and values above the mean $+ 2.33$ SD of the results in the control subjects for BPO, PVO, APO, AXO, BPA, PVA, APA, AXA were considered to be positive. Meanwhile, the fetal cord sera were used as negative control.

Drugs and chemicals

Benzylpenicillin-sodium, ampicillin-sodium, phenoxomethylpenicillin and amoxicillin were purchased from HuaBei Chemical Co, Hebei, China. 6-Aminopenicillanic acid (6-APA) and phenylacetic acid (PHA) were purchased from Sigma Chemical Co, St. Louis, MO, USA. Phenoxacetic acid (PHOA), *N*-phenylglycine (NPG), *n*-(α -Hydroxyphenyl) glycine (PHPG) were purchased from Acros Organics Chemical Co, Geel, Belgium.

RAST inhibition test

To analyze the correlation between the chemical structure and the cross-reactivity of penicillins in terms of IgE level, the inhibition test was studied as previously described (10). Briefly, the inhibitors were dissolved in PBS pH7.2 and the pH of the solutions was adjusted to 6.5–7.5. Serum (20 μ L) was incubated for 2 h at room temperature with 20 μ L of inhibitor solution and after adding the determinant paper discs, the serum was incubated for a further 3 h at room temperature. After this, the discs were washed three times with PBS containing 0.05% Tween-20 and 0.005% sodium azide before incubation overnight with ^{125} I-anti-human IgE. The discs were washed again as above and counted in a Gamma counter (China National Nuclear Corporation, Beijing, China). The percentage inhibition was calculated according to the formula:

$$\% \text{Inhibition} = \frac{[(\text{Counts NIS} - \text{Counts IS}) / \text{Counts NIS}] \times 100\%}{}$$

where NIS refers to the noninhibited serum and IS to the inhibited serum.

Statistical methods

Differences between the two and/or three groups were analyzed with chi-square test. A value of $P < 0.05$ was considered statistically significant. All the analyses were performed using the SPSS (10.0) statistics program (Statistical Product and Service Solutions, Chicago, IL, USA).

RESULTS

Specific serum IgE antibodies

The rate of positive response for specific IgE antibodies in the 133 penicillin allergic, but PG skin test-negative patients was 59.40% (79/133). With the eight different antigenic determinants, the rates of positive response for antibodies against the major and minor determinants were 39.10% (52) and 42.86% (57) respectively. There were no significant differences, between the two groups of determinants (Table 1).

Cross-reactivity

Of the four penicillin drugs, the rates of positive response for specific IgE antibodies to one of PG, PV, AP, AX were 34.59% (46/133), 30.83% (41/133), 23.31% (31/133), 30.83% (41/133) respectively. Positive responses for specific IgE antibody to PG only were 10 (7.52%), significantly lower than the cross-reaction positive cases 36(27.07%) to PG ($P < 0.01$). Cases of positive responses only against

PV (8), AP (10) and AX (7), were also observed. The results showed that partial cross-reactivity existed between PG and other penicillins. From Table 2, we can clearly see that the cumulative positive rate increased significantly with increasing number of specific IgE antibodies used.

The structure involved in IgE antibody recognition

Amongst the cross-reactive sera, three sera (serum L1, L2 and L3) were chosen as representative examples for further studies, based on the RAST results. The clinical characteristics of the three subjects are presented in Table 3.

Results of RAST inhibition studies for serum L1 are shown in Fig. 1. With the drug-solid phases, PG, PV, PHA and PHOA showed good inhibitory activity, whereas 6-APA showed markedly lower activity.

The values of RAST results in serum L2 to PVO were four or more. The RAST Inhibition data for

Table 2. The rate of positive response to specific IgE against four penicillin drugs in the PG skin test-negative patients with penicillin allergy

Group	PG	PV	AP	AX	Total
A (%)	10 (7.52)	8 (6.02)	10 (7.52)	7 (5.26)	35 (26.32)
B (%)	36 (27.07)	33 (26.19)	21 (19.05)	34 (25.56)	44 (33.08)
C (%)	46 (34.59)	41 (30.83)	31 (23.31)	41 (30.83)	79 (59.40)

A, positive cases only to specific drug indicated; B, cross-reaction positive cases; C, positive cases with inclusion of specific drug shown.

PG, benzylpenicillin-sodium; AP, ampicillin-sodium; PV, phenoxymethylpenicillin; AX, amoxicillin.

Table 1. The rate of positive specific immunoglobulin (Ig) E response against the major and minor antigenic determinants in 133 in the PG skin test-negative patients with penicillin allergy

Group	Major antigenic determinants				Minor antigenic determinants			
	BPO	PVO	APO	AXO	BPA	PVA	APA	AXA
Specific positive cases (%)	30 (22.56)	21 (15.79)	22 (16.54)	11 (8.27)	28 (21.05)	25 (18.80)	20 (15.04)	23 (17.29%)
Total positive cases (%)	52 (39.10)				57 (42.86)			

APO, ampicilloyl; APA, ampicillanyl; AXA, amoxicillanyl; AXO, amoxicilloyl; BPA, benzylpenicillanyl; BPO, benzylpenicilloyl; PVA, phenoxomethylpenicilloyl; PVO, phenoxomethylpenicillol; PG, benzylpenicillin G.

No.	Age (years)	Sex	Drugs	Allergic response	Skin testing	Positive specific IgE antibodies
L1	46	M	PG	Urticaria	–	BPO, PVO, APO, AXO, BPA, PVA, APA, AXA
L2	19	F	PG	Urticaria	–	BPO, PVO, APO, AXO, BPA, PVA, APA, AXA
L3	75	M	PG	Anaphylaxis	–	BPO, PVO, APO

'+' means positive, '–' means negative.
F, female; M, male.

Table 3. Clinical characteristics of patients included in this study

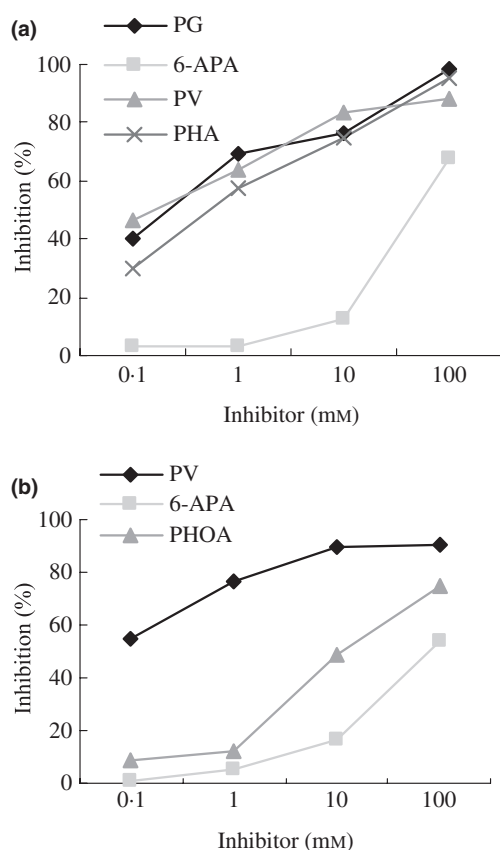


Fig. 1. Inhibition of IgE antibody binding in serum L1. to benzylpenicilloyl-polylysine paper discs (a) and phenoxymethylpenicilloyl-polylysine paper discs (b) by benzylpenicillin G (PG), penicillin V (PV), phenylacetic acid (PHA), 6-aminopenicillanic acid (6-APA), phenoxacetic acid (PHOA) at 0.1, 1, 10, 100 mmol/L.

serum L2 using PVO-PLL-paper disc solid phases show (Fig. 2) that PG, PV, AP and AX had good inhibitory activity, despite the variable side-chains: PHA, PHOA, *N*-phenylglycine (NPG), *n*-(α -Hydroxyphenyl) glycine (PHPG). 6-APA was the

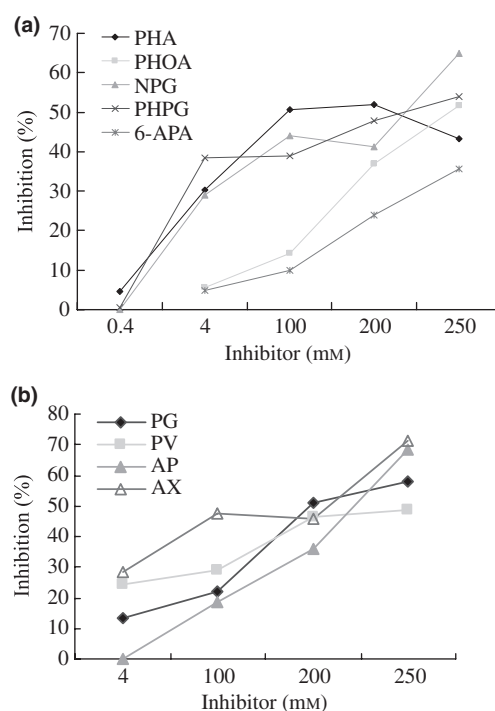


Fig. 2. (a) Inhibition of IgE antibody binding in serum L2 to phenoxymethylpenicilloyl-polylysine (PVO-PLL) discs by. Phenylacetic acid (PHA), phenoxacetic acid (PHOA), *N*-phenylglycine (NPG), *n*-(α -Hydroxyphenyl) glycine (PHPG), 6-aminopenicillanic acid (6-APA) at 4, 100, 200, 250 mmol/L. (b) Inhibition of IgE antibody binding in serum samples L2 to discs PVO-PLL by penicillin G (PG), penicillin V (PV), ampicillin-sodium (AP), amoxycillin (AX) at 4, 100, 200, 250 mmol/L.

weakest inhibitor showing only 4–36% inhibition within the concentration range 0.4–250 mm.

The RAST result for serum L3 to PVO was 4:16. Using PVO-PLL-paper disc solid phases (Fig. 3) show that all the compounds tested produced similar responses except at the highest inhibitor concentration (250 mmol/L).

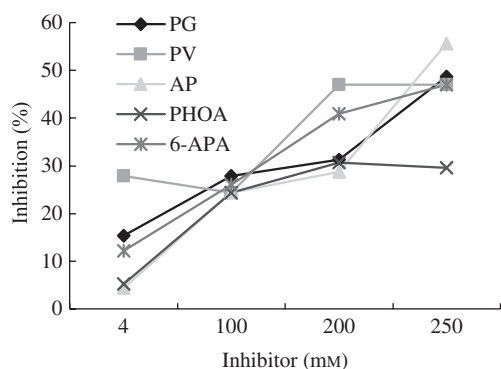


Fig. 3. Inhibition of IgE antibody binding in serum L3 to phenoxymethylpenicilloyl-polylysine discs by benzylpenicillin G (PG), ampicillin-sodium (AP), penicillin V (PV), phenoxacetic acid (PHOA), 6-aminopenicillanic acid (6-APA) at 4, 100, 200, 250 mmol/L.

DISCUSSION

Skin testing with penicilloyl polylysine (PPL) and MDM represents the first-line method for diagnosing beta-lactam hypersensitivity (11–13). In China, people take skin tests with benzylpenicillin-sodium, as there are no commercial sources of MDM. When the skin test results are negative, people would be thought to be tolerant of PG. However, evidence exists to indicate that a negative skin test does not exclude hypersensitivity (14–16). Our previous study reported that the combined use of major and minor determinants in RAST would improve recognition frequency of IgE antibodies (6). To improve the sensitivity and specificity of RAST, we examined eight kinds of specific IgE antibodies in serum, including major

and minor antigenic determinants to four drugs of penicillins (PG, PV, AP, AX). The rates of positive response for the specific IgE antibodies against one of PG, PV, AP and AX in 133 patients, who were penicillin allergic, but PG skin test-negative, were 34.59% (46), 30.83% (41), 23.31% (31) and 30.83% (41) respectively. Furthermore, the total rate of positive response for specific IgE antibodies against the four drugs combined was 59.40% (79). The rate of positive response using specific IgE increased from 39.10% to 59.40% with the use of minor antigenic determinants. This suggests that the minor antigenic determinant may play an important role in penicillin allergy in those with negative skin test to PG.

As originally described, the classical determinant recognized by IgE antibodies is the binuclear heterocyclic structure of the betalactam ring, the benzylpenicilloyl moiety. However, some reactions result from recognition of a side-chain group with or without the participation of all or part of the adjacent β -lactam ring (15, 17, 18). Recently, Zhao *et al.* (10) reported that allergic cross-reactions between penicilins and cephalothins. The side-chains of PG and cephalothin are apparently as different as that between the benzyl and (thienyl) methyl groups. Small features such as the methylene-containing structure may act as allergenic determinants. Two general points are immediately obvious from our RAST inhibition studies. The first is that the percentage of cross-reactivity between PG and other penicillins to subject L1, L2 or L3 is high. The second point is the binding site of the specific IgE antibody is different in serums L1, L2

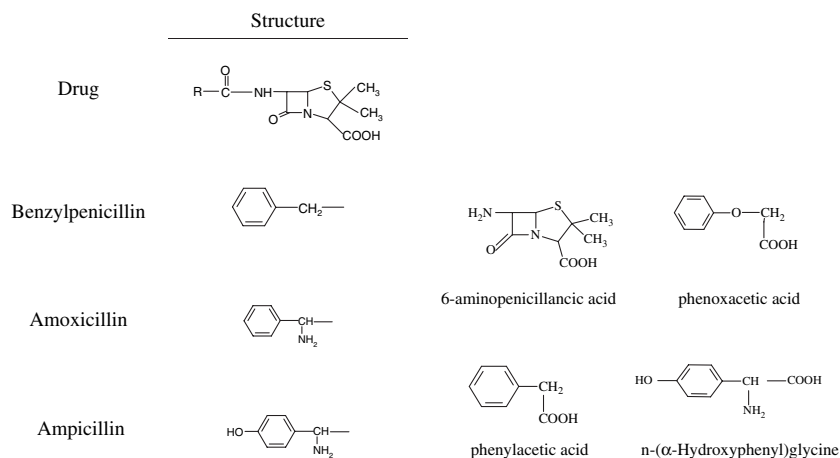


Fig. 4. Structures of some compounds used in radioallergosorbent test inhibition studies.

and L3. The results from serum L1 clearly showed that 6-APA was the weakest inhibitor, regardless of drug-solid phases (BPO-, PVO-PLL) used. Similarly for serum L2, PG, PV, AP or AX, all showed better inhibitory potencies than 6-APA, the significantly weaker inhibitor. For serum L3, 6-aminopenicillanic acid (6-APA) seemed to be an important contributor to the cross-reactivity of drugs. These molecules representing side-chain groups of PV and AX were not as structurally similar as molecules with side-chain groups of PG (19). However, there were similar parts such as the phenyl or benzyl groups in the side-chains (Fig. 4) and these may be the structures involved in antibody recognition.

Overall, the results show that RAST is a valuable method for evaluating persons who are skin test negative to PG, but yet are suspected of being allergic to penicillins. It provides additional information complementary to those of *in vivo* studies. Our results also indicates that the side chains of penicillins could induce specific responses with a variable degree of crossreactivity among different penicillins, but further research should be conducted to better define the exact structures acting as allergenic determinants.

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