

Differing patterns of wheal and flare skin reactivity in patients allergic to the penicillins

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We compared the immediate wheal and flare skin test reactivity to major and minor antigenic determinants from penicillin G, ampicillin, and methicillin in 240 patients. In 57 patients with positive skin tests to one or another of these drugs, a marked variability in the patterns of reactivity was noted. Among the major determinants, 24 patients reacted to only one drug, 5 to all three drugs, and 8 to two of the drugs. Among the minor determinants, 20 patients reacted to only one drug, 5 to all three drugs, and 9 reacted to two drugs. These results show that a positive skin test to one penicillin drug is not necessarily associated with positive reactions to all the penicillin drugs, indicating that skin test reagents to semisynthetic penicillins as well as to penicillin G may be useful.

The introduction of the semisynthetic penicillins raised the hope that these drugs could be given safely to patients who were allergic to penicillin G. However, reactions to the new drugs in patients allergic to penicillin G were soon reported.¹⁻⁶ Presently most physicians consider that a history of penicillin allergy is a contraindication to the use of the semisynthetic penicillins,⁷ and the literature⁸ accompanying these drugs also list hypersensitivity reactions to any of them as contraindications to their use. The presence of skin-sensitizing antibody, as detected by immediate wheal and erythema skin tests, correlates with immediate and accelerated reactions caused by penicillin G,^{9, 10} and skin tests to penicillin G are valuable predictive tests for allergy to this drug. The value of skin tests with the semisynthetic penicillins has been less thoroughly examined.^{6, 11-14} In this study we have compared the wheal and flare skin reactivity in patients with allergic reactions to penicillin G, methicillin, or ampicillin to determine whether any patterns of sensitivity could be detected. The results indicate that patients reactive to one penicillin may not react to another and that patterns of skin reactivity exist.

MATERIALS AND METHODS

Skin test reagents

"Major" and "minor" determinant skin test reagents were prepared for penicillin G (Pen G), ampicillin (Amp), and methicillin (Meth). Penicillin G was purchased from Sigma Chemical Company, St. Louis, Missouri; methicillin and ampicillin were gifts from Bristol Laboratories, Syracuse, New York. Poly-L-lysine (PL), molecular weight approximately 100,000,

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was purchased from Pilot Laboratories, Watertown, Massachusetts, and contained 478 lysine residues per molecule (manufacturer's analysis). The Pen G-PL conjugate was prepared by the method described by Levine.¹⁵ PL was reacted with penicillin G in aqueous solution at pH 11.5 for 90 minutes with 1 mole of drug per mole of epsilon amino group of lysine. Ampicillin and methicillin were reacted with PL in the same manner. The PL-drug conjugates were then succinylated three times with a threefold molar excess of succinic anhydride. Conjugates were exhaustively dialyzed in the cold, initially with an ion-exchange resin (AG1x8) (Biorad Laboratories, Richmond, California) outside of the dialysis bag and finally against 0.15M NaCl. All conjugates were analyzed by duplicate Kjeldahl analysis. Penicilloyl analyses were performed on the conjugates by the penamaldade method.¹⁶ The molar extinction coefficient for ampicillin was not known, and, in calculations to determine the number of penicilloyl groups introduced, the coefficient for penicillin G was used. From 20 to 35 per cent of epsilon amino groups of PL were substituted by the various penicillins when reacted as described above (Pen G-PL, 27 per cent; Meth-PL, 22 per cent; Amp-PL, 32 per cent). The conjugates were diluted in 0.01M K_2HPO_4 - KH_2PO_4 , 0.15M NaCl, pH 7.4 (phosphate-buffered saline, PBS), to a concentration of 1×10^{-6} M of the whole conjugate prior to use in skin testing. PL conjugates were stored frozen at -20° C. Analysis of these frozen conjugates after one year revealed only a slight drop (7 to 14 per cent) in their penicilloyl content.

A minor determinant mixture was prepared for each drug by mixing the crystalline drug in aqueous solution with the alkaline hydrolysis product (presumably the homologous penicilloate) as described by Voss and colleagues.¹⁷ The alkaline hydrolysis product was prepared by reacting the drug in aqueous solution with 1N NaOH at pH 11.5 for 90 minutes after which the pH was adjusted to 7.4 with 1N HCl. The crude mixture was diluted with PBS so that the final concentration was 1×10^{-2} M for each component (assuming 100 per cent yield of the corresponding penicilloic acid). The minor determinant reagents were stored frozen and thawed for use at 4 to 6 week intervals.

All reagents were sterilized by passage through a Millipore filter (pore size 0.22μ), and bacterial cultures were performed to assure sterility. After thawing, the reagents were stored at 4° C. Part way through our study we suspected that the plain drug in the ampicillin minor determinant mixture might deteriorate.* A patient with a recent anaphylactic reaction to ampicillin had a negative direct skin test to the minor determinant mixture for ampicillin, but a skin test with freshly dissolved sodium ampicillin was positive. After this observation, in addition to the minor determinant mixture described above, each drug by itself was prepared fresh just prior to performing the skin tests. The powdered drug was diluted with PBS so that the final concentration was 1.68×10^{-2} M. Thus, there were 3 reagents for each drug: the PL-drug conjugate, the minor determinant mixture, and the freshly prepared drug. Control reagents were the diluent (PBS) and PL, 1×10^{-6} M. In reporting the results, the freshly prepared drug was considered as one of the "minor" determinants.

Of the 240 patients tested, the first 96 or 40 per cent were tested with the PL reagent and the minor determinant mixture for each drug but not the freshly prepared drug, while the last 144 or 60 per cent were tested with all the reagents. Because the patterns of skin test reactivity were essentially the same, we have combined the results from the two groups.

Skin test procedure

Scratch tests were done first with each reagent. The skin of the volar surface of the forearm was abraded but not broken, and one drop of the skin test reagent was placed on the abraded skin. Tests were read 15 minutes later, and a positive reaction was a wheal 3 mm. or greater with a surrounding zone of erythema. Only if scratch tests were negative were intradermal tests performed.

Intradermal immediate wheal and erythema skin tests were done over the deltoid area

*Sodium ampicillin is an alkaline antibiotic, and the pH of solutions of this drug are usually greater than 8. At this pH, degradation to the corresponding penicilloic acid would be favored.

of the upper arm. Approximately 0.005 to 0.01 ml. were injected with a 1 ml. disposable tuberculin syringe and a 0.5 inch, 27 gauge needle. The initial bleb raised by injection was 2 to 3 mm. in diameter; infrequently the initial bleb was up to 4 mm. in size. A positive reaction, measured at 15 minutes, was a wheal 1 to 2 mm. greater than the initial bleb with a surrounding zone of erythema. If the wheal at 15 minutes was the same size as the initial bleb, the skin test was considered negative. We noted in some instances that the wheal increased in size but there was no erythema, and at other times erythema was present without a measurable increase in wheal size. Both of these were considered as negative responses. If the resultant wheal at 15 minutes was 1 to 2 mm. greater than the initial bleb, it was graded +1. A +2 test was a wheal 3 to 5 mm. larger than the initial bleb, +3 was 6 to 8 mm. larger, and +4 was 9 mm. greater than the initial bleb. All patients with positive skin tests did not react to either control.

Patients

All subjects were interviewed by the authors and included patients (1) who had recent suspected allergic reactions to one of these drugs, (2) who gave a history suggestive of penicillin allergy and required treatment with one of the penicillins, or (3) who gave a history suggestive of penicillin allergy and who agreed to have the skin tests performed.

RESULTS

Skin tests were performed on 240 patients. Among this group 57 had positive skin tests. Twenty-three were positive to the major determinant reagents alone, 20 were positive to the minor determinant reagents alone, and 14 were positive to both major and minor determinants. Among patients with positive tests, the frequency of reactions to the major determinants alone, the minor determinants alone, and both major and minor determinants is remarkably similar to that reported by Levine and co-workers¹⁸ in an earlier report on penicillin G skin tests.

We have listed the skin test results in patients with positive reactions in Tables I and II. The patterns of major determinant skin reactions (37 pa-

TABLE I. Major determinant skin reactions in 37 patients

Patient No.	Skin test reagent		
	Pen G-PL	Meth-PL	Amp-PL
20	+	-	-
2	-	+	-
2	-	-	+
5	+	+	+
7	+	+	-
1	+	-	+

TABLE II. Minor determinant skin reactions in 34 patients

Patient No.	Skin test reagent		
	Pen G	Meth	Amp
16	+	-	-
1	-	+	-
3	-	-	+
5	+	+	+
2	+	+	-
6	+	-	+
1	-	+	+

tients) are shown in Table I. Twenty patients were positive to Pen G-PL alone, 2 to Meth-PL alone, and 2 to Amp-PL alone. Five of the 37 reacted to all three PL-drug conjugates. Seven patients reacted to Pen G-PL and Meth-PL but not Amp-PL. The patterns of reactivity to the minor determinants (34 patients) are shown in Table II. Sixteen patients reacted to penicillin G alone, one to methicillin alone, 3 to ampicillin alone, and 5 of the 34 to all three drugs. Six patients reacted to the minor determinants of penicillin G and ampicillin but not methicillin. Results from individual patients with positive reactions are shown in Table III. Examples of patients reactive to only one drug, to all three drugs, to two of the drugs, to minor determinants only, and to major determinants only are listed. Patients 6 and 7 had positive minor determinant skin tests to penicillin G and ampicillin but negative reactions to methicillin. This pattern was found in 6 patients and was very striking in several, such as Patient No. 7 who had markedly positive scratch tests to penicillin G and ampicillin and a negative intradermal test to methicillin.

We had the opportunity of treating two patients with a drug to which the skin tests were negative despite positive skin tests to one of the other penicillin drugs.

Case reports

Patient N. G. was a 56-year-old man who developed a rash in December, 1965, while being treated with methicillin after insertion of a Starr-Edwards prosthesis in the aortic valve. In the summer of 1969, he was treated elsewhere with ampicillin orally without difficulty. In October of 1969, intradermal skin tests were positive to the minor determinants of penicillin G (+2). They were negative to ampicillin, and he was again treated with ampicillin without a reaction.

Patient R. P. was a 20-year-old man who had a rash after antibiotic therapy with ampicillin and lincomycin in June of 1967. He was seen here in March of 1968 for treatment of osteomyelitis. He had positive intradermal skin tests to ampicillin (+1) but negative tests to methicillin and was treated with methicillin without a reaction.

TABLE III. Examples of patterns of wheal and flare skin test reactivity

Patient	PL-conjugates			Minor determinants			Type of allergic reaction
	Pen G	Meth	Amp	Pen G	Meth	Amp	
1	+3	0	0	0	0	0	Hives after penicillin*
2	0	+2	0	0	0	0	Petechial rash after penicillin
3	0	0	+2	0	0	0	Rash after nafcillin
4	+4	+4	+4	0	0	0	Urticaria after penicillin
5	+2	+1	0	0	0	0	Hives after methicillin and penicillin G
6	+4	0	0	+4	0	+2	Immediate reaction to penicillin
7	0	0	0	Sc† +4	0	Sc +4	Anaphylaxis from oral penicillin
8	0	0	0	+4	+2	+4	Hives after penicillin
9	+2	+1	0	+2	0	0	Hives after penicillin
10	0	0	0	+2	0	0	Rash after methicillin
11	+4	+3	+3	0	0	+4	Hives 10 minutes after procaine penicillin G
12	0	0	0	Sc +4	Sc +2	Sc +4	Extreme sensitivity to penicillin G; hives and wheezing when in room where penicillin G used

*In patients with reactions to "penicillin" we could not determine which type of penicillin was involved.

†Sc = scratch tests; other tests were intradermal.

Of the 57 patients with positive skin tests, 20 were positive only to the drug suspected of causing the reaction, 24 patients were positive to this drug as well as others of the drugs tested, and 13 patients did not react to the suspected drug but were positive to one or both of the other drugs. Explanations for this latter finding are discussed below.

DISCUSSION

We have studied wheal and erythema skin test reactivity to three penicillin drugs: penicillin G, methicillin, and ampicillin, and our results indicate that there is a marked variability in skin test responses. Many patients reacted to only one of the drugs, others reacted to all three of the drugs, and some to two only. That certain patients reacted to all three drugs is in keeping with the earlier reports of Stewart,⁶ who described 5 patients with positive skin tests to both penicillin G and methicillin, and Parker and Thiel,¹² who demonstrated skin test reactions to several drug-polylysine conjugates in patients with skin test reactions to penicillin G. Others^{13, 14} have also found positive immediate skin tests to semisynthetic penicillin in patients with penicillin G allergy. Other patients, however, who reacted to only one or two of the drugs tested demonstrate that patients with positive tests to one of the penicillins are not necessarily positive to all of them. This view is supported by the results of Parker and Thiel¹² in one patient, by those of Shibata and co-workers,²² and by the findings of Luton.¹¹ The latter¹¹ reported on 8 patients with prior anaphylactic reactions to penicillin G, positive skin tests to penicillin G, and negative skin tests to methicillin. These patients were given 1 Gm. of methicillin intramuscularly and had no allergic reactions. We observed 2 patients who had positive skin tests to one of the penicillins but negative skin tests to another penicillin which was then used in therapy. Neither patient had any reaction. The studies of Levine and Zolov⁹ as well as our own¹⁰ have demonstrated that skin tests for major and minor determinants are valuable for prediction of penicillin G allergy, and presumably these findings are applicable in the case of any one of the semisynthetic penicillins. Our results suggest that within the group of penicillin drugs skin test reagents may be used to determine whether a patient can receive a given penicillin despite allergy to another, but further studies are needed.

The variable reactions in our patients suggest that skin-sensitizing antibodies may make subtle distinctions between the penicillin drugs. The formulas for the three drugs studied are shown in Fig. 1. The beta lactam ring and the thiazolidine ring are the same in all three drugs, but the side chains differ. Although the structure of the major antigenic determinant, the penicilloyl groups, is known for these drugs, little information concerning the structure of "minor" antigenic determinants exists.¹⁹ Although Levine²⁰ concluded that a large part of rabbit antibody to the benzylpenicilloyl (BPO) determinant is adapted to the entire group, Atsumi and associates²¹ have reported that antibody to the BPO group can be subfractionated into populations having different reactivities with the side chain and nuclear portions of the BPO group. They suggested that antibodies adapted to the penicilloyl nucleus would display greater cross-reactivity among semisynthetic penicillins than antibody adapted

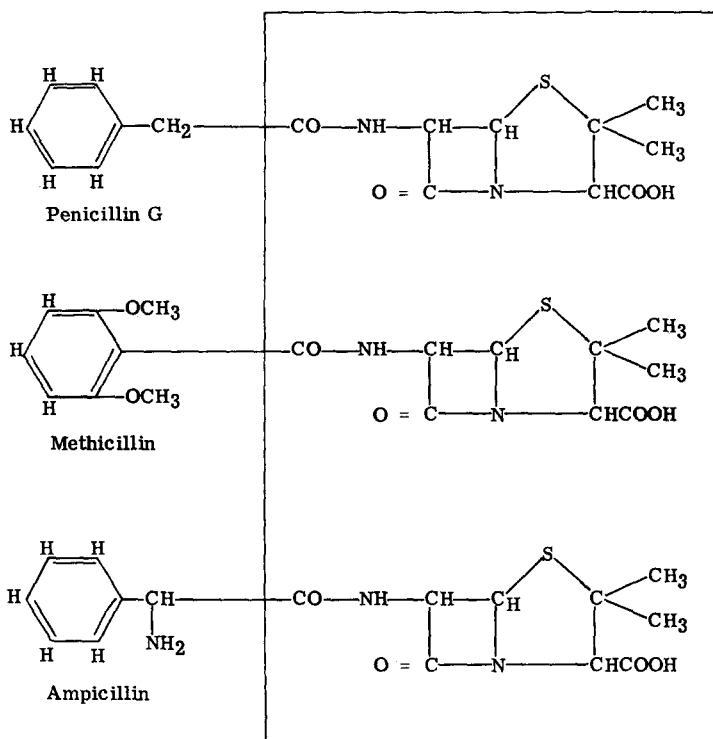


FIG. 1. Formulas for the drugs tested. The box encloses the portion of each drug which is shared by the others.

to the side chains. In an analysis of the heterogeneity of human skin-sensitizing antibodies to penicillin, Shibata and his associates²² presented evidence suggesting that as many as eight kinds of antibody molecules, differing in their binding properties, existed, and our results are in keeping with their findings.

Thirteen of our patients had negative skin tests to the suspected drug but positive tests to one of the other drugs. There are several possible explanations for this anomalous finding:

1. The patients did not correctly identify the implicated drug, the general term penicillin being used to refer to all the penicillin drugs. For example, one patient reported "penicillin allergy," and we subsequently learned that the allergic reaction had been to ampicillin.

2. This finding might represent an example of the "original antigenic sin." The concept that patients immunized with one antigen will respond with antibodies to that antigen when challenged with a cross-reacting antigen was originally demonstrated with influenza virus,²³ and Levine and associates²⁴ have suggested that the original antigenic sin can also be committed with skin-sensitizing antibody in penicillin allergy. Seven of the 13 patients had allergic reactions to methicillin or ampicillin, negative skin tests to these drugs, and positive skin tests to penicillin G.

3. Possibly a given semisynthetic penicillin might form a "minor" determinant more rapidly than does penicillin G. This might explain a skin reac-

tion to a given semisynthetic penicillin in the face of a negative response to penicillin G.

4. We did the skin tests at only one concentration. Some patients might have had positive skin tests to some of the drugs if they were tested with higher concentrations of reagents.²⁵

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