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Diagnosing Nonimmediate Reactions to Penicillins by in vivo Tests

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Key Words

Penicillins · Challenges · Intradermal tests ·
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Abstract

Background: Maculopapular and urticarial rashes are nonimmediate manifestations common during penicillin treatment; the former often represent cell-mediated hypersensitivity. Our objectives were to assess the incidence of allergy in adults reporting nonimmediate manifestations during penicillin therapy and to evaluate the diagnostic potential of patch tests, delayed-reading skin tests and challenges in such cases. **Methods:** We used prick and intradermal tests as well as patch tests with penicillin determinants, ampicillin, amoxicillin and any other suspect penicillins. We also performed challenges with the suspect antibiotics. **Results:** Such antibiotics were aminopenicillins in 93.1% of 259 patients, most of whom had suffered from maculopapular rashes followed by piperacillin (4.2%). Three subjects displayed immediate skin test positivity. Ninety-four subjects showed patch test and delayed intradermal test positivity to the culprit penicillin (90 to aminopenicillins and 4 to piperacillin) and were considered as having had delayed hypersensitivity reactions. Five of the 8 subjects who dis-

played delayed intradermal test positivity and patch test negativity to the suspect penicillin underwent challenges, 2 reacted positively to the responsible aminopenicillin. Among the remaining 154 with negative results in allergologic tests, 125 agreed to undergo challenges; only 3 reacted. In all, 98 patients – 93 of whom had experienced maculopapular rashes – displayed delayed hypersensitivity (94 to aminopenicillins and 4 to piperacillin). **Conclusions:** Both patch and intradermal tests are useful in evaluating nonimmediate reactions to penicillins, particularly maculopapular rashes. Patch test and delayed intradermal positivity together indicate delayed hypersensitivity. Intradermal testing appears to be slightly more sensitive than patch testing.

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Introduction

Penicillins cause several kinds of allergic reactions classifiable as immediate or nonimmediate according to the time interval between drug administration and their onset [1, 2]. The former occur within the 1st hour after drug administration and are manifested clinically by urticaria and/or angioedema, anaphylactic shock, rhinitis and bronchospasm [3, 4]. Nonimmediate reactions are those

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occurring more than 1 h after drug administration. The main nonimmediate reactions are delayed-appearing urticaria/angioedema and maculopapular or morbilliform rashes [3, 4]; the latter are common particularly during treatment with ampicillin (AM) and amoxicillin (AX) [5–7].

In the last few years, increasing attention has been paid to nonimmediate hypersensitivity reactions to systemically administered drugs, with clinical, immunohistological and laboratory studies suggesting that a type IV (cell-mediated) pathogenic mechanism may be involved [8–13].

Hertl et al. [14] isolated CD8+ T cell clones from the peripheral blood of patients who had suffered from maculopapular eruptions because of delayed hypersensitivity to penicillins. Other researchers [15, 16] have found increased percentages of circulating cutaneous lymphocyte-associated antigen-positive T cells in patients with penicillin-induced nonimmediate eruptions compared with healthy controls. In addition, the presence of the activation marker HLA-DR in the cutaneous lymphocyte-associated antigen-positive T cell population of patients was also increased. Recent studies [17, 18] provided further evidence that cytotoxic T cells containing perforin and granzyme B play an essential role in maculopapular exanthems induced by several drugs, including β -lactams like AX, cefazolin and ceftriaxone.

Moreover, since 1966 there have been studies suggesting that the delayed reading of intradermal skin tests and/or patch tests can be an effective way of diagnosing nonimmediate allergic reactions to systemically administered β -lactams, such as maculopapular and urticarial rashes [1, 2, 19–29]. In some cases, challenges with suspect β -lactams have confirmed allergologic test positivity [2, 20, 23–26, 29]. Many of these studies regarding patients who developed delayed hypersensitivity reactions to β -lactams indicate that these conditions are often associated with late responses to both intradermal (more than 6 h after injection) and patch testing [2, 23–25, 27, 28].

However, only few studies have been carried out with large samples of subjects previously classified as nonimmediate reactors to penicillins [2, 22, 24]; the percentage of positive responses to patch tests with aminopenicillins (AM and AX) was, respectively, 35.9 [22] and 40.2% [2], that of positive responses to unspecified penicillins 41.4% [24]. In two of these studies, patients were also assessed with delayed-reading intradermal tests, showing a very different percentage of positive responses: 38 out of 40 (95%) [24] versus 62 out of 144 (43%) [2]. In the former study, however, the amount (0.1 ml) of reagents used for

intradermal testing was larger than that (0.02 ml) used in the latter. Many other studies investigated subjects selected on the basis of positive responses to allergologic tests [21, 23, 29]; therefore, their results are not comparable.

In the present study, a specific diagnostic protocol with the standard techniques – skin tests, patch tests and challenges – was applied to patients with nonimmediate reactions to penicillins in an attempt to clarify their pathogenic mechanism. Such a protocol has been described in a previous study [2], in which 144 subjects of the present study who suffered from nonimmediate reactions to aminopenicillins were first evaluated.

Materials and Methods

We studied all patients seen by our staff between January 1994 and April 2001 reporting nonimmediate reactions during penicillin therapy. We carefully questioned patients to identify the suspect antibiotic. Symptoms were classified on the basis of medical records or, failing that, according to patients' descriptions. In particular, maculopapular rashes on different areas of the skin and skin wheal and flare reactions were distinguished. Those subjects who initially could not specify their cutaneous reactions did so after we had shown typical pictures of maculopapular and urticarial eruptions. After the informed consent of the patients (or the parents of those under 18 years of age), they underwent the following immunoallergologic tests.

Prick and Intradermal Skin Tests

They were carried out using the following: (1) Penicilloylpolylysine (PPL; Allergopen, Reinbeck, Germany). The undiluted solution was obtained by reconstituting the lyophilized contents of a vial containing 0.175 mg PPL plus 20 mg mannitol in 5 ml buffer. A 1:10 dilution of this solution in 0.9% NaCl was initially used; when results were negative, testing was repeated with the undiluted solution. (2) Minor determinant mixture (Allergopen), containing 0.6 mg benzylpenicillin (BP) and 0.5 mg benzylpenicilloate per milliliter, initially diluted 1:100 in 0.9% NaCl. Tests were repeated with the undiluted dilution when initial results were negative. (3) BP diluted in 0.9% NaCl and administered at concentrations of 100 and 10,000 IU/ml. (4) AM and AX, both at concentrations of 1 and 20 mg/ml, after dilution in 0.9% NaCl. (5) Piperacillin, at concentrations of 1 and 20 mg/ml, after dilution in 0.9% NaCl, was also used in subjects with adverse reactions to this penicillin.

Reactions were considered positive when a wheal larger than 3 mm in diameter was present 15–20 min later. When negative results were observed, 0.02 ml of the hapten solution was injected intradermally on volar forearm skin, and readings were made at 20 min. Results were considered positive when wheals larger than 5 mm were present. Positive controls for prick and intradermal tests were performed with histamine (10 and 1 mg/ml, respectively); normal saline was used as a negative control. Reading of late reactions to intradermal tests was done after 48 and 72 h; any infiltrated erythema with a diameter larger than 5 mm was considered a positive reaction.

Control intradermal tests with AM, AX, and piperacillin were also administered to 30 healthy subjects who had previously been

treated with one or more of these penicillins without any adverse effects.

Patch Tests

They were administered with (1) BP, (2) AM, (3) AX (5% in petrolatum; FIRMA, Florence, Italy), and piperacillin (at a concentration of 200 mg/ml in 0.9% NaCl).

All reagents were applied to uninvolved skin on the interscapular region of the patient's back, using acrylate adhesive strips with small plates attached for test allergens (Curatest, Lohmann, Neuwied, Germany). Occlusion time was 48 h. Readings were made as recommended by Wilkinson et al. [30], 15 min after the removal of the strips and 24 h later. Control patch tests in 30 healthy subjects who had previously tolerated one or more of these penicillins were also performed.

Radioallergosorbent Test

In vitro assays for antigen-specific IgE to penicilloyl G, penicilloyl V, AM and AX were performed using the Phadebas RAST (Pharmacia, Uppsala, Sweden) in the first 165 subjects, 144 of whom with adverse reactions to aminopenicillins.

Challenges

Subjects who were negative in all of the above tests or displayed delayed intradermal test positivity and patch test negativity underwent challenges with one of the following penicillins: AM (500 mg) or AX (500 mg) orally, or BP (1,000,000 IU), benzathine penicillin (1,200,000 IU) or piperacillin (1 g) intramuscularly. Each patient was challenged with the penicillin suspected in his or her case. We used 500 mg of AM and AX as in our previous studies [2, 25]. With regard to the other penicillins, we chose the single dose used in the therapeutic courses during which patients had experienced their cutaneous eruptions. We administered an initial dose of one hundredth of the therapeutic one (indicated in parentheses above). In cases with negative results, 1 week later we administered a dose of one tenth and, if the result was again negative, after another week a full dose. Informed consent was obtained from all subjects, who were admitted to a day hospital for 12 h for administration of the challenge.

On the basis of the results of our previous study [25], we felt it was unjustified to perform challenges that could cause suffering in patients displaying patch test and delayed intradermal test positivity to the suspect drug. In fact, all the 18 patients of the study who displayed this pattern of positivity and agreed to undergo oral challenges with the culprit aminopenicillins reacted positively.

Results

We examined a total of 259 adults (188 females, 71 males), ranging in age from 13 to 84 years (mean age: 37.02 ± 16.38 years) with a history of nonimmediate reactions to β-lactams. Our workup was performed 46.82 ± 73.90 months (range: 1–540) after the most recent adverse reaction.

Two hundred and forty-one subjects (93.1%) reported adverse reactions to aminopenicillins, 11 (4.2%) to piperacillin, 4 (1.5%) to benzathine penicillin and 3 (1.1%) to

Table 1. Manifestations reported by the subjects and results of allergologic evaluations

Symptoms	Patients	Patients with DH	Patients with IH
Maculopapular rash	173	93 (53.7)	1 (0.6)
Urticaria	33	–	1 (3)
Erythema	22	4 (18.2)	1 (4.5)
Angioedema	13	–	–
Urticaria/angioedema	13	–	–
Local reaction	1	1 (100)	–
Other manifestations	4	–	–
Total	259	98 (37.8)	3 (1.1)

Figures in parentheses represent percentage.
DH = Delayed hypersensitivity; IH = immediate hypersensitivity.

BP. AX was the suspect aminopenicillin in 107 patients, followed by AM in 59 and bacampicillin (BC) in 48. Twenty-seven subjects had suffered from distinct reactions to different aminopenicillins in separate episodes (16 to AM/AX, 6 to AX/BC, 4 to AM/BC, 1 to AX/AM/BC).

The most frequent clinical manifestations described by the subjects were maculopapular rashes (173 cases) followed by urticaria and urticaria-angioedema (table 1). One patient suffered from linear IgA bullous dermatosis, a subepidermal blistering disease characterized by linear deposition of IgA along the basement membrane zone, which can be provoked by many different drugs, even in the same patient [31].

Three subjects displayed immediate skin test positivity. They had experienced, respectively, an urticarial reaction 2 h after a benzathine penicillin intramuscular injection, an erythema 4 h after BC, and a maculopapular rash during the 6th day of AX therapy; all displayed intradermal test positivity to PPL, and 1 patient also a slight radioallergosorbent test (RAST) positivity to penicilloyl V (0.68 kU/l). The patient with maculopapular rash may have had a false-positive response to the immediate-reading intradermal test; however, he was not challenged.

Ninety-four subjects showed patch test and delayed intradermal test positivity to the culprit penicillins (90 to aminopenicillins and 4 to piperacillin), which indicates delayed hypersensitivity [25]. Eight patients displayed delayed intradermal test positivity and patch test negativity to aminopenicillins, and 154 were negative in allergologic tests.

Table 2. Patterns of skin test and patch test reactivity in 94 patients with delayed hypersensitivity to aminopenicillins

Patients	Intradermal-test delayed response				Patch test response		
	MDM	BP	AM	AX	BP	AM	AX
62	-	-	+	+	-	+	+
12	-	+	+	+	+	+	+
9	-	+	+	+	-	+	+
6	+	+	+	+	+	+	+
1	+	+	+	+	-	+	+
3	-	-	+	+	-	-	-
1	-	+	+	+	-	-	-

MDM = Minor determinant mixture.

With regard to aminopenicillins, patch test and delayed intradermal test positivity to AM and AX constituted the main pattern of reactivity, followed by patch test and delayed intradermal test positivity to BP, AM and AX (table 2). All the 9 subjects with delayed intradermal test positivity to BP, AM and AX and patch test positivity only to AM and AX reacted to intradermal tests with the BP concentration of 10,000 IU/ml, and 2 of them also to 100 IU/ml. All patients with delayed reactions to intradermal skin tests with AM and AX reacted to both the 1- and 20-mg concentrations.

Of the 4 patients who had suffered from maculopapular eruptions during aminopenicillin therapy and displayed delayed intradermal test positivity and patch test negativity, one tolerated the suspect aminopenicillin; another reacted to the challenge; the other 2 were not challenged because of, respectively, ulcerative rectocolitis and a slight maculopapular rash that appeared 12 h after intradermal tests. We diagnosed the last 3 subjects as having had delayed hypersensitivity reactions (the one with ulcerative rectocolitis because she had experienced two distinct reactions to AM and AX in separate episodes which had persisted for about 2 weeks after the antibiotic had been withdrawn and steroid treatment started).

Three of the 4 patients with aminopenicillin-induced nonmaculopapular eruptions, who showed delayed intradermal test positivity and patch test negativity, consented to undergo oral challenges. Of the 2 subjects who had suffered from erythematous rashes, 1 reacted positively 5 h after AX administration (5 mg), developing the same type of eruption; the other tolerated AM. The third subject, who suffered from recurrent urticaria and had experienced an urticarial rash on the 3rd day of AX thera-

py, did not react to the challenge; laboratory tests detected 760 IU/ml of antibodies against HBsAg.

Of the 141 patients (74 with maculopapular rashes and 67 with nonmaculopapular manifestations associated with aminopenicillin therapy) presenting negative results in allergologic tests (both immediate and delayed-type skin tests and RASTs), 117 agreed to submit to challenges with the suspect antibiotic (AX in 81 and AM in 36). Only 1 of the 64 patients with maculopapular rashes reacted to 50 mg of AM; in this patient, laboratory tests detected a high titer of antibodies against Epstein-Barr virus: 170 AU/ml of IgG against viral capsid antigen (normal range: 0-20) and 198 AU/ml of IgG against Epstein-Barr nuclear antigen (normal range: 0-20), indicating a previous infection.

Among the 53 subjects with nonmaculopapular manifestations, two reacted: one who suffered from linear IgA bullous dermatosis and another who had experienced oral and vaginal ulcers during aminopenicillin therapy; both suffered from the same reaction as before.

Overall we found 94 cases (39%) of delayed hypersensitivity among the 241 subjects who had experienced adverse reactions to aminopenicillins: 90 of the 166 (54.2%) with maculopapular rashes and 4 of the 19 (21%) with an erythema. With regard to subjects who had reacted to penicillins other than aminopenicillins, 4 displayed delayed intradermal test and patch test positivity to piperacillin (3 of them also to AM and AX); 3 of the 4 had suffered from maculopapular eruptions, and 1 from a local reaction to intramuscular piperacillin. Of the 13 subjects with negative results in allergologic tests, 8 agreed to undergo challenges (5 with piperacillin, 3 with benzathine penicillin); none reacted.

Discussion

Patch tests and delayed-reading intradermal tests are useful tools in evaluating nonimmediate reactions to penicillins, particularly morbilliform rashes: 93 (53.7%) of the 173 subjects with maculopapular rashes were diagnosed as having had a delayed hypersensitivity on the basis of these tests (table 1).

Intradermal testing produced a slightly larger number of delayed positive responses than patch testing. In fact, there were 94 subjects who presented both delayed intradermal test and patch test positivity and 8 with patch test negativity and delayed intradermal test positivity. The reasons for this phenomenon, which has been observed by others [23, 24, 28], are not clear, but it may be connected

with the fact that the haptens bind different molecules within the skin. Moreover, the drug being tested may be unable to penetrate into the epidermis, or the concentration used in patch testing may be too low to elicit a positive reaction. However, false-positive delayed intradermal responses exist. Therefore, in selected cases, such patterns of skin responses could be assessed using provocation tests with culprit penicillins. The RAST did not prove to be useful, since the only positive case also displayed an immediate response to the intradermal test with PPL.

Both delayed intradermal test positivity plus patch test positivity and delayed intradermal test positivity confirmed by the challenge indicate a type IV hypersensitivity. With regard to 94 subjects with such hypersensitivity to aminopenicillins, 65 of them (69.1%) reacted only to AM and AX (table 2). Therefore, in patients reporting reactions to aminopenicillins it is important to perform skin tests with these β -lactams in addition to penicillin determinants (PPL, BP and minor determinant mixture). Other *in vivo* studies have shown similar results in patients suffering from nonimmediate reactions to aminopenicillins [2, 13, 22, 25, 26], confirming the importance of side chain determinants in such patients, as demonstrated by Voorhost and Sparreboom [32]. It is interesting to note that the 4 subjects with delayed hypersensitivity to piperacillin also displayed negative results to penicillin determinants.

Delayed hypersensitivity to β -lactams, particularly aminopenicillins, is a long-lasting condition, which does not appear to be influenced by the time interval between the last adverse reaction and allergologic testing [2, 25]. Therefore, it is unlikely that patients who have experienced delayed hypersensitivity reactions, particularly maculopapular rashes, and display negative testing results will have lost their hypersensitivity.

In some studies, which evaluated subjects with maculopapular eruptions during penicillin therapy, negative results in patch and/or intradermal tests using an appropriate panel were confirmed by the negativity of provocation tests with the suspect penicillin [2, 25, 27, 33].

In the present study, all but one of the 65 challenged subjects who reported a maculopapular rash during penicillin therapy and had negative results in allergologic tests responded negatively to challenges with the suspect antibiotic. All patients with nonimmediate urticarial and/or angioedematous reactions who had both negative intradermal and patch test results and agreed to undergo challenges tolerated the suspect penicillin. In effect, drug-associated urticaria, usually interpreted as a manifestation of

allergy, may be caused by the infectious organism itself [34]. Urticarial (as well as maculopapular) eruptions may also result from interaction between viruses and antibiotics, as in infectious mononucleosis [35]. According to the hypothesis of Pichler et al. [10], during viral infections, expression of major histocompatibility complex molecules in the tissue is stimulated, probably by high interferon- γ levels. This would improve direct binding of penicillins to these molecules and thus boost drug presentation. Reactive T cells would be stimulated in the tissue and provoke allergic symptoms. However, cases of urticarial delayed hypersensitivity reactions to β -lactams have been diagnosed on the basis of delayed intradermal test or patch test positivity [13, 19, 26, 28, 29].

In some studies [13, 23, 26], there were also patients with negative results in allergologic investigations who reacted to the provocation test with the suspect β -lactam by experiencing nonimmediate cutaneous eruptions, particularly maculopapular and urticarial. In particular, Luque et al. [13] assessed 19 patients with nonimmediate reactions (12 urticaria and 7 exanthema) to β -lactams using delayed-reading intradermal tests, lymphocyte transformation tests and provocation tests: 11 of the 19 (57.9%) were positive to lymphocyte transformation tests; 7 of the 19 (36.8%) displayed positive responses to intradermal tests and were not challenged, and the remaining 12 reacted to the challenge. Therefore, considering the data of the present study as well as those of the literature, false-negative results in patch testing and delayed-reading intradermal testing are possible.

Furthermore, a preliminary study performed in the USA did not find any positive results in 15 subjects with delayed cutaneous reactions to penicillins, who, however, did not undergo challenges with the suspect antibiotic [36]. Therefore, results of a diagnostic protocol including both delayed-reading intradermal tests and patch tests might vary according to the population assessed. Such a possibility is supported by the demonstration that delayed hypersensitivity to aminopenicillins is related to major histocompatibility complex genes [37].

In cases of positive reactions to challenges with suspect drugs in patients displaying negative results in skin and patch tests, avoidance of penicillin therapy should be advised even though the pathogenic mechanism remains undetermined.

In conclusion, it is difficult to attribute a nonimmediate cutaneous eruption to either infection or drug intake only on the basis of the clinical history. Our diagnostic workup allowed us to clarify the nature of the manifestations experienced by 229 (88.4%) of the 259 patients stud-

ied. Immediate hypersensitivity was diagnosed in 3 subjects (1.1%) and delayed hypersensitivity in 98 (37.8%), while in 125 (48.3%) negative challenge results were a reasonable indication that allergy was not involved; 3 sub-

jects (1.1%) reacted to challenges despite negative results in allergologic tests. Definitive diagnoses could not be provided for the remaining 30 (11.6%), who did not consent to undergo challenges.

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