Allergy 2007: 62: 53–58

DOI: 10.1111/j.1398-9995.2006.01272.x

Original article

A comparison of the performance of two penicillin reagent kits in the diagnosis of β-lactam hypersensitivity

Background: Skin testing with penicilloyl polylysine (PPL) and minor determinant mixture (MDM) represents the first-line method for diagnosing β-lactam hypersensitivity. However, in 2004, Allergopharma and Hollister-Stier announced their decision to stop the production of penicillin reagents (Allergopen® and PrePen®, respectively) within 1 year. Therefore, we decided to compare PPL and MDM from Allergopharma (Allergopen) with those from Diater (DAP®).

Methods: We skin-tested 195 subjects with both Allergopen and DAP reagents, as well as with other β-lactams; 74 (group A) had had immediate reactions to β-lactams and 74 (group B) nonimmediate reactions, while 47 (group C) underwent prophylactic tests.

Results: One hundred two patients (52.3%) had positive skin tests; 29 (14.9%) were positive to PPL and/or MDM. Of the 102 skin-test-positive patients, 44 belonged to group A, 57 to B and 1 to C; the last was positive only to Allergopen PPL (PPL-A) and tolerated the benzylpenicillin challenge. Minor determinant mixture reagents produced identical results in the 148 patients of groups A and B, 22 (14.9%) of which were positive. Both PPL reagents produced negative results in 139 of these 148 patients and positive ones in 5; one subject was positive to DAP PPL (PPL-D) and negative to PPL-A, while three patients were positive to PPL-A and negative to PPL-D; two of the latter tolerated benzylpenicillin challenges.

Conclusions: Minor determinant mixture reagents produced identical results in all 195 patients. Results of skin testing with PPL reagents were concordant in 190 (97.4%) of them. Therefore, DAP reagents are a reliable alternative to Allergopen ones.

A. Romano[1,2], M. Viola[1], P. J. Bousquet[3], F. Gaeta[1], R. Valluzzi[1], C. Caruso[1], P. Demoly[3]

[1]Department of Internal Medicine and Geriatrics, UCSC-Allergy Unit, Complesso Integrato Columbus, Rome, Italy; [2]IRCCS Oasi Maria S.S., Troina, Italy; [3]Exploration des allergies et INSERM U454, Hôpital Arnaud de Villeneuve, Montpellier Cedex, France

Key words: β-lactams; cephalosporins; penicillins; reagents; skin tests.

Prof. Antonino Romano
Department of Internal Medicine and Geriatrics
Allergy Unit
Complesso Integrato Columbus
Via G. Moscati, 31
00168 Rome, Italy

Accepted for publication 11 October 2006

Diagnostic protocols for evaluating subjects with either immediate (i.e. occurring within the first hour after drug administration) or nonimmediate (i.e. occurring more than 1 h after drug administration) hypersensitivity reactions to β-lactams have recently been devised by the European Network for Drug Allergy (ENDA), the European Academy of Allergology and Clinical Immunology interest group on drug hypersensitivity (1, 2). In both ENDA protocols—as well as in the American practice parameters (3)—skin testing with penicilloyl polylysine (PPL) and minor determinant mixture (MDM) represents the first-line method for diagnosing hypersensitivity reactions to β-lactams. Such protocols recommend the use of benzylpenicillin, amoxicillin, ampicillin and any other suspect β-lactam, in addition to PPL and MDM.

Abbreviations: ENDA, European Network for Drug Allergy; IgE, immunoglobulin E; MDM, minor determinant mixture; NSAIDs, nonsteroidal anti-inflammatory drugs; PPL, penicilloyl polylysine.

Moreover, two recent studies emphasized the importance of skin testing with PPL and MDM in diagnosing β-lactam hypersensitivity (4, 5). Bousquet et al. (4) observed positive skin tests in 136 (16.5%) of 824 patients with histories suggestive of a β-lactam allergy; 20 (14.7%) of them were positive only to PPL and/or MDM. Matheu et al. (5) diagnosed a hypersensitivity in 44 (9.5%) of 463 patients with adverse reactions to β-lactams; 21 (47.7%) of the sensitive patients displayed positive skin tests only to PPL and/or MDM. However, these two studies did not distinguish sufficiently between immediate and nonimmediate reactions. With regard to the diagnosis of the latter, the contribution of skin testing with PPL and MDM is very limited. In a study of ours (6), only 7 (7.4%) of 94 patients with a cell-mediated hypersensitivity to aminopenicillins were positive to MDM and none to PPL; all seven patients were also positive to ampicillin and amoxicillin.

As far as the diagnosis of immediate reactions is concerned, the percentage of positive responses to skin tests with the classic penicillin reagents (PPL, MDM and

benzylpenicillin) varies, depending on the responsible β-lactams and populations evaluated. In two recent studies regarding subjects with immediate hypersensitivity reactions to cephalosporins (7, 8), the rate of positive responses to penicillin determinants was 11.8% (9 of 76 patients) and 1.6% (2 of 127), respectively. On the contrary, 125 (43.1%) of 290 adults with immediate reactions to penicillins assessed by Torres et al. (9) presented skin-test positivity to PPL and/or MDM. With regard to children, Atanasković-Marković et al. (10) evaluated 1170 subjects with immediate reactions to penicillins and/or cephalosporins, diagnosing a hypersensitivity in 682; 591 (86.7%) of the latter were skin-test positive to at least one penicillin reagent.

Nevertheless, in 2004, Allergopharma (Hamburg, Germany) and Hollister-Stier (Spokane, WA, USA) announced their decision to stop the production of both PPL and MDM (Allergopen® and PrePen®, respectively) within 1 year. Therefore, we decided to compare PPL and MDM from Allergopharma with those from Diater S.A. (DAP®, Madrid, Spain), which have been available on the market since 2003. To address this question, a large group of subjects was evaluated by skin tests with both Allergopen and DAP reagents.

Methods

Patient selection

We studied all subjects with histories of hypersensitivity reactions to β-lactams seen in the allergy units of Complesso Integrato Columbus and Oasi Maria Santissima between June 2004 and June 2005. We also re-evaluated some patients with a cell-mediated hypersensitivity to penicillins. Symptoms were classified on the basis of medical records or, failing that, according to patients' descriptions. Patients requiring prophylactic allergologic tests to β-lactams because of histories of adverse reactions to non-β-lactam antibiotics or non-steroidal anti-inflammatory drugs (NSAIDs) were also included.

Prior to the study, all subjects received information about possible risks of allergologic tests, and a written informed consent was obtained from each patient or the parents of those under 18 years of age. The protocol was approved by the respective institutional review boards.

Skin and patch testing

In patients with histories of immediate reactions to β-lactams (group A), skin tests with penicillin reagents were performed on two different days (11). On the first day, prick and intradermal tests were carried out using Allergopen PPL (PPL-A, final concentration: 5×10^{-5} mmol/l), DAP PPL (PPL-D; final concentration: 1.07×10^{-2} mmol/l), Allergopen MDM (MDM-A, benzylpenicillin and sodium benzylpenicilloate; final concentration: 2×10^{-2} mmol/l), DAP MDM (MDM-D, benzylpenicillin, sodium benzylpenicilloate and benzylpenicilloic acid; final concentration: 1.5 mmol/l) and benzylpenicillin (Pharmacia, Milan, Italy) up to 10 000 IU/ml.

Ampicillin (Amplital; Pharmacia), amoxicillin (Ibiamox; IBI, Aprilia, Italy) and any responsible semisynthetic penicillin at concentrations of 1 and 20 mg/ml, after dilution in normal saline, were used on the second day.

Any responsible cephalosporin was used at a concentration of 2 mg/ml in 0.9% NaCl on the third day. For injectable β-lactams, we used the intravenous form under sterile conditions, while for noninjectable ones we prepared solutions with the powder contained in capsules or tablets, as previously described (7).

Tests were conducted and readings were taken according to the ENDA recommendations (1, 12).

Patients with nonimmediate reactions to β-lactams (group B) were evaluated with a diagnostic algorithm (2, 13), which combines skin tests and patch tests.

In the first evaluation (first day), prick and intradermal skin tests were carried out using PPL-A, PPL-D, MDM-A and MDM-D, as well as benzylpenicillin. Ampicillin, amoxicillin and any suspect β-lactam were used in the second evaluation (third day). The final concentrations of these reagents were the same ones used for evaluating subjects with immediate reactions.

Readings of late reactions to intradermal tests were taken after 48 and 72 h; any infiltrated erythema with a diameter larger than 5 mm was considered a positive reaction (6).

In the first evaluation, patch tests were administered with benzylpenicillin, ampicillin, amoxicillin (5% in petrolatum) (FIRMA, Florence, Italy), and any other suspect β-lactam (at a concentration of 200 mg/ml in 0.9% NaCl), in addition to skin tests with penicillin reagents. Patients with a previously diagnosed cell-mediated hypersensitivity who agreed to undergo re-evaluations were not patch-tested with benzylpenicillin.

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 & Rauscher GmbH & Co. KG, Rengsdorf, Germany). Occlusion time was 48 h. Readings were taken, as recommended by Brockow et al. (12), 15 min after removal of the strips and 24 h later.

Patients who underwent predictive tests before a prescribed β-lactam course (group C) were tested with penicillin reagents, ampicillin, amoxicillin, cefuroxime (Curoxim; Glaxo Wellcome, Verona, Italy) and ceftriaxone (Rocefin; Roche, Milan, Italy), with the procedures and at the concentrations used for group A.

In vitro tests

In group A subjects, assays (UniCAP®; Pharmacia Diagnostics AB, Milan, Italy) for specific immunoglobulin E (IgE) to penicilloyl G, penicilloyl V, ampicilloyl, amoxicilloyl and cefaclor were performed. A positive result was defined as a value ≥0.35 kU/l. Blood samples were obtained when patients were evaluated and sera were kept at −20°C until assayed.

Challenges

Subjects with histories of hypersensitivity reactions to β-lactams who were negative in allergologic tests were requested to undergo challenges with the suspect drugs. Patients who displayed contradictory results to allergologic tests with Allergopen and DAP reagents and were negative to benzylpenicillin were requested to undergo challenges with the latter.

We administered an initial dose of one hundredth of the therapeutic one. In cases with negative results, we administered a dose of one tenth and, if the result was again negative, a full dose. The amount of the latter was 500 mg for ampicillin and amoxicillin (orally) while it was 1 000 000 IU for benzylpenicillin and 1 g for piperacillin (intramuscularly). With regard to cephalosporins, the amount was 200 mg for cefpodoxime, 400 mg for cefixime and ceftibuten, 500 mg for cefaclor and cephalexin (orally) and 1 g for the remaining cephalosporins (intramuscularly). The time interval

between each dose was 1 h in case of immediate reactions and 1 week in case of nonimmediate reactions. Informed consent was obtained from all subjects who were admitted to a day hospital for 12 h for administration of the challenge.

Patients of group C displaying negative results in allergologic tests were challenged orally using divided doses of cefuroxime axetil (Oraxim; Malesci, Florence, Italy): one fourth of the therapeutic dose of 500 mg (125 mg) was administered initially; the remaining three fourths (375 mg) were given 1 h later, if no symptoms had developed with the initial administration.

Results

We examined a total of 195 subjects (154 females, 41 males) with a mean age of 43.5 ± 19.8 years (range 3–90 years). Among them, 74 (group A, 37.9%) had histories of immediate reactions to β-lactams and 74 (group B, 37.9%) of nonimmediate reactions; 49 of the latter with a previously diagnosed cell-mediated hypersensitivity to penicillins were re-evaluated. The remaining 47 patients (group C, 24.1%) underwent prophylactic tests with β-lactams because they had had adverse reactions to either NSAIDs (33 patients) or non-β-lactam antibiotics (14 patients), mainly quinolones (8 patients), and their doctors had required such tests.

With regard to the 148 subjects with adverse reactions to β-lactams, our work-up was performed with intervals ranging from 1 to 372 months after the most recent reaction (Tables 1 and 2).

These 148 patients had experienced a total of 223 reactions to β-lactams (Tables 1 and 2). The majority (88 patients, 59.5%) had suffered only one reaction, whereas 60 subjects had had distinct reactions to either the same β-lactam (48 patients) or different β-lactams (12 patients) in separate episodes; four of the latter patients had experienced three distinct reactions. The responsible β-lactams are shown in Tables 1 and 2. One hundred six (71.6%) subjects reported adverse reactions to penicillins, 40 (27.0%) to cephalosporins and 2 (1.4%) to both penicillins and cephalosporins. The clinical manifestations are shown in Tables 1 and 2. Most patients of group A had suffered anaphylactic shocks, while most of those of group B maculopapular rashes.

Skin tests and specific IgE assays (UniCAP) indicated that 48 (64.9%) of the 74 group A patients had experienced type I hypersensitivity reactions: 14 displayed positive results in both skin tests and UniCAP, 30 were skin-test positive and UniCAP negative, and 4 were UniCAP positive and skin-test negative.

Of the 44 patients with positive skin-test responses, 24 reacted only to semisynthetic penicillins and/or cephalosporins and 4 only to penicillin reagents (3 of whom only to PPL and/or MDM), while the remaining 16 displayed different patterns of skin reactivity (Table 3).

Of 26 patients with negative allergologic tests, 20 agreed to submit to challenges with suspect β-lactams and tolerated them.

Table 1. Clinical characteristics of the 74 patients of group A

	All patients ($n = 74$)
Mean age (years) ± SD	43.02 ± 18.73
Women, *n* (%)	51 (68.91)
Median time interval* (range)	6 (1–324)
Culprit β-lactams, *n* (%)	All reactions ($n = 116$)
Penicillins	
Amoxicillin	33 (17 plus clavulanic acid) (28.45)
Ampicillin	23 (19.83)
Bacampicillin	10 (8.62)
Piperacillin	4 (3.45)
Benzathine penicillin	3 (2.59)
Benzylpenicillin	1 (0.86)
Cephalosporins	
Ceftriaxone	17 (14.65)
Cefaclor	8 (6.9)
Ceftazidime	5 (4.31)
Cefazolin	3 (2.59)
Cefotaxime	3 (2.59)
Cefonicid	2 (1.72)
Cephaloridine	1 (0.86)
Cefpodoxime	1 (0.86)
Cefodizime	1 (0.86)
Cephalexin	1 (0.86)
Manifestations, *n* (%)	All reactions ($n = 116$)
Anaphylactic shock	62 (53.45)
Urticaria	29 (25)
Urticaria and angio-oedema	14 (12.07)
Angio-oedema	9 (7.76)
Hypotension	2 (1.72)

* Time (months) elapsed between the most recent reaction and the allergologic evaluation.

Among group B subjects, 57 presented patch-test and/or delayed intradermal-test positivity to at least one of the β-lactam reagents tested, indicating a cell-mediated hypersensitivity.

Table 4 shows the patterns of skin-test and patch-test reactivity of such patients. Positive patch tests and delayed intradermal responses to ampicillin and amoxicillin constituted the main pattern of reactivity. Eleven patients displayed patch-test negativity and delayed intradermal-test positivity to either ampicillin and amoxicillin or other responsible drugs (one to piperacillin and one to ceftriaxone). Ten patients presented delayed intradermal-test positivity to both MDM reagents, together with positive responses to benzylpenicillin, ampicillin and amoxicillin. There were two patients who displayed positive patch tests and delayed intradermal responses to ampicillin and amoxicillin, as well as immediate positive responses only to PPL-A (Table 4); both accepted the benzylpenicillin challenges and tolerated them.

The 49 patients with a cell-mediated hypersensitivity to penicillins, who were retested from 1 year to more than 10 years after the first allergologic examination, continued to be positive.

Table 2. Clinical characteristics of the 74 patients of group B

	All patients ($n = 74$)
Mean age (years) ± SD	45.79 ± 20.12
Women, *n* (%)	56 (75.67)
Median time interval* (range)	96 (1–372)
Culprit β-lactams, *n* (%)	All reactions ($n = 107$)
Penicillins	
Amoxicillin	47 (11 plus clavulanic acid; 1 plus dicloxacillin) (43.92)
Ampicillin	28 (3 plus cloxacillin; 2 plus sulbactam) (26.17)
Benzylpenicillin	13 (12.15)
Bacampicillin	4 (3.74)
Piperacillin	2 (1.86)
Cephalosporins	
Ceftazidime	3 (2.80)
Ceftriaxone	2 (1.86)
Cephalexin	2 (1.86)
Cefazolin	1 (0.94)
Cefodizime	1 (0.94)
Cefixime	1 (0.94)
Cefprozil	1 (0.94)
Ceftibuten	1 (0.94)
Cefuroxime	1 (0.94)
Manifestations, *n* (%)	All reactions ($n = 107$)
Maculopapular rash	59 (55.14)
Maculopapular rash and angio-oedema	23 (21.49)
Urticaria	10 (9.35)
Urticaria and angio-oedema	8 (7.48)
Erythema	4 (3.74)
Angio-oedema	3 (2.80)

* Time (months) elapsed between the most recent reaction and the allergologic evaluation.

Table 3. Patterns of skin-test reactivity in 44 of 48 patients* with immediate hypersensitivity to β-lactams

PPL-A	PPL-D	MDM-A	MDM-D	BP	AM	AX	Other culprit drugs	No. of patients
−	−	−	−	−	−	−	+†	14
−	−	−	−	−	−	+	−	5
−	−	−	−	−	+	+	−	4
+	+	−	−	−	−	−	−	2
+	+	−	−	+	−	−	−	1
+	−	+	+	−	−	−	−	1
−	−	+	+	+	+	+	−	6
−	−	−	−	+	+	+	−	3
−	−	+	+	+	+	−	−	2
+	+	−	−	+	+	+	−	1
−	+	+	+	+	+	+	−	1
+	+	+	+	+	+	+	−	1
−	−	−	−	−	+	+	+ (cefaclor)	1
−	−	−	−	+	+	+	+ (cefonicid)	1
−	−	+	+	−	+	+	+ (ceftriaxone)	1

PPL-A, Allergopen PPL; PPL-D, DAP PPL; MDM-A, Allergopen MDM; MDM-D, DAP MDM; BP, benzylpenicillin; AM, ampicillin; AX, amoxicillin.
* The remaining four were skin-test negative and UniCAP positive.
† 7 ceftriaxone, 2 cefazolin, 2 cefotaxime, 2 cefaclor, 1 cefodizime.

Of 17 patients with negative allergologic tests, 14 accepted challenges with suspect β-lactams and did not react.

One of the 47 patients of group C was skin-test positive only to PPL-A; he accepted the benzylpenicillin challenge and tolerated it.

All 47 group C patients tolerated the cefuroxime axetil challenges.

Overall, 106 (54.4%) of our 195 subjects had positive allergologic tests (skin tests and/or UniCAP) to at least one of the β-lactam reagents tested; 29 (28.4%) of the 102 patients with skin-test positivity were positive to PPL and/or MDM; one of them belonged to group C.

Skin testing with MDM reagents produced concordant results in all the 195 subjects: it was negative to both MDM-A and MDM-D in 173 and positive in 22. Of the latter, 12 belonged to group A and 10 to group B (Tables 3 and 4).

With regard to PPL reagents, skin tests were negative to both PPL-A and PPL-D in 185 patients and positive to both reagents in 5. Of the remaining five patients, one of group A (Table 3), two of group B (Table 4) and one of group C were positive to PPL-A and negative to PPL-D, while one of group A (Table 3) was positive to PPL-D and negative to PPL-A; neither of the two group A patients underwent the benzylpenicillin challenge because of skin-test positivity to benzylpenicillin (Table 3).

None of the 195 subjects evaluated suffered a systemic reaction to test reagents.

Discussion

After Allergopharma and Hollister-Stier ceased production of penicillin reagents, there was the danger that physicians would be set back more than 25 years in managing patients with histories of penicillin allergy, which is the most frequently reported drug allergy. Therefore, *in vivo* comparison with the Diater reagents was a necessity. Such reagents have been sold in Spain since 2003 as an allergen for prick and intradermal tests. The company is currently working to obtain authorization for other countries as well. In this study, we observed a good concordance between Allergopen and DAP reagents. In effect, MDM-A and MDM-D produced identical results in all 195 patients, 22 of whom were positive to both reagents. Results of skin testing with PPL were concordant in 190 (97.4%) of the 195 subjects. Thus, our results confirm those of a recent study by Rodriguez-Bada et al. (14), which also compared Allergopen and DAP reagents in 22 penicillin-allergic subjects using both *in vivo* and *in vitro* tests.

With the exception of three cases, we did not perform provocation tests in positive patients aimed at assessing the specificity of skin tests because of ethical reasons. When provocation tests are used as the gold standard for classifying subjects as allergic or not, there exists a variable risk of severe reactions in skin-test-positive patients. However, we did perform provocation tests with benzylpenicillin in three of the five patients who

Table 4. Patterns of skin-test and patch-test reactivity in 57 patients with delayed hypersensitivity to β-lactams

Intradermal test delayed response								Patch test response				
PPL-A	PPL-D	MDM-A	MDM-D	BP	AM	AX	Other culprit drugs	BP*	AM	AX	Other culprit drugs	No. of patients
−	−	−	−	−	+	+	−	−	+	+	−	22
−	−	−	−	+	+	+	−	−	+	+	−	12
−	−	+	+	+	+	+	−	−	+	+	−	7
−	−	+	+	+	+	+	−	+	+	+	−	2
+†	−	−	−	−	+	+	−	−	+	+	−	2
−	−	−	−	−	−	−	+ (ceftriaxone)	−	−	−	+	1
−	−	−	−	−	+	+	−	−	−	−	−	7
−	−	−	−	−	−	−	+ (ceftriaxone, piperacillin)	−	−	−	−	2
−	−	+	+	+	+	+	−	−	−	−	−	1
−	−	−	−	+	+	+	−	−	−	−	−	1

PPL-A, Allergopen PPL; PPL-D, DAP PPL; MDM-A, Allergopen MDM; MDM-D, DAP MDM; BP, benzylpenicillin; AM, ampicillin; AX, amoxicillin.
* Patch tests with benzylpenicillin were not performed in the 49 subjects with a previous diagnosis of cell-mediated hypersensitivity.
† Immediate positive responses to intradermal tests.

presented contradictory results to allergologic tests with Allergopen and DAP reagents; one of these three patients belonged to group C and displayed a positive response only to PPL-A; the remaining two belonged to group B and presented skin-test positivity to PPL-A, together with patch-test and delayed intradermal-test positivity to ampicillin and amoxicillin (Table 4). These patients were challenged, because the first had no history of hypersensitivity reactions to β-lactams and in a previous study of ours (15) patients with patch test and delayed intradermal-test positivity to ampicillin and amoxicillin and negative results to penicillin reagents had tolerated benzylpenicillin challenges. All of the three aforesaid patients tolerated provocation tests with benzylpenicillin; therefore, skin testing with PPL-D appears to be more specific than with PPL-A. The remaining two of these five patients, who presented positive results to PPL-A and negative ones to PPL-D, were not requested to undergo benzylpenicillin challenges because they were also skin-test positive to both MDM-A and MDM-D (Table 3).

The sensitivities of Allergopen and DAP reagents are very similar. In effect, both MDM-A and MDM-D were positive in 22 (14.9%) of the 148 patients with histories of either immediate or nonimmediate reactions to β-lactams (groups A and B). Allergopen PPL was positive in 8 (5.4%) of the 148 patients of groups A and B (including the two patients of group B who tolerated benzylpenicillin challenges), while PPL-D was positive in 6 (4.1%) of these 148 subjects.

In the present study, the rate of positive responses to PPL and/or MDM observed among patients with either IgE-mediated or cell-mediated hypersensitivity (28 of 105, 26.7%) is lower than that found in studies by Bousquet et al. (4) and Matheu et al. (5), which was slightly above 45% and 70%, respectively. The rate of positive responses to only PPL and/or MDM found in the present study (2.9%) is also lower than the 14.7% and 47.7%, respectively, found in the aforesaid studies (4, 5). Such different results may derive from the samples assessed. In our study, 40 (27.0%) of the 148 subjects with histories of β-lactam allergy (groups A and B) had reacted to cephalosporins, and the rate of positive responses to penicillin determinants in such patients is generally low (7, 8). Moreover, the 74 group B subjects had experienced nonimmediate reactions, and 49 of them had a previous diagnosis of cell-mediated hypersensitivity to penicillins and were re-evaluated. In a previously mentioned study (6), which evaluated 259 patients with nonimmediate reactions to penicillins, only seven (2.7%) were positive to MDM and none to PPL. With regard to negative responses to PPL in patients with a cell-mediated hypersensitivity to penicillins, such negative results may be related to the nature of the carrier; in effect, polylysine is a nonimmunogenic carrier, as observed by Levine in delayed reactions to penicillins (16).

In the present study, 3 (4.1%) of the 74 patients with histories of immediate hypersensitivity reactions to β-lactams displayed positive responses only to skin tests with PPL and/or MDM. According to recent guidelines (1), these three patients should have been provoked in the absence of PPL and MDM reagents. Thus, the present study confirms that PPL and MDM are useful reagents in diagnosing an IgE-mediated hypersensitivity to β-lactams, as recently stated by the ENDA (17).

On the contrary, the contribution of skin testing with PPL and MDM in diagnosing cell-mediated hypersensitivity reactions to β-lactams is very limited. Indeed, although we observed 10 patients with delayed intradermal-test positivity to MDM, all 57 patients (including the aforesaid 10) with type IV hypersensitivity presented positive responses to patch tests and/or delayed intradermal tests with responsible β-lactams (Table 4).

In the present study, the 49 patients with a cell-mediated hypersensitivity to penicillins who were retested continued to be positive. These results agree with those of a previous study (18), which re-evaluated with patch tests 23 patients with a cell-mediated hypersensitivity and did not observe any negativization. Two other studies (19,

20), which retested such patients from 1 year to more than 10 years after the first allergologic examination, found a rate of negativization of 6.7% (1 of 15) and 4.6% (1 of 21), respectively. Considering this persistence, we recalled patients with such hypersensitivity in order to compare Allergopen and DAP reagents.

In the aforementioned study (18), 2 of the 23 patients retested displayed a new positivity to benzylpenicillin; both subjects denied use of this drug in the interval between allergologic evaluations. In the present study, therefore, we did not perform patch tests with benzylpenicillin in the 49 patients re-evaluated.

Conclusion

In skin testing, DAP reagents are a reliable and safe alternative to Allergopen ones, with very similar sensitivity. The specificity of PPL-D appears to be higher, at least in the small sample that we evaluated.

References

1. Torres MJ, Blanca M, Fernandez J, Romano A, de Weck A, Aberer W et al. Diagnosis of immediate allergic reactions to beta-lactam antibiotics. Allergy 2003;**58**:961–972.
2. Romano A, Blanca M, Torres MJ, Bircher A, Aberer W, Brockow K et al. Diagnosis of non-immediate reactions to β-lactam antibiotics. Allergy 2004;**39**:1153–1160.
3. Bernstein IL, Gruchalla RS, Lee RE, Nicklas RA, Dykewicz MS, Shepherd GM et al. Disease management of drug hypersensitivity: a practice parameter. Ann Allergy Asthma Immunol 1999;**83**:665–700.
4. Bousquet PJ, Co-Minh HB, Arnoux B, Daures JP, Demoly P. Importance of mixture of minor determinants and benzylpenicilloyl poly-L-lysine skin testing in the diagnosis of β-lactam allergy. J Allergy Clin Immunol 2005;**115**:1314–1316.
5. Matheu V, Pérez-Rodriguez E, Sánchez-Machin I, de la Torre F, García-Robaina JC. Major and minor determinants are high-performance skin tests in β-lactam allergy diagnosis. J Allergy Clin Immunol 2005;**116**:1167–1168.
6. Romano A, Viola M, Mondino C, Pettinato R, Di Fonso M, Papa G et al. Diagnosing nonimmediate reactions to penicillins by in vivo tests. Int Arch Allergy Immunol 2002;**129**:169–174.
7. Romano A, Guéant-Rodriguez RM, Viola M, Amoghly F, Gaeta F, Guéant JL. Diagnosing immediate reactions to cephalosporins. Clin Exp Allergy 2005;**35**:1234–1242.
8. Antunez C, Blanca-Lopez N, Torres MJ, Mayorga C, Perez-Inestrosa E, Montañez MI et al. Immediate allergic reactions to cephalosporins: evaluation of cross-reactivity with a panel of penicillins and cephalosporins. J Allergy Clin Immunol 2006;**117**:404–410.
9. Torres MJ, Romano A, Mayorga C, Moya MC, Guzman AE, Reche M et al. Diagnostic evaluation of a large group of patients with immediate allergy to penicillins: the role of skin testing. Allergy 2001;**56**:850–856.
10. Atanasković-Marković M, Ćirković Veličković T, Gavrović-Jankulović M, Vučković O, Nestorović B. Immediate allergic reactions to cephalosporins and penicillins and their cross-reactivity in children. Pediatr Allergy Immunol 2005;**16**:341–347.
11. Romano A, Guéant-Rodriguez RM, Viola M, Pettinato R, Guéant JL. Cross-reactivity and tolerability of cephalosporins in patients with immediate hypersensitivity to penicillins. Ann Intern Med 2004;**141**:16–22.
12. Brockow K, Romano A, Blanca M, Ring J, Pichler W, Demoly P. General considerations for skin test procedures in the diagnosis of drug hypersensitivity. Allergy 2002;**57**:45–51.
13. Romano A, Quaratino D, Di Fonso M, Papa G, Venuti A, Gasbarrini G. A diagnostic protocol for evaluating nonimmediate reactions to aminopenicillins. J Allergy Clin Immunol 1999;**103**:1186–1190.
14. Rodriguez-Bada JL, Montañez MI, Torres MJ, Mayorga C, Canto G, Perez-Inestrosa E et al. Skin testing for immediate hypersensitivity to betalactams: comparison between two commercial kits. Allergy 2006;**61**:947–951.
15. Romano A, Di Fonso M, Papa G, Pietrantonio F, Federico F, Fabrizi G et al. Evaluation of adverse cutaneous reactions to aminopenicillins with emphasis on those manifested by maculopapular rashes. Allergy 1995;**50**:113–118.
16. Levine BB. Immunologic mechanisms of penicillin allergy: a haptenic model system for the study of allergic diseases of man. N Engl J Med 1966;**275**:1115–1125.
17. Torres MJ, Blanca M and the European Network for Drug Allergy (ENDA) and the EAACI interest group on drug hypersensitivity. Importance of skin testing with major and minor determinants of benzylpenicillin in the diagnosis of allergy to betalactams. Statement from the European Network for Drug Allergy concerning AllergoPen withdrawal. Allergy 2006;**61**:910–911.
18. Romano A, Di Fonso M, Pietrantonio F, Pocobelli D, Giannarini L, Del Bono A et al. Repeated patch testing in delayed hypersensitivity to beta-lactam antibiotics. Contact Dermatitis 1993;**28**:190.
19. Patriarca G, Schiavino D, Nucera E, Milani A. Positive allergological tests may turn negative with no further exposure to the specific allergen: A long-term, prospective, follow-up study in patients allergic to penicillin. J Investig Allergol Clin Immunol 1996;**6**:162–165.
20. Torres MJ, Sánchez-Sabaté E, Álvarez J, Mayorga C, Fernández J, Padial A et al. Skin test evaluation in nonimmediate allergic reactions to penicillins. Allergy 2004;**59**:219–224.