ORIGINAL ARTICLE

Prospective, Multicenter Clinical Trial to Validate New Products for Skin Tests in the Diagnosis of Allergy to Penicillin

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Abstract

Background: Allergy to penicillin is the most commonly reported type of drug hypersensitivity. Diagnosis is currently confirmed using skin tests with benzylpenicillin reagents, ie, penicilloyl-polylysine (PPL) as the major determinant of benzylpenicillin and benzylpenicillin, benzylpenicilloate and benzylpenilloate as a minor determinant mixture (MDM).
Objective: To synthesize and assess the diagnostic capacity of 2 new benzylpenicillin reagents in patients with immediate hypersensitivity reactions to ß-lactams: benzylpenicilloyl octa-L-lysine (BP-OL) as the major determinant and benzylpenilloate (penilloate) as the minor determinant.
Methods: Prospective multicenter clinical trial performed in 18 Spanish centers. Efficacy was assessed by detection of positive skin test results in an allergic population and negative skin test results in a nonallergic, drug-exposed population. Sensitivity, specificity, and negative and positive predictive values were determined.
Results: The study sample comprised 94 allergic patients: 31 (35.23%) presented anaphylaxis, 4 (4.55%) anaphylactic shock, 51 (58.04%) urticaria, and 2 (2.27%) no specific condition. The culprit ß-lactams were amoxicillin in 63 cases (71.60%), benzypencillin in 14 cases (15.89%), cephalosporins in 2 cases (2.27%), other drugs in 3 cases (3.42%), and unidentified agents in 6 cases (6.82%). The results of testing with BP-OL were positive in 46 cases (52.3%); the results of testing with penilloate were positive in 33 cases (37.5%). When both reagents were taken into consideration, sensitivity reached 61.36% and specificity 100%. Skin testing with penilloate was significantly more often negative when the interval between the reaction and the study was longer.
Conclusions: The sensitivity of BP-OL and penilloate was 61%. Considering that amoxicillin was the culprit drug in 71% of reactions, these results indicate that most patients were allergic to the whole group of penicillins. These data support the use of benzylpenicillin determinants in the diagnosis of allergy to ß-lactams, even in predominantly amoxicillin-allergic populations.

Key words: Hypersensitivity. ß-Lactams. Penicillin determinants. Skin tests. Clinical trial.

Resumen

Antecedentes: La alergia a penicilina es la más frecuente de las reacciones de hipersensibilidad a medicamentos. El determinante mayor de la penicilina, Benzylpeniciloil (PPL) y los determinantes menores (MDM), compuestos de Bencilpenicilina, Bencilpeniciloato y Bencilpeniloato se han utilizado en pruebas cutáneas para el diagnóstico de la alergia a Penicilinas.
Objetivos: Sintetizar y evaluar la capacidad diagnóstica de 2 nuevos reactivos de Benzylpenicilina, benzylpenicilloyl octa-L-lisina (BP-OL) y Bencilpeniloato (Penilloate), en pacientes con reacciones de hipersensibilidad inmediatas a Betalactámicos.
Métodos: Para ello se ha llevado a cabo un ensayo prospectivo y multicéntrico en 18 centros hospitalarios españoles. La eficacia se evaluó mediante la detección de la positividad en pruebas cutáneas en una población de pacientes alérgicos y la negatividad en las mismas pruebas en un grupo control de pacientes expuestos a Penicilina. Se determinó la sensibilidad, especificidad, y valores predictivos negativos y positivos de los mismos.

Resultados: Se incluyeron 94 pacientes alérgicos a los betalactámicos: 31 (35.23%) presentaban anafilaxia, 4 (4.55%) shock anafiláctico, 51 (58.04%) urticaria y 2 (2.27%) historia no determinada. El medicamento responsable fue: amoxicilina en 63 casos (71,60%), pencilina G en 14 casos (15,89%), cefalosporinas en 2 casos (2,27%), otros betalactámicos en 3 casos (3,42%) y no identificado en 6 casos (6,82%). 46 pacientes (52,3%) fueron positivos a BP-OL y 33 (37,5%) a Penilloate. Considerando ambos reactivos, la sensibilidad alcanza el 61,36% con una especificidad del 100%. Las pruebas cutáneas a Penilloate fueron significativamente más negativas cuando el intervalo entre la reacción y el estudio era mayor.
Conclusiones: La sensibilidad del BP-OL y Penilloate fue del 61% y considerando que la amoxicilina fue la responsable en el 71% de las reacciones, estos resultados indican que la mayoría de los pacientes eran alérgicos al grupo completo de penicilinas. Estos datos soportan la necesidad de seguir utilizando los determinantes de la Penicilina en el diagnóstico de los pacientes alérgicos a Betalactámicos, incluso en poblaciones donde predomina la amoxicilina.

Palabras clave: Hipersensibilidad inmediata. Betalactámicos. Determinantes de la Penicilinas. Pruebas cutáneas. Ensayo clínico.

Introduction

Allergy to penicillin is the most commonly reported type of drug hypersensitivity, accounting for at least 10% of all reactions [1,2]. Anaphylactic shock is recorded in 0.01% of these cases, and 9% of anaphylactic reactions are fatal if penicillin is administered [3]. However, approximately 85% to 90% of people reporting penicillin allergy can tolerate penicillins [4,5]. In fact, the results of skin tests and provocation tests have shown that drug hypersensitivity reactions occur in less than 25% of patients with a history suggesting drug allergy and that these reactions were confirmed in 8.4% of patients allergic to ß-lactams [6]. Hypersensitivity to ß-lactams has major consequences in terms of the safety, durability, and effectiveness of treatment and of the confirmation of the presence or absence of drug allergy [2,6,7].

Skin testing with the major and minor antigenic determinants of penicillin is used to confirm the diagnosis of penicillin hypersensitivity. The procedure is recommended by European guidelines [7,8] and American guidelines [9,10]. The major determinant is formed by the conjugation of benzylpenicillin (BP) to the polylysine reagent to form the penicilloyl determinant penicilloyl-polylysine (PPL) [11]; the minor determinants consist of BP, benzylpenicilloate, and benzylpenilloate, which make up the minor determinant mixture (MDM) [12].

The sensitivity of BP determinants is affected by several factors, and the percentage of positive responses to major and minor determinants can vary in different populations, thus reflecting different patterns of consumption, prescription habits, and genetic factors [13-17]. Moreover the number of responders to PPL and MDM has decreased over time, as shown in studies performed in the same population [13,15]. Initially, 77.7% of patients had positive responses to skin tests with PPL, MDM, or both; this percentage dropped to 42.1% (PPL) and 22.1% (MDM) after 10 years. Nevertheless, major and minor determinants of BP continue to play a key role in diagnosis, as they induce a positive response in 46% of patients with positive skin test results to penicillins; in addition, 14% of patients are positive only to these 2 reagents [16].

The aim of the present study was to synthesize and evaluate the diagnostic capacity of 2 new BP reagents—diagnostic allergy penicillin (DAP), a purer and more stable benzylpenicilloyl octa-L-lysine (BP-OL), and the most stable minor determinant, sodium benzylpenilloate (penilloate)—in patients with immediate hypersensitivity reactions to ß-lactams in order to obtain marketing authorization from Spanish and European medicines agencies.

Material and Methods

Trial Design

We performed a prospective multicenter clinical trial (Eudra CT 2008 003309 15) to validate the diagnostic capacity of DAP (BP-OL and penilloate) following the Guideline on clinical evaluation of diagnostic agents (CPMP/EWP/1119/98/Rev 1) of the European Medicines Agency. The study included patients who were allergic and nonallergic to ß-lactams from 18 Spanish centers. Efficacy was assessed by detection of positive skin test results in an allergic population and negative skin test results in nonallergic individuals exposed to the drug. The sensitivity, specificity, and negative and positive predictive values were determined.

The trial was approved by the ethics committee of each participating center and by the Spanish Agency for Medicine and Health Care Products and was conducted according to the principles of the Declaration of Helsinki, Good Clinical Practice, and local regulations. All patients and controls provided written informed consent.

Study Groups

The study population was divided into cases and controls. To be included, patients had to be aged >18 years and have experienced an immediate hypersensitivity reaction to any ß-lactam with clinical symptoms of allergy (including urticaria, anaphylaxis, or anaphylactic shock). The reaction had to be confirmed by positive skin test results to a penicillin determinant or negative skin test results but positive provocation test result to a penicillin derivative during the year before the study started. The control group included patients who had received and tolerated treatment with penicillin during the previous year or treatment with at least penicillin V a month before the DAP challenge. Patients included in the control group did not show any intolerance or allergic reactions to the aforementioned antibiotics and met the remaining inclusion criteria.

Skin tests were performed following the recommendations of the European Network for Drug Allergy (ENDA) [18,19]. The drugs used were PPL and MDM (Diater), BP (Normon), amoxicillin (Glaxo Smithkline Beecham), and the culprit drug if different. In those cases where skin test results were negative, a provocation test with the culprit ß-lactam was carried out following ENDA recommendations [20].

Preparation of Reagents

Benzylpenicilloyl-octa-L-lysine (Figure 1A): Octalysine was dissolved in sterile water for injection and the pH adjusted to 10.0. Sodium benzylpenicillin solution was added, the pH adjusted to 11.5 then decreased to 3.6, and the solution was centrifuged. After purification, the solution was freeze-dried. The resulting product was characterized by proton nuclear magnetic resonance (^{1}H-NMR). The final purified product (0.04 mg/mL) and mannitol (20 mg/mL) were dissolved with phosphate buffer, sterilized, filtered off, and lyophilized to yield a dry white powder. The vial with this content was closed and vacuum-sealed before reconstitution for skin tests. The content of active substance was analyzed using high-performance liquid chromatography (HPLC).

Benzylpenilloate (Figure 1B): BP (Sandoz) was dissolved in water and stabilized at a pH of 12 before undergoing acid hydrolysis at a pH of 4. This solution was maintained at 70°C to 80°C for 2 hours and then at 4°C for 24 hours. The precipitate was filtered off, washed (pH 4), and freeze-dried. The resulting product was characterized using ^{1}H-NMR. The final purified product (0.5 mg/mL) and mannitol (20 mg/mL) were dissolved with phosphate buffer, sterilized, filtered off, and lyophilized to yield a dry white powder. The vial with this content was closed and vacuum-sealed before reconstitution for skin tests. The content of active substance was analyzed using HPLC.

Chemical Characterization and Analysis of the Reagents

The ^{1}H-NMR experiments were carried out in a Bruker AV-300 spectrometer; chemical shifts were externally referenced to solvent residual signal and given in ppm.

The purity of the compounds was analyzed by reverse phase HPLC using a UV detector at 220 nm. Samples were tested in an Alliance 2695 HPLC Separations Module (Waters) equipped with a Sunfire C18 3.5-µm (75 × 4.6 mm) chromatographic column (Waters). Samples were eluted at a flow rate of 1 mL/min and with a mobile phase consisting of water/acetonitrile (0.1% trifluoroacetic acid). The gradient program was as follows: 5% acetonitrile at 0 minutes; 28% acetonitrile at 5 minutes, 35% acetonitrile at 8 minutes, 44%

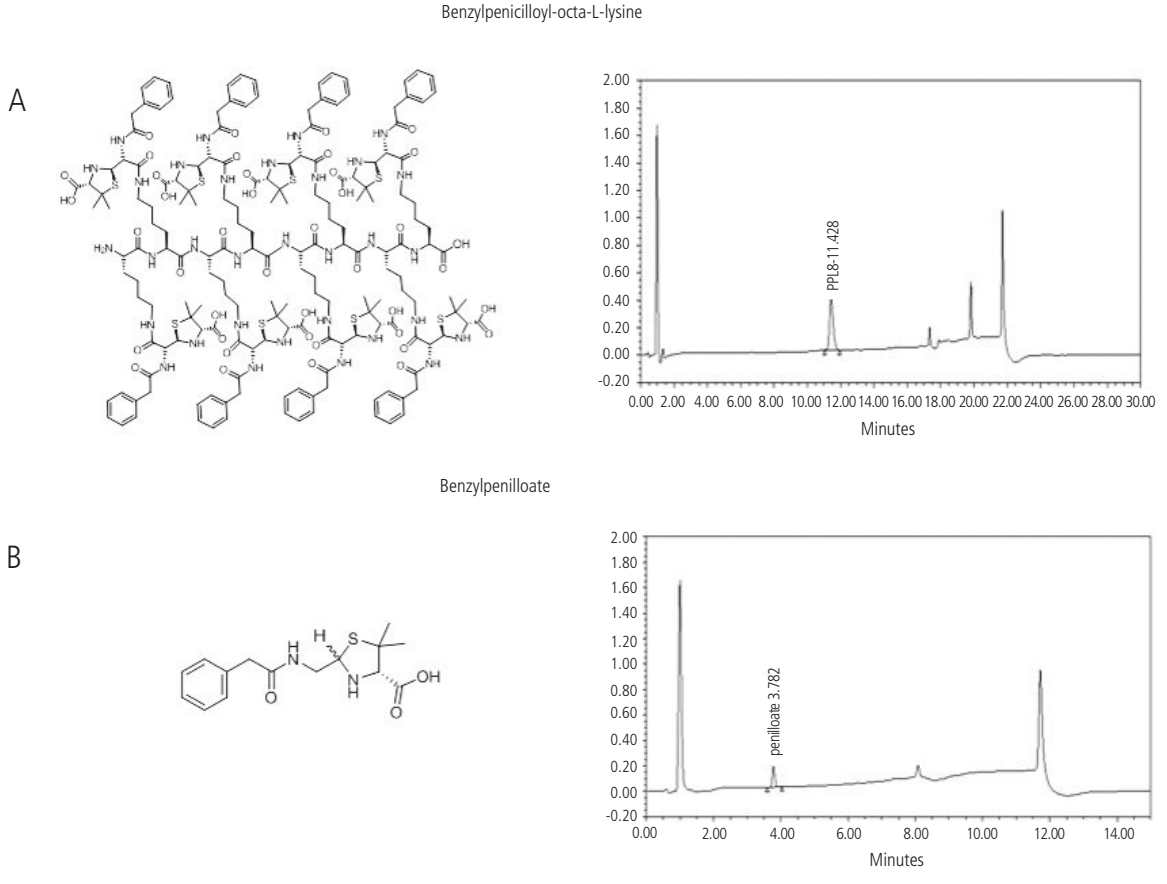

Figure 1. Chemical structure and chromatogram of benzylpenicilloyl-octa-L-lysine (A) and benzylpenilloate (B).

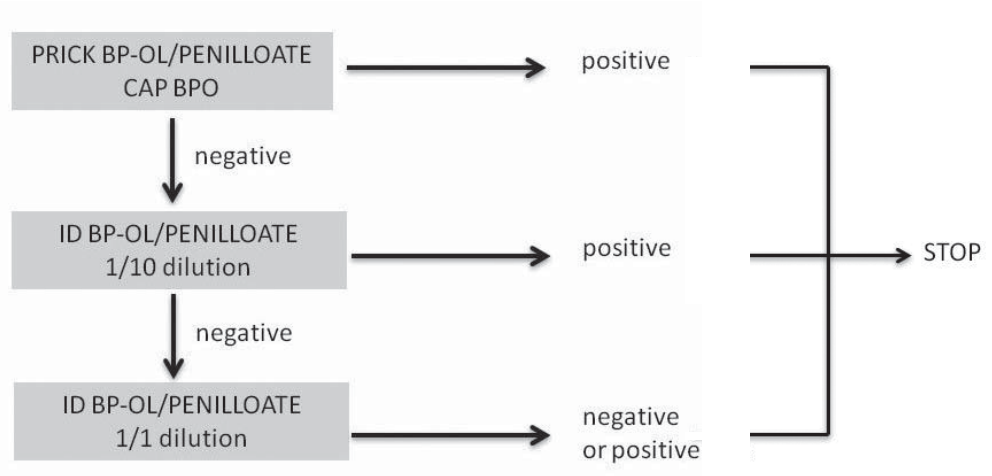

Figure 2. Diagnostic algorithm of the clinical trial. BP-OL indicates benzylpenicilloyl octa-L-lysine; ID, intradermal.

acetonitrile at 9 minutes, 70% acetonitrile at 15 minutes, 100% acetonitrile at 16 minutes, and isocratic elution with 5% acetonitrile from 20-30 minutes.

Skin Tests

Figure 2 shows the diagnostic algorithm of the study. Skin prick and intradermal tests were carried out as previously described [7] using 0.02-0.03 mL of solution prepared daily. The reagents were BP-OL and benzylpenilloate, at a maximum concentration of 0.04 mg/mL and 0.5 mg/mL, starting at 1/10 in the intradermal tests.

In skin prick testing, a positive response was defined as a wheal larger than 3 mm surrounded by erythema with a negative response to the saline control. In the intradermal tests, the wheal area was marked initially and 20 minutes after testing, and an increase in diameter >3 mm surrounded by erythema was considered a positive result. Patients were followed for 1 week to monitor the presence of delayed responses to skin testing or the onset of adverse effects.

In Vitro Specific Immunoglobulin E Antibody Determination

Immunoglobulin (Ig) E antibody determination was performed using the CAP-FEIA assay (Phadia) with C1 (benzylpenicilloyl) following the manufacturer's instructions. The results were obtained by direct comparison with standards run in parallel, with a value ≥0.35 kU_A/L considered positive [21].

Statistical Analysis

The sample size was calculated based on previously reported data [15]: sensitivity of 77% and specificity of 99% (both with a 95%CI) and precision of 90%. Under these conditions, the diagnosis was confirmed in at least 75% of the patients, and the sample size was estimated at 138 participants (69 allergic and 69 nonallergic). Sensitivity, specificity, and positive and negative predictive values were then estimated with the new determinants. Normally distributed quantitative variables were compared using the Mann-Whitney test. All *P* values were 2-tailed and accompanied by their respective 95%CI. Statistical significance was set at $P \leq .05$. The statistical analysis was performed using EPIDAT v 3.1.

Results

Description of the Products

The chemical characterization of both compounds was carried out using ^{1}H-NMR. The spectra showed the signals corresponding to the products, and their structures are described in Figure 1.

The purity of the products (BP-OL and penilloate) was analyzed using HPLC. Figure 1A shows a peak corresponding to BP-OL at a retention time of 11.4 minutes; Figure 1B shows a peak corresponding to penilloate at a retention time of 3.8 minutes. These data illustrate the high purity of the corresponding compounds.

Population Evaluated

A group of 94 patients with confirmed immediate hypersensitivity reactions to ß-lactams were assessed. During the study, 6 cases were excluded for the following reasons: 1 did not comply with the study requisites, 2 had dermographism and therefore the skin tests showed false-positive results, and 3 did not complete the study. The clinical and demographic characteristics of patients are described in Table 1.

The mean age was 46.94 years (95%CI, 43.84-51.41), 34 were men (38.64%), and the time interval between the reaction and the study was 2484.74 days (95%CI, 1777.62-3355.23). As for clinical symptoms, 31 cases (35.23%) developed anaphylaxis, 4 (4.55%) anaphylactic shock, and 51 (58.04%)

Table 1. Clinical and Demographic Characteristics of the Patients Included in the Study

Patient	Age	Sex	Reaction	Drug	Episodes	Diagnosis	Last Reaction to Study, d
1	62	Male	AS	AX	2	ST (+)	365
2	56	Male	U	AX/PV	2	ST (+)	1825
3	51	Male	AN	AX	2	ST (+)	2920
4	37	Female	AN	AX	3	ST (+)	730
5	39	Male	U/AN	AX	2	ST (+)	2920
6	73	Male	U	AX	1	ST (+)	2920
7	44	Male	U	AX	1	ST (+)	365
8	58	Male	U	BP	1	ST (+)	5475
9	41	Female	A	AX	1	ST (+)	1825
10	61	Female	A	AX	1	ST (+)	730
11	33	Male	AS	AX	1	ST (+)	1825
12	55	Female	AN	AX	1	ST (+)	150
13	21	Female	AN	BP	1	ST (+)	2190
14	48	Male	AN	AX-CV	1	ST (+)	1095
15	68	Female	U	AX	1	ST (+)	1460
16	74	Female	U	AX-CV	1	ST (+)	365
17	58	Male	AS	AX-CV	1	ST (+)	480
18	55	Female	U	AX-CV	1	ST (+)	510
19	20	Male	U	AX-CV	1	ST (+)	630
20	34	Female	AN	BL	1	ST (+)	10 950
21	77	Male	U	AX-CV	1	ST (+)	940
22	34	Male	U	AX-CV	1	ST (+)	2190
23	33	Female	U	BL	1	ST (+)	12 045
24	61	Male	AN	AX-CV	1	ST (+)	10 220
25	43	Female	AS	AX-CV	1	ST (+)	2765
26	35	Female	AN	BP	1	ST (+)	5110
27	33	Female	U	BP	1	ST (+)	12 045
28	64	Male	U	BP	1	ST (+)	6935
29	57	Male	U	AX	1	ST (+)	275
30	29	Female	U	BP	1	ST (+)	10 220
31	50	Female	U	AX	1	ST (+)	9125
32	26	Female	U	AX-CV	1	ST (+)	120
33	27	Female	U	AX-CV	1	ST (+)	60
34	53	Male	U	AX-CV	1	ST (+)	420
35	55	Male	U	AX-CV	1	ST (+)	300
36	45	Male	U	AX	1	ST (+)	120
37	21	Female	AN	AX	2	ST (+)	150
38	48	Female	AN	AX-CV	1	ST (+)	330
39	70	Female	AN	BP/AX	2	ST (+)	180
40	60	Female	AN	AX-CV	1	ST (+)	180
41	26	Female	U	AX	1	ST (+)	9490
42	21	Female	AN	BP	1	ST (+)	2555
43	17	Female	U	AX	1	ST (+)	1800
44	19	Female	U	AX	1	ST (+)	90
45	71	Female	U	AX	1	ST (+)	90
46	43	Female	AN	AX-CV	1	ST (+)	255
47	54	Female	U	BP	1	ST(–)/DPT (+)	8
48	32	Female	AN	AX-CV	1	ST (+)	450
49	17	Female	U	AX-CV	1	ST (+)	90
50	54	Female	U	AX	1	ST (+)	510
51	79	Male	AN	AX	1	ST (+)	730
52	39	Female	U	PV	1	ST (+)	1000
53	52	Male	U	BP	1	ST (+)	7300

Table 1. Continued

Patient	Age	Sex	Reaction	Drug	Episodes	Diagnosis	Last Reaction to Study, d
54	76	Male	U	AX-CV	1	ST (+)	420
55	34	Female	U	AX	1	ST (+)	510
56	71	Male	U	BL	1	ST (+)	2500
57	23	Female	U	AX-CV	1	ST (+)	1095
58	69	Female	AN	AX-CV	1	ST (+)	30
59	42	Female	AN	CPS/CPS	2	ST (+)	200
60	42	Male	AN	CPS	1	ST (+)	600
61	69	Male	U	BL	1	ST (+)	930
62	35	Female	U	AX-CV	1	ST (+)	60
63	27	Female	U	AX	1	ST (+)	6020
64	66	Male	U	AX-CV	1	ST (+)	180
65	45	Female	AN	AX	1	ST (+)	12
66	38	Female	AN	BP	1	ST (+)	13 870
67	33	Male	UK	BL	1	ST (+)	9125
68	28	Male	U	AX	1	ST (+)	30
69	54	Male	AN	BP	1	ST (+)	30
70	40	Female	AN	AX-CV	1	ST (+)	150
71	45	Female	AN	AX-CV	1	ST (+)	22
72	52	Female	U	AX	1	ST (+)	7300
73	37	Male	AN	AX-CV	1	ST (–)/DPT (+)	120
74	74	Male	AN	AX	1	ST (+)	7300
75	20	Female	U	BL	1	ST (+)	1700
76	29	Male	U	BP	1	ST (+)	7300
77	25	Female	U	AX	1	ST (+)	365
78	67	Female	U	AX	1	ST (+)	4015
79	42	Female	U	AX	1	ST (+)	4015
80	60	Female	U	AX-CV	1	ST (+)	365
81	70	Female	U	AX-CV	1	ST (+)	510
82	77	Female	U	AX-CV	1	ST (+)	780
83	37	Female	AN	AX	1	ST (+)	120
84	46	Female	AN	AX	1	ST (+)	90
85	79	Female	U	AX-CV	1	ST (+)	420
86	65	Male	U	AX-CV	1	ST (+)	60
87	56	Female	AN	BP	1	ST (+)	3650
88	25	Male	UK	BP	1	ST (+)	6935

Abbreviations: AN, anaphylaxis; AS, anaphylactic shock; AX, amoxicillin; AX-CV, amoxicillin-clavulanic acid; BL, nonidentified ß-lactam; BP, benzylpenicillin; CPS, cephalosporin; DPT, drug provocation test; PV, penicillin V; ST, skin test; U, urticaria; UK, unknown.

urticaria. In 2 cases (2.27%), the reaction, although immediate, was not identified. Eighty patients (90.91%) developed 1 episode, 7 (7.95%) developed 2 episodes, and 1 (1.14%) developed 3 episodes. The ß-lactams involved in the reaction were amoxicillin in 63 cases (71.60%), cephalosporins in 2 cases (2.27%), penicillin V in 1 case (1.14%), penicillin G in 14 cases (15.89%), amoxicillin and penicillin V in 1 case (1.14%), amoxicillin and BP in 1 case (1.14%), and unidentified agents in 6 cases (6.82%).

The control group (n=79) comprised individuals with confirmed good tolerance to penicillin V during the previous year and until a month before the study. This group was matched for sex and age with the cases.

Skin Test Results

The skin test results are shown in Table 2. The results were positive to PPL in 65 cases (73.9%) and to MDM in 41 cases (46.6%). Using the new determinants, the results were positive to BP-OL in 46 cases (52.3%) and to penilloate in 33 cases (37.5%). The mean (SD) time interval between reaction and performance of the clinical trial was 2484.74 (3485.83) days, and the time interval between the first skin test and the clinical trial was 71.18 (88.01) days. We analyzed the relationship between both time intervals and the presence of positive or negative results to skin testing with BP-OL and penilloate. The only differences recorded were with penilloate, for which the

Table 2. Skin Tests Results With the Classic PPL, MDM, and BLs in the Clinical Trial Recruitment (ST I) and With the New Reagents BPO-L and Penilloate (ST II)

Patient	Drug	Skin Tests 1			Interval ST I-ST II, d	Skin Tests II		Interval Reaction to Study, d
		PPL	MDM	Other		BP-OL	Penilloate	
1	AX	+	+		1	+ (ID 1/10)	–	365
2	AX/PV	+	+		1	+ (ID 1/10)	–	1825
3	AX	+	+		1	+ (ID 1/10)	+ (ID 1/10)	2920
4	AX	–	+	BP +	1	–	–	730
5	AX	–	+	AX +	15	–	–	2920
6	AX	+	–		1	+ (ID 1/10)	–	2920
7	AX	+	–		7	+ (ID 1/10)	–	365
8	BP	+	–		40	+ (P)	–	5475
9	AX	–	+		5	–	–	1825
10	AX	–	+		1	–	–	730
11	AX	+	+		4	+ (ID 1/1)	+ (ID 1/1)	1825
12	AX	+	+		7	+ (ID 1/10)	+ (ID 1/10)	150
13	BP	+	–		1	+ (P)	–	2190
14	AX-CV	–	+		7	+ (ID 1/10)	+ (ID 1/10)	1095
15	AX	+	–		90	–	+ (ID 1/1)	1460
16	AX-CV	+	+		20	–	–	365
17	AX-CV	+	+		30	+ (ID 1/10)	+ (ID 1/10)	480
18	AX-CV	+	–	BP +	10	+ (ID 1/10)	+ (ID 1/1)	510
19	AX-CV	+	–		1	–	–	630
20	BL	+	+		35	–	–	10 950
21	AX-CV	–	+	AX +/BP +	10	–	+ (ID 1/1)	940
22	AX-CV	–	+	AX +	150	–	–	2190
23	BL	+	–	AX +	5	+ (ID1/10)	–	12 045
24	AX-CV	–	+	AX +/BP +	35	–	+ (ID 1/1)	10 220
25	AX-CV	–	+	AX +	20	–	+ (ID 1/1)	2765
26	BP	+	–		60	+ (P)	+ (P)	5510
27	BP	+	+	BP +	30	+ (ID 1/1)	+ (ID 1/1)	12 045
28	BP	+	–		210	+ (ID 1/1)	–	6935
29	AX	+	+	AX +	10	–	+ (P)	275
30	BP	+	+		240	+ (ID1/10	+ (ID 1/1)	10 220
31	AX	+	–		365	+ (P)	–	9125
32	AX-CV	+	–		60	–	–	120
33	AX-CV	–	–	AX +/BP +	40	–	–	60
34	AX-CV	+	–	AX +	60	–	–	420
35	AX-CV	–	+	AX +/BP +	60	–	–	300
36	AX	+	+		1	+ (P)	+ (P)	120
37	AX	+	–	AX +	30	–	–	180
38	AX-CV	+	–	AX +	30	–	–	330
39	BP/AX	–	–	AX +	40	–	–	180
40	AX-CV	+	+	AX +	60	+ (P)	+ (ID 1/10)	180
41	AX	+	+		6	–	+ (ID 1/1)	9490
42	BP	+	–		24	+ (ID 1)	+ (ID 1/10)	2555
43	AX	+	–		15	+ (ID 1)	–	1800
44	AX	–	–	AX +	35	–	–	90
45	AX	+	+	AX +	30	+ (ID 1/10)	–	90
46	AX-CV	+	+		35	+ (ID 1/10)	–	255
47	BP	ND	ND	BP+	3	–	–	8
48	AX-CV	+	+		130	+ (ID 1)	–	450
49	AX-CV	+	–		30	–	–	90
50	AX	–	–	AX +/BP +	1	–	–	510
51	AX	+	+		30	+ (ID 1)	+ (ID 1/1)	730

Table 2. Continued

Patient	Drug	Skin Tests 1			Interval ST I-ST II, d	Skin Tests II		Interval Reaction to Study, d
		PPL	MDM	Other		BP-OL	Penilloate	
52	PV	+	+		300	+ (ID 1/10)	+ (ID 1/10)	1000
53	BP	+	+		210	–	+ (ID 1/1)	7300
54	AX-CV	+	–		30	+ (P)	+ (ID 1/10)	420
55	AX	–	+	AX +/BP +	120	–	–	510
56	BL	+	–		120	+ (ID 1/10)	+ (ID 1/10)	2500
57	AX-CV	+	–		150	–	–	1095
58	AX-CV	+	–		5	+ (P)	+ (ID 1/10)	30
59	CPS/CPS	+	+	AX +	40	+ (ID 1/10)	+ (ID 1/10)	200
60	CFP	+	+		30	+ (ID 1)	+ (ID 1/1)	600
61	BL	+	–		270	+ (P)	–	930
62	AX-CV	+	–		30	+ (ID 1/10)	–	60
63	AX	+	–		180	–	+ (ID 1/1)	6020
64	AX-CV	+	–		60	+ (ID 1)	–	180
65	AX	–	–	AX +	2	–	–	12
66	BP	+	–		200	–	–	13 870
67	BL	+	–	Ax +	1	+ (ID 1)	–	9125
68	AX	–	–	AX +	15	–	–	30
69	BP	+	–		15	–	–	30
70	AX-CV	+	+		100	+ (ID 1/10)	–	150
71	AX-CV	+	+		7	+ (ID 1)	+ (ID 1/1)	22
72	AX	+	+		20	+ (ID 1)	+ (ID 1/1)	7300
73	AX-CV	–	–	AX +/BP +	1	–	–	120
74	AX	–	+	AX +/BP +	90	–	+ (ID 1/10)	7300
75	BL	–	+		180	+ (ID 1/10)	+ (ID 1/10)	1700
76	BP	+	+		180	+ (ID 1)	+ (ID 1/1)	7300
77	AX	+	–		330	–	–	365
78	AX	+	–	AX +	180	+ (ID 1)	–	4015
79	AX	+	–		300	–	–	4015
80	AX-CV	–	+	BP +	165	–	–	365
81	AX-CV	+	–		160	+ (ID 1/10)	–	510
82	AX-CV	+	–		260	+ (ID 1/10)	–	780
83	AX	+	–		30	–	–	120
84	AX	–	–	AX +	30	–	–	90
85	AX-CV	+	–		120	+ (ID 1)	–	420
86	AX-CV	+	–		20	+ (ID 1/10)	+ (P)	60
87	BP	+	–		60	–	–	3650
88	BP	+	+		120	–	–	6935

Abbreviations: AX, amoxicillin; AX-CLV, amoxicillin-clavulanic acid; BL, nonidentified ß-lactam; BP-OL, benzylpenicilloyl octa-L-lysine; CPS, cephalosporin; ND, not done; PV, penicillin V; BP, benzylpenicillin.

results were significantly more often negative when the interval between the reaction and the study was longer (Figure 3). There was a statistically significant difference between positivity to PPL and BP-OL ($P<.05$).

When both reagents were taken into consideration, sensitivity reached 61.36% (95%CI, 50.62-72.11), with a specificity of 100% (95%CI, 99.37-100). The results for the different parameters are shown in Table 3. No adverse reaction or delayed response to skin testing was observed in any of the 167 patients who comprised the total population of this clinical trial.

The test was carried out on both arms of 73.37% of the study patients; the result of the diagnostic test was the same for both arms (precision of 100%).

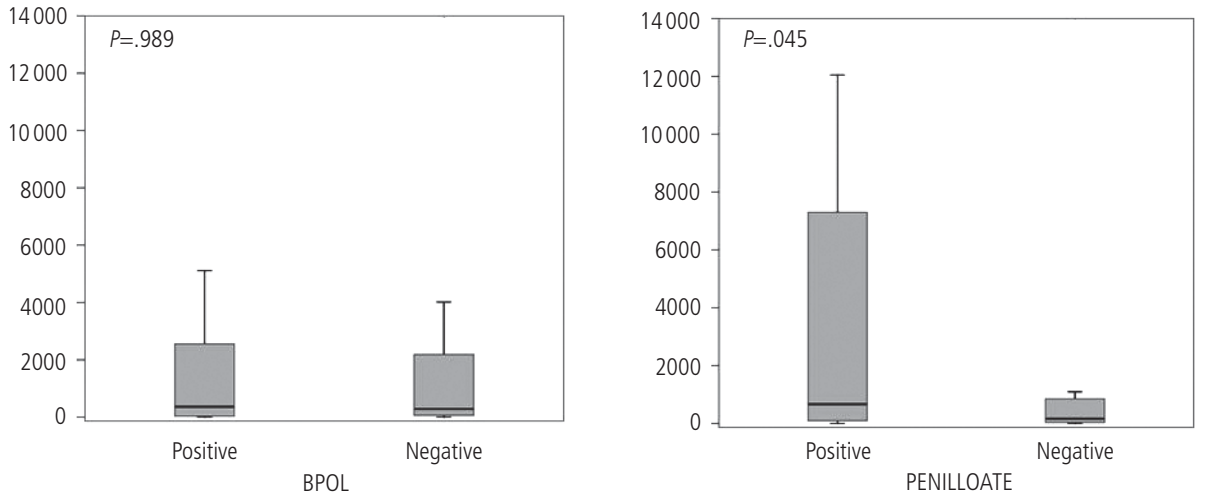

Figure 3. Box-plot analysis of the interval between the reaction and the study in patients with positive and negative results for both BP-OL and benzylpenilloate. BPOL indicates benzylpenicilloyl-octa-L-lysine.

Table 3. Sensitivity, Specificity, and Negative and Positive Predictive Values of the New Reagents

	Parameter	95%CI
Sensitivity	61.36%	50.62%-72.11%
Specificity	100%	99.37%-100%
Negative predictive value	69.91%	61.01%-78.81%
Positive predictive value	100%	99.07%-100%

In Vitro Results

The sensitivity of in vitro testing using BP was nearly 3 times lower than that detected by skin testing (25.00% vs 61.63%), whereas the specificity reached 100% with both methods. Moreover, the combination of a negative skin test result and positive in vitro test result was not detected.

Discussion

We assessed the diagnostic capacity of new products for skin testing in a well-defined group of patients with immediate allergic reactions to ß-lactams and a control group. We obtained a sensitivity of 61%. The results of the present study support the recommendations of other authors [16,22], who stated that in addition to BP-derived determinants, other determinants are required to confirm a diagnosis of allergy to ß-lactams; consequently, BP-derived determinants are still necessary.

Comparisons between the BP major determinants PPL and BP-OL showed that 73.9% of patients had positive results with PPL, whereas 52.3% had positive results with BP-OL. This discrepancy may be explained by the difference in timing of skin testing between PPL and BP-OL, which, although less than 1 year, could have affected the rate of positivity. This decrease in sensitivity over time has previously been reported with skin testing and in vitro tests with ß-lactams [23,24].

Moreover, the analysis of minor determinants revealed a decrease from 46.6% with MDM to 37.5% with penilloate. With these determinants, the time interval between the reaction and the study had a marked effect on the possibility of detecting a positive response, because the evaluation with penilloate was always performed after that with MDM, as previously described [23,24]. In addition, MDM contains BP, and this could affect the results. Thus, as reported by Romano et al [25], skin testing revealed that a low percentage (<5%) of patients are allergic to ß-lactams but positive to BP alone. However, although BP alone seems to contribute to total sensitivity to MDM, the stability of the product is higher when penilloate is used alone. This requirement is necessary for improving the quality and standardization of the product in order to obtain marketing authorization from European and American agencies.

It is remarkable that both BP-OL and penilloate were safe, with no systemic adverse reactions in any of the patients. This finding is important, considering that the percentage of systemic symptoms induced by skin testing with penicillin has been reported to be 1.3% of all tested patients and 8.8% of patients with positive skin test results [26]. Of note, in the present study, 100% specificity was obtained with both reagents in the control group.

Finally, comparisons between skin tests and in vitro IgE determination showed that, as previously described [27], the former are more sensitive. Skin testing with the new reagents increased sensitivity in more than 36.63% of cases. In contrast to the results reported by other authors [28], we found no cases of patients with a negative skin test result and positive in vitro result [28].

Our results show that testing with the new major and minor determinants of BP, BP-OL, and penilloate is highly sensitive and specific. In addition, the fact that we observed

no systemic reactions indicates that their safety profile is good. Although these determinants remain necessary for diagnosis, even in a population where amoxicillin is the ß-lactam most often inducing the reaction, more studies are needed in the general population, even in predominantly amoxicillin-allergic populations.

Acknowledgments

We thank Ian Johnston for help with the English language version of the manuscript.

All of the authors had full access to the data, which are the property of Diater Laboratorios. Diater Laboratories is responsible for the integrity of data and the accuracy of the data analysis.

Funding

The study was funded in part by the FIS-Thematic Networks and Cooperative Research Centers (RIRAAF/RD07/0064) and by Diater laboratories.

Conflicts of Interest

All potential conflicts of interest have been disclosed.

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Manuscript received September 27, 2012; accepted for publication February 8, 2013.

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