

Original article

Skin testing for immediate hypersensitivity to betalactams: comparison between two commercial kits

Introduction: Skin testing with major and minor determinants of benzylpenicillin is the recommended standard practice to evaluate subjects with immediate hypersensitivity to betalactams. The withdrawal of these products from the market has set us back to the early days, before the introduction of reagents for *in vivo* testing.

Objectives: To compare a recently released kit of benzylpenicillin conjugated to poly-l-lysine (PPL) and minor determinants mixture (MDM) with the previously existing kit in a positive control group of subjects sensitized to major and/or minor determinants of benzylpenicillin.

Methods: Skin tests with both kits were made in a group of positive subjects previously diagnosed with immediate hypersensitivity to penicillins and with positive results to PPL and/or MDM and in a negative control group. Radio-allergosorbent test (RAST) inhibition assays with a pool of sera and individual samples were carried out to compare the inhibition capacity of PPL and MDM of both kits.

Results: Of 22 cases selected from our historical group, 14 were positive: eight to PPL, three to MDM and three to both. These results were equivalent for both kits. RAST inhibition studies showed similar potencies in the inhibition of PPL and MDM.

Conclusions: Both tests show similar results in terms of RAST inhibition assays and skin tests sensitivity and specificity in the groups selected. The new assay can be used for the same purpose and indications as the previous test.

J. L. Rodríguez-Bada¹,
M. I. Montañez³, M. J. Torres¹,
C. Mayorga¹, G. Canto²,
E. Perez-Inestrosa³, R. Suau³,
M. Blanca¹

¹Research Unit for Allergic Diseases, Allergy Service, Carlos Haya Hospital, Málaga, Spain;

²Allergy Service, 12 de Octubre Hospital, Madrid, Spain; ³Department of Organic Chemistry, Malaga University, Spain

Key words: allergy; betalactams; determinants; diagnosis; skin tests.

Maria José Torres Jaén
Laboratorio de Investigación
Hospital Civil, pabellón 5, sótano
29009 Malaga
Spain

Accepted for publication 17 April 2006

Allergic reactions to betalactams (BL) are the most common cause of adverse drug reactions mediated by specific immunological mechanisms, with immediate reactions being the most frequent and best studied (1, 2). Skin testing with the major determinant of benzylpenicillin coupled with poly-l-lysine and the so-called minor determinant mixture (MDM), composed of benzylpenicillin and benzylpenicilloic acid, has long been used for the diagnosis of patients with an immediate immunoglobulin E (IgE) response to penicillins. These reagents are part of the standard skin testing reagents used for the diagnosis of immediate hypersensitivity to BL (3–11).

Benzylpenicilloyl-poly-l-lysine (PPL), distributed in the USA by Urban Kremmer, was removed from the market in the early 1990s. In 2005, the other product still available (Allergopharma kit) was also withdrawn by Merk in Europe and the rest of the world, thereby returning us to a situation similar to that in the pre-1960s. Despite changes in some countries in the use of BL and the appearance of side-chain-specific reactions, the use of

PPL and MDM is still necessary for the diagnostic evaluation (12, 13). In an earlier study, published by our group, 54.7% of all patients with a positive skin test to BL determinants (including aminopenicillins) were positive to one or more determinants generated by benzylpenicillin (PPL and/or MDM) (14).

A new kit with major and minor determinants of benzylpenicillin has recently been commercialized in Spain for skin testing, following the general approach described elsewhere (3, 11). The aim of this study was to compare this new kit with the previous widely used kit by performing skin tests and immunochemical studies. The results indicate that these new conjugates are equal in potency, sensitivity and specificity to the previously existing kit and do not differ in terms of applicability.

Patients and methods

Patients and controls

Patients diagnosed with an immediate allergic reaction to BL using the diagnostic procedure described in the European Network for Drug Allergy protocol were evaluated (13). Inclusion criterion

Abbreviations: PPL, benzylpenicilloyl-poly-l-lysine reagent for skin testing; MDM, minor determinant mixture reagent for skin testing.

included a positive skin test to PPL and/or MDM during the two previous years.

Forty skin-test-negative patients with good tolerance to benzylpenicillin were used as negative controls. The study was approved by the relevant institutional review boards, and informed consent for the diagnostic procedures was obtained from the patients and controls.

Chemical characterization of the Diater reagents

PPL and MDM provided by Diater Laboratories (Madrid, Spain) were characterized by reverse-phase HPLC. MDM samples for quantitative analysis were prepared by dissolving the content of one vial in 1 ml acetonitrile : water (50% v/v) mixture and filtered thorough nylon filters. 20 µl of sample was eluted [Luna (150 × 4.6 mm) 5 µm Phenomenex column] by using a linear 4.5–93% acetonitrile gradient in 2.5% formic acid for 30 min at a flow rate of 1 ml/min. Elution was monitored at 280, 254 and 210 nm.

Benzylpenicilloyl-poly-L-lysine samples were prepared as described (15). About 30 µl of diluted sample was chromatographed in a HPLC Alliance HT (Waters) equipped with a X-Terra MS (Waters Corp, Milford, MA, USA) C₁₈ 3.5 µm (2.1 × 50 mm) column using a linear 0–100% acetonitrile gradient in 0.1% Trifluoroacetic acid with a 0.5 ml/min flow rate, coupled to a Water Micromass ZQ mass detector (Waters Corp, Manchester, UK).

Skin test

Skin testing was carried out as previously described (13), using PPL (5×10^{-5} M) and MDM (2×10^{-2} M), provided by Allergopharma (Reinbeck, Germany) and Diater. In both prick and intradermal testing, a minimum wheal area of 3 mm or an increase of area > 3 mm was considered positive, with a negative response to the saline control [for more detail see references (13 and (16)).

In vitro specific IgE antibody determination

This was performed by radioallergosorbent test (RAST) as described (17). Briefly, 30 µl of patient sera were incubated with the disc with PPL conjugates. Washed radiolabelled anti-IgE antibody (Pharmacia Diagnostics, Uppsala, Sweden) was then added and incubated overnight. The discs were then washed and their radioactivity was measured in a gamma counter, Cobra II auto-gamma (Packard BioScience Company, Frankfurt, Germany). Results were calculated as a percentage of the maximum. Samples were considered positive if they were > 2.5% of label uptake, which was the mean + 2 SD of the negative control group.

RAST inhibition

Cross-inhibition studies were made using PPL in the solid phase. This was carried out as reported (18) by incubating sera from patients allergic to BL (with RAST values > 7% label uptake) with PPL and MDM from Allergopharma and Diater laboratories, at two 10-fold-concentrations, 6×10^{-6} and 6×10^{-7} M for PPL and 2×10^{-2} and 2×10^{-3} M for MDM. Comparison of the inhibition capacity of the reagents was made at 50% inhibition.

Statistical studies

The coefficient of variation for each measurement for the RAST inhibition assays was made with a pool of sera to establish the mini-

imum variation accepted as different cross reactivity. Values > 15% were considered as different and the coefficient of variation of each sample was within 10% of the variation. Comparison in the percentage of positive cases to the kits was made by chi-square analysis.

Results

Minor determinant mixture reagents from Diater were made by basic hydrolysis, to yield benzylpenicilloate, and by subsequent acid degradation, to give the corresponding benzylpenilloate. The MDM composition mixture was carefully analysed by HPLC and only three peaks at 9.44, 10.65 and 13.47 min were detected and quantified. The chemical structure of these compounds was unambiguously assigned by the interpretation of their Mass Spectrum, for which 308, 352 and 334 amu (atomic mass units) were observed, corresponding to their respective molecular weights.

The PPL HPLC chromatogram revealed two peaks at 5.82 and 6.20 min, integrating for 21% and 68% areas, respectively. The mass spectrum of these peaks revealed that the major component corresponded to octalysine, in which eight benzylpenicilloyl groups were attached, and the minor component to octalysine, bound with seven benzylpenicilloyl groups.

Radioallergosorbent test inhibition studies using three individual cases, with a low, medium and high percentage of RAST binding, are shown in Fig. 1. Greater than 50% inhibition was found in all the sera, with the highest concentration of the inhibitor, PPL or MDM, in the fluid phase. This percentage inhibition tended to decrease with the lower concentration, the decrease being greater with the PPL reagent. Comparison between the PPL and MDM reagents from Allergopharma and Diater showed that the percentage inhibition detected was parallel and almost exactly the same for the three sera evaluated. Similar results were obtained using a pool of sera from the positive cases (data not shown).

We evaluated 22 patients, 10 men and 12 women, diagnosed with an immediate allergic reaction to BL and with positive skin tests to PPL or MDM during the two previous years, as tested with the Allergopharma reagents. The mean age was 46.9 years (range, 20–69) and the mean time interval between the onset of the reaction and the skin tests performed for this study was 1296 days (range, 30–7300 days). Eight patients had developed urticaria, eleven anaphylaxis, and three an anaphylactic shock. Amoxicillin was the drug involved in the reaction in 18 patients, benzylpenicillin in two and un-recalled penicillin in two.

These patients were all originally skin test positive to PPL and/or MDM using the Allergopharma reagents. Nine were positive to PPL, 10 to MDM and three to both PPL and MDM. RAST to PPL was positive in eight patients, all of whom were also skin test positive (Table 1). At the time of the evaluation for this study using the Allergopharma reagents, eight patients were

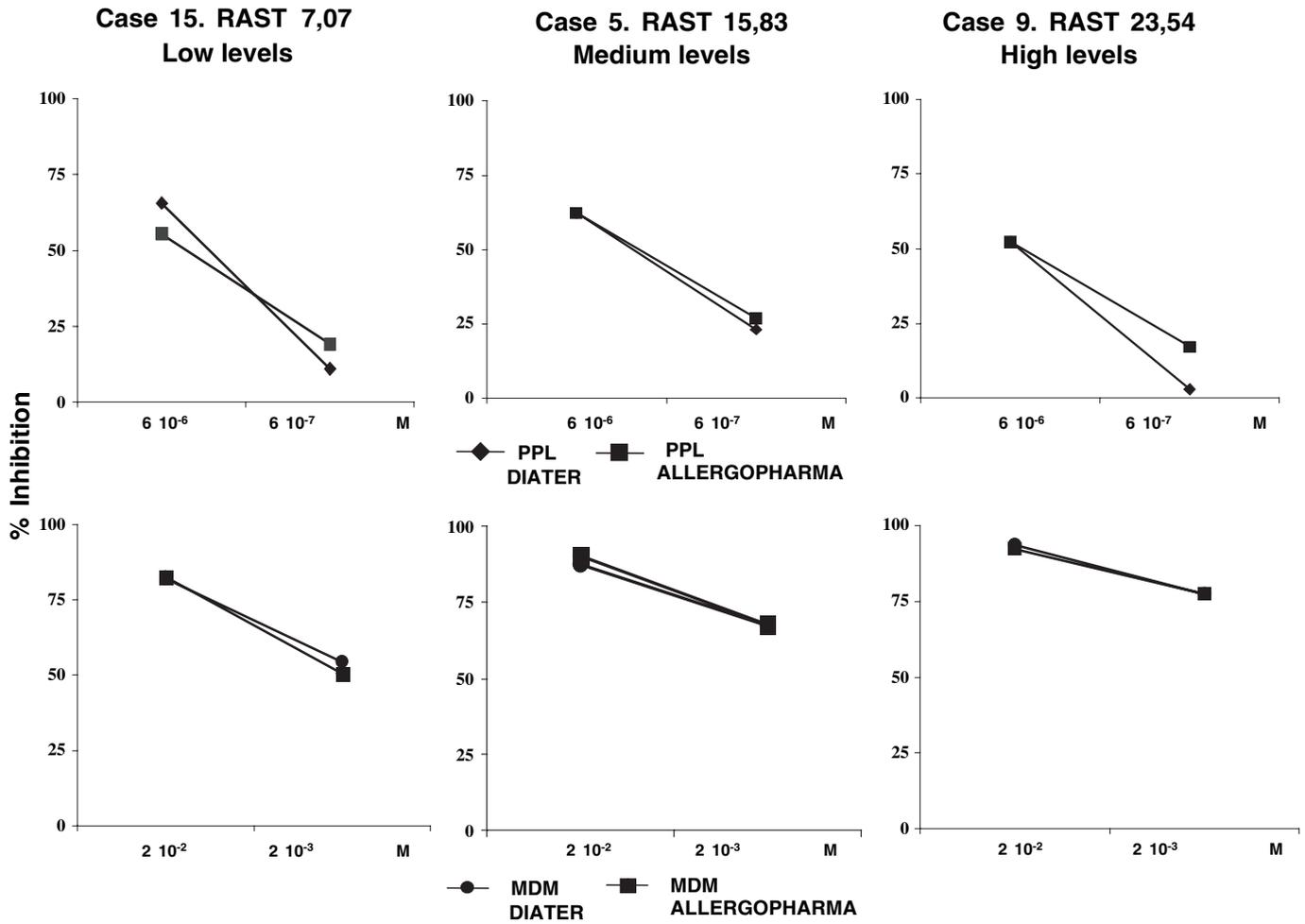


Figure 1. Radioallergosorbent test (RAST) inhibition assays using benzylpenicilloyl-poly-l-lysine (PPL) in the solid phase and PPL and minor determinant mixture (MDM) from Allergopharma and Diater in the fluid phase. Three cases are shown with different RAST results (low, medium and high).

negative and 14 remained positive (eight to PPL, three to MDM and three to both determinants). Table 1 also shows the results obtained using PPL and MDM provided by Diater. Eight of the patients were skin test negative and 14 positive (eight to PPL, three to MDM and three to both determinants). Comparison between the Allergopharma and Diater reagents showed almost identical results for both haptens. The only difference concerned case 16, who was skin prick negative and intradermal test positive at one-tenth dilution with Allergopharma and skin prick positive with Diater. Skin test with PPL and MDM from Diater laboratories was negative in the negative control group.

Discussion

After > 50 years of use, skin testing with major and minor determinants of benzyl penicillin following the

classical recommendations has proved to be safe, with the incidence of side effects < 1% (19, 20). Figures regarding resensitization are also low or negligible. A recent study by Nugent *et al.* showed that 2.5% of the initially negative cases converted to positive after skin testing with PPL, benzylpenicillin and MDM (21).

The withdrawal of PPL in the 1990s in USA, followed by its recent withdrawal in Europe in 2005, has created a situation similar to that in the late 1960s and early 1970s. We are only aware of one company currently providing a substitute for this product for *in vivo* diagnostic use. Although the manufacturer's information indicates that the product is valid for *in vivo* diagnosis, no comparative studies with the pre-existing reagents have been carried out. Accordingly, we undertook a comparative study between the Diater kit and the Allergopharma kit, obtained from our few remaining stocks. The results indicated that the reagents from both companies were equivalent in the skin test response to PPL, MDM, or

Table 1. Drug involved, time interval, RAST with PPL and ST results with PPL and MDM from Allergopharma and Diater

ID	Drug	Interval (days)	RAST PPL	ST PPL Allergopharma	ST MDM Allergopharma	ST PPL Diater	ST MDM Diater
1	AX	30	1.97	ID-	5 × 4 mm*	ID-	4 × 4 mm*
2	AX	30	0	ID-	ID-	ID-	ID-
3	AX	1460	14.7	3 × 4 mm*	ID-	4 × 5 mm*	ID-
4	AX	1460	0.53	4 × 4 mm**	ID-	4 × 4 mm**	ID-
5	BP	730	15.83	3 × 4 mm*	ID-	3 × 5 mm*	ID-
6	AX	60	16.35	6 × 4 mm	ID-	5 × 5 mm	ID-
7	AX	730	2.3	ID-	ID-	ID-	ID-
8	AX	730	1.26	ID-	ID-	ID-	ID-
9	AX	30	23.54	ID-	3 × 4 mm*	Prick-	4 × 4 mm*
10	AX	365	0.28	ID-	ID-	ID-	ID-
11	AX	7300	0.58	ID-	ID-	ID-	ID-
12	PENI	5475	1.17	5 × 4 mm*	ID-	4 × 4 mm*	ID-
13	AX	365	4.04	6 × 6 mm	ID-	5 × 6 mm	ID-
14	AX	730	1.45	ID-	ID-	ID-	ID-
15	AX	5475	7.07	4 × 4 mm	5 × 5 mm	5 × 4 mm	5 × 4 mm
16	AX	2190	0.44	ID-	4 × 5 mm**	3 × 3 mm**	3 × 5 mm*
17	AX	30	1.22	4 × 5 mm	4 × 4 mm	5 × 3 mm	4 × 4 mm
18	PENI	365	12.73	5 × 3 mm	ID-	4 mm	ID-
19	AX	124	8.36	5 × 4 mm	3 × 4 mm	4 × 5 mm	4 × 4 mm
20	AX	30	2.9	4 × 4 mm	ID-	3 × 5 mm	ID-
21	AX	79	0.5	ID-	ID-	ID-	ID-
22	BP	730	1.56	ID-	ID-	ID-	ID-

AX, amoxicillin; BP, benzylpenicillin; ID, intradermal–negative; MDM, minor determinant mixture; PENI, unrecalled penicillin; PPL, benzylpenicilloyl-poly-L-lysine; RAST, radioallergosorbent test; ST, skin tests.

*Positive by prick testing; **Positive by intradermal testing (1/10).

both, with 63% of the cases being positive. Interestingly, because our cases were taken from previously available positive controls from our data base, the percentage of patients that became negative was also similar for both kits. Of 12 subjects positive to PPL, 11 still remained positive, but of the 10 MDM, only three were still positive ($P < 0.001$). Comparison of the RAST results for PPL showed that the skin test was more sensitive than the *in vitro* tests and that those cases that became skin test negative were also RAST negative. Considering the initial number of positive cases, we were able to confirm that a considerable percentage remained positive; this is in agreement with previous studies of natural history of sensitivity, indicating that those cases positive to PPL are those who become negative later (22).

The results of the RAST inhibition studies showed a similar behaviour for all the sera, either PPL or MDM, with the maximum concentration producing at least 50% inhibition in all the sera. Although we have provided the chemical characteristics of the Diater reagents, similar data for the Allergopharma product have been omitted because of the unavailability of the appropriate compound for these studies.

To summarize, the results of this study indicate that the kit currently provided by Diater laboratories for the *in vivo* diagnosis of immediate hypersensitivity to BL is at least as sensitive as the previously existing Allergopharma kit, giving similar results in a positive control group. RAST inhibition studies showed that the capacity of the Diater kit to inhibit the IgE specific response was also similar. **The Diater kit can therefore be recommended for the *in vivo* diagnosis of allergy to penicillins.**

Conflict of interest

The authors have no conflict of interest concerning the data reported in this study.

Acknowledgments

We thank Ian Johnstone for help with the final English language version of this manuscript. This work was supported by Grants Fondo de Investigación Sanitaria (PIO31165) and Junta de Andalucía (197/04).

References

1. Weiss ME, Adkinson NF. Immediate hypersensitivity reactions to penicillin and related antibiotics. *Clin Allergy* 1988;**18**:515–540.
2. Blanca M, Garcia J, Vega JM, Miranda A, Carmona MJ, Terrados S et al. New aspects of allergic reactions to betalactams: cross-reactions and unique specificities. *Clin Exp Allergy* 1994;**24**:399–407.
3. De Weck AL, Blum G. Recent clinical and immunological aspects of penicillin allergy. *Int Archs Allergy Appl Immunol* 1965;**27**:231–256.
4. Adkinson NF Jr, Thompson WL, Maddrey WC, Lichtenstein L. Routine use of penicillin skin testing on an inpatient service. *N Eng J Med* 1971;**285**:22–24.
5. Green GR, Roseblum AH, Sweet LC. Evaluation of penicillin hypersensitivity: Value of clinical history and skin testing with penicilloyl-polylysine and penicillin G. A cooperative prospective study of the penicillin study group of the American Academy of Allergy. *J Allergy Clin Immunol* 1977;**60**:339–345.
6. Van Dellen RG, Walsh WE, Peters GA, Gleich GJ. Differing patterns of wheal and flare skin reactivity in patients allergic to the penicillins. *J Allergy* 1971;**47**:230–236.
7. Solley GO, Gleich GJ, Van Dellen RG. Penicillin allergy: clinical experience with a battery of skin test reagents. *J Allergy Clin Immunol* 1982;**69**:238–244.
8. Mendelson LM, Ressler C, Rosen JP, Selcow JE. Routine elective penicillin allergy skin testing in children and adolescents: study of sensitization. *J Allergy Clin Immunol* 1984;**73**:76–81.
9. Sullivan TJ, Wedener HJ, Shatz GS, Yecies LD, Parker CW. Skin testing to detect penicillin allergy. *J Allergy Clin Immunol* 1981;**68**:171–180.
10. Gadde J., Spence M., Wheeler B., Adkinson NF Jr. Clinical experience with penicillin skin testing in a large inner-city STD clinic. *JAMA* 1993;**27**:2456–2463.
11. Macy E, Ritcher PK, Falkoff R, Zeiger R. Skin testing with penicilloate and penilloate prepared by an improved method: amoxicillin oral challenges in patients with negative skin test responses to penicillin reagents. *J Allergy Clin Immunol* 1997;**100**:586–591.
12. 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 beta-lactam allergy. *J Allergy Clin Immunol* 2005;**115**:1314–1316.
13. Torres MJ, Blanca M, Fernandez J, Romano A, Weck A, Aberer W et al. Diagnosis of immediate allergic reactions to beta-lactam antibiotics. *Allergy* 2003;**58**:961–972.
14. Blanca M, Perez E, Garcia J, Miranda A, Fernandez J, Vega JM et al. Anaphylaxis to amoxicillin but good tolerance for benzylpenicillin. *Allergy* 1988;**43**:508–510.
15. Levine BB, Ovary Z. Studies of the mechanism of the formation of the penicillin antigen III. The N(D-(Benzyl penicilloyl)) group as an antigenic determinant responsible for hypersensitivity to penicillin G. *J Exp Med* 1961;**114**:875–904.
16. Brockow K, Romano A, Blanca A, Ring J, Pichler W, Demoly P et al. General considerations for skin test procedures in the diagnosis of drug hypersensitivity. *Allergy* 2002;**57**:45–51.
17. Blanca M, Mayorga C, Perez E, Suau R, Juarez C, Vega JM et al. Determination of IgE antibodies to the benzylpenicilloyl determinant. A comparison between poly-L-lysine and human serum albumin as carrier. *J Immunol Methods* 1992;**153**:99–105.
18. Moreno F, Blanca M, Mayorga C, Terrados S, Moya MC, Perez E et al. Studies of the specificities of IgE antibodies found in sera from subjects with allergic reactions to penicillins. *Int Arch Allergy Immunol* 1995;**108**:74–81.
19. Lockey RF, Benedict LM, Turkeltaub PC, Bukantz SC. Fatalities from immunotherapy (IT) and skin testing (ST). *J Allergy Clin Immunol* 1987;**79**:660–677.
20. Valyasevi MA, Van Dellen RG. Frequency of systematic reactions to penicillin skin tests. *Ann Allergy Asthma Immunol*, 2000;**85**:363–365.
21. Nugent JS, Quinn JM, McGrath CM, Hrnrcir DE, Boleman WT, Freeman TM. Determination of the incidence of sensitization after penicillin skin testing. *Ann Allergy Asthma Immunol* 2003;**90**:398–403.
22. Blanca M, Torres MJ, Garcia JJ, Romano A, Mayorga C, de Ramon E et al. Natural evolution of skin test sensitivity in patients allergic to betalactam antibiotics. *J Allergy Clin Immunol* 1999;**103**:918–924.