

Continued need of appropriate betalactam-derived skin test reagents for the management of allergy to betalactams

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Summary

Immediate allergic reactions to betalactams (BLs) are due to IgE antibodies that recognize the ring-derived penicilloyl determinant or side-chain structures of common BLs. The presence of specific IgE antibodies can be demonstrated by skin testing, the determination of specific IgE antibodies in sera or their binding to basophils with subsequent activation upon contact with penicillins *in vitro*. Skin tests are still the most sensitive technique followed by *in vitro* tests, which may sometimes yield useful complementary information. The diversity of the response to BLs has meant that in some instances, in addition to benzylpenicillin-derived determinants, testing for amoxicillin, cephalosporins or other BLs may also be required to establish the diagnosis. The recent withdrawal from the market of BL-derived materials for skin testing will have a serious effect on public health, resulting in a return to the pre-1960 era before these reagents became available. Because of their greater sensitivity, these skin tests cannot yet be replaced by *in vitro* tests. Furthermore, skin tests are the most readily available form of allergy testing for physicians. This paper reviews the results of skin tests in BL allergy and provides evidence for their continued need.

Keywords allergy, betalactam, diagnosis, skin test

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Introduction

Allergic reactions were reported shortly after the introduction of penicillins as therapeutic agents [1]. By the 1950s, increasing reports of anaphylactic reactions and other side-effects testified to a serious public health problem [1]. Fifty years later, penicillins and the other members of the betalactam (BL) family are still the drugs most commonly involved in allergic drug reactions. This trend is expected to continue in the future [2, 3]. Immunological tests have been used since early times to evaluate subjects with allergic reactions. Skin tests were the first to be applied and both immediate and delayed responses were soon observed [4, 5].

Skin testing with BL is currently a source of public health concern because of the sudden lack of availability world-wide of appropriate reagents for *in vivo* testing. Benzylpenicilloyl-polylysine (BPO-PLL), also known as penicilloyl-polylysine (PPL), was first developed in 1960/1961 [6–8] and afforded a major improvement to the

diagnosis of penicillin allergy at the time; this was soon followed by the so-called minor determinant mixture (MDM) [9]. Unfortunately, BPO-PLL was removed from the US market in 2004 by Hollister-Stier while Allergopharma ceased production of PPL and of MDM in Europe in 2005. This means that these essential reagents are no longer available to the majority of allergologists. This review highlights the usefulness of skin tests with recommendations about their adaptation to the current clinical requirements relating to allergy to penicillins and the other BL antibiotics.

Continued need for skin testing

Immediate reactions to BL are still the most important cause of allergic drug reactions [2, 3]. Ten percent of ambulatory and hospitalized patients report allergies to medications containing BL antibiotics [2]. The incidence of anaphylactic reactions varies from 0.004% to 0.015% [5]. The use of alternative agents may adversely affect the

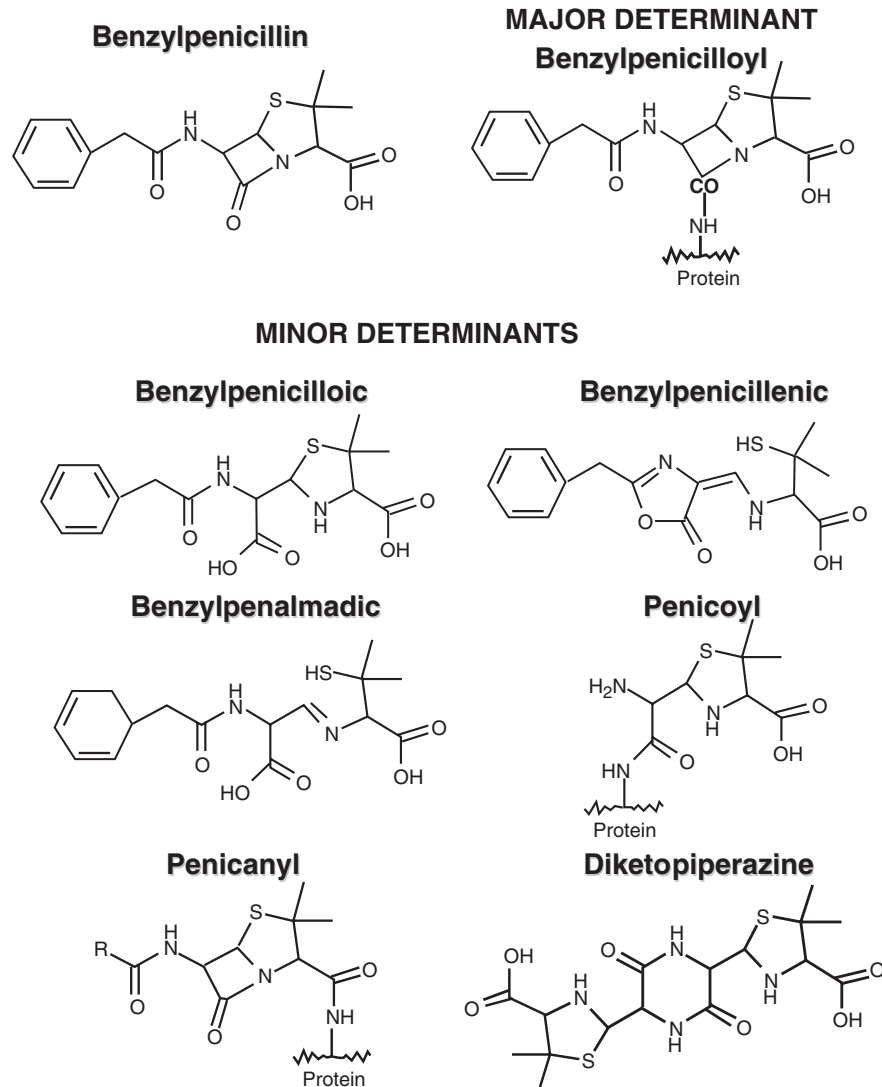


Fig. 1. Chemical structures of the penicillin molecule and proposed major and minor determinants.

ability to overcome anti-microbial resistance and the choice of other antibiotics, such as vancomycin or quinolones, is more costly and more likely to have adverse effects [10, 11].

BLs are also often prescribed in the absence of definite clinical indications, leading to unnecessary high-exposure rates [12, 13]. Allergic reactions may be merely coincidental to BL administration, with no causal relationship. Subjects are often labelled as allergic to BL with no additional investigations, even though they are not in fact allergic [14]. On the other hand, anaphylaxis to BL is underreported [15]. Amoxicillin (AX) is considered nowadays the most frequent cause of anaphylaxis to BL [3, 16, 17]. For many physicians, penicillin skin testing has become a component of antibiotic prescriptions [18]. Thus, it will be a major public health setback if optimal skin testing to BL is no longer possible.

Historical perspective

The BL ring binds covalently to the free amino groups of proteins, forming stable conjugates [19], such as the so-called BPO determinant [20]. It was soon shown that the bulk component of the immunological response was directed towards this structure [4, 21], hence the denomination of the major determinant, although other reactivities of the penicillin molecule had also been described [6, 22]. Later, it was noticed that some individuals with anaphylactic reactions did not respond to this penicilloyl structure but reacted to Benzylpenicillin (BP) directly applied to the skin. This observation led to the discovery of other so-called MDM [9, 23]. Fewer than 10% of antibody responses appear to be due to these minor determinants [24]. Figure 1 shows the chemical structures of the penicillin molecule and the major and minor determinants.

For many years, PPL was sold in the USA as a research reagent 'not for human use' by Kremers-Urban, as the FDA obstinately refused to register it, requesting much additional information (toxicity studies, etc.) as relevant for a new drug. Considering the restricted market and the fact that this chemically well-defined reagent was injected intra-dermally into humans in nanogram quantities, these requests seemed somewhat abusive. More so, as at the same time allergologists were still allowed to use their own dust (dirt) extracts as skin test reagents. Under pressure from the National Institute of Allergy and Infectious Diseases (NIAID) and the American Academy of Allergy, which had sponsored a large-scale study [25], the FDA relented. PPL was introduced onto the US market by Kremers-Urban in 1973. In Europe, PPL had been produced and distributed for about 10 years as a research reagent by the Institute of Clinical Immunology of the University of Bern, Switzerland. It was finally registered, first for the French market by the Laboratoire des Staller-gènes in 1974, and subsequently taken over as PPL and MDM by Allergopharma around 1986. Since then, a large number of studies plus innumerable individual reports using both determinants have been published.

Consolidated experience in skin testing with penicillins

The first large study was carried out by Green *et al.*, who evaluated 3000 subjects, of whom 1718 had symptoms compatible with penicillin allergy. PPL plus BP were used and 19% of the cases studied were positive to one or more penicillin reagents. Among the patients who were positive, 54% were only positive to PPL, 22% to BP, and 25% to BPO and BP [25]. At that time, the recommended concentration was established at 5×10^{-5} M for PPL in Europe and 6×10^{-5} in USA and 10 000 U/mL for BP.

In 1981, Sullivan reported a study involving 740 patients, 63% of whom had a positive skin test to one or more penicillin determinants. In this study, in addition to BP, Penicilloic acid (PA) was also used. The percentage of subjects responding to PPL was 21%, to MDM 42%, and to PPL plus MDM 45.2%. Interestingly, cases only positive to BP or to PA were observed, with 14.6% of cases responding only to PA [26]. The use of ampicillin (AMP) for skin testing added no additional information as all AMP-positive cases were also positive to some BP determinants.

In a study carried out by Solley *et al.* with 778 subjects, in addition to major and minor determinants of BP, AMP and methicillin were also used. PPL was positive in 8.2% of the cases, BP in 20.5%, PA in 10.2%, and both BP and PA (MDM) in 38.9% [27]. Interestingly, in this study skin testing with the unmodified penicillins contributed to 11.6% of the cases and their conjugation to PLL added a further 13.9%, representing a total of 25.5%.

The largest study reported was published by Gadde *et al.*, with 5063 subjects, of whom 776 had a previous

history of penicillin allergy. Of these, 7.1% had positive skin tests to penicillin determinants; 75% were positive to PPL, 10% to MDM, and 14% to BPO and MDM [28]. In summary, in all these studies there was a variable degree of response to major and minor determinants of BP, with the response to PPL alone or in association with a response to MDM being positive in over 50% of the cases evaluated. The predictive value of a positive test indicated that 50% of the patients could develop another clinical reaction after re-exposure while the predictive value of a negative test indicated that in the event of further administration of BP, there would be good tolerance, or at least just a minimal clinical response [2].

Since the late 1980s, a number of further studies appeared determining the value of major and minor determinants of BP plus the response to AX and AMP minor determinants. The role of PPL and/or MDM in diagnosis became lower than 50%, with an increasing role for AX over the years [29–33]. This most probably reflects the increasing use of AX vs. the older penicillins in the United States and Western Europe. Nevertheless, a recent retrospective study in France on 824 patients consulting between 1996 and 2004 for BL allergy revealed 136 with positive skin tests [32]. Among those, 14.7% were positive only to PPL and/or MDM and in an additional 27.6–32%, positive skin tests to some other BL were confirmed by positive tests to PPL and/or MDM. Accordingly, the withdrawal of PPL and MDM would have had the consequence of leaving 20/136 patients undiagnosed, including some with anaphylactic reactions. The situation may become even worse in some other regions of the world where exposure to the older BP still seems to be prevalent, as judged from skin testing of the population [33], in which skin tests to PPL, MDM and BP combined yielded as much as 88.1% positive cases.

In Spain, a new PPL and MDM kit has been recently commercialized (Diater Laboratories, Madrid, Spain), with equivalent results that can be used for the same purpose and indications as the previous test [34].

Skin testing with other betalactams

Cephalosporins are currently the second most important group of BL antibiotics and their chemical structures are more heterogeneous than those of penicillins. Increasing evidence supports the role of this group of antibiotics in the induction of IgE-mediated responses [35, 36]. Although conjugation of cephalosporins to albumin and other proteins is less efficient, these compounds are also able to produce hapten-carrier conjugates [36].

The first reactions to cephalosporins were attributed to cross-reactivity with penicillins [37], but increasing evidence has shown that some patients can respond to cephalosporins while tolerating penicillin derivatives [38–45]. Skin testing with cephalosporins raises a number of problems; most oral cephalosporins are poorly soluble,

skin tests are not widely standardized and different concentrations have been used by different groups. Concentrations from 10 to 30 mg/mL have been recommended for intra-dermal testing with some cephalosporins [46]. Data suggest that different patterns of positive responses may occur; while some cases develop cross-reactivity between similar side chains [40, 43, 47], others experience a selective skin response to one cephalosporin only, and in others cross-reactivity between penicillins and cephalosporins seems to occur [48, 49].

Comparison between skin tests, specific immunoglobulin E determinations and other biological tests

In addition to skin testing, various *in vitro* methods are also available for the diagnosis of immediate hyper-sensitivity to BL. These methods can generally be considered complementary, or in some instances alternative. There is a sizeable group of patients with a history of penicillin allergy and negative skin tests but positive drug provocation tests and positive biological tests *in vitro* [50]. Originally performed by radioimmunoassay, the first technique was designated RAST [51, 52]; nowadays, the fluoroimmunoassay is the most widely used test [53].

In a strict sense, no detailed comparative studies have yet been carried out. Most studies compared results of *in vivo* tests, usually BP conjugated to PLL and BP or BP/PA, to an *in vitro* conjugate of BP with human serum albumin (HSA). The *in vitro* equivalent of BP, PA, or other free determinants has never been used. Kraft et al. [54–56] found an excellent correlation between skin tests to BPO and RAST to BPO, but in cases with skin tests only positive to minor determinants the results were variable. Similar results were reported by Basomba et al. [57]. This has been attributed to a high degree of *in vitro* cross-reactivity between MDM and the major determinants [55]. However, it must be pointed out that the specificity of anti-penicillin IgE antibodies can be assessed by comparing free determinants with conjugates in RAST inhibition tests [58].

The conjugation of BPO to PLL has also been used, and although this gave better results than with HSA, an important number of cases still remained skin-test positive but *in vitro* negative [50]. For side-chain-specific antibodies in particular, PLL or an amino spacer have been considered better carriers than HSA. In general, with all these carriers, a good correlation has been found between the clinical situation and the results of the skin test and the *in vitro* test [59, 60]. In a study involving subjects who were skin-test positive to either BPO and/or MDM, *in vitro* testing was positive to BP in 68% of cases and to AX in 52%, but when both haptens were used the positivity increased to 74%. On the other hand, in subjects with a selective response to AX, the sensitivity of the test was 41% [53]. Thus, if only the IgE *in vitro* test is performed, an important number of cases will be missed.

However, cases who were *in vitro* positive but *in vivo* negative have also been reported [50]. In a study carried out in 290 cases, 13.8% were only RAST positive [61]. Whether or not this demonstrates pathological sensitization remains unknown, as these skin test negative – RAST-positive patients have not been BL challenged.

Other *in vitro* biological tests exploit the capacity of basophils from BL-allergic patients to become activated and to release histamine and/or sulphidoleucotrienes (CAST) in the presence of the hapten. In such tests, peripheral blood cells are first stimulated *in vitro* with the hapten. Basophil activation is determined by flow cytometry from the expression of the CD63 molecule on the cell membrane,¹ and the CAST assay quantitates the leucotrienes released in the supernatant. Recently, data have been reported about the usefulness of these two tests [62–64]. In patients with a positive history and a positive skin test, the basophil activation test is positive in about 48–51% of cases, while CAST is positive in 43–47%. When both tests are combined, positivity increases to 68%, as shown in a recent multi-centre study performed by the European Network for Drug Allergy (ENDA) with 150 patients [65]. In the same patients, IgE determination was positive in 38%. Although the new biological tests can give useful complementary information, skin testing remains the method of choice and the first to perform, unless otherwise indicated.

Possible adverse effects of skin testing

After more than 50 years, skin testing with BL determinants has proved safe in the hands of experienced personnel and although fatalities have been described, they concerned patients in whom no prick test was done before the intra-dermal tests [66, 67]. Skin testing with BP determinants has shown a low incidence of side-effects, in the order of 0.12–1% [68]. However, as MDM-positive skin responses are mainly associated with anaphylaxis, systemic reactions may occur. In a recent retrospective, case-control study involving 147 skin-test-positive patients, 13 (8.8%) experienced a systemic reaction during the tests. These 13 reactors were compared with non-reactors (135 patients who had a positive skin test with no systemic reactions). Anaphylaxis (69%) and a chronology of less than 1 h after the drug intake (91%) were significantly more frequent in reactors as compared with non-reactors (43% and 35%, respectively) [69].

Methods for performing skin tests

The two main methods used for the diagnosis of immediate hyper-sensitivity to BL are prick and intra-dermal testing [68, 70]. Although patch testing is used by some,

¹Basophil activation tests commercially available as BASOTEST (Orpegen, Heidelberg, Germany) or FLOW CAST (Bülmann, Allschwil, Switzerland).

this is not considered to be a useful method for IgE-mediated reactions. Some perform patch tests in situations where the type of clinical reaction reported by the patient is uncertain [71, 72].

Prick testing is performed by pricking the skin with an appropriate needle or lancet through the allergen solution. If the response is negative, intra-dermal tests are then carried out. This is done by the injection of 0.02–0.05 mL of the hapten solution, raising a small bleb that is initially delineated. These are usually performed on the volar forearm, but may involve other parts of the body such as the back.

The drug to test has to be freshly re-constituted and taken directly from the vial. Mandatory reagents used are PPL and MDM. The maximum concentrations recommended for PLL are 5×10^{-5} M and for MDM 2×10^{-2} M. In the USA, the concentration of PPL is 6×10^{-5} M. Because MDM is not available in the USA for everyday practice, most authors have used BP at a maximum concentration of 10 000 IU/mL.

The appearance of side-chain-specific reactions to AX has necessitated the use of this antibiotic for testing. This is particularly relevant in some European countries, such as Spain or France, although it has also been reported to be relevant in other countries [68]. Concerning AMP, no specific reactions have been reported with this antibiotic, and it can therefore be considered optional [73, 74]. The maximum concentrations recommended for aminopenicillins are 20–25 mg/mL.

Cephalosporin skin testing is also becoming progressively more necessary, not only to evaluate primary sensitization but also cross-reactivity. Great variability exists with cephalosporins, especially with prick testing. In most instances, concentrations up to 2 mg/mL are considered non-irritant, although studies in negative control groups show that concentrations as high as 50 mg/mL can be used [46].

In patients reporting symptoms of severe reactions or in patients with special risk factors, intra-dermal testing and even prick testing must be done first with several 10-fold dilutions that are gradually increased until the appearance of a positive response or up to the maximum recommended dose [68].

Readings are made 15–20 min after application of the hapten. In the prick test, a weal larger than 3 mm in diameter accompanied by erythema with a negative response to the control saline is considered positive. In intra-dermal testing, the weal area is marked initially and 15–20 min after testing. An increase in the diameter greater than 3 mm is considered positive. Patients should be advised of the possibility of a late reaction that may initiate or be patent as early as 6–8 h after drug application, with a maximal response at 24–48 h. Although not common, this mainly occurs with intra-dermal testing and at maximum doses, though it may also occur with prick testing. Systemic reactions resembling those reported by

the patients, though generally of lesser intensity, may require the skin test to be performed by experienced personnel and in an adequate emergency-ready setting. With classic BP determinants (BPO, MDM), the reported frequency of side-effects is low and reactions usually mild, varying from 0.1% to 0.7% [25, 26, 28, 75]. In patients with positive skin tests, figures of 13% have been reported [62].

Skin testing and re-sensitization

Penicillin allergy is a slowly subsiding phenomenon [56, 76, 77]. The natural history of BL hyper-sensitivity tells us that allergic subjects tend to become negative after drug avoidance for a variable time, a matter of 6–12 months for specific IgE [56] and of a few years for skin sensitivity [26, 78]. Specific T cell reactivity, on the other hand, usually persists for many years [79, 80]. In theory, although a very low concentration of drug is used in skin tests, a possibility for re-sensitization still exists. This has been reported after slight contact with penicillins, at concentrations even lower than those used in skin testing [81].

Most studies involving re-sensitization include both skin tests plus a drug provocation test. This implies that in the event of re-sensitization, it cannot be easily attributed to the role of skin testing [26]. Nevertheless, in most studies, and even including drug provocation, figures are usually low [82, 76]. In a study carried out by Nugent *et al.* [83] in a group of 329 cases, 2.5% of the initially negative cases converted to positive after skin testing with PPL, BP, and MDM.

Conclusions

Despite the advent of various complementary and alternative *in vitro* diagnostic methods, skin testing remains a central need for the diagnosis of BL allergy, a public health problem of continued importance. Some recent studies already show that the withdrawal of PPL and MDM will have deleterious effects on the diagnosis of BL allergy. Public health authorities and physicians should mobilize for continued access by the community to these required skin test reagents.

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