Diagnosing the Hypersensitivity to Betalactams

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Betalactam (BL) antibiotics are the most frequent elicitors of drug hypersensitivity reactions (HR). Although benzylpenicillin (BP) was the first BL implicated in hypersensitivity reactions, amoxicillin (AX) now being the most frequent drug inducing reactions (1). Clavulanic acid is emerging drug involved in a significant number of cases (1,2).

Although HRs can be produced by any of the four immunological mechanisms of Gell and Coombs (3), in the allergy practice we mainly deal with type I and type IV reactions. As a working classification they can be grouped as immediate (appearing within one hour of drug intake) and non-immediate reactions (appearing more than one hour after drug intake).

Early reports showed a frequency of allergic reactions to penicillins ranging from 0.7-10%, with the frequency of anaphylaxis being 0.015-0.004% (4). Studies carried out in large series of patients with cutaneous symptoms showed that 19% were finally diagnosed as being allergic to BLs (5).

Depending on their chemical structure BLS are classified in different classes :penicillin and cephalosporins, monobactams, carbapenems, oxacephems and clavams. The common basic structure consists of a four-member BL ring that in penicillins is condensed with a five-member thiazolidine ring and in cephalosporins with a six-member dihydrothiazine ring. Penicillins have one side chain (R1) and cephalosporins two (R1 and R2), with substitution at the R1 and R2 side chains resulting in various antibiotics. These changes, although is some minor, ca be discriminated as different by the immunological system with relevant clinical consequences (6).

Immediate reactions appear within a period ranging from minutes to one hour after BL administration. The clinical entities are anaphylaxis and urticaria (7).

Within non immediate reactions we actually include accelerated and delayed reactions under the Levine classification (8). Symptoms appear from 24-48 hours after drug administration, although symptoms can initiate within one hour of drug intake (9-11).

The most frequent entity is maculopapular exanthema, followed by urticaria (12,13). Other reactions are more severe and include acute generalized exanthematic pustulosis, drug hypersensitivity syndrome with eosinophilia and systemic symptoms/drug-induced hypersensitivity syndrome (DRESS/DIHS), and bullous eruptions such as erythema multiforme, Stevens-Johnson syndrome, and toxic epidermal necrolysis, fixed drug eruption and contact dermatitis (14,15).

BLs bind spontaneously to endogenous proteins that can be recognized by the immunological system (16,17). The most common structure was benzylpenicilloyl (BPO), which results from the opening of the BL ring by an amino group of the protein, considered during many years the major antigenic determinant. Other determinants identified were benzyl penicillin and benzyl penicilloic, known as minor determinants mixture (MDM) (18,19). In most of cephalosporins, the R2 side chain is lost after

opening of the BL ring, with the R1 and part of the BL ring being recognized by IgE antibodies (20). Cross-reactivity can be explained in terms of similarity of the R1 side chain (21,22). Studies using polyclonal IgE antibodies have shown that although differences in the chemical structures are relevant for the antigenic determinant, the whole structure that includes the protein carrier is necessary for the constitution of the antigen. The relevant parts of the penicillin are the common BL ring, including the thyazolidine and the side chain (23).

Immediate reactions may occur with much lower amounts that therapeutic doses like those used for skin testing or traces present in foods (24,25). The IgE response to BLs is not a long-lasting phenomenon. IgE antibodies decrease over time at a variable rate, depending on the specificity of the antibody. Antibodies to side chain AX determinants become negative before those recognizing the BPO determinant (26).

In non immediate reactions there is involvement of different subsets of T cells in the inflammatory response, tissue damage and repair process. The skin has a mononuclear cell infiltrate composed mainly of T cells, expressing activation markers (CD25, CD69 and HLADR) and the skin homing receptor CLA in both CD4 and CD8 subsets (27,28).

For establishing a diagnosis, in immediate reactions the recommended procedure is skin testing with the PPL and MDM (benzylpenicillin and benzylpeniloic) (29). In countries where AX is the most important drug involved in sensitization, this is also required for diagnosis (30,31). When any other BLs are involved in the reaction and skin tests to PPL, MDM and AX are negative, skin testing with the culprit BL is recommended (2,30,31). Because clavulanic is actually a drug with increasing incidence in production of immediate hypersensitivity reactions within the BLs group, this is now available for in vivo diagnosis.

General procedures have been described by the European Academy of Allergy and Clinical Immunology (30-32). For those severe cases prick testing is recommended first following by intradermal if negative response. A rate of 1.3% of systemic symptoms has been reported in all tested patients and 8.8% in those with a positive skin, with a history of anaphylaxis being a risk factor (25). Precautions must be taken, particularly in severe cases, reducing the hapten concentration, using each determinant separately in time, or even considering performing an *in vitro* test first.

Methods widely used for in vitro testing are based on the immunoassay (33). A commercial platform for routine analysis is the CAP System FEIA method (Phadia, Thermofisher, USA). The specificity of this method ranges from 83.3-100% and the sensitivity from 12.5-25%, depending on the clinical manifestations (34). Another procedure being progressively uses is based on the capacity of these cells to release histamine after activation by a free drug, with a sensitivity of 48.6% and a specificity of 93% (35). Both *in vitro* tests, although less sensitive than skin testing, have proved to be complementary, with some cases being skin test negative and in vitro test positive (36).

DPT can be considered for those patients who are skin test and *in vitro* test negative, have no risk factors and for whom diagnosis is mandatory (37). It is estimated that up to 30% of the patients with immediate allergic reactions to BL will fail to be diagnosed if DPT is not done (68). This is usually performed as a single-blind placebo-controlled test under strict hospital surveillance with emergency room facilities (30,31). The drug is administered at increasing doses, with a minimum interval of 30-60 minutes between each administration if good tolerance is established at the previous dose, until the full therapeutically dose is reached.

For non immediate reactions skin testing is indicated in patients with exanthematic or urticarial reactions. Intradermal and/or patch tests with a late reading at 24-48 h have usually been recommended for the diagnosis of non-immediate reactions to BL.

Intradermal testing is done in the same way as for immediate reactions, with readings at 48 and 72 h, considering as positive any infiltrated erythema with a diameter >5 mm (38). These reactions should be documented by the diameter of the erythema and the papulation/infiltrate, as well as a morphological description (erythematous swelling, erythematous infiltrate, only erythema, eczema with papulation with or without vesicles). Patch tests can be done with BP, AM, AX and the culprit BL, using a concentration of 5% in petrolatum. Readings should be made according to the European Environmental and Contact Dermatitis Research Group patch test classification, 15 min after removal of the strips and 24 h and 48 h later.

For in vitro testing, the lymphocyte transformation test (LTT), although it is not routinely recommended, can be used in vitro for the evaluation of non-immediate reactions. In a study carried out by our group, 57% of patients had a positive LTT to at least one of the penicillins tested (39).

Although skin and *in vitro* testing have been proposed for the diagnostic evaluation of non-immediate reactions to BL major limitations exist, due to the low sensitivity of and difficulties in training personnel to perform the LTT (9,10). Because exanthematic reactions are mild in nature, and in many instances subjects may have good tolerance, DPT is recommended. The methodology, as for immediate reactions, consists of the administration of the BL at increasing doses under a careful clinical observation, paying particular attention to symptoms that may usually start after more than one hour, giving increasing doses up to a maximum amount of one fifth of the therapeutic dose. If good tolerance exists in this first step, at least 48 hours later increasing doses are usually given up to a full therapeutic dose. DPT is contraindicated in the case of severe reactions, such as DRESS/DIHS, bullous eruptions, acute generalized exanthematic pustulosis or hematologic reactions.

Due to similarities in their structures cross-reactivity exist between different penicillins and even between penicillins and cephalosporins (7,23,25). The discovery of side chain specific determinants in immediate reactions and the observation that in non-immediate reactions to BLs subjects with a positive response to aminopenicillins could tolerate other compounds like BP and penicillin V, led to the idea that cross-reactivity is not equal amongst all BLs and that the immunological mechanism and the primary drug inducing the sensitization need to be taken into account (12,13,16,17). The rate of cross reactivity with cephalosporins in patients with a primary allergy to penicillins is around 10% (40). It is assumed that first generation cephalosporins can cross-react with penicillins, because their structural features are more similar to those of penicillin, while second and third generation cephalosporin are less likely to induce cross-reactivity, owing to differences in their chemical structure. Cross-reactivity increases to more than 30% in those cases were penicillins and cephalosporins share the same side chain, as occurs between amoxicillin and cefadroxil (41). Cross-reactivity between other BL groups seems to be very low. A rate of 0.9% has been reported between imipenem and penicillins (42).

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Table 1. Gell and Coombs classification of hypersensitivity reactions to drugs.

TYPE OF REACTION	MECHANISMS	CLINICAL SYMPTOMS
Type I **Immediate**	*IgE-mediated*	Urticaria Angioedema Anaphylaxis Anaphylactic shock Bronchial asthma Rhinitis
Type II **Cytotoxic**	*Antibody-mediated*	Immune hemolitic anemia Trombocytopenia Blood diseases Organ-specific reaction
Type III **Immunocomplex**	*Immunocomplex-mediated*	Serum sickness-like syndrome Vasculitis Organ-specific reaction
Type IV **Delayed**	*T cell-mediated*	Maculopapular exanthema Delayed urticaria Stevens–Johnson syndrome Toxic epidermal necrolysis Organ-specific reactions Acute generalized exanthematic pustulosis DRESS/DHIS Fixed drug eruption Contact eczema

Table 2. Classification of penicillins and cephalosporins.

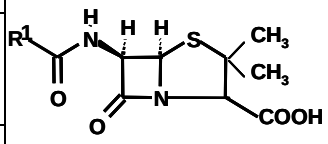
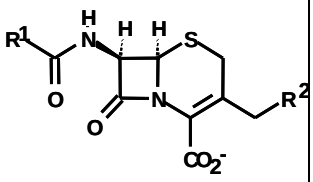
GROUP	COMPOUNDS		STRUCTURE
PENICILLINS			
Natural	Penicillin G Penicillin V		
Aminopenicillins	Amoxicillin Ampicillin	Bacampicillin	
Penicillinase-Resistant	Methicillin Oxacillin Cloxacillin	Nafcillin, Dicloxacillin	
Carboxypenicillins	Carbenicillin Ticarcillin		
Acylaminopenicillins	Azlocillin Mezlocillia	Piperacillin	
CEPHALOSPORINS			
First Generation	Cefadroxil Cefalexin Cephalotin Cefapirine	Cefazoline Cefprozil Cefradine	
Second Generation	Cefaclor Cefamandole Cefmetazole Cefminox Cefonicid Ceforanide	Cefotetan Cefotiam Cefoxitín Cefuroxime Loracarbacef	
Third Generation	Cefdinir Cefetamet Cefixime Cefodizime Cefoperazone Cefotaxime Cefpodoxime	Ceftizoxime Cefpiramide Cefsulodine Ceftazidime Ceftibuten Ceftriaxone	
Fouth generation	Cefepime Cefpirome		

Table 3. Reagents and concentrations recommended for skin testing and drug provocation testing.

REAGENT	SKIN TESTING	DRUG PROVOCATION TEST
PPL	$5x10^{-5}$ mMol/l	Not done
MDM	$2x10^{-2}$ mMol/l	Not done
Benzylpenicillin	10.000 IU/ml	10^3, 10^4, 10^5, $5x10^5$ IU/ml Cumulative dose ($6x10^5$ IU/ml)
Amoxicillin	20 mg/ml	5, 50, 100, 150, 200 mg Cumulative dose (500 mg)
Ampicillin	20 mg/ml	5, 50, 100, 150, 200 mg Cumulative dose (500 mg)
Cephalosporins	2 mg/ml	5, 50, 100, 150, 200 mg Cumulative dose (500 mg)
Clavulanic	20 mg/ml	Not done