Diagnosis of Clavulanic Acid Allergy Using Basophil Activation and Leukotriene Release by Basophils

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Abstract

Clavulanic acid is a potent inhibitor of ß-lactamase that is increasingly prescribed in association with amoxicillin. We report 2 cases of patients who experienced pruritus, wheals, and angioedema after oral intake of amoxicillin/clavulanic acid. Routine skin tests for ß-lactam antibiotics and specific immunoglobulin (Ig) E were negative in both patients. Analysis of CD63 expression by the basophil activation test (BAT) using flow cytometry and of sulphidoleukotriene (sLT) release by basophils using the cellular allergen stimulation test (CAST) revealed significant positive responses with amoxicillin/clavulanic acid and with clavulanic acid, and negative responses with amoxicillin and other ß-lactam antibiotics. In addition, cultured CD3+CD4+ cells showed a significant increase in the expression of CD69, CD25, and HLA-DR in the presence of clavulanic acid. Both patients tolerated therapeutic doses of amoxicillin.
BAT and CAST are useful ex vivo procedures for the detection of specific IgE-mediated allergy to clavulanic acid, especially for patients with negative skin test results.

Key words: Clavulanic acid. ß-lactam antibiotics. Basophil activation test. Sulphidoleukotrienes. Lymphocyte activation markers.

Resumen

El ácido clavulánico es un inhibidor potente de ß-lactamasas, que se prescribe en asociación con amoxicilina. Describimos dos casos de pacientes que presentaron urticaria, prurito y angioedema tras la ingestión de amoxicilina/ácido clavulánico. El estudio alergológico habitual mediante pruebas cutáneas con determinantes mayores y menores de antibióticos ß-lactámicos y la determinación de IgE específica resultó negativo en ambos pacientes. Tanto los Test de Activación de basófilos (TAB) como los Tests de producción de sulfidoleucotrienos por basófilos (CAST) resultaron positivos en presencia de amoxicilina/ácido clavulánico y ácido clavulánico, y negativos con amoxicilina y otros antibióticos ß-lactámicos. Además, los linfocitos CD3+CD4+ mostraron un aumento en la expresión de CD69, CD25 y HLA-DR en presencia de ácido clavulánico. Ambos pacientes toleraron dosis terapéuticas de amoxicilina.
BAT y CAST son dos técnicas *ex vivo* útiles para la detección de respuestas IgE específicas a ácido clavulánico, especialmente en pacientes con pruebas cutáneas negativas.

Palabras clave: Ácido clavulánico. Antibióticos ß-lactámicos. Test de Activación de Basófilos. Sulfidoleucotrienos. Marcadores de activación linfocitaria.

Introduction

Clavulanic acid (CA) is a ß-lactam antibiotic with weak antibacterial activity, but it is a potent inhibitor of ß-lactamase and is therefore increasingly prescribed in daily medical practice in combination with amoxicillin (AX). Despite its widespread use, there are only a few reported cases of allergic reactions to CA, and in most of them an immunoglobulin (Ig) E-mediated mechanism has been suggested [1-4]. However, possible type IV hypersensitivity reactions have been described [5-7].

Skin prick tests and intradermal tests are the main diagnostic methods used to confirm clinical suspicion after immediate

allergic reactions to ß-lactam antibiotics. In vitro techniques to quantify serum-specific IgE are not very reliable diagnostic techniques, because they present lower sensitivity than in vivo tests. Previous studies have demonstrated that quantification of in vitro basophil activation using the basophil activation test (BAT) carried out by flow cytometry can be reliable for measuring IgE-dependent, allergen-specific responses in patients who are allergic to ß-lactams [8]. We present 2 cases of selective immediate hypersensitivity to CA. The BAT and the cellular allergen stimulation test (CAST), which determines the release of sulphidoleukotriene (sLT) by basophils, were used to confirm the presence of specific IgE.

Case Descriptions

Patient 1: One hour after taking the first capsule of amoxicillin-clavulanic acid (AX/CA) (500 mg/125 mg) (Augmentine, GlaxoSmithKline, Madrid, Spain) for a lower respiratory tract infection, a 47-year-old man experienced systemic pruritus and generalized urticaria. He received dexchlorpheniramine and methylprednisolone and had a rapid recovery. Two years before the consultation, he had received AX/CA twice, with similar but milder reactions. There was no personal or family history of atopy.

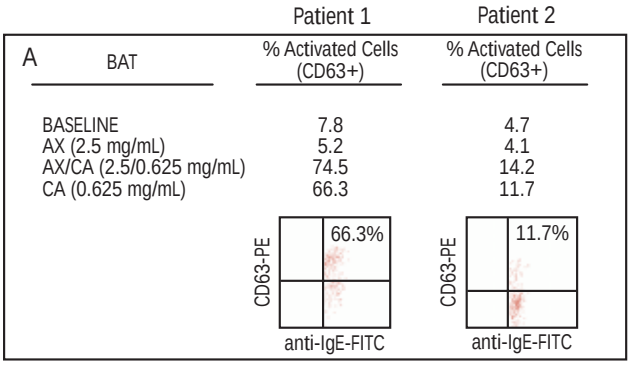
Patient 2: Ten minutes after ingestion of AX/CA (500 mg/125 mg) for an upper respiratory tract infection, a 43-year-old woman presented a wheal on her neck that disappeared spontaneously. After this reaction the patient tolerated a 7-day course of AX. Twenty days later she experienced symptoms of acute sinusitis, and 10 minutes after swallowing a capsule of AX/CA (500 mg/125 mg) she presented abdominal pain, cervical pruritus, generalized urticaria, and oral angioedema. The reaction subsided within 2 hours with dexchlorpheniramine and methylprednisolone. There was no personal or family history of atopy.

Routine skin tests were performed as described elsewhere [9] with AX, AX/CA, penicillin G sodium, ampicillin, benzylpenicilloyl poly-L-lysine (PPL) and minor determinant mixture (MDM). The results of all these skin tests were negative in both patients. Skin tests with CA were not performed because the powder formulation available was only suitable for in vitro diagnostic use. Serum determinations of specific IgE (CAP-FEIA, Pharmacia, Uppsala, Sweden) to ampicillin, penicillin G sodium, penicillin V potassium, and AX were also negative in both patients (< 0.35 kU_A/L).

Flow cytometric analysis of CD63 expression by basophils was performed after in vitro allergen-specific stimulation, as described elsewhere [8]. Both patients showed significant positive responses with AX/CA and with CA and negative responses with AX (Figure), ampicillin, PPL, and MDM (data not shown). All antibiotics were tested at 2 different final concentrations [8], and CA at 0.625 and 0.156 mg/mL, after being tested on a pool of control subjects, in order to verify the lack of nonspecific activation.

To evaluate the functional response of basophils, we determined the antigen-specific sLT production of these cells (CAST-ELISA, Bühlmann Laboratories, Allschwil, Switzerland) using the same antibiotic concentrations as for the BAT. As in BAT, these concentrations were selected after being previously tested on a pool of control subjects in order to exclude concentrations that cause nonspecific leukotriene release. Both patients showed a stimulation index for sLT release (concentration of sLT released after contact with the antigen/concentration of sLT released without stimulus) greater than 3 with AX/CA and CA (Figure), and were considered positive (only releases over 100 pg/mL were considered significant). AX, ampicillin, PPL, and MDM did not cause significant specific leukotriene production (data not shown).

In addition, the expression of activation markers (CD69, CD25, and HLA-DR) on the surface of $CD3^+CD4^+$ cells and their upregulation by ß-lactam antibiotics were studied using the lymphocyte stimulation test. In both patients, the stimulation index for these molecules (percentage of positive cultured cells stimulated with the antigen/percentage of positive cultured cells not stimulated with the antigen) showed



B CAST-sLT	pg/ml	SI	pg/ml	SI
BASELINE	539	1.0	140	1.0
AX (2.5 mg/mL)	408	< 1.0	194	1.4
AX/CA (2.5/0.625 mg/mL)	1732	3.2	949	6.8
CA (0.625 mg/mL)	> 4333	> 8.0	1380	9.8

C LST-CA (0.625 mg/mL) CD3 + CD4 + cells	SI 4h	SI 24h	SI 48h	SI 4h	SI 24h	SI 48h
CD69+	5.3	–	–	27.0	–	–
CD25+	–	5.5	4.0	–	10.7	23.6
HLA-DR+	–	3.6	6.4	–	25.8	3.0

Figure. In vitro results of patients 1 and 2. A, Basophil activation test (BAT). Percentages of CD63+ cells expressed in response to amoxicillin (AX), amoxicillin/clavulanic acid (AX/CA), and clavulanic acid (CA). The plots show the expression of CD63-PE against anti-IgE-FITC in response to CA. B, the cellular allergen stimulation test (CAST) was used to measure release of sulphidoleukotriene (sLT) by basophils. Results given in picograms of sLT per milliliter and as the stimulation index (SI: antibiotic value divided by the baseline response). C, Lymphocyte stimulation test. Expression of activation markers at 4, 24, and 48 hours by $CD3^+CD4^+$ cells expressed in response to CA. Results given as the SI.
In all 3 tests, the concentrations of antibiotics were selected after being previously tested on a pool of control subjects in order to exclude concentrations that cause nonspecific activation.

a significant increase in the expression of CD69 (early T-cell activation antigen) within 4 hours of culture. Furthermore, surface CD25 and HLA-DR were upregulated within 24-48 hours when CA was added to the culture (Figure). A similar response was obtained with AX/CA and no response was obtained from culture with other ß–lactam antibiotics (data not shown).

Control samples obtained from patients with an immediate allergic reaction to AX and positive skin test results with AX were included in all in vitro assays. In these cases, BAT, CAST, and activation marker analysis showed positive results in response to AX and AX/CA, and were negative with CA (data not shown).

Oral challenge tests were performed after patients gave their informed consent. Both patients tolerated therapeutic doses of AX. Patient 1 had systemic pruritus, generalized urticaria, and facial angioedema after ingestion of AX/CA (500 mg/125 mg). In patient 2, challenge testing with AX/CA was not performed due to the increased severity of symptoms during the second exposure.

Discussion

CA is a ß-lactamase inhibitor derived from 6-aminopenicillanic acid, which has a complex structure with an oxazolidine ring instead of the thiazolidine structure of penicillins. After breaking down into several small haptenic groups, CA is able to react with blood proteins. Nevertheless, CA was formerly judged to be of low immunogenicity and does not seem to cross-react with penicillins [1,10].

Very few immediate allergic reactions to CA have been reported, and this may be due to the very low allergenic potential of CA [1-4]. Although some of these reactions are reported to have induced immediate skin responses, skin test results have been negative in other patients (as occurred in the 2 cases we report).

Previous studies have demonstrated that IgE-dependent, allergen-specific responses in patients who are allergic to ß-lactam antibiotics can be efficiently measured by a flow-assisted analysis of basophils activated in vitro using the BAT [8]. In the cases we report, BAT, supported by the quantification of sLT release, confirmed the clinical suspicion of sensitization to CA in 2 patients with immediate adverse reactions to the combination of AX and CA, and a negative response in skin tests. In addition, the results of the lymphocyte stimulation test indicated that specifically sensitized T lymphocytes had recognized the CA antigen, and this may have eventually produced an allergen-specific IgE response.

BAT and CAST are easily accessible and rapid in vitro techniques. Our findings demonstrate that they are also reliable procedures for the detection of IgE-mediated allergy to CA, especially for patients with negative skin test results, and that they could prevent the risk of anaphylaxis that is inherent in challenge tests.

To the best of our knowledge, this is the first study to use BAT and CAST as techniques that closely reproduce the in vivo pathway leading to symptoms in order to confirm the diagnosis of CA allergy.

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