

Short communication

Cysteinyl-leukotriene release test (CAST) in the diagnosis of immediate drug reactions

Background: The diagnosis of allergic reactions to drugs is difficult. Most skin tests are not standardized, and *in vitro* tests are needed to avoid provocation tests. Cross-linking of IgE on basophils is known to cause the release of both cysteinyl leukotriene (Cys-LT) and histamine. We aimed to evaluate the diagnostic utility (sensitivity, specificity, and efficiency) of measurement of sulfidoleukotrienes in drug allergy.

Methods: We performed a prospective study in 55 patients with proven immediate adverse reactions to drugs (30 to β -lactams, six to acetaminophen, and 19 to aspirin) and 64 drug-exposed nonallergic controls. Positive diagnosis was established by history, skin tests, and, if needed, oral provocation tests. Cys-LT release was determined after drug-allergen stimulation by the cellular antigen stimulation test (CAST®) technique. Histamine release was also assessed on the same samples by enzyme immunoassay. Spontaneous and anti-Fc ϵ RI α -induced mediator release was also studied in all subjects. Sensitivity, specificity, and efficiency were calculated.

Results: Net Cys-LT release was over the maximal threshold given by the manufacturer in 19/55 patients and in 9/64 controls. Net histamine release was over 5% of total histamine content in 28/55 patients and 34/64 controls. The efficiency of both tests was low.

Conclusions: Thus, in most cases, the *in vitro* Cys-LT test has little or no diagnostic utility and is not superior to histamine release.

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In a recent study (1), adverse drug reactions appeared to be the fourth leading cause of death in the USA; of over 106 000 deaths in 1994, almost 25% were probably of allergic nature. However, there is no existing extensive epidemiologic study with documented cases, as numerous reactions with symptoms suggestive of allergy, but without an allergic mechanism, are often considered to be real drug allergies (2, 3). The diagnosis of drug allergies and pseudoallergies is difficult and is currently based on clinical history and skin testing for some drugs. Provocation tests certainly have the best sensitivity but are potentially harmful. They can only be performed under the most rigorous surveillance due to the potentially life-threatening reactions.

Therefore, *in vitro* tests would be of tremendous interest to establish the culprit agent, especially when the patient is receiving several drugs simultaneously. These biologic tests include serum drug-specific anti-

bodies (4, 5), histamine release (6, 7), Cys-LT release (8, 9), histamine and tryptase measurements in body fluids (10, 11), basophil membrane markers (12), and T-cell-based assays (proliferation, activation, and clones) (13). However, none of them are validated in sufficient number of patients and against a formal diagnosis. Cross-linking of IgE on basophils is known to cause release of both sLT (8, 9), and histamine (6, 7), particularly when pretreated with the cytokines IL-3, IL-5, or GM-CSF (14). These mediators can also be liberated by non-IgE-dependent mechanisms (8, 9), and, in both situations, their generation by blood cells can be measured *in vitro*. The Cys-LT release test (CAST) (8) is used in many laboratories for the diagnosis of drug allergy and aspirin intolerance (19) but has not been fully validated.

In this pilot study, we evaluated the CAST in 55 patients with proven drug allergy or pseudoallergy and 64 drug-exposed, nonallergic controls. We compared the specificity and sensitivity of the CAST with that of histamine release to test whether Cys-LT release is more accurate.

Abbreviations. CAST: cysteinyl-leukotriene release test; Cys-LT: cysteinyl leukotriene; GM-CSF: granulocyte macrophage colony stimulating factor; IL: interleukin.

Material and methods

Patients and drug allergy diagnosis

Fifty-five patients (21 males, 34 females), aged 8–81 years (median: 38.0; 25–75th percentiles: 25.0–47.0), attended our drug allergy consultation. Patients had anaphylactic shock (8) or urticaria/angioedema occurring within 1 h after drug intake (49). The period of time elapsing from the clinical reaction to the blood sampling ranged from 6 to 1200 weeks (median: 44; 25–75th percentiles: 24–261). The diagnosis of drug allergy and intolerance was carefully evaluated.

For β -lactam reactions, we always performed skin prick tests (to penicilloyl-polylysine 0.035 mg/ml, minor determinant mixture 1.1 mg/ml, benzylpenicillin 1.1 mg/ml, amoxicillin 25 mg/ml, and ampicillin 25 mg/ml), followed by intradermal tests (when prick tests were negative), in accordance with studies previously published (7, 15). In patients with negative skin tests, provocation tests were performed (7). For acetaminophen and aspirin reactions, provocation tests were carried out. Provocation tests were single-blind, placebo-controlled challenges, performed after informed consent, starting with 0.1–1% of the daily dose of the culprit drug with 5- to 10-fold increments at 30-min intervals until reaching the therapeutic dose. The test was immediately stopped when allergic symptoms occurred and the reaction treated. For all patients, blood was withdrawn 1–2 h before undergoing the drug challenge.

Sixty-four subjects (22 males, 42 females) ranging from 8 to 70 years of age (median [25–75th percentiles], 36.5 years [28.0–53.0]) were used as the control group. They had no history of drug allergy but were exposed for therapeutic reasons to the drug further tested *in vitro*.

Cys-LT release *in vitro*

Release of Cys-LT produced *in vitro* by isolated peripheral blood leukocytes, after drug allergen stimulation, was measured by the cellular antigen stimulation test (CAST) technique (8). The reagents for isolation and stimulation of cells, except if mentioned, were contained in the Bühlmann CAST-ELISA kit (Bühlmann Laboratories AG, Allschwil, Switzerland). Venous blood was drawn into heparinized tubes before the skin tests and the oral provocation test. Blood specimens were stored at 20–25°C for 2–3 h, before the leukocytes were isolated by dextran sedimentation: in a polypropylene tube, one volume of dextran solution was added to four volumes of homogenized blood, and the mixture was gently vortexed at low speed. After 90-min incubation at room temperature, the upper phase was transferred into a second tube and centrifuged for 15 min at 130 g at 20–25°C. The supernatant was discarded and the cells resuspended in four volumes of stimulation buffer (PBS containing Ca^{2+} and Mg^{2+} and IL-3). Stimulation was carried out in duplicates with 200 μl of cell suspension and 50 μl of stimulation buffer (spontaneous release), or 50 μl of monoclonal antibody directed against the affinity IgE receptor Fc ϵ RI α as positive control or 50 μl of drug allergen working concentrations (final concentrations: 0.2, 2.0, 20, and 200 $\mu\text{g/ml}$ for unconjugated drugs and 0.02, 0.2, 2.0, and 20 μM for benzylpenicilloyl-polylysine, and amoxycilloyl-polylysine, according to the manufacturer's instructions). After a 40-min incubation period at 37°C, the cells were centrifuged for 3 min at 1000 g, at 2–8°C, and 200 μl of supernatants was collected carefully to avoid picking up cells and frozen at –20°C until measurements were performed. Cys-LT (LTC₄/D₄/E₄) were measured by ELISA as provided in the CAST-ELISA kit.

Results were expressed as the ratio of the Cys-LT net release to the maximal value of the cutoff ranges, calculated from 10 or more nonallergic donors, provided by the manufacturer for every drug. These values (pg/ml) were 161 for β -lactams, 152 for aspirin, and 285 for acetaminophen.

Histamine release

Histamine release was measured on the same sample prepared for the CAST by a procedure previously described in detail (7, 16). Results were expressed as a percentage of total histamine content after correction for spontaneous release, and the net histamine release was calculated as follows: (anti-IgE- or drug-induced release – spontaneous release)/(total histamine content – spontaneous release). The limit of positivity was set at 5%, as previously accepted (17).

Statistical analysis

Sensitivity (Se), specificity (Sp), and efficiency were calculated according to Galen & Gambino (18).

Results

Characteristics of the patients

For β -lactam-allergic patients, none of the prick tests were positive. Intradermal tests were positive for 12 patients allergic to penicillins (with penicilloyl-polylysine and/or penicillin minor determinant mixture and/or amoxicillin and/or ampicillin reagents) and six patients allergic to cephalosporins (one with the minor determinant mixture and five with amoxicillin reagent). Skin-test-negative patients allergic to penicillin (5/17) and cephalosporin (7/13) and the other patients underwent a provocative challenge with the culprit drug, which was always positive. None of the patients reacted to the placebo challenge. They experienced the same (but milder) symptoms as in the original reaction within 15–60 min. The symptoms rapidly disappeared after 40–60 mg of prednisolone and cetirizine 10 mg.

Cys-LT and histamine releases

Net Cys-LT release (Fig. 1A) was over the maximal threshold given by the manufacturer in 19/55 patients (median ratio [25–75th percentiles] of 0.66 [0.24–1.47]), and in 9/64 controls (0.51 [0.31–0.70]).

Net histamine release (Fig. 1B) was over 5% of total histamine content in 28/55 patients (median percent release [25–75th percentiles] of 5.05 [2.10–11.30]) and 34/64 controls (5.40 [1.90–14.10]).

Combining both assays did not add any diagnostic power (Table 1).

Discussion

In this pilot study, we attempted to evaluate the diagnostic value of the CAST in immediate allergic and pseudoallergic reactions to drugs. The results do not support the use of the test as a screening method for the diagnosis of immediate drug adverse reactions. Using the method recommended by the manufacturer in an unselected patient population, we observed that the CAST gave slightly better results than histamine release. The results found for histamine release in this study are

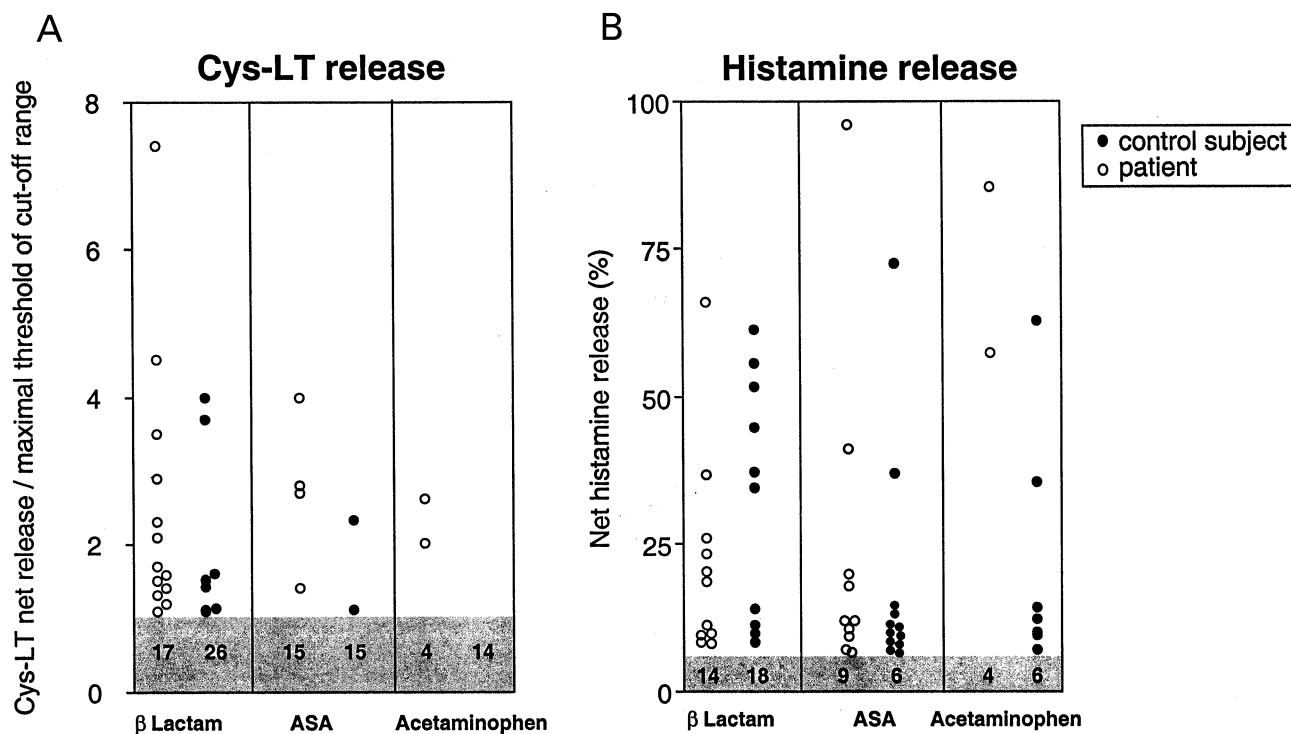


Figure 1A. Net Cys-LT release results in 55 drug-allergic and aspirin-intolerant patients and 64 control subjects. There was no difference between the different groups. Results were expressed as the ratio of the Cys-LT net release to the maximal value of the cutoff ranges given by the manufacturer for every drug. These values (pg/ml of Cys-LT) were 161 for β -lactams, 152 for aspirin, (ASA) and 285 for acetaminophen. The ratio value for the positive limit for the three drugs studied was over 1 (shaded area).

Figure 1B. Net histamine release results in 55 drug-allergic and aspirin-intolerant patients and 64 control subjects. There was no difference between the different groups. Results were expressed as percentage of total histamine content after correction for spontaneous release, and the net histamine release was calculated as follows: (anti-IgE- or drug-induced release – spontaneous release)/(total histamine content – spontaneous release). The limit of positivity was set at 5% (shaded area).

very similar to those previously published (7). We then tried to determine whether the addition of CAST and histamine release tests would improve the diagnosis efficiency, but the results were still poor.

Table 1. Sensitivity, specificity, and efficiency values in percentages of Cys-LT and histamine release tests in 55 patients with proven immediate adverse drug reaction and 64 controls

Number of subjects	Drugs		
	β -Lactams	Aspirin	Acetaminophen
Patients	30	19	6
Controls	33	17	14
Cys-LT release (CAST)			
Sensitivity	43%	21%	33%
Specificity	79%	88%	100%
Efficiency	62%	53%	80%
Histamine release (HR)			
Sensitivity	53%	53%	33%
Specificity	55%	35%	43%
Efficiency	54%	44%	40%
Cys-LT and histamine release (CAST + HR)			
Sensitivity	48%	37%	33%
Specificity	67%	62%	71%
Efficiency	58%	49%	60%

The CAST was supposed to be efficient with aspirin and penicillin according to previous studies (9, 20). Kubota et al. (9) found that the CAST assay was positive in 5/8 aspirin-intolerant patients and 8/12 patients with diclofenac reactions. Recently, Sanz et al. (20) found that 5/7 patients with β -lactam sensitivity had a positive CAST. However, these studies were carried out in a small group of patients and controls and could not give a definite assessment of the efficiency of the CAST for β -lactams. Other studies performed with aspirin or aspirin plus C5a stimulation showed an increasing sensitivity of the CAST. Czech et al. (21) were the first to report that the low sensitivity of the CAST in aspirin-intolerant patients was significantly increased by the C5a-induced generation of Cys-LT when compared to normal controls. However, this aspirin preincubation of peripheral blood leukocytes did not exert a significant increase in Cys-LT in intolerant patients. In the larger study of May et al. (22), leukocytes from aspirin-intolerant patients were tested with aspirin or aspirin plus C5a. The results showed that aspirin itself induced a slight but significant increase of released Cys-LT, whereas aspirin plus C5a induced a more prominent and higher significant increase of these mediators.

The low sensitivity of the CAST in our study may be owing to the following reasons:

- 1) The reactive compound may not be the drug itself but one of its metabolites (23).
- 2) The injectable soluble form of the drug may not be available (ready to be tested on blood basophils).
- 3) The mechanism may not be IgE-dependent and/or may be nondependent on these mediators (or on the cells releasing these mediators).

Another obvious reason for low sensitivity is the fact that the hypersensitivity has been gradually lost. Actually, it has already been found that skin test reactivity decreases with time in the case of β -lactams (24). Even if, in the present study, the diagnosis of drug allergy was made by the most stringent criteria (including provocation tests), the patients were studied at a variable distance from the drug reaction; if they had been tested closer to it, the results of the CAST might have differed.

However, although we wanted to use the *in vitro* test in the conditions of clinical practice, in future studies, this parameter should be considered closely. Moreover, patients with positive skin tests should be distinguished from those with negative skin tests and positive oral challenge.

Basophils are activated by full allergens, but not by haptens, and we used the parent drug of acetaminophen and most β -lactams together with some conjugates (benzylpenicilloyl-polylysine and amoxycilloyl-polylysine provided by the manufacturer). Finally, in aspirin reactions, although the involvement of Cys-LT is clear, the use of the CAST as a diagnostic test is not supported by our results.

Improvement of the CAST has already been proposed in some research laboratories (21, 22). In preincubation with C5a and aspirin, it was found that the sensitivity of the CAST for the diagnosis of aspirin sensitivity was higher when using aspirin alone (21). As currently used in clinical practice, the CAST is not efficient enough for the diagnosis of immediate reactions to β -lactams or aspirin. However, optimizing the CAST and/or focusing on some patients only may make this test of value. Such improvements should be carried out before proposing the current CAST for diagnosis of any drug-allergic or pseudoallergic reactions.

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