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Clinical Usefulness of Cellular Antigen Stimulation Test in Detection of Aspirin Allergy

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SUMMARY Acetylsalicylic acid, commonly known as aspirin, can induce some hypersensitive reactions with clinical symptoms such as urticaria, angioedema, acute bronchospasm, and rarely anaphylactic shock. At present, detection of aspirin allergy is still rather difficult and requires an adequate clinical history and sensitive in vivo and in vitro tests. The aim of the study was to evaluate the diagnostic utility of cellular antigen stimulation test (CAST) in the detection of allergic reaction mediated by aspirin. Fifty patients (39 women and 11 men) with a history of hypersensitivity reaction to aspirin were included in the study. Positive scratch test to aspirin was found in 72% (36/50) and positive CAST in 58% (29/50) of patients. Both skin scratch test and CAST positive results were recorded in 48% (24/50%) and negative results in 20% (9/45) of patients. The level of agreement between skin scratch test and CAST was fair with Cohen's kappa of 0.269 (0.95% CI 0.004-0.533). The observed between-test agreement was 66%. It is concluded that CAST-ELISA might be of value as an additional test for the detection of aspirin nonallergic hypersensitivity in suspected individuals.

KEY WORDS: acetylsalicylic acid; scratch test; cellular antigen stimulation test

INTRODUCTION

There is no doubt that allergies to drugs are complex diseases. Drugs can elicit a wide variety of different immune and nonimmune reactions (1). Acetylsalicylic acid (ASA), commonly known as aspirin, can cause reactions which are not IgE-mediated. Although IgE is not involved in such non-allergic hypersensitivity, mast cells and basophils may be triggered to release mediators, essentially histamine and sulfidoleukotrienes (sLT) (2). The latter, SULFIDOLENKOTRIENES as important newly-formed mediators in allergies, can cause bronchoconstriction, increased vascular permeability, enhanced mucous secretion, and decreased mucocilliary transport (3). Moreover, these clinical symptoms provoked by ASA are a consequence of cyclo-oxygenase inhibition leading to diversion of arachidonic acid synthesis to lipo-oxygenase pathways with preferential generation of sLT (4). The increased population exposure to aspirin has resulted in a relevant number of allergic reactions every year. Detection of aspirin hypersensitivity in daily routine is still rather difficult and is based on adequate clinical history and provocation tests, essentially skin tests and lymphocyte transformation test (LTT). Yet, these tests or procedures are not always sensitive enough and differ in their specificity, safety, reproducibility and efficiency. Provocation tests have the best sensitivity but re-

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quire hospitalization of patients and may also be potentially harmful (5). In some cases skin testing cannot be performed or may involve additional risk. Also, results of LLT may fail to produce clear answers. As mentioned earlier, leukotriene metabolism is deeply involved in aspirin intolerance. These mediators can be liberated by cross-linking of IgE on basophils or by non-IgE dependent mechanisms. Until now, there has been no *in vitro* test based on mediator release (with a few exceptions), which could be used as a diagnostic tool. The cellular antigen stimulation test (CAST)-ELISA test, as currently used in clinical practice, was applied to measure the production of sLT (LTC_4 and its metabolites LTD_4 and LTE_4) released by reactive cells after *in vitro* stimulation with a specific allergen. The aim of the study was to evaluate the diagnostic sensitivity of CAST in a group of patients allergic to aspirin according to clinical history and skin scratch test (SCR).

PATIENTS AND METHODS

Patients

Fifty patients, 39 (78%) women and 11 (22%) men, mean age 37 (range 3-78) years, with a history of hypersensitivity reaction to aspirin were included in the study. The clinical reactions after drug ingestion developed as urticaria and/or angioedema in 22 (44%), dermatitis atopica in 12 (24%), rhinitis in five (10%) and medicamentous exanthema in 11 (22%) patients. SCR and CAST were performed mainly during the period from 1 to 240 months after allergic reactions. All patients avoided any medication for 3-10 days prior to testing.

Diagnostic tests

SCR was performed on the volar side of the forearm with a previously prepared 10% aspirin solution. Test result was read within 20-30 minutes (early response), and compared with positive control (histamine in a concentration of 1:10000) and negative control (buffer solution). Skin reaction to aspirin was considered positive when the size of the initial wheal increased in diameter by 3 mm or greater than 3 mm. The CAST-ELISA was done according to the protocol issued by Bühlmann laboratories (6). Briefly, the test consists of three steps: 1) leukocyte isolation, 2) cell stimulation, and 3) leukotriene determination. In the first and second steps, peripheral blood leukocytes are isolated after dextran sedimentation of blood samples and prestimulated with IL-3. Cell suspension was aliquoted to separate tubes for basal level release (spontaneous release), stimulation control, and for each drug to be tested. As a positive control, the patient's leukocytes were stimulated with anti-IgE receptor antibody. After the incubation period (40 minutes at 37º C), the samples were centrifuged and the supernatant was either frozen at -20º C until measurement or immediately tested for sLT concentration by ELISA. ELISA was performed using precoated microtiter plates (2 x 96 wells). Blanking reagent, ELISA controls, standards, and cell supernatants (100 µL) in duplicate were added to the plates. Enzyme conjugate (sLT conjugated to alkaline phosphatase) and anti sLT antibody were also added to each well (50 µL), and the plates were incubated for 2 hours at 18-28º C on a plate rotator. After washing, the sub-

Table 1. Sensitivity of skin scratch test (SCR) and cellular allergen stimulation test (CAST) in determination of aspirin hypersensitivity

Test result	Overall sensitivity	Sensitivity according to clinical diagnosis			
		Urticaria/	Atopic	Drug	Rhinitis
SCR +	36/50 (72%)	16/22 (73%)	11/13 (85%)	4/10 (40%)	5/5 (100%)
CAST+	29/50 (58%)	12/22 (54%)	10/13 (77%)	2/10 (20%)	5/5 (100%)
SCR +					
CAST+	24/50 (48%)	9/22 (41%)	9/13 (69%)	1/10 (10%)	5/5 (100%)
SCR +					
CAST-	12/36 (27%)	7/22 (32%)	2/13 (15%)	3/10 (30%)	
SCR -					
CAST+	5/14 (36%)	3/22 (14%)	1/13 (8%)	1/10 (10%)	
SCR -					
CAST-	9/45 (20%)	3/22 (14%)	1/13 (8%)	5/10 (50%)	

+ positive test result; - negative test result

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strate solution (200 µL) was added to the plates and incubated for 30 minutes at room temperature. At the end, stop solution was added (50 µL) and color absorbance was expressed as pg/mL of the sLT released. The values were extrapolated to the sLT standard curve. Results were considered positive if exceeding the cut-off range value, 152 pg/mL for aspirin, calculated after subtraction of background. Results were statistically analyzed using Cohen′s kappa test.

RESULTS

Results of SCR and CAST recorded in patients with hypersensitivity reactions to aspirin are shown in Table 1. Positive SCR results to ASA were found in 72% (36/50), and positive CAST results in 58% (29/50) of patients. Both SCR and CAST positive results were found in 48% (24/50), and negative results in 20% (9/45) of patients. Negative CAST results were found in 33% (12/36) of patients with positive SCR, and negative SCR test results in 36% (5/14) of patients with positive CAST. The lowest test sensitivity for both tests was observed in patients which hypersensitivity to aspirin clinically expressed as medicamentous exanthema. The overall concordance of SCR and CAST test sensitivity was fair with kappa of 0.269 (0.95%CI 0.004-0.533). The observed agreement between the tests was 66%.

DISCUSSION

Diagnostic sensitivity of CAST (58%) in our study group of patients with a history of aspirin hypersensitivity was concordant with diagnostic sensitivity of SCR (72%). Similar diagnostic sensitivity of CAST has been reported in patients with hypersensitivity to nonsteroidal anti-inflammatory drugs (NSAIDs) and in aspirin-intolerant patients with airway-related and skin symptoms (4,8-10). On the contrary, some other authors did not confirm enhanced sLT production by blood leukocytes isolated from aspirin-intolerant individuals upon incubation with aspirin *in vitro* (9,11,12). Such a discrepancy can be explained by random effect recorded in a relatively small number of patients, i.e. inhomogeneity of the groups studied (asthma and urticaria), and by use of a high aspirin concentration that may unmask some intracellular mechanisms related to the higher eosinophilia in aspirin-intolerant asthma.

Aspirin-intolerant individuals in our study group were tested at different time points after hypersensitivity reaction to aspirin had occurred. We occasionally observed positive SCR and CAST results even 20 years after the first drug exposure. It means that specific basophil reactivity in some aspirin-intolerant individuals may persist for a much longer time than three weeks to three months, the time suggested as optimal time interval for testing for hypersensitivity reaction to aspirin (13,14). Interestingly enough, Luque *et al.* (15) have described T-cell proliferative response to the drug *in vitro* 10 or more years after the occurrence of hypersensitivity reaction to the drug.

We conclude that the determination of sLT release from the patients' leukocytes upon incubation with aspirin *in vitro* might be a valuable tool for confirmation of nonallergic aspirin hypersensitivity in suspected individuals.

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