BASOPHIL ACTIVATION TEST VERSUS HISTORY AND SKIN TESTS IN PATIENTS WITH SUSPECTED NSAIDS-INDUCED HYPERSENSITIVITY

CRISTINA PETRIŞOR[1], MIHAELA COCIŞ[1], NADIA GHERMAN-IONICĂ[2], MANUELA SFICHI[3], NATALIA HAGĂU[1]

[1]2nd Anesthesia and Intensive Care Department, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca
[2]Immunology Department, Iuliu Haţieganu University of Medicine and Pharmacy Cluj-Napoca
[3] Immunopathology Department, Emergency County Hospital Cluj-Napoca

Abstract

Aims. *Non-steroidal antiinflammatory drugs (NSAIDs) might be responsible for drug-induced hypersensitivity. In vitro diagnosis includes the basophil activation test (BAT). The aim of our study was to determine the concordance of BAT versus history and skin tests in patients with suspected NSAIDs-induced hypersensitivity.*

Patients and methods. *42 patients with suspected NSAIDs-induced hypersensitivity were tested in vivo (skin tests) and in vitro (BAT). 11 controls were also tested. BAT was performed with Flow2Cast technique. Cohen kappa index was used to assess the agreement of BAT versus history and skin tests. U Mann-Whitney test was used to determine the difference between mean values for numerical data.*

Results. *22 patients had negative skin tests and 20 patients had positive skin tests. Kappa indexes were 0.17 (0.05-0.29) for BAT versus history and 0.48 (0.23-0.74) for BAT versus skin tests. 3 of the 22 patients with negative skin tests had positive BAT (13.63%). Mean values for BAT stimulation indexes were 1.19 for controls, 2.07 for the patients with negative skin tests and 4.59 for the patients with positive skin tests ($p<0.01$ [U Mann Whitney Test]).*

Conclusion. *The slight concordance between BAT and history and the lack of statistical difference between controls and patients with negative skin tests suggest that drug reactivity diminishes with time or that hypersensitivity is not IgE-mediated in these patients. BAT may avoid 13,63% challenge tests in patients with negative skin tests. BAT discriminates well between patients with positive skin tests and both controls and patients with negative skin tests.*

Keywords: basophil activation test, flow cytometry, drug allergy, hypersensitivity.

Introduction

Non-steroidal antiinflammatory drugs (NSAIDs) are frequently responsible for drug-induced hypersensitivity reactions. They account for 20-25% of all hypersensitivity reactions to drugs and are the second cause of drug hypersensitivity reactions [1,2].

There are currently two types of immediate-type hypersensitivity reactions caused by NSAIDs: multiple NSAIDs-induced (related to cox-1 inhibition) and single drug induced (IgE-mediated) [2].

Aspirin/NSAIDs hypersensitivity syndrome implies intolerance to several drugs. The precise molecular and cellular mechanisms of clinical hypersensitivity to multiple NSAIDs and the nature of the main effector cells involved is not yet clear. Basophil activation may be envolved and may be related to the pharmacological effects related to cox-1 inhibition [1,3,4]. BAT has proved to be a useful confirmatory *in vitro* test for NSAIDs hypersensitivity [1,3,4].

IgE-mediated immediate-type hypersensitivity is well described for several NSAIDs, mainly pyrazolones like metamizol and propyphenazone, paracetamol and diclofenac [2,5,6].

The *in vitro* diagnosis of both NSAIDs hypersensitivity and IgE-mediated selective hypersensitivity reactions includes the basophil activation test (BAT)

Adress for correspondence: petrisor.cristina@umfcluj.ro

[4,5,7,8,9,10]. BAT is well correlated with positive challenge tests [1]. The concentrations used for *in vitro* tests varied considerably in the past, as well as patients' selection criteria and tehnical differences in the performance of flow cytometry.

In the context of several methodological and population differences, the aims of our study were: (i) to determine the concordance of BAT results versus clinical history and allergologic skin tests in patients with NSAIDs-induced immediate-type hypersensitivity reaction; (ii) to determine the rates of BAT positivity in patients with negative skin tests; and (iii) to determine whether BAT discriminates well between patients and controls.

Material and methods

With the approval of the Research Ethics Committee of the University Hospital of Cluj-Napoca and subjects' informed consent, 42 patients with suspected NSAIDs-induced hypersensitivity were tested *in vivo* (skin tests) and *in vitro* (BAT). The inclusion criterion was history suggestive of an immediate-type hypersensitivity reaction caused by a single NSAID. Eleven controls with negative history and negative skin tests were also tested. Exclusion criteria were current steroid medication, H1 or H2 antihistamines or antidepressants.

In vivo tests, the skin prick test (SPT) and the intradermal test (IDT), were performed using commercially available solutions of NSAIDs. The concentrations used were: metamizol 500 mg/ml (SPT) and 5 mg/ml (IDT), diclofenac 25 mg/ml (SPT) and 2.5 mg/ml (IDT), paracetamol 10 mg/ml (SPT) and 1 mg/ml (IDT). The SPT was considered positive when the wheal diameter was superior to 3mm within 20 minutes. For IDT, the wheal area was obtained initially by injecting 0.01-0.02 ml of drug dilution and was measured 20 minutes after testing. The doubling of the initial injection wheal represented a positive result.

In vitro flow cytometry-based analysis of activated basophils (BAT) was performed with Flow2Cast technique (Bühlmann Laboratories, Switzerland). We used Cell Quest programme (FACSCalibur BD Analyzer) to detect the up-regulation of CD63 marker on the basophils after stimulation with NSAIDs and double staining with two monoclonal antibodies, anti- CCR3-PE (human chemokine receptor labeled with phycoerythrin) and anti-CD63-FITC (or Gp53, a glycoprotein expressed on activated basophils). The NSAIDs concentrations used in BAT were those recommended by the manufacturer (metamizol 25 µg/ml, paracetamol 5 µg/ml and diclofenac 12.5 µg/ml) and concentrations 2-log scales lower. We analysed the stimulation index (SI) calculated as the percentage of activated basophils after stimulation with NMBA divided by the negative control (the percentage of spontaneously activated basophils) for both patients and controls. The result of BAT was considered positive when the SI for at least one drug concentration was higher ≥2 and when the percentage of activated basophils was above 5% after stimulation withNSAIDs, in order to rule out small unspecific activations [1,3,4,7].

Cohen *kappa* index was used to assess the agreement between the *in vitro* tests and history. U Mann Whitney test was used to establish the statistical significance of the difference between mean values for numerical data (continous variables). Two-tailed Fisher exact test was used to establish the level of significance for the differences in the positivity rates for patient groups (categorial variables).

Results

Eleven control subjects with negative skin tests and without previous history of NSAIDs-induced immediate-type hypersensitivity reactions were tested together with 42 patients with positive history. Considering a SI ≥2 as positive BAT, none of the subjects in the control group (Group A) had positive BAT (Table I).

Table I. Skin tests and BAT results for controls. SI BAT= stimulation index for the basophil activation test.

Group A = controls					
	Substance	**Skin tests**	**SI BAT**	**BAT result**	**Clinical symptoms**
1	algocalmin	neg	0.55	neg	-
2	algocalmin	neg	0.66	neg	-
3	algocalmin	neg	0.80	neg	-
4	algocalmin	neg	0.86	neg	-
5	algocalmin	neg	0.87	neg	-
6	algocalmin	neg	1.45	neg	-
7	algocalmin	neg	1.55	neg	-
8	algocalmin	neg	1.56	neg	-
9	diclofenac	neg	1.58	neg	-
10	paracetamol	neg	1.59	neg	-
11	algocalmin	neg	1.71	neg	-

All patients were tested for the culprit NSAIDs. From the 42 patients with NSAIDs-induced immediate-type hypersensitivity reactions, 22 patients had negative skin tests (Group B) and 20 presented positive skin tests (Group C) (Table II).

Cohen *kappa* indexes were 0.17 (0.05-0.29) for BAT versus history and 0.48 (0.23-0.74) for BAT versus skin tests.

With a threshold for BAT positivity SI ≥2, there were 3 positive BAT results for the patients with negative skin tests (13.63%) and 11 positive BAT results for the patients with positive skin tests (55%). There is no statistical difference between the rates of BAT positivity for groups A and B, but the difference between BAT positivity rates for both group A and group B versus group C is highly significant (Fisher exact test, $p<0.01$).

The mean values for BAT SI were 1.19 for controls (Group A), 2.07 for the patients with negative skin tests (Group B) and 4.59 for the patients with positive skin tests (Group C). The mean values for BAT SI were higher for patients with positive skin tests when compared to those of group A and B (Figure 1).

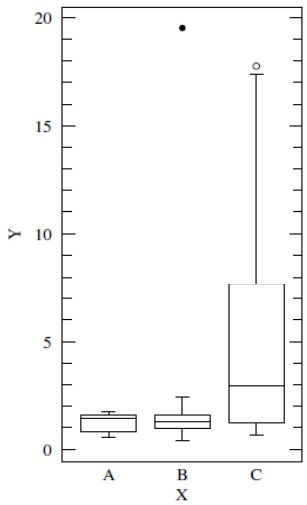

Figure 1. Box plot for BAT stimulation indexs (SI) on the Y-axis for groups A (subjects with negative history and negative skin tests), B (patients with positive history and negative skin tests) and C (patients with positive history and skin tests).

Assessing the statistical significance for the differences between mean values for SI among the three subject groups, p-value for group A versus group B was 0.38, while for both groups A and B versus group C p-values were <0.01 (U Mann Whitney).

Discussion

Non-steroidal anti-inflammatory drugs are common causes of drug hypersensitivity, with half of the reactions being life-threatning [7,9]. Detection of the culprit drug is a prerequisite for effective prevention [7]. Retrospective diagnosis includes *in vivo* tests (skin tests and drug challenge tests) and *in vitro* tests (detection of drug specific IgE and flow cytometry-assisted basophil activation tests).

Allergologic skin tests are the current refference test, but their diagnostic accuracy is not absolute [6]. Challenge tests remain the golden standard tests [2], but their performance is time consuming. Drug challenge tests are contraindicated in patients who have experienced severe reactions as they might be dangerous by exposing the patients to the culprit drug and eventually inducing anaphylaxis [1,7].

Thus, reliable *in vitro* test are neccessary to confirm the diagnosis of NSAIDs-induced immediate-type hypersensitivity reactions. For NSAIDs, there are currently no standardised *in vitro* assays to detect drug-specific IgE antibodies [9].

BAT closely resembles the *in vivo* pathway leading to symptoms [11]. The basophil activation tests rely on the quantification of CD63 marker up-regulation on the surface of circulating basophils after exposure to the culprit drug (the allergen) *in vitro*. CD63 is an intracellular activation marker which is expressed on the surface of circulating basophils after exposure to the allergen and is detected on the cellular membrane using flow cytometry assays [9]. BAT has been used for the diagnosis of NMBAs and antibiotic-induced anapylaxis [12,13,14], as well as for NSAIDs [4,5,7,8,9,10]. BAT may provide a safe, convenient and rapid method for the diagnosis of hypersensitivity to NSAIDs [7].

The diagnostic reliability of BAT has been investigated [4,5,7,8,9,10]. Reported sensitivities vary widely among studies, from 11.7-41.7% to 63.3 [1,4], with 93.3-100% specificity [4,5,7,8,9]. The low BAT sensitivities may be attributable to the fact that mast cells and eosinophils are also involved in hypersensitivity

Table II. Patients' clinical, skin test and BAT results. SI = stimulation index; BAT = basophil activation test.

Group B = patients with negative skin tests					Group C = patients with positive skin test				
	Substance	SI BAT	BAT result	Clinical symptoms		Substance	SI BAT	BAT result	Clinical symptoms
1	algocalmin	0.39	neg	urticaria	**1**	algocalmin	0.63	neg	urticaria
2	paracetamol	0.43	neg	urticaria	**2**	algocalmin	0.68	neg	hypotension
3	algocalmin	0.67	neg	angioedema	**3**	algocalmin	0.74	neg	urticaria
4	algocalmin	0.83	neg	angioedema	**4**	algocalmin	1.13	neg	angioedema
5	algocalmin	0.88	neg	angioedema	**5**	algocalmin	1.17	neg	shock
6	paracetamol	1.01	neg	urticaria	**6**	algocalmin	1.32	neg	shock
7	algocalmin	1.02	neg	urticaria	**7**	algocalmin	1.73	neg	urticaria
8	algocalmin	1.04	neg	shock	**8**	algocalmin	1.90	neg	angioedema
9	paracetamol	1.13	neg	angioedema	**9**	algocalmin	1.94	neg	urticaria
10	algocalmin	1.15	neg	angioedema	**10**	paracetamol	2.84	poz	shock
11	algocalmin	1.21	neg	shock	**11**	algocalmin	3.09	poz	angioedema
12	algocalmin	1.32	neg	shock	**12**	paracetamol	3.14	poz	urticaria
13	paracetamol	1.46	neg	angioedema	**13**	algocalmin	3.50	poz	shock
14	algocalmin	1.47	neg	angioedema	**14**	algocalmin	4.15	poz	urticaria
15	algocalmin	1.48	neg	urticaria	**15**	diclofenac	7.49	poz	urticaria
16	diclofenac	1.49	neg	urticaria	**16**	algocalmin	7.72	poz	bronchospasm
17	algocalmin	1.56	neg	hypotension	**17**	algocalmin	9.23	poz	urticaria
18	paracetamol	1.57	neg	hypotension	**18**	paracetamol	9.89	poz	urticaria
19	algocalmin	1.89	neg	urticaria	**19**	algocalmin	11.83	poz	shock
20	algocalmin	2.06	poz	hypotension	**20**	algocalmin	17.74	poz	urticaria
21	algocalmin	2.13	poz	urticaria					
22	algocalmin	19.53	poz	hypotension					

reactions [7]. In our study, we found a moderate agreement between BAT and skin tests, suggesting that further research is needed in order to improve BAT sensitivity for NSAIDs.

In previous studies, there were heterogenous criteria for the patients' and controls' inclusion criteria, for the concentrations used in the performance of skin testing and in flow cytometry, as well as tehnical differences and different interpretation of the results [1]. Moreover, population differences have been pointed out [7].

High drug doses seem to induce nonspecific basophil activation and allergic patients react at lower concentrations than controls in BAT [3,7]. As basophil nonspecific activation appears in a dose-dependent manner at higher drug concentrations, we used three concentrations in BAT for each drug, starting from the concentrations recommended by the manufacturer and one and 2-log scales lower than those. None of the control subjects had a positive BAT, thus nonspecific activation was excluded in our study.

BAT discriminates well between patients with positive skin test and both controls and patients with negative skin tests. BAT was more frequently positive and basophils were more strongly activated in patients with positive skin tests. Clinical histories are not sufficient to diagnose true NSAIDs hypersensitivity [10]. The slight concordance between BAT and history and the lack of significant statistical difference for BAT between controls and patients with positive history and negative skin tests suggest that drug reactivity diminishes with time or that the reactions are not IgE-mediated. In patients with negative skin tests the definitive diagnosis can only be established after the challenge tests which carry the risk of inducing an allergic reaction. BAT represents a risk-free *in vitro* test that might avoid the challenge tests when there is a positive history and the skin tests are false negative in 13.63% patients.

In conclusion, the slight concordance between BAT and history and the lack of statistical difference between controls and patients with positive history and negative skin tests suggest that drug reactivity diminishes with time or that hypersensitivity is not IgE-mediated in these patients. BAT may avoid 13,63% challenge tests in patients with negative skin tests. BAT discriminates well between patients with positive skin tests and both controls and patients with negative skin tests.

Acknowledgements

The financial support from the National Plan II, Priority Domain Partnership Romania, under number 41-062/2007, was highly appreciated. None of the authors has any competing interests to declare.

References

1. Gamboa P, Sanz ML, Caballero MR, et al. The flow cytometic determination of basophil activation induced by aspirin and other non-steroidal anti-inflammatory drugs (NSAIDS) is useful for in vitro diagnosis of the NSAIDS hypersensitivity syndrome. Clin Exp Allergy, 2004; 34:1448-1457.

2. Kowalski ML, Makowska JS, Blanca M, et al. Hypersensitivity to nonsteroidal anti-inflammatory drugs (NSAIDs) – classification, diagnosis and management: review of the EAACI/ENDA and GA2LEN/HANNA. Allergy, 2011; 66:818–829.

3. De Weck AL, Sanz ML, Gamboa M, et al. NSAIDS hypersensitivity syndrome. A multicenter study. II. Basophil activation by non-steroidal anti-inflammatory drugs and its impact on pathogenesis. J Investig Allergol Clin Immunol, 2010; 20(1):39-57.

4. Sanz ML, Gamboa P, De Weck AL. A New Combined Test with Flowcytometric Basophil Activation and Determination of Sulfidoleukotrienes Is Useful for In Vitro Diagnosis of Hypersensitivity to Aspirin and other Nonsteroidal Anti-Inflammatory Drugs. Int Arch Allery Immunol, 2005; 136:58-72.

5. Gomez E, Blanca-Lopez N, Torres M. Immunoglobulin E-mediated immediate allergic reactions to dipyrone: value of basophil activation test in the identification of patients. Clin Exp Allergy, 2009; 39:1217-1224.

6. Himly M, Jahn-Schmid B, Pitterschatscher K, et al. IgE-mediated immediate-type hypersensitivity reactions to the pyrazolone drug propyphenazone. J Allergy Clin Immunol, 2003; 114(4):882-888.

7. Kim M, Joo Cho Y. Flow cytometry-assisted basophil activatrion test as a safe diagnostic tool for aspirin/NSAID hypersensitivity. Allergy Asthma Immunol Res, 2012; 4(3):137-142.

8. Rodriguez-Trabado A, Camara-Mijon C, Ramos-Cantarino A. Basophil activation test for the in vitro diagnosis of nonsteroidal anti-inflammatory drug hypersensitivity. Allergy Asthma Proc, 2008; 29(3):241-249.

9. Gamboa PM, Sanz ML, Caballero MR, et al. Use of CD63 expression as a marker of in vitro basophil activation and leukotriene determination in metamizol allergic patients. Allergy, 2003; 58:312-317.

10. Abuaf N, Rostane H, Barbara J, et al. Comparison of CD63 Upregulation Induced by NSAIDs on Basophils and Monocytes in Patients with NSAID Hypersensitivity. J Allergy (Cairo), 2012; 2012:580873.

11. Ebo DG, BridtsCH, Hagendorens MM, et al. Basophil Activation Test by Flow Cytometry: Present and Future Applications in Allergology. Clinical Cytometry, 2008; 74B:201-210.

12. Ebo DG, Bridts CH, Hagendorens MM, Mertens CH, De Clerck LS, Stevens WJ. Flow-assisted diagnostic management of anaphylaxis from rocuronium bromide Allergy, 2006: 61:935-939.

13. Kvedariene V, Kamey S, Ryckwaert Y, et al. Diagnosis of neuromuscular blocking agent hypersensitivity reactions using cytofluorimetric analysis of basophils. Allergy, 2006: 61:311-315.

14. De Weck AL, Sanz ML, Gamboa PM, and members of ENDA (European Network for Drug Allergy). Diagnosis of immediate-type ß-lactam allergy in vitro by flow-cytometric basophil activation test and sulfi doleukotriene production: a multicenter study. J Investig Allergol Clin Immunol, 2009; 19:91-109.