

ORIGINAL ARTICLE

Immunoglobulin E-mediated immediate allergic reactions to dipyrone: value of basophil activation test in the identification of patients

E. Gómez*, N. Blanca-Lopez†, M. J. Torres‡, G. Requena‡, C. Rondon‡, G. Canto§, M. Blanca‡ and C. Mayorga*

*Research Laboratory, Fundacion IMABIS-Carlos Haya Hospital, Málaga, Spain, †Allergy Service, 12 de Octubre Hospital, Madrid, Spain, ‡Allergy Service, Carlos Haya Hospital, Málaga, Spain and §Allergy Unit, Infanta Leonor Hospital, Madrid, Spain

Clinical & Experimental Allergy

Summary

Background Pyrazolones are a major cause of immediate IgE-mediated reactions to drugs in many countries.

Objective The aim of the study was to evaluate a group of patients with this type of reaction by basophil activation test (BAT), focusing on the influence on positivity of the time between the reaction and this study.

Methods The study included 51 patients with selective immediate allergic reactions to pyrazolones and 56 controls. Patients were defined by skin testing or a drug provocation test and BATs with pyrazolones were carried out in all cases. Patients who were BAT positive were followed-up for 30 months to establish the rate of negativization.

Results BAT was positive in 28 (54.9%) cases. BAT sensitivity was higher in those who were skin-test positive (85.7%) compared with those who were skin-test negative (33.3%). The time between the initial reaction and this study was significantly shorter in those who were skin-test positive ($P = 0.005$) and those who were BAT positive ($P = 0.017$). Follow-up of the BAT-positive patients showed a decrease over time, with 60% of these patients becoming negative after 6 months.

Conclusions BAT is a useful complement to skin testing for the evaluation of immediate allergic reactions to pyrazolones. Although not optimal, BAT sensitivity was also positive in patients with a negative skin test and it is a reasonable alternative in patients with severe reactions who may develop symptoms after skin testing. The time of performance of the test is critical to obtain a positive response.

Keywords basophils, hypersensitivity, IgE, pyrazolones, selective

Submitted 13 August 2008; revised 12 January 2009; accepted 30 January 2009

Correspondence:

Maria José Torres Jaén, Allergy Service, Carlos Haya Hospital, Pl. Hospital Civil, 29009 Malaga, Spain. E-mail: mjtortesj@gmail.com

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most common cause of adverse drug reactions, with an important number of reactions induced by hypersensitivity mechanisms [1]. The most common clinical entities are asthma and urticaria, sometimes accompanied by anaphylaxis. The mechanism involved in urticaria and asthma is similar and based on the inhibition of cyclooxygenase-1 enzymes in NSAID-sensitive patients [2]. This diminishes the synthesis of prostaglandin E₂, normally acting as a brake on the production of cysteinyl leukotrienes [3], leading to an exacerbated production of leukotrienes and cross-intolerance with other NSAIDs [4].

Alternatively, patients with hypersensitivity reactions to NSAIDs can react to just one drug and have good tolerance to other NSAIDs from the same group or from different groups [5, 6]. In these selective responders, an IgE mechanism has been proposed [7–10]. Drugs implicated include paracetamol [11], acetyl salicylic acid (ACA) [7], diclofenac [8], ketorolac [12], pyrazolones [8–10, 13, 14] and cyclooxygenase-2 selective inhibitors [15], with the possible existence of selective responses to all available NSAIDs. Pyrazolones are the most frequent drug involved in this category, although no systematic studies have estimated its prevalence. Owing to the poor sensitivity of *in vivo* methods and the lack of experimental prototypes for *in vitro* assays [4], the diagnosis of selective allergic

reactions to pyrazolones is often based on a drug provocation test (DPT) [5, 6]. The sensitivity of skin prick testing has proved to be low and intradermal skin testing has the potential risk of side-effects [9, 11, 14]. For *in vitro* testing, while experimental prototypes to propylphenazone in humans have been reported [10], for dipyrone, only a non-experimental prototype has so far been developed.

The basophil activation test (BAT) using flow cytometry is based on the determination of activated basophils in the presence of the specific hapten by measuring the CD63 expression on the cell surface. This method enables the diagnosis of immediate allergic reactions to drugs without risk, circumventing the need to develop complex conjugates required for *in vitro* testing [9, 16–18]. Although nowadays BAT is not a primary diagnostic test, it can be complementary to other diagnostic methods, particularly when, for various reasons, they cannot be performed or show doubtful results. BAT has been indicated in betalactams [16, 17, 19] and in muscle relaxants [20, 21] but heterogeneous results have been obtained in NSAIDs in terms of sensitivity and specificity [22–24]. The main purpose of this study was to evaluate a group of patients with immediate selective reactions to pyrazolones, assessing the value of BAT and focusing on the influence on positivity of the time between the reaction and this study.

Materials and methods

Patients

We evaluated patients with confirmed selective immediate allergic reactions to pyrazolones. The diagnosis was established by the presence of clinical symptoms of anaphylaxis or immediate urticaria after pyrazolone intake and a positive skin test. If the skin test was negative, at least two episodes with a pyrazolone derivative were required for diagnosis. If just a single reaction occurred, a DPT was carried out in patients with urticaria. To rule out an idiosyncratic reaction we assessed tolerance to ASA in all cases. As controls, we selected subjects with good tolerance to both pyrazolones and ASA, confirmed by a negative DPT.

The study was conducted according to the Declaration of Helsinki principles and was approved by the Ethics Committee of Carlos Haya Hospital. All subjects included in the study were informed orally about the study and signed the corresponding informed consent.

Skin testing

Skin testing was carried out as described [9], using metamizol 0.1 mg/mL (Boehringer Ingelheim, Barcelona, Spain). In the skin prick tests, a weal larger than 3 mm with a negative response to the control saline was

considered positive. In the intradermal tests the weal area was marked initially and 20 min after testing, and an increase in diameter greater than 3 mm was considered positive [25].

Drug provocation test

In patients with just one episode of urticaria, a DPT was carried out to confirm the diagnosis, as previously described [5]. Four doses (10, 50, 125 and 250 mg) were administered at 1 h intervals on the first day, and, if no response, one dose (575 mg), on the second day. In all cases, good tolerance to ASA was assessed in order to confirm selectivity, as described [5]: two doses (50 and 100 mg) were administered on the first day, and, if negative, another two doses (250 and 500 mg) on the second day.

Basophil activation by flow cytometry

Hundred microlitres of heparinized whole blood was aliquoted per test and 20 µL of stimulation buffer was added and incubated for 10 min at 37 °C in a water bath. After this, 100 µL of the washing solution was added to the negative control tube, 100 µL of the fMLP (chemotactic peptide *N*-formyl-Met-Leu-Phe) and anti-human IgE antibodies (BD Pharmingen San Diego, CA, USA) to the positive control tubes and 100 µL of two final drug concentrations to the different samples. Metamizol (Boehringer) was used at 2.5 and 0.25 mg/mL. These optimal concentrations were chosen based on dose-response curves (Fig. 1). Concentrations higher than those described above are cytotoxic [9, 22] and concentrations below decrease sensitivity. The samples were

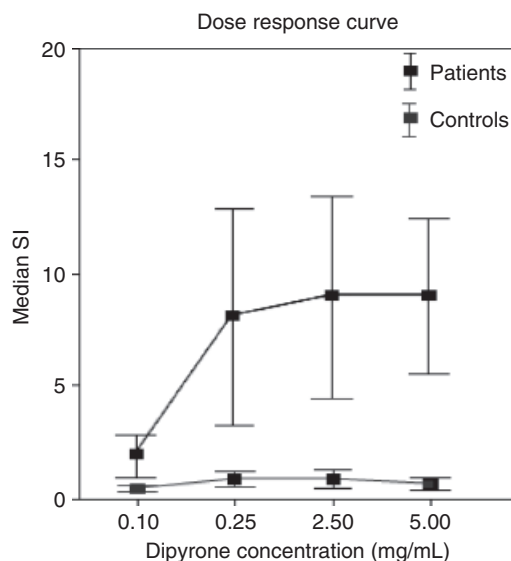


Fig. 1. Dose-response curve for positive ($N=28$) and negative ($N=48$) responders to dipyrone. Results are expressed as the SI (means $\pm$ SEM).

incubated for 30 min at 37 °C in a water bath. The degranulation was stopped by incubating the samples on ice for 5 min and then 3 µL of the monoclonal antibodies anti-IgE FITC and CD63 PE (Caltag Laboratories, Burlingame, CA, USA) was added to each tube. Then, 2 mL of pre-warmed lysing solution was added and the tube was incubated for 10 min at room temperature. After centrifuging and washing, the cells were analyzed in a flow cytometer by acquiring at least 250–500 basophils per sample. Results were considered as positive when the stimulation index (SI), calculated as the ratio between the percentage of degranulated basophils with the different haptens and the negative control, was greater than 2 in at least one of the dilutions mentioned above. We selected this value after the performance of a ROC curve generated after comparison of BAT to dipyrone between patients and controls (Fig. 2). When the percentage of spontaneously activated basophils was <2.5%, we required an additional condition, i.e. that the percentage of basophils activated after contact with the antigen should be ≥5%, as previously described [16]. Non-responder patients, considered as those that had negative results to anti-IgE positive control and drugs, were not included in the study.

Statistical analysis

Comparisons for quantitative variables without a normal distribution were carried out by the Mann–Whitney and Kruskal–Wallis tests. Comparisons for qualitative variables were carried out by means of the χ^2 test. The evolution of BAT tests was analyzed by survival analysis (Kaplan–Meier test using log–rank statistics). ROC curve analyses were performed to calculate the optimal cut-off value corresponding to the best sensitivity and specificity.

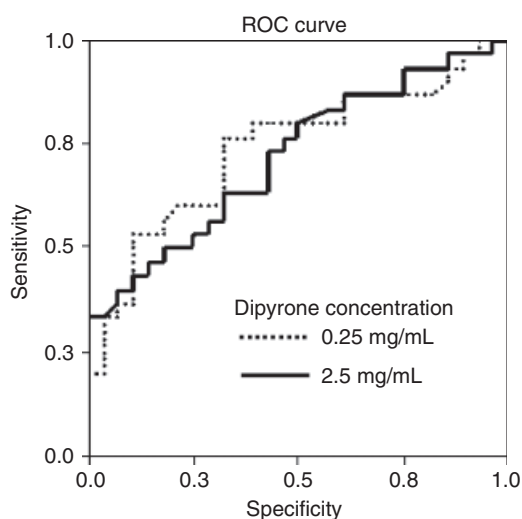


Fig. 2. ROC curve generated after comparison of basophil activation test (BAT) to dipyrone of patients vs. controls.

All reported *P*-values represent two-tailed tests, with values <0.05 considered statistically significant. The statistical analysis was performed using the SPSS program, version 15.0.

Results

Over the 4-year period 2002–2006, a total of 51 patients with immediate selective reactions to dipyrone agreed to be included in the study, as well as 56 subjects with good tolerance to NSAIDs as controls. The clinical characteristics of these patients, as well as the skin test and BAT results to dipyrone, are shown in Table 1. There were 35 women and 16 men, the median age was 39 years [interquartile range (IR): 28–50], 38 had developed anaphylaxis (74.5%) and 13 urticaria (25.5%), with a median interval between the reaction and the study of 8 months (IR: 3–12). Twenty-one were skin-test positive to dipyrone (four by prick). Five cases had a positive DPT after dipyrone administration, one case reacted with 10 mg, three with 50 mg and one with 125 mg; all were urticarial reactions.

The median SI for the higher pyrazolone concentration (2.5 mg/mL) was 1.35 (IR: 0.75–5.24) and for the lower concentration (0.25 mg/mL) it was 2.07 (IR: 1.14–4.64). Analysis of the sensitivity of the BAT to dipyrone showed that 28 patients were BAT positive at at least one of the concentrations (54.9%). Twenty-one patients were skin-test positive (41.17%). Eighteen (35.29%) patients were both BAT and skin-test positive, 3 (5.8%) skin-test positive and BAT negative and 10 (19.6%) skin-test negative and BAT positive.

The control group included 56 subjects, and the median age was 37 years (IR: 26.5–49.0), with no significant differences with the patients. Skin tests to dipyrone were negative in all cases. The median SI for the higher pyrazolone concentration (2.5 mg/mL) was 0.7 (IR: 0.42–1.39) and for the lower concentration (0.25 mg/mL) it was 0.81 (IR: 0.55–1.58). Eight subjects (14.28%) were BAT positive at at least one of the concentrations; giving a specificity of 85.71%.

Comparison of the SI of the BAT to dipyrone between patients and controls is shown in Fig. 3. Significant differences were found for both concentrations used, 0.25 mg/mL (*P* = 0.001) and 2.5 mg/mL (*P* = 0.002).

The comparison of the BAT and skin test results showed that the SI was significantly higher in those who were skin-test positive (85.7%) compared with those who were skin-test negative (33.3%), at both concentrations: 0.25 mg/mL (*P* = 0.002) and 2.5 mg/mL (*P* < 0.001) (Fig. 4).

The time interval between the reaction and the performance of the study was significantly shorter in patients who tested positive, for both skin testing (*P* = 0.005) and BAT (*P* = 0.017) (Fig. 5). The median time interval in the

Table 1. Clinical characteristics of the study patients and results of skin testing and basophil activation test to pyrazolones

Patient	Sex	Age	Reaction	Episodes	Interval (month)	Skin test (weal size)	DPT dipyrone	DPT ASA	BAT (SI) 2.5 mg/mL	BAT (SI) 0.25 mg/mL	BAT
1	F	33	Urticaria	1	26	Negative	Positive	Negative	1.07	1.61	Negative
2	F	27	Anaphylaxis	2	1	Positive*(3×3 mm)	Not done	Negative	7.68	3.35	Positive
3	F	58	Anaphylaxis	1	14	Positive (5×7 mm)	Not done	Negative	11.38	4.28	Positive
4	M	57	Anaphylaxis	1	10	Positive (6×6 mm)	Not done	Negative	0.87	1.33	Negative
5	F	29	Urticaria	1	8	Negative	Positive	Negative	1.90	8.72	Positive
6	F	31	Anaphylaxis	2	4	Negative	Not done	Negative	2.63	2.32	Positive
7	F	18	Urticaria	3	36	Negative	Not done	Negative	0.25	0.27	Negative
8	M	27	Anaphylaxis	2	12	Negative	Not done	Negative	0.69	6.57	Positive
9	F	22	Anaphylaxis	3	3	Negative	Not done	Negative	1.66	1.62	Negative
10	F	48	Urticaria	2	35	Negative	Not done	Negative	1.13	0.89	Negative
11	M	17	Anaphylaxis	2	9	Negative	Not done	Negative	0.57	0.39	Negative
12	F	38	Urticaria	1	2	Negative	Positive	Negative	0.74	0.66	Negative
13	M	32	Anaphylaxis	1	5	Positive (7×7 mm)	Not done	Negative	26.52	29.19	Positive
14	F	36	Anaphylaxis	2	12	Negative	Not done	Negative	0.90	2.10	Positive
15	F	69	Anaphylaxis	1	8	Positive (8×7 mm)	Not done	Negative	12.69	13.30	Positive
16	M	40	Anaphylaxis	1	10	Positive (7×5 mm)	Not done	Negative	3.13	4.64	Positive
17	M	43	Anaphylaxis	1	3	Positive*(3×4 mm)	Not done	Negative	6.82	1.31	Positive
18	M	39	Urticaria	1	5	Positive*(4×4 mm)	Not done	Negative	0.42	0.42	Negative
19	F	48	Anaphylaxis	1	2	Positive (9×9 mm)	Not done	Negative	10.94	12.03	Positive
20	F	46	Anaphylaxis	2	109	Negative	Not done	Negative	0.04	1.31	Negative
21	F	50	Anaphylaxis	3	10	Negative	Not done	Negative	0.91	2.29	Positive
22	F	37	Urticaria	2	24	Negative	Not done	Negative	0.93	1.14	Negative
23	M	47	Urticaria	2	35	Negative	Not done	Negative	0.81	1.32	Negative
24	F	23	Anaphylaxis	2	4	Negative	Not done	Negative	0.44	0.66	Negative
25	M	28	Anaphylaxis	2	5	Negative	Not done	Negative	5.24	9.51	Positive
26	F	52	Anaphylaxis	2	36	Negative	Not done	Negative	1.22	1.23	Negative
27	F	48	Anaphylaxis	1	1	Positive*(3×3 mm)	Not done	Negative	3.87	4.07	Positive
28	F	56	Anaphylaxis	3	4	Negative	Not done	Negative	4.65	2.59	Positive
29	M	49	Anaphylaxis	1	1	Positive (5×7 mm)	Not done	Negative	1.35	5.66	Positive
30	F	31	Anaphylaxis	11	3	Negative	Not done	Negative	1.99		Negative
31	F	80	Anaphylaxis	1	1	Positive (5×5 mm)	Not done	Negative	1.73	2.07	Positive
32	F	37	Anaphylaxis	2	26	Negative	Not done	Negative	0.97	1.52	Negative
33	F	22	Anaphylaxis	1	1	Positive (3×4 mm)	Not done	Negative	6.55	2.98	Positive
34	F	18	Anaphylaxis	1	14	Positive (5×6 mm)	Not done	Negative	10.23	3.99	Positive
35	F	59	Anaphylaxis	2	10	Positive (5×5 mm)	Not done	Negative	.75	1.45	Negative
36	F	66	Urticaria	1	8	Negative	Positive	Negative	1.99	7.25	Positive
37	M	71	Anaphylaxis	2	4	Negative	Not done	Negative	2.68	2.12	Positive
38	F	18	Anaphylaxis	2	36	Negative	Not done	Negative	0.19	0.24	Negative
39	F	31	Anaphylaxis	3	12	Negative	Not done	Negative	0.78	5.98	Positive
40	F	41	Anaphylaxis	2	3	Negative	Not done	Negative	1.55	1.70	Negative
41	F	53	Urticaria	3	35	Negative	Not done	Negative	1.23	0.99	Negative
42	F	25	Urticaria	2	9	Negative	Not done	Negative	0.47	0.35	Negative
43	M	62	Urticaria	1	2	Negative	Positive	Negative	0.74	0.56	Negative
44	M	49	Anaphylaxis	1	5	Positive (6×6 mm)	Not done	Negative	10.23	15.01	Positive
45	F	57	Anaphylaxis	2	12	Negative	Not done	Negative	0.90	2.22	Positive
46	M	37	Anaphylaxis	1	8	Positive (5×4 mm)	Not done	Negative	11.35	12.20	Positive
47	F	22	Anaphylaxis	1	10	Positive (5×5 mm)	Not done	Negative	2.98	3.89	Positive
48	F	39	Anaphylaxis	1	3	Positive (4×4 mm)	Not done	Negative	5.99	1.30	Positive
49	M	41	Anaphylaxis	1	5	Positive (4×5 mm)	Not done	Negative	0.42	0.55	Negative
50	F	39	Anaphylaxis	1	2	Positive (5×4 mm)	Not done	Negative	10.94	11.99	Positive
51	M	25	Urticaria	1	9	Negative	Positive	Negative	0.12	1.31	Negative

*Skin tests were positive by prick; shaded BAT results are those considered as positive (SI > 2).

BAT, basophil activation test; DPT, drug provocation test

skin-test positive patients was 5 months (IR: 2–10) and in the skin-test negative patients it was 9.5 months (4–28.5). For BAT, the median time interval in the patients with a positive result was 6.5 months (IR: 2.25–10) and in those with a negative result it was 10 months (IR: 4–35).

We also performed a survival analysis to evaluate prospectively the role of the time interval between the reaction and the study. We followed the 28 BAT-positive patients over 30 months and found that the BAT decreased quickly over time (Fig. 6), faster at the higher concentration. Notably, at 6 months, 60% of the patients had become negative at both concentrations.

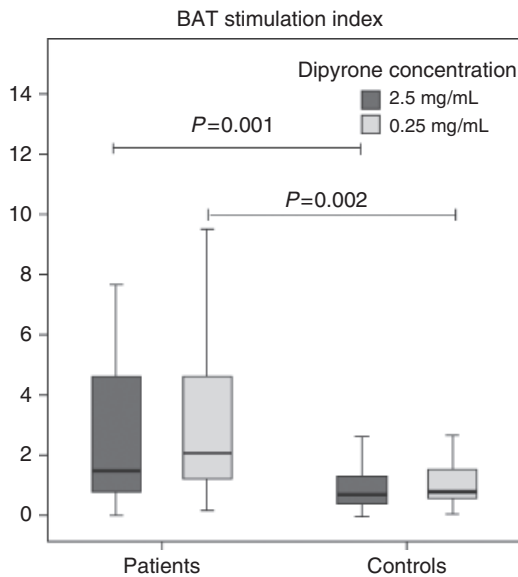


Fig. 3. Comparison of the basophil activation test with dipyrone at two concentrations in patients and controls.

Discussion

In this study, we focused on the evaluation of a well-characterized group of patients with immediate selective reactions to pyrazolones. Attempts have been made to develop *in vitro* assays to quantify IgE antibodies to pyrazolones. Animal studies using different pyrazolone derivatives with the production of experimental antibodies have found that different structures can be recognized with a low degree of cross-reactivity [26]. Concerning IgE antibodies in humans, their *in vitro*

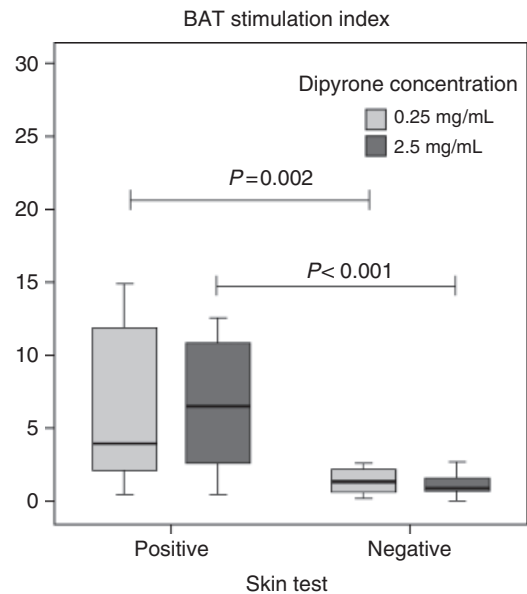


Fig. 4. Comparison of the basophil activation test with dipyrone at two concentrations in patients who were skin-test positive or negative to dipyrone.

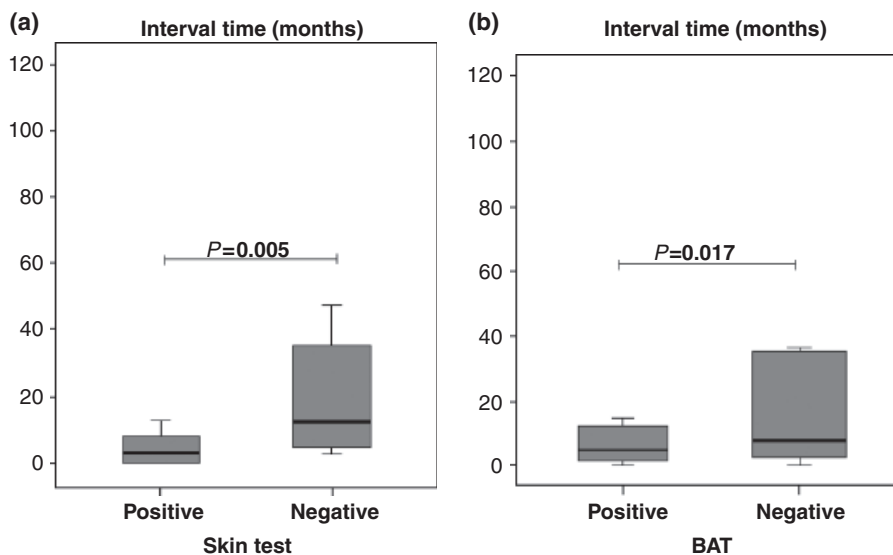


Fig. 5. Influence of the time interval between the reaction and the study on the positivity of skin testing (a) and basophil activation test (b).

presence has only been clearly demonstrated with propi-phenazone [10]. Concerning dipyrone, different metabolites formed either by alkaline hydrolysis [27] or by reaction of biotransformations [28] have been identified, although the IgE recognition of these structures has not been demonstrated.

Considering these difficulties, although the BAT represents a good alternative to identify patients with immediate selective reactions, few studies have yet been carried out. Gamboa *et al.* [9] reported the value of this method in a group of well-defined subjects with immediate selective allergic reactions to dipyrone, with a few differences compared with our results. The sensitivity was lower (42.3%) but the specificity was 100%. These differences may be due to the time interval between the study and the reaction, which was longer (16.9 months) in the Gamboa study than ours (8 months) and the fact that the per-

tage of patients with anaphylaxis in our group was higher (74.5% vs. 57.7%). Concerning specificity, these drugs, like other analgesic and anti-inflammatory compounds, can interact pharmacologically with basophils and induce non-specific histamine release in controls, thereby justifying the figures reported in our study. These data have been shown in subjects with idiosyncratic reactions to NSAIDs, where many of these drugs, including paracetamol, induce false positive results [22].

In this study, we found that the shorter the time interval between the reaction and the study, the more likelihood of having a positive skin test and/or BAT. Skin testing enabled us to identify 41% of the cases. One reason for this low percentage is the time interval between the reaction and the study. This has been shown in subjects with immediate hypersensitivity reactions to betalactams, where the time interval contributes to the positivity of the skin test [29]. In fact, in our study, the time interval between the reaction and the performance of skin testing in those who were skin-test negative was twice that of those who were skin-test positive.

An adequate procedure to evaluate this is to follow-up positive cases over time by repeating the test. In general, few prospective studies have been carried out. Data concerning specific IgE determined by immunoassay have been reported with betalactams [16] and chlorhexidine [30], with differences in the number of patients assessed, the surveillance period and the methods used. In our study, 60% of the patients who were BAT positive to dipyrone became negative after 6 months of follow-up. This differs from a study with betalactams [19], where the rate of negativization was longer since 60% negativization took 18 months. Longer rates of negativization have also been found with neuromuscular blocking agents in BAT, in which data showed that the sensitivity of the methods was about 85% when the patients were evaluated within 3 years of the reaction but decreased dramatically to 47% if the evaluation was performed after 4 years [20]. This may be due to the fact that the response to betalactams is more consistent and long-lasting than that to other drugs [22, 28, 30].

Although one of the reasons for a negative result was the time interval between the reaction and the study, eight

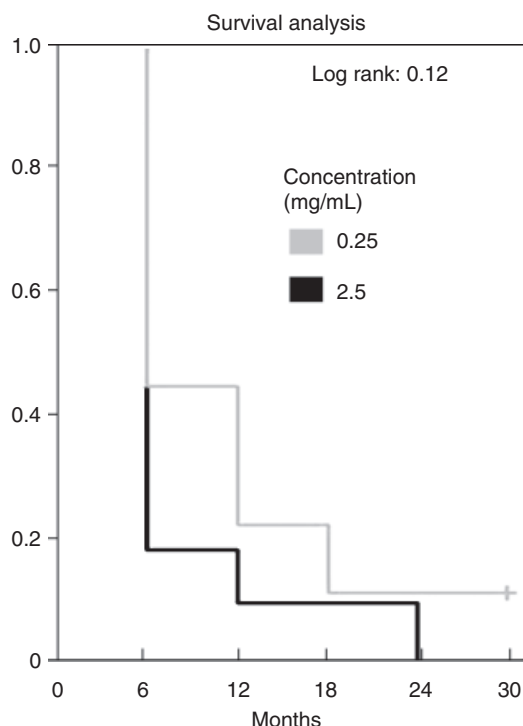


Fig. 6. Basophil activation test survival analysis over a 30-month period.

Table 2. Results of drug provocation test to pyrazolones

Patient	Previous reaction	Last dose (mg)	Cumulative dose (mg)	Time of appearance of symptoms (minutes)	Symptoms
1	Urticaria	10	10	15	Weals and erythema on arms and legs
5	Urticaria	50	60	60	Weals and pruritus on chest and elbows
12	Urticaria	10	10	20	Facial erythema and angioedema
36	Urticaria	125	185	60	Maculopapulae and erythema on neck and chest
43	Urticaria	10	10	10	Generalized itching and angioedema
51	Urticaria	50	60	40	Weals on face, neck and abdomen

cases were negative even though this period was short, suggesting that other factors, such as the identification of the adequate metabolite, may be necessary to identify these patients.

In summary, based on the selective response to dipyrone and good tolerance to other non-chemically related NSAID and the BAT negativization rate, specific IgE reactions to dipyrone occur, as has also been shown by others. The combination of skin testing and BAT can be used as a diagnostic tool. BAT could be the first choice, especially in cases at risk, although the time interval seems to be critical for test sensitivity. Identification of the adequate metabolite and further improvements are required for a better diagnosis of these patients.

Acknowledgements

We thank Ian Johnstone for help with the final English language version of this manuscript.

Sources of funding: This work was supported by grants from Spanish Health Ministry (FIS) (PIO31165) and FIS network RIRAAF (RD07/0064).

References

- 1 Faich GA. Adverse-drug-reaction monitoring. *N Engl J Med* 1986; **314**:1589–92.
- 2 Szczeklik A, Gryglewski RJ, Czerniawska-Mysik G. Relationship of inhibition of prostaglandin biosynthesis by analgesics to asthma attacks in aspirin-sensitive patients. *Br Med J* 1975; **1**:67–9.
- 3 Szczeklik A, Stevenson D. Aspirin-induced asthma: advances in pathogenesis, diagnosis and management. *J Allergy Clin Immunol* 2003; **111**:913–21.
- 4 Stevenson DD, Simon RA. Sensitivity to aspirin and nonsteroidal anti-inflammatory drugs. In: Middleton E, ed. *Allergy. Principles and practice*, 5th edn, 1998, De Young L, St Louis, Missouri, pp. 1225–34.
- 5 Quirarte J, Blanco C, Castillo R, Delgado J, Carrillo T. Intolerance to nonsteroidal antiinflammatory drugs: results of controlled drug challenges in 98 patients. *J Allergy Clin Immunol* 1996; **98**:678–85.
- 6 Carmona MJ, Blanca M, Garcia A *et al.* Intolerance to piroxicam in patients with adverse reactions to nonsteroidal antiinflammatory drugs. *J Allergy Clin Immunol* 1992; **90**:873–9.
- 7 Blanca M, Perez E, Garcia JJ *et al.* Angioedema and IgE antibodies to aspirin: a case report. *Ann Allergy* 1989; **62**:295–8.
- 8 Harrer A. Diclofenac-specific monoclonal antibodies show variable cross-reactivity to diclofenac metabolites. *EAACI Abstract book* 2007: 35–6.
- 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**:312–7.
- 10 Himly M, Jahn-Schmid B, Pittertschatscher K *et al.* IgE-mediated immediate-type hypersensitivity to the pyrazolone drug propyphenazone. *J Allergy Clin Immunol* 2003; **111**:882–8.
- 11 de Paramo BJ, Gancedo SQ, Cuevas M, Camo IP, Martin JA, Cosmes EL. Paracetamol (acetaminophen) hypersensitivity. *Ann Allergy Asthma Immunol* 2000; **85**:508–11.
- 12 Scala E, Giani M, Pirrotta L *et al.* Selective severe anaphylactic reaction due to ketorolac tromethamine without nonsteroidal anti-inflammatory drug intolerance. *J Allergy Clin Immunol* 2001; **107**:557.
- 13 Czerniawska-Mysik G, Szczeklik A. Idiosyncrasy to pyrazolone drugs. *Allergy* 1981; **36**:381–4.
- 14 Kowalski ML, Bienkiewicz B, Woszczek G, Iwaszkiewicz J, Poniatowska M. Diagnosis of pyrazolone drug sensitivity: clinical history versus skin testing and in vitro testing. *Allergy Asthma Proc* 1999; **20**:347–52.
- 15 Fontaine C, Bousquet PJ, Demoly P. Anaphylactic shock caused by a selective allergy to celecoxib, with no allergy to rofecoxib or sulfamethoxazole. *J Allergy Clin Immunol* 2005; **115**:633–4.
- 16 Torres MJ, Padial A, Mayorga C *et al.* The diagnostic interpretation of basophil activation test in immediate allergic reactions to betalactams. *Clin Exp Allergy* 2004; **34**:1768–75.
- 17 Sanz ML, Gamboa PM, Antepara I *et al.* Flow cytometric basophile activation test by detection of CD63 expression in patients with immediate-type reactions to betalactam antibiotics. *Clin Exp Allergy* 2002; **32**:277–86.
- 18 Ebo DG, Bridts CH, Hagendorens MM, Aerts NE, De Clerck LS, Stevens WJ. Basophil activation test by flow cytometry: present and future applications in allergology. *Cytometry B Clin Cytom* 2008; **74**:201–10.
- 19 Fernández TD, Torres MJ, Blanca-López N *et al.* Negativization rates of IgE radioimmunoassay and basophil activation test in immediate reactions to penicillins. *Allergy* 2009; **64**:242–8.
- 20 Kvedariene V, Kamey S, Ryckwaert Y *et al.* Diagnosis of neuromuscular blocking agent hypersensitivity reactions using cytofluorimetric analysis of basophils. *Allergy* 2006; **61**:311–5.
- 21 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**:935–9.
- 22 Gamboa P, Sanz ML, Caballero MR *et al.* The flow-cytometric determination of basophil activation induced by aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) is useful for in vitro diagnosis of the NSAID hypersensitivity syndrome. *Clin Exp Allergy* 2004; **34**:1448–57.
- 23 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 Allergy* 2005; **136**:58–72.
- 24 De Weck AL, Sanz ML, LENDA Group, *et al.* Diagnosis of hypersensitivity to non-steroidal anti-inflammatory drugs (NSAIDs) in vitro by flowcytometry (Flow-CAST) and sulfidoleukotriene assay. A multicentric study. Vienna, EAACI Congress, 2006, p. 654.
- 25 Brockow K, Romano A, Blanca M *et al.* General considerations for skin test procedures in the diagnosis of drug hypersensitivity. *Allergy* 2002; **57**:45–51.
- 26 Schneider CH, Kasper MF, de Weck AL, Rolli H, Angst BD. Diagnosis of antibody-mediated drug allergy. Pyrazolinone and

- pyrazolidinedione cross-reactivity relationships. *Allergy* 1987; **42**:597–603.
- 27 Vasileva-Aleksandrova P, Aleksandrov A. Study of the interaction of noramidopyrine methanesulfonate sodium (Analgin) with tetrazolium salts in alkaline medium. *Pharmazie* 1990; **45**:348–9.
 - 28 Schiebel HM, Gries O, Schuppel R, Schulze P. Identification of conjugated metabolites of pyrazolone drugs in human urine by pyrolysis-mass spectrometry. *J Anal Appl Pyrol* 1987; **10**: 267–81.
 - 29 Blanca M, Torres MJ, García JJ *et al*. Natural evolution of skin test sensitivity in patients allergic to beta-lactam antibiotics. *J Allergy Clin Immunol* 1999; **103**:918–24.
 - 30 Garvey LH, Krøigaard M, Poulsen LK *et al*. IgE-mediated allergy to chlorhexidine. *J Allergy Clin Immunol* 2007; **120**: 409–15.