

***In vitro* evaluation of IgE-mediated hypersensitivity reactions to quinolones**

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Keywords

allergy; basophil activation test; IgE; quinolones; radioimmunoassay.

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Abstract

Background: Hypersensitivity IgE-mediated reactions to quinolones are not easy to diagnose, with skin testing inducing false positive results. The aim of the study was to evaluate the *in vitro*-specific IgE response in patients with immediate allergic reactions to quinolones.

Methods: We evaluated 38 patients with confirmed immediate allergic reactions to quinolones. Those with anaphylaxis were considered allergic by clinical history, once other possible causes were ruled out, and those with urticaria by drug provocation. Sepharose-radioimmunoassay (RIA) and basophil activation test (BAT) with ciprofloxacin, moxifloxacin and levofloxacin were performed.

Results: The quinolones involved were moxifloxacin ($N = 24$), ciprofloxacin ($N = 11$) and levofloxacin ($N = 3$). Sepharose-RIA was positive in 12 cases (31.57%) and BAT in 27 (71.05%). With Sepharose-RIA, 8 (21%) were positive to ciprofloxacin, 7 (18.4%) to moxifloxacin and 7 (18.4%) to levofloxacin. With BAT, 23 (60.5%) were positive to ciprofloxacin, 12 (31.6%) to moxifloxacin and 8 (21%) to levofloxacin. The specificity of the Sepharose-RIA was demonstrated by inhibition tests. To confirm that the BAT results observed were IgE mediated, the PI3K inhibitor wortmannin was used, with this compound inhibiting the BAT when stimulated with anti-IgE and the different quinolones, but not when fMLP was used as the basophil stimulator. Sepharose-RIA and BAT were repeated in positive cases 1 year later, detecting a decrease in all cases, with four becoming negative.

Conclusion: Immediate hypersensitivity reactions to quinolones do occur, with moxifloxacin being the drug most frequently involved. The BAT is a useful method for diagnosing patients. Specific IgE was demonstrated by Sepharose-RIA and inhibition assay.

Quinolones are safe and well-tolerated antibiotics that are increasingly prescribed because of their effectiveness against Gram-positive and Gram-negative bacteria. Most adverse effects are mild, involving the gastrointestinal or central nervous systems, although cardio-, hepato- and phototoxicity also occur (1).

Hypersensitivity reactions have also been reported, the most frequent being immediate urticaria and anaphylaxis (2–10). Based on spontaneous reports of adverse drug reactions, the

frequency of anaphylaxis associated with quinolone administration has been estimated at 1.8–2.3 per 10 million days of treatment (2). In the particular case of ciprofloxacin, anaphylaxis has been reported to occur in 1.2 per 100 000 prescriptions (3). In a study of cases reported in Germany over a 10-year period (1993–2004), moxifloxacin was the quinolone involved in 54% of the cases, followed by levofloxacin in 15% and ciprofloxacin in 13% (4).

The diagnosis of immediate allergic reactions to quinolones is still a matter of debate. The value of skin prick and intradermal testing is controversial, and although some authors consider skin testing useful (9, 10), others have shown an important percentage of false positive results

Abbreviations

BAT, basophil activation test; DPT, drug provocation test; sepharose-RIA, sepharose radioimmunoassay; SI, stimulation index.

(6, 11, 12). These differences may be explained by the capacity of some quinolones to induce direct histamine release (12). The diagnostic approach, therefore, is the drug provocation test (DPT), which is not free of risk if we consider that the reaction can be severe. This enhances the need to develop sensitive *in vitro* methods that could allow us to diagnose IgE-mediated reactions.

The presence of *in vitro*-specific IgE to quinolones has been reported, using sepharose radioimmunoassay (Seph-rose-RIA), with a sensitivity of 54.5% (8). Two more recent studies, performed with fewer cases, have shown contradictory results regarding the use of basophil activation tests (BAT) in the diagnosis of immediate reactions to quinolones (13, 14). In this study, we evaluated the *in vitro*-specific IgE response in a group of 38 patients with confirmed immediate allergic reactions to quinolones by two different approaches, Sepharose-RIA and BAT, showing that both methods were useful although the sensitivity of the BAT was higher.

Methods

Patients and controls

All patients referred to the Allergy Services of three Spanish Hospitals with an allergic reaction after administration of a quinolone derivative over a 3-year period (2006–2008) were eligible for inclusion in the study. Different clinical categories were established: anaphylaxis and anaphylactic shock, defined according to the criteria of Sampson (15), and urticaria when manifestations were limited to the skin and consisted of pruritic, erythematous cutaneous elevations that blanched with pressure at various sites on the body.

In the allergological work-up, skin testing was not performed because of the low sensitivity and specificity described in the literature (6, 11, 12). Patients with anaphylaxis and anaphylactic shock were considered allergic by the clinical history, once other possible causes were ruled out. In those with urticaria, a DPT was performed to confirm the diagnosis. The controls consisted of 35 cases with confirmed good tolerance to quinolones, 10 who had total IgE > 1000 kIU/l and 25 with total IgE < 100 kIU/l.

The study was approved by the relevant institutional review boards, and informed consent for the diagnostic procedures was obtained from the patients and controls.

Total IgE

This was measured by CAP-FEIA following the manufacturer's recommendations (Phadia, Uppsala, Sweden).

Drug provocation test

Single-blind placebo-controlled DPT was carried out using ciprofloxacin (Ciprofloxacino Normon, Madrid, Spain), moxifloxacin (Actira, Bayer, Barcelona, Spain) or levofloxacin (Tavanic, Sanofi-Avantis, Barcelona, Spain) under strict hospital surveillance, as described (16). This test consisted of the administration of the suspected quinolone at 30-min

intervals at increasing doses till reaching the full therapeutic dose or symptoms of a drug reaction occurred. For ciprofloxacin and levofloxacin, the doses administered were 5, 50, 100, 150 and 200 mg (cumulative dose of 505 mg) and for moxifloxacin 5, 50, 100, 100 and 150 mg (cumulative dose of 405 mg). Informed consent for the performance of DPT was obtained from all patients.

Reagents for *in vitro* assays

The quinolones used were moxifloxacin (Sigma-Aldrich, St. Louis, MO, USA) and levofloxacin and ciprofloxacin (Fluka, Oakville, Ontario, Canada).

Basophil activation test

This was performed as previously described with some modifications (17). In brief, 100 µl of heparinized whole blood and 20 µl of stimulation buffer (NaCl at 0.78%, KCl at 0.037%, CaCl₂ at 0.078%, MgCl₂ at 0.033%, HSA at 0.1%, HEPES at 1 M and IL-3 at 10 µg/ml) were added per test and incubated for 10 min at 37°C. After this, 100 µl of the two drug concentrations was added to different samples. As a negative control, 100 µl of washing solution was added. We used two positive controls (100 µl), one non-IgE-mediated *N*-formyl-leucyl-phenylalanine (fMLP, Orpegen Pharma, 200× stock solution, 0.4 mM) and the other an anti-human IgE (BD Pharmingen, 0.5 mg/ml).

The two optimal concentrations for each drug (ciprofloxacin: 2 and 0.2 mg/ml; moxifloxacin: 0.2 and 0.1 mg/ml; levofloxacin: 4 and 2 mg/ml) were chosen based on dose-response curves and cytotoxicity studies (Fig. 1A). The samples were incubated for 30 min at 37°C. The degranulation was stopped by incubating on ice for 5 min, after which anti-IgE FITC and CD63 PE (Caltag Laboratories, Burlingame, CA, USA) were added to each tube. Then, 2 ml of prewarmed lysing solution was added and incubated for 10 min at room temperature. After one wash, the cells were analyzed in a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA, USA), acquiring at least 250–500 basophils per sample. Results were considered as positive when the percentage of CD63 was 5% over spontaneous activation observed for the negative control, and the stimulation index (SI), calculated as the ratio between the percentage of degranulated basophils with the haptens and the spontaneous basophil activation, was > 2 in at least one of the dilutions (17–19).

To confirm that basophil activation was IgE mediated, we analyzed the wortmannin inhibitory effect at different concentrations (5, 1, 0.1 µM) (18). Wortmannin acts as a specific inhibitor of phosphatidylinositol 3-kinase (PI3-K), which has been shown to be one of the important kinases activated by FcεRI receptor cross-linking involved in IgE-mediated stimulation of human basophils (20). This was used with the positive controls (fMLP and anti-IgE) and the different haptens ciprofloxacin, moxifloxacin and levofloxacin at the same concentrations described previously. We also followed up the BAT-positive patients at least 1 year after the first determination to evaluate the specific IgE clearance.

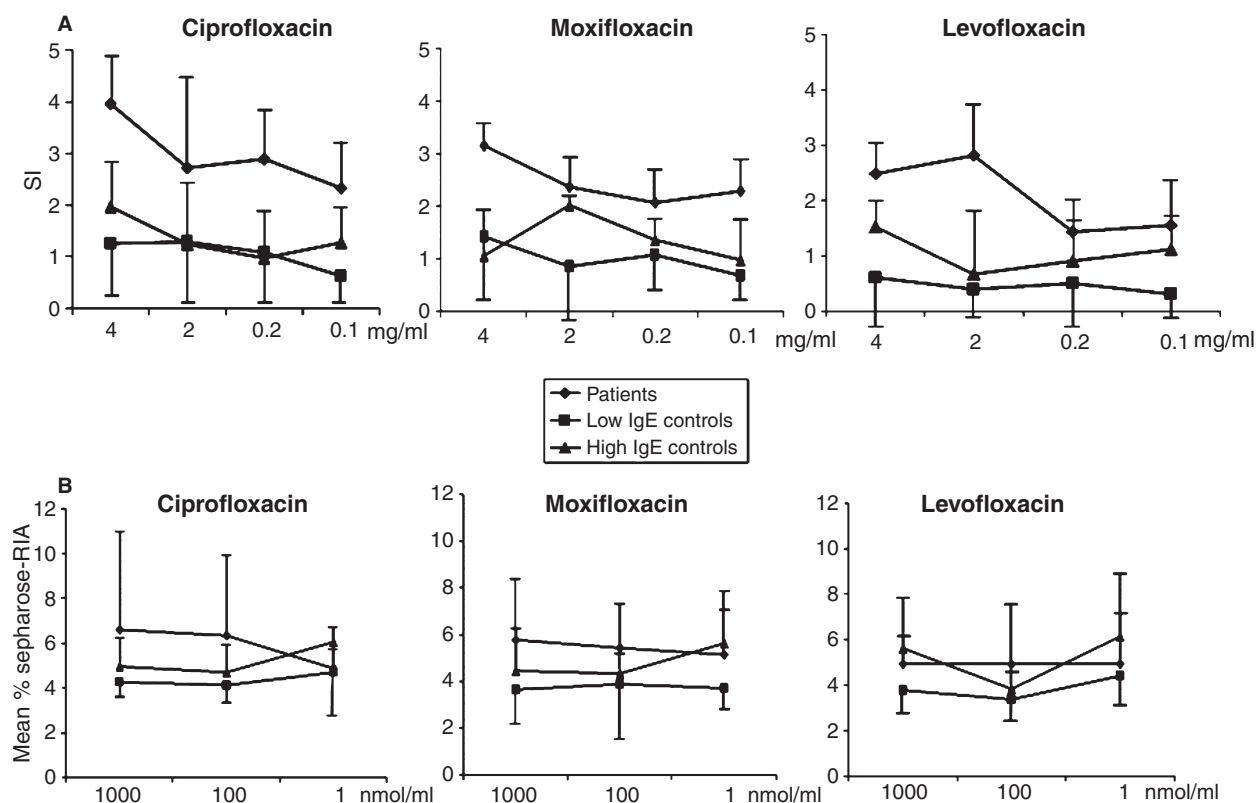


Figure 1 (A) Basophil activation test dose-response curve for ciprofloxacin, moxifloxacin and levofloxacin in patients and controls. Results are expressed as stimulation index (SI). (B) Sepharose-

radioimmunoassay dose-response curve for ciprofloxacin, moxifloxacin and levofloxacin in patients and controls. Results are expressed as % of cpm respect to maximum cpm.

Radioimmunoassay

For Sepharose-RIA, ciprofloxacin, moxifloxacin and levofloxacin at different concentrations (1, 100 and 1000 nmol/ml) were conjugated to sepharose epoxy-activated 6B (Phadia) as described in (8). After doing dose-response curves with each drug, we considered 100 nmol/ml as the optimal concentration (Fig. 1B).

The procedure was as follows: 50 μ l of the patient's sera was incubated with 100 μ l of sepharose at 60 mg/ml. After washing, 50 μ l of radiolabeled anti-IgE antibody (kindly provided by ALK-Abello, Madrid, Spain) was added and incubated overnight by shaking. After washing, the radioactivity was measured in a Cobra II auto-gamma counter (Packard BioScience Co, Frankfurt, Germany). We calculated the percentage of Sepharose-RIA as the ratio to the percentage of the maximum (cpm in 50 μ l of radiolabeled anti-IgE antibody) in each patient. The results were reported as the ratio of the percentage of Sepharose-RIA bound in the patients to the average percentage bound in the control subjects. We considered results to be positive when this ratio was higher than 2.

Specificity was confirmed with an inhibition assay, as described in (8). Briefly, the serum (50 μ l) was diluted as prescribed, incubated for 1 h with 50 μ l of the drug at three

different concentrations (1, 100 and 1000 nmol/ml), and then sepharose bound to quinolones at 100 nmol/ml was added. The test was subsequently carried out as for the IgE determination described earlier. The results were expressed as percentage inhibition.

Statistical studies

Comparisons for quantitative variables without normal distributions were made by the Mann-Whitney tests and between qualitative variables by the chi-square test. Correlation between quantitative variables was performed by Spearman rank analysis. All reported *P*-values represented two-tailed tests, with values ≤ 0.05 considered statistically significant. The statistical analysis was performed using the SPSS program version 11.5 (SPSS Inc., Chicago, IL, USA).

Results

The final study included 38 patients with confirmed immediate allergic reactions to quinolones (Table 1). Twenty-four were women (63.2%) and the median age was 43.5 (IR: 31.7–55.7). The clinical symptoms were urticaria in 12 (31.6%), anaphylaxis in 16 (42.1%) and anaphylactic shock in 10 (26.3%). The median interval between the reaction and the

Table 1 Clinical characteristics, Sepharose-radioimmunoassay (RIA) and basophil activation test (BAT) results of the patients

Patients	Age	Gender	Reaction	Drug	IgE (kIU/l)	Time* (months)	Sepharose-RIA			BAT			%CD63		
							CIP	MOX	LEV	CIP	MOX	LEV	CIP	MOX	LEV
1	30	M	URT	CIP	43	5	0.96	0.92	1.31	21.6	3.4	1.9	65.59	10.54	5.89
2	18	F	URT	CIP	1850	1	3.27	0.99	0.52	4.45	0.85	1.08	24.26	4.64	5.89
3	43	F	URT	CIP	172	9	1.24	1.37	1.55	1.52	1.98	0.87	9.35	12.22	5.34
4	42	F	URT	CIP	494	6	1.02	0.77	1.2	0.99	0.51	0.21	6.1	3.15	1.32
5	28	F	URT	CIP	75	5	0.78	1.55	0.27	0.84	1.58	1.19	4.76	8.92	6.7
6	53	F	ANA	CIP	41	7	1.13	1.56	0.94	3.83	0.48	0.17	26.86	3.33	1.16
7	37	F	SHOCK	CIP	114	4	0.9	0.88	1.11	3.77	0.82	0.34	20.31	4.42	1.83
8	66	F	SHOCK	CIP	212	6	6.47	2.38	3.34	1.05	0.21	0.5	3.41	0.68	1.62
9	44	F	SHOCK	CIP	27	6	0.78	1.52	0.97	4.62	0	2.69	26.25	0	15.28
10	27	F	SHOCK	CIP	514	1	0.58	1.17	0.74	0.4	0.55	0.99	1.97	2.71	4.86
11	58	M	SHOCK	CIP	112	3	0.92	0.34	0.92	0.75	0.71	0.43	4.12	3.9	2.37
12	40	F	URT	MOX	58	2	1.22	1.21	1.81	2.35	0.88	2.12	10.99	4.11	9.92
13	78	F	URT	MOX	67	1	1.23	1	0.64	2.08	1.06	1.84	12.27	6.21	10.84
14	60	M	URT	MOX	13	1	1.13	1.33	1.41	2.89	0.88	0.54	18.31	5.6	3.54
15	26	F	URT	MOX	125	4	4.08	4.54	1.95	9.99	3.55	0.37	55.17	19.57	2.03
16	51	M	URT	MOX	87	12	2.25	0.9	1.3	0.34	2.66	1.2	2.42	18.99	9.99
17	59	F	URT	MOX	21	6	1.97	2.31	2.26	3.28	1.49	10	16.67	7.56	50
18	35	M	ANA	MOX	113	10	1.07	1.3	0	5.14	0.9	0.5	24.51	4.23	2.67
19	64	M	ANA	MOX	506	1	1.53	1.53	1.66	7.52	2.01	0.78	48.12	12.86	4.99
20	32	M	ANA	MOX	203	4	0.89	0.93	1.53	2.45	4.61	1.03	7.6	14.29	3.2
21	54	M	ANA	MOX	3	11	0.93	0.93	0.53	3.07	2.86	1.05	35.29	32.83	12.05
22	51	M	ANA	MOX	15	8	1.53	1.39	1.05	0.34	2.67	1.4	1.58	12.44	6.52
23	73	M	ANA	MOX	127	4	1.22	2.31	1.11	2.55	1.65	1.59	15.93	10.28	9.93
24	31	F	ANA	MOX	258	5	0.88	1.15	0.98	0.73	0.19	0.05	3.59	0.91	0.23
25	51	F	ANA	MOX	1030	3	1	1.62	1.32	0.77	0.19	0.2	5	1.21	1.3
26	18	M	ANA	MOX	21	2	0.73	0.72	0.99	4.05	4.63	3.87	23.57	26.93	22.52
27	36	F	ANA	MOX	34	2	2.07	2.22	2.75	3.15	2.69	2.67	21.76	18.59	18.39
28	30	M	ANA	MOX	18	10	1.88	1.69	2.06	1.21	8.67	1.33	5.34	38.4	5.88
29	58	F	ANA	MOX	34	3	1.43	1.15	1.03	1.57	1.03	0.03	8.15	5.34	0.15
30	73	F	ANA	MOX	39	6	1.4	0.24	0.57	1.14	0.17	0.95	8.02	1.19	6.68
31	30	F	SHOCK	MOX	43	3	2.58	3.23	1.97	4.59	1.66	0.9	19.69	0.38	3.86
32	50	F	SHOCK	MOX	18	3	1.37	1.02	1.12	2.3	1.05	1.96	11.50	5.29	9.83
33	51	F	SHOCK	MOX	4210	1	1.57	0.34	0.27	0.88	1.98	0.72	4.83	10.82	3.94
34	44	F	SHOCK	MOX	74	7	1.78	1.95	3.5	5.93	0.65	5.86	31.77	3.51	31.64
35	55	F	SHOCK	MOX	41	7	1.53	1.62	0.95	1.87	9.9	4.46	11.14	58.9	26.49
36	32	M	URT	LEV	320	1	2.39	1.87	2.61	8.35	1.09	2.24	47.83	6.25	12.82
37	35	M	ANA	LEV	8	10	1.17	1	0.93	3.57	2.07	1.32	5.99	3.47	2.21
38	42	F	ANA	LEV	201	4	2.48	2.85	3.17	2.29	4.18	5.58	12.5	22.81	30.43

M, male; F, female; URT, urticaria; ANA, anaphylaxis; SHOCK, anaphylactic shock; CIP, ciprofloxacin; MOX, moxifloxacin; LEV, levofloxacin.

*Time between adverse reaction and study. Text highlighted in gray indicates positive results.

study was 4 (IR:2–7) months. The median total IgE was 74.50 (IR:32.25–205.25). The drugs involved in the reaction were moxifloxacin in 24 patients (63.2%), ciprofloxacin in 11 (28.9%) and levofloxacin in 3 (7.9%). In patients with anaphylaxis and anaphylactic shock, 20 cases (52.6%) had taken quinolones alone and six together with other drugs (two ibuprofen, two omeprazol, one paracetamol, one metamizol). In these six cases, good tolerance to these drugs was assessed by DPT. In those cases with urticaria, the diagnosis was confirmed by a DPT.

Results of the BAT and Sepharose-RIA for each quinolone and for the individual cases are shown in Table 1. Sepharose-RIA was positive in 12 cases (31.57%), BAT in 27

(71.05%) and the combination of both tests showed positive results in 28 (73.68%). In Sepharose-RIA, eight cases (21%) were positive to ciprofloxacin, 7 (18.4%) to moxifloxacin and 7 (18.4%) to levofloxacin. In BAT, 23 cases (60.5%) were positive to ciprofloxacin, 12 (31.6%) to moxifloxacin and 8 (21%) to levofloxacin. Comparison of BAT and Sepharose-RIA results depending on the clinical symptoms showed no statistical differences. There was no correlation between the total IgE levels or time interval between the reaction and the study and the presence of a positive Sepharose-RIA or BAT.

Concerning the two drugs most frequently involved in the reaction, we found that in those cases where ciprofloxacin was the culprit drug BAT to ciprofloxacin was positive in

45% and Sepharose-RIA in 18.2%, with no increase in sensitivity when including results obtained with moxifloxacin, levofloxacin or both. When the culprit drug was moxifloxacin, BAT sensitivity to moxifloxacin was 41.7% and Sepharose-RIA 17%; however, when including results obtained in BAT with ciprofloxacin, sensitivity increased to 79.2% for BAT and to 29.2% for Sepharose-RIA, with no increase in sensitivity after adding levofloxacin. In controls, BAT was positive in 3 of 25 cases (12%) to at least one quinolone and none with Sepharose-RIA (0%).

The specificity of the Sepharose-RIA was analyzed by inhibition tests in two patients (8 and 31) (Fig. 2). The serum from patient eight showed the highest percentage of inhibition with ciprofloxacin, which was the culprit quinolone, followed by levofloxacin and moxifloxacin, with no inhibition detected with amoxicillin. In patient 31, in whom the culprit quinolone was moxifloxacin, the percentage inhibition was similar with moxifloxacin and ciprofloxacin, followed by levofloxacin, with no inhibition detected with amoxicillin.

In patients 9 and 20, we determined whether the BAT results observed were IgE mediated using the PI3K inhibitor wortmannin (Fig. 3). We found that this compound inhibited the BAT when stimulated with anti-IgE and the different

quinolones, but not when fMLP was used as the basophil stimulator. Moreover, when we repeated BAT and Sepharose-RIA in some positive cases some time later we found that in all these cases the results decreased, and even became negative in four cases (Fig. 4). This confirmed the IgE clearance in these patients.

Discussion

Quinolones are synthetic antibiotics with a good safety profile, although hypersensitivity reactions, mainly potentially severe immediate-type reactions, have been reported (2–10). In this manuscript, we present data on a group of 38 patients with immediate allergic reactions to quinolones, where an IgE mechanism was demonstrated. The drug most frequently involved in the reaction was moxifloxacin (63.2%), followed by ciprofloxacin (28.9%). These results agree with those obtained in a Spanish population (10), where of the total group of patients ($N = 17$) evaluated because of an immediate reaction to quinolones, seven were induced by moxifloxacin, five by ciprofloxacin, two by norfloxacin, two by ofloxacin and one by pefloxacin; and also with those obtained in a different population, where moxifloxacin was the quinolone

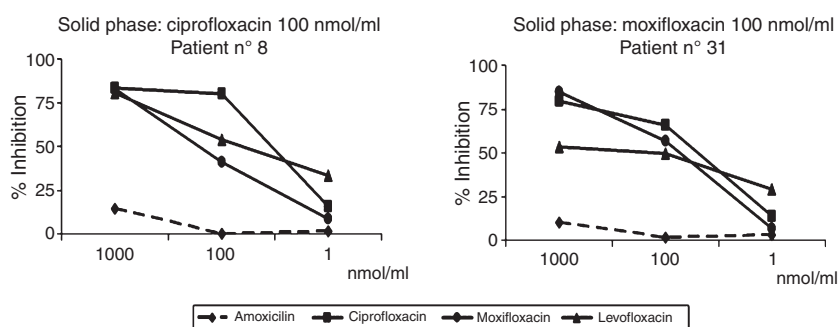


Figure 2 Sepharose-radioimmunoassay (RIA) inhibition assay of two patients using ciprofloxacin (left) and moxifloxacin (right) in the solid phase, and ciprofloxacin, moxifloxacin, levofloxacin and amoxicillin in

the fluid phase. Results are expressed as percentage of Sepharose-RIA inhibition.

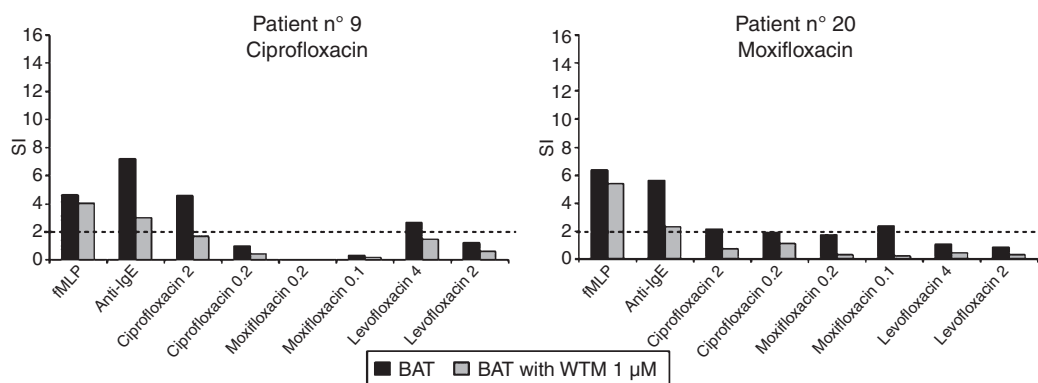


Figure 3 Wortmannin inhibitory effect in basophil activation in two positive cases, positive controls (fMLP and anti-IgE) and with cipro-

floxacin, moxifloxacin and levofloxacin at different concentrations measured in mg/ml.

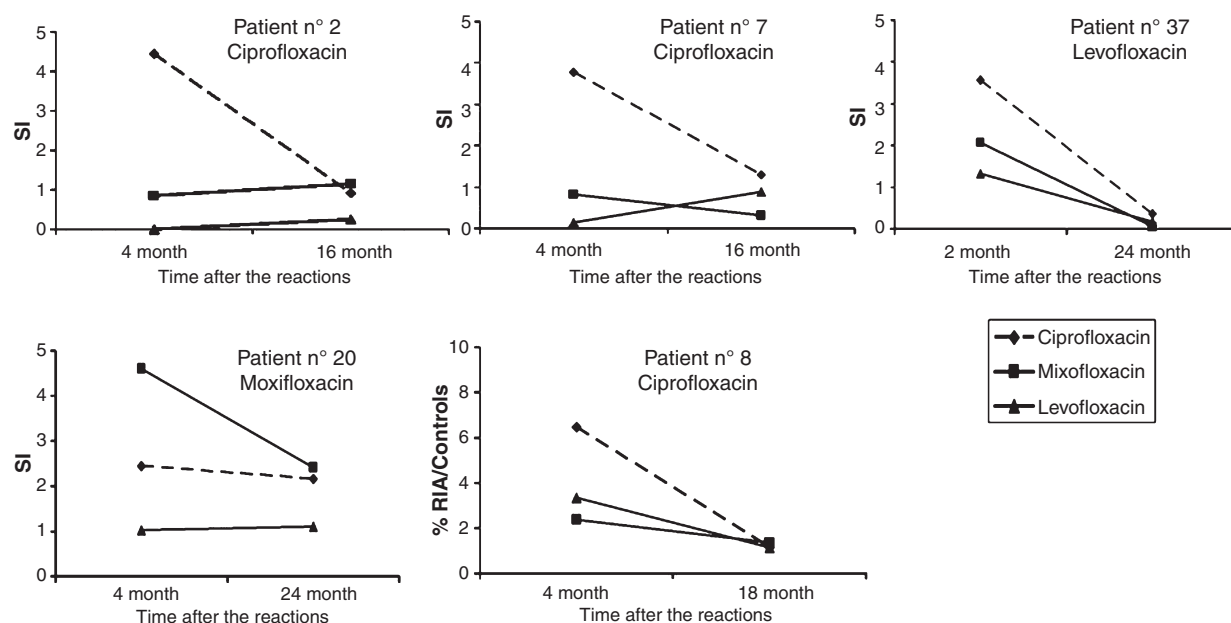


Figure 4 Evolution over time of BAT (four cases) and Sepharose-radioimmunoassay (RIA) (one case). In each case, two determinations were performed. The first time when the patient was diagnosed

and the second at least 1 year later. Results are expressed as stimulation index (SI) for BAT and as % Sepharose-RIA individual patient/mean Sepharose-RIA controls for Sepharose-RIA.

most frequently involved in anaphylaxis over a 10-year period (1993–2004) which coincided with the increased commercialization of moxifloxacin (4). Whether moxifloxacin immediate hypersensitivity reactions will increase in other populations is at present not known.

The diagnosis of immediate reactions to quinolones is a subject of debate. Although some authors consider skin prick and intradermal tests as good diagnostic methods (9, 10), many others have detected false positive results in controls (6, 10–12), attributed to nonspecific histamine release by quinolones because of mast cell activation (10, 12). This, together with the lack of a specific *in vitro* test for the detection of specific IgE antibodies to quinolones, has resulted in DPT being used as an alternative for the diagnosis of immediate allergic reactions to quinolones. However, DPT is not free of risk, especially in severe reactions, which are not infrequent, as occurred in our study where two-thirds of the cases had had anaphylaxis or anaphylactic shock. In our study, DPT was only performed in those cases with urticarial reactions to confirm the diagnosis and in more severe cases just to rule out the involvement of concomitant drugs in the reaction, such as non steroidal anti-inflammatory drugs (NSAIDs).

The difficulty in diagnosing immediate hypersensitivity reactions to quinolones using skin testing, as has been described previously, reinforces the need for *in vitro* approaches. Only one study has analyzed the presence of specific IgE *in vitro* using a Sepharose-RIA to quinolones, diagnosing 54.5% of the patients with immediate reactions to quinolones (8). These data contrast with our results, where we only detected 28.9% of the positive cases. In both studies, the interval between the acute reaction and the study was short and both used a similar

clinical diagnostic approach. The differences may therefore be attributed to the fact that the drug involved in the reaction and the type of reaction were different; in fact, in our study two-thirds of the cases concerned severe reactions, the drugs most frequently involved being moxifloxacin in 63.2% and ciprofloxacin in 28.9%. In the study by Manfredi et al. (8), urticaria was the most frequent reaction (85%), with just 13% of cases having anaphylactic shock, and the drugs most frequently involved were ciprofloxacin (23%) and cinoxacin (23%). Additionally, total IgE may affect RIA-sepharose sensitivity; in fact, in the study by Manfredi et al. (8), specific IgE to quinolones disappeared more slowly in those with higher total IgE. To avoid total IgE interference in Sepharose-RIA values, we analyzed the specific IgE antibodies to quinolones in the control group both with high total IgE and low total IgE levels. In fact, in the present study no positive results were detected in controls, and no correlation was seen between total IgE and Sepharose-RIA at the optimal quinolone concentration.

More recently, two studies have been published using BAT for the diagnosis of immediate allergic reactions to quinolones, with different results (13, 14). While in one study, BAT appeared to be useful for diagnosis (13), the other found no positive result (14). However, in both studies the sensitivity of BAT could not be calculated because of the low number of patients (five and four cases, respectively) included. Moreover, no comparisons were made with other *in vitro* methods, and no specificity studies including tolerant controls were carried out (13, 14). In the present study, BAT was positive in an important percentage of cases (71.05%), more than double that of the sensitivity with the Sepharose-RIA, with

no improvement in sensitivity when both methods were used. Moreover, in 69% of the cases with severe reactions ($N = 26$ cases) BAT was positive, which is important as it enables DPT to be avoided for diagnosis.

Because basophils can be activated by nonspecific IgE-mediated mechanisms, it is important to verify that the results are produced by specific IgE antibodies. For this purpose, we studied the effect of wortmannin, which is a potent and specific inhibitor of PI3K and has been used to demonstrate the role of this enzyme in diverse signal transduction processes, for example in regulating histamine release by basophils and mast cells by the PI3K. This enzyme is activated through the cross-linking of IgE on high-affinity IgE receptors (FcεRI) and induces the phosphorylation and activation of phospholipase, an increase in intracellular free calcium and histamine release (21, 22).

We observed that this molecule induced BAT inhibition when stimulated with anti-IgE and with haptens but not when fMLP was used as the basophil activator. This has also been shown with other drugs such as clavulanic acid (23). Furthermore, the BAT and Sepharose-RIA decreased, even becoming negative, indicating a specific IgE response to haptens (23–26).

The sensitivity of the BAT and Sepharose-RIA differed depending on the culprit drug. Those cases where ciprofloxacin was the culprit drug showed no improvement in sensitivity after adding the results obtained with moxifloxacin or levofloxacin. However, when moxifloxacin was the culprit drug, the sensitivity of the BAT increased from 41.7% to 79.2% and Sepharose-RIA from 17% to 29.2% after adding other quinolone results. This phenomenon has been extensively studied with betalactams, where there are cases that develop the reaction with amoxicillin but their IgE antibodies only recognize benzylpenicillin (27, 28). Using BAT, it has been shown that from 11.5% to 15.55% of cases that developed an immediate reaction to amoxicillin were only positive to benzylpenicillin (17, 18). Similar results have been obtained with RAST, where it has been seen that patients develop a reaction to amoxicillin or a cephalosporin and have IgE antibodies that recognize only benzylpenicillin (27). These results indicate that IgE antibodies to BL seem to be related to previous exposure, even if no previous reaction occurred. This may be related with the ability of the immune system to mount an anamnestic response (27). In subsequent responses, specificities may be more related to previous expo-

sure than to the inducing agent. The fact that patients developing a reaction to moxifloxacin may only recognize ciprofloxacin may indicate that as the latter was introduced on to the market previously, patients were originally sensitized to this drug. This is the reason why a patient developing a reaction to moxifloxacin is considered positive although he only recognized ciprofloxacin, as has been considered in different manuscripts on BL. Other possible explanations, such as cross-reactivity or co-existing sensitivity, are less plausible.

In the present study, 63.6% of the patients were positive with more than one quinolone in Sepharose-RIA and 48.2% in BAT. Moreover, we performed a Sepharose-RIA inhibition assay, detecting different percentages of inhibition with different quinolones, highest with the quinolone involved in the reaction and negative with a nonrelated antibiotic, such as betalactams. These data confirm previous reports indicating not only the specificity of the IgE response but also the existence of wide cross-reactivity between quinolones (6, 8, 10, 13, 28, 29).

In conclusion, immediate hypersensitivity reactions to quinolones do exist, with moxifloxacin being the drug most frequently involved in the reaction in our study population. The BAT is a useful method for diagnosing patients, especially in those patients with severe reactions where drug provocation testing may increase the risk.

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Conflict of interest

None of the authors has any conflict of interest, nor have they received any money for the present study. Research is part of their daily activities. All the authors had full access to all the data (including statistical reports) and can take responsibility for the integrity of the data and the accuracy of the data analysis. The study was funded by FIS-Thematic Networks and Co-operative Research Centres: RIRAAF (RD07/0064).

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