

Fluoroquinolone Photodegradation Influences Specific Basophil Activation

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Key Words

Allergy • Basophil activation • Immunoglobulin E • Quinolones • Photodegradation • Hapten-protein conjugates

Abstract

Fluoroquinolones (FQs) are photoreactive drugs, but it is not known whether laboratory light exposure can influence the induction of photoproducts and modify in vitro test results. The basophil activation test (BAT) has proven to be useful for evaluating immunoglobulin E (IgE)-mediated hypersensitivity to FQs, with a higher percentage of positive responders with ciprofloxacin (CIP) than with moxifloxacin (MOX). We studied the effect of laboratory light on CIP and MOX degradation, and drug-protein conjugate formation, and its influence on the BAT for evaluating IgE-mediated hypersensitivity to FQs. The results showed an important decrease in fluorescence emission intensity under light compared to dark conditions for MOX, and that BAT positivity was lower in light (17.9%) than in dark (35.7%) conditions. No changes were found for CIP in either fluorescence emission intensity or BAT results (46.4% in both conditions). We can conclude that light exposure is a critical factor in BAT results when photolabile drugs like MOX are used. Therefore, light is important when interpreting in vitro results.

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Introduction

Quinolones have been used for more than 30 years to treat a wide range of infections. UV radiation induces their photodegradation, which is modulated by the nature and position of the substituents attached to the quinolone skeleton [1, 2]. For example, the presence of a halogen, as in fluoroquinolones (FQs), seems to be associated with a higher phototoxic potential [3, 4]. Photodecomposition may involve a variety of photochemical processes, such as generation of singlet oxygen, production of superoxide, defluorination, decarboxylation at C-3, or oxidation of the amino group at C-7 [1, 2, 4].

Generally FQs are well tolerated [5], although the last decade has witnessed an increasing number of immediate hypersensitivity reactions (IHR) induced by FQs, with urticaria and anaphylaxis being the most frequently reported reactions [6–8]. These observations, especially the occurrence of more severe reactions, have been associated with the introduction of moxifloxacin (MOX) for therapeutic use [6]. In fact, in a group of patients diagnosed with IHR to FQs, MOX was involved in more than 60% of the cases with more severe reactions, followed by ciprofloxacin (CIP) in 30% and, to a much lower extent, levofloxacin [8].

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Evidence supporting an immunoglobulin E (IgE) mechanism for IHR has been provided by the detection of specific antibodies, by both immunoassay and basophil activation tests (BAT), with different patterns of cross-reactivity among FQs [7, 8]. Despite these findings, the true nature of the haptenic substructure (from the parent drug or its metabolites) recognized by the immune system remains unknown. The BAT is an adequate model for studying IgE-mediated reactions to FQs because, in addition to sensitized basophils, it enables study of the hapten, both free and protein bound, as well as its metabolites.

Previous evidence from well-validated CIP and MOX IHR cases suggests that basophil activation occurs with CIP more often than with MOX, even in those cases where MOX was the culprit drug [8]. Because each FQ exhibits chemical differences, our hypothesis was that they may behave differently upon light exposure, which may influence the formation of drug-protein conjugates and therefore interfere with the basophil activation. To test this hypothesis, we investigated how light exposure can affect the BAT results in patients with IgE-mediated hypersensitivity reactions and controls with good tolerance to these FQs.

Material and Methods

The stability of the FQs when exposed to laboratory light was checked by spectrophotometric and fluorometric measurements in an aqueous solution and in supernatants obtained from the BAT. These supernatants were divided into high molecular weight fractions ($>3,000$ Da), containing the drug bound to the serum proteins, and low molecular weight fractions ($<3,000$ Da), with the free drug or its metabolites, before analysis.

The BAT was done as described [8] under light and dark conditions with whole blood from patients with confirmed immediate hypersensitivity to CIP ($n = 15$) or MOX ($n = 13$) and quinolone-tolerant controls ($n = 20$). Results were considered positive when the stimulation index (SI), calculated as the ratio between the percentage of degranulated basophils with the haptens and the spontaneous basophil activation, was greater than 3. Detailed information about the photochemical and biological studies is available in the online supplementary material (for all online suppl. material, see www.karger.com/doi/10.1159/000343023).

Results

Photostability of CIP and MOX

Absorption spectra of CIP and MOX showed a wide wavelength band reaching up to 400 nm (online suppl. fig. S1A and online suppl. material). Emission studies

were performed by excitation at 320 and 337 nm for CIP and MOX, respectively, displaying different emission bands centered at 420 nm for CIP and 460 nm for MOX. Neither CIP nor MOX exhibited significant spectroscopic changes under light or dark conditions in aqueous solution, indicating a low photodegradation (online suppl. fig. S1B).

We then analyzed the effect of laboratory light on FQ degradation and on their capability to form drug-protein conjugates in whole blood, the medium used in BAT. The emission data of the low and high molecular weight fractions showed few, if any, differences in fluorescence intensity for CIP under light or dark conditions, either in free or protein fractions (fig. 1). However, remarkable differences were observed for MOX in both fractions, with an important decrease in fluorescence emission intensity upon light exposure, indicating drug photodegradation.

BAT Results

Twenty-eight patients with confirmed IHR to CIP and MOX and 20 controls with confirmed good tolerance to FQs were evaluated (table 1 and online suppl. material). Figure 2 shows the dose-response curve with four different concentrations of CIP and MOX, in light and dark conditions, in 16 allergic patients and 15 controls. The optimal concentrations were found to be 0.2 and 2 mg/ml for both drugs, and these concentrations were used throughout the study.

Table 1 shows the results of the BAT for CIP and MOX in light and dark conditions for the individual cases. For CIP, BAT was positive in 13 cases (46.4%) under light conditions and in 13 cases (46.4%) under dark conditions. For MOX, BAT was positive in 5 cases (17.9%) under light conditions and in 10 cases (35.7%) under dark conditions. Results were positive to either of the two FQs in 13 cases (46.4%) under light conditions and in 16 cases (57.1%) under dark conditions. Online supplementary figure S2 shows the dot plot in light and dark conditions of two representative cases.

Analysis of the results depending on the FQ involved in the reaction showed that, in those cases where CIP was the culprit drug ($n = 15$), BAT was positive to CIP in 5 cases (33.33%) in light and 6 cases (40%) in dark conditions, to MOX in 6 cases (40%) in light and 4 cases (26.66%) in dark conditions, and to either of the two quinolones in 5 cases (33.33%) in light and 8 cases (53.33%) in dark conditions.

In those cases where MOX was the culprit drug ($n = 13$), BAT was positive to CIP in 8 cases (61.53%) in light and 7 cases (53.84%) in dark conditions, to MOX in 2 cases (15.38%) in light and 6 cases (46.15%) in dark condi-

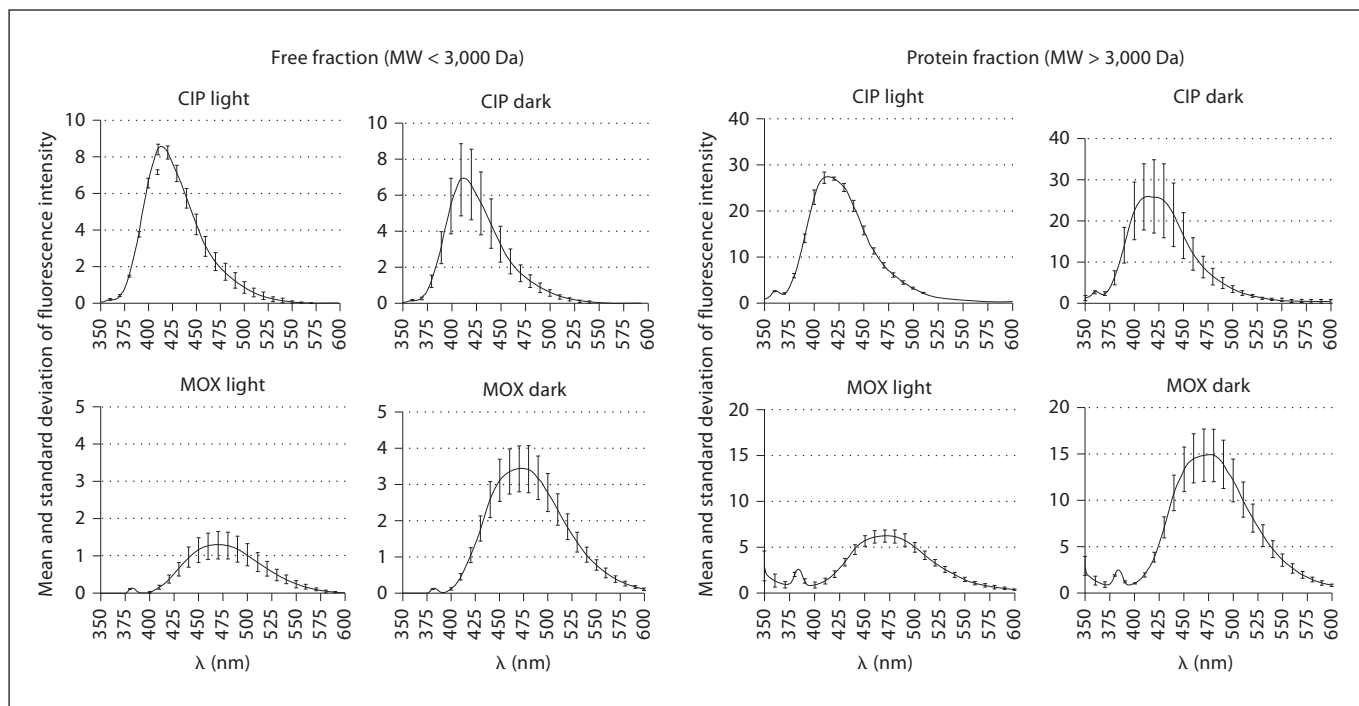


Fig. 1. Mean and standard deviation of emission fluorescence spectra of CIP and MOX in light and dark conditions, obtained from two different fractions, greater and lower than 3,000 Da.

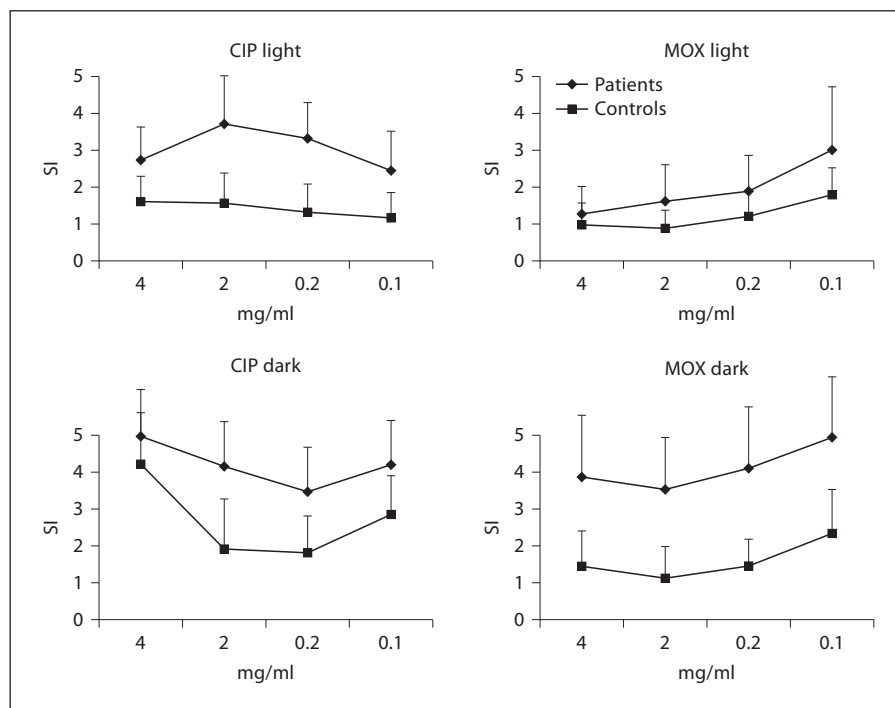


Fig. 2. BAT dose-response curves for CIP and MOX in 16 patients, 8 with a reaction to MOX and 8 with a reaction to CIP, and 15 controls in light and dark conditions. The results are expressed as SI.

Table 1. Clinical characteristics and BAT results of the patients

Pa- tient No.	Age years	Reaction	Time ^a months	Drug	%CD63 CIP		%CD63 MOX		SI CIP		SI MOX	
					light	dark	light	dark	light	dark	light	dark
1	60	Urticaria	2	MOX	47.27	53.5	15.57	29.79	8.16	9.24	2.69	5.15
2	74	Anaphylactic shock	12	MOX	5.72	41.9	13.96	3.9	0.73	5.33	1.78	0.50
3	67	Urticaria	3	MOX	35.39	5.95	2.91	3.83	8.87	1.49	0.73	0.96
4	58	Anaphylaxis	12	MOX	19.54	1.95	4.07	18.82	3.32	0.33	0.93	3.20
5	67	Anaphylactic shock	2	MOX	40.47	42.86	23.96	18.77	6.30	6.68	3.73	2.92
6	24	Anaphylaxis	3	MOX	26.27	59.55	12.94	99.98	4.99	8.74	2.42	18.72
7	31	Anaphylaxis	12	MOX	23.3	40.94	6.11	48.21	4.13	6.27	1.08	8.55
8	44	Anaphylactic shock	7	MOX	18.75	3.81	4.39	9.85	2.83	0.58	0.71	1.49
9	65	Anaphylaxis	3	MOX	7.83	12.4	10.58	10.05	1.87	2.97	2.53	2.41
10	59	Urticaria	14	MOX	24.12	20.59	5.96	21.55	4.04	3.45	1	3.61
11	18	Anaphylaxis	1	MOX	5.05	0	3.63	0.56	0.71	0	0.51	0.08
12	45	Anaphylaxis	1	MOX	1.41	0.70	0.75	1.27	0.30	0.15	0.16	0.27
13	63	Anaphylaxis	4	MOX	31.27	23.42	22.92	15.62	5.50	4.12	4.03	3.08
14	41	Anaphylaxis	3	CIP	25.45	11.61	21.23	16.17	2.99	1.37	2.50	1.90
15	39	Anaphylaxis	12	CIP	30.26	51.19	10.95	9.86	5.31	8.98	1.92	1.73
16	16	Anaphylaxis	2	CIP	1.56	23.81	0.79	3.18	0.32	4.94	0.16	0.66
17	58	Anaphylaxis	10	CIP	3.19	15.24	4.97	24.64	0.59	2.83	0.92	4.58
18	16	Anaphylaxis	10	CIP	9.72	39.82	9.68	75.51	1.72	7.06	1.72	13.39
19	39	Anaphylaxis	3	CIP	23.53	3.23	33.33	24.29	3.95	0.54	5.59	4.08
20	53	Anaphylaxis	1	CIP	17.06	2.67	18.6	1.86	3.12	0.49	3.40	0.34
21	23	Anaphylaxis	16	CIP	34.88	19.31	19.43	20.43	6.82	3.78	3.80	4
22	37	Anaphylaxis	10	CIP	7.74	4.01	10.9	4.63	1.37	0.71	1.93	0.82
23	41	Urticaria	1	CIP	2.98	4.12	3.5	8.15	0.63	0.87	0.74	1.72
24	35	Urticaria	12	CIP	12.59	31.92	7.51	6.15	2.23	5.65	1.33	1.09
25	67	Anaphylaxis	3	CIP	11.2	11.19	5.99	6.7	2.33	2.33	1.25	1.39
26	22	Anaphylaxis	5	CIP	4.3	8.87	8.37	9.94	0.83	1.71	1.61	1.92
27	47	Urticaria	12	CIP	6.5	7.75	6.48	3.74	1.22	1.45	1.21	1.34
28	57	Urticaria	3	CIP	18.1	18.46	0.93	1.69	3.20	3.26	0.16	0.37

Basophil activation is considered positive when SI is greater than 3. ^a Time between the adverse reaction and study.

tions, and to either of the two FQs in 8 cases (61.53%) in both light and dark conditions.

In controls (n = 20), under light conditions, BAT was positive in 2 cases to CIP, in 1 case to MOX, and in 2 cases to at least one FQ; in dark conditions, BAT was positive in 1 case to CIP, in 2 cases to MOX, and in 2 cases to at least one FQ. As a result, the specificity was 90% in both light and dark conditions.

Discussion

The presence of IgE antibodies to FQs has been demonstrated by immunoassays, including inhibition studies, although they do not enable us to determine the hap-

ten determinant involved [7, 8]. Recently, BAT has proven to be a useful tool for evaluating IgE responses to these drugs, though the results seem to depend on the FQ used in the test and are lower with MOX [8]. This, together with the fact that in recent years there has been an increase in the number of MOX reactions (in most cases severe), [6, 8] makes it important to analyze in depth the factors influencing these different behaviors. Based on the photolability of FQs [1–3, 9] our hypothesis was that the differences found in the BAT assay between the FQs may be explained by changes induced under light exposure during the in vitro test procedure, which influences FQ degradation differently, producing a lower amount of drug-protein conjugates.

The results obtained from fluorescence emission studies suggest that during BAT both FQs are able to bind to blood proteins, although free drug also remains. The data show an important photodegradation under laboratory light conditions, especially for MOX; as a consequence, lower drug-protein conjugates are also obtained. These results could be explained in terms of photostability since both FQs, although they present the same basic structure, show a different photochemical behavior due to different substituents [1, 2]. Even though the presence of a fluorine atom in C-6 makes both drugs somewhat photoreactive, the degree to which the molecules are photolabile is modulated by substituents and is directly related to the electronegativity of the substituent at C-8 [3, 4]. When we analyzed the photostability in aqueous solution we observed a similar behavior for both FQs. However, important differences, with a high MOX degradation, were found when the same experiments were done in whole blood, mimicking the BAT conditions. This may be explained by the fact that the photochemical behavior also depends on the characteristics of the medium, particularly in biological environments [1, 2]. Thus, in this study the complexity of the blood samples showed the unexpected facet of the reactivity of these FQs, which makes it difficult to formulate a hypothesis about the mechanisms involved.

These data explain why BAT positivity under light exposure was lower with MOX (17.9%) than with CIP (46.4%), with no patients being positive solely to MOX, as previously reported [8]. However, when the BAT results were analyzed under dark conditions, there was an increase in the number of positive cases to at least one FQ, from 46.4 to 57.1%.

The results obtained in BAT with the lower response observed with MOX in light (17.9%) compared to dark (35.7%) conditions and no changes in positivity for CIP (46.4%) correlate with the different photobehavior observed in these FQs, with degradation after light stimulation found only in MOX. Thus, in order to improve the sensitivity of BAT with MOX, this assay should be carried out under dark conditions to avoid drug photodegradation and possible misleading results.

Analysis of the results depending on the culprit drug showed that in patients in whom MOX was responsible, in light conditions only 15.38% were BAT positive to this drug while 61.53% were positive to CIP. Thus, although the culprit drug was MOX, most cases were positive to CIP, as in the study by Aranda et al. [8]. This phenomenon was not detected when CIP was the culprit drug. The reason for this was that all of the cases that were positive

to MOX were also positive to CIP, indicating that although the reaction was induced by MOX, IgE recognition was in part directed to CIP. Similar results have been published for patients with IHR to amoxicillin or amoxicillin-clavulanic acid where IgE mainly recognized benzylpenicillin [10, 11]. This seems to indicate that IgE antibodies are related to the drug of first exposure (benzylpenicillin and CIP), even if no previous reaction occurred, thus reflecting an anamnestic immune response [10, 11]. The occurrence of this phenomenon is expected to decrease over time as MOX consumption increases compared to CIP, as demonstrated with betalactams where benzylpenicillin is no longer the structure most often recognized [11, 12].

Finally, the question remains of whether the lower sensitivity for MOX found in BAT could also affect other *in vitro* tests, such as the radioimmunoassay. This may be the case since a lower sensitivity was found with sepharose-RIA to MOX (18%) compared to that achieved with CIP (21%) in the study by Aranda et al. [8], although further research is needed to analyze whether this phenomenon may influence other *in vivo* or *in vitro* tests.

Summarizing, the data reported here suggest that MOX is sensitive to ambient laboratory light present during the performance of an *in vitro* assay such as BAT, producing higher drug photodegradation and, as a consequence, lower amounts of drug-protein conjugates. This shows that light exposure is a critical factor in the results of the BAT when photolabile drugs are used and it is important to bear this in mind when interpreting *in vitro* results.

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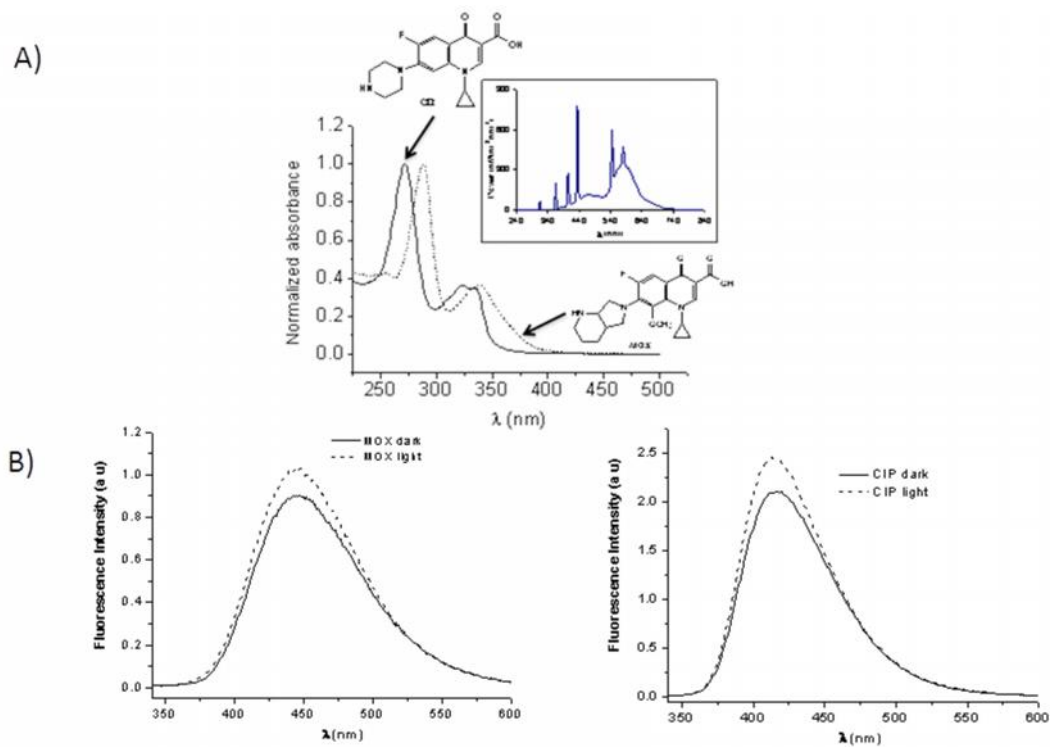


Figure S1. (A) UV-vis absorption spectra of ciprofloxacin (—) and moxifloxacin (-----). Insert: Emission spectrum of the fluorescent laboratory lamps. (B) Emission fluorescence spectra of CIP ($\lambda_{\text{exc}} = 320$ nm) and MOX ($\lambda_{\text{exc}} = 337$ nm) at 20 minutes of light exposure and under dark conditions. All measurements were performed in milliQ water.

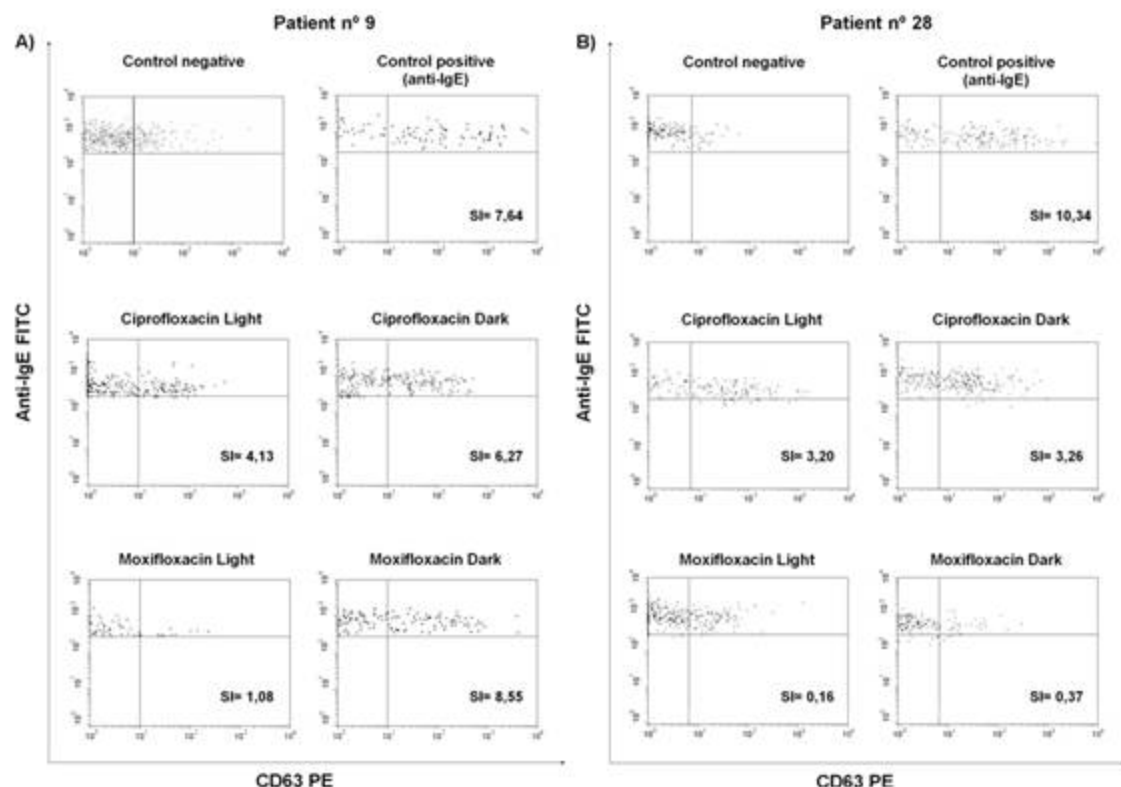


Figure S2. Dot plot graphs of basophil activation expressed as SI from two patients, case 9 induced by moxifloxacin and case 28 induced by ciprofloxacin, with ciprofloxacin and moxifloxacin at 2 mg/mL in light and dark conditions.

ONLINE SUPPLEMENTARY MATERIAL

Fluoroquinolone photodegradation influences the specific basophil activation.

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METHODS

1 Photostability studies

To check the stability of fluoroquinolones when exposed to laboratory light, spectrophotometric and fluorometric measurements were performed in aqueous solution and in whole blood.

CIP and MOX were dissolved in water to achieve a final concentration of 3×10^{-5} M, transferred to two vials, one submitted to irradiation for 20 minutes with fluorescent neon lamps, with the same conditions as in the working place, and the other kept in the dark. For absorption and fluorescence measurements, the samples were placed in 10x10 mm² quartz cells of 4 mL capacity with a septum cap. All experiments were carried out in aerated aqueous solution (milliQ) at room temperature. Absorption spectra were recorded on a HP 8452A Diode Array Spectrophotometer. Steady-state fluorescence experiments were performed using a Jasco FP-750 spectrofluorometer, equipped with a monochromator in the wavelength range of 350-600 nm.

In order to analyze the effect of the laboratory light on fluoroquinolone degradation and the ability of fluoroquinolones to form drug-protein conjugates in the particular characteristics of blood, we evaluated the supernatant obtained from the BAT, as described below, under both light and dark conditions, in five healthy controls. Two different fractions, with MW higher or lower than 3000 Da, were obtained from the supernatant by using Microcon YM-3 filters (Millipore, Carrigtwohill CO, Ireland). The <3000 Da fraction contains the free drug or its metabolites and the >3000 Da fraction contains the drug bound to the serum proteins.

Absorption and fluorescence measurements of both fractions of the two fluoroquinolones were performed under the different conditions described above, although in serum. Samples with molecular weight fractions >3000 Da were diluted with 2 mL of PBS buffer. Those samples <3000 Da were diluted with PBS and absorbances were adjusted at <0.06 for CIP and <0.02 for MOX at 320 nm and 337 nm excitation wavelengths, respectively.

2 Basophil activation test (light and dark conditions)

This was performed as described [1]. 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. After this, as a negative control, 100 µL of washing solution was added and as a positive control, 100 µL of anti-human IgE (BD Pharmingen, 0.5 mg/ml) was used. One hundred microliters of the two fluoroquinolones, MOX (Sigma-Aldrich, St. Louis, MO, USA) and CIP

(Fluka, Oakville, Ontario, Canada), at concentrations selected by dose-response curves, was added and incubated for 30 min at 37°C. The degranulation was stopped by incubating on ice, after which anti-IgE FITC and CD63 PE (Caltag Laboratories, Burlingame, CA, USA) were added to each tube and incubated for 20 min. Then, 2 mL of pre-warmed 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) by acquiring at least 1000 basophils per sample and interpreted using the CellQuest software.

The BAT was carried out in parallel under laboratory light and dark conditions. The latter was performed as described above, but with the tubes containing the drugs and samples wrapped with aluminum foil to keep out the light.

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 greater than 3.

3 Patients and controls

The patients included in the study for the BAT analysis were evaluated at the Allergy Services of Carlos Haya and Infanta Leonor Hospitals because of an immediate allergic reaction after administration of CIP or MOX. Different clinical categories were established: anaphylaxis and anaphylactic shock, defined according to the criteria of Sampson [2], and urticaria when manifestations were limited to the skin, in the absence of systemic symptoms, and they 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 [3]. Patients with anaphylaxis and anaphylactic shock were considered allergic by unequivocal clinical history, once other possible causes were ruled out. In those with urticaria, the diagnosis was confirmed by a drug provocation test (DPT). The controls consisted of 20 cases with confirmed good tolerance to fluoroquinolones (CIP and MOX).

Single-blind placebo-controlled DPT was carried out using CIP (Ciprofloxacin Normon, Madrid, Spain) and MOX (Actira, Bayer, Barcelona, Spain) under strict hospital surveillance, as described [4]. This consisted of the administration of the suspected fluoroquinolone at 30-min intervals at increasing doses till reaching the full therapeutic dose or appearance of symptoms of a drug reaction. For CIP the doses administered were 5, 50, 100, 150 and 200 mg (cumulative dose of 505 mg) and for MOX 5, 50, 100, 100 and 150 mg (cumulative dose of 405 mg).

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

4 Mean clinical characteristics of the group of patients (N=28).

The mean age of all the patients was 45.2 years (IR: 32-59.75), the mean interval between the reaction and the study was 6.4 months (IR: 2.25-12), and the clinical symptoms were anaphylaxis in 18 (64.3%), urticaria in 7 (25%), and anaphylactic shock in 3 (10.7%). Of the patients with anaphylaxis and anaphylactic shock, 17 had taken fluoroquinolone alone and 4 together with other drugs (two ibuprofen, one omeprazole, one paracetamol). In these four cases, good tolerance to these drugs was assessed by DPT. In the patients with urticaria, the diagnosis was confirmed by a DPT.

In the group of patients where MOX was the culprit drug (N=13) the mean age was 51.94 years (IR:37.5-66), the mean interval between the study and reaction was 5.84 months (IR:2-12), and the clinical symptoms were urticaria in 3 (23.1%), anaphylaxis in 7 (53.8%), and anaphylactic shock in 3 (23.1%). In the group of patients where CIP was the culprit drug (N=15) the mean age was 39.4 years (IR:23-53), the mean interval between the study and reaction was 6.87 months (IR:3-12), and the clinical symptoms were urticaria in 4 (26.7%) and anaphylaxis in 11 (73.3%).

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