Brief Communication
Allergy Asthma Immunol Res. 2012 November;4(6):367-369.
http://dx.doi.org/10.4168/aair.2012.4.6.367
pISSN 2092-7355 • eISSN 2092-7363

AAIR
Allergy, Asthma & Immunology Research

Immunologic Evaluation of Ofloxacin Hypersensitivity

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Quinolone hypersensitivity, most of which is immediate type, is rare but has increased in recent years. The pathogenic mechanisms underlying immediate reactions are not defined clearly. This study was aimed to observe the clinical characteristics of immediate hypersensitivity to ofloxacin and to investigate the pathogenic mechanism with detection of serum specific IgE to ofloxacin using an enzyme-linked immunoasorbent assay (ELISA). We recruited 5 patients with immediate hypersensitivity reactions to ofloxacin (group I), and as control groups, 5 subjects with ciprofloxacin hypersensitivity (group II) and 20 healthy subjects with no history of drug allergy. Serum specific-IgE to ofloxacin-human serum albumin (HSA) conjugate was detectable in four group I subjects (80%) and three group II subjects (60%). The ELISA inhibition test showed significant inhibition with both ofloxacin-HSA conjugate and free ofloxacin in a dose-dependent manner. As to ciprofloxacin, significant inhibition was noted upon addition of free ciprofloxacin in one subject, while minimal inhibition was noted in the other. We confirmed that an IgE-mediated response is a major pathogenic mechanism of ofloxacin hypersensitivity. Cross reactivity between ofloxacin and ciprofloxacin was noted with individual difference.

Key Words: Cross reactivity; enzyme-linked immunosorbent assay; IgE; immediate hypersensitivity; ofloxacin

INTRODUCTION

Quinolones are relatively well tolerated antibiotics, with even the most adverse effects being mild.[1] Hypersensitivity reactions to quinolones, most of which are immediate-type, seem to have increased in recent years. Ofloxacin, a commonly prescribed quinolone, is used in various forms, including as an injection, tablet, eye drops or ointment. Moreover, it has been prescribed as a second-line anti-tuberculosis agent in Asia, because of its wide antimicrobial spectrum and convenience of use. However, the mechanism underlying ofloxacin hypersensitivity remains unknown. In this study, to evaluate IgE-mediated mechanisms, we detected serum-specific IgE to ofloxacin-human serum albumin (HSA) conjugate using an enzyme-linked immunoasorbent assay (ELISA) in patients with ofloxacin hypersensitivity.

MATERIALS AND METHODS

We recruited 5 patients with immediate hypersensitivity reactions to ofloxacin (group I), and as control groups, 5 subjects with ciprofloxacin hypersensitivity (group II) and 20 non-atopic healthy subjects. A skin prick test (SPT) was performed with ofloxacin at a concentration of 10 mg/mL in distilled water. Normal saline and 1 mg/mL histamine were used as negative and positive controls, respectively. SPT was considered positive when a wheal larger than 3 mm with surrounding erythema was present 15 minutes after exposure.

Total serum IgE levels were measured using the ImmnoCAP System (Phadia, Uppsala, Sweden). Atopy was defined as a positive SPT for at least one common aeroallergens (Bencard Co., Bredford, UK). To detect serum specific IgE and IgG antibodies, ofloxacin-HSA conjugate was prepared in our laboratory and ELISA was performed as described previously.[2,3] In brief, microplates (Corning, New York, NY, USA) were coated with the ofloxacin-HSA conjugate (10 μg/mL) and were incubated with

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Received: March 19, 2012; Revised: April 23, 2012; Accepted: May 11, 2012
• There are no financial or other issues that might lead to conflict of interest.

sera of patients or controls. Then goat anti-human IgE antibody (Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD, USA), alkaline phosphate-conjugated rabbit anti-goat IgG antibody (ReserveAPTM; Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD, USA) and p-nitro-phenyl phosphate (PNPP; St. Louis, MO, USA) substrate were added in order. Alkaline phosphate-conjugated rabbit anti-human IgG antibody (Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD, USA) was used to detect serum specific IgG to ofloxacin-HSA conjugate. The positive cutoff value of the ELISA was determined as the mean plus three standard deviations (SD) of the healthy controls. To evaluate the specificity of IgE binding and possible cross reactivity with ciprofloxacin, an IgE ELISA inhibition test was performed with serial addition (1-100 μg/mL) of ofloxacin-HSA conjugate and the free forms of ofloxacin and ciprofloxacin using individual serum specimens as described previously.[3]

RESULTS

All of group I and four group II subjects were female and atopy was found in four group I subjects. The major clinical symptoms were urticaria/angioedema (three subjects) and anaphylaxis (two subjects) in group I subjects. None had a positive SPT to ofloxacin. Serum specific-IgE to ofloxacin-HSA conjugate was detectable in four group I subjects (80%) and three group II subjects (60%, Fig. 1), while none had serum specific-IgG1 antibody. Serum-specific IgG4 to ofloxacin-HSA conjugate was noted in one group I and two group II subjects who tested positive for serum-specific IgE antibody (Table). The ELISA inhibition test showed significant inhibition with both ofloxacin-HSA conjugate and free ofloxacin in a dose-dependent manner (Fig. 2). Regarding ciprofloxacin, significant inhibition was noted upon addition of free ciprofloxacin in patient 3 of group I (Fig. 2A), while minimal inhibition was noted in patient 1of group I (Fig. 2B).

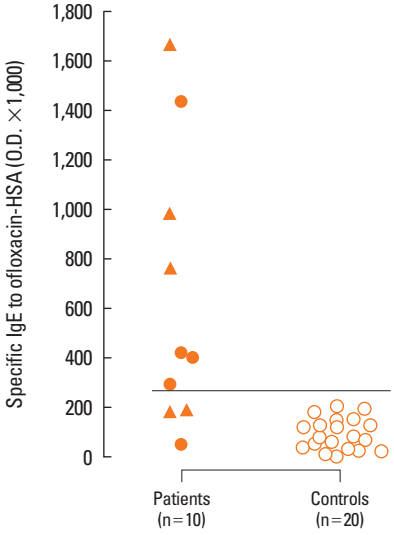

Fig. 1. Serum specific IgE levels to ofloxacin-human serum albumin (HSA) conjugate by ELISA in patients with ofloxacin (●) and ciprofloxacin (▲) hypersensitivity and healthy controls (○). Horizontal bar indicates the cut-off value for positive specific IgE bindings.

DISCUSSION

Immediate hypersensitivity reactions to quinolones are not

Table. Clinical characteristics and immunologic findings of the study subjects

Patient (no.)	Gender	Age (yr)	Type of reaction	Total IgE (KU/L)	Specific IgE to ofloxacin-HSA conjugate by ELISA		
					IgE	IgG1	IgG4
Group I*							
1	F	47	ANA	52	+	-	+
2	F	38	ANA	33	+	-	-
3	F	19	URT	759	+	-	-
4	F	33	ANGIO	319	+	-	-
5	F	61	URT/ANGIO	354	-	-	-
Group II†							
1	M	38	ANA	87	+	-	+
2	F	54	ANA	100	+	-	+
3	F	22	ANA	180	+	-	-
4	F	48	URT	288	-	-	-
5	F	45	URT	55	-	-	-

*Subjects with ofloxacin hypersensitivity; †Subjects with ciprofloxacin hypersensitivity.
F, female; M, male; OFL, ofloxacin; CIP, ciprofloxacin; URT, urticaria; ANGIO, angioedema; ANA, anaphylaxis; ELISA, enzyme-linked immunosorbent assay; +, positive; -, negative.

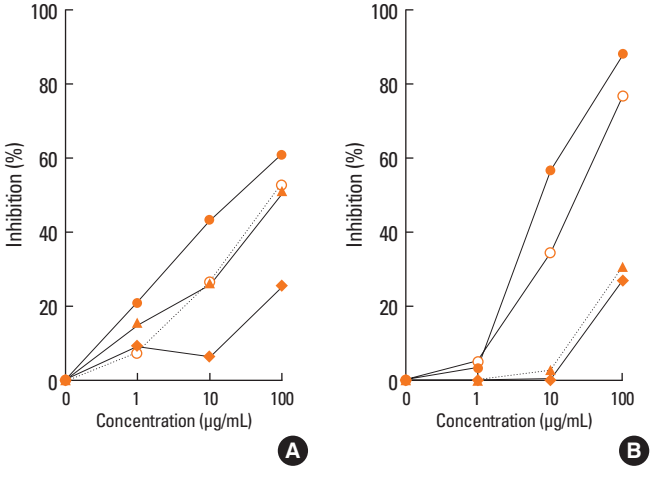

Fig. 2. The IgE ELISA inhibition test results with serial additions of ofloxacin-HSA conjugate (●), free forms of ofloxacin (○) and ciprofloxacin (▲), and human serum albumin alone (■). Significant inhibitions were noted upon addition of free ciprofloxacin in patient 3 of group I (A), while minimal inhibition was noted in patient 1 of group II (B).

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common in frequency from 0.4% to 2%.[4,5] A literature review revealed that ciprofloxacin was the most frequently implicated because of its high consumption, followed by ofloxacin and cinoxacin. The most frequent reactions were urticaria and anaphylaxis.[6,7] In this study, we report the clinical features of five patients with ofloxacin hypersensitivity compared to five with ciprofloxacin hypersensitivity by detection of serum-specific IgE antibodies.

As a method of diagnosis of quinolone hypersensitivity, skin test results have been contradictory and unreliable,[8] giving false positive responses in healthy controls by inducing direct histamine release.[6,9] In this study, all group I subjects exhibited negative SPT results to the maximum ofloxacin concentration. A few studies have reported the presence of serum-specific IgE to ciprofloxacin and moxifloxacin using radioimmunoassay,[6,10] however, the method has a risk of radiation exposure. The present study, to the best of our knowledge, is the first to demonstrate a high positive rate (80%) of serum-specific IgE to ofloxacin-HSA conjugate using ELISA, and to confirm IgE binding specificity by ELISA inhibition tests. Although the drug challenge test is the confirmative diagnostic method, it is not sufficiently risk-free to be performed in daily practice. The basophil activation test has been suggested as an alternative *in vitro* test of quinolone hypersensitivity, however, the sensitivity ranges from 0%[4] to 71%.[11] Based on these findings, measurement of serum-specific IgE to ofloxacin-HSA conjugate using ELISA may be a useful and reliable *in vitro* method for diagnosing ofloxacin hypersensitivity, obviating the need for challenge tests.

The basic structure of quinolones is a nitrogen-containing eight-member heterocyclic aromatic compound with a carboxylic group at position 3 and a ketone group at position 4. The addition of a fluorine substituent at position 6 and a piperazinyl moiety at position 7 resulted in ciprofloxacin and the addition of a methyl substituent on the piperazine ring led to ofloxacin. IgE antibodies interact mainly with the side chains at positions 2-6,[7] and frequent cross reactivity among structurally similar quinolones has been suggested.[4,12,13] In this study, three (60%) group II subjects had high serum-specific IgE to ofloxacin-HSA conjugate, and they showed significant inhibition with additions of both ofloxacin and ofloxacin-HSA conjugate (data not shown). Of the group I subjects having high serum-specific IgE to ofloxacin-HSA conjugate, significant inhibitions were noted with the free forms of ciprofloxacin and ofloxacin in patient 3, whereas only minimal inhibitions were noted with free ciprofloxacin in patient 1, indicating that ofloxacin has immunologic cross reactivity with ciprofloxacin, which differ between individuals. These data suggest the utility of ELISA and ELISA inhibition testing for evaluating cross reactions with structurally similar quinolones in patients with ofloxacin hypersensitivity.

Few studies have investigated the role of specific IgG in antibiotics allergies.[9] In this study, some group I and II subjects had high specific IgG4 levels, suggesting a parallel immune response with specific IgE, but without a pathologic role.

In conclusion, we suggest that an IgE-mediated response to the hapten part of ofloxacin is the major pathogenic mechanism underlying ofloxacin hypersensitivity. In addition, cross reactivity with ciprofloxacin was noted, although this differed between individual subjects.

ACKNOWLEDGMENTS

This study was supported by a grant from the Korea Science and Engineering Foundation (KOSEF) funded by the Korean government (MEST, 2009-0078646).

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