INTRODUCTION

Quinolones are relatively well tolerated antibiotics, with even the most adverse effects being mild. 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 the pathogenic mechanism with detection of serum specific IgE to ofloxacin using an enzyme-linked immunoabsorbent 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. 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

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. In brief, microplates (Corning, New York, NY, USA) were coated with the ofloxacin-HSA conjugate (10 μg/mL) and were incubated with
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 not detected 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 1 of group I (Fig. 2B).

DISCUSSION

Immediate hypersensitivity reactions to quinolones are not

| 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.
common in frequency from 0.4% to 2%. 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. 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, giving false positive responses in healthy controls by inducing direct histamine release. 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, 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% to 71%. 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 carbocyclic 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, and frequent cross reactivity among structurally similar quinolones has been suggested. 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. 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).

REFERENCES

1. Bertino J Jr, Fish D. The safety profile of the fluoroquinolones. Clin Ther 2000;22:798-817; discussion 797.
2. Suh YJ, Lee YM, Choi JH, Suh CH, Nahm DH, Park HS. Heterogeneity of IgE response to cefotetan pivoxil was noted in 2 patients with cefotetan-induced occupational asthma. J Allergy Clin Immunol 2003;112:209-10.
3. Kim JE, Kim SH, Jin HJ, Hwang EK, Kim JH, Ye YM, Park HS. IgE Sensitization to Cephalosporins in Health Care Workers. Allergy Asthma Immunol Res 2012;4:85-91.
4. Demoleas SE, Davies GE. Quinolones. Oral antibiotics of the future. J Am Podiatr Med Assoc 1988;78:522-5.
5. Dembry LM, Farrington JM, Andriele VT. Fluoroquinolone antibiotics: adverse effects and safety profiles. Infect Dis Clin Pract (Baltim Md) 1999;8:421-8.
6. Aranda A, Mayorga C, Ariza A, Doña I, Rosado A, Blanca-Lopez N, Andreu I, Torres MJ. In vitro evaluation of IgE-mediated hypersensitivity reactions to quinolones. Allergy 2011;66:247-54.
7. Manfredi M, Severino M, Testi S, Macchia D, Ermini G, Pichler WJ, Campi P. Detection of specific IgE to quinolones. J Allergy Clin Immunol 2004;113:155-60.
8. Reaño M, Vives R, Rodríguez J, Daroca P, Canto G, Fernández J. Ciprofloxacin-induced vasculitis. Allergy 1997;52:599-600.
9. Dávila I, Diez ML, Quirce S, Frij J, De La Hoz B, Lazaro M. Cross-reactivity between quinolones. Report of three cases. Allergy 1993;48:388-90.
10. Seitz CS, Bröcker EB, Trautmann A. Diagnostic testing in suspected fluoroquinolone hypersensitivity. Clin Exp Allergy 2008;39:1738-45.
11. Schmid DA, Campi P, Pichler WJ. Hypersensitivity reactions to quinolones. Curr Pharm Des 2006;12:3313-26.
12. González I, Lobera T, Blasco A, del Pozo MD. Immediate hypersensitivity to quinolones: moxifloxacin cross-reactivity. J Invest Allergol Clin Immunol 2005;15:146-9.
13. Lobera T, Audicana MT, Alarcón E, Longo N, Navarro B, Muñoz D. Allergy to quinolones: low cross-reactivity to levofloxacin. J Investig Allergol Clin Immunol 2010;20:607-11.