Acta Derm Venereol (Stockh) 1997; 77: 285–288

# Cutaneous Adverse Reaction to Ciprofloxacin: Demonstration of Specific Lymphocyte Proliferation and Cross-reactivity to Ofloxacin *In vitro*

ANDREA C. RÖNNAU[1], BERNHARDT SACHS[1], SHERKO VON SCHMIEDEBERG[1,2], NICOLAS HUNZELMANN[3], THOMAS RUZICKA[2], ERNST GLEICHMANN[1] and HANS-CHRISTIAN SCHUPPE[2]

*[1]Division of Immunology, Medical Institute of Environmental Hygiene at Heinrich-Heine-University, Departments of Dermatology, [2]Heinrich-Heine-University, Duesseldorf and [3]Albert-Magnus-University, Cologne, Germany*

**Ciprofloxacin (CPFX) is a widely used fluoroquinolone antibiotic, inducing cutaneous adverse drug reactions in about 1 to 2% of the treated patients. Conclusive diagnosis of drug allergy, however, still remains a major problem in daily clinical practice. Here, we present 2 patients with drug allergy to CPFX. In both cases the clinical suspicion for CPFX as the causative agent was confirmed in vitro by means of the lymphocyte transformation test, whereas epicutaneous patch tests remained negative. In vivo, a small percentage of the drug is biotransformed to the three major metabolites desethylene-, sulfo- and oxociprofloxacin. Though structurally closely related to their mother compound, these metabolites failed to induce in vitro lymphocyte proliferation in both patients. On the other hand, in vitro cross-reactivity to ofloxacin, another fluorinated quinolone, could be demonstrated, which to our knowledge has not previously been reported.** ***Key words: T-cell reactivity; drug metabolism; quinolones; lymphocyte transformation test.***

(Accepted November 29, 1996.)

Acta Derm Venereol (Stockh) 1997; 77: 285–288.

H.-C. Schuppe, Department of Dermatology, Heinrich-Heine-University, Moorenstrasse 5, 40225 Duesseldorf, Germany.

Ciprofloxacin (CPFX) is a fluorinated quinolone antibiotic (Fig. 1) with potent activity against a broad spectrum of bacterial pathogens. Quinolones impair bacterial DNA metabolism by inhibiting the enzyme DNA gyrase, which is present only in bacterial cells (1). Due to its excellent tissue penetration, it is used in a wide variety of clinical specialities and has been administered to over 100 million patients (2). The first world-wide clinical study on the efficacy and safety of CPFX was conducted by Schacht et al. (3) in 1988 and revealed an overall incidence of adverse reactions of 10.2%. Skin reactions – most commonly rashes and pruritus – affected 0.8 to 1.9% of the patients and approximately 12% of all severe adverse drug reactions involved the skin (3–7).

Cross-sensitivity between quinolones is a known, but rather sporadically cited phenomenon. With regard to CPFX, only 4 reports can be found in the literature (8–11). Studies using $^{14}$C-labelled CPFX showed that proportions of approximately 19% after oral and 12% after intravenous administration are excreted as metabolites in urine and faeces (12). These metabolites are structurally highly related to their mother compound (Fig. 1) and differ only with respect to their piperacinyl moiety. Four metabolites have been identified: desethylene- (M1), sulfo- (M2), oxo- (M3), and formyl-CPFX (M4) (12). In case of suspected drug hypersensitivity, in vitro test systems like the lymphocyte transformation test (LTT) can be a helpful tool in confirming sensitisation without subjecting the patient to the risk of re-challenge. Here, we present 2 patients with clinically suspected drug allergy to CPFX and describe the results of in vivo and in vitro allergy testing. In contrast to epicutaneous patch tests, specific lymphocyte reactivity to CPFX in vitro allowed the demonstration of sensitisation in both patients.

## MATERIALS AND METHODS

*Case 1*

A 77-year-old female was admitted to a district hospital for physical therapy after implantation of a total hip endoprothesis. The patient developed a urinary tract infection and was treated initially with piperacillin. Subsequently, due to persistent fever, her treatment was changed to oral CPFX 1 g/day. On the third day of CPFX treatment she developed discrete blepharoedema and a maculopapular eruption spreading from the trunk, across the arms and legs, which was accompanied by intense itching. There were no signs of systemic reactions. CPFX was withdrawn immediately and after a short course of high dose systemic prednisolone combined with topical glucocorticosteroids the skin lesions gradually declined. Concurrent medication consisted of a variety of drugs, all of which had been taken throughout the episode of adverse drug reaction and during recovery. It remained unclear whether the patient had previously taken CPFX. Her medical history included a bleeding duodenal ulcer, cerebral stroke with residual right-sided symptoms, and insulin-dependent diabetes mellitus type IIb with distal sensory polyneuropathy and bladder dysfunction. Physical examination proved the cardiovascular, respiratory and gastrointestinal systems to be normal. Red blood cell count showed a mild normochromic, normocytic anaemia. The differential blood count revealed marked eosinophilia (4,900 /μl) and slight monocytosis (1,200 /μl) with normal counts of neutrophils, basophils, and lymphocytes. C-reactive protein and blood sedimentation rate were elevated at 29.7 mg/l (range $<5$ mg/l) and 40 mm/h, respectively. Numerous bacteria with some leukocytes and erythrocytes were found in the urine sediment. Other laboratory findings, including coagulation factors, blood chemistry, liver enzymes and renal function parameters, were within normal limits.

Allergy testing was performed 1 month after recovery. The results were as follows:

1) Patch tests with CPFX and ofloxacin (both 10% in saline) were negative, as was a standard series of frequent European allergens.
2) Prick tests with CPFX and ofloxacin (both 10% in saline) were negative, as was a standard series of drugs and preservative substances.
3) Total serum IgE was below 35 U/ml; IgE screening for atopy (SX1, Pharmacia) was negative.

*Case 2*

A 51-year-old female was admitted to the neurology department for severe pain in her neck and shoulder region. Nuclear magnetic resonance imaging revealed spondylodiscitis in segment C6/7. The patient was started on ceftriaxone (2 g/day) intravenously with genta-

 ISSN 0001-5555

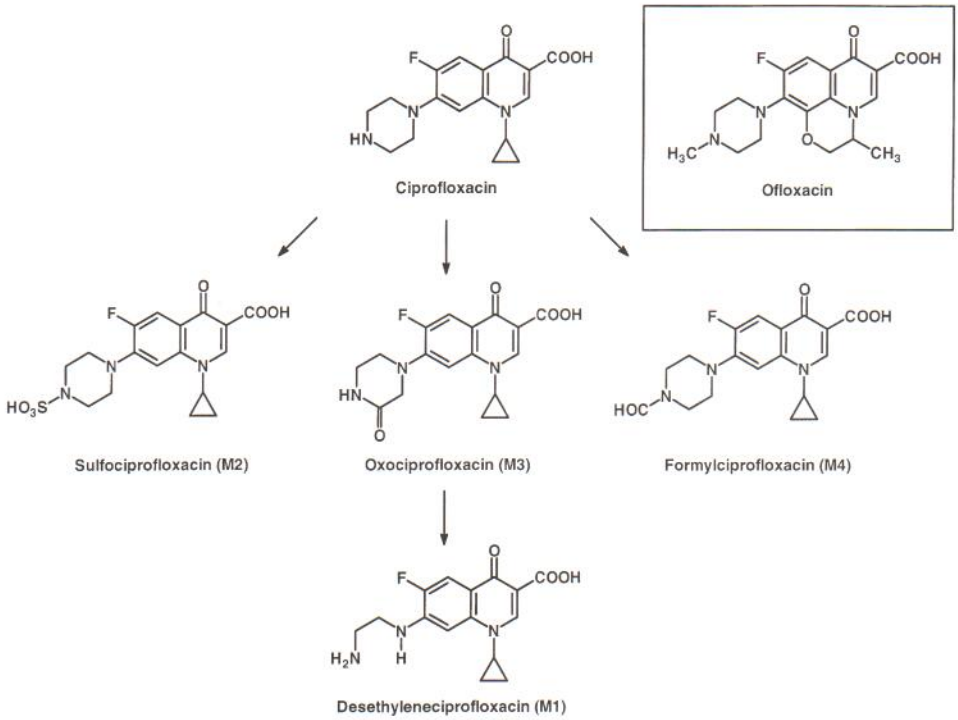


*Fig. 1.* Ciprofloxacin and its metabolites; ofloxacin.

mycin (240 mg/day) and oxacillin (12 g/day) added during the following day. After transfer to the orthopaedic department intravenous antibiotic therapy was switched to flucloxacillin (6 g/day) with gentamycin (160 mg/day) re-added on day 9. Seven days into this regimen, she developed angioedema and generalised urticaria. Weals and angioedema disappeared within 3 h after intravenous antihistamines. Due to persisting inflammatory signs antibiotic therapy was continued with CPFX. Thirty minutes after intravenous CPFX the patient experienced another episode of allergic drug reaction with identical immediate-type hypersensitivity symptoms. Upon re-examination the patient remembered that she had taken CPFX 1 year before, but without any adverse effects. Parallel medication consisted of ranitidine and diazepam, which were still well tolerated after recovery. Despite interruption of antibiotic therapy, sedimentation rate returned to normal and radiological follow-up showed no progression of the vertebral body erosion.

Allergy testing was performed 4 months after recovery. The results were as follows:

1) Epicutaneous tests with CPFX, gentamycin and flucloxacillin (all 10% in glycerine) were negative, as was a standard series of drug components.
2) Prick testing with benzylpenicilloyl-polylysine and the "minor determinant mixture" (benzylpenicillin, benzylpenicilloate) was negative, as was intracutaneous testing with benzylpenicilloyl-polylysine. Intracutaneous application of the "minor determinant mixture" resulted in a positive reaction of an 8-mm weal with a 14-mm flare compared to 12 and 30 mm for the histamine control.
3) Prick testing with CPFX was positive, with a 6-mm weal and 14-mm flare (histamine control: 7 and 30 mm, respectively).
4) Prick testing with gentamycin and flucloxacillin was negative.
5) Total serum IgE was 30 U/ml; specific serum IgE determined for penicilloyl G, ampicillin and amoxycillin was not detectable.

*Lymphocyte transformation test (LTT)*

Peripheral blood mononuclear cells (PBMC) were isolated from heparinised (40 U/ml) whole blood on Ficoll® gradient (1.077 g/ml) (Ficoll-Paque®, Pharmacia, Uppsala, Sweden) according to the manufacturer's instructions. Isolated PBMC were washed twice in phosphate-buffered saline (PBS) and suspended in RPMI 1640 medium (Life Technologies, Paisely, Scotland) containing 5% autologous plasma, L-glutamine (2 mM), pyruvate (1 mM), HEPES buffer (25 mM), non-essential amino acids, penicillin (10 U/ml), and streptomycin (10 µg/ml) (complete medium; all supplements from Life Technologies, Paisely, Scotland). $1 \times 10^5$ PBMC/200 µl were cultured for 7 days in 96-well round-bottom plates (Greiner, Essen, Germany) as sixfold cultures at 37°C and 7% $CO_2$ in humidified atmosphere. During the last 17 h, 25 µl $^3$H-thymidine with $1{,}85 \times 10^4$ Bq were added (ICN, Irvine, USA). Cells were harvested with a PHD$^{RM}$ cell harvester (Cambridge Technology, UK) on filters coated with a solid scintillator (Ready filter with Xtalscint$^{TM}$, Beckman, Fullerton, USA). Incorporated radioactivity was measured as counts per minute (cpm) in a scintillation counter (LS 600 IC, Beckman Instruments, Fullerton, USA). Lymphocyte proliferation was expressed as stimulation index (SI), which is the ratio of the mean values of cpm from cultures with and without test compound. Values exceeding 2 were considered as positive results and investigated for statistical significance by means of Welch's modified *t*-test, comparing drug-treated cultures with untreated control cultures ($*p<0.05$, $**p<0.01$).

Stock solutions of the drugs were always freshly prepared. CPFX and ofloxacin concentrations were directly diluted from commercial preparations (Ciprobay®, Bayer, Leverkusen, Germany; Tarivid®, Hoechst, Frankfurt, Germany). For some cultures, pure CPFX (Sigma, St. Louis, USA) dissolved in 0.9% saline, subsequently diluted with complete medium, was used. Due to poor solubility in aqueous medium, desethylene- (M1) and oxo-CPFX (M3) (kindly donated by Bayer, Leverkusen, Germany) had to be dissolved initially in 0.1 M acetic acid and 0.1 M sodium hydroxide, respectively. Stock solutions of 10 mg/ml were subsequently diluted to final concentrations in complete medium. Solvent contents in highest metabolite concentrations were then $1 \times 10^{-4}$ M. Sulfo-CPFX (M2) (kindly donated by Bayer, Leverkusen, Germany) was directly soluble in RPMI. Flucloxacillin was purchased as Staphylex® from SmithKline Beecham, Munich, Germany, and gentamycin as Refobacin® infusion solution from Merck, Darmstadt, Germany. Both were employed in eight concentrations ranging from 0.01 to 500 µg/ml. Toxicity controls

were performed as co-incubations of the drugs in various concentrations together with 5 μg/ml concanavalin A (ConA). PBMC of 3 healthy volunteers served as controls. Two of them had had no previous CPFX exposure; one had previously taken CPFX without any complications.

## RESULTS

### *Case 1*

In a first LTT, 1 week after recovery, PBMC of this patient proliferated in response to 1, 5, and 20 μg/ml CPFX with SIs of 4.0, 6.1, and 2.2, respectively. In a second LTT, 2 months later, the SI at 1 μg/ml CPFX dropped to 2.7 and no proliferation was seen in response to other concentrations (Fig. 2). Ofloxacin (Fig. 4, data shown for first LTT) as well as the metabolites desethylene-, sulfo- and oxo-CPFX (M1-M3) in concentrations of 0.1, 1, 5, and 10 μg/ml did not lead to specific lymphocyte stimulation in the two LTTs (data not shown).

### *Case 2*

The second patient was tested 19 weeks after her allergic reaction. Cultured with CPFX in concentrations ranging from 0.01 to 50 μg/ml, PBMC proliferated in a dose-dependent manner with positive SIs of 4.5 and 2.8 at concentrations of 1 and 5 μg/ml, respectively (Fig. 3). PBMC of this patient did not respond to the metabolites (M1-M3) of CPFX either (data not shown). Incubation with ofloxacin demonstrated a distinct proliferation, with a sixfold increase in $^{3}$H-thymidine incorporation at a concentration of 20 μg/ml (Fig. 4). With culture series of flucloxacillin and gentamycin positive SIs of 6.1 and 3.3, respectively, were achieved, both at a concentration of 10 μg/ml (data not shown).

### *Controls*

None of the 3 control subjects revealed unspecific proliferation to the drugs or metabolites (Figs. 2–4; data not shown for metabolites, flucloxacillin and gentamycin). Toxicity controls with CPFX led to heterogeneous results, in that mitogen-induced proliferative response was dose-dependently inhibited in concentrations above 5 μg/ml CPFX in one volunteer (65% inhibition compared with the ConA control for 10 and 20 μg/ml CPFX, 24% inhibition for 50 μg/ml CPFX), whereas no inhibition up to 50 μg/ml CPFX occurred in another subject. Similar discrepancies have been described in the literature (13). The metabolites (M1-M3) and ofloxacin had no inhibitory effect on the proliferative response of PBMC in mitogenesis assays at the highest concentrations used in the LTT (data not shown).

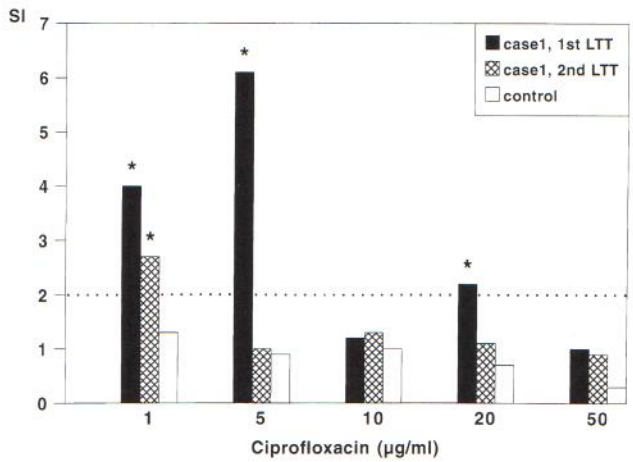


*Fig. 2.* LTT in CPFX-induced drug-allergy (case 1): PBMC of the patient and control were incubated for 7 days as sixfold cultures with 1 to 50 μg/ml CPFX. Lymphocyte proliferation is expressed as a ratio of the mean values of cpm from cultures with and without test compound (SI). *Asterisks* indicate a significant difference relative to the respective medium control (Welch's modified *t*-test; $*p<0.05$).

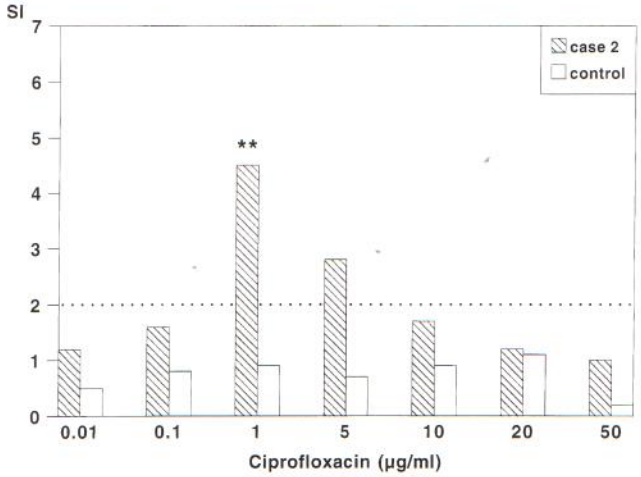


*Fig. 3.* LTT in CPFX-induced drug-allergy (case 2): PBMC of the patient and control were incubated for 7 days as sixfold cultures with 0.01 to 50 μg/ml CPFX. Lymphocyte proliferation is expressed as a ratio of the mean values of cpm from cultures with and without test compound (SI). *Asterisks* indicate a significant difference relative to medium control (Welch's modified *t*-test; $**p<0.01$).

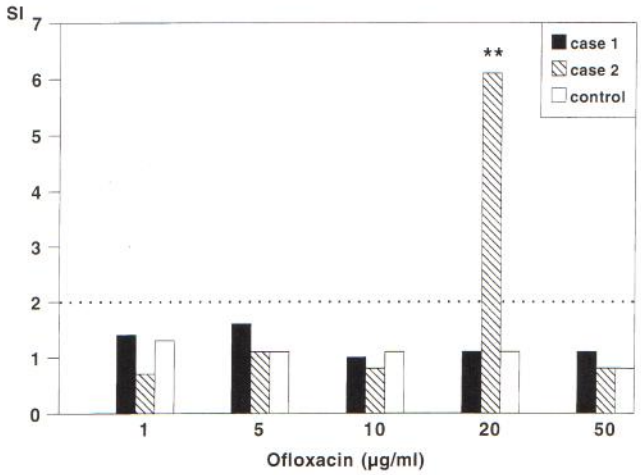


*Fig. 4.* LTT in CPFX-induced drug-allergy (cases 1 and 2): PBMC of the patients and control were incubated for 7 days as sixfold cultures with 1 to 50 μg/ml ofloxacin. Neither of the patients had previously been in contact with ofloxacin. Lymphocyte proliferation is expressed as a ratio of the mean values of cpm from cultures with and without test compound (SI). *Asterisks* indicate a significant difference relative to the respective medium control (Welch's modified *t*-test; $**p<0.01$).

## DISCUSSION

Conclusive diagnosis of drug allergy still remains a major problem in daily clinical practice. In case of delayed-type hypersensitivity reactions, the LTT is still the only available in vitro test for detecting sensitisation at the cellular level. Other in vitro assays depend directly or indirectly on antibody production, which is not necessarily the final route of allergy. In order to increase LTT sensitivity and specificity it is essential to determine optimal culture conditions, including appropriate solvent, drug concentration and the requirement of a metabolising system for each individual substance tested. Since only small series of patients have been investigated for selected substances, knowledge about these conditions is lacking for many drugs.

In the present study we confirmed two clinically suspected cases of drug allergy to CPFX using the LTT. The highest SIs were achieved at 1 and 5 μg/ml CPFX – concentrations, corresponding to peak serum values of 2.5 μg/ml CPFX 1 h after oral treatment with 500 mg CPFX (14). In both patients epicutaneous patch tests with CPFX remained negative. This phenomenon has been previously reported in cases of CPFX allergy confirmed by oral provocation (10, 11). Therefore, epicutaneous application of CPFX might be inappropriate to detect sensitisation induced by systemic treatment. Prick testing with CPFX was negative in the first patient, as expected for delayed-type hypersensitivity, but positive in the second patient, indicating either a specific IgE-mediated mechanism or an unspecific histamine release caused by CPFX. Both explanations are conceivable: the induction of IgE-responses is T-cell dependent (15), and positive LTTs in patients with IgE-mediated drug allergy have been published (16). Nonetheless positive prick tests with CPFX in unsensitised control persons have been described (9) and are consistent with our experience (unpublished data).

Cross-allergy is a well-known phenomenon with regard to β-lactam antibiotics, but with quinolones reports are rare. However, our second patient showed a distinct proliferation of PBMC in vitro, not only to CPFX but also to ofloxacin. This patient had never been in contact with ofloxacin before, suggesting true cross-reactivity at the T-cell level. Remarkably the two patients did not react to the metabolites (M1-M3), although these are structurally highly related to their mother compound (Fig. 1). Formyl-CPFX (M4) was not available and therefore not tested. An explanation for this non-reactivity might be the ability of memory T-cells to differentiate between the minor differences of mother compound and metabolites. This would correspond to observations by Stejskal et al. (17), who showed that lymphocytes from patients with drug-induced occupational allergy were able to discriminate between the stereoisomers quinine and quinidine or differences in the side-chains of penicillin. If so, non-reactivity towards metabolites would match, because sensitisation to those may not have been established, due to peak serum concentrations ranging approximately tenfold below those of the original compound (12). Furthermore, chemically reactive intermediates could be formed, which directly bind to surrounding proteins, thereby escaping further metabolism to those compounds tested (M1-M3). If these intermediates are shared by ofloxacin and CPFX, the observed cross-allergy could be explained.

In summary, 2 cases of suspected drug allergy to CPFX were confirmed by means of the LTT, whereas epicutaneous patch tests remained negative. Furthermore, in vitro cross-reactivity between CPFX and ofloxacin – two fluorinated quinolones – was demonstrated, which, to our knowledge, has not previously been reported. We conclude that in cases of suspected drug allergy to CPFX the LTT can be a useful diagnostic tool.

## ACKNOWLEDGEMENTS

This work was supported by Grant 01 GB 9406 from *Bundesministerium für Bildung, Forschung und Technologie* (BMFT), Bonn, Germany, for co-operation between university clinics and extrauniversity institutes performing basic research.

The authors wish to thank Marty B.F. Wulferink for helpful discussion and Jaime Davis-Dobbertin for critically reading the manuscript.

## REFERENCES

1. Stahlmann R. Safety profiles of quinolones. J Antimicrob Chemother 1990; 26 (Suppl. D): 31–44.
2. Norrby SR, Lietman PS. Safety and tolerability of fluoroquinolones. Drugs 1993; 45 (Suppl. 3): 59–64.
3. Schacht P, Arcieri G, Branolte J, Bruck H, Chysky V, Griffith E, et al. Worldwide clinical data on efficacy and safety of ciprofloxacin. Infection 1988; 16 (Suppl.1): 29–41.
4. Reiter C, Pfeiffer M, Hullman RN. Brief report: safety of ciprofloxacin based on phase IV studies ("Anwendungsbeobachtung") in the Federal Republic of Germany. Am J Med 1989; 87 (Suppl. 5A): 103–106.
5. Arcieri G, Becker N, Esposito B, Griffith E, Heyd A, Neumann C, et al. Safety of intravenous ciprofloxacin. Am J Med 1989; 87 (Suppl. 5a): 92–97.
6. Schacht P, Arcieri G, Hullmann R. Safety of oral ciprofloxacin. An update based on clinical trial results. Am J Med 1989; 87 (Suppl. 5a): 98–102.
7. Zürcher K, Krebs A. Other antiinfectious drugs. In: Zürcher K, Krebs A, eds. Cutaneous drug reactions. Basel: Karger, 1992: 82–83.
8. Correia O, Delgado L, Barros MA. Bullous photodermatosis after lomefloxacin. Arch Dermatol 1994; 130: 808–809.
9. Davila I, Diez ML, Quirce S, Fraj J, De La Hoz B, Lazaro M. Cross-reactivity between quinolones. Report of three cases. Allergy 1993; 48: 388–390.
10. Alonso MD, Martin JA, Quirce S, Davila I, Lezaun A, Sanchez Cano M. Fixed eruption caused by ciprofloxacin with cross-sensitivity to norfloxacin. Allergy 1993; 48: 296–297.
11. Kawada A, Hiruma M, Morimoto K, Ishibashi A, Banba H. Fixed drug eruption induced by ciprofloxacin followed by ofloxacin. Contact Dermatitis 1994; 31: 182–183.
12. Beermann D, Scholl H, Wingender W, Förster D, Beubler E, Kukovetz WR. Metabolism of ciprofloxacin in man. Current Clinical Practice Series 1985; 34: 141–146.
13. Shalit I. Immunological aspects of new quinolones. Eur J Microbiol Infect Dis 1991; 10: 262–266.
14. Garraffo R, Lapalus P, Dellamonica P, Bernad E, Etesse H. Study of the bioequivalence of ciprofloxacin 500 mg orally versus 200 mg i.v. Chemioterapia 1987; 6 (Suppl. 2): 298–300.
15. Plaut M, Zimmerman EM. Allergy and mechanisms of hypersensitivity. In: Paul WE, ed. Fundamental immunology. New York: Raven Press, 1993: 1401–1403.
16. Brander C, Mauri-Hellweg D, Bettens F, Rolli HP, Goldman M, Pichler WJ. Heterogeneous T-cell responses to beta-lactam-modified self-structures are observed in penicillin-allergic individuals. J Immunol 1995; 155: 2670–2678.
17. Stejskal VDM, Olin RG, Forsbeck M. The lymphocyte transformation test for diagnosis of drug-induced occupational allergy. J Allergy Clin Immunol 1986; 77: 411–426.