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

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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 vitro, a small percentage of the drug is biotransformed to the three major metabolites desethyl-, sulfu- and oxo-ciprofloxacin. 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.

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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 specialties 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, no reports can be found in the literature (8–11). Studies using 14C-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 piperacilano moiety. Four metabolites have been identified: desethyl(CPFX (M1), M2), sulfu-(M2), oxo-(M3), and formyl-CFX (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 endoprosthesis. 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 monocytesis (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 leucocytes 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 spondylothesis in segment C6/7. The patient was started on ceftriaxone (2 g/day) intravenously with genta-
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 fluocoxacin (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 angio-
edema 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 fluocoxacin (all 10% in glycerine) were negative, as was a standard series of drug components.
2) Prick testing with benzylpenicillloyl-polysyline and the "minor determinant mixture" (benzylpenicillin, benzylpenicilloate) was negative, as was intracutaneous testing with benzylpenicillloyl-
polysyline. Intracutaneous application of the "minor determinant mixture" resulted in a positive reaction in 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 fluocoxacin was negative.
5) Total serum IgE was 30 U/ml. Specific serum IgE determined for penicillloyl G, ampicillin and amoxycillin was not detectable.

Lymphocyte transformation test (LTT)
Peripherial 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 manu-

![Diagram](image)

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

facturer's instructions. Isolated PBMC were washed twice in phosphate-buffered saline (PBS) and suspended in RPMI 1640 medium (Life Technologies, Paisley, 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 strepto-
mycin (10 µg/ml) (complete medium; all supplements from Life Technologies, Paisley, Scotland). 1×10⁷ PBMC/200 µl were cultured for 4 days in 96-well round-bottom plates (Grenner, Essen, Germany) as sixfold cultures at 37°C and 7% CO₂ in humidified atmosphere. During the last 17 h, 25 µl [³H]-thymidine with 1.85×10⁶ Bq were added (ICN, Irvine, USA). Cells were harvested with a PHD™ cell harvester (Cambridge Technology, UK) on filters coated with a solid seinitillator (Ready filter with Xtsbcint®M, 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, desethyline- (M1) and oxa-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 1×10⁻⁴ M. Sulfo-CPFX (M2) (kindly donated by Bayer, Leverkusen, Germany) was directly soluble in RPMI. Fluocoxacin 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

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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 CFX exposure; one had previously taken CFX 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 CFX with SIs of 4.0, 6.1, and 2.2, respectively. In a second LTT, 2 months later, the SI at 1 μg/ml CFX 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 desethyl-, sulfo- and oxo-CFX (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 CFX 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 CFX either (data not shown). Incubation with ofloxacin demonstrated a distinct proliferation, with a sixfold increase in 3H-thymidine incorporation at a concentration of 20 μg/ml (Fig. 4). With culture series of fluvoxacillin 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, fluvoxacillin and gentamycin). Toxicity controls with CFX led to heterogeneous results, in that mitogen-induced proliferative response was dose-dependently inhibited in concentrations above 5 μg/ml CFX in one volunteer (65% inhibition compared with the ConA control for 10 and 20 μg/ml CFX, 24% inhibition for 50 μg/ml CFX), whereas no inhibition up to 50 μg/ml CFX 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).
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 SLs 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 quindine or differences in the side-chains of penicillin. If so, non-reactivity towards metabolites would match, because sensitisation to these 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.

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