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Population Pharmacokinetics and Penetration into Prostatic, Seminal, and Vaginal Fluid for Ciprofloxacin, Levofloxacin, and Their Combination

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Key Words
Body fluid penetration · Chronic bacterial prostatitis · Ejaculate · Pharmacokinetic drug-drug interaction · Population pharmacokinetic modeling · Prostatic fluid · Sperm cells · Vaginal fluid

Abstract
Background: Our objectives were to assess the pharmacokinetic interaction and body fluid penetration of ciprofloxacin and levofloxacin. ***Methods:*** This study was a single-dose open randomized three-way crossover in 15 healthy volunteers receiving 500 mg oral levofloxacin, 500 mg oral ciprofloxacin, or 250 mg levofloxacin and 250 mg ciprofloxacin co-administered. Serum, urine, and body fluid concentrations were determined by high-performance liquid chromatography and analyzed via population pharmacokinetic modeling. ***Results:*** Modeling indicated that ciprofloxacin inhibited the renal reabsorption of levofloxacin. Ciprofloxacin increased the net renal clearance of levofloxacin by 13%, as its estimated affinity for a putative tubular reabsorption transporter was 12-fold higher (Km: 568 μM) compared to levofloxacin (Km: 6,830 μM). Levofloxacin increased the bioavailability of ciprofloxacin by 12% and achieved significantly ($p < 0.05$) higher concentrations at 3 h in ejaculate, prostatic, seminal, and vaginal fluid compared to ciprofloxacin. ***Conclusion:*** Modeling suggested that ciprofloxacin inhibited the tubular reabsorption of levofloxacin due to a 12-fold higher affinity for a putative tubular reabsorption transporter compared to levofloxacin. This pharmacokinetic interaction was not clinically relevant.

Introduction

Drug transporters play a critical role in the absorption and disposition of many drugs and pharmacokinetic (PK) interactions involving drug transporters may affect the effectiveness and side effects. Many in vitro and animal studies emphasize the importance of drug transporters (including P-glycoprotein) for quinolone antibiotics at the renal, hepatobiliary, and intestinal sites [1–9]. Fluoroquinolones are both subject to renal tubular secretion and reabsorption, and either of these processes may be subject to drug-drug interactions [9–15]. Most data on these

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transporters come from in vitro or animal studies. We are not aware of human clinical trials that assessed the PK interaction between two quinolone antibiotics.

Ciprofloxacin and levofloxacin are commonly used quinolones and are substrates of renal, hepatobiliary, and intestinal transporters [9, 11, 12, 16]. Given their chemical similarity, mutual PK interactions of ciprofloxacin and levofloxacin under co-administration seem possible.

Co-administration of two drugs, here fluoroquinolones, can also facilitate PK studies comparing their body fluid penetration. This may be helpful for difficult clinical situations when control groups are not possible and for difficult to obtain tissues or body fluids. For the assessment of tissue penetration under simultaneous dosing of both drugs, prior data on the potential extent of interaction or mechanistic information on the disposition of both drugs should be available to support the design of the tissue penetration study. To minimize a potential interaction in the rate and extent of tissue penetration, administration of low doses should be helpful, as low drug concentrations are less likely to show an interaction or exceed a Michaelis-Menten constant of a saturable process.

Population PK modeling [17, 18] is the method of choice to determine the extent, time course, and potential site(s) and mechanism(s) of a PK interaction, and to account for the between-subject variability in PK parameters. As ciprofloxacin and levofloxacin share the same primary target, DNA-gyrase in Gram-negative bacteria, the combination of ciprofloxacin and levofloxacin is not expected to be clinically beneficial and was primarily studied to assess the mechanisms of PK interaction.

Our primary objective was to determine the extent of interaction between oral ciprofloxacin and oral levofloxacin and to develop a mechanism-based population PK model for the time course, potential site(s) and mechanism(s) of interaction. Secondly, the penetration of levofloxacin and ciprofloxacin into prostatic, seminal, and vaginal fluid and into sperm cells was assessed.

Materials and Methods

Study Design

This study was a single-dose open-label controlled randomized phase-I three-period six-sequence crossover trial. The washout period between the two study periods was 7 days. Fourteen subjects received treatments A and B. Treatment C included these 14 subjects receiving treatments A and B and 1 additional subject who did not receive treatments A and B. Therefore, 15 subjects received treatment C to increase the sample size of the body fluid penetration arm, and 14 subjects were available for an intra-individual comparison of treatments A versus C and B versus C. For the 15 healthy volunteers (7 females/8 males), average ± SD weight was 65.7 ± 10.2 kg, age 23 ± 6 years, and height 172.9 ± 9.4 cm.

The study protocol was approved by the ethics committee of the Bavarian Medical Association (reference number: 99006). Written informed consent was obtained from all volunteers before inclusion in the study. The study was performed following the revised version of the Declaration of Helsinki and all relevant national and international guidelines. Details on the clinical procedures and drug analysis and on treatments A and B were described by Wagenlehner et al. [19]. Unless stated otherwise, these procedures also apply to treatment C.

Subjects received either an oral tablet of 500 mg levofloxacin (treatment A) or of 500 mg ciprofloxacin (treatment B). For treatment C that was not reported previously, subjects received simultaneously one tablet of 250 mg ciprofloxacin (Bayer Vital GmbH, Leverkusen, Germany) and one tablet of 250 mg levofloxacin (Sanofi Aventis, Berlin, Germany) orally with 240 ml low-carbonated calcium-poor mineral water. Venous blood samples were obtained immediately prior to drug administration and at 0.33, 0.67, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h afterwards. Urine was collected before drug administration, and at 0–3, 3–6, 6–12, 12–24, 24–36, and 36–48 h after dosing. For treatments A and B, urine was also collected at 48–72, 72–96, and 96–120 h.

Collection of Body Fluids

In male volunteers, prostatic fluid was obtained by prostatic massage at 3 h after drug administration (for treatment C only). Immediately thereafter, ejaculate was obtained by masturbation. Ejaculate samples were left at room temperature until liquefaction and were then divided: One aliquot was taken without further treatment for sperm cell count (Fuchs Rosenthal counting chamber) and for measurement of drug concentrations. The other aliquot was used for measurement of drug concentrations in cell-free seminal fluid and in sperm cells.

In female subjects, a pre-weighed vaginal tampon was placed 2.5 h after drug administration, which remained for 30 min (for treatment C only). After removal the vaginal tampons were weighed. The volunteers were not allowed to empty their bladder before sampling of prostatic fluid, ejaculate, or vaginal fluid. All samples were immediately processed, stored at –20°C, and assayed within 2 months.

Sample Preparation of Body Fluids

Prostatic Fluid and Ejaculate

Ejaculate and prostatic fluid (50 μl) samples were precipitated by addition of 25 μl internal standard solution (pipemidic acid). After centrifugation, 15 μl were analyzed by high-performance liquid chromatography [19].

Cell-Free Seminal Fluid and Sperm Cells

The ejaculate (50 μl) was pipetted on a silicone layer (100 μl) and centrifuged using a Microfuge (Beckman, Krefeld, Germany) at 10,000 rpm for approximately 1 min. The aqueous layer at the bottom of the vial contained 20% perchloric acid (50 μl). To this 50 μl perchloric acid containing the sperm cells, 25 μl internal standard solution (pipemidic acid) were added. After centrifugation, 15 μl were analyzed using high-performance liquid chromatography [19].

Table 1. Bioanalytical performance data for body fluid assays

	Linear range, μg/ml		r^2	Spiked quality controls for levofloxacin, %		Spiked quality controls for ciprofloxacin, %	
	levofloxacin	ciprofloxacin		intra-assay precision	range of accuracy	intra-assay precision	range of accuracy
Prostatic and cell-free seminal fluid	0.0204–8.39	0.0202–8.27	≥0.999	0.9–5.7	96.8–100.8	2.1–6.5	96.0–102.4
Ejaculate	0.0205–8.39	0.0191–8.07	≥0.999	0.5–2.9	98.5–101.4	3.9–5.1	97.9–103.6
Sperm cells	0.0172–8.07	0.0170–7.96	≥0.999	0.3–3.5	100.1–102.7	0.5–9.1	97.8–104.5
Vaginal fluid	0.0482–9.94	0.0476–9.80	≥0.999	1.7–5.2	102.8–106.6	1.6–6.2	104.7–108.9

r^2 = Coefficient of determination for levofloxacin and ciprofloxacin.

Vaginal Secretion

To each of the samples, 15 ml phosphate buffer (pH 7.5) containing the internal standard (enrofloxacin) and chloroform/diethyl ether (8:2, 20 ml) were added and mixed for 15 min. After removing of the liquid phases, the tampon was extracted with 20 ml of the extraction solution described above. The liquid phases were combined and centrifuged (10 min, 3,600 rpm). The organic layer was evaporated to dryness using nitrogen at room temperature. The residual was re-dissolved using 200 μl mobile phase; 50 μl were analyzed by high-performance liquid chromatography [19].

Drug Analysis in Body Fluids

Drug concentrations in prostatic and cell-free seminal fluid, ejaculate, sperm cells, and vaginal fluid samples were measured in comparison to calibration rows in cell-free seminal fluid, ejaculate, sperm cells, or vaginal fluid. Calibration standards were prepared by adding defined amounts of standard solution of levofloxacin or ciprofloxacin to cell-free seminal fluid, ejaculate, sperm cells, or vaginal fluid collected on pre-weighed vaginal tampons. All fluids used to prepare calibration rows were tested and shown not to contain any interfering drugs.

Spiked quality control samples were prepared for determination of intra-assay variation by adding defined amounts of the stock solution or the spiked control of higher concentration to defined amounts of cell-free seminal fluid, ejaculate, or sperm cells (all tested not to contain any of the study drugs). For vaginal fluid, defined amounts of a buffer calibration standard was added to vaginal fluid collected on pre-weighed vaginal tampons. No interference was observed for levofloxacin, ciprofloxacin, or the internal standard in prostatic and cell-free seminal fluid, ejaculate, sperm cells, and vaginal fluid samples. Weighted linear regression (1/peak height ratio) was performed for calibration. The performance data of the bioanalytical assay for the body fluids were excellent (table 1). The lower limits of quantification were identical to the lowest calibration levels shown in table 1.

Non-Compartmental PK Analysis

PK parameters were determined by standard non-compartmental analysis in WinNonlin™ Professional (version 4.0.1, Pharsight Corp., Mountain View, Calif., USA). Concentration ratios were calculated by dividing the body fluid concentration through the serum concentration at the respective sampling time. Only serum and urine data were included in the population PK modeling analysis, since the time course of tissue penetration could not be determined based on body fluid data collected at one time point.

Population PK Analysis

Estimation and Model Discrimination

We applied nonlinear mixed-effects modeling in NONMEM VI (level 1.2, NONMEM Project Group, Icon Development Solutions, Ellicott City, Md., USA) [20]. The first order conditional estimation method with the interaction estimation option (FOCE+I) and the ADVAN6 differential equation solver were used for all modeling (including the bootstrap analysis). Models were assessed and compared via visual predictive checks (as described previously [21]), the objective function in NONMEM, individual curve fits, and other standard diagnostic plots. Uncertainty of parameter estimates was assessed via non-parametric bootstrap techniques with 400 replicates as previously described [21, 22].

Scaling of PK Parameters

Allometric scaling was used to scale all clearance and volume terms [23–26]. The $F_{WTCL,i}$ is the fractional change in clearance and maximum rate of a transport process (Vmax) and $F_{WTV,i}$ is the fractional change in volume of distribution for the i-th subject with total body weight WT_i compared with a standard weight WT_{STD} of 70 kg:

$$F_{WTCL,i} = \left(\frac{WT_i}{WT_{STD}}\right)^{0.75} \quad (1)$$

$$F_{WTV,i} = \frac{WT_i}{WT_{STD}} \quad (2)$$

The assumptions of this allometric model are that volume of distribution scales linearly with WT (allometric exponent: 1.0) and clearance scales slightly less than linearly with WT (allometric exponent: 0.75). Clearance by glomerular filtration was calculated by the Cockcroft and Gault equation [27] based on the unbound fraction ($f_{unbound}$) of each quinolone (0.70 for ciprofloxacin [28, 29] and 0.69 for levofloxacin [30]).

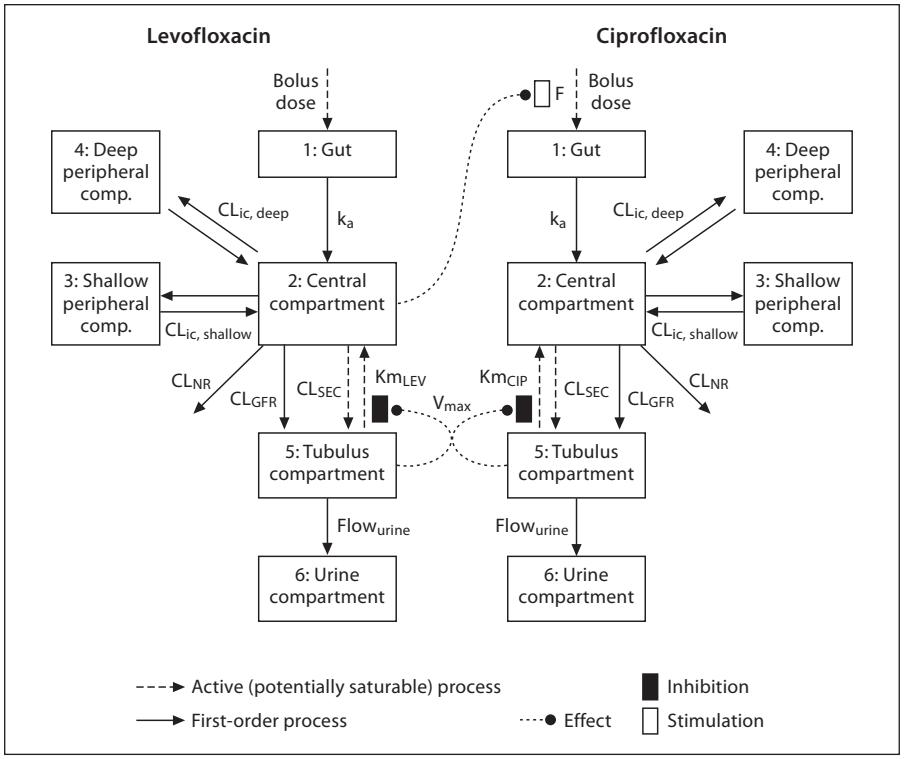

Fig. 1. Structural model for oral levofloxacin and ciprofloxacin and their interaction at the tubular and intestinal sites (see table 3 for parameter explanations).

Structural Model

One-, two-, and three-compartment disposition models with first-order absorption with or without a lag time were considered for both quinolones. To account for tubular secretion and reabsorption, a renal tubulus compartment and a urine compartment were included. Active renal tubular secretion and an active reabsorption process were considered. Differential equations for ciprofloxacin were (all initial conditions were zero, parameters are explained beneath the equations and in fig. 1; see also table 3):

$$\frac{dA1}{dt} = -ka \cdot A1 \quad (3)$$

$$\frac{dA2}{dt} = ka \cdot A1 - \left(CL_{NR} + CL_{GFR} + CL_{SEC} + CL_{ic,shallow} + CL_{ic,deep}\right) \cdot C2 + CL_{ic,shallow} \cdot C3 + CL_{ic,deep} \cdot C4 + CL_{reabs,CIP} \cdot C5 \quad (4)$$

$$\frac{dA3}{dt} = CL_{ic,shallow} \cdot \left(C2 - C3\right) \quad (5)$$

$$\frac{dA4}{dt} = CL_{ic,deep} \cdot \left(C2 - C4\right) \quad (6)$$

$$\frac{dA5}{dt} = \left(CL_{GFR} + CL_{SEC}\right) \cdot C2 - \left(CL_{reabs,CIP} + Flow_{urine}\right) \cdot C5 \quad (7)$$

$$\frac{dA6}{dt} = Flow_{urine} \cdot C5 \quad (8)$$

The same equations with a different set of PK parameters were used for levofloxacin. The A_{NN} represent the amounts of drug and C_{NN} the molar drug concentrations in compartment NN. Compartment numbers are 1 for gut, 2 for central compartment, 3 for shallow peripheral compartment, 4 for deep peripheral compartment, 5 for tubulus compartment, and 6 for urine. Models with a relative extent of bioavailability (F) for ciprofloxacin given with levofloxacin compared to ciprofloxacin given alone were evaluated. To implement a relative bioavailability, the bolus dose entering the A1 compartment was multiplied by F. Urine flow rate ($Flow_{urine}$) was set to 0.058 L/h according to literature data [31, 32] and was scaled allometrically with an exponent of 0.75.

PK Interaction Models

Based on the results from non-compartmental analysis, we considered models with a different extent of bioavailability or different non-renal clearances for co-administration compared to separate administration of each quinolone. Additionally, interaction models with a mechanism-based inhibition of tubular reabsorption or of tubular secretion were assessed. For a competitive interaction of ciprofloxacin and levofloxacin, the reabsorption clearance for ciprofloxacin ($CL_{reabs,CIP}$) was:

$$CL_{reabs,CIP} = \frac{Vmax}{Km_{CIP} \cdot \left(1 + \frac{C5_{LEV}}{Km_{LEV}}\right) + C5_{CIP}} \quad (9)$$

The $C5_{CIP}$ is the ciprofloxacin and $C5_{LEV}$ the levofloxacin concentration in the tubulus compartment and the Km are the concentrations of the respective quinolone that result in 50% of the maximum rate of reabsorption (Vmax). The reabsorption clearance for levofloxacin ($CL_{reabs,LEV}$) was:

$$CL_{reabs,LEV} = \frac{Vmax}{Km_{LEV} \cdot \left(1 + \frac{C5_{CIP}}{Km_{CIP}}\right) + C5_{LEV}} \quad (10)$$

Table 2. PK parameters of levofloxacin and ciprofloxacin from non-compartmental analysis in healthy volunteers after oral administration alone or in combination

	Levofloxacin			Ciprofloxacin		
	levofloxacin alone (treatment A)	simultaneous levofloxacin + ciprofloxacin (treatment C)	ratio of treatment C/ treatment A	ciprofloxacin alone (treatment B)	simultaneous ciprofloxacin + levofloxacin (treatment C)	ratio of treatment C/ treatment B
Peak serum concentration, $mg \cdot l^{-1}$	6.08 (3.93–7.74)	3.23 (1.99–4.00)	53.1[b]	2.30 (1.40–2.95)	1.46 (0.793–1.83)	63.8[a, b]
Terminal half-life in serum, h	5.64 (4.41–7.34)	5.96 (4.77–8.38)	105.8	4.61 (2.41–5.40)	4.52 (3.74–5.29)	98.0
Terminal half-life in urine, h	14.3 (10.1–21.4)	7.04 (5.42–9.13)	49.1[a, c]	12.9 (3.18–23.8)	5.98 (4.01–8.21)	46.4[a, c]
Apparent total clearance, $l \cdot h^{-1}$	10.8 (7.03–13.7)	11.4 (6.90–14.9)	105.1[a]	50.9 (40.0–72.3)	46.5 (30.0–67.0)	91.4[a]
Renal clearance, $l \cdot h^{-1}$	8.43 (5.95–11.2)	9.55 (6.16–12.5)	113.3[a]	18.8 (16.8–23.8)	19.6 (14.7–26.1)	104.3
Apparent non-renal clearance, $l \cdot h^{-1}$	2.01 (1.09–3.25)	1.84 (0.738–3.04)	91.4[a]	32.2 (20.3–52.1)	24.7 (13.7–43.5)	76.9[a]
Fraction excreted unchanged in urine, %	81.8 (69.6–86.7)	84.2 (77.2–87.9)	102.9	38.6 (25.9–50.3)	46.7 (34.4–54.4)	121.1

Data presented as medians (min.–max.).
[a] The p value from ANOVA on the log scale was <0.05 for this comparison. [b] ANOVA was performed using dose-normalized peak concentrations. [c] Urine sampled up to 120 h for treatments A and B and up to 48 h for treatment C.

These equations assume that ciprofloxacin and levofloxacin compete for the same reabsorption transporter and that Vmax is the same between both quinolones. As we did not have mechanistic data on reabsorption transporters, we made these simplifying assumptions following the rule of parsimony. All concentrations and doses were entered in molar units for modeling. Similar equations for uncompetitive, non-competitive, and mixed inhibition were considered [33, 34].

Parameter Variability and Residual Error Model

The between-subject variability was described by an exponential parameter variability model and residual error by a proportional error model for serum concentrations and by a combined additive and proportional error model for amounts excreted in urine during each urine collection interval.

Simulations

Simulations were performed in Berkeley Madonna (version 8.3.11, University of California at Berkeley, Calif., USA) based on the mean PK parameter estimates.

Statistical Analysis

Differences in the tissue penetration between levofloxacin and ciprofloxacin were evaluated by paired t tests. ANOVA was used for statistical comparisons of non-compartmental PK parameters. Owing to the small sample size, these results were considered as descriptive. All analyses were performed on a logarithmic scale with an α of 0.05.

Results

Levofloxacin and ciprofloxacin were well tolerated by the healthy subjects. There were no clinical or laboratory adverse events reported. No subject withdrew because of adverse events.

Non-Compartmental Analysis

Non-compartmental analysis indicated that the apparent non-renal clearance of ciprofloxacin was 23% lower (p = 0.028, ANOVA; table 2) for co-administration of 250 mg ciprofloxacin and 250 mg levofloxacin compared to 500 mg ciprofloxacin given alone. As renal clearance of ciprofloxacin was unchanged, the apparent total clearance for ciprofloxacin was significantly lower (p = 0.03) when ciprofloxacin was co-administered with levofloxacin compared to ciprofloxacin given alone. For levofloxacin, renal clearance was 13% higher (p = 0.006) for levofloxacin co-administered with ciprofloxacin compared to levofloxacin given alone. The dose normalized peak concentration of ciprofloxacin was higher (p = 0.01; table 2) under co-administration.

Population PK Modeling

Three compartment disposition models with first-order absorption and an absorption lag-time (fig. 1) were superior to one- and two-compartment models and provided unbiased and precise individual and population fits for serum and urine data of both quinolones (fig. 2). The final model included a simplified, semi-physiological description of the renal drug elimination. Either antibiotic had a tubulus compartment between the central and urine compartment. Efflux from the tubulus compartment into the urine compartment was set to urine flow rate. The input into the tubulus compartment was determined by passive glomerular filtration and renal tubular secretion (fig. 1). An active reabsorption process from the tubulus to the central compart-

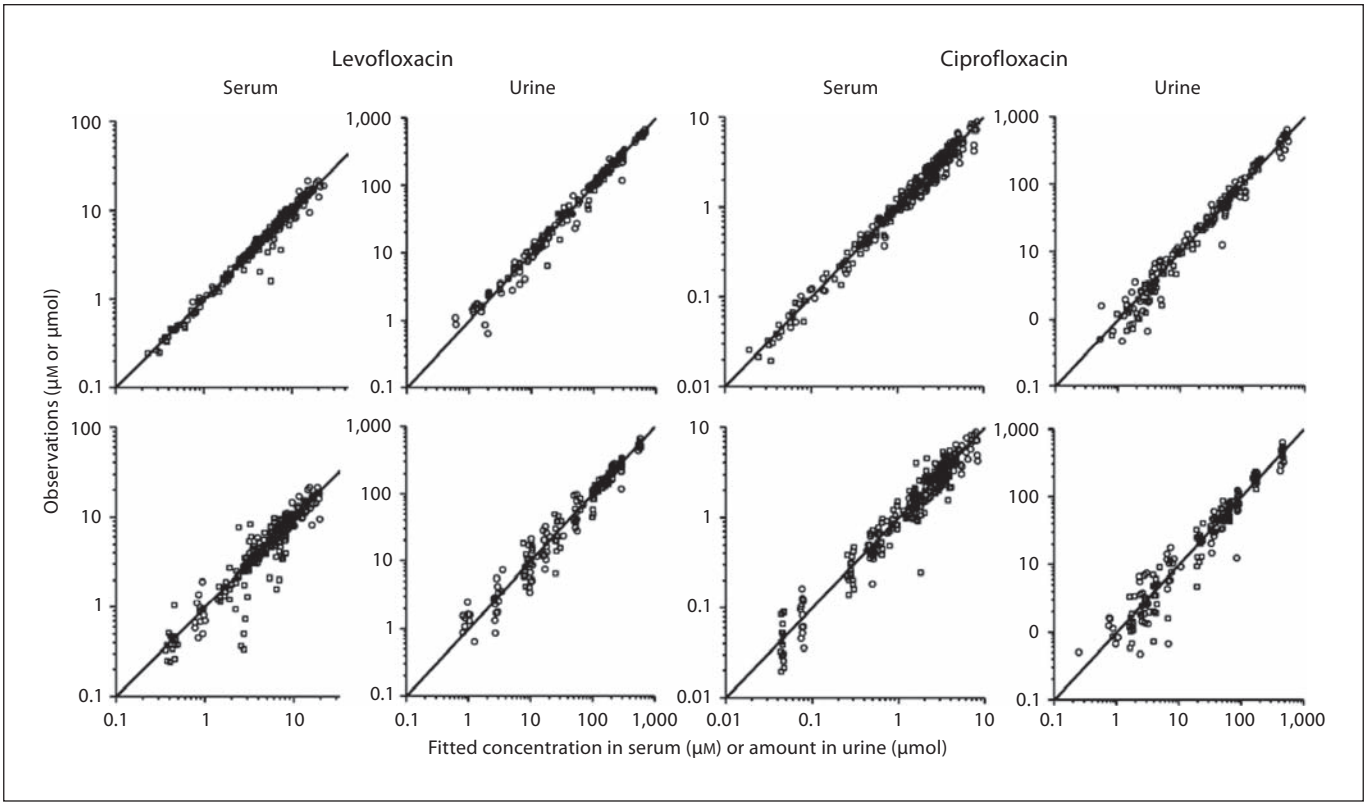

Fig. 2. Observed versus predicted serum concentrations and amounts excreted unchanged in urine for levofloxacin and ciprofloxacin after separate (circles) and simultaneous (squares) administration and line of identity; top row: individual predictions (fits), bottom row: population predictions.

ment was included to describe the interaction between both quinolones.

Ciprofloxacin was estimated to have a 12-fold higher affinity (see ratio of Km values in table 3) for the putative reabsorption transporter. Therefore, ciprofloxacin inhibited the reabsorption of levofloxacin and increased the renal clearance of levofloxacin in agreement with the results from non-compartmental analysis. The visual predictive checks (fig. 3) showed that the final model adequately predicted the central tendency and variability of the observations for both drugs and at both dose levels in serum and urine.

Mechanism of Interaction

Compared to the final model with a competitive interaction (table 3), the objective function was worse by 4.6 for a model with uncompetitive interaction and worse by 1.5 for a model with noncompetitive interaction. A model with a mixed interaction had a similar objective function as the competitive model. As these objective function differences were small and as all these models had essentially indistinguishable diagnostic plots, we selected a model with competitive interaction as final model. This was the simplest model and this model yielded excellent diagnostic plots. Estimation of different Vmax for each quinolone yielded no significant improvement in the objective function. Specifying tubular secretion as a saturable process and including an interaction between levofloxacin and ciprofloxacin for tubular secretion did not improve the objective function significantly compared to the final model.

Modeling identified a 12% increased extent of bioavailability of ciprofloxacin co-administered with levofloxacin (treatment C) as the most likely reason for the 9% lower apparent nonrenal clearance for ciprofloxacin from non-compartmental analysis. The objective function of the final model with different extents of ciprofloxacin bioavailability was better by 14.6 ($p < 0.001$) compared to a model with two different nonrenal clearances for ciprofloxacin and better by 14.8 ($p < 0.001$)

Table 3. Population PK parameter estimates (10–90th percentiles from nonparametric bootstrap) for levofloxacin and ciprofloxacin after oral administration in healthy volunteers

	Symbol	Unit	Levofloxacin		Ciprofloxacin	
			estimate	between-subject variability[a]	estimate	between-subject variability[a]
Absorption						
Half-life of absorption	$t_{1/2}(k_a)$	min	19.9 (14.1–30.2)	0.596 (0.299–0.800)	14.0 (6.33–28.1)	0.330 (0.018–0.651)
Absorption lag time	Tlag	min	13.0 (10.3–16.1)	0.394 (0.185–0.587)	12.4 (8.80–16.7)	0.279 (0.004–0.524)
Relative extent of bioavailability	F				1.12 (1.02–1.24)[e]	
Elimination						
Nonrenal clearance[b]	CL_{NR}	$l \cdot h^{-1}$	1.86 (1.65–2.05)	0.165 (0.001–0.213)	33.7 (29.8–37.5)	0.178 (0.137–0.204)
Glomerular filtration clearance[b]	CL_{GFR}	$l \cdot h^{-1}$	5.17[g]		5.25[g]	
Tubular secretion clearance[b]	CL_{SEC}	$l \cdot h^{-1}$	5.65 (4.99–8.32)	0.237 (0.129–0.300)	27.4 (21.8–84.5)	
Maximum rate of reabsorption[b]	Vmax	$\mu mol \cdot h^{-1}$	43.4 (15.8–692)[d, j]		43.4 (15.8–692)[d, j]	
Concentration resulting in 50% of Vmax[b]	Km	μM	6,830 (3,349–131 · 10^3)[i]		568 (265–2,281)[i]	
Estimated volume of tubulus compartment[c]	V_{Tub}	ml	22.9 (17.9–37.9)[d]		22.9 (17.9–37.9)[d]	
Distribution						
Volume of central compartment[c]	V1	l	56.3 (46.1–63.3)	0.160 (0.111–0.187)[f]	149 (89.4–177)	0.178 (0.131–0.215)[f]
Volume of shallow peripheral compartment[c]	V2	l	33.8 (29.1–41.6)	0.160 (0.111–0.187)[f]	78.4 (58.9–119)	0.178 (0.131–0.215)[f]
Volume of deep peripheral compartment[c]	V3	l	9.63 (8.52–11.0)	0.160 (0.111–0.187)[f]	56.7 (43.6–72.6)	0.178 (0.131–0.215)[f]
Intercompartmental clearance to V2[b]	$CL_{ic,shallow}$	$l \cdot h^{-1}$	23.6 (18.1–29.6)		44.1 (25.5–74.6)	
Intercompartmental clearance to V3[b]	$CL_{ic,deep}$	$l \cdot h^{-1}$	0.441 (0.327–0.593)		2.68 (2.12–3.36)	
Serum concentration						
Proportional error		%	0.133 (0.106–0.155)[h]		0.186 (0.160–0.199)[h]	
Amount in urine						
Proportional error		%	0.178 (0.154–0.198)		0.225 (0.189–0.275)	
Additive error		μmol	0.462 (0.004–0.679)		0.733 (0.04–0.968)	

[a] Estimates are apparent coefficients of variation for the between-subject variability.

[b] Estimates are group estimates for subjects with a standard weight of 70 kg using an allometric exponent of 0.75.

[c] Estimates are group estimates for subjects with a standard weight of 70 kg using linear scaling by weight.

[d] Parameter shared between levofloxacin and ciprofloxacin.

[e] Relative extent of bioavailability of 250 mg ciprofloxacin under co-administration with 250 mg levofloxacin compared to 500 mg ciprofloxacin given alone.

[f] Variability was estimated as variability of volume of distribution at steady state.

[g] Clearance (CL_{GFR}) was fixed to the glomerular filtration rate predicted by the Cockcroft and Gault formula times fraction unbound (69% for levofloxacin and 70% for ciprofloxacin) for subjects with a typical weight of 70 kg, age of 18 years, and serum creatinine concentration of 0.949 mg/dl for males and 0.807 mg/dl for females.

[h] In addition to the proportional error, an additive error term for serum concentrations was estimated to be very small and was eventually fixed to zero.

[i] The ratio of Km values between both quinolones yields the approximately 12-fold higher affinity for ciprofloxacin compared to levofloxacin for the putative reabsorption transporter.

[j] The apparent, net renal clearance ($CL_{R,net,0-12h}$) over the first 12 h was calculated as: $CL_{R,net,0-12h} = (A_{Filtered} + A_{Secreted} - A_{Reabsorbed})/AUC_{0-12h,plasma}$ with $A_{Filtered}$, $A_{Secreted}$, and $A_{Reabsorbed}$ representing the amount of drug filtered, secreted and reabsorbed from 0 to 12 h. The apparent, average reabsorption clearance during the first 12 h ($CL_{Reabs,0-12h}$) was calculated as $CL_{Reabs,0-12h} = CL_{GFR} + CL_{SEC} - CL_{R,net,0-12h}$. The $CL_{Reabs,0-12h}$ for a typical subject was 0.86 l/h for 500 mg levofloxacin given alone and 10 l/h for 500 mg ciprofloxacin given alone.

compared to a model with the same extent of bioavailability for ciprofloxacin given with or without levofloxacin. The estimated relative bioavailability of levofloxacin co-administered with ciprofloxacin was 0.993 compared to levofloxacin given alone and was subsequently fixed to 1.

Body Fluid Penetration

For both drugs, body fluid concentrations were higher in seminal fluid and ejaculate compared to prostatic fluid and vaginal fluid (table 4). Both quinolones had low and variable concentrations in sperm cells. One subject had an at least 7-fold higher concentration for both antibiotics in vaginal fluid compared to all other subjects. Therefore, vaginal fluid data are presented and were analyzed with and without the data from this subject. At 3 h after the dose, levofloxacin achieved significantly higher concentrations compared to ciprofloxacin in all studied body fluids except sperm cells.

Body fluid to serum concentration ratios (table 5) were >1 for all subjects in ejaculate and seminal fluid. Ratios for ejaculate and seminal fluid were approximately 2.6-

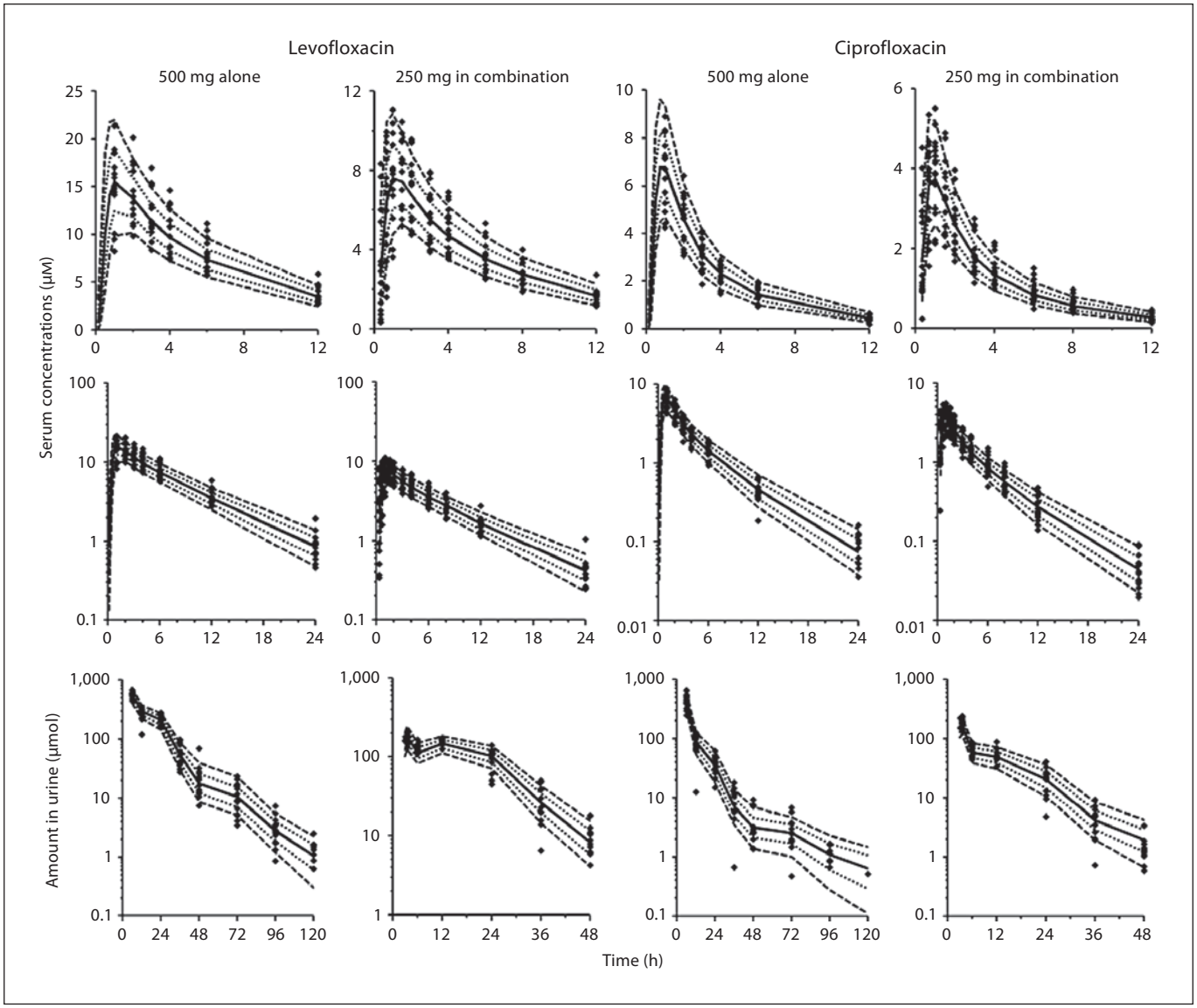

Fig. 3. Visual predictive check for levofloxacin in serum and urine and ciprofloxacin in serum and urine. Markers are observations; lines are model predictions: medians (continuous lines), IQR (dotted lines), and 10–90% percentiles (dashed lines). Ideally, 50% of the observations should fall outside the IQR and 10% of the observations should fall outside the 80% prediction interval on each side. For ciprofloxacin, 1 mg/l is equivalent to approximately 3.02 μmol/l; for levofloxacin, 1 mg/l is equivalent to approximately 2.77 μmol/l.

fold higher for ciprofloxacin relative to levofloxacin. For both antibiotics, ratios for prostatic fluid and vaginal fluid were <1, and the lowest ratios were observed for sperm cells.

Simulated Time Course of Concentration Ratios

For a simulation of 250 mg ciprofloxacin co-administered with 250 mg levofloxacin (fig. 4), concentrations in the shallow peripheral compartments were in equilibrium with the central compartment after approximately 3 h, whereas equilibration between the deep peripheral and the central compartment required approximately 3 to 4 days after a single dose.

Simulated ciprofloxacin concentrations in the tubulus compartment exceeded the Km for the putative tubular reabsorption transporter (568 μmol/l; table 3) for ap-

Table 4. Body fluid concentrations at 3 h after oral co-administration of 250 mg levofloxacin and 250 mg ciprofloxacin in healthy subjects

	n	Body fluid concentration, mg/l				Plasma conc. at 3 h, mg/l		Paired t test[e] p value
		median and range		geometric mean and % CV		geometric mean and % CV		
		levofloxacin	ciprofloxacin	levofloxacin	ciprofloxacin	levofloxacin	ciprofloxacin	
Prostatic fluid	8/7	0.854 (0.664–1.49)	0.141 (0.072–0.403)[b]	0.891 (31)	0.155 (65)	1.70 (16) 1.70 (14)[f]	0.530 (26) 0.551 (14)[f]	<0.001[d]
Ejaculate	8	3.07 (1.95–6.13)	2.23 (1.68–6.03)	3.21 (40)	2.63 (55)			0.014
Seminal fluid	8	3.27 (1.96–6.17)	2.29 (1.69–5.99)	3.25 (39)	2.59 (55)			0.010
Sperm cells	5[c]	0.044 (0.038–0.400)	0.048 (0.030–0.315)	0.091 (107)	0.083 (100)	1.62 (15)[g]	0.462 (13)[g]	0.252
Vaginal fluid (n = 7)[a]	7	0.750 (0.393–8.90)[a]	0.167 (0.095–12.0)[a]	1.01 (161)	0.287 (241)	2.56 (9) 2.46 (8)[f]	0.693 (22) 0.640 (11)[f]	0.003
Vaginal fluid (n = 6)[a]	6	0.741 (0.393–1.21)	0.167 (0.095–0.266)	0.703 (42)	0.154 (38)	2.59 (9)[g]	0.608 (36)[g]	<0.001

[a] One subject had a substantially higher concentration in vaginal fluid both for ciprofloxacin and for levofloxacin (presumably due to urinary contamination of the tampon). Therefore, the results are shown both with and without this subject.

[b] The concentration in prostatic fluid was below the quantification limit for ciprofloxacin for 1 subject.

[c] No sample was available for concentration measurement for 2 subjects. One subject had a concentration below the quantification limit both for levofloxacin and ciprofloxacin.

[d] n = 8 for levofloxacin and n = 7 for ciprofloxacin. Statistical comparison performed for n = 7.

[e] Comparison of the body fluid concentrations of levofloxacin and ciprofloxacin in tissue. Analysis was performed on log scale.

[f] Values represent model-fitted concentrations.

[g] Geometric mean and CV for the same number of subjects who had body fluid concentrations available.

Table 5. Body fluid to serum concentration ratios at 3 h after oral co-administration of 250 mg levofloxacin and 250 mg ciprofloxacin in healthy subjects

	n	Body fluid to serum concentration ratio (–)				paired t test p value
		median and range		geometric mean and % CV		
		levofloxacin	ciprofloxacin	levofloxacin	ciprofloxacin	
Prostatic fluid	8/7	0.506 (0.409–0.774)	0.248 (0.152–0.718)[b]	0.523 (22)	0.295 (69)	0.010[d]
Ejaculate	8	1.85 (1.24–3.17)	4.85 (2.93–10.2)	1.89 (35)	4.96 (44)	<0.001
Seminal fluid	8	1.90 (1.25–3.07)	4.77 (2.88–9.73)	1.91 (34)	4.89 (43)	<0.001
Sperm cells	5[c]	0.031 (0.026–0.237)	0.101 (0.075–0.697)	0.057 (106)	0.179 (101)	<0.001
Vaginal fluid (n = 7)[a]	7	0.300 (0.137–3.79)[a]	0.305 (0.120–20.2)[a]	0.395 (167)	0.414 (245)	0.873
Vaginal fluid (n = 6)[a]	6	0.282 (0.137–0.440)	0.244 (0.120–0.328)	0.271 (42)	0.216 (41)	0.026

[a] One subject had a substantially higher concentration in vaginal fluid both for ciprofloxacin and for levofloxacin (presumably due to urinary contamination of the tampon). Therefore, the results are shown both with and without this subject.

[b] The concentration in prostatic fluid was below the quantification limit for ciprofloxacin for 1 subject.

[c] No sample was available for concentration measurement for 2 subjects. One subject had a concentration below the quantification limit both for levofloxacin and ciprofloxacin.

[d] n = 8 for levofloxacin and n = 7 for ciprofloxacin. Statistical comparison performed for n = 7.

proximately 3 h. The predicted peak concentrations of levofloxacin in the tubulus compartment were more than 5-fold lower than the Km of 6,830 μmol/l for reabsorption of levofloxacin. This suggested levofloxacin did not inhibit the reabsorption of ciprofloxacin.

Simulations from the model indicated that the typical apparent total clearance of levofloxacin changed by 5% or less for oral levofloxacin doses from 125 to 1,000 mg and ciprofloxacin doses from 125 to 1,000 mg. For 250 mg of each quinolone, the model predicted an increase in the apparent total clearance of levofloxacin to 1.024 times the estimate for levofloxacin alone. The median (range) for this ratio in the 14 subjects studied was 1.051 (0.888–1.179) and the geometric mean was 1.039 (9% CV). Total

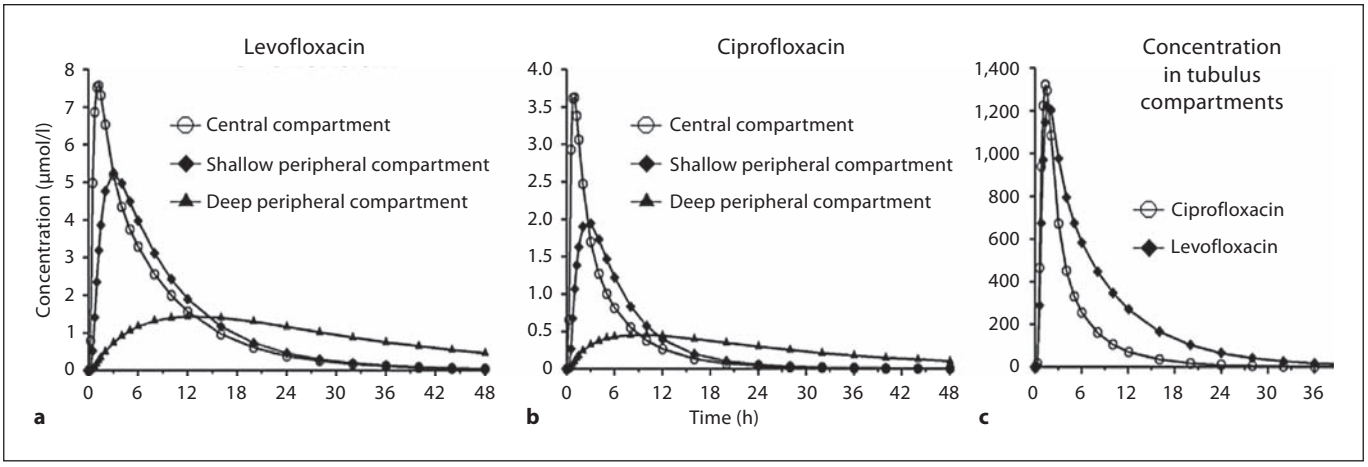

Fig. 4. Simulated concentrations for levofloxacin (**a**) and ciprofloxacin (**b**) and the tubulus (**c**) after a single oral dose of 250 mg levofloxacin and 250 mg ciprofloxacin (co-administered). For ciprofloxacin, 1 mg/l is equivalent to approximately 3.02 μmol/l; for levofloxacin, 1 mg/l is equivalent to approximately 2.77 μmol/l.

clearance of ciprofloxacin was less than 5% higher under co-administration of 125 to 1,000 mg oral ciprofloxacin with 1,000 mg oral levofloxacin compared to the respective ciprofloxacin dose given alone. Therefore, the effect of levofloxacin on the relative bioavailability of ciprofloxacin (+12% with 250 mg levofloxacin) had a more pronounced effect on ciprofloxacin exposure than the interaction at the renal tubule.

Discussion

This study focused on characterizing the extent, sites, potential mechanism, and time course of the interaction between ciprofloxacin and levofloxacin. If these quinolones show a mutual inhibition of elimination, then clearance changes over time and the assumptions of standard non-compartmental analysis are not being met for the co-administration treatment [35]. We applied a semi-physiological population PK model that could handle the full time course of interaction. This model suggested ciprofloxacin inhibited the reabsorption of levofloxacin from the renal tubulus compartment, since ciprofloxacin had a 12-fold higher affinity (see Km values in table 3) for a putative reabsorption transporter. This increased the renal clearance of levofloxacin during co-administration with ciprofloxacin. Levofloxacin has been shown to undergo reabsorption from the renal tubule in humans and in rats [10, 11]. Little is known about the specific transporters that potentially contribute to this reabsorption. Reabsorption of levofloxacin is not affected by tetraethylammonium, cimetidine, or *p*-aminohippurate in rat [11].

The final model (fig. 1) yielded simulated peak concentrations in the tubulus compartment for ciprofloxacin that were approximately 2.3 times higher than the Km for the reabsorption transporter (fig. 4c; table 3), whereas this peak concentration was approximately 18% of the Km for levofloxacin.

Population PK modeling could not clearly distinguish whether a competitive, uncompetitive, noncompetitive, or mixed interaction best describes the data, probably because the overall extent of interaction was small at the studied doses. As we only had interaction data at one dose level, the competitive interaction model was chosen as the simplest model following the rule of parsimony. Interaction studies over a range of doses and various dosage regimens, such as intermittent dosing and continuous infusion of both quinolones, would support a better identification of the mechanism of interaction. It is a limitation of this study that we did not perform transporter studies to better support the mechanism of interaction. The assumption that Vmax of the reabsorption transporter is the same for ciprofloxacin and levofloxacin also presents a potential limitation of the model. However, the interaction model should give a reasonable estimate for the relatively small extent of interaction at clinical doses.

A competitive interaction at the renal tubules for levofloxacin and ciprofloxacin is in agreement with literature data. Quinolones are subject to active tubular secretion

[12, 36–41] and levofloxacin is known to undergo tubular reabsorption, which may also play a role for ciprofloxacin [11, 42]. Additionally, temafloxacin shows a nonlinear increase in renal clearance with urine flow [36]. Given the high quinolone concentrations in the renal tubules, an interaction in tubular reabsorption seems possible. A competitive interaction model represents the case of two structurally similar quinolone molecules competing for the same reabsorption transporter.

As a second site of interaction, the relative extent of bioavailability of ciprofloxacin was increased by 12% (table 3) under co-administration with 250 mg levofloxacin compared to ciprofloxacin alone. Modeling identified this increased bioavailability as the most likely reason for the decreased apparent nonrenal clearance of ciprofloxacin during simultaneous administration of both quinolones. This might be explained by levofloxacin inhibiting a transporter secreting ciprofloxacin from mucosal cells into the gastrointestinal lumen [43] as observed for the inhibition of intestinal secretion of ciprofloxacin by grepafloxacin [44]. Based on studies on active intestinal secretion [7, 45–47], levofloxacin might have inhibited the intestinal secretion of ciprofloxacin. Alternatively, bioavailability for the 250 mg ciprofloxacin dose might have been higher, as this smaller dose might be dissolved and absorbed more efficiently in the gastrointestinal tract. However, Plaisance et al. [48] found a similar extent of bioavailability for 200 and 750 mg oral ciprofloxacin. A physicochemical interaction of both quinolones in the gastrointestinal tract seems less likely [49]. Further studies are required to assess the mechanism of this interaction.

The final model had excellent predictive performance (fig. 3). Saturation of renal reabsorption clearance in the model caused a $\leq$10% change in apparent total clearance at oral doses of 250 versus 1,000 mg ciprofloxacin or levofloxacin, when each quinolone was given separately. Therefore, the model is in agreement with literature data which show that levofloxacin [12, 30, 49, 50] and ciprofloxacin [39, 48, 51–53] display linear PK over the dose range employed in the present study. Serum and urine data over a much wider dose range are probably required to identify a potential saturation of tubular secretion clearance. Tubular secretion presents another potential site of interaction of these quinolones. As peak ciprofloxacin concentrations for the 500 mg dose in this study were approximately 2.8-fold lower than the Michaelis-Menten constant for saturable renal clearance (6.45 mg/l) estimated by Landersdorfer et al. [54], this study was unlikely to find a notable saturation of tubular secretion clearance. Due to the small extent of interaction, the present dataset did not support estimation of models with saturable tubular secretion in addition to a saturable reabsorption.

The PK parameter estimates (tables 2, 3) were in good agreement with estimates for levofloxacin [55–59] and ciprofloxacin [60, 61] from the literature. As a potential limitation of this study, the relatively high number of model parameters and small sample size (n = 15) might have increased the uncertainty of some parameter estimates such as Vmax and Km, as shown by the confidence intervals in table 3.

Levofloxacin and ciprofloxacin are key antibiotics for treatment of urogenital infections. However, emergence of resistance is becoming increasingly important in many countries, and not only for urogenital infections by *E. coli* [62–64]. Ciprofloxacin and levofloxacin both primarily target DNA gyrase in *E. coli* and *P. aeruginosa* [65–67], and therefore one would not expect a clinical benefit for the combination of these two quinolones. However, studying this combination was helpful to assess the mechanisms of PK interaction. Studying the combination of two quinolones allows one to potentially saturate a transporter by the drug with higher affinity for a transporter. Given the small extent of interaction between ciprofloxacin and levofloxacin, this interaction was not clinically relevant.

As the second aim of this study, the penetration of both quinolones into body fluids relevant for urogenital infections was assessed. Fluoroquinolones usually achieve tissue and body fluid concentrations that are higher than those of most β-lactams due to their physicochemical properties [68–74]. Our group applied body fluid penetration models for other quinolones and β-lactams [75–81]. Studies of unbound antibiotic concentrations in these body fluids would be preferable [82–84]; however, we are not aware of validated assays for unbound antibiotic concentrations in these body fluids and studied total concentrations using validated assays (table 1). The protein binding of ciprofloxacin and levofloxacin in serum is similar and relatively low (approximately 30%). Therefore, even a moderately higher or lower protein binding in body fluids compared to serum will not substantially affect the unbound body fluid concentrations. Also the physicochemical properties of ciprofloxacin and levofloxacin are comparable. For levofloxacin pKa_1 is 6.05, pKa_2 8.22, and the isoelectric point 7.14. For ciprofloxacin, pKa_1 is 6.09, pKa_2 8.74, and the isoelectric point 7.42. The octanol/water partition coefficient (log D) is –0.50 for levofloxacin and –1.24 for ciprofloxacin [49].

While PK/pharmacodynamic target values for quinolones in these body fluids (table 4) are unknown, this study contributes information from a PK perspective. Direct comparison of tissue homogenate concentrations with MICs are usually considered meaningless [84], because such crude concentrations are a mixture of different entities like plasma, interstitial, intracellular and transcellular fluids (glandular secretion). Concentrations in well defined body fluids may well have clinical relevance. The transcellular penetration of antimicrobials into the prostatic and seminal fluids shows great differences between substances [85] and therefore a different ability to eradicate pathogens from these sites can be assumed. Additionally, determinations of drug concentrations in sperm cells could be clinically relevant in regard to teratogenicity.

The body fluid penetration data in table 5 are in good agreement with the results from literature on quinolones. The median body fluid to serum concentration ratios of various quinolones ranged from 0.10 to 1.29 for prostatic fluid [71, 75–77, 80, 81, 85–87], from 1.0 to 7.1 for seminal fluid [71, 75–77, 80, 81, 85], and from 1.0 to 8.4 for ejaculate [77, 81]. Our experimental procedure did not involve micturition before sampling of body fluids, since we wanted to minimize the risk for contamination of body fluids with urine. Such contamination would have caused artificially elevated body fluid concentrations. The present results on sperm cell penetration (table 5) were in good agreement with the low sperm cell concentrations of gatifloxacin [81]. While the present study does not contribute data on the rate of tissue penetration, modeling of the penetration of levofloxacin [88] and ciprofloxacin [89] into prostate tissue showed that the rate of equilibration between prostate tissue and serum is fast for both quinolones.

In summary, this study indicated that ciprofloxacin increased the renal clearance of levofloxacin by 13% and that levofloxacin decreased the apparent nonrenal clearance of ciprofloxacin by 23% for co-administration of 250 mg oral ciprofloxacin and 250 mg oral levofloxacin compared to the respective quinolones given alone. Population PK analysis suggested that ciprofloxacin inhibited the tubular reabsorption of levofloxacin, since ciprofloxacin was estimated to have a 12-fold higher affinity for this transporter. Levofloxacin was estimated to increase the extent of bioavailability of ciprofloxacin by 12%. Simulations from the model over a wide dose range suggested that the extent of interaction is not clinically relevant. Levofloxacin achieved significantly higher concentrations compared to ciprofloxacin in prostatic fluid, vaginal fluid, ejaculate, and seminal fluid. Further studies are warranted to assess the time course of (unbound) fluoroquinolone concentrations in these body fluids and to compare the effectiveness of ciprofloxacin and levofloxacin in urogenital infections.

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The data of treatments A and B have been published without any data from treatment C and without any population PK modeling as a research paper [96].

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