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Ex Vivo Antibacterial Properties of Rufloxacin Compared with Those of Norfloxacin in a Study with Healthy Volunteers

L. AGUILAR,[1]* I. P. BALCABAO,[2] P. SALVÁ,[3] M. MARTÍN,[2] J. COSTA,[3] J. PRIETO,[2] AND R. DAL-RÉ[1]

Medical Department, SmithKline Beecham Pharmaceuticals,[1] and Microbiology Department, Universidad Complutense,[2] Madrid, and Clinical Pharmacology Department, Hospital Universitario Germans Trias i Pujol, Badalona,[3] Spain

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Twelve adult males participated in a randomized crossover phase I clinical trial comparing serum bactericidal titers (SBTs), urine bactericidal titers (UBTs), and urine killing rates (UKRs) against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213, after the administration of single 400-mg doses of rufloxacin and norfloxacin at different times up to 72 h postdose. SBTs were significantly higher ($P < 0.05$) against *E. coli* from 8 to 48 h and against *S. aureus* from 4 to 24 h with rufloxacin. UBTs for *E. coli* were higher ($P < 0.05$) for norfloxacin at early sample times (0 to 8 h) but higher for rufloxacin ($P < 0.05$) at sample times from 16 h on for both *E. coli* and *S. aureus*. Similar UKRs were obtained for both quinolones for 0 to 2 h and 8 to 12 h, but the UKR was maintained for 72 h with rufloxacin. The high and sustained mean levels of rufloxacin in urine (>35 μg/ml), median UBTs (>32 for *E. coli* and 16 for *S. aureus*) and UKRs for *E. coli* suggest prolonged urine antibacterial activity (for at least 72 h) and its use as a single 400-mg dose in the treatment of uncomplicated cystitis.

Rufloxacin is a broad-spectrum quinolone (24) that is less active in vitro than norfloxacin against *Escherichia coli* (33) and that exhibits a prolonged elimination half-life as a major pharmacokinetic feature (35). Its in vitro activity (7, 33), in conjunction with its pharmacokinetic profile (11), suggest that rufloxacin may well be of clinical use in the treatment of urinary tract infections (33).

Rufloxacin and norfloxacin differ in both their pharmacokinetics and their in vitro activities. Measurement of ex vivo bactericidal activity allows for a direct comparison of pharmacodynamic properties (12) in the evaluation of new drugs (2). The area under the bactericidal curve (AUBC) is a sensitive index of the pharmacodynamic effects of a drug (17) and may provide an accurate guide to dosage (8) for those agents exhibiting concentration-dependent killing (25).

Taking into account the fact that the measurement of the antibacterial activity in urine correlates directly with the outcome of infection (16) more than the determination of MICs and MBCs and the levels of antibiotics in urine (4) do, we evaluated the efficacy of rufloxacin versus that of norfloxacin by assessing by urine bactericidal titers (UBTs), serum bactericidal titers (SBTs), and the rate of killing in urine (UKRs) to explore the potential clinical use of rufloxacin in the treatment of urinary tract infections.

MATERIALS AND METHODS

Subjects and study design. Twelve healthy male volunteers participated in the randomized crossover phase I clinical trial described here. They received single oral doses (400 mg) of rufloxacin and norfloxacin separated by a 14-day washout period. The protocol was approved by the Clinical Trials Committee of Hospital Universitario Germans Trias i Pujol. Written informed consent was obtained from all subjects before their inclusion in the study.

The volunteers had the following characteristics (mean ± standard deviation): age, 24.1 ± 2.7 years; height, 174.9 ± 6.7 cm; weight, 70.9 ± 7.4 kg. General physical examination and laboratory tests (complete blood counts, blood chemistry tests, and urinalyses) were performed before entry into the study, daily (for physical examination) for 3 days, and a week after the administration of each antibiotic.

Drug dosing and sampling procedure. During each treatment period, drugs were orally administered with 150 ml of water. Serum samples (15 ml) were collected before (0 h) and at 2, 4, 6, 8, 12, 24, 48, and 72 h after drug administration. Serum was separated for bioassay and for determination of SBTs. Urine samples were collected prior to drug administration (0 h) and at intervals of 0 to 2, 2 to 4, 4 to 8, 8 to 12, 12 to 16, 16 to 24, 24 to 36, 36 to 48, 48 to 60, and 60 to 72 h after dosing for bioassay and for determination of UBTs in all volunteers and UKRs in randomly selected volunteers. Serum and an aliquot of the recorded excreted volumes of urine were stored at −20°C until testing.

Microbiological determinations. The rufloxacin and norfloxacin used in the study were provided by the manufacturers (Mediolanum, Milan, Italy, and Merck Sharp & Dohme, Madrid, Spain, respectively). In vitro susceptibility testing was performed five times each for *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 by standardized methods (20).

SBTs and UBTs were determined against reference strains *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 by the microdilution technique (36) with microtiter plates. Serum and urine samples from each volunteer were diluted in noninactivated human serum or urine, respectively, obtained from each volunteer before drug administration and containing 20% Iso-Sensitest broth (Oxoid, Basingstoke, United Kingdom). The final volume of each well was 100 μl. The final inoculum was 10^5 CFU/ml. The inoculated plates were incubated at 37°C for 18 h and were subcultivated in antibiotic-free Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.), which was incubated at 37°C for 18 h. The bactericidal endpoints were defined as the highest dilution of serum or urine killing 99.9% of the original inocula.

UKRs for *E. coli* ATCC 25922 were determined in urine obtained from six randomly selected volunteers at sample times of 0 to 2, 8 to 12, and 60 to 72 h after the administration of both the rufloxacin and the norfloxacin doses. A modification of the method used by Krogstad and Moellering (18) was used with a 2-ml volume of sample, to which the same volume of Iso-Sensitest broth was added. The mixtures were homogenized in 25-ml sterile polystyrene tubes, to which 400 μl of a culture of *E. coli* in the logarithmic growth phase was added, giving a final inoculum of 10^7 CFU/ml. All of the tubes were incubated at 37°C in a shaking bath. Appropriate decimal dilutions were made in sterile saline solution for the determination of viable bacterial counts at 0, 1, 2, 3, and 4 h from the start of incubation. Aliquots of 20 μl of each dilution were subcultivated in Iso-Sensitest agar, and the plates were incubated at 37°C for 18 to 24 h. CFU counting was performed to measure the number of viable bacteria per milliliter at each time.

Rufloxacin and norfloxacin levels were determined by bioassay. The indicator organisms were *E. coli* ISF 432 for rufloxacin (11) and *E. coli* ATCC 25922 for norfloxacin; the organisms were inoculated in antibiotic agar N1 (Difco) plates. A total of 60 μl of each sample was deposited into 10-mm-diameter wells in the inoculated plates, and the plates were then incubated at 37°C for 18 h. For serum assays, rufloxacin and norfloxacin standards from 10 to 0.25 μg/ml were prepared in pooled serum obtained from volunteers before administration of the drugs. Rufloxacin standards of 100, 50, 25, 10, and 5 μg/ml and norfloxacin standards of 400, 200, 100, 50, and 25 μg/ml in pooled urine obtained from the volunteers

* Corresponding author. Mailing address: Medical Department, SmithKline Beecham Pharmaceuticals, Valle de la Fuenfría No. 3, 28034 Madrid, Spain. Phone: 34-1-734.60.00. Fax: 34-1-372.14.90.

TABLE 1. Levels of drug in serum and SBTs

Time (h)	Rufloxacin				Norfloxacin			
	No. of serum samples[a]	Level in serum (μg/ml)[b]	SBT[c]		No. of serum samples[a]	Level in serum (μg/ml)[b]	SBT[c]	
			E. coli (n = 12)	*S. aureus* (n = 12)			*E. coli* (n = 12)	*S. aureus* (n = 12)
2	12	4.6 ± 0.7	8 (4–16)	2 (2–8)	12	1.1 ± 0.3	8 (4–16)	2 (<2–8)
4	12	4.2 ± 0.7	4 (4–16)	2 (<2–4)	12	0.7 ± 0.4	4 (2–8)	2 (<2–4)
6	12	4.2 ± 0.9	4 (4–16)	2 (2–8)	9	0.5 ± 0.2	2 (<2–8)	<2 (<2–2)
8	12	4.0 ± 0.8	4 (2–8)	2 (<2–4)	8	0.3 ± 0.1	2 (<2–4)	<2 (<2–2)
12	12	3.7 ± 0.7	4 (2–8)	1 (<2–4)	4	0.2 ± 0.1	<2 (<2–2)	<2 (<2–<2)
24	12	3.3 ± 0.8	3 (2–8)	1 (<2–4)	1	0.1	<2	<2
48	12	2.2 ± 0.9	2 (<2–4)	<2 (<2–2)	0			
72	10	0.9 ± 0.5	<2 (<2–2)	<2 (<2–<2)	0			

[a] Number of serum samples with antibiotic concentrations over the detection limit (0.50 and 0.12 μg/ml for rufloxacin and norfloxacin, respectively).
[b] Values are means ± standard deviations.
[c] Values are medians (ranges).

before drug administration were used in urine assays. The Microstat program (Ecosoft, Inc., Indianapolis, Ind.) was used to determine the assay regression line (standard curve) and to extrapolate the antibiotic concentrations from the corresponding inhibition zone diameters. Reproducibility between days was 3, 6, and 6% for concentrations of 0.62, 2.5, and 5 μg/ml, respectively, for the bioassay of rufloxacin in serum; 4, 5, and 10% at concentrations of 1.5, 6.25, and 50 μg/ml, respectively, for the bioassay of rufloxacin in urine; 2, 4, and 8% at concentrations of 0.5, 1.7, and 2.3 μg/ml, respectively, for the assay of norfloxacin in serum; and 8, 8, and 12% at concentrations of 25, 100, and 400 μg/ml, respectively, for the assay of norfloxacin in urine. The lower limits of detection were 0.50 and 1.25 μg/ml for assays of rufloxacin in serum and urine, respectively, and 0.12 and 0.5 μg/ml for assays of norfloxacin in serum and urine, respectively.

Pharmacokinetic analysis. Pharmacokinetic analysis was performed by using the MK model program (9) as the software package for pharmacokinetic data modeling and the PKCALC program for pharmacokinetic data analysis (28), assuming complete bioavailability for these two drugs, which are intended only for oral administration. The concentrations of rufloxacin and norfloxacin in plasma were well-fitted into a one-compartment open model with first-order absorption (9). The log likelihood criterion with the MK model (an extended least-squares modeling program) allowed the choice of the one-compartment model (9).

Statistical analysis. The comparison of the bactericidal titers in serum and urine was performed by analysis of the two-way variance (treatment and phase of the trial) by repeated measures. In relation to determination of the UKR, the rates of decrease of the initial inoculum [$100 - (100\ \text{inoculum}_{x\ \text{h}}/\text{inoculum}_{0\ \text{h}})$] were compared by two-way variance analysis for repeated series and by Student's t test.

RESULTS

Rufloxacin and norfloxacin were well tolerated by the volunteers, with no clinically significant changes in the variables studied. Three volunteers experienced mild adverse events: in two volunteers following the administration of the norfloxacin dose (elevation of serum glutamic pyruvic transaminase levels and abdominal pain) and in one volunteer after the intake of rufloxacin (sensation of abdominal distension). The relationship to the study medication was assessed as unrelated in the first volunteer and was probably unrelated in the other two volunteers.

Modal MICs and MBC were 1 and 2 μg of rufloxacin per ml, respectively, for *E. coli* ATCC 25922, 4 and 4 μg of rufloxacin per ml, respectively, for *S. aureus* ATCC 29213, 0.125 and 0.25 μg of norfloxacin per ml, respectively, for *E. coli* ATCC 25922, and 0.5 and 1 μg of norfloxacin per ml, respectively, for *S. aureus* ATCC 29213.

The levels of the drugs in serum and SBTs over time are presented in Table 1. Before drug administration, the levels of drug in serum and SBTs were below the limit of detection (SBT, <2). Rufloxacin was found to be present at higher levels in serum ($P < 0.001$) than norfloxacin at all sampling times. SBTs against *E. coli* ATCC 25922 were significantly higher ($P < 0.01$) for rufloxacin than for norfloxacin at sample times of 8, 12, 24, and 48 h, while against *S. aureus* ATCC 29213, the SBT of rufloxacin was significantly ($P < 0.05$) higher than that of norfloxacin at 4, 6, 8, 12, and 24 h.

The levels of the drugs in urine and UBTs are given in Table 2. Before drug administration, the levels of the drugs in urine

TABLE 2. Levels of drug in urine and UBTs

Time (h)	Rufloxacin				Norfloxacin			
	No. of urine samples[a]	Level in urine (mg/ml)[b]	UBT[c]		No. of urine samples[a]	Level in urine (mg/ml)[b]	UBT[c]	
			E. coli (n = 12)	*S. aureus* (n = 12)			*E. coli* (n = 12)	*S. aureus* (n = 12)
0–2	12	44.7 ± 19.7	64 (4–512)	16 (2–512)	12	167.0 ± 100.0	256 (128–1,024)	128 (32–256)
2–4	12	44.1 ± 12.1	64 (16–256)	16 (8–64)	12	85.0 ± 42.3	256 (64–256)	64 (8–256)
4–8	12	36.0 ± 5.2	64 (16–64)	16 (2–32)	12	58.8 ± 27.4	128 (32–256)	32 (4–128)
8–12	12	43.5 ± 22.2	32 (16–512)	16 (2–128)	12	41.1 ± 21.9	96 (32–256)	8 (4–32)
12–16	12	43.5 ± 12.3	64 (16–128)	16 (8–64)	12	26.6 ± 19.5	32 (16–128)	8 (4–32)
16–24	12	45.2 ± 10.5	64 (16–256)	16 (4–128)	12	15.6 ± 14.0	16 (4–64)	4 (<2–32)
24–36	12	48.3 ± 22.3	64 (4–1,024)	16 (2–256)	12	4.8 ± 2.4	6 (<2–16)	2 (<2–8)
36–48	12	47.0 ± 8.1	64 (32–128)	16 (4–64)	11	2.8 ± 2.1	2 (<2–16)	<2 (<2–2)
48–60	12	37.2 ± 9.3	48 (4–128)	16 (2–32)	4	1.0 ± 0.5	<2 (<2–2)	<2 (<2–2)
60–72	12	38.5 ± 8.9	64 (16–128)	16 (8–16)	1	1	<2	<2

[a] Number of urine samples with antibiotic concentrations over the detection limit (1.25 and 0.5 μg/ml for rufloxacin and norfloxacin, respectively).
[b] Values are means ± standard deviations.
[c] Values are medians (ranges).

and UBTs were below the limit of detection (UBT, <2). Significantly ($P < 0.05$) higher levels of norfloxacin were found in urine at 0 to 2, 2 to 4, and 4 to 8 h, and from 12 to 72 h significantly higher levels of rufloxacin were found in urine. Similarly, bactericidal activity was higher ($P < 0.05$) for norfloxacin at early sample times (0 to 2, 2 to 4, and 4 to 8 h), whereas UBTs were higher ($P < 0.01$) for rufloxacin at sample times from 16 h on when the test strain was *E. coli* ATCC 25922. Bactericidal activity was similar for both quinolones at early sample times when *S. aureus* ATCC 29213 was used, but they were significantly higher ($P < 0.05$) for rufloxacin at sample times from 16 h on.

Pharmacokinetic and pharmacodynamic parameters are presented in Table 3. Norfloxacin was more rapidly absorbed than rufloxacin, with the time to the maximum concentration in serum occurring at 2 h for norfloxacin versus 3.5 h for rufloxacin. With the same oral dose, a higher maximum concentration in serum was achieved with rufloxacin (4.9 μg/ml versus 1.1 μg/ml, for norfloxacin). The mean terminal half lives in serum were 28 h for rufloxacin and 2.8 h for norfloxacin, with a greater area under the concentration-time curve from time zero to infinity ($AUC_{0-\infty}$) for rufloxacin in serum (238.7 versus 5.9 μg · h/ml for norfloxacin) and a greater area under the concentration-time curve from 0 to 72 h (AUC_{0-72}) for rufloxacin in urine (221,105 versus 166,037 μg · h/ml for norfloxacin).

With respect to pharmacodynamic parameters, similar magnitudes of urine AUBCs were obtained for both quinolones for each strain tested, but a higher serum AUBC was obtained for rufloxacin with *E. coli* ATCC 25922 (171 versus 29 for norfloxacin) and *S. aureus* ATCC 29213 (45 versus 8 for norfloxacin).

The rate of killing in urine samples (Table 4) was similar for sample times of 0 to 2 and 8 to 12 h for both drugs at the different CFU counting points (1 to 4 h), resulting in a reduction of approximately 99.9% of the initial inoculum ($\cong 10^7$ CFU/ml) after 4 h of incubation. With rufloxacin this rate of killing was maintained at sample times of 60 to 72 h, but this was not the case for norfloxacin; at 60 to 72 h no norfloxacin was detected in the urine of these six volunteers.

TABLE 3. Pharmacokinetic and pharmacodynamic parameters[a]

Drug and compartment	C_{max} (μg/ml)[b]	T_{max} (h)[b]	$t_{1/2}$ (h)[b]	AUC (μg · h/ml)[b]	CL/F (ml/min/kg)[b]	V/F (liters/kg)[b]	MRT (h)[b]	A_e (μg)[b]	AUBC[c]	
									E. coli	*S. aureus*
Rufloxacin										
Serum	4.9 ± 0.8	3.5 ± 2.11	28.0 ± 15.5	238.7 ± 91.5	0.5 ± 0.2	1.0 ± 0.2	43.3 ± 20.6		171 (82–320)	45 (18–178)
Urine		8.8 ± 4.8		221,105.2 ± 88,004.7			28.2 ± 3.6	9,618.5 ± 5,580.6	613 (369–3,231.4)	167 (131.48–1,288.2)
Norfloxacin										
Serum	1.1 ± 0.3	2.0 ± 0	2.8 ± 2.1	5.9 ± 3.1	20.1 ± 10.0	4.2 ± 1.2	5.3 ± 2.7		29 (20–92)	8 (0–34)
Urine		4.3 ± 2.8		166,037.5 ± 66,311.9			7.8 ± 1.1	23,776.0 ± 14,103.2	844.62 (351.64–1,916.4)	265.02 (66.54–898.44)

[a] C_{max}, maximum concentration of drug in serum; T_{max}, time to maximum concentration of drug in serum; $t_{1/2}$, half-life; AUC, area under the concentration-time curve (time zero to infinity for serum and time zero to 72 h for urine); CL/F, clearance/bioavailability; V/F, volume of distribution/bioavailability; MRT, mean residence time; A_e, maximum quantity excreted in urine in relation to time to maximum concentration of drug in serum.

[b] Values are means ± standard deviations.

[c] Values are medians (ranges).

DISCUSSION

The pharmacokinetic parameters of rufloxacin found in the present study were similar to those found by others (15), and minor differences may be due to the different detection systems used (15). Similarly, differences in rufloxacin levels measured by high-performance liquid chromatography and bioassay have been reported (11). The major pharmacokinetic differences between these two quinolones after the administration of the same oral dose were the 4.5 times higher maximum concentration of rufloxacin in serum and the 40 times greater serum AUC of rufloxacin versus those of norfloxacin. This latter pharmacokinetic factor leads to at least 5.6 times greater serum AUBC of rufloxacin for the two strains tested, although in vitro susceptibility favors norfloxacin (four to eight times on the basis of the MBC).

The levels of drugs in serum correlate poorly with the response to bacteriuria (30). By using a cutoff ≥ 8 for SBTs as adequate for a trough level (21), the results obtained in the present study suggest that neither of the two drugs tested are adequated for use in the treatment of systemic infections: we obtained an SBT of ≥ 8 only at the first sample time (2 h) for rufloxacin against *E. coli* ATCC 25992. The same conclusion was obtained when a 24-h area under inhibitory curve ($AUC_{0-24\ h}$/MIC) of ≥ 125 was used as the breakpoint for the probability of clinical and microbiological cure (6), since the area under

TABLE 4. UKRs at three sample times for *E. coli* ATCC 25922 after 1 to 4 h of incubation

Time (h)	Mean ± SD (range) UKR (% decrease of initial inoculum)[a]					
	Rufloxacin			Norfloxacin		
	0–2 h	8–12 h	60–72 h	0–2 h	8–12 h	60–72 h
1	88.37 ± 7.64 (73.70–94.25)	87.27 ± 8.88 (71.16–96.61)	90.31 ± 4.70 (83.40–96.02)	89.68 ± 4.83[b] (84.86–98.55)	95.85 ± 3.0[b] (91.49–99.22)	0
2	98.33 ± 1.02 (96.69–99.29)	98.39 ± 0.98 (97.12–99.37)	98.66 ± 0.91 (96.98–99.34)	97.81 ± 0.91[b] (96.84–99.38)	99.27 ± 0.57[b] (98.62–99.85)	0
3	99.63 ± 0.15 (99.42–99.85)	99.72 ± 0.14 (99.52–99.87)	99.75 ± 0.52 (99.55–99.89)	99.44 ± 0.22 (99.07–99.69)	99.68 ± 0.33 (99.02–99.90)	0
4	99.86 ± 0.1 (99.68–99.94)	99.92 ± 0.03 (99.90–99.96)	99.86 ± 0.08 (99.72–99.95)	99.83 ± 0.06 (99.75–99.92)	99.89 ± 0.02 (99.86–99.93)	0

[a] UKRs were determined with urine from six volunteers. The initial inoculum was $\cong 10^7$ CFU/ml.
[b] $P < 0.05$.

the inhibitory curve is less than 125 for both drugs (87.27 versus 47.44 for *E. coli* and 21.82 versus 11.86 for *S. aureus* for rufloxacin and norfloxacin, respectively). This fact may have clinical implications because the susceptibilities of clinical isolates (33) are similar to those of the strains tested in the present study.

The protein bindings of rufloxacin and norfloxacin are 80% (11) and 14% (14), respectively. Considering that only the free drug is microbiologically active (3), the use of broth as a diluent might produce a false elevation in SBT (23). To avoid this problem, the use of human serum has been recommended (1, 27). In the present trial, each volunteer's own pretreatment serum or urine was used as the diluent in determining SBTs and UBTs, respectively, in order to simulate the in vivo conditions. The endogenous bactericidal activity of serum (19) can be ruled out since bactericidal titers before drug administration were <2.

Similar urine AUCs were found (the urine AUC for rufloxacin was 1.3 times that for norfloxacin) because of the sustained levels ($\cong$40 μg/ml) all along the sampling interval for rufloxacin and the very high norfloxacin concentration at early sampling times. Although MBCs favor norfloxacin (eight times lower than that of rufloxacin for the *E. coli* strain and four times lower than that of rufloxacin for the *S. aureus* strain), urine AUBCs were similar for both drugs and each strain. The explanation of this fact may be in the relation between the estimated bactericidal titers (drug level/MBC) and the experimental titers. While experimental bactericidal titers of norfloxacin in serum and urine for both strains and that of rufloxacin for the *S. aureus* strain were similar to the expected ones (1 dilution of the experimental median titers above or below the value of the expected titer), the bactericidal titers of rufloxacin for *E. coli* ATCC 25922 that were actually determined were approximately four times higher than the expected ones for all of the urine samples and for the first serum sample. This higher, unexpected UBT of rufloxacin against *E. coli* can be explained by the presence of low concentrations ($\cong$5 μg/ml) in urine of the active *N*-desmethyl derivative (15) that were greater than the MIC. The similar activities of the derivative and rufloxacin against *E. coli* (33) may result in a sinergistic effect against this strain. On the other hand, an increase in the expected bactericidal activity was not observed against *S. aureus*, probably because of the absence of a sinergistic effect because the derivative has activity about eight times less than that of rufloxacin against *S. aureus* isolates (33), and therefore, the concentrations of the derivative in urine are below its MIC. Other quinolones have metabolites with lower levels of activity than the parent compound and that are present in smaller amounts (<1%) (34).

Although single-dose therapy that achieves high concentrations in urine that last for at least 12 to 24 h eliminates bladder infection (29), therapy for 3 days or longer is more effective (10), especially when a single dose is used against bacteria of the genus *Staphylococcus* such as *S. saprophyticus* (26). If we consider adequate a UBT similar to the SBT ($\geq$8) (21), rufloxacin achieves eight times the adequate median bactericidal titer for at least 72 h against the *E. coli* strain and two times the adequate median bactericidal titer against the *S. aureus* strain during the same period, while norfloxacin achieves it for 16 to 24 h against the *E. coli* strain and 8 to 12 h against the *S. aureus* strain.

From the bactericidal killing kinetic point of view, all samples taken from the six volunteers at 0 to 2 h and 8 to 12 h reduced the initial inoculum of *E. coli* ATCC 25922 by $\cong$99.9% after 4 h of incubation. Conceptually, in the norfloxacin group we observed a significantly ($P < 0.05$) greater bactericidal

effect of samples obtained from 8 to 12 h, which contained lower drug levels, compared with that of samples obtained from 0 to 2 h, which contained higher drug levels. This ex vivo paradoxical effect may reflect the same effect detected in vitro (22, 32), because for norfloxacin, samples obtained from 0 to 2 h had higher drug levels than the optimum bactericidal concentrations (30 to 60 times the MIC of the selected compound) (5). The killing rate of urine samples obtained from 0 to 2 and 8 to 12 h was maintained in the samples obtained from 60 to 72 h with rufloxacin but not with norfloxacin, because nondetectable levels of norfloxacin were found in these volunteers at the interval of 60 to 72 h.

E. coli and *S. saprophyticus* are the main etiological microorganisms of uncomplicated cystitis in 80 and 5 to 15% of cases, respectively (13). Quinolone treatment of this entity is more effective when it lasts for 3 days or longer (10), particularly in patients infected with *S. saprophyticus* (26) and those with higher rates of early recurrences (31). From the results of the present phase I clinical trial, we conclude that a 400-mg single oral dose of rufloxacin provides at least 3 days of antimicrobial activity in urine against *E. coli* ATCC 25922, as determined from both UBT and UKR measurements and UBTs against *S. aureus*. Clinical trials are warranted to see if these results have clinical implications, especially with respect to *E. coli* recurrences and the eradication of *S. saprophyticus*, taking into account the fact that measurement of urine antibacterial activity correlates directly with outcomes of infection (16).

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