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Suspicion of Quinolone Active Metabolite Following Discrepancy between Predicted and Experimental Urine Bactericidal Activities

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The prediction of urine antibacterial activity from pharmacological and microbiological parameters was assessed by using experimental urine levels and urine bactericidal titers determined up to 72 h after a 400-mg single dose of two quinolones in a phase I study. The area under the bactericidal curve (AUBC) was accurately predicted for norfloxacin but significantly ($P < 0.001$) underestimated for rufloxacin (actual value was four times higher than the predicted value against *Escherichia coli* and two times higher against *Staphylococcus aureus*). In vitro susceptibility differences between the two strains predicted the ex vivo AUBC differences for norfloxacin but not for rufloxacin, where ex vivo differences were greater than expected. Urine bactericidal titers for up to 72 h were accurately predicted for norfloxacin against *E. coli* and *S. aureus* and for rufloxacin against *S. aureus*, but experimental activity for up to 48 h was four times higher ($P < 0.001$) than the predicted activity for rufloxacin against *E. coli*. In the case of norfloxacin, the duration of adequate urine antibacterial activity against *S. aureus* was overestimated. Inaccurate estimations of ex vivo antibacterial activity of a suspected active metabolite (as with rufloxacin) when an adequate cutoff is not established may have dosing implications.

The end point of antimicrobial treatment of a urinary tract infection is elimination of bacteria from the urinary tract, since symptoms disappear spontaneously even in the presence of bacteriuria (21). Disappearance of bacteriuria correlates with susceptibility of the etiological agent to the concentration of the antibiotic in urine (22), and results depend on high sustained urinary concentrations of the antibiotic (21). The problem arises when these concepts (high, sustained) are applied to urine antibacterial activity, a measurement that correlates directly with the outcome of infection (10). Urine bactericidal titers are a logical measuring tool for the magnitude of urine antibacterial activity, the area under the bactericidal curve (AUBC) being a sensitive index of the pharmacodynamic effect of a drug (11) for those agents exhibiting concentration-dependent killing, at least with serum determinations (16).

When serum bactericidal titers are considered, a cutoff of ≥1:8 is considered adequate (13), but few studies have addressed the question of optimal bactericidal activity of quinolones in serum (24) and none have addressed the problem of activity in urine. In a previous in vitro pharmacokinetic model with enoxacin, titers of ≥1:10 correlated with eradication of test organisms over a 28-h period of exposure to the quinolone (4). With respect to the duration of antibacterial activity, although antibiotics that achieve high urinary concentrations for at least 24 h may be used in single doses (giving basically 1-day therapy with regard to antimicrobial activity in urine) (21), different studies and authors suggest that 3-day therapy is a better treatment for cystitis (14, 18).

This study attempted to determine if predicted urine antibacterial activity, calculated from pharmacokinetic and microbiological parameters, correlates with experimental antibacterial activity. This information can be used to assess the dosing interval and duration of therapy.

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MATERIALS AND METHODS

We reviewed the data obtained in a randomized, crossover, controlled phase I study performed in April 1992 with healthy volunteers in which 400-mg single doses of rufloxacin and norfloxacin were administered to 12 volunteers with a 7-day washout period (2). Urine samples were collected prior to dosing and at the following intervals: 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. Urine bactericidal titers against *Escherichia coli* ATCC 25922 (rufloxacin, MIC = 1 μg/ml and minimal bactericidal concentration [MBC] = 2 μg/ml; norfloxacin, MIC = 0.125 μg/ml and MBC = 0.25 μg/ml) and *Staphylococcus aureus* ATCC 29213 (rufloxacin, MIC = 4 μg/ml and MBC = 4 μg/ml; norfloxacin, MIC = 0.5 μg/ml and MBC = 1 μg/ml) were measured.

MBCs and drug levels by bioassay using *E. coli* ISF 432 for rufloxacin and *E. coli* ATCC 25922 for norfloxacin were determined by standard methods (2, 12). Experimental urine bactericidal titers were determined by diluting the posttreatment urine sample in a liquid medium composed of 20% Iso-Sensitest broth and 80% pretreatment urine obtained from the subject. The final inoculum was 10^5 CFU/ml. Microdilution was the technique used for the determination (27).

Predicted antibacterial titers were calculated by the method described by Drusano et al. (6) and displayed as $1/2^n$, where n was the number of halvings (dilutions) for which the resultant antimicrobial concentration remained above the MBC. Experimental and predicted AUBCs were calculated from a plot of experimental and predicted urine bactericidal titers versus time without dividing by the number of hours of each interval and using as time points the middle value of each collection time period. The trapezoidal rule and the PKCALC program (20) were used for the calculation.

Statistical analysis was performed with the Wilcoxon signed-rank test for comparison of data on paired samples. A significance level of 0.001 was established for multiple comparisons (Bonferroni method) in order to have an overall significance level of $P < 0.05$.

RESULTS

Tables 1 and 2 show the predicted and experimental urine bactericidal titers (expressed as the denominator of the dilution), the AUBCs, and the ratio of experimental/predicted AUBCs and titers against *E. coli* and *S. aureus*, respectively. Experimental titers from 2 to 48 h were significantly higher ($P < 0.001$) than those predicted for rufloxacin against *E. coli* but not for norfloxacin against either strain at any sample time

TABLE 1. Predicted, experimental, and ratio of experimental/predicted urine bactericidal titers and AUBCs of norfloxacin and rufloxacin for *E. coli* ATCC 25922[a]

Drug and parameter	Time (h)	Predicted value	Experimental result	Experimental/ predicted
Rufloxacin				
UBT	0–2	16 (4–32)	64 (4–512)	4 (1–16)**
	2–4	16 (8–16)*	64 (16–256)*	4 (2–16)**
	4–8	16 (8–16)*	64 (16–64)*	4 (2–8)**
	8–12	16 (8–32)*	32 (16–512)*	3 (2–16)
	12–16	16 (8–32)*	64 (16–128)*	4 (2–8)
	16–24	16 (8–32)*	64 (16–256)*	4 (2–16)**
	24–36	16 (4–32)	64 (4–1,024)	4 (1–32)**
	36–48	16 (16–32)*	64 (32–128)*	4 (2–8)
	48–60	16 (4–16)	48 (4–128)	3 (1–8)
	60–72	16 (8–16)	64 (16–128)	4 (1–8)
AUBC		1,116 (784–1,680)*	4,116 (2,240–22,400)*	4 (2–18)**
Norfloxacin				
UBT	0–2	384 (128–1,024)	256 (128–1,024)	0.8 (0–2)**
	2–4	256 (64–512)	256 (64–256)	1 (1–2)**
	4–8	192 (32–256)	128 (32–256)	1 (1–2)**
	8–12	128 (32–256)	96 (32–256)	1 (0–2)
	12–16	64 (16–128)	32 (16–128)	0.8 (0–4)
	16–24	32 (16–128)	16 (4–64)	0.5 (0–1)**
	24–36	16 (4–32)	6 (0–16)	0.5 (0–1)**
	36–48	4 (0–16)	2 (0–16)	0.5 (0–1)[b]
	48–60	0 (0–4)	0 (0–2)	0 (0–1)[c]
	60–72	0 (0–2)	0 (0–2)	1 (1–1)[d]
AUBC		3,848 (1,408–7,288)	2,320 (1,048–4,992)	0.7 (0–2)**

[a] *, $P < 0.001$ for predicted versus experimental (intragroup). **, $P < 0.001$ for experimental/predicted rufloxacin versus norfloxacin. Values are expressed as medians; numbers in parentheses are ranges. Except where indicated, $n = 12$. UBT, urine bactericidal titer.
[b] Ten volunteers with predicted titer of >0.
[c] Four volunteers with predicted titer of >0.
[d] One volunteer with predicted titer of >0.

or for rufloxacin against *S. aureus* (where significant differences were found only from 2 to 4 h). Experimental AUBCs with both strains were significantly higher than those predicted for rufloxacin but not for norfloxacin. This increase in the experimental AUBCs over the predicted AUBCs was significantly higher for rufloxacin than for norfloxacin. When the ratio of experimental/predicted urine bactericidal titers is considered, our results show that experimental ex vivo activity exceeded predicted activity against both strains with rufloxacin, whereas with norfloxacin, the ratios are ≤1; i.e., experimental bactericidal activity never exceeded the predicted one. This increase in experimental activity over predicted activity with rufloxacin was significantly higher ($P < 0.001$) than that with norfloxacin against *E. coli* but not against *S. aureus.*

DISCUSSION

Urine bactericidal titers were correctly predicted in this study with norfloxacin and with rufloxacin when *S. aureus* was the organism tested. The crossover design of the study, the use of precise standardized methodology with the same organisms, and the dilution of each volunteer's urine samples with pretreatment urine of the same volunteer could have contributed to this accurate prediction. In contrast, significantly higher experimental titers were determined with rufloxacin against *E. coli*. This could be attributed to the synergistic action of supra- or subinhibitory concentrations of the *N*-desmethyl derivative of rufloxacin with the suprainhibitory concentrations of the parent compound. This metabolite has the same in vitro activity against *E. coli* as the parent drug (26), and suprainhibitory concentrations are obtained in urine (9). The similar predicted and experimental ex vivo activities in the other three cases (norfloxacin against both organisms and rufloxacin against *S. aureus*) may be explained by the absence of active metabolite in the case of norfloxacin (25) and the low in vitro activity of the rufloxacin metabolite (eight times lower than that of the parent compound) against *S. aureus* (26). However, in the latter case, experimental titers were higher than those predicted, showing a lesser degree of ex vivo synergism between rufloxacin and its metabolite against *S. aureus* than against *E. coli.*

The significantly higher experimental/predicted ex vivo activity ratio (calculated from the individual titers or AUBCs) for rufloxacin versus norfloxacin strongly suggests the contribution of the metabolite of rufloxacin to this urine bactericidal activity. Furthermore, as the AUBC is a means for assessing the effect of combined antimicrobial agents and as synergy can be defined as an AUBC for combined drugs which is significantly greater than that for each one alone (23), the significant differences found between predicted and experimental AUBCs with the two strains tested in the case of rufloxacin can be attributed to the active metabolite, even though for dose-dependent antimicrobial agents, the fact of progressive dilution in the bactericidal test can minimize this increase (23). In terms of in vitro susceptibility, if in vitro bactericidal activity is two times higher for *E. coli* than for *S. aureus* with rufloxacin (MBCs of 2 versus 4 μg/ml), ex vivo bactericidal activity should maintain the same proportion, as occurs with the predicted rufloxacin AUBCs (558 versus 1,116). This proportion, probably due to the active metabolite, is not maintained in rufloxa-

TABLE 2. Predicted, experimental, and ratio of experimental/predicted urine bactericidal titers and AUBCs of norfloxacin and rufloxacin for *S. aureus* ATCC 29213[a]

Drug and parameter	Time (h)	Predicted value	Experimental result	Experimental/ predicted
Rufloxacin				
UBT	0–2	8 (2–16)	16 (2–512)	2 (1–32)
	2–4	8 (4–8)*	16 (8–64)*	2 (2–8)
	4–8	8 (4–8)	16 (2–32)	2 (1–4)
	8–12	8 (4–16)	16 (2–128)	2 (1–8)
	12–16	8 (4–16)	16 (8–64)	2 (1–8)
	16–24	8 (4–16)	16 (4–128)	2 (1–16)
	24–36	8 (2–16)	16 (2–256)	2 (1–16)
	36–48	8 (8–16)	16 (4–64)	2 (1–8)
	48–60	8 (2–8)	16 (2–32)	2 (1–4)
	60–72	8 (4–8)	16 (8–16)	2 (1–2)
AUBC		558 (392–840)*	1,096 (720–7,200)*	2.1 (1–9)**
Norfloxacin				
UBT	0–2	96 (32–256)	128 (32–256)	1 (1–4)
	2–4	64 (16–128)	64 (8–256)	0.5 (1–4)
	4–8	48 (8–64)	32 (4–128)	0.5 (0–2)
	8–12	32 (8–64)	8 (4–32)	0.5 (0–1)
	12–16	16 (4–32)	8 (4–32)	0.5 (0–2)
	16–24	8 (4–32)	4 (0–32)	0.4 (0–2)
	24–36	4 (0–8)	2 (0–8)	1 (0–2)[b]
	36–48	1 (0–4)	0 (0–2)	0.3 (0–1)[c]
	48–60	0 (0–0)	0 (0–0)	
	60–72	0 (0–0)	0 (0–0)	
AUBC		956 (352–1,816)	736 (200–2,552)	0.7 (0–2)**

[a] *, $P < 0.001$ predicted versus experimental (intragroup). **, $P < 0.001$ experimental/predicted rufloxacin versus norfloxacin. Values are expressed as medians; numbers in parentheses are ranges. Except where indicated, $n = 12$. UBT, urine bactericidal titer.
[b] Eleven volunteers with predicted titer of >0.
[c] Six volunteers with predicted titer of >0.

cin experimental ex vivo activity, where a nearly 4:1 proportion is shown (4,116 versus 1,096). In the case of norfloxacin, the proportion of the in vitro bactericidal activity between *E. coli* and *S. aureus* (4:1) is maintained in the predicted and experimental AUBCs.

Although the *N*-desmethyl derivative should also be present when levels in urine (that afterwards were used to calculate predicted titers) were measured, we consider that the determined levels reflected only the concentration of the parent compound in urine, because generally, synergism cannot be shown in diffusion methods (1, 3). In agar diffusion tests, there is a relationship between the zone of inhibition and the potency of the antimicrobial agent (3), the distance reached by a particular concentration being proportional to the antimicrobial agent in the reservoir (1). However, when two drugs are present in the reservoir, the zone of inhibition simply reflects the activity of the predominant active antimicrobial compound, and therefore, synergism cannot be shown (3). In this case, rufloxacin and its metabolite have a similar susceptibility to *E. coli* (the reading microorganism), but considering that concentrations of rufloxacin should be about eight times higher than those of the metabolite (40 μg/ml [2] versus, theoretically, 5 μg/ml [9]), rufloxacin could be considered the predominant antimicrobial agent, and therefore, the inhibition zones simply reflect its concentration.

When concentrations in serum are considered, the antibiotic actions of β-lactams for controlling experimental infections can be considered time dependent (7), whereas quinolones can be considered level dependent, as a high peak/MIC or area under the curve/MIC ratio is necessary for clinical efficacy (parameters easily achieved with single daily doses) (8). When urine antibiotic concentrations are considered, 3 days is the favored duration of treatment, as no efficacy differences between quinolones are found with treatments lasting longer (15), despite the suggested 24-h urine antimicrobial activity allowing a single dose (21) and the quinolone pharmacodynamic parameters (the level-dependent action and the high urine peak/MIC easily obtained after a single dose of quinolone [2]).

When the concepts of high (>1:10) and sustained urine bactericidal activity and 12- to 24-h high urine concentrations to eliminate bladder infections (21) were related, both quinolones showed experimental and predicted titers against *E. coli* above the cutoff for at least 24 h. In the case of *S. aureus*, experimental activity is adequate with rufloxacin and higher than the predicted activity, which is below the cutoff. With norfloxacin, predicted activity against *S. aureus* is adequate for 16 h, whereas experimental activity is adequate for up to 8 h. Predicted values may overestimate the actual antibacterial activity, which may lead to establishing expanded dosing intervals against bacteria of the genus *Staphylococcus*, for which single-dose therapy is less effective (17). If we consider the need for 3-day urine activity, the optimal situation is achieved only with rufloxacin (experimentally against both strains, and using the predicted model against only *E. coli*, underestimating ex vivo activity against *S. aureus*).

This study shows that the antibacterial activity in urine predicted from pharmacokinetic and microbiological parameters does not correlate with the magnitude and duration of the experimental activity. This difference is due to higher experimental activity in the presence of quinolone active metabolites (rufloxacin) or to lower duration of experimental activity in the case of quinolones with short half-lives (norfloxacin). These findings may have dosing implications when the need for 1- or 3-day effective antibacterial activity is considered, as shown by the similar clinical and bacteriological results obtained in a clinical trial on uncomplicated cystitis that compared a 400-mg single dose of rufloxacin with 400 mg of norfloxacin given twice daily for 3 days (5) and the higher efficacy obtained with 3-day versus single-dose norfloxacin therapy due to *Staphylococcus saprophyticus* failures in the latter study group (19).

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