Antibacterial Activity of Lomefloxacin in a Pharmacokinetic In Vitro Model

CORNELIA RUSTIGE and BERND WIEDEMANN*

Pharmazeutische Mikrobiologie der Universität Bonn, An der Immenburg 4, 5300 Bonn, Federal Republic of Germany

Received 26 October 1989/Accepted 7 March 1990

An in vitro model simulating two-compartment pharmacokinetics was used to study the antibacterial activity of lomefloxacin after single oral doses of 200 and 400 mg. Lomefloxacin produced reliable bactericidal activity against gram-negative aerobic bacteria and staphylococci. Bacterial strains for which MICs were <0.5 μg/ml were inhibited with both dosing schedules. Doubling the dose from 200 to 400 mg increased the bactericidal activity only against Pseudomonas aeruginosa. Against Enterococcus faecalis lomefloxacin showed no effect. Selection of resistant variants during the simulation of treatment was observed with Staphylococcus aureus and P. aeruginosa.

Lomefloxacin is a new difluorinated quinolone with a broad spectrum of antibacterial activity. Its in vitro activity is similar to those of norfloxacin and enoxacin against gram-negative bacteria, but it has relatively weak activity against Enterococcus faecalis (2). The pharmacokinetic properties of this drug include good oral absorption and a prolonged serum elimination half-life ranging from 7 to 8 h in human volunteers (4).

The antibacterial activity of antibiotics in vitro is most commonly assessed by determination of the MIC in cultures in which bacteria are exposed to constant concentrations of the substances for about 18 h. In vivo, however, antibiotic concentrations change according to the pharmacokinetics of the particular drug (7). Therefore, the therapeutic value of antibacterial agents cannot be judged by MIC data alone or by comparison of MICs with pharmacokinetic data. A more reliable judgment can be given in vitro models, which permit bactericidal activity to be measured under concentrations that appear to occur in vivo (9).

In this study, we compared the activity of two different doses of lomefloxacin against gram-negative and gram-positive bacteria, simulating the concentration-time curves obtained in humans after single oral doses of 200 or 400 mg.

MATERIALS AND METHODS

For simulation of the concentration-time curves of lomefloxacin, we used the in vitro model of Grasso et al. (3) with slight variations. This model is set up in a thermostatic room at 37°C. It involves four flasks, one as a reservoir for antibiotic-free broth, one as an absorption compartment (B) containing dose D of the antibiotic dissolved in sterile culture broth at volume V_B, one as a central compartment (A) containing the bacterial culture of the test strain at volume V_A, and one for the eliminated culture medium. All are serially connected by tubing in the path of which are peristaltic pumps that maintain a constant flow rate F through the system. The flow rate is set to mimic the elimination half-life of the drug in human subjects. To maintain exponentially decreasing drug concentrations, the flow rates into and out of the central compartment must be identical. The flow rate in this system depends on the volumes in A and B: F = k_B \cdot V_A = k_D \cdot V_B. The concentration-time curves obtained in humans were mathematically adapted to reproduce the two-compartment pharmacokinetics of lomefloxacin in this in vitro model (Table 1). Three different flow rates were used to model the absorption phase to peak concentration in the central compartment, the α (distribution) phase, and the β (elimination) phase. To closely mimic the biexponential decline of the concentration in the central compartment, we used the model of Grasso et al. (3) after the time to maximum concentration of drug without an absorption compartment.

The pharmacokinetic parameters were determined on the basis of data taken from Morrison et al. (4). Lomefloxacin (G. D. Searle & Co., Neu Isenburg, Federal Republic of Germany) was kindly provided by the manufacturer. The tests were performed with Iso-Sensitest broth (ph 7.2) (Oxoid, Ltd., Wesel, Federal Republic of Germany) as the test medium. All strains used in this investigation are shown in Table 2. The central compartment was inoculated with an overnight culture to obtain exponentially growing bacteria at 10⁷ CFU/ml after 1 h of incubation when treatment was started. Samples were drawn at 1-h intervals for bacterial counts and drug concentration measurements. Bacteria were counted by making 10-fold dilutions of the samples with sterile, chilled 0.9% NaCl solution and then plating 50 μl on China blue-lactose agar. To determine bacterial counts below 10⁵ CFU/ml, 1-ml samples were filtered through a 0.2-μm-pore-size filter (type SM 11407, size 47; Sartorius-Membranfilter GmbH, Göttingen, Federal Republic of Germany) and cultured on agar. Plates and filters were read after sufficient incubation time to develop colonies at 37°C.

<table>
<thead>
<tr>
<th>TABLE 1. Pharmacokinetic parameters used for simulation of the concentration-time curves of a single oral dose of 200 or 400 mg of lomefloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter (units)</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>C_max (μg/ml)</td>
</tr>
<tr>
<td>T_max (min)</td>
</tr>
<tr>
<td>t_1/2 (min)</td>
</tr>
<tr>
<td>k_B (min⁻¹)</td>
</tr>
<tr>
<td>AUC (μg · min/ml)</td>
</tr>
</tbody>
</table>

* Abbreviations: C_max, maximum concentration of drug in serum; T_max, time to maximum concentration of drug in serum; t_1/2, half-life; k_B, elimination rate constant; AUC, area under the simulated concentration-time curve.
The concentrations were determined by an agar diffusion test with Iso-Sensitest agar as the test medium and *Klebsiella pneumoniae* (CR1) as the indicator organism. Standards were prepared by using Iso-Sensitest broth. Plates were incubated overnight at 37°C, and then inhibition zones were measured. The lomefloxacin assay was linear over a range of 0.125 to 1 μg/ml; the coefficient of variation was 5.7% at 0.125 μg/ml, 7.5% at 0.25 μg/ml, 4.06% at 0.5 μg/ml, and 1.58% at 1 μg/ml. The MIC for the bacterial strains was determined by the microdilution method with an inoculum of 10⁵ CFU/ml (5) taken from samples at time zero and after 24 h (Table 2).

The comparison of kill curves with different shapes is facilitated by the use of the area above the kill curve (AAC; CFU [log difference]/millilliters · hour) in a given time interval (A. Jansen and B. Wiedemann, Paper at the Congress, Deutschen Gesellschaft für Hygiene und Mikrobiologie, Kiel, Federal Republic of Germany, 1988). The area above all experimental kill curves was calculated from 0 to 12 h by the trapezoidal method as used for the calculation of the area under the concentration-time curve in pharmacokinetic studies. Areas above the zero line are calculated as negative, indicating bacterial growth; areas below the zero line are calculated as positive, indicating reduction of the number of viable cells during the experiment.

### RESULTS

**Drug concentrations.** The concentrations of lomefloxacin measured during the experiments correlated well with the data from human volunteers. We did not find a significant difference between the calculated human plasma concentration curve and the periodically determined concentrations in the system (Fig. 1).

**Antibacterial effect.** While the nalidixic acid-resistant (Nx7) strain of *Escherichia coli* was not inhibited by the 200-mg dosage, *Enterobacter cloacae*, *Serratia marcescens*, *K. pneumoniae*, and the nalidixic acid-susceptible strain of *E. coli* were rapidly reduced in numbers by 4 to 6 orders of magnitude (Fig. 2). The inhibited strains did not show any regrowth until 12 h. *K. pneumoniae* and the nalidixic acid-susceptible strain of *E. coli* remained 3 to 4 orders of magnitude below the inoculum at 24 h. The bacterial count of *E. cloacae* and *S. marcescens* increased after 12 h and reached counts above the inoculum after 24 h.

The doubling of the dose to 400 mg resulted in similar reductions in bacterial cell counts (Fig. 3). The highly susceptible strains of the family *Enterobacteriaceae* did not regress even after 24 h. The nalidixic acid-resistant strain of *E. coli* was slightly reduced in numbers under these conditions. Gram-positive bacteria did not seem to be influenced...
FIG. 2. Activity of lomefloxacin against members of the Enterobacteriaceae after a single oral dose of 200 mg. All cultures were inoculated with $1 \times 10^7$ to $2 \times 10^7$ CFU/ml. Symbols: ○, E. coli Nrx W3110; ○, E. cloacae 025149b; *, K. pneumoniae 2180; □, S. marcescens WT16004; +, E. coli W3110.

by an increase of the dose (Fig. 4). While E. faecalis was not inhibited at all, the bacterial cell count of Staphylococcus aureus was slowly but continuously reduced by 4 orders of magnitude with both dosing schedules within 12 h, although this strain regrew after 24 h. The bacterial cell count of Pseudomonas aeruginosa decreased by 2 orders of magnitude. No regrowth was observed until 12 h. At 24 h after dosing, however, the bacterial cell count was ten times that of the inoculum.

DISCUSSION

Lomefloxacin shows favorable pharmacokinetic properties. Peak levels in serum occur within 1 h and increase
linearly with dose (4). The serum elimination half-life in human volunteers ranges from 7 to 8 h. After 24 h, mean concentrations range between 0.12 μg/ml after a single 200-mg dose and 0.3 μg/ml after the 400-mg dose. The prolonged serum elimination half-life of the drug is responsible for the maintenance of concentrations in serum above the MICs for most of the bacterial strains for about 12 h.

From the AAC data shown in Table 3, we conclude that an increase of the dose of lomefloxacin from 200 to 400 mg does not significantly improve its activity against members of the Enterobacteriaceae and S. aureus. Slight differences in the AAC values are within the error of the experiment. Obviously, the 200-mg dose already has the optimum effect for the above-mentioned strains. The 400-mg dose increased the AAC value for P. aeruginosa from 11.42 to 27.47. Another doubling of the dose to 800 mg further increased the effect to 30.13 (data not shown). During the simulation of lomefloxacin treatment, we did not select resistant variants when members of the Enterobacteriaceae were used (Table 2).

However, at both doses we selected resistant strains of S. aureus. For these, which were cultured at the end of the experiment, the MIC increased fourfold. Similarly, the MIC for P. aeruginosa increased 16-fold after its exposure to the 400-mg dose. The concentration of lomefloxacin during the 200-mg dose is below the MIC for the P. aeruginosa strain. Therefore, regrowth occurs without selection of resistant variants with the lower dose.

The high increase of the MIC (16-fold) of quinolones suggests that the mutation is the result of a point mutation of the gyrA gene. Further support comes from MIC data with β-lactam antibiotics and aminoglycosides, since it has been shown that penetration mutants usually have a parallel increase in the MIC of all these antibiotics (6). The MICs for the lomefloxacin-resistant strains were identical to the MICs of these antibiotics compared with the susceptible strain (MIC of gentamicin, 2 μg/ml; and MIC of piperacillin, 4 μg/ml).

Lomefloxacin has relatively weak activity, like norfloxacin and enoxacin, against enterococcal and streptococcal species (2). Against E. faecalis it shows no effect. The bactericidal activity of lomefloxacin against the nalidixic acid-resistant strain of E. coli is similar to that of enoxacin after a single oral dose of 200 mg (B. Wiedemann, Proc. 15th Int. Congr. Chemother., abstr. no. 9, 1987).

In vitro and in vivo experiments have demonstrated that lomefloxacin has a broad antibacterial spectrum with particularly good activity against gram-negative aerobic bacteria and staphylococci. The MICs for 90% of strains tested of most members of the Enterobacteriaceae are ≤1 μg/ml (8). Our study suggests that bacterial strains for which MICs are <1 μg/ml are well inhibited by both dosing schedules within the first dosing interval. Once-daily dosing may be sufficient to treat systemic infections caused by the more susceptible pathogens. According to the results of Stone et al. (8), a larger dose, or twice-daily dosing, may be required for infections caused by less susceptible pathogens such as
those for which the MIC is >1 μg/ml. Our results, however, showed that an increase in dosage does not significantly improve the killing ability of the drug against less susceptible pathogens, but a twice-daily application may prevent the development of resistant mutants.

In this in vitro model, host defense mechanisms were not involved; thus, only the interaction of the antibiotic with the bacterial population was analyzed. In contrast to conventional in vitro tests, these models offer the benefit of bacterial exposure to drug under dynamic conditions. They might help to improve the quality of in vitro laboratory predictions of clinical responses to antibiotic treatment and offer an alternative method for the study of dosing and administration of antimicrobial agents (1).

LITERATURE CITED