Pharmacokinetics and Bactericidal Activities of One 800-Milligram Dose versus Two 400-Milligram Doses of Intravenously Administered Pefloxacin in Healthy Volunteers

O. PETITJEAN,1 B. PANGON,2 N. BRION,3 M. TOD,1,4 C. CHAPLAIN,2 V. LE GROS,3 K. LOUCHAH1, AND P. ALLOUCH2

Département de Pharmacotoxicologie, Hôtel Avicenne, 125, route de Stalingrad, 93009 Bobigny cedex,1 Service de Microbiologie, Centre Hospitalier de Versailles, 78157 Le Chesnay,2 and VERSUS, 75009 Paris,3 France

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Pefloxacin pharmacokinetics and serum bactericidal activities (SBA) against Escherichia coli and Staphylococcus aureus were compared after intravenous infusion of either a single 800-mg dose or twice-daily 400-mg doses into 16 healthy volunteers. Plasma pefloxacin concentrations were measured for up to 60 h, and SBAs were determined 1, 12, and 24 h after the start of the infusion. The mean areas under the concentration-versus-time curve for plasma were not different (138 versus 136 h mg/liter). The mean clearances, volumes of distribution, and half-lives were also comparable. The mean (± standard deviation) maximal concentration after the 800-mg infusion was 12.11 ± 1.35 versus 6.51 ± 0.73 mg/liter after the first 400-mg infusion and 7.42 ± 0.76 mg/liter after the second 400-mg infusion. Mean trough concentrations at 24 h were significantly different: 2.77 ± 0.63 (800 mg) versus 1.93 ± 0.49 (400 mg twice) mg/liter (P = 0.0007). Mean SBAs against E. coli after 800 mg of pefloxacin were higher than 1/128 (1 h), 1/32 (12 h), and 1/16 (24 h). Mean SBAs against S. aureus under the same conditions were higher than 1/64 (1 h), 1/16 (12 h), and 1/8 (24 h). Mean SBAs at 1 and 12 h were significantly higher after the 800-mg infusion than after the 400-mg infusion but were similar at 24 h for both regimens. Comparison of SBAs according to National Committee for Clinical Laboratory Standards criteria showed a similar adequacy at 24 h for both regimens against both strains. Administration of 800 mg of pefloxacin once a day is bioequivalent to 400 mg twice a day, and bactericidal activity of the 800-mg infusion is not less than that of two 400-mg infusions.

Pefloxacin is a fluorinated quinoline that is especially active in vitro against gram-negative bacteria and staphylococcal strains (8). Its excellent penetration into various tissues and fluids allows its use in a wide range of infections (8). Pefloxacin is usually given as 400 mg twice a day (b.i.d.) intravenously or per os, resulting in similar maximum concentrations in plasma of approximately 10 mg/liter owing to its complete bioavailability (7). Pefloxacin is metabolized extensively to form the active N-demethyl pefloxacin (nor pefloxacin) and inactive N-oxide metabolites (9). The elimination half-life (t1/2) of pefloxacin following administration of single doses ranged from 8 to 13 h, increasing to 14 to 15 h after multiple dosing (7).

Thus, administration of 800 mg of pefloxacin once a day instead of 400 mg b.i.d. could be clinically effective and should improve compliance with treatment. The aim of this study was to compare pefloxacin pharmacokinetics and serum bactericidal activity (SBA) in 16 healthy volunteers after intravenous infusion of either a single 800-mg dose or two 400-mg doses given at a 12-h interval.

MATERIALS AND METHODS

Subjects. Sixteen healthy male Caucasian volunteers were enrolled in this study. Their mean weight (± standard deviation) was 66.9 ± 4.9 kg (range, 62 to 75 kg), their mean height was 176.6 ± 4.8 cm (range, 169 to 185 cm), and their mean age was 25.7 ± 3.2 years (range, 21 to 31 years).

No medication intake was permitted during the last 2 weeks prior to drug infusion. The study protocol was approved by the local Ethics Committee, and informed consent was obtained.

Administration of drug. Pefloxacin mesylate (Rhône-Poulenc-Rorer) was given according to a randomly distributed crossover design. Each volunteer overtly received either 800 mg once a day or 400 mg b.i.d. Pefloxacin was infused intravenously for 1 h. A 1-week washout period was established between the two sequences. In the 800-mg series, blood samples were taken before infusion and then at 20, 40, 60, 65, 70, 80, 90, 120, 150, and 180 min and 4, 5, 7, 9, 12, 16, 24, 36, 48, and 60 h after the start of the infusion. In the 400-mg series, blood samples were taken before infusion and then at 20, 40, 60, 65, 70, 80, 90, 120, 150, and 180 min and at 4, 5, 7, 9, and 12 h after the start of the first 400-mg infusion; they were also taken at 20, 40, 60, 65, 70, 80, 90, 120, 150, and 180 min and at 4, 5, 7, 9, 12, 16, 24, 36, and 48 h after the start of the second 400-mg infusion.

Plasma was separated and immediately stored at −20°C until pefloxacin was assayed for. Serum was separated from clotted blood in the samples taken just before infusion and at 1, 12 (i.e., before reinfusion in the 400-mg group), and 24 h after the start of the infusion for bactericidal activity determination.

Drug assay. The pefloxacin concentrations in plasma were measured by high-pressure liquid chromatography (10). The limit of quantification was 0.1 mg/liter. Inter-run precision was 6.1% at 0.15 mg/liter, 3.9% at 6.0 mg/liter, and 4.1% at 11 mg/liter. The major pefloxacin metabolites (nor pefloxacin and pefloxacin N-oxide) did not interfere with the assay.

SBA. Two reference strains were tested: Escherichia coli
IP 7624 and *Staphylococcus aureus* IP 7625. The MICs of pefloxacin were 0.03 and 0.25 mg/liter, respectively, for *E. coli* and *S. aureus* (4).

Serum bactericidal titers were determined by using a modified version of the micromethod reported by Prober et al. (14). Serial twofold dilutions of serum (from 1/2 to 1/1,024), made with heat-inactivated lyophilized human serum (Lyotrol NX Biomérieux) as the diluent, were deposited in the wells of microtiter plates, and an inoculum of 10° CFU/ml was added, with a final total volume of 100 μl. After 18 h of incubation at 37°C, 10 μl from each well was transferred onto Mueller-Hinton agar supplemented with Ca²⁺ and Mg²⁺ (Diagnostics Pasteur) and incubated for 18 h at 37°C. The bactericidal titer was the titer that resulted in a 99.9% reduction in CFU based on the CFU of the initial inoculum. The final inoculum was 10⁶ per spot.

Titers are expressed as the inverse of the serum dilution; for example, 1/2 and 1/4, etc., are recorded as 2 and 4, etc., and 1 means no activity. Mean serum bactericidal titers were compared by using variance analysis after conversion of the titers to log base 2.

Results are also expressed according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) (11); titer ≥ 32, adequate; 32 > titer > 2, intermediate; titer ≤ 2, inadequate. Results were compared by using the χ² test.

**Data analysis.** Concentration-versus-time data were fitted to a biexponential model after zero-order input rate by using the SIPHAR program (Simed, Créteil, France). The choice between alternative models was made on the basis of the Schwartz criterion (16).

Nonlinear regression analysis was performed by using weighted least squares with a weight of 1/\(C^2\), where \(C\) is concentration of the drug. Several parameters were considered for the pharmacokinetic analysis. The maximal concentration, \(C_{\text{max}}\), was determined at the end of the infusion. The experimental area under the concentration-versus-time curve at 60 h (AUC₆₀) was calculated by the log-trapezoidal rule up to the last sampling time. The total AUC at infinity (AUCₙ) was obtained by adding \(C/k_e\) to AUC₆₀, where \(C\) is the concentration of the last sample and \(k_e\) is the slope of the terminal phase. Clearance was calculated as \(CL = D/AUCₙ\), where \(D\) is the dose. The half-life of the elimination phase was \(t_{1/2} = \ln 2/k_e\). The volume of distribution at steady state (\(V_{SS}\)) was calculated by moment analysis (12). Pharmacokinetic parameters were compared by using Latin square analysis of variance. The significance level was 0.05. Moreover, AUCs were compared by using Westlake’s confidence interval analysis.

**RESULTS**

Mean plasma pefloxacin concentrations obtained with both dosage schedules are shown in Fig. 1. Pharmacokinetic parameters are given in Table 1. Mean AUCₙₛ were very similar (138 versus 136 h · mg/liter); the difference was not statistically significant, and the confidence interval for the difference was only 6.2%. Likewise, mean CL, \(V_{SS}\), and \(t_{1/2}\) were comparable. When normalized to a dose of 400 mg, the \(C_{\text{max}}\) observed after an 800-mg infusion was slightly different from that after the first 400-mg infusion (6.05 ± 0.67 versus 6.51 ± 0.73 mg/liter; \(P = 0.055\)), but the confidence interval for the difference was 13.0%. The concentration at 24 h (\(C_{24}\)) which would be the concentration before readministration in both schedules, was significantly different: 1.93 ± 0.49

(800 mg) versus 2.77 ± 0.63 (400 mg b.i.d.) mg/liter (\(P = 0.0007\)).

The differences observed between the pharmacokinetic profiles after each mode of administration must be interpreted in the light of bactericidal activity results. Serum samples obtained prior to pefloxacin infusion did not show any bactericidal activity. Mean serum bactericidal titers measured 1, 12, or 24 h after the start of the infusion are reported in Table 2. Bactericidal activities of residual \(C_{24}\) h were not significantly different in the two series for both strains. Conversely, bactericidal titers at 1 and 12 h were significantly higher after the 800-mg infusion than after the 400-mg infusion. These results were evaluated according to NCCLS criteria (Table 3). At 12 h, SBAs against *E. coli* were adequate in 14 subjects after the 800-mg infusion versus 6 subjects after the 400-mg infusion. Against *S. aureus*, SBAs at 12 h were adequate in six subjects after the 800-mg infusion versus none after the 400-mg infusion. Comparison of bactericidal activity according to NCCLS criteria at 1 or 24 h showed no significant differences between the two schedules.

**DISCUSSION**

Pefloxacin pharmacokinetics in healthy volunteers after repeated intravenous administrations (400 mg b.i.d.) were previously studied by Frydman et al. (7). Their results were very similar to those obtained in the present study, as they found a \(C_{\text{max}}\) of 5.80 ± 1.59 mg/liters, \(t_{1/2}\) of 11.0 ± 2.6 h, CL of 8.29 ± 2.86 liters/h/1.73 m², and \(V_{SS}\) of 134.0 ± 23.81 liters.

Nonlinearity in pefloxacin pharmacokinetics has been investigated for time dependency (7) and dose dependency (2). Time dependency results in some accumulation of pefloxacin during repeated 400-mg intravenous or oral treatments: the ratio of AUC₁₂₅₆₇₈ (16th dose)/AUC₁₂₅₆₇₈ (1st dose) was 1.37 ± 0.20, while the theoretical value of this ratio is 1.00 in linear pharmacokinetics. The precise mechanisms of this time dependency are not known, but a possible saturable
process in the metabolic pathway was hypothesized (7). Such a mechanism could also lead to some dose dependency in pefloxacin pharmacokinetics. In their study of dose dependency, Barre et al. (2) concluded linearity of pefloxacin pharmacokinetics in the 200- to 800-mg range, but only three subjects were tested. The present data, obtained with 16 healthy volunteers after intravenous administration of 400 mg twice or 800 mg once, enabled us to confirm their findings. The absence of any significant difference between the different pharmacokinetic parameters evaluated in both schedules showed that pefloxacin pharmacokinetics were linear in the 400- to 800-mg range. Moreover, the Cmax observed in the present study after 800 mg of pefloxacin (12.1 ± 1.35 mg/liter) was higher than the Cmax after 400 mg every 12 h for 16 doses in the study by Frydman et al. (9.55 ± 1.63 mg/liter). If saturation of pefloxacin metabolism were to occur, this process would be concentration dependent and should have been observed in our study. Thus, it seems that saturation of pefloxacin metabolism is unlikely. Rather, time dependency of pefloxacin kinetics could be explained by self- or metabolite-mediated inhibition of metabolism. It should be noted that among the fluoroquinolones, increase in t1/2 and decrease in clearance after administration of multiple doses have been described for ciprofloxacin, which is the most-metabolized fluoroquinolone after pefloxacin (respectively, 40 and 85%) (1). Moreover, many cytochrome P-450 inhibitors have an imidazole ring or a nitrogen ring in their structures: allopurinol, cimetidine, isoniazid, ketoconazole, metronidazole, and phenylbutazone are well-known inhibitors (13). The piperazin or quinoline ring of fluoroquinolones could be responsible for inhibition by the enzymes of their own metabolism. Since the mechanism of pefloxacin pharmacokinetic time dependency remains controversial, the accumulation ratio of 800 mg of pefloxacin given once daily for several days cannot be predicted.

Bioavailability of pefloxacin is considered to be nearly 100% (2, 10, 12, 18), and primary pharmacokinetic parameters (CL and VSS) are not significantly different after oral or 1-h intravenous administration. Thus, it is expected that pharmacokinetic bioequivalence between the two schedules (400 mg twice versus 800 mg once) observed after a 1-h intravenous infusion would also hold after oral administration.

Finally, from a pharmacokinetic point of view, 800 mg once daily and 400 mg b.i.d. of pefloxacin were bioequivalent, the only significant difference being the C24 h for pefloxacin. However, the pefloxacin concentration kinetics of the two schedules were not superimposable, and the difference in their profiles had to be interpreted in terms of bactericidal activity.

With this aim in view, SBAs against two reference strains were used to determine the bioequivalence of the two regimens. Serum bactericidal titers were measured 1, 12, and 24 h after the start of the infusion. Serum bactericidal titers have been widely used since 1947 for antibiotic treatment monitoring in patients with endocarditis. The correlation between SBA and clinical outcome has been thoroughly investigated (20, 21); SBA was shown to be predictive of bacteriological eradication but not of bacteriological failure or clinical evolution. Thus, Wolfson and Swartz (22) proposed SBA as a tool for the comparison of new antibiotics with reference drugs.

Our results obtained after a 400-mg infusion of pefloxacin were similar to those of Van der Auwera et al. (19): serum bactericidal titers at 12 h were 1/16 and 1/8 against E. coli and S. aureus, respectively. Comparison of mean serum bactericidal titers for each dosage regimen showed that 12 h after an 800-mg infusion of pefloxacin, these titers are higher than 1/32 against E. coli and 1/16 against S. aureus, and the difference with the 400-mg infusion is significant. Although the C24 h were not statistically different for the two regimens, they were not clinically different, as evidenced by the identical SBAs. These results demonstrate that the bactericidal activity of an 800-mg dose of pefloxacin given once a day should not be less than that of 400 mg of pefloxacin given b.i.d.

These results support the association of pefloxacin with

### Table 1. Pharmacokinetic parameters of pefloxacin

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Cmax (mg/liter)</th>
<th>AUCA0 (h·mg/liter)</th>
<th>AUCA1 (h·mg/liter)</th>
<th>t1/2 (h)</th>
<th>CL (liters/h)</th>
<th>VSS (liters)</th>
<th>C24 h (mg/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>400 mg b.i.d.</td>
<td>6.51 ± 0.73</td>
<td>129 ± 28</td>
<td>138 ± 34</td>
<td>12.4 ± 2.3</td>
<td>5.77 ± 1.52</td>
<td>95.1 ± 11.1</td>
<td>2.77 ± 0.63</td>
</tr>
<tr>
<td></td>
<td>(4.65-7.35)</td>
<td>(75-195)</td>
<td>(76-223)</td>
<td>(8.8-15.0)</td>
<td>(4.47-7.50)</td>
<td>(80.3-119.0)</td>
<td>(1.50-4.30)</td>
</tr>
<tr>
<td>800 mg once daily</td>
<td>7.42 ± 0.76</td>
<td>131 ± 22</td>
<td>136 ± 25</td>
<td>12.2 ± 1.7</td>
<td>6.03 ± 1.26</td>
<td>97.9 ± 8.9</td>
<td>1.93 ± 0.49</td>
</tr>
<tr>
<td></td>
<td>(6.25-8.75)</td>
<td>(85-153)</td>
<td>(87-159)</td>
<td>(9.7-15.3)</td>
<td>(4.35-9.08)</td>
<td>(83.7-115.0)</td>
<td>(1.05-2.85)</td>
</tr>
<tr>
<td>p&lt;sup&gt;fd&lt;/sup&gt;</td>
<td>0.055</td>
<td>0.825</td>
<td>0.785</td>
<td>0.33</td>
<td>0.057</td>
<td>0.22</td>
<td>0.0007</td>
</tr>
<tr>
<td>Westlake confidence interval (%)</td>
<td>13.0</td>
<td>5.3</td>
<td>6.2</td>
<td>5.0</td>
<td>42.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Values are means ± standard deviations. Values in parentheses are ranges.

b First infusion.

c Second infusion.

d Comparison of Cmax after 800-mg infusion with Cmax after 400-mg infusion, with values normalized to 400 mg.

### Table 2. Mean SBAs against two test strains expressed as inverses of serum dilutions

<table>
<thead>
<tr>
<th>Strain</th>
<th>Time (h)</th>
<th>SBA after dose of:</th>
<th>400 mg</th>
<th>800 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>1</td>
<td>148&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>45&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>1</td>
<td>115&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>23&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> P < 0.0005 compared with 400-mg dose at 1 h.

<sup>b</sup> P < 0.01 compared with 400-mg dose at 12 h.

<sup>c</sup> P < 0.05 compared with 400-mg dose at 1 h.

<sup>d</sup> P < 0.0005 compared with 400-mg dose at 12 h.
TABLE 3. Comparison of bactericidal titers according to NCCLS criteria

<table>
<thead>
<tr>
<th>Strain</th>
<th>Interpretation</th>
<th>400-mg dose</th>
<th>800-mg dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 h 12 h 24 h</td>
<td>1 h 12 h 24 h</td>
</tr>
<tr>
<td>E. coli</td>
<td>Adequate</td>
<td>15 6 6 16</td>
<td>14a 6 14</td>
</tr>
<tr>
<td></td>
<td>Intermediate</td>
<td>1 9 10 0</td>
<td>2e 10</td>
</tr>
<tr>
<td></td>
<td>Inadequate</td>
<td>0 1 0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>S. aureus</td>
<td>Adequate</td>
<td>10 0 1 12</td>
<td>6b 2</td>
</tr>
<tr>
<td></td>
<td>Intermediate</td>
<td>6 9 4 4</td>
<td>4 9</td>
</tr>
<tr>
<td></td>
<td>Inadequate</td>
<td>0 7 11 11</td>
<td>0 1b 9</td>
</tr>
</tbody>
</table>

* P = 0.01 compared with 400-mg dose at h 12.

b P = 0.005 compared with 400-mg dose at h 12.

aminoglycosides once a day. Indeed, an increasing number of in vitro, animal, and clinical studies support once-daily administration of aminoglycosides (3). Since aminoglycosides are frequently used in combination with quinolones, it is important to determine which one among the commercially available fluoroquinolones is the most suitable for once-daily administration. Pefloxacin, whose elimination t1/2 is 10 to 12 h (versus 7 h for ofloxacin and 4 to 5 h for ciprofloxacin) (15), was a priori the best candidate provided that its kinetics were linear, and 800 mg once daily was bioequivalent to 400 mg b.i.d. Moreover, fluoroquinolones exhibit concentration-dependent bactericidal activity (17) and a prolonged postantibiotic effect (23) against gram-negative bacilli and time-dependent bactericidal activity against staphylococci (5, 6). Thus, 800 mg of pefloxacin once a day could improve bacteriological and clinical efficacies in gram-negative bacillus infections, whereas pefloxacin at 400 mg b.i.d. could be preferable in staphylococcal infections. However, clinical outcome and tolerance of once-daily pefloxacin administration remain to be evaluated by clinical trials.

REFERENCES