Multiple-Dose Pharmacokinetics of Sparfloxacin and Its Influence on Fecal Flora

M. RITZ,1 H. LODE,1* M. FABBENDER,1 K. BORNER,2 P. KOEPPPE,3 AND C. E. NORD4

Department of Pulmonary and Infectious Diseases, City Hospital Berlin-Zehlendorf;1 and Institute of Clinical Chemistry and Clinical Biochemistry2 and Department of Radiology,3 Klinikum Steglitz, Freie Universität Berlin, Berlin, Germany, and National Institute of Bacteriology, Stockholm, Sweden4

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In a randomized, double-blind, placebo-controlled, multiple-dose pharmacokinetic study, the safety and effect on intestinal flora of sparfloxacin (SPX) were determined in 12 healthy male volunteers (8 received SPX and 4 received a placebo). Following fasting and oral administration of 400 mg on day 1 and 200 mg on days 2 to 8, concentrations of the free drug in serum, urine, and feces were measured by high-performance liquid chromatography; serum and urine were also evaluated by a microbiological assay. All results, except those for renal excretion, exclude the glucuron conjugate metabolite. A mean peak concentration in serum (400-mg dose) of 0.56 ± 0.13 mg/liter was measured 5.52 ± 0.98 h after administration. Pharmacokinetic parameters (measured by high-performance liquid chromatography) were based on an open, one-compartment model and resulted in the following day 1 (calculated for the 200-mg dose), day 4 (recalculated for a single dose), and day 8 values (mean ± standard deviation): area under the curve, 16.4 ± 2.3 (day 1) and 18.3 ± 5.1 (day 4) mg · h/liter; elimination half-life, 18.3 ± 3.9 h; steady-state volume of distribution, 4.7 ± 1.4 (day 1) and 4.3 ± 1.2 (day 8) liters/kg; apparent total clearance, 201 ± 31 (day 1) and 190 ± 51 (day 4) ml/min; renal clearance, 19.1 ± 5.8 (day 1) and 23.2 ± 19.4 (day 4) ml/min. Recovery in urine on day 1 was 5.89% ± 1.4% of the dose in 24 h for the parent compound and 18.4% ± 6.8% for the SPX glucuronide. The concentrations of SPX in stool declined from 739.6 ± 484.4 mg/kg on day 2 and 671.7 ± 474.8 mg/kg on day 4 to 476.4 ± 239.7 mg/kg on day 8. These results demonstrate moderate-to-good bioavailability of SPX in addition to a long elimination half-life, a high volume of distribution, and mainly nonrenal elimination. The fecal flora was analyzed for aerobic and anaerobic bacteria before, during, and after drug administration. SPX resulted in a decrease of Escherichia coli, a moderate decrease of enterococci, and a slight decrease of bifidobacteria. Except for E. coli, the numbers of fecal bacteria returned to pretreatment values by day 28 after first dosing. Overall tolerability of SPX was good. Adverse reactions in volunteers were mild and self-limited and were as follows in the SPX-treated group: gastrointestinal disturbances, four cases; soft stool, two cases; pruritus, one case; and cephalgia, three cases. No gastrointestinal disturbances were noted in the placebo-treated group; side effects in this group were as follows: mild cephalgia, one case; transient myalgia, one case.

The new fluoroquinolones are a rapidly expanding group of antimicrobial agents. They show potent activity against aerobic gram-negative pathogens and are typically less active towards gram-positive organisms (21, 26, 30, 31). Sparfloxacin (SPX) is a new investigational difluorinated quinolone (13, 20). Studies on its antibacterial activity by several researchers (8, 10, 15, 19, 28) have revealed a broad spectrum of activity characterized by improved activity towards gram-positive cocci (7), including Streptococcus pneumoniae, methicillin-susceptible Staphylococcus aureus, Mycoplasma spp., Chlamydia spp., Mycobacterium spp., glucose nonfermenters, and anaerobes. According to Nakamura et al. (19), most pathogenic organisms are usually inhibited in vitro by SPX concentrations below 0.1 mg/liter and in vivo activities of SPX are generally superior to those of other quinolones. Many oral antibiotics have a significant effect on fecal flora. Normal bacterial species may be eliminated, allowing colonization by resistant species (22). It is therefore important to investigate the effect of any new antimicrobial compound on normal fecal flora. The objectives of our trial were to determine the pharmacokinetic properties of SPX after administration of single and multiple doses, to determine its effect on the intestinal flora in volunteers, and to assess tolerance to SPX over an 8-day application period.

MATERIALS AND METHODS

Volunteers. Twelve healthy male volunteers with no history of multiple drug allergies, especially to quinolones, participated in the study. Their mean age was 30.9 ± 5.7 years, their mean body weight was 77.9 ± 7.8 kg, their mean body surface area was 1.97 ± 0.10 m², and their mean creatinine clearance was 111.3 ± 21.5 ml/min/1.73 m², which was calculated as follows: clearance = [(drug concentration in urine · volume of urine [24 h] · 1.73)/(drug concentration in serum · 1.440 · body surface area)]. A battery of laboratory tests, including hematology, biochemistry, illicit drug testing, urinalysis, and physical examinations before and after the study period, produced normal results. Use of any medication, including antibiotic therapy, prior to the study and diarrhea or soft stool at the start of the study were further criteria for noninclusion. Intake of coffee, tea, cocoa, and nuts was not allowed during the study period, and smoking was restricted to a maximum of five cigarettes per day. Written consent in accordance with the regulations in the Federal Republic of Germany was obtained; the study was approved by the local ethics committee.

Dosage and administration. The test subjects received a 400-mg single oral dose of SPX (RP 64206, batch T 89006;
Rhone-Poulenc Rorer) on day 1 and single 200-mg daily oral doses up to day 8. Treatments were randomized by Rhone-Poulenc IBP in balanced blocks, with two-thirds of the subjects on the SPX regimen and one-third receiving a placebo. All trials were started after an overnight fast, which continued for another 4 h after drug administration. Ad libitum water consumption was allowed starting 2 h after drug administration. The study medication was administered in the form of white, film-coated tablets, each weighing 100 mg, together with 100 ml of tap water. None of the test subjects discontinued taking their medication during the trial.

Samples. Blood samples for the pharmacokinetic study were drawn from a peripheral vein on days 1 and 4 just before treatment intake and after 2, 6, 10, and 24 h. Blood specimens were allowed to clot at room temperature, were protected from light, and were subsequently centrifuged at 3,000 x g for 10 min, and then serum was separated. Urine samples were collected on days 1 and 8 from 0 to 6, 6 to 12, and 12 to 24 h after drug administration in air-tight glass bottles and on days 11, 21, and 28 as single samples. Random fecal samples for microbiology were collected 7 and 4 days prior to the study and 2, 4, 8, 11, 21, 28, and 56 days after the first treatment. After division of the blood and urine samples for bioassay and liquid chromatography and collection of the fecal samples for microbiological investigation, samples were stored at -80°C and all assays were performed within 3 months. Transport of deep-frozen fecal specimens from Berlin, Germany, to Stockholm, Sweden, was done by plane in special ice-containing polystyrene packages and took less than 20 h.

High-performance liquid chromatography (HPLC) of SPX. SPX was measured by an HPLC method involving reversed-phase chromatography and fluorometric detection. A specific and sensitive analytical method for determination of SPX in serum and urine has been described in detail elsewhere (5). This method is based on the high polarity and weak fluorescence of SPX. Since the main metabolite of SPX, a glucon conjugate, does not fluoresce, urinary excretion of the metabolite was evaluated by UV absorbance detection. The detection limits were 0.05 mg/liter in serum, 0.46 mg/liter in urine, and 0.14 mg/liter in stool. The day-to-day coefficient of variation ranged between 10.4 and 3.6% for serum (c [concentration range in milligrams per liter], 0.30 to 0.91), from 7.8 to 5.0% for urine (c, 6.40 to 20.60), and from 4.7 to 2.4% for stool (c, 0.55 to 1.50). Within-day coefficients of variation ranged from 5.0 to 2.7% for serum (c, 0.55 to 1.50), from 5.8 to 3.4% for urine (c, 6.00 to 13.0), and from 4.6 to 2.6% for stool (c, 1.02 to 4.67). Concentration versus peak area curves were linear in the following ranges: between 0.1 and 2.0 mg/liter for serum, 1.56 and 50.0 mg/liter for urine, and 0.5 and 20 mg/kg for stool.

Bioassay of SPX. As a microbiological assay a conventional agar plate diffusion method was performed (4; as modified by Reeves and Bywater [24]). Serum and urine assays were performed on Difco agar with Bacillus subtilis ATCC 6633 as the indicator strain. Serum samples were assayed against standards prepared in activity-free pooled human serum. Phosphate buffer (pH 7.0) was used for predilution of serum and urine standards. All assays were performed in triplicate. The detection limit in both urine and serum was 0.06 mg/liter. The coefficient of variation between concentrations of 0.156 and 2.0 mg/liter varied between 2.6 and 6.6%.

Analysis of fecal flora. Analysis of fecal flora was carried out at the National Bacteriological Laboratory, Stockholm, Sweden. A 1-g sample of stool was homogenized in 9 ml of prereduced peptone-yeast extract medium, and prereduced diltuents were used in all of the anaerobic microbiological assays. Tenfold serial dilutions were made to a dilution of 10⁻⁸. The samples were inoculated and processed as described by Heimdahl and Nord (11). After incubation, total colony counts were determined from aerobic and anaerobic blood agar plates. Different colony types were counted, isolated in pure culture, and identified, as were the different colonies appearing on the selective media. All fecal samples were assayed for the presence of Clostridium difficile and one of its toxins by the method described by Aronsson et al. (1). Counts were given as log numbers of bacteria per gram of feces.

Protein binding. Protein binding in serum was determined with pooled serum containing 5, 2.5, and 2 mg of SPX per liter. The measurements were done with the micropartition MPS-1 system for separation of free SPX from the protein-bound solute (Amicon, Witten, Germany). Separation was provided by filtration through an anisotropic, hydrophilic ultrafiltration membrane. Separation was done at 22°C, and incubations were done at 37°C; the centrifuge speed was 1,500 x g, and the centrifugation time was 30 min. The quantitative partitioning capability is reflected by retention of >99% of serum protein and <5% of l-thyroxine. The drug did not adsorb to the filter, and the ultrafiltration partitioning of SPX in buffer was 100%. The coefficients of variation were 2.1% at 5 mg/liter, 1.1% at 2.5 mg/liter, and 0.9% at 2.0 mg/liter.

Pharmacokinetic calculations. Considering the limitation of data collection, the pharmacokinetic parameters were calculated by assuming a simple open one-compartment model for n applications. Calculation was done after curve fitting by the least-squares method (25), and the sum of squared relative deviations was minimized (14). Biomatematical calculations were performed by standard methods (3, 9), and all of the sampling and dosing history was used to fit the data. The pharmacokinetic results of day 1 were calculated for the 200-mg dose, and those for day 4 were recalculated for a single dose by subtraction of the accumulated and extrapolated concentrations. All results were adjusted to 70 kg of body weight, and clearance values were normalized to a body surface of 1.73 m². The maximum concentration of SPX serum was determined by computer fitting, and the area under the concentration-time curve was calculated by assuming a one-compartment model, as well as noncompartmentally.

RESULTS

Comparison of HPLC and bioassay. There was an excellent correlation between HPLC and bioassay results. Method comparison was done by bivariate analysis of regression (2), which is described by the following equations: c (bioassay) = 1.035 * c (HPLC) - 0.06 for serum and c (bioassay) = 1.092 * c(HPLC) - 0.09 for urine. Because of the excellent correlation, only HPLC results were used for the pharmacokinetic calculations presented here.

Concentrations in serum. Mean concentrations in serum on day 1 (in milligrams per liter) declined from 0.94 ± 0.23 after 1 h and 0.98 ± 0.27 after 6 h to 0.80 ± 0.21 after 10 h. A mean concentration in serum of 0.45 ± 0.09 mg/liter was still measurable 24 h after administration. On day 8, the concentrations decreased from 0.88 ± 0.27 mg/liter after 2 h to 0.87 ± 0.23 mg/liter after 6 h, 0.75 ± 0.20 mg/liter after 10 h, and 0.42 ± 0.11 mg/liter after 12 h ("effective" values, adjusted for the dosage history, and the calculated and recalculated values determined as described above are shown in Fig. 1).

Mean serum protein binding of 56 ± 2% was found.

Concentrations of SPX in stool. Since the samples for assay of SPX in feces were not quantitatively collected, SPX concentrations in stool samples were calculated by assumption of a daily average stool weight of 125 g/24 h. The concentration of
SPX in stool declined from 759.6 ± 484.4 mg/kg on day 2 to 671.7 ± 474.8 mg/kg on day 4 and 476.4 ± 239.7 mg/kg on day 8. However, we must point out that quantitative collections of fecal samples were not done.

**Urinary excretion.** Mean SPX concentrations (milligrams per liter) in urine on day 1 ranged from 17.3 ± 8.5 within the first 6 h to 13.6 ± 7.2 in the 12- to 24-h interval. SPX recovery from urine (0 to 24 h) was low, at 5.89 ± 1.4% of the dose on day 1 and 6.69 ± 3.2% of the dose on day 8. Renal excretion of SPX glucuronide on day 1 was 18.4 ± 6.8% of the dose.

**Pharmacokinetic parameters.** The day 1 results were calculated for the 200-mg dose, and the day 4 results were recalculated for a single dose. Since the recalculated peak concentration of SPX in serum and area under the concentration-time curve of days 1 and 4 are in good agreement, the chosen dosage regimen adequately accounted for SPX accumulation. The peak concentrations of SPX in serum were 0.56 ± 0.13 mg/liter on day 1 and 0.61 ± 0.16 mg/liter on day 4. The areas under the concentration-time curves were 16.4 ± 2.3 mg h/liter on day 1 and 18.3 ± 5.1 mg h/liter on day 4, indicating moderate-to-good bioavailability. The estimated volume of distribution (V), which far exceeds the extracellular volume, was very high, at 47 ± 1.4 liters/kg on day 1 and 4.3 ± 1.2 liters/kg on day 8. The elimination phase was slow, with a calculated terminal half-life of 18.3 ± 2.41 h for day 1. Renal clearances were 19.1 ± 5.8 ml/min on day 1 and 23.2 ± 19.4 ml/min on day 8, significantly below the apparent total clearances of 201 ± 31 ml/min on day 1 and 190 ± 51 ml/min on day 8. The pharmacokinetic analysis results are summarized in Table 1.

**Fecal flora analysis.** The bacteria investigated in this study were actinomyces, anaerobic gram-positive cocci, bacilli, members of the family Bacteroidaceae, bifidobacteria, staphylococci, streptococci, clostridia, corynebacteria, *E. coli*, eubacteria, enterococci, lactobacilli, propionibacteria, and veillonelae; yeasts were examined as well. In our study, analysis of fecal flora revealed a significant (>2 log10) reduction in *E. coli* bacteria, from approximately 10^10 to 10^7 g of feces to less than 10^2 g of feces during the period of SPX administration to day 21, and the counts did not return to the pretreatment values until day 56. There was a moderate reduction in counts of enterococci, but this reduction was transient and counts did return to pretreatment values between days 28 and 56. A slight decrease of bifidobacteria was seen on days 4 to 8 of the study (Fig. 2). *C. difficile* and one of its toxins were not detected in any sample before or after dosing. No changes in fecal flora were seen in the placebo group.

**Safety and tolerance.** Overall tolerance was good. In the SPX-treated group, the side effects noted were celphalagia (three cases) and pruritus (one case); for most volunteers with mild gastrointestinal disturbances, soft stool (two cases) and meteorism (four cases) lasted only for 1 or 2 days (mainly on days 4 and 5 of the application period). One subject reported soft stool and meteorism from days 4 to 7. Adverse reactions in the placebo-treated group were celphalagia (one case) and myalgia (one case). All reactions were mild and self-limited. The results of laboratory tests remained normal for all of the volunteers during and after the study period.

**DISCUSSION**

The major pharmacokinetic feature in our study was the prolonged elimination half-life. Although sampling was done only up to 24 h with a limited number of samples in the elimination phase, this result was close to those of other studies (13, 18, 27). The reason for slow elimination of SPX could be enterohepatic circulation (about 40%), as was shown in animal studies [19] and the pronounced nonrenal elimination [23]. Limited data suggest that minor enterohepatic circulation also occurs with ciprofloxacin (13). The long elimination half-life could be an advantage, resulting in bactericidal concentrations for prolonged periods, which would make once-a-day treatment possible.

The present study demonstrates that elimination of SPX is mainly nonrenal. Extrarenal elimination routes have been confirmed by a study with 14C-labeled SPX in rats (16), revealing recoveries in urine and feces of 13 to 21 and 80 to 89% of a given oral dose, respectively. In a phase one study, about 10% of the dose (200 mg given orally) was excreted unchanged, 25% was excreted in urine and as a glucuronide, and 56% was excreted unchanged in feces (18).

SPX is characterized by a high V of 331 ± 101 liters/70 kg, but accurate information on the V can be obtained only

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**TABLE 1. Pharmacokinetics of SPX as determined by HPLC**

<table>
<thead>
<tr>
<th>Dose (mg/day)</th>
<th>Cmax (mg/liter)</th>
<th>t1/2 (h)</th>
<th>AUC0-24 (mg h/liter)</th>
<th>V (liters/70 kg)</th>
<th>Renal CL (ml/min)</th>
<th>Total CL (ml/min)</th>
<th>Recovery in urine (%/24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>400, 1</td>
<td>0.56 ± 0.13a</td>
<td>18.3 ± 3.9</td>
<td>16.4 ± 2.3b</td>
<td>331 ± 101</td>
<td>19.1 ± 5.8</td>
<td>201 ± 31</td>
<td>5.89 ± 1.4</td>
</tr>
<tr>
<td>200, 4</td>
<td>0.61 ± 0.16c</td>
<td>18.3 ± 3.9</td>
<td>18.3 ± 5.1c</td>
<td>303 ± 86</td>
<td>23.2 ± 19.4</td>
<td>190 ± 51</td>
<td>6.69 ± 3.2a</td>
</tr>
</tbody>
</table>

* Abbreviations: Cmax, maximum concentration of SPX in serum; t1/2, terminal half-life; AUC0-24, total area under the serum concentration-time curve.

a If applicable, recalculated for 200-mg dose.

b If applicable, recalculated for a single dose (i.e., by subtracting accumulation).

c Day 8.

d ND, not determined.
following intravenous administration and therefore this result must be considered to be only an estimate. However, high vs are a specific characteristic of quinolones (28). This value is substantially greater than that of total body water and strongly suggests that the drug is concentrated in certain tissues. It has been reported previously (22) that SPX is distributed in many tissues at levels similar to or higher than that in plasma, except for some tissues, like the brain.

Many investigators have studied the effects of quinolones on the normal intestinal flora. As might be suggested by their antimicrobial profile, they produce far more effects on aerobic gram-negative flora. As repeatedly demonstrated, quinolones drastically reduce numbers of members of the family Enterobacteriaceae and have less of an effect on enterococci, little effect on the anaerobic flora, and none on staphylococci (6, 12, 30, 31). It might be suggested that after oral administration, SPX would be more likely to affect bowel flora because of its high levels in feces, enterohepatic circulation, secretion into bile, and extended antimicrobial activity spectrum. Despite these facts, our results reveal only a moderate effect of SPX on the gut flora, comparable to those of the other quinolones (17).

Clinical adverse effects of quinolones are usually mild, including gastrointestinal disturbances (3 to 8.4% of adverse reactions), central nervous system problems (0.9 to 4.7%), and allergic reactions (0.5 to 2.2%), and laboratory test abnormalities, such as elevations in hepatic enzymes (1.8 to 2.7%) (29, 31). In our study, gastrointestinal disturbances, such as meteorism and soft stool, occurred in five volunteers in the SPX-treated group but in none of the placebo-treated group.

REFERENCES


