Single-Dose Pharmacokinetics of Ro 17-2301 (AMA-1080), a Monocyclic β-Lactam, in Humans

ERHARD WEIDEKAMM,1* KLAUS STOECKEL,1 HERMANN-JOSEF EGGER,1 AND WALTER H. ZIEGLER2

Pharmaceutical Research Department, F. Hoffmann-La Roche & Co., Ltd., CH-4002 Basel,2 and Department of Clinical Pharmacology, University Hospital Zurich, CH-8032 Zurich,2 Switzerland

Received 17 May 1984/Accepted 13 August 1984

Ro 17-2301 (AMA-1080) is a new N-sulfonated monocyclic β-lactam that is highly active against gram-negative bacteria, especially against Enterobacteriaceae and Haemophilus, Neisseria, and Pseudomonas spp. (Kondo et al., Proc. Int. Congr. Chemother. 13th, Vienna, Austria, p. 561-565, 1983). The single-dose pharmacokinetics of this compound were studied in six healthy male volunteers who received intravenous infusions of 500, 1,000, and 2,000 mg. A good linear correlation ($r^2 = 0.99$) was found between the dose infused and the resulting area under the plasma concentration-time curve. Maximal plasma concentrations of 36, 78, and 150 µg/ml appeared after doses of 500, 1,000, and 2,000 mg, respectively. The mean terminal elimination half-life was 1.8 h (range, 1.4 to 2.3 h), the apparent volume of distribution at steady state was 17 liters, and the total systemic clearance was 150 ml/min. Within 72 h 78% to 89% of the dose was recovered intact from urine. After administration of $^{14}$C-labeled Ro 17-2301, 96% of the radioactivity was found in the urine and 3% was found in the feces. The concomitant administration of probenecid did not affect the renal clearance or urinary excretion of this β-lactam, an indication that the renal elimination of this substance is only by glomerular filtration. Ro 17-2301 was 18% bound to human plasma protein, and this binding was independent of concentration between 25 and 400 µg/ml. Based on these data, the pharmacokinetics of this monocyclic β-lactam should be predictable in the foreseen dose ranges.

In 1981 the first N-sulfonated monocyclic β-lactam antibiotic, sulfazecin, was discovered by Takeda Chemical Industries (3). The chemical modification of this molecule led to Ro 17-2301 (AMA-1080) (Fig. 1), a potent antibacterial compound which shows high resistance to hydrolysis by both chromosomal and plasmid-mediated β-lactamases. The in vivo and in vitro activities of Ro 17-2301 are comparable to those of aztreonam, cefoperazone, cefazidime, cefmenoxime, and cefsporulin (M. Kondo, S. Kishimoto, M. Ochiai, K. Okonogi, and A. Omada, Proc. Int. Congr. Chemother. 13, Vienna, Austria, p. 561-565, 1983; K. Okonogi and M. Kuno, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 23rd, Las Vegas, Nev., abstr. no. 325, 1983; J. Siegel, P. Ryan, D. April, and J. Christenson, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 23rd, Las Vegas, Nev., abstr. no. 580, 1983; R. L. Then, and P. Angehrn, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 23rd, Las Vegas, Nev., abstr. no. 579, 1983). Because Ro 17-2301 has good potential for clinical use in humans, this study was conducted to characterize its disposition and pharmacokinetics. Various doses of the antibiotic were administered intravenously (i.v.) to human volunteers. The pharmacokinetics were linear for doses between 500 and 2,000 mg. Elimination was primarily by glomerular filtration, the half-life was 2 h, and distribution was throughout the extracellular water.

MATERIALS AND METHODS

Subjects. In total, eight healthy male volunteers (two for mass balance, six for dose proportionality study) aged 23 to 27 years participated in these clinical trials. Before enrollment and after completion of the study, each subject received a complete physical examination, a 12-lead electrocardiogram, and a series of laboratory tests (hematology, blood chemistry, urinalysis). All volunteers gave informed, written consent before entering this study, and protocols were approved by the institutional ethical review committee of the Department of Medicine, University Hospital Zurich.

Drug administration. Either 500, 1,000, or 2,000 mg of lyophilized Ro 17-2301 (disodium salt) was dissolved in 20 ml of sterile water. These doses were infused into the arm vein of each of six subjects, using a Harvard syringe infusion pump. The duration of infusion was 20 min. The time interval between the administration of the different doses was at least 2 weeks. In a second study the same subjects received an infusion of 1,000 mg of Ro 17-2301; however, this time 1,000 mg of probenecid (corresponding to two tablets of Benemid; Merck, Sharp & Dohme) ca. was administered orally 2 h before the infusion of Ro 17-2301. Additional doses of 500 mg of probenecid were given 10 min before and 4 h after the start of infusion.

The urinary and fecal excretion of total radioactivity and Ro 17-2301 was measured after the infusion of 1,000 mg (ca. 100 μCi) of a mixture of $^{14}$C-labeled and unlabeled drug. The mixture was prepared by cocrystallization of the two compounds. Before dilution the activity of the radioactive substance was 42.3 μCi/mg and the radiochemical purity amounted to 96%; the position of the label is indicated by the asterisk in Fig. 1.

Sample collection. In addition to the blank samples collected before drug infusion, 9-ml blood samples were collected into citrate-containing Vacutainers (Becton Dickinson & Co.) 5, 10, and 15 min after the start of drug infusion and exactly at the end of the infusion (20 min). Additional samples were collected 5, 10, 20, and 30 min and 1, 1.5, 2, 3, 4, 5, 6, 8, 9, and 10 h after the end of infusion. Within 30 min of collection, each blood sample was centrifuged at 1,000 × g for 15 min, and the plasma was carefully transferred to glass tubes and stored at −20°C or analyzed immediately. Urine samples were collected at 0 to 2, 2 to 4, 4 to 6, 6 to 8,
and 8 to 24 h and stored at -20°C until analysis. Plasma and urine samples were titrated to pH 6, using 0.1 M phosphate buffer (pH 3) to avoid degradation of Ro 17-2301 at pH values above 7.5. In addition to the blank samples before drug administration, all feces excreted over the following intervals were collected and frozen at -20°C: 0 to 24, 24 to 48, 48 to 72, and 72 to 96 h.

**Protein binding.** To minimize the degradation of Ro 17-2301 in plasma at a pH of >7.5 during protein-binding studies, the following precautions were taken: (i) only plasma of freshly drawn blood was used (7), (ii) control samples of plasma and ultrafiltrate were kept for the duration of the ultrafiltration at 37°C, (iii) immediately after dialysis or ultrafiltration samples were buffered at pH 6 with 0.1 M phosphate buffer (pH 3) and analyzed, and (iv) all binding data from ultrafiltration were calculated with reference to the control samples. To determine the extent of Ro 17-2301 binding to purified human serum albumin (HSA), a solution of 36 g of HSA (Sigma Chemical Co.) per liter in 0.134 M phosphate buffer, pH 7.5, was used. The HSA samples were processed identically to the plasma samples (see above). The plasma or albumin solutions were spiked with various amounts of Ro 17-2301 that were dissolved in small volumes (<10%, vol/vol) of pH 7.4 isotonic phosphate buffer.

Equilibrium dialysis was performed with Teflon dialysis cells (Dianorm) and Union Carbide dialysis tubing (pre-soaked in buffer), with a molecular weight cutoff of 10,000. Plasma, 1 ml, was dialyzed against 1 ml of phosphate buffer, pH 7.4. The cells were rotated at 37°C for 4 h. Aliquots from both sides of the membrane were removed, adjusted to pH 6 with 0.1 M phosphate buffer (pH 3), and assayed for Ro 17-2301.

Ultrafiltration was performed by using the Emit Free Liquid System I for weak acids (Syva). A 1-ml plasma or albumin solution of Ro 17-2301 was centrifuged at 37°C until 350 μl of ultrafiltrate was obtained (30 min, 2,000 × g). The ultrafiltrate was buffered to pH 6 as described above and assayed for Ro 17-2301.

**Assay procedures.** A 200-μl portion of plasma or urine, previously adjusted to pH 6, was pipetted into metal cylinders on large agar plates containing medium I (Difco Laboratories). *Escherichia coli* 1346 was the test organism. Inhibition zones were measured after incubation for 9 h at 37°C and the unknown concentrations of Ro 17-2301 were determined by using the curve-fitting program (no. SD-03 A) of the Hewlett-Packard HP 97 calculator. The limit of detection, an inhibition zone approximately three times the inner diameter of the cylinder (6 mm), was 0.25 μg/ml for a 200-μl specimen. In the concentration range of 0.25 to 90 μg/ml, a linear correlation ($r^2 = 0.9993$) existed between inhibition zone diameter and plasma concentration of Ro 17-2301. The precision (coefficient of variation of replicate analyses) was ±6.8% ($n = 12$). This bioassay correlates well with a high-pressure liquid chromatography method (H.-J. Egger, G. Fischer, E. Weidekamm, and D. Kronenberger, Proc. Int. Congr. Chemother. 13th, Vienna, Austria, 85/39–85/44, 1983).

Total radioactivity was measured in samples of plasma, urine, and feces and converted to microgram equivalents of Ro 17-2301. Each plasma (200 μl) and urine (100 μl) sample was mixed with 18 ml of Bruno-Christian solution (1) and counted in a liquid scintillation counter (Isocap 300). Feces were thoroughly mixed with the same amount of water (by weight); 200-mg aliquots were oxidized together with cellulose in an IN 4101 Oximat (ICN Intertechnique) before determination of radioactivity. All measurements of radioactivity were in triplicate and were corrected for background counts and quenching.

The samples from the protein-binding study (plasma, ultrafiltrate, dialysate) were analyzed by a specific high-pressure liquid chromatography assay (Egger et al., 13th ICC, 1983). Briefly, the sample workup included buffering of the biological samples to pH 6.0 with phosphate buffer, protein precipitation with acetonitrile, and purification of the aqueous phase containing the β-lactam by extraction with methylene chloride. An aliquot of the aqueous phase was analyzed by ion pair reversed-phase chromatography with UV detection. The detection limit for Ro 17-2301 in 0.5-ml specimens was 1 μg/ml (plasma, ultrafiltrate, dialysate) with a precision (coefficient of variation of replicate analyses) of <2%. For determination of Ro 17-2301 concentrations in plasma and urine, the bioassay was preferred because of its lower detection limits and the simplicity of sample handling.

**Pharmacokinetic evaluation.** The pharmacokinetic analysis of the plasma concentrations used a two-compartment open model. Biexponential equation 1 was fitted individually to the drug concentration-time data of each subject, using the nonlinear least-squares computer program NONLIN (5):

$$C_p = \frac{k_0 (k_{21} - \alpha)(1 - e^{-t/T})}{V_1 \alpha (\alpha - \beta)} e^{-\alpha t} + \frac{k_0 (\beta - k_{21})(1 - e^{-\beta t})}{V_1 \beta (\alpha - \beta)} e^{-\beta t}$$

(1)

This equation describes the model-predicted plasma concentration ($C_p$) at any time ($t$) during infusion and postinfusion; $k_0$ is the infusion rate in units of amount per time, $V_1$ is the apparent volume of distribution of the central compartment, $k_{21}$ is the intercompartmental transfer rate constant, and $\alpha$ and $\beta$ are hybrid distribution and elimination rate constants, respectively. $T = t$ during infusion, and after infusion $T$ is constant and equal to the time that the infusion ceased. A weighting factor of 1/(measured concentration) was chosen for the curve fitting. Equations 2 and 3 were used to calculate $A$ and $B$, the coefficients of the exponentials that would have been observed if the doses were administered as a bolus:

$$A = \frac{\text{dose} (\alpha - k_{21})}{V_1 (\alpha - \beta)}$$

(2)

and

$$B = \frac{\text{dose} (k_{21} - \beta)}{V_1 (\alpha - \beta)}$$

(3)
The area under the concentration-time curve (AUC_{0-\infty}) was calculated as follows:

\[ AUC_{0-\infty} = \frac{A}{\alpha} + \frac{B}{\beta} \]  

(4)

The AUC_{0-\infty} values were used to calculate the total systemic clearance (Cl_t):

\[ Cl_t = \frac{dose_{i.v.}}{AUC_{0-\infty}} \]  

(5)

Renal clearance of total (bound plus unbound) drug (Cl_{fr}) during selected time intervals was obtained from equation 6

\[ Cl_{fr} = \frac{X_{mt} - t_2}{AUC_{t_1 - t_2}} \]  

(6)

where \( X_m \) represents the amount of drug excreted during the time interval \( t_1 - t_2 \) and AUC_{t_1-t_2} is the area under the drug concentration-time curve for the same interval as determined by the trapezoidal rule. Moreover, an average Cl_{fr} was calculated by:

\[ Cl_{fr} = f_r \cdot Cl_{fr} \]  

(7)

where \( f_r \) is the fraction of the dose which was ultimately excreted into the urine. Renal clearance of unbound (free) drug (Cl_{fr}) was calculated according to

\[ Cl_{fr} = \frac{Cl_{fr}}{f_r} \]  

(8)

where \( f_r \) is the fraction unbound in plasma.

The apparent volume of distribution at steady state (V_{ss}) was calculated by a model-independent method (4)

\[ V_{ss} = \frac{\text{infused dose} \times AUMC}{AUC^2} \frac{\text{infused dose} \cdot T}{2 \cdot AUC} \]  

(9)

where AUMC is the area under the first-moment curve and \( T \) is the time of infusion.

**Statistical analysis.** The rank test according to Friedman (2, 8) was used to examine the influence of dose on the parameters elimination half-life \( t_{1/2p} \), \( V_{ss} \), \( Cl_{fr} \), \( Cl_t \), \( f_r \) (fraction of dose excreted in urine), and the influence of time on Cl_{fr} for the individual time interval values. The Wilcoxon rank test (2, 8) was used to test for parameter differences for \( t_{1/2p} \), \( V_{ss} \), \( Cl_{fr} \), \( Cl_t \), and \( f_r \) after the coadministration of probenecid.

**RESULTS**

Plasma concentrations. Figure 2 shows semilogarithmic plots of the mean plasma concentrations versus time data of six subjects after single-dose i.v. infusions of 500, 1,000, and 2,000 mg of Ro 17-2301 and 1,000 mg of Ro 17-2301 in the presence of probenecid. The individual concentration versus time data could, without exception, in all subjects be described by equation 1. The mean values of the pharmacokinetic parameters at the three doses are given in Table 1. Maximum plasma concentrations, measured at the end of infusion, were 35.5, 77.7, and 150.0 \( \mu g/ml \) after doses of 500, 1,000, and 2,000, mg, respectively. The area under the plasma concentration-time curve \( (AUC_{0-\infty}) \) increased linearly \( (r^2 = 0.99) \) with a dose of up to 2,000 mg. The \( t_{1/2B} \) ranged between 1.4 and 2.3 h, was independent of dose \( (P > 0.05) \), and averaged 1.8 ± 0.2 h. The apparent volume of distribution at steady state \( (V_{ss}) \) was between 15.3 and 21.0 liters (mean ± standard deviation, 16.9 ± 1.5 liters) and was
VOL. 26, 1000 1.77 plus dose (P (Cls) tl/2(), Cls, of dose percentage probenecid 2,000 55.7 the for total mean 1,000-56.0 doses. 78 and taken. 247 2,000 mg of 646 Urinary concentration. In the later was degraded after and start of 21.3 versus 17.0 liters; P < 0.05. Urinary excretion. Total urinary excretion, expressed as percentage of dose recovered intact in the urine within 24 h, was 78.0 ± 10.3, 85.2 ± 3.2, and 84.7 ± 3.2, respectively, after the 500-, 1,000-, and 2,000-mg i.v. doses (Table 2). We observed no influence of dose on total urinary excretion (P > 0.05) (Table 2). In addition, there was also no influence of time on the renal clearance (Clf) of Ro 17-2301 (Table 3). The mean values of Clf (±1 standard deviation) from Table 1 are 115.2 ± 19.1, 125.6 ± 6.9, and 123.5 ± 12.9 ml/min for the 500-, 1,000-, and 2,000-mg doses, respectively. The corresponding mean values of renal clearance with reference to free (unbound) drug (Clf) are 142.7, 156.2, and 152.5 ml/min, respectively, for the 500-, 1,000-, and 1,500-mg doses. Excretion balance. Within 72 h, 94 and 98% of the administered radioactivity (ca. 100 µCi) was recovered in the urine, and within 96 h 2.2 and 3.5% was found in the feces. A total of 78 and 89% of the radioactivity in urine was unchanged drug. The radioactivity in urine collected between 0 and 6 h was mostly unchanged Ro 17-2301, whereas in the later intervals the fraction of metabolite(s) increased continuously. When the respective AUCin values were measured for total radioactivity and parent compound in the plasma, 78 and 86% was unchanged Ro 17-2301. The plasma concentration versus time curves of total radioactivity and unchanged Ro 17-2301 were roughly identical up to 4 h after the start of infusion; they subsequently diverged continuously. Protein binding. The plasma protein binding of Ro 17-2301 was similar by equilibrium dialysis and ultrafiltration when the precautions described in Materials and Methods were taken. Ro 17-2301 degrades in alkaline plasma (pH > 7.5), and even in fresh human plasma up to 15% of Ro 17-2301 degraded after 4 h at 37°C. The binding of Ro 17-2301 to human plasma was concentration independent in the range of the observed total plasma concentrations (25 to 400 µg/ml); the fraction of Ro 17-2301 unbound (fp) in plasma was 0.82 ± 0.03. The binding to HSA (36 g/liter) was the same as in plasma over the concentration range 25 to 400 µg/ml, with fp = 0.81 ± 0.03.

**DISCUSSION**

The plasma concentration-time data of Ro 17-2301 in six healthy volunteers could be adequately described by a two-compartment open model. The dose-proportional increase in AUCin indicated that the pharmacokinetics of Ro 17-2301 are dose-concentration independent (linear) over the range studied. The dose-independent values for total body clearance (Clf) suggest that both renal (Clf) and nonrenal (Clf) clearances are constant and dose independent, since Clf = Clf + Clf. In addition, Clf was measured directly over different time intervals after the three different doses, and it was indeed a constant, dose- and time-independent value which averaged 120 ml/min (Table 3). Consequently, Clf must also be dose independent. The average value for Clf was 26 ml/min, 18% of the Clf. The modification of the mean Clf for plasma protein binding (fp = 0.82) results in an average renal clearance of free (unbound) drug (Clf) of about 150 ml/min. This Clf value is close to the average glomerular filtration rate in humans (~125 ml/min), an indication that the renal elimination of Ro 17-2301 is exclusively by glomerular filtration and that tubular secretion plays an insignificant role in the renal elimination process. If tubular secretion is not involved, probenecid should have no effect on the renal clearance of Ro 17-2301; the data in Table 1 support this interpretation. After the administration of 2,000 mg of probenecid over a period of 6 h (see Materials and Methods), Clf, AUCin, t1/2(0), and Clf of Ro 17-2301 were not altered. In contrast to these findings, probenecid delayed the excretion of aztreonam, a structurally related monocyclic beta-lactam (for structure see reference 10), by reducing its tubular secretion (E. A. Swabb, A. A. Sugarman, and M. A. Leitz, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 22nd, Miami, Fla., abstr. no. 140, 1982).

**TABLE 1. Pharmacokinetic parameters of Ro 17-2301 (AMA-1080) after i.v. infusion (20 min) of ascending doses**

<table>
<thead>
<tr>
<th>Dose infused (mg)</th>
<th>Parameter (mean ± SD; n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t1/2(g) (h)</td>
</tr>
<tr>
<td>500</td>
<td>1.76 ± 0.25</td>
</tr>
<tr>
<td>1,000</td>
<td>1.77 ± 0.14</td>
</tr>
<tr>
<td>2,000</td>
<td>1.93 ± 0.22</td>
</tr>
<tr>
<td>1,000 plus 2,000 mg of probenecid</td>
<td>1.99 ± 0.15</td>
</tr>
</tbody>
</table>

**TABLE 2. Urinary recovery of Ro 17-2301**

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th>% of dose recovered (mean ± SD; n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-2</td>
</tr>
<tr>
<td>500</td>
<td>48.9 ± 12.5</td>
</tr>
<tr>
<td>1,000</td>
<td>56.0 ± 10.9</td>
</tr>
<tr>
<td>2,000</td>
<td>55.7 ± 3.9</td>
</tr>
<tr>
<td>1,000 plus 2 g of probenecid</td>
<td>44.6 ± 11.3</td>
</tr>
</tbody>
</table>
TABLE 3. Renal clearance of Ro 17-2301 during selected intervals

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th>Mean Cl\textsubscript{k} of Ro 17-2301 ± SD (ml/min; n = 6) at time (h) after dosing:</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>123 ± 13 126 ± 29 114 ± 18 112 ± 38</td>
</tr>
<tr>
<td>1,000</td>
<td>124 ± 19 117 ± 49 134 ± 63 106 ± 23</td>
</tr>
<tr>
<td>2,000</td>
<td>123 ± 11 118 ± 23 121 ± 46 121 ± 30</td>
</tr>
</tbody>
</table>

There was a significant influence of probenecid on $V_{SS}$ which cannot yet be explained. The most obvious explanation for the increase in $V_{SS}$ would have been the displacement of Ro 17-2301 from its plasma protein-binding sites by probenecid (6). This seems unlikely, however, since the protein binding of the drug is only 18%. In this situation even total displacement of Ro 17-2301 could account for only 50% of the observed $V_{SS}$ increase (6). Moreover, a binding change is inconsistent with the lack of effect of probenecid on clearance, a parameter that is also sensitive to changes in binding.

Nonrenal clearance (Cl\textsubscript{k,n}) is one-sixth the Cl\textsubscript{k}. The nonrenal pathway is unknown; it is probably either biliary excretion or metabolism. The low recovery of the administered radioactivity in the feces (2.2 to 2.5%) favors the metabolic pathway, as does the presence of non-Ro 17-2301 radioactivity in the urine. Because the molecule is negatively charged at all physiological pH values, enterohepatic circulation is unlikely.

As aztreonam, Ro 17-2301 degrades in alkaline plasma (pH > 7.5). The degradation product is in both cases probably a beta-lactam ring open form (D. Janicke, presentation at the 1984 Buffalo Pharmaceutical Symposium). To minimize Ro 17-2301 degradation during the protein-binding measurements, we initially used the ultrafiltration method for its shorter running time. There was, however, good agreement between the protein-binding data of both equilbrium dialysis and ultrafiltration. Thus, the degradation product(s) does not seem to interfere with the protein binding of Ro 17-2301.

The steady-state volume of distribution of Ro 17-2301 (16.9 liters) was very similar to the extravascular water volume (~15 liters for a 70-kg man). Moreover, its binding preference for HSA suggests that this drug binds in both the vascular and the extravascular-extracellular spaces mainly to this protein. In this respect, Ro 17-2301 resembles other beta-lactam antibiotics, especially the cephalosporins (9). K. Stoeckel and J. Koup, Am. J. Med., in press). The similarity in distributional space (extracellular water) and binding preference (HSA) gives Ro 17-2301 a pharmacokinetic profile that corresponds closely to that of cephalosporins with low protein binding and elimination by renal glomerular filtration without tubular secretion (e.g., cefslodin, ceftriaxime, moxalactam, cefpazidime [9]). The half-life of Ro 17-2301 (1.8 h) is therefore similar to the half-lives known for this group of cephalosporins (1.9 to 2.3 h [9]).

ACKNOWLEDGMENTS

We acknowledge the expert technical assistance of Danielle Kronenberger, Renée Portmann, Vreny Trüb, and Guy Fischer.

LITERATURE CITED