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Altered Pharmacokinetic Disposition of Ciprofloxacin and Vancomycin after Single and Multiple Doses in Rabbits

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The pharmacokinetic disposition of vancomycin and ciprofloxacin was assessed in rabbits before the efficacy of these compounds in experimental staphylococcal endocarditis was compared. Ciprofloxacin was given in single intravenous bolus doses of 25 and 35 mg/kg and also in a multiple-dose regimen of 35 mg/kg every 6 h. Vancomycin was given in a similar manner in single doses of 17.5 and 25 mg/kg and in a multiple-dose regimen of 17.5 mg/kg every 6 h. Serum was sampled frequently after injections and analyzed by microbiologic assay for drug concentration. The pharmacokinetic parameters of clearance and steady-state volume of distribution were calculated by compartment-independent methods. These studies revealed that clearance of ciprofloxacin was reduced significantly after multiple doses (7.42 ± 0.85 [standard deviation] versus 6.09 ± 0.71 liters/h, $P < 0.01$). Although the half-life and volume of distribution increased after multiple dosing, the differences were not statistically significant. The disposition of vancomycin following single doses was significantly altered after the 25-mg/kg dose compared with the 17.5-mg/kg dose. Half-life, clearance, and volume of distribution changed from 1.27 ± 0.2 to 1.60 ± 0.21 h ($P < 0.05$), 0.54 ± 0.05 to 0.39 ± 0.04 liters/h ($P < 0.01$), and 0.37 ± 0.04 to 0.31 ± 0.03 liters/kg ($P < 0.05$), respectively. The disposition of ciprofloxacin was not altered with increases in dose size, and the disposition of vancomycin was not altered after multiple doses. If such alterations in the pharmacokinetic disposition of antimicrobial agents are unanticipated, the higher and more prolonged than expected serum concentrations may have an effect on the outcome of experimental infections.

Ciprofloxacin is a new fluorinated quinolone antimicrobial agent which will soon be released in oral form. The antibacterial spectrum of the compound includes aerobic gram-positive and gram-negative bacteria, including methicillin-resistant staphylococci and *Pseudomonas aeruginosa* (14). Although several quinolone congeners are under development, ciprofloxacin possesses the most potent in vitro activity (14, 19). After oral or intravenous (i.v.) administration, serum and tissue concentrations are achieved which are potentially effective in a wide variety of infectious diseases (11, 18).

At least two studies with humans have reported alterations in the pharmacokinetic disposition of ciprofloxacin after repeated oral dosing (1, 4). Both studies found significant increases in half-life and decreases in clearance of the drug after 7 to 13 days of treatment. Other investigators have not observed this phenomenon (3, 8).

To accurately assess the appropriate dosage regimen for a study comparing ciprofloxacin with vancomycin in the treatment of experimental staphylococcal endocarditis, we performed pharmacokinetic studies in male New Zealand White rabbits. The purpose of the study was to detect any changes in the pharmacokinetic disposition of ciprofloxacin or vancomycin with changes in dose size or multiple dosing. Significant alterations in the disposition of these drugs would affect achievable serum and tissue concentrations and could affect the outcome of the experimental infection.

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MATERIALS AND METHODS

Preparation of drug solutions. Ciprofloxacin powder (provided by Miles Pharmaceuticals, West Haven, Conn.) was dissolved in 5% glucose in water at 25 and 35 mg/ml. Vancomycin (Eli Lilly & Co., Indianapolis, Ind.) was dissolved in 5% glucose in water at 17.5 and 25 mg/ml. After preparation, all solutions were sterilized by passage through filters (0.2-μm pore size; Gelman Sciences, Ann Arbor, Mich.). All drug solutions were stored in sterile containers at 4°C until used.

Pharmacokinetic studies. Male New Zealand White rabbits weighing 2 to 3 kg (Langshaw Farms, Augusta, Mich.) were given single i.v. bolus injections of ciprofloxacin at doses of 25 and 35 mg/kg. Five rabbits received each dose, and serum samples for the determination of antimicrobial agent concentrations were obtained by venipuncture 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, and 12.0 h after drug administration. A multiple-dose study was performed with ciprofloxacin in a group of five rabbits at a dose of 35 mg/kg i.v. every 6 h, for a total of 13 doses. Animals were weighed every 24 h, and the dose administered was adjusted for weight as needed. Postdose and predose (trough) serum samples for the determination of drug concentrations were obtained daily, with postdose samples obtained 15 min after drug administration and trough samples obtained just prior to administration of a scheduled dose. At the time of administration of the 13th dose, a trough serum sample was obtained, followed by drug administration and the drawing of serum samples for the determination of antibiotic concentra-

TABLE 1. Mean serum concentrations of ciprofloxacin and vancomycin

Drug and dosage[a] (mg/kg)	Mean concn in serum (μg/ml) ± SD at:										
	0.25 h	0.5 h	0.75 h	1 h	1.5 h	2 h	3 h	4 h	6 h	8 h	12 h
Ciprofloxacin											
25, S.D.	6.0 ± 1.2	3.9 ± 0.6	2.8 ± 0.6	2.3 ± 0.2	1.7 ± 0.4	1.1 ± 0.2	0.7 ± 0.2	0.5 ± 0.1	0.22 ± 0.02	0.08 ± 0.02	0.02 ± 0.01
35, S.D.	10.3 ± 1.0	6.2 ± 0.5	4.3 ± 0.4	3.5 ± 0.5	2.0 ± 0.3	1.6 ± 0.4	0.8 ± 0.2	0.44 ± 0.15	0.23 ± 0.08	0.1 ± 0.06	0.03 ± 0.01
35, M.D.	8.1 ± 2.2	6.0 ± 0.7	4.2 ± 0.7	3.8 ± 0.5	2.4 ± 0.2	1.9 ± 0.1	1.0 ± 0.2	0.75 ± 0.08	0.33 ± 0.13	—[b]	0.09 ± 0.03
Vancomycin											
17.5, S.D.	50.2 ± 3.0	40.2 ± 5.0	32.8 ± 1.9	28.2 ± 2.9	24.0 ± 1.4	16.7 ± 2.7	12.0 ± 2.4	5.5 ± 0.7	2.0 ± 0.7	—	—
25, S.D.	85.2 ± 10.0	70.4 ± 4.8	58.5 ± 3.4	52.0 ± 4.3	45.4 ± 7.1	31.4 ± 5.9	23.4 ± 4.0	16.6 ± 2.4	5.9 ± 0.9	2.6 ± 1.1	—
17.5, M.D.	54.6 ± 6.4	43.7 ± 4.3	35.5 ± 5.3	28.4 ± 4.3	21.5 ± 4.4	15.4 ± 2.1	9.4 ± 1.8	5.3 ± 1.7	1.8 ± 0.3	—	—

[a] S.D., Single dose; M.D., multiple doses.
[b] —, No sample obtained.

tions 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, and 12.0 h later.

As for ciprofloxacin, vancomycin was given in single i.v. bolus injections at 17.5 and 25 mg/kg. Five rabbits received each dose, and serum samples for the determination of drug concentrations were obtained 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, and 6.0 h after drug administration (an 8-h sample was obtained for animals receiving 25 mg/kg). A multiple-dose study was performed in a group of five rabbits as described for ciprofloxacin, at a dose of 17.5 mg/kg i.v. every 6 h. Animals were weighed daily, and the dose administered was adjusted for weight as needed. Serum samples were obtained on the same schedule as for ciprofloxacin, except that following the 13th dose, a 12-h sample was not obtained.

Drug concentrations in serum. Ciprofloxacin concentrations in serum were determined by bioassay with an agar well diffusion method as modified by Reeves and Bywater (13). This method uses antibiotic medium 11 (Difco Laboratories, Detroit, Mich.) seeded with *Klebsiella pneumoniae* ATCC 10031 as the assay organism. The lower assay limit was 0.02 μg/ml. Assay variability was approximately 8.5%. Vancomycin concentrations in serum were also determined by bioassay, using a paper disk diffusion method for concentrations of ≥5 μg/ml (15); for sera containing lower concentrations, an agar well diffusion method was used. The agar used for both vancomycin assay procedures was antibiotic medium 5 (Difco) seeded with *Bacillus subtilis* ATCC 6633 as the assay organism. The lower assay limit was 1.0 μg/ml. Assay variability was approximately 4%. Pooled normal rabbit serum was used to prepare standards and dilute serum samples as needed for the bioassay techniques. All serum samples and standards were assayed in quadruplicate.

Pharmacokinetic analysis. Serum ciprofloxacin and vancomycin concentration versus time curves were described by biexponential and monoexponential models, respectively. Elimination half-life was calculated by dividing the natural logarithm of 2 by the terminal elimination rate constant estimated from the model. The other pharmacokinetic parameters of each drug were determined by compartment-independent methods (10). The area under the plasma concentration versus time curve (AUC) for ciprofloxacin was calculated by the trapezoidal rule. The AUC for the multiple-dose data was calculated from time zero to the last measured datum point, whereas the AUC for single-dose data was calculated from time zero to infinity. The AUC beyond the last measured point was estimated by dividing the predicted value of the last point by the terminal elimination rate constant. The AUC for vancomycin was calculated by dividing the predicted time zero concentration (C_0) by the elimination rate constant.

Estimates of plasma clearance (CL) and steady-state volume of distribution (V_{ss}) were calculated from the noncompartmental equations CL = dose/AUC and V_{ss} = [dose (AUMC)]/(AUC)^2, where AUMC is the area under the first moment of the serum concentration versus time curve calculated by the trapezoidal rule.

Statistical analysis. Comparison of mean data was performed by an analysis of variance. $P \leq 0.05$ was considered significant.

RESULTS

Serum concentration data for ciprofloxacin and vancomycin are detailed in Table 1. Pharmacokinetic analysis of these data (Table 2) revealed a significant change in the clearance of ciprofloxacin after multiple doses. The clearance decreased from 7.42 liters/h after the 35-mg/kg single dose to 6.09 liters/h after 13 doses given every 6 h. Although the half-life and volume of distribution both increased with multiple dosing, the differences were not significant owing to the variance of the data. All of the calculated parameters of vancomycin were significantly altered when the single dose was increased from 17.5 to 25 mg/kg (ca. 40% increase). Half-life increased from 1.27 to 1.60 h. CL decreased from 0.54 to 0.39 liter/h, and V_{ss} decreased from 0.37 to 0.31 liter/kg.

TABLE 2. Pharmacokinetic parameters of ciprofloxacin and vancomycin[a]

Drug and dosage (mg/kg)	Half-life (h)	CL (liters/h)	V_{ss} (liters/kg)
Ciprofloxacin			
25, S.D.	1.85 (0.25)	7.85 (0.90)	4.66 (0.65)
35, S.D.	1.90 (0.27)	7.42 (0.85)	4.06 (0.61)
35, M.D.	2.49 (0.64)	6.09 (0.71)	5.65 (1.48)
F	3.36	6.92	1.7
P	NS[b]	<0.01	NS
Vancomycin			
17.5, S.D.	1.27 (0.20)	0.54 (0.05)	0.37 (0.04)
25, S.D.	1.60 (0.21)	0.39 (0.04)	0.31 (0.03)
17.5, M.D.	1.27 (0.07)	0.51 (0.06)	0.36 (0.03)
F	6.14	11.78	4.75
P	<0.05	<0.01	<0.05

[a] See Table 1, footnote *a*. Values in parentheses are standard deviations.
[b] NS, Not significant.

The disposition of ciprofloxacin was not altered with increases in dose size, and the disposition of vancomycin was not altered after multiple doses.

DISCUSSION

Other investigators using the rabbit for experimental infections have limited their pharmacokinetic studies to determination of serum and tissue concentrations and estimations of half-life. The half-lives of ciprofloxacin in New Zealand White rabbits have been reported to be 1.8 and 2.2 h after 40 mg/kg every 8 h given subcutaneously and 9.7 h after 50 mg/kg every 8 h given intramuscularly (5, 12, 16). The values of 1.8 and 2.2 h are consistent with our value of 2.5 h after multiple 35-mg/kg i.v. doses. The serum concentration data are not directly comparable because, unlike the other investigators, we administered ciprofloxacin by i.v. bolus injection. Regardless, serum concentrations of the drug in these other studies were approximately 50% of those found during our investigation. This is probably due to the need for absorption from the intramuscular or subcutaneous site.

A recent study of experimental endocarditis in rabbits used vancomycin at a dose of 30 mg/kg every 12 h by i.v. injection (7). The estimated half-life of vancomycin was 1.9 h, which is consistent with the value of 1.6 h we observed after a 25-mg/kg dose. The peak concentration 15 min after the injection of vancomycin in the aforementioned paper (7) was found to be 179 μg/ml. This is quite different from our value of 85 μg/ml at the same time point. There is no evident explanation for this other than the assay method, since the animals were the same and the drug dose and method of administration were very similar. However, two additional studies that used 15 to 30 mg/kg of vancomycin i.v. found concentrations of 42 to 55 μg/ml approximately 0.5 to 1 h after the dose (2, 17). These numbers are consistent with our value of 52 μg/ml at 1 h.

Investigations with mice and rats have demonstrated that duration above the MIC in serum and endocardial vegetation correlates best with outcome of the experimental infection (6, 9). For example, Frimodt-Moller et al. (6) assessed the effect of pharmacokinetic characteristics of various antimicrobial agents on their 50% effective dose (ED_{50}) in pneumococcal infection in mice. They found that the only significant correlation was between the log ED_{50} and the duration above the MIC for serum concentrations of each drug. In their study even small changes in duration above the MIC (2 to 3 h) resulted in 10-fold differences in ED_{50}s. Changes of this magnitude in duration above the MIC were observed in our study. For example, the MIC for the methicillin-resistant isolate of *Staphylococcus aureus* used to induce endocarditis was 0.4 μg of ciprofloxacin per ml. The time that serum concentrations remained above this value after the 35-mg/kg single dose was 4 h. In contrast, this concentration was exceeded for nearly 6 h when multiple doses of the drug were given.

The vancomycin MIC for the *S. aureus* isolate was 0.8 μg/ml. Serum concentrations of vancomycin above this value were maintained for nearly 8 h after the 17.5-mg/kg dose and for about 11 h after the 25-mg/kg dose. If the concentrations achieved after the 25-mg/kg dose had been proportionately (1.4 times) larger and not enhanced due to decreased clearance, serum concentrations above 0.8 μg/ml would have been present for only slightly longer than 8 h.

It is not known whether these somewhat higher and more prolonged serum concentrations would significantly affect the outcome of the experimental infection, but it is possible. Further investigation is necessary to determine whether different durations above the MIC with the same drug will result in a different outcome of therapy of experimental infection in rabbits. It would appear to be useful to more accurately assess the pharmacokinetic characteristics of drugs used in experimental infections. The assumption of linear pharmacokinetic disposition from single-dose studies may be inappropriate. Experimental efficacy studies with multiple doses may benefit from concurrent concentration monitoring to avoid bias from altered kinetic disposition.

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