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Ciprofloxacin versus Vancomycin in the Therapy of Experimental Methicillin-Resistant *Staphylococcus aureus* Endocarditis

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We compared the efficacy of ciprofloxacin with that of vancomycin by using the rabbit model of methicillin-resistant *Staphylococcus aureus* endocarditis. Endocarditis was treated with ciprofloxacin (25 mg/kg [body weight] intravenously every 8 h) or vancomycin (17.5 mg/kg intravenously every 6 h) for 3 days. Vancomycin and ciprofloxacin were equally efficacious in clearing bacteremia. Both reduced vegetation bacterial counts by 5 $\log_{10}$ CFU/g and renal and splenic bacterial counts by more than 3 $\log_{10}$ CFU/g as compared with untreated control rabbits after 26 h of infection ($P < 0.001$). Both antimicrobial agents were able to eradicate the infectious process in an equivalent proportion of animals. No methicillin-resistant *S. aureus* that was recovered from ciprofloxacin-treated rabbits developed resistance to ciprofloxacin during therapy. Peak concentrations of ciprofloxacin in the sera of rabbits with endocarditis were significantly higher than those predicted by single-dose studies in uninfected rabbits. This finding was likely due to changes in the pharmacokinetics of the drug with multiple dosing and in infected versus uninfected rabbits. This study demonstrated that intravenously administered ciprofloxacin is as efficacious as vancomycin is in an in vivo model of a serious systemic methicillin-resistant *S. aureus* infection.

Methicillin-resistant *Staphylococcus aureus* is an important community and nosocomially acquired pathogen (4, 10, 11). Vancomycin is the most reliable antimicrobial agent for the treatment of infections caused by this organism, but tolerance to the drug in methicillin-resistant *S. aureus* has been described previously (MBC-to-MIC ratio of at least 32) and has been correlated with treatment failure (14). The existence of tolerance and the potential for the emergence of resistance to vancomycin makes the development of new antimicrobial agents with activity against methicillin-resistant *S. aureus* a worthwhile goal.

Ciprofloxacin, a fluorinated 4-quinolone antimicrobial agent, has been shown to have excellent in vitro activity against methicillin-resistant *S. aureus* (12, 13). We undertook an in vivo study comparing the efficacy of ciprofloxacin with that of vancomycin by using the rabbit model of endocarditis and used a clinical isolate of methicillin-resistant *S. aureus* as the test organism. This model affords a severe test of antimicrobial efficacy in a serious systemic infection.

MATERIALS AND METHODS

Organism. The methicillin-resistant *S. aureus* strain used (MRSA 494) was an isolate from a patient with endocarditis. Methicillin resistance was demonstrated with an oxacillin disk and by determining the methicillin MIC and MBC for the organism by a microdilution method, as described below.

In vitro studies. MICs and MBCs of methicillin, vancomycin, and ciprofloxacin for MRSA 494 were determined in quadruplicate by a broth microdilution method using supplemented Mueller-Hinton broth (7). The MIC was defined as the lowest concentration of antimicrobial agent that prevented visible growth after 18 to 20 h of incubation at 35°C. The MBC was defined as the lowest concentration of antimicrobial agent that killed ≥99.9% of the inoculum after 18 to 20 h of exposure, demonstrated by subculture of portions of the microtiter dilutions onto Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.).

Animal studies. Male New Zealand White rabbits (Langshaw Farms, Augusta, Mich.) that weighed 2.0 to 3.3 kg were used. Pharmacokinetic studies were performed in uninfected rabbits with single intravenous bolus injections of vancomycin (17.5 mg/kg [body weight]; $n = 5$) or ciprofloxacin (25 mg/kg; $n = 5$). Multiple blood samples were obtained for 8 (vancomycin) to 12 (ciprofloxacin) h after administration of antimicrobial agents. Vancomycin and ciprofloxacin serum concentration versus time curves were described by one-compartment and two-compartment bolus models, respectively. Elimination half-life was calculated by dividing the natural logarithm of 2 by the terminal elimination rate constant estimated from the model.

For the production of endocarditis, rabbits were anesthetized by intramuscular injection of xylazine and ketamine. The left carotid artery was cannulated, and a catheter was advanced across the aortic valve (1). Each rabbit was inoculated intravenously with 10^6 CFU of MRSA 494 suspended in 1 ml of 0.85% NaCl 3 days after catheter placement. This procedure reliably produced endocarditis in all animals with properly positioned catheters.

Twenty-six hours after bacterial challenge, all animals had 1 ml of blood withdrawn aseptically from an ear vein for culture. Serial dilution and plating techniques were used to determine CFU per milliliter of blood. Inclusion in the study required that this initial blood culture be positive and that proper placement of the catheter across the aortic valve be verified at autopsy.

After initial blood cultures were obtained, animals were randomized into control (no treatment), vancomycin, or

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ciprofloxacin treatment groups. Vancomycin-treated animals received 17.5 mg/kg intravenously every 6 h, and ciprofloxacin-treated animals received 25 mg/kg intravenously every 8 h. Controls were sacrificed at the time therapy was initiated in animals that received antimicrobial agents, with sacrifice followed by autopsy.

Antimicrobial agents were administered for 3 days, with the dose adjusted for weight on a daily basis. Serum for measurement of peak (15 min after administration of both antimicrobial agents) and trough (just before a scheduled dose) antimicrobial agent concentrations and peak serum inhibitory and bactericidal titers (SIT and SBT; see below) was obtained at the time of dose 1 on day 2. Blood for culture was obtained before dose 1 on day 3.

After 3 days of therapy, randomly selected animals from both treatment groups were sacrificed 8 to 10 h after the final vancomycin dose or 10 to 12 h after the final ciprofloxacin dose. Blood for culture was obtained at the time of sacrifice. Other antimicrobial agent-treated animals were monitored for 7 days posttherapy to assess for potential eradication of endocarditis. Blood for culture was obtained from each of these animals 3 and 7 days posttherapy, followed by sacrifice. All animals that had received antimicrobial therapy survived until the time of elective sacrifice.

Immediately after sacrifice, animals were autopsied in an aseptic manner. All aortic valvular and left ventricular vegetations and a 250- to 300-mg section of left kidney and spleen, including areas with infarction or abscess formation or both, if visible, were removed. Vegetations and tissues were weighed wet and then homogenized in the presence of 1 ml of sterile 0.85% NaCl with a sterile mortar and pestle. Serial dilution and plating techniques were used to determine the number of CFU present, and results were expressed as CFU per gram. We were able to detect as few as 10 CFU per vegetation or tissue section. Because of this sensitivity limit, tissues or vegetations found to be sterile were considered to contain 10 CFU for numerical and statistical purposes. However, if all vegetations and tissues removed from animals sacrificed 7 days posttherapy were sterile, infection in those animals was considered eradicated. It was assumed that residual bacteria present would grow to detectable numbers during that time period.

Antimicrobial agent concentrations in serum. Ciprofloxacin concentrations in serum were determined by bioassay with an agar well diffusion method (2) as modified by Reeves and Bywater (8). This method used 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. Vancomycin concentrations in serum also were determined by bioassay with a disk diffusion method for levels ≥5.0 μg/ml (9). For levels below this, an agar well diffusion method was used. This method used antibiotic medium 5 (Difco) seeded with *Bacillus subtilis* ATCC 6633 as the assay organism. The lower assay limit was 1.0 μg/ml. Pooled normal rabbit serum was used to prepare standards and dilute serum samples as needed, and all serum samples and standards were assayed in quadruplicate.

SIT and SBT. Serum samples obtained at the time of peak antimicrobial agent concentration were used to determine the SIT and SBT. A microdilution method modified by the use of supplemented Mueller-Hinton broth as the diluent was used because vancomycin and ciprofloxacin are minimally protein bound (15). The SIT was defined as the highest dilution of serum that prevented visible growth after 18 to 20 h of incubation at 35°C. The SBT was defined as the highest dilution of serum that killed ≥99.9% of the inoculum after 18 to 20 h of exposure, demonstrated by subculture of portions of the microtiter dilutions onto Trypticase soy agar.

Resistance to ciprofloxacin. To ascertain whether residual MRSA 494 isolates from ciprofloxacin-treated animals persisted because of the development of resistance, all such organisms were plated onto Trypticase soy agar containing 5 μg of ciprofloxacin per ml. Based on achievable concentrations of ciprofloxacin in rabbits with endocarditis (see below), resistance at this level was thought to be relevant clinically.

Statistical analysis. All comparisons of means were made by one-way analysis of variance. All comparisons of relative frequencies were made by the Fisher exact test.

RESULTS

In vitro studies. The geometric mean MICs and MBCs of methicillin, vancomycin, and ciprofloxacin for MRSA 494 were 21.8 and 59.5, 0.4 and 0.4, and 0.3 and 0.6 μg/ml, respectively.

Animal studies. Pharmacokinetic studies revealed that the highest concentration of either antimicrobial agent in serum occurred 0.25 h after administration. This sampling time therefore was defined as the time of peak antimicrobial agent concentration. Mean ± standard deviation peak concentration in serum and elimination half-life, as determined in single-dose studies, were 50.2 ± 3.0 μg/ml and 1.3 ± 0.2 h for vancomycin and 6.0 ± 1.2 μg/ml and 1.9 ± 0.3 h for ciprofloxacin, respectively.

No significant difference was found in the degree of initial bacteremia (mean ± standard deviation $\log_{10}$ CFU/milliliter) for animals randomized to receive vancomycin (2.26 ± 0.72; $n = 31$) or ciprofloxacin (2.49 ± 0.82; $n = 36$) or for controls sacrificed 26 h after bacterial challenge (2.63 ± 0.75; $n = 17$). Mean ± standard deviation peak and trough antimicrobial agent concentrations in serum and geometric mean peak SIT and SBT achieved on day 2 of therapy were 51.0 ± 12.4 and 3.1 ± 1.9 μg/ml and 1:105 and 1:77, respectively, for vancomycin-treated animals. For those that received cirprofloxacin, the corresponding values were 10.8 ± 2.3 and 0.3 ± 0.1 μg/ml and 1:45 and 1:34. Of interest was the finding that mean peak ciprofloxacin levels were significantly higher than those expected in the single-dose pharmacokinetic studies we performed ($P < 0.001$). Additionally, peak SITs and SBTs for animals that received vancomycin were significantly higher than those for animals that received ciprofloxacin ($P < 0.001$).

After 2 days of therapy, there was no significant difference in blood culture positivity between the treatment groups. One animal from each group continued to show a low-level bacteremia (1.0 $\log_{10}$ CFU/ml for both; $P = 0.50$).

At elective sacrifice after 3 days of therapy, 0 of 22 vancomycin-treated and 1 of 27 ciprofloxacin-treated rabbits were bacteremic ($P = 0.55$). The bacteremia in this animal was low grade (1.0 $\log_{10}$ CFU/ml). Vegetation and tissue bacterial counts are shown in Table 1. There was a highly significant reduction in bacterial counts in animals treated with either antimicrobial agent, and no difference was observed between the treatment groups.

Eighteen treated rabbits were monitored for 7 days posttherapy; nine had received ciprofloxacin and nine had received vancomycin. Blood cultures were sterile in all 18 rabbits at 3 days posttherapy. At 7 days posttherapy, seven of nine ciprofloxacin-treated and nine of nine vancomycin-treated animals had sterile blood cultures ($P = 0.24$); vegetations were sterile in six of nine ciprofloxacin-treated and

TABLE 1. Counts of MRSA 494 in vegetations and tissues

Treatment group	Mean ± SD $\log_{10}$ CFU/g[a]		
	Vegetation	Kidney	Spleen
Ciprofloxacin (27 rabbits)	3.37 ± 1.58 (14)	2.01 ± 0.93 (21)	1.56 ± 0.23 (24)
Vancomycin (22 rabbits)	3.56 ± 1.67 (12)	1.91 ± 1.01 (18)	1.79 ± 0.97 (21)
No treatment (controls; 17 rabbits)	8.45 ± 0.70	5.27 ± 1.55	5.15 ± 0.58

[a] Numbers in parentheses are the number of rabbits rendered culture negative. No significant difference in bacterial counts between the ciprofloxacin and vancomycin treatment groups was observed. Significant differences were noted between the ciprofloxacin-treated animals and the controls and between the vancomycin-treated animals and the controls in all cases ($P < 0.001$).

eight of nine vancomycin-treated animals ($P = 0.25$). Considering vegetations and tissues together, five of nine ciprofloxacin-treated and seven of nine vancomycin-treated animals had sterile cultures and thus were considered to have their infections eradicated ($P = 0.24$).

Resistance to ciprofloxacin. We found no MRSA 494 isolates from rabbits treated with ciprofloxacin able to grow on Trypticase soy agar containing 5 μg of ciprofloxacin per ml.

DISCUSSION

The purpose of this study was to compare the therapeutic efficacy of ciprofloxacin to that of vancomycin in an in vivo model of a serious methicillin-resistant *S. aureus* infection. Both antimicrobial agents were found to be of equal efficacy in clearing bacteremia, in reducing vegetation and tissue bacterial counts, and in eradicating methicillin-resistant *S. aureus* endocarditis in rabbits. These findings support the contention that ciprofloxacin should be a viable alternative to vancomycin for the treatment of human infections caused by methicillin-resistant *S. aureus*.

Peak concentrations of ciprofloxacin in serum achieved in our study were higher than those reported to date in humans after parenteral administration (3, 5, 16, 17). This is the result of the small doses used in published studies. We found these high peak levels to be associated with a significant degree of inhibition and killing of the test organism, as assessed by SITs and SBTs. Of note was the fact that the higher SITs and SBTs found in rabbits that received vancomycin as compared with those found in rabbits treated with ciprofloxacin did not correlate with an improved microbiologic outcome. Peak concentrations of ciprofloxacin in serum in the range we produced should be attainable in humans with the use of larger parenteral doses, although close monitoring for possible adverse reactions associated with such concentrations would be warranted.

Our finding that peak concentrations of ciprofloxacin in the serum of animals with endocarditis were significantly higher than those predicted by single-dose studies was likely due to alterations in the pharmacokinetics of the drug with multiple dosing and in infected versus uninfected animals. Further studies are required to elucidate what alteration(s) may have occurred in infected rabbits. Pharmacokinetic studies we have done with multiple dosing in uninfected rabbits have shown a prolongation in elimination half-life and a decrease in clearance of the drug as compared with single-dose studies (unpublished data). Such changes will lead to higher than predicted drug levels over time. These findings underscore the importance of additional pharmacokinetic analyses of antimicrobial agents beyond single-dose studies and close monitoring of drug concentrations in serum during therapy of experimental infections in animals. Changes in drug disposition that may become evident with multiple dosing, if not compensated for by adjustment in dosage interval or dosage size, could influence the outcome of experimental infections.

We found that the emergence of resistance to ciprofloxacin at or above 5 μg/ml did not occur during our 3-day treatment course. However, the development of resistance to the drug during therapy of a human *S. aureus* infection has been described, with an increase in MIC from 0.5 to 4 μg/ml observed (6). This raises some concern regarding the frequency at which this event might occur when the use of ciprofloxacin becomes more widespread. In patients being treated with ciprofloxacin for infections caused by *S. aureus*, serial isolates (if available) should undergo careful testing to detect increases in MIC or MBC which could herald treatment failure.

This study has shown that ciprofloxacin is as efficacious as vancomycin is in the therapy of an experimental methicillin-resistant *S. aureus* infection. Comparative studies of the efficacy of ciprofloxacin versus that of vancomycin in methicillin-resistant *S. aureus* infections in humans would be the next logical step in the evaluation of this potent antimicrobial agent.

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