Effect of Peritoneal Dialysis Fluid and pH on Bactericidal Activity of Ciprofloxacin

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Ciprofloxacin is active in vitro against most bacteria that cause peritonitis associated with peritoneal dialysis. We compared the effects of pH (5.5 and 7.4) and medium (dialysis fluid) on the bactericidal activity of ciprofloxacin, tobramycin, vancomycin plus rifampin, and rifampin against *Pseudomonas aeruginosa*, *Escherichia coli*, and three strains of staphylococci. The bactericidal activity of ciprofloxacin was not significantly affected by pH or medium, in contrast to the activity of tobramycin, which was decreased by low pH.

Peritonitis remains the major complication of continuous ambulatory peritoneal dialysis (CAPD) (7, 9, 10, 13). Empiric therapy for CAPD peritonitis generally consists of an aminoglycoside and an antistaphylococcal agent (e.g., oxacillin, vancomycin, or a cephalosporin) given either intraperitoneally or parenterally. In this study, we investigated the activity of ciprofloxacin, a new broad-spectrum oral quinoline antibiotic, in peritoneal dialysis fluid. Ciprofloxacin has been shown to have excellent activity against the major pathogens associated with CAPD peritonitis, including methicillin-resistant staphylococci (1, 12), and aerobic gram-negative bacilli, including *Pseudomonas aeruginosa* (2, 3, 5, 16). Because previous studies showed a loss of activity of some antibiotics in dialysis fluid due to the low pH and high osmolarity of the dialysate solution (11, 14), we examined the bactericidal activity of ciprofloxacin in dialysis solution at various pHs against strains of bacteria that commonly cause CAPD peritonitis.

Two isolates of *Staphylococcus epidermidis* (methicillin resistant by disk susceptibility test) recovered from patients with peritonitis during CAPD were tested in addition to *P. aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, and *Escherichia coli* ATCC 25922. Ciprofloxacin (Miles Laboratories, Inc., West Haven, Conn.), rifampin (Merrell-Dow Pharmaceuticals Inc., Cincinnati, Ohio), and tobramycin and vancomycin (Eli Lilly & Co., Indianapolis, Ind.) solutions were made daily and diluted in the test media being used. MICs (Table 1) were determined in triplicate by microtiter dilution in cation-supplemented Mueller-Hinton broth (8). Test media for killing curves included Mueller-Hinton broth at a pH of 7.4 and buffered to 5.5 with 0.1 N HCl and 1.5% Dianeal (Travenol Laboratories, Deerfield, Ind.) (pH 5.5), as well as buffered to pH 7.4 with 0.1 N NaOH, and dialysis effluent obtained after an overnight dwell from patients who had not received any antibiotics for at least 1 month and who were not experiencing any signs or symptoms of peritonitis. The effluent (pH 7.4) was filtered through a sterile membrane (pore size, 0.45 μm; Millipore Corp., Bedford, Mass.) before use to remove cells and other particulate matter. Time-kill assays were performed in duplicate. A 20-ml volume of broth, dialysate, or effluent was inoculated with an overnight culture (18 h) of the test organism to yield a final concentration of 10^7 CFU/ml. Antibiotic(s) was added to achieve a final concentration of five times the MIC for the organism tested. Controls con-

taining no antibiotic were assayed simultaneously. We chose a concentration of five times the MIC for the test organism because we demonstrated a concentration-dependent increase in the bactericidal activity of ciprofloxacin which was maximal at this concentration. The resultant concentrations (0.04 to 2.5 μg/ml) are within a clinically achievable range in peritoneal dialysis fluid after a single oral dose of 750 mg of ciprofloxacin (S. Kowalsky, R. Echols, E. McCormick, and E. Andrews, Program Abstr. 25th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1005, 1985). When combinations of antibiotics were tested, individual antibiotics were assayed simultaneously. Tubes were incubated in a 37°C water bath. After vigorous agitation, samples were removed at 0, 0.5, 1, 2, 3, 4, 6, and 24 h after inoculation and plated by using a spiral plater (Spiral Systems Instruments Inc., Bethesda, Md.) onto plates (150 by 15 mm) of Mueller-Hinton agar (6).

Time-kill-curve results are shown as the average of duplicate assays. The results of assays for all organisms in Mueller-Hinton broth at both pHs are not shown in order to simplify the figures. These curves were coincident with those obtained for dialysis fluid at their respective pHs, except for the growth controls, for which Mueller-Hinton broth resulted in consistently better growth than did dialysis fluid. The time-kill kinetics of ciprofloxacin and tobramycin against *E. coli* are shown in Fig. 1. Tobramycin was adversely affected by the low pH. Bactericidal activity was reduced by a factor of 10^2 to 10^3 in the samples tested at pH 5.5 compared with pH 7.4. The killing of *E. coli* by tobramycin in dialysis effluent was intermediate to that in fresh dialysate at pH 7.4 and 5.5. The bactericidal activity of ciprofloxacin was slightly affected by the low pH of the unbuffered dialysate, although not as significantly as was that of tobramycin. There was also a moderate increase in bactericidal activity in the effluent compared to that in the dialysate. The pH-dependent activity of tobramycin against

TABLE 1. MICs for test organisms

Organism	MIC (μg/ml)			
	Ciprofloxacin	Tobramycin	Vancomycin	Rifampin
Pseudomonas aeruginosa	0.1	1.0		
Escherichia coli	0.008	0.25		
Staphylococcus aureus	0.25		0.5	0.008
Staphylococcus epidermidis 1	0.5		1.0	0.008
Staphylococcus epidermidis 2	0.5		1.0	0.016

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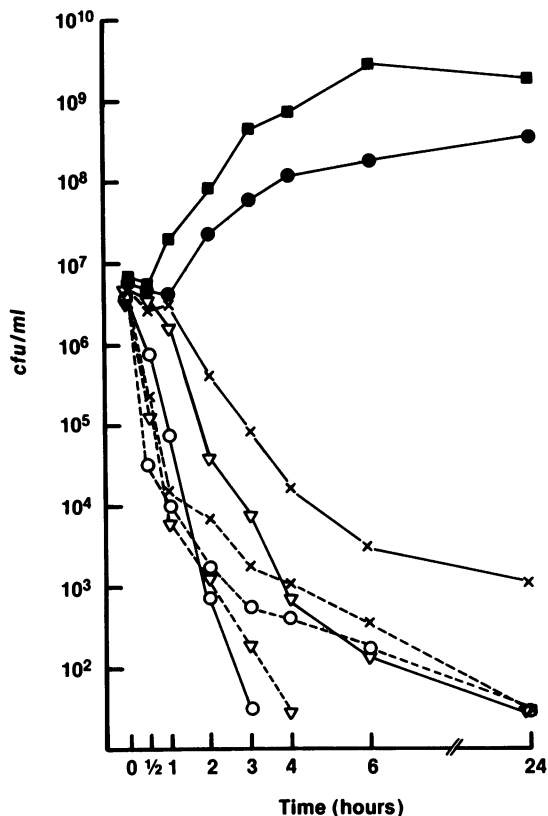FIG. 1. Killing curves of tobramycin (1.25 μg/ml) and ciprofloxacin (0.04 μg/ml) against *E. coli*. ■, Effluent growth control; ●, dialysate (pH 7.4) growth control; ∇—∇, effluent plus tobramycin; x—x, dialysate (pH 5.5) plus tobramycin; O—O, dialysate (pH 7.4) plus tobramycin; ∇---∇, effluent plus ciprofloxacin; x---x, dialysate (pH 5.5) plus ciprofloxacin; O---O, dialysate (pH 7.4) plus ciprofloxacin.
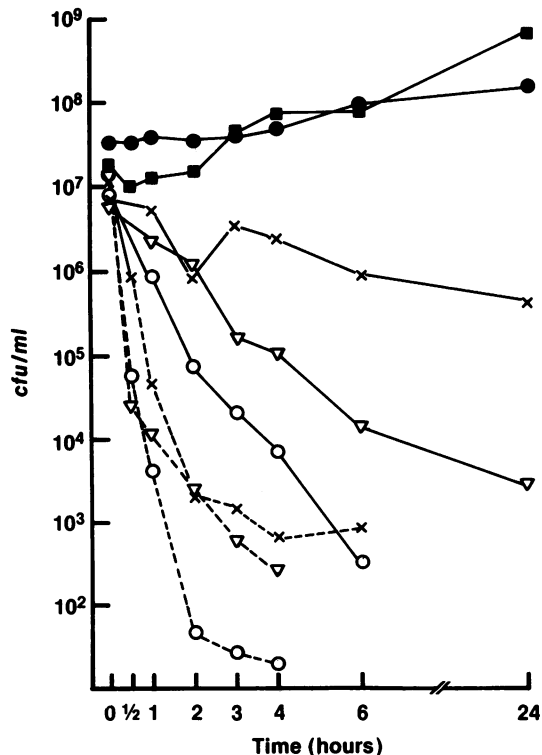

FIG. 2. Killing curves of tobramycin (5 μg/ml) and ciprofloxacin (0.5 μg/ml) against *P. aeruginosa*. For an explanation of the symbols, see the legend to Fig. 1.
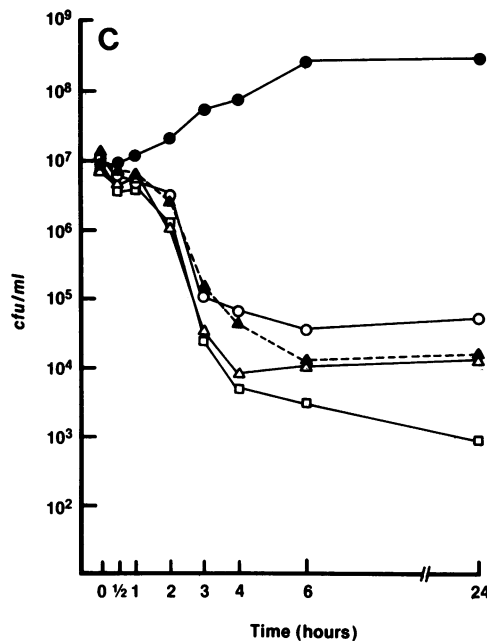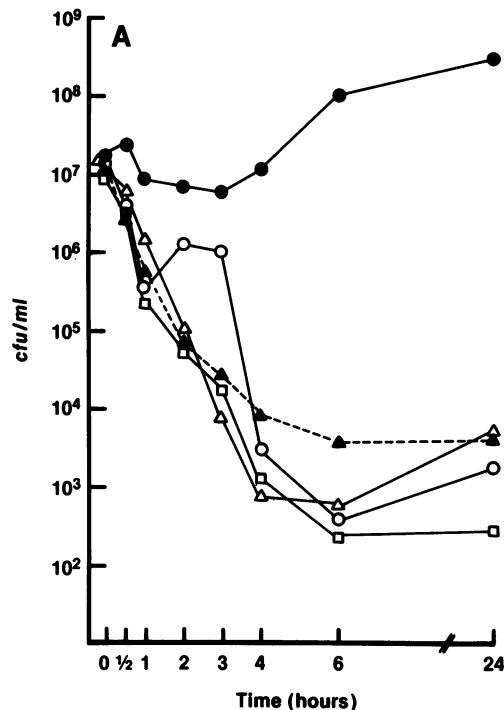

FIG. 3. (*Continued on next page*)

P. aeruginosa is shown in Fig. 2. As was seen with *E. coli*, there was a significant decrease in bactericidal activity at the lower pH. Ciprofloxacin showed rapid bactericidal activity against *P. aeruginosa*, with >99.9% killing in 2 h. Similar to *E. coli*, there was a slight loss of bactericidal activity with decrease in pH. The bactericidal activity of ciprofloxacin against the three strains of staphylococci in dialysis effluent is shown in Fig. 3. Killing was less rapid than with *E. coli* or *P. aeruginosa*, but the activity of ciprofloxacin was equal to that observed with vancomycin, rifampin, and the combination of vancomycin and rifampin. No effect on the rate of bactericidal activity by pH or medium was noted. There was
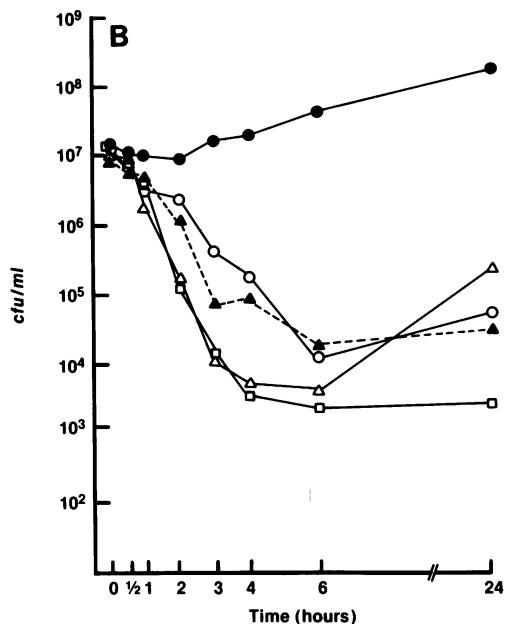
FIG. 3. (A) Killing curves of vancomycin (2.5 μg/ml; O), rifampin (0.04 μg/ml; △), vancomycin (2.5 μg/ml) plus rifampin (0.04 μg/ml; □), and ciprofloxacin (1.25 μg/ml; ▲) against *S. aureus*. ●, Growth. (B) Killing curves of vancomycin (5.0 μg/ml; O), rifampin (0.04 μg/ml; △), vancomycin (5.0 μg/ml) plus rifampin (0.04 μg/ml; □), and ciprofloxacin (2.5 μg/ml; ▲) against methicillin-resistant *S. epidermidis* 1. ●, Growth. (C) Killing curves of vancomycin (5.0 μg/ml; O), rifampin (0.08 μg/ml; △), vancomycin (5.0 μg/ml) plus rifampin (0.08 μg/ml; □), and ciprofloxacin (2.5 μg/ml; ▲) against methicillin-resistant *S. epidermidis* 2. ●, Growth.

no significant regrowth with vancomycin, ciprofloxacin, or the combination of vancomycin plus rifampin; however, sporadic regrowth was observed with rifampin alone.

Shalit et al. (11) recently described the effect of dialysate fluid on the activity of various antibiotics against *P. aeruginosa* and noted that aminoglycoside activity was adversely affected by both low pH and dialysis effluent fluid when MIC and MBC testing was performed. They could not show a similar effect in a time-kill assay, possibly due to the different methodology (continuous aeration and agitation). The reason for the loss of bactericidal activity of tobramycin in dialysis effluent was previously described with *S. aureus* (14). The reduced membrane potential in low-pH media has been hypothesized as the mechanism for decreasing aminoglycoside activity in such media (4). Ciprofloxacin exhibited a slight loss of activity in the low-pH dialysis fluid, which was corrected with pH adjustment to 7.4. No mechanism for the effect of pH on the activity of ciprofloxacin has been proposed, although the phenomenon has been described (17). Our studies demonstrated that the in vitro activity of ciprofloxacin equals or exceeds that of tobramycin against both *P. aeruginosa* and *E. coli* regardless of the pH of the test medium. For *P. aeruginosa*, ciprofloxacin resulted in a 2- to 3-$\log_{10}$-greater decrease in inoculum than did tobramycin in all situations tested. The bactericidal activity of tobramycin was also adversely affected by dialysis effluent, a medium that is at physiological pH and osmolarity (11).

Effluent did not appear to have any effect on the bactericidal activity of rifampin, vancomycin, or ciprofloxacin.

Because both vancomycin and aminoglycosides require parenteral administration (either intraperitoneally or intravenously), the apparent advantage of orally administered ciprofloxacin could be realized given the common practice of outpatient treatment of CAPD peritonitis (15). With its demonstrated in vitro bactericidal activity against many of the organisms associated with CAPD peritonitis, we believe clinical trials with ciprofloxacin in CAPD peritonitis are indicated.

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