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In Vitro Antibacterial Activities of Antibiotics Against *Pseudomonas aeruginosa* in Peritoneal Dialysis Fluid

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Intraperitoneal antibiotics are used to treat *Pseudomonas aeruginosa* peritonitis, a serious complication of continuous ambulatory peritoneal dialysis. However, *P. aeruginosa* killing is often inefficient despite low MBCs. Broth dilution MIC/MBC and time kill curves of tobramycin, amikacin, netilmicin, azlocillin, piperacillin, ceftazidime, cefsulodin, and ciprofloxacin were determined in peritoneal dialysis fluid (PDF), buffered PDF, fluid recovered from patients on continuous ambulatory peritoneal dialysis (RPF), and cation-supplemented Mueller-Hinton broth. MBCs of all antibiotics were 8 to 16 times greater in PDF and RPF than in Mueller-Hinton broth or buffered PDF. Use of the time kill curve technique and Mueller-Hinton broth showed that aminoglycosides killed ≥99.9% of *P. aeruginosa* at 1 h, ciprofloxacin killed ≥99.9% at 2 h, and β-lactams killed ≥99.9% at 6 h. In contrast, killing was not demonstrated in PDF by any drug at 6 h and by aminoglycosides only at 24 h. Bactericidal activity was optimal in RPF for ciprofloxacin at 1 h and for aminoglycosides at 2 h; bactericidal activity was not demonstrated in RPF with any β-lactam (no kill by penicillins; <99% kill by cephalosporins). Slow bacterial growth, increased protein binding, and glucose concentrations and other inhibitors may interfere with β-lactam activity in RPF. These considerations and reported clinical failures and toxicity of aminoglycoside therapy warrant further study of quinolones and drug combinations in *P. aeruginosa* peritonitis.

Continuous ambulatory peritoneal dialysis (CAPD), used in the treatment of patients with end-stage renal failure, is often complicated by peritonitis (4, 5, 7, 10), which occurs once per 11.3 to 13.1 patient-months (3, 9). The causative organisms are mainly gram-positive cocci (*Staphylococcus epidermidis*, *Staphylococcus aureus*, *Streptococcus* spp.), accounting for 50 to 65% of all episodes, and aerobic gram-negative bacilli (*Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella* spp.), accounting for 15 to 30% of the cases (5, 6). In one study, *P. aeruginosa* was the most frequently encountered gram-negative organism, accounting for 38.5% of gram-negative peritonitis and 9.6% of all cases (6). Peritonitis caused by *P. aeruginosa* has been associated with abscess formation, peritoneal fibrosis, and a high failure rate of antibiotic therapy, often necessitating removal of the intraperitoneal (i.p.) catheter (4, 6).

Many authors recommend i.p. administration of aminoglycosides for the treatment of gram-negative peritonitis (3, 5, 10, 12). The i.p. doses commonly used are generally chosen to achieve concentrations similar to their usual peak concentrations in serum (i.e., 6 to 8 μg of gentamicin or tobramycin per ml of dialysate) (3–6, 10). Unfortunately, such regimens have been associated with sustained, potentially toxic concentrations of aminoglycosides in serum (4, 5) and in one clinical study failed to eradicate *P. aeruginosa* despite the antibacterial activity predicted by in vitro testing (6).

Few studies have addressed the activity of antibiotics against *P. aeruginosa* in peritoneal dialysis fluid (PDF) (1) or in recovered peritoneal fluid (RPF) (peritoneal fluid recovered from patients undergoing CAPD) (J. J. Couperus, I. Roy, and H. A. Elder, Program Intersci. Conf. Antimicrob. Agents Chemother. 21st, Chicago, Ill., abstr. no. 101, 1981). These reports indicate decreased activity of aminoglycosides in PDF and RPF in comparison to activity in broth. The present study was designed to evaluate the in vitro activity of various antibiotics active against *P. aeruginosa*, including several investigational compounds, in PDF, buffered PDF (↑ pH PDF), and from RPF.

MATERIALS AND METHODS

Bacteria. Two strains of *P. aeruginosa* ATCC 27853 and an isolate recovered from a CAPD patient with *P. aeruginosa* peritonitis were used.

Antibiotics. Tobramycin (Eli Lilly & Co., Indianapolis, Ind.), amikacin (Bristol Laboratories, Syracuse, N.Y.), netilmicin (Schering Corp., Kenilworth, N.J.), azlocillin (Miles

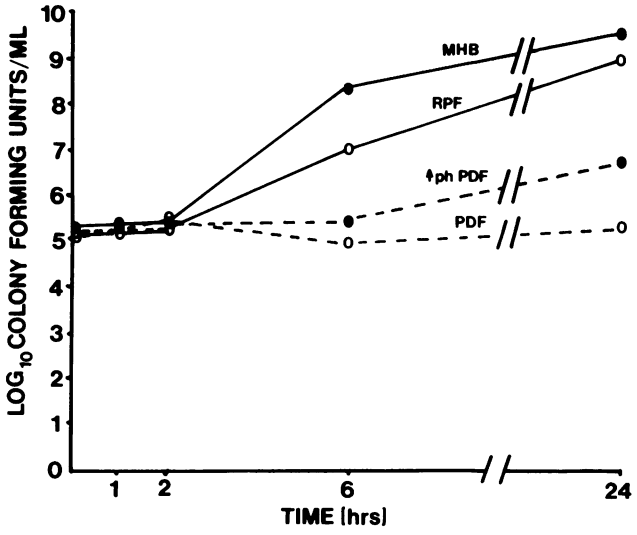

FIG. 1. Growth kinetics of *P. aeruginosa* ATCC 27853 without antibiotics.

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TABLE 1. MBCs (μg/ml) of various drugs against *P. aeruginosa* ATCC 27853[a]

Fluid	MBC (μg/ml) of:							
	Tobramycin	Amikacin	Netilmicin	Azlocillin	Piperacillin	Ceftazidime	Cefsulodin	Ciprofloxacin
MHB with Ca^{2+} and Mg^{2+}	2	8	16	8–16	8–16	4	8	0.25
PDF (pH, 5.5)	16–32	128–256	32	>256	>256	64	64	8–16
↑ pH PDF (pH, 7.4)	1–2	4–8	8–16	32	64	4–8	8–16	0.25–0.5
RPF (pH, 7.4)	16–32	64	64–128	256	>256	128	128–256	2

[a] Values are presented as the MBC (or range of MBCs) obtained from four independent determinations.

Pharmaceuticals, West Haven, Conn.), piperacillin (Lederle Piperacillin, Inc., Carolina, Puerto Rico), ceftazidime (Glaxo, Greenford, England), cefsulodin (Abbott Laboratories, North Chicago, Ill.), and ciprofloxacin (Miles Pharmaceuticals) were obtained as dry powders. Each drug was dissolved in an appropriate diluent, filter sterilized, and stored at −70°C.

Growth media. The growth media used in this study were Mueller-Hinton broth supplemented with Ca^{2+} and Mg^{2+} (MHB), 1.5% Dianeal (Travenol Laboratories, Inc., Deerfield, Ill.), PDF (pH, 5.5), PDF buffered with 0.1 N NaOH to pH 7.4 (↑ pH PDF), and RPF, composed of sterile, antibiotic-free peritoneal fluid, obtained immediately after a 6-h dialysis from patients undergoing CAPD. A differential cell count and bacteriological cultures were obtained from each RPF sample to ensure sterility and lack of pleocytosis (<50 leukocytes per mm^3 of RPF). The pH, osmolarity, protein content, and glucose, Ca^{2+}, and Mg^{2+} concentrations were determined in each growth medium by using the following methods: pH, by hydrogen ion selective electrode (Fisher Scientific Co., Pittsburgh, Pa.); osmolarity, by vapor pressure Osmometer (Wescor Inc., Logan, Utah); protein content, by tetrachloroacetic acid precipitation; glucose, by the glucose oxidase method (Beckman-Astra, Brea, Calif.); Ca^{2+}, by colorimetric assay with *O*-cresolpthalein complexone (Beckman-Astra); Mg^{2+}, by colorimetric assay with calmagite (Gilford Diagnostics, Cleveland, Ohio).

Susceptibility testing. MICs and MBCs were determined by the tube broth dilution method with an inoculum of 5×10^5 CFU/ml (16). The MBC was defined as 99.9% killing. Time kill curves were calculated from growth in 50-ml flasks containing 10 ml of media incubated on a rotary shaker at 37°C and sampled at 0, 1, 2, 6, and 24 h after inoculation (10^5 CFU/ml). The following antibiotic concentrations (μg/ml) were used in each of the above-mentioned growth media: tobramycin, 8; amikacin, 25; netilmicin, 10; azlocillin, 200; piperacillin, 200; ceftazidime, 100; cefsulodin, 100; and ciprofloxacin, 2.

RESULTS

MBCs of antibiotics in various fluids against *P. aeruginosa* ATCC 27853 are shown in Table 1. Similar results were obtained with the clinical isolate of *P. aeruginosa*. The MBCs of all the antibiotics tested were 8 to 16 times greater in PDF and RPF than in ↑ pH PDF and MHB. MICs were not determined owing to lack of visible turbidity in PDF, ↑ pH PDF, and RPF.

A comparison of several features of PDF and RPF is outlined in Table 2. Of note are the pH differences, high glucose concentrations, and the protein content in RPF.

The rate of antibacterial activity of different antibiotics against *P. aeruginosa* strains in various fluids is illustrated in Fig. 1 through 6. The time kill curves obtained with amikacin and netilmicin were similar to those of tobramycin, whereas those of azlocillin were similar to those of piperacillin.

The data illustrated in Fig. 2 through 6 show that the most efficient bactericidal rate in RPF was achieved by ciprofloxacin (≥99.9% kill at 1 h) and by aminoglycosides (≥99.9% kill at 2 h). This was comparable to their activity in MHB (control). Bactericidal activity was not detected in RPF with any β-lactam, in contrast to adequate killing in MHB (≥99.9% kill at 6 h). Killing was not demonstrated in PDF by any drug at 6 h, and only by aminoglycosides at 24 h.

DISCUSSION

The lack of bactericidal activity of β-lactams against *P. aeruginosa* in commercial PDF and RPF compared with standard broth can be attributed to several factors.

Slow bacterial growth, and hence reduced cell wall synthesis, has been associated in vitro with decreased bactericidal activity of β-lactams and other cell-wall-active drugs (8). This is supported by our data, which showed that maximal rates of bactericidal activity in the control broth (2 to 6 h after inoculation) correlated with the time of maximal rate of bacterial growth. Similarly, a decreased growth rate of *P. aeruginosa* in the three different types of fluids tested, i.e., PDF, ↑ pH PDF, and RPF, compared with MHB correlated with decreased bactericidal activity of β-lactams in these fluids.

Penicillin activity is adversely affected by low pH (13). This fact is reflected by the higher MBCs in the acidic PDF. However, buffering PDF to a pH of 7.4 had only a minimal effect on the rate of bactericidal activity of the β-lactams as measured by the kinetic studies. Factors such as rotational incubation, aeration, or volume differences may have contributed to this discrepancy. In addition, the pH of the RPF

TABLE 2. Comparison between Dianeal PDF and RPF

Fluid	pH	Total protein (mg/100 ml)	Osmolarity (mosM/liter)	Glucose (mg/100 ml)	Ca^{2+} (mEq/liter)	Mg^{2+} (mEq/liter)
PDF	5.5	0	346	1,500	3.5	1.5
RPF[a]	7.4–7.54	156–211	279–298	241–325	2.1–3.1	1.2–1.6

[a] Values are presented as the range obtained from four independent determinations.

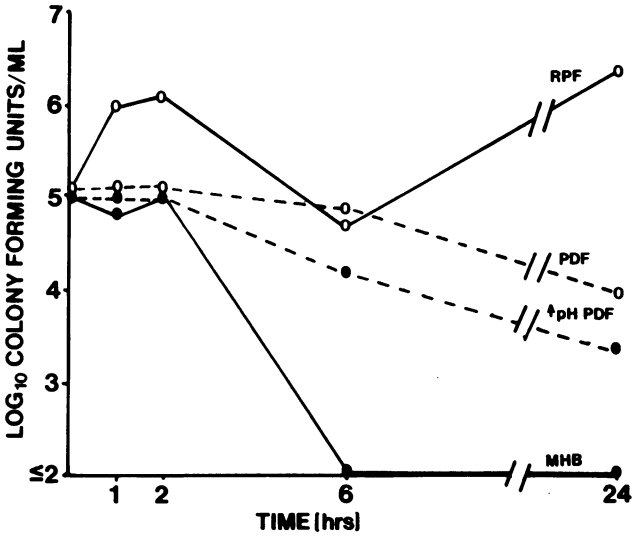

FIG. 2. Growth kinetics of *P. aeruginosa* ATCC 27853 in media containing piperacillin (200 μg/ml).

ranged from 7.4 to 7.54; thus, lack of bactericidal activity in this fluid cannot be attributed to a pH effect.

There is evidence of an adverse effect of high glucose concentration on penicillin activity (14). The glucose concentrations in PDF, ↑ pH PDF, and RPF were well above the physiological serum range (Table 2) and therefore could have contributed to the inhibition of β-lactam activity.

Decreased bactericidal activity of β-lactams in urine with increased osmolarity has been reported (15). Although this factor may have played a role in inhibiting antibiotic activity in PDF and ↑ pH PDF (osmolarity, 346 mosM/liter), it could not have in iso-osmolar RPF.

The peritoneal effluent derived from patients undergoing CAPD contains a high concentration of urea and other waste products. Although these components were not measured in the present study, they may have had an effect on bacterial growth and β-lactam activity. This requires further investigation.

The activity of the aminoglycosides and the carboxyquinolone derivative, ciprofloxacin, were decreased in PDF, most probably because of the low pH of this solution (2, 11). This is further supported by the marked improvement in activity of these compounds in buffered PDF and in RPF (pH, 7.4 to 7.54).

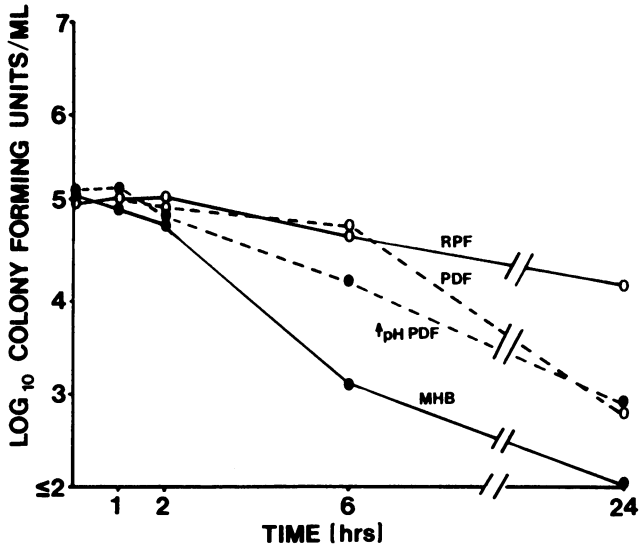

FIG. 4. Growth kinetics of *P. aeruginosa* ATCC 27853 in media containing cefsulodin (100 μg/ml).

The favorable bactericidal activity of aminoglycosides in RPF as measured by the time kill curve technique is of interest in view of the high MBCs of these drugs in the same fluid as measured by the tube broth dilution method. Factors that may have contributed to the enhanced aminoglycoside activity in the kinetic studies include constant mixing of drug and organisms and potentially improved oxygenation in the shaking flasks during kinetic studies, as opposed to the stationary tubes when MBCs were tested.

The present in vitro study implies that the i.p. administration of anti-pseudomonas β-lactams in dialysis fluid for the treatment of CAPD-related *P. aeruginosa* peritonitis might be inadequate. The use of aminoglycosides in this setting,

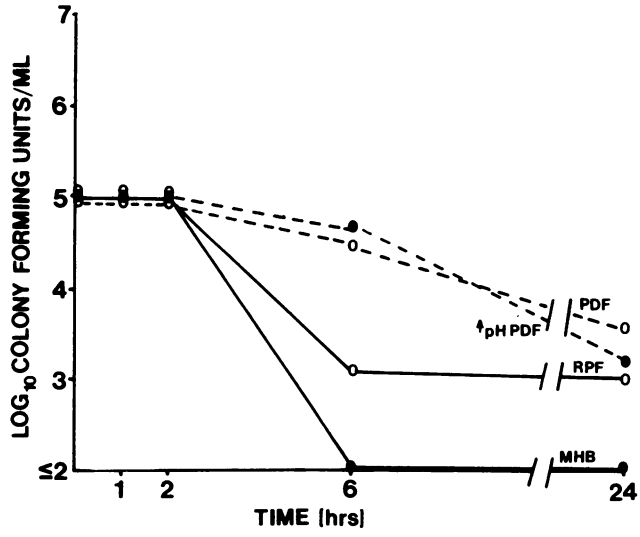

FIG. 3. Growth kinetics of *P. aeruginosa* ATCC 27853 in media containing ceftazidime (100 μg/ml).

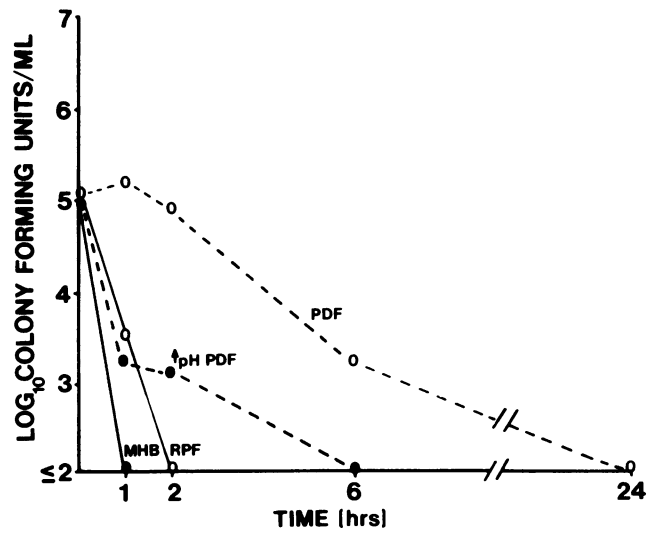

FIG. 5. Growth kinetics of *P. aeruginosa* ATCC 27853 in media containing tobramycin (8 μg/ml).

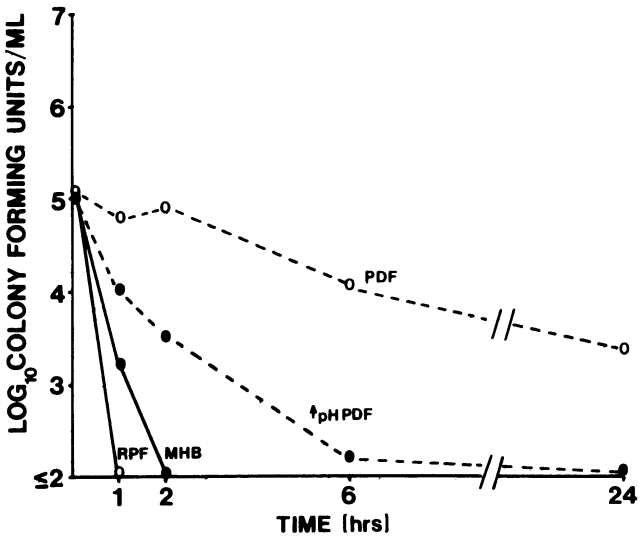

FIG. 6. Growth kinetics of *P. aeruginosa* ATCC 27853 in media containing ciprofloxacin (2 μg/ml).

although supported by favorable in vitro bactericidal effect, is problematic, mainly owing to potentially toxic concentrations of these compounds in serum with prolonged i.p. administration in CAPD patients (4) and to clinical failures when i.p. aminoglycosides were used alone for treatment of *P. aeruginosa* peritonitis (6). New quinolone derivatives may be useful for treating CAPD-related *P. aeruginosa* peritonitis owing to their favorable in vitro activity in RPF, good toxic-therapeutic index, and absorption after oral administration.

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