Bacterial Growth and Killing in Chronic Ambulatory Peritoneal Dialysis Fluids

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We determined the ability of *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Escherichia coli* to survive and grow in peritoneal dialysis fluids from patients undergoing chronic ambulatory peritoneal dialysis. Staphylococci did not survive in commercially available dialysis solutions but grew readily in peritoneal effluents obtained from patients after the dialysis dwell time. The number of CFU doubled 6 and 13 times in 24 h for *S. epidermidis* and *S. aureus*, respectively. *E. coli* grew well in both the pre- and postdialysis peritoneal fluid. Peritoneal macrophages as well as peripheral blood leukocytes inhibited bacterial growth in peritoneal dialysis fluid. However, 10^6 phagocytes per ml were minimally required to obtain a bacteriostatic effect. The addition of serum to peritoneal dialysis fluid increased the antibacterial activity of macrophages and blood leukocytes. The capacity of the aminoglycoside antibiotic tobramycin to reduce bacterial CFU in peritoneal dialysis fluid was only 10% of its bactericidal capacity in standard Mueller-Hinton broth. Peritoneal dialysis fluid had no effect on the antibacterial activity of imipenem.

Bacterial peritonitis is the major complication limiting the use of chronic ambulatory peritoneal dialysis (CAPD) for the treatment of patients with end-stage renal disease. Even with currently improved techniques, peritonitis will occur at least once in more than 50% of CAPD patients, with an overall rate of one episode of peritonitis every 6 to 12 patient months (9, 21). *Staphylococcus epidermidis*, *Staphylococcus aureus*, and gram-negative aerobic bacteria are largely responsible for the infections. The route of infection in CAPD peritonitis is generally thought to be via the indwelling peritoneal catheter or its tunnel through the abdominal wall (4, 21). Little more is known about the pathogenesis of CAPD peritonitis. We recently reported on the antibacterial defense mechanisms of the peritoneal cavity in these patients (19). However, more studies on bacterial as well as host factors are needed to fully understand the events that lead from accidental contamination of the peritoneal cavity to frank peritonitis. In this study we determined some of the growth characteristics of *S. epidermidis*, *S. aureus*, and *Escherichia coli* in CAPD fluids. In addition, the influence of phagocytic cells, serum, and antibiotics on bacterial multiplication were investigated.

The results indicate that, although commercially available CAPD solutions may not support bacterial growth, these fluids are modified during the intraperitoneal dwell time to become suitable milieu for bacterial multiplication. The data further suggest that peritoneal macrophages, when present in sufficient numbers, are able to suppress bacterial growth. On the other hand, the action of some antibiotics may be severely compromised in CAPD fluids.

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MATERIALS AND METHODS

Bacterial inoculum. Clinical strains of *S. epidermidis* and *E. coli* (serotype O22:H16) and the Cowan I strain of *S. aureus* were used in this study. These three bacterial species are responsible for the majority of peritonitis episodes during CAPD (19, 21). Bacteria were maintained on blood agar plates at 4°C. For each experiment, several colonies of bacteria were subcultured into 10 ml of Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) in a shaking incubator set at 37°C. After overnight growth the bacteria were washed twice with sterile phosphate-buffered saline (PBS, pH 7.4) and suspended in PBS to a concentration of ca. 5×10^8 CFU/ml. Further dilutions were made in the appropriate experimental growth medium.

Media. The following media were tested for their capacity to support bacterial growth: (i) PBS; (ii) commercially available CAPD solutions containing 1.5, 2.5, or 4.25% glucose, pH 5.1 to 5.5 (Dianeal; Travenol Laboratories, Deerfield, Ill.); (iii) cell-free peritoneal dialysis effluent pooled from five discarded exchange bags after centrifugation, pH 7.2 to 7.6 (30 min at $1,600 \times g$). The exchange bags were from five noninfected CAPD patients treated at the Regional Kidney Disease Program, Hennepin County Medical Center, Minneapolis, Minn. Mueller-Hinton broth (Difco) was used as growth control medium.

Phagocytic cells and serum. Human peritoneal macrophages (PMΦ) were isolated from peritoneal effluent from noninfected CAPD patients after an overnight dwell time. The peritoneal cells in these fluids were harvested by centrifugation, washed, and prepared essentially as previously described (19).

The final cell suspensions contained a mean (range) of 86% (78 to 91%) PMΦ, 8% (3 to 15%) lymphocytes, 4% (0 to 9%) polymorphonuclear neutrophil leukocytes (PMN), and 2% (0 to 6%) eosinophils. By trypan blue exclusion, greater than 95% of the PMΦ were viable.

Peripheral blood PMN were recovered from heparinized venous blood of healthy donors by methods modified from Böyum (1), which have been previously described in detail (12).

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Purity and viability by trypan blue exclusion of the PMN suspension exceeded 95%. Sera from five healthy donors were pooled and stored at -70°C . Just before use, a portion of serum was thawed and added to the incubation mixtures. PMN and sera from healthy donors were used to provide comparative data based on many previous studies (12, 19).

Antibiotics. Tobramycin and imipenem were used in this study and were kindly donated by Eli Lilly & Co., Indianapolis, Ind., and the Merck Institute, Rahway, N.J., respectively.

Experimental design. Bacterial growth and killing were determined in sterile capped polystyrene tubes (Falcon, Oxnard, Calif.) containing various 1-ml mixtures of bacteria, phagocytic cells, serum, and antibiotics. Before constituting the mixtures, bacteria, phagocytic cells, and antibiotics were suspended in the medium under study. The initial bacterial inoculum was prepared to be ca. 0.5×10^3 to 1.0×10^3 CFU/ml, whereas the number of phagocytic cells and the concentrations of antibiotics and serum were varied. The tubes were incubated at 37°C on a slowly rotating rack (Rotorack; Fisher Scientific, Chicago, Ill.). Immediately after constituting the mixtures (zero time) and at specified intervals thereafter, 100- μl samples were removed from the tubes, diluted appropriately in sterile PBS, and plated onto agar (antibiotic medium no. 2; Difco). The agar plates were incubated at 37°C for 24 to 48 h and then counted. In experiments with phagocytes, the samples were immediately diluted in ice-cold water and likewise plated onto agar. The observed reductions in bacterial CFU primarily reflect the association of bacteria with and subsequent killing by phagocytes (19).

Statistical analysis. The colony counts per milliliter in the different mixtures were compared by Student's *t*-test. The null hypotheses were rejected at ≤ 0.05 . Mean values are reported $\pm$ standard error of the mean.

RESULTS

Ability of CAPD fluids to support bacterial growth. When peritonitis is to occur, bacteria would be required to survive and grow in the milieu of the CAPD peritoneal cavity. To mimic these conditions in vitro, we added bacteria to commercial dialysis solutions as well as peritoneal dialysis effluents in tubes that were slowly rotated at 37°C . A relatively low number of microorganisms (400 to 800 CFU/ml) was used to inoculate the tubes since bacterial CFU are

in this range during CAPD peritonitis and may be even lower at the time of contamination (15).

Staphylococcal CFU rapidly decrease in commercial dialysis solutions and also in PBS (Table 1). In contrast, staphylococci readily multiplied in the pooled peritoneal effluent obtained from CAPD patients after a dialysis exchange (Table 1). *S. epidermidis* CFU doubled twice and six times after 5 and 24 h of incubation, respectively. *S. aureus* CFU likewise doubled 3 and 13 times after 5 and 24 h of incubation in the pooled dialysis effluent (Table 1). Unlike the staphylococci, *E. coli* were able to grow in commercial dialysis solution (ca. 10 generations in 24 h), but better growth was recorded when *E. coli* were inoculated into pooled dialysis effluent (ca. 13 generations in 24 h; Table 1). In addition, multiplication of *E. coli* in pooled peritoneal dialysis effluent was already apparent after 1 h of incubation, but staphylococci needed more than 2 h before multiplication became evident (Fig. 1). No significant differences in bacterial survival or growth were noted when commercial dialysis solutions with different concentrations of glucose were compared (data not shown).

Bacterial survival in the presence of phagocytes. During the dialysis dwell time, phagocytic cells, primarily PM Φ , appear in the peritoneal dialysis solution of CAPD patients. To investigate the influence of such phagocytes on bacterial multiplication in the peritoneal cavity, we added increasing numbers of PM Φ to vials containing bacteria in pooled peritoneal dialysis effluent. These PM Φ effectively prevent bacterial growth and reduce the number of microorganisms in dialysis effluent (Fig. 2).

However, at least 10^6 PM Φ per ml of effluent are needed to consistently prevent bacterial growth over a 5-h incubation period (Fig. 2).

Since PM Φ are largely replaced by PMN as the predominant phagocytic cell during episodes of acute peritonitis (7, 15), we also determined the capacity of donor PMN to inhibit bacterial growth in the CAPD milieu. PMN were as effective as PM Φ in inhibiting bacterial growth in pooled peritoneal dialysis effluent. Again, 10^6 PMN per ml of effluent were needed to obtain a bactericidal effect (Fig. 3). The number of *S. epidermidis* CFU was affected to a greater extent than the number of *S. aureus* CFU by the presence of PM Φ or PMN (Fig. 2 and 3). The inhibitory effects of PM Φ and PMN on the growth of *E. coli* were similar to those observed for *S. aureus*. Thus, the *E. coli* CFU remained less than 10^3 per ml over a 5-h incubation period in the presence of 10^6 PMN or

TABLE 1. Bacterial growth in peritoneal dialysis fluids, PBS, and Mueller-Hinton broth

Growth medium	Mean growth (CFU/ml) $\pm$ SEM ^a								
	<i>S. epidermidis</i> at (h):			<i>S. aureus</i> at (h):			<i>E. coli</i> at (h):		
	0	5	24	0	5	24	0	5	24
PBS (pH 7.4)	587 $\pm$ 54	<25	<25	740 $\pm$ 69	<25	<25	975 $\pm$ 71	475 $\pm$ 53	$1.8 \times 10^3 \pm 0.2 \times 10^3$
Peritoneal dialysis solution (Dianeal)	456 $\pm$ 67	<25	<25	679 $\pm$ 73	<25	<25	1,050 $\pm$ 83	$1.2 \times 10^4 \pm 0.1 \times 10^4$	$8.0 \times 10^5 \pm 0.8 \times 10^5$
Peritoneal dialysis effluent	432 $\pm$ 52	$1.8 \times 10^3 \pm 0.3 \times 10^3$	$2.0 \times 10^4 \pm 0.7 \times 10^4$	670 $\pm$ 82	$5.8 \times 10^3 \pm 1.2 \times 10^3$	$5.0 \times 10^6 \pm 0.6 \times 10^6$	675 $\pm$ 78	$7.7 \times 10^3 \pm 0.8 \times 10^3$	$6.6 \times 10^6 \pm 0.5 \times 10^6$
Mueller-Hinton broth	425 $\pm$ 83	$6.5 \times 10^4 \pm 1.6 \times 10^4$	$1.8 \times 10^9 \pm 0.4 \times 10^9$	394 $\pm$ 73	$3.5 \times 10^4 \pm 1.0 \times 10^4$	$1.2 \times 10^9 \pm 0.3 \times 10^9$	969 $\pm$ 78	$6.4 \times 10^6 \pm 0.6 \times 10^6$	$6.6 \times 10^9 \pm 0.8 \times 10^9$

^a Based on at least five separate experiments.

FIG. 1. Bacterial growth in pooled peritoneal dialysis effluent at 37°C. Symbols indicate mean $\pm$ SEM CFU of *E. coli*, *S. aureus*, and *S. epidermidis* based on five or more separate experiments.

PM Φ . Consistently reduced numbers of *E. coli* CFU at 5 h were only found when 5×10^6 PMN or PM Φ had been added to the tubes (data not shown).

Effect of serum on bacterial survival. In addition to phagocytic cells, serum proteins diffuse into the peritoneal cavity during the dialysis dwell time (15). Although some of these

FIG. 2. Inhibitory effect of PM Φ on the growth of *S. epidermidis* and *S. aureus* in pooled peritoneal dialysis effluent. Significant inhibition ($P < 0.05$) occurred at 5×10^5 PM Φ per ml for *S. epidermidis* growth and at 1×10^6 PM Φ per ml for *S. aureus* growth.

FIG. 3. Inhibitory effect of PMN on the growth of *S. epidermidis* and *S. aureus* in pooled peritoneal dialysis effluent. Significant inhibition ($P < 0.05$) occurred at 5×10^5 PMN per ml for *S. epidermidis* growth and at 1×10^6 PMN per ml for *S. aureus* growth.

proteins such as immunoglobulins and complement factors may enhance the phagocytic process through bacterial opsonization, the level of opsonins in CAPD effluents is very low (19). We therefore studied the effect of additional serum on bacterial growth in pooled peritoneal dialysis effluent. Ten percent serum had no influence on *S. aureus* growth in peritoneal dialysis effluent. However, a synergistic bacteriostatic effect was observed when serum and phagocytic cells were both present in the incubation mixtures (Table 2).

Effect of antibiotics in peritoneal dialysis effluent. Episodes of bacterial peritonitis in CAPD patients are treated with antimicrobial agents given either intraperitoneally or by other routes. Penicillins, cephalosporins, and aminoglycosides are commonly used antibiotics (10, 14, 15). We studied the bactericidal activity of a beta-lactam antibiotic, imipenem, and an aminoglycoside, tobramycin, on *S. aureus* growth in pooled peritoneal dialysis effluent. The antibac-

TABLE 2. Synergistic inhibition of *S. aureus* multiplication by serum and phagocytes

Growth conditions ^a	Mean <i>S. aureus</i> (CFU $\pm$ SEM) at (h): ^b	
	0	5
PDE	545 $\pm$ 48	4,429 $\pm$ 624
PDE + 10% serum	467 $\pm$ 78	4,725 $\pm$ 613
PDE + 5×10^5 PMN	512 $\pm$ 19	4,100 $\pm$ 350
PDE + 5×10^5 PM Φ	500 $\pm$ 95	3,450 $\pm$ 715
PDE + 10% serum + 5×10^5 PMN	425 $\pm$ 10	689 $\pm$ 63 ^c
PDE + 10% serum + 5×10^5 PM Φ	431 $\pm$ 63	994 $\pm$ 237 ^c

^a PDE, Pooled peritoneal dialysis effluent.

^b Three separate experiments.

^c Significant ($P < 0.05$) compared with growth in PDE without phagocytes, without serum, or both.

terial activity of the antibiotics in Mueller-Hinton broth was used as a control. Surprisingly, the activity of tobramycin was reduced by 90% when peritoneal dialysis effluent was used as the medium instead of Mueller-Hinton broth (Fig. 4). In contrast, no difference in *S. aureus* killing rate was found for imipenem in dialysis fluids and Mueller-Hinton broth. The minimal inhibitory and bactericidal concentrations of imipenem were 0.01 and 0.1 $\mu\text{g/ml}$, respectively, in Mueller Hinton broth as well as in peritoneal dialysis effluent (not shown in Fig. 4).

DISCUSSION

Current understanding of the pathogenesis of CAPD peritonitis is restricted to the potential routes of contamination of the peritoneal cavity. Most episodes of peritonitis seem to be secondary to accidental breaks in the sterile technique of the exchange of bags, fluid leaks through cracks in the lines or bags, and infection tracking along the catheter tunnel in the abdominal wall (5). Entry of microorganisms, especially gram-negative enterobacterial species, into the peritoneal cavity by transmural migration through the bowel is another potential route of infection (18). The aim of this study was to explore some of the events after peritoneal contamination that may be important in the development of clinically apparent infection.

Although bacterial survival and multiplication in CAPD fluids would, in the face of frank peritonitis, seem to occur, results of this study indicate that the fate of bacteria invading the peritoneal cavity depends on multiple factors. Thus, commercially available solutions used for CAPD did not support survival of *S. epidermidis* or *S. aureus*. *E. coli*, on the other hand, survived and even multiplied in these solutions.

In sharp contrast, staphylococci grew readily in CAPD effluents obtained from patients after the dialysis dwell time.

FIG. 4. Staphylocidal activity of tobramycin in peritoneal dialysis effluent compared with its activity in Mueller-Hinton broth.

The CAPD fluid apparently changes after intra-abdominal instillation during the dialysis time from a nonsupportive medium to a medium that supports staphylococcal growth. Thus, the survival of bacteria contaminating the peritoneal cavity is dependent upon the microbial species as well as on the actual composition of the peritoneal cavity fluid at the time of inoculation. Apart from the diffusion of many different host proteins into the CAPD fluid, changes in the acidity of the fluid, osmolarity, and cation content may also be important in this respect (3). More studies are needed that further delineate the factors in CAPD fluids that determine bacterial survival and death.

Perhaps more relevant to the final outcome of bacterial contamination of the peritoneal cavity is the presence of host defense mechanisms at this site. The results of this study would indicate that peritoneal macrophages can phagocytize and kill microorganisms in peritoneal dialysis effluent. In this way, peritonitis subsequent to accidental contamination may be prevented. Opsonins such as those present in serum were shown to augment the phagocytic response. However, it is not known how rapidly macrophages appear in the dialysis solution nor has the rate of diffusion into the peritoneal cavity of critical opsonins been determined. Sufficient numbers may never be reached since 0.5×10^6 to 1.0×10^6 macrophages per ml were needed to obtain a consistent bacteriostatic effect over a 5-h incubation period. Such numbers of peritoneal cells are not routinely found in the effluents of noninfected CAPD patients; rather, 1×10^4 to 5×10^4 cells per ml are present, and greater than 1×10^5 cells per ml of effluent is generally taken as a sign of peritoneal infection (5, 6, 13, 19). Only during episodes of infection are 0.5×10^6 to 12×10^6 cells per ml of peritoneal effluent found. The cells are then predominantly PMN mobilized from the bloodstream (7, 15). Although PMN and sera from healthy donors were used in lieu of those of CAPD patients, our data suggest that such a cellular response could potentially constitute an adequate phagocytic challenge against the invading microorganisms. We are presently investigating the antibacterial activities of PMN and opsonins from peripheral blood of uremic CAPD patients as well as exudate PMN and opsonins that appear in the peritoneal dialysates during episodes of infection in these patients.

Current CAPD techniques, however, mandate the exchange of peritoneal effluent-containing phagocytes and opsonins for dialysis solutions completely devoid of such antimicrobial mechanisms. In addition, we and other investigators have found that the commercial dialysis solutions adversely affect the phagocytic and bactericidal capacities of PMN and PMN (18, 19). Hence, these perturbations in local antibacterial defense mechanisms may be important in the pathogenesis of peritonitis in CAPD patients.

The use of antibiotics for the treatment of CAPD peritonitis has received increased attention (6, 13). Many studies have delineated the pharmacokinetics of antibiotics given via various routes (6, 8, 11, 17). Our limited observations on the action of two antimicrobial agents on *S. aureus* growth in dialysis fluids indicate that some antibiotics may behave differently in the patient than in standard laboratory tests.

Although we have not attempted to elucidate the cause(s), the composition of CAPD fluids greatly reduced the activity of the aminoglycoside tobramycin. Aminoglycoside antibiotics are known to be very sensitive to the pH of the medium, divalent cation levels, the oxidation-reduction potential, and the presence of host-derived factors in the medium (2, 16, 20). CAPD fluids differ considerably from standard Mueller-Hinton broth in these factors. It would thus be potentially

advantageous to treat CAPD peritonitis with antimicrobial agents with proven efficacy in CAPD fluids.

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