In Vitro Study of Bacterial Growth in Continuous Ambulatory Peritoneal Dialysis Fluids

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We examined the in vitro survival of bacteria in continuous ambulatory peritoneal dialysis effluents of patients with clinical peritonitis and those without peritonitis. Standard strains of coagulase-negative staphylococci (CNS), Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa were inoculated into the fluids, and portions were plated for bacterial counts at 0.5, 4, 24, 48, 72, and 96 h. Unused dialysate fluid was also inoculated simultaneously. Our results show that CNS increased minimally up to 48 h in the noninfected continuous ambulatory peritoneal dialysis effluents and decreased by 96 h, whereas survival was only minimal in the infected effluent. S. aureus showed trends similar to those of CNS, but differences in survival in infected and noninfected effluents were less marked. By contrast, E. coli and P. aeruginosa increased by greater than 1,000-fold in all solutions tested. Based on the above findings, it is likely that a proportionate number of culture-negative cases of peritonitis are due to gram-positive cocci, especially CNS, which are not retrievable by standard culture techniques because of poor survival rate.

Continuous ambulatory peritoneal dialysis (CAPD) patients with clinical evidence of peritonitis pose a problem for both the clinician and the microbiologist when the infecting organism cannot be isolated (5). The yield of positive cultures might be increased by several technical modifications; e.g., different buffers have different effects on bacterial growth (1). The culturing of larger volumes and use of membrane filtrations, pour plates, and antibiotic removal devices (2, 6, 7) are, however, time-consuming. Several studies to date show that approximately 10 to 20% of clinically infected CAPD effluents are culture negative (3, 5). The rationale of this study was to evaluate in vitro differences in bacterial growth patterns in clinically infected and noninfected CAPD fluids. Our observations over a 48- to 96-h time frame indicate that, under identical conditions, gram-positive organisms survive poorly, whereas gram-negative organisms, such as Escherichia coli and Pseudomonas aeruginosa, multiply more than 1,000-fold.

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MATERIALS AND METHODS

Bacterial strains. Standard organisms from the American Type Culture Collection were used as test strains. These were coagulase-negative staphylococci (CNS), Staphylococcus epidermidis ATCC 12228, Staphylococcus aureus ATCC 25923, E. coli ATCC 25922, and P. aeruginosa ATCC 27853. Isolated colonies from an 18-h growth on blood agar were inoculated into tryptic soy broth and grown at 36°C for 3 to 4 h. The turbidity was then adjusted visually to 0.5 McFarland BaSO4 standard (ca. 107 to 108 CFU/ml). Aliquots of 0.01 ml of the above solution were diluted in 10 ml of distilled water (1 to 1000), and 0.001 ml of this solution was plated to obtain the exact number of CFU per milliliter.

Test solutions. The following solutions were used for in vitro bacterial seeding: (i) pooled dialysate effluents from eight episodes of five patients on CAPD collected at a time when they had clinical peritonitis and before antibiotic therapy was instituted; (ii) pooled dialysate effluents from nine episodes of seven clinically noninfected CAPD patients; (iii) unused dialysate fluid; and (iv) tryptic soy broth for positive control. The initial glucose concentration of all CAPD effluents and unused dialysate fluid was 2.5%.

Other data. The Gram stains and total leukocyte counts of all the dialysate effluents were studied. The pH of these fluids was measured on a pH meter (Zeromatic SS-3; Beckman Instruments, Inc.); glucose, urea, creatinine, and albumin were determined by a multiple-system analyzer (Dupont ACA III). Osmolarity was determined by freezing point depression (Advanced Digimatic Osmometer, model 3011). The fluids (10 to 50 ml) were then membrane filtered (Millipore Corp.); the filtrates were kept frozen and used for in vitro seeding at a later date. Neutrophil-macrophage phagocytic factors affecting bacterial growth were therefore excluded. Routine bacteriological culture by direct plating of a standard loopful of the fluid and putting 1 ml of the fluid into thioglycolate, together with cultures of the Millipore filters directly on chocolate agar, was carried out.

Experimental procedure. Pooled samples (10 ml) of each of the above solutions were individually inoculated with 10 μl of the bacterial suspensions. The solutions were incubated at 36°C, and portions were plated on blood agar at 1/2, 4, 24, 48, 72, and 96 h. The CFU per milliliter were calculated from the colony counts after an 18-h incubation at 36°C. All tests were performed at least in duplicate.

Statistical analysis. The colony counts per milliliter of the gram-positive versus gram-negative organisms in all fluids, namely, unused dialysate and pooled clinically infected and noninfected fluids were individually compared by the two-tailed Student t test.

RESULTS

The growth patterns of gram-positive and gram-negative organisms differed significantly in all fluids tested (P < 0.01). There was relatively better survival of CNS in the nonperitonitis CAPD fluid compared with the clinically
infected CAPD and unused dialysate (Fig. 1). By contrast, although the rates of growth differed to some extent, both E. coli and P. aeruginosa increased consistently over the 96-h time frame in all three groups (Fig. 2). The number of S. aureus in both groups of CAPD fluids initially increased up to 48 h; thereafter, the number decreased with time, but small numbers of residual organisms were present at 72 and 96 h. Unused dialysate allowed the least survival of both CNS and S. aureus. The growth of all three groups of organisms in control tryptic soy broth was >10⁵ CFU/ml in 24 h.

The leukocyte counts of the noninfected CAPD fluids were less than 10/mm³, compared with a range from >100 to 4,300/mm³ in infected effluents. The Gram stain showed neutrophils but no organisms.

Results of the routine bacterial cultures showed no growth in six of the eight clinically infected effluents. The two positive cultures were moderate growths (10 to 15 CFU per plate, inoculum of 0.01 ml) on plate media and confluent growth on the filters of Proteus mirabilis and Citrobacter freundii. In addition, results of the Millipore filtration showed four additional positive cultures of five colonies or fewer of gram-positive cocci, three cultures of CNS, and one culture of S. aureus. All clinically noninfected effluents were sterile.

The glucose concentration of the pooled, clinically infected CAPD effluents was 644 mg/dl, compared with 1,332 mg/dl in the control nonperitonitis group and may be of significance for further study. The urea nitrogen concentration in the clinically infected CAPD ranged from 31 to 137 mg/dl (mean, 70 mg/dl) and that in the nonperitonitis group averaged 44 mg/dl.

The mean creatinine concentration was 10.4 mg/dl in the clinically infected CAPD fluids and 7.4 mg/dl in the noninfected control group. The pH range in both CAPD effluent groups was 7.0 to 8.6, whereas that of unused dialysate was 5.4. The osmolality of unused dialysate was 332 mosM/kg, that of infected pooled dialysate was 327 mosM/kg, and that of pooled nonperitonitis effluents was 336 mosM/kg. The albumin concentration was less than 0.9 g/liter in both sets of effluents.

**DISCUSSION**

A frequent complication in CAPD patients is bacterial peritonitis; these episodes account for approximately 20% of catheter failures (4). Hence, isolating the etiologic agent is of utmost importance, and difficulties are encountered because a mobile fluid environment is maintained in the peritoneal cavity. Important variables are type of fluid, required dwell time, and to a great extent, the expertise of the patient performing the exchange. Whether peritoneal seeding is from transmural migration from the bowel or catheter tunnel infection, survival and growth of bacteria introduced into the peritoneal cavity would lead to peritonitis. Our in vitro data indicate that gram-positive cocci survive and grow poorly in CAPD effluents, particularly in infected CAPD effluents. This finding is of notable importance with CNS. In contrast, both gram-negative bacilli tested, E. coli and P. aeruginosa, increased markedly.

Various biochemical parameters of infected and noninfected CAPD effluents were studied to evaluate factors that may promote or inhibit growth of various organisms. CAPD effluents from infected patients had lower glucose levels in comparison with clinically uninfected effluents and unused dialysate fluid. In addition, a low pH of 5.4 in unused dialysate may be a contributing factor to the inhibition of growth of gram-positive organisms in this fluid.

Verbrugh et al. (8) studied survival of S. epidermidis, S. aureus, and E. coli in unused dialysate and uninfecteD CAPD effluents over 24 h in a similar in vitro system. They found that commercially available unused CAPD fluid did not
support survival of *S. epidermidis* or *S. aureus* but allowed *E. coli* to multiply. Also, their observation that there was a better survival rate of all three organisms at 24 h in uninfected CAPD was similar to ours. However, we find that once the fluid is clinically infected, CNS decreased markedly on in vitro seeding. To compare this with an in vivo situation, once CNS enter the peritoneal cavity and infection sets in, the infected CAPD effluent, a poor growth-promoting medium, provides a poor milieu for the survival of CNS. The numbers are then further diluted by exchanges with fresh peritoneal dialysis fluid, which also offers a poor growth environment. With only a 4- to 6-h exchange during which by-products are accumulating, the CNS may start to grow. If the dwell time is 12 h, as in overnight dialysis, there may be some growth initially but a subsequent decrease in the numbers of organisms present. Since the development of infection to set in from the original nidus must be sufficient to produce symptoms or a cloudy fluid, the first CAPD effluent received by the microbiology laboratory for culture may represent a dwell time of greater than 24 h.

Vas (6) suggested that organisms may not be cultured because of the dilution of organisms in large volumes of CAPD fluid; therefore, concentration of specimens should increase the yield significantly. In a recent study (7), Vas et al. have also shown a higher yield after use of antibiotic-removing devices. In some instances, filtration is impractical because fibrin and cellular material clog the Millipore filters; this specialized technique is time-consuming. All these specialized techniques add to the cost. Our studies suggest that the major factor in the high incidence of culture-negative peritonitis, besides dilution of small numbers of organisms, is the inherent poor survival of gram-positive organisms in CAPD fluid.

Poor survival of CNS after 24 to 48 h in infected CAPD fluid may cause infections by CNS to be self-limited, and symptomatology may subside spontaneously. Careful questioning of patients on CAPD, regarding transient episodic abdominal pain, may be of value in evaluating this observation.

In conclusion, from our in vitro observations a few significant points emerge. (i) Gram-negative organisms, such as *E. coli* and *P. aeruginosa*, multiply and grow in unused and patient CAPD effluents whether clinically infected or not. (ii) Survival of gram-positive cocci, particularly CNS, is poor after 48 h in clinically infected CAPD effluents. (iii) In general, gram-positive cocci do not multiply vigorously in CAPD fluids, and this may account for a significant portion of culture-negative peritonitis. (iv) Therapy directed against gram-positive cocci may be a valuable consideration in culture-negative peritonitis.

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LITERATURE CITED


