Factors Affecting Staphylococcus epidermidis Growth in Peritoneal Dialysis Solutions

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Staphylococcus epidermidis is the most frequent cause of peritonitis complicating continuous ambulatory peritoneal dialysis. We studied factors that might influence the growth of S. epidermidis in commercially available peritoneal dialysis solution (PDS). Test strains were inoculated into PDS and incubated overnight at 37°C. Samples were removed at appropriate intervals, bacterial counts were performed, and growth curves were constructed. We studied the effects of various osmolarities, the neutralization and acidification of fresh and spent PDS, and the effect of intraperitoneal dwell time on the ability of PDS to support growth of S. epidermidis. In fresh PDS, numbers of bacteria remained constant after 24 h. No significant differences in growth were observed among PDS with 0.5, 1.5, 2.5, and 4.25% glucose. Neutralizing acidic fresh PDS had no effect on bacterial growth. However, growth did occur in spent PDS. PDS which was recovered after only 2 h in the peritoneal cavity supported growth to the same extent as did PDS recovered after 4 to 6 h. Mean log₁₀ changes after 24 h of incubation were as follows: for fresh PDS, −1.3; after 2 h dwell time, 2.9; after 4 h dwell time, 1.9; and after 6 h dwell time, 1.3. Acidification of spent PDS to less than pH 6.35 produced less rapid growth; mean log₁₀ increases after 24 h of incubation were 1.9 for pH 7.75, 1.6 for pH 6.35, 0.6 for pH 5.75, and 0.7 for pH 4.95. Fresh PDS of all available osmolarities neither supported the growth of S. epidermidis nor was bactericidal. Spent PDS supported bacterial growth, and this growth was partly independent of the neutralization which occurred during the dialysis.

Continuous ambulatory peritoneal dialysis, first described by Popovich et al. (14), is an accepted alternative to hemodialysis. Peritonitis remains the major complication and the major cause of the high cost of this technique, despite an incidence which has declined to roughly 0.4 infections per patient per year (4, 10, 11). In approximately 70% of these infections, gram-positive aerobic cocci, which arise from the patient’s own cutaneous or nasopharyngeal flora, are isolated (10). Almost 20% of infections involve gram-negative enteric bacilli, and the remainder are due to unusual organisms such as fungi or mycobacteria. Staphylococcus epidermidis and Staphylococcus aureus account for 60% of all infections (10, 11, 15).

In peritoneal dialysis, S. epidermidis, an organism of low virulence, presumably gains access to the peritoneal cavity through the surgical incision or through the catheter lumen. The presence of both the catheter, which acts as a foreign body, and peritoneal dialysis solution (PDS) may render host defenses inadequate to control continued bacterial growth. The catheter itself may provide a site of attachment for bacteria (12). Cellular defense mechanisms may be less effective on the surface of a foreign body, and the presence of PDS within the peritoneal cavity has been shown to have an inhibitory effect on cellular defense mechanisms (10). It is also possible that the PDS may serve as a culture medium to stimulate bacterial growth.

PDS composition is substantially altered after intraperitoneal instillation. The normally acidic PDS rapidly equilibrates towards neutrality, and urea, creatinine, amino acids, and protein dialyze into the solution. The protein may amount to 1 g/liter of dialysis fluid. During episodes of peritonitis, the peritoneal membrane is significantly altered, increasing protein loss and probably altering other components (11, 15, 16).

In this study we attempted to assess the ability of fresh and spent PDS to support the growth of S. epidermidis and to examine factors which influence the growth of S. epidermidis in PDS.

MATERIALS AND METHODS

Three strains of S. epidermidis were isolated from three patients with peritonitis and maintained on agar slants. These strains were confirmed as S. epidermidis by using the API Staph-Ident system (1, 3). Colonies of S. epidermidis were streaked on blood agar and incubated overnight at 37°C to ensure a pure growth. Colonies of the S. epidermidis strain were then transferred to 10 ml of Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) and incubated at 37°C for 18 h. By using a no. 1 McFarland barium sulfate standard, an inoculum was prepared in saline and added to appropriate volumes of prewarmed PDS (Dianeal, Baxter Laboratories). This yielded an approximate bacterial concentration of 10⁷ to 10⁹ CFU/ml. Volumes (250 ml) of fresh PDS or PDS recovered from the peritoneal cavities of patients undergoing continuous ambulatory peritoneal dialysis were used. The spent PDS was first filtered through a membrane filter (0.2 μm; Millex-GS, Millipore Corp., Bedford, Mass.) to ensure sterility and to remove cellular elements. Samples (1 ml) of inoculated PDS were recovered after 0, 2, 4, 6, and 24 h of incubation. Before dilution and plating, the CFUs in the inocula were dispersed by mixing with a Vortex mixer (The Vortex Manufacturing Co., Cleveland, Ohio). Each 1-ml sample was serially diluted with normal saline. Samples (100 μl) of selected dilutions (usually 10⁻³, 10⁻⁴, and 10⁻⁵) were plated in triplicate on Mueller-
Hinton agar by using the Parker stick method. After incubation overnight at 37°C, CFUs (in the range of 30 to 300 per plate) were counted by using a Quebec colony counter and growth curves were constructed.

The dialysis solution was modified in various ways to determine the effects of these changes on bacterial growth. The effect of osmolarity on bacterial growth in fresh PDS was examined for two different pH ranges. Samples of fresh PDS with 0.5, 1.5, 2.5, and 4.25% glucose were used as media at pHs 5.2 and 7.4, respectively. NaOH solution (1 M) was used to adjust the pH upwards. The effect of intraperitoneal dwell time on bacterial growth was examined by using 2.5% PDS recovered from a patient's peritoneal cavity 2, 4, and 6 h postinfusion as media for bacterial growth.

Spent PDS has a pH of approximately 7.75. The effect of pH on bacterial growth in spent PDS was examined by modifying pH in spent (6-h dwell time) PDS from 7.75 to 6.35, 5.75, and 4.95 by using 1 M HCl.

An assay of 22 amino acids and derivatives was conducted on spent PDS recovered from a continuous ambulatory peritoneal dialysis patient 2, 4, and 6 h postinfusion. Samples were analyzed on a Beckman 121 MB amino acid analyzer (Beckman Instruments, Inc., Fullerton, Calif.) with Bension D-X8.25 resin and a single-column, three-buffer lithium method as described previously (13). Total protein was assayed on the Hitachi 705 autoanalyzer by using the biuret method (18).

Data collection and work-up involved selecting the serial dilution plates with the highest number of CFU within the 30 to 300 limits. Then, by using either three or six counts (depending upon whether the experiment had been repeated), means and standard deviations were calculated. The means were used to plot growth curves, and the standard deviations of the 24-h samples were used to conduct a one-way analysis of variance utilizing Tukey's w procedure for multiple comparisons (17). This statistical procedure produced a critical difference value for the pooled

standard deviations of the 24-h samples. For all calculations, P was less than 0.05.

RESULTS

All results are based on combined data from the three strains used in the study.

Fresh PDS. After 24 h of incubation in fresh PDS containing 0.5, 1.5, 2.5, and 4.25% glucose at pH 5.2, there were no significant mean log_{10} changes in bacterial colony counts (Fig. 1). At pH 7.4, the mean log_{10} changes were not significantly different for 1.5, 2.5, and 4.25% glucose solutions. In 0.5% solution, there was a log_{10} decrease of 0.7. Thus, bacterial growth did not change with different osmolarities at either pH.

Fresh versus spent PDS. There was a significant difference between bacterial colony counts in the fresh PDS and all three of the spent PDS (Fig. 2). At 24 h, there was a mean log_{10} decrease of 1.3 in the fresh PDS. The 2-, 4-, and 6-h spent PDS showed a mean log_{10} increase from inoculum levels of 1.9, 1.9, and 1.3, respectively. The three spent PDS samples supported bacterial growth equally well and significantly better than did the fresh samples.

pH in spent PDS. In spent 2.5% glucose PDS at pH 6.35 and pH 7.75, there were mean log_{10} colony count increases of 1.6 and 1.9 above the inoculum level (Fig. 3). At pH 4.95 and pH 5.75, mean log_{10} colony counts increased by 0.7 and 0.6, respectively. Spent PDS at all four pH values supported growth at 24 h, but the solutions at a more nearly neutral pH showed significantly higher levels of growth.

Amino acid accumulation. Amino acid analysis of spent PDS recovered from the peritoneal cavity of a continuous ambulatory peritoneal dialysis patient 2, 4, and 6 h
postinfusion showed that 75% of the final 6-h concentration was present after 2 h (Table 1).

**DISCUSSION**

Two recent studies examined the growth of *S. epidermidis* and other organisms in fresh and spent PDS (5, 8). Diskin et al. (5) suggested that fresh PDS was bactericidal. Fluornoy et al. (8) found that fresh PDS was bacteriostatic. These studies have inconsistent conclusions; a major problem is the definition used for bactericidal. Diskin et al. (5) and Appleby and John (2) suggested that a 1-log drop in CFU is evidence of bactericidal activity. However, neither of these studies confirmed complete absence of CFU during the time of incubation, which extended up to 24 h.

The present study showed that there was no growth of *S. epidermidis* in fresh PDS and confirmed the observation of Diskin et al. (5) and Fluornoy et al. (8) that there is actually a 1- to 1.5-log decrease in CFU during a 24-h incubation period. This indicates that fresh PDS is at least bacteriostatic but does not have the ability to completely eliminate bacterial CFU during the 24-h period. This study did not show any significant difference when pH or osmolarity (as determined by glucose concentration) was altered. There was no evidence of increased bactericidal activity in fresh PDS at lower pHs or at different osmolarities. The findings consistently showed 1- to 2-log increases in bacterial growth in spent PDS. Reducing the pH of the spent PDS reduced the growth of *S. epidermidis* in vitro. At no time did growth parallel that of neutral or acidic fresh PDS. Duwe et al. (6) have shown in vivo that the pH of the PDS neutralized within 30 min after it had been instilled in the peritoneal cavity. Therefore, pH would likely have played a very small role in the inhibition of growth in vivo unless the PDS was changed every hour.

From the previous studies, a number of substances have been postulated to be growth-promoting factors. Diskin et al. (5) showed that when serum was added to fresh PDS, it no longer suppressed the growth of bacteria. However, addition of urea alone to the fresh PDS did not promote growth. Fluornoy et al. (8) relied on the analysis of spent PDS. The former study was limited by the additives used, and the latter was restricted by the compounds analyzed in the PDS.

The results of the present study show that pH played a critical role but was probably not an important factor in vivo. Also, osmolarity did not play a major role in the growth promotion of *S. epidermidis*.

The spent PDS was analyzed for protein and amino acid content. A protein analysis detected no albumin in the spent PDS. However, amino acid analysis showed a high concentration of most amino acids. The amino acids entered the PDS and equilibrated within 2 h. The growth-promoting ability of spent PDS was also apparent in 2 h. It is likely that a number of compounds may act as growth-promoting factors. These compounds enter the PDS within the first 2 h and may be serum proteins, although smaller subunits such as amino acids also seem to promote growth. Early studies on the requirements of *Staphylococcus albus* showed the need for amino acid supplement, usually in the form of casein amino acid (9). A more recent study found that *S. epidermidis* required arginine, valine, and proline most frequently for growth (7). These were all present in spent PDS by 2 h. To identify the exact requirement would require the addition of amino acids singly and in combination with fresh PDS. This observation, coupled with recognition of the equilibration phase of amino acids, may help in the future treatment of peritoneal infection by allowing the physician to time exchanges and to take advantage of the bacteriostatic activity of fresh PDS. This becomes impractical if the equilibration takes less than 1 h, as in the case of pH.

In conclusion, this study confirmed the findings of other studies, namely, that fresh PDS is bacteriostatic. There is no evidence at this time that fresh PDS is bactericidal (as reported by Diskin et al. [5]). Spent PDS supported the growth of *S. epidermidis*, and this growth-promoting ability was decreased by reducing the pH of the solution. The growth-promoting factor appeared to be protein or amino acids and was present in the PDS in sufficient quantities by 2-h dwell time.

<table>
<thead>
<tr>
<th>Amino acid or derivative</th>
<th>Concn (nmol/ml) of PDS recovered at the following times postinfusion (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 h</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>15.2 (69)</td>
</tr>
<tr>
<td>Threonine</td>
<td>75.0 (76)</td>
</tr>
<tr>
<td>Serine</td>
<td>69.4 (78)</td>
</tr>
<tr>
<td>Asparagine</td>
<td>35.1 (63)</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>20.7 (87)</td>
</tr>
<tr>
<td>Glutamine</td>
<td>401.4 (75)</td>
</tr>
<tr>
<td>Proline</td>
<td>162.8 (76)</td>
</tr>
<tr>
<td>Glycine</td>
<td>436.8 (74)</td>
</tr>
<tr>
<td>Alanine</td>
<td>411.0 (81)</td>
</tr>
<tr>
<td>Valine</td>
<td>90.2 (73)</td>
</tr>
<tr>
<td>Methionine</td>
<td>17.0 (81)</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>41.3 (77)</td>
</tr>
<tr>
<td>Leucine</td>
<td>53.7 (81)</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>23.2 (68)</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>31.3 (84)</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>9.9 (100)</td>
</tr>
<tr>
<td>Lysine</td>
<td>103.4 (70)</td>
</tr>
<tr>
<td>Histidine</td>
<td>41.6 (70)</td>
</tr>
<tr>
<td>Arginine</td>
<td>69.7 (67)</td>
</tr>
</tbody>
</table>

* Percentage of maximum concentration.
ACKNOWLEDGMENTS

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LITERATURE CITED