Bacteriological Aspects of Selective Decontamination of the Digestive Tract as a Method of Infection Prevention in Granulocytopenic Patients

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We describe the bacteriological results of a controlled clinical trial of selective decontamination of the digestive tract as a method of infection prevention in granulocytopenic patients. Selective elimination of Enterobacteriaceae and Pseudomonadaceae species was accomplished by the oral administration of nalidixic acid, co-trimoxazole, or polymyxin. Yeasts were eliminated selectively by amphotericin B or nystatin treatment. The drugs used in this study were chosen because of their capacities to selectively eliminate gram-negative rods and yeasts without affecting the anaerobic part of the gut flora which is responsible for colonization resistance. Compared with the control group, the selectively decontaminated patients had significantly fewer (P < 0.0005) gram-negative rods or yeasts or both in their throat swab cultures and in their feces. This reduction may explain the clinical effectiveness of selective decontamination.

Aerobic gram-negative rods and yeasts may cause life-threatening infections in granulocytopenic patients. Many reports have shown that the gastrointestinal tract is a reservoir of these potentially pathogenic microorganisms (3, 21, 22, 27, 39). Elimination of microorganisms in this region can result in a reduction in the infection frequency in granulocytopenic patients. This can be done by oral administration of nonabsorbable antibiotics, such as gentamicin and vancomycin (resulting in total decontamination) (3, 7, 11, 15, 17, 18, 22, 25, 26, 30, 39). However, this procedure eradicates both the aerobic organisms and the anaerobic organisms in the gut flora (4, 22). Anaerobes rarely cause infections in granulocytopenic patients (1, 13, 20); they may even have a beneficial influence by preventing infections caused by aerobic bacteria. In concert with host factors, anaerobes limit the growth of aerobes. This mechanism, which regulates the colonization pattern of the digestive tract (many details of which are not yet known), is called colonization resistance (32, 36).

In animal experiments, it has been found that the number of anaerobes is important for colonization resistance (40). Therefore, in studies with animals a method of infection prevention was developed which selectively eradicated aerobic gram-negative rods and yeasts but kept the anaerobic part of the gut flora intact (16, 35, 37). This procedure was called selective decontamination of the digestive tract (SDD). SDD is only feasible with antimicrobial drugs which have (i) a narrow spectrum, not affecting anaerobes (nalidixic acid, polymyxin, amphotericin B, and nystatin), and (ii) a proportionally stronger bactericidal effect on anaerobes than on aerobes, so that selective elimination of gram-negative rods can be attempted by administering well-defined oral doses of the drug (co-trimoxazole [37] and neomycin [12, 14]).

Recently, we described the results of a prospectively randomized study of 105 granulocytopenic patients in whom the prophylactic value of this SDD treatment was investigated (9, 10, 29). In the 53 SDD-treated patients, the treatment resulted in a significant reduction in severe infections compared with a control group which did not receive any prophylactic treatment (29).

We report here bacteriological results for the 53 SDD-treated patients and for the 52 control patients. The success of the SDD treatment and the effect of this procedure on the colonization resistance are discussed.

MATERIALS AND METHODS

Patients. Adult patients with acute leukemia (myeloid or lymphoid) or aplastic anemia with less than 1000 granulocytes per mm3 of blood were allocated at random either to the SDD treatment group or to the
control group not receiving prophylactic treatment. No isolation measures were used for either group; all patients were treated in normal bedrooms under ward conditions and received nonsterilized hospital food. Patient characteristics are shown in Table 1.

**SDD.** The choice of antimicrobial drugs for SDD was based on the influence of the drugs on colonization resistance. Animal experiments showed that the drugs used in this study were safe in this respect (12, 16, 35, 37). This was confirmed later in humans (14).

SDD was directed both at *Enterobacteriaceae* and *Pseudomonadaceae* species and at yeasts. *Staphylococcus* and *Streptococcus* species were not eliminated selectively. Elimination of gram-negative rods was accomplished by treatment with nalidixic acid (8 g/day), co-trimoxazole (2,880 mg/day; i.e., 2,400 mg of sulfa-methoxazole per day and 480 mg of trimethoprim per day), or polymyxin (800 mg/day). The susceptibilities of the isolated gram-negative rods and the known or presumed hypersensitivities of the patients determined which drugs were administered for selective decontamination. This procedure is shown in Fig. 1. Occasionally, a combination of two of the antimicrobial drugs was administered if resistance of the gram-negative rods isolated during SDD treatment required it.

Elimination of yeasts was accomplished by treatment with amphotericin B (2 g/day) or nystatin (6 × 10⁶ IU/day). All drugs in SDD treatments were given orally as tablets, except amphotericin B, which was administered as a suspension. The dose of co-trimox-azoze was divided into three portions; all other drugs in SDD treatments were administered four times a day. SDD treatment was continued until the peripheral granulocyte count was more than 1,000 cells per mm³ of blood three times in succession. Blood counts were performed three times each week. Other reasons for termination of a study were death of the patient and discharge from the hospital.

**Bacteriological surveillance.** Because the patients were not isolated and therefore might have become colonized by nosocomial resistant bacteria, we monitored the potentially pathogenic flora of the patients. Routine bacteriological culturing was restricted to throat and fecal specimens; thus, we monitored the beginning and the end of the gastrointestinal tract. All routine cultures were incubated aerobically; cultures were taken three times a week in both patient groups from the moment of randomization, during SDD treatment, and, if the patient remained in the hospital, up to 1 week thereafter.

**Throat swabs.** Throat swabs were cultured on sheep blood agar, MacConkey agar (Oxoid), yeast isolation agar (Merck), and Levinthal agar (Oxoid), and also in brain heart infusion broth (Oxoid). After overnight incubation at 37°C, each brain heart infusion broth culture was inoculated onto sheep blood, MacConkey, yeast isolation, and Levinthal agars.

**Feces.** A 1-g portion of feces was serially diluted (1:10) in brain heart infusion broth and incubated for 18 h at 37°C. Thereafter, the dilutions with growth were inoculated onto MacConkey agar, yeast isolation agar, and kanamycin aesculin azide agar (Oxoid). In this way the concentrations of the aerobic gram-negative rods, yeasts, and enterococci could be determined. Anal swabs, which were used occasionally when no feces were available, were streaked onto MacConkey, yeast isolation, and kanamycin aesculin azide agars and placed into brain heart infusion broth for enrichment. After overnight incubation, each broth culture was inoculated onto MacConkey, yeast isolation, and kanamycin aesculin azide agars. All solid media except MacConkey and kanamycin aesculin azide agars were incubated for at least 42 h at 37°C.

**Food.** Food samples (especially vegetables and unheated foods) were taken at random and cultured for gram-negative rods and other potentially pathogenic bacteria.

**Identification and biotyping of *Enterobacteriaceae* and *Pseudomonadaceae* species.** We made pure cultures of all of the different colonies of gram-negative rods. These cultures were identified by using the API 20E system (5, 23). Each API 20E profile number was called a biotype. Biotypes that were isolated from samples taken from patients when they were admitted were referred to as endogenous, and biotypes isolated later were called exogenous. “Common” biotypes were those biotypes which were isolated most frequently from the throat swabs and the fecal specimens from SDD-treated and control patients during the study period.

**Susceptibility to antimicrobial drugs.** The susceptibility patterns of all biotypes, *Staphylococcus aureus*, and beta-hemolytic streptococci were determined by agar diffusion (2) on diagnostic sensitivity test agar (Oxoid) with susceptibility test tablets (Neo-sensitabs; Rosco). We also tested the susceptibilities of *Enterobacteriaceae* and *Pseudomonadaceae* species to nalidixic acid, co-trimoxazole, and polymyxin.

Enterococci isolated before the SDD treatment started were tested for susceptibility to co-trimoxazole.

**Monitoring colonization resistance.** To monitor the effect of the SDD treatment on colonization resistance, we measured the concentration of enterococci in the feces (33). Most enterococci are resistant to the drugs used for SDD, as are anaerobes.

**Statistical analysis.** Statistical analysis was done by the two-sided chi-square test.

<table>
<thead>
<tr>
<th>Table 1. Patient characteristics</th>
<th>Length of study (weeks) with a granulocyte count of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;100 cells per mm³</td>
</tr>
<tr>
<td><strong>Group</strong></td>
<td><strong>Acute myeloid leukemia</strong></td>
</tr>
<tr>
<td>SDD</td>
<td>19</td>
</tr>
<tr>
<td>Control</td>
<td>18</td>
</tr>
</tbody>
</table>
RESULTS

Patients. A total of 113 patients were divided randomly; 8 patients were excluded from our evaluation. In one SDD-treated and one control patient, the protocol was not followed properly. Four SDD-treated and two control patients died within a few days after randomization. Of the 105 remaining patients, 53 were assigned to the SDD treatment group, and 52 were assigned to the control group and thus did not receive any prophylactic treatment. At the moment of randomization, there was no difference between the SDD-treated group and the control group in the number of patients with potentially pathogenic bacteria in the throat swab cultures (Table 2) and in the feces (Table 3).

SDD. The majority of the patients received oral amphotericin B for the suppression of yeasts; only four patients were treated with nystatin for a total of 46 days. For selective elimination of gram-negative rods, co-trimoxazole and nalidixic acid were used most frequently (for 711 days [29 patients] and 602 days [36 patients], respectively). In 12 patients both drugs were used for different reasons (resistant strains, side effects), but never in combination. Changing to polymyxin or adding polymyxin was done when resistant Enterobacteriaceae or Pseudomonadaceae species were isolated from two or more consecutive fecal samples. In six patients polymyxin was added to the SDD treatment schedule for this reason; this drug was used for a total of 149 days.

Bacteriological surveillance. (i) Throat swabs. At the moment of randomization, Enterobacteriaceae and Pseudomonadaceae species were isolated from the throat swabs from 13 of the patients in the SDD group, and yeasts were cultured from the throat swabs from 26 patients in this group (Table 2). Haemophilus influenzae was not isolated. Excluding the first week of the study, gram-negative rods and yeasts were cultured from 3.7 and 27.3%, respectively, of the throat swabs from the SDD patients. The incidence of these microorganisms in the throat swabs of the SDD patients was significantly reduced compared with the incidence in the control group (Table 2). SDD treatment appeared not to influence the number of cultures containing S. aureus and Streptococcus pyogenes (Table 2).

(ii) Feces. All patients had gram-negative rods in their initial fecal cultures. After nalidixic acid treatment was started, gram-negative rods could not be cultured after an average period of 6.9 ± 3.9 days; for co-trimoxazole, this period was 8.9 ± 4.6 days. In patients who received
polymyxin, the first fecal sample obtained after drug treatment was negative; i.e., the effect of this drug was achieved in 1 to 3 days.

In 15 patients SDD treatment was completely successful; all of the fecal samples obtained after the first negative fecal sample remained free of gram-negative rods for a total time of 208 days (range, 4 to 32 days).

In 13 patients no negative fecal sample or only one negative fecal sample was obtained because of a short observation period (less than 14 days; reasons for termination, granulocyte concentration of >1,000 cells per mm², death, or discharge from the hospital). In the other 25 SDD-treated patients gram-negative rods were cultured from one (14 patients) or more (11 patients) fecal samples during SDD treatment.

The 406 fecal samples obtained from the SDD group after the first 1 week of the study were compared with the 325 samples from the control group. The percentage of fecal cultures containing gram-negative rods differed significantly between the two groups (25.6 versus 95.1%) (Table 3).

Yeasts, which were cultured from the initial fecal samples from 14 SDD-treated patients, disappeared in 4.1 ± 3.9 days after treatment with the antifungal drugs was started. Thereafter, all of the fecal samples obtained from 39 patients remained free of yeasts. In the other 14 patients, yeasts were cultured from one (eight patients) or more fecal samples. Yeasts were cultured from 8.4% of all the fecal specimens (omitting those obtained during the first 1 week of each study period). This implied a significant reduction compared with the percentage of fecal samples containing yeasts in the control group (Table 3).

(iii) Food. Gram-negative rods were found in low concentrations in foods (<10³ cells per g).

Identification and biotyping of Enterobacteriaceae and Pseudomonadaceae species. From the stool specimens of the 25 SDD patients who were positive for Enterobacteriaceae and Pseudomonadaceae species, a total of 108 API 20E biotypes were isolated after the days on which the first negative fecal samples were obtained. Usually, gram-negative rods were found in low concentrations in these samples (Fig. 2), and one biotype was found in each sample; occasionally, more than one biotype was found in a sample.

To evaluate whether gram-negative rods were eliminated or only suppressed by SDD treatment, we compared 121 biotypes which were isolated during (108 biotypes) and after (13 biotypes) the period of SDD treatment with the bacteria isolated when the patients were admitted. Of these 121 biotypes, 93 (77%) were exogenous (i.e., nosocomial in origin). Of the 28 biotypes which were also isolated when the patients were admitted, 16 (57%) were common, and 22 (24%) of the 93 exogenous biotypes mentioned above were common. Although also isolated when patients were admitted, the common biotypes could have been nosocomial in origin.

Susceptibilities of Enterobacteriaceae and Pseudomonadaceae species and enterococci. Of the 108 biotypes isolated during SDD treatment and after the first negative fecal sam-

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Table 2. Culture results of throat swabs

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of patients</th>
<th>Gram-negative rods</th>
<th>Yeasts</th>
<th>Gram-positive cocci*</th>
<th>No. of cultures 1 week after randomization</th>
<th>Gram-negative rods</th>
<th>Yeasts</th>
<th>Gram-positive cocci*</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDD</td>
<td>53</td>
<td>13</td>
<td>26</td>
<td>14</td>
<td>432</td>
<td>3.7</td>
<td>27.3</td>
<td>7.2</td>
</tr>
<tr>
<td>Control</td>
<td>52</td>
<td>13</td>
<td>27</td>
<td>10</td>
<td>388</td>
<td>29.6</td>
<td>42.6</td>
<td>9.0</td>
</tr>
</tbody>
</table>

* S. aureus and S. pyogenes.

b P < 0.0005 by the chi-square test.

Table 3. Culture results for feces

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of patients</th>
<th>Gram-negative rods</th>
<th>Yeasts</th>
<th>Enterococci</th>
<th>No. of cultures 1 week after randomization</th>
<th>Gram-negative rods</th>
<th>Yeasts</th>
<th>Enterococci</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDD</td>
<td>53</td>
<td>53</td>
<td>14</td>
<td>52</td>
<td>406</td>
<td>25.6</td>
<td>8.4</td>
<td>91.4</td>
</tr>
<tr>
<td>Control</td>
<td>52</td>
<td>52</td>
<td>11</td>
<td>51</td>
<td>325</td>
<td>95.1</td>
<td>21.5</td>
<td>92.6</td>
</tr>
</tbody>
</table>

* P < 0.0005 by the chi-square test.
plies were obtained, 78 were susceptible to the drugs used for SDD treatment at the moment of occurrence of the positive culture. In no case could these biotypes be cultured from the next fecal sample. This was also true of 11 of the 30 remaining biotypes, which were resistant to the drugs used for SDD treatment. In order to eliminate 19 of the resistant biotypes, adjustment of the treatment was necessary. This was either because a gram-negative bacterium persisted for a longer period in the flora of a patient or because it was found at a high concentration in the fecal cultures. Enterococci isolated before SDD was started were resistant to co-trimoxazole.

Colonization resistance. Regarding the preservation of colonization resistance, the concentrations of the enterococci and the aerobic gram-negative rods in the feces were compared in the two groups (Fig. 3). Because antimicrobial agents other than the SDD drugs given to the patients in the case of a (suspected) infection might influence colonization resistance (31, 34), we included in this evaluation only those samples which were obtained during periods when no systemic antimicrobial therapy was being administered. Although the concentration of gram-negative rods decreased rapidly after the start of SDD treatment, the concentration of the enterococci did not change.

DISCUSSION

Our results show that it is possible to eliminate selectively susceptible Enterobacteriaceae and Pseudomonadaceae species from the gastrointestinal tract with nalidixic acid, co-trimoxazole, or polymyxin. Fecal cultures became free of gram-negative rods 1 week after the start of nalidixic acid or co-trimoxazole treatment, but with polymyxin this was achieved within a few days. The rapid effect of polymyxin has been described previously for Salmonella carriers (8).

As all patients had gram-negative rods in their feces when they were admitted (Table 3), the bacteriological effect of the SDD treatment could be measured best by frequent monitoring of the fecal flora. In this way the moment of disappearance of Enterobacteriaceae and Pseudomonadaceae species from the feces and the maintenance of this condition could be determined rather accurately. Because it took about 1 week of treatment to free the fecal samples of the SDD patients of gram-negative rods and yeasts, the samples obtained after the first week of study were compared in the SDD and control groups. Gram-negative rods could still be cultured from 25.6% of the fecal samples in the SDD group. This was probably due to the fact that our patients received nonsterilized food and were not isolated in any way. Our randomly chosen food samples support the hypothesis that food is a main source of Enterobacteriaceae (6, 24) and Pseudomonadaceae species (24, 28). Oral contamination with small numbers of gram-negative rods from food, beverages, and other environmental sources sometimes resulted in positive fecal and throat swab cultures. However, in no case did we observe colonization (persistance of a gram-negative bacterium in the gastrointestinal tract for more than 1 week). It was remarkable that there was also a significant reduction in the number of throat swabs containing gram-negative rods, despite the brief presence of the swallowed SDD drugs in the oropharynx. Fractions of the two absorbable SDD drugs (especially co-trimoxazole) may have been excreted into the saliva and thus may have added to the SDD effect in the oropharynx.

Yeasts were found more frequently in throats (Table 2) than in feces (Table 3). At the moment of randomization, about 50% of the patients had yeasts in their oropharyngeal cultures (Table 2) and about 25% had yeasts in their feces (Table 3). After amphotericin B administration was started, the number of throat swabs and fecal samples containing yeasts decreased significantly. This reduction in positive throat swab cultures is remarkable if one realizes that this drug has only a short stay and thus a short contact time in the oropharynx. This reduction cannot be explained by an additional systemic effect since amphotericin B and nystatin are nonabsorbable drugs.

The bacteriological efficacy of SDD (i.e., the reduction in the number of aerobic gram-nega-
tive rods and yeasts) is in accordance with the clinical effect of this treatment, as described previously (9, 29).

Biotyping of Enterobacteriaceae species has been used for epidemiological purposes (38). In our study this technique suggested that with SDD treatment we were perhaps eliminating gram-negative bacteria rather than suppressing them, since the majority of the biotypes isolated during and after the period of SDD treatment differed from the biotypes isolated when the patients were admitted. However, small numbers of gram-negative bacteria may have been missed by our tests. Of the 28 biotypes isolated both when the patients were admitted and during treatment, 16 were common. This means that about 50% of these bacteria were ubiquitous, which makes it difficult to draw conclusions about their endogenous or exogenous origins.

The biotypes which were isolated during SDD treatment after the first negative fecal samples were obtained were, in general (72%), susceptible to the drugs used at the time when they were isolated. Moreover, 11 of 30 resistant biotypes disappeared without adjustment of the treatment, possibly as a consequence of intact colonization resistance. However, to what degree colonization resistance remained intact during SDD was difficult to estimate. Anaerobes of various species are apparently involved in colonization resistance, and for optimal colonization resistance many of these species are required (40). Because of the complexity of anaerobic culturing and species determination, we made no attempt to perform an anaerobic inventory of the feces.

In rodents an enlarged cecum and an increased concentration in the oropharynx and in the feces ("bacterial overgrowth") of an orally administered resistant bacterium both indicate decreased colonization resistance (19, 33). However, these parameters obviously cannot be used in humans. Instead, the concentration of enterococci was used as the next best parameter. During SDD treatment the concentration of enterococci remained stable and did not differ from that in the control group (Fig. 3). In addition, during SDD treatment most of the transient gram-negative rods found in the "positive" fecal cultures disappeared without adjustment of the treatment. From these two findings, we concluded that colonization resistance was not greatly disturbed. We believe that this intact colonization resistance is of great significance in the apparently effective prevention of infections by SDD treatment when isolation procedures are not used. The need for care in a protective environment appears to be definitely decreased if colonization resistance is maintained. However, because in conventional hospital environments the patients may become contaminated continuously by (small numbers of) potentially pathogenic bacteria (among which resistant strains could be present), frequent bacteriological monitoring is considered a prerequisite in this kind of infection prophylaxis.

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