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Selective Suppression of Alimentary Tract Microbial Flora as Prophylaxis During Granulocytopenia

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Oral nonabsorbable antibiotics have been used to suppress the rectal flora in granulocytopenic patients. Problems with these therapies, i.e., compliance, acquisition of undesirable flora, and cost, motivated the search for an alternative therapy which would increase compliance and effectively reduce the *Enterobacteriaceae* without creating a microbial vacuum. Trimethoprim-sulfamethoxazole was found to be easily taken, to suppress the *Enterobacteriaceae*, and to maintain the anaerobic rectal flora for biological stability of the rectal ecosystem. However, concurrent use of parenteral antibiotics profoundly influenced rectal flora and temporarily destroyed the colonization resistance afforded by the anaerobes.

Infections frequent among granulocytopenic patients with cancer usually arise along the alimentary canal or respiratory tract and are caused by locally colonizing organisms of which about one half are hospital acquired (9). The oral nonabsorbable antibiotic combination of gentamicin, vancomycin, and nystatin will suppress most of the aerobic and anaerobic bacteria as well as most yeasts, provided the patients can continuously tolerate and ingest these prophylactic antibiotics (8).

It has been our opinion that infections are reduced when these prophylactic drug combinations are used outside of laminar air flow reverse isolation (8, 10); however, patients taking oral nonabsorbable antibiotics often acquire, become colonized with, and subsequently become infected with organisms resistant to these antibiotics (2). This would be expected in view of the microbial vacuum created; however, it might be possible to suppress the potentially pathogenic aerobic flora without suppressing the anaerobic flora and thus to allow for the preservation of the colonization resistance afforded by the anaerobes in the gastrointestinal tract. Such an approach has appeal because the group of organisms known to cause infection most frequently in granulocytopenic patients is the *Enterobacteriaceae*, whereas anaerobes rarely cause infection in these patients. Would it be possible to effectively suppress this one group of organisms without upsetting the balanced ecosystem along the intestinal tract? The drug trimethoprim-sulfamethoxazole (TMP/SMZ), which is effective against *Enterobacteriaceae* and does not have broad anaerobic coverage, offers the possibility for such selective suppression. Previous studies by Naff (7) and Knothe (4) indicated that the *Enterobacteriaceae* were reduced in stool samples from patients receiving TMP/SMZ, whereas the recovery of anaerobes was not significantly affected. It remained to be determined whether similar results could be obtained in cancer patients and whether the anaerobes would provide effective prevention of overgrowth by other microflora.

We have recently reported the clinical comparison of TMP/SMZ plus nystatin (TMP/SMZ+N) with gentamicin plus nystatin (G+N) as one aspect of an infection prevention program for granulocytopenic patients with leukemia (12). In the study, two concurrent evaluations were conducted comparing (i) the effect of TMP/SMZ with that of placebo on the rectal flora of patients undergoing cytotoxic drug therapy of small cell cancer of the lung and (ii) the effect of TMP/SMZ+N with that of G+N on the rectal flora of patients with acute leukemia receiving remission induction chemotherapy. By studying the total enteric flora of patients on these regimens we compared the effect of the various antibiotic regimens on (i) the suppression of the *Enterobacteriaceae*, (ii) the maintenance of the anaerobic flora as a mechanism for colonization resistance, (iii) the acquisition and colonization by potential pathogens, and (iv) the prevention of overgrowth by inherently resistant organisms.

MATERIALS AND METHODS

Patient population and antibiotic prophylaxis. Patients with small cell carcinoma of the lung undergoing identical initial induction chemotherapy were pro-

spectively randomized in a double-blind study to receive TMP/SMZ (160/800 mg) or an identical placebo orally every 12 h. Patients with acute nonlymphocytic leukemia undergoing reinduction were prospectively randomized to receive either gentamicin liquid (200 mg) plus nystatin tablets (4×10^6 U) and nystatin suspension (10^6 U) (G+N) orally every 4 h, or TMP/SMZ tablets (160/800 mg orally every 12 h (plus nystatin suspension (10^6 U orally every 4 h) (TMP/SMZ+N). Prophylactic antimicrobial agents were initiated 24 to 72 h before cancer chemotherapy (see reference 12 for additional details of the clinical trial methodology). For the purposes of the microbiological data reported here, the patients with leukemia were studied for 6 weeks and the patients with small cell carcinoma were studied for 5 weeks unless prophylactic therapy was discontinued due to tumor remission, death, or noncompliance. Rectal flora studies were carried out on eight patients in each group (32 total patients) as laboratory capabilities permitted; hence, the results described herein represent a subgroup of the larger patient population evaluated for clinical infection prophylaxis.

Microbiological sample collection. Anaerobic rectal cultures were taken weekly by the same microbiologist with emphasis on proper transportation and culture of anaerobes. The anaerobic transport system consisted of a stoppered vial containing 1 ml of reduced transport fluid within a larger vial which also contained several palladium catalyst pellets. The transport systems were prepared in a Coy anaerobic chamber and removed from the chamber immediately before use. A reduced swab from a dual-tube anaerobic transport system (Scott Laboratories, Fiskeville, R.I.) was used to obtain the rectal sample. The rectal swab was placed in the reduced transport fluid in the inner vial, and both tubes were gassed with a hydrogen mixture before closing. Specimen collection was prompt, and transport to the anaerobic chamber was completed within 15 min.

Organism quantitation and identification. The samples were diluted in reduced transport fluid inside the Coy anaerobic chamber. Viable anaerobic and facultative anaerobic organisms were quantitatively cultured in the chamber by spreading samples of the dilutions on anaerobic blood agar plates (England Laboratories, Hyattsville, Md.), which were then incubated for 7 days at 37°C. Afterwards the samples were removed from the chamber, and similar methods were used to quantify the aerobic flora on selective media (KF Streptococcus Agar, Rogosa SL Agar, Desoxycholate Agar [Difco Laboratories, Detroit, Mich.], Pseudosel Agar and Mycosel Agar [BBL Microbiology Systems, Cockeysville, Md.], and Mannitol Salt Agar [Inolex, Glenwood, Ill]). These plates were incubated at 37°C under 5% CO_2 for 48 h except the Mycosel Agar, which was incubated at 25°C for 7 days. Microorganisms, both aerobic and anaerobic, were not identified to species but grouped according to their growth characteristics on the selective media.

Particle counts. Microscopic particle counts of the original specimen collection tubes were performed using a Petroff-Hauser chamber with a dark-field condenser. Particle counts were compared with viable counts to determine the percentage of viable bacteria per particle.

RESULTS

Eight patients were entered in each of the four arms of the study (Table 1). Data collection from patients was discontinued when the patient went off the protocol due to lack of compliance, tumor remission, or death. To evaluate only the effects of prophylactic antibiotics on the rectal flora, patients were removed from further analysis as soon as therapeutic antibiotics were given during the 6-week study period. The patients with leukemia generally had more profound and extended granulocytopenia than did those with lung cancer. This was evident by the fewer samples obtained from leukemia patients before the initiation of therapeutic antibiotics; i.e., these patients often had febrile episodes necessitating parenteral broad-spectrum antibiotics. Thus the majority of leukemia patients were no longer evaluated after 2 to 3 weeks (Table 1).

We decided to study the viable recoveries from rectal swabs taken in a uniform manner by the same microbiologist rather than a stool sample because of the consistency of specimen acquisition and transport offered by the rectal swab. Differences in rates of specimen processing can be critical when quantitating anaerobes. A comparison of the mean bacterial recovery from rectal swabs of patients with small cell carcinoma who received TMP-SMZ or placebo is given in Table 2. The placebo group had rectal bacterial recovery rates which were consistent with other published reports (5). There were no significant changes in the percent viable bacterial recovery in patients on TMP-SMZ as compared to placebo. This was a result of persistence of the anerobic flora despite TMP-SMZ therapy. The *Enterobacteriaceae*, however, were markedly reduced in these patients, except for one individual who continued to carry at TMP-SMZ-resistant *Escherichia coli* strain throughout TMP-SMZ administration. Both groups had equally low recovery rates for yeasts, which averaged less than 0.01% of the total viable flora.

A comparison of the mean bacterial recovery from rectal swabs of patients with leukemia receiving G+N or TMP-SMZ+N (Table 3) indicates that gentamicin therapy reduced the total

TABLE 1. *Patients in study*

Patients	Prophylactic regimen	No. of patients in study at week:						
		Zero	1	2	3	4	5	6
Small cell carcinoma of the lung	Placebo	8	8	7	7	7	7	
	TMP/SMZ	8	8	8	7	7	7	
Acute leukemia	TMP/SMZ+N	8	8	6	2	0	0	0
	G+N	8	5	3	0	0	0	0

TABLE 2. *Mean bacterial recovery from rectal swabs from patients with small cell carcinoma of the lung treated with TMP-SMZ or placebo*

Treatment	Time of sample	Total particle count (10^8)	Total viable organisms (10^7)	% Viable recovery	Isolates (% of total viable organisms)			
					Anaerobes	*Enterobacteriaceae*	Enterococci	Yeasts
TMP-SMZ	Before drug	2.1	6.7	32.7	87.2	1.2	0.2	<0.01
	During treatment	7.3	15.0	20.7	88.4	0.01[a]	4.4	<0.01
Placebo	Before drug	3.0	8.3	27.6	91.0	3.7	0.04	<0.01
	During treatment	3.0	8.0	26.8	89.0	3.8	0.1	<0.01

[a] Excluding one patient who carried a TMP-SMZ-resistant *E. coli* strain.

TABLE 3. *Mean bacterial recovery from rectal swabs from patients with acute leukemia treated with G+N or TMP-SMZ+N*

Treatment	Time of sample	Total particle count (10^8)	Total viable organisms (10^7)	% Viable recovery	Isolates (% of total viable organisms)			
					Anaerobes	*Enterobacteriaceae*	Entrococci	Yeasts
G+N	Before drug	2.7	6.0	21.9	88.2	2.8	0.14	<0.01
	During treatment	2.6	2.3	9.7	59.7[a]	0.0005	0	<0.01
TMP-SMZ+N	Before drug	4.3	10.0	23.7	95.6	0.3	0.2	<0.01
	During treatment	2.8	5.7	20.6	93.4	0.0007	1.3	<0.01

[a] One patient's anaerobic flora was eliminated and replaced with *S. epidermidis*.

viable recovery of microorganisms including *Enterobacteriaceae*, enterococci, and especially the anaerobes. One patient who received G+N had a virtual elimination of the anaerobic flora with subsequent heavy colonization by *Staphylococcus epidermidis*. The TMP-SMZ+N regimen was again effective in reducing the *Enterobacteriaceae* but did not significantly reduce the total recovery rates nor the proportion of anaerobes in the rectal flora.

Evaluation of the isolated effect of the prophylactic antibiotics on the rectal flora in the patients with leukemia was hampered by the frequent necessity for therapeutic treatment with parenteral antibiotics during febrile episodes. These additional antibiotics (usually ticarcillin + aminoglycoside) markedly reduced the rectal flora (Table 4). The percentage of viable anaerobic bacteria was equivalent during the base-line period, but after the G+N therapy anaerobic flora was reduced. This marked reduction in viable anaerobes when G+N was administered was further lowered with the addition of parenteral antibiotics. The reduction in anaerobic flora coincided with the acquisition of *Pseudomonas aeruginosa* in one patient and multiply resistant diphtheroids in another. Dramatic shifts in the microbial populations comprising the rectal flora were seen in two patients and three patients in whom yeasts and *S. epidermidis*, respectively, constituted greater than 50% of the viable microorganisms.

TABLE 4. *Mean percent of viable anaerobic bacteria*[a]

Prophylactic antibiotic regimen	Before antibiotics	Oral antibiotics	Oral antibiotics and parenteral antibiotics (if needed)
G+N	19.3 (8)[b]	5.8 (8)	5.0[c] (20)
TMP-SMZ+N	22.7 (8)	19.2 (16)	11.1[d] (32)

[a] As a percentage of the total particle count.

[b] Parentheses indicate sample size.

[c] One patient acquired *P. aeruginosa*; two patients experienced population shifts resulting in >50% yeast; three patients experienced population shifts resulting in >50% *S. epidermidis*; and one patient acquired a resistant diphtheroid.

[d] One patient experienced population shifts resulting in >50% yeast; two patients experienced population shifts resulting in >50% *Lactobacillus*; one patient experienced population shifts resulting in >50% *S. epidermidis*; two patients acquired a resistant diphtheroid.

TMP-SMZ+N by itself had little effect on the recovery of viable anaerobes, but when parenteral antibiotics were added, anaerobes were reduced, although not as much as with the G+N combination. During the combined therapy of TMP-SMZ+N and therapeutic antibiotics, two

patients acquired multiply resistant diphtheroids and four patients experienced population shifts with 50% of the viable flora being either yeast, *Lactobacillus*, or *S. epidermidis*.

DISCUSSION

Life-threatening infections in granulocytopenic patients have been shown to be caused principally by bacteria that colonize the patient's alimentary tract (9). One half of these organisms are acquired by the patient during hospitalization (9). One approach to infection prevention is the use of antibiotic prophylaxis to suppress the alimentary canal microbial flora. To achieve this end, oral nonabsorbable antibiotic regimens that basically suppress the total microbial flora have been used (1, 8). Another approach is to use selective microbial suppression in an attempt to eliminate the potentially more dangerous pathogens (3, 6, 11, 12; M. Gurwith, J. Bruton, B. Lank, G. Harding, and A. Ronald, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 17th, New York, N.Y., abstr. no. 409, 1977).

Gentamicin, a commonly used prophylactic antibiotic, has a broad spectrum, is nonabsorbable, and has been reported to achieve almost total suppression of the intestinal flora when administered in combination with vancomycin and nystatin (1). Our data showed that when gentamicin was administered with nystatin only, it suppressed the *Enterobacteriaceae* and other facultative anaerobes and had a varied individual effect on the anaerobic flora. This overall effect resulted in a generalized suppression of the intestinal flora including anaerobes, with the virtual elimination of *Enterobacteriaceae* and enterococci. *S. epidermidis* persisted, and this is consistent with a general increase in infections with this organism (12; J. Wade, S. Schimpff, K. Newman, V. Young, and P. Wiernik, Clin. Res. **28**: 381A, 1980). A major problem with the G+N regimen is the lack of patient compliance because of nausea, vomiting, and unpleasant taste. This compliance problem is of significant concern because the patients who discontinue the antibiotics while still granulocytopenic are at risk of rapid regrowth in the alimentary tract by gentamicin-resistant *P. aeruginosa* (2) or by facultatively anaerobic potential pathogens with subsequent infection (8). Therefore the total elimination of the intestinal flora is best reserved for a protected environment, e.g., laminar air flow room.

TMP-SMZ was effective in suppressing the rectal *Enterobacteriaceae* in the patients with both leukemia and small cell carcinoma with the exception of one patient with small cell carcinoma who was colonized with a TMP/SMZ-resistant *E. coli* strain throughout TMP/SMZ prophylaxis. Resistant strains have occasionally been reported in patients receiving TMP/SMZ, and screening for susceptibility of the patient's coliforms before prophylaxis may be of value. The recoveries of other organisms were not significantly affected. The preservation of the anaerobic flora of patients with leukemia on TMP-SMZ+N would have contributed to maintenance of the colonization resistance of the alimentary tract of these patients. This protection, along with the lack of compliance problems of patients on TMP-SMZ, contributed to the fact that the number of samples for study on the TMP/SMZ+N regimen was twice as many as those on the G+N regimen.

A number of clinical trials (3, 6; Gurwith et al., 17th ICAAC, abstr. no. 409, 1977), including one from this center (12), have suggested that TMP/SMZ+N is effective as a prophylactic regimen for granulocytopenic patients. We emphasize that infection incidence is reduced, but by no means is this technique a panacea. Although the data are strongly suggestive of a partial prophylactic benefit, the observation from this study that parenteral antibiotics such as ticarcillin and amikacin will reduce rectal anaerobes raises a serious cautionary note. Population shifts and acquisition of new organisms, including potential pathogens, occurred during treatment periods. This important finding may lead to questions of interpretations of any data which have not taken parenteral therapy into account. The use of TMP/SMZ may be an adequate approach for patients who receive intensive myelosuppressive therapy, but where granulocytopenia can be expected to be both profound (<100/ml) and prolonged (>14 days), the administration of parenteral antibiotics will probably negate colonization resistance and seriously compromise the value of this approach.

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