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Impact of Cefoperazone Therapy on Fecal Flora

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To evaluate the effects of parenteral cefoperazone therapy upon human fecal flora, fecal specimens obtained from four patients before and during therapy (as well as after therapy for one patient) were cultured quantitatively for facultative, aerobic, and anaerobic bacteria and for fungi. Cefoperazone therapy was associated with major changes in fecal flora. There was suppression to undetectable levels or an appreciable reduction in all anaerobic bacteria as well as suppression of all initially detected *Enterobacteriaceae*. During therapy, there was acquisition or an increase in counts of *Candida* spp., so that these became the most numerous fecal microorganisms in all patients. In addition, *Pseudomonas* spp. and coagulase-negative *Staphylococcus* sp. were acquired by three patients. These marked alterations in flora have potentially important consequences.

Therapy with a variety of antimicrobial agents has been shown to produce changes in the normal fecal microflora (2, 14). Although the role of the normal flora is still poorly understood, there is evidence that alterations in flora may have important consequences.

Cefoperazone is a new agent of the cephalosporin class which, because it is active against a very broad spectrum of microorganisms (7) and is excreted in large part via the biliary tract (10), might be expected to produce appreciable changes in the intestinal flora. Thus, we evaluated the effects of parenteral cefoperazone therapy upon human fecal flora.

MATERIALS AND METHODS

Four hospitalized, adult male patients with soft tissue or bone infections for which parenteral cefoperazone was judged to be appropriate therapy were chosen to have total fecal flora studies performed. Although each patient had received antimicrobial agents in the past, no antimicrobial therapy had been given in the preceding 2 weeks. All received cefoperazone, 4 g daily, for 12 to 30 days. Written, informed consent was obtained from each patient.

Fecal specimens from all patients were collected before therapy and on day 8 of therapy. In addition, for patient 1, fecal specimens were collected on day 22 of therapy and at 3 weeks after cessation of cefoperazone. Specimens were collected in nonsterile plastic containers and were transported immediately to an anaerobic chamber in which they were processed. Approximately 1 g of homogenized specimen was weighed, diluted 10-fold in 0.5% yeast extract solution containing glass beads, and emulsified in a Vortex mixer. Serial 10-fold dilutions were made in yeast extract, and volumes of 0.1 ml of selected dilutions (the lowest dilution being 1:10) were plated by a rotator-pipette method onto the following media for aerobic incubation: brucella blood agar (Difco Laboratories, Detroit, Mich.) with 5% sheep blood supplemented with vitamin K_1 and hemin (BAK) (13); mannitol-salt agar (Clinical Standards Laboratories, Carson, Calif.); MacConkey agar (Clinical Standards Laboratories); Pfizer selective enterococcus agar (Pfizer Diagnostics Div., New York, N.Y.); cetrimide agar (Eastman Kodak Co., Rochester, N.Y.); and Sabouraud agar (Difco) with chloramphenicol. The following media were inoculated for anaerobic incubation: BAK, *Bacteroides*-bile-esculin agar, kanamycin–vancomycin–laked-blood agar, rifampin-blood agar, *Bifidobacterium* agar, cycloserine-cefoxitin-egg yolk-fructose agar, and egg yolk-neomycin agar (for ethanol-treated dilutions) as described previously (13). With these methods, the lowest detectable number of microorganisms is 2 $\log_{10}$ per g.

Aerobic plates were incubated for 24 to 48 h and anaerobic plates were incubated for 48 to 96 h before examination. Colonies of differing morphologies were counted; representatives of each morphological type were then picked for isolation and identification. Anaerobic organisms were identified by standard procedures (6, 13). Facultative gram-negative bacilli were identified with API 20E test strips (Analytab Products, Plainview, N.Y.). Other aerobic and facultative isolates were identified by standard methods (8). Cefoperazone susceptibility tests were performed on facultative and aerobic isolates by the Kirby-Bauer disk diffusion method, with susceptibility defined as a zone of inhibition of 18 mm or more in diameter around a 75-μg disk (as recommended by the Roerig Division of Pfizer Pharmaceuticals, New York, N.Y.). Fecal specimens were assayed for the presence of cytotoxic activity neutralized by *Clostridium sordellii* antitoxin in HeLa cell tissue culture by methods previously reported (9). Concentrations of cefoperazone in feces

were measured by high-pressure liquid chromatography. Additional fecal specimens obtained during and after cefoperazone therapy were cultured on selective medium for *Clostridium difficile* (3) and assayed for cytotoxin.

RESULTS

Results of fecal cultures and fecal assays for cefoperazone are given in Table 1. Whereas total microorganism counts decreased somewhat (a change of <3 $\log_{10}$) and total counts of facultative or aerobic organisms increased somewhat, the most notable effect of cefoperazone therapy was suppression to undetectable levels (to <2 $\log_{10}$ in three patients) or an appreciable reduction (>4 $\log_{10}$ in the fourth patient) of the normal anaerobic flora.

In all patients there was also suppression to undetectable levels of all *Enterobacteriaceae*, and in three patients there was concomitant ingrowth of one or more species of *Pseudomonas*. Interestingly, three of the five acquired species of *Pseudomonas* were cefoperazone susceptible (zones of inhibition of 18 mm or more in diameter), and two had intermediate zones of inhibition (17 and 15 mm in diameter).

Three patients acquired coagulase-negative *Staphylococcus* sp., and two acquired *Corynebacterium* sp. The three patients who did not originally harbor fungi acquired *Candida glabrata* during therapy. For all four patients, the most numerous fecal microorganism during therapy was a species of *Candida*.

In three patients, no fecal anaerobic bacteria were detected on day 8 of therapy; in the fourth patient, the anaerobic flora was reduced to 6.1 $\log_{10}$ *Clostridium* sp. and 7.2 $\log_{10}$ *Propionibacterium* sp. Of interest, *C. difficile*, initially present in the fecal specimen of patient 3 (who had no associated gastrointestinal symptoms at that time), was among the anaerobic flora which was eradicated during cefoperazone therapy.

Patient 1 had two follow-up fecal cultures (on day 22 of therapy and then at 3 weeks after discontinuation of cefoperazone, while receiving an oral cephalosporin). The cefoperazone-induced changes which were still present on day 22 of therapy were the persistence of *Candida* sp. as the most numerous fecal microorganisms and the continued presence of *Pseudomonas* spp. There were, however, some fecal anaerobes (4.1 $\log_{10}$ *Lactobacillus* sp. and 7.3 $\log_{10}$ anaerobic gram-positive cocci) isolated on day 22 of therapy, although none had been detected on day 8. Three weeks after cefoperazone was discontinued, despite the oral cephalosporin therapy, the fecal flora of this patient was virtually the same as the pretherapy flora, with the exceptions of the acquisition of *C. difficile* and the acquisition of *Bacteroides* spp. (the absence of fecal *Bacteroides* sp. before therapy is unusual and presumed to be due to previous extensive antimicrobial therapy).

All four patients developed diarrhea, without fecal leukocytes, while receiving cefoperazone. This was a minor problem for patient 3, who had 4 to 6 soft bowel movements per day, but patients 1 and 2 had 8 to 10 liquid stools daily. Patient 4, although he had only two to three bowel movements daily, developed nocturnal fecal incontinence. None of the patients experienced abdominal pain or tenesmus. Symptoms resolved in three patients within 4 days after cessation of cefoperazone. For patient 1, who received an additional 4-week course of oral cephalosporin therapy after discontinuation of cefoperazone, diarrhea persisted until all antimicrobial therapy was stopped. No other enteric pathogens were detected in patient 1. They were not sought in the other patients. Sigmoidoscopy was not performed in any patient. None of the patients felt that the diarrhea warranted discontinuation of cefoperazone therapy, although for patient 4 the diarrhea might have been a significant problem had he required a prolonged course of treatment.

Fecal cultures for *C. difficile* and assays for cytotoxin (performed one to four times per patient) were negative for all patients during therapy. However, patients 1, 2, and 4 acquired *C. difficile* (at 3 weeks, 2 months, and 5 weeks, respectively) after discontinuation of cefoperazone. Two of these patients, 1 and 2, while receiving subsequent cephalosporin therapy, had diarrhea, with fecal cultures positive for *C. difficile* (not quantitated for patient 1; counts of 5 $\log_{10}$ for patient 2) but with negative fecal cytotoxin assays. After all antimicrobial therapy was stopped, their symptoms resolved, and *C. difficile* was no longer detected in their feces. Patient 4 did not receive additional antimicrobial therapy, and his acquisition of *C. difficile* (with counts of 6 $\log_{10}$ per g of feces [wet weight]; negative fecal cytotoxin assay) was not associated with symptoms.

Concentrations of cefoperazone in the feces of our patients during therapy were extremely high, ranging from 10.7 to 21.7 mg/g of feces (dry weight) (Table 1).

DISCUSSION

The alterations in fecal flora which we observed in our cefoperazone-treated patients are the most marked that have been reported in association with the use of a single antimicrobial agent. However, the significance of changes in intestinal microecology is not yet fully understood, and conclusions as to clinical importance cannot be drawn from our study of a small number of patients.

TABLE 1. Levels of cefoperazone[a] and counts of fecal microorganisms

Microorganism(s)	Counts[b]									
	Patient 1				Patient 2		Patient 3		Patient 4	
	Before[c]	During[c]		3 wk after[c]	Before	During (day 8)	Before	During (day 8)	Before	During (day 8)
		Day 8	Day 22							
Total	10.9	9.4	8.6	10.9	11.6	8.9	11.7	8.9	11.4	8.7
Total facultative and aerobic	8.9	9.4	8.6	9.6	6.5	8.9	8.3	8.9	7.0	8.7
Escherichia coli	8.1	ND[d]	ND	9.1	4.7	ND	7.3	ND	6.8	ND
Klebsiella pneumoniae	7.0	ND	ND	7.3	ND	ND	7.6	ND	ND	ND
Citrobacter sp.	6.2	ND	ND	8.5	ND	ND	ND	ND	ND	ND
Enterobacter cloacae	ND	ND	ND	ND	2.7	ND	ND	ND	ND	ND
Morganella sp.	ND	ND	ND	8.8	ND	ND	ND	ND	ND	ND
Proteus mirabilis	5.2	ND	ND	ND	6.5	ND	ND	ND	ND	ND
Pseudomonas spp., cefoperazone susceptible	ND	3.9	4.1	ND	ND	4.8	ND	3.2	ND	ND
Pseudomonas sp., intermediate susceptibility	ND	4.1	3.4	ND	ND	ND	ND	ND	ND	ND
Pseudomonas maltophilia	ND	ND	ND	ND	ND	3.3	ND	ND	ND	ND
Staphylococcus sp., coagulase negative	ND	5.2	5.1	ND	ND	5.4	ND	ND	ND	7.0
Group D *Streptococcus* sp.	8.8	3.9	4.0	9.2	11.8	4.1	7.7	ND	6.4	4.9
Corynebacterium sp.	ND	5.8	ND	ND	ND	3.2	ND	ND	ND	ND
Candida albicans	6.5	9.4	8.6	4.6	ND	ND	ND	ND	ND	ND
Candida glabrata	ND	ND	ND	ND	ND	8.9	ND	8.9	ND	8.7
Total anaerobic	10.9	ND	7.3	10.9	11.6	ND	11.7	ND	11.4	7.2
Bacteroides fragilis group	ND	ND	ND	9.7	11.2	ND	11.2	ND	11.2	ND
Other *Bacteroides* spp.	ND	ND	ND	ND	10.4	ND	11.4	ND	8.0	ND
Lactobacillus spp.	7.4	ND	4.1	7.3	6.4	ND	ND	ND	9.2	ND
Eubacterium spp.	9.7	ND	ND	9.8	11.0	ND	10.4	ND	10.6	ND
Bifidobacterium spp.	ND	ND	ND	ND	9.7	ND	9.9	ND	9.3	ND
Anaerobic gram-positive cocci	9.5	ND	7.3	9.9	11.0	ND	ND	ND	10.5	ND
Megasphaera elsdenii	10.9	ND	ND	ND	ND	ND	ND	ND	ND	ND
Veillonella sp.	ND	ND	ND	ND	ND	ND	10.5	ND	ND	ND
Clostridium difficile	ND	ND	ND	8.8	ND	ND	9.6	ND	ND	ND
Other *Clostridium* spp.	9.0	ND	ND	10.8	9.7	ND	10.2	ND	10.6	6.1
Propionibacterium sp.	ND	ND	ND	ND	ND	ND	ND	ND	ND	7.2

[a] Cefoperazone levels, expressed as milligrams per gram of feces (dry weight), were as follows on day 8 of therapy: patient 1, 14.0; patient 2, 21.7; and patient 3, 10.7.

[b] Counts of microorganisms expressed as $\log_{10}$ per gram of feces (dry weight).

[c] Time of specimen in relation to cefoperazone therapy.

[d] ND, None detected (counts of <2 $\log_{10}$ per g).

Although it is possible that the marked alterations in fecal flora contributed to the diarrhea noted by our patients (for example, by altering bacterial metabolism of bile acids), there are other possible mechanisms for this symptom, such as direct stimulation of intestinal smooth muscle (reported by Takai et al. to occur in experimental animals with concentrations of cefoperazone comparable to those found in the feces of our patients [15]) or changes in intestinal water transport (such as those which have been reported in association with other antimicrobial therapy [4]).

We found no evidence that *C. difficile* is a cause of diarrhea during cefoperazone therapy. However, the possible roles of cefoperazone as either a potential therapeutic agent for the eradication of *C. difficile* (as occurred in one of our patients) or, alternatively, as an agent predisposing to the development of *C. difficile*-induced disease after discontinuation of therapy are interesting considerations which merit further investigation. Asymptomatic acquisition of *C. difficile* after antimicrobial therapy has been recognized recently as not uncommon (17). Additional studies are needed to identify the factors which differentiate asymptomatic colonization from disease production by *C. difficile*.

We cannot explain the high incidence of diarrhea observed in our patients. One patient (patient 3) had such mild symptoms that his change in bowel habits would not have been noticed

without specific inquiry. Two patients had histories of excessive alcohol intake for many years, and another had long-standing diabetes mellitus with diabetic gastropathy. Perhaps these underlying conditions or the relatively long courses of therapy given to our patients contributed to their developing diarrhea. Alternatively, this symptom may be a more common side effect of cefoperazone therapy than is appreciated, noted in our study because of the special interest in the gastrointestinal tract. Except for patient 1, who had multiple evaluations for a variety of enteric pathogens, patients were not evaluated for intestinal pathogens other than *C. difficile*. However, the prompt resolution of diarrhea after discontinuation of cefoperazone for the three patients other than patient 1 suggests a causal relationship, whatever the mechanism.

In addition to the local effects on gastrointestinal physiology which may occur with antimicrobial therapy, there are other potentially important consequences which may result from alterations of the intestinal flora. For example, there is evidence that the normal flora may act as a natural defense (often termed "colonization resistance" [16]) against infection with enteric pathogens such as *Salmonella* spp. and *Shigella* spp. (5) as well as provide protection against antimicrobial agent-associated colitis caused by *C. difficile* (1). In addition, members of the intestinal microflora attain importance when they serve as a reservoir of potential pathogens (11); thus, antimicrobial agent-induced colonization of the bowel by resistant bacteria and fungi may predispose patients to subsequent endogenous infections with these organisms (11, 12, 18). It is of interest that in addition to acquiring resistant microorganisms, three of our patients had intestinal colonization with cefoperazone-susceptible *Pseudomonas* sp. during therapy.

Another role of the intestinal flora, that of synthesizing vitamin K, may be affected adversely by antimicrobial agent-induced changes (2). Thus, although our patients had no bleeding problems or abnormal clotting studies, the potential for bleeding diatheses in patients with marked alterations in flora should be considered.

In contrast, there are clinical settings, such as preparation for intestinal surgery and "intestinal decontamination" for severely neutropenic patients, in which suppression of the intestinal flora is indicated. Whether retention of anaerobic flora is desirable in these situations (to maintain colonization resistance) is not certain. Perhaps cefoperazone should be evaluated in these conditions.

Clinicians should be aware of the marked changes in intestinal flora which may accompany the use of cefoperazone, considering, for example, that in patients who develop infection during or immediately after therapy, *Pseudomonas* spp. or *Candida* spp. may be likely pathogens. In addition, there is the opportunity and need to evaluate which, if any, of the many possible consequences attend the temporary marked suppression of the normal flora. Clearly, further study is needed to define properly the role of the normal flora and the importance of alterations in intestinal microecology.

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