ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 1986, p. 175–178
0066-4804/86/010175-04$02.00/0

Vol. 29, No. 1

Effect of Moxalactam on Human Fecal Microflora

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Received 28 February 1985/Accepted 23 October 1985

Five healthy male adults received 2 g of moxalactam every 12 h for 7 days. The alterations of fecal microflora were investigated before, during, and after treatment with moxalactam. On day 7 of treatment, the number of total bacteria was decreased in all subjects. There was marked suppression of the obligate anaerobic bacteria and enterobacteria to undetectable levels, but the counts of *Streptococcus* spp. and *Lactobacillus* spp. increased. On day 7, two subjects had *Clostridium innocuum* and *Clostridium ramosum* in their feces but not *Clostridium difficile*. On day 7 after treatment, the counts of *Streptococcus* spp., enterobacteria, *Lactobacillus* spp., and *Clostridium* spp. in all subjects were still not normal.

Several antibiotics are known to cause considerable changes in normal human fecal flora and sometimes also to permit overgrowth of resistant bacteria (7, 17). Some fecal bacteria may produce toxins and can cause pathogenic conditions. Moxalactam, a 1-oxa-β-lactam derivative, was developed by Shionogi & Co., Osaka, Japan. It was demonstrated that this drug is effective against both aerobic and anaerobic bacteria (18).

Although moxalactam might be expected to produce appreciable changes in the human fecal flora, there are few published data concerning the effect of this drug on the fecal flora (2). This study was undertaken to determine the changes in normal human fecal flora when moxalactam was administered intravenously.

Five healthy male subjects between the ages of 32 and 44 years and weighing 62 to 76 kg (mean, 67.8 ± 5.4 kg) participated in this study. All subjects received moxalactam in a 2-g dose every 12 h for 7 days. The drug was administered in 50 ml of saline solution over a period of 30 min.

Freshly voided fecal samples were collected from the five individuals before intravenous moxalactam administration, on the last day (day 7) of administration, and on day 7 after administration. Collected fecal specimens were placed in a sterile transport medium (15) filled with CO_2 gas. Samples sealed in the medium were stored at 4°C until use. The specimens were cultured by a previously described method (14). After thorough mixing, a series of 10-fold dilutions (10^{-1} to 10^{-8}) was made under anaerobic conditions. The diluent used was prepared in the same manner as described elsewhere (13). The following media were used for anaerobic incubation: Eggerth-Gagnon agar (14), glucose-blood-liver agar (14), bifidobacteria-selective agar (14), eubacteria-selective agar (13), neomycin-brilliant green-taurocholate-blood agar (14), neomycin-Nagler agar (12), modified veillonellae-selective agar (14), modified lactobacilli-selective agar (14), and cycloserine-cefoxitin-egg yolk-fructose agar (8). The samples were incubated at 37°C for 3 days in anaerobic steel wool jars (16) filled with oxygen-free CO_2. For the isolation of aerobic bacteria, the media used were Trypticase soy (TS) agar with 5% blood (14), deoxycholate-hydrogen sulfide-lactose agar (Eiken Chemical Co. Ltd., Tokyo, Japan) (14), triphenyltetrazolium chloride-acridine orange-thallous sulfate-esculin-crystal violet agar (14), phenylethyl alcohol-egg yolk suspension agar (14), potate glucose agar (14), and nalidixic acid-cetrimide agar (Eiken). Triphenyltetrazolium chloride-acridine orange-thallous sulfate-esculin-crystal violet agar, phenylethyl alcohol-egg yolk suspension agar, potato glucose agar, and nalidixic acid-cetrimide agar were incubated at 37°C for 48 h, while TS agar and deoxycholate-hydrogen sulfide-lactose agar were incubated at 37°C for 24 h. In addition, the dilutions were heated in an 80°C water bath for 10 min prior to inoculation to detect sporeformers. The heated dilutions were cultured on the surfaces of Eggerth-Gagnon agar, glucose-blood-liver agar, and TS agar as nonselective media and neomycin-Nagler agar as the selective medium.

After incubation, each plate was examined for bacterial counts. Colonies from the anaerobic agar were subcultured on Eggerth-Gagnon agar or glucose-blood-liver agar in duplicate to allow aerobic and anaerobic incubation. Microorganisms growing only on the anaerobic agar were identified as strictly anaerobic bacteria. All of the strains isolated were maintained on Eggerth-Gagnon liver agar slants (14) filled with 100% CO_2 gas. Colonies from TS agar were also subcultured on TS agar aerobically. The aerobic isolates were kept on TS agar slants (14). Both slants were stored at 4°C. Transfers were usually made at 1-month intervals. Strictly anaerobic bacteria isolated from the feces were identified by methods described previously (4, 9). Enterobacteria, staphylococci, and *Candida* spp. were classified by the API systems (Analytab Products, Inc., Plainview, N.Y.). The other aerobic bacteria were identified by conventional methods (6, 11). For the isolates identified, the bacterial count per gram of wet feces was calculated and converted into a logarithmic equivalent. A few strains could not be fit into any of the established species; these were designated as spp. The total viable count was calculated from the sum of the counts of each bacterial species. With these methods, the lowest detectable number of microorganisms was 2 $\log_{10}$/g of wet feces. The bacterial counts were analyzed statistically by Student's t test. The concentration of moxalactam in the feces was determined by the band culture method (10).

The total bacteria had decreased on day 7 ($P < 0.001$) of treatment but were at normal levels on day 7 after treatment.

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TABLE 1. Fecal bacteria and yeasts isolated from five subjects receiving moxalactam

Organism	Bacterial counts at indicated time relative to moxalactam treatment (no. of subjects infected/no. of subjects tested)[a]		
	Before	During (day 7)	After
Bacteroides	10.7 ± 0.2[b] (5/5)	(0/5)	10.8 ± 0.1[b] (5/5)
B. buccae-oris group	9.4 ± 0.7 (3/5)	(0/5)	9.2 ± 0.4 (2/5)
B. fragilis group	10.7 ± 0.1 (5/5)	(0/5)	10.4 ± 0.3 (5/5)
B. distasonis	9.9 ± 0.8 (4/5)	(0/5)	10.0 ± 0.2 (5/5)
B. fragilis	9.9 ± 0.4 (4/5)	(0/5)	9.8 ± 0.1 (4/5)
B. thetaiotaomicron	9.4 ± 0.2 (2/5)	(0/5)	(0/5)
B. uniformis	9.2 (1/5)	(0/5)	9.2 (1/5)
B. vulgatus	10.7 ± 0.1 (5/5)	(0/5)	10.4 ± 0.3 (5/5)
B. eggerthii	9.2 (1/5)	(0/5)	(0/5)
B. oralis	9.3 ± 0.2 (2/5)	(0/5)	(0/5)
B. ureolyticus	9.3 (1/5)	(0/5)	8.7 (0/5)
Bacteroides spp.[c]	9.8 ± 0.2 (5/5)	(0/5)	9.0 ± 0.3[d] (2/5)
Bifidobacterium	10.2 ± 0.5 (5/5)	(0/5)	10.6 ± 0.2 (4/5)
B. adolescentis	10.2 ± 0.4 (5/5)	(0/5)	10.6 ± 0.2 (5/5)
B. bifidum	8.5 ± 0.6 (2/5)	(0/5)	9.1 (1/5)
B. longum	9.7 ± 0.6 (3/5)	(0/5)	8.8 (1/5)
Bifidobacterium spp.[c]	9.8 ± 0.1 (2/5)	(0/5)	(0/5)
Clostridium	7.5 ± 0.8 (5/5)	9.4 ± 0.3[e] (2/5)	9.7 ± 0.5[d] (4/5)
C. beijerinckii	6.7 (1/5)	(0/5)	(0/5)
C. bifermentans	3.3 (1/5)	(0/5)	(0/5)
C. butyricum	3.6 (1/5)	(0/5)	4.7 (1/5)
C. clostridiiforme	8.8 (1/5)	(0/5)	(0/5)
C. coccoides	5.6 ± 0.7 (2/5)	(0/5)	(0/5)
C. innocuum	5.8 ± 0.4 (4/5)	9.4 ± 0.3[e] (2/5)	9.4 ± 0.7[e] (4/5)
C. paraputrificum	7.2 (1/5)	(0/5)	4.5 (1/5)
C. perfringens	3.0 (1/5)	(0/5)	4.5 ± 0.4 (3/5)
C. ramosum	7.6 ± 1.1 (3/5)	8.7 (1/5)	8.8 ± 0.9 (3/5)
C. sartagoformum	(0/5)	(0/5)	8.2 (1/5)
C, sphenoides	7.2 ± 0.3 (2/5)	(0/5)	(0/5)
C. tertium	6.7 ± 3.2 (2/5)	(0/5)	5.8 (1/5)
Clostridium spp.[c]	7.6 ± 2.1 (4/5)	(0/5)	7.1 ± 2.2 (3/5)
Coprococcus sp.[f]	9.7 (1/5)	(0/5)	(0/5)
Eubacterium	10.4 ± 0.4 (5/5)	(0/5)	10.1 ± 0.5 (5/5)
E. aerofaciens	10.3 ± 0.5 (5/5)	(0/5)	10.1 ± 0.2 (5/5)
E. biforme	9.3 (1/5)	(0/5)	(0/5)
E. lentum	9.8 ± 0.3 (2/5)	(0/5)	(0/5)
E. moniliforme	9.3 (1/5)	(0/5)	(0/5)
E. rectale	9.3 ± 0.6 (2/5)	(0/5)	(0/5)
Eubacterium spp.[c]	9.2 ± 0.7 (5/5)	(0/5)	(0/5)
Fusobacterium	9.5 ± 0.3 (4/5)	(0/5)	9.6 ± 0.1 (2/5)
F. mortiferum	9.4 ± 0.3 (2/5)	(0/5)	9.6 (1/5)
F. russii	9.7 (1/5)	(0/5)	(0/5)
F. varium	9.6 ± 0.2 (2/5)	(0/5)	(0/5)
Fusobacterium spp.[c]	9.7 ± 0.3 (3/5)	(0/5)	(0/5)
Megasphaera elsdenii	8.4 (1/5)	(0/5)	9.7 (1/5)
Mitsuokella multiacidus	9.9 (1/5)	(0/5)	(0/5)
Peptostreptococcus	10.1 ± 0.3 (5/5)	(0/5)	10.1 ± 0.4 (5/5)
P. anaerobius	9.2 (1/5)	(0/5)	(0/5)
P. micros	9.3 ± 0.5 (2/5)	(0/5)	(0/5)
P. productus	9.9 ± 0.4 (3/5)	(0/5)	9.9 ± 0.3 (3/5)
Peptostreptococcus spp.[c]	9.6 ± 0.4 (2/5)	(0/5)	10.2 ± 0.3 (2/5)
Ruminococcus gnavus	9.3 (1/5)	(0/5)	(0/5)
Veillonella parvula	5.5 ± 2.1 (3/5)	(0/5)	8.4 ± 0.9[e] (5/5)
Total anaerobes	11.1 ± 0.2 (5/5)	9.1 ± 0.6 (2/5)	11.1 ± 0.2 (5/5)
Bacillus subtilis	8.4 ± 0.3 (2/5)	(0/5)	(0/5)
Citrobacter freundii	5.7 ± 0.7 (2/5)	(0/5)	8.3 (1/5)
Escherichia coli	7.4 ± 1.0 (5/5)	(0/5)	8.7 ± 0.4[e] (5/5)
Klebsiella pneumoniae	7.5 ± 1.1 (2/5)	(0/5)	8.0 ± 0.5 (2/5)
Lactobacillus	5.4 ± 2.5 (5/5)	7.5 ± 2.1 (5/5)	8.2 ± 0.7[e] (5/5)
L. gasseri	5.8 ± 1.1 (2/5)	7.3 ± 2.4 (4/5)	8.2 ± 0.7[e] (5/5)
L. reuteri	4.1 ± 1.6 (2/5)	8.1 ± 0.2 (2/5)	7.5 ± 1.2 (3/5)
L. salivarius subsp. *salivarius*	3.5 (1/5)	(0/5)	(0/5)
Lactobacillus spp.[c]	4.8 (1/5)	(0/5)	7.2 (1/5)
Proteus mirabilis	(0/5)	(0/5)	7.6 (1/5)

Continued

TABLE 1—*Continued*

Organism	Bacterial counts at indicated time relative to moxalactam treatment (no. of subjects infected/no. of subjects tested)[a]		
	Before	During (day 7)	After
Pseudomonas aeruginosa	4.1 (1/5)	(0/5)	4.4 ± 0.6 (2/5)
Staphylococcus epidermidis	3.2 ± 0.5 (2/5)	(0/5)	3.8 ± 0.8 (2/5)
Streptococcus	6.1 ± 1.2 (5/5)	9.9 ± 0.4[c] (5/5)	9.1 ± 0.9[d] (5/5)
S. durans	6.8 ± 0.3 (2/5)	(0/5)	(0/5)
S. faecalis	5.9 ± 1.1 (5/5)	9.9 ± 0.4[c] (5/5)	9.0 ± 0.9[d] (5/5)
S. faecium	5.2 ± 0.6 (2/5)	9.1 ± 0.1[c] (2/5)	6.6 (1/5)
S. sanguis	7.0 (1/5)	(0/5)	(0/5)
Streptococcus spp.[e]	6.6 ± 0.8 (2/5)	(0/5)	8.1 (1/5)
Candida	3.3 ± 0.6 (4/5)	3.8 ± 0.9 (4/5)	4.2 ± 0.5 (3/5)
C. albicans	3.2 ± 0.6 (4/5)	3.8 ± 0.9 (4/5)	4.2 ± 0.5 (3/5)
Candida spp.[e]	2.5 ± 0.5 (3/5)	(0/5)	3.9 (1/5)
Total aerobes and yeasts	8.3 ± 0.6 (5/5)	10.0 ± 0.5[d] (5/5)	9.4 ± 0.6[c] (5/5)
Total bacteria	11.1 ± 0.2 (5/5)	10.0 ± 0.5[d] (5/5)	11.1 ± 0.1 (5/5)

[a] Moxalactam was administered at 4 g per day during the experimental periods.
[b] Bacterial counts were expressed as mean ± standard deviation of $\log_{10}$ per gram of wet feces.
[c] Some isolates could not be identified to the species level with currently accepted identification protocols and presently recognized species.
[d] Statistically significant at $P < 0.01$ when compared with the pretreatment values (Student's t test was used for the bacterial counts).
[e] $P < 0.001$.
[f] One isolate (one species).

After the drug treatment, the total counts in all subjects showed a recovery to pretreatment normal levels.

On day 7 of treatment, the incidence of *Bacteroides vulgatus*, *Bacteroides* spp., *Bifidobacterium adolescentis*, *Eubacterium aerofaciens*, and *Eubacterium* spp. was lower than that pretreatment (Table 1). In two of the subjects, however, the counts of *Clostridium* sp. were markedly increased. The *Clostridium* sp. were identified as *Clostridium innocuum* and *Clostridium ramosum*. On the other hand, the aerobic bacteria were significantly ($P < 0.001$) increased on day 7. The counts of *Streptococcus* spp. ($P < 0.001$) and *Lactobacillus* spp. were higher in this experimental period than in the pretreatment period. Significantly increased numbers of *Streptococcus faecalis* and *Streptococcus faecium* were observed on day 7. The counts of *Candida* spp. changed in all subjects. Especially, there were marked increases in *Candida* sp. in two subjects. On day 7 of treatment, the concentration of moxalactam in the feces was 110.4 ± 73.9 μg/g.

By 1 week after administration, the total aerobes ($P < 0.01$) and the counts of *Streptococcus* spp. ($P < 0.001$), enterobacteria ($P < 0.05$), *Lactobacillus* spp. ($P < 0.05$), *Clostridium* spp. ($P < 0.05$), and veillonellae had increased but had not recovered to the pretreatment levels. On the other hand, the counts of *Bacteroides* spp., *Eubacterium* spp., *Peptostreptococcus* spp., and *Bifidobacterium* spp. had recovered to normal levels. The numbers of *C. innocuum*, *Bacteroides* spp., *S. faecalis*, *Lactobacillus gasseri*, *Veillonella parvula*, and *Escherichia coli* were significantly higher on day 7 after treatment than pretreatment. The concentration of moxalactam in the feces decreased to undetectable levels (<1 μg/g).

The intravenous administration of moxalactam induces marked changes in the fecal flora, with suppression of strict anaerobic bacteria and enterobacteria. Similar changes were recently reported with moxalactam except for lactobacilli and *Pseudomonas* spp. (2). It has also been reported that the fecal microflora of patients given cefoperazone (1) or ceftriaxone (3) was dominated by members of the family *Streptococcaceae* and clostridia but not by obligate anaerobes and enterobacteria. Our results with intravenous administration of moxalactam were in close agreement with these reports.

In the present study, the significant reduction of strict anaerobes, particularly *Bacteroides fragilis* group, *Bifidobacterium* spp., *Eubacterium* spp., and *Peptostreptococcus* spp., in stool specimens during treatment was in close agreement with the report of Allen et al. (2). The increased numbers of *Streptococcus* spp. and *Lactobacillus* spp. in the fecal microflora during treatment may be due to the resistance of these microorganisms to moxalactam. It is of interest that two of our subjects had intestinal colonization with moxalactam-resistant *Clostridium* spp. during treatment. None of the *Clostridium* spp. isolated during this study were identified as *Clostridium difficile*.

A period of 7 days after moxalactam administration was not sufficient for the fecal flora to recover to the pretreatment levels. Normalization of aerobic bacteria and *Clostridium* spp. may require another week. However, the numbers and incidences of *B. fragilis* group, *Bifidobacterium adolescentis*, *Eubacterium aerofaciens*, and *Peptostreptococcus productus*, which were the predominant fecal bacteria before treatment, were normalized on day 7 after treatment. On the other hand, significantly increased numbers of *C. innocuum*, *V. parvula*, *S. faecalis*, *E. coli*, and *L. gasseri* were observed after the treatment. On day 12 after cefoperazone administration, a reduction in the number of bifidobacteria and an increase in the number of *Eubacterium* spp., *Clostridium* spp., *Lactobacillus* spp., and *Streptococcus* spp. were reported (5). Our results were closely related with regard to the increased number of *Clostridium* spp. and *Streptococcus* spp. These alterations after the treatment may be considered a common effect of the new β-lactam antibiotics on human fecal microflora.

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