EFFECT OF PARENTERAL CEFEPIME ON HUMAN FECAL FLORA

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Alteration of fecal flora was studied in six healthy male volunteers receiving cefepime (CFPM), a new cephalosporin, at a dose of one gram twice a day for five days. The treatment caused little change in fecal flora except pronounced decrease or elimination in *Escherichia coli* and *Lactobacillus* spp.; these organisms were normalized after cessation of cefepime administration. Overgrowth of *Candida* spp. and *Pseudomonas* spp. was not seen in all volunteers. A small number of *Clostridium difficile* were detected temporarily in only one of six volunteers, who developed no loose feces nor diarrhea.

Key words : Cefepime, Fecal flora, *Bacteroides fragilis* group, *Clostridium difficile*

Introduction

Cefepime (CFPM) is a new parenteral aminothiazol methoxyimino cephalosporin having the 3-side chain methyl substitution with N-methylpyrrolidine. It was synthesized at Bristol-Myers Research Institute, Tokyo, Japan. This compound has been bacteriologically characterised by strong activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* as well as against major members of the family Enterobacteriaceae[1~4]

It is known that administration of antimicrobial agents can eliminate or reduce normal intestinal flora. The alteration of flora may cause the overgrowth of *Clostridium difficile*, *S. aureus*, *P. aeruginosa*, and so on, the emergence of resistance in some bacteria in normal intestinal flora, and impairment of the resistance of the intestinal tract to colonization[5,6]. In recent years interest is growing in the investigation of the impact of newly developed antimicrobial agents on human intestinal flora, in keeping with the expansion of their antimicrobial activity. The present study examined the effect of CFPM on the fecal flora in human volunteers.

Materials and Methods

1. Human volunteers

Six fully informed healthy male volunteers, who had received no antimicrobial agents in the preceding three months, were enrolled in the study (Table 1). All subjects were given CFPM by drip infusion route at a dosage of 1.0g twice a day for 5 days.

2. Collection of specimens and culture technique

The methods used have been described elsewhere[7,8]. Fecal specimens were obtained from the volunteers before initiation of the drugs, on day 3 (during therapy), day 9 (after therapy), day 17 and one month after treatment began. Collected specimens were immediately transported at 4℃ in a sterile plastic container which was kept in anaeromate (Nissui Seiyaku). Fecal culture was done within eight hours after collection. A portion of each specimen was emulsified in prereduced anaerobic dilution buffer A[7] containing glass beads. Serial 10-fold dilutions were made for quantitative culture in the dilution buffer. Selected dilutions were placed onto different nonselective and selective media[7,8] as shown in Tables 2 (for anaerobes) and 3 (for aerobes). Only for *C. difficile* detection,

Table 1. Human volunteers studied

Volunteer	Age (y)	Sex	Weight (kg)	Height (cm)
A	25	male	52.5	167.0
B	28	male	62.0	170.0
C	35	male	67.7	168.0
D	23	male	66.0	185.7
E	21	male	62.4	171.0
F	32	male	71.0	176.0
Mean±SD	27.3±5.4		63.6±6.4	173.0±7.0

Table 2. Media used for anaerobic fecal flora

Medium	Incubation period (days)	purpose
Glucose-Blood-Liver (BL) agar	3–6	total count
Modified GAM agar	3–6	total count
Bacteroides Bile Esculin (BBE) agar	3	*Bacteroides fragilis* group
Modified FM agar	3	*Fusobacterium* spp.
Bifidobacteria-Selective (BS) agar	3	*Bifidobacterium* spp.
Rifampicin-Brucella Blood agar	3	lecithinase negative clostridia & *Eubacterium* spp.
CW Egg York agar	2	lecithinase positive clostridia
Cycloserine Cefoxitin Mannitol agar (CCMA)	2	*Clostridium difficile*

Table 3. Media used for aerobic fecal flora

Medium	Incubation period (days)	Purpose
Trypticase Soy Blood agar	3	total count
Staphylococcus No. 110 agar	2	*Staphylococcus* spp.
EF agar	2	*Enterococcus* spp.
Modified Drygalsky agar	2	Enterobacteriaceae
NAC agar	2	*Pseudomonas* spp.
Lactobacillus-Selective agar	3	*Lactobacillus* spp.
Candida GE agar	2	*Candida* spp.

dilution and inoculation of fecal specimens were processed anaerobically in an anaerobic chamber (Hirasawa) under 82% N_2, 10% CO_2 and 8% H_2. The following media were incubated in an anaerobic chamber : Glucose-Blood-Liver agar (BL agar, Nissui Seiyaku), Modified GAM agar (Nissui Seiyaku), Bacteroides Bile Esculin agar (BBE agar, Kyokutou Seiyaku), Modified FM agar (Nissui Seiyaku), Bifidobacteria-Selective agar (BS agar), Rifampicin-Brucella Blood agar, CW Egg Yolk agar (Nissui Seiyaku), Cycloserine Cefoxitin Mannitol agar (CCMA, Nissui Seiyaku), and Lactobacillus-Selective agar. Aerobically cultured media were Trypticase Soy II 5% Sheep Blood agar (BBL), BTB agar (Nissui Seiyaku), NAC agar (Eiken Kagaku), Staphylococcus No. 110 agar (Nissui Seiyaku), EF agar (Nissui Seiyaku), and Candida GE agar (Nissui Seiyaku). Incubation periods of media are shown in Tables 2 and 3. With these methods, the lowest detectable number of microorganisms is 2 $\log_{10}$ per gram of wet feces.

Each colony having a distinct morphology on the media were microscopically examined and counted after Gram stain. Representatives of each colony type were isolated for identification, which was performed by standard methods as previously described[9~11]. Identification of species of *Bacteroides fragilis* group and genus *Fusobacterium* were done by RAP ID/ANA (Amco). Species of Enterobacteriaceae and genus *Enterococcus* were determined by both Vitek system (GPI and GPC card, Amco) and API system (20E and Strept, Asuka Junyaku).

An aliquote of each fecal specimen was dried and the wet/dry feces weight ratio was determined as previously described[8]. Bacterial counts of microorganisms in feces were eventually expressed as the common logarithmic number of microorganims per

Table 4. Effect of five-day treatment with cefepime on anaerobic fecal microflora in six healthy volunteers

Organism	Log mean bacterial counts ± SD/g of dry feces (no. of pos. cases)				
	Before	3 days[a]	9 days	17 days	1 month
Total anaerobes	11.00±0.22 (6)	11.46±0.63 (6)	11.32±0.29 (6)	11.63±0.39 (6)	11.43±0.31 (6)
Bacteroides fragilis group	10.23±0.56 (6)	10.73±0.98 (6)	10.82±0.51 (6)	10.50±0.92 (6)	10.31±0.69 (6)
Fusobacterium spp.	8.10±1.16 (5)	7.32±1.13 (6)	8.47±0.89 (4)	7.94±1.25 (3)	7.34±0.66 (4)
Bifidobacterium spp.	10.02±0.46 (6)	10.31±0.81 (6)	9.82±0.79 (6)	10.25±0.45 (6)	10.57±0.45 (6)
Lec.[b] pos. clostridia	ND[c] (0)	ND (0)	3.48±0.40 (3)	4.07 (1)	4.69±1.53 (3)
Lec. neg. clostridia & *Eubacterium* spp.	8.77±0.54 (6)	8.52±0.76 (6)	9.10±1.13 (6)	8.17±0.76 (6)	8.01±0.99 (6)

[a] Days after beginning of treatment
[b] Lec.: lecithinase.
[c] ND: not detected (less than 2 $\log_{10}$/g of wet feces)

Table 5. Effect of five-day treatment with cefepime on aerobic fecal microflora in six healthy volunteers

Organism	Log mean bacterial counts ±SD/g of dry feces (no. of pos. cases)				
	Before	3 days[a]	9 days	17 days	1 month
Total aerobes	9.39±0.67 (6)	7.52±1.25 (6)	7.27±0.85 (6)	8.29±0.81 (6)	8.10±0.57 (6)
Staphylococcus spp.	4.06±0.54 (4)	3.57±0.11 (3)	3.48±0.46 (4)	3.16±0.68 (2)	3.67±0.41 (4)
Enterococcus spp.	6.76±0.86 (6)	6.19±1.62 (6)	5.43±1.57 (6)	5.74±1.68 (6)	5.28±1.17 (6)
Enterobacteriaceae	7.45±0.23 (6)	5.58±1.64 (4)	5.97±2.16 (6)	6.80±1.40 (6)	7.27±1.10 (6)
Pseudomonas spp.	ND[b] (6)	ND (0)	2.90 (1)	5.35 (1)	ND (0)
Lactobacillus spp.	9.39±0.67 (6)	7.52±1.25 (6)	6.38±1.75 (6)	6.67±2.06 (6)	7.52±2.23 (6)
Candida spp.	3.90±0.83 (4)	4.37±1.90 (2)	3.85±1.19 (3)	3.40±0.77 (4)	3.67±0.70 (3)

[a] Days after beginning of treatment
[b] ND: not detected (less than 2 $\log_{10}$/g of wet feces)

gram of dry feces.

3. *C. difficile* toxin detection

C. difficile D-1 toxin (toxin A) in fecal specimens was immunologically measured by using D-1 toxin latex kit (Shionogi).

Results

1. Impact on the fecal microflora (genus and group levels)

Tables 4 and 5 show mean total counts and counts of major genera and groups of anaerobes and aerobes, respectively, in six healthy volunteers before, during and after the administration of CFPM.

Mean total counts of anaerobic bacteria were not influenced by the 5-day administration of CFPM by drip infusion. Among anaerobic bacteria, counts of *B. fragilis* group, *Fusobacterium* spp., *Bifidobacterium* spp., lecithinase-negative clostridia and *Eubacterium* spp. did not change either during or after treatment. However, lecithinase-positive clostridia, which was not originally present in the fecal flora, was detected in small numbers in five volunteers after termination of treatment ; four volunteers had these clostridia temporarily and in only one case was this a constant finding.

The mean counts of total aerobic organisms, Enterobacteriaceae, and *Lactobacillus* spp. were decreased slightly by the administration of CFPM. One month after beginning of treatment, however, these organisms returned to levels similar to those found before treatment except *Lactobacillus* spp. counts of which was less than 5 $\log_{10}$ in two volunteers. *Pseudomonas* spp. which were not detected

Table 6. The effect of cefepime on *Bacteroides* spp. and *Fusobacterium* spp. in human feces

Organism	Bacterial counts[a]														
	Volunteer A					Volunteer B					Volunteer C				
	Before	3 days[b]	9 days	17 days	1 month	Before	3 days	9 days	17 days	1 month	Before	3 days	9 days	17 days	1 month
Bacteroides															
B. fragilis		9.03	9.97		10.30		9.48		11.05		10.14	9.12	10.78		8.99
B. vulgatus	10.96	9.51	10.66	9.54	10.78	9.99	9.48	9.32	11.15	11.17	9.43	9.59		8.97	9.29
B. distasonis			10.27	8.76				9.02					10.18	8.67	8.99
B. ovatus															
B. thetaiotaomicron						9.99	9.78	9.80				9.42	10.18	8.97	
B. uniformis		8.73	9.97	9.24			10.08	9.02	10.45	10.57	9.74	9.59	10.78	9.27	9.68
Fusobacterium															
F. mortiferm	9.29	8.24	8.70	6.50	6.80										
F. varium	9.11			6.32	6.62	7.22	6.85	9.52	9.22	8.21					
Organism	Volunteer D					Volunteer E					Volunteer F				
	Before	3 days	9 days	17 days	1 month	Before	3 days	9 days	17 days	1 month	Before	3 days	9 days	17 days	1 month
Bacteroides															
B. fragilis	10.43	11.68	10.62	10.80	9.94	8.92	11.73	10.53	9.53	8.88		9.43			
B. vulgatus			10.02	10.02	10.06						8.85	9.73	9.74	10.87	9.39
B. distasonis		11.20	10.32			8.92		10.53		9.06	9.15	9.43	9.74	10.57	
B. ovatus				10.02											
B. thetaiotaomicron	10.13	10.90	10.32	10.02	9.46	8.45	11.04				9.45	9.90	9.44	11.04	8.99
B. uniformis		10.90	10.02		9.76	8.75	11.51	11.23	8.75	9.28	9.15	9.73	10.04	11.04	9.17
Fusobacterium															
F. mortiferm	8.20	6.68	8.28	7.89	7.46		6.39	7.39		6.68	8.90	9.18			

[a] Log_{10} per gram of dry feces
[b] Days after beginning of treatment

before treatment, were found in small numbers in two volunteers on one occasion only after termination of therapy. No volunteer had an increase in counts of *Candida* spp.

2. Effect of CFPM on *Bacteroides* spp. and *Fusobacterium* spp. (species levels)

After commencement of treatment, in five of six volunteers some species of *B. fragilis* group organisms appeared which had not been detected in fecal culture before treatment (Table 6). After discontinuation of treatment, these species still persisted in three of the five volunteers. The species of *B. fragilis* group organisms detected before treatment were not present in three of six volunteers one month after the CFPM administration.

Fusobacterium mortiferm, isolated in three of six volunteers before treatment, disappeared in only one volunteer after treatment (Table 6). CFPM had little effect on fecal *Fusobacterium varium*.

3. Effect of CFPM on fecal Enterobacteriaceae and *Enterococcus* spp. (species level)

Escherichia coli, which was the sole species isolated from all six volunteers before CFPM administration, apparently decreased by counts of 3 log_{10} or more in four of six volunteers (Table 7). *Citrobacter freundii*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter cloacae*, *Enterobacter taylorae* and *Hafnia alvei* were detected as transient organisms after treatment.

Enterococcus faecium was a predominant fecal

Table 7. The effect of cefepime on *Enterobacteriaceae* and *Enterococcus* spp. in human feces

Organism	Bacterial counts[a]														
	Volunteer A					Volunteer B					Volunteer C				
	Before	3 days[b]	9 days	17 days	1 month	Before	3 days	9 days	17 days	1 month	Before	3 days	9 days	17 days	1 month
Escherichia															
E. coli	7.23	4.26	7.17	5.14	6.12	7.84		7.87	9.15	8.52	7.21	7.67	6.60	6.79	5.72
Citrobacter															
C. freundii								7.87					6.60		
Klebsiella															
K. pneumoniae								7.27					7.30	5.84	
K. oxytoca								7.27							
Enterobacter															
E. cloacae													7.08		
E. taylorae									8.25						
Hafnia															
H. alvei										7.57					
Enterococcus															
E. faecalis	4.71		5.58										5.35		
E. faecium	4.89	3.33	5.76	3.50	4.76			7.60			7.48	5.96		4.58	7.19
E. avium							7.64	7.42	8.15	5.95					
E. durans					4.58	7.06									

Organism	Volunteer D					Volunteer E					Volunteer F				
	Before	3 days	9 days	17 days	1 month	Before	3 days	9 days	17 days	1 month	Before	3 days	9 days	17 days	1 month
Escherichia															
E. coli	7.55		3.90	5.67	7.71	7.61	6.10	6.00	6.64	7.19	7.43	4.27	2.87	7.17	8.07
Citrobacter															
C. freundii				4.72											
Klebsiella															
K. pneumoniae									6.10	6.82					
Enterococcus															
E. faecalis			5.68					3.51		3.06	6.32		3.35		
E. faecium	7.33				5.07	6.09	7.87	3.81	7.08	3.66	6.62	6.17		5.38	4.72
E. avium		6.16	5.68					3.51							
E. durans						6.69									

[a] Log_{10} per gram of dry feces
[b] Days after beginning of treatment

species in *Enterococcus* spp. before, during and after treatment and was not affected by CFPM administration (Table 7). Other *Enterococcus* species were unstable fecal organisms.

4. *C. difficile* and its toxin in fecal specimens

C. difficile was isolated in only one volunteer with low counts on days 9 and 17 after treatment (Table 8). No loose feces or diarrhea was noted in this volunteer. Its toxin was not detected from any fecal specimens.

Table 8. Isolation of *Clostridium difficile* and detection of its D-1 toxin in feces of the healthy volunteers administrated cefepime for 5 days

Volunteer		Isolation of *C. difficile* and detection of D-1 toxin				
		Before	3 days[a]	9 days	17 days	1 month
A	toxin	–	–	–	–	–
	isolation	–[b]	–	–	–	–
B	toxin	–	–	–	–	–
	isolation	–	–	–	–	–
C	toxin	–	–	–	–	–
	isolation	–	–	–	–	–
D	toxin	–	–	–	–	–
	isolation	–	–	–	–	–
E	toxin	–	–	–	–	–
	isolation	–	–	4.70[c]	3.18	–
F	toxin	–	–	–	–	–
	isolation	–	–	–	–	–

[a] Days after beginning of treatment
[b] Less than 2 $\log_{10}$/g of wet feces
[c] Logarithmic bacterial counts/g of dry feces

Discussion

In this study, CFPM was found to have no profound effect after 5 days administration on fecal anaerobes and aerobes except *E. coli* and *Lactobacillus* spp., counts of which were normalized after termination of the CFPM administration. Overgrowth of *C. difficile* was noted in only one volunteer; its counts were low and no toxin of *C. difficile* was detected in fecal specimens. The increase or overgrowth of *Candida* spp. and *Pseudomonas* spp. was not observed.

Antimicrobial activity of CFPM covers a variety of aerobes of the families Enterobacteriaceae, *Staphylococcus* spp. and *Pseudomonas* spp.[1~4]. However, this compound has little activity against the anaerobes, *B. fragilis* and *C. difficile*[2]. A pharmacokinetic study showed that i.v. CFPM was excreted in urine with a high recovery rate[2]. The present results on the impact of CFPM on human fecal microflora could be related to these characteristics of the compound.

The present results with CFPM can be favorably compared to those of cefotaxime, an aminothiazolyl methoxyimino cephalosporin, studied in children[12]; the latter compound caused a profound decrease or elimination of *E. coli* but had no significant effect on other aerobes and anaerobes. Ceftazidime treatment was noted to produce significant decrease in counts of members of the family Enterobacteriaceae[13,14] but not to effect *Enterococcus* spp.[13]; also 41% of anaerobes were eradicated by the administration of the compound[13]. In contrast to these cephalosporins, cefoperazone[15~17], moxalactam[18] and ceftriaxone[13] have pronounced effects on fecal anaerobic and aerobic microflora.

In conclusion, CFPM had less effect on fecal microflora than other cephalosporins and caused little colonization and superinfection by *Candida* spp. *Pseudomonas* spp. and *C. difficile*.

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注射剤 Cefepime の人腸内細菌叢に及ぼす影響

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新規セファロスポリン系抗生剤 cefepime (CFPM) 1 g を 1 日 2 回投与された 6 名健康男子の糞便内フローラの変化を検討した。本剤の投与は *Escherichia coli* と *Lactobacillus* spp. の顕著な減少もしくは消失を除けば，糞便内フローラにほとんど変化をもたらさなかった。減少もしくは消失したこれらの細菌は，CFPM の投与終了後には正常化した。*Candida* spp. や *Pseudomonas* spp. の異常増殖はすべての症例で認められなかった。少量の *Clostridium difficile* が 6 名中 1 名において一過性に検出されたが，この症例に軟便や下痢はみられなかった。

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