ANTIBACTERIAL ACTIVITY OF CEFMINOX AGAINST ANAEROBES

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The antibacterial activity of cefminox (CMNX) against anaerobic bacteria was studied *in vitro*. The results are as follows: 1. CMNX exerted antibacterial activity against a wide range of anaerobes, excluding *Clostridium innocuum*. The antibacterial activity of CMNX against *Bacteroides fragilis* was comparable to that of latamoxef and superior to cefoxitin, but CMNX's activity against anaerobic cocci was slightly inferior to cefoxitin's; 2. A comparison of the MICs and MBCs of CMNX indicated that this drug exerts a complete bactericidal effect at a concentration which inhibits the growth of bacteria; 3. CMNX was found to be stable to the β-lactamases produced by *B. fragilis*; 4. CMNX exerted an antibacterial activity against *C. difficile*.

Cefminox (CMNX) is a new cephamycin antibiotic. It has been known that this agent shows antibacterial activity against various aerobic Gram-positive and Gram-negative bacteria. Its activity is especially higher than conventional cephamycins against such Gram-negative bacteria as *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus* and *Serratia marcescens*. Moreover, CMNX is as stable as conventional cephamycins to the actions of the β-lactamases produced by these bacteria.[1,2] However, with regard to the antibacterial activity of CMNX against anaerobic bacteria, only *Bacteroides fragilis* has been studied as a test anaerobe.

This paper reports the results of studies on the antibacterial activity of CMNX against anaerobic bacteria in comparison with latamoxef, cefoxitin, cefmenoxime and ceftizoxime, and also on the stabilities of these antibiotics to the actions of β-lactamases produced by *B. fragilis*.

Materials and Methods

Bacterial Strains

Gram-positive and Gram-negative bacteria maintained in the Institution's culture collection and those isolated from clinical specimens were employed in the present study.

Tested Antibiotics

Cefminox (CMNX; 772 μg/mg; Meiji Seika Kaisha, Ltd.). Cefoxitin (CFX; 936.5 μg/mg; Nippon Merck-Banyu Co., Ltd.). Cefmenoxime (CMX; 958 μg/mg; Takeda Pharm. Co., Ltd.). Ceftizoxime (CZX; 863 μg/mg; Fujisawa Pharm. Co., Ltd.). Latamoxef (LMOX; 924 μg/mg; Shionogi Pharm. Co., Ltd.).

Susceptibility Test Method

The susceptibility of the anaerobes to the drugs was determined according to the method established by the Investigation Committee of MIC Determination for Anaerobes, Japan Society of Chemotherapy.[3] The MIC determination plate medium was prepared using GAM agar (Nissui), to which menadione was added at 10 μg/ml. Menadione was added at 0.1 μg/ml to GAM bouillon (Nissui), and this liquid medium was used for overnight culture of the anaerobes. Anaerobic culturing was carried out in an anaerobic glove box (CO_2 10%; H_2 10%; N_2 80%).

Determination of Minimum Bactericidal Concentration (MBC)

Anaerobes were cultured in the GAM liquid medium at 37°C for 24 hours. The cultures were then diluted and inoculated at a final cell concentration of $10^4 \sim 10^5$ cfu/ml to the fresh GAM liquid medium containing a test antibiotic at various concentrations. After culturing at 37°C for 24 hours, the MIC was determined. One loopful of each culture (*ca.* 10 μl) was spread onto GAM plates containing no antibiotics, and the plates were incubated at 37°C for 24 hours. The MBC was defined as the lowest antibiotic concentration in the GAM liquid medium in which a test anaerobe had been cultured and showed no growth on the antibiotic-free GAM plate.

Stability to β-Lactamase

Preparation of Crude Enzyme Solution: Five strains of *B. fragilis* were cultured in GAM bouillon, and the log-phase cells (6-hour culture) were collected. The cells were then sonicated and centrifuged (at 15,000 rpm for 60 minutes), and the resulting supernatants were used as crude enzyme solutions.

Antibiotics Tested: The following antibiotics were compared for stability to the actions of the above β-lactamases. Cefminox, cefazolin (CEZ), cefpiramide (CPM), cefoperazone (CPZ), ceftazidime (CAZ), cefotaxime (CTX) and cefoxitin.

Determination of β-Lactamase Activity: Macroiodometry[4] was employed.

Results

Antibacterial Spectrum

Tables 1 and 2 present the results of studies on the anaerobic antibacterial spectrum of CMNX in comparison with LMOX, CMX, CZX and CFX.

CMNX inhibited the growth of all 50 reference strains of 35 species — except for the one strain of *Clostridium innocuum* ATCC 14501 — at a concentration of 6.25 μg/ml at a starting cell density of 10^6 cfu/ml, and at 12.5 μg/ml at 10^8 cfu/ml. CMNX was thus found to have a broad antibacterial spectrum covering a wide range of anaerobes.

When comparison was made of the antibacterial activities of CMNX and the other antibiotics, CMNX's activity against *B. fragilis* strains was inferior to LMOX but superior to CFX. However, against all other strains, CMNX's antibacterial activity was superior to the activities of LMOX and CFX.

Distribution of Susceptibility of Clinical Isolates to Antibiotics

The distributions of the susceptibility of the clinical isolates to CMNX and the other control antibiotics are compiled in Tables 3 ~ 11.

The MIC values of CMNX for 27 strains of *B. fragilis* ranged from 0.78 to 3.13 μg/ml at a starting cell density of both 10^6 and 10^8 cfu/ml; the MIC distribution peak occurred at 0.78 μg/ml. When the susceptibility of *B. fragilis* strains to CMNX was compared to their susceptibility to the other antibiotics, the order was LMOX>CMNX>CZX>CMX>CFX at 10^6 cfu/ml, while it was LMOX>CMNX>CFX>CMX>CZX at 10^8 cfu/ml.

The MIC values of CMNX for 9 strains of *B. melaninogenicus* ranged — except for one strain — from 0.10 to 0.78 μg/ml when a starting cell density of 10^6 cfu/ml was employed, while it ranged — except for one strain — from 0.10 to 1.56 μg/ml when 10^8 cfu/ml was employed. When a comparison was made among the antibiotics with regard to the susceptibility of *B. melaninogenicus*, the order was CZX>LMOX>CMX>CMNX=CFX at 10^6 cfu/ml, while it was CZX>CMX>CMNX=LMOX=CFX at 10^8 cfu/ml.

Table 1. The activity of CMNX and other cephem antibiotics against reference strains of anaerobes. 1.

Organism	CMNX		LMOX		CMX		CZX		CFX	
	10^6*	10^8*	10^6	10^8	10^6	10^8	10^6	10^8	10^6	10^8
P. magnus ATCC 29328	0.05	0.78	0.39	3.13	≤0.025	0.78	0.20	6.25	0.10	0.39
P. asaccharolyticus WAL 3218	0.20	0.39	0.20	0.20	0.05	0.20	0.10	0.10	0.20	0.20
P. anaerobius ATCC 27337	0.20	3.13	1.56	3.13	≤0.025	0.39	0.20	0.20	0.78	0.78
S. parvulus VPI 0546	0.20	0.20	3.13	6.25	≤0.025	0.39	0.20	0.39	1.56	1.56
S. intermedius ATCC 27335	6.25	12.5	12.5	12.5	0.05	0.10	0.39	0.39	6.25	6.25
S. constellatus ATCC 27823	6.25	12.5	12.5	25	≤0.025	0.20	0.78	1.56	6.25	6.25
S. mutans ATCC 25175	3.13	6.25	3.13	3.13	≤0.025	0.05	0.05	0.10	1.56	1.56
E. limosum ATCC 8486	0.20	0.20	6.25	6.25	≤0.025	0.05	3.13	6.25	0.78	0.78
E. cylindroides ATCC 27803	≤0.025	50	6.25	6.25	≤0.025	1.56	0.78	1.56	0.20	6.25
E. plauti VPI 0310	0.20	6.25	0.78	6.25	≤0.025	1.56	3.13	6.25	1.56	6.25
E. plauti VPI 0311	6.25	12.5	1.56	6.25	1.56	12.5	6.25	>100	6.25	12.5
P. acnes* ATCC 11827	≤0.025	0.39	≤0.025	0.39	≤0.025	0.05	≤0.025	0.05	≤0.025	0.10
P. acnes ATCC 11828	≤0.025	0.78	≤0.025	3.13	≤0.025	0.20	≤0.025	0.20	≤0.025	0.39
C. sordellii ATCC 9714	0.78	0.78	1.56	1.56	0.20	0.20	0.10	0.10	0.39	0.39
C. perfringens WAL 3503	0.10	1.56	0.05	3.13	0.78	3.13	0.05	3.13	0.78	1.56
C. perfringens ATCC 3624	0.05	25	≤0.025	100	≤0.025	100	≤0.025	50	0.39	6.25
C. sporogenes ATCC 3584	0.20	0.78	0.78	3.13	1.56	6.25	50	>100	0.39	0.39
C. histolyticum ATCC 19401	0.20	0.20	0.39	0.78	3.13	3.13	25	25	0.78	0.78
C. novyi ATCC 19402	0.10	0.10	0.20	0.78	0.10	0.10	0.10	0.20	0.10	0.10
C. ramosum ATCC 25582	1.56	3.13	6.25	6.25	0.20	0.20	6.25	6.25	3.13	6.25
C. tertium ATCC 19405	0.78	0.78	3.13	3.13	3.13	12.5	100	>100	0.78	0.78
C. innocuum ATCC 14501	>100	>100	100	>100	6.25	12.5	12.5	25	50	50
C. clostridiiforme ATCC 25537	≤0.025	0.39	≤0.025	1.56	≤0.025	1.56	1.56	6.25	1.56	6.25

* Inoculum; cfu/ml.

Abbreviation: *P*, *Peptostreptococcus*; *S*, *Streptococcus*; *E*, *Eubacterium*; *P**, *Propionibacterium*; *C*, *Clostridium*.

Table 2. The activity of CMNX and other cephem antibiotics against reference strains of anaerobes. 2.

Organism	CMNX		LMOX		CMX		CZX		CFX	
	10^6	10^8	10^6	10^8	10^6	10^8	10^6	10^8	10^6	10^8
B. fragilis ATCC 25285	0.78	0.78	0.39	0.78	3.13	3.13	0.78	6.25	3.13	6.25
B. fragilis GM 7000	0.78	0.78	0.39	0.78	1.56	3.13	3.13	6.25	3.13	6.25
B. vulgatus ATCC 29327	0.39	0.39	0.10	0.20	0.78	3.13	≤0.025	0.78	0.78	1.56
B. vulgatus ATCC 8482	1.56	3.13	1.56	3.13	6.25	50	0.20	6.25	6.25	6.25
B. distasonis GM 7007	1.56	1.56	0.78	6.25	3.13	12.5	0.78	6.25	12.5	12.5
B. distasonis ATCC 8503	0.20	0.39	1.56	1.56	0.20	0.78	0.10	0.39	0.78	1.56
B. thetaiotaomicron ATCC 29741	3.13	3.13	3.13	6.25	12.5	50	1.56	6.25	6.25	12.5
B. thetaiotaomicron WAL 2926	3.13	6.25	3.13	6.25	25	50	1.56	12.5	12.5	25
B. thetaiotaomicron WAL 3304	3.13	6.25	3.13	6.25	25	100	3.13	50	6.25	25
B. ovatus ATCC 8483	6.25	12.5	6.25	50	25	100	6.25	25	6.25	25
B. uniformis ATCC 8492	0.39	0.39	0.05	0.78	3.13	6.25	3.13	6.25	0.78	0.78
B. capillosus ATCC 29799	≤0.025	3.13	≤0.025	3.13	≤0.025	3.13	≤0.025	6.25	3.13	12.5
B. asaccharolyticus ATCC 25260	0.10	0.20	0.20	0.39	≤0.025	0.20	≤0.025	0.78	0.20	0.39
B. asaccharolyticus GAI 0415	≤0.025	0.10	≤0.025	0.20	≤0.025	0.05	≤0.025	0.05	≤0.025	0.20
B. asaccharolyticus GAI 0414	0.39	0.39	≤0.025	0.78	≤0.025	0.10	≤0.025	0.20	0.20	0.39
B. melaninogenicus JKI 8	0.39	0.39	0.39	0.39	0.05	0.10	≤0.025	0.10	0.20	0.39
B. melaninogenicus GAI 0411	0.39	1.56	1.56	1.56	≤0.025	1.56	0.39	3.13	1.56	3.13
F. nucleatum ATCC 10953	0.10	0.10	0.39	0.39	0.10	0.20	0.20	0.39	0.20	0.20
F. nucleatum F 1	0.10	0.10	0.39	1.56	0.05	0.10	≤0.025	0.39	0.10	0.39
F. nucleatum Fev. 1	0.10	0.10	0.39	0.39	≤0.025	0.10	0.10	0.39	0.05	0.20
F. nucleatum ATCC 25586	0.10	0.78	0.39	0.78	≤0.025	0.39	0.05	0.39	0.20	0.39
F. varium ATCC 8501	0.78	1.56	6.25	12.5	3.13	12.5	3.13	25	3.13	6.25
F. mortiferum VPI 4249	0.78	1.56	3.13	6.25	0.39	100	1.56	100	1.56	6.25
F. mortiferum VPI 5696	0.20	0.39	0.78	1.56	0.39	50	12.5	>100	0.78	1.56
F. naviforme VPI 4877	≤0.025	0.10	0.10	0.20	≤0.025	≤0.025	≤0.025	≤0.025	≤0.025	0.05
F. gonidiaformans VPI 0482A	≤0.025	0.10	≤0.025	0.20	≤0.025	≤0.025	≤0.025	≤0.025	≤0.025	0.10
V. parvula ATCC 10790	0.20	0.39	0.78	3.13	0.39	0.39	0.20	0.20	0.20	0.39

Inoculum; cfu/ml.

Abbreviation: *B, Bacteroides; F, Fusobacterium; V, Veillonella.*

Table 3. MIC distribution of CMNX and other cephem antibiotics to 27 isolates of *Bacteroides fragilis*.

	Is*	MIC (μg/ml)													
		0.025	0.05	0.10	0.20	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	>100
CMNX	10^6						16	8	3						
	10^8						16	7	4						
LMOX	10^6				1	7	14		5						
	10^8					3	16	3		5					
CFX	10^6								1	17	9				
	10^8								1	13	12	1			
CZX	10^6					5	11	6			5				
	10^8								2	4	2	7	7	1	4
CMX	10^6							4	18			1	4		
	10^8									2	3	12	5		5

* Is: Inoculum size (cfu/ml).

Table 4. MIC distribution of CMNX and the other cephem antibiotics to 9 isolates of *Bacteroides melaninogenicus*.

	Is*	MIC (μg/ml)													
		0.025	0.05	0.10	0.20	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	>100
CMNX	10^6			3	3	1	1				1				
	10^8			3	1	3		1				1			
LMOX	10^6		6	1	1				1						
	10^8			4	2	1		1		1					
CFX	10^6		2		4	1	1	1							
	10^8			1	1	5		1	1						
CZX	10^6	6	2				1								
	10^8	4	2		2				1						
CMX	10^6	6	1	1					1						
	10^8	5		1	1	1					1				

* Is: Inoculum size (cfu/ml).

Table 5. MIC distribution of CMNX and other cephem antibiotics to 14 isolates of *Veillonella parvula*.

	Is*	MIC (μg/ml)													
		0.025	0.05	0.10	0.20	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	>100
CMNX	10^6			1	5	5		2	1						
	10^5				3	6	1	2	2						
LMOX	10^6			1			2	2	4	2	2	1			
	10^5				1			2	2	5	3		1		
CFX	10^6			1	2	2	5	2	1	1					
	10^5			1		3	2	5	1	2					
CZX	10^6		1		3	1		7	2						
	10^5			1	2		1	7	3						
CMX	10^6			1	2	3	5	1	2						
	10^5				1	3	2	6	1	1					

* Is: Inoculum size (cfu/ml).

Table 6. MIC distribution of CMNX and other cephem antibiotics to 10 isolates of *Peptostreptococcus magnus*.

	Is*	MIC (μg/ml)													
		0.025	0.05	0.10	0.20	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	>100
CMNX	10^6				1		7	2							
	10^5						2	7	1						
LMOX	10^6				1	2	6	1							
	10^5						5	5							
CFX	10^6			1	9										
	10^5				5	5									
CZX	10^6		1		1	3	4	1							
	10^5						1	3	3	3					
CMX	10^6				1		4	5							
	10^5						1	4	5						

* Is: Inoculum size (cfu/ml).

Table 7. MIC distribution of CMNX and other cephem antibiotics to 12 isolates of *Peptostreptococcus asaccharolyticus*.

	Is*	MIC (μg/ml)													
		0.025	0.05	0.10	0.20	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	>100
CMNX	10^6		1	5	6										
	10^8			1	11										
LMOX	10^6		7	4			1								
	10^8		2	8		1		1							
CFX	10^6	11	1												
	10^8	4	7			1									
CZX	10^6	11				1									
	10^8	11					1								
CMX	10^6	1	7	4											
	10^8		1	11											

* Is: Inoculum size (cfu/ml).

Table 8. MIC distribution of CMNX and other cephem antibiotics to 8 isolates of *Peptostreptococcus anaerobius*.

	Is*	MIC (μg/ml)													
		0.025	0.05	0.10	0.20	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	>100
CMNX	10^6						1	4	3						
	10^8							5	3						
LMOX	10^6							1	6	1					
	10^8							1	6	1					
CFX	10^6				1	6	1								
	10^8				1	6	1								
CZX	10^6		4	3			1								
	10^8		2	5			1								
CMX	10^6				6	1	1								
	10^8				5	2	1								

* Is: Inoculum size (cfu/ml).

Table 9. MIC distribution of CMNX and other cephem antibiotics to 7 isolates of *Peptostreptococcus prevotii*.

	Is*	MIC (μg/ml)													
		0.025	0.05	0.10	0.20	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	>100
CMNX	10^6	1	4		2										
	10^8		1	3	1	2									
LMOX	10^6	1	2	1	1	2									
	10^8		1	1	1	1	3								
CFX	10^6	3	2	2											
	10^8		4	3											
CZX	10^6	4	1		1	1									
	10^8	2	1	1	1		2								
CMX	10^6	4			2	1									
	10^8	1	2	2		2									

* Is: Inoculum size (cfu/ml).

Table 10. MIC distribution of CMNX and other cephem antibiotics to 16 isolates of *Clostridium difficile*.

	Is*	MIC (μg/ml)													
		0.025	0.05	0.10	0.20	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	>100
CMNX	10^6								16						
	10^8								16						
LMOX	10^6												6	10	
	10^8													16	
CFX	10^6											1	4	11	
	10^8												1	15	
CZX	10^6												1		15
	10^8														16
CMX	10^6									1		11	4		
	10^8											1	15		

* Is: Inoculum size (cfu/ml).

Table 11. MIC distribution of CMNX and other cephem antibiotics to 14 isolates of *Clostridium perfringens*.

	Is*	MIC (μg/ml)												
		0.025	0.05	0.10	0.20	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	>100
CMNX	10^6			3	2	1	4	2	2						
	10^8				1	2	1	1	5			1	1		2
LMOX	10^6		1	1	6	2	1	3							
	10^8			1	1	2	1	3	2				2		2
CFX	10^6					4	3	4	1		2				
	10^8						3	3	2	4			1		1
CZX	10^6		1	1	2	2	1	1	4						2
	10^8				1	1	2	1	4	1	1				3
CMX	10^6	3		1		1	4	2	3						
	10^8			1		1	2	2	2	3					3

* Is: Inoculum size (cfu/ml).

In the case of 14 strains of *Veillonella parvula*, the MIC values of CMNX were 0.10~3.13 μg/ml at 10^6 cfu/ml and 0.20~3.13 μg/ml at 10^8 cfu/ml. The order of the susceptibility to CMNX and the other antibiotics was CMX>CZX>CMNX=CFX>LMOX at 10^6 cfu/ml and CMNX=CMX>CZX=CFX>LMOX at 10^8 cfu/ml.

The MIC values of CMNX for 10 strains of *Peptostreptococcus magnus* ranged from 0.20 to 1.56 μg/ml at 10^6 cfu/ml and from 0.78 to 3.13 μg/ml at 10^8 cfu/ml. The order of the susceptibility was CFX>LMOX=CZX>CMNX=CMX at 10^6 cfu/ml, while it was CFX>CMNX=LMOX>CMX>CZX at 10^8 cfu/ml.

The MIC ranges of CMNX for 12 strains of *P. asaccharolyticus* were 0.05~0.20 μg/ml at 10^6 cfu/ml and 0.10~0.20 μg/ml at 10^8 cfu/ml. The order of the susceptibility was CFX≒CZX>LMOX≒CMX>CMNX at 10^6 cfu/ml and CZX>CFX>CMX>CMNX>LMOX at 10^8 cfu/ml.

The MIC ranges of CMNX for 8 strains of *P. anaerobius* were 0.78~3.13 μg/ml at 10^6 cfu/ml and 1.56~3.13 μg/ml at 10^8 cfu/ml. The order of the susceptibility was CZX>CMX>CFX>CMNX>LMOX at 10^6 cfu/ml and CZX>CMX≒CFX>CMNX>LMOX at 10^8 cfu/ml.

The MIC ranges of CMNX for 7 strains of *P. prevotii* were ≤0.025~0.20 μg/ml at 10^6 cfu/ml and 0.05~0.39 μg/ml at 10^8 cfu/ml. The order of susceptibility was CFX>CMNX>CZX=CMX=LMOX at 10^6 cfu/ml and CFX>CMX=CMNX>CZX=LMOX at 10^8 cfu/ml.

The MICs of CMNX for all 16 strains of *C. difficile* were 3.13 μg/ml at both 10^6 and 10^8 cfu/ml. Also, the order of the susceptibility was the same at both 10^6 and 10^8 cfu/ml, that is, CMNX>CMX>LMOX=CFX>CZX.

In the case of 14 strains of *C. perfringens*, the MIC values of CMNX ranged from 0.10 to 3.13 μg/ml at 10^6 cfu/ml and from 0.20 to 100 μg/ml at 10^8 cfu/ml. The order of the susceptibility was LMOX>CMNX=CMX>CFX>CZX at 10^6 cfu/ml, while it was CFX>CMNX=LMOX=CZX=CMX at 10^8 cfu/ml.

Comparison of MIC and MBC for Various Anaerobes

Table 12 compares the MIC and MBC values of CMNX, LMOX and CMX for 11 strains of 9 anaerobic species.

In the case of CMNX, the MIC values were exactly the same as the corresponding MBC values for 10 of the tested strains (excluding *Fusobacterium mortiferum* VPI 4249). On the other hand, in

Table 12. Correlations between MICs and MBCs of CMNX and other cephem antibiotics (μg/ml).

Organism	CMNX		LMOX		CMX	
	MIC	MBC	MIC	MBC	MIC	MBC
B. fragilis ATCC 25285	0.78	0.78	0.39	0.78	3.13	6.25
B. fragilis GM 7000	0.78	0.78	0.39	0.39	3.13	3.13
B. fragilis GAI 0511	0.78	0.78	0.39	0.78	12.5	25
B. thetaiotaomicron ATCC 29741	3.13	3.13	3.13	3.13	12.5	50
B. vulgatus ATCC 29327	0.39	0.39	0.20	0.20	1.56	3.13
B. distasonis ATCC 8503	0.20	0.20	1.56	1.56	0.20	0.20
F. varium ATCC 8501	1.56	1.56	6.25	12.5	3.13	6.25
F. mortiferum VPI 4249	0.78	1.56	3.13	6.25	3.13	25
C. sordellii ATCC 9714	0.78	0.78	1.56	3.13	0.20	0.20
P. magnus ATCC 29328	0.39	0.39	0.39	0.39	0.39	0.39
S. intermedius ATCC 27335	6.25	6.25	6.25	12.5	0.20	0.20

Table 13. Stability of CMNX and other cephem antibiotics to β-lactamases from *Bacteroides fragilis* isolates.

Source of enzyme	Substrate profile (relative V_{max})						
	CMNX	CEZ	CPM	CPZ	CAZ	CTX	CFX
B. fragilis GAI 0763	<1	100	34	35	23	8	<1
B. fragilis GAI 0511	<1	100	44	25	32	18	<1
B. fragilis GAI 0556	<1	100	54	32	22	11	<1
B. fragilis GAI 0548	<1	100	65	43	24	13	<1
B. fragilis GAI 0830	<1	100	38	24	15	7	<1

the case of LMOX and CMX, the MBC values were 2 or more times larger than the corresponding MIC values in 6 of the 11 strains.

Stability of CMNX to β-Lactamase of *B. fragilis*

The stability of CMNX to the β-lactamases obtained from 5 strains of *B. fragilis* was compared with the stabilities of CEZ, CPM, CPZ, CAZ, CTX and CFX. The rate of hydrolysis of CEZ by each β-lactamase preparation was defined as 100. The results are presented in Table 13.

As was the case with CFX, CMNX underwent almost no hydrolysis by the β-lactamase preparations obtained from *B. fragilis*.

Discussion

Cefminox (CMNX), a newly-developed antibiotic of the cephamycin group, was studied *in vitro* for its antibacterial activity against anaerobes in comparison with CFX, LMOX, CZX and CMX.

The antibacterial activity of CMNX against *B. fragilis*, which is frequently isolated from clinical specimens, was somewhat inferior to that of LMOX but superior to CFX. Especially, in comparison with the fact that CFX demonstrated lower MICs than CZX and CMX at a starting cell density of 10^8 cfu/ml but higher MICs than CZX at 10^6 cfu/ml, CMNX demonstrated lower MICs than CZX and CMX at both 10^6 and 10^8 cfu/ml.

The antibacterial activity of CMNX against *C. difficile*, which is known as a causative bacterium of diarrhoea and of pseudomembranous colitis occurring in connection with chemotherapy, was better than the activity of any of the other tested antibiotics, and an MIC of 3.13 μg/ml was obtained for each of the tested strains. Compared with CFX, CMNX's antibacterial activity tended to be better against *B. fragilis* and *C. difficile*, but poorer against anaerobic cocci (*P. magnus, P. asaccharolyticus, P. anaerobius* and *P. prevotii*).

Compared with LMOX, the antibacterial activity of CMNX tended to be inferior against *B. fragilis, B. melaninogenicus, P. magnus* and *P. asaccharolyticus*, but superior against *V. parvula, P. anaerobius* and *P. prevotii*.

The MIC values of CMNX for the anaerobes were exactly the same as its MBC values in 10 of the 11 strains tested, and thus CMNX exerted a complete bactericidal effect at its minimum inhibitory concentrations.

In addition, CMNX as well as CFX was found to be very stable to the action of the β-lactamases produced by *B. fragilis*.

On the basis of the above results, CMNX can be considered to have a high potential as a therapeutic agent for the treatment of anaerobic infections, especially those in which *B. fragilis* is involved.

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