IN VITRO AND *IN VIVO* EVALUATIONS OF BMY-28100, A NEW ORAL CEPHALOSPORIN

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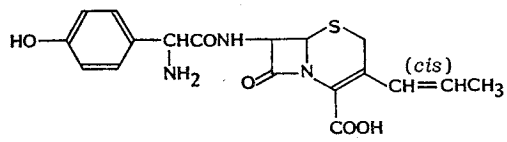
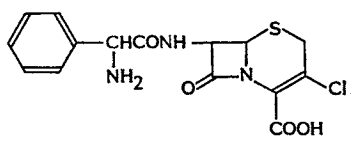
A new semisynthetic oral cephalosporin, BMY-28100, was evaluated for *in vitro* and *in vivo* antibacterial activities in comparison with cefaclor and cephalexin. BMY-28100 showed *in vitro* activity 3- and 10-fold more potent than that of cefaclor against *Staphylococcus aureus* and *Streptococcus pneumoniae*, respectively. BMY-28100 was slightly better than cefaclor and about 4 times more active than cephalexin against *Haemophilus influenzae* and *Neisseria gonorrhoeae*. *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* were comparably susceptible to BMY-28100 and cefaclor. The bactericidal activity of BMY-28100 against *S. aureus*, *E. coli* and *P. mirabilis* was equal to or twice as high as MIC value, which was similar to that of cefaclor. The stability of BMY-28100 against penicillinases was nearly comparable to that of cefaclor, whereas cefaclor was somewhat unstable to cephalosporinases. BMY-28100 was about twice as active as cefaclor against three Gram-positive bacterial infections. BMY-28100 was also more potent against infections of *H. influenzae* and *P. mirabilis*, but slightly less active against *E. coli* Juhl than cefaclor. Blood level parameters of BMY-28100 were significantly superior to those of cefaclor and slightly better than cephalexin in mice and rats. The urinary recovery of BMY-28100 was somewhat higher and comparable to that of cefaclor and cephalexin, respectively. BMY-28100 was more stable than cefaclor in human and calf sera at 37°C.

BMY-28100 (Fig. 1), 7-[*R*-α-amino-α-(4-hydroxyphenyl)acetamido]-3-[(*Z*)-1-propenyl]ceph-3-em-4-carboxylic acid, is a new oral cephalosporin which was synthesized in Bristol-Myers Research Institute, Ltd., Tokyo[1]. In preliminary comparison, the compound has been found effective against a variety of Gram-positive and Gram-negative organisms *in vitro* and *in vivo* after oral administration[2]. These studies have been expanded, and in this report BMY-28100 has been evaluated with respect to its *in vitro*

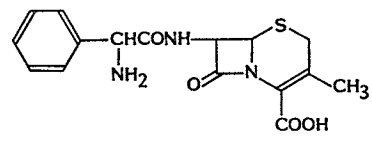
Fig. 1. Chemical structure of cephalosporins.



BMY-28100



Cefaclor (CCL)



Cephalexin (CEX)

antibacterial activity, stability against β-lactamases and in the serum, pharmacokinetics in rodents and *in vivo* efficacy in bacterial infections in mice. BMY-28100 was compared with cefaclor and cephalexin.

Materials and Methods

Cephalosporins

BMY-28100 was prepared at Bristol-Myers Research Institute, Ltd., Tokyo. Cefaclor (Shionogi-Lilly) and cephalexin (Shionogi-Lilly) were obtained from commercial sources.

Bacteria

The test organisms, 56 strains of Gram-positive and 81 strains of Gram-negative bacteria were clinical isolates and standard strains of our laboratory. The β-lactamase-producing strains of bacteria were supplied by Dr. S. MITSUHASHI of Gunma University, Dr. T. SAWAI of Chiba University and by the Microbiological Research Department of Bristol-Myers Company.

Determination of Minimum Inhibitory Concentrations (MICs)

MICs were determined on solid medium by the standard 2-fold agar dilution method[3]. Mueller-Hinton agar (Difco) was used in these assay except for fastidious bacteria which were tested by gonococcus agar (Eiken). Overnight broth cultures served as the source of inoculum. A volume of approximately 0.003 ml of the diluted culture containing 10^6 cells/ml was applied to the surface of the antibiotic-containing agar plates with a multi-inoculator. After incubation at 37°C for 18 hours, plates were examined for colony development, and the MIC, the lowest concentration of antibiotic causing no visible growth, was recorded.

Bactericidal Activity

The bactericidal activity of the cephalosporins was determined by exposing various organisms to a 2-fold series of antibiotic concentrations in Mueller-Hinton broth. Initial cell concentrations were adjusted to about 10^5 cells/ml. Quantitative killing curve studies were performed by incubating the broths at 37°C for 24 hours. Samples were removed at 1, 2, 4, 6, 8 and 24 hours and the number of viable bacteria was determined. The lowest concentration of antibiotics that reduced the initial cell count by 99.9% after overnight incubation was designated as the minimum bactericidal concentration (MBC).

Stability to β-Lactamases

The β-lactamases used in this study were purified by the method described previously[4]. Stability to various β-lactamases was determined by spectrophotometric assay by measuring the absorbance at the absorption maximum of each compound as previously reported[5,6]. Difference in extinction between BMY-28100 and corresponding hydrolyzed one was measured at 282 nm.

Protective Effect

Organisms were cultured overnight at 37°C in heart infusion broth and suspended in 5% hog mucin (American Laboratory, Omaha, Neb., U.S.A.). Male *dd*Y-mice weighing 19 to 24 g were infected intraperitoneally with about 100 times of the median lethal dose of the pathogen. Five mice at each dose level were individually given an antibiotic solution orally just before the bacterial challenge. The 50% protective dose (PD_{50}, mg/kg) was calculated by the method of LITCHFIELD and WILCOXON[7], from survival rate recorded on 7 days after the bacterial infection.

Blood Level and Urinary Recovery in Mice and Rats

Male *dd*Y-mice, weighing 18 to 22 g, and male Wister-rats, 150 to 200 g, were given antibiotic solution by oral administration. The antibiotic activity of blood samples collected from the orbital sinus was assayed by the paper disc - agar diffusion method. *Micrococcus luteus* PCI-1001 was used for assay organism. A group of five male *dd*Y-mice or male Wister-rats given antibiotic solution orally, was kept in a metabolic cage and urine specimens were collected during 24 hours after administration. Antibiotic activity of urine samples was assayed by the same method in blood level experiment.

Stability in Serum

Stability in human and calf sera was determined at 37°C. The initial concentration was 100 μg/ml in 90% serum and 10% pH 7.0 phosphate buffer solution and residual antibiotic activity was determined by the paper disc-agar diffusion assay.

Results

Antibacterial Spectrum

The activity of BMY-28100, cefaclor and cephalexin against standard strains of bacteria stocked in our laboratory is shown in Table 1. BMY-28100 was about 2 to 4 times more active than cefaclor against 4 strains of *Staphylococcus aureus*, whereas the activity of cephalexin was inferior to that of cefaclor. Against most of the Gram-negative organisms tested, BMY-28100 was equivalent to cefaclor and about 4 to 8 times better than cephalexin. BMY-28100 showed good activity against *Providencia rettgeri*, it was 4 times as active as cefaclor. The strains of *Morganella morganii* and *Enterobacter aerogenes* were slightly susceptible to BMY-28100, but not to cefaclor and cephalexin. *Enterobacter cloacae*, *Serratia marcescens* and *Pseudomonas aeruginosa* were resistant to all of the cephalosporins tested. Table 2 shows the *in vitro* activities of these oral cephalosporins against 115 aerobic bacteria. BMY-28100 was most active against Gram-positive organisms among the cephalosporins tested. It was about 3 times as active as cefaclor against 19 strains of *S. aureus* and about 10 times more active than cefaclor against *Streptococcus pyogenes* and *Streptococcus pneumoniae*. Furthermore, BMY-28100 demonstrated an appreciable level of activity against *Enterococcus faecalis* which was resistant to cefaclor and cephalexin,

Table 1. Antibacterial spectrum of BMY-28100, cefaclor and cephalexin against standard strains of bacteria.

Test organism	MIC (μg/ml)		
	BMY-28100	CCL	CEX
Staphylococcus aureus FDA 209P JC-1	0.4	1.6	3.1
S. aureus Smith	0.4	0.8	1.6
S. aureus Terajima	0.1	0.4	0.2
S. aureus MS 353	0.2	0.8	1.6
Bacillus subtilis ATCC 6633	0.4	0.4	0.8
Escherichia coli NIHJ JC-2	1.6	1.6	6.3
E. coli K-12 C600	1.6	1.6	6.3
Klebsiella pneumoniae PCI-602	0.4	0.4	3.1
Salmonella typhimurium IID 971	0.8	0.8	6.3
S. typhi 901	0.4	0.4	3.1
S. schottmuelleri 8006	0.8	0.8	3.1
S. enteritidis G14	0.4	0.4	1.6
Proteus mirabilis IFO 3849	1.6	1.6	12.5
P. vulgaris OX 19	25	25	25
P. vulgaris HX 19	6.3	6.3	12.5
Providencia rettgeri IFO 3850	0.8	3.1	6.3
Morganella morganii IFO 3848	25	50	>100
Enterobacter aerogenes ATCC 13048	25	>100	>100
E. cloacae 963	100	>100	>100
Serratia marcescens IAM 1184	100	>100	>100
Pseudomonas aeruginosa IFO 3445	>100	>100	>100
P. aeruginosa PAO 1	>100	>100	>100

CCL: Cefaclor, CEX: cephalexin.

Table 2. *In vitro* antibacterial activity of BMY-28100, cefaclor and cephalexin against aerobic bacteria.

Test organism (No. of strains)	Compound	MIC (μg/ml)	
		Range	Geometric mean
Staphylococcus aureus (19)	BMY-28100	0.2~3.1	0.74
	CCL	0.2~12.5	1.8
	CEX	0.4~12.5	2.4
Streptococcus pyogenes (6)	BMY-28100	0.05	0.05
	CCL	0.4	0.40
	CEX	0.8	0.80
S. pneumoniae (6)	BMY-28100	0.05~0.2	0.11
	CCL	0.4~3.1	1.6
	CEX	0.8~3.1	1.8
Enterococcus faecalis (15)	BMY-28100	0.8~25	9.9
	CCL	6.3~>100	>100
	CEX	6.3~>100	87
E. faecium (5)	BMY-28100	25	25
	CCL	100~>100	>100
	CEX	>100	>100
Haemophilus influenzae (10)	BMY-28100	0.4~0.8	0.65
	CCL	0.8~1.6	0.98
	CEX	3.1	3.1
Neisseria gonorrhoeae (8)	BMY-28100	0.013~6.3	0.68
	CCL	0.05~25	1.0
	CEX	0.05~12.5	1.4
N. meningitidis (5)	BMY-28100	0.4	0.40
	CCL	0.8	0.80
	CEX	0.8	0.80
Escherichia coli (13)	BMY-28100	0.8~25	4.1
	CCL	0.8~100	6.3
	CEX	3.1~50	11
Klebsiella pneumoniae (11)	BMY-28100	0.8~100	3.0
	CCL	0.8~100	2.5
	CEX	1.6~100	6.3
Proteus mirabilis (10)	BMY-28100	0.8~1.6	1.3
	CCL	1.6~3.1	2.0
	CEX	12.5~25	15

CCL: Cefaclor, CEX: cephalexin.

although the strains of *Enterococcus faecium* were resistant to these three cephalosporins. Against *Haemophilus influenzae* and *Neisseria gonorrhoeae*, the activities of BMY-28100 were slightly superior to those of cefaclor and cephalexin. BMY-28100 was twice as active as cefaclor and cephalexin against *Neisseria meningitidis* and comparable to cefaclor against *Escherichia coli*. The strains of *Klebsiella pneumoniae* were also similarly susceptible to BMY-28100 and cefaclor, but cephalexin was 4 times weaker than BMY-28100. The activity of BMY-28100 against *Proteus mirabilis* was somewhat better than that of cefaclor and about 10 times superior to cephalexin.

Bactericidal Activity

The bactericidal activity of BMY-28100 was compared with that of cefaclor and cephalexin against three test organisms (Fig. 2). The MIC value of BMY-28100 against *S. aureus* Smith was 0.8 μg/ml

Fig. 2. Bactericidal activity of BMY-28100, cefaclor and cephalexin in Mueller-Hinton broth. CCL: Cefaclor, CEX: cephalexin.

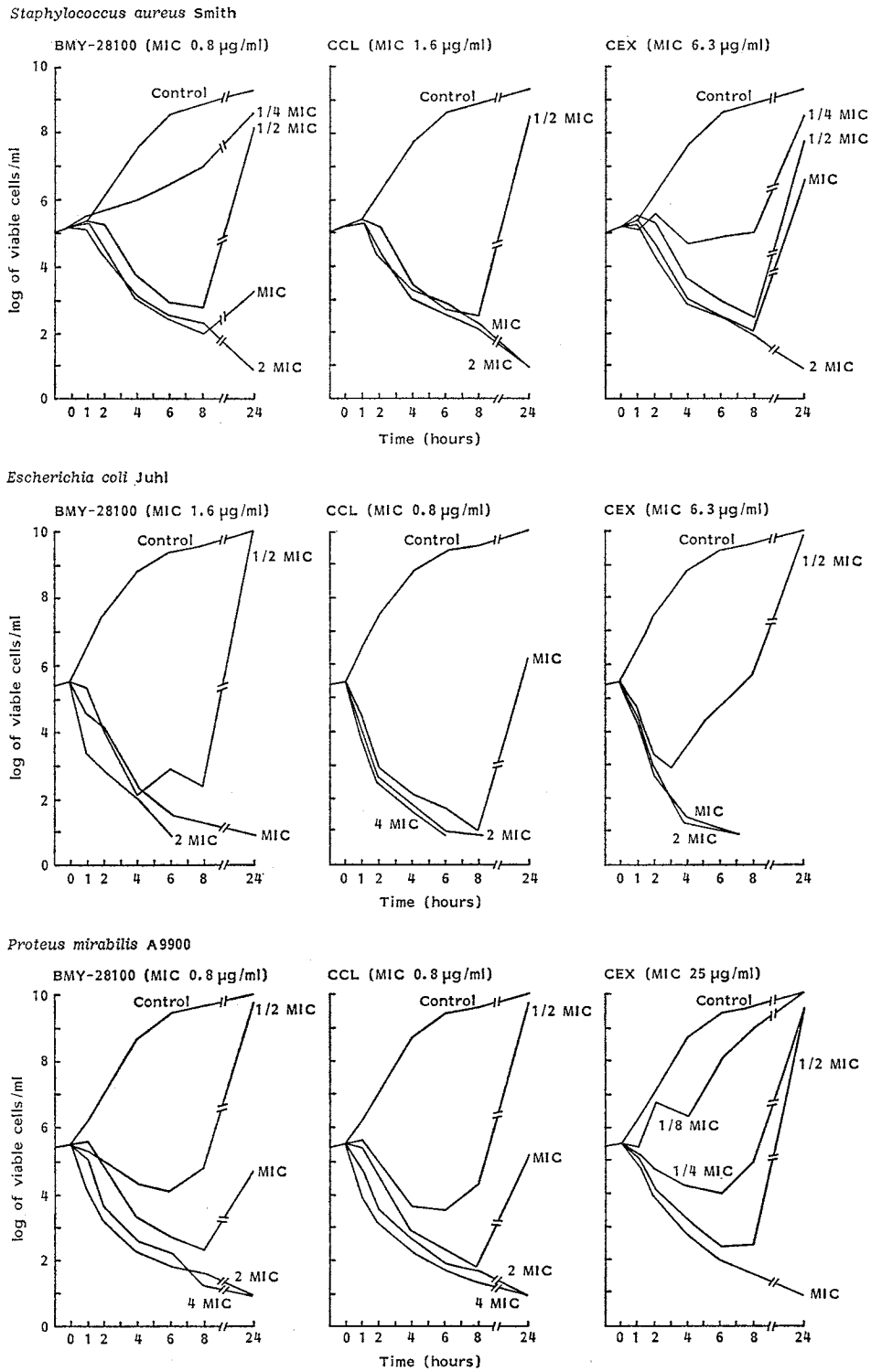


Table 3. Relative stability of BMY-28100, cefaclor and cephalexin against β-lactamases.

Enzyme source	RHR (%)				
	PCG	CER	BMY-28100	CCL	CEX
Penicillinase:					
Escherichia coli W3630/Rms 212	100	34	4	5	<1
E. coli ML1410 RGN 14	100	14	5	<1	<1
E. coli ML1410 RGN 823	100	32	8	6	<1
Pseudomonas aeruginosa Ml/Rms139	100	<1	<1	<1	<1
Cephalosporinase:					
Enterobacter cloacae GN7471		100	20	96	28
Citrobacter freundii GN7391		100	19	86	33
Cefuroxime-hydrolyzing β-lactamase:					
Proteus vulgaris GN7919		100	82	264	21
Bacteroides fragilis 308		100	16	76	4

RHR: Relative hydrolysis rate by β-lactamases. PCG: Benzylpenicillin, CER: cephaloridine, CCL: cefaclor, CEX: cephalexin.

Table 4. Protective effect of BMY-28100, cefaclor and cephalexin against experimental infections in mice.

Test organism	PD_{50} (mg/kg, po)		
	BMY-28100	CCL	CEX
Staphylococcus aureus Smith	0.09	0.17	0.42
S. aureus BX-1633	1.3	2.2	17
Streptococcus pyogenes A20201	0.07	0.14	0.74
Haemophilus influenzae A9729	1.1	1.6	18
Escherichia coli Juhl	1.1	0.8	8.0
Proteus mirabilis A9554	1.1	1.8	13

CCL: Cefaclor, CEX: cephalexin.

and its MBC, the MBC, was 1.6 μg/ml, whereas the MIC value of cefaclor, 1.6 μg/ml corresponded to its MBC. Against both Gram-negative bacteria, the bactericidal activity of BMY-28100 was same as that of cefaclor and 2- to 16-fold better than that of cephalexin.

Stability to β-Lactamases

The β-lactamase stability of BMY-28100 was assayed by determining relative rates of hydrolysis by various β-lactamases (Table 3). BMY-28100, cefaclor and cephalexin were resistant to hydrolysis by 4 types of penicillinases. BMY-28100 and cephalexin were moderately hydrolyzed by cephalosporinases from *E. cloacae* and *Citrobacter freundii*, whereas the hydrolysis rate of cefaclor was nearly equivalent to that of cephaloridine. Against cefuroxime-hydrolyzing β-lactamases of *Proteus vulgaris* and *Bacteroides fragilis*, cephalexin was most resistant among the cephalosporins tested, and cefaclor was most sensitive to Richmond type C cefuroxime-hydrolyzing β-lactamase[8].

Protective Activity

Table 4 shows the *in vivo* therapeutic acivities of BMY-28100 administered orally to the mice infected with a variety of bacteria compared with those of cefaclor and cephalexin. BMY-28100 showed the best activity against three Gram-positive bacterial infections, it was about twice as active as cefaclor and 5- to 10-fold superior to cephalexin. Against Gram-negative organisms, BMY-28100 was equal to or slightly better than cefaclor, whereas cephalexin was 7 to 16 times inferior to BMY-28100.

Table 5. Pharmacokinetic parameters of BMY-28100, cefaclor and cephalexin after oral administration to mice and rats.

Compound	Dose (mg/kg, po)	Blood level parameters[a]			Recovery in urine[b] (%)
		Cmax (μg/ml)	$T_{1/2}$ (hours)	AUC (μg·hours/ml)	
Mice (n=10)					
BMY-28100	50	27±1.3	0.83±0.06	34±2.0	73
CCL	50	28±1.7	0.60±0.04	22±1.9	60
CEX	50	27±2.1	0.58±0.04	26±1.4	75
Rats (n=5)					
BMY-28100	80	15±2.0	2.1±0.20	65±2.3	46
	40	14±1.3	1.9±0.18	41±1.6	46
CCL	80	16±1.0	2.0±0.15	43±2.4	39
	40	7.5±0.57	2.0±0.11	21±0.60	38
CEX	80	17±1.1	2.7±0.08	63±5.3	57
	40	12±1.6	2.5±0.73	47±3.7	52

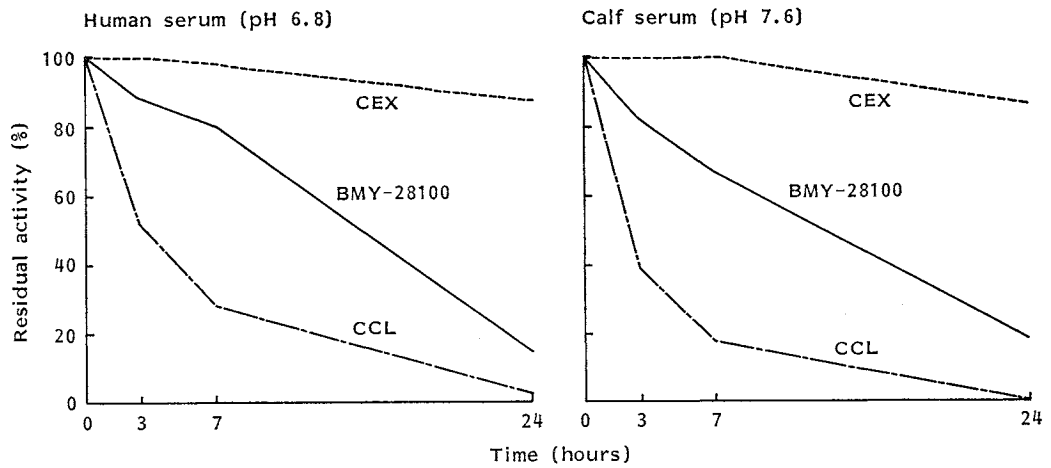
Data represents the mean±SE.

[a] Blood collection (time after drug administration), mice: 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5 and 3 hours, rats: 0.5, 1, 2, 3, 4, 5, 6 and 7 hours.

[b] 0 to 24 hours collection period.

CCL: Cefaclor, CEX: cephalexin.

Fig. 3. Stability of BMY-28100, cefaclor and cephalexin in serum at 37°C.
CCL: Cefaclor, CEX: cephalexin.



Blood Level and Urinary Recovery

The blood level and urinary excretion of BMY-28100 were examined in mice and rats after oral administration and the results are shown in Table 5. The half-life value of BMY-28100 in mice was longer than those of cefaclor and cephalexin and its AUC value was also significantly greater than that of cefaclor. The AUC values of BMY-28100 were also greater than those of cefaclor and comparable to those of cephalexin in rats. The urinary recovery rates of BMY-28100 were slightly higher than those of cefaclor in both mice and rats.

Stability in the Serum

As shown in Fig. 3, BMY-28100 was significantly more stable to cefaclor in both human and calf sera, though cephalexin was the most stable among the cephalosporins tested.

Discussion

BMY-28100 was compared with cefaclor and cephalexin in the *in vitro* and *in vivo* antibacterial activities and bioavailability in mice and rats. The compound showed better *in vitro* activity than cefaclor and cephalexin which was currently available for clinical use. Especially, against Gram-positive organisms, such as *S. aureus*, *S. pyogenes*, *S. pneumoniae* and *E. faecalis*, BMY-28100 was 3 to 20 times more active than cefaclor and cephalexin. Against Gram-negative organisms, BMY-28100 had slightly better or comparable activity to cefaclor, which has been reported to have more potent activity than cephalexin, cephaloglycin and cephradine against Gram-negative bacteria[9,10]. The bactericidal activity of BMY-28100 was almost equivalent to that of cefaclor, its MBC values were equal to or twice as high as MIC values. The stability of cefaclor to various types of β-lactamases has been reported by NEU and FU[11]. In our experiments, BMY-28100 was slightly more stable than cefaclor against cephalosporinase and cefuroxime-hydrolyzing β-lactamase. The *in vivo* therapeutic efficacy of BMY-28100 was well correlated to its *in vitro* activity, and was better than that of cefaclor against five out of six Gram-positive and Gram-negative bacterial infections. The pharmacokinetic profiles of cefaclor in laboratory animals and human volunteers[12,13] have been reported. BMY-28100 showed blood level parameters superior to those of cefaclor and comparable to those of cephalexin in rodent animals. The stability in the serum of oral cephalosporins was also examined, since the instability of cefaclor in the serum and buffer solution was reported by FOGLESONG *et al.*[14]. BMY-28100 was significantly more stable than cefaclor in both human and calf sera.

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