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In vitro and *In vivo* Evaluations of LB10517, a Novel Parenteral Broad-Spectrum Cephalosporin

Hye-Kyong Song[1], Takeshi Nishino[2], Mi-Kyeong Seo[1], Mu-Yong Kim[1], Yong-Hee Lee[1], In-Chull Kim[1] and Jin-Hwan Kwak[1]

[1]Biotech Research Institute, LG Chem Research Park, LG Chemical Ltd., Tae-jon 305-380, Korea and [2]Microbiology Department, Kyoto Pharmaceutical University, Kyoto 607, Japan

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The *in vitro* activity of LB10517, a new catechol-substituted cephalosporin, was compared with those of E-1077, cefpirome and ceftazidime against 1034 clinical isolates collected in Japan. LB10517 showed a broad-spectrum antibacterial activitity against a wide range of gram-positive and gram-negative bacteria including non-glucose fermenting rods, *Pseudomonas aeruginosa*. Against the methicillin-susceptible strains of *Staphylococcus aureus* (MSSA) and *Streptococcus pyogenes*, the MIC_{90} values of LB10517 which required to inhibit 90% of the strains were 3.13 μg/ml and 0.1 μg/ml, respectively. It was as active as E-1077 but more active than cefpirome and ceftazidime. Methicillin-resistant strains of *S. aureus* (MRSA) and *Enterococcus* spp. were highly resistant to all the test compounds. LB10517 was highly active against most members of the family *Enterobacteriaceae*, 90% of which were inhibited at a concentration of less than 0.78 μg/ml, except for *Enterobacter cloacae* (1.56 μg/ml) and *Serratia marcescens* (3.13 μg/ml). Its activity was comparable to those of E-1077 and cefpirome but it was greater than that of ceftazidime. Against *Pseudomonas aeruginosa*, LB10517 showed the most potent antibacterial activity among the compounds tested. Ninety percent of *P. aeruginosa* isolates were susceptible at the concentration of 0.39 μg/ml. Its activity was 32- to 128-fold higher than those of E-1077, cefpirome and ceftazidime. Against imipenem- or ofloxacin-resistant *P. aeruginosa*, LB10517 with MIC_{90}s of 6.25 μg/ml and 3.13 μg/ml, respectively, showed 16-fold more potent activity than the other test compounds. LB10517 showed a relatively high plasma level and long plasma elimination half-life in rats ($t_{1/2}(\beta)$, 52 min) and dogs ($t_{1/2}(\beta)$, 103 min).

Key words : LB10517, Cephalosporin, Catechol, *tonB*, MIC

INTRODUCTION

Research is continuing in new cephalosporins with the aim of expanding the spectrum of activity, improving pharmacokinetic properties to permit less frequent dosing, overcoming problems of resistance and reducing the incidence of side effects in humans. Even though a number of new cephalosporins which have broad spectrum activity for the therapeutic use in respiratory and urinary tract infections were launched already or under clinical development in many places of the world, few cephalosporins have significant activity against *Pseudomonas aeruginosa*. To discover anti-pseudomonal β-lactams with broad spectrum antibacterial activity, several catechol-substituted β-lactams were synthesized. The use of a catechol group on beta-lactam structures enhanced the drug's penetration into bacterial cells such as *P. aeruginosa* via the *tonB* dependent iron transport system.

LB10517, {7-[(Z)-2-(2-aminothiazol-4yl)-2-(S)-(α-carboxyl-3,4-dihydroxy benzyl oxyimino) acetamide]-3-[(E)-3-(4,6-diamino-1-pyrimidino)-1-propen-1-yl]-3-cephem-4-carboxylate}, is a novel catechol-substituted parenteral cephalosporin synthesized at LG Chemical Ltd. (Fig. 1). Although most of already known catecholic cephalosporins have poor antibacterial activity against gram-positive bacteria (Arisawa *et al.*, 1991, Erwin *et al.*, 1991, Nakagawa *et al.*, 1987, Silley *et al.*, 1990), LB10517 showed well-balanced broad spectrum activity against both gram-positive and gram-negative bacteria. The spectrum of LB10517 activity includes members of the family *Enterobacteriaceae*, *Pseudomonas aeruginosa*, *Staphylococci* and *Streptococcus* spp. (Kwak *et al.*, 1994).

Correspondence to: Jin-Hwan Kwak, Biotech Research Institute, LG Chem Research Park, LG Chemical Ltd.,104-1 Moonji-Dong, Yuseong-Ku, Tae-jon 305-380, Korea

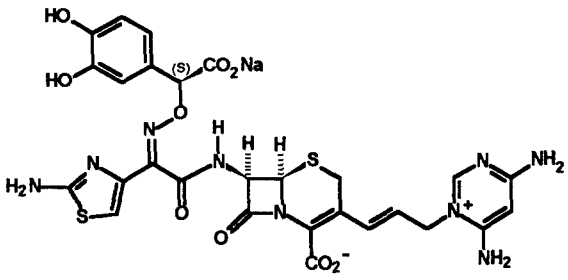


Fig. 1. Chemical structure of LB10517.

In this paper, we compared the *in vitro* activities of LB10517 with those of E-1077 (Watanabe *et al.*, 1992) cefpirome (Seibert *et al.*, 1983) and ceftazidime against clinical isolates collected in Japan. In addition, morphological alteration and pharmacokinetic studies were described.

MATERIALS AND METHODS

Antimicrobial agents

LB10517 was synthesized at Biotech Research Institute, LG Chem Research Park, LG Chem Ltd. E-1077 and cefpirome were provided by Esai Co., Ltd. and Hoechst Japan, Tokyo, respectively. Ceftazidime (Glaxo) and ceftriaxone (Sigma) were obtained commercially as standards.

Test organisms

The bacterial strains used in this study were originally isolated from clinical specimens of humans. These were collected at several hospitals in Japan. All isolates were maintained as stock cultures at -70°C.

Susceptibility studies

The MICs of LB10517 and reference compounds against 1034 clinical isolates were determined by the agar dilution method recommended by the Japan Society of Chemotherapy (Committee for Revision for MIC Determination Method, 1981). Sensitivity test agar (Eiken) was used for all organisms except for the following : *Streptococci* (sensitivity test agar supplemented with 10% defibrinated horse blood). *Haemophilus influenzae* (sensitivity test agar supplemented with 5% Fildes enrichment), *Moraxella* (*Branhamella*) *catarrhalis* (chocolate sensitivity test agar supplemented with 5% defibrinated horse blood), and *Neisseria* spp. (chocolate sensitivity test agar supplemented with 10% defibranated horse blood). Fresh cultures of the bacterial strains were diluted, and 5 μl of each bacterial suspension, corresponding to about 10^4 CFU, was spotted (Microplanter; Sakuma Seisakusho, Tokyo, Japan) onto agar plates that contained two-fold serial dilutions of antibiotics. The MIC was considered to be the lowest concentration that completely inhibited visible growth on agar plates after incubation for 18 h at 37°C. For *Neisseria* spp., incubation was carried out in a candle jar. The MIC_{50} and MIC_{90} were the concentrations of a drug required to inhibit 50% and 90% of the strains, respectively.

Morphological Changes

For the morphological alteration study, the overnight cultures of *E. coli* 3190Y, *P. aeruginosa* 1912E and *S. aureus* giorgio were diluted 100-fold into fresh Mueller-Hinton broth, grown at 35°C for 3 h, and then added with LB10517 at various concentrations. After additional incubation for 3 h, the cells were fixed and morphological changes of the cells was examined by a microscope (Hata *et al.*, 1992).

Pharmacokinetic studies

For the pharmacokinetic study on LB10517 in rats, five male SD rats weighing 220 to 260 g were used. Rats anesthetized by ethyl ether were cannulated with polyethylene tubes into the right femoral vein and femoral atery, and were kept in Ballmann cages. Test drug in distilled water was administered intravenously via a femoral vein at a dose of 20 mg/kg. Blood samples were collected from femoral artery at 1, 2.5, 5, 10, 20, 40, 60, 120 and 180 min after administration and plasma samples were seperated from heparinized blood by centrifugation at 4,500 X g for 10 min at 4°C. Antibiotic concentration was measured by the bioassay (Bennett *et al.*, 1966). Mueller-Hinton Medium (Difco) seeded with susceptibility test organisms was used for bioassay by agar diffusion test. Drug concentrations of each plasma samples were calculated by the method of Bennett, using standard curves in which the logarithms of the concentrations were proportional to the areas of the inhibition zones. Pharmacokinetic parameters were calculated by applying the two-compartment model.

For the pharmacokinetic study of LB10517 in dogs, two male Beagle dogs were injected intraveneously via a cephalic vein with a single dose of 20 mg/kg. The blood samples were obtained at 1, 15, 30, 60, 120, 240 and 360 min after injection. The pharmacokinetic data of each drug in dogs were calculated as above.

RESULTS

In vitro antibacterial activity

The antibacterial activities of LB10517, E-1077, cefpirome and ceftazidime against clinical isolates are shown in Table I. LB10517 showed expanded broad spectrum antibacterial activities against both gram-po-

Table I. Comparative *in vitro* activity of LB10517 against 1034 clinical isolates collected in Japan

Organism No. of strains	Antimicrobial Agents	MIC(μg/ml) Range	50%	90%
MSSA [58]	LB10517	0.10-50	0.39	3.13
	Ceftazidime	6.25->100	12.5	50
	Cefpirome	0.20-50	0.78	6.25
	E-1077	0.20-12.5	0.78	3.13
MRSA [36]	LB10517	1.56->100	100	>100
	Ceftazidime	50->100	>100	>100
	Cefpirome	12.5-100	50	100
	E-1077	1.56-100	25	50
S. epidermidis [53]	LB10517	0.10->100	1.56	100
	Ceftazidime	1.56-100	6.25	50
	Cefpirome	0.05-12.5	0.39	6.25
	E-1077	0.20-25	1.56	12.5
S. pyogenes [50]	LB10517	0.025-0.10	0.05	0.1
	Ceftazidime	0.20-0.78	0.39	0.39
	Cefpirome	<0.006-<0.006	<0.006	<0.006
	E-1077	0.05-0.10	0.05	0.1
S. pneumoniae [47]	LB10517	0.025-6.25	0.1	0.78
	Ceftazidime	0.10-12.5	0.39	1.56
	Cefpirome	<0.006-0.39	<0.006	0.025
	E-1077	0.025-1.56	0.05	0.2
E. faecelis [45]	LB10517	3.13-25	12.5	25
	Ceftazidime	25->100	>100	>100
	Cefpirome	1.56-50	6.25	25
	E-1077	1.56-12.5	3.13	12.5
E. faecium [39]	LB10517	6.25->100	>100	>100
	Ceftazidime	100->100	>100	>100
	Cefpirome	0.78->100	100	>100
	E-1077	12.5->100	>100	>100
E. avium [39]	LB10517	6.25->100	25	>100
	Ceftazidime	50->100	>100	>100
	Cefpirome	0.39->100	50	>100
	E-1077	6.25->100	25	>100
E. coli [50]	LB10517	0.012-0.20	0.05	0.2
	Ceftazidime	0.05-6.25	0.2	1.56
	Cefpirome	<0.006-0.78	0.012	0.025
	E-1077	0.012-12.5	0.025	0.05
C. freundii [40]	LB10517	0.05-3.13	0.1	0.78
	Ceftazidime	0.10->100	0.39	100
	Cefpirome	<0.006-3.13	0.025	0.78
	E-1077	0.025-50	0.1	0.78
K. pneumoniae [49]	LB10517	0.05-1.56	0.1	0.2
	Ceftazidime	0.05-0.78	0.05	0.78
	Cefpirome	<0.006-12.5	0.012	0.2
	E-1077	<0.006-100	<0.006	0.2
E. cloacae [39]	LB10517	0.012-6.25	0.1	1.56
	Ceftazidime	0.10->100	0.39	100
	Cefpirome	0.012-6.25	0.05	3.13
	E-1077	0.012-1.56	0.05	1.56
E. aerogenes [40]	LB10517	0.025-0.78	0.05	0.39
	Ceftazidime	0.05-50	0.1	12.5
	Cefpirome	0.012-0.78	0.05	0.39
	E-1077	0.12-0.20	0.025	0.2
S. marcescens [40]	LB10517	0.05-0.25	0.2	3.13
	Ceftazidime	0.10-100	0.39	50
	Cefpirome	0.025-100	0.20	12.5
	E-1077	0.10->100	0.39	12.5
P. mirabilis [40]	LB10517	0.05-0.78	0.1	0.2
	Ceftazidime	0.10-1.56	0.1	0.2
	Cefpirome	0.025-0.78	0.05	0.2
	E-1077	0.05-1.56	0.1	0.2
P. vulgaris [39]	LB10517	0.1-3.13	0.2	0.78
	Ceftazidime	0.10-3.13	0.2	0.78
	Cefpirome	0.1-50	0.39	12.5
	E-1077	0.39-100	3.13	50
P. rettgeri [38]	LB10517	<0.006-0.20	0.25	0.05
	Ceftazidime	<0.006-12.5	0.05	1.56
	Cefpirome	<0.006-3.13	<0.006	0.2
	E-1077	<0.006-0.20	<0.006	0.025
M. morganii [40]	LB10517	0.05-3.13	0.2	0.39
	Ceftazidime	0.025-100	0.05	12.5
	Cefpirome	<0.006-0.78	<0.006	0.2
	E-1077	<0.006-0.39	<0.006	0.2
H. influenzae [53]	LB10517	0.2-1.56	0.39	0.78
	Ceftazidime	0.05-0.78	0.2	0.39
	Cefpirome	<0.006-0.78	0.025	0.05
	E-1077	0.025-0.78	0.05	0.2
P. aeruginosa [29]	LB10517	0.025-3.13	0.05	0.39
	Ceftazidime	0.78-100	6.25	25
	Cefpirome	1.56-100	12.5	50
	E-1077	0.78-100	6.25	12.5
P aeruginosa IMP-r[36]	LB10517	0.2-12.5	1.56	6.25
	Ceftazidime	1.56->100	12.5	100
	Cefpirome	3.13->100	25	>100
	E-1077	0.78->100	6.25	100
P. aeruginosa OFLX-r[39]	LB10517	0.39-12.5	0.78	3.13
	Ceftazidime	0.78-100	6.25	50
	Cefpirome	3.13->100	25	100
	E-1077	0.78-50	6.25	50
A. calcoaceticus [37]	LB10517	0.20-6.25	0.78	3.13
	Ceftazidime	0.78-25	6.25	25
	Cefpirome	0.20-25	1.56	3.13
	E-1077	0.39-50	3.13	6.25
Neisseria spp. [12]	LB10517	0.25-0.39	0.05	0.2
	Ceftazidime	<0.006-0.39	0.05	0.2
	Cefpirome	<0.006-0.05	<0.006	0.012
	E-1077	<0.006-0.10	0.012	0.025
M. catarrhalis [46]	LB10517	0.2-3.13	0.78	1.56
	Ceftazidime	0.025-0.39	0.05	0.2
	Cefpirome	0.012-1.56	0.39	0.78
	E-1077	0.012-1.56	0.2	0.78

sitive and gram-negative bacteria including non-glucose fermenting rods, *P. aeruginosa*. Against methicillin-susceptible strains of *Staphylococcus aureus*

(MSSA), LB10517 with a MIC_{90} of 3.13 μg/ml was as active as E-1077 but much more active than ceftazidime. Methicillin-resistant strains of *S. aureus* (MRSA) were highly resistant to all compounds. LB 10517 was less active than the reference compounds against *Staphylococcus epidermidis*. Against *Streptococcus pyogenes*, LB10517 exhibited high inhibitory activity (MIC_{90}, 0.1 μg/ml), as did E-1077, and was four-fold more active than ceftazidime. But against *Streptococcus pneumoniae*, cefpirome was most active among the test compounds. The activity of LB 10517 against *Enterococcus faecalis* was almost the same as that of cefpirome. However, all cephalosporins hardly showed activity against other species of *Enterococci*, such as *Enterococcus avium* and *Enterococcus faecium*. LB10517 was highly active against most members of the family *Enterobacteriaceae*, 90% of which were inhibited at a concentration of less than 0.78 μg/ml, except for *Enterobacter cloacae* (1.56 μg/ml) and *S. marcescens* (3. 13 μg/ml). Against *E. coli*, LB10517 with a MIC_{90} of 0. 2 μg/ml showed four-fold less active than E-1077. LB 10517 showed comparable avtivity to those of E-1077 and cefpirome against *Citrobacter freundii*, *Klebsiella pneumoniae* and *Enterobacter aerogenes* but it was much more potent than ceftazidime. The activity of LB10517 against *E. cloacae* was similar to that of E-1077 but more active than those of cefpirome and ceftazidime. Against *S. marcescens*, LB10517 were four- to eight-fold more active than E-1077 and cefpirome. Against *Proteus mirabilis*, LB10517 with a MIC_{90} of 0.2 μg/ml had similar activity to the reference compounds. But LB10517 showed 16- to 64-fold more potent activity than E-1077 and cefpirome against *Proteus vulgaris*. Against *Providencia rettgeri* and *Morganella morganii*, LB10517 was as active as E-1077 and cefpirome but 32-fold more active than ceftazidime. Against *Haemophilus influenzae*, cefpirome and E-1077 were two- or four-old more active than LB10517, but LB10517 was as active as ceftazidime. Another important antibacterial feature of LB10517 was their potent activity against *P. aeruginosa*. The MIC_{90} of LB 10517 against *P. aeruginosa* was 0.39 μg/ml, and its activity was 32- to 128-fold more potent than those of all the reference compounds. Especially, LB10517 was active against imipenem- or ofloxacin-resistant *P. aeruginosa*. The MIC_{90}s against these strains were 6.25 μg/ml and 3.13 μg/ml, respectively. LB10517 with a MIC_{90} of 3.13 μg/ml, showed similar activity to that of cefpirome against *Acinetobacter calcoaceticus* but was twice as active as E-1077. Against *Neisseria* spp., LB 10517 was as active as ceftazidime but less active than E-1077 and cefpirome. Against *Moraxella catarrhalis*, LB10517 was two fold less active than E-1077 and cefpirome.

Morphological changes

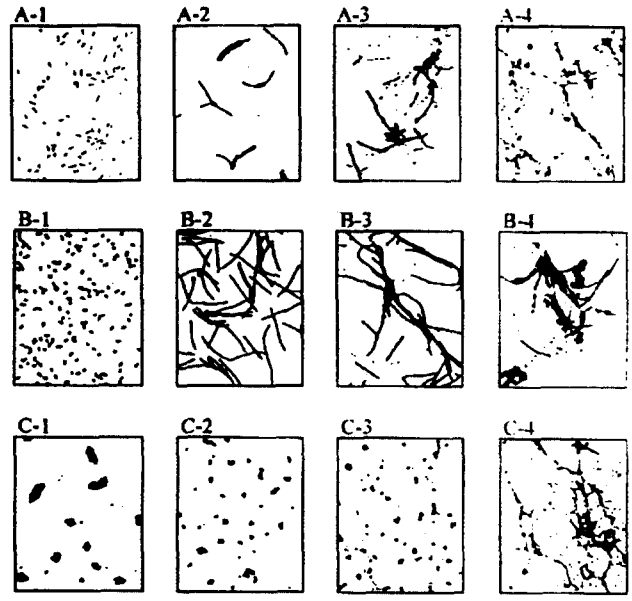


Fig. 2. Morphological changes of *E. coli* 3190Y, *P. aeruginosa* 1912E, and *S. aureus* giorgio grown in Mueller-Hinton broth containing LB10517 for 3 h at 37 °C. Lanes: A-1 through A-4, *E. coli* 3190Y; A-1, no addition; A-2, 0.008 μg/ml (1/4×MIC); A-3, 0.031 μg/ml (1×MIC); A-4, 0.13 μg/ml (4×MIC); B-1 through B-4, *P. aeruginosa* 1912E; B-1, no addition; B-2, 0.13 μg/ml (1/4×MIC); B-3, 0.5 μg/ml (1×MIC); B-4, 2 μg/ml (4×MIC); C-1 through C-4, *S. aureus* giorgio; C-1, no addition; C-2, 0.13 μg/ml (1/4×MIC); C-3, 0.5 μg/ml (1×MIC); C-4, 2 μg/ml (4×MIC). Magnification, ×1000

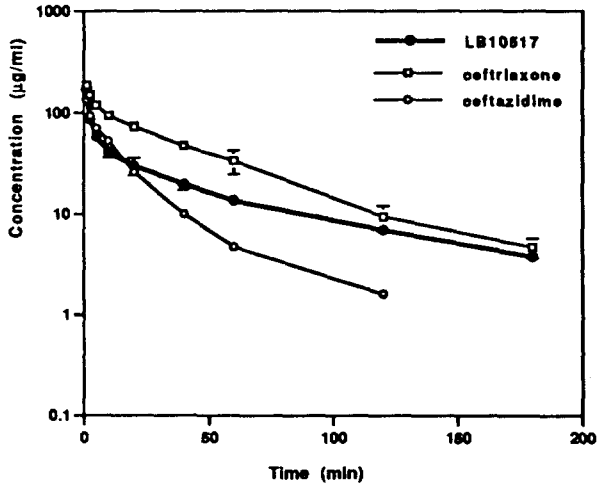
The morphological changes induced by LB10517 in *E. coli* 3190Y, *P. aeruginosa* 1912E and *S. aureus* giorgio were examined by the differential interference microscopy. Figure 2 showed the morphological alterations of *E. coli*, *P. aeruginosa* and *S. aureus* exposed to the various concentrations of LB10517 for 3 h. LB10517 induced the formation of filamentatous cells and the subsequent cell lysis in *E. coli* and *P. aeruginosa*, and induced cell lysis in *S. aureus* at the concentration of 1 X MIC. Cell populations reduced dramatically above 1 X MIC.

Pharmacokinetic studies

The pharmacokinetic properties of LB10517 in rats and dogs are shown in Figures 3 and 4, respectively. The serum elimination half-life in rats and dogs was estimated from the serum concentrations measured after single i.v. injection. LB10517 showed relatively high plasma level and long plasma elimination half life in rats ($t_{1/2}(\beta)$, 52 min) and dogs ($t_{1/2}(\beta)$, 103 min).

DISCUSSION

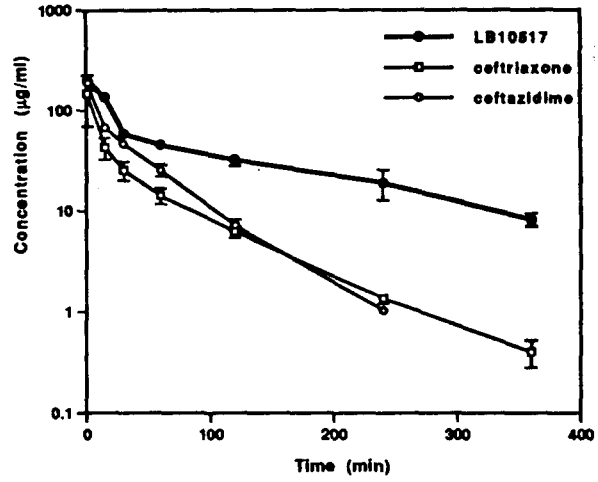
LB10517 had well balanced and broad spectrum antibacterial activity against both gram-positive and



	LB10517	Ceftriaxone	Ceftazidime
AUC [μg min/ml]	2480	5475	1714
$T_{1/2}$ (β) [min]	52	39	20
CL [ml/min]	5.19	3.27	10.7
Vd_{ss}[ml]	345.2	173.2	227.5

* AUC : Area Under the Curve
* CL : Clearance
* Vd_{ss} : Volume of Distribution at Steady-State

Fig. 3. Pharmacokinetic parameters of LB10517, ceftraixone and ceftazidime in rats



	LB10517	Ceftriaxone	Ceftazidime
AUC [μg min/ml]	8095	5081	5887
$T_{1/2}$ (β) [min]	103	58	48
CL [ml/min]	2.50	3.93	3.47
Vd_{ss}[ml]	336.7	259.7	202.6

* AUC : Area Under the Curve
* CL : Clearance
* Vd_{ss} : Volume of Distribution at Steady-State

Fig. 4. Pharmacokinetic parameters of LB10517, ceftraixone and ceftazidime in dogs

gram-negative bacteria. This catecholic cephalosporin LB10517 with a catechol moiety at 7 position, can utilize the *tonB* dependent iron transport system in addition to porin proteins to enter the bacterial periplasmic space. This dual mode of entry may afford increased potency during infections via the iron-deficient state that *tonB* gene product would initiate the active transport of iron-chelated catechols across the bacterial outer membrane (Aisen *et al.*, 1980, Barclay *et al.*, 1985, Curtis *et al.*, 1988, Hartmann *et al.*, 1980, Weinberg *et al.*, 1978). This characteristic may offer an advantage in overcoming the resistance of *P. aeruginosa* and the other G (-) bacterial mutants that have strong permeability barrier. Because few cephalosporins are active for *P. aeruginosa*, a new cephalosporin with broad-spectrum and potent anti-pseudomonal activity will be important therapeutic and empiric treatment regimens for the increased refractory pseudomonal infections, especially in immunocompromised patients. Actually LB10517 with a MIC_{90} of 0.39 μg/ml was very active against clinical isolates of *P. aeruginosa*. It was 64-fold more active than ceftazidime which has the most potent anti-pseudomonal activity among the commercialized cephalosporins. LB10517 with MIC_{90}s of 6.25 μg /ml and 3.13 μg/ml, respectively, was also active against imipenem or ofloxacin resistant *P. aeruginosa*. The *in vivo* efficacy of LB10517 in systemic infection clearly reflected its *in vitro* activity against *P. aeruginosa* (Kwak *et al.*, 1994).

LB10517 had improved pharmacokinetic properties compared with those of ceftriaxone and ceftazidime in animals. LB10517 showed a possibility of once a day dose regimen in humans, equivalent to ceftriaxone. Since recent research on β-lactam antibiotics is focussing on the development of new cephalosporins which are resistant to the hydrolysis by β-lactamases and have good pharmacokinetic properties to reduce dose frequency in humans, a catecholic cephalosporin LB10517 with apparent advantages in antibacterial activities and pharmacokinetic profiles can be a good candidate for clinical development. Further toxicological studies are therefore warranted.

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