ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 1996, p. 1825–1831 Vol. 40, No. 8
0066-4804/96/$04.00+0

In Vitro and In Vivo Activities of LB10522, a New Catecholic Cephalosporin

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Received 1 December 1995/Returned for modification 13 February 1996/Accepted 27 May 1996

In vitro activity of LB10522 was compared with those of cefpirome, ceftazidime, ceftriaxone, and cefoperazone against clinical isolates. Against gram-positive bacteria, LB10522 was most active among the compounds tested. It was fourfold more active than cefpirome against methicillin-susceptible *Staphylococcus aureus* and *Enterococcus faecalis*. LB10522 was highly effective against most members of the family *Enterobacteriaceae* tested. Ninety percent of isolates of *Escherichia coli*, *Klebsiella oxytoca*, *Proteus vulgaris*, *Proteus mirabilis*, and *Salmonella* spp. were inhibited at a concentration of ≤0.5 μg/ml. These activities were comparable to those of cefpirome. Against *Pseudomonas aeruginosa*, LB10522 with a MIC at which 90% of the isolates are inhibited of 2 μg/ml was 16- and 32-fold more active than ceftazidime and cefpirome, respectively. LB10522 exhibited excellent protective effects against bacterial infections in mice, as reflected by its in vitro activity. The 50% effective doses of LB10522 were lower than those of cefpirome and ceftazidime against systemic infections caused by *Staphylococcus aureus* giorgio, *Streptococcus pneumoniae* III, *Pseudomonas aeruginosa* 1912E, *Escherichia coli* 851E, *Proteus mirabilis* 1315E, *Serratia marcescens* 1826E, and *Acinetobacter calcoaceticus* Ac-54. LB10522 was very resistant to hydrolysis by various β-lactamases such as TEM-3, TEM-7, SHV-1, FEC-1, and P-99. LB10522 did not induce β-lactamase in *Enterobacter cloacae* 1194E, although most of the reference cephalosporins acted as inducers of β-lactamase in this strain. Time-kill study showed that LB10522, at concentrations of two or four times the MIC, had a rapid bactericidal activity against *Staphylococcus aureus* 6538p, *Escherichia coli* 851E, and *Pseudomonas aeruginosa* 1912E.

A number of new cephalosporins which have an extended broad-spectrum antibacterial activity for the therapeutic use in respiratory and urinary-tract infections have been launched or are currently under clinical development. However, few cephalosporins have significant activity against *Pseudomonas aeruginosa*, which causes refractory infections in immunocompromised patients. To discover antipseudomonal cephalosporins with a broad-spectrum antibacterial activity, several catechol-substituted cephalosporins have been synthesized (1–3, 6, 7, 10–13, 16, 21). It has been reported that the introduction of a catechol group on β-lactam structures enhanced the capability of drugs to penetrate into gram-negative bacteria, especially *P. aeruginosa*, by utilizing the *tonB*-dependent iron transport system (5, 20). In contrast to its improved activities against gram-negative organisms, most of the already known catecholic cephalosporins have poor antibacterial activities against gram-positive bacteria.

LB10522 {7-[(Z)-2-(2-aminothiazol-4-yl)-2-(S)-(a-carboxyl-3,4-dihydroxybenzyloxyimino)acetamide]-3-[(E)-3-(4-amino-1-pyrimidino)-1-propen-1-yl]-3-cephem-4-carboxylate} is a parenteral cephalosporin that has a catechol moiety at the 7-oxime side chain (Fig. 1). This compound exhibited a well-balanced and broad-spectrum antibacterial activity against gram-positive and gram-negative bacteria. In particular, LB10522 showed potent activities against *Staphylococcus aureus* and *P. aeruginosa.*

In this paper, we describe in vitro and in vivo activities of LB10522 compared with those of cefpirome, ceftazidime, ceftriaxone, and cefoperazone. In addition, the stabilities to various β-lactamases and a time-kill study are reported.

(This work was presented in part at the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy [9, 17].)

MATERIALS AND METHODS

Antimicrobial agents. LB10522 and cefpirome were synthesized at Biotech Research Institute, LG Chemical Ltd. Commercially available antibiotics were obtained as reference compounds: ceftazidime (Glaxo), cefotaxime (Hoechst), ceftriaxone (Sigma), cefoperazone (Sigma), cephaloridine (Sigma), cefoxitin (Sigma), and cefsulodine (Sigma).

Test organisms. The bacterial strains used in this study were originally isolated from human clinical specimens. These were collected at several hospitals in Korea between 1991 and 1994. All isolates were stored frozen at −70°C.

Susceptibility tests. MICs of LB10522 against clinical isolates and β-lactamase-producing resistant strains were determined by the agar dilution method described by the National Committee for Clinical Laboratory Standards (15). Mueller-Hinton (MH) medium (Difco Laboratories, Detroit, Mich.) was used for testing aerobic and facultative organisms. For *Streptococcus pneumoniae* and *Streptococcus pyogenes*, MH broth was supplemented with 5% defibrinated sheep blood. Test strains were grown for 18 h in MH broth. These overnight cultures were diluted with the same fresh medium to a density of approximately 10^7 CFU/ml and applied to MH agar plates containing an antimicrobial agent serially

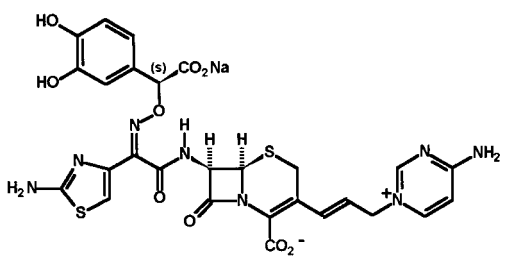

FIG. 1. Chemical structure of LB10522.

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TABLE 1. Comparative in vitro activities of LB10522 against clinical isolates[a]

Microorganism(s)[b] (no. of strains) and antimicrobial agent	MIC range	MIC_{50}	MIC_{90}
MSSA (80)			
LB10522	0.13–2	0.5	0.5
Cefpirome	0.063–16	1	2
Ceftazidime	0.13–128	8	8
Ceftriaxone	0.031–32	8	8
Cefoperazone	0.25–16	8	16
MRSA (88)			
LB10522	0.25–>128	128	>128
Cefpirome	0.063–>128	128	128
Ceftazidime	0.13–>128	>128	>128
Ceftriaxone	0.063–>128	>128	>128
Cefoperazone	2–>128	>128	>128
MSSE (61)			
LB10522	0.063–4	0.25	0.5
Cefpirome	0.25–4	0.5	2
Ceftazidime	4–32	8	16
Ceftriaxone	1–16	4	8
Cefoperazone	1–32	4	8
MRSE (87)			
LB10522	0.063–>128	8	128
Cefpirome	0.13–>128	4	128
Ceftazidime	1–>128	32	>128
Ceftriaxone	0.5–>128	32	>128
Cefoperazone	2–>128	16	>128
Streptococcus pyogenes (19)			
LB10522	≤0.008–≤0.008	≤0.008	≤0.008
Cefpirome	≤0.008–0.016	≤0.008	0.016
Ceftazidime	0.13–0.25	0.13	0.13
Ceftriaxone	0.016–0.031	0.016	0.031
Cefoperazone	0.063–0.13	0.13	0.13
Enterococcus faecalis (40)			
LB10522	2–>128	8	32
Cefpirome	8–>128	32	>128
Ceftazidime	128–>128	>128	>128
Ceftriaxone	>128–>128	>128	>128
Cefoperazone	32–>128	128	>128
Escherichia coli (151)			
LB10522	0.063–16	0.13	0.25
Cefpirome	0.016–16	0.13	0.25
Ceftazidime	0.063–128	0.25	1
Ceftriaxone	≤0.008–128	0.063	0.13
Cefoperazone	0.063–>128	4	32
Enterobacter cloacae (52)			
LB10522	0.13–64	2	32
Cefpirome	0.031–32	1	16
Ceftazidime	0.063–>128	8	64
Ceftriaxone	0.016–>128	8	>128
Cefoperazone	0.25–>128	16	>128
Enterobacter aerogenes (37)			
LB10522	0.25–32	0.25	4
Cefpirome	0.063–32	0.25	2
Ceftazidime	0.13–128	0.5	16
Ceftriaxone	0.063–128	0.13	32
Cefoperazone	0.5–>128	2	128
Citrobacter freundii (23)			
LB10522	0.063–8	0.5	4
Cefpirome	0.031–16	0.13	4
Ceftazidime	0.13–>128	0.5	128
Ceftriaxone	0.063–128	0.13	64
Cefoperazone	0.016–>128	2	128
Klebsiella pneumoniae (91)			
LB10522	0.063–16	0.25	4
Cefpirome	0.016–128	0.13	8
Ceftazidime	0.031–>128	0.25	32
Ceftriaxone	≤0.008–>128	0.063	32
Cefoperazone	0.063–>128	2	>128
Klebsiella oxytoca (49)			
LB10522	0.063–32	0.063	0.13
Cefpirome	0.016–64	0.031	0.063
Ceftazidime	0.031–>128	0.13	0.25
Ceftriaxone	≤0.008–128	0.063	0.063
Cefoperazone	0.063–>128	2	4
Proteus vulgaris (20)			
LB10522	0.031–2	0.063	0.5
Cefpirome	0.031–8	0.13	0.25
Ceftazidime	0.016–64	0.063	0.13
Ceftriaxone	≤0.008–8	0.016	0.063
Cefoperazone	1–64	2	8
Proteus mirabilis (26)			
LB10522	0.063–1	0.063	0.13
Cefpirome	0.063–1	0.13	0.13
Ceftazidime	0.031–8	0.063	0.13
Ceftriaxone	≤0.008–4	≤0.008	0.016
Cefoperazone	1–8	2	8
Serratia marcescens (104)			
LB10522	0.25–>128	1	8
Cefpirome	0.063–>128	0.25	8
Ceftazidime	0.063–>128	1	32
Ceftriaxone	0.13–>128	8	64
Cefoperazone	1–>128	32	>128
Morganella morganii (31)			
LB10522	0.25–32	0.5	8
Cefpirome	0.031–32	0.25	16
Ceftazidime	0.25–128	2	64
Ceftriaxone	0.031–128	0.25	64
Cefoperazone	4–>128	8	>128
Salmonella spp. (34)			
LB10522	0.031–4	0.063	0.13
Cefpirome	0.031–0.5	0.063	0.13
Ceftazidime	0.063–64	0.25	0.25
Ceftriaxone	0.031–0.25	0.063	0.13
Cefoperazone	0.5–>128	0.5	16
Yersinia spp. (8)			
LB10522	0.031–4	0.031	
Cefpirome	0.016–32	0.016	
Ceftazidime	0.031–128	0.063	
Ceftriaxone	≤0.008–64	0.016	
Cefoperazone	0.063–>128	0.13	
Pseudomonas aeruginosa (156)			
LB10522	0.063–128	0.5	2
Cefpirome	2–>128	32	64
Ceftazidime	1–128	8	32
Ceftriaxone	1–>128	128	>128

Continued

TABLE 1—*Continued*

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TABLE 1—*Continued*

Microorganism(s)[b] (no. of strains) and antimicrobial agent	MIC range	MIC_{50}	MIC_{90}
Cefoperazone	8–>128	128	>128
Stenotrophomonas maltophilia (26)			
LB10522	1–>128	128	>128
Cefpirome	0.063–>128	>128	>128
Ceftazidime	0.25–>128	128	>128
Ceftriaxone	1–>128	>128	>128
Cefoperazone	2–>128	>128	>128
Acinetobacter calcoaceticus (51)			
LB10522	0.25–>128	4	32
Cefpirome	0.13–>128	8	64
Ceftazidime	0.13–64	8	32
Ceftriaxone	0.031–>128	32	128
Cefoperazone	0.016–>128	>128	>128
Flavobacterium spp. (9)			
LB10522	0.13–>128	64	
Cefpirome	0.063–>128	32	
Ceftazidime	0.13–>128	32	
Ceftriaxone	0.063–>128	64	
Cefoperazone	0.5–>128	64	

[a] The data are in micrograms per milliliter.
[b] MSSA and MRSA, methicillin-susceptible and -resistant *Staphylococcus aureus*, respectively; MSSE and MRSE, methicillin-susceptible and -resistant *Staphylococcus epidermidis*, respectively.

diluted by use of an automatic MIC-2000 multipin inoculator (Dynatech Laboratories, Inc., Alexandria, Va.) to yield 10^4 CFU per spot. MIC was considered to be the lowest concentration that completely inhibited growth on agar plates, disregarding a single colony or a faint haze caused by the inoculum.

Time-kill study. Test organisms incubated in MH broth for 18 h at 35°C were diluted with fresh broth to approximately 10^5 CFU/ml, and the diluted cultures were preincubated for 2 h. Each drug was added to the cultures at the concentrations of 1/4, 1/2, 1, 2, and 4 times the MIC. Aliquots (100 μl) of the cultures were removed at 0, 2, 4, 6, and 24 h of incubation, and several dilutions were prepared in saline as needed. Drug carryover effects were reduced by 100-fold dilution of the sample with agar. The number of viable cells was determined on a drug-free MH agar plate. MBC was determined also by subculturing 100 μl of culture broth onto an antibiotic-free MH agar plate and defined as the lowest concentration which induced a >99.9% reduction in CFU after 18 h of incubation at 35°C (14).

In vivo activity. In vivo activity of LB10522 was determined against systemic infections in mice. Test organisms for infection were cultured on tryptic soy agar medium (Difco) at 35°C for 18 h and were suspended in gastric mucin (Difco). Male ICR mice (Biotech Research Institute) weighing 19 to 21 g in four groups of six mice were infected intraperitoneally with 0.5 ml of a bacterial suspension corresponding to an inoculum range of 5 to 10 times the minimal lethal dose of bacteria. Four dose levels were used for each antibiotic, depending on in vitro antimicrobial activity of the compounds. Mice were subcutaneously injected twice, at 1 and 4 h postinfection, with various dose regimens of antibiotics. Mortality was recorded for 7 days, and the median effective dose needed to protect 50% of mice (ED_{50}) was calculated by the method of Bliss (4). All untreated mice died within 2 days after infection.

Stability to β-lactamases. To determine β-lactamase stability, cell-free sonic extracts of β-lactamase-producing strains were prepared from mid-exponential-phase cultures in tryptone soy broth. The rates of hydrolysis of the drugs by several β-lactamases were assayed in a temperature-controlled UV spectrophotometer (Shimadzu UV-2000) at 35°C. All antibiotic solutions were prepared immediately before use in 50 mM sodium phosphate buffer, and the reaction was monitored at a wavelength which gave the maximum change in absorbance after hydrolysis. The stabilities of the drugs to hydrolysis by β-lactamases were expressed as the rate of hydrolysis relative to that of cephaloridine, which was assigned a value of 100%. The kinetic parameters, such as K_m, K_i, and V_{max}, of the compounds for TEM-9 β-lactamase were also determined from hydrolytic rates at various substrate concentrations by use of a Lineweaver-Burk plot. V_{max}/K_m can be taken as a measure of efficiency of hydrolysis, as defined by Pollock (18).

TABLE 2. Protective effect of LB10522 against systemic infections in mice

Microorganism (inoculum [CFU/mouse], mucin [%]) and compound[a]	MIC (μg/ml)	ED_{50} [mg/kg/dose] (95% confidence interval)
Staphylococcus aureus giorgio (5×10^7, 7.5)		
LB10522	0.5	0.7 (0.27–1.53)
Cefpirome	0.5	0.8 (0.25–2.31)
Ceftazidime	4	12.1 (5.10–25.30)
Streptococcus pneumoniae III (2.1×10^3, SS[b])		
LB10522	0.016	2.39 (0.00–9.03)
Cefpirome	0.016	4.16 (0.46–17.80)
Ceftazidime	0.13	39.10 (12.60–1070)
Streptococcus pyogenes 77A (2.8×10^4, SS)		
LB10522	≤0.008	1.47 (0.68–3.57)
Cefpirome	≤0.008	0.18 (0.09–0.33)
Ceftazidime	0.031	9.65 (4.56–94.10)
Pseudomonas aeruginosa 1912E (2×10^6, 7.5)		
LB10522	0.5	2.69 (0.86–5.91)
Cefpirome	4	10.9 (6.48–17.00)
Ceftazidime	1	8.42 (2.28–21.00)
Escherichia coli 851E (10^6, 5.0)		
LB10522	0.031	0.82 (0.34–1.68)
Cefpirome	0.031	2.02 (0.50–7.78)
Ceftazidime	0.063	1.46 (0.01–3.52)
Proteus mirabilis 1315E (5×10^7, 5.0)		
LB10522	0.13	1.43 (0.64–2.95)
Cefpirome	0.25	6.51 (—[c])
Ceftazidime	0.063	2.22 (0.54–40.3)
Serratia marcescens 1826E (5×10^7, 7.5)		
LB10522	0.25	0.16 (—)
Cefpirome	0.063	0.62 (0.01–1.07)
Ceftazidime	0.5	2.84 (0.41–30.6)
Morganella morganii 1375E (10^7, 5.0)		
LB10522	0.13	0.54 (0.11–4.10)
Cefpirome	0.031	0.35 (0.11–0.82)
Ceftazidime	0.063	4.00 (—)
Acinetobacter calcoaceticus Ac-54 (5×10^7, 7.5)		
LB10522	1	0.12 (0.05–0.24)
Cefpirome	4	0.97 (0.22–2.09)
Ceftazidime	4	1.43 (0.30–5.52)

[a] Subcutaneously administrated at 1 and 4 h after infection.
[b] SS, 0.9% saline solution instead of mucin.
[c] —, confidence limits could not be calculated.

Induction of β-lactamase. An overnight culture of *Enterobacter cloacae* 1194E was diluted 20-fold into 10 ml of fresh brain heart infusion broth (Difco) and incubated with shaking at 35°C. After 2.5 h of incubation (mid-log phase), test compounds were added as inducers. Incubation was continued for 2 h, and the cells were harvested and washed twice with 50 mM phosphate buffer (pH 7.0). The cells were suspended in the same buffer and disrupted with sonicator in ice water. Cell debris and unbroken cells were removed by centrifugation, and the resulting supernatant fluid was used as a crude enzyme. β-Lactamase activity was

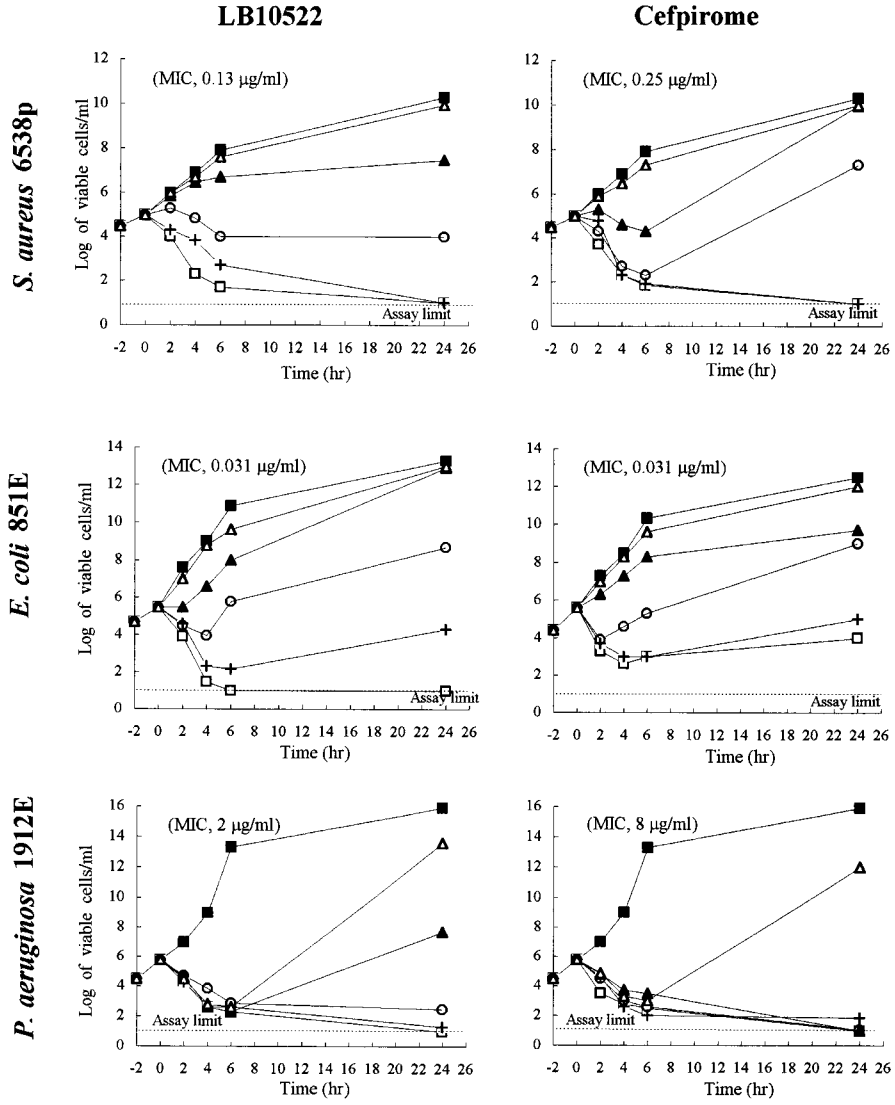

FIG. 2. Bactericidal activities of LB10522 and cefpirome against *Staphylococcus aureus* 6538p, *E. coli* 851E, and *P. aeruginosa* 1912E. ■, untreated control; △, 1/4× MIC; ▲, 1/2× MIC, ○, 1× MIC; +, 2× MIC; □, 4× MIC.

determined with 100 μM cephaloridine as the substrate (19). The protein concentration was estimated by the method of Bradford (4a) with bovine serum albumin as the standard.

RESULTS

In vitro antibacterial activity. In vitro activities of LB10522 against 1,243 clinical isolates were compared with those of cefpirome, ceftazidime, ceftriaxone, and cefoperazone. Table 1 summarized MIC ranges and MICs for 50 and 90% of the strains tested (MIC_{50}s and MIC_{90}s, respectively). LB10522 showed an extended broad spectrum of antibacterial activity against gram-positive and gram-negative bacteria including non-glucose-fermenting rods, *P. aeruginosa.*

Against methicillin-susceptible strains of *Staphylococcus aureus*, LB10522 with a MIC_{90} of 0.5 μg/ml was fourfold more active than cefpirome. LB10522 was also more active than the other cephalosporins tested against methicillin-susceptible *Staphylococcus epidermidis*, *Streptococcus pyogenes*, and *Enterococcus faecalis*. However, methicillin-resistant strains of *Staphylococcus aureus* and *Staphylococcus epidermidis* (MRSE) were highly resistant to all cephalosporins tested.

LB10522 was active against most members of the family *Enterobacteriaceae*, 90% of which were inhibited at the concentration of less than 0.5 μg/ml, except for *Enterobacter cloacae*, *Enterobacter aerogenes*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Serratia marcescens*, and *Morganella morganii*. Against *E. coli* and *Proteus mirabilis*, MIC_{90}s of LB10522 were 0.25 and 0.13 μg/ml, respectively, and were equal to those of cefpirome. Against *Proteus vulgaris*, LB10522 with a MIC_{90} of 0.5 μg/ml was twofold less active than cefpirome. LB10522 inhibited 90% of isolates in *Klebsiella oxytoca* and *Salmonella* spp. at the concentration of 0.13 μg/ml and was comparable to cefpirome in activity. However, *Enterobacter cloacae* was not well inhibited by the compounds tested. MIC_{90}s of LB10522, cefpirome, and ceftazidime against this species were 32, 16, and 64 μg/ml, respectively, but more than 50% of the strains were susceptible to LB10522 and cefpirome. Against *Enterobacter aerogenes*, *Citrobacter freundii*, and *Klebsiella pneu-*

TABLE 3. Stabilities of LB10522 and other cephalosporins to various β-lactamases

β-Lactamase (type[a])	Source	Relative rate of hydrolysis at 50 μM[b]				
		LB10522	Cefpirome	Ceftazidime	Ceftriaxone	Cefotaxime
TEM-3 (2be)	*Escherichia coli*	0.98	29	2.9	130	130
TEM-5 (2be)	*Escherichia coli*	20	20	14	26	25
TEM-7 (2be)	*Escherichia coli*	3.7	55	4.2	3.3	5
TEM-9 (2be)	*Escherichia coli*	40	65	76	32	21
FEC-1 (2e)	*Proteus vulgaris*	2.1	5.7	1.5	52	27
SHV-1 (2b)	*Klebsiella aerogenes*	0.41	2	NA[c]	0.65	0.77
P99 (1)	*Enterobacter cloacae*	0.057	0.21	0.0029	0.049	0.047
PCase I (2b)	*Bacillus cereus*	7.2	3.8	3.4	91	260
PCase II (2d)	*Bacillus cereus*	8.6	4.7	2.3	95	320
PCase III (2d)	*Enterobacter cloacae*	0.22	0.44	0.043	0.44	0.4
PCase IV (2c)	*Enterobacter cloacae*	NA	NA	NA	NA	NA

[a] According to reference 4b.
[b] The enzyme activity was determined by the spectrophotometric assay at 35°C and is expressed relative to the rate for cephaloridine, which was assigned a value of 100.
[c] NA, the rate of hydrolysis was too slow to determine.

moniae, MIC_{90} of LB10522 was 4 μg/ml. LB10522 was as active as cefpirome but 4- to 32-fold more active than ceftazidime, ceftriaxone, and cefoperazone. LB10522 had activity similar to that of cefpirome but was more active than the other cephalosporins against *Serratia marcescens* and *M. morganii*. The characteristic feature of LB10522 was its good activity against *P. aeruginosa*. MIC_{90} of LB10522 against *P. aeruginosa* was 2 μg/ml, and its activity was 16- to 64-fold better than those of the reference compounds. Against *Acinetobacter calcoaceticus*, the activity of LB10522 was similar to that of cefpirome but more potent than that of the other cephalosporins. *Stenotrophomonas maltophilia* and *Flavobacterium* spp. were highly resistant to all compounds tested.

Time-kill study. The bactericidal activity of LB10522 compared with that of cefpirome is shown in Fig. 2. LB10522, at concentrations of two and four times the MIC, had a rapid bactericidal activity against *Staphylococcus aureus* ATCC 6538p, *E. coli* 851E, and *P. aeruginosa* 1912E, and regrowth after 24-h incubation was not detected. This bactericidal effect was comparable to that of cefpirome. MBCs of LB10522 in MH broth were two to four times as high as MICs for all test strains.

In vivo activity in mice. The protective effects of LB10522 against systemic infections induced by nine different pathogenic strains are compared with those of cefpirome and ceftazidime in Table 2. LB10522 exhibited the most potent protective effects against systemic bacterial infections except for *Streptococcus pyogenes* and *M. morganii*. Against *Staphylococcus aureus* giorgio infection, the ED_{50}s of LB10522, cefpirome, and ceftazidime were 0.7, 0.8, and 12.1 mg/kg of body weight, respectively. Against *Streptococcus pneumoniae* III infection, LB10522 with an ED_{50} of 2.39 mg/kg was comparable to cefpirome (4.16 mg/kg) but 16-fold more active than ceftazidime (39.1 mg/kg). The ED_{50}s of LB10522, cefpirome, and ceftazidime against *Streptococcus pyogenes* 77A infection were 1.47, 0.18, and 9.65 mg/kg, respectively. LB10522 was less active than cefpirome, but it was more potent than ceftazidime. Against *P. aeruginosa* 1912E infection, LB10522 with an ED_{50} of 2.69 mg/kg was more active than cefpirome (10.9 mg/kg) and ceftazidime (8.42 mg/kg). Against *E. coli* 851E, the ED_{50} of LB10522 was 0.82 mg/kg, which was about half of those of cefpirome and ceftazidime. The ED_{50}s of LB10522, cefpirome, and ceftazidime against *Proteus mirabilis* 1315E were 1.43, 6.51, and 2.22 mg/kg, respectively. LB10522 (ED_{50}, 0.16 mg/kg) was also more active than cefpirome (0.62 mg/kg) and ceftazidime (2.84 mg/kg) against *Serratia marcescens* 1826E infection. Against *M. morganii* 1375E, the ED_{50}s of LB10522, cefpirome, and ceftazidime were 0.54, 0.35, and 4.00 mg/kg, respectively. LB10522 was slightly less active than cefpirome but eight times more active than ceftazidime. LB10522 with an ED_{50} of 0.12 mg/kg was more active than cefpirome (0.97 mg/kg) and ceftazidime (1.43 mg/kg) against *A. calcoaceticus* Ac-54 infection. These results showed that LB10522 had more potent in vivo activities than those expected from in vitro activities.

Stability to β-lactamases. The susceptibility of LB10522 to enzymatic hydrolysis was evaluated by measuring the relative hydrolysis rates for several types of β-lactamases. Table 3 shows that LB10522 was more stable than the reference compounds to hydrolysis by β-lactamases. The enzyme kinetic pa-

TABLE 4. Kinetic parameters of LB10522 and other cephalosporins for TEM9 β-lactamase

Antimicrobial agent	V_{max} (μmol/min/mg)	K_m (mM)	K_i (mM)	Physiological efficiency (V_{max}/K_m)	Relative rate of hydrolysis at 50 μM (μmol/min/mg)	MIC (μg/ml)
Nitrocefin	0.83	0.2		4.3	8	ND[a]
LB10522	2.45	0.086	0.096	29	40	2
Cefpirome	26	0.82	0.4	31	65	8
Ceftazidime	ND	ND	>2[b]	ND	227	>128
Ceftriaxone	1.1	0.02	0.056	56	32	4
Cefotaxime	0.74	0.027	0.11	27	21	2
Cephaloridine	9.4	0.16	0.47	59	100	32

[a] ND, not determined.
[b] Unmeasurable because of poor substrate affinity.

TABLE 5. Comparative activity of LB10522 against β-lactamase-producing strains

Microorganism	Enzyme	MIC (μg/ml)				
		LB10522	Cefpirome	Ceftazidime	Ceftriaxone	Cefoperazone
Staphylococcus aureus MS15009/I258	NI[a]	0.25	8	4	32	32
Citrobacter diversus 2046E	NI	0.13	1	0.5	16	128
Escherichia coli						
ML4901/Rms213	NI	0.5	0.13	4	4	8
ML1410 RGN14	TEM-1	0.016	0.031	0.063	0.031	1
1193E	TEM-1	0.031	0.063	0.25	0.063	8
ML1410 RGN823	TEM-2	0.031	0.13	0.25	0.031	16
3455E	TEM-3	0.13	2	8	8	16
3739E	TEM-5	0.5	0.5	8	2	8
3457E	TEM-7	0.13	4	16	0.25	0.5
2639E	TEM-9	2	8	>128	4	32
ML1410 RGN238	OXA-1	0.031	0.031	0.13	0.031	0.13
3140	TEM[b]	1	4	8	4	32
3151	TEM[b]	0.5	0.25	2	0.25	4
E182	TEM[b]	8	16	32	128	128
E191	TEM[b]	0.5	0.5	0.5	4	8
Enterobacter cloacae						
GN 7471	NI	64	16	>128	16	32
1194E	CHR IND+VE	4	2	128	>128	32
P99	P99	32	4	64	128	128
Klebsiella aerogenes						
1976E	SHV-1	0.13	0.25	0.25	0.13	32
1082E	K1+	0.063	1	0.5	8	>128
Proteus vulgaris						
GN 76	FEC-1	0.063	0.13	0.031	≤0.008	1
Pseudomonas aeruginosa						
GN 918	NI	0.25	8	4	64	32
Klebsiella pneumoniae						
4058	TEM[b]	2	8	32	8	128
4059	TEM[b]	0.13	0.031	0.13	0.031	0.5
F159	TEM[b]	4	2	128	8	64
F161	TEM[b]	4	16	64	32	128
F162	TEM[b]	4	2	8	16	32
F163	TEM[b]	4	2	16	32	32
F169	TEM[b]	4	8	64	64	64
F170	TEM[b]	4	2	128	16	64
Enterobacter aerogenes						
D108	TEM[b]	4	4	2	32	>128
D109	TEM[b]	0.5	2	2	16	128
D111	TEM[b]	8	2	2	16	>128

[a] NI, not identified.
[b] TEM β-lactamase from clinical isolates was identified by PCR.

rameters of the drugs for TEM-9 β-lactamase were determined by measuring K_i, V_{max}, and K_m (Table 4). The physiological efficiency (V_{max}/K_m) of LB10522 was similar to those of cefpirome and cefotaxime.

In vitro antibacterial activities of LB10522 against representative β-lactamase-producing bacterial strains are compared with those of the reference cephalosporins in Table 5. LB10522 had potent antibacterial activity against the β-lactamase-producing resistant strains. High stability of LB10522 to various β-lactamases is likely to be the major reason for its excellent activity against resistant strains.

Induction of β-lactamase. Figure 3 shows the activities of the enzyme induced in *Enterobacter cloacae* 1194E by LB10522 and reference compounds. Although most of the test compounds induced β-lactamase even at low concentrations below their MICs, LB10522 and ceftazidime did not induce β-lactamase at high concentrations. The induction pattern was dose dependent for the compounds. LB10522 was the weakest inducer among the compounds tested.

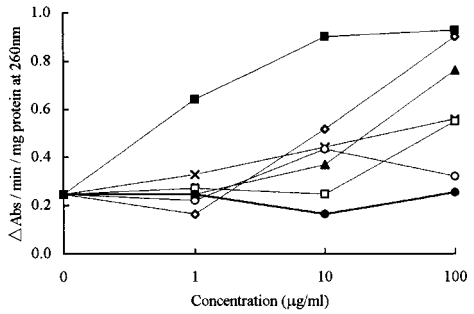

FIG. 3. Induction of β-lactamase in *Enterobacter cloacae* 1194E by LB10522 and other cephalosporins. β-Lactamase activity was determined by the spectrophotometric method measuring the decrease in absorbance (ΔAbs) in a temperature-controlled spectrophotometer at 30°C. Cephaloridine (100 μM) was used as a substrate. ■, cefotoxin; ◇, cephaloridine; ▲, cefpirome; ×, cefsulodine; □, ceftriaxone; ○, ceftazidime; ●, LB10522.

DISCUSSION

LB10522 is a new catecholic cephalosporin with antibacterial activity which is significantly better than those of currently available cephalosporins. This compound had a well-balanced and broad-spectrum antibacterial activity against gram-positive and gram-negative bacteria. Although most of the already known catecholic cephalosporins have weak activity against gram-positive bacteria, LB10522 showed more potent antibacterial activity against these strains than cefpirome. Against systemic infections in mice caused by various gram-positive and gram-negative bacteria, LB10522 showed an excellent protective effect, and its in vivo efficacy correlated well with in vitro activity. It has been reported that catecholic cephalosporins with a catechol moiety at the C-3 or C-7 position of the β-lactam ring can utilize the *tonB*-dependent iron transport system in addition to porin proteins to enter the bacterial periplasmic space (5, 8, 20, 21). This dual mode of entry may afford an increased potency during infections via the iron-deficient state in which the *tonB* gene product would initiate the active transport of iron-chelated catechols across the bacterial outer membrane. LB10522 maintained a relatively high concentration in plasma and showed a long half-life in plasma in animals (9). Thus, the excellent in vivo activity of LB10522 in mice may be attributable to its characteristic entry into bacterial cells in low-iron environments and its good pharmacokinetic profiles in animals. Compared with the broad-spectrum cephalosporins, LB10522 was more stable to hydrolysis by various clinically important β-lactamases such as TEMs, present in members of the family *Enterobacteriaceae*. Because of its high stability to various β-lactamases, LB10522 showed potent activities against β-lactamase-producing resistant bacteria, which are major reasons of resistance to β-lactam antibiotics.

The results in these studies, together with the preliminary safety study, suggest that LB10522 is a promising new parenteral cephalosporin and deserves further development because of its fascinating mechanism of action compared with other classical cephalosporins.

REFERENCES

1. **Arisawa, M., Y. Sekine, S. Shimizu, H. Takano, P. Angehrn, and R. L. Then.** 1991. In vitro and in vivo evaluation of Ro 09-1428, a new parenteral cephalosporin with high antipseudomonal activity. Antimicrob. Agents Chemother. **35:**653–659.
2. **Arnould, J. C., A. Bertrandie, T. G. C. Bird, D. Boucherot, F. Jung, J. J. Lohmann, and A. Olivier.** 1992. Synthesis and structure-activity relationship of cephalosporins with C-3′ catechol-containing residues. J. Med. Chem. **35:**2631–2642.
3. **Aszodi, J., D. Humbert, A. Bonnefoy, P. Collette, P. Mauvais, and J. F. Chantot.** 1994. RU 59863, a novel catechol-substituted vinylogous cephalosporin: *in-vitro* antibacterial activity, abstr. F99, p. 161. *In* Abstracts of the 34th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
4. **Bliss, C. I.** 1985. The statistics in bioassay. Academic Press, Inc., New York.

4a. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:**248–254.

4b. **Bush, K., G. A. Jacoby, and A. A. Medeiros.** 1995. A functional classification scheme for β-lactamases and its correlation with molecular structure. Antimicrob. Agents Chemother. **39:**1211–1233.

5. **Curtis, N. A. C., R. L. Eisenstadt, S. J. East, R. J. Cornford, L. A. Walker, and A. J. White.** 1988. Iron-regulated outer membrane proteins of *Escherichia coli* K-12 and mechanism of action of catechol-substituted cephalosporins. Antimicrob. Agents Chemother. **32:**1879–1886.
6. **Erwin, M. E., R. N. Jones, M. S. Barrett, B. M. Briggs, and D. M. Johnson.** 1991. In vitro evaluation of GR69153, a novel catechol-substituted cephalosporin. Antimicrob. Agents Chemother. **35:**929–937.
7. **Jones, R. N., and M. E. Erwin.** 1992. In vitro evaluation of Ro 09-1227, a novel catechol-substituted cephalosporin. Antimicrob. Agents Chemother. **36:**233–238.
8. **Hartmann, A., and V. Braun.** 1980. Iron transport in *Escherichia coli*: uptake and modification of ferrichrome. J. Bacteriol. **143:**246–255.
9. **Kim, M.-Y., H.-K. Song, J.-I. Oh, M.-K. Seo, Y.-H. Lee, I.-C. Kim, and J.-H. Kwak.** 1995. LB10522, a new catechol-substituted cephalosporin. II. β-Lactamase stabilities and biological activities, abstr. F42, p. 120. *In* Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
10. **Maejima, T., M. Inoue, and S. Mitsuhashi.** 1991. In vitro antibacterial activity of KP-736, a new cephem antibiotic. Antimicrob. Agents Chemother. **35:**104–110.
11. **Mochizuki, H., H. Yamada, Y. Oikawa, K. Murakami, J. Ishiguro, H. Kosuzume, N. Arizawa, and E. Mochida.** 1988. Bactericidal activity of M14659 enhanced in low-iron environments. Antimicrob. Agents Chemother. **32:** 1648–1654.
12. **Nakagawa, S., M. Sanada, K. Matsuda, T. Hashizume, Y. Asahi, R. Ushijima, N. Ohtake, and N. Tanaka.** 1989. In vitro and in vivo antibacterial activities of BO-1341, a new antipseudomonal cephalosporin. Antimicrob. Agents Chemother. **33:**1423–1427.
13. **Nakagawa, S., M. Sanada, K. Matsuda, N. Hazumi, and N. Tanaka.** 1987. Biological activity of BO-1236, a new antipseudomonal cephalosporin. Antimicrob. Agents Chemother. **31:**1100–1105.
14. **National Committee for Clinical Laboratory Standards.** 1992. Methods for determining bactericidal activity of antimicrobial agents. M26-T. National Committee for Clinical Laboratory Standards, Villanova, Pa.
15. **National Committee for Clinical Laboratory Standards.** 1993. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 3rd edition. Approved standard M7-A3. National Committee for Clinical Laboratory Standards, Villanova, Pa.
16. **Ogino, H., K. Iwamatsu, K. Katano, S. Nakabayashi, T. Yoshida, T. Tsuruoka, S. Inouye, and S. Kondo.** 1990. New aminothiazolylglycylcephalosporins with a 1,5-dihydroxy-4-pyridone-2-carbonyl group. I. Synthesis and biological activity of cephalosporin derivatives leading to M0703. J. Antibiotics **43:**174–188.
17. **Oh, J.-I., K.-S. Baek, H.-K. Song, M.-Y. Kim, Y.-Z. Kim, I.-C. Kim, and J.-H. Kwak.** 1995. LB10522, a new catechol-substituted cephalosporin. I. *In vitro* and *in vivo* antibacterial activities, abstr. F41, p. 120. *In* Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
18. **Pollock, M. R.** 1986. Purification and properties of penicillinase from two strains of *Bacillus licheniformis*: a chemical, physicochemical and physiological comparison. Biochem. J. **94:**666–675.
19. **Shinzo, K., S. Arai, S. Hayashi, and K. Fujimoto.** 1986. β-Lactamase stability of cefpirome (HR810), a new cephalosporin with a broad antimicrobial spectrum. Antimicrob. Agents Chemother. **30:**713–718.
20. **Silley, P., J. W. Griffiths, D. Monsey, and A. M. Harris.** 1990. Mode of action of GR69153, a novel catechol-substituted cephalosporin, and its interaction with the *tonB*-dependent iron transport system. Antimicrob. Agents Chemother. **34:**1806–1808.
21. **Watanabe, N.-A., T. Nagasu, K. Katsu, and K. Kitoh.** 1987. E-0702, a new cephalosporin, is incorporated into *Escherichia coli* cells via the *tonB*-dependent iron transport system. Antimicrob. Agents Chemother. **31:**497–504.