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In Vitro and In Vivo Activities of LJC10,627, a New Carbapenem with Stability to Dehydropeptidase I

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The activity of LJC10,627 was compared with the activities of imipenem and other antibiotics. LJC10,627 was more active against most members of the family *Enterobacteriaceae*, *Pseudomonas* spp., and *Acinetobacter* spp. but slightly less active than imipenem against staphylococci and streptococci. LJC10,627 showed stability to mouse dehydropeptidase I and was more effective in vivo than imipenem plus cilastatin against gram-negative bacterial infections and as effective against staphylococcal infections.

Several carbapenem antibiotics have been discovered over the past 10 years; however, to date only imipenem has been commercialized (3, 4, 9, 10). LJC10,627 (L-627) [(1*R*, 5*S*,6*S*)-2-[(6,7-dihydro-5*H*-pyrazolo[1,2-a][1,2,4,]triazolium-6-yl)]thio-6-[(*R*)-1-hydroxyethyl]-1-methyl-carbapenem-3-carboxylate] is a new carbapenem developed by Lederle (Japan), LTD., Tokyo, Japan (Fig. 1). The introduction of the methyl radical at the 1-β position of the carbapenem skeleton gives LJC10,627 stability against hydrolysis by kidney dehydropeptidase I, making the coadministration of a dehydropeptidase inhibitor unnecessary. Preliminary reports indicate that LJC10,627, like imipenem, exhibits potency against a broad bacterial spectrum, has favorable pharmacokinetic properties, and is stable to common β-lactamases, but unlike imipenem it is stable to hydrolysis by dehydropeptidase I (2, 8, 11).

This study compares the activity of LJC10,627 with those of imipenem, ciprofloxacin, amikacin, ceftazidime, and piperacillin against aerobic pathogens and with that of metronidazole against *Bacteroides fragilis* group isolates. The in vivo effectiveness of LJC10,627 was compared with that of the combination of imipenem plus cilastatin.

A total of 198 recent clinical isolates that included resistant strains was tested. MICs against aerobic bacteria were determined by broth microdilution using the methodology recommended by the National Committee for Clinical Laboratory Standards (6). The medium used was cation-supplemented Mueller-Hinton broth with a volume of 0.1 ml per well and an inoculum density of 5×10^5 CFU/ml. Mueller-Hinton broth supplemented with 5% defibrinated sheep blood was used for nonenterococcal streptococci. MICs were recorded after 18 h of incubation at 35°C in ambient air (6). Wilkins-Chalgren agar supplemented with 5% lysed sheep blood was used to test the *B. fragilis* group isolates (7). The plates were incubated at 35°C in an anaerobic chamber (Coy Laboratories, Ann Arbor, Mich.) for 48 h. The effects of inoculum and supplementation of the media with serum on the activities of the antibiotics were evaluated by using the microdilution methodology described above.

The stabilities of LJC10,627, imipenem, and imipenem plus cilastatin to dehydropeptidase I were determined by using a crude extract of mouse kidney dipeptidase. The extract was prepared by using a modification of the method of Campbell et al. (1). Aqueous homogenates of mouse kidney were extracted with butanol and then the enzyme protein was precipitated by cold acetone. The antibiotics (200 μl) were mixed with an equal volume of the crude enzyme preparation or H_2O to give a final antibiotic concentration of 0.5 μg/ml. The mixtures were incubated at 35°C, and 50 μl aliquots were withdrawn after 0, 1, 3, 5, 7, and 24 h. Biological activities of the various aliquots were assayed by an agar well diffusion method with *Escherichia coli* LL 300 as the indicator organism.

The therapeutic effects of LJC10,627 and imipenem plus cilastatin were determined against acute lethal systemic infections with gram-negative or gram-positive bacteria. Female mice (strain CD-1, 20 ± 2 g each; Charles River Laboratories) were challenged by intraperitoneal injection of bacteria suspended in Trypticase soy broth or 5% hog gastric mucin. Four to five antibacterial dose levels, in 0.5 ml of 0.2% aqueous agar, were administered subcutaneously 0.5 h after infection. The 7-day survival ratios from three separate tests (5 mice per dose level) were pooled for the estimation of the median effective dose by probit analysis (5).

The activities of the antibiotics against the 198 clinical isolates are summarized in Table 1. LJC10,627 was more active than imipenem, ceftazidime, piperacillin, and amikacin against most enteric organisms but was less active than ciprofloxacin. The MIC of LJC10,627 for 90% of the *Serratia marcescens* isolates tested was equal to that of imipenem and ceftazidime. LJC10,627 was four times more active than imipenem, two times less active than ciprofloxacin, and more active than the other beta-lactam antibiotics and amikacin against *Pseudomonas aeruginosa*. LJC10,627 and imipenem demonstrated greater activities than any of the antibiotics tested against *Acinetobacter* spp. Neither LJC10,627 nor imipenem exhibited activity against *Xanthomonas maltophilia*. Against the *B. fragilis* group, the activity of LJC10,627 was similar to those of imipenem and metronida-

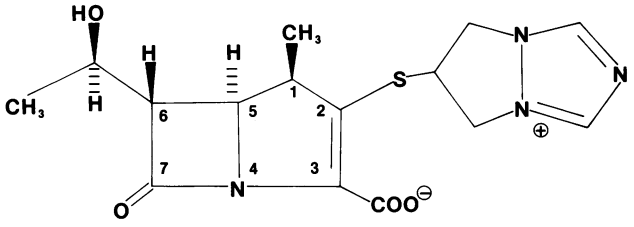

FIG. 1. Chemical structure of LJC10,627.

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TABLE 1. In vitro activities of LJC10,627 and comparative agents against 198 clinical isolates

Organism (no. tested)	Antibiotic	MIC (μg/ml)[a]		
		Range	50%	90%
Escherichia coli (10)	LJC10,627	0.03–0.25	0.03	0.25
	Imipenem	0.12–0.50	0.12	0.25
	Ciprofloxacin	≤0.015–0.03	≤0.015	0.03
	Amikacin	1.00–8.00	2.00	8.00
	Ceftazidime	≤0.12–0.25	≤0.12	0.25
	Piperacillin	2.00–>128.00	128.00	128.00
Klebsiella pneumoniae (10)	LJC10,627	0.06–1.00	0.12	0.25
	Imipenem	0.12–1.00	0.25	0.50
	Ciprofloxacin	≤0.015–0.50	0.03	0.06
	Amikacin	0.12–16.00	1.00	2.00
	Ceftazidime	≤0.12–1.00	0.25	1.00
	Piperacillin	0.50–>128.00	128.00	128.00
Enterobacter cloacae (10)	LJC10,627	≤0.015–0.50	0.12	0.50
	Imipenem	0.25–2.00	0.50	1.00
	Ciprofloxacin	≤0.015–0.12	0.03	0.06
	Amikacin	0.50–2.00	1.00	2.00
	Ceftazidime	0.25–>128.00	16.00	>128.00
	Piperacillin	128.00–>128.00	>128.00	>128.00
Enterobacter aerogenes (10)	LJC10,627	0.03–1.00	0.25	0.50
	Imipenem	0.50–2.00	1.00	2.00
	Ciprofloxacin	≤0.015–0.12	≤0.015	0.03
	Amikacin	1.00–16.00	2.00	16.00
	Ceftazidime	0.12–>128.00	16.00	128.00
	Piperacillin	2.00–>128.00	128.00	>128.00
Serratia marcescens (10)	LJC10,627	0.25–2.00	0.50	1.00
	Imipenem	0.50–2.00	0.50	1.00
	Ciprofloxacin	0.03–0.12	0.06	0.06
	Amikacin	0.50–8.00	1.00	4.00
	Ceftazidime	≤0.12–2.00	0.25	1.00
	Piperacillin	2.00–>128.00	128.00	>128.00
Proteus group (indole +) (10)	LJC10,627	0.12–4.00	1.00	2.00
	Imipenem	1.00–4.00	2.00	4.00
	Ciprofloxacin	≤0.015–0.25	≤0.015	0.25
	Amikacin	1.00–8.00	2.00	4.00
	Ceftazidime	≤0.12–128.00	2.00	64.00
	Piperacillin	64.00–>128.00	128.00	>128.00
Citrobacter diversus (10)	LJC10,627	0.03–0.06	0.06	0.06
	Imipenem	0.12–0.50	0.12	0.25
	Ciprofloxacin	≤0.015–0.06	≤0.015	0.03
	Amikacin	0.25–2.00	1.00	2.00
	Ceftazidime	0.12–64.00	0.50	2.00
	Piperacillin	8.00–>128.00	>128.00	>128.00
Citrobacter freundii (10)	LJC10,627	≤0.015–0.25	0.12	0.12
	Imipenem	0.50–1.00	0.50	1.00
	Ciprofloxacin	≤0.015–0.50	≤0.015	0.12
	Amikacin	0.12–2.00	1.00	2.00
	Ceftazidime	≤0.12–>128.00	128.00	>128.00
	Piperacillin	4.00–>128.00	>128.00	>128.00
Pseudomonas aeruginosa (10)	LJC10,627	0.25–2.00	0.50	1.00
	Imipenem	1.00–8.00	2.00	4.00
	Ciprofloxacin	0.06–1.00	0.12	0.50
	Amikacin	2.00–16.00	8.00	16.00
	Ceftazidime	2.00–128.00	4.00	64.00
	Piperacillin	2.00–>128.00	128.00	>128.00

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TABLE 1—*Continued*

Organism (no. tested)	Antibiotic	MIC (μg/ml)[a]		
		Range	50%	90%
Xanthomonas maltophilia (10)	LJC10,627	16.00–>16.00	>16.00	>16.00
	Imipenem	16.00–>16.00	>16.00	>16.00
	Ciprofloxacin	0.50–2.00	1.00	2.00
	Amikacin	2.00–>64.00	64.00	>64.00
	Ceftazidime	0.25–>128.00	16.00	32.00
	Piperacillin	64.00–>128.00	>128.00	>128.00
Acinetobacter calcoaceticus (10)	LJC10,627	≤0.015–0.25	0.06	0.25
	Imipenem	≤0.015–0.25	0.06	0.25
	Ciprofloxacin	≤0.015–16.00	0.25	16.00
	Amikacin	0.50–16.00	4.00	8.00
	Ceftazidime	≤0.12–64.00	2.00	8.00
	Piperacillin	≤0.12–>128.00	128.00	>128.00
Enterococcus spp. (14)	LJC10,627	1.00–8.00	2.00	4.00
	Imipenem	0.25–1.00	0.50	0.50
	Ciprofloxacin	0.50–4.00	0.50	4.00
	Amikacin	32.00–>64.00	>64.00	>64.00
	Ceftazidime	16.00–>128.00	>128.00	>128.00
	Piperacillin	0.50–16.00	2.00	8.00
Streptococcus pneumoniae (10)	LJC10,627	≤0.015–0.06	≤0.015	0.06
	Imipenem	≤0.015–0.03	≤0.015	0.03
	Ciprofloxacin	0.06–1.00	0.25	0.50
	Amikacin	2.00–64.00	32.00	64.00
	Ceftazidime	≤0.12–1.00	≤0.12	0.25
	Piperacillin	≤0.12–0.50	≤0.12	0.25
Staphylococcus aureus, oxacillin resistant (10)	LJC10,627	4.00–>16.00	>16.00	>16.00
	Imipenem	2.00–>16.00	>16.00	>16.00
	Ciprofloxacin	0.25–16.00	0.50	4.00
	Amikacin	2.00–>64.00	16.00	32.00
	Ceftazidime	64.00–>128.00	>128.00	>128.00
	Piperacillin	64.00–>128.00	128.00	>128.00
Staphylococcus aureus, oxacillin susceptible (10)	LJC10,627	0.06–0.25	0.06	0.12
	Imipenem	≤0.015–0.03	≤0.015	0.03
	Ciprofloxacin	0.50–1.00	0.50	1.00
	Amikacin	2.00–4.00	4.00	4.00
	Ceftazidime	8.00–16.00	8.00	16.00
	Piperacillin	4.00–64.00	16.00	64.00
Coagulase-negative staphylococci, oxacillin resistant (10)	LJC10,627	8.00–>16.00	>16.00	>16.00
	Imipenem	4.00–>16.00	>16.00	>16.00
	Ciprofloxacin	0.06–0.50	0.25	0.25
	Amikacin	1.00–64.00	8.00	32.00
	Ceftazidime	16.00–>128.00	64.00	>128.00
	Piperacillin	8.00–>128.00	64.00	>128.00
Coagulase-negative staphylococci, oxacillin susceptible (13)	LJC10,627	0.03–4.00	0.06	1.00
	Imipenem	≤0.015–1.00	≤0.015	0.03
	Ciprofloxacin	0.12–0.50	0.25	0.50
	Amikacin	0.12–8.00	2.00	8.00
	Ceftazidime	4.00–128.00	8.00	32.00
	Piperacillin	0.50–>128.00	16.00	>128.00
Bacteroides fragilis group (21)	LJC10,627	0.06–1.00	0.25	0.50
	Imipenem	≤0.015–0.50	0.12	0.50
	Metronidazole	≤0.12–0.50	0.25	0.50
	Piperacillin	1.00–>256.00	128.00	256.00

[a] 50% and 90%, MIC for 50 and 90% of isolates, respectively.

TABLE 2. In vitro activity of LJC10,627 against strains producing plasmid-mediated β-lactamases

Organism	Enzyme	MIC (μg/ml)					
		LJC10,627	Imipenem	Ciprofloxacin	Amikacin	Ceftazidime	Piperacillin
Escherichia coli	OXA-2	0.12	0.25	≤0.015	1.00	1.00	16.00
Escherichia coli	OXA-3	0.06	0.25	≤0.015	2.00	0.25	16.00
Escherichia coli	OXA-4	0.06	0.12	≤0.015	2.00	0.25	64.00
Pseudomonas aeruginosa	OXA-6	0.25	1.00	0.12	4.00	2.00	64.00
Escherichia coli	OXA-7	0.03	0.12	≤0.015	2.00	≤0.12	128.00
Pseudomonas aeruginosa	PSE-1	0.25	1.00	0.06	2.00	1.00	128.00
Pseudomonas aeruginosa	PSE-2	0.50	1.00	0.12	4.00	2.00	64.00
Pseudomonas aeruginosa	PSE-3	0.25	1.00	0.12	4.00	2.00	64.00
Pseudomonas aeruginosa	PSE-4	0.25	1.00	0.12	4.00	1.00	128.00
Pseudomonas aeruginosa	LCR-1	0.50	1.00	0.12	4.00	2.00	64.00
Pseudomonas aeruginosa	CARB-4	0.25	1.00	0.12	4.00	2.00	>128.00
Aeromonas spp.	AER-1	0.12	0.25	≤0.015	1.00	0.25	32.00
Escherichia coli	TEM-1	0.06	0.12	≤0.015	1.00	0.25	>128.00
Escherichia coli	TEM-2	0.12	0.25	≤0.015	1.00	1.00	>128.00
Escherichia coli	SHV-1	0.06	0.12	≤0.015	2.00	0.50	>128.00
Klebsiella pneumoniae	CTX-1	0.03	0.12	1.00	8.00	32.00	>128.00
Escherichia coli	HMS-1	0.03	0.06	≤0.015	1.00	0.25	>128.00

zole and greater than that of piperacillin. In general, LJC10,627 was less active than imipenem against most gram-positive isolates including oxacillin-susceptible *Staphylococcus* spp., *Streptococcus pyogenes*, *Streptococcus pneumoniae*, and *Enterococcus* spp. Imipenem was the most active agent tested against *Streptococcus* and *Enterococcus* spp., while LJC10,627 ranked second in potency against the streptococci. Oxacillin-resistant *Staphylococcus* spp. were inhibited only by ciprofloxacin.

The activity of LJC10,627 against strains containing various plasmid-mediated β-lactamases (kindly supplied by A. Medeiros, Miriam Hospital, Providence, R.I.) was compared with those of the other antibiotics (Table 2). LJC10,627 and imipenem exhibited good activities against these strains, including an isolate producing CTX-1, a cefotaxime-hydrolyzing enzyme. LJC10,627 showed better inherent activity (lower MICs) than imipenem against most of these strains.

On the basis of in vitro antimicrobial activity, LJC10,627 was not susceptible to hydrolysis by mouse kidney dehydropeptidase I when incubated in the presence of the enzyme for 24 h. In contrast, imipenem was partially inactivated within 1 h and totally inactivated after 3 h of incubation with the enzyme. Imipenem plus cilastatin was partially inactivated in 3 to 5 h and complete inactivation was observed after 7 h of incubation with the enzyme (Fig. 2).

The MICs of LJC10,627 and imipenem for one strain each of *Klebsiella pneumoniae*, *P. aeruginosa*, *Acinetobacter calcoaceticus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Enterococcus* spp. were not significantly affected (≤2 dilutions) when the inoculum size was increased from 10^5 to 10^7 CFU/ml. A pronounced inoculum effect (MICs increased by more than 3 dilutions) against *Serratia marcescens*, *Enterobacter cloacae*, and *E. coli* was observed for both antibiotics. A similar effect was also observed for LJC10,627, but not imipenem, against a strain of *Enterobacter aerogenes*. Supplementation of Mueller-Hinton II broth with 50% inactivated normal human serum did not affect the activity of LJC10,627 or imipenem except against a strain of *Serratia marcescens*, with the MICs of LJC10,627 and imipenem increasing by 2 and 3 dilutions, respectively.

LJC10,627 was very effective in protecting mice against infections with *Staphylococcus aureus*, *E. coli* and *P. aeruginosa* (Table 3). Compared with the activity of imipenem plus cilastatin, LJC10,627 was two times more potent against *E. coli* 311 and *P. aeruginosa* 12-4-4. The therapeutic effect of LJC10,627 was comparable to that of imipenem plus cilastatin against an infection caused by *Staphylococcus aureus* Smith, with a median effective dose for both of 0.03 mg/kg.

Our results indicate that LJC10,627 is a very effective antibacterial agent with a broad spectrum of activity. It is more active in vitro than imipenem against most members of

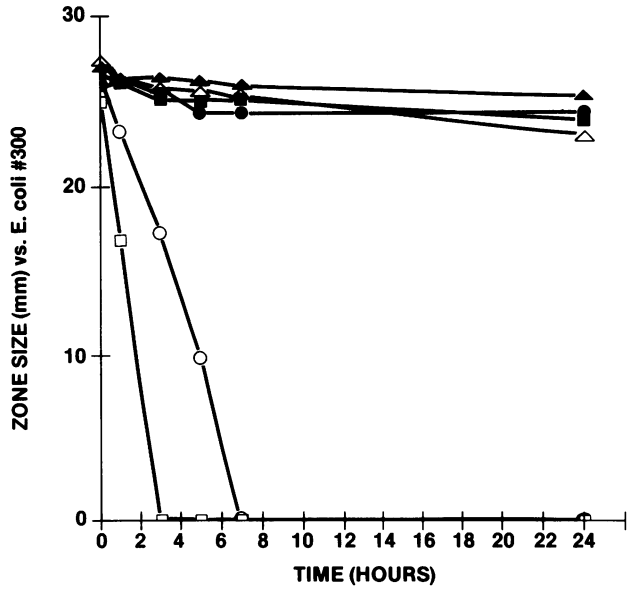

FIG. 2. Comparison of the susceptibilities to hydrolysis of LJC10,627, imipenem, and imipenem plus cilastatin (Primaxin) by mouse kidney dehydropeptidase I enzyme. △, LJC10,627 plus enzyme; ▲, LJC10,627 plus H_2O; □, imipenem plus enzyme; ■, imipenem plus H_2O; ○, Primaxin plus enzyme; ●, Primaxin plus H_2O.

TABLE 3. Protective effects of LJC10,627 against systemic infections in mice

Organism	Challenge dose	Antibiotic	ED_{50}[a] (95% confidence limit)	MIC (μg/ml)
Staphylococcus aureus Smith	1.4×10^6 (5% mucin)	LJC10,627	0.029 (0.023–0.036)	0.03
		Imipenem[b]	0.027 (0.022–0.034)	≤0.015
Escherichia coli 311	2.4×10^6	LJC10,627	0.26 (0.19–0.35)	0.03
		Imipenem	0.52 (0.39–0.70)	0.12
Pseudomonas aeruginosa 12-4-4	8.6×10^5 (5% mucin)	LJC10,627	0.17 (0.14–0.22)	0.25
		Imipenem	0.29 (0.23–0.36)	0.5

[a] ED_{50}, 50% Effective dose, probit method. Values are given in milligrams per kilogram.
[b] 50% Effective doses were determined with imipenem plus cilastatin (Primaxin). MICs were determined with imipenem standard powder.

the family *Enterobacteriaceae* and *P. aeruginosa*. It is slightly less active than imipenem against gram-positive aerobic cocci. An inoculum effect was observed with selected strains of *Serratia marcescens*, *Enterobacter* spp., and *E. coli*. Its activity was not significantly altered by supplementation of the medium with serum. The in vivo effectiveness of this agent mirrored its in vitro activity. LJC10,627 was more effective than imipenem plus cilastatin in protecting mice against acute lethal infections with *E. coli* and *P. aeruginosa* and as effective against infection with *Staphylococcus aureus*. LJC10,627 was less susceptible to hydrolysis by mouse kidney dehydropeptidase I than imipenem, thus offering in vivo protection as a single agent and not requiring the addition of a dehydropeptidase inhibitor. This new carbapenem merits further in vitro and in vivo investigation as well as pharmacokinetics studies to determine its clinical potential.

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