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In Vitro Activity of LJC10,627, a New Carbapenem Antibiotic with High Stability to Dehydropeptidase I

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The in vitro activity of LJC10,627, a new carbapenem, was compared with those of imipenem and ceftazidime. LJC10,627 had broad-spectrum activity against gram-positive and gram-negative clinical isolates. The MICs of this compound for 90% of members of the family *Enterobacteriaceae* tested (MIC_{90}s), including strains resistant to ceftazidime, ranged from 0.1 to 25 μg/ml. LJC10,627 inhibited *Pseudomonas aeruginosa* at an MIC_{90} of 3.13 μg/ml; it thus was twofold more active than imipenem. This compound inhibited *Haemophilus*, *Neisseria*, and *Branhamella* species at MIC_{90}s of 3.13, 0.1, and 0.1 μg/ml, respectively. LJC10,627 was two- to fourfold less active than imipenem against methicillin-susceptible *Staphylococcus aureus* and *Staphylococcus epidermidis* at MIC_{90}s of 0.1 and 0.39 μg/ml. However, the compound was found to be twofold more active than imipenem against *Bacteroides fragilis* at an MIC_{90} of 1.56 μg/ml. LJC10,627 was very stable to various β-lactamases except for *Xanthomonas maltophilia* oxyiminocephalosporinase type II. LJC10,627 was minimally hydrolyzed by swine renal dehydropeptidase I; its residual activity was 93.0% after 2 h. Killing kinetics of this compound for *Escherichia coli* and *Pseudomonas aeruginosa* showed that bactericidal action occurred at concentrations above the MIC (0.05 and 0.39 μg/ml, respectively). LJC10,627 had a high affinity for penicillin-binding proteins 2, 4, and 1B(s) of *Escherichia coli* and *Pseudomonas aeruginosa* and penicillin-binding proteins 1 and 4 of *Staphylococcus aureus*.

Imipenem, a carbapenem compound, was developed as an antibiotic for treatment of severe infections caused by *Pseudomonas aeruginosa* or members of the family *Enterobacteriaceae* (4, 6). Although this compound is very active against these pathogens, it is hydrolyzed by dehydropeptidases present in renal tissue (3). LJC10,627 {(4*R*, 5*S*, 6*S*)-3-[(6,7-dihydro-5*H*-pyrazolo[1,2-*a*][1,2,4]triazol-4-ium-6-yl)thio]-6-[(*R*)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo(3,2,0)hept-2-ene-2-carboxylate} is a new carbapenem antibiotic with a methyl radical introduced at the 1β site and a triazolium radical introduced at the side chain of the second site (Fig. 1).

In this report we describe the in vitro antimicrobial activity, stability to β-lactamases, stability to dehydropeptidase I (DHP-I), bactericidal activity, and affinity for penicillin-binding proteins (PBPs) of LJC10,627 and the morphological changes of bacterial cells following exposure to this compound.

(This work was presented at the 29th Interscience Conference on Antimicrobial Agents and Chemotherapy, September 1989, Houston, Tex. [M. Hikida, M. Yoshida, K. Nishiki, Y. Furukawa, K. Ubukata, M. Konno, and S. Mitsuhashi, Program Abstr. 29th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 221, 1989].)

MATERIALS AND METHODS

Antibiotics. LJC10,627 was synthesized as a sodium salt at the Chemical and Formulation Laboratories, Lederle (Japan) Ltd., Saitama, Japan. Standard laboratory antibiotics of imipenem and ceftazidime were provided by Banyu Pharmaceuticals Co. Ltd., Tokyo, Japan, and Nippon Glaxo Co. LTd., Tokyo, Japan, respectively. LJC10,627 and ceftazidime were dissolved in a 1/15 M phosphate buffer (pH 7.0). Imipenem was dissolved in a 1/10 M 3-(*N*-morpholino)propanesulfonic acid (MOPS) buffer (pH 7.0) on the day of use.

Organisms. Clinical isolates that have been collected since 1988 at Teikyo University Hospital were mainly used for susceptibility testing. For strains of some species that were not present among these clinical isolates, clinical isolates collected at the Episome Institute from all over Japan were used. These included methicillin-resistant staphylococci, ceftazidime-resistant members of the family *Enterobacteriaceae*, and *Pseudomonas* spp. Each strain was stored at −80°C in a mixture of glycerol and heart infusion broth (Eiken Chemical Co., Ltd., Tokyo, Japan) until use.

Antimicrobial susceptibility tests. Antimicrobial activity was measured in Mueller-Hinton (MH) agar (Eiken Chemical Co., Ltd.) by an agar dilution method. The susceptibilities of streptococci were determined with MH agar supplemented with 10% sheep blood, and those of *Haemophilus* and *Neisseria* species were determined with chocolate MH agar in the presence of 5% CO_2. For *Bacteroides fragilis*, GAM agar (Eiken Chemical Co., Ltd.), which was incubated in jars (GasPak; BBL Microbiology Systems, Cockeysville,

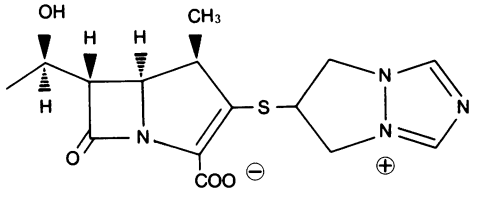

FIG. 1. Structure of LJC10,627 {(4*R*,5*S*,6*S*)-3-[(6,7-dihydro-5*H*-pyrazolo[1,2-*a*] [1,2,4]triazol-4-ium-6-yl)thio]-6-[(*R*)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo(3,2,0)hept-2-ene-2-carboxylate}.

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TABLE 1. Comparative in vitro activities of LJC10,627 and other antimicrobial agents against gram-negative organisms

Organism (no. of isolates)	Antimicrobial agent	MIC (μg/ml)[a]		
		Range	50%	90%
Escherichia coli (144)	LJC10,627	0.02–0.1	0.05	0.1
	Imipenem	0.1–0.2	0.2	0.2
	Ceftazidime	0.02–0.78	0.1	0.2
Klebsiella pneumoniae (92)	LJC10,627	0.05–0.78	0.2	0.78
	Imipenem	0.1–1.56	0.2	0.78
	Ceftazidime	0.02–0.78	0.2	0.39
Klebsiella oxytoca (28)	LJC10,627	0.05–0.78	0.2	0.78
	Imipenem	0.2–0.78	0.39	0.78
	Ceftazidime	0.02–0.78	0.1	0.2
Enterobacter aerogenes (19)	LJC10,627	0.05–0.78	0.39	0.78
	Imipenem	0.2–0.78	0.78	0.78
	Ceftazidime	0.1–100	0.39	100
Enterobacter cloacae (54)	LJC10,627	0.05–0.78	0.1	0.39
	Imipenem	0.1–1.56	0.39	0.78
	Ceftazidime	0.1–100	0.39	100
Citrobacter freundii (95)	LJC10,627	0.02–0.78	0.1	0.39
	Imipenem	0.1–1.56	0.2	0.78
	Ceftazidime	0.1–>100	0.39	100
Serratia marcescens (99)	LJC10,627	0.39–50	3.13	25
	Imipenem	0.78–50	3.13	12.5
	Ceftazidime	0.2–>100	3.13	>100
Proteus mirabilis (101)	LJC10,627	0.1–6.25	0.78	3.13
	Imipenem	0.39–12.5	1.56	6.25
	Ceftazidime	0.01–3.13	0.05	0.1
Proteus vulgaris (95)	LJC10,627	0.2–3.13	1.56	1.56
	Imipenem	0.2–3.13	1.56	3.13
	Ceftazidime	0.05–1.56	0.1	0.2
Morganella morganii (68)	LJC10,627	0.39–3.13	1.56	1.56
	Imipenem	0.78–6.25	3.13	6.25
	Ceftazidime	0.1–25	0.39	12.5
Pseudomonas aeruginosa (116)	LJC10,627	0.2–25	0.78	3.13
	Imipenem	0.39–50	1.56	6.25
	Ceftazidime	0.1–>100	3.13	12.5
Pseudomonas cepacia (45)	LJC10,627	0.39–3.13	1.56	3.13
	Imipenem	0.78–6.25	3.13	6.25
	Ceftazidime	0.39–3.13	0.78	1.56
Acinetobacter calcoaceticus (35)	LJC10,627	0.05–0.2	0.1	0.2
	Imipenem	0.02–0.39	0.2	0.2
	Ceftazidime	1.56–25	6.25	12.5
Haemophilus influenzae, ampicillin susceptible (75)	LJC10,627	0.39–3.13	0.78	3.13
	Imipenem	0.39–6.25	1.56	3.13
	Ceftazidime	0.05–0.39	0.1	0.2
Haemophilus influenzae, ampicillin resistant (12) (≥6.25 μg/ml)	LJC10,627	0.39–12.5	0.78	3.13
	Imipenem	0.39–25	1.56	3.13
	Ceftazidime	0.05–0.39	0.1	0.2
Branhamella catarrhalis (41)	LJC10,627	0.01–0.1	0.05	0.1
	Imipenem	0.01–0.2	0.02	0.1
	Ceftazidime	0.01–0.1	0.05	0.1
Neisseria gonorrhoeae, ampicillin susceptible (19)	LJC10,627	≤0.006–0.1	0.02	0.1
	Imipenem	≤0.006–0.2	0.1	0.2
	Ceftazidime	≤0.006–0.39	0.02	0.2
Neisseria gonorrhoeae, ampicillin resistant (7) (≥6.25 μg/ml)	LJC10,627	≤0.006–0.1	0.05	0.1
	Imipenem	0.01–0.1	0.1	0.1
	Ceftazidime	0.01–0.05	0.01	0.05

[a] 50% and 90%, MICs for 50 and 90% of strains tested, respectively.

TABLE 2. Comparative in vitro activities of LJC10,627 and other antimicrobial agents against gram-positive organisms

Organism (no. of isolates)	Antimicrobial agents	MIC (μg/ml)[a]		
		Range	50%	90%
Staphylococcus aureus, methicillin susceptible (44)	LJC10,627	0.02–0.78	0.05	0.1
	Imipenem	0.01–0.1	0.01	0.02
	Ceftazidime	6.25–50	6.25	12.5
Staphylococcus aureus, methicillin resistant (71)	LJC10,627	1.56–100	25	50
	Imipenem	0.1–100	12.5	50
	Ceftazidime	100–>100	>100	>100
Staphylococcus epidermidis, methicillin susceptible (68)	LJC10,627	0.02–1.56	0.05	0.39
	Imipenem	0.01–0.39	0.02	0.1
	Ceftazidime	3.13–>100	6.25	25
Staphylococcus epidermidis, methicillin resistant (44)	LJC10,627	0.2–100	3.13	50
	Imipenem	0.05–100	1.56	50
	Ceftazidime	6.25–>100	25	>100
Streptococcus pyogenes (46)	LJC10,627	≤0.006–0.05	≤0.006	≤0.006
	Imipenem	≤0.006–0.02	≤0.006	≤0.006
	Ceftazidime	0.05–3.13	0.1	0.1
Streptococcus agalactiae (32)	LJC10,627	0.02–0.05	0.02	0.05
	Imipenem	0.01–0.02	0.01	0.02
	Ceftazidime	0.39–0.78	0.39	0.78
Streptococcus pneumoniae (29)	LJC10,627	≤0.006–0.02	0.01	0.01
	Imipenem	≤0.006–0.01	≤0.006	0.01
	Ceftazidime	0.2–0.78	0.2	0.39
Enterococcus faecalis (95)	LJC10,627	0.78–25	3.13	3.13
	Imipenem	0.39–6.25	0.78	1.56
	Ceftazidime	12.5–>100	100	>100
Bacteroides fragilis (27)	LJC10,627	0.1–3.13	0.39	1.56
	Imipenem	0.2–25	0.78	3.13
	Ceftazidime	1.56–>100	25	>100

[a] 50% and 90%, MICs for 50 and 90% of strains tested, respectively.

Md.) at 35°C for 48 h, was used. Overnight cultures in MH broth were diluted in broth to give a final inoculum of 10^4 CFU per spot. All of the agar plates were incubated at 37°C for 24 h except for those containing staphylococci, which were incubated at 32°C for 48 h. The MIC was defined as the lowest concentration of antibiotic that inhibited visible microbial growth on the agar. All assays were run with the following control strains: *Escherichia coli* NIHJ JC1, *Staphylococcus aureus* FDA 209P, and *Pseudomonas aeruginosa* NCTC 10490.

TABLE 3. Stabilities of LJC10,627, imipenem, and ceftazidime to β-lactamases

Enzyme source	Type[a]	Relative rate of hydrolysis[b]		
		LJC10,627	Imipenem	Ceftazidime
Escherichia coli ML4901(Rms212)	PCase type 1	0.02	0.02	<0.01
Escherichia coli ML4901(Rms213)	PCase type II	0.24	0.23	0.04
Escherichia coli ML4901(Rms139)	PCase type IV	<0.01	0.05	<0.01
Staphylococcus aureus ML15009(PI258)	PCase type V	<0.01	<0.01	<0.01
Escherichia coli GN5482	CSase	0.02	0.07	0.05
Enterobacter cloacae GN7471	CSase	0.07	0.05	0.02
Citrobacter freundii GN7391	CSase	<0.01	0.01	<0.01
Serratia marcescens GN10857	CSase	0.23	0.27	0.08
Pseudomonas aeruginosa GN10362	CSase	0.63	0.82	<0.01
Xanthomonas maltophilia GN12873	CXase type I	0.13	0.13	2.03
Xanthomonas maltophilia GN12873	CXase type II	18.4	44.3	2.06

[a] Abbreviations: PCase, penicillinase; CSase, cephalosporinase; CXase, oxyiminocephalosporinase.
[b] Relative rates of hydrolysis were calculated on benzylpenicillin (penicillin G) as 100 for penicillinases and oxyiminocephalosporinase type II and on cephaloridine as 100 for cephalosporinases and oxyiminocephalosporinase type I.

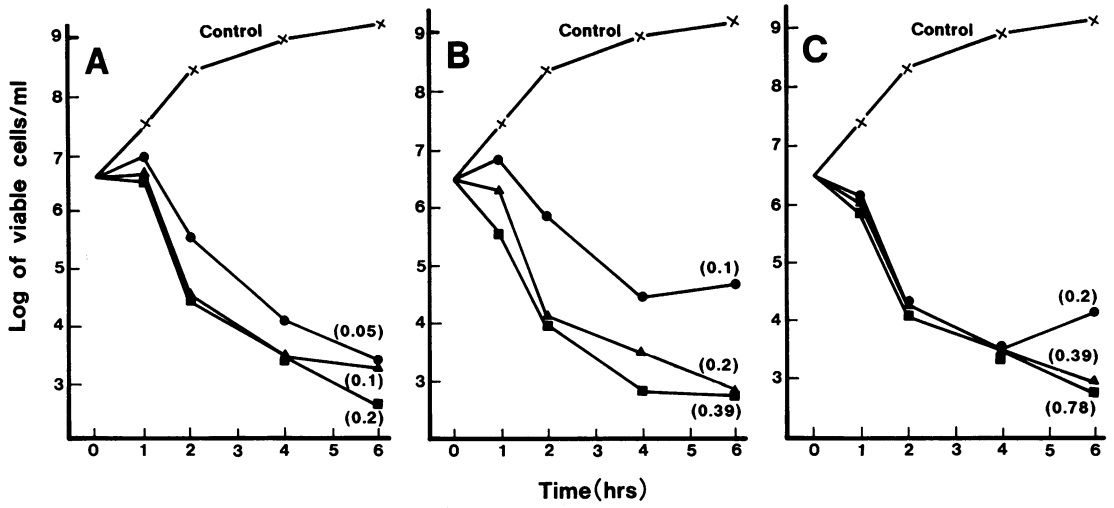

FIG. 2. Bacterial killing kinetics curves of LJC10,627 (A), imipenem (B), and ceftazidime (C) at the MICs (●), 2× MICs (▲), and 4× MICs (■) against *Escherichia coli* TK2241. Numbers in parentheses refer to the concentration of antibiotics.

β-Lactamase assays. β-Lactamases from various strains were prepared by a previously described method (10). The stability of each compound for various β-lactamases was determined by a spectrophotometric assay (11). The molecular absorbancy difference (Δε) and the specific wavelength for LJC10,627 were 5.73/mM per cm and 293 nm, respectively. The relative rate of hydrolysis was determined as an initial rate at a concentration of 100 μM of each compound.

Stability to renal DHP-I. The stability of LJC10,627 and imipenem to renal DHP-I was measured with a purified enzyme extracted from swine kidney cortex (1). As a substrate, each compound was adjusted to give a final concentration of 50 μg/ml and was then added to the enzyme solution in 50 mM MOPS buffer (pH 7.2). The reaction mixture was incubated at 35°C for 2 h and then diluted with an equal volume of methanol. The residual antibiotic activity in the supernatant after centrifugation at 1,000 × *g* for 20 min was determined by a bioassay method by using *Staphylococcus aureus* Terajima. Standard curves were calculated by using inactivated enzyme as a control.

Killing kinetics. Bacterial killing by LJC10,627 and comparative compounds was determined at concentrations equal to the respective MIC, 2× MIC, and 4× MIC. A total of 1 ml of bacteria cultured overnight in MH broth was inoculated into 9 ml of fresh broth and then incubated on a shaker at 37°C for 2 h. After dilution in MH broth to obtain a final inoculum of 10^6 CFU/ml, 100 μl of the bacterial suspension was inoculated into 9.9 ml of MH broth containing each of the antibiotics to be tested. The tubes were then incubated without shaking, and samples were removed at timed intervals. To eliminate antibiotic carry-over, 1 ml of each of the samples was centrifuged at 2,000 × *g* for 10 min. The sedimented cells were suspended in the original volume of MH broth and serially diluted 10-fold with broth, and then 100 μl of each of the samples was plated in triplicate on MH agar. The plates were incubated overnight at 37°C, and the number of colonies that grew were counted.

Phase-contrast microscopy. A 0.5-ml sample of an overnight culture was inoculated into 9.5 ml of MH broth and cultured with shaking at 37°C for 2 h. The cells were diluted

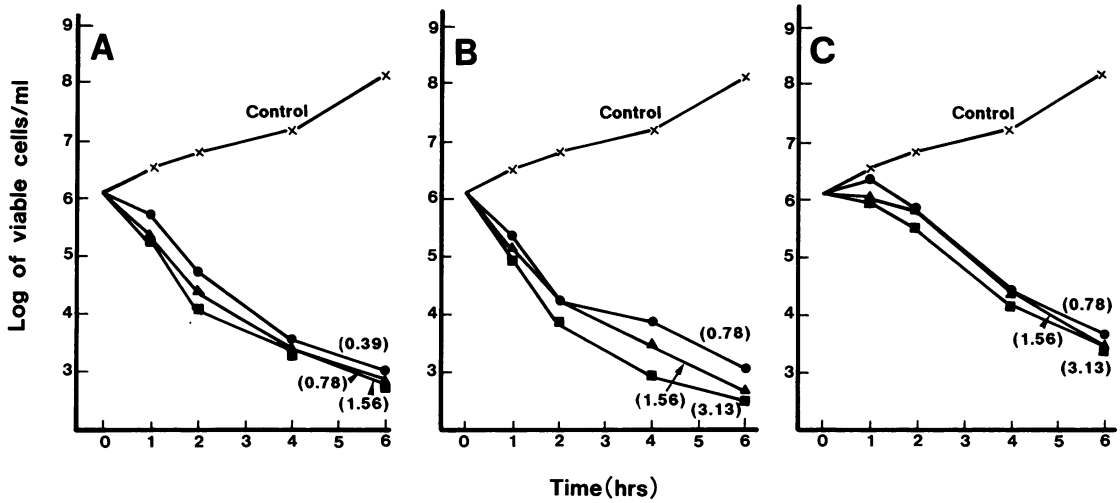

FIG. 3. Bacterial killing kinetics curves of LJC10,627 (A), imipenem (B), and ceftazidime (C) at the MICs (●), 2× MICs (▲), and 4× MICs (■) against *Pseudomonas aeruginosa* TK2306.

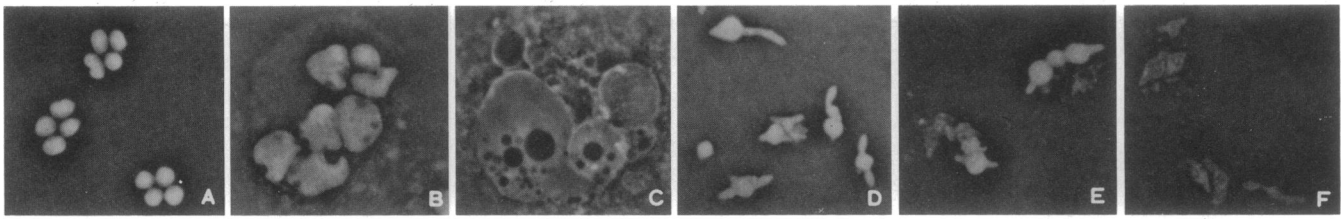

FIG. 4. Morphological changes of cells of *Escherichia coli* TK2241 (A through C) and *Pseudomonas aeruginosa* TK2306 (D through F) when they were exposed to the MIC of LJC10,627. Observation was performed by phase-contrast microscopy. Times of exposure to LJC10,627 were as follows: 1 h (A), 3 h (B), 6 h (C), 1 h (D), 3 h (E), and 6 h (F).

with broth to 10^6 CFU/ml, and 5 μl of the dilution was seeded onto glass slides coated with 200 μl of MH agar containing various concentrations of LJC10,627, imipenem, or ceftazidime. The glass slides were covered with cover slips and then incubated at 37°C. Observations was made under ×1,000 magnification with a phase-contrast microscope (MD1; Nikon Co. Ltd., Tokyo, Japan).

Affinity for PBPs. Affinities of LJC10,627, imipenem, and ceftazidime for PBPs of *Escherichia coli* JE1011, *Pseudomonas aeruginosa* NCTC 10490, and *Staphylococcus aureus* FDA 209P were determined by a previously described method (9). The relative affinity of each compound for PBPs was determined as follows. Membrane fractions were preincubated at 30°C for 10 min with 5 μl of nonradioactive compounds diluted to various concentrations and postincubated with 5 μl of 1 mM [^{14}C]benzylpenicillin for another 10 min.

RESULTS

Antimicrobial activities. Table 1 shows the in vitro activity of LJC10,627 against gram-negative aerobes compared with those of imipenem and ceftazidime. The MICs of LJC10,627 for 90% of the strains (MIC_{90}) of *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter aerogenes*, *Enterobacter cloacae*, and *Citrobacter freundii* were ≦0.78 μg/ml; it was thus twofold more active than imipenem. The MIC_{90} of LJC10,627 for *Serratia marcescens* was 25 μg/ml, which was twofold greater than that of imipenem but fourfold lower than that of ceftazidime. LJC10,627 was about twofold more active than imipenem against *Proteus mirabilis*, *Proteus vulgaris*, and *Morganella morganii* at MIC_{90}s of 3.13, 1.56, and 1.56 μg/ml, respectively, but 8- to 32-fold less active than ceftazidime. LJC10,627 also inhibited clinical isolates of *Enterobacter aerogenes*, *Enterobacter cloacae*, and *Citrobacter freundii* resistant to ceftazidime at >100 μg/ml at MICs similar to those for ceftazidime-susceptible strains.

At an MIC_{90} of 3.13 μg/ml, LJC10,627 was twofold more active than imipenem against *Pseudomonas aeruginosa* and *Pseudomonas cepacia*. In addition, it also inhibited *Pseudomonas aeruginosa* isolates resistant to ceftazidime at >100 μg/ml at an MIC of 25 μg/ml. LJC10,627 and imipenem were equally active against *Acinetobacter calcoaceticus* at MIC_{90}s of 0.2 μg/ml, which made them 64-fold less active than ceftazidime. LJC10,627 was active against ampicillin-resistant and ampicillin-susceptible *Haemophilus influenzae* strains at MIC_{90}s of 3.13 μg/ml, which were almost equal to those of imipenem but about eightfold greater than those of ceftazidime. Penicillinase-producing and nonproducing *Neisseria gonorrhoeae* and *Branhamella catarrhalis* strains were inhibited by LJC10,627 at an MIC_{90} of 0.1 μg/ml, which was equivalent to that of imipenem.

Table 2 shows the comparative in vitro activities of LJC10,627 and other antimicrobial agents against gram-positive organisms. LJC10,627 inhibited methicillin-susceptible *Staphylococcus aureus* and *Staphylococcus epidermidis* at MIC_{90}s of 0.1 and 0.39 μg/ml, respectively; these values were 4-fold greater than those of imipenem but about 128-fold lower than those of ceftazidime. However, LJC10,627 and imipenem exhibited low activities at MIC_{90}s of 50 μg/ml against methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*. Ceftazidime had even lower activity against methicillin-resistant staphylococci. The MIC_{90}s of LJC10,627 against *Streptococcus pyogenes*, *Streptococcus agalactiae*, and *Streptococcus pneumoniae* were ≦0.006, 0.05, and 0.01 μg/ml, respectively. These values were almost equal to those of imipenem and 16- to 32-fold lower than those of ceftazidime. Against the isolates of *Enterococcus faecalis* resistant to ceftazidime at >100 μg/ml, the MIC_{90} of LJC10,627 was 3.13 μg/ml, which was twofold greater than that of imipenem. LJC10,627 was twofold more active than imipenem against *Bacteroides fragilis* at an MIC_{90} of 1.56 μg/ml.

Stability to β-lactamases. The comparative stabilities of LJC10,627 and the other antimicrobial agents to various β-lactamases are shown in Table 3. LJC10,627 was very stable against plasmid- and chromosomal-mediated β-lactamases. However, one enzyme, oxyiminocephalosporinase type II produced from *Xanthomonas maltophilia* GN12873, slightly hydrolyzed LJC10,627 at a relative rate of 18.4. This rate was lower than that of imipenem.

Stability to DHP-I. The stabilities of LJC10,627 and imipenem to swine renal DHP-I were measured by a bioassay at a substrate concentration of 50 μg/ml. The residual antibiotic activity of LJC10,627 after incubation with the enzyme at 37°C for 2 h was 93.0%. By contrast, the residual activity of imipenem was ≦0.1%.

Killing kinetics. The bacterial killing kinetics of LJC10,627 were compared with those of imipenem and ceftazidime at concentrations equal to the MIC, 2× MIC, and 4× MIC of each antibiotic. Figure 2 shows the killing kinetics obtained with clinical isolate *Escherichia coli* TK2241 in the presence of these antibiotics. All of the antibiotics produced a bactericidal response at values greater than the MIC. LJC10,627 produced a 3-log-unit decrease of viable cells (CFU) after 5.5 h at 0.05 μg/ml (MIC) and by 3.5 h at concentrations ≧0.1 μg/ml (2× MIC). No regrowth was observed at concentrations above 2× MIC in 24 h. The killing kinetics of these compounds for *Pseudomonas aeruginosa* TK2306 are shown in Fig. 3. LJC10,627 produced bactericidal action at concentrations ≧0.39 μg/ml (MIC), and the 3-log-unit CFU decrease occurred in about 5 h.

Morphological changes. The morphological changes of cells of *Escherichia coli* TK2241 and *Pseudomonas aerugi-*

TABLE 4. Affinities of LJC10,627, imipenem, and ceftazidime for PBPs from *Escherichia coli* JE1011 and *Pseudomonas aeruginosa* NCTC 10490

Strain	Antibiotic	MIC (μg/ml)	ID_{50} (μg/ml) for PBP:					
			1A	1B(s)	2	3	4	5 and 6
Escherichia coli JE1011	LJC10,627	0.05	2.7	1.4	<0.1	25	<0.1	50
	Imipenem	0.1	0.2	0.4	<0.1	21	0.1	1.1
	Ceftazidime	0.05	1.7	1.8	>25	<0.1	50	>50
Pseudomonas aeruginosa NCTC 10490	LJC10,627	0.2	0.24	0.5	<0.1	0.2	<0.1	15
	Imipenem	0.78	1.1	0.4	<0.1	0.3	<0.1	2.7
	Ceftazidime	0.78	0.2	3.4	>50	<0.1	1.0	>50

nosa TK2306 following exposure to LJC10,627 are shown in Fig. 4. When the *Escherichia coli* cells were cultured on agar containing 0.05 μg of the compound per ml (MIC), they changed from bacilli to large spherical forms (Fig. 4A). This was followed by cell lysis (Fig. 4B and C). A similar cell lysis was observed with *Pseudomonas aeruginosa* when the cells were cultured on agar containing 0.39 μg of LJC10,627 per ml (MIC) (Fig. 4D through F).

Affinity for PBPs. Table 4 shows the concentrations of LJC10,627, imipenem, and ceftazidime required to inhibit [^{14}C]benzylpenicillin binding by 50% (ID_{50}) for the PBPs of *Escherichia coli* JE1011 and *Pseudomonas aeruginosa* NCTC 10490. LJC10,627 was highly bound to PBPs 2 and 4 of these strains at concentrations of <0.1 μg/ml. The ID_{50}s were equal to those of imipenem. LJC10,627 also bound to PBP 1B(s) of *Escherichia coli* at an ID_{50} of 1.4 μg/ml and the PBP 1B of *Pseudomonas aeruginosa* at an ID_{50} of 0.5 μg/ml. These affinities were slightly lower than those of imipenem. LJC10,627 showed relatively high binding to PBP 1A of *Pseudomonas aeruginosa* at an ID_{50} of 0.24 μg/ml in comparison with the ID_{50} of imipenem of 1.1 μg/ml. LJC10,627 was not susceptible to PBPs 3, 5, and 6 of *Escherichia coli* or PBPs 5 and 6 of *Pseudomonas aeruginosa*.

The affinities of LJC10,627 for PBPs of *Staphylococcus aureus* (Table 5) were especially high, like those of imipenem, to PBPs 1 and 4 at ID_{50}s of <0.1 μg/ml. It showed more than 10-fold lower affinities for PBPs 2 and 3 than those of imipenem.

DISCUSSION

Imipenem, a derivative of thienamycin (4), has been reported to have potent activity against a broad spectrum of aerobic and anaerobic organisms (6). This compound is stable to all but a few of the various types of β-lactamases (5). However, it is hydrolyzed by human renal DHP-I (3). SM7338 is a new carbapenem compound which is relatively stable to DHP-I (2). In addition, its in vitro activity is greater than that of imipenem against most members of the family *Enterobactericeae* and *Pseudomonas* species, but it is less active than imipenem against gram-positive aerobes (2, 7, 8).

TABLE 5. Affinities of LJC10,627, imipenem, and ceftazidime for PBPs from *Staphylococcus aureus* FDA 209P

Antibiotic	MIC (μg/ml)	ID_{50} (μg/ml) for PBP:			
		1	2	3	4
LJC10,627	0.1	<0.1	2.8	25	<0.1
Imipenem	0.02	<0.1	0.2	0.1	<0.1
Ceftazidime	12.5	1.0	3.1	3.8	>25

In this report we describe the in vitro antimicrobial activity, β-lactamase stability, renal DHP-I stability, killing kinetics, and PBP affinity of LJC10,627. Overall, this new compound was more active than imipenem against the members of the family *Enterobacteriaceae*, *Pseudomonas* species, and *Bacteroides fragilis* but was less active than imipenem against gram-positive aerobes. These results were supported by the following findings. (i) LJC10,627 produced bactericidal action against *Escherichia coli* and *Pseudomonas aeruginosa*, (ii) cell lysis occurred in cells swollen by exposure to this compound, and (iii) morphological changes corresponded with the PBP affinity of the compound.

Nevertheless, the affinity of LJC10,627 for PBP 1B(s) was slightly lower than that of imipenem. The MIC of LJC10,627 for *Pseudomonas aeruginosa* was less affected by EDTA treatment than was that of imipenem (data not shown). From this result, we speculate that this compound has a potent ability to permeate into cells.

In pharmacokinetics, by using [^{14}C]LJC10,627, about 74% of the original compound was excreted into the urine of rats and no bioactive metabolites were observed (N. Yamashita, T. Hirai, T. Nakazawa, T. Watanabe, T. Kuroda, K. Kawashima, M. Hikida, Y. Furukawa, and T. Honda, 29th ICAAC, abstr. no. 223, 1989). Moreover, it has also been reported that LJC10,627 alone is more effective than imipenem plus cilastatin in protecting mice against infections with *Escherichia coli* and *Pseudomonas aeruginosa* (P. J. Petersen, W. J. Weiss, N. V. Jacobus, and R. T. Testa, 29th ICAAC, abstr. no. 222, 1989).

Recently, we confirmed that this compound is very stable to human renal DHP-I (unpublished data). These results suggest that further pharmacological and clinical studies should be conducted with LJC10,627 to determine its clinical usefulness.

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