ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 1992, p. 481–483
0066-4804/92/020481-03$02.00/0

Vol. 36, No. 2

Renal Dehydropeptidase-I Stability of LJC 10,627, a New Carbapenem Antibiotic

MUNEO HIKIDA,[1]* KOUSUKE KAWASHIMA,[1] KATSUYUKI NISHIKI,[1] YASUO FURUKAWA,[1] KAZUAKI NISHIZAWA,[2] ISAO SAITO,[3] AND SADAHITO KUWAO[4]

Biological Research Laboratories, Lederle (Japan), Ltd., 1-6-34, Kashiwa-cho, Shiki-shi, Saitama 353,[1] Department of Urology, Nerima Hospital, 2-41, Asahigaoka, Nerima-ku, Tokyo 176,[2] Department of Urology, Tokyo Kyosai Hospital 2-3-8, Nakameguro, Meguro-ku, Tokyo 153,[3] and Department of Pathology, School of Medicine, Kitasato University, 1-15-1, Kitasato, Sagamihara-shi, Kanagawa 228,[4] Japan

Received 18 June 1991/Accepted 28 November 1991

LJC 10,627 is a new parenteral carbapenem antibiotic. LJC 10,627 stability against human renal dehydropeptidase-I was compared with that of imipenem. Hydrolysis of this compound was not detectable by spectrophotometrical assay. Even after a 2-h incubation of antibiotics with this enzyme at 30°C, the concentration of LJC 10,627 remained at 92.3% of the initial concentration, whereas imipenem completely disappeared. Thus, it was found that this compound was highly stable against human renal dehydropeptidase-I. Furthermore, LJC 10,627 had a low affinity for this enzyme, as indicated by the high K_i value (0.38 mM).

Since the discovery of thienamycin, carbapenems have been well known for exhibiting very potent antimicrobial activities against a broad spectrum of bacteria (1, 5). All of the carbapenem derivatives including imipenem, however, have been found to undergo metabolism by a renal tubular brush border dipeptidase, dehydropeptidase-I (DHP-I) (4, 11) and to be relatively unstable in aqueous solution. These features result in low concentrations in urine, which is one of the major hindrances to the clinical application of these drugs. To overcome these defects, a compound which possesses potent broad-spectrum antimicrobial activity and is stable enough for clinical practice without coadministration of DHP-I inhibitor has been much desired.

LJC 10,627, a new 1-β-methyl carbapenem antibiotic, has an extremely wide spectrum of in vitro antibacterial activity, including activity against most aerobic and anaerobic gram-positive and gram-negative bacteria (9). In this paper, we report a remarkable stability of LJC 10,627 against human and animal DHP-Is.

LJC 10,627 (1-β-methyl compound; Fig. 1), LJC 10,723 (1-unsubstituted LJC 10,627; Fig. 1), imipenem, and glycyldehydrophenylalanine were prepared in our laboratory. The other reagents used in this study were obtained from either Sigma Chemical Co. (St. Louis, Mo.) or Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). Normal portions of human kidney tissue were collected from grossly excised tissues of renal tumors. Renal DHP-Is were purified from porcine, cynomolgus monkey, and human kidney cortices by the procedures reported by Campbell et al. (2, 3), and their activities were assayed by using glycyldehydrophenylalanine as a substrate. The respective molecular weights of the purified enzymes obtained from the renal cortices, estimated from their electrophoretic mobilities in a sodium dodecyl sulfate-polyacrylamide gel, were about 47,000, 72,000 and 66,000, being almost identical to those given in previous reports (2, 3).

TABLE 1. Kinetics of hydrolysis of LJC 10,627 by renal DHP-Is from porcine, monkey, and human kidneys

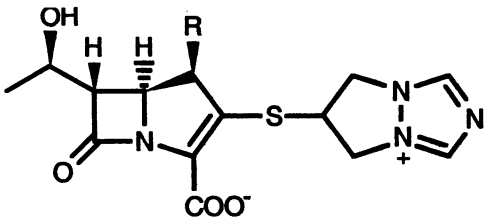
Substrate	Swine DHP-I			Monkey DHP-I			Human DHP-I		
	K_m (mM)	V_{max}[a]	V_{max}/K_m	K_m (mM)	V_{max}	V_{max}/K_m	K_m (mM)	V_{max}	V_{max}/K_m
Glycyldehydrophenylalanine	0.100	100	1,000	0.133	100	751.9	0.139	100	719.4
LJC 10,627	0.435	0.160	0.37	1.111	1.172	1.06	0.38[b]		
LJC 10,723	5.000	9.506	1.90	0.455	0.410	0.90	0.526	0.061	0.12
Imipenem	0.167	2.376	14.23	0.097	0.241	2.49	0.217	0.288	1.33

[a] Given relative to an arbitrary value of 100 for hydrolysis of glycyldehydrophenylalanine.
[b] K_i (millimolar). Glycyldehydrophenylalanine was used as a substrate.

The hydrolytic rates of the compounds were monitored by spectrophotometric measurement of decrease in the light absorbance (10) specific for each compound. The millimolar extinction coefficients per centimeter ($\Delta\varepsilon$) were as follows; LJC 10,627, 5.73 at 293 nm; LJC 10,723, 8.02 at 295 nm; imipenem, 8.00 at 299 nm (4); and glycyldehydrophenylalanine, 15.3 at 275 nm (4). All reactions were carried out in 50 mM MOPS [3-(*N*-morpholino)propanesulfonic acid] buffer (pH 7.0) at 30°C and initiated by addition of the tested compound.

The Michaelis constant (K_m) and maximum rate (V_{max}) of each hydrolytic reaction were determined from a Line-

* Corresponding author.



R = CH_3 LJC10,627
R = H LJC10,723

FIG. 1. Structures of LJC 10,627 and LJC 10,723.

weaver-Burk plot between substrate concentrations of 25 and 200 μM. The dissociation constants of enzyme-inhibitor complexes (K_i) were determined by the Dixon plot. These determinations were carried out in duplicate. These kinetic parameters of the enzymes are shown in Table 1. With the swine enzyme, the V_{max} of LJC 10,627 was about 15 times lower than that of imipenem; in contrast, with the monkey enzyme, the V_{max} of LJC 10,627 was approximately five times higher than that of imipenem. On the other hand, no hydrolysis of LJC 10,627 was detected after incubation with human DHP-I. Concerning the K_m value, LJC 10,627 exhibited considerably greater values for all of the three enzyme preparations than did the other test compounds. Furthermore, the V_{max}/K_m ratios of LJC 10,627 for the three enzymes are all smaller than those of imipenem. The K_i value of LJC 10,627 was 0.38 mM. This result suggests that LJC 10,627 has a low affinity for human DHP-I. These enzymological kinetic studies suggested a remarkable stability of LJC 10,627 against the hydrolytic action of human DHP-I compared with imipenem and led us to try the stability test with a longer incubation.

The test compound (at a final concentration of 100 μM) was added to the enzyme solution in 50 mM MOPS buffer (pH 7.0). The enzyme activity used was 2.6 U/ml. One unit of the enzyme was defined as the amount of enzyme which hydrolyzed 1 μM glycyldehydrophenylalanine per min at 30°C in 50 mM MOPS buffer (pH 7.0). Aliquots of the reaction mixture were collected at 0.25, 0.5, 1, 2, and 4 h after incubation and then mixed with 2 volumes of acetonitrile by vortexing. Chloroform was added to a final concentration of 50% (vol/vol) and mixed well. The remaining drug concentration in the supernatant fluid after centrifugation at 5,000 × g for 10 min was determined by high-pressure liquid chromatography (HPLC). The HPLC system consists of a Waters 600E, a Waters 712 WISP, and a Jasco 870 UV detector. The chromatographic conditions used are shown in Table 2. After incubation at 30°C for 2 h, the residual amount of LJC 10,627 decreased by only about 8% of the initial amount, while that of imipenem decreased by 79% after only 0.5 h (Fig. 2). The datum points shown in the figure are the means of two independent experiments for each compound. The kinetic profile of the reactions of LJC 10,723 with the three enzymes differs from those of both LJC 10,627 and imipenem. It indicates that high stability against DHP-I is attributable not only to 1-β-methylation but also to the presence of a triazolium radical at the side chain of the second position. Further support for this notion may be provided by the fact that meropenem (SM-7338), a derivative of 1-β-methyl-carbapenem, is only four times more stable than imipenem for human renal DHP-I according to a comparison of the V_{max}/K_m ratios (8). In comparison with the present result, meropenem seems to be more unstable against human renal DHP-I in spite of the presence of a 1-β-methyl base in its chemical structure.

TABLE 2. HPLC condition of LJC 10,627 and imipenem

Parameter	Value for[a]:	
	LJC 10,627	Imipenem
Guard column	Inertsil ODS-2, 4.6 (i.d.) by 10 mm	TSKgel ODS-80T_M, 4.6 (i.d.) by 10 mm
Analytical column	Inertsil ODS-2, 4.6 (i.d.) by 150 mm	TSKgel ODS-80T_M, 4.6 (i.d.) by 250 mm
Mobile phase	A	B
Flow rate	0.9 ml/min	0.75 ml/min
Detection	UV (295 nm)	UV (298 nm)
Injection vol	20 μl	20 μl
Retention time	6.3 min	11.7 min

[a] i.d., inside diameter; A, acetonitrile–50 mM acetate buffer, pH 5.5 = 2/100; B, methanol–100 mM phosphate buffer, pH 7.0 = 5/100.

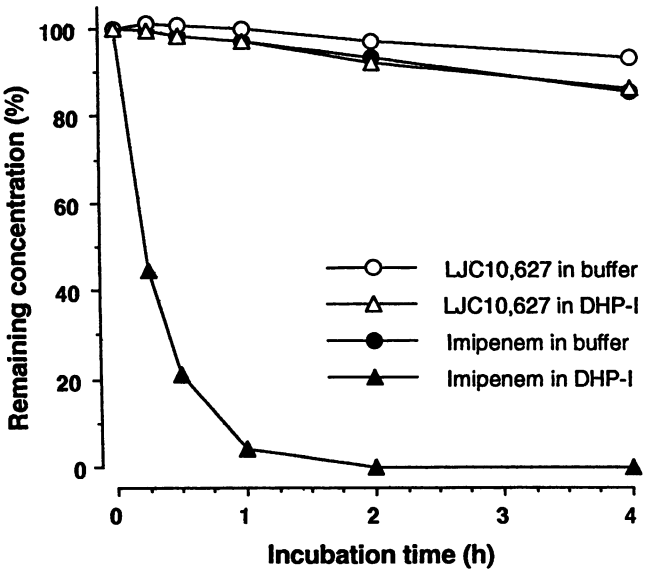


FIG. 2. Stability of LJC 10,627 against human renal DHP-I.

It is well known that inactivation of carbapenems readily occurs because of metabolic degradation in vivo. This is one of the major obstacles against their successful introduction to the clinical field. Some advance in the stability of carbapenems has been brought about by formation of meropenem and panipenem (CS-533), which, compared with imipenem, are durable against the decomposing actions of DHP-I and/or β-lactamase (6, 7) without needing a DHP-I inhibitor. LJC 10,627 appears to have achieved further advances with respect to stability. This new compound deserves further studies and clinical trials to determine its role in modern chemotherapy.

REFERENCES

1. **Birnbaum, J., F. M. Kahan, H. Kropp, and J. S. MacDonald.** 1985. Carbapenems, a new class of beta-lactam antibiotics. Am. J. Med. **78**(Suppl. 6A):3–21.
2. **Campbell, B. J.** 1970. Renal dipeptidase. Methods Enzymol. **19**:722–729.
3. **Campbell, B. J., L. J. Forrester, W. L. Zahler, and M. Burks.** 1984. β-Lactamase activity of purified and partially character-

ized human renal dipeptidase. J. Biol. Chem. **259**:14586–14590.
4. **Kropp, H., J. G. Sundelof, R. Hajdu, and F. M. Kahan.** 1982. Metabolism of thienamycin and related carbapenem antibiotics by the renal dipeptidase, dehydropeptidase-I. Antimicrob. Agents Chemother. **22**:62–70.
5. **Mitsuhashi, S.** 1983. *In-vitro* and *in-vivo* antibacterial activity of imipenem against clinical isolates of bacteria. J. Antimicrob. Chemother. **12**(Suppl. D):53–64.
6. **Neu, H. C., N.-X. Chin, G. Saha, and P. Labthavikul.** 1986. In vitro activity against aerobic and anaerobic gram-positive and gram-negative bacteria and β-lactamase stability of RS-533, a novel carbapenem. Antimicrob. Agents Chemother. **30**:828–834.
7. **Neu, H. C., A. Novelli, and N.-X. Chin.** 1989. In vitro activity and β-lactamase stability of a new carbapenem, SM-7338. Antimicrob. Agents Chemother. **33**:1009–1018.
8. **Tanio, T., H. Nouda, E. Tada, T. Kohzuki, M. Kato, M. Fukasawa, T. Okuda, and S. Kamidono.** 1987. Program Abstr. 27th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 758.
9. **Ubukata, K., M. Hikida, M. Yoshida, K. Nishiki, Y. Furukawa, K. Tashiro, M. Konno, and S. Mitsuhashi.** 1990. In vitro activity of LJC 10,627, a new carbapenem antibiotic with high stability to dehydropeptidase I. Antimicrob. Agents Chemother. **34**:994–1000.
10. **Waley, S. G.** 1974. A spectrophotometric assay of β-lactamase action on penicillins. Biochem. J. **139**:780–789.
11. **Wise, R.** 1986. In vitro and pharmacokinetic properties of the carbapenems. Antimicrob. Agents Chemother. **30**:343–349.