ELSEVIER

Journal of Chromatography B, 688 (1997) 95–99

JOURNAL OF CHROMATOGRAPHY B

Determination of a new carbapenem derivative, DA-1131, in plasma and urine by high-performance liquid chromatography

So Hee Kim[a], Jong Won Kwon[b], Junnick Yang[b], Myung Gull Lee[a,*]

[a]*College of Pharmacy, Seoul National University, San 56-1, Shinlim-Dong, Kwanak-Gu, Seoul 151-742, South Korea*
[b]*Research Laboratories, Dong-A Pharmaceutical Company, Ltd., 47 Sanggal-Ri, Kiheung-Up, Yongin-Gun, Kyunggi-Do 449-900, South Korea*

Received 2 June 1995; revised 29 May 1996; accepted 10 June 1996

Abstract

A high-performance liquid chromatographic method was developed for the determination of a new carbapenem, DA-1131 (I), in human plasma and urine and in rat blood and tissue homogenates. The method involved deproteinization of the biological samples with 1 volume each of 0.04 *M* $Ba(OH)_2$ and $ZnSO_4$ aqueous solution. A 50-μl aliquot of the supernatant was injected onto a C_{18} reversed-phase column. The mobile phase employed was 0.015 *M* KH_2PO_4–acetonitrile (9:1, v/v) with a pH of 5.0. The flow-rate was 0.8 ml/min. The column effluent was monitored by a ultraviolet detector at 300 nm. The retention time of I was 8.0 min. The detection limits of I in human plasma and urine were 0.1 and 0.5 μg/ml, respectively. The coefficients of variation of the assay were generally low (below 8.39%) for human plasma and urine, and rat blood and tissue homogenates. No interferences from endogenous substances were observed.

Keywords: Carbapenem derivative

1. Introduction

Imipenem and meropenem, carbapenem derivatives, have potent anti-bacterial activity against a broad spectrum of microorganisms. High-performance liquid chromatographic (HPLC) analyses of imipenem [1] and meropenem [2] in biological samples have been reported. Recently, Research Laboratories of Dong-A Pharmaceutical (Yongin-Gun, South Korea) developed a new carbapenem derivative, DA-1131, (1*R*,5*S*,6*S*)-[(2*S*,4*S*)-2-[(*E*)-3-methansulfonyl amino-1-propenyl] pyrrolidine-4-ylthiol-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylic acid (I, Fig. 1). Compound I is currently being evaluated in preclinical trials.

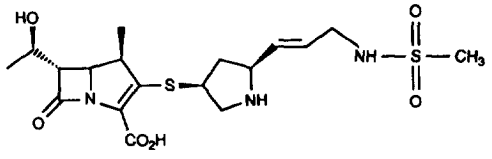

Fig. 1. Chemical structure of I.

Stability, tissue metabolism, tissue distribution and blood partition of I have recently been published [3]. The degradation half-lives of I were 0.260, 1.23, 2.20, 7.16, 14.2, 10.5, 31.3, 21.3, 0.262, 0.241 and 1.45 h for solutions of pH values 1–11, respectively. Compound I was also unstable after incubation in human plasma, rat liver homogenate and human gastric juice. However, I seemed to be stable in

*Corresponding author.

PII S0378-4347(96)00268-X

human plasma for up to 12 h storage at −20°C. The plasma-to-blood cell concentration ratios were independent of blood concentrations of I when the whole blood was incubated for up to 120 min; the mean values were 5.56±1.47, 5.80±2.19 and 4.61±1.82 at rabbit blood concentrations of 2, 10 and 20 μg/ml, respectively.

This paper describes the HPLC method with simple sample preparation procedure for the determination of I in human plasma and urine, and rat blood and tissue homogenates.

2. Experimental

2.1. Chemicals

Compound I was kindly supplied by Research Laboratories of Dong-A. Other chemicals were of reagent grade or HPLC grade and used without further purification.

2.2. Preparation of standard solutions

Stock solution of I was prepared by dissolving I powder in distilled water. Appropriate dilutions of the stock solution were made with distilled water. Standard solutions of I in water, plasma and urine were prepared by spiking appropriate volume (less than 10 μl per ml) of the diluted stock solution to give the final concentrations of 50, 10, 5, 1, 0.5 and 0.1 μg/ml. Rat tissues were homogenized with 4 volumes of distilled water using a tissue homogenizer (Ultra-Turrax, T25, Janke and Kunkel, IKA-Labortechnik, Staufeni, Germany) and immediately centrifuged at 2000 *g* for 20 min. Two volumes of distilled water were added to rat blood to facilitate the hemolysis of blood cells and increase the reproducibility of I from whole blood [4,5]. After vortex-mixing and centrifugation, the supernatant was collected. Standard solutions of I in tissue homogenates (including blood samples) of 0.1, 0.2 and 1 μg/ml were similarly prepared. The HPLC assay results on 50, 1 and 0.1 μg/ml for plasma and 50, 1 and 0.5 μg/ml for urine are listed in Table 1, and on 0.1 and 1.0 μg/ml for tissue homogenates are listed in Table 2. Response factors were calculated by dividing the peak height (mm) of I by its concentrations (μg/ml). Recoveries (%) in plasma, urine and tissue homogenates were calculated by dividing the peak height of I in the biological samples by that in water [6]. Accuracy was calculated by dividing mean measured concentration by spiked concentration.

2.3. Sample preparation

To 50 μl of the biological samples were added 50 μl of each of 0.04 *M* $Ba(OH)_2$ and $ZnSO_4$ aqueous solution. After vortex-mixing and centrifugation at 14 000 *g* for 1 min, 50 μl of the supernatant was injected directly onto the HPLC column. Although I was unstable in biological samples and pH solutions [3], I was fairly stable in the present HPLC condition (the absolute recovery of I in water after addition of

Table 1
Response factors and recoveries of I at various concentrations in human plasma and urine

Concentration (μg/ml)	Response factor[a]	Recovery[b] (%)	Accuracy[c] (%)
Human plasma			
50	27.3±1.38 (5.04)	93.9	102
1	26.8±0.708 (2.65)	93.3	99.9
0.1	26.1±0.460 (1.76)	92.6	97.5
Human urine			
50	24.6±0.406 (1.65)	84.0	103
1	23.8±0.601 (2.53)	87.0	99.8
0.5	23.4±0.626 (2.67)	88.4	98.4

Values in parentheses are within-day C.V. (%); $n=5$.
[a] Compound I peak height (mm) divided by its concentration (μg/ml); mean±S.D..
[b] Recovery compared with water.
[c] (Mean measured concentration/spiked concentration)×100.

Table 2
Response factors and recoveries of I at various concentrations in rat blood and its tissue homogenates

Tissue[a]	Concentration (μg/ml)	Response factor[b]	Recovery[c] (%)	Accuracy[d] (%)
Blood	1	14.6±0.548 (3.75)	50.4	103
	0.1	14.6±1.10 (7.56)	51.4	103
Liver	1	20.5±0.686 (3.35)	70.7	100
	0.1	20.3±1.59 (7.82)	71.8	99.1
Lung	1	22.9±0.335 (1.46)	79.1	105
	0.1	21.0±0.115 (0.549)	74.4	95.9
Heart	1	28.6±0.596 (2.09)	98.5	101
	0.1	27.1±0.404 (1.49)	95.7	95.6
Brain	1	11.6±0.170 (1.46)	40.1	93.0
	0.1	13.0±0.656 (5.04)	46.0	104
Kidney	1	14.3±0.200 (1.40)	49.4	97.5
	0.1	15.4±0.153 (0.994)	54.4	105
Muscle	1	25.4±0.210 (0.829)	87.5	98.6
	0.1	25.2±0.513 (2.04)	89.0	97.8
Stomach	1	25.2±0.734 (2.91)	87.0	100
	0.1	24.7±0.379 (1.53)	87.5	98.3
Intestine	1	25.4±0.297 (1.17)	87.5	100
	0.1	24.0±0.700 (2.92)	84.9	95.1
Spleen	1	23.0±0.329 (1.43)	79.5	96.2
	0.1	24.1±1.24 (5.16)	85.1	100

Values in parentheses are within-day C.V. (%); $n=3$.
[a] Tissue samples were homogenized with 4 volumes of distilled water.
[b] Compound I peak height (mm) divided by its concentration (μg/ml); mean±S.D..
[c] Recovery compared with water.
[d] (Mean measured concentration/spiked concentration)×100.

$Ba(OH)_2$ and $ZnSO_4$ was essentially complete). The biological samples were immediately stored in the −70°C freezer (Model Revco ULT 1490 D-N-S, Western Mednics, CA, USA) and $Ba(OH)_2$ and $ZnSO_4$ were added just before HPLC analysis. Compound I was stable in plasma for up to 12 h storage at −20°C, at least 3 months storage at −70°C and in urine for up to 12 h at room temperature.

2.4. HPLC apparatus

The HPLC system consisted of a Model 7125 injector (50-μl loop, Rheodyne, Cotati, CA, USA), a Model 1330 pump (Bio-Rad, Japan Servo, Tokyo, Japan), a reversed-phase column (Capcell pak, C_{18}, 25 cm×4.69 mm I.D., particle size 5 μm, Shiseido, Tokyo, Japan), a Model 1306 UV detector (Bio-Rad) and a Model 1200 recorder (Linear, Reno, NV, USA). The mobile phase, 0.015 *M* KH_2PO_4–acetonitrile (9:1, v/v) with a pH of 5.0, was run at a flow-rate of 0.8 ml/min and the column effluent was monitored by the UV detector set at 300 nm. The retention time of I was approximately 8.0 min.

3. Results and discussion

The UV absorption maximum of I occurred at 300 nm and this was therefore used for the HPLC analysis. Fig. 2 shows typical chromatograms of drug-free human plasma, drug standards in human plasma and rat plasma after intravenous administration of I; the corresponding chromatograms for urine, and rat stomach homogenates are shown in Figs. 3 and 4, respectively. No interferences from endogenous substances were observed in any of the biological samples. The peak of I was symmetrical and eluted at approximately 8.0 min.

The detection limits for I in human plasma and urine were 0.1 and 0.5 μg/ml, respectively (Table 1), based on a signal-to-noise ratio of 3.0. The mean

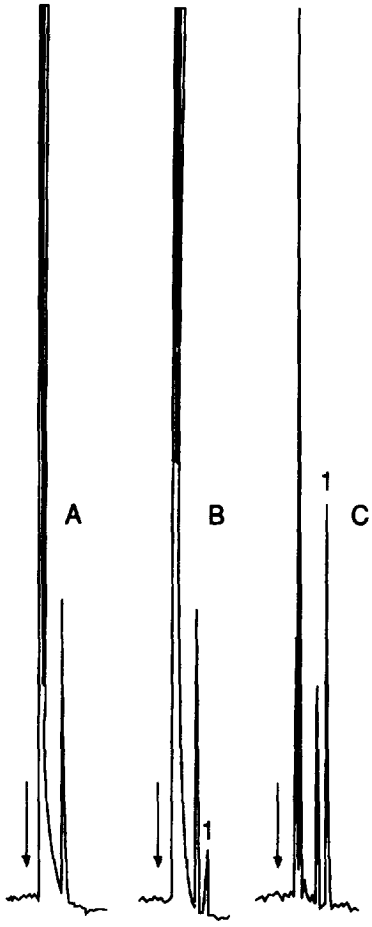

Fig. 2. Chromatograms after deproteinization of drug-free human plasma (A), human plasma spiked with 0.5 μg/ml of I (B) and plasma obtained from a rat at 60 min after 1-min intravenous administration of I, 50 mg/kg (C). Peak: 1=I (8.0 min). The arrows mark the point of injection. The detector sensitivity was set at 0.01 AUFS and the recorder sensitivity was set at 10 mV (A and B) and 20 mV (C).

within-day coefficients of variation (C.V.) of I in human plasma and urine were 2.85% (range 1.76–5.04%) and 2.85% (range 1.65–4.75%), respectively. The mean between-day C.V. values for the analysis of the same samples on three consecutive days were 2.30 and 4.29% in human plasma and urine, respectively. Mean analytical recoveries of spiked I from human plasma and urine were 92.8% (range 91.3–93.9%) and 87.0% (range 84.0–89.3%), respectively. The accuracy [(measured concentration/spiked concentration)×100] of I was 97.5–103% for human plasma and urine (Table 1).

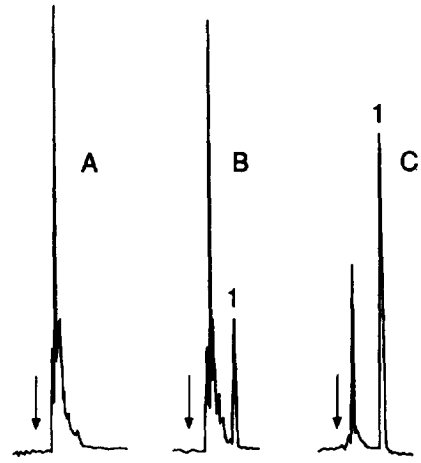

Fig. 3. Chromatograms after deproteinization of drug-free human urine (A), human urine spiked with 5.0 μg/ml of I (B) and urine obtained from a rat collected between 0–8 h after 1-min intravenous administration of I, 50 mg/kg (C). Peak: 1=I (8.0 min). The arrows mark the point of injection. The detector sensitivity was set at 0.01 AUFS and the recorder sensitivity was set at 50 mV (A and B) and 500 mV (C).

The HPLC assay method was also successful for the analysis of I in rat tissues (Table 2). The detection limits for I were 0.1 μg/ml for all tissues studied. The mean within-day C.V. values for I ranged from 0.549% (lung) to 7.82% (liver). The mean analytical recoveries of spiked I ranged from 40.1% (brain) to 98.5% (heart). However, the recovery from blood (50.4–51.4%), brain (40.1–46.0%) and kidney (49.4–54.4%) were relatively low. The exact reason for the low recovery is unknown. This might be due to the coprecipitation of I during the deproteinization procedures and this could result from binding and/or adsorption of I to the endogenous compounds in blood, brain and kidney. The accuracy ranged from 93.0% to 105%.

Compound I, 50 mg/kg, was administered (the total injection volume was approximately 1 ml) for 1 min via the jugular vein of a Sprague–Dawley rat (335 g). Approximately 0.12 ml of blood was collected at appropriate time intervals and a 50-μl aliquot of plasma samples was collected. The arterial plasma concentration–time profile of I is shown in

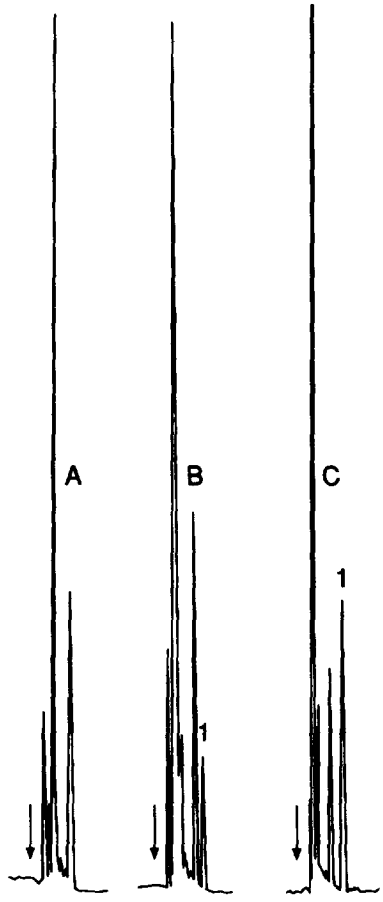

Fig. 4. Chromatograms after deproteinization of drug-free rat stomach homogenate (A), rat stomach homogenate spiked with 1.0 μg/ml of I (B), and stomach homogenate obtained from a rat at 30 min after 1-min intravenous administration of I, 50 mg/kg (C). Peak: 1 = I (8.0 min). The arrows mark the point of injection. The detector sensitivity was set at 0.01 AUFS and the recorder sensitivity was set at 10 mV.

Fig. 5. The terminal half-life, total body clearance, apparent volume of distribution at steady state (V_{SS}) and mean residence time of I were 15.2 min, 12.4 ml/min/kg, 170 ml/kg and 13.7 min, respectively.

The tissue concentrations of I were also measured at 30 min after 1-min intravenous administration of I, 50 mg/kg, to a rat; the values were 31.5 μg/ml for plasma and 11.0, 4.63, 5.65, 1.08, 20.6, 3.05, 5.38, 5.89, 4.62, 2.39, 2.10 and 6.13 μg/g tissue for the liver, lung, heart, brain, kidney, muscle, stomach, small intestine, large intestine, spleen, fat and mesentery, respectively.

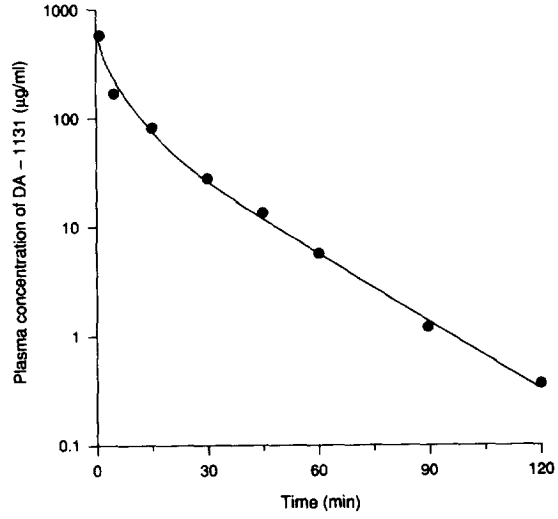

Fig. 5. Arterial plasma concentration–time profile of I after 1-min intravenous administration of I, 50 mg/kg, to a Sprague–Dawley rat.

Acknowledgments

This research was in part supported by the Korea Ministry of Science and Technology (HAN Project), 1994–1995.

References

[1] G. Carlucci, L. Biordi, C. Vicentini and M. Bologna, J. Pharm. Biomed. Anal., 8 (1990) 283.
[2] L.Å. Burman, I. Nilsson-Ehle, M. Hutchson, S.J. Haworth and S.R. Norrby, J. Antimicrob. Chemother., 27 (1991) 219.
[3] S.H. Kim, W.B. Kim and M.G. Lee, Res. Commun. Mol. Pathol. Pharmacol., 90 (1995) 347.
[4] M.G. Lee, C.Y. Lui, M.-L. Chen and W.L. Chiou, Int. J. Clin. Pharmacol. Ther. Toxicol., 22 (1984) 530.
[5] W.G. Shin, M.G. Lee, M.H. Lee and N.D. Kim, Biopharm. Drug Dispos., 13 (1992) 305.
[6] S.H. Lee and M.G. Lee, Biopharm. Drug Dispos., 16 (1995) 547.