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Short communication

High-performance liquid chromatographic analysis of DA-7867, a new oxazolidinone, in human plasma and urine and in rat tissue homogenates

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Abstract

An HPLC method was developed for the determination of a new oxazolidinone, DA-7867 (I), in human plasma and urine and in rat tissue homogenates. To 100 μl of biological sample, 300 μl acetonitrile and 50 μl methanol containing 10 μg/ml DA-7858 (the internal standard) were added. After vortex-mixing and centrifugation, the supernatant was evaporated under a gentle stream of nitrogen. The residue was reconstituted in 100 μl of the mobile phase and a 50-μl aliquot was injected directly onto the reversed-phase (C_{18}) column. The mobile phase, 20 m*M* KH_2PO_4:acetonitrile (75:25, v/v) was run at a flow rate of 1.5 ml/min and the column effluent was monitored by a UV detector set at 300 nm. The retention times of I and DA-7858 were approximately 6.5 and 8.7 min, respectively. The detection limits of I in human plasma and urine and in rat tissue homogenates were 20, 20, and 50 ng/ml, respectively.

Keywords: DA-7867; Oxazolidinones

1. Introduction

During the 1980s and 1990s, the emergence and wide spread of methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE) (*Enterococcus faecium* and *Enterococcus faecalis*), vancomycin/glycopeptide-intermediate *S. aureus* (VISA/GISA), penicillin-resistant *Streptococcus pneumoniae* (PRSP), and multidrug-resistant (MDR) coagulase-negative staphylococci were reported [1]. The oxazolidinones, such as linezolid (PNU-100766), eperezolid (PNU-100592), PNU-177553, DuP-721, DuP-105, VRC-3808, RWJ-334181, RWJ-337813, and AZ02563 were synthesized [2] to overcome the aforementioned problem of emerging resistance in G(+) bacteria. The oxazolidinones inhibit bacterial protein synthesis [1,2].

Recently, a new oxazolidinone, DA-7867 (I, (*S*)-[*N*-3-(4-(2-m(1-methyl-5-tetrazolyl)-pyridine-5-yl)-3-fluorophenyl)-2-oxo-5-oxazolidinyl]methyl acetamide, a basic compound having a molecular weight of 411.39 Da; Fig. 1) has been developed (Research Laboratory of Dong-A Pharmaceutical Company, Yongin, South Korea). Compound I had good anti-

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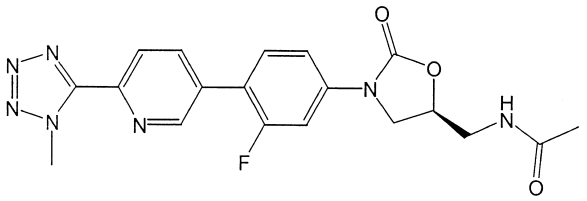
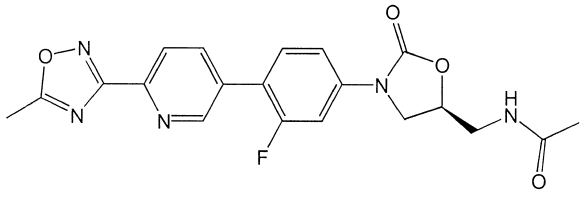


I



DA-7858

Fig. 1. Chemical structures of I and DA-7858 (the internal standard).

bacterial activities against G(+) bacteria including resistant strains such as MRSA, VRE, and PRSP and some G(−) strains including *Haemophilus influenzae* and *Moraxella catarrhalis* [3–5]. Compound I showed 4- to 8-fold better antibacterial activities compared to linezolid against G(+) and G(−) pathogens including MDR bacteria [3–5]. The following data on DA-7867 were obtained (internal report). The log partition coefficients (compared with octanol) of I were 0.9, 1.6, 1.6, 1.8, and 1.5 for pH solutions of 1, 3, 5, and 7 and distilled water, respectively. Solubility of I in pH solutions of 1, 3, 5, and 7 and distilled water was 93.0, 15.8, 14.4, 7.5, and 12.5 μg/ml, respectively. Solubility of I in 10, 25, and 30% ethanol, 10, 25, and 50% glycerin and 10, 25, and 50% polyethylene glycol 400 was 47, 194, 2244, 16, 24, 48, 58, 162, and 639 μg/ml, respectively. Powder of I was stable for up to 9 months storage at 25, 40, and 60 °C when stored in a closed, light-protected container; more than 99% was recovered. Solution of I (5 μg/ml) was also stable for up to 7 days storage at 25 °C when stored in a closed, light-protected container in pHs of 1, 3, 5, 7, and 9 and distilled water; the recovery of spiked amount of I was 100, 100, 100, 100, 96.7, and 100% for pH solutions of 1, 3, 5, 7, and 9 and distilled water, respectively. Compound I is being evaluated in preclinical study as a new oxazolidinone antibiotic.

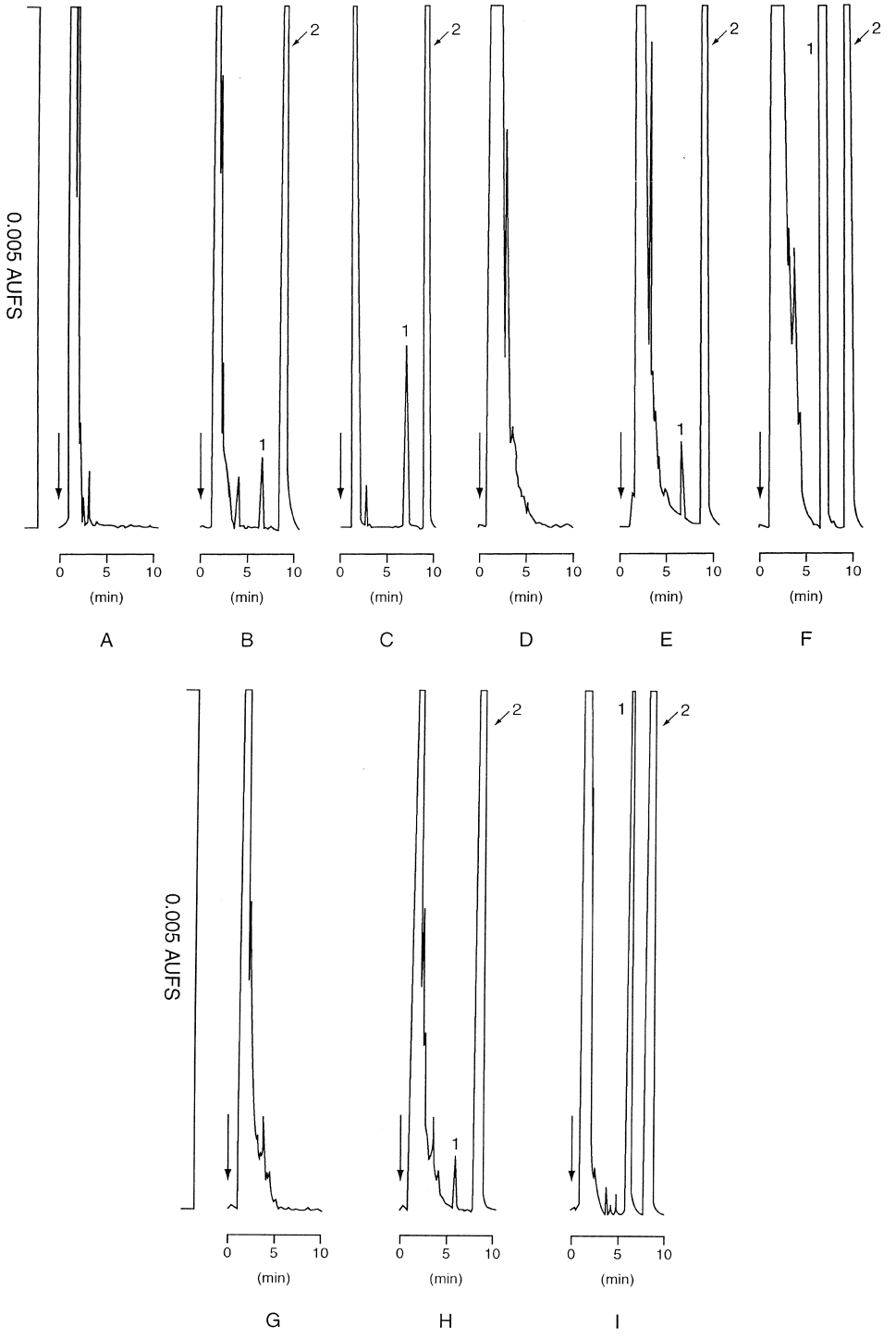
High-performance liquid chromatographic (HPLC) analyses of linezoid (PNU-100766) have been reported [6–9]. The purpose of this paper is to report the HPLC method for the determination of I in human plasma and urine and rat tissue homogenates.

2. Experimental

2.1. Chemicals

Compound I (purity of 99.9% based on HPLC data) and DA-7858 ((*S*)-[*N*-3-(4-(2-(5-methyl-1,2,4-oxadiazol-3-yl)-pyridine-5-yl)-3-fluorophenyl)-2-oxo-5-oxazolidinyl] methyl acetamide (purity of 99.6% based on HPLC data), the internal standard; Fig. 1) were supplied by the Research Laboratory of Dong-A Pharmaceutical Company. *N*,*N*-Dimethylacetamide (DMA) and polyethylene glycol 400 (PEG 400) were from Sigma (St. Louis, MO, USA) and Duksan Chemical (Seoul, South Korea), respectively. Potassium phosphate, monobasic and acetonitrile were purchased from Dongnam Chemical (Seoul, South Korea) and Honeywell International, Burdick and Jackson (Muskegon, MI, USA). The other chemicals were of reagent or HPLC grade and, therefore, were used without further purification.

Fig. 2. Chromatograms of drug-free human plasma (A), human plasma spiked with 0.1 μg/ml of I and 10 μg/ml of DA-7858 (B), plasma collected from a male Sprague−Dawley rat at 48 h after 1-min intravenous administration of 10 mg/kg of I (0.276 μg/ml) (C), drug-free human urine (D), human urine spiked with 0.1 μg/ml of I and 10 μg/ml of DA-7858 (E), urine collected from a male Sprague−Dawley rat between 0 and 48 h after 1-min intravenous administration of 10 mg/kg of I (21.5 μg/ml) (F), drug-free rat lung homogenate (G), rat lung homogenate spiked with 0.1 μg/ml of I and 10 μg/ml of DA-7858 (H), and rat lung homogenate collected from a male Sprague−Dawley rat 30 min after 1-min intravenous administration of 20 mg/kg of I (11.2 μg/ml) (I). Peaks: 1, I (6.5 min); 2, DA-7858 (the internal standard) (8.7 min). The arrow marks the point of injection. The detector's sensitivity was set at 0.005 AUFS (absorption unit full scale) and recorder's sensitivity was set at attenuation 16. The chart speed was 15 cm/h.


0.005 AUFS
1
2
0 5 10
(min)
A
B
C
D
E
F
G
H
I

2.2. Preparation of stock and standard solutions

A stock solution of I was prepared in dimethyl sulfoxide (DMSO; 1 mg/ml). Appropriate dilutions of the stock solution were made with DMSO. Standard solutions of I in human plasma and urine and in rat tissue homogenates (1 g of each rat tissue or organ was homogenized (Ultra-Turrax, T25, Janke and Kunkel, IKA-Labortechnik, Staufen, Germany) with four volumes of 0.9% NaCl-injectable solution, centrifuged for 10 min at 9000 *g* and the supernatant collected) were prepared by spiking with an appropriate volume (less than 10 μl per ml of biological fluids) of the variously diluted stock solutions giving final concentrations of 0.02, 0.05, 0.1, 0.2, 0.5, 2, 10, and 50 μg/ml for human plasma, 0.02, 0.05, 0.1, 0.5, 1, 2, 10, and 50 μg/ml for human urine, and 0.05, 0.1, 1, and 10 μg/ml for rat tissue homogenates.

2.3. Sample preparation

A 50-μl aliquot of DA-7858 (internal standard, 10 μg/ml in methanol) and a 300 μl acetonitrile (to deproteinize biological sample [10]) were added to a 100 μl of biological sample. After vortex-mixing and centrifugation at 9000 *g* for 5 min, the supernatant was transferred into a clean Eppendorf tube and evaporated under a gentle stream of nitrogen. The residue was reconstituted in a 100-μl aliquot of the mobile phase and a 50-μl aliquot was injected directly onto the HPLC column.

2.4. HPLC apparatus

The HPLC system consisted of a model AS-1559 autosampler (Jasco, Tokyo, Japan), a model 2250 pump (Bischoff, Leonberg, Germany), a reversed-phase (C_{18}) column (RP-18; 15 cm length×4.6 mm I.D.; particle size 3.5 μm; Hichrome, Berkshire, UK), a model UV/VIS-151 detector (Gilson, Middleton, WI, USA) and a model Chromatocorder 21 integrator (SIC, Tokyo, Japan). The mobile phase, 20 m*M* KH_2PO_4:acetonitrile (75:25, v/v), was run at a flow rate of 1.5 ml/min. The column effluent was monitored by a UV detector set at 300 nm.

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

Theoretical concentration (μg/ml)	Response factor		Accuracy[c] (%)	
	Within-day (n=3)	Between-day (n=3)	Within-day (n=3)	Between-day (n=3)
Human plasma				
50	0.183 (2.28)[a]	0.184 (0.688)	101 (2.28)	102 (0.688)
	19 600 (0.759)[b]	19 700 (0.735)	102 (0.759)	103 (0.735)
0.05	0.183 (5.66)	0.177 (0.690)	101 (5.66)	97.9 (0.690)
	19 500 (2.58)	18 700 (3.89)	102 (2.58)	97.4 (3.38)
0.02	0.179 (2.14)	0.181 (1.13)	99.2 (2.14)	100 (1.13)
	18 700 (1.93)	19 200 (2.70)	97.2 (1.93)	99.9 (2.70)
Human urine				
50	0.206 (2.48)	0.191 (7.22)	104 (2.48)	95.7 (7.22)
	26 000 (4.75)	25 500 (2.27)	105 (4.75)	104 (2.27)
0.05	0.198 (3.44)	0.200 (2.23)	104 (3.44)	100 (2.23)
	23 100 (4.70)	24 000 (6.36)	93.5 (4.70)	98.2 (6.36)
0.02	0.212 (1.83)	0.210 (1.17)	104 (5.00)	105 (1.17)
	26 100 (3.62)	26 100 (0.417)	106 (3.62)	107 (0.417)

Values in parentheses are coefficients of variation, C.V.s (%).
[a] [Drug peak height (in cm) divided by its concentration (μg/ml)]/[internal standard peak height (in cm)]; mean (ml/μg).
[b] [Drug peak height (in cm) divided by its concentration (μg/ml)]; mean (cm/μg per ml).
[c] (Mean observed concentration/theoretical concentration)×100; mean.

3. Results and discussion

Fig. 2 shows typical chromatograms of drug-free human plasma, drug standard in human plasma, plasma collected at 48 h after intravenous administration of 10 mg/kg of I to a rat, drug-free human urine, drug standard in human urine, urine collected between 0 and 48 h after intravenous administration of 10 mg/kg of I to a rat, drug-free rat lung homogenate, drug standard in rat lung homogenate, and rat lung collected at 30 min after intravenous administration of 20 mg/kg of I to a rat using the deproteinization method. No interferences from endogenous substances were observed in any of the biological samples.

The retention times of I and internal standard were approximately 6.5 and 8.7 min, respectively. The detection limits of I in human plasma and urine were both 20 ng/ml (Table 1) based on a signal-to-noise ratio of 3.0. The mean within-day and between-day (3 consecutive days) coefficients of variation (C.V.s) for both response factors ([drug peak height (in cm) divided by its concentration (μg/ml)]/[internal standard peak height (in cm)]) and accuracies [(mean observed concentration/theoretical concentration)× 100] in human plasma and urine at 0.02, 0.05, and 50 μg/ml of I are listed in Table 1. The mean within-day C.V.s for response factor of I in human plasma and urine were 1.76% (range 0.210–5.66%) and 3.24% (range 0.655–7.76%), respectively, within the

Table 2
Response factors and accuracies of I at various concentrations in rat tissue homogenates

Tissue	Theoretical concentration (μg/ml)	Response factor	Accuracy[c]	Tissue	Theoretical concentration (μg/ml)	Response factor	Accuracy[c]
Liver	10	0.136[a] (1.54)	102 (1.54)	Stomach	10	0.118[a] (0.674)	101 (0.675)
		15 300[b] (1.36)	99.8 (1.36)			14 100[b] (0.325)	105 (0.325)
	0.05	0.133 (1.54)	99.4 (1.54)		0.05	0.115 (8.02)	99.0 (8.02)
		15 600 (2.08)	102 (2.08)			13 100 (8.33)	98.0 (8.33)
Lung	10	0.129 (1.62)	101 (1.62)	Small intestine	10	0.128 (1.00)	93.3 (1.00)
		14 800 (4.93)	101 (4.93)			15 500 (1.32)	95.7 (1.32)
	0.05	0.129 (3.10)	101 (3.10)		0.05	0.142 (5.65)	104 (5.65)
		14 700 (5.28)	99.8 (5.28)			17 700 (7.72)	109 (7.72)
Heart	10	0.107 (7.57)	100 (7.57)	Large intestine	10	0.117 (1.02)	98.8 (1.02)
		12 100 (2.62)	98.4 (2.62)			14 300 (1.92)	105 (1.92)
	0.05	0.103 (2.99)	96.7 (2.99)		0.05	0.124 (7.95)	104 (7.95)
		12 400 (3.14)	101 (3.14)			12 900 (3.55)	94.9 (3.55)
Brain	10	0.116 (1.60)	102 (1.60)	Spleen	10	0.122 (1.99)	99.4 (1.99)
		12 600 (3.74)	98.7 (3.74)			13 800 (2.61)	90.8 (2.61)
	0.05	0.114 (7.65)	100 (7.65)		0.05	0.123 (4.01)	102 (4.01)
		12 700 (8.98)	99.1 (8.98)			16 200 (1.42)	107 (1.42)
Kidney	10	0.127 (0.442)	98.5 (0.442)	Mesentery	10	0.135 (8.01)	103 (8.01)
		14 200 (1.14)	94.1 (1.14)			17 100 (1.83)	106 (1.83)
	0.05	0.129 (0.852)	100 (0.852)		0.05	0.150 (1.46)	102 (1.46)
		16 200 (0.620)	108 (0.620)			14 900 (0.754)	92.4 (0.754)
Muscle	10	0.106 (5.25)	93.2 (5.25)	Fat	10	0.142 (1.84)	91.3 (1.84)
		12 200 (2.83)	92.6 (2.83)			16 600 (5.22)	95.3 (5.22)
	0.05	0.120 (1.96)	106 (1.96)		0.05	0.155 (5.31)	99.8 (5.31)
		14 300 (2.67)	108 (2.67)			17 100 (2.71)	98.2 (2.71)

Values in parentheses are C.V.s (%); $n=3$.
[a] [Drug peak height (in cm) divided by its concentration (μg/ml)]/[internal standard peak height (in cm)]; mean (ml/μg).
[b] [Drug peak height (in cm) divided by its concentration (μg/ml)]; mean (cm/μg per ml).
[c] (Mean observed concentration/theoretical concentration)×100; mean.

concentration range of 0.02–50 μg/ml. The corresponding values for between-day were 0.613% (range 0.0350–1.13%) and 2.67% (range 0.302–7.22%). The within-day accuracies of I were 99.2–103% and 96.7–104% for human plasma and urine, respectively, within the concentration range of 0.02–50 μg/ml. The corresponding values for between-day were 97.5–102% and 95.1–105%.

This HPLC method was employed for the determination of I in rat tissues (liver, lung, heart, brain, kidney, muscle, stomach, small intestine, large intestine, spleen, mesentery, and fat) (Table 2). The detection limit of I was ~50 ng/ml for all rat tissues (or organs) studied (Table 2). The mean within-day C.V.s for response factor in rat tissues ranged from 0.442 (kidney at 10 μg/ml) to 8.02% (stomach at 0.05 μg/ml) within the concentration range of 0.05–10 μg/ml. The accuracy of I in rat tissues ranged from 91.3% (fat at 10 μg/ml) to 106% (kidney at 0.05 μg/ml) within the concentration range of 0.05–10 μg/ml.

Although an internal standard (DA-7858) is freely available upon request to the Research Laboratory of Dong-A Pharmaceutical Company, it is not easy to synthesize the DA-7858 in another laboratory. Moreover, sample preparation was simple; deproteinization with acetonitrile was employed. Hence, the present assay data on I were recalculated without using an internal standard, and are also listed in Tables 1 and 2. The mean within-day C.V.s for response factor of I in human plasma and urine were 1.63% (range 0.758–2.59%) and 4.83% (range 1.59–11.3%), respectively. The corresponding values for between-day were 1.62% (range 0.377–3.89%) and 2.75% (range 0.417–6.36%). The within-day accuracies of I were 96.5–103% and 93.5–105% for human plasma and urine, respectively. The corresponding values for between-day were 96.2–103% and 95.8–107%. The mean within-day C.V.s for response factor in rat tissues ranged from 0.249% (large intestine at 1 μg/ml) to 13.1% (spleen at 1 μg/ml). The accuracies of I in rat tissues ranged from 90.8% (spleen at 10 μg/ml) to 109% (small intestine at 0.05 μg/ml).

This HPLC method was successfully applied to pharmacokinetic studies of I in five rats (Fig. 3). After 1-min intravenous administration of 10 mg/kg of I (I was dissolved in DMA:PEG 400:distilled water 3:5:2, v/v/v) to five male Sprague–Dawley rats, the plasma concentrations of I decayed in a polyexponential fashion with a mean terminal half-life of 783 min. Compound I was cleared slowly in rats; the time-averaged total body clearance was relatively slow (0.860 ml/min per kg) and terminal half-life was relatively long (783 min). The amount of I recovered from each tissue 30 min after intravenous administration of 20 mg/kg of I to a rat was 30.7, 11.2, 12.4, 0.642, 20.8, 16.9, 8.80, 14.3, 4.75, 10.5, 6.02, and 4.86 μg/g for liver, lung, heart, brain, kidney, muscle, stomach, small intestine, large intestine, spleen, mesentery, and fat, respectively, and the plasma concentration was 50.5 μg/ml. The above data indicated that the affinity of rat tissues for I was not great and this could be supported by the relatively small value of volume of distribution at steady state (V_{ss}) of I, 844 ml/kg.

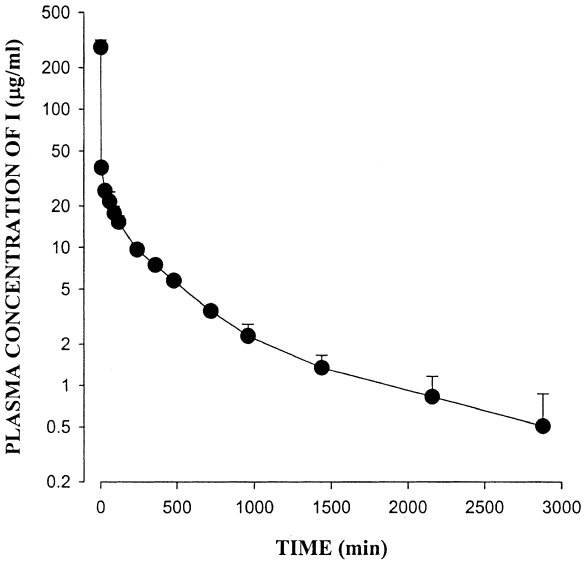


Fig. 3. Mean arterial plasma concentration–time profile of I after 1-min intravenous administration of 10 mg/kg of I to five male Sprague–Dawley rats. Bars represent standard deviations.

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