L-656,575 (OCP-9-176): A NOVEL OXACEPHEM

PHARMACOKINETICS AND EXPERIMENTAL CHEMOTHERAPY†

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L-656,575 is a new oxacephem that, based on studies in rhesus monkeys, is expected to have a moderately long half-life in humans. After administration of a 10-mg/kg dose by the intramuscular route to rhesus monkeys, peak serum concentrations of 32~54 μg/ml were seen at about 30 minutes, and the half-life was estimated to be 63 minutes. Urinary recovery of administered dose was >94% in 6 hours. In mice given a 20-mg/kg dose by the subcutaneous route, a peak serum concentration of 22.9 μg/ml was observed at 15 minutes after dosing, and the half-life in serum was about 18 minutes. Urinary recovery of the dose was 59% in 6 hours. In another study in mice, administration of probenecid did not extend the half-life of L-656,575, suggesting that the antibiotic is excreted primarily by glomerular filtration in this species. Binding to human plasma proteins was 30% at drug concentrations from 25~100 μg/ml. L-656,575 also was shown to be efficacious in experimental bacteremias due to Gram-positive and Gram-negative pathogens in mice, thus confirming the broad spectrum of activity demonstrated for L-656,575 *in vitro*.

L-656,575 (OCP-9-176) is a novel 2-methyloxacephalosporin that has potent *in vitro* antibacterial activity against a wide spectrum of aerobic clinical isolates[1~4].

In the present study, the serum pharmacokinetics and urinary excretion in mice and rhesus monkeys, and experimental chemotherapy in mouse bacteremias of L-656,575 as compared to ceftazidime are summarized.

Materials and Methods

Antibiotics

L-656,575 was supplied by Meiji Seika Kaisha, Ltd., Yokohama, Japan; it was prepared in 0.067 M SORENSEN's buffer pH 7.0. The commercial preparation of ceftazidime (Glaxo, Fortaz, ceftazidime for injection) was used; it was diluted in sterile distilled water to the desired concentration.

Animals

Female CD-1 mice (Charles River Breeding Laboratories, Wilmington, MA) weighing 20 g were used. They were housed in a temperature-controlled environment and fed Purina Formulab Chow No. 5008 and tap water *ad libitum*.

Three male rhesus monkeys (Charles River Breeding Laboratories, Key Lois, FL) were used. When not on test, they were housed in individual cages and were fed Purina Monkey Chow No. 5037 and fruit twice-daily. Tap water was available *ad libitum*. On the day before a test, they were transferred under ketamine anesthesia to chairs designed to restrain the animals with minimal discomfort.

Pharmacokinetics in Mice

The serum pharmacokinetics and urinary excretion of L-656,575 were determined in mice and

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compared with those of ceftazidime. Groups of 5 mice each were given a 20-mg/kg subcutaneous dose of an antibiotic. Immediately after dosing, a group of mice was placed in a metabolism cage designed to collect urine free of fecal contamination. At a specified time after the dose, blood and urine samples were collected from a group, and the antibiotic content of the serum and urine was determined by bioassay (see Microbiological Assay).

Pharmacokinetics in Rhesus Monkeys

The pharmacokinetics of L-656,575 also were determined in 3 male rhesus monkeys and compared with ceftazidime in a crossover design. The monkeys weighed, on average, 3.8 kg at the time of the L-656,575 study and 4.1 kg at the time of the ceftazidime study. Each monkey was given a 10-mg/kg intramuscular dose of the antibiotic into the *vastus lateralis* muscle. At specified times after dosing, blood samples were drawn from the femoral vessels. Urine was collected in a flask set in ice water for the intervals 0~6 hours and 6~24 hours. Antibiotic content of serum and urine was determined by bioassay.

Microbiological Assay

Standard disk-diffusion procedures were used to determine total bioactivity in serum and urine samples. The bioassay for L-656,575 employed *Escherichia coli* MB 4353 (Merck Culture Collection) in nutrient agar supplemented with yeast extract. *Proteus mirabilis* MB 838 was the assay organism used for analysis of ceftazidime. Standard curves were prepared in normal serum of the test species and in SORENSEN's buffer pH 7.2 at concentrations ranging from 0.625~5 μg/ml. Serum samples were diluted in normal mouse or monkey serum; urine was diluted in SORENSEN's buffer pH 7.2. After incubation at 37°C, zones of inhibition were measured with the aid of an image analyzer. The potency of each sample was calculated from the regression line computed for the appropriate standard curve. Sensitivity of the two assay procedures was 0.3 μg/ml.

Chemotherapeutics in Mice

The therapeutic efficacy of L-656,575 as compared to ceftazidime was demonstrated in experimental bacteremias in mice. Groups of 5 mice were infected intraperitoneally with a suitable dilution of the pathogen in brain heart infusion or in 5% hog gastric mucin. Therapy was administered by the subcutaneous route immediately after infection and again 6 hours later. At least four, 4-fold dilutions of each antibiotic were tested. The mice were observed for 7 days and the number of survivors on that day was used to calculate the median effective dose (ED_{50}) and the median lethal dose of the pathogen (LD_{50}) by the method of KNUDSEN and CURTIS[5]. All bacterial cultures used in this study were of human origin.

Human Plasma Binding Studies

Binding to human plasma proteins was determined as described in PELAK *et al.*[6]. Briefly, the plasma/antibiotic mixture was placed in the upper chamber of an Amicon Centricon-10 microconcentrator and centrifuged at 4,500×*g* for 30 minutes. The ultrafiltrate was analyzed in a Gilford UV/VIS "Response" scanning spectrophotometer.

The serum binding was calculated as follows:

$$\frac{\text{Peak absorption, buffer} - \text{Peak absorption, ultrafiltrate}}{\text{Peak absorption, buffer}} \times 100 = \% \text{ Bound}$$

Peak absorption for L-656,575 was at 230 nm.

Results and Discussion

Pharmacokinetics in Mice

The pharmacokinetics of L-656,575 in mice given a single 20-mg/kg subcutaneous dose compared favorably with those of ceftazidime (Table 1). The observed peak serum concentration (C_{MAX}) of L-656,575 was 22.9 μg/ml at 15 minutes after dosing. The area under the serum concentration/time

curve extrapolated to infinity (AUC) was 13.7 μg·hours/ml, and the half-life, estimated from the terminal linear portion of the serum concentration/time curve ($T_{1/2}$), was 17.6 minutes. Comparable values for ceftazidime were: C_{MAX}=21.1 μg/ml, AUC=16.5 μg·hours/ml and $T_{1/2}$=22.9 minutes. Urinary recovery of administered dose in 6 hours was 59.0% for L-656,575 and 59.8% for ceftazidime.

Renal tubular secretion does not appear to be a major route of elimination of L-656,575 in the mouse. In a study in which mice were administered a 500-mg/kg oral dose of probenecid immediately before the 20 mg/kg subcutaneous dose of L-656,575, there was no increase in the serum concentrations, in the area under the serum concentration/time curve or in the half-life (Table 2). The only effect of probenecid was to delay urine output for about 1 hour. Thereafter, urine output was normal, and urinary recovery of the L-656,575 dose was slightly higher in the groups that were given probenecid than in those given L-656,575 alone.

Table 1. Pharmacokinetics of L-656,575 in mice given a single 20-mg/kg subcutaneous dose: Comparison with ceftazidime[a].

Time (minutes)	Serum concentration (μg/ml[b])	
	L-656,575	Ceftazidime
15	22.9	21.1
30	15.4	18.2
60	4.2	6.1
120	0.4	1.0
240	<0.3	<0.3
360	<0.3	<0.3
$AUC_{0-\infty}$ (μg·hours/ml)	13.7	16.5
$t_{1/2}^{\beta}$ (minutes)	17.6	22.9

[a] Six groups of five 20-g CD1 female mice were used for each antibiotic.

[b] Determined as total bioactivity in a standard disk-diffusion assay using *Escherichia coli* MB 4353 as the assay organism for L-656,575 and *Proteus mirabilis* MB 838 for ceftazidime. Sensitivity of each bioassay was 0.3 μg/ml.

Pharmacokinetics in Rhesus Monkeys

Results of these studies are summarized in Table 3. Peak serum concentrations of L-656,575 were observed at about 30 minutes after dosing and ranged from 32~54 μg/ml. The area under the serum concentration/time curve averaged 85.4 μg·hours/ml and the half-life in serum was estimated to be about 63 minutes. The drug was eliminated rapidly into the urine; 94~100% of the administered dose was recovered in 6 hours.

Following a 10-mg/kg intramuscular dose of ceftazidime, peak serum concentrations occurred at 30 minutes after dosing and ranged

Table 2. Effect of probenecid on serum concentrations and urinary excretion of L-656,575 in mice given a 20-mg/kg subcutaneous dose.

Time (minutes)	Serum concentration (μg/ml[a])		Time (hours)	Urinary recovery (% of dose)	
	L-656,575[b]	L-656,575+ BEN		L-656,575	L-656,575+ BEN
15	19.2	18.6			
30	15.2	15.7	0~0.5	8.3	NS
60	5.7	6.0	0~1	40.4	19.5
120	0.8	0.6	0~2	52.0	76.6
240	<0.3	<0.3	0~4	48.7	67.2
360	<0.3	<0.3	0~6	45.1	59.3
$AUC_{0-\infty}$ (μg·hours/ml)	14.4	14.3			
$t_{1/2}^{\beta}$ (minutes)	20.9	18.2			

[a] Determined as total bioactivity in a standard disk-diffusion assay using *Escherichia coli* MB 4353 as the assay organism. Sensitivity of the assay was 0.3 μg/ml.

[b] Groups of five 20-g CD1 female mice were used. The L-656,575 dose was administered immediately after a 500-mg/kg oral dose of probenecid (BEN).

NS: No urine excreted.

Table 3. Pharmacokinetics of L-656,575 in rhesus monkeys following a single 10-mg/kg intramuscular dose: Comparison with ceftazidime (CAZ) in a crossover design.

Time (minutes)	Serum concentration (μg/ml[a])					
	Monkey 83-233		Monkey 83-234		Monkey 83-235	
	L-656,575	CAZ	L-656,575	CAZ	L-656,575	CAZ
15	30.6	23.9	41.3	20.0	49.5	13.2
30	29.5	24.6	53.3	20.7	54.0	20.0
60	32.4	17.5	35.3	16.9	35.8	16.2
120	17.2	4.5	21.3	10.4	20.1	11.8
240	2.9	2.1	3.5	2.6	3.7	2.9
360	0.8	0.6	0.9	0.8	1.0	1.1
$AUC_{0-\infty}$ (μg·hours/ml)	71.4	43.4	91.4	46.1	93.5	46.8
$t_{1/2}\beta$ (minutes)	64.3	66.1	61.0	85.4	63.3	70.3

Time (hours)	Urinary recovery (% of administered dose)					
	Monkey 83-233		Monkey 83-234		Monkey 83-235	
	L-656,575	CAZ	L-656,575	CAZ	L-656,575	CAZ
0~6	98	50	94	114	109	100
0~24	103	63	102	117	114	108

[a] Determined as total bioactivity in a standard disk-diffusion assay using *Escherichia coli* MB 4353 as the assay organism for L-656,575 and *Proteus mirabilis* MB 838 for ceftazidime. Sensitivity of each bioassay was 0.3 μg/ml.

Table 4. Comparison of the therapeutic efficacy of L-656,575 and ceftazidime in experimental bacteremias in mice.

Pathogen	Challenge cfu	Dose[a] LD_{50}	L-656,575		Ceftazidime	
			MIC[b] (μg/ml)	ED_{50}[c] (mg/kg)	MIC (μg/ml)	ED_{50} (mg/kg)
Staphylococcus aureus MB 2865	4.4×10^2	317	4	3.1	4	5.2
Enterobacter cloacae MB 2646[d]	7.7×10^7	25	4	1.6	128	50
Escherichia coli MB 2891[d]	1.1×10^2	11	0.5	0.8	64	1.5
Klebsiella pneumoniae MB 4005	3.8×10^4	137	0.06	3.8	0.03	1.6
Proteus mirabilis MB 2830[d]	1.7×10^6	2,890	0.25	0.2	0.06	0.08
Pseudomonas aeruginosa MB 2835[d]	8.6×10^7	110	4	35.7	1	11.8

[a] Infection was established by intraperitoneal injection of an appropriate dilution of the pathogen in brain heart infusion (*K. pneumoniae* and *P. aeruginosa*) or in 5% hog gastric mucin. cfu were determined by standard plate counting techniques and the number of median lethal doses (LD_{50}) by the method of KNUDSEN and CURTIS.

[b] The MIC was determined in an agar dilution assay against an inoculum of 10^5 cfu/spot.

[c] Therapy was administered by the subcutaneous route at 0 and 6 hours after infection. The median effective dose (ED_{50}) was calculated by the method of KNUDSEN and CURTIS on the 7th day post infection.

[d] β-Lactamase producer.

from 20~24.6 μg/ml. The average area under the serum concentration/time curve was 45.4 μg·hours/ml and the serum half-life was estimated to be about 74 minutes. Ceftazidime appeared in high concentrations in urine; 100% of the administered dose was recovered in 6 hours from 2 monkeys, but only 50% of the dose was recovered in the 6-hour urine sample of the third monkey. We have no explanation for this discrepancy.

Thus, in monkeys given a 10-mg/kg intramuscular dose, peak serum concentrations of L-656,575 and the area under the serum concentration/time curve were about twice as great as those of ceftazidime, but the serum half-life of ceftazidime was slightly longer than that of L-656,575, 63 minutes vs. 74 minutes for ceftazidime. Both drugs appeared in urine in high concentrations, and 100% of the administered dose was recovered in 24 hours, in most cases.

Chemotherapeutics in Mice

L-656,575 was as efficacious as ceftazidime against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *P. mirabilis* and *E. coli* (Table 4). It protected mice infected with *Enterobacter cloacae*, a β-lactamase producing organism that is resistant to ceftazidime and also many other cephalosporins[6]. The ED_{50} was 1.6 mg/kg as compared to 50 mg/kg for ceftazidime. L-656,575 compared favorably with ceftazidime when used as therapy against a *Pseudomonas aeruginosa* bacteremia. The ED_{50} of L-656,575 was 35.7 mg/kg as compared to 11.8 mg/kg for ceftazidime. In general, the MIC was a very good predictor of the therapeutic efficacy of each agent.

Human Plasma Binding Studies

When determined by using ultrafiltration followed by UV spectrophotometry, binding to human plasma proteins of L-656,575 was approximately 30% (range: 26~33%) at drug concentrations from 25~100 μg/ml. This low serum protein binding as well as glomerular filtration may explain the favorable serum half-life and pharmacokinetics of L-656,575.

Conclusions

The pharmacokinetics of L-656,575 were similar to those observed with ceftazidime in both mice and monkeys. Based on the studies in monkeys, L-656,575 is expected to have a moderately long $T_{1/2}$ in humans. In experimental bacteremias in mice, L-656,575 was effective vs. both Gram-positive and Gram-negative pathogens, thus confirming the broad spectrum of activity demonstrated for L-656,575 *in vitro*.

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