QUATERNARY HETEROCYCLYLAMINO β-LACTAMS

VI. *IN VITRO* AND *IN VIVO* ANTIBACTERIAL PROPERTIES OF L-642,946 AND L-652,813, A LONG ACTING CEPHEM

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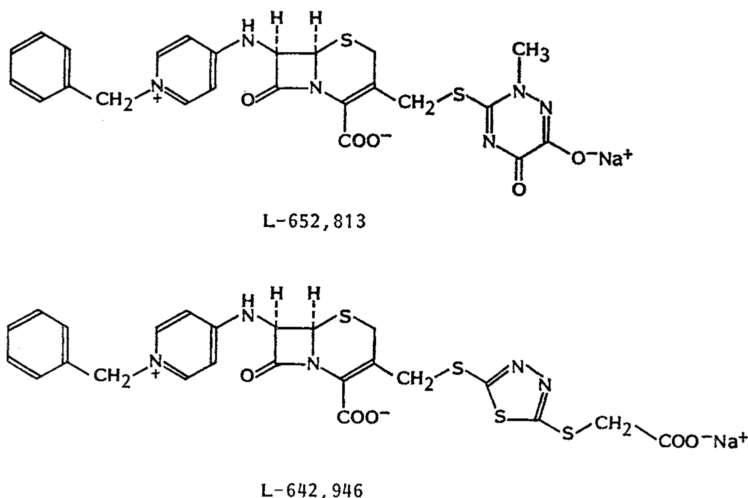
Two newly described quaternary heterocyclylamino β-lactams, L-642,946 and L-652,813, were shown to exhibit potent activity against a broad spectrum of aerobic and anaerobic bacteria *in vitro*. The activity of these agents *in vitro* translated well to chemotherapeutic activity in experimental bacteremias in mice.

Substitution of the thiadiazine moiety of L-642,946 with a triazine moiety effected a marked change in the pharmacokinetics of the new derivative, L-652,813. In mice given a 20 mg/kg subcutaneous dose, the peak serum concentration and the half-life of L-652,813 were about three times greater than those of L-642,946 and the area under the serum concentration/time curve was increased by about 5-fold. The pharmacokinetics of L-652,813 in mice and in rhesus monkeys more closely resembled those of ceftriaxone which carries the same triazine moiety on the C-3 side chain.

L-642,946 and L-652,813 (Fig. 1), represent two new members of a series of quaternary *N*-heterocyclylamino β-lactams[1,2], that carry a novel 7-amidine-linked side chain and bind preferentially (after mecillinam[3], clavulanic acid[4], and imipenem[4]) to penicillin binding protein 2 (PBP-2) of *Escherichia coli*[5]. L-652,813 was synthesized with the hope of extending the serum half-life of L-642,946, a highly water soluble, potent broad spectrum antibacterial agent. When the thiadiazine moiety of L-642,946 was replaced with the triazine moiety, the half-life in serum of mice indeed was extended.

Fig. 1. Structures of quaternary heterocyclylamino β-lactams.



This paper summarizes the antibacterial activity *in vitro* of these compounds against a wide variety of human and animal pathogens, efficacy against experimental septicemias in mice, and the serum pharmacokinetics and urinary excretion in mice and rhesus monkeys.

Materials and Methods

Antibiotics

L-652,813, L-642,946 and cefoxitin were prepared at the Merck Sharp and Dohme Research Laboratories. Ceftriaxone was supplied by Hoffmann-La Roche, Inc., and cefuroxime by Glaxo, Ltd.

Animals

Female CDl mice purchased from Charles River Breeding Laboratories, Wilmington, MA were used. They were fed Purina Formula Chow No. 5008, provided with tap water *ad libitum* and housed in a temperature-controlled environment.

Male rhesus monkeys were purchased from Buckshire Corporation, Perkasie, PA., and were housed in individual cages. They were fed Purina Chow No. 5037 and fruit twice daily. 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.

Cultures

Cultures used for evaluation *in vitro* included bacterial pathogens of human and animal origin as well as selected laboratory strains carrying chromosomal or R-plasmid mediated β-lactamases. Aerobic and anaerobic cultures were maintained at -70°C on Trypticase Soy agar (BBL) slants or in 15% skim milk.

The aerobic cultures were transferred into Trypticase Soy broth (BBL), incubated for 18 to 20 hours at 35°C and diluted further for testing. The anaerobic test cultures were restored from skim milk suspensions by subculturing to Brain Heart Infusion (BHI) (Difco) plus yeast extract (YE). Three subsequent transfers at 48-hour intervals were made, the first in BHI+YE broth and the second and third in Thiogycollate-135C (Thio-135C) broth. The anaerobic broths were supplemented with hemin (5 μg/ml), menadione (0.5 μg/ml) and sodium bicarbonate (1 mg/ml, Thio-135C only). Culture dilutions were prepared in unsupplemented Thio-135C broth and were used as the inocula for susceptibility testing.

Bacterial strains used in the *in vivo* studies were of human or animal origin and were selected because of their virulence for mice. They were maintained in the lyophilized state and were reconstituted in BHI before use. Identifying numbers are those of the Merck stock culture collection.

Minimal Inhibitory Concentration (MIC)

The minimal inhibitory concentrations were determined using an agar dilution method. Each compound was dissolved in sterile distilled water at a concentration of 1.28 mg/ml and subsequent 2-fold serial dilutions were made in water. One-ml of each antibiotic-containing solution was mixed with 9 ml of molten agar in 15×100 mm petri dishes. Nutrient agar was used for the aerobes and Wilkins - Chalgren agar (with addition of hemin and menadione) was employed for the anaerobes. The drug-agar plates were inoculated with the cultures using a Denley multipoint inoculator designed to deliver 1 μl directly onto the agar surface. A final inoculum of 10^4 or 10^6 colony forming units (cfu)/spot was used for the aerobes and about 10^6 cfu/spot for the anaerobes. The aerobes were incubated at 35°C for 18~20 hours. The anaerobes were incubated in a Forma anaerobic chamber under strict anaerobic conditions provided by an atmosphere of 10% hydrogen, 8% carbon dioxide and 82% nitrogen. The minimun inhibitory concentration (MIC), in each case, was defined as the lowest concentration of drug showing no distinct growth or less than five discrete colonies/spot.

Chemotherapeutic Studies

In the mouse protection tests, 19~22 g CD1 female mice were infected intraperitoneally (ip) with 0.5 ml of an appropriate dilution of the pathogen in BHI for *Klebsiella pneumoniae* and in 5% hog

gastric mucin (Wilson Laboratories, Division of Inolex Corp., Park Forest South, IL, U.S.A.) for all other pathogens. Under these conditions all infected untreated mice died within 48 hours. The antibiotics were dissolved in sterile distilled water or in SORENSEN's buffer pH 7.2 and administered by subcutaneous (sc) injection in a volume of 0.5 ml immediately after infection and again 6 hours later. At least four 4-fold dilutions of each antibiotic were tested. All animals were observed for a period of 7 days after which the median effective dose (ED_{50}) was calculated by the method of KNUDSEN and CURTIS[6].

Chemoprophylaxis

Prolongation of therapeutic efficacy was evaluated in a mouse systemic infection model. Antibiotics were administered as a single dose by the subcutaneous route to groups of five CD1 female mice at 5 hours or 2 hours before infection or immediately after infection. The infection was established by the intraperitoneal injection of a suitable dilution in BHI of *K. pneumoniae* MB 4005. The median effective dose (ED_{50}) was calculated by the method of KNUDSEN and CURTIS and was based on the number of survivors on the 10th day after infection.

Pharmacokinetics in Mice

Separate groups of five 20 g CD1 female mice were given a single subcutaneous dose of an antibiotic equivalent to 20 mg/kg body weight and 0.5 ml water by gavage to stimulate urine flow. The mice were placed in metabolism cages fitted with devices to collect the urine. At specified intervals of time, one group of mice consisting of five animals was bled by heart puncture. The pooled blood was allowed to clot at room temperature. The serum was separated by centrifugation and stored in the frozen state until assayed. The urine was collected from each group, the volume recorded and the sample frozen for bioassay. The serum half-life estimated from the terminal linear portion of the serum concentration/time curve ($T_{1/2}$), the area under the serum concentration/time curve extrapolated to infinity (AUC) and the plasma clearance rate (Clp) were calculated with the aid of a computer program.

Pharmacokinetics in Rhesus Monkeys

The serum pharmacokinetics and urinary excretion of L-652,813 and ceftriaxone were compared in a crossover design using three male rhesus monkeys. There was a 4-week rest period between drug doses. Monkeys weighed, on average, 5.55 kg at the time of the L-652,813 dose and 5.62 kg at the time of the ceftriaxone dose.

Each antibiotic was prepared in sterile distilled water at a concentration of 40 mg/ml. Solution of L-652,813 was facilitated by the addition of $NaHCO_3$; the pH of this solution was 8. The pH of the ceftriaxone solution was 6.5. This concentration (40 mg/ml) allowed the drug dose to be delivered in a volume of 1.2~1.6 ml.

The monkeys were given a single 10 mg/kg dose by intramuscular (im) injection into the left thigh muscle. Blood samples were drawn at 0.25, 0.5, 1, 2, 4 and 6 hours after dosing. The serum was removed after centrifugation and stored at −20°C until assayed. Urine was collected after 6 hours and after 24 hours. The volume was recorded and an aliquot was frozen for bioassay.

Microbiological Assay

A standard disk diffusion assay was performed using Antibiotic Medium No. 1 seeded with *E. coli* MB 4269 as the assay organism for L-652,813 and L-642,946. *K. pneumoniae* MB 1264 seeded in Tryptic Soy agar (Difco) was the assay organism for ceftriaxone. Serum samples were diluted in normal mouse or monkey serum and urine 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 assay varied from approximately 0.2~0.8 μg/ml.

Human Plasma Binding Studies

Antibiotic solutions at a concentration of 1.0 mg/ml were diluted in SORENSEN's buffer pH 7.2 and in human plasma to a final concentration of 100 μg/ml. Citrated human plasma purchased from

Sera-Tec Biologicals (North Brunswick, N.J., U.S.A.) was adjusted to pH 7.3~7.5 by bubbling carbon dioxide or oxygen through the plasma.

The drug/buffer and drug/plasma mixtures were incubated for 30 minutes at 35°C. Two ml volumes were then placed in the upper chambers of Amicon Centricon-10 microconcentrators fitted with YM membranes which retain molecules above 10,000 molecular weight. After centrifugation for 30 minutes at 4,500×g in a Centra-7R centrifuge in a 45° fixed angle rotor, the ultrafiltrates and the buffer solutions were diluted 1/10 with SORENSEN's buffer.

The samples were placed in a Gilford UV/VIS "Response" scanning spectrophotometer and the absorption spectrum was determined for each antibiotic in buffer and in ultrafiltrate by scanning between 200~320 nm.

The serum binding was calculated as follows:

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

Results and Discussion

In Vitro Studies

The two heterocyclylamino β-lactam antibiotics, L-642,946 and L-652,813, both demonstrated a broad spectrum of activity against aerobic and anaerobic bacteria.

Results obtained when L-652,813, L-642,946, ceftriaxone and cefuroxime were tested against an inoculum of 10^4 cfu/spot in nutrient agar against a panel of 17 aerobic bacteria are presented in Table 1. L-652,813 was as potent as the other drugs against *Staphylococcus aureus* and superior to the others against Enterobacteriaceae. None of the compounds tested was active against *Streptococcus faecalis* or *Pseudomonas aeruginosa*.

Both L-652,813 and L-642,946, unlike ceftriaxone and cefuroxime, were active against *Enterobacter cloacae* MB 2646 and *Klebsiella oxytoca* MB 4354 suggesting that these new compounds are resistant

Table 1. Comparison of the antibacterial activity of L-652,813, L-642,946, ceftriaxone and cefuroxime against selected strains of aerobic bacteria.

Organism	MB number	MIC (μg/ml)[a]			
		L-652,813	L-642,946	Ceftriaxone	Cefuroxime
Staphylococcus aureus	2868	1	8	4	1
S. aureus	2865	1	4	2	1
Streptococcus faecalis	2864	>128	>128	32	128
Escherichia coli TEM 2(+)	4351	≤0.008	≤0.008	≤0.008	1
E. coli	2891	≤0.008	0.06	4	32
Salmonella typhimurium	3860	≤0.008	0.03	0.125	8
Enterobacter cloacae P99(−)	2647	≤0.008	≤0.125	0.015	4
E. cloacae P99(+)	2646	0.25	1	128	>128
E. aerogenes	2828	≤0.008	0.125	1	32
Klebsiella oxytoca K1(+)	4354	0.5	4	32	>128
K. pneumoniae	4005	≤0.008	≤0.008	0.015	0.5
Morganella morganii Sm(R)	2833	≤0.008	0.015	0.03	16
Proteus vulgaris	2829	≤0.008	≤0.008	≤0.008	8
P. mirabilis Gm(R)	2830	≤0.008	0.015	≤0.008	1
Pseudomonas aeruginosa RPL11(+)	3350	>128	128	128	>128
P. aeruginosa	2835	128	128	16	>128
Serratia marcescens	2840	0.03	0.125	4	>128

[a] Agar-dilution test, inoculum 10^4 cfu/spot, nutrient agar, incubation at 35°C for 18~20 hours.

Table 2. Antibacterial activity of L-652,813, L-642,946, ceftriaxone, cefuroxime and cefoxitin against selected anaerobes.

Organism	MB number	MIC (μg/ml)[a]				
		L-652,813	L-642,946	Ceftriaxone	Cefuroxime	Cefoxitin
Actinomyces naeslundi	4053	1	0.25	0.5	0.06	0.015
Eubacterium limosum	3344	1	8	0.5	2	0.5
Propionibacterium acnes	2249	0.06	NT	0.125	0.015	0.03
Peptostreptococcus anaerobius	3282	1	0.5	16	0.03	0.5
Clostridium perfringens	4418	1	NT	0.5	0.125	0.5
C. ramosum	4272	4	NT	0.5	NT	16
C. difficile Clind	4273	NT	1	NT	128	64
C. difficile Fox	4380	64	NT	128	128	64
Bifidobacterium dentium	4427	>128	16	4	16	64
Bacteroides fragilis	4324	16	4	2	8	4
B. fragilis	3214	64	16	>128	>128	8
B. fragilis	4360	4	16	2	8	32
B. fragilis	4419	1	8	4	16	8
B. distasonis	4361	4	16	128	128	32
B. distasonis	3445	>128	>128	128	>128	128
B. ovatus	3248	2	32	128	128	16
B. thetaiotaomicron	4362	32	32	128	16	32
B. thetaiotaomicron	4420	2	16	64	32	16
B. asaccharolyticus	4271	0.125	NT	≤0.008	0.125	0.125
Fusobacterium mortiferum	3345	4	32	128	32	32
Veillonella alcalescens	1952	16	2	0.5	4	8

[a] Agar-dilution assay, multipoint inoculator, Wilkins - Chalgren agar, inoculum 10^6 cfu/spot, incubation at 35°C for 48 hours in anaerobic atmosphere.
NT: Not tested.

to hydrolysis by the chromosomal cephalosporinases of these organisms. They were also resistant to the R-plasmid mediated enzyme of *E. coli* MB 4351.

Like other β-lactams of this class[2)], L-652,813 and L-642,946 gave higher MICs when tested in Trypticase Soy agar (data not shown).

L-652,813 also demonstrated good activity against anaerobic bacteria. The Gram-positive cocci were susceptible to all five drugs tested with the exception of *Peptostreptococcus anaerobius* which was resistant to ceftriaxone (MIC=16 μg/ml). The *Clostridium* sp. were also susceptible except for the *Clostridium difficile* strain which was resistant to all the cephalosporins tested. L-652,813 showed good activity against *Bacteroides* sp. It was, however, the least active drug against *Veillonella alcalescens*.

Chemotherapeutic Trials

Excellent therapeutic effectiveness of L-642,946 and L-652,813 was demonstrated against experimental systemic infections in mice when the drugs were administered parenterally.

Results of these studies in comparison with ceftriaxone and cefuroxime are shown in Table 3. The ED_{50} values are from single trials in which all antibiotics were tested simultaneously.

In general, the activity of L-652,813 appeared to be 2~4-fold greater than L-642,946 in protecting mice against a number of Gram-positive and Gram-negative pathogens. One exception was the *Proteus mirabilis* MB 2830 infection against which L-652,813 was about 4-fold less active. The potent

Table 3. Comparison of the therapeutic efficacy of L-652,813, L-642,946, ceftriaxone and cefuroxime in experimental bacteremias[a] in mice.

Pathogen	MB number	ED_{50} (mg/kg×2 sc doses)[b]			
		L-652,813	L-642,946	Ceftriaxone	Cefuroxime
Staphylococcus aureus	2865[c]	3.6	13.3	1.9	0.4
Escherichia coli	2891[c]	0.005	0.09	0.78	24.9
Klebsiella pneumoniae	4005	3.9	7.6	0.16	122.6
Proteus mirabilis	2830[c]	5.7	20.6	0.013	7.2
P. mirabilis	3125	6.3	1.9	0.006	1.3
P. vulgaris	2829[c]	0.01	0.024	0.024	13.26

[a] Infection was established by the intraperitoneal injection of an appropriate dilution of the pathogen in brain heart infusion broth (for *Klebsiella pneumoniae*) and 5% hog gastric mucin for all others. Challenge doses contained 19～317 LD_{50}s.

[b] Medium effective dose (ED_{50}) was calculated by the method of KNUDSEN and CURTIS; drugs administered subcutaneously at 0 and 6 hours after infection.

[c] β-Lactamase producing strain.

activity *in vitro* of L-642,946 and L-652,813 was translated well to potent activity *in vivo*. The MICs determined in nutrient agar were good indicators of the ED_{50}s.

Ceftriaxone was significantly more active than the subject compounds against both *P. mirabilis* bacteremias. Cefuroxime, the most active agent against *Staphylococcus aureus*, was much less active against *E. coli*, *K. pneumoniae* and *P. vulgaris*.

Chemoprophylaxis

In a single trial using mice infected with *Klebsiella pneumoniae* MB 4005, L-652,813 was less effective than ceftriaxone but more effective than L-642,946 when therapy was given as a single sc dose immediately after infection (Table 4).

When therapy was administered at 2 hours or 5 hours before infection, L-642,946 did not protect mice; the ED_{50} values were >200 mg/kg. L-652,813 was efficacious when given 2 hours before infection. The ED_{50} was 6.24 mg/kg compared to 2.94 mg/kg when dosed immediately after infection. When L-652,813 was administered at 5 hours before infection, a high dose was required to protect mice. The ED_{50} was 96 mg/kg.

Table 4. Effect of single dose therapy on chemoprophylaxis against *Klebsiella pneumoniae* bacteremia in mice[a].

Antibiotic	Therapy time (hour)	ED_{50} (mg/kg sc)	MIC[b] (μg/ml)
L-652,813	0	2.94	≤0.008
	−2	6.24	
	−5	96.0	
L-642,946	0	5.0	≤0.008
	−2	>200.0	
	−5	>200.0	
Ceftriaxone	0	0.16	0.06
	−2	0.29	
	−5	1.29	

[a] Groups of 5 CD1 female mice were treated with a single subcutaneous dose of the antibiotic at the times indicated. All mice were infected at 0 hour with *K. pneumoniae* MB 4005. The challenge dose contained 318 LD_{50}.

[b] Determined in an agar dilution assay at 10^6 cfu/spot in nutrient agar.

ED_{50} values for ceftriaxone were 1.29 mg/kg when dosed 5 hours before infection, 0.29 mg/kg when dosed 2 hours before infection, and 0.16 mg/kg when administered immediately after the infecting dose.

Although $T_{1/2}$ values for L-652,813 and ceftriaxone were similar, the peak serum concentration and AUC for L-652,813 were about half those of ceftriaxone while the Clp was twice that of ceftriaxone (see Pharmacokinetics in Mice below). These latter factors appeared to have had a greater impact on chemotherapeutic outcome in these trials than did the $T_{1/2}$.

Pharmacokinetics in Mice

L-652,813 was rapidly absorbed and distributed in mice following a 20 mg/kg sc dose. A peak serum concentration of 44.6 μg/ml was observed at 15 minutes after dosing and measurable amounts of activity remained in serum through 6 hours after the dose (Table 5). Serum half-life ($T_{1/2}$) was 70 minutes, the area under the serum concentration/time curve (AUC) was 73.8 mg·hours/liter and the plasma clearance rate (Clp) was 4.5 ml/minute/kg.

L-642,946, reached a peak serum concentration of 15.2 μg/ml at 15 minutes after dosing, but no activity was detected in serum at 4 hours after a 20 mg/kg sc dose. The $T_{1/2}$ was 21 minutes, the AUC was 13.8 mg·hours/liter and the Clp was 24.2 ml/minute/kg.

For comparison, data from studies with ceftriaxone, a long-acting cephalosporin in humans, are also presented in Table 3. Concentrations in serum reached a peak of 87.7 μg/ml at 30 minutes after the 20 mg/kg dose and declined slowly to a concentration of 2.1 μg/ml at 6 hours. The AUC was 145 mg·hours/ml, the Clp was 2.3 ml/minute/kg and the $T_{1/2}$ was 64 minutes in this single test.

High concentrations (>200 μg/ml) of L-652,813 were found in urine from 1 hour through 6 hours after dosing and 53% of the dose was recovered in 6 hours. These values were similar to those obtained with ceftriaxone (concentrations >200 μg/ml from 1~6 hours and 41% of the dose recovered in urine in 6 hours) and greater than those found following a comparable dose of L-642,946 (concentrations of about 60~120 μg/ml and only 15% of the dose recovered in urine in 6 hours).

None of these cephalosporins was absorbed following oral administration to mice; less than 1%

Table 5. Antibiotic concentrations in serum and urine of mice following a 20 mg/kg subcutaneous dose.

Antibiotic	Serum (μg/ml[a] at minute)						AUC[b] (mg·hours/liter)	$T_{1/2}$ (minutes)	Clp (ml/minute/kg)
	15	30	60	120	240	360			
L-652,813	44.6	40.5	32.1	14.7	2.3	0.7	73.8	70	4.5
L-642,946	15.2	14.6	6.3	0.9	<0.2	<0.2	13.8	21	24.2
Ceftriaxone	83.7	87.7	56.6	23.2	7.7	2.1	145.0	63	2.3

Antibiotic	Urine (μg/ml[a] at hour)				Recovery (μg/6 hours)	Dose recovered in 6 hours (%)
	0–1	0–2	0–4	0–6		
L-652,813	420	248	262	316	1,063	53.2
L-642,946	96	70	76	57	303	15.2
Ceftriaxone	277	250	242	198	820	41.0

[a] Determined as total bioactivity by a disk-diffusion method using *Klebsiella pneumoniae* MB 1264 (for ceftriaxone) and *Escherichia coli* MB 4269 (for L-652,813 and L-642,946) as assay organisms.

[b] Area under the serum concentration/time curve extrapolated to infinity (AUC), the serum half-life estimated from the terminal linear portion of the serum concentration/time curve ($T_{1/2}$) and the plasma clearance rate (Clp) were calculated using a computer program.

of the 20 mg/kg oral dose was recovered in urine in 6 hours.

Pharmacokinetics in Rhesus Monkeys

Concentrations of drug in serum, area under the serum concentration/time curve (AUC), the half-life in serum ($T_{1/2}$) and the plasma clearance rate (Clp) for L-652,813 and ceftriaxone are shown in Table 6.

Following a single 10 mg/kg im dose, the average peak serum concentration of ceftriaxone was slightly higher than that of L-652,813 (70.3 μg/ml vs. 61.9 μg/ml), the AUC was greater (215 vs. 163 mg·hours/liter), the $T_{1/2}$ was 1.5 times longer (105 vs. 70 minutes) and the Clp was slightly slower (0.70 vs. 0.97 ml/minute/kg) in these monkeys.

Concentrations of drug in urine were similar for both antibiotics (Table 7). High concentrations (253~649 μg/ml) were found in 0~6 hours samples and lower levels (21~54 μg/ml) were present

Table 6. Serum concentrations of drug in rhesus monkeys following a 10 mg/kg intramuscular dose.

	L-652,813			Ceftriaxone		
	Monkey number			Monkey number		
	11078	11079	11148	11078	11079	11148
Time (minutes)	(μg/ml)[a]			(μg/ml)[a]		
15	44.7	75.2	59.0	62.6	36.9	70.2
30	51.6	52.1	45.7	64.5	54.6	82.1
60	49.1	53.8	48.3	51.3	64.3	76.7
120	30.2	33.6	32.6	38.4	61.0	41.7
240	13.1	25.7	25.0	17.2	33.8	24.4
360	5.3	6.3	6.2	6.8	17.5	10.6
AUC[b] (mg·hours/liter)	152	190	177	187	303	248
$T_{1/2}$[c] (minutes)	91.5	58.9	59.4	89.2	125.8	99.3
Clp[d] (ml/minute/kg)	1.1	0.9	0.9	0.9	0.5	0.7

[a] Determined by bioassay.
[b] AUC: Area under the serum concentration/time curve extrapolated to infinity.
[c] $T_{1/2}$: Serum half-life estimated from the terminal linear portion of the serum concentration/time curve.
[d] Clp: Plasma clearance rate.

Table 7. Urinary excretion of drug in rhesus monkeys following a 10 mg/kg intramuscular dose.

	L-652,813			Ceftriaxone		
	Monkey number			Monkey number		
	11078	11079	11148	11078	11079	11148
Time: 0~6 hours						
μg/ml[a]	253.3	649.0	261.5	511.0	362.0	375.5
Total mg	16.2	16.9	11.2	9.7	9.4	14.6
% Dose excreted	33.8	30.1	18.1	21.1	15.7	22.9
Time: 6~24 hours						
μg/ml	21.0	53.6	46.0	27.5	54.4	30.4
Total mg	3.4	8.9	13.2	2.6	7.9	3.5
% Dose excreted	7.2	15.9	21.2	5.7	13.1	5.5
Time: 0~24 hours						
Total mg	19.6	25.8	24.4	12.3	17.3	18.1
% Dose excreted	41.0	46.0	39.3	26.8	28.8	28.4

[a] Determined by bioassay.

in 6～24 hours urines. However, the total amount of drug excreted in urine in 24 hours was greater for L-652,813 (39～46% of dose) than for ceftriaxone (27～29% of dose).

In man, about 40% of a dose of ceftriaxone is eliminated through the bile and about 60% in the urine in 48 hours[7]. In rats given a 20 mg/kg iv dose of ceftriaxone, urinary recovery was 32.0% and biliary recovery, 61.8% in 24 hours[8]. The low urinary recovery of ceftriaxone in these monkeys along with the change in stool consistency (one monkey had diarrhea and the other two had very soft, but formed stools at 24 hours after dosing) suggest a high extrarenal elimination of ceftriaxone in this species also. No other untoward effects were noted with ceftriaxone; none were seen with L-652,813.

Interestingly, substitution of the 3-carbamoyloxymethyl grouping in cefoxitin with the triazine grouping from ceftriaxone did not provide a cefoxitin with a longer serum half-life in mice or monkeys (unpublished data).

Plasma Protein Binding

When determined by using ultrafiltration followed by UV spectrophotometry, the binding to human plasma proteins was 60% for both L-642,946 and L-652,813 and about 90% for ceftriaxone.

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