IN VIVO ANTIBACTERIAL ACTIVITY OF FK482, A NEW ORALLY ACTIVE CEPHALOSPORIN

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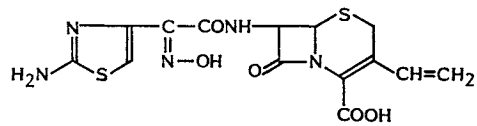
The therapeutic activities of orally administered FK482 were compared with those of reference antibiotics against systemic and local infections with a variety of bacteria in mice and rabbits. In systemic infections in mice, oral FK482 was almost as effective as oral cefaclor (CCL) and more effective than oral cephalexin (CEX) against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* infections. However, FK482 afforded superior protective activity when given subcutaneously against *E. coli* infection in mice, and this activity was more potent than that of subcutaneously given CCL. In comparison with CCL, the reason that the *in vivo* activity of orally given FK482 against mouse systemic infections was weaker than had been anticipated from its potent *in vitro* activity was due to its poor oral absorption in mice. In local infections in rabbits, a species in which FK482 was better absorbed than in mice, FK482 was more effective than CCL, CEX or amoxicillin (AMPC). Against pneumonia induced by *S. aureus* or *Streptococcus pyogenes*, FK482 was as effective as AMPC and more effective than CCL in reducing the number of viable bacteria in the lungs of infected rabbits. In the oral treatment of experimental ascending pyelonephritis in rabbits, FK482 was superior to CCL and AMPC against methicillin-resistant *S. aureus* infection, as effective as AMPC and more effective than CCL against *Enterococcus faecalis* infection, and as effective as cefixime (CFIX) and more effective than CCL and AMPC against *E. coli* infection in reducing the number of viable bacteria in the kidneys and urine.

We recently began developing FK482[1] (Fig. 1), a new cephem antibiotic for oral use, which combines potent antibacterial activities against Staphylococci and *Enterococcus faecalis* with broad spectrum, potent antibacterial and bactericidal activities against Gram-negative bacteria and excellent β-lactamase stability of cefixime (CFIX). In this study, the *in vivo* activities of FK482 were compared with those of cefaclor (CCL), cephalexin (CEX), CFIX and amoxicillin (AMPC).

Fig. 1. Chemical structure of FK482.



Materials and Methods

Antibiotics

The antibiotics used in this study were FK482 and CFIX (Fujisawa Research Laboratories, Osaka, Japan), CCL and CEX (Eli Lilly and Company, Indianapolis, U.S.A.), and AMPC (Beecham Labo-

ratories, Betchworth, UK).

Bacterial Strains

Clinical isolates of various species of bacteria used were obtained from several hospitals in Japan.

Antibiotic Susceptibility Testing

The MICs of the test antibiotics were determined by the agar dilution method described in our accompanying paper[1].

Protective Effect on Systemic Infections in Mice

Male ICR-strain mice, aged 4 weeks were used in groups of 10. Each challenge organism was cultured overnight on Trypticase soy agar at 37°C and suspended in 5% bacteriological mucin (Nakarai Chemical Ltd., Kyoto, Japan) at conventional inoculum sizes. A cell suspension of 0.5 ml was injected intraperitoneally and the test antibiotics suspended in 0.5% methylcellulose solution were given orally or subcutaneously in single doses 1 hour after challenge. The mice were observed for 4 days. The protective effect of the test drugs was expressed in terms of ED_{50} (mg/kg) values which were calculated by the Probit method[2].

Therapeutic Effect on Experimental Pneumonia in Rabbits

Pneumonia was established in rabbits by the techniques of KANNANGARA *et al.*[3]. Japanese white rabbits (male, 1.4~2.2 kg, $n=3\sim18$) were anesthetized by intraperitoneal injection of 12 mg of pentobarbital sodium. The ventral portion of the neck was shaved and cleansed with 70% ethanol. The trachea was exposed by a midline vertical incision in the neck, and 15 cm, 20 gauge polyethylene tubing with 22 gauge needle (Igarashi Ika Kogyo Co., Ltd., Tokyo, Japan) was introduced by a single puncture through the upper tracheal rings. The tubing was then introduced into the tracheobrochial tree as far as possible, and the needle was withdrawn with the tubing left in the trachea. 0.5 ml of the bacterial inoculum (*Staphylococcus aureus* 2548: 4.2×10^9 cfu, *Streptococcus pyogenes* S23: $3.5\sim5.5\times10^8$ cfu) was introduced through the trachea. The tubing was then removed, and the incision was closed with metallic skin clips. The animals recovered from the anesthesia soon after procedure and were transferred to their cages. The rabbits were given a single oral dose of 20 mg/kg of the antibiotic 4 hours after challenge and thereafter twice a day on days 2 and 3 for *S. aureus* infection, and a single dose of 5 mg/kg of the antibiotic twice a day for 3 days starting 24 hours after challenge for *S. pyogenes* infection. The rabbits were sacrificed 4 days after challenge, and the lungs were homogenized in physiological saline, appropriately diluted, and the viable cell counts were determined by conventional plating techniques.

Therapeutic Effect on Experimental Pyelonephritis in Rabbits

Japanese white rabbits (male, 1.8~2.2 kg, $n=3\sim11$) were anesthetized by intraperitoneal injection of 12 mg of pentobarbital sodium. The hair of the lower abdomen and left flank was removed with electric clippers, and the operative site cleansed with 70% ethanol. Using sterile instruments, a 3-cm incision was made near the bladder and paralleled to the costal margin. A small segment of the ureter, approximately 2 cm distal to the bladder, was freed from the surrounding tissues and ligated with silk thread after intraurethral injection of 0.1 or 0.2 ml of the bacterial inoculum (*S. aureus* 4041: 3.0×10^4 cfu, *E. faecalis* 0112: $3.2\sim4.0\times10^4$ cfu, *Escherichia coli* 3056: $0.9\sim3.6\times10^3$ cfu) with a 26 gauge needle. The peritoneum was closed with silk sutures and the skin incision closed with metal clips. A dose of 20 mg/kg of antibiotic was administered orally twice a day for 3 days from 24 hours after challenge for *S. aureus* or *E. coli* infections, or for 2 days from 24 hours after challenge for *E. faecalis* infection. The animals were killed 4 days after challenge with *S. aureus* and *E. coli*, and 3 days after challenge with *E. faecalis*. The blood, kidneys, liver, bladder tissue and urine in the pelvis and the bladder were aseptically removed. The kidneys were macroscopically examined for abscess and the grade of severity of abscess were expressed as +++, ++, +, ± and −. The organs were then homogenized in physiological saline, and appropriately diluted. The viable cell counts were determined by conventional plating techniques.

Serum Levels and Urinary Excretion in Mice and Rabbits

Male ICR-strain mice aged 4 to 5 weeks were used in groups of 10 and male Japanese white rabbits

weighing 2.6 to 2.8 kg were used in groups of 5. The antibiotics were given orally in a single dose of 20 mg/kg. At specified intervals after dosing, blood samples were collected by heart puncture from mice and from the ear vein of rabbits, allowed to clot and centrifuged for 15 minutes. For collection of urine, each mouse was housed in a metabolism cage, and the urine samples were collected over a period of 24 hours after dosing. For rabbits, the urine samples were collected through a catheter at 0 to 3 and 3 to 6 hours, and in a metabolism cage at 6 to 24 hours after dosing. The concentrations of the test antibiotics were determined by the disc-plate diffusion technique using *Providencia stuartii* ATCC 43665 as the test organism for FK482, *E. coli* ATCC 39188 for CFIX and *Bacillus subtilis* ATCC 6633 for CCL, CEX and AMPC.

Statistical Analyses

All statistical analyses were performed with Student's t-test[4] for paired differences.

Results

Protective Effect of FK482 and Reference Antibiotics after Oral Dosing on Systemic Infection in Mice

The protective effect of orally given FK482 on systemic infections in mice was compared with that of the reference antibiotics (Table 1). Against *S. aureus* infection, FK482 was almost as effective as CCL and AMPC, and more effective than CEX. Against systemic infections with *E. coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*, FK482 was almost as effective as CCL and more effective than CEX, but less effective than CFIX. FK482 had stronger protective effect than AMPC against *E. coli* infection and similar effect to AMPC against *P. mirabilis* infection. These results show that the protective activity of FK482 after oral dosing in mice did not reflect its potent *in vitro* activity.

Table 1. Protective effect of FK482 and reference antibiotics after oral dosing on systemic infections in mice.

Organism	Mucin	Challenge dose (cfu/mouse, ip)	Antibiotic[a]	ED_{50} (mg/kg)	MIC[b] (μg/ml)
Staphylococcus aureus 2490	+	4.3×10^8	FK482	0.55	0.20
			CCL	0.37	1.56
			CEX	1.75[c]	3.13
			AMPC	0.31	0.20
Escherichia coli 29	+	1.2×10^7	FK482	4.15	0.10
			CFIX	0.64[d]	0.10
			CCL	2.37	0.78
			CEX	17.9[c]	6.25
			AMPC	7.08[c]	1.56
Klebsiella pneumoniae 1	+	1.5×10^6	FK482	2.44	0.20
			CFIX	0.24[d]	0.05
			CCL	1.14	0.78
			CEX	6.62[c]	6.25
Proteus mirabilis 4	+	2.1×10^7	FK482	2.09	0.10
			CFIX	0.06[d]	$\leqq$0.025
			CCL	2.78	1.56
			CEX	35.8[c]	12.5
			AMPC	3.21	1.56

[a] Antibiotic was given orally 1 hour after challenge; [b] agar dilution method (stamp method, 10^3 cfu/spot), Mueller-Hinton agar; [c] statistical significances refer to Student's t-test for paired differences, significant difference ($P<0.05$), FK482>reference antibiotic; [d] FK482<reference antibiotic.

CCL; Cefaclor, CEX; cephalexin, AMPC; amoxicillin, CFIX; cefixime.

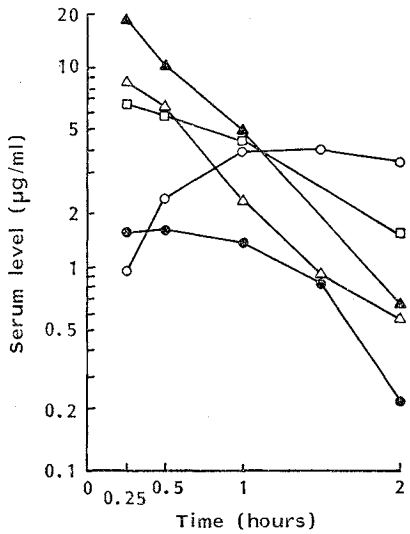
Table 2. Comparative protective effect of FK482 and cefaclor (CCL) after oral and subcutaneous dosing on systemic infection in mice.

Organism	Dosing route	Antibiotic[a]	ED_{50} (mg/kg)	MIC[b] (μg/ml)
Escherichia coli 29	po	FK482	4.15	0.10
		CCL	2.37	0.78
	sc	FK482	0.10	0.10
		CCL	0.79[c]	0.78

[a] Antibiotic was given orally or subcutaneously 1 hour after challenge; [b] agar dilution method (stamp method, 10^3 cfu/spot), Mueller-Hinton agar; [c] statistical significances refer to Student's t-test for paired differences, significant difference from FK482 ($P<0.05$).

Fig. 2. Serum levels and urinary recovery of FK482 and reference antibiotics after an oral dose of 20 mg/kg in mice.

● FK482, ○ cefixime (CFIX), △ cefaclor (CCL), ▲ cephalexin (CEX), □ amoxicillin (AMPC).



Antibiotic	Urinary recovery (%)
FK482	9.8
CFIX	13.0
CCL	68.6
CEX	65.2
AMPC	33.5

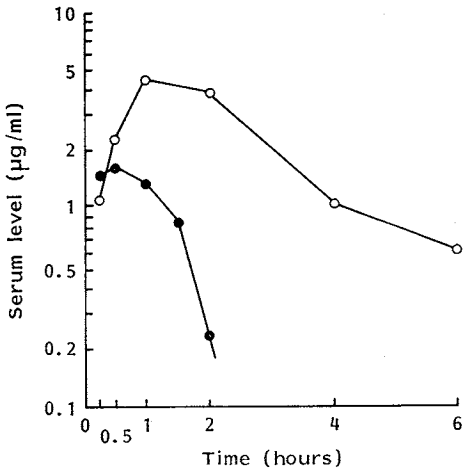
Protective Effect of FK482 and CCL after Subcutaneous Dosing on Systemic Infection in Mice

The protective activity of FK482 given subcutaneously was compared with that of CCL (Table 2). Against systemic infection with *E. coli*, FK482 given subcutaneously was about 40 times more effective than that given orally and 8 times more effective than CCL given subcutaneously. These results suggest that the protective activity of subcutaneous FK482 well reflected its potent *in vitro* activity, in comparison with CCL.

Serum Levels and Urinary Recovery of FK482 and Reference Antibiotics after Oral Dosing in Mice

To investigate why the protective activity of oral FK482 against systemic infection in mice did not reflect its potent *in vitro* activity, the serum levels and urinary recovery of FK482 in mice given the drug in oral doses of 20 mg/kg were compared with those of the reference antibiotics. The serum levels and urinary recovery rates of FK482 after oral dosing were the lowest of the test drugs (Fig. 2). Therefore, it is thought that the unexpectedly weak *in vivo* activity of FK482 was due to its poor absorp-

Fig. 3. Serum levels and urinary recovery of FK482 after an oral dose of 20 mg/kg in mice and rabbits.
○ Rabbit, ● mouse.



Animal	Urinary recovery (%)
Mouse	9.8
Rabbit	45.8

tion after oral dosing in mice.

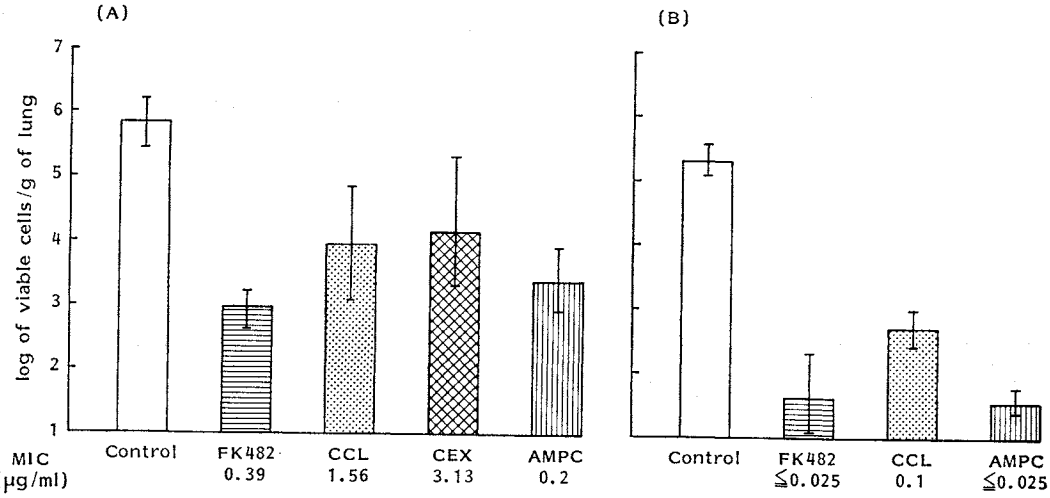
Comparative Serum Levels and Urinary Recovery of FK482 after Oral Dosing in Mice and Rabbits

In a preliminary pharmacokinetic study, there were considerable differences in the absorption of orally dosed FK482 among animals. That is, the absorption of FK482 after oral dosing in mice was low, but in rabbits was higher. Fig. 3 shows the serum levels and urinary recovery of FK482 after an oral dose of 20 mg/kg in mice and rabbits. The peak serum levels of FK482 in mice and rabbits were 1.58 and 4.22 μg/ml; serum half-lives were 0.4 and 1.1 hours; the area under the serum concentration curve (AUC) (0~∞) were 2.17 and 13.5 μg·hour/ml; urinary recoveries (0~24 hours) were 9.8 and 45.8%; and absolute bioavailabilities were 12.6 and 32.3%, respectively. These results show that the absorption of FK482 was far higher in rabbits than in mice. We therefore thought it of interest to investigate the therapeutic efficacy of FK482 against local infections in rabbits.

Therapeutic Efficacy of Orally Dosed FK482 and Reference Antibiotics against Experimental Pneumonia in Rabbits

The therapeutic efficacy of FK482 and the reference antibiotics against pneumonia induced by *S. aureus* 2548 and *S. pyogenes* S23 in rabbits is shown in Fig. 4. Against *S. aureus* pneumonia, the decrease of viable cells in the lungs of FK482-treated rabbits was more marked than in the reference drug-treated animals, although only the FK482-treated group showed a significant difference ($P<0.05$) from the non-treated control group and there was no significant difference between the FK482-treated group and the reference drug-treated group. That is, the viable cell counts (log cfu/g) in the lungs after oral treatment were 3.01±0.29 for FK482 ($n=3$), 3.99±0.87 for CCL ($n=3$), 4.19±0.94 for CEX ($n=3$), 3.45±0.50 for AMPC ($n=3$) and 5.89±0.44 for the non-treated control ($n=3$). Against *S. pyogenes* pneumonia, FK482 was as effective as AMPC and more effective than CCL in reducing the number of viable bacteria in the lungs, although there was no significant difference between FK482-treated group and the reference drug-treated group; the viable cell counts were 1.66± 0.65 for FK482 ($n=5$), 2.74±0.28 for CCL ($n=8$), 1.58±0.22 for AMPC ($n=8$) and 5.42±0.24 for

Fig. 4. Therapeutic efficacy of FK482 and reference antibiotics after oral dosing against experimental pneumonia in rabbits.



(A) *Staphylococcus aureus* 2548 (4.2×10^9 cfu): treatment, 20 mg/kg, po, 4 hours after challenge and thereafter twice a day on days 2 and 3; (B) *Streptococcus pyogenes* S23 ($3.0\sim5.5\times10^8$ cfu): treatment, 5 mg/kg, po, twice a day for 3 days starting 24 hours after challenge.

CCL; Cefaclor, CEX; cephalexin, AMPC; amoxicillin.

Vertical bars indicate the SE.

Table 3. Therapeutic efficacy of FK482 and reference antibiotics after oral dosing against experimental urinary tract infection induced by *Staphylococcus aureus* 4041 (MRSA)[a] in rabbits.

		FK482[b]	CCL[b]	AMPC[b]	Control
log viable cell count/g or ml[c]	Infected left kidney	2.68±0.51	4.91±0.42[d]	5.47±0.43[d]	6.47±0.22[d]
	Right kidney	<0.8	2.94±0.29	1.19±0.20	5.59±0.29
	Urine in pelvis	<1.0	5.12±0.60	5.45±0.99	7.66±0.50
	Urine in bladder	<1.0	<1.0	<1.4	4.69±0.44
	Bladder tissue	2.24±0.46	1.36±0.23	2.31±0.52	3.87±0.84[d]
	Liver	<1.4	<1.0	<1.4	2.05±0.18
	Blood	<1.0	<1.0	<1.0	<1.0
Grade of abscess[c]	Cortex	±	+~++	+~++	++~+++
	Medulla	±	+~++	+~++	++~+++
	Pelvis	−~±	+~++	+~++	+++
MIC (μg/ml)		3.13	>100	50	

[a] Challenge, 3.2×10^4 cfu, into left ureter; [b] treatment, 20 mg/kg, po, twice a day for 3 days from 24 hours after challenge; [c] observation, 4 days after challenge; [d] statistical significances refer to Student's t-test for paired differences, significant difference from FK482 ($P<0.05$).

CCL; Cefaclor, AMPC; amoxicillin.

the non-treated control ($n=18$). These results show that oral dosing with FK482 in rabbits gave therapeutic activity against experimental pneumonia which reflected well its potent *in vitro* activity.

Therapeutic Efficacy of FK482 and Reference Antibiotics against Ascending Pyelonephritis in Rabbits

Against ascending pyelonephritis induced by methicillin-resistant *S. aureus* 4041, orally dosed FK482 ($n=5$) had significantly stronger activity than CCL ($n=5$) and AMPC ($n=5$) in reducing the

Table 4. Therapeutic efficacy of FK482 and reference antibiotics after oral dosing against experimental urinary tract infection induced by *Enterococcus faecalis* 0112[a] in rabbits.

		FK482[b]	CCL[b]	AMPC[b]	Control
Log viable cell count/g or ml[c]	Infected left kidney	4.46±0.23	5.78±0.29[d]	4.05±0.36	8.25±0.24[d]
	Right kidney	<1.3	1.74±0.36	<0.8	2.00±0.74
	Urine in pelvis	5.60±0.58	6.55±0.51[d]	4.94±0.42	9.18±0.09[d]
	Urine in bladder	<1.0	<1.5	<1.0	<1.0
	Bladder tissue	<1.2	4.03±1.02	<1.3	<1.8
	Liver	<1.5	2.39±0.22	<1.1	2.96±0.24
	Blood	<1.0	<1.0	<1.0	<1.0
Grade of abscess[c]	Cortex	+	+~++	±~++	++~+++
	Medulla	+	++	+	+++
	Pelvis	++	++~+++	+~++	+++
MIC (μg/ml)		6.25	>100	0.78	

[a] Challenge, $3.2\sim4.0\times10^4$ cfu, into left ureter; [b] treatment, 20 mg/kg, po, twice a day for 2 days from 24 hours after challenge; [c] observation, 3 days after challenge; [d] statistical significances refer to Student's t-test for paired differences, significant difference from FK482 ($P<0.05$).

CCL; Cefaclor, AMPC; amoxicillin.

Table 5. Therapeutic efficacy of FK482 and reference antibiotics after oral dosing against experimental urinary tract infection induced by *Escherichia coli* 3056[a] in rabbits.

	log viable cell count/g or ml[c]				
	FK482[b]	CFIX[b]	CCL[b]	AMPC[b]	Control
Infected left kidney	3.67±0.54	3.40±0.35	6.80±0.24[d]	5.91±0.23[d]	8.53±0.14[d]
Right kidney	1.79±0.62	<1.0	1.08±0.04	2.87±0.74[d]	2.76±0.62[d]
Urine in pelvis	4.32±0.67	3.17±0.66	8.98±0.25[d]	7.40±1.17[d]	8.84±0.23[d]
Urine in bladder	<1.0	<1.0	2.16±0.80	<1.0	6.18±0.32
Bladder tissue	<1.0	<1.0	2.75±0.41	1.93±0.71	5.79±0.64
Liver	2.95±0.34	<1.0	2.48±0.26	2.16±0.61	3.02±0.32
Blood	<1.0	<1.0	<1.0	<1.0	<1.0
MIC (μg/ml)	0.20	0.20	1.56	3.13	

[a] Challenge, $0.9\sim3.6\times10^3$ cfu, into left ureter; [b] treatment, 20 mg/kg, po, twice a day for 3 days from 24 hours after challenge; [c] observation, 4 days after challenge; [d] statistical significances refer to Student's t-test for paired differences, significant difference from FK482 ($P<0.05$).

CFIX; Cefixime, CCL; cefaclor, AMPC; amoxicillin.

number of viable bacteria in the kidneys, urine of the pelvis and in the severity of abscess in the kidneys (Table 3). Against *E. faecalis* 0112 pyelonephritis, FK482 ($n=6$) was almost as active as AMPC ($n=6$) and more active than CCL ($n=6$) or the control ($n=6$) in reducing the number of viable bacteria in the organs and urine, and in the severity of abscess in the kidneys (Table 4). Against *E. coli* 3056 pyelonephritis, FK482 ($n=5$) was almost as effective as CFIX ($n=7$) and more effective than CCL ($n=8$), AMPC ($n=3$) or the control ($n=11$) in reducing the number of viable bacteria in the organs and urine. These results suggest that the *in vivo* activity of FK482 against ascending pyelonephritis in rabbits, in which FK482 was better absorbed than in mice, reflected well its potent *in vitro* activity.

Discussion

In vitro studies on FK482 showed that the drug combined potent antibacterial activity against Staphylococci, Streptococci and *E. faecalis* with the broad spectrum, potent antibacterial activity

against Gram-negative bacteria, and excellent stability to β-lactamases as like as CFIX. We compared the therapeutic efficacies of FK482 and commercially available oral β-lactam antibiotics in experimental infections in animals. In a pharmacokinetic study[5], there were considerable differences in the absorption of the compound after oral dosing in different animals.

That is, mice and rats showed low absorption (absolute bioavailability: 12.6 and 15.3%, respectively), rabbits showed moderate absorption (32.3%) and dogs showed high absorption (72.3%).

Therefore, we studied the *in vivo* antibacterial activity of FK482 in experimental infections in mice and rabbits. In mouse systemic infections, the protective activities afforded by FK482 after oral dosing were almost equal to those of CCL, despite the better *in vitro* activities of FK482. However, the protective activities of FK482 after subcutaneous dosing were superior to those of CCL. These results suggested that the reason for the weaker *in vivo* activity of orally given FK482 against mouse systemic infections was due to its poor absorption after oral dosing in mice. On the other hand, in local infections such as pneumonia and ascending pyelonephritis in rabbits, in which FK482 was better absorbed than in mice, the therapeutic efficacy of FK482 after oral dosing reflected well its potent *in vitro* activity. Therefore, we can say that the clinical efficacy of FK482 against infections will depend on its bioavailability after oral dosing in human. FK482 merits further pharmacokinetic and toxicological studies.

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