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In Vivo Antibacterial Activity of FK041, a New Orally Active Cephalosporin

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The therapeutic activities of orally administered FK041 were evaluated in mouse models of systemic and local infections with a variety of bacteria and were compared with those of cefdinir (CFDN) and cefditoren pivoxil (CDTR-PI). FK041 exhibited potent therapeutic activity against lethal systemic infections induced by intraperitoneally inoculated *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae* with 50% effective doses (ED_{50}) in the range of 0.20 to 0.36 mg/kg and was more active than CFDN and CDTR-PI. This result correlated well with its *in vitro* activity. The therapeutic effects of FK041 and reference drugs on murine local infections were evaluated in an *in vivo* pharmacokinetic model simulating human plasma concentrations for oral administration of 50 mg, 100 mg, and 200 mg. Against murine subcutaneous abscess induced by *S. aureus*, FK041 was as effective as CFDN and significantly more effective than CDTR-PI in reducing the number of recoverable viable bacteria in the skin at the infection sites. The efficacy of FK041 against murine pneumonia with *H. influenzae* was comparable to that of CDTR-PI and was superior to that of CFDN in reducing viable bacteria activity in the lungs. These results strongly suggest that FK041 has potential for clinical use against various bacterial infections.

FK041, a new cephem antibiotic for oral use, has been shown to have some advantages over the currently available oral cephalosporins[1,2]. For example, it demonstrated a potent and broad spectrum of activity against a wide variety of clinical isolates of Gram-positive and Gram-negative bacteria[1]. When compared with CFDN[1,3,4], FK041 also displayed improved activities against clinically important major pathogens, including *H. influenzae*, in addition to more potent antibacterial activity, better oral absorbability, and excellent stability to β-lactamase hydrolysis. To evaluate its *in vivo* activity, the therapeutic effects of FK041 on murine systemic infections were compared with those of cefdinir (CFDN)[4] and cefditoren pivoxil (CDTR-PI)[5]. Moreover, in order to predict its clinical efficacy, the therapeutic effects of FK041 were compared with those of the reference drugs against local infection models in mice with an *in vivo* pharmacokinetic model in which human plasma concentrations after oral administration of FK041 were reproduced in murine plasma.

Materials and Methods

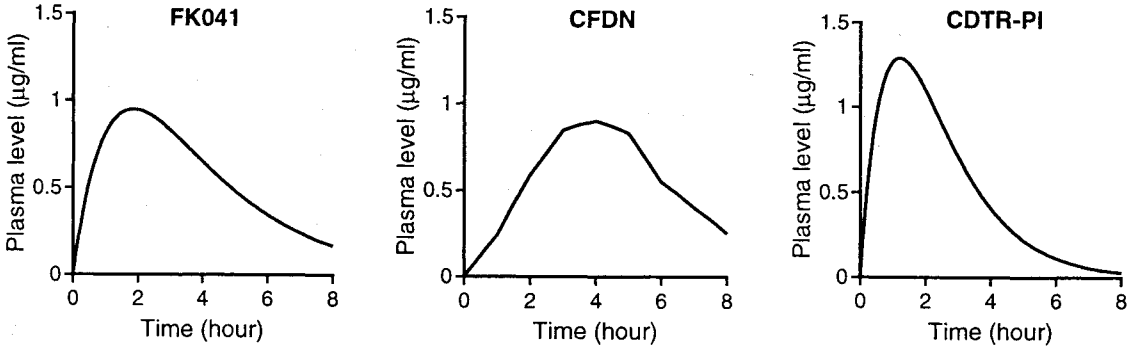
Antibiotics

FK041, CFDN, and CDTR-PI were prepared in the Medicinal Chemistry Research Laboratories of Fujisawa Pharm. Co., Ltd., Osaka, Japan.

Animals

ICR-strain male mice aged 4 weeks (Japan SLC Co.) were used. Mice were housed in cages and food and water were given at libitum.

Fig. 1. The dosage regimens of each antibiotic for adaptation in humans: plasma levels in humans after a single oral dosing of 100 mg.



Bacteria

The strains used for the experiments were as follows: Methicillin-sensitive *Staphylococcus aureus* Smith and *S. aureus* 47 (MSSA), *Escherichia coli* 29, *Klebsiella pneumoniae* 1, and *Haemophilus influenzae* 3001. These strains were previously screened for pathogenicity.

Antimicrobial Susceptibility Tests

MIC tests were carried out by serial twofold dilution in Mueller-Hinton agar (Difco Laboratories, Detroit, MI, USA) in Petri dishes. This medium supplemented with heated 5% defibrinated horse blood (chocolate agar) was used for *H. influenzae*. *E. coli* and *K. pneumoniae* were precultured in Mueller-Hinton broth (Difco); *S. aureus* was precultured in Trypticase soy broth (BBL Microbiology System, Cockeysville, MD, USA); *H. influenzae* was precultured in Trypticase soy broth plus 5% Fildes enrichment (Oxoid, Ltd., Hampshire, UK). Overnight cultures of these organisms were diluted 100-fold with the respective medium, and an inoculum containing 10^4 cfu was applied with a multipoint replicating apparatus to agar plates containing serial twofold dilutions of each antibiotic. These plates were incubated at 37°C for 18 hours. For *H. influenzae*, incubation was carried out in an atmosphere of 5% CO_2. The MIC was read as the lowest drug concentration required to inhibit visible growth of the organism.

Lethal Systemic Infection

The *in vivo* efficacy of FK041 was examined by a modification of the previously reported method[4]. Male ICR-strain mice were used in groups of 8. *S. aureus* Smith, *E. coli* 29, and *K. pneumoniae* 1 were cultured overnight on Trypticase soy agar at 37°C. Each test strain was suspended in 5% bacteriological mucin (Nacalai Tesque Inc., Kyoto, Japan) to be given at about 1 to 5 minimum lethal dose. A cell suspension of 0.5 ml was injected intraperitoneally and the test antibiotics suspended in 0.5% methylcellulose solution were given orally 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 the 50% effective doses (ED_{50}) estimated by the probit method[6].

Therapeutic Effect in an *In Vivo* Pharmacokinetic Model

(1) Dose and dosing regimens: The doses and dosing schedules of the drugs were designed so that plasma concentrations in mice may approximate the time-related concentrations in human plasma after oral administration of 50, 100, and 200 mg. Human plasma concentrations of each antibiotic after 100 mg oral administration were referred to data reported previously[7~9]. The doses for oral administration with 50 and 200 mg were calculated by multiplying each dose ratio by 100 mg. Since plasma clearance in mice was much faster than that in humans, frequent subcutaneous administration were necessary to reproduce the human plasma concentrations in mice plasma. Each dose was calculated by a mathematical model[10]. The dosage regimens are shown in Fig. 1 and Table 1.

(2) Subcutaneous abscess: Groups of 5 mice were used. *S. aureus* 47 precultured on brain heart infusion agar (Difco) at 37°C for 18 hours was suspended in 2% Cytodex 1 (Pharmacia Biotech AB, Uppsala, Sweden), and 0.1 ml (4.6×10^4 cfu) was injected subcutaneously into the mice. The infected mice were subcutaneouly treated with 13 administrations of the drugs, as shown in Table 1, starting at 1 hour after infection at a site remote to the infection.

Table 1. The dosage regimens of each antibiotic for adaptation in humans: fractionized dosases and dosing schedules in mice to approximate the time-related concentrations in human plasma produced by a single oral dosing of 100 mg.

Injection point (hr)	Dose (mg/kg)		
	FK041	CFDN	CDTR-PI
0	0.331	0.028	0.285
0.5	0.603	0.078	0.383
1.0	0.588	0.184	0.242
1.5	0.580	0.338	0.165
2.0	0.555	0.452	0.111
2.5	0.522	0.546	0.075
3.0	0.484	0.610	0.050
3.5	0.443	0.645	0.034
4.0	0.402	0.622	0.023
4.5	0.362	0.549	0.016
5.0	0.492	0.801	0.014
6.0	0.511	0.662	0.009
7.0	0.385	0.489	0.004

Pieces of shaved skin at the infection sites were aseptically removed at 8 and 24 hours after the initial dosing, and the viable bacteria were counted after homogenization by a conventional plating method.

(3) Pneumonia: Groups of 6 mice each were immunosuppressed with intraperitoneal cyclophosphamide at 200 mg/kg 4 days before infection and were injured on their tracheal surfaces with 0.025 ml of 1% formalin intranasally given at 1 day before infection. *H. influenzae* 3001 precultured in brain heart infusion broth (Difco) supplemented with 5% Fildes enrichment (Oxoid) at 37°C for 18 hours in 5% CO_2 atmosphere was suspended in brain heart infusion broth, and 0.05 ml (2.8×10^8 cfu) was intranasally inoculated in the anesthetized mice. The infected mice were subcutaneously treated with 13 administrations of the drugs, as shown in Table 1, starting at 1.5 hour after infection. The mice were sacrificed at 7 and 24 hours after the initial dosing, and the lungs were aseptically removed. Each sample was homogenized and the number of viable bacteria was counted by the conventional plating method.

Statistical Analysis

Turkey-type multiple comparison was used to determine the significant differences between the drug-treated and non-treated groups in log10 values of viable counts in local infections.

Results and discussion

Therapeutic Effects of FK041 on Lethal Systemic Infection

The therapeutic effects of FK041 were compared with those of CFDN and CDTR-PI against lethal systemic infection models in mice induced by 3 clinically common pathogens (Table 2). FK041 exhibited excellent therapeutic effects against all of the systemic infection models induced by *S. aureus*, *E. coli*, and *K. pneumoniae* with ED_{50}s in the range of 0.20 to 0.36 mg/kg, and FK041 was the most active of the test agents against these infection models. In our preceding paper on the *in vitro* evaluation of FK041, it was also shown to be superior to CFDN and CDTR against clinical isolates of *S. aureus*, *E. coli*, and *K. pneumoniae*. Our results showed that the *in vivo* results with FK041 when compared with CDTR-PI gave better than expected *in vivo* protection on the basis of the *in vitro* MICs. Whereas FK041 showed from 0 to 4 times better *in vitro* activity, the *in vivo* superiority over CDTR-PI ranged from 4 to 41 times better activity. Although the Cmax of CDTR-PI is higher

Table 2. Protective effect of FK041 after oral dosing on systemic infections in mice.

Organism	Challenge dose (cfu/mouse)	Antibiotic[a]	ED_{50} (mg/kg)	MIC[b] (μg/ml)
Staphylococcus aureus Smith	2.6×10^7	FK041	0.35	0.39
		CFDN	0.90	0.78
		CDTR-PI	14.5	1.56
Escherichia coli 29	2.0×10^6	FK041	0.36	≦0.025
		CFDN	2.20	0.1
		CDTR-PI	1.45	≦0.025
Klebsiella pneumoniae 1	1.5×10^5	FK041	0.20	0.1
		CFDN	1.30	0.2
		CDTR-PI	5.22	0.2

[a] Antibiotic was given orally 1 hour after ip challenge. [b] Agar dilution method (stamp method, 10^4 cfu/spot, Mueller-Hinton agar).

Table 3. Therapeutic effect of FK041 after oral dosing on subcutaneous abscess with *Staphylococcus aureus* 47[a] in mice.

Antibiotic	Dose equivalent to human[b] (mg)	MIC[c] (μg/ml)	Log of cfu/abscess at time after initial dosing (mean ± SE)	
			8 hours	24 hours
FK041	50	0.39	4.63 ± 0.03^d	4.91 ± 0.16^d
	100		4.37 ± 0.06^d	3.97 ± 0.18^d
	200		4.37 ± 0.15^d	3.49 ± 0.07^d
CFDN	50	0.78	4.94 ± 0.16^d	5.97 ± 0.33^e
	100		4.63 ± 0.03^d	4.20 ± 0.32^d
	200		nd[f]	nd[f]
CDTR-PI	50	0.78	nd[f]	nd[f]
	100		6.91 ± 0.18^e	7.55 ± 0.29^e
	200		6.72 ± 0.24^e	7.28 ± 0.18^e
Control			6.33 ± 0.31	6.82 ± 0.11

[a] Mice were subcutaneously inoculated with 0.1 ml of *S. aureus* 47 (4.6×10^4 cfu) on their dorsal regions; [b] as shown in Fig. 1, antibiotics were subcutaneously administered at sites remote from the infection sites so that plasma concentrations in mice may approximate the concentrations in human plasma after oral dosing of 50, 100, and 200 mg, respectively; [c] agar dilution method (stamp method, 10^4 cfu/spot, Mueller-Hinton agar); statistical significance refers to one way layout analysis of variance and Turkey-Kramer multiple comparison test, [d] significant difference ($p<0.01$) from control, [e] significant difference ($p<0.01$) from FK041; [f] not determined.

than that of FK041 after orally administrated at same dose, the T-half of CDTR-PI is shorter than that of FK041[7]. When compared with the time above MIC on the basis of each MIC, the time above MIC of FK041 is longer than that of CDTR-PI (Data is not shown). The time above MIC is the pharmacodynamic parameter that best indicates the efficacy of β-lactam[10]. This discrepancy may be attributed to the different pharmacokinetics of each drug.

Therapeutic Effects of FK041 in an *In Vivo* Pharmacokinetic Model

The *in vivo* efficacy of FK041 against lethal systemic infection in mice correlated well with its *in vitro* activity. This result, however, does not necessarily mean that FK041 is clinically effective against bacterial infections in human. If there is a discrepancy in pharmacokinetic properties between mice and humans, the results obtained in mice can not be readily extrapolated to man. In order to account for this possibility, and to predict efficacy in humans more accurately , we designed an *in vivo* pharmacokinetic model in which drug concentrations in human plasma are reproduced in mouse plasma[10]. The therapeutic activities of FK041 against experimental models of subcutaneous abscess and pneumonia were evaluated with this *in vivo* pharmacokinetic model and were compared with those of CFDN and CDTR-PI. Fig. 1 shows plasma concentrations in humans after a single oral administration of 100 mg of each test drug and Table 1 indicates treatment regimens in mice to simulate the time-related concentrations in human plasma.

Against subcutaneous abscess with *S. aureus* 47, FK041 exhibited a dose dependent therapeutic effect and significantly reduced the viable cell counts in the skin to 4.91, 3.97, and 3.49 log10 at the respective doses equivalent to 50, 100, and 200 mg in humans, when compared with 6.82 log10 in the control mice at 24 hours after the initial dosing (Table 3). The therapeutic effect of FK041 was significantly superior to that of CDTR-PI, which did not significantly reduce the viable cell counts at any dose tested. In addition, the number of recovered bacteria from infected mice treated with 50 mg equivalent of FK041 was significantly lower than with CFDN at 24 hours after the initial dosing. These

Table 4. Therapeutic effect of FK041 after oral dosing on pneumonia with *Haemophilus influenzae* 3001[a] in mice.

Antibiotic	Dose equivalent to human[b] (mg)	MIC[c] (μg/ml)	Log of cfu/lung at time after initial dosing (mean ± SE)	
			7 hours	24 hours
FK041	50	0.2	6.70 ± 0.23	6.36 ± 0.22
	100		5.49 ± 0.03[d]	5.33 ± 0.10[d]
	200		5.34 ± 0.15[d]	5.23 ± 0.12[d]
CFDN	50	0.78	6.78 ± 0.11	6.57 ± 0.27
	100		6.35 ± 0.16[d, e]	6.19 ± 0.28
	200		nd[f]	nd
CDTR-PI	50	0.025	nd	nd
	100		5.69 ± 0.23[d]	5.62 ± 0.18[d]
	200		5.43 ± 0.11[d]	5.12 ± 0.15[d]
Control			7.35 ± 0.08	7.13 ± 0.04

[a] Mice treated with cyclophosphamide (200 mg/kg, ip) and 1% formalin (25 μl/mouse, intranasally) 4 days before infection were intranasally inoculated with 25 μl of *H. influenzae* 3001 (3.1×10^7 cfu); [b] as shown in Fig. 1, antibiotics were subcutaneously administered so that plasma concentrations in mice may approximate the concentrations in human plasma after oral dosing of 50, 100, and 200 mg, respectively; [c] agar dilution method (stamp method, 10^4 cfu/spot, Mueller-Hinton agar supplemented with 5% horse blood (chocolate agar)); statistical significance refers to one way layout analysis of variance and Turkey-Kramer multiple comparison test, [d] significant difference ($p<0.01$) from control, [e] significant difference ($p<0.01$) from FK041; [f] not determined.

results suggest that FK041 may be more potent than CFDN and CDTR-PI against human skin infections induced by *S. aureus*.

Table 4 shows the efficacy of FK041 in a mouse model of pneumonia induced by *H. influenzae*. The viable cell counts in the lungs were significantly decreased to 5.33 and 5.23 log10 after a single oral administration of FK041 at the respective doses equivalent to 100 and 200 mg in humans, when compared with 7.13 log10 in the control mice at 24 hours after the initial dosing. FK041 was significantly superior to CFDN in reduction of the viable cell counts recovered from the infected mice treated with 100 mg equivalent at 7 hours after the initial dosing. There was no significant difference in the number of recovered bacteria between FK041 and CDTR-PI at any dose tested. These results suggest that oral treatment with FK041 at 100 mg/day may be effective against human respiratory infection with *H. influenzae* and that the therapeutic efficacy of FK041 may be superior to that of CFDN.

In conclusion, FK041 exhibited more potent therapeutic effects in several experimental infections in mice than CFDN, in accordance with the results of *in vitro* activity determinations, which indicated that FK041 was more potent than CFDN against a wide variety of Gram-positive and negative bacteria. These results suggest that FK041 may be effective in the clinical treatment of bacterial infection.

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