IN VITRO AND *IN VIVO* ANTIBACTERIAL ACTIVITIES OF FR-31564, A NEW PHOSPHONIC ACID ANTIBIOTIC

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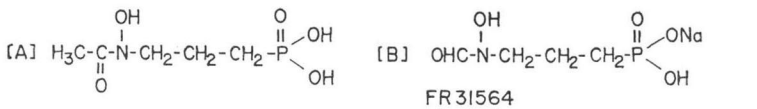
FR-31564, a new phosphonic acid antibiotic, was active against most Gram-negative bacteria except *Serratia marcescens* and glucose-nonfermenting Gram-negative rods excluding *Pseudomonas aeruginosa*. The antibacterial activity *in vitro* of FR-31564 was stronger than that of fosfomycin especially against *Escherichia coli, Klebsiella pneumoniae, Enterobacter* species and *P. aeruginosa*. FR-31564 also was active against Gram-negative bacteria resistant to β-lactam antibiotics and against gentamicin-resistant strains of *P. aeruginosa*. The antibacterial activity *in vitro* of FR-31564, like that of fosfomycin, was enhanced when 10% rabbit blood was added to the nutrient agar. The therapeutic efficacy of FR-31564 in experimental infections in mice was superior to that of fosfomycin in infections due to most Gram-negative bacteria used, and was similar to that of gentamicin in infections due to *Citrobacter freundii, Proteus rettgeri* and *Proteus inconstans* B. The protective effect of FR-31564, particularly in the *P. aeruginosa* infection, was superior to that of other control drugs including gentamicin.

Streptomyces rubellomurinus produces an antibiotic possessing Gram-negative antibacterial activity[1] with the chemical structure given in Fig. 1-A. FR-31564, a synthetic analog with the structure shown in Fig. 1-B, is superior in antibacterial activity to its parent compound[2]. This paper presents a laboratory assessment of the antimicrobial activities, *in vitro* and *in vivo*, of FR-31564.

Fig. 1. Chemical structure of new phosphonic acid antibiotics.



3-(N-Acetyl-N-hydroxyamino) propylphosphonic acid

Monosodium 3-(N-formyl-N-hydroxyamino)propylphosphonate

Materials and Methods

1. Antibiotics

FR-31564 and fosfomycin were prepared by the Research Laboratories of Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan; ampicillin (ABPC) by Beecham Laboratories, Betchworth, England; cefazolin (CEZ) by Fujisawa Pharmaceutical Co., Ltd.; kanamycin (KM) by Meiji Seika Co., Ltd., Tokyo, Japan; gentamicin (GM) by Schering Corporation, Bloomfield, U.S.A.; amikacin (AMK) by Banyu Pharmaceutical Co., Ltd., Tokyo, Japan; piperacillin (T-1220) by Toyama Chemical Co., Ltd., Toyama, Japan; and ticarcillin (TIPC) by Beecham Laboratories, Betchworth, England.

2. Bacterial strains

Standard strains of pathogenic bacteria maintained in the Fujisawa Research Laboratories were used. Clinical isolates were supplied by several hospitals in Japan.

3. Measurement of antibacterial activity *in vitro*

The antibacterial activity of the test antibiotics was determined by the agar dilution method. Unless otherwise specified, each strain was cultured in nutrient broth (Eiken, Japan) at 37°C for 20 hours. These cultures were diluted 1,000-fold and inoculated with a multiple inoculator onto nutrient agar (Difco, Detroit, U.S.A.) containing graded concentrations of the test drugs. The minimum inhibitory concentrations (MICs) were determined after incubation at 37°C for 18~20 hours. To determine the effect of blood on MICs of the test antibiotics, nutrient agar (Difco) supplemented with 10% defibrinated rabbit blood was used as the test medium. Nutrient broth (Eiken, Japan) supplemented with 10% horse serum was used for preculture of *Streptococcus* species. Nutrient agar (Difco) with 10% chocolatized rabbit blood was used to determine the activity of antibiotics against *Streptococcus* species and *Neisseria gonorrhoeae*.

4. Experimental infections in mice

Male ICR-strain mice, weighing 23±1.5 g were used in groups of 10. Each challenge organism

Table 1. Antibacterial spectra of FR-31564 and fosfomycin.

Organism	MIC; μg/ml			
	FR-31564		Fosfomycin	
	NA	NA+10% blood[c]	NA	NA+10% blood[c]
S. aureus 209P JC-1	100	50	12.5	1.56
S. epidermidis 1	200	200	6.25	1.56
B. subtilis ATCC 6633	3.13	12.5	1.56	50
M. luteus PCI-1001	0.1	0.2	3.13	0.78
S. pyogenes S-23[a]	50	100	1.56	6.25
S. faecalis 6783[a]	>400	>400	25	25
N. gonorrhoeae Oka[b]	12.5	12.5	3.13	3.13
E. coli NIHJ JC-2	25	3.13	12.5	3.13
E. coli 18	6.25	3.13	12.5	6.25
K. pneumoniae NCTC-418	12.5	3.13	100	3.13
C. freundii 3	3.13	1.56	1.56	0.2
E. aerogenes 10	12.5	6.25	12.5	12.5
E. cloacae 4	6.25	1.56	100	12.5
S. marcescens 4	50	50	1.56	1.56
P. mirabilis 75	6.25	12.5	1.56	1.56
P. vulgaris IAM-1025	1.56	1.56	0.78	3.13
P. rettgeri 3	0.78	3.13	0.78	3.13
P. inconstans 21	12.5	25	6.25	25
P. morganii 55	50	50	50	50
P. aeruginosa IAM-1095	3.13	3.13	100	50
P. maltophilia ATCC 13637	200	200	25	50
P. cepacia ATCC 25416	400	>400	>400	>400
A. calcoaceticus 4	50	25	12.5	50
A. faecalis 1	200	200	25	50
S. flexneri Ia EW-8	1.56	1.56	0.78	3.13
S. sonnei I EW-33	100	3.13	50	6.25
S. enteritidis 1891	0.39	1.56	1.56	1.56
S. typhi O-901	0.2	0.78	0.2	0.2
S. paratyphi A-1015	6.25	25	25	50
S. typhimurium 1406	3.13	0.78	6.25	0.78

Condition: Nutrient agar (NA), stamp method, 37°C, 20 hours, 1,000-fold dilution.

[a] Supplemented with 10% rabbit blood.

[b] Supplemented with 10% rabbit blood, 5% CO_2.

[c] Rabbit blood.

was suspended in 2.5 or 5% gastric mucin (INC Pharmaceuticals Inc., Cleveland, U.S.A.) at conventional inoculum sizes. A 0.5-ml cell suspension of the organism was injected intraperitoneally and the test antibiotics were given subcutaneously twice at 1 and 3 hours after challenge. The mice were observed for 4 days and the therapeutic effect of the test drugs was expressed in terms of ED_{50} (mg/kg) values calculated by the Probit method[8].

Results

1. Antibacterial Spectrum.

The antibacterial spectrum of FR-31564 comparing it with that of fosfomycin is given in Table 1. FR-31564 exerted antibacterial activity against most of the Gram-negative bacteria tested except *S. marcescens* and glucose-nonfermenting Gram-negative rods excluding *P. aeruginosa* and also against *P. aeruginosa*, *C. freundii* and *Enterobacter* species that are relatively resistant to β-lactam antibiotics. Unlike fosfomycin, FR-31564 was inactive against Gram-positive bacteria. The antibacterial activity of FR-31564 against several organisms including *E. coli* and *K. pneumoniae*, like that of fosfomycin, was enhanced by adding 10% rabbit blood to the test medium.

2. Influence of Various Culture Conditions on Antibacterial Activity (Table 2).

a) Test media. The antibacterial activity of FR-31564 was influenced by the test media as was that of fosfomycin. The use of nutrient agar gave relatively lower MICs for both FR-31564 and fosfomycin than did the use of other conventional media.

b) Inoculum size. The antibacterial activity of FR-31564 was influenced similarly to that of fosfomycin by inoculum size, *i.e.*, the antibacterial activity of both drugs decreased markedly when large inocula were used with nutrient agar medium. As a result, the MICs of FR-31564, like fosfomycin, were determined under standard conditions utilizing an inoculum achieved by 1,000-fold dilution of the preculture.

c) pH of the test media. MICs of FR-31564 were not influenced by pH (pH 6~9) of nutrient agar. In contrast, the antibacterial activity of fosfomycin decreased at pH 8~9.

d) Rabbit serum, defibrinated rabbit blood and glucose-6-phosphate. The antibacterial activity of FR-31564 like that of fosfomycin

Table 2. Influence of various factors on antimicrobial activity of FR-31564 and fosfomycin.

Factor		MIC; μg/ml	
		FR-31564	Fosfomycin
Medium	Nutrient agar (Difco)	25	12.5
	MUELLER-HINTON agar (Difco)	100	50
	Heart infusion agar (Difco)	50	25
	Brain heart infusion agar (Difco)	200	200
	Trypticase soy agar (BBL)	200	25
Inoculum size	10^{0}	>400	200
	10^{-1}	400	50
	10^{-2}	200	25
	10^{-3}	25	12.5
	10^{-4}	12.5	12.5
	10^{-5}	6.25	3.13
Medium pH	6.0	25	25
	7.0	25	12.5
	8.0	50	100
	9.0	50	200
Serum Blood G-6-P	Nutrient agar alone	25	12.5
	with 10% rabbit serum	25	6.25
	with 10% rabbit blood	1.56	1.56
	with glucose-6-phosphate (5 μg/ml)	1.56	1.56

Organism: *E. coli* NIHJ JC-2

Table 3. Antimicrobial activity of FR-31564 and other antibiotics against clinical isolates.

Organism	Drug	MIC; μg/ml		Organism	Drug	MIC; μg/ml	
		Mean	Distribution range			Mean	Distribution range
S. aureus (50 strains)	FR-31564	>400	100 ~ >400	*E. aerogenes* (50 strains)	FR-31564	6.70	0.2 ~ 400
	Fosfomycin	8.72	1.56~ 25		Fosfomycin	27.5	3.13~ >400
	Ampicillin	0.313	≦0.1 ~ 0.78		Piperacillin	10.9	1.56~ >400
	Cefazolin	0.258	0.2 ~ 0.39		Amikacin	1.92	0.78~ 12.5
	Kanamycin	3.96	0.39~ >400		Gentamicin	2.61	1.56~ 6.25
E. coli (100 strains)	FR-31564	5.79	0.2 ~ 200	*E. cloacae* (100 strains)	FR-31564	1.80	≦0.1 ~ 100
	Fosfomycin	15.3	1.56~ 100		Fosfomycin	64.6	0.78~ >400
	Ampicillin	19.9	0.78~ >400		Piperacillin	8.13	≦0.1 ~ >400
	Piperacillin	11.7	0.78~ >400		Amikacin	1.65	0.39~ 6.25
	Cefazolin	1.83	0.78~ 25		Gentamicin	1.97	0.78~ 12.5
K. pneumoniae (100 strains)	FR-31564	3.82	0.2 ~ 25	*C. freundii* (100 strains)	FR-31564	2.72	0.39~ >400
	Fosfomycin	38.2	6.25~ >400		Fosfomycin	3.00	0.78~ 100
	Piperacillin	8.31	0.78~ >400		Piperacillin	9.41	0.78~ 400
	Cefazolin	2.54	1.56~ >400		Amikacin	1.58	0.39~ 100
					Gentamicin	2.61	0.78~ 50
P. mirabilis (50 strains)	FR-31564	5.22	1.56~ 25	*P. aeruginosa* (50 strains)	FR-31564	9.74	1.56~ 400
	Fosfomycin	3.21	0.78~ 100		Fosfomycin	18.4	1.56~ 400
	Ampicillin	0.443	≦0.1 ~ 6.25		Ticarcillin	25.3	0.78~ >400
	Piperacillin	0.3	≦0.1 ~ 50		Piperacillin	4.67	0.39~ 100
	Cefazolin	1.29	0.78~ 6.25		Gentamicin	16.0	6.25~ 400
P. vulgaris (50 strains)	FR-31564	4.42	≦0.1 ~ 100	*S. marcescens* (50 strains)	FR-31564	33.9	0.39~ 400
	Fosfomycin	2.72	≦0.1 ~ 100		Fosfomycin	2.88	0.2 ~ 400
	Piperacillin	0.523	≦0.1 ~ 6.25		Piperacillin	14.8	1.56~ >400
	Amikacin	1.72	0.39~ 6.25		Amikacin	3.74	1.56~ 12.5
	Gentamicin	2.34	≦0.1 ~ 25		Gentamicin	3.64	1.56~ 100
P. rettgeri (50 strains)	FR-31564	8.78	0.39~ 100	*Salmonella* spp. (50 strains)	FR-31564	4.01	0.39~ 400
	Fosfomycin	9.03	0.78~ 200		Fosfomycin	3.17	0.39~ 25
	Piperacillin	2.44	≦0.1 ~ 100		Ampicillin	0.576	≦0.1 ~ >400
	Amikacin	1.48	0.39~ 12.5		Cefazolin	2.27	0.78~ 400
	Gentamicin	4.12	0.39~ 100		Kanamycin	5.59	0.78~ >400
P. morganii (50 strains)	FR-31564	125	25 ~ 400	*Shigella* spp. (50 strains)	FR-31564	3.79	≦0.1 ~ 25
	Fosfomycin	39	12.5 ~ >400		Fosfomycin	3.4	≦0.1 ~ 50
	Piperacillin	4.01	0.39~ 200		Ampicillin	0.481	≦0.1 ~ 1.56
	Amikacin	1.77	0.39~ 25		Cefazolin	0.849	≦0.1 ~ 1.56
	Gentamicin	2.18	0.39~ 50		Kanamycin	1.77	≦0.1 ~ 6.25
P. inconstans B (50 strains)	FR-31564	10.4	≦0.1 ~ 100				
	Fosfomycin	9.09	0.78~ >400				
	Piperacillin	3.08	≦0.1 ~ 200				
	Amikacin	4.30	0.39~ 12.5				
	Gentamicin	16.7	3.13~ 200				

Condition: Nutrient agar (Difco), 37°C, 20 hours
Inoculum: 1,000-fold dilution, stamp method

was enhanced by adding glucose-6-phosphate to nutrient agar as an inducer of the hexose-6-phosphate transport system and also by adding defibrinated rabbit blood containing the glucose-6-phosphate transport system. Interestingly the activity of FR-31564, unlike that of fosfomycin, was not affected by rabbit serum.

3. Susceptibility of Clinical Isolates to FR-31564

Table 3 compares the mean MICs of FR-31564 with those of other antibiotics for a large number of clinical strains. There was a marked difference between the antibacterial activity of FR-31564 and that of fosfomycin against *Staphylococcus aureus*. FR-31564 with a mean MIC of >400 μg/ml, was inactive against 50 strains of *S. aureus* in contrast to fosfomycin with a mean MIC of 8.72 μg/ml. The mean MICs of FR-31564 against 100 strains each of *E. coli* and *K. pneumoniae* were 5.79 and 3.82 μg/ml respectively, considerably lower than those of fosfomycin, ampicillin and piperacillin. The mean MICs of FR-31564 for 50 strains each of *Proteus mirabilis, Proteus vulgaris* and *P. rettgeri* were 5.22, 4.42 and 8.78 μg/ml respectively. The antibacterial activity of FR-31564 against these organisms was similar to or slightly less active than that of fosfomycin and was less active than that of cefazolin and the other control drugs. The mean MICs of FR-31564 for 50 strains each of *Proteus morganii* and *P. inconstans* B were 125 and 10.4 μg/ml respectively. The antibacterial activity of FR-31564 was less than that of fosfomycin against *P. morganii* and almost the same as that of fosfomycin against *P. inconstans* B. The mean MICs of FR-31564 for 50 strains of *Enterobacter aerogenes* and 100 strains each of *Enterobacter cloacae* and *C. freundii* were 6.70, 1.80 and 2.72 μg/ml respectively. The MICs of FR-31564 against *Enterobacter* species were lower than those of fosfomycin but were almost the same against *Citrobacter*. For 50 strains each of *P. aeruginosa* and *S. marcescens*, the mean MICs of FR-31564 were 9.74 and 33.9 μg/ml respectively. FR-31564 was more active than fosfomycin against *P. aeruginosa*, but was less active than fosfomycin against *S. marcescens*. For 50 strains each of *Salmonella* species and *Shigella* species, the respective mean MICs of FR-31564 were 4.01 and 3.79 μg/ml, and were almost the same as those of fosfomycin.

4. Influence of Blood on Antibacterial Activity of FR-31564

Since the antibacterial activity of FR-31564 against *E. coli* NIHJ JC-2 (Table 2) is enhanced in the presence of blood, we studied the effect of blood on the antibacterial activity of FR-31564 against various clinical isolates (Table 4). The mean MICs of FR-31564 for 50 strains each of *E. coli* and *K. pneumoniae* in the absence of blood were 5.37 and 6.34 μg/ml respectively. However, the antibacterial activity of FR-31564 was enhanced in the presence of blood, and the MICs were 0.85 and 2.18 μg/ml respectively. Likewise, the antibacterial activity of FR-31564 against *P. rettgeri, P. morganii, P. inconstans* B, *E. aerogenes, E. cloacae, C. freundii* and *Shigella sonnei* II was enhanced in the presence of blood. A similar effect of blood with FR-31564 was also obtained with fosfomycin for most of the organisms tested. The results of the antibacterial activity of FR-31564 in the presence or absence of blood showed that FR-31564 was active against *E. coli, K. pneumoniae, P. mirabilis,* the indole-positive *Proteus* group, *Enterobacter* species, *C. freundii, P. aeruginosa, Salmonella* species and *Shigella* species but was inactive against pathogenic Gram-positive bacteria including *S. aureus* as well as *S. marcescens* and glucose-nonfermenting Gram-negative rods excepting *P. aeruginosa*.

5. Antibacterial Activity of FR-31564 against Gram-negative Bacteria Resistant to Other Antibiotics

Table 5 shows the antibacterial activity of FR-31564 against 6 species of clinical isolates resistant

Table 4. Antimicrobial activity of FR-31564 and fosfomycin in the presence of 10% rabbit blood.

Organism	Mean MIC (Distribution range); μg/ml			
	FR-31564		Fosfomycin	
	NA[a]	NA+10% blood[b]	NA	NA+10% blood
S. aureus (6 strains)	>400 (>400)	>400 (>400)	25 (12.5~100)	1.97 (0.78~3.13)
E. coli (50 strains)	5.37 (0.39~200)	0.85 (0.39~6.25)	17.4 (6.25~50)	1.15 (0.39~25)
K. pneumoniae (50 strains)	6.34 (0.78~50)	2.18 (0.78~12.5)	30.8 (0.78~>400)	3.96 (0.2~50)
P. mirabilis (7 strains)	9.29 (3.13~25)	11.3 (6.25~12.5)	6.25 (3.13~50)	3.13 (0.78~12.5)
P. vulgaris (7 strains)	7.62 (1.56~200)	8.41 (1.56~25)	2.56 (0.78~12.5)	2.32 (0.78~12.5)
P. rettgeri (7 strains)	10.3 (0.78~50)	5.66 (3.13~6.25)	41.0 (12.5~>400)	15.2 (6.25~25)
P. morganii (21 strains)	126 (50~400)	19.8 (6.25~50)	59.0 (25~200)	46.8 (12.5~100)
P. inconstans B (7 strains)	55.2 (1.56~>400)	22.6 (3.13~400)	50 (0.78~>400)	37.2 (3.13~>400)
E. aerogenes (50 strains)	6.70 (0.2~400)	1.98 (0.2~25)	27.5 (3.13~>400)	5.15 (0.39~50)
E. cloacae (50 strains)	2.61 (0.2~50)	0.85 (0.2~6.25)	45.4 (3.13~>400)	8.36 (0.39~100)
C. freundii (50 strains)	2.88 (0.78~200)	1.25 (≦0.1~25)	4.48 (0.78~100)	0.65 (0.2~6.25)
P. aeruginosa (7 strains)	5.13 (1.56~12.5)	15.2 (6.25~50)	20.5 (12.5~25)	45.3 (25~50)
S. marcescens (6 strains)	>400 (100~>400)	178 (50~>400)	11.1 (3.13~50)	4.64 (0.39~12.5)
S. sonnei II (7 strains)	12.5 (6.25~50)	0.86 (0.39~3.13)	6.90 (1.56~12.5)	1.16 (0.78~3.13)
S. flexneri 2a (6 strains)	0.55 (≦0.1~1.56)	0.35 (0.2~3.13)	2.21 (1.56~6.25)	0.22 (0.2~0.39)
S. typhi (3 strains)	0.39 (≦0.1~3.13)	1.97 (0.39~12.5)	0.49 (≦0.1~3.13)	4.96 (1.56~12.5)

[a] NA: Nutrient agar (Difco)
[b] 10% blood; 10% defibrinated rabbit blood
Inoculum: 1,000-fold dilution, 37°C, 20 hours, stamp method

Table 5. Antimicrobial activity of FR-31564 and fosfomycin against antibiotic resistant strains of clinical isolates.

Organism	Mean MIC; μg/ml		
	Control antibiotic	FR-31564	Fosfomycin
E. coli (17 strains) ampicillin resistant	>400	4.80	17.0
K. pneumoniae (4 strains) cefazolin resistant	82	2.20	12.5
P. mirabilis (4 strains) ampicillin resistant	160	2.60	1.80
E. aerogenes (13 strains) piperacillin resistant	100	4.50	19.0
C. freundii (15 strains) piperacillin resistant	160	2.40	6.00
P. aeruginosa ticarcillin resistant (7 strains)	210	12.0	23.0
gentamicin resistant (7 strains)	100	7.20	14.0

Condition: Nutrient agar (Difco), 37°C, 20 hours, stamp method.
Inoculum size: 1,000-fold dilution

to other drugs. FR-31564 was active against 17 strains of *E. coli* highly resistant to ampicillin (mean MIC: >400 μg/ml), the MIC being 4.8 μg/ml. Four strains of cefazolin-resistant *K. pneumoniae* (mean MIC: 82 μg/ml) were susceptible to FR-31564, the mean MICs of FR-31564 and fosfomycin being 2.20 and 12.5 μg/ml respectively. FR-31564 was active against 4 strains of ampicillin-resistant *P. mirabilis* (mean MIC: 160 μg/ml), 13 strains of piperacillin-resistant *E. aerogenes* (mean MIC: 100 μg/ml) and 15 strains of piperacillin-resistant *C. freundii* (mean MIC: 160 μg/ml) with MICs of 2.60, 4.50 and 2.40 μg/ml respectively. FR-31564 was active against all 7 strains each of ticarcillin- and gentamicin-resistant *P. aeruginosa* (mean MIC: 210 and 100 μg/ml respectively), the mean MICs of FR-31564

Table 6. Protective effect of FR-31564 and other antibiotics on experimental infection in mice.

Organism	Mucin (%)	Drug	ED_{50}; mg/kg	MIC; μg/ml
E. coli 23 (5.5×10^6/mouse)	2.5	FR-31564	1.89	50
		Fosfomycin	*15.4	12.5
		Piperacillin	0.667	1.56
		Ticarcillin	*7.07	6.25
		Ampicillin	3.85	1.56
		Cefazolin	1.74	1.56
		Gentamicin	1.02	0.39
K. pneumoniae 74 (7×10^6/mouse)	5	FR-31564	0.587	3.13
		Fosfomycin	*26.0	12.5
		Piperacillin	*7.21	1.56
		Cefazolin	*1.87	1.56
		Gentamicin	0.351	0.39
P. mirabilis 60 (1.6×10^6/mouse)	5	FR-31564	4.59	6.25
		Fosfomycin	3.48	1.56
		Piperacillin	**1.52	0.39
		Ticarcillin	2.66	3.13
		Ampicillin	**<0.87	0.39
		Cefazolin	6.06	1.56
P. vulgaris 8 (9.5×10^6/mouse)	5	FR-31564	0.728	3.13
		Fosfomycin	*2.76	1.56
		Piperacillin	0.904	0.39
		Ticarcillin	*6.72	3.13
P. rettgeri 34 (2.1×10^5/mouse)	5	FR-31564	0.162	3.13
		Fosfomycin	*4.75	3.13
		Piperacillin	*0.874	0.2
		Gentamicin	*0.707	0.78
P. morganii 64 (2.4×10^6/mouse)	5	FR-31564	36.5	200
		Fosfomycin	13.9	25
		Piperacillin	55.1	25
		Gentamicin	**0.287	1.56
P. inconstans B 21 (3.6×10^7/mouse)	5	FR-31564	1.12	3.13
		Fosfomycin	*18.9	6.25
		Piperacillin	*7.25	0.78
		Gentamicin	1.17	1.56
E. aerogenes 9 (6.8×10^5/mouse)	5	FR-31564	7.68	6.25
		Fosfomycin	12.7	6.25
		Piperacillin	9.11	25
		Gentamicin	**0.356	0.78
E. cloacae 63 (4×10^6/mouse)	5	FR-31564	8.14	12.5
		Fosfomycin	*>217	>400
		Piperacillin	**1.29	0.78
		Gentamicin	**0.423	1.56
C. freundii 13 (7×10^5/mouse)	5	FR-31564	0.924	0.78
		Fosfomycin	*6.07	3.13
		Piperacillin	*14.5	25
		Gentamicin	0.841	0.78
P. aeruginosa 26 (1.2×10^6/mouse)	5	FR-31564	1.85	3.13
		Fosfomycin	*92.5	6.25
		Piperacillin	*75.0	3.13
		Ticarcillin	*121	12.5
		Gentamicin	*10.2	25

Mouse: JCL ICR strain, male, 4 W (23.5 ± 1.5 g), 10/group
Infection: Mucin suspension, 0.5 ml/mouse, i.p.
Therapy: 1 and 3 hours after challenge, s.c.
Significant difference; *: FR-31564 > antibiotic **: FR-31564 < antibiotic

were 12.0 and 7.2 μg/ml, respectively. Moreover, FR-31564 was more active than fosfomycin against these resistant organisms except ampicillin-resistant strains of *P. mirabilis*.

As shown above, FR-31564 was active against Gram-negative bacteria resistant to β-lactam antibiotics such as ampicillin-, cefazolin-, piperacillin- and ticarcillin-, and gentamicin-resistant strains of *P. aeruginosa*: These results show that no cross-resistance was seen between FR-31564 and the other antibiotics tested.

6. Protective Effect of FR-31564 on Experimental Infection in Mice

The therapeutic effect of FR-31564 after subcutaneous dosing was studied in experimental infections in mice (Table 6). The therapeutic effect of FR-31564 was superior to that of fosfomycin against infections due to 8 of the 11 species of bacteria tested except *P. mirabilis, P. morganii* and *E. aerogenes*. Namely, the protective effect of FR-31564 against infections due to *E. coli* strain 23 and *K. pneumoniae* strain 13 was similar to that of gentamicin and was more potent than that of fosfomycin and piperacillin. Antibiotic FR-31564 was effective against infection due to *E. cloacae* strain 63, whereas fosfomycin was not effective against infection due to the same bacteria. The activity of FR-31564 was similar to fosfomycin activity against infection due to *P. mirabilis* strain 60, but was less effective than fosfomycin against infection due to *P. morganii* strain 64.

However, the protective effect of FR-31564 was superior to that of fosfomycin against all infections due to *P. vulgaris* strain 8, *P. rettgeri* strain 34, *P. inconstans* B strain 21 and was similar to that of gentamicin against infections due to the latter 2 pathogens. The protective effect of FR-31564 against *P. aeruginosa* strain 26 was the most potent of the 5 drugs including gentamicin.

Discussion

FR-31564 is an antibiotic of the same chemical class as fosfomycin since FR-31564 contains phosphonic acid in the molecule. In spite of their chemical similarities, FR-31564 differs from fosfomycin in both antibacterial spectrum and potency. FR-31564 was inactive against Gram-positive pathogens and was comparatively less active than fosfomycin against *S. marcescens*. In contrast, the antibacterial activity of FR-31564 against several Gram-negative bacteria including *P. aeruginosa* was more potent than that of fosfomycin. There was cross-resistance between FR-31564 and fosfomycin in some clinical isolates spontaneously resistant to the antibiotics. However, no cross-resistance was seen in some clinical isolates[4]. These findings suggest certain differences exist in the mechanisms of bacterial resistance to the two drugs. FR-31564 and fosfomycin are both transported into bacterial cells by the L-α-glycerophosphate system. Interestingly FR-31564 was incorporated into the cells more efficiently than was fosfomycin in those organisms which were more susceptible to FR-31564 than to fosfomycin[4]. Finally, FR-31564 differed substantially from fosfomycin in antibacterial activity *in vivo*. The therapeutic efficacy of FR-31564 on murine infections due to Gram-negative bacteria was superior to that of fosfomycin.

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