

In Vitro and In Vivo Antibacterial Activity of FR-31564, a Phosphonic Acid Antimicrobial Agent

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The in vitro and in vivo activity of FR-31564 [sodium hydrogen 3-(*N*-hydroxyformamido)propylphosphonate] against gram-positive and -negative aerobic and anaerobic bacteria was investigated and compared with that of fosfomycin, cephalexin, carbenicillin, and trimethoprim-sulfamethoxazole. The in vitro activity of FR-31564 was markedly enhanced when combined with glucose 6-phosphate or fructose 6-phosphate, but not when combined with ribose phosphate, adenosine monophosphate, or glycerol phosphate. In vitro activity of FR-31564 also was enhanced by human or horse blood, but not by human serum. The type of medium had a great effect on the minimal inhibitory concentration, with the lowest minimal inhibitory concentrations achieved on nutrient agar, 8- to 16-fold less than with Mueller-Hinton, heart infusion, or Trypticase soy agars. FR-31564 was more active than fosfomycin, cephalexin, carbenicillin, or trimethoprim-sulfamethoxazole against *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Enterobacter cloacae*, *E. aerogenes*, and *Citrobacter*. It was less active than fosfomycin against *Serratia marcescens* and *Proteus mirabilis* and did not inhibit gram-positive cocci or anaerobic species. FR-31564 inhibited a number of *E. coli*, *K. pneumoniae*, and some *Pseudomonas aeruginosa* strains resistant to the other agents. In the presence and absence of human blood FR-31564 showed bactericidal activity, and *P. aeruginosa* exposed to FR-31564 for 3 h showed a 6-h lag in regrowth. FR-31564 administered by the subcutaneous route was more active in protecting mice challenged with *P. aeruginosa* than was fosfomycin, carbenicillin, or cefoperazone. It was as active by the oral route in protecting mice challenged with *E. coli* as was fosfomycin, ampicillin, cephalexin, or trimethoprim-sulfamethoxazole.

In spite of the proliferation of new semisynthetic penicillins and cephalosporins, there remains a need for new antimicrobial agents which could be utilized in both parenteral and oral forms. There has been increased resistance to antimicrobial agents even in organisms found in the outpatient setting. Extensive studies with fosfomycin (1-cis,1,2-epoxypropylphosphonic acid) have shown that this compound, produced by strains of *Streptomyces rebulomurinus*, has excellent gram-negative antibacterial activity (1) and also inhibits some gram-positive species. The synthesis of FR-31564 [sodium hydrogen 3-(*N*-hydroxyformamido)propylphosphonate] (Fig. 1) caused us to investigate the in vitro and in vivo antibacterial activity of this agent against a large number of bacteria isolated from infections arising both in the inpatient and outpatient setting.

MATERIALS AND METHODS

Antimicrobial agents. FR-31564 was a gift of Fujisawa Pharmaceutical Co., Osaka, Japan. Ampicillin

and carbenicillin were obtained from Beecham Laboratories, and cefoperazone was from Toyama Chemical Co. Trimethoprim (TMP) and sulfamethoxazole (SMX) were obtained from Roche Laboratories. Cephalexin was obtained from Eli Lilly & Co., and gentamicin was from Schering Corp. Glucose 6-phosphate (G6P), ribose 5-phosphate, fructose 6-phosphate, β -glycerol phosphate, and adenosine 5'-monophosphate were purchased from Sigma Chemical Co.

Bacterial strains. Fresh clinical isolates from specimens of sputum, urine, wounds, and blood submitted to the diagnostic laboratories of the Columbia-Presbyterian Medical Center were used. Isolates were stored on slants of Trypticase soy agar (BBL Microbiology Systems) at room temperature in the dark until used.

Measurement of antibacterial activity. The antibacterial activity of the test antimicrobial agent was determined by the agar dilution method. Overnight broth cultures of organisms were diluted 10^{-3} and inoculated with a multiple-point inoculator that would deliver 0.01 ml onto nutrient agar (Difco Laboratories) which contained twofold-increasing concentrations of antibiotic. The minimal inhibitory concentration (MIC) was determined after 18 h of incubation at

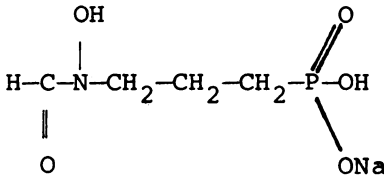


FIG. 1. FR-31564, sodium hydrogen 3-(N-hydroxyformamido)propylphosphonate.

35°C. Broth dilution in nutrient broth was used to determine the minimal bactericidal concentration (MBC). Samples (0.1 ml) from each clear broth tube were inoculated onto agar plates. The MBC was defined as the concentration of antimicrobial agent which caused absence of growth or growth of fewer than five colonies. The activity of FR-31564 was also determined on Mueller-Hinton (BBL), heart infusion (Difco), and Trypticase soy agars with the same inoculum of 10^4 colony-forming units. The activity of FR-31564 under anaerobic conditions was determined in GasPak jars (BBL) by utilizing prereduced nutrient agar medium supplemented with 5% sheep blood and vitamin K for 48 h at 35°C. Activity of FR-31564 against streptococci and *Neisseria* was determined on chocolatezated 10% rabbit blood nutrient agar. The activity of TMP-SMX was determined in the presence of 0.1 U of thymidine phosphorylase (Burroughs-Wellcome Co.) per ml.

Killing curve comparisons of agents were performed by using an inoculum from organisms in the exponential phase of growth which were incubated in flasks on a gyratory shaker. Samples were removed at specified times, immediately diluted in broth, and plated at several dilutions to determine colony-forming unit survival. In experiments determining killing in the presence of human blood, control samples were run in the same concentration of heparinized human blood.

Animal experiments. Male, ICR strain mice, weighing 20 ± 2 g were used in groups of 10 animals per organism per dose of antimicrobial agent. Each challenge organism was suspended in 5% gastric mucin (INC Pharmaceuticals Inc.). A 0.5-ml suspension of the organism to be tested was injected intraperitoneally. The test antibiotics were administered by either oral or subcutaneous injection at 1 and 3 h after the bacterial challenge. Mice were observed daily for 4 days, and the therapeutic effect of the test antimicrobial agents was expressed in terms of 50% effective dose values (in milligrams per kilogram) calculated by the Probit method.

RESULTS

The activity of FR-31564 and the other agents tested is shown in Table 1. Activity is shown for FR-31564 and fosfomycin in the presence and absence of G6P. FR-31564 inhibited 50% of *Escherichia coli* at a concentration of 1.56 $\mu\text{g}/\text{ml}$, which was 4-fold more active than fosfomycin and cephalixin and 16-fold more active than carbenicillin, but 2-fold less active than TMP-SMX. In the presence of G6P, FR-31564 in-

hibited 90% of the *E. coli* at 0.78 $\mu\text{g}/\text{ml}$. In the presence of G6P the activity of fosfomycin also was enhanced, but FR-31564 was still the more active compound—90% inhibited by 0.78 μg of FR-31564/ml compared with 1.56 μg of fosfomycin per ml. FR-31564 had activity against *Klebsiella pneumoniae* similar to its activity against *E. coli*, but it was markedly more active than fosfomycin with 90% of isolates inhibited by 6.25 $\mu\text{g}/\text{ml}$; whereas ≥ 100 μg of fosfomycin, carbenicillin, or TMP-SMX per ml was needed to inhibit 90% of isolates. *Proteus mirabilis* isolates were inhibited by low concentrations of FR-31564, but fosfomycin was more active. *Proteus vulgaris* isolates also were inhibited by FR-31564, and it was more active than carbenicillin or TMP-SMX. In contrast, *Morganella morganii* isolates were uniformly resistant to FR-31564, whereas many of these isolates were inhibited by carbenicillin. Although 90% of *Providencia rettgeri* and *P. inconstans* were inhibited by FR-31564 at 25 and 12.5 $\mu\text{g}/\text{ml}$, respectively, these concentrations were higher than those required for many other organisms. *Enterobacter cloacae* isolates were very susceptible to FR-31564, with 50% inhibited by 0.2 $\mu\text{g}/\text{ml}$ and 90% inhibited by 1.56 $\mu\text{g}/\text{ml}$. The activity of FR-31564 was further enhanced by G6P, with 90% inhibited by 0.1 $\mu\text{g}/\text{ml}$. FR-31564 was 8-fold more active than fosfomycin against *E. cloacae* and more than 1,000-fold more active than carbenicillin. FR-31564 also was the most active agent tested against *Enterobacter aerogenes*, 16-fold more active than carbenicillin. Although FR-31564 and fosfomycin inhibited *Citrobacter* isolates, equally well at low concentrations, FR-31564 was the more active compound, inhibiting 90% at 6.25 $\mu\text{g}/\text{ml}$, compared with 50 $\mu\text{g}/\text{ml}$ for fosfomycin. Both compounds inhibited carbenicillin- and cephalixin-resistant *Citrobacter* isolates. FR-31564 was much less active than fosfomycin against *Serratia marcescens* isolates, with 90% inhibited by 3.13 μg of fosfomycin per ml, whereas 50 μg of FR-31564 per ml was required to inhibit 90%. Indeed, fosfomycin was more active than TMP-SMX. FR-31564 inhibited 50% of *Pseudomonas aeruginosa* at 6.25 $\mu\text{g}/\text{ml}$ compared with 50 $\mu\text{g}/\text{ml}$ for carbenicillin and 12.5 $\mu\text{g}/\text{ml}$ for cefoperazone and gentamicin. These isolates were multiresistant, as the high MICs indicate, and do not reflect the exact potential of the agent against *P. aeruginosa*, since the mean FR-31564 MIC against 30 subsequent isolates was 0.39 $\mu\text{g}/\text{ml}$. FR-31564 did not inhibit *Acinetobacter*. FR-31564 and fosfomycin had similar activity against *Salmonella*, including *S. typhi*, the majority of which were ampicillin and carbenicillin resistant due to the presence of β -lactamases. Addition of G6P markedly enhanced

TABLE 1. Antimicrobial activity of FR-31564 and other agents

Organism (no. of isolates)	Agent	MIC ($\mu\text{g/ml}$)		
		Range	50% Inhibited	90% Inhibited
<i>Escherichia coli</i> (31)	FR-31564	0.1-25 ($<0.025-6.25$) ^a	1.56 (0.39)	6.25 (0.78)
	Fosfomycin	0.39-25 (0.1-12.5)	6.25 (0.39)	12.5 (1.56)
	Cephalexin	0.78->100	6.25	12.5
	Carbencillin	3.13->100	25	>100
	TMP-SMX ^b	0.05->100	0.78	12.5
<i>Klebsiella pneumoniae</i> (31)	FR-31564	0.39->100 (0.05-3.13)	1.56 (0.20)	6.25 (0.39)
	Fosfomycin	6.25->100 (0.1-12.5)	25 (0.78)	>100 (1.56)
	Cephalexin	12.5->100	12.5	25
	Carbencillin	12.5->100	>100	>100
	TMP-SMX	0.78-100	3.13	100
<i>Proteus mirabilis</i> (22)	FR-31564	0.2-6.25 (0.39-3.13)	0.78 (0.78)	3.13 (1.56)
	Fosfomycin	0.1-12.5 (0.1-12.5)	0.39 (0.20)	0.78 (0.78)
	Cephalexin	6.25->100	6.25	6.25
	Carbencillin	0.78->100	1.56	100
	TMP-SMX	0.05->100	0.10	12.5
<i>Proteus vulgaris</i> (11)	FR-31564	0.39->100 (0.39-50)	0.78 (1.56)	6.25 (6.25)
	Fosfomycin	0.78-6.25 (0.39-6.25)	3.13 (1.56)	3.13 (6.25)
	Cephalexin	1.56-100	25	100
	Carbencillin	1.39->100	3.13	>100
	TMP-SMX	0.10->100	1.56	12.5
<i>Morganella morganii</i> (12)	FR-31564	100->100 (25->100)	100 (50)	>100 (>100)
	Fosfomycin	3.13->100 (6.25->100)	25 (50)	100 (50)
	Cephalexin	12.5->100	>100	>100
	Carbencillin	1.56->100	3.13	>100
	TMP-SMX	0.39->100	0.78	25
<i>Providencia rettgeri</i> (13)	FR-31564	0.39-50 (0.39-50)	6.25 (3.13)	25 (50)
	Fosfomycin	1.56-25 (0.78-50)	3.13 (3.13)	12.5 (12.5)
	Cephalexin	0.78-100	25	100
	Carbencillin	0.39->100	0.78	>100
	TMP-SMX	0.05->100	6.25	100
<i>Proteus inconstans</i> (21)	FR-31564	1.56->100 (1.56->100)	12.5 (6.25)	12.5 (12.5)
	Fosfomycin	0.39->100 (≤ 0.025 ->100)	3.13 (6.25)	6.25 (12.5)
	Cephalexin	0.78-100	25	50
	Carbencillin	1.56->100	>100	>100
	TMP-SMX	0.20-50	0.78	1.56
<i>Enterobacter cloacae</i> (28)	FR-31564	0.05-3.13 ($\leq 0.025-0.39$)	0.20 (0.10)	1.56 (0.10)
	Fosfomycin	0.39-100 (0.05-6.25)	12.5 (1.56)	25 (3.13)

TABLE 1—Continued

Organism (no. of isolates)	Agent	MIC ($\mu\text{g/ml}$)		
		Range	50% Inhibited	90% Inhibited
	Cephalexin	50->100	>100	>100
	Carbenicillin	6.25->100	50	>100
	TMP-SMX	0.78->100	3.13	>100
<i>Enterobacter aerogenes</i> (28)	FR-31564	0.20-12.5 (0.5-0.39)	0.78 (0.10)	3.13 (0.39)
	Fosfomycin	3.13-25 (0.10-6.25)	12.5 (0.78)	25 (1.56)
	Cephalexin	3.13->100	>100	>100
	Carbenicillin	1.56->100	25	>100
	TMP-SMX	0.78->100	6.25	50
<i>Citrobacter</i> (21)	FR-31564	0.10->100 (≤ 0.025 -1.56)	3.13 (0.39)	6.25 (0.39)
	Fosfomycin	0.10->100 (0.05-50)	3.13 (0.10)	50 (0.78)
	Cephalexin	6.25-100	6.25	25
	Carbenicillin	6.25->100	>100	>100
	TMP-SMX	0.39->100	1.56	12.5
<i>Serratia marcescens</i> (21)	FR-31564	6.25-50 (3.13-25)	12.5 (25)	50 (25)
	Fosfomycin	0.20-12.5 (0.10-12.5)	0.39 (0.20)	3.13 (1.56)
	Cephalexin	>100	>100	>100
	Carbenicillin	1.25->100	>100	>100
	TMP-SMX	1.56-50	3.13	12.5
<i>Pseudomonas aeruginosa</i> (71)	FR-31564	0.78->100 (0.39->100)	6.25 (3.13)	>100 (>100)
	Fosfomycin	1.56->100 (1.56->100)	12.5 (12.5)	>100 (>100)
	T-1551	1.56-50	12.5	25
	Carbenicillin	1.56->100	50	>100
	TMP-SMX	3.13->100	100	>100
	Gentamicin	0.30->100	12.5	>100
<i>Acinetobacter</i> (11)	FR-31564	6.25->100 (6.25->100)	100 (100)	>100 (>100)
	Fosfomycin	6.25->100 (6.25->100)	50 (50)	100 (100)
	Cephalexin	6.25->100	100	>100
	Carbenicillin	1.56->100	6.25	>100
	TMP-SMX	3.13->100	6.25	>100
<i>Salmonella</i> (17)	FR-31564	0.39-12.5 (0.20-6.25)	6.25 (0.39)	12.5 (0.78)
	Fosfomycin	1.56-100 (0.20-6.25)	6.25 (0.39)	12.5 (0.78)
	Cephalexin	6.25	6.25	6.25
	Carbenicillin	3.13->100	>100	>100
	TMP-SMX	1.56->100	100	>100
<i>Shigella</i> (11)	FR-31456	3.13-12.5 (0.10-0.78)	6.25 (0.39)	6.25 (0.78)
	Fosfomycin	6.25-50 (0.20-0.78)	25 (0.39)	25 (0.78)
	Cephalexin	3.13-12.5	3.13	6.25
	Carbenicillin	3.13->100	3.13	>100
	TMP-SMX	0.39->100	25	>100

^a Parentheses indicate that the assay was performed in the presence of 5 μg of G6P per ml.

^b TMP-SMX agents were present at a ratio of 20:1, and the numerical value represents the concentration of TMP.

the activity of both FR-31564 and fosfomycin against *Salmonella*. FR-31564 was more active than fosfomycin against *Shigella*. Activity was markedly enhanced by G6P.

The activity of FR-31564 and fosfomycin in the presence of G6P against selected other species are compared in Table 2. FR-31564 did not inhibit streptococci, *Streptococcus pyogenes*, *S. agalactiae*, or *S. faecalis*, whereas fosfomycin inhibited 50% of these organisms at concentrations of 12.5 µg/ml or less. FR-31564 also did not inhibit *Staphylococcus aureus* or *S. epidermidis*, whether or not the isolates were β-lactamase producers. Although FR-31564 did not inhibit *Neisseria gonorrhoeae*, it did inhibit 50% of *Haemophilus influenzae* at 0.78 µg/ml. Some *Pseudomonas maltophilia* strains were inhibited, but *P. cepacia* strains were not. *Yersinia enterocolitica* was inhibited. *Clostridium perfringens*, *B. fragilis*, peptostreptococci, and peptococci were not inhibited.

The effects of various growth conditions upon the in vitro activity of FR-31564 were investigated. Table 3 demonstrates that both FR-31564 and fosfomycin were subject to an inoculum effect for *E. coli*, *K. pneumoniae*, *E. cloacae*, *P. rettgeri*, and *P. aeruginosa*, but that the inoculum effect was more pronounced for fosfomycin. *Morganella* isolates were not susceptible to FR-31564, even at a low inoculum. Table 4 demonstrates that enriched media such as Mueller-Hinton, heart infusion, or Trypticase soy agar caused a marked increase in MIC values, but the effect was less with FR-31564 than with fosfomycin. Although fosfomycin was distinctly less active at pH 7.9 (Table 5) than at pH 5.9, there was less of a pH effect with FR-31564. The most important influence upon the antibacterial activity of FR-31564 was the presence of G6P (Table 6). In the presence of 5 µg of G6P per ml the MIC was eightfold less for *E. coli*, *K. pneumoniae*, *S. typhimurium*, and *Shigella sonnei* (not shown), whereas for *P. rettgeri* and *E. cloacae* the reduction in MIC was fourfold. No reduction in MIC occurred with *Morganella*, *P. aeruginosa*, or *S. marcescens*. Among the other compounds tested, only fructose 6-phosphate produced the same effect as G6P, but ribose 5-phosphate, adenosine 5'-monophosphate, or glycerol phosphate did not increase the activity of FR-31564. There was no further reduction in the MICs when the concentrations of G6P was increased to 20 µg/ml, and at a G6P concentration of 20 µg/ml organisms resistant to FR-31564, such as *Morganella*, remained resistant. FR-31564 inhibited organisms under both aerobic and anaerobic conditions. Twenty-five organisms were tested, and examples are given in

Table 7. FR-31564 was more active against *E. coli* and *K. pneumoniae* anaerobically than it was aerobically. *S. marcescens*, *P. rettgeri*, *P. mirabilis*, and *E. cloacae*, in contrast, either showed similar inhibition aerobically and anaerobically, or were more effectively inhibited under aerobic conditions. Performance of tests in the presence of 10% CO₂ did not alter the activity of FR-31564.

The activity of FR-31564 against all of the organisms tested (with the exception of *P. aeruginosa*) was enhanced in the presence of 10% fresh human blood (Table 8). Sheep blood and horse blood enhanced the activity of FR-31564 against some species (*E. coli* and *K. pneumoniae*) but not against other species. Pooled 50% normal human serum actually decreased the activity of both FR-31564 and fosfomycin (Table 9). However, the MBCs were lower in the presence of serum for some of the organisms (data not shown). There was a 2- to 32-fold difference between MICs and MBCs for most organisms. The presence or absence of G6P did not show any consistent effect on this difference between the MIC and MBC (Table 10).

The activity of FR-31564 against bacteria resistant to other agents is shown in Table 11. FR-31564 inhibited carbenicillin-, cephalixin-, or fosfomycin-resistant *E. coli*, *K. pneumoniae* isolates resistant to TMP-SMX or fosfomycin and *P. mirabilis* isolates resistant to carbenicillin and TMP were inhibited, as were *E. cloacae* isolates resistant to TMP-SMX, carbenicillin, or fosfomycin. However, of 17 strains of *P. aeruginosa* resistant to gentamicin, only 7 (41%) were inhibited by FR-31564. Of these gentamicin-resistant *Pseudomonas*, fosfomycin inhibited only five (29%) and carbenicillin inhibited two (11%) of these strains, but cefoperazone inhibited all but one at 6.25 to 25 µg/ml (data not shown).

Killing curves of the activity of FR-31564 and fosfomycin are shown in Fig. 2. In the presence of nutrient broth, FR-31564 prevented growth but did not reduce the number of colony-forming units. Although fosfomycin reduced the number of colony-forming units by 4.5 logs at 3 h, there was complete regrowth of the organism. In contrast, in the presence of human blood, FR-31564, fosfomycin, and carbenicillin all caused complete eradication of the organisms. To determine whether FR-31564 had to remain continually in the presence of the organisms, we exposed *P. aeruginosa* (Fig. 3) for 3 h to FR-31564, fosfomycin, and carbenicillin and then washed out the antimicrobial agents. FR-31564 exerted an inhibitory effect for 6 h after this exposure, as did carbenicillin, whereas the inhibitory effect with fosfomycin lasted only 2 h.

TABLE 2. Comparative activity of FR-31564 and fosfomycin against selected species

Organism (no. of isolates)	Agent	MIC ($\mu\text{g/ml}$) in the presence of G6P		
		Range	50% Inhibited	90% Inhibited
<i>Haemophilus influenzae</i> (5)	FR-31564	0.39->100	0.78	>100
	Fosfomycin	0.78-12.5	6.25	12.5
<i>Neisseria gonorrhoeae</i> (5)	FR-31564	>100	>100	>100
	Fosfomycin	25-50	50	50
<i>Pseudomonas maltophilia</i> (6)	FR-31564	0.78-100	25	100
	Fosfomycin	0.78-100	25	100
<i>Yersinia enterocolitica</i> (2)	FR-31564	12.5-50	12.5	50
	Fosfomycin	25-150	25	50
<i>Staphylococcus aureus</i> (8)	FR-31564	50->100 (50->100)	>100 (>100)	>100 (>100)
	Fosfomycin	0.78-6.26 (0.20-3.13)	6.25 (0.78)	6.25 (3.13)
<i>Staphylococcus epidermidis</i> (6)	FR-31564	50->100 (50->100)	>100 (>100)	>100 (>100)
	Fosfomycin	3.13-25 (1.56-12.5)	3.13 (1.56)	12.5 (3.13)
<i>Streptococcus faecalis</i> (10)	FR-31564	>100 (>100)	>100 (>100)	>100 (>100)
	Fosfomycin	12.5-25 (6.25-25)	12.5 (12.5)	25 (25)
<i>Streptococcus pyogenes</i> (6)	FR-31564	12.5->100 (1.56->100)	>100 (>100)	>100 (>100)
	Fosfomycin	1.56-6.25 (0.78-6.25)	1.56 (3.13)	3.13 (6.25)
<i>Streptococcus agalactiae</i> (5)	FR-31564	>100 (>100)	>100 (>100)	>100 (>100)
	Fosfomycin	1.56-3.13 (0.2-1.56)	1.56 (0.2)	3.13 (1.56)
<i>Streptococcus bovis</i> (5)	FR-31564	>100 (>100)	>100 (>100)	>100 (>100)
<i>Streptococcus pneumoniae</i> (5)	FR-13564	>100	>100	>100
<i>Listeria monocytogenes</i> (5)	FR-31564	>100	>100	>100
<i>Clostridium perfringens</i> (5)	FR-31564	>100	>100	>100
<i>Peptostreptococci</i> (2)	FR-31564	>100	>100	>100
<i>Peptococci</i> (2)	FR-31564	>100	>100	>100
<i>Clostridium difficile</i> (2)	FR-31564	>100	>100	>100
<i>Bacteroides fragilis</i> (13)	FR-31564	>100 (>100)	>100 (>100)	>100 (>100)
	Fosfomycin	>100 (>100)	>100 (>100)	>100 (>100)
<i>Pseudomonas cepacia</i> (3)	FR-31564	>100	>100	>100
	Fosfomycin	>100	>100	>100

Protective effect of FR-31564 on experimental infection in mice. The protective effect of FR-31564 given by the subcutaneous route in an experimental infection due to *P. aeruginosa* and to *E. coli* was compared with the protection given by fosfomycin, carbenicillin, cefoperazone, ampicillin, gentamicin, and TMP-SMX (Table 12). The 50% effective dose values

for FR-31564 were significantly lower than those of fosfomycin, carbenicillin, or cefoperazone when *P. aeruginosa* was the infecting organism and the route of drug administration was subcutaneous. FR-31564 also was significantly more effective orally than fosfomycin, even though the MICs were identical. In contrast, FR-31564 was no more effective by either the subcutaneous or oral route than the other agents when *E. coli* was the infecting organism.

TABLE 3. Effect of inoculum size on MIC

Organism	Inoculum (CFU) ^a	MIC (μg/ml)	
		FR-31564	Fosfomycin
<i>E. coli</i>	10 ³	0.39	3.13
	10 ⁵	1.56	25
	10 ⁷	6.25	>100
<i>K. pneumoniae</i>	10 ³	0.10	3.13
	10 ⁵	0.78	25
	10 ⁷	3.13	>100
<i>E. cloacae</i>	10 ³	0.20	100
	10 ⁵	0.39	>100
	10 ⁷	0.78	>100
<i>M. morgani</i>	10 ³	25	6.25
	10 ⁵	25	12.5
	10 ⁷	50	25
<i>P. rettgeri</i>	10 ³	0.20	6.25
	10 ⁵	1.56	12.5
	10 ⁷	12.5	25
<i>P. aeruginosa</i>	10 ³	3.13	1.56
	10 ⁵	6.25	6.25
	10 ⁷	12.5	50

^a Based upon dilutions and plating for colony-forming units (CFU).

DISCUSSION

Although fosfomycin has not been utilized clinically in the United States, it has proved to be a useful clinical compound in some other countries. FR-31564 is an antimicrobial agent of

TABLE 5. Effect of pH of the medium on MIC

Organism	Agent	MIC (μg/ml) at pH:		
		5.9	6.9	7.9
<i>E. coli</i>	FR-31564	0.78	1.56	6.25
	Fosfomycin	25	25	100
<i>K. pneumoniae</i>	FR-31564	6.25	1.56	6.25
	Fosfomycin	25	25	100
<i>E. cloacae</i>	FR-31564	0.78	0.39	1.56
	Fosfomycin	>100	>100	>100
<i>M. morgani</i>	FR-31564	25	25	50
	Fosfomycin	6.25	12.5	100
<i>P. rettgeri</i>	FR-31564	0.39	0.78	0.39
	Fosfomycin	6.25	12.5	25
<i>P. aeruginosa</i>	FR-31564	3.13	6.25	6.25
	Fosfomycin	3.13	3.13	12.5

TABLE 4. Effect of medium on MIC

Organism	Agent	MIC (μg/ml) on the following agar:			
		Nutrient	Mueller-Hinton	Heart infusion	Trypticase soy
<i>E. coli</i>	FR-31564	1.56	25	25	50
	Fosfomycin	25	>100	>100	>100
<i>K. pneumoniae</i>	FR-31564	0.78	25	>100	>100
	Fosfomycin	25	>100	>100	>100
<i>E. cloacae</i>	FR-31564	0.39	3.13	6.25	12.5
	Fosfomycin	>100	>100	>100	>100
<i>P. morgani</i>	FR-31564	25	50	25	100
	Fosfomycin	12.5	>100	>100	>100
<i>P. rettgeri</i>	FR-31564	1.56	6.25	6.25	25
	Fosfomycin	12.5	>100	>100	>100
<i>P. aeruginosa</i>	FR-31564	6.25	6.25	25	12.5
	Fosfomycin	6.25	50	50	50

TABLE 6. Effect of addition of sugar phosphates or nucleotides on MIC

Organism	MIC ($\mu\text{g/ml}$) on medium containing:					
	No addition	G6P ^a	Fructose 6-phosphate ^b	Ribose 5-phosphate ^b	Adenosine 5-monophosphate ^b	Glycerol phosphate ^b
<i>E. coli</i>	1.56	0.2	0.2	1.56	1.56	1.56
<i>K. pneumoniae</i>	1.56	0.2	0.2	3.13	1.56	1.56
<i>E. cloacae</i>	0.39	0.1	0.2	0.39	1.56	0.39
<i>M. morgani</i>	25	25	50	25	25	50
<i>P. rettgeri</i>	1.56	0.78	0.78	1.56	0.78	1.56
<i>P. aeruginosa</i>	6.25	6.25	6.25	3.13	6.25	6.25

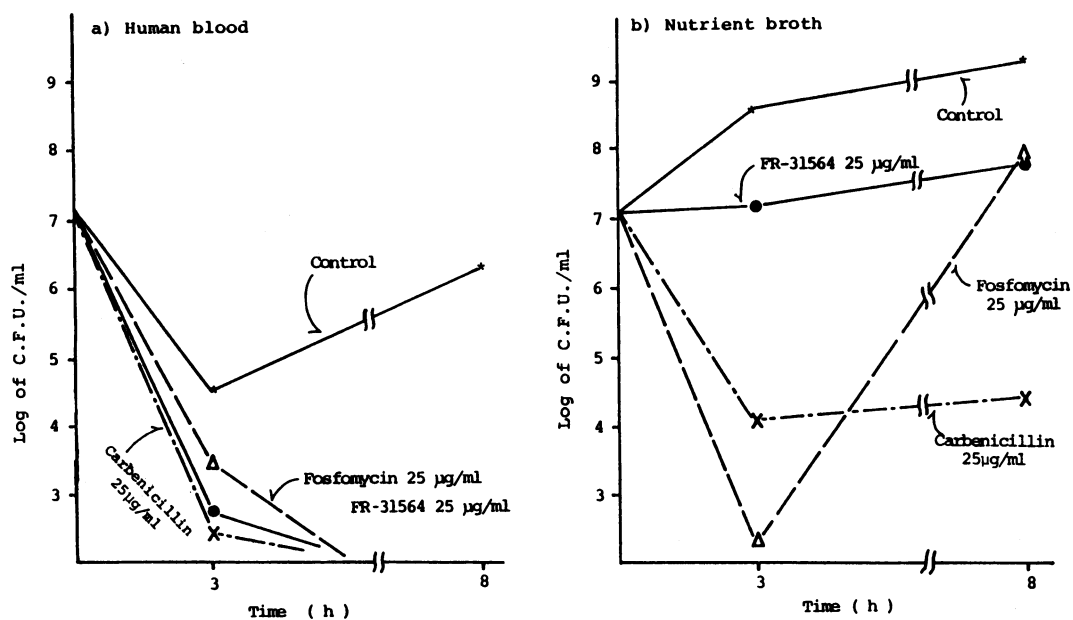
^a At 5 $\mu\text{g/ml}$.^b At 20 $\mu\text{g/ml}$.

FIG. 2. Effect of incubation in nutrient broth containing FR-31564, carbenicillin, or fosfomycin in the presence or absence of human blood upon the survival of *E. coli*. *E. coli* 2 was incubated with 25 μg of each agent per ml on a gyratory shaker, and samples were removed and plated on nutrient agar at the times noted. MICs for this strain were 12.5 $\mu\text{g/ml}$ for carbenicillin and 25 $\mu\text{g/ml}$ for fosfomycin and FR-31564.

the same family since it contains phosphonic acid in the molecule. Although FR-31564 and fosfomycin are similar chemically, their antibacterial spectra are quite different since fosfomycin inhibits gram-positive species, all of which are resistant to FR-31564 (3). In contrast, FR-31564 is appreciably more active against a number of the *Enterobacteriaceae* than is fosfomycin. Both agents are relatively inactive against *Morganella* and *Acinetobacter*, and FR-31564 did not inhibit *Serratia*, whereas fosfomycin did. FR-31564 also was more active than fosfomycin against *P. aeruginosa*. The compound, like fosfomycin, is more active in the presence of G6P and probably is transported into the cells by the glycerol phosphate transport system. It is not clear whether all resistance to FR-31564 is due to lack of the 2-β-glycerol phosphate transport system, as Kojo et al. (2) have shown to be true for resistant *P. aeruginosa*. We are currently investigating resistance in other strains of bacteria. The increased activity of FR-31564 over fosfomycin would appear to be due to its more rapid uptake (2).

The marked influence of testing conditions upon MIC values makes it necessary to be care-

TABLE 7. Effect of oxygen on the activity of FR-31564

Organism	MIC (MBC), µg/ml	
	Anaerobic	Aerobic
<i>E. coli</i>	0.8 (0.2)	6.3 (0.4)
<i>K. pneumoniae</i>	1.6 (0.05)	1.6 (0.2)
<i>S. marcescens</i>	3.1 (3.1)	3.1 (1.6)
<i>P. rettgeri</i>	0.8 (0.8)	0.8 (0.8)
<i>E. cloacae</i>	0.2 (0.1)	0.2 (0.2)
<i>P. mirabilis</i>	3.1 (1.6)	1.6 (0.8)

TABLE 8. Effect of blood on the inhibitory activity of FR-31564

Organism	Agent	MIC (µg/ml) on medium containing:			
		No addition	10% human blood	10% sheep blood	10% horse blood
<i>E. coli</i>	FR-31564	1.56	0.78	0.78	0.39
	Fosfomycin	25	1.56	1.56	0.78
<i>K. pneumoniae</i>	FR-31564	1.56	0.78	0.78	0.78
	Fosfomycin	12.5	1.56	6.25	12.5
<i>E. cloacae</i>	FR-31564	0.39	0.20	0.39	0.39
	Fosfomycin	100	6.25	25	25
<i>M. morganii</i>	FR-31564	25	12.5	100	100
	Fosfomycin	12.5	25	25	25
<i>P. rettgeri</i>	FR-31564	0.78	0.39	0.78	0.78
	Fosfomycin	12.5	12.5	12.5	12.5
<i>P. aeruginosa</i>	FR-31564	3.13	3.13	3.13	6.25
	Fosfomycin	3.13	3.13	6.25	3.13

TABLE 9. Activity of FR-31564 and fosfomycin in the presence of 50% human serum^a

Organism	Agent	MIC (µg/ml) on medium containing:	
		No addition	50% human serum
<i>E. coli</i>	FR-31564	1.56	12.5
	Fosfomycin	25	>100
<i>K. pneumoniae</i>	FR-31564	1.56	6.25
	Fosfomycin	12.5	>100
<i>E. cloacae</i>	FR-31564	0.39	3.13
	Fosfomycin	100	>100
<i>M. morganii</i>	FR-31564	25	50
	Fosfomycin	12.5	>100
<i>P. rettgeri</i>	FR-31564	0.78	1.56
	Fosfomycin	12.5	100
<i>P. aeruginosa</i>	FR-31564	3.13	6.25
	Fosfomycin	3.13	12.5

^a Experiments were performed on nutrient agar (Difco) at 37°C for 20 h with an inoculum of 10⁸ cells.

TABLE 10. Comparison of MIC and MBC

Organism	MIC (MBC), µg/ml, on medium containing:	
	No addition	G6P
<i>E. coli</i>	6.3 (100)	0.8 (6.3)
<i>K. pneumoniae</i>	12.5 (25)	0.8 (12.5)
<i>E. cloacae</i>	6.3 (100)	0.2 (6.3)
<i>P. mirabilis</i>	6.3 (>100)	6.3 (>100)
<i>P. rettgeri</i>	6.3 (>100)	3.1 (>100)
<i>P. aeruginosa</i>	12.5 (>100)	6.3 (>100)

TABLE 11. Activity of FR-31564 against bacteria resistant to other agents

Organism	MIC ($\mu\text{g/ml}$)				
	FR-31564	Fosfomycin	Cephalexin	Carbenicillin	TMP-SMX
<i>E. coli</i> 3476	0.78	6.25	6.25	>100	6.25
<i>E. coli</i> 3889	0.78	12.5	>100	>100	6.25
<i>E. coli</i> 4567	1.56	6.25	6.25	>100	100
<i>E. coli</i> 5167	1.56	25	12.5	100	12.5
<i>K. pneumoniae</i> 1076	1.56	12.5	12.5	>100	>100
<i>K. pneumoniae</i> 5789	1.56	>100	25	>100	1.56
<i>P. mirabilis</i>	0.78	0.39	6.25	>100	>100
<i>P. inconstans</i>	3.13	3.13	50	>100	1.56
<i>E. cloacae</i> 4264	0.1	0.39	>100	>100	100
<i>E. cloacae</i> 4914	1.56	>100	25	50	3.1

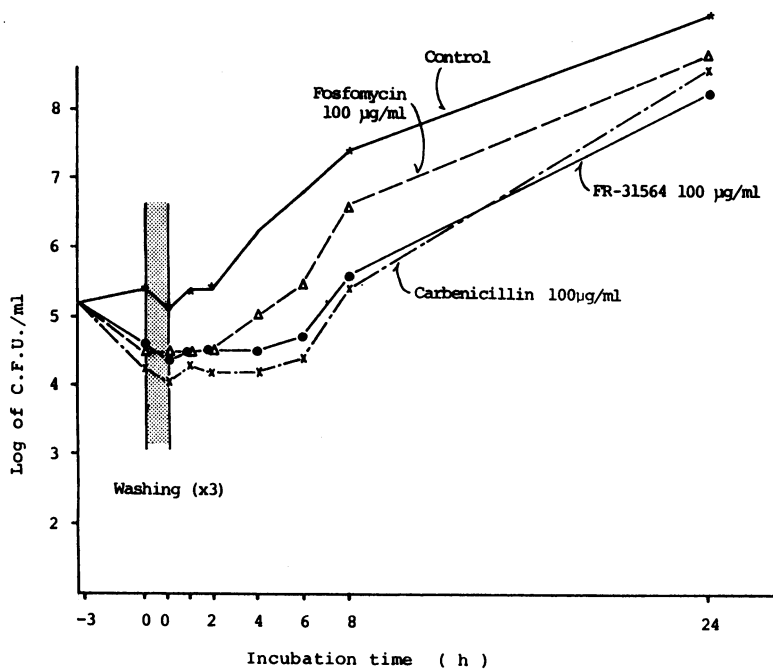


FIG. 3. Effect of 3 h of exposure of *P. aeruginosa* 5223 to 100 μg of carbenicillin, fosfomycin, or FR-31564 per ml. The MIC of all three agents was 25 $\mu\text{g/ml}$. Organisms were washed with 0.05 M potassium phosphate buffer (pH 7) three times and resuspended in nutrient broth.

TABLE 12. Protective effect of FR-31564 and other antibiotics on infections in mice

Organism (inoculum)	Route of administration of antibiotic	Agent	50% Effective dose (mg/kg)	95% Confidence limit	MIC ($\mu\text{g/ml}$)
<i>P. aeruginosa</i> (2.1×10^6)	Subcutaneous	FR-31564	13.6	7.62-24.1	6.25 (6.25) ^a
		Fosfomycin	139 ^b	79.0-317	6.25 (6.25)
		Carbenicillin	129 ^b	89.0-262	12.5
		Cefoperazone	44.0 ^b	24.7-78.2	1.56
		Gentamicin	8.14	85.97-11.7	12.5
<i>E. coli</i> (1.5×10^7)	Subcutaneous	FR-31564	3.17	0.71-8.98	6.25 (0.39)
		Fosfomycin	6.98	1.48-22.3	6.25 (0.20)
		Ampicillin	1.33	0.14-3.48	0.78
		Cefoperazone	0.91 ^c	0.10-1.56	0.10
		TMP-SMX	2.28	1.05-4.43	0.20
<i>P. aeruginosa</i> (3.1×10^6)	Oral	FR-31564	11.2	1.21-27.1	6.25 (6.25)
		Fosfomycin	102 ^b	56.2-235	6.25 (6.25)
<i>E. coli</i> (1.9×10^7)	Oral	FR-31564	2.70	0-6.98	6.25 (0.39)
		Fosfomycin	8.30	2.14-18.1	6.25 (0.20)
		Ampicillin	5.40	0.54-13.2	0.78
		Cephalexin	3.24	0.26-6.08	3.13
		TMP-SMX	<3.19	Not calculated	0.20

^a Parentheses indicate that the experiment was done in the presence of 5 μg of G6P per ml.

^b FR-31564 significantly more effective than other agent, $P < 0.01$.

^c FR-31564 significantly less effective than other agent, $P < 0.01$.

ful in performing inhibition tests with the agent since use of standard media such as Mueller-Hinton or Trypticase soy agar would cause the majority of species to appear resistant. The agent is active in an anaerobic environment and over a wide pH range.

In the presence of human blood FR-31564 acts as an effective antimicrobial agent with enhancement of its antibacterial activity. Indeed, it shows excellent animal protection against *P. aeruginosa* and *E. coli* infections, indicating that its activity in vivo may be appreciably greater than that which would be anticipated from the results of in vitro experiments. Preliminary experiments by our group show that it acts

synergistically with both β -lactams and aminoglycosides. If the compound has reasonable pharmacokinetics, it may prove useful in selected infections due to bacteria resistant to other agents.

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