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Alafosfalin, a New Inhibitor of Cell Wall Biosynthesis: In Vitro Activity Against Urinary Isolates in Japan and Potentiation with β-Lactams

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A new phosphonopeptide, alafosfalin, was evaluated for in vitro antibacterial activity and for synergism with β-lactams, using 475 Japanese clinical isolates from urinary tract infections. Alafosfalin was found to be highly active against *Escherichia coli* and moderately active against *Serratia, Klebsiella, Enterobacter,* and *Citrobacter,* but less active against gram-positive organisms than were β-lactams such as cephazolin or ampicillin and inactive against indole-positive *Proteus, Pseudomonas,* and *Acinetobacter.* Potentiation with the two β-lactams (fractional inhibitory concentration ≦ 0.5) was found in 10 to 40% of susceptible strains in 4:1 and 1:4 combinations, and to a lesser extent in those species or genera that were insensitive to alafosfalin alone. No cross resistance was seen between alafosfalin and the β-lactams or any other commonly used antibacterial agents tested. Effect on selected ampicillin-resistant strains, differential sensitivity to alafosfalin among resistant strains of various types, and sensitivity of alafosfalin-insensitive *E. coli* and *Klebsiella* to other antibiotics are also discussed.

Alafosfalin (formerly alaphosphin; Ro 03-7008), L-alanyl-L-1-aminoethylphosphonic acid, is a novel phosphonopeptide designed and synthesized by Hassall et al. (5) and Allen et al. (1) which inhibits the biosynthesis of bacterial cell walls by a mechanism differing from that elucidated for cell wall-active antibiotics such as β-lactams and D-cycloserine (2, 4). When sensitive bacteria are exposed to alafosfalin, the compound is actively transported into the cells by means of LL-dipeptide permeases and produces, by intracellular hydrolysis, alanine and aminoethylphosphonic acid. The latter acts on several steps of cell wall biosynthesis (1, 4), thereby accounting for its antibacterial activity as well as the synergism observed with other cell wall-acting antibiotics.

The present study was undertaken to evaluate the in vitro activity of alafosfalin alone and of the combinations with β-lactams against 475 strains (11 genera) of urinary tract infection (UTI) bacteria isolated in Japan.

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MATERIALS AND METHODS

Test organisms. All the organisms used were clinical isolates at 18 hospitals in the Kanto District of Japan. They were recovered in June to September 1976 from used Urotubes (Roche Products Ltd., Welwyn, England), identified by using Enterotubes and Oxi/Ferm tubes as reported previously (8, 9), and tested within 6 months. A total of 475 strains were used for the present study: 144 *Escherichia coli*, 9 *Citrobacter* sp., 46 *Proteus* sp., 16 *Enterobacter* sp., 85 *Serratia* sp., 29 *Klebsiella* sp., 54 *Pseudomonas* sp., 15 *Acinetobacter* sp., 4 *Achromobacter* sp., 2 *Alcaligenes* sp., 1 *Flavobacter* sp., 37 *Staphylococcus* sp., and 33 *Streptococcus faecalis* (enterococci). All strains were inoculated from an agar slant to Tryptosoy broth (lot no. 5L038; Nissui) cultured overnight at 37°C and used for the test by dilution with sterile saline.

Determination of minimal inhibitory concentration (MIC). Susceptibility to alafosfalin, penicillin G (PCG), ampicillin (AMP), and cephazolin (CEZ), and to combinations of it and these β-lactams, was determined by the agar dilution method.

Alafosfalin was obtained from Roche, and the preparations of β-lactams used were as follows: penicillin G (lot no. GLD42; Meiji Seika Kaisha Ltd.), AMP sodium salt (PA-S; lot no. 54·36), and CEZ sodium (Cefamezin, lot no. ZH-7240; Fujisawa Pharmaceutical Co., Ltd.).

Since it is known that alafosfalin is antagonized by the presence of small peptides and to a lesser extent by alanine and glutamic acid (2), a defined minimal medium, staphylococcal defined medium (5), was used throughout the present study. Test organisms grown overnight were inoculated onto the surface of a staphylococcal defined medium agar plate containing each drug at 14 different concentrations by using a replicating device (Multi-Typing Apparatus; Rikoh Shoji Co.).

The replicator delivered approximately 2 to 3 μl of a 10^2 (for gram-positive cocci) or 10^3 (for gram-negative rods) dilution of an original suspension (10^9 cells per ml) of each strain in Tryptosoy broth (overnight culture). The MIC was defined as the lowest concentration of antibiotics or antibacterial agents that prevented any colony from growing after 18 h of incubation at 37°C. A test of significance for differences in the MIC distribution of each drug with each strain or genus was performed by the rank sum test (10). Unless otherwise stated, strains against which the MIC of a drug was >25 μg/ml were judged as resistant to the antibiotic tested.

The fractional inhibitory concentration (FIC) was calculated by the method of Elion et al. (6) with the individual strain for each combination. It was judged as highly positive and positive in potentiation when the FIC index was <0.5 and 1.0 to 0.5, respectively. In this calculation, an MIC > 100 is arbitrarily assumed as 200 μg/ml.

RESULTS

In vitro activity of alafosfalin against UTI isolates. The overall activity of alafosfalin against 475 UTI isolates is given in Table 1, expressed by the range of the MIC and by the MIC necessary to inhibit 50% (MIC_{50}) and 90% of strains tested in comparison with two established β-lactams, AMP and CEZ.

E. coli were the most susceptible to alafosfalin among all species and genera tested, confirming previous reports (1, 2). Thus, among 144 isolates, 93 and 86% of the strains were inhibited by 12.5 and 1.56 μg/ml, respectively, whereas the MIC_{50}

TABLE 1. *In vitro activity of alafosfalin on UTI isolates compared with that of AMP and CEZ*

Organism (no. of isolates)	Drug	MIC (μg/ml of medium)		
		Range	For (% of strains):	
			50	90
E. coli (144)	Alafosfalin	0.025->100	0.39	12.5
	AMP	0.78->100	3.13	>100
	CEZ	0.39->100	1.56	3.13
Klebsiella sp. (29)	Alafosfalin	0.78->100	6.25	>100
	AMP	≦0.012->100	100	>100
	CEZ	0.78->100	3.13	25
Citrobacter sp. (9)	Alafosfalin	0.78->100	12.5	>100
	AMP	0.39->100	>100	>100
	CEZ	0.39->100	>100	>100
Serratia sp. (85)	Alafosfalin	0.1->100	12.5	100
	AMP	0.78->100	100	>100
	CEZ	0.39->100	100	>100
Enterobacter sp. (16)	Alafosfalin	0.78- 50	12.5	25
	AMP	50->100	>100	>100
	CEZ	1.56->100	>100	>100
Indole(+) *Proteus* (28)	Alafosfalin	1.56->100	>100	>100
	AMP	0.78->100	100	>100
	CEZ	0.2->100	100	>100
P. mirabilis (18)	Alafosfalin	3.13->100	>100	>100
	AMP	1.56->100	3.13	>100
	CEZ	3.13->100	12.5	>100
Pseudomonas aeruginosa (44)	Alafosfalin	0.78->100	>100	>100
	AMP	25->100	>100	>100
	CEZ	50->100	>100	>100
Acinetobacter sp. (15)	Alafosfalin	25->100	>100	>100
	AMP	3.13->100	50	>100
	CEZ	1.56->100	>100	>100
Staphylococcus sp. (37)	Alafosfalin	≦0.012->100	25	>100
	AMP	≦0.012->100	0.78	>100
	CEZ	0.05->100	1.56	>100
Streptococcus faecalis (33)	Alafosfalin	≦0.012->100	6.25	25
	AMP	0.39->100	1.56	6.25
	CEZ	0.39->100	12.5	25
Pseudomonas sp. (other than *P. aeruginosa*) (10)	Alafosfalin	0.2->100	>100	>100
	AMP	25->100	>100	>100
	CEZ	1.56->100	>100	>100

was 0.39 μg/ml. The majority of *Klebsiella* sp., *Enterobacter* sp., *Citrobacter* sp., and *Serratia* sp. were also inhibited by 12.5 μg/ml; the latter three were totally insensitive to the β-lactams compared here. Different species of the genera *Enterobacter* and *Serratia* were equally sensitive (*E. cloacae, E. hafniae, E. aerogenes, E. agglomerans, S. marcescens, S. liquefaciens,* and *S. rubidaea*). Against all the above genera, the activity of alafosfalin was superior to that of CEZ and AMP, which were used as standards, whereas alafosfalin was less active against gram-positive cocci such as *S. faecalis* and staphylococci than was AMP. Thus, 88% of *S. faecalis* isolates were inhibited by alafosfalin at 12.5 μg/ml, a concentration which inhibited only 49% of *S. aureus* and *S. epidermidis* strains. Alafosfalin was inactive against most nonfermentative gram-negative rods like *Pseudomonas, Acinetobacter, Alcaligenes,* and *Flavobacterium. Proteus* isolates were also insensitive to alafosfalin, except for *P. mirabilis* in which a minority (28%) were found to be susceptible to 12.5 μg of alafosfalin per ml.

When results of all UTI isolates tested were combined regardless of genus, alafosfalin was active against 74% of the gram-positive cocci, 65% of the gram-negative bacilli, and 66% of the total UTI isolates collected (8).

Susceptibility of antibiotic-resistant strains to alafosfalin. More than 87% of the gram-negative bacteria used were found to be resistant to one or more commonly used antibacterial agents (MIC ≧ 25 μg/ml), e.g., AMP, CEZ, kanamycin, gentamicin, tetracycline, or nalidixic acid. The susceptibility to alafosfalin of such resistant isolates was calculated for each genus (Table 2). It was found that the MIC_{50} of alafosfalin against strains tested in seven genera was below 12.5 μg/ml, with the exception of *Staphylococcus* resistant to other antibiotics. The strains sensitive to alafosfalin occupied almost 70% of the total isolates tested. Three different patterns in the susceptibility to alafosfalin of such resistant strains were found. Thus: (i) in *E. coli, S. faecalis,* and *Enterobacter* sp., alafosfalin was equally active against strains either resistant or sensitive to any antibiotics; (ii) in *Staphylococcus* sp. and *Serratia* sp., antibiotic-susceptible strains were significantly more susceptible to alafosfalin than were strains which were resistant to at least one of the antibiotics used in this study; and (iii) alafosfalin was selectively more active against a certain type of resistant strain (i.e., tetracycline-resistant strains of *Klebsiella* and *E. coli*).

When the relationship of resistant markers to alafosfalin resistance was looked at with *E. coli,* it was noted that most of the strains multiresistant to AMP, CEZ, kanamycin, and nalidixic acid were also resistant to alafosfalin (67%). The percentages of kanamycin-resistant and nalidixic acid-resistant *E. coli* were high in those strains which were alafosfalin resistant. A similar situation was found with those strains of *Klebsiella* resistant to at least one of the β-lactams tested. On the other hand, with *Citrobacter* and *Enterobacter,* most of the kanamycin-resistant strains were sensitive to alafosfalin. With *Staphylococcus* sp., kanamycin-resistant strains were also, in general, less associated with alafosfalin resistance, although the high resistance to alafosfalin by (i) strains resistant to β-lactams tested, resistant to erythromycin, and resistant to tetracycline and (ii) strains resistant to tetracycline alone was noticeable. With *S. faecalis,* it was found that the most frequently occurring resistant strains, i.e., those resistant to kanamycin and gentamicin, were all sensitive to alafosfalin.

The above results indicated that alafosfalin displayed the lowest cross resistance with the other common antibiotics examined, including the β-lactams. Thus 51 of the 144 *E. coli* isolates tested were AMP resistant (MIC ≧ 25 μg/ml; Table 2). The MIC distribution to alafosfalin in these and the AMP-sensitive strains was identical (Fig. 1), whereas CEZ was clearly less active against AMP-resistant strains, indicating a degree of cross resistance. This was more clearly seen in the population analysis of typical resistant strains (Fig. 2). AMP-resistant strain no. 154 contained a population of 10^{-5} CEZ-resistant clones, which contrasted with a value of 10^{-9} in AMP-sensitive strains (e.g., strain no. 437). The alafosfalin-resistant population was less than 10^{-8}, regardless of susceptibility to AMP. Furthermore, alafosfalin in combination with CEZ decreased the resistant population in strain no. 154 to the level of the AMP-sensitive strains (no. 154-A and 154-B in Fig. 2c). Similar effects were also seen with the AMP-susceptible strain no. 437 (Fig. 2b), though to a lesser extent. The frequency of resistant clones to alafosfalin itself (10^{-8} to 10^{-9}) in alafosfalin-sensitive strains was much less than that (10^{-5} to 10^{-6}) reported for another phosphonic acid containing the antibiotic fosfomycin (7).

Synergism with β-lactam antibiotics. The susceptibility of UTI isolated to the combination of alafosfalin with three β-lactam antibiotics was examined in the ratios of 1:4 and 4:1. Figure 3 shows the MIC distribution of the combinations in comparison with that of the β-lactam antibiotics alone. A clear potentiation of each β-lactam was observed in every combination at both ratios against those strains which were either susceptible to alafosfalin or almost insensitive to these β-lactams. On the other hand, no synergism was

TABLE 2. *Differential susceptibility to alafosfalin among UTI isolates susceptible or resistant to other drugs*

Organism (no. of strains)	Drug[a]	Susceptibility to alafosfalin in:							
		Susceptible strains[b]				Resistant strains[c]			
		No. of strains	MIC (μg/ml)			No. of strains	MIC (μg/ml)		
			Range	For (% of strains): 50	For (% of strains): 90		Range	For (% of strains): 50	For (% of strains): 90
E. coli (144)	PCG	42	0.05–25	0.39	12.5	102	0.024–>100	0.78	12.5
	AMP	93	0.024–50	0.39	12.5	51	0.05–>100	0.78	12.5
	CEZ	142	0.024–>100	0.39	12.5	2	0.39–12.5	0.39	12.5
	KM	118	0.024–>100	0.39	12.5	26	0.05–>100	0.78	>100
	TC	78	0.024–>100	0.39	25	66	0.05–>100	0.39	12.5
	NA	127	0.024–>100	0.78	12.5	17	0.1–>100	0.78	>100
	GM	143	0.024–>100	0.39	12.5	1	>100	>100	>100
	EM	45	0.1–12.5	0.78	1.56	99	0.24–>100	0.39	25
Citrobacter sp. (9)	PCG	1	0.78	0.78	0.78	8	1.56–>100	12.5	>100
	AMP	1	0.78	0.78	0.78	8	1.56–>100	12.5	>100
	CEZ	2	0.78–6.25	0.78	6.25	7	1.56–>100	12.5	>100
	KM	4	0.78–>100	12.5	>100	5	1.56–>100	6.25	>100
	TC	4	0.78–>100	1.56	>100	5	3.13–>100	12.5	>100
	NA	8	1.56–>100	12.5	>100	1	0.78	0.78	0.78
	GM	9	0.78–>100	12.5	>100	0	—	—	—
	EM	0	—[d]	—	—	9	0.78–>100	1.56	>100
Enterobacter sp. (16)	PCG	0	—	—	—	16	0.78–50	12.5	25
	AMP	0	—	—	—	16	0.78–50	12.5	25
	CEZ	6	6.25–25	12.5	25	10	0.78–50	12.5	25
	KM	9	6.25–25	12.5	25	7	0.78–50	12.5	>100
	TC	8	6.25–25	12.5	25	8	0.78–50	12.5	>100
	NA	11	0.78–50	12.5	25	5	6.25–25	12.5	25
	GM	16	0.78–50	12.5	25	0	—	—	—
	EM	0	—	—	—	16	0.78–50	12.5	25
Serratia sp. (85)	PCG	4	0.39–25	1.56	25	81	0.1–>100	12.5	100
	AMP	9	0.1–12.5	1.56	12.5	76	3.13–>100	25	>100
	CEZ	14	0.1–25	6.25	25	71	1.56–>100	25	>100
	KM	23	0.1–>100	12.5	>100	62	0.2–>100	12.5	100
	TC	17	0.2–>100	12.5	>100	68	0.1–>100	12.5	>100
	NA	26	0.1–>100	12.5	50	59	0.2–>100	12.5	>100
	GM	73	0.1–>100	12.5	50	12	3.13–>100	50	>100
	EM	0	—	—	—	85	0.1–>100	12.5	>100
Klebsiella sp. (29)	PCG	1	12.5	12.5	12.5	28	0.78–>100	6.25	>100
	AMP	1	12.5	12.5	12.5	28	0.78–>100	6.25	>100
	CEZ	24	0.78–>100	6.25	25	5	6.25–>100	12.5	>100
	KM	25	0.78–>100	6.25	25	4	6.25–>100	12.5	>100
	TC	16	0.78–>100	12.5	>100	13	0.78–>100	3.13	25
	NA	22	0.78–>100	6.25	25	7	3.13–>100	12.5	>100
	GM	29	0.78–>100	6.25	>100	0	—	—	—
	EM	0	—	—	—	29	0.78–>100	6.25	>100
Staphylococcus sp. (37)	PCG	24	≤0.012–>100	12.5	50	13	0.39–>100	>100	>100
	AMP	29	≤0.012–>100	12.5	>100	8	6.25–>100	>100	>100
	CEZ	30	≤0.012–>100	25	>100	7	25–>100	>100	>100
	KM	25	≤0.012–>100	6.25	>100	12	1.56–>100	25	>100
	TC	25	≤0.012–>100	12.5	50	12	0.1–>100	>100	>100
	NA	11	≤0.012–>100	25	>100	26	0.1–>100	25	>100
	GM	33	≤0.012–>100	25	>100	4	25–>100	>100	>100
	EM	20	≤0.012–50	6.25	25	17	0.39–>100	>100	>100
S. faecalis (33)	PCG	31	≤0.012–>100	6.25	25	2	3.13–>100	3.13	>100
	AMP	30	≤0.012–>100	6.25	25	3	3.13–>100	3.13	>100
	CEZ	18	≤0.012–>100	1.56	>100	15	1.56–>100	6.25	25
	KM	18	≤0.012–>100	3.13	>100	15	0.2–>100	6.25	25
	TC	23	≤0.012–>100	6.25	25	10	0.39–>100	6.25	12.5
	NA	4	1.56–>100	3.13	>100	29	≤0.012–>100	6.25	25
	GM	26	≤0.012–>100	6.25	>100	7	6.25–25	6.25	25
	EM	21	≤0.012–>100	6.25	12.5	12	0.2–>100	12.5	>100

[a] PCG, Penicillin G; KM, kanamycin; GM, gentamicin; NA, nalidixic acid; TC, tetracycline; EM, erythromycin.
[b] Strains susceptible to drug indicated at left.
[c] Strains resistant to drug indicated at left.
[d] —, strains were neither susceptible nor resistant.

evident with gram-positive cocci. The difference between the combination and the antibiotic alone was found to be significant in many cases, by the rank sum test (10) (Table 3). Table 4 shows the percentage of total strains against which the FIC index was ≦ 0.5. Synergy of up to 31% was observed between alafosfalin and two β-lactams in *E. coli*, *Klebsiella*, *Enterobacter*, and *Citrobacter*. Total synergistic cases, i.e., strains against which the index was <1.0 (B in Table 4), were observed in more than 55% of strains in these genera when a combination of AMP and alafosfalin (4:1) was used. The synergy was less remarkable in *Serratia* and *S. faecalis*. It is to be noted that synergy by the alafosfalin combination with the β-lactams was observed in up to 54% of strains belonging to indole-positive *Proteus* and of nonfermentative rods (Table 4), against which either alafosfalin or each β-lactam alone was almost inactive (Table 1). Thus, in 54 and 20% of the strains of *Acinetobacter* sp. was found a potentiation (FIC index < 1.0) in combinations of AMP and CEZ with alafosfalin, respectively (1:4) (Table 4).

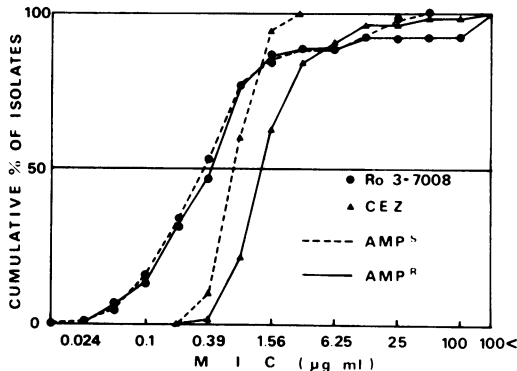

FIG. 1. *Comparison of Ro 03-7008 and CEZ activity toward AMP-resistant (——) and AMP-susceptible (- - - - -) strains.*

DISCUSSION

Alafosfalin was shown in this study to have a broad spectrum of antibacterial activity against

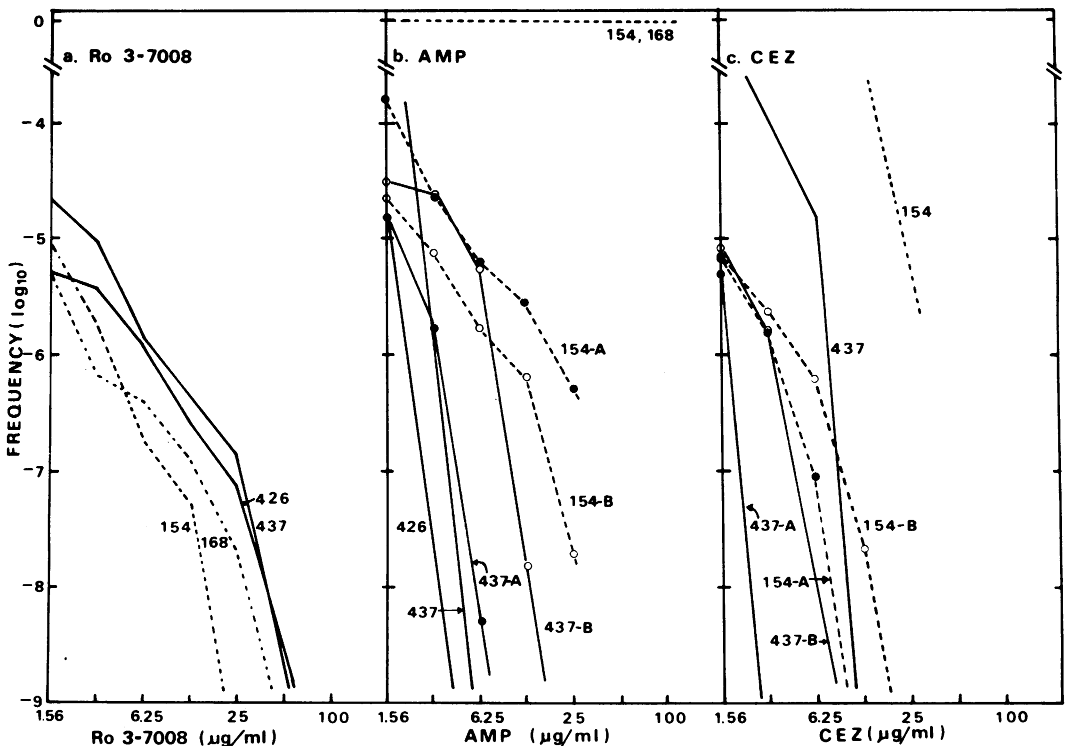

FIG. 2. *Population distribution of resistant clones to each concentration of Ro 03-7008 (a), AMP (b), and CEZ (c), within AMP-susceptible (——) (strain no. 437 and 425) and AMP-resistant (- - - - -) (strain no. 154 and 168) E. coli isolates. A and B indicate, respectively, a 10:1 and 1:1 combination of each drug with Ro 03-7008; 10^9 cells were plated on selection staphylococcal defined medium agar containing each concentration of antibiotics, and viable cells were counted.*

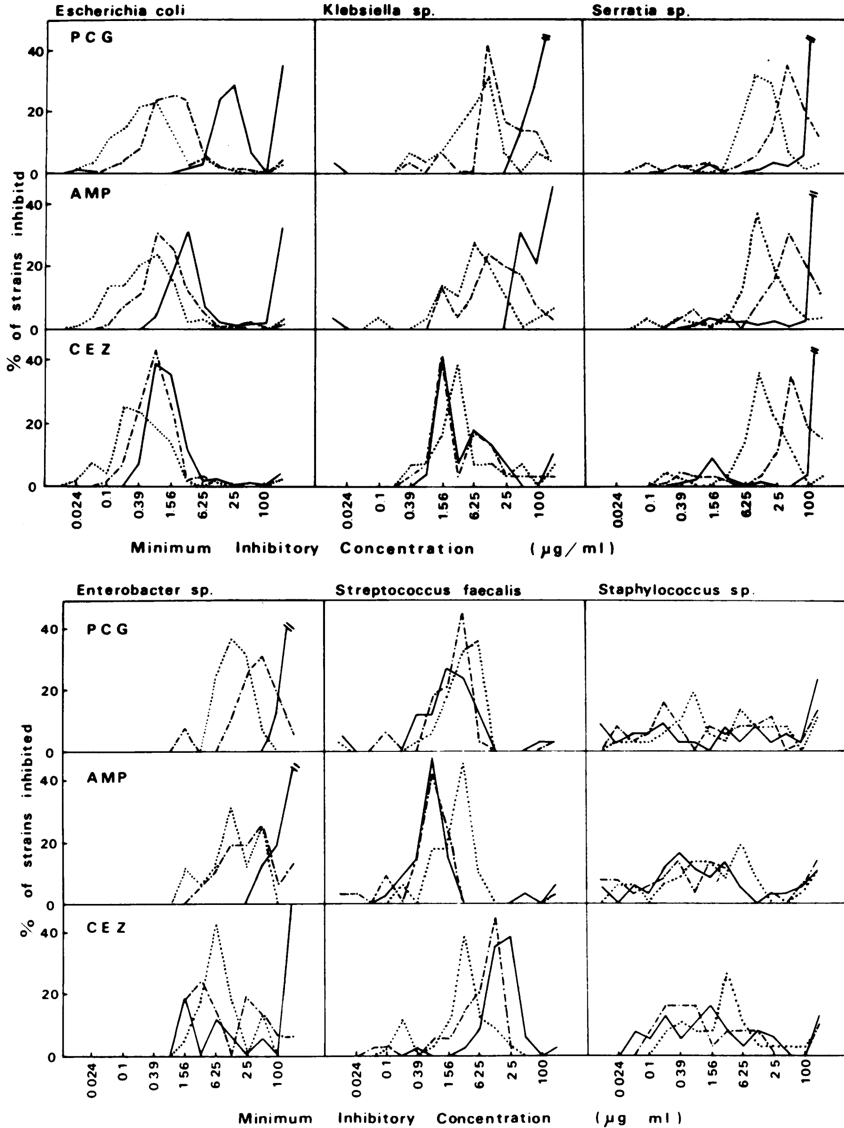

FIG. 3. Comparison of MIC distribution of penicillin G (PCG), AMP, and CEZ alone (——) with that of the same antibiotics in combination with Ro 03-7008. Ratio of each β-lactam to Ro 03-7008 was 4:1 (·–·–·) or 1:4 (· · · · ·).

both gram-negative and -positive UTI pathogens. The highly potent in vitro activity of the drug against *E. coli* was noteworthy, whereas against gram-positive cocci it exhibited a similar or somewhat lower activity than the β-lactams compared with it (Table 1). Alafosfalin was also active against *Serratia, Citrobacter,* and *Enterobacter* strains that are totally resistant to such β-lactams as AMP or CEZ, indicating a possible role in clinical medicine. It should be borne in mind, however, that the MIC determination utilized a defined agar, since alafosfalin was totally inactive on any rich media (2). This fact may reasonably suggest that it would exert its best activity in vivo in a poor nutritional environment such as urine. Should this prove correct, the present results indicate that the majority of common pathogens in UTI, including *E. coli, Klebsiella, Citrobacter, Enterobacter,* and *Serratia,* would be susceptible to alafosfalin at concentrations of <25 μg/ml, easily achieved by a single oral administration (3).

TABLE 3. *Comparison of MIC_{50} of combinations with those of each single drug*[a]

Species or genera (no. of strains)	MIC_{50} (μg/ml)										
	Alafosfalin alone	AMP alone	Alafosfalin/AMP				CEZ alone	Alafosfalin/CEZ			
			4:1	Significance[b]	1:4	Significance[b]		4:1	Significance[b]	1:4	Significance[b]
E. coli (21)	0.39	>100	0.98	S***	1.95	S***	1.56	0.98	S***	0.98	N
Klebsiella sp. (23)	6.25	100	7.81	S***	15.63	S***	3.13	3.91	N	1.95	N
Citrobacter sp. (9)	12.5	>100	15.63	S*	31.25	S*	>100	7.81	S*	15.63	N
Serratia sp. (25)	12.5	>100	15.63	S***	62.5	S***	>100	15.63	S***	62.5	S***
Enterobacter sp. (16)	12.5	>100	15.63	S***	31.25	S***	>100	7.81	S*	7.81	N
Staphylococcus sp. (23)	25	1.56	3.91	I*	1.95	N	1.56	3.91	I**	0.98	N
S. faecalis (19)	3.13	0.78	1.91	I***	0.98	N	12.5	3.91	S***	7.81	S**

[a] The combination is superior (S) or inferior (I) to each drug (AMP or CEZ) alone by $P < 0.01$ (***), $P < 0.05$ (**), or $P < 0.2$ (*); N means that the difference is statistically nonsignificant. Statistical significance was examined by the rank sum test (9), using all MICs on each strain. Note that the significance is not a mere comparison of two MIC_{50} columns, and therefore the same MIC_{50} can give a different P value when the sensitivity distribution among strains differs widely in scattering.

TABLE 4. *Percentage of strains potentiated with alafosfalin (Ro 03-7008)*

UTI isolate (no. of strains)	% Potentiated with[a]							
	AMP/alafosfalin				CEZ/alafosfalin			
	4:1		1:4		4:1		1:4	
	A	B	A	B	A	B	A	B
E. coli (144)	16	55	18	38	8	51	16	38
Citrobacter sp. (9)	22	55	11	22	22	33	11	33
Enterobacter sp. (16)	31	62	13	26	25	38	19	38
Serratia sp. (85)	14	38	9	30	9	30	11	25
Klebsiella sp. (29)	28	59	10	31	7	28	14	59
P. mirabilis (17)	12	24	12	24	0	29	6	30
Indole (+) *Proteus* (29)	3	10	3	17	3	17	3	17
Pseudomonas sp. (54)	0	2	2	4	0	6	0	6
Acinetobacter sp. (15)	7	14	7	54	7	7	13	20
Staphylococcus sp. (34)	15	39	24	48	15	39	18	39
S. faecalis (33)	18	30	3	21	12	61	24	57

[a] Values in columns A and B refer to strains against which the FIC index was ≤0.5 and >1.0, respectively.

Synergism between alafosfalin and β-lactams was evident with a wide range of genera and species (Tables 3 and 4; Fig. 3), confirming the previous report (1, 2). It is suggested from the absence of cross resistance between alafosfalin and β-lactams (Table 2; Fig. 1) that the proposed multiblockade of cell wall synthesis could contribute to this phenomenon (1, 4). The extent of the synergism varied with the strains tested, but in general more than 50% of the susceptible strains showed FIC indexes of less than 1.0 (Table 4). It is noteworthy that a minority of strains insensitive to alafosfalin alone also exhibited a clear potentiation with β-lactams so as to widen the susceptible spectrum. This fact leads one to anticipate that, if any of the β-lactams active against these species was used as the partner, a higher activity could be expected in combination with alafosfalin. That more than 70% of UTI isolates resistant to established antibiotics are susceptible to alafosfalin is a great advantage to the phosphonopeptide.

Among 144 *E. coli* strains examined, 11 strains (7.6%) were found to be naturally resistant to alafosfalin. The mechanisms of the resistance should be clarified for better clinical use of this unique antibacterial agent. Our preliminary study showed that the uptake of alafosfalin in these naturally resistant strains was roughly one-fourth to one-half of that in the susceptible strains. By contrast, the resistant clones selected in the laboratory did not show any uptake of the drug. Despite the level of natural resistance, the high activity and extremely low incidence of cross resistance with existing agents suggest a potential clinical role for alafosfalin either alone or as a combination product with β-lactam antibiotics.

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