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In Vitro Activity of Fludalanine Combined with Pentizidone Compared with Those of Other Agents

R. WISE* AND J. M. ANDREWS

Department of Medical Microbiology, Dudley Road Hospital, Birmingham, United Kingdom

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The in vitro activity of fludalanine (MK641) combined with pentizidone (MK642) so as to give a fludalanine/D-cycloserine ratio of 1:1 was compared with the activities of ampicillin, ticarcillin, cefuroxime, ceftazidime, and trimethoprim against 452 recent isolates and known β-lactam- and trimethoprim-resistant strains. In addition, the in vitro activity of fludalanine-pentizidone on four different media, including a defined medium (DFN-2), was studied. The MIC of fludalanine-pentizidone against 90% of *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *Providencia stuartii*, *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *Staphylococcus aureus*, and fecal streptococci was 4 μg or less per ml on DFN-2, and activity was somewhat reduced on the other media. *Proteus* spp. and *Pseudomonas aeruginosa* (90% MIC, ≤64 μg/ml) and *Bacteroides* spp. (90% MIC, 16 μg/ml) were less susceptible. Generally, fludalanine-pentizidone was less active than ceftazidime and comparable in activity to cefuroxime. β-Lactamase-producing and trimethoprim-resistant strains tended to be susceptible to fludalanine-pentidizone. In the absence of human serum, the MBC of fludalanine-pentizidone was similar to the MIC. In the presence of increasing concentrations of human serum, there tended to be a greater difference between the MIC and MBC.

Antimicrobial agents, often of low molecular weight, which are analogs of bacterial cell wall precursors have enjoyed specialized but limited clinical use. Cycloserine, a D-alanine-D-alanine synthetase of *Streptomyces orchidaceous*, is active against a wide range of organisms, including *Mycobacterium tuberculosis* (4). Fosfomycin, produced from several *Streptomyces* spp., is also active against a wide range of bacteria, is an analog of phosphoenolpyruvate (6), and is widely used in certain parts of the world. Alaphosphin, a phosphonopeptide, no longer under development, acts as an alanine analog (1). A new development is the combination designated MK641/642 (F. M. Kahan and H. Kropp, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 15th, Washington, D.C., abstr. no. 100, 1975) (Fig. 1), which is a combination of fludalanine (MK641, 2-^{2}H-3-fluoro-D-alanine) and pentizidone (MK642, sodium-D-4[(2-oxo-3-pentene-4-*yl*)-amino]-3-isoxazolidinone hemihydrate), the latter being a prodrug which is hydrolyzed spontaneously and converted to D-cycloserine. These two compounds are being developed for oral administration in a fixed MK641/MK642 ratio of 3:2.1, which is equivalent to a fludalanine/D-cycloserine ratio of 3:1; it is reported that this combination has a wide antibacterial spectrum (H. Kropp, F. M. Kahan, and H. B. Woodruff, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 15th, Washington, D.C., abstr. no. 101, 1975). It is expected that after oral administration, the ratio of the two components in serum will be about 1:1, at which ratio antibacterial synergy is reported to be maximal (information from Merck Sharp & Dohme).

In this study, we compared the activity of a 1:1 ratio of the combination with those of other broad-spectrum agents. In particular, we investigated the effect that different laboratory media have upon activity; preliminary studies have shown that the activity of fludalanine-pentizidone is antagonized by the alanine and glycine content of certain standard bacteriological testing media (information from Pre-clinical Brochure, Merck Sharp & Dohme Research Laboratories, Rahway, N.J., 1983).

* Corresponding author.

MATERIALS AND METHODS

Strains and antimicrobial agents. A total of 452 strains were examined, of which 421 were recent clinical isolates from this hospital. The remaining strains were known to be resistant to β-lactam antimicrobial agents and trimethoprim.

The antimicrobial agents investigated in this study were obtained from the following sources: fludalanine-pentizidone, Merck Sharp & Dohme, Hoddesdon, England; ampicillin and ticarcillin, Beecham Research Laboratories, Brentford, England; cefuroxime and ceftazidime, Glaxo Research Laboratories, Greenford, England; trimethoprim, Burroughs Wellcome, Beckenham, England. The fludalanine-pentizidone was used throughout to yield a fludalanine/D-cycloserine ratio of 1:1 (wt/wt), and the results were expressed as a single number with both agents present at the indicated concentration. Fludalanine was supplied as the pure substance. Pentizidone was hydrolyzed by 0.25 N hydrochloric acid to D-cycloserine according to the recommendations of Merck Sharp & Dohme.

Media. Susceptibility testing of the strains was performed with Isosensitest (pH 7.2, Oxoid Ltd., Basingstoke, England) for ampicillin, ticarcillin, cefuroxime, ceftazidime, and trimethoprim. For fludalanine-pentizidone, the following media were used: Isosensitest, direct sensitivity test (DST) (pH 7.4; Oxoid), Mueller-Hinton (pH 7.4 lot no. 28927128; Oxoid), and DFN-2 media (pH 7.2), which is a mixture of basal salts, vitamins, and known concentrations of amino acids supplied by Merck Sharp & Dohme Research Laboratories, Rahway, N.J. This latter media was prepared by adding 7.8 g of DFN-2 supplement to 250 ml of water, filter sterilizing the mixture, and then adding (at 55°C) 250 ml of autoclaved 1.6% agar (no. 1; Oxoid).

Susceptibility testing. The antimicrobial activities of the compounds were determined by an agar plate dilution method. The above media were supplemented as follows: 5% horse blood plus 10% IsoVitaleX (BBL, Becton-Dickinson, Wembley, U.K.) to support growth of streptococci (including *Streptococcus pneumoniae*), *Haemophilus influenzae*, and *Neisseria gonorrhoeae* and 5% horse blood to support growth of *Bacteroides* spp.

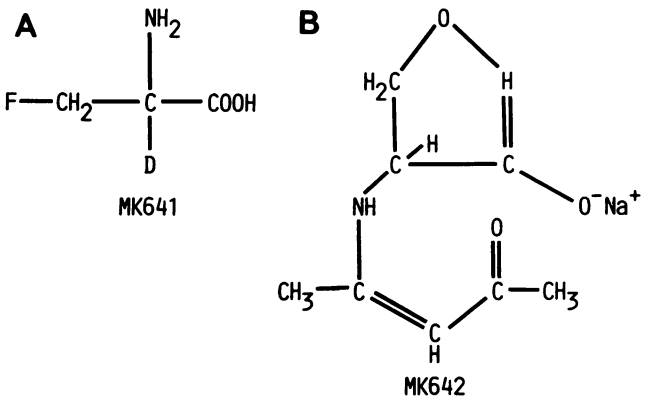

FIG. 1. Structures of fludalanine (A) and pentizidone (B). D, Deuterium (^2H).

The inocula were prepared as follows. For all strains except streptococci (including *Streptococcus pneumoniae*), *N. gonorrhoeae*, *H. influenzae*, and *Bacteroides* spp., the organisms were grown overnight in nutrient broth yielding viable counts of about 10^9 CFU per ml. Streptococci were grown in Todd-Hewitt broth; *H. influenzae*, *Streptococcus pneumoniae*, and *N. gonorrhoeae* were grown in Levinthal broth; and *Bacteroides* spp. were grown in Wilkins-Chalgren broth (Oxoid). For each broth, comparable viable counts were obtained.

The inocula were obtained by transferring 1 μl of an undiluted or a 1:100 dilution of the overnight culture to the surface of the antibiotic-containing agar by a multipoint inoculating device (Denley-Tech Ltd., Billingshurst, England). The final inoculum sizes on the plate surface were 10^4 and 10^6 CFU.

All plates were incubated in air at 37°C for 24 h, except the anaerobes, which were incubated in an anaerobic cabinet with an atmosphere of 80% nitrogen, 10% carbon dioxide, and 10% hydrogen, and *H. influenzae* and *N. gonorrhoeae*, which were incubated in air plus 10% CO_2. The MIC of the antibiotic was defined as the concentration in agar at which there was a reduction (by counting) to no more than two colonies in the original inoculum. In the case of large inocula, a slight haze of growth was ignored.

The effect of human serum and urine on the MIC and MBC of fludalanine-pentizidone was studied in two strains each of *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, Lancefield group D streptococci, and *Staphylococcus aureus*. An overnight broth culture of these organisms was inoculated into 1 ml of DFN-2 defined media broth (made as above without addition of agar) with 0, 20, and 70% human serum and also 100%, pooled, antibiotic-free human urine (pH 5.4) and decreasing concentrations of fludalanine-pentizidone. The final inoculum was about 10^5 CFU/ml. After 24 h of incubation, the MIC was defined as the concentration of fludalanine-pentizidone at which there was no visible growth. The tubes were then subcultured by transferring 0.01 ml onto MacConkey agar (for gram-negative bacilli) or blood agar (for the gram-positive cocci) and incubating the cultures in air at 37°C for a further 24 h. The MBC was defined as the lowest concentration of fludalanine-pentizidone in the original broth at which there was no growth after subculture; this was equivalent to a lethality rate of 99%.

RESULTS

Table 1 shows the results obtained from 426 of the 452 strains tested with the inocula at 10^4 CFU and the effect of the four different laboratory media upon the activity of fludalanine-pentizidone. Against the *Enterobacteriaceae*, the combination had a modest degree of activity compared with the other agents studied. With the exception of *Proteus* spp. and *Serratia* spp., when the DFN-2 medium was used, the MICs were equal to or less than 8 μg/ml. The *Proteus* and *Serratia* spp. were about fourfold less susceptible. Also tested, but not shown in Table 1, were five strains of *Citrobacter freundii* (mode MIC of fludalanine-pentizidone on DFN-2 medium, 1 μg/ml), six strains of *Acinetobacter* spp. (mode MIC, 32 μg/ml), five strains of *Shigella sonnei* (mode MIC, 1 μg/ml), and two *Salmonella* spp. (MICs, 2 and 4 μg/ml). Ceftazidime was the most active compound tested, and, generalizing, fludalanine-pentizidone was equivalent in activity to cefuroxime. However, known characterized β-lactamase-producing *Enterobacteriaceae* strains which were included were as susceptible to fludalanine-pentizidone as were the non-β-lactamase producers. For example, included among the *E. coli* was a strain containing the TEM-1 β-lactamase which was resistant to ampicillin and ticarcillin (MIC, >128 μg/ml) and susceptible to 4 μg of fludalanine-pentizidone per ml (on DFN-2 medium). Similarly, a strain of *Enterobacter cloacae* possessing the P99 cephalosporinase was resistant to cefuroxime (MIC, >128 μg/ml) but susceptible to fludalanine-pentizidone (MIC, 8 μg/ml on DFN-2 medium). Included among the *E. coli* tested were four strains which were highly resistant to trimethoprim (MIC, ≥256 μg/ml) but which were susceptible to 1 to 4 μg of fludalanine-pentizidone per ml.

The different media did affect the apparent susceptibility of the *Enterobacteriaceae* to fludalanine-pentizidone; the strains tended to be two- to eightfold more susceptible on the DFN-2 medium than on the other media studied. In the case of *Providencia stuartii*, the strains appeared to be at least 16-fold more resistant on the Isosensitest, Mueller-Hinton, and DST media than on the DFN-2 medium.

Fludalanine-pentizidone appeared to have little activity against *Pseudomonas aeruginosa*, except that 4 of 49 strains were susceptible to 8 mg or less per ml when DFN-2 medium was used. Ceftazidime was the most active compound tested against this species.

Ceftazidime was also the most active compound against the strains of *H. influenzae* and *N. gonorrhoeae* tested. The β-lactamase-producing strains of both these species were as susceptible to fludalanine-pentizidone as were the nonproducers, whereas reduced susceptibility to ampicillin and ticarcillin β-lactamase producers was found. The choice of medium had little effect upon the susceptibility of *H. influenzae* to fludalanine-pentizidone, but strains of *N. gonorrhoeae* were generally two- to eightfold less susceptible on Mueller-Hinton, Isosensitest, and DST media than on DFN-2 medium. All of the agents studied showed poor activity against *Bacteroides* spp. The strain of *Bacteroides thetaiotaomicron* was resistant (MIC, >128 μg/ml) to all the β-lactams tested but was susceptible to 16 μg of fludalanine-pentizidone per ml on all but the DST medium (MIC, 32 μg/ml).

Trimethoprim was about two to eight times more active than fludalanine-pentizidone against *Staphylococcus aureus*. The activity of fludalanine-pentizidone was notable in that there was a very narrow range of susceptibility; the β-lactamase-producing and methicillin-resistant strains were as susceptible as were the other strains studied. The activity was most pronounced on the DFN-2 medium; it was fourfold greater on DFN-2 than on the other three media which were used.

TABLE 1. MICs inhibiting cumulative percentage of isolates

Species	No. of isolates	Antibiotic (medium)[a]	MIC (μg/ml)		
			Range	50%	90%
E. coli	50	Fludalanine-pentizidone (Isosens)	4–16	4	8
		Fludalanine-pentizidone (DST)	4–16	4	8
		Fludalanine-pentizidone (MH)	4–32	4	8
		Fludalanine-pentizidone (DFN-2)	0.5–8	1	2
		Ampicillin	0.12–>128	32	>128
		Ticarcillin	0.5–>128	4	>128
		Cefuroxime	0.25–32	4	8
		Ceftazidime	0.03–>128	0.12	4
		Trimethoprim	0.06–>128	0.25	1
Klebsiella spp.	50	Fludalanine-pentizidone (Isosens)	4–16	8	16
		Fludalanine-pentizidone (DST)	8–32	16	16
		Fludalanine-pentizidone (MH)	8–32	8	32
		Fludalanine-pentizidone (DFN-2)	1–4	2	4
		Ampicillin	2–>128	32	>128
		Ticarcillin	4–>128	>128	>128
		Cefuroxime	0.5–128	2	16
		Ceftazidime	0.03–4	0.06	0.25
		Trimethoprim	0.25–>128	0.5	128
Enterobacter spp. (4 *E. aerogenes* and 5 *E. cloacae*)	9	Fludalanine-pentizidone (Isosens)	4–16	8	16
		Fludalanine-pentizidone (DST)	8–32	8	32
		Fludalanine-pentizidone (MH)	8–16	8	16
		Fludalanine-pentizidone (DFN-2)	0.5–2	1	2
		Ampicillin	8–>128	64	>128
		Ticarcillin	2–>128	8	>128
		Cefuroxime	4–>128	8	>128
		Ceftazidime	0.12–4	0.25	4
		Trimethoprim	0.25–>128	1	>128
Proteus mirabilis	49	Fludalanine-pentizidone (Isosens)	8–≥64	32	32
		Fludalanine-pentizidone (DST)	8–≥64	16	32
		Fludalanine-pentizidone (MH)	8–≥64	32	32
		Fludalanine-pentizidone (DFN-2)	4–≥64	8	16
		Ampicillin	0.5–>128	1	>128
		Ticarcillin	0.25–>128	0.5	>128
		Cefuroxime	0.5–8	0.5	4
		Ceftazidime	0.03–0.12	0.03	0.06
		Trimethoprim	0.25–>128	1	>128
Indole-positive *Proteus* spp. (25 *P. vulgaris*, 18 *P. morganii*, and 4 *P. rettgeri*)	47	Fludalanine-pentizidone (Isosens)	4–≥64	32	≥64
		Fludalanine-pentizidone (DST)	8–≥64	16	≥64
		Fludalanine-pentizidone (MH)	8–≥64	32	≥64
		Fludalanine-pentizidone (DFN-2)	2–≥64	8	≥64
		Ampicillin	0.12–>128	64	>128
		Ticarcillin	0.25–>128	1	32
		Cefuroxime	1–>128	32	>128
		Ceftazidime	0.03–2	0.03	0.25
		Trimethoprim	0.25–>128	1	>128
Serratia spp. (15 *S. marcescens*, 2 *S. liquifaciens*)	17	Fludalanine-pentizidone (Isosens)	16–≥64	≥64	≥64
		Fludalanine-pentizidone (DST)	≥64	≥64	≥64
		Fludalanine-pentizidone (MH)	16–≥64	≥64	≥64
		Fludalanine-pentizidone (DFN-2)	4–≥64	8	32
		Ampicillin	4–128	32	128
		Ticarcillin	0.5–8	4	8
		Cefuroxime	4–>128	64	>128
		Ceftazidime	0.015–8	0.12	0.25
		Trimethoprim	0.12–>128	2	16
Providencia stuartii	19	Fludalanine-pentizidone (Isosens)	16–≥64	16	32
		Fludalanine-pentizidone (DST)	≥64	≥64	≥64
		Fludalanine-pentizidone (MH)	16–≥64	≥32	≥64
		Fludalanine-pentizidone (DFN-2)	1–8	4	4
		Ampicillin	4–>128	128	>128
		Ticarcillin	0.5–>128	1	>128
		Cefuroxime	0.25–64	1	16
		Ceftazidime	0.06–1	0.12	0.5
		Trimethoprim	0.5–>128	4	>128

TABLE 1—*Continued*

Species	No. of isolates	Antibiotic (medium)[a]	MIC (μg/ml)		
			Range	50%	90%
P. aeruginosa	49	Fludalanine-pentizidone (Isosens)	32–≥64	≥64	≥64
		Fludalanine-pentizidone (DST)	32–≥64	≥64	≥64
		Fludalanine-pentzidone (MH)	32–≥64	≥64	≥64
		Fludalanine-pentizidone (DFN-2)	1–≥64	≥64	≥64
		Ampicillin	8–>128	>128	>128
		Ticarcillin	1–>128	16	>128
		Cefuroxime	8–>128	>128	>128
		Ceftazidime	0.06–32	1	16
		Trimethoprim	1–>128	>128	>128
H. influenzae (including 9 β-lactamase positive)	25	Fludalanine-pentizidone (Isosens)	2–8	4	8
		Fluralanine-pentizidone (DST)	2–16	4	16
		Fludalanine-pentizidone (MH)	4–16	8	16
		Fludalanine-pentizidone (DFN-2)	2–8	4	8
		Ampicillin	0.12–8	0.5	4
		Ticarcillin	0.12–8	0.25	2
		Cefuroxime	0.5–16	1	2
		Ceftazidime	0.03–1	0.06	0.25
		Trimethoprim	0.12–64	0.12	64
N. gonorrhoeae (including 12 β-lactamase positive)	27	Fludalanine-pentizidone (Isosens)	2–16	8	8
		Fludalanine-pentizidone (DST)	2–16	16	16
		Fludalanine-pentizidone (MH)	2–16	8	16
		Fludalanine-pentizidone (DFN-2)	0.25–2	1	2
		Ampicillin	0.015–64	0.25	8
		Ticarcillin	<0.015–16	0.25	8
		Cefuroxime	<0.015–0.12	0.03	0.12
		Ceftazidime	<0.015–0.06	<0.015	0.03
		Trimethoprim	16–64	32	32
Bacteroides spp. (23 *B. fragilis*, 1 *B. ovatus*, and 1 *B. thetaiotaomicron*)	25	Fludalinine-pentizidone (Isosens)	8–32	8	16
		Fludalanine-pentizidone (DST)	8–≥64	8	32
		Fludalanine-pentizidone (MH)	8–32	8	32
		Fludalanine-pentizidone (DFN-2)	4–≥64	8	16
		Ampicillin	4–>128	8	>128
		Ticarcillin	4–>128	8	>128
		Cefuroxime	1–>128	8	>128
		Ceftazidime	2–>128	8	>128
		Trimethoprim	8–64	16	64
Staphylococcus aureus (including 10 methicillin resistant)	30	Fludalanine-pentizidone (Isosens)	2–4	4	4
		Fludalanine-pentizidone (DST)	4	4	4
		Fludalanine-pentizidone (MH)	2–4	4	4
		Fludalanine-pentizidone (DFN-2)	0.5–4	0.5	1
		Ampicillin	0.03–32	1	32
		Ticarcillin	0.5–64	4	32
		Cefuroxime	0.5–>128	4	32
		Ceftazidime	8–64	16	64
		Trimethoprim	0.12–1	0.5	0.5
Lancefield group D streptococci	10	Fludalanine-pentizidone (Isosens)	8–32	16	16
		Fludalanine-pentizidone (DST)	4–32	16	16
		Fludalanine-pentizidone (MH)	4–32	16	16
		Fludalanine-pentizidone (DFN-2)	4–32	8	16
		Ampicillin	0.25–1	0.5	1
		Ticarcillin	16–64	32	64
		Cefuroxime	2–>128	128	>128
		Ceftazidime	16–>128	>128	>128
		Trimethoprim	0.12–1	0.12	0.5
Streptococcus pneumoniae	19	Fludalanine-pentizidone (Isosens)	2–8	4	8
		Fludalanine-pentizidone (DST)	2–8	4	8
		Fludalanine-pentizidone (MH)	2–8	2	8
		Fludalanine-pentizidone (DFN-2)	2–8	4	8
		Ampicillin	<0.015–2	0.015	0.03
		Ticarcillin	0.12–32	0.25	8
		Cefuroxime	<0.015–0.5	0.03	0.25
		Ceftazidime	0.06–2	0.12	2
		Trimethoprim	1–>128	4	>128

[a] The fludalanine-pentizidone MIC is expressed as a single number; both agents were present in a 1:1 ratio at the stated concentration. Abbreviations: Isosens, Isosensitest agar; MH, Mueller Hinton agar.

TABLE 2. Effect of increased percentage of human serum and urine on the MIC and MBC of fludalanine-pentizidone

Strain		Plate MIC (μg/ml)	Concn (μg/ml) at the following human serum %						Concn (μg/ml) with urine	
			0		20		70			
			MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Escherichia coli	1	1	4	4	16	64	8	>128	8	64
Escherichia coli	2	1	4	4	4	128	8	64	8	8
Klebsiella pneumoniae	1	2	16	16	4	16	128	>128	16	64
Klebsiella pneumoniae	2	4	4	8	8	>128	128	>128	8	16
Proteus mirabilis	1	8	32	64	16	32	4	>128	32	128
Proteus mirabilis	2	8	32	64	16	16	4	>128	32	128
Group D streptococci	1	4	16	32	8	16	4	32	16	32
Group D streptococci	2	8	16	16	8	16	16	>128	32	64
Staphylococcus aureus	1	0.5	4	8	1	2	8	32	32	>128
Staphylococcus aureus	2	0.5	2	2	0.5	2	4	16	16	16

Five strains each of Lancefield groups A and B streptococci were studied (not shown in Table 1) and the MICs of fludalanine-pentizidone on DFN-2 medium were between 0.5 and 1 μg/ml (compared with between 2 and 4 μg/ml on the other three media). Lancefield group D streptococci were less susceptible to fludalanine-pentizidone; the activity was about 16-fold less than that of ampicillin.

Three strains of *Streptococcus pneumoniae* known to be resistant to trimethoprim (MIC, ≥128 μg/ml) were susceptible to between 2 and 8 μg/ml of fludalanine-pentizidone. Two strains known to have a reduced susceptibility to benzylpenicillin (MIC, 0.25 and 1 μg/ml) were as susceptible to fludalanine-pentizidone as strains which were highly susceptible to benzylpenicillin.

An increase in inoculum from 10^4 to 10^6 CFU had remarkably little effect upon the activity of fludalanine-pentizidone against the whole range of bacteria tested; at most, a twofold reduction in activity was seen. In contrast, a marked inoculum effect (commonly, a 4- to 16-fold reduction in activity) was seen with ampicillin and ticarcillin when β-lactamase-producing strains were tested.

The effects of increased amounts of serum upon the MIC and MBC of fludalanine-pentizidone are shown in Table 2. Additionally, the MIC obtained by the plate method (inoculum, 10^4 CFU) is compared with the broth-derived MIC (inoculum, 10^5 CFU/ml), and it can be seen that the activity in broth (without serum) is two- to eightfold less than in agar. In the absence of serum, the MIC is the same as or similar to the MBC. In the presence of increasing amounts of serum, the MIC of *E. coli* and *K. pneumoniae* tended to increase but those of *Proteus mirabilis* decreased. In addition, a greater difference between the MIC and MBC was noted at higher concentrations of serum. The activity of fludalanine-pentizidone in pooled human urine was similar to (or less than, especially in the case of *Staphylococcus aureus*) that in broth.

DISCUSSION

Preliminary pharmacokinetic studies suggest that 300 mg of fludalanine (MK641) should be administered with 210 mg of pentizidone (MK642) to yield a fludalanine/cycloserine ratio of 1:1 in serum. At 1 to 2 h postadministration, the expected levels in serum would then be about 10 μg/ml (information from Pre-clinical Brochure, Merck Sharp & Dohme, 1983), and levels in urine would be considerably higher. If an arbitrary breakpoint for this dose of the combination of 8 μg/ml is taken and if the susceptibility measurement of the DFN-2 medium is used, then fludalanine-pentizidone can be considered to have a moderately broad spectrum of activity as compared with the newer β-lactams. The spectrum would include the majority of *Enterobacteriaceae* (except *Proteus* spp. and *Serratia* spp.), *N. gonorrhoeae*, *Staphylococcus aureus* (including methicillin-resistant strains), and Lancefield groups A and B streptococci. *P. aeruginosa*, *Bacteroides* spp., and fecal streptococci would have to be considered resistant at this dose for the treatment of systemic (versus urinary tract) infections, and *H. influenzae* and *Streptococcus pneumoniae* would be on the borderline between resistant and susceptible.

This study confirmed that the susceptibility testing of fludalanine-pentizidone is medium dependent, with DFN-2 medium showing the compound at its most active state and with activity in urine or in the presence of serum being lower than that on DFN-2 medium. This phenomenon has been observed with other similar agents. The activity of alaphosphin is highly dependent upon the presence of peptone, casein hydrolysate, various amino acids, and pH (2); the need to use a special defined medium for testing this compound was discussed. Similarly in the case of fosfomycin, the standardization of in vitro testing has been a problem (3). This is related to fosfomycin transport into the bacterial cell which is induced by the presence of glucose-6-phosphate and inhibited by glucose and phosphates (5). It is noteworthy that fewer medium variations were observed when horse blood was added (to support growth of streptococci, *H. influenzae*, *N. gonorrhoeae*, and *Bacteroides* spp.); presumably, this was related to the fact that DFN-2 was then less defined, and there was thus greater similarity among the media.

Fludalanine has been shown to irreversibly inactivate alanine racemase at concentrations at or above the MIC; with increasing concentrations, however, the agent then becomes incorporated into the bacterial cell in place of D-alanine. This phenomena is known as self-reversal (15th ICAAC, abstr. no. 100). Cycloserine inhibits this phenomena and also acts on D-alanyl-D-alanine synthetase. However, both components are antagonized by the unphysiologically high levels of amino acids (especially L-alanine and glycine) found in many laboratory media (15th ICAAC, abstr. no. 101). The DFN-2 medium duplicates the concentrations of free alanine, glycine, glucose, and pH found in human serum (data from Merck Sharp & Dohme, Rahway, N.J.). Results of early clinical trials suggest that this combination is effective in systemic infections, and further studies are awaited with interest. However, problems in interpreting laboratory susceptibility tests may be anticipated.

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