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In Vitro Activity of Ro 13-9904, a New β-Lactamase-Stable Cephalosporin

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The minimal inhibitory concentration (MIC) of Ro 13-9904 against 245 clinical isolates was determined by an agar dilution method. The activity of Ro 13-9904 against most *Enterobacteriaceae* was similar to that of cefotaxime; it was slightly more active than cefotaxime against *Proteus mirabilis*, *Providencia* species, and *Serratia marcescens*, but slightly less active against *Klebsiella* species. Ro 13-9904 was twofold more active than cefotaxime and threefold more active than ticarcillin against ticarcillin-susceptible *Pseudomonas aeruginosa*, with a mean MIC of 7.2 μg/ml; isolates highly resistant to ticarcillin were inhibited by a mean MIC of 17.2 μg/ml. Ro 13-9904 was fourfold more active than ampicillin against susceptible *Haemophilus influenzae* and was equally active against β-lactamase-producing isolates. Ro 13-9904 was highly active against pneumococci and moderately active (MIC, 4 μg/ml) against *Staphylococcus aureus* isolates, whether they were susceptible or resistant to penicillin G. Oxacillin-resistant *S. aureus* and *Streptococcus faecalis* were completely resistant to Ro 13-9904 (MIC, >128 μg/ml).

Ro 13-9904 {(6R,7R)-7-[2-(2-amino-4-thiazolyl)-2-(Z-methoxyimino)-acetamido]-3-[(2,5-dihydro-6-hydroxy-2-methyl-5-oxo-as-triazine-3-yl) thiomethyl] ceph-3-em-4-carboxylic acid} combines high antibacterial potency, stability to a wide variety of β-lactamases, and favorable pharmacokinetic properties (2, 3). According to the manufacturers (Hoffmann-LaRoche, Inc., Switzerland), Ro 13-9904 has a terminal half-life of 6 to 7 h and a protein binding of 95%. In this study we compared the minimal inhibitory concentrations (MICs) of Ro 13-9904 with those of cephalothin, ampicillin, ticarcillin, and cefotaxime against various penicillin- and cephalothin-susceptible and -resistant isolates.

MATERIALS AND METHODS

Antibiotics. Antibiotic reference powders with known potency were obtained from the following manufacturers: Ro 13-9904 from Hoffmann-LaRoche; cephalothin from Eli Lilly Research Laboratories; ampicillin and ticarcillin from Beecham Research Laboratories; and cefotaxime from Hoechst A.G.

Bacterial isolates. The strains examined were isolated recently from specimens submitted to our clinical laboratory. For some species, isolates were selected so as to include some strains that were susceptible and other strains that were resistant to β-lactam antibiotics. Most resistant strains produced β-lactamase, according to the results of the chromogenic cephalosporin nitrocefin test (1).

Susceptibility tests. A total of 204 isolates of gram-negative bacilli and 4 isolates of gram-positive cocci were tested for antimicrobial susceptibility by using an agar dilution technique with diagnostic sensitivity test agar (Oxoid). The diagnostic sensitivity test agar was supplemented with 5% lysed horse blood to support the growth of *Haemophilus influenzae* and of streptococci. Overnight cultures in Trypticase soy broth (BBL Microbiology Systems) were further diluted and inoculated with an automatic multipoint inoculator, which delivered final inocula of 10^4 to 10^5 colony-forming units. Plates with antibiotic concentrations ranging from 128 to 0.008 μg/ml were prepared fresh daily. The MIC was the lowest concentration of antibiotic which allowed no visible growth or only one single colony after incubation at 36°C for 18 h.

RESULTS

Ro 13-9904 and cefotaxime had essentially the same activity against all of the isolates (Table 1). They were less active than ampicillin against penicillin-susceptible *Staphylococcus aureus*, but retained their activity against strains resistant to penicillin. Both were inactive against oxacillin-resistant isolates of *S. aureus* and against enterococci. They were four times more active than ampicillin against pneumococci, 20 times more active than ampicillin against susceptible *H. influenza*, and equally active against isolates resistant to ampicillin. All of the ampicillin-resistant isolates produced β-lactamase.

The activities of Ro 13-9904 and cefotaxime against the *Enterobacteriaceae* were also similar. Both antibiotics were about 100-fold more active than cephalothin against cephalothin-susceptible isolates of *Escherichia coli*, *Klebsiella* species, and *Proteus mirabilis*. The MICs of Ro 13-9904 and of cefotaxime were 4 to 10 times

TABLE 1. *Comparative activities of Ro 13-9904 and various penicillins and cephalosporins against various penicillin- and cephalothin-sensitive and -resistant isolates*

Organism (no. of isolates)	Compound	MIC (μg/ml)		
		Range	For % of isolates	
			50	90
S. aureus				
Penicillin G susceptible[a] (6)	Ro 13-9904	1–4	4	ND[b]
	Ampicillin	0.25–2	0.5	ND
	Cefotaxime	0.5–2	2	ND
Penicillin G resistant (6)	Ro 13-9904	4	4	ND
	Ampicillin	8–16	8	ND
	Cefotaxime	2	2	ND
Oxacillin resistant (6)	Ro 13-9904	16–>128	128	ND
	Ampicillin	8–64	32	ND
	Cefotaxime	16–>128	64	ND
S. faecalis (12)	Ro 13-9904	128–>128	128	>128
	Ampicillin	2–4	2	2
	Cefotaxime	128–>128	128	>128
S. pneumoniae (11)	Ro 13-9904	0.008–0.03	0.015	0.03
	Ampicillin	0.03–0.12	0.06	0.12
	Cefotaxime	0.008–0.015	0.015	0.015
H. influenzae				
Ampicillin susceptible (20)	Ro 13-9904	0.015	0.015	0.015
	Ampicillin	0.25–0.5	0.25	0.5
	Cefotaxime	0.015–0.03	0.015	0.015
Ampicillin resistant (8)	Ro 13-9904	0.015	0.015	ND
	Ampicillin	4–16	8	ND
	Cefotaxime	0.015–0.03	0.015	ND
Escherichia coli				
Cephalothin susceptible (15)	Ro 13-9904	0.03–0.06	0.06	0.06
	Cephalothin	4–16	8	16
	Cefotaxime	0.015–0.12	0.06	0.06
Cephalothin resistant (5)	Ro 13-9904	0.06–1	1	ND
	Cephalothin	32–>128	>128	ND
	Cefotaxime	0.06–2	1	ND
Klebsiella species				
Cephalothin susceptible (10)	Ro 13-9904	0.03–0.12	0.06	0.12
	Cephalothin	2–8	4	4
	Cefotaxime	0.015–0.12	0.03	0.03
Cephalothin resistant (10)	Ro 13-9904	0.015–4	0.12	2
	Cephalothin	32–>128	64	>128
	Cefotaxime	0.008–0.5	0.06	0.25
P. mirabilis				
Cephalothin susceptible (16)	Ro 13-9904	0.008	0.008	0.008
	Cephalothin	2–8	4	8
	Cefotaxime	0.008–0.03	0.008	0.015
Cephalothin resistant (5)	Ro 13-9904	0.008–0.12	0.03	ND
	Cephalothin	64–>128	>128	ND
	Cefotaxime	0.008–0.5	0.06	ND
Proteus (indole positive) (15)	Ro 13-9904	0.008–2	0.008	0.25
	Cephalothin	8–>128	>128	>128
	Cefotaxime	0.008–1	0.015	0.25
Providencia species (10)	Ro 13-9904	0.008–0.25	0.03	0.25
	Cephalothin	1–>128	64	>128
	Cefotaxime	0.008–1	0.12	1

TABLE 1. *Comparative activities of Ro 13-9904 and various penicillins and cephalosporins against various penicillin- and cephalothin-sensitive and -resistant isolates*

Organism (no. of isolates)	Compound	MIC (μg/ml)		
		Range	For % of isolates	
			50	90
Enterobacter species (10)	Ro 13-9904	0.06–32	0.12	0.5
	Cephalothin	32->128	64	>128
	Cefotaxime	0.06–16	0.25	1
Citrobacter freundii (10)	Ro 13-9904	0.03–1	0.12	1
	Cephalothin	2->128	64	128
	Cefotaxime	0.06–1	0.12	1
S. marcescens (10)	Ro 13-9904	0.12–1	0.5	1
	Cephalothin	>128	>128	>128
	Cefotaxime	0.12–2	1	2
Acinetobacter species (10)	Ro 13-9904	1–16	8	8
	Ticarcillin	1–32	8	32
	Cefotaxime	1–16	8	16
P. aeruginosa				
Ticarcillin susceptible (20)	Ro 13-9904	1–32	8	16
	Ticarcillin	0.5–128	32	32
	Cefotaxime	1–64	16	32
Ticarcillin resistant (10)	Ro 13-9904	8–64	16	32
	Ticarcillin	256->1,024	>1,024	>1,024
	Cefotaxime	32–128	64	128
Pseudomonas species[c] (10)	Ro 13-8804	1–128	4	128
	Ticarcillin	4->128	32	128
	Cefotaxime	1–64	4	16

[a] For explanation see text.
[b] ND, not determinable.
[c] *P. maltophilia* (3), *P. cepacia* (2), *P. putida* (2), *P. stutzeri* (2), *P. putrefaciens* (1).

higher against the strains resistant to cephalothin than against the susceptible ones.

Ro 13-9904, with a mean MIC of 7.2 μg/ml, was twice as active as cefotaxime and three times as active as ticarcillin against ticarcillin-susceptible *Pseudomonas aeruginosa*. The mean MIC of Ro 13-9904 against ticarcillin-resistant isolates was 17.2 μg/ml. There was no direct relationship between the degree of resistance to ticarcillin and activity of Ro 13-9904. For eight of nine strains highly resistant to ticarcillin (MIC, >1,024 μg/ml), Ro 13-9904 had MICs between 8 and 16 μg/ml. For the ninth strain, the MIC was 32 μg/ml. In contrast, for three other strains requiring an MIC of ticarcillin between 64 and 256 μg/ml, Ro 13-9904 had MICs of between 32 and 64 μg/ml.

Ro 13-9904 and cefotaxime had similar activities against *Acinetobacter* species and against *Pseudomonas* species, such as *P. cepacia*, *P. stutzeri*, *P. putrefaciens*, *P. putida*, and *P. maltophilia*. Two of three strains of the latter two species were relatively resistant to both drugs.

DISCUSSION

Ro 13-9904 has a high order of activity against most gram-negative bacilli and gram-positive cocci, with the exception of *Streptococcus faecalis* and oxacillin-resistant *S. aureus*. Strains of *P. aeruginosa* were moderately susceptible; *P. maltophilia* was rather resistant. The activity of Ro 13-9904 is very similar to that of cefotaxime: like the latter drug, Ro 13-9904 remained very active against isolates with acquired resistance against other β-lactam antibiotics (ampicillin, cephalothin, and ticarcillin). These results are in good agreement with the data published by Shannon et al. (3).

The in vitro activities of Ro 13-9904 and cefotaxime are very similar. What distinguishes Ro 13-9904 is its unusually long half-life (8 h) and its penetration into tissue fluid (2). Ro 13-9904 merits further study.

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