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Antibacterial Activity of DL 473, a C_3-Substituted Rifamycin Derivative

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DL 473 is a 3-[(4-cyclopentyl-1-piperazinyl)iminomethyl] rifamycin SV derivative which inhibited staphylococci, streptococci (including *Streptococcus faecalis*, *Listeria* species, and *Bacteroides* species. DL 473 was less active than rifampin against these species. DL 473 did not inhibit *Enterobacteriaceae* nor most *Pseudomonas* species. A combination of DL 473 and vancomycin or nafcillin tested against staphylococci was primarily additive and antagonism was not encountered.

There has been an increased interest in the activity of rifamycin compounds against bacteria other than mycobacteria. Rifampin enters white cells and has been used to treat endocarditis in combination with anti-cell wall antibiotics (5). Arioli and colleagues (1) have reported on the activity of piperazinyl hydrazones of 3-formyl rifamycin SV. They synthesized a 3-[(4-cyclopentyl-1-piperazinyl)iminomethyl] rifamycin SV (DL 473) which has been reported to have a long half-life in animals and to inhibit mycobacteria and chlamydia (4, 6, 7). I wished to study the in vitro activity of DL 473 and to compare its activity against various bacteria with that of rifampin.

DL 473 was a gift from V. Arioli, Research

TABLE 1. In vitro activity of DL 473

Organism (no. of strains)	Range	MIC (μg/ml)[a]	
		50%	90%
Acinetobacter calcoaceticus (5)	≥100	≥100	≥100
Aeromonas (5)	6.3–25	12.5	25
Bacteroides fragilis (23)	0.4–1.6	0.4	1.6
Bacteroides, Other (17)	0.1–1.6	0.4	1.6
Bordetella bronchiseptica (1)	50		
Enterobacter cloacae (5)	50–100	50	100
Escherichia coli (7)	3.1–25	12.5	25
Kelbsiella pneumoniae (5)	50–100	50	100
Proteus mirabilis (5)	50–100	50	100
Providencia stuartii (5)	25	25	25
Pseudomonas aeruginosa (14)	50–200	50	200
Pseudomonas alcaligenes (2)	100	100	100
Pseudomonas cepacia (6)	1.6–50	25	50
Pseudomonas diminuta (2)	6.3		
Pseudomonas fluorescens (2)	25–50		
Pseudomonas stutzeri (1)	50		
Serratia marcescens (6)	50–100	100	100
Serratia liquefaciens (5)	50	50	50
Streptococcus agalactiae (4)	0.01–0.4	0.1	0.2
Streptococcus bovis (5)	0.2–0.4	0.4	0.4
Streptococcus faecium (2)	0.2		
Streptococcus mitis (4)	0.1–0.4	0.1	0.2
Streptococcus pyogenes (10)	0.01–0.4	0.1	0.2
Streptococcus pneumoniae (10)	0.01–0.4	0.1	0.2
Yersinia enterocolitica (2)	100		

[a] 50% and 90%, MIC at which 50 and 90% of the strains, respectively, were inhibited.

TABLE 2. Comparative in vitro activities of DL 473 and other agents

Organism (no. of strains)	Agent	Range	MIC (μg/ml)[a]	
			50%	90%
Listeria monocytogenes (17)	DL473	0.2–1.6	0.2	0.4
	Rifampin	0.01–0.4	0.05	0.2
	Ampicillin	0.8–6.3	1.6	3.1
	Vancomycin	0.4–3.1	0.8	3.1
Staphylococcus aureus (35)[b]	DL473	0.01–12.5	0.05	0.2
	Rifampin	0.01–12.5	0.01	0.02
	Methicillin	0.8–>100	6.3	12.5
	Vancomycin	9.1–6.3	1.6	3.1
Staphylococcus aureus (12) (methicillin resistant)	DL473	0.01–12.5	0.05	0.2
	Rifampin	0.01–12.5	0.01	0.02
	Methicillin	12.5–>100	50	100
	Vancomycin	0.4–6.3	1.6	3.1
Staphylococcus epidermidis (25)	DL473	0.01–50	0.05	0.2
	Rifampin	0.01–50	0.01	0.02
	Methicillin	0.2–4	2	4
	Erythromycin	0.05–4	0.4	4
	Vancomycin	0.1–6.3	1.6	6.3
Staphylococcus epidermidis (15) (methicillin resistant)	DL473	0.1–50	0.1	0.2
	Rifampin	0.01–50	0.05	0.1
	Methicillin	12.5–>100	50	>100
	Vancomycin	0.1–6.3	1.6	6.3
Streptococcus faecalis (17)	DL473	0.8–3.1	0.8	3.1
	Rifampin	0.4–3.1	0.8	1.6
	Ampicillin	0.8–6.3	1.6	3.1
	Vancomycin	0.8–3.1	1.6	3.1

[a] 50% and 90%, MIC at which 50 and 90% of the strains, respectively, were inhibited.
[b] All β-lactamase-producing isolates.

Laboratories Gruppo Lepetit, Milan, Italy. Rifampin was obtained from Dow Chemical Co., Midland, Mich. Erythromycin was obtained from Abbott Laboratories, North Chicago, Ill., gentamicin from Schering-Plough Corp., Kenilworth, N.J., and ampicillin and methicillin from Beecham Research Laboratories, Ltd., Brentford, Middlesex, England.

All bacteria has been isolated from patients hospitalized at the Columbia-Presbyterian Medical Center in New York City. Organisms had been stored on slants or frozen at −70°C for periods of 2 weeks to 5 years. Bacteria had been identified by standard techniques.

Solutions of antimicrobial agents were prepared fresh daily for each experiment. Minimal inhibitory concentrations (MICs) were determined by the agar dilution technique. Mueller-Hinton agar was used. Organisms were applied with a spot replicating device that delivered 10^5 CFUs. Incubation was at 35°C for 18 h. Streptococci and *Listeria* species were tested on agar which contained 5% sheep blood. Broth dilutions were performed in 1-ml tubes, using as final inoculum 10^5 CFUs in Mueller-Hinton broth incubated at 35°C for 18 h. Minimal bactericidal concentrations (MBCs) were determined by plating 0.1 ml from clear tubes of broth to agar. The MBC was the concentration at which there was no growth. Synergy studies were performed on agar utilizing twofold dilutions of drugs in various concentrations. Synergy was defined as a fractional inhibitory index of <0.5, additive effect was defined as a fractional inhibitory index of 0.5 to 1.0, and antagonism was defined as a fractional inhibitory index of >2.

The overall activity of DL 473 is shown in Table 1. In general, the compound had very poor activity against aerobic gram-negative bacilli such as *Escherichia coli, Klebsiella* species, *Serratia* species, etc. It also was not active against *Pseudomonas aeruginosa* or other *Pseudomonas* species, or against *Acinetobacter* species. In contrast, the activity of DL 473 against

TABLE 3. Effect of an inoculum size of 10^7 CFU versus 10^5 CFU on the in vitro activity of DL 473

Organism	No. of strains tested	No. identical	No. greater by a fold of:			
			2	4	8	>8
Staphylococcus aureus	8	0	1	5	2	0
Staphylococcus epidermidis	6	3	1	0	0	2
Streptococcus faecalis	11	0	1	6	2	2

TABLE 4. Synergy of DL 473 with other antibiotics against staphylococci

Organism	Agent	No. tested	No. showing synergy	No. showing additive effect	No. showing antagonism
Staphylococcus aureus	Vancomycin	22	7	15	0
	Nafcillin	22	0	22	0
Staphylococcus epidermidis	Vancomycin	8	3	5	0
	Nafcillin	8	0	8	0

anaerobic species such as *Bacteroides fragilis* and other *Bacteroides* species such as *Bacteroides thetaiotamicron, Bacteroides melaninogenicus*, and *Bacteroides vulgatus* was excellent, with 90% of the isolates inhibited by 1.6 μg/ml. DL 473 also inhibited most streptococcal species, such as group A and group B, at concentrations ≤0.4 μg/ml.

The comparative activities of DL 473, rifampin, and other agents tested against staphylococci, *Streptococcus faecalis*, and *Listeria* is shown in Table 2. DL 473 was four- to eightfold less active than rifampin against *Staphylococcus aureus*. For example, the DL 473 MIC against *S. aureus* was 0.1 μg/ml compared to a rifampin MIC of 0.025 μg/ml, or a DL 473 MIC of 0.8 μg/ml compared to 0.025 μg/ml. However, DL 473 did inhibit methicillin-resistant isolates, all at ≤0.2 μg/ml, and it also inhibited erythromycin-resistant *S. aureus*. The activity of DL 473 against *Staphylococcus epidermidis* was virtually identical to its activity against *S. aureus*. The comparable rifamycin MICs would be 0.001 and 0.025 μg/ml compared to 0.1 and 0.8 μg/ml for DL 473. DL 473 inhibited methicillin- and erythromycin-resistant *S. epidermidis*. DL 473 was comparable to ampicillin and vancomycin against *S. faecalis* and more active than those two compounds against *Listeria* species, but less active than rifampin. Rifampin was also two- to eightfold more active than DL 473 when tested against single isolates of *Streptococcus faecium, Streptococcus mitis*, and *Streptococcus bovis*.

The inoculum size had an effect upon the activity of DL 473. Table 3 demonstrates that there was great variability in the effect of increasing inoculum size to 10^7 from 10^5 CFUs. Several isolates showed a greater than eightfold rise in MIC, but the most common increase was a fourfold increase in MIC values. The difference between MIC and MBC values for five isolates each of *S. aureus, S. epidermidis*, and *S. faecalis* ranged from no increase to an eightfold increase; for example, organisms with MICs of 0.01 μg/ml had MBC values of 0.01, 0.025, and 0.1 μg/ml. The MIC of DL 473 in the presence of 50% normal human serum for the aforementioned organisms was 3.1 μg/ml compared to 0.01 μg/ml in Mueller-Hinton broth.

Table 4 shows the effect of combination of DL 473 with vancomycin or nafcillin when tested against staphylococci. Against *S. aureus* and *S. epidermidis* the combination of DL 473 and nafcillin was additive, and neither synergy nor antagonism was noted. In contrast, vancomycin and DL 473 acted in a synergistic manner for 32 and 42% of the *S. aureus* and *S. epidermidis* strains, respectively. Antagonism was not seen. An example of synergy against *S. aureus* was a DL 473 MIC of 0.2 μg/ml and a vancomycin MIC of 1.6 μg/ml with an MIC of the combined agents 0.025 μg/ml. We did not find antagonism as has been reported for some combinations of rifampin and vancomycin or penicillins (8), but I did not determine bactericidal synergy concentrations.

The results of this study extend the earlier observations of Arioli et al. (1). The in vitro activity of DL 473, although less than that of rifampin, is excellent against staphylococci, streptococci (including *S. faecalis*), *Listeria* species and *Bacteroides* species. DL 473 has shown better 50% effective dose values in mice than rifampin, which may be due to either its higher serum levels or longer half-life. The longer half-life may be a combination of greater protein binding and altered biliary excretion (2). Clearly, the in vitro characteristics of the compound support further clinical studies of the compound in humans to determine whether its pharmacokinetic advantages (3) may be of value in human infections.

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