ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 1976, p. 146-149
Vol. 10, No. 1

Printed in U.S.A.

In Vitro Antimicrobial Activity of Cinoxacin Against 2,968 Clinical Bacterial Isolates

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Received for publication 9 February 1976

Cinoxacin demonstrated effective in vitro antimicrobial activity against the *Enterobacteriaceae*, but negligible activity against *Pseudomonas aeruginosa* and gram-positive cocci. The activity of cinoxacin was slightly greater than that of nalidixic acid.

A number of synthetic organic acids containing cinnoline ring structures have been investigated for antimicrobial activity (3, 8). Of these compounds, cinoxacin (1-ethyl-1,4-dihydro-4,oxo-[1,3]dioxolo[4,5-g]cinnogine-3-carboxylic acid) was chosen for clinical and expanded in vitro investigation. Cinoxacin (Lilly compound 64715) possesses many characteristics similar to nalidixic acid and oxolinic acid. Preliminary in vitro bacterial susceptibility studies indicate a similar gram-negative antibacterial activity to that of nalidixic acid (2, 5, 7).

This study presents in vitro broth dilution susceptibility data for cinoxacin against 2,968 clinical bacterial isolates plus a direct comparison of cinoxacin and nalidixic acid minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) results against the most prevalent urinary tract isolates.

MATERIALS AND METHODS

Bacterial cultures. Organisms used in the study were obtained from the Clinical Microbiology Division of the Kaiser Foundation Hospital Laboratories, Oregon Region. A total of 2,968 bacterial isolates including 1,621 urinary tract isolates conforming to the quantitative criteria of Kass (4) were tested. Each isolate was identified by the replicator plate method described by Fuchs (1), utilizing 17 to 24 biochemical tests.

Susceptibility testing. MICs of all antimicrobial agents were determined by the broth microdilution technique. Mueller-Hinton broth (Difco) was used in previously prepared well plastic trays (8 by 10; Micro-Media Systems, Inc., Campbell, Calif.). The antimicrobial agents were injected into the wells in 100-μl volumes. Organisms were grown to stationary phase (10^9 organisms/ml) in brain heart infusion broth and then diluted 1:100 in sterile water. An automatic tray inoculator delivered 5 μl of this dilution to each 100-μl well; final inoculum concentration was 5×10^5 organisms/ml.

In the inoculum size studies, organisms were inoculated as above, using similar equipment and three inoculum concentrations, 10^7, 10^5, and 10^3 organisms/ml.

MIC end points were defined as the lowest broth concentration totally inhibiting organism growth (clear well) after 15 to 18 h of incubation at 35 C. Determination of the MBC was performed by subculturing 1 μl of both turbid and clear wells into Mueller-Hinton broth. The MBC was defined as the lowest subcultured broth concentration revealing no growth (clear well) after overnight incubation at 35 C. This represents a three-log minimum-kill (99.9%) end point.

RESULTS AND DISCUSSION

The cumulative percentage of gram-negative bacilli inhibited by increasing cinoxacin concentrations is shown in Table 1. Among the 2,139 strains tested, *Escherichia coli*, *Citrobacter diversus*, *Proteus morganii*, and *Proteus vulgaris* exhibited the greatest susceptibility. Ten of the thirteen tabulated *Enterobacteriaceae* species showed greater than 90% inhibition of strains at 16 μg/ml. Only *Proteus mirabilis*, *Proteus rettgeri*, and *Providencia* species demonstrated a relative resistance to cinoxacin. All *P. morganii* and *P. vulgaris* strains were inhibited by 4 μg/ml in contrast to 67 and 0% for *Proteus rettgeri* and *Providencia* species, respectively. Only 12 of 210 pseudomonas isolates had MIC values of 64 μg/ml or less. However, 43 of the 57 non-*Enterobacteriaceae*, nonpseudomonas gram-negative isolates had MIC values of 64 μg/ml or less.

The data in Table 2 confirm the poor cinoxacin antimicrobial activity against gram-positive organisms. Of note, however, was the 26 to 28% inhibitory response of staphylococcus isolates. Only 2 of 191 streptococcus strains had MIC values of 64 μg/ml or less.

The comparison of cinoxacin and nalidixic acid MICs and MBCs is shown in Table 3. The six organism species tested represent six of the

TABLE 1. *Susceptibility (MICs) of clinical isolates of gram-negative bacilli to cinoxacin*

Organism	No. of isolates	Cumulative % susceptible at MIC of:						
		1.0	2	4	8	16	32	64
Escherichia coli	1,383	25	78	96	99	99	99	99
Citrobacter freundii	28		25	61	97	100		
C. diversus	11	27	73	91	100			
Klebsiella pneumoniae	166	1	25	81	99	100		
Enterobacter cloacae	50	6	24	72	88	96		98
E. aerogenes	17		6	65	88	94	100	
E. agglomerans	15	27	47	73	100			
Serratia marcescens	11	18		73	100			
Proteus mirabilis	123	1	9	30	76	87	89	90
P. morganii	13	77	100					
P. rettgeri	12	17	34	67	73	75	92	100
P. vulgaris	5	20	80	100				
Providencia species	6					50	83	100
Pseudomonas aeruginosa	197		1	1	2			3
Pseudomonas species[a]	13	8					16	44
Acinetobacter anitratus (Herellea)	25				4	8	20	64
Acinetobacter lwoffi	9				11	33	55	100
Moraxella species	9	22		44			88	
Others[b]	46	30	74	87			89	91

[a] Includes *Pseudomonas maltophilia* (6), *P. stutzeri* (3), *P. cepacia* (2), and *Pseudomonas* species (2).

[b] Includes *Pasteurella multocida* (4), *Aeromonas hydrophilia* (3), enteropathogenic *E. coli* (3), *Shigella* species (22), *Salmonella enteritidis* (7), and one strain each of EF4, Ve type 2, Vd, 11K type 1, *Achromobacter xylosoxidans*, *Flavobacterium* species, and gram-negative bacillus NOS.

TABLE 2. *Susceptibility (MICs) of clinical isolates of gram-positive cocci to cinoxacin*

Organism	No. of isolates	Cumulative % susceptible at MIC of:						
		1	2	4	8	16	32	64
Staphylococcus aureus	415					1	3	26
S. epidermidis	223					1	3	28
Streptococcus faecalis	172							1
Streptococcus[a] group D (not *S. faecalis*)	7							
Streptococcus, beta-hemolytic[b]	4							
Streptococcus, viridans group	8							13

[a] Includes *S. faecium* (5), *S. durans* (1), and *S. bovis* (1).

[b] Includes *S. agalacteae* (1) and beta streptococcus not group A, B, or D (3).

eight more frequently encountered urinary tract pathogens (*Staphylococcus epidermidis* and *Enterobacter* species not included). In direct comparison, cinoxacin was slightly more active than nalidixic acid against the group.

The effect of the inoculum size on the cinoxacin MIC results is shown in Table 4. Slight increases in the mean MIC values were demonstrated for all tested species with increasing inoculum concentrations. However, four- to eightfold increase in MBCs was found for both cinoxacin and nalidixic acid with an inoculum size of 10^7 organisms/ml (not shown). At the highest concentration tested (64 μg/ml), 87% of the 1,621 urinary tract isolates were inhibited by cinoxacin. This concentration was considerably below the 250- to 500-μg/ml concentration of drug easily reached in the urine on usual dosages (6). With a higher testing range, the percentage of organisms inhibited may approach those of nitrofurantoin (94% inhibited at 128 μg/ml) and trimethoprim/sulfamethoxazole (98% inhibited at 4/76 μg/ml). If the gram-positive urinary microbes were eliminated, the cinoxacin efficacy of 96% at 64 μg/ml was comparable to that of the other two antimicrobial agents tested in parallel.

Cinoxacin appears to have definite advantages over nalidixic acid, including higher serum and urine concentrations, expanded *Enterobacteriaceae* antimicrobial activity, and a greater homogeneity of susceptible bacterial populations (8). More studies are needed to clarify the significance of antagonizing factors

TABLE 3. *Comparison of MICs and MBCs of cinoxacin and nalidixic acid for six prevalent urinary tract pathogens*

Organism	No. of isolates	Antibiotic[a]	MIC or MBC	Cumulative % susceptible at concn (μg/ml) of:						
				1	2	4	8	16	32	64
Escherichia coli	25	CX	MIC	8	72	92	100			
			MBC	4	44	88	100			
		NA	MIC	16	76	88	100			
			MBC	12	68	88	100			
Klebsiella pneumoniae	25	CX	MIC			76	96	100		
			MBC			68	92	100		
		NA	MIC			42	88	100		
			MBC			32	84	100		
Proteus mirabilis	25	CX	MIC		12	84	92		96	100
			MBC		4	84	92			100
		NA	MIC			56	100			
			MBC			48	96	100		
Indole-positive *Proteus* species[b]	10	CX	MIC	20	90	100				
			MBC		80	100				
		NA	MIC	10	60	70	90	100		
			MBC	10	40	70	90	100		
Pseudomonas aeruginosa	10	CX	MIC							20
			MBC							10
		NA	MIC						30	
			MBC							20
Streptococcus faecalis	25	CX	MIC							
			MBC							
		NA	MIC							
			MBC							

[a] CX, Cinoxacin, NA, nalidixic acid.
[b] Includes *P. morganii* (4), *P. rettgeri* (4), and *P. vulgaris* (2).

TABLE 4. *Effect of inoculum size on cinoxacin in MICs for prevalent urinary tract pathogens*

Organism (no.)	Inoculum size	No. of isolates with MIC at:							
		≤1	2	4	8	16	32	64	>64
Escherichia coli (10)	10^3	1	6	3					
	10^5	1	5	2	2				
	10^7		6	2	2				
Klebsiella pneumoniae (10)	10^3			9		1			
	10^5			8	1	1			
	10^7			7	2	1			
Proteus mirabilis (10)	10^3		6	2			2		
	10^5		2	5	1		1	1	
	10^7		1	5	2		1	1	
Indole-positive *Proteus* species[a] (10)	10^3	5	5						
	10^5	2	7	1					
	10^7	2	6	2					
Pseudomonas aeruginosa (10)	10^3							2	8
	10^5							2	8
	10^7								10
Streptococcus faecalis (10)	10^3							1	9
	10^5								10
	10^7								10

[a] Indole-positive strains: *P. morganii* (4), *P. rettgeri* (4), and *P. vulgaris* (2).

(8), effects of high cinoxacin concentration on heretofore resistant strains, and in vivo utility in urinary and non-urinary infection.

ACKNOWLEDGMENTS

We thank Eli Lilly & Co. for the financial grant to investigate cinoxacin.

Additional appreciation is given to Rollie Packer, MT(ASCP), for technical help.

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