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Influence of Medium and Method on the In Vitro Susceptibility of *Pseudomonas aeruginosa* and Other Bacteria to Ciprofloxacin and Enoxacin

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Ciprofloxacin and enoxacin were two- to fourfold less active against *Pseudomonas aeruginosa* in calcium- and magnesium-supplemented broth compared with unsupplemented broth regardless of inoculum size, presence of serum, or use of inhibitory or bactericidal endpoints ($P < 0.01$). The effect of cation supplementation was less pronounced and less consistent for *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*.

Several new fluorinated piperazinyl quinolone compounds have been shown to have potent antibacterial effects, most notably against gram-negative bacilli (7). However, available pharmacokinetic and microbiological data suggest that the differences between achievable serum or tissue concentrations in vivo and inhibitory or bactericidal concentrations for certain pathogens (e.g., *Pseudomonas aeruginosa*) may be small. Therefore, relatively small changes in activity found in tests performed by standardized methods in media that more accurately reflect in vivo conditions could be important. The objective of this study was to evaluate the in vitro activities of two new quinolone agents, ciprofloxacin (Miles Pharmaceuticals, West Haven, Conn.) and enoxacin (Warner-Lambert Pharmaceuticals, Ann Arbor, Mich.) in four different media using standardized susceptibility-testing methods.

Broth dilution studies were conducted by the microtechnique with two inoculum sizes (8, 11). Three liquid media were used: Mueller-Hinton broth (MHB; Difco Laboratories, Detroit, Mich.), MHB supplemented with calcium (2.5 mM/liter) and magnesium (1.1 mM/liter) (MHB-S), and MHB-S combined 1:1 with fresh heat-inactivated human serum (MHB-S/HS) (18). The MBC was read as the lowest concentration of drug that resulted in 99.9% reduction of the initial inoculum size after transfer of 10 μl of media from clear wells and incubation for 18 to 24 h (13). The activities of ciprofloxacin and enoxacin were also determined by agar dilution with Mueller-Hinton agar (MHA). Inocula were applied to the surface of MHA containing ciprofloxacin or enoxacin in 10-μl drops. The geometric mean $\log_{10}$ (± the standard deviation) inoculum sizes used in tests on MHA were 5.9 (±0.4) and 3.9 (±0.4) CFU per spot and 7.2 (±0.4) and 5.1 (±0.3) CFU/ml in liquid media. Eight clinical isolates and an American Type Culture Collection control strain of *P. aeruginosa* were studied. The strains of *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* studied each included an American Type Culture Collection control strain and two clinical isolates. All *S. aureus* strains were susceptible to methicillin. All *S. aureus* were tested in triplicate, and over 1/3 of other isolates were tested in duplicate in each medium; the variation was ±1 twofold dilution (95% confidence interval). The MIC and MBC ($\log_2$ transformed values) in each medium, the magnitude of increase in MIC or MBC, and the ratios of MBC to MIC for each drug in MHB-S and MHB-S/HS were compared with data obtained in MHB by using the Wilcoxon signed-rank test for matched pairs. Differences were considered significant at the $P < 0.05$ level for a two-tailed test.

The activities of ciprofloxacin and enoxacin against nine strains of *P. aeruginosa* in the four media tested are summarized in Table 1. Cation supplementation of MHB with or without human serum significantly reduced the activities of both drugs ($P < 0.01$). MICs and MBCs showed a median two- to fourfold increase at both inoculum sizes tested. The magnitudes of reduction of activity in supplemented broth were similar for ciprofloxacin and enoxacin ($P > 0.05$). The median MICs obtained by the agar dilution technique were two times higher than in MHB for ciprofloxacin ($P < 0.05$), whereas no significant difference was found with enoxacin (Table 1).

Both quinolones were highly active against non-*Pseudomonas* strains, with all strains inhibited by 1 μg of ciprofloxacin and 2 μg of enoxacin per ml. The results obtained with the laboratory reference strains when tested with the larger inocula are presented in Table 2. Compared with *P. aeruginosa*, the effect of cation supplementation on the activities of the quinolones was quantitatively less pronounced and less consistent among the nine strains of *E. coli*, *K. pneumoniae*, and *S. aureus*. With the three strains of *E. coli* tested, the MICs determined the MHB-S were consistently two times higher than in unsupplemented MHB for both drugs and both inoculum sizes; however, this increase was not observed in the presence of serum. The activities of the quinolones against *K. pneumoniae* and *S. aureus* were not substantially affected by cation supplementation. Compared with the respective median MICs and MBCs determined in MHB, there was either no change or only a twofold change in MHB-S, MHB-S/HS, or MHA.

Both drugs appeared to be bactericidal against all bacteria in the three liquid media; on the average, the MBC was only one twofold dilution (range, 0 to 4) higher than the MIC. There was no effect of media on the ratio of MBC to MIC for ciprofloxacin or enoxacin ($P > 0.05$).

The activities of ciprofloxacin and enoxacin against all species were not substantially influenced by a 100-fold increase in inoculum size (Table 1). The median MIC (or

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TABLE 1. Activities of ciprofloxacin and enoxacin in MHB and on MHA and effect of cation supplementation with (MHB-S/HS) or without (MHB-S) human serum against strains of *P. aeruginosa*

Inoculum size and drug	Media (range) MIC or MBC (μg/ml) in MHB		Median fold increase (range) in MIC or MBC (μg/ml) compared with MHB in:				
	MIC	MBC	MHB-S[a]		MHB-S/HS[a]		MHA
			MIC	MBC	MIC	MBC	MIC
~10^7 CFU/ml							
Ciprofloxacin	0.25 (0.125–1)	0.5 (0.125–1)	2 (1–8)	4 (2–16)	2 (0.5–4)	2 (1–8)	2 (0.5–8)[b]
Enoxacin	1 (0.5–4)	4 (1–8)	4 (2–4)	2 (1–8)	4 (1–8)	2 (0.25–4)	2 (0.5–2)[c]
~10^5 CFU/ml							
Ciprofloxacin	0.125 (0.03–0.5)		4 (2–4)		4 (1–4)		2 (0.5–8)[b]
Enoxacin	1 (0.25–4)		2 (2–4)		2 (1–8)		1 (0.5–4)[c]

[a] The decrease in activity was statistically significant ($P < 0.05$) compared with activity in MHB for all comparisons.
[b] The decrease in activity was statistically significant ($P < 0.05$) compared with activity in MHB.
[c] The decrease in activity was not statistically significant ($P > 0.05$) compared with activity in MHB.

TABLE 2. In vitro activity of ciprofloxacin (C) and enoxacin (E) against four laboratory reference strains in four media tested at inoculum sizes of 10^7 CFU/ml in liquid media and 10^6 CFU per spot on MHA

Organism	Drug	MIC (MBC) (μg/ml) with:			
		MHB	MHB-S	MHB-S/HS	MHA
P. aeruginosa ATCC 27853	C	0.5 (1)	1 (2)	1 (1)	2
	E	4 (4)	8 (8)	4 (8)	8
E. coli ATCC 25922	C	0.008 (0.008)	0.016 (0.016)	0.008 (0.008)	0.016
	E	0.062 (0.062)	0.125 (0.125)	0.125 (0.125)	0.062
K. pneumoniae ATCC 13883	C	0.031 (0.031)	0.062 (0.062)	0.008 (0.016)	0.062
	E	0.25 (0.5)	0.25 (0.5)	0.125 (0.125)	0.25
S. aureus ATCC 29213	C	0.25 (0.25)	0.5 (0.5)	1 (2)	0.5
	E	1 (1)	1 (2)	2 (2)	1

MBC; data not shown) was only one twofold dilution higher (range 0 to 3) with the larger inoculum in each of the four media.

Reduction of antibiotic activity against *P. aeruginosa* with magnesium and calcium supplementation has been reported for various drugs, such as aminoglycosides (10, 16), polymyxins (6, 12), and ceftazidime (5). The results of the present study demonstrate that magnesium and calcium supplementation of MHB also results in statistically significant increases in the MICs and MBCs of two new quinolones against *P. aeruginosa*. However, this increase appears to be lower than that observed with aminoglycosides (10, 16).

It has been shown that the activities of ciprofloxacin and enoxacin against aerobic gram-positive and gram-negative bacteria are decreased in urine and at pH <7 (2–4, 14, 15). Reeves et al. (14) and Ratcliffe and Smith (N. T. Ratcliffe and J. T. Smith, 4th Mediterranean Congress of Chemotherapy, abstr. no. 608, 1984) have suggested that reduction of quinolone activity in urine may be due to high urinary concentrations of magnesium. Our data show that magnesium and calcium concentrations similar to that observed in serum also may result in alterations in the activities of ciprofloxacin and enoxacin, particularly against *P. aeruginosa*. In contrast to the data of Ratcliffe and Smith, we did not note a disproportionate rise in MIC and MBC in cation-supplemented media.

The presence of serum or a 100-fold increase in inoculum size had little effect on the in vitro activities of ciprofloxacin and enoxacin against most major pathogens, which is consistent with previous reports (2, 4, 9, 14, 15).

The reduction of in vitro activity in cation-supplemented broth may be of consideration in the development of standards for in vitro susceptibility testing of quinolones against bacteria usually inhibited at or near the susceptibility breakpoint (e.g., *P. aeruginosa*). For example, using the preliminary breakpoint for susceptibility of an MIC ≤ 4 μg/ml for enoxacin (17), all nine strains of *P. aeruginosa* tested with an inoculum of 10^5 would be considered susceptible in MHB; however, only seven of these strains would be considered susceptible in MHB-S. In contrast, all strains would be considered susceptible (1) to ciprofloxacin (MIC ≤ 1 μg/ml) in all of the media tested. Further studies correlating the in vitro susceptibility patterns of bacteria in unsupplemented and cation-supplemented media with clinical results are required to determine the role of medium supplementation in predictions of in vivo efficacy in the treatment of infections due to *P. aeruginosa* with these new quinolones.

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