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Temafloxacin Disk Potency and Tentative Interpretive Criteria for Susceptibility Tests

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Temafloxacin disk susceptibility test criteria were evaluated by testing 697 bacterial isolates. Either 5- or 10-μg disks could be used satisfactorily. A 5-μg temafloxacin disk with zone size breakpoints of ≤12 mm for resistance (MIC, >4.0 μg/ml) and ≥16 mm for susceptibility (MIC, ≤2.0 μg/ml) is recommended.

Temafloxacin is a difluoroquinolone antimicrobial agent that is being evaluated in clinical trials. Temafloxacin has been known as A-62254, which is the hydrochloride salt of A-63004. Temafloxacin is structurally similar to the aryl quinolone difloxacin (A-56619), but temafloxacin is two to four times more potent (2, 9). The spectrum of antibacterial activity of temafloxacin has been found to be similar to that of ofloxacin, but temafloxacin is slightly less potent (2, 3). In an experimental mouse model (9), oral administration of temafloxacin has been reported to be effective in treating mice challenged with different enteric bacilli, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and different streptococci. Peak levels in mouse serum were six times higher than those obtained with comparable doses of ciprofloxacin but lower than those observed with difloxacin (9).

In 36 normal healthy human volunteers, an average peak level in serum of 6.9 μg/ml was observed after 600-mg orally administered doses, i.e., the twice-daily dosage that is used for treating respiratory tract or skin and soft tissue infections (D. J. Hardy, personal communication). On the basis of such human pharmacokinetic data, it seems reasonable to categorize bacteria with MICs of ≤2.0 μg/ml as being susceptible and those with MICs of >4.0 μg/ml as being resistant. These are the tentative MIC breakpoints that have been used for interpreting in vitro test results with most other fluoroquinolone agents. Such breakpoints should be considered tentative until clinical experience confirms their appropriateness or suggests that modifications need to be made.

To help guide the selection of patients who are entered into ongoing clinical studies, zone size interpretive criteria for disk susceptibility tests are needed. In this report, we describe early in vitro studies that were carried out to select the most appropriate disk content for temafloxacin disks and to propose zone size interpretive standards.

Temafloxacin was provided by Abbott Laboratories (North Chicago, Ill.), as were 5- and 10-μg temafloxacin disks. Broth microdilution and disk diffusion susceptibility tests were performed by the procedures recommended by the National Committee for Clinical Laboratory Standards (10, 11). For the broth dilution tests, Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) was supplemented with 50 mg of calcium and 25 mg of magnesium per liter (10). If needed for growth of the species being tested, 2 to 3% lysed horse blood was added to the microdilution trays and 5% defibrinated sheep blood was added to the agar medium for disk tests. MICs were recorded after 16 to 18 h of incubation at 35°C in ambient air. Tests were performed with 697 bacterial isolates, including 263 members of the family *Enterobacteriaceae* which were also tested against nalidixic acid.

Table 1 includes data accumulated with the enteric bacilli and evaluates cross-resistance and cross-susceptibility to temafloxacin and nalidixic acid. Only 8 of the 263 isolates (3%) were resistant to temafloxacin (MIC, >4.0 μg/ml), and all of those strains were also very resistant to nalidixic acid (MIC, >128 μg/ml). Eighteen other strains were highly resistant to nalidixic acid but were not resistant to temafloxacin. All of the nalidixic acid-susceptible isolates were also susceptible to temafloxacin. Nalidixic acid-resistant enteric bacilli tend to have increased MICs of temafloxacin. In vitro tests with one agent cannot be used to predict susceptibility or resistance to the other.

The results of in vitro studies with temafloxacin are summarized in Table 2. Nalidixic acid-resistant enteric bacilli were similar to *P. aeruginosa* in their susceptibility to temafloxacin (geometric mean MICs, 2.1 and 1.4 μg/ml, respectively). Nalidixic acid-susceptible enteric bacilli and *Acinetobacter* spp. were much more susceptible to temafloxacin (geometric mean MICs, 0.15 and 0.13 μg/ml, respectively). Staphylococci, including methicillin-resistant strains, were very susceptible to temafloxacin (geometric mean MICs, 0.13 to 0.18 μg/ml). For enterococci and *Listeria* spp., mean MICs were about two times higher than those for streptococci (1.4 and 1.1 versus 0.56 μg/ml).

Table 2 also describes the range of zone diameters with 5- and 10-μg temafloxacin disks. The zone diameters were directly compared, and the following regression formula was

TABLE 1. Cross-resistance and cross-susceptibility to temafloxacin and nalidixic acid among 263 members of the family *Enterobacteriaceae*[a]

Nalidixic acid MIC (μg/ml) categories[b]	Total no. of isolates	No. in each temafloxacin MIC category			Geometric mean MIC (μg/ml)
		>4.0 μg/ml (R)	4.0 μg/ml (I)	≤2.0 μg/ml (S)	
>128 (R)	26	8	4	14	2.9
32–128 (R)	8	0	1	7	0.84
16 (I or S)	2	0	1	1	—[c]
≤8.0 (S)	227	0	0	227	0.14

[a] Includes 12 *Citrobacter* spp., 22 *Escherichia coli*, 2 *Cedecea lapagei*, 62 *Enterobacter* spp., 25 *Klebsiella* spp., 33 *Serratia* spp., 31 *Proteus* spp., 10 *Morganella morganii*, 33 *Providencia* spp., 10 *Salmonella enteritidis*, 13 *Shigella* spp., and 10 *Yersinia enterocolitica* isolates.

[b] R, Resistant; I, intermediate; S, susceptible.

[c] —, Geometric mean MICs were not calculated for two temafloxacin MICs (0.5 and 4.0 μg/ml).

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TABLE 2. Temafloxacin susceptibility test results with 697 microorganisms that were used for evaluating 5- and 10-μg disk tests

Microorganism (no. of isolates tested)	MIC (μg/ml) range[a] (geometric mean)	Zone diam (mm) range[a] (mean)	
		5-μg disks	10-μg disks
Enterobacteriaceae			
Nalidixic acid susceptible (229)[b]	≤0.03–4.0 (0.15)	15–32 (24.4)	20–37 (27.2)
Nalidixic acid resistant (34)	0.12–>16 (2.1)	6–26 (13.8)	8–30 (17.5)
Pseudomonas species			
P. aeruginosa (30)	0.5–8.0 (1.4)	10–24 (19.0)	17–30 (23.6)
P. maltophilia (12)	0.25–8.0 (2.2)	10–33 (21.2)	17–37 (26.8)
Other species (15)[c]	0.06–16 (0.57)	6–33 (23.3)	15–37 (28.1)
Acinetobacter calcoaceticus (14)	0.06–0.5 (0.13)	19–27 (23.8)	21–28 (25.5)
Other gram-negative bacilli (10)[d]	0.12–>16 (2.1)	6–29 (15.4)	6–31 (18.3)
Branhamella catarrhalis (20)	≤0.03 (≤0.03)	28–37 (31.6)	30–40 (32.6)
Staphylococcus species			
Methicillin-susceptible *S. aureus* (100)	≤0.03–2.0 (0.13)	20–32 (26.5)	21–37 (28.8)
Other species (40)[e]	0.06–0.5 (0.18)	24–32 (33.2)	27–35 (31.1)
Methicillin resistant (30)[f]	0.06–0.25 (0.16)	21–32 (26.2)	24–37 (28.6)
Enterococcus species (20)[g]	0.5–8.0 (1.4)	8–4 (16.8)	13–28 (21.3)
Streptococcus species (90)[h]	0.12–2.0 (0.56)	14–32 (21.0)	18–36 (24.4)
Listeria monocytogenes (10)	0.5–2.0 (1.1)	16–22 (20.2)	22–27 (25.7)
Corynebacterium jekeium (10)	0.5–1.0 (0.62)	25–29 (26.5)	28–32 (29.6)
Bacillus species (10)	0.06–0.5 (0.14)	18–25 (21.1)	19–28 (22.6)

[a] Minimum and maximum MICs or zone diameters. Only one value is listed when all MICs were the same.
[b] Nalidixic acid MIC, ≤16 μg/ml (Table 1).
[c] Includes six *P. cepacia*, three *P. stutzeri*, two *P. fluorescens*, two *P. putida*, and two *P. acidovorans* isolates.
[d] Includes six *Achromobacter xylosoxidans*, two *Aeromonas* spp., and two *Flavobacterium* spp. isolates.
[e] Includes 4 *S. haemolyticus*, 4 *S. hominis*, 4 *S. simulans*, 4 *S. saprophyticus*, 4 *S. warneri*, and 20 unidentified species isolates.
[f] Includes 20 *S. aureus* and 10 coagulase-negative species isolates.
[g] Includes 10 *E. faecalis*, 6 *E. faecium*, 2 *E. durans*, and 2 *E. hirae* isolates.
[h] Includes 20 *S. agalactiae*, 20 *S. pyogenes*, 10 *S. bovis*, 20 *S. pneumoniae* (10 penicillin-resistant strains), and 10 serogroup C and 10 serogroup G streptococcal isolates.

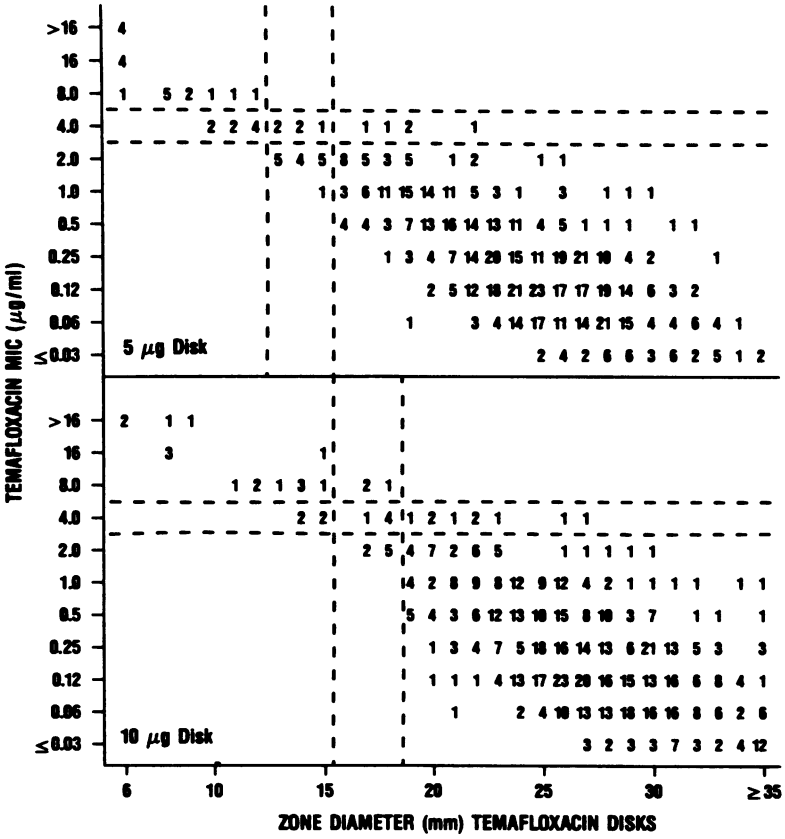

FIG. 1. Correlation of temafloxacin MICs and zones of inhibition around 5- and 10-μg temafloxacin disks. The broken lines represent tentative interpretive breakpoints.

TABLE 3. Proposed disk test criteria for six different fluoroquinolone compounds with the same MIC breakpoints[a]

Antimicrobial agent and reference	Zone size (mm) interpretive criteria			
	5-μg disk		10-μg disk	
	Resistance	Susceptibility	Resistance	Susceptibility
Temafloxacin[b]	≤12	≥16	≤15	≥19
Ofloxacin (5, 8)	≤12	≥16		
Fleroxacin (7)	≤15	≥19		
Pefloxacin (6)	≤15	≥19		
Enoxacin (4)			≤14	≥19
Amifloxacin (1)			≤16	≥20

[a] Includes quinolones with MIC breakpoints of ≤2.0 μg/ml for susceptibility and >4.0 μg/ml for resistance.

[b] Temafloxacin interpretive criteria are those determined for the current data base.

calculated: $y = 3.65 + 1.02x$ ($r = 0.95$). The average zone around 10-μg disks was 26.3 mm (6 to 41 mm), whereas the zones around 5-μg disks averaged 23.3 mm (6 to 37 mm). With the majority of strains, there was a 3-mm difference in the zone sizes for the two disk potencies. Unusually large zones were uncommon, i.e., 90% of the zones around 5-μg disks were ≤29 mm in diameter, and with 10-μg disks, 90% of the strains gave zones of ≤31 mm. These data were also examined to evaluate the ability of the two disks to discriminate between resistant and susceptible populations. Figure 1 displays scattergrams which compare MICs with concomitantly determined zone diameters for each of the 697 isolates. Zone size interpretive criteria for the susceptible category were ≥19 mm for 10-μg disks and ≥16 mm for 5-μg disks. In both cases, a 3-mm range of zone sizes included the intermediate category. With those interpretive criteria, there were no false-resistance or false-susceptibility disk tests with either disk potency. With the 5-μg disk, there were 28 (4%) minor discrepancies (intermediate with one method but resistant or susceptible with the other method). With the 10-μg disk, there were 23 (3.3%) minor discrepancies. Either disk potency could be used with equal confidence.

Since the two disk potencies were equally effective in terms of their abilities to separate susceptible and resistant populations, we compared criteria for the two temafloxacin disks with those that have been developed for similar fluoroquinolone compounds (1, 4–8). In vitro test criteria for two chemically related drugs may differ if the agents diffuse at different rates or if there are significant differences in anticipated concentrations in serum or tissue. In most cases, those two factors are essentially identical, and similar interpretive criteria and disk potencies can be used for both drugs in order to avoid creating an artificial advantage for one drug over the other. Table 3 lists the interpretive criteria that we determined for the two temafloxacin disk potencies. These criteria are contrasted with the disk contents and zone size criteria that have been independently determined for other quinolone agents with the same MIC breakpoints (MIC, ≤2.0 μg/ml for susceptibility). Amifloxacin (1) and enoxacin (4) disks contain 10 μg of drug, and susceptibility breakpoints are ≥19 or ≥20 mm. We identified similar breakpoints for tests which use 10-μg temafloxacin disks. The use of 5-μg disks has been recommended for testing pefloxacin (6), fleroxacin (7), and ofloxacin (5, 8). Zone size breakpoints for pefloxacin and fleroxacin disks are ≤15 and ≥19 mm, whereas 3-mm-smaller breakpoints (≤12 and ≥16 mm) have been described for tests with 5-μg ofloxacin and temafloxacin disks.

Because temafloxacin and ofloxacin have very similar spectra of activity (2, 3), it seems reasonable to select the same disk potency for both agents. Identical zone size interpretive criteria apply to the two disks. Unfortunately, the possibility of using a 10-μg ofloxacin disk has not been seriously considered (8). The disk potency and zone size criteria for ofloxacin and temafloxacin should be kept the same unless there is some overwhelming reason to apply different criteria. We are not aware of any reason to assume that the two agents will differ in their in vitro characteristics or clinical applications.

In summary, we recommend that a 5-μg temafloxacin disk be used with zone size breakpoints of ≤12 mm for resistance and ≥16 mm for susceptibility. A 10-μg temafloxacin disk with appropriately adjusted zone size breakpoints would also perform satisfactorily, but at this time there is no obvious reason to consider use of the more potent disk.

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