REPRINT

ISSN 1120-009X

Volume 17, Number 1
February 2005

Journal of Chemotherapy

Interpretive Criteria for Disk Diffusion Susceptibility Testing of Ulifloxacin, the Active Metabolite of Prulifloxacin

E.S.I.F.T. srl - Firenze

Journal of Chemotherapy Vol. 17 - n. 2 (138-142) - 2005

Interpretive Criteria for Disk Diffusion Susceptibility Testing of Ulifloxacin, the Active Metabolite of Prulifloxacin

M.P. MONTANARI [1] - L. FERRANTE [1] - E. TILI [1] - I. COCHETTI [1] - V. ROSSI [2] - P.E. VARALDO [1]

[1] Institute of Microbiology and Biomedical Sciences, Marche Polytechnic University Medical School, Ancona, Italy.
[2] ACRAF SpA, Pomezia (Roma), Italy.

Corresponding author: Pietro E. Varaldo, M.D., Institute of Microbiology and Biomedical Sciences, Marche Polytechnic University Medical School, Via Ranieri, Monte d'Ago, 60131 Ancona, Italy. Tel. +39071.2204694; Fax +39071.2204693; E-mail: pe.varaldo@univpm.it

Summary

Prulifloxacin, a new fluoroquinolone, is a prodrug whose active compound, ulifloxacin, is derived from its transformation after oral administration and intestinal absorption. Based on early pharmacokinetic and pharmacodynamic data, the following MIC breakpoints have tentatively been proposed: ≤1 µg/ml, susceptible; 2 µg/ml, intermediate; and ≥4 µg/ml, resistant. In this report, ulifloxacin MIC vs. zone diameter scattergrams and discrepancy rates were analyzed in 461 freshly isolated clinical strains (237 *Enterobacteriaceae*, 101 nonfermenters, and 123 Gram-positive bacteria). In agreement with the guidelines of the National Committee for Clinical Laboratory Standards, a modification of the error rate-bounded method was used to select disk diffusion test breakpoints. The following zone diameter breakpoints were chosen and are proposed herein for the interpretation of ulifloxacin disk (5 µg) test results: ≤15 and ≥19 mm for *Enterobacteriaceae*, ≤16 and ≥20 mm for nonfermenters, and ≤14 and ≥18 mm for Gram-positive bacteria. By applying these breakpoint values, no very major errors were detected, while major and minor errors were largely below the accepted discrepancy rates.

Key words: **Prulifloxacin, ulifloxacin, quinolone antibiotics, fluoroquinolones, breakpoints, error rate-bounded method, interpretive criteria, discrepancy rates, disk diffusion susceptibility testing.**

INTRODUCTION

Ulifloxacin, a fluoroquinolone formerly called NM394 [1-3] or AF 3013 [5], is the active compound derived from the transformation of the prodrug prulifloxacin (also called NM441 or AF 3012) after its oral administration and intestinal absorption. Its broad spectrum of activity covers enterobacteria, nonfermenters, enteropathogenic bacteria (including *Campylobacter jejuni*), respiratory pathogens (*Haemophilus influenzae*, *Moraxella catarrhalis*, β-haemolytic streptococci), and methicillin-susceptible staphylococci [1-6]. Compared with ciprofloxacin, its activity (MIC and MBC tests) is similar or greater against gram-positive bacteria and greater against gram-negative bacteria [5]. Very recently, the microbiological and pharmacological profile of this new fluoroquinolone and its therapeutic efficacy have been reviewed [7].

At present, prulifloxacin is going to be marketed in Italy and other European countries. The introduction of new antimicrobial agents necessitates the development of appropriate methods for the *in vitro* recognition of microorganisms that may not respond to treatment. This includes the optimization and standardization of susceptibility tests and the estab-

ISSN 1120-009X

lishment of interpretive criteria for MIC and disk diffusion methods.

Following once daily oral administration of prulifloxacin (600 mg), peak serum concentrations at steady state reach about 2 μg/ml, and the serum elimination half-life is approximately 12 h [3,8,9]. MIC interpretive criteria have yet to be established for ulifloxacin, but based on early pharmacokinetic and pharmacodynamic data [3,8,9] and the breakpoints proposed by manufacturer [10], microorganisms for which the MIC is ≤1 μg/ml could be considered tentatively susceptible; those for which the MIC is ≥4 μg/ml could be categorized as resistant; and those for which the MIC is 2 μg/ml could be classified as intermediate. These tentative interpretive MIC breakpoints might be confirmed or altered after additional clinical studies will be completed. However, before that can happen, susceptibility testing criteria are needed in order to enroll patients into the clinical trials.

In this report, disk diffusion susceptibility tests with 5-μg ulifloxacin disks were evaluated. Zone diameters corresponding to the tentative MIC breakpoints noted above were selected as provisional interpretive criteria.

MATERIALS AND METHODS

Ulifloxacin was provided by Angelini ACRAF (Pomezia, Italy), and ciprofloxacin was purchased from Sigma-Aldrich, (Milan, Italy). Ulifloxacin and ciprofloxacin disks (5 μg) were manufactured by Oxoid Italiana (Garbagnate Milanese, MI, Italy).

A total of 461 freshly isolated clinical strains, namely 237 cultures of *Enterobacteriaceae*, 101 of glucose nonfermenting Gram-negative rods, and 123 of Gram-positive bacteria, were selected in order to have an appropriate variety of clinically relevant species and a broad range of MICs.

Broth microdilution and disk diffusion tests were performed following the methods recommended by the National Committee for Clinical Laboratory Standards (NCCLS) [11,12]. Cation-adjusted Mueller-Hinton broth and Mueller-Hinton agar were used as test media, supplemented with 2-3% lysed horse blood and 5% defibrinated sheep blood, respectively, when pneumococci were tested. Quality control strains included *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *S. aureus* ATCC 25923 (disk diffusion only) *Streptococcus pneumoniae* ATCC 49619, *Enterococcus faecalis* ATCC 29212. All plates and microplates were incubated overnight at 35°C in normal atmosphere, while plates with pneumococci were incubated in 5-7% CO_2.

The NCCLS guidelines and relevant discrepancy rates [13] were essentially referred to for the development of interpretive criteria and breakpoints for ulifloxacin.

RESULTS

A comparison of the MICs of ulifloxacin and ciprofloxacin for all test strains is detailed in *Table 1*. In agreement with our previous findings [5], the *in vitro* activity of ulifloxacin resulting from MIC assays appeared to be similar to or greater than that of ciprofloxacin. Consistent and comparable results were obtained with disk diffusion tests.

MIC vs. zone diameter scattergrams for each of the three bacterial groups tested (*Enterobacteriaceae*, nonfermenters, and Gram-positive bacteria) are provided in *Figures 1* to *3*, and discrepancy rates are listed in *Table 2*. By adjusting the proposed interpretive breakpoints until the number of very major and major discrepancies were held to a minimum, the following zone diameter breakpoints were chosen: ≤15 and ≥19 mm for *Enterobacteriaceae*, ≤16 and ≥20 mm for nonfermenters, ≤14 and ≥18 mm for Gram-positive bacteria. By applying these breakpoint values, no very major errors (false-susceptible disk diffusion test results) were detected. Only three major errors (false-resistant disk tests) were found, all largely below the accepted discrepancy rates [13]: one (*Enterobacteriaceae*, ≤I - 2 category, where I is the tentative intermediate MIC value of 2 μg/ml) constituted a discrepancy rate of 0.6% vs. an accepted discrepancy rate of <40%, and two (nonfermenters, I ± 1 category) constituted a discrepancy rate of 8.7% vs. an accepted discrepancy rate of <10%. Minor errors were represented by 10 isolates among *Enterobacteriaceae*, 5 among nonfermenters, and 4 among Gram-positive bacteria (all in the I ± 1 category), yielding discrepancy rates of 30.3%, 21.7%, and 15.4%, respectively, vs. an accepted discrepancy rate of <40%.

DISCUSSION

As a rule, the susceptible and resistant breakpoints for the disk diffusion test are determined by correlating the disk diffusion inhibitory zone diameters with MICs. In the past this has often been accomplished by regression analysis. The ability of regression analysis to suggest appropriate zone diameter breakpoints is dependent upon a fairly even distribution of organisms at each MIC tested, particularly in the range of the intermediate MIC ± 2 to 3 twofold dilutions [14]. With many of the newer antibiotics, however, resistant organisms are few, and the MIC distribution is heavily weighted toward very susceptible MICs, resulting in an unreliable regression line. To overcome this problem the error rate-bounded method of Metzler and De Haan [15] as modified by Brunden *et al.* [16] can be used to select disk diffusion test breakpoints, and this method has been accepted by the NCCLS [13,17].

Prulifloxacin, by virtue of its broad antibacterial activity and considering that it can be given orally

TABLE 1 - *Comparative activities of ulifloxacin and ciprofloxacin against 461 bacterial clinical isolates.*

Organism (n. tested)	Antimicrobial agent	MIC (μg/ml)		
		Range	50%	90%
Escherichia coli (68)	Ulifloxacin	≤0.015-128	≤0.015	2
	Ciprofloxacin	≤0.015-128	≤0.015	4
Klebsiella spp. (39)	Ulifloxacin	≤0.015-64	≤0.015	2
	Ciprofloxacin	≤0.015-128	≤0.015	4
Enterobacter spp. (28)	Ulifloxacin	≤0.015-32	0.03	8
	Ciprofloxacin	≤0.015-128	0.03	32
Serratia spp. (25)	Ulifloxacin	≤0.015-32	0.03	1
	Ciprofloxacin	≤0.015-32	0.03	4
Citrobacter spp. (14)	Ulifloxacin	≤0.015-4	0.03	4
	Ciprofloxacin	≤0.015-32	0.03	8
Proteus spp. (24)	Ulifloxacin	≤0.015-128	0.06	1
	Ciprofloxacin	≤0.015-128	0.03	4
Providencia spp. (24)	Ulifloxacin	≤0.015-128	1	64
	Ciprofloxacin	≤0.015->128	0.125	128
Morganella spp. (16)	Ulifloxacin	≤0.015-128	0.03	8
	Ciprofloxacin	≤0.015-128	0.03	32
Total *Enterobacteriaceae* (237)	Ulifloxacin	≤0.015-128	0.03	16
	Ciprofloxacin	≤0.015->128	0.03	32
Pseudomonas aeruginosa (73)	Ulifloxacin	0.03-64	0.25	16
	Ciprofloxacin	0.03-128	1	64
Acinetobacter spp. (23)	Ulifloxacin	0.03-128	4	128
	Ciprofloxacin	0.03-128	4	128
Stenotrophomonas maltophilia (5)	Ulifloxacin	0.5-64		
	Ciprofloxacin	0.25-32		
Total nonfermenters (101)	Ulifloxacin	0.03-128	1	64
	Ciprofloxacin	0.03-128	1	64
Staphylococcus aureus, methicillin susceptible (35)	Ulifloxacin	0.06-1	0.125	0.5
	Ciprofloxacin	0.12-1	0.25	0.5
Staphylococcus aureus, methicillin resistant (37)	Ulifloxacin	0.12-128	8	64
	Ciprofloxacin	0.25->128	16	64
Coagulase negative staphylococci (17)	Ulifloxacin	0.06-64	0.06	1
	Ciprofloxacin	≤0.015-128	0.03	4
Enterococcus spp. (17)	Ulifloxacin	0.25-128	1	32
	Ciprofloxacin	0.25-128	1	64
Streptococcus pneumoniae (17)	Ulifloxacin	0.25-16	1	2
	Ciprofloxacin	0.25-32	1	8
Total Gram-positive bacteria (123)	Ulifloxacin	0.6-128	1	32
	Ciprofloxacin	≤0.015->128	4	64

once a day, might become an important agent to add to the physician's armamentarium. To help guide ongoing clinical trials, we propose that the breakpoints resulting from the present study (≤15 and ≥19 mm for *Enterobacteriaceae*, ≤16 and ≥20 mm for nonfermenters, ≤14 and ≥18 mm for Gram-positive bacteria) be used for interpreting ulifloxacin disk test results.

TABLE 2 - *MIC-zone diameter discrepancy rates for ulifloxacin (5-µg disks) with 461 bacterial clinical isolates.*

Organisms (proposed zone diameter breakpoints, mm)	MIC range	N. of isolates	N. of discrepancies (% discrepancy rate)		
			Very major	Major	Minor
Enterobacteriaceae	≥I + 2	32	0	NA	0
(≤15, ≥19)	I ± 1	33	0	0	10 (30.3)
	≤I - 2	172	NA	1 (0.6)	0
	Total	237	0	1 (0.4)	10 (4.2)
Nonfermenters	≥I + 2	32	0	NA	0
(≤16, ≥20)	I ± 1	23	0	2 (8.7)	5 (21.7)
	≤I - 2	46	NA	0	0
	Total	101	0	2 (1.9)	5 (4.9)
Gram-positive bacteria	≥I + 2	37	0	NA	0
(≤14, ≥18)	I ± 1	26	0	0	4 (15.4)
	≤I - 2	60	NA	0	0
	Total	123	0	0	4 (3.2)

NA, not applicable
I, tentative intermediate MIC value (2 µg/ml).

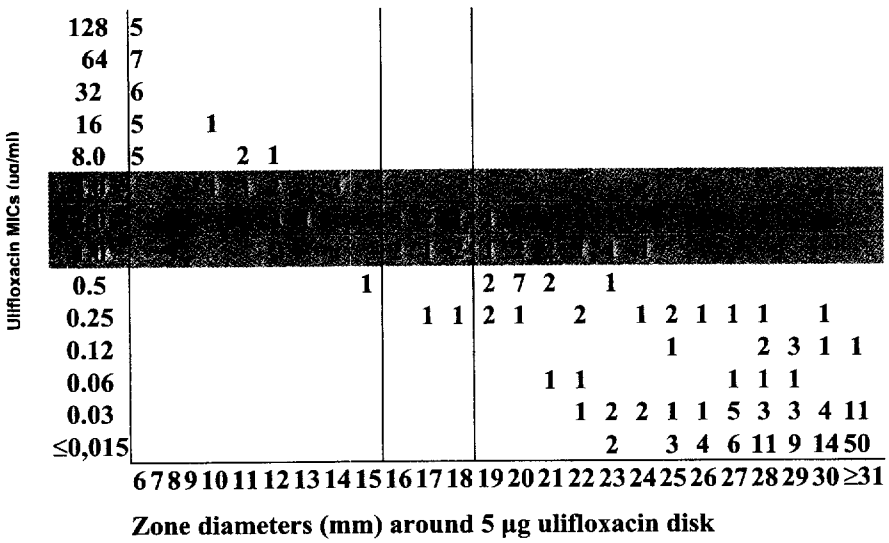

FIGURE 1 - Ulifloxacin MICs vs. zone diameters with 237 *Enterobacteriaceae* isolates. Horizontal and vertical lines represent suggested MIC breakpoints (≤1 µg/ml and ≥4 µg/ml) and best-fit zone diameter breakpoints (≤15 mm and ≥19 mm), respectively. Numbers for individual MIC-zone diameter combinations are numbers of isolates.

REFERENCES

[1] Ozaki M, Matsuda M, Tomii Y *et al.* In vitro antibacterial activity of a new quinolone, NM441. Antimicrob Agents Chemother 1991; 35 (12): 2490–2495.

[2] Ozaki M, Matsuda M, Tomii Y *et al.* In vivo evaluation of NM441, a new thiazeto-quinoline derivative. Antimicrob Agents Chemother 1991; 35 (12): 2496–2499.

[3] Nakashima M, Uematsu T, Kosuge K *et al.* Pharmacokinetics and safety of NM441, a new quinolone, in healthy male volunteers. J Clin Pharmacol 1994; 34 (9): 930–937.

[4] Yoshida T, Mitsuhashi S. Antibacterial activity of NM394, the active form of prodrug NM441, a new quinolone. Antimicrob Agents Chemother 1993; 37 (4): 793–800.

[5] Montanari MP, Mingoia M, Varaldo PE. In vitro antibacterial activities of AF 3013, the active metabolite of prulifloxacin, against nosocomial and community Italian isolates. Antimicrob Agents Chemother 2001; 45 (12): 3616-3622.

[6] Prats G, Roig C, Mirò E, Navarro F, Mirelis B. In vitro activity of the active metabolite of prulifloxacin (AF 3013) compared with six other fluoroquinolones. Eur J Clin Microbiol Infect Dis 2002; 21 (4): 328-334.

[7] Keam SJ, Perry CM. Prulifloxacin. Drugs 2004; 64 (19), 2221-2234.

[8] Fattore C, Cipolla G, Gatti G *et al.* Pharmacokinetic interactions between theophylline and prulifloxacin in healthy volunteers. Clin Drug Invest 1998; 16 (5): 387-392.

[9] Picollo R, Brion N, Gualano V *et al.* Pharmacokinetics

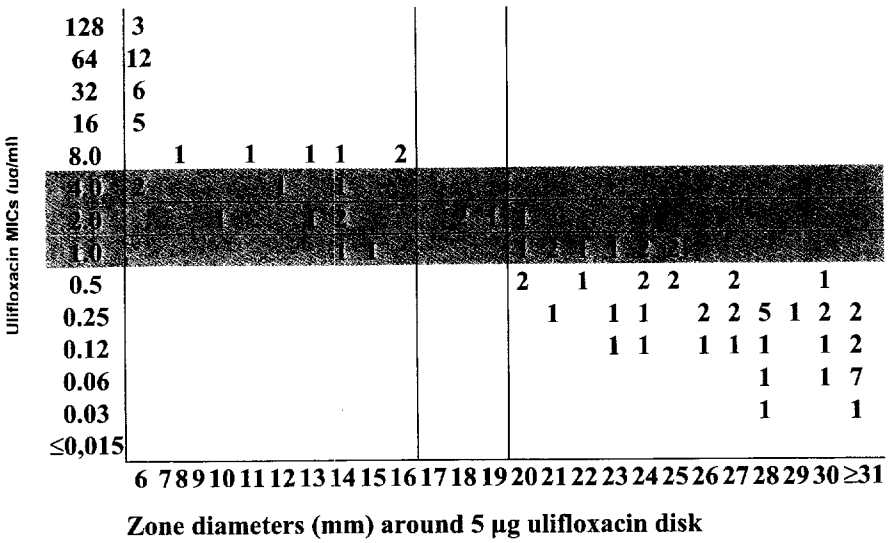

FIGURE 2 - Ulifloxacin MICs vs. zone diameters with 101 glucose nonfermenting Gram-negative isolates. Horizontal and vertical lines represent suggested MIC breakpoints (≤1 µg/ml and ≥4 µg/ml) and best-fit zone diameter breakpoints (≤16 mm and ≥20 mm), respectively. Numbers for individual MIC-zone diameter combinations are numbers of isolates.

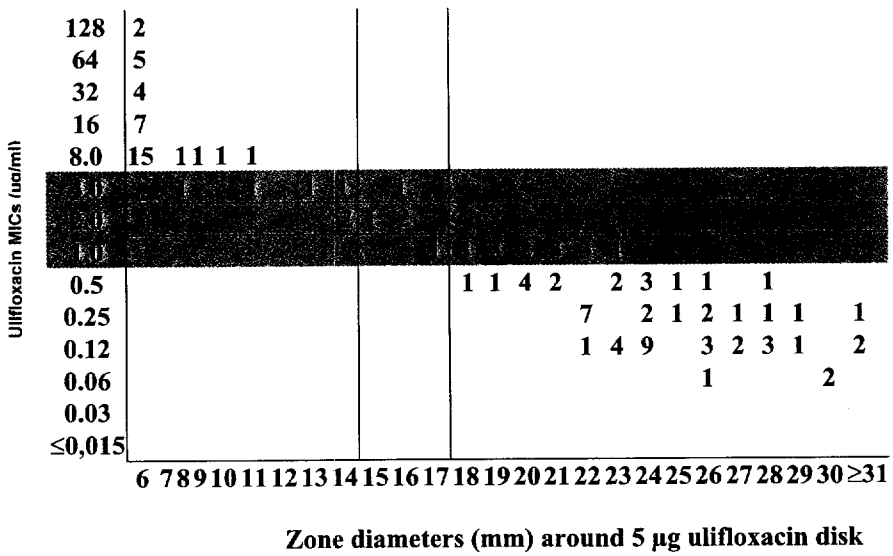

FIGURE 3 - Ulifloxacin MICs vs. zone diameters with 123 Gram-positive isolates. Horizontal and vertical lines represent suggested MIC breakpoints (≤1 µg/ml and ≥4 µg/ml) and best-fit zone diameter breakpoints (≤14 mm and ≥18 mm), respectively. Numbers for individual MIC-zone diameter combinations are numbers of isolates.

and tolerability of prulifloxacin after single oral administration. Arzneim-Forsch/Drug Res 2003; 53 (3): 201-205.

[10] Angelini ACRAF S.p.A. Investigator's brochure: Unidrox® prulifloxacin 600 mg tablets. Rome: ACRAF S.p.A. 2004.

[11] National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 6th ed. Approved standard M7-A6. Wayne, PA: NCCLS 2003.

[12] National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility tests. 8th ed. Approved standard M2-A8. Wayne, PA: NCCLS 2003.

[13] National Committee for Clinical Laboratory Standards. Development of *in vitro* susceptibility testing criteria and quality control parameters. 2nd ed. Approved standard M23-A2. Wayne, PA: NCCLS 2001.

[14] Fuchs PC, Barry AL, Brown SD. Selection of zone size interpretive criteria for disk diffusion susceptibility tests of three antibiotics against *Streptococcus pneumoniae*, using the new guidelines of the National Committee for Clinical Laboratory Standards. Antimicrob Agents Chemother 2002; 46 (2): 398-401.

[15] Metzler DM, DeHaan RM. Susceptibility test of anaerobic bacteria: statistical and clinical considerations. J Infect Dis 1974; 130 (6): 588-594.

[16] Brunden MN, Zurenko GE, Kapik B. Modification of the error-bounded classification scheme for use with two MIC breakpoints. Diagn Microbiol Infect Dis 1992; 15 (2): 135-140.

[17] National Committee for Clinical Laboratory Standards. Development of *in vitro* susceptibility testing criteria and quality control parameters. Approved standard M23-A. Villanova, PA: NCCLS 1994.

Journal of Chemotherapy

Published under the auspices of the Italian Society of Chemotherapy

Subscription Information:

See our website: www.jchemother.it or contact:

Administrative Office

E.S.I.F.T. srl
Via Carlo del Greco, 36 - 50141 Firenze, Italy
Telephone: +39055.453647 - Fax +39055.4554821

Director: Prof. Teresita Mazzei

The ***Journal of Chemotherapy*** is indexed in: Medline (Index Medicus), Current Contents/Clinical Practice®, Research Alert Direct®, Chemical Abstracts, Embase/Excerpta Medica, Biosis and is available on Adonis CD-ROM. See our website: **http://www.jchemother.it**

Authorization of the Law Court of Florence No. 3022 of the 22nd. March, 1982.

Printer «PuntoStampa» - Firenze (Italia) - May 2005