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Contemporary Activity of Grepafloxacin: Re-Evaluation of Antimicrobial Features of a Potent Fluoroquinolone

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AMENDED ABSTRACT

Objective: To re-evaluate the potency and usable spectrum of activity for grepafloxacin against contemporary pathogens collected from clinical infections in 2001-2002. These results will update the grepafloxacin role compared to other quinolone agents introduced since 1999, in preparation for expanded in vitro and in vivo investigations against resistant (R) strains.
Methods: A total of 931 strains of recently isolated bacterial pathogens were tested by reference NCCLS methods compared to 25 other agents including four marketed fluoroquinolones (FQ). The organisms included: *Escherichia coli* (EC; 52), *Klebsiella pneumoniae* (KPN; 51), *Enterobacter cloacae* (ECL; 55), *Pseudomonas aeruginosa* (PSA; ciprofloxacin-R, 52; and ciprofloxacin-S, 50), methicillin-R *S. aureus* (MRSA; 104), methicillin-susceptible (S) *S. aureus* (MSSA; 58), coagulase-negative staphylococci (CoNS; 50), beta-haemolytic streptococci (BHS; 52), *Streptococcus pneumoniae* (SPN; 167), *Haemophilus influenzae* (HI; 105), *Moraxella catarrhalis* (MCAT; 100) and *Legionella pneumophila* (35).
Results: Grepafloxacin activity was comparable to ciprofloxacin, levofloxacin and gatifloxacin against EC, KPN and ECL (MIC_{90}, 0.03-2 µg/ml; R=0.0-7.7%). For PSA, grepafloxacin was active against ciprofloxacin-S (MIC_{50}, 2 µg/ml), but not ciprofloxacin-R (MIC_{90}, >8 µg/ml) isolates. Against MSSA, grepafloxacin S rate was 91.4%, equal to levofloxacin; none of the FQs were active against MRSA or CoNS. Gatifloxacin and grepafloxacin had the same MIC_{90} against BHS (0.25 µg/ml) and penicillin-susceptible SPN (0.25 µg/ml). Grepafloxacin and other FQ activities were not influenced by penicillin R in SPN. Grepafloxacin was very active against HI (MIC_{90}, 0.03 µg/ml), MCAT (0.03 µg/ml) and *Legionella* spp. (0.5 µg/ml).
Conclusions: These recent results indicate that grepafloxacin has retained its potent spectrum against Enterobacteriaceae, methicillin-S staphylococci, and the pathogens causing community-acquired respiratory tract infections. Additional potency was observed versus ciprofloxacin-S PSA and other streptococci. As issues of adverse drug reactions are more accurately evaluated and minimized, it remains clear that grepafloxacin continues to be an excellent candidate FQ for ambulatory care practice settings.

INTRODUCTION

Grepafloxacin is a fluoroquinolone synthesized by Otsuka Pharmaceuticals, Tokyo, Japan, and characterized by a N-1 cyclopropyl group, a C-5 methyl group, and a C-7 piperazinyl moiety with an attached methyl group. Earlier studies have documented the potent in vitro activity of this compound against a wide range of clinically important bacterial species, especially Gram-positive cocci and atypical organisms.

Grepafloxacin was first marketed in Germany in August 1997 and has been used by more than 400,000 patients worldwide. In the United States (US), it was approved by the FDA for oral treatment of mild to moderate infections, including community-acquired infections, acute bacterial exacerbations of chronic bronquitis, and nongonococcal urethritis and cervicitis caused by *Chlamydia trachomatis*.

The safety profile of grepafloxacin has been favorably characterized in a number of preclinical and clinical studies, as well as in postmarketing evaluation. However, it was withdrawn from the US market due to reports of Q-T interval prolongation. The purpose of this study was to re-evaluate the potency and spectrum of grepafloxacin against contemporary pathogens collected from clinical infections in 2001-2002, if it becomes a candidate for market re-entry.

MATERIALS AND METHODS

Organisms: A total of 931 clinical isolates were collected in 2001 and 2002 predominantly from US medical centers. All isolates were tested for grepafloxacin by the NCCLS agar dilution method and these results were compared to those of 25 antimicrobial agents (13 reported here), including four fluoroquinolones, tested by NCCLS reference broth microdilution methods.

Agar dilution: Reagent grade grepafloxacin powder was provided by Otsuka Pharmaceuticals Co., Ltd. (Tokyo, Japan). A stock solution of the antimicrobial agent was made in sterile saline and final dilution schedule ranged from 0.004 to 8 µg/ml. Approximately 10^4 CFU were applied to the agar surface using an inoculum-replicating device. A control plate containing no antimicrobial agent was placed at the beginning and end of the dilution series to assure there was no drug compound carry over.

Broth microdilution: A 50 ml aliquot from the bacterial suspension adjusted to a 0.5 McFarland standard suspension was pipetted into 10 ml of the appropriate media, mixed adequately, and dispensed into the wells of a commercial dry form panel (TREK Diagnostics, Cleveland, OH) using an auto-inoculator to a final concentration of approximately 3-5 x 10^5 CFU/ml. All plates and panels were incubated at 35ºC in an ambient air environment for 16-20 hours for the Gram-negative isolates and 20-24 hours for the Gram-positive and fastidious species. The MIC values were interpreted according to NCCLS criteria (2003).

Quality control (QC): QC was performed by testing *S. aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *S. pneumoniae* ATCC 49619, *H. influenzae* ATCC 49247 and 49766, *E. coli* ATCC 25922, and *P. aeruginosa* ATCC 27853 with each series of tests.

Table 1. Contemporary activity update for grepafloxacin tested against Gram-negative organisms (500 strains).

Antimicrobial agent (no. tested)	MIC (µg/ml) 50%	90%	Range	% by category[a] Susceptible	Resistant
E. coli (52)					
Grepafloxacin	0.016	0.03	0.008->8	92.3	7.7
Ciprofloxacin	≤0.03	≤0.03	≤0.03->4	92.3	7.7
Gatifloxacin	≤0.03	0.06	≤0.03->4	92.3	5.8
Levofloxacin	≤0.03	0.06	≤0.03->4	92.3	7.7
Nalidixic Acid	2	4	1->32	92.3	7.7
Amoxicillin/Clavulanate	4	16	≤2->16	86.5	3.8
Cefepime	≤0.12	≤0.12	≤0.12-2	100.0	0.0
Ceftriaxone	≤0.25	≤0.25	≤0.25-32	98.1	0.0(3.8)[b]
Cefuroxime	4	8	1->16	73.1	3.8
Gentamicin	≤2	≤2	≤2->8	98.1	1.9
Imipenem	0.12	0.12	≤0.06-0.5	100.0	0.0
Piperacillin/Tazobactam	2	4	≤0.5-32	98.1	0.0
Trimethoprim/Sulfamethoxazole	≤0.5	>2	≤0.5->2	80.8	19.2
K. pneumoniae (51)					
Grepafloxacin	0.03	1	0.016-1	100.0	0.0
Ciprofloxacin	≤0.03	1	≤0.03-2	98.0	0.0
Gatifloxacin	0.06	1	≤0.03-1	100.0	0.0
Levofloxacin	0.06	1	≤0.03-1	100.0	0.0
Nalidixic Acid	4	>32	2->32	76.5	19.6
Amoxicillin/Clavulanate	≤2	8	≤2->16	94.1	2.0
Cefepime	≤0.12	≤0.12	≤0.12-8	100.0	0.0
Ceftriaxone	≤0.25	≤0.25	≤0.25->32	92.2	2.0(7.8)[b]
Cefuroxime	2	8	0.5->16	84.3	7.8
Gentamicin	≤2	4	≤2->8	90.2	7.8
Imipenem	0.12	0.25	≤0.06-1	100.0	0.0
Piperacillin/Tazobactam	2	8	1->64	94.1	5.9
Trimethoprim/Sulfamethoxazole	≤0.5	>2	≤0.5->2	86.3	13.7
E. cloacae (55)					
Grepafloxacin	0.03	2	0.016->8	85.5	5.4
Ciprofloxacin	≤0.03	1	≤0.03->4	90.9	5.4
Gatifloxacin	0.06	1	≤0.03->4	94.5	3.6
Levofloxacin	≤0.03	1	≤0.03->4	94.5	3.6
Nalidixic Acid	4	>32	2->32	76.4	18.2
Amoxicillin/Clavulanate	>16	>16	16->16	0.0	98.2
Cefepime	≤0.12	2	≤0.12-4	100.0	0.0
Ceftriaxone	0.5	>32	≤0.25->32	72.7	12.7
Cefuroxime	16	>16	4->16	7.3	43.6
Gentamicin	≤2	≤2	≤2->8	94.5	3.6
Imipenem	0.25	1	≤0.06-1	100.0	0.0
Piperacillin/Tazobactam	4	64	1->64	78.2	5.5
Trimethoprim/Sulfamethoxazole	≤0.5	>2	≤0.5->2	89.1	10.9
P. aeruginosa					
ciprofloxacin-susceptible (52)					
Grepafloxacin	0.25	2	0.06->8	-[c]	-
Gatifloxacin	1	2	≤0.03-4	90.4	0.0
Levofloxacin	0.5	2	≤0.03-4	94.2	0.0
Amikacin	4	8	0.5->32	94.2	1.9
Aztreonam	4	16	0.25->16	82.7	1.9
Cefepime	2	8	0.25-16	98.1	0.0
Ceftazidime	2	8	≤2->16	94.2	1.9
Gentamicin	≤2	4	≤2->8	92.3	1.9
Imipenem	1	2	0.25->8	94.2	1.9
Piperacillin/Tazobactam	8	32	≤0.5->64	98.1	1.9
Tobramycin	0.5	1	≤0.12-8	98.1	0.0
ciprofloxacin-resistant (50)					
Grepafloxacin	>8	>8	0.25->8	-	-
Gatifloxacin	>4	>4	4->4	0.0	92.0
Levofloxacin	>4	>4	4->4	0.0	94.0
Amikacin	8	16	0.5->32	96.0	2.0
Aztreonam	16	>16	0.25->16	42.0	40.0
Cefepime	8	>16	2->16	52.0	24.0
Ceftazidime	4	>16	≤1->16	62.0	34.0
Gentamicin	4	>8	≤2->8	54.0	26.0
Imipenem	2	>8	0.12->8	66.0	20.0
Piperacillin/Tazobactam	16	>64	≤0.5->64	68.0	32.0
Tobramycin	1	>16	0.25->16	76.0	22.0
H. influenzae (105)[f]					
Grepafloxacin	0.016	0.03	≤0.004-0.03	100.0	-
Ciprofloxacin	≤0.03	≤0.03	≤0.03	100.0	-
Gatifloxacin	≤0.03	≤0.03	≤0.03	100.0	-
Levofloxacin	≤0.03	≤0.03	≤0.03	100.0	-
Amoxicillin/Clavulanate	0.5	1	0.12-8	98.1	1.9
Ampicillin	2	>4	≤0.5->4	46.7	50.0
Ceftriaxone	≤0.008	≤0.008	≤0.008-0.06	100.0	-
Cefuroxime	1	2	≤0.06->8	96.2	1.0
Clarithromycin	8	16	≤0.25-32	83.8	1.9
Tetracycline	≤2	≤2	≤2	100.0	0.0
Trimethoprim/Sulfamethoxazole	≤0.5	4	≤0.5->4	83.8	12.4
M. catarrhalis (100)[e]					
Grepafloxacin	0.016	0.03	0.008-0.03	100.0	-
Ciprofloxacin	≤0.03	0.06	≤0.03-0.06	100.0	-
Gatifloxacin	≤0.03	≤0.03	≤0.03-0.06	100.0	-
Levofloxacin	≤0.03	≤0.03	≤0.03-0.06	100.0	-
Amoxicillin/Clavulanate	≤2	≤2	≤2	100.0	0.0
Penicillin[f]	4	>4	≤0.03->4	9.0	91.0
Ceftriaxone	≤0.25	0.5	≤0.008-2	100.0	0.0
Cefuroxime	1	2	0.12-4	100.0	0.0
Clarithromycin	≤0.25	≤0.25	≤0.25	100.0	0.0
Tetracycline	≤2	>4	≤2->16	88.0	1.0
Trimethoprim/Sulfamethoxazole	≤0.5	≤0.5	≤0.5-4	95.0	2.0
L. pneumophila (35)					
Grepafloxacin	0.5	0.5	0.25-0.5	-	-
Levofloxacin	0.12	0.12	0.03-0.12	-	-
Erythromycin	1	1	0.12-2	-	-

a. Susceptibility criteria of the NCCLS.
b. Number in parenthesis indicates the percentage of ESBL phenotypes (NCCLS, 2003).
c. - = indicates that no published interpretive criteria are available.
d. Includes five ß-lactamase negative, ampicillin-resistant strains.
e. Susceptibility criteria of the NCCLS as applied to *H. influenzae*.
f. Susceptibility rates were based on the ß-lactamase test results.

Table 2. Contemporary activity update for grepafloxacin tested against Gram-positive organisms (431 strains).

Antimicrobial agent (no. tested)	MIC (µg/ml) 50%	90%	Range	% by category[a] Susceptible	Resistant
S. aureus					
methicillin-susceptible (58)					
Grepafloxacin	0.06	1	0.016->8	91.4	8.6
Ciprofloxacin	0.25	4	0.12->4	87.9	10.3
Gatifloxacin	0.12	0.5	≤0.03->4	94.8	5.2
Levofloxacin	0.12	2	≤0.03->4	91.4	5.2
Amoxicillin/Clavulanate	≤2	≤2	≤2-4	100.0	0.0
Ceftriaxone	2	4	≤0.25-4	100.0	0.0
Clindamycin	0.12	0.25	≤0.06->8	94.8	5.2
Erythromycin	0.5	>8	0.25->8	75.9	24.1
Linezolid	2	2	1-2	100.0	-[d]
Penicillin	4	32	≤0.015->32	17.2	82.8
Quinupristin/Dalfopristin	0.25	0.5	0.12-0.5	100.0	0.0
Vancomycin	1	1	0.5-2	100.0	0.0
methicillin-resistant (104)					
Grepafloxacin	>8	>8	0.06->8	3.8	96.2
Ciprofloxacin	>4	>4	0.25->4	1.9	98.1
Gatifloxacin	4	>4	0.06->4	24.0	42.3
Levofloxacin	>4	>4	0.12->4	3.8	77.9
Amoxicillin/Clavulanate	16	>16	4->16	8.7[c]	91.3
Ceftriaxone	>32	>32	0.5->32	2.9[c]	69.2
Clindamycin	>8	>8	≤0.06->8	16.5	83.5
Erythromycin	>8	>8	0.25->8	1.0	99.0
Linezolid	2	2	0.5-4	100.0	-
Penicillin	32	>32	4->32	0.0[c]	100.0
Quinupristin/Dalfopristin	0.5	0.5	0.12-1	100.0	0.0
Vancomycin	1	1	0.5-2	100.0	0.0
Coagulase-neg. staphylococci (50)					
Grepafloxacin	2	>8	0.06->8	48.0	48.0
Ciprofloxacin	4	>4	0.06->4	44.0	56.0
Gatifloxacin	1	>4	0.06->4	60.0	24.0
Levofloxacin	2	>4	0.12->4	50.0	44.0
Amoxicillin/Clavulanate	≤2	16	≤2->16	72.0[d]	28.0
Ceftriaxone	16	>32	1->32	44.0[d]	20.0
Clindamycin	0.12	>8	≤0.06->8	78.0	22.0
Erythromycin	>8	>8	≤0.06->8	30.0	70.0
Linezolid	1	1	0.5-2	100.0	-
Oxacillin	4	>8	≤0.06->8	20.0[d]	80.0
Quinupristin/Dalfopristin	0.25	0.5	≤0.06-0.5	100.0	0.0
Vancomycin	1	2	0.5-2	100.0	0.0
ß-haemolytic streptococci (52)					
Grepafloxacin	0.12	0.25	0.06-1	94.2	0.0
Ciprofloxacin	0.5	0.5	0.25-4	-	-
Gatifloxacin	0.25	0.25	0.12-1	100.0	0.0
Levofloxacin	0.5	0.5	0.25-2	100.0	0.0
Cefepime	≤0.12	≤0.12	≤0.12-0.25	100.0	0.0
Ceftriaxone	≤0.25	≤0.25	≤0.25-0.5	100.0	0.0
Clindamycin	≤0.06	≤0.06	≤0.06-8	98.1	1.9
Erythromycin	≤0.06	1	≤0.06-8	88.5	11.5
Linezolid	1	1	0.5-1	100.0	0.0
Penicillin	≤0.015	≤0.015	≤0.015-0.03	100.0	0.0
Quinupristin/Dalfopristin	0.12	0.12	≤0.06-0.25	100.0	0.0
Tetracycline	≤4	>8	≤4->8	86.5	13.5
Vancomycin	0.25	0.5	≤0.12-0.5	100.0	0.0
S. pneumoniae					
penicillin-susceptible (52)					
Grepafloxacin	0.25	0.5	0.06-0.5	100.0	0.0
Ciprofloxacin	1	2	0.25-4	-	-
Gatifloxacin	0.25	0.5	0.12-0.5	100.0	0.0
Levofloxacin	1	1	0.25-1	100.0	0.0
Amoxicillin/Clavulanate	≤0.06	≤0.06	≤0.06	100.0	0.0
Cefepime	≤0.06	0.12	≤0.06-0.5	100.0	0.0
Ceftriaxone	0.03	0.06	≤0.008-0.5	100.0	0.0
Cefuroxime	≤0.06	0.25	≤0.06-2	96.2	0.0
Clindamycin	≤0.06	≤0.06	≤0.06->8	96.2	3.8
Erythromycin	≤0.25	≤0.25	≤0.25->32	90.4	9.6
Linezolid	0.5	1	0.25-2	100.0	-
Trimethoprim/Sulfamethoxazole	≤0.5	1	≤0.5->4	84.6	7.7
Vancomycin	0.25	0.5	≤0.06-0.5	100.0	-
penicillin-intermediate (52)					
Grepafloxacin	0.25	0.25	0.12-2	98.1	1.9
Ciprofloxacin	1	2	0.5->4	-	-
Gatifloxacin	0.25	0.5	0.12-1	100.0	0.0
Levofloxacin	1	1	0.5-2	100.0	0.0
Amoxicillin/Clavulanate	0.25	1	≤0.06-2	100.0	0.0
Cefepime	0.25	1	≤0.06-4	96.2	1.9
Ceftriaxone	0.25	1	0.015-2	98.1	0.0
Cefuroxime	0.5	4	0.12-4	67.3	19.2
Clindamycin	≤0.06	≤0.06	≤0.06->8	90.0	10.0
Erythromycin	≤0.25	8	≤0.25->32	51.9	46.2
Linezolid	0.5	1	0.25-1	100.0	-
Trimethoprim/Sulfamethoxazole	≤0.5	>4	≤0.5->4	51.9	34.6
Vancomycin	0.25	0.5	0.25-1	100.0	-
penicillin-resistant (63)					
Grepafloxacin	0.25	0.5	0.12-1	98.4	0.0
Ciprofloxacin	1	2	0.5-4	-	-
Gatifloxacin	0.25	0.25	0.12-0.5	100.0	0.0
Levofloxacin	1	1	0.5-2	100.0	0.0
Amoxicillin/Clavulanate	2	8	1-8	55.6	30.2
Cefepime	1	2	0.5-4	76.4	1.6
Ceftriaxone	1	4	0.5-8	79.4	12.7
Cefuroxime	8	>8	4->8	0.0	100.0
Clindamycin	≤0.06	>8	≤0.06->8	79.0	21.0
Erythromycin	8	>32	≤0.25->32	25.8	74.2
Linezolid	0.5	1	0.25-2	100.0	-
Trimethoprim/Sulfamethoxazole	>4	>4	≤0.5->4	15.9	76.2
Vancomycin	0.25	0.5	≤0.06-0.5	100.0	-

a. Susceptibility criteria of the NCCLS.
b. - = indicates that no published interpretive criteria are available.
c. Oxacillin result predicts susceptibility for all ß-lactams, e.g. 100.0% resistant for these strains.
d. The susceptibility rate for all ß-lactam agents would be predicted from the oxacillin test result.

RESULTS

- All *K. pneumoniae* isolates (MIC_{90}, 1µg/ml), 92.3% of *E. coli* (MIC_{90}, 0.03 µg/ml), and 85.5% of *Enterobacter* spp. isolates (MIC_{90}, 2 µg/ml) were susceptible (S) to grepafloxacin (Table 1).
- Grepafloxacin (MIC_{50}, 0.25 µg/ml) was two- to four-fold more potent against ciprofloxacin-susceptible *P. aeruginosa* strains than either gatifloxacin (MIC_{50}, 1 µg/ml) or levofloxacin (MIC_{50}, 0.5 µg/ml) (Table 1).
- Ciprofloxacin-resistant *P. aeruginosa* isolates showed cross-resistance to all fluoroquinolones evaluated. Amikacin (MIC_{90}, 16 µg/ml) was the most active compound (96.0% susceptible) tested against this pathogen (Table 1).
- *H. influenzae* and *M. catarrhalis* isolates were very susceptible to all fluoroquinolones (grepafloxacin MIC_{90}, 0.03 µg/ml; 100%S).
- Against *L. pneumophila*, levofloxacin was four-fold more potent than grepafloxacin (MIC_{50} and MIC_{90}, 0.12 µg/ml versus 0.5 µg/ml, respectively), and eight-fold more potent than erythromycin (MIC_{50} and MIC_{90}, 1 µg/ml).
- Against the methicillin-susceptible *S. aureus*, grepafloxacin (MIC_{50}, 0.06 µg/ml, 91.4% S) was four-fold more potent than ciprofloxacin (MIC_{50}, 0.25 µg/ml) and two-fold more potent than levofloxacin or gatifloxacin (MIC_{50}, 0.12 µg/ml) (Table 2).
- Grepafloxacin (MIC_{50}, 0.12 µg/ml) was as potent as gatifloxacin and two-fold more potent than ciprofloxacin or levofloxacin against ß-haemolytic streptococci (Table 2).
- Grepafloxacin and other fluoroquinolones activities were not influenced by penicillin-resistance in *S. pneumoniae* isolates. Penicillin-resistant isolates showed a MIC_{50} of 0.25 µg/ml for both grepafloxacin and gatifloxacin, 1 µg/ml for levofloxacin and 2 µg/ml for ciprofloxacin (Table 2).

CONCLUSIONS

- All fluoroquinolones evaluated presented similar in vitro activity against Enterobacteriaceae (85.5-100.0% S); however, susceptibility rates to other compounds, such as cefuroxime, amoxicillin/clavulanate and trimethoprim/sulfamethoxazole were relatively low for these key pathogens.
- Grepafloxacin was very active against pathogens responsible for community-acquired respiratory tract infections, including *H. influenzae*, *M. catarrhalis*, *S. pneumoniae* and methicillin-susceptible *S. aureus.*
- The results of this study of recent isolates indicates that grepafloxacin has retained its potency and spectrum against most clinically important and indicated pathogens.

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