Garenoxacin Activity Tested Against *S. pneumoniae, H. influenzae* and *M. catarrhalis* with Elevated or Resistant Fluoroquinolone MIC Values

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Abstract

Objective: To assess the garenoxacin (GRN) potency against a vast number of international respiratory tract infection (RTI) pathogens, especially versus phenotypic (high MIC) or genotypic (sequence change) QRDR mutants. A total of 40,423 isolates from six continents were analyzed (1999-2005).

Methods: *S. pneumoniae* (SPN; 18,887 strains), *H. influenzae* (HI; 15,555) and *M. catarrhalis* (MCAT; 5,981) were susceptibility (S) tested by CLSI broth microdilution methods against GRN and 25 comparison agents. Phenotypic QRDR mutants (PQMs) were defined by a ciprofloxacin (CIPRO) or levofloxacin (LEVO) SPN MIC at >= 4mg/L; or CIPRO MIC of >= 0.25 mg/L for HI and MCAT. 124 SPN strains in 3 S groups (1. CIPRO and LEVO MIC >= 4mg/L; 2. CIPRO>= 4 mg/L and LEVO <= 2mg/L; 3. CIPRO and LEVO <= 2mg/L) had QRDR sequences determined (gyr A,B; par C,E).

Results: Penicillin, macrolides and ceftriaxone resistance (R) rates for SPN were 18.1, 28.4 and 0.8%, respectively. CIPRO (2.6% R) and LEVO (0.9% R) rates were low as were PQM occurrences among HI (19 strains, 0.1%) and MCAT (15 strains, 0.2%). GRN remained the most active fluoroquinolone (>90%S) against all mutant isolates, but HI isolates were only 78.9-84.2% S (see table). QRDR sequencing demonstrated average numbers of mutations at 2.7, 1.5 and 1.1 for S groups 1, 2, and 3, respectively; GRN $MIC_{50/90}$ results are 0.5/2, 0.06/0.12 and 0.06/0.12 mg/L. Highest GRN MIC results (>= 1 mg/L) were associated with >= 3 QRDR mutations. (See Table)

	GRN MIC (mg/L)		% at	
Organism group (no. tested)	50%	90%	<=1mg/L	<=2mg/L
SPN, all (18,887)	0.06	0.06	>99.9	>99.9
CIPRO MIC @ >= 4 mg/L (448)	0.06	1	97.5	99.2
LEVO MIC @ >= 4 mg/L (171)	1	2	90.6	97.1
HI, all (15,555)	<=0.008	0.03	>99.9	>99.9
CIPRO MIC @ >=0.25 mg/L (19)	0.12	8	78.9	84.2
MCAT, all (5,981)	0.016	0.03	100.0	100.0
CIPRO MIC @ >=0.25 mg/L (15)	0.12	0.5	100.0	100.0

Conclusions: GRN maintains clinically usable activity (MIC, <= 1 mg/L) against important community-acquired RTI pathogens having R to presently marketed fluoroquinolones and against those isolates with documented QRDR mutations. Continued development of this novel des-F(6)quinolone agent appears desirable.

Introduction

Garenoxacin is a novel des-F(6)-quinolone that lacks the C6-position fluorine and has a unique difluoromethoxy substitution at position C8. These alterations resulted in a drug with improved potency against both DNA gyrases and topoisomerase IV. Garenoxacin has been described as highly active against important Gram-positive and –negative pathogens including: Enterobacteriaceae, staphylococci, streptococci (*S. pneumoniae*, viridans group species and ß-haemolytic streptococci), *Acinetobacter* spp. and some other Gram-negative non-fermentative bacilli, *Haemophilus influenzae*, *Moraxella catarrhalis*, atypical respiratory tract pathogens (Mycoplasmas, *C. pneumoniae*, and *Legionella* spp.), many enterococci and anaerobes, especially Gram-positive species. These features are complemented by a favorable pharmacokinetic/pharmacodynamic profile, leading to a high AUC/MIC ratio. This high AUC/MIC ratio leads to a greater probability of favorable target attainment that has been associated with successful bacterial eradication and minimization of mutational events among indicated species (low MPC values). These elements of spectrum and potency favor garenoxacin applications for CA-RTI (hospitalized or ambulatory patients).

Since garenoxacin features high potency and breadth of spectrum against common CA-RTI pathogens (40,423 strains tested since 1999), a subset of strains having elevated MIC values to currently utilized fluoroquinolones was selected for further studies. Garenoxacin MICs versus those organisms and the determination of QRDR mutations was used to establish the role of this investigational des-F(6)-quinolone against emerging resistant pathogen types.

Materials and Methods

Bacterial strains. The organisms were consecutively collected isolates processed in a central laboratory system (JMI Laboratories, North Liberty, Iowa, USA; Women's and Children's Hospital, Adelaide, Australia) using common reference test reagents. Isolates were derived from a variety of clinical sources (Program Objectives) such as: bloodstream, community-acquired or nosocomial respiratory tract infections, skin and soft tissue infections, urinary tract infections and selected patient populations. In this investigation, the isolates were obtained from a wide variety of medical centers each year in North America (≥ 25 sites in the USA and Canada), Latin America (10 nations) and Europe (≥ 25 sites). The distribution of tested organisms was three CA-RTI species (*S. pneumoniae* [18,887], *H. influenzae* [15,555], *M. catarrhalis* [5,981]) with elevated fluoroquinolone MIC results as follows:

1. *S. pneumoniae* – ciprofloxacin or levofloxacin MIC at ≥ 4 mg/L
2. *H. influenzae* or *M. catarrhalis* – ciprofloxacin MIC at ≥ 0.25 mg/L.

A total of 488 *S. pneumoniae*, 19 *H. influenzae*, and only 15 *M. catarrhalis* strains were the principal subject of this presentation; e.g. 2.6, 0.1 and 0.2% of isolates, respectively.

Susceptibility testing methods: All MIC values were generated using broth microdilution methods (CLSI M7-A7) with panels produced by TREK Diagnostics (Cleveland, Ohio, USA). Mueller-Hinton broth was supplemented where indicated with 2-5% lysed horse blood (fastidious streptococci) and HTM components (*Haemophilus* species). Concurrent quality assurance was maintained via use of CLSI-recommended strains: *Escherichia coli* ATCC 25922 and 35218, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923 and 29213, *H. influenzae* ATCC 49247 and 49766, and *S. pneumoniae* ATCC 49619. All quality control results were within published MIC ranges (CLSI M100-S16). More than 30 antimicrobial agents were processed each year with selected agents (18, Table 1) compared to garenoxacin in this presentation. A breakpoint for garenoxacin susceptibility and resistance at ≤1/≥4 mg/L was used for comparison purposes only.

Molecular methods. The QRDR was assessed for mutations in *gyrA* or *gyrB* and *parC* or *parE* by PCR amplification and sequence analyses. *S. pneumoniae* and *H. influenzae* isolates with elevated garenoxacin MIC results (2 - >4 mg/L) were processed to detect mutations as were selected isolates with fluoroquinolones MICs ≥ three log_2 dilutions above the wild-type MIC distributions. A total of 124 isolates had QRDR sequences determined.

Results

- Garenoxacin was very active against all clinical isolates of *S. pneumoniae* (MIC_{90}, 0.06 mg/L; >99.9% inhibited by ≤ 1 mg/L), *H. influenzae* and *M. catarrhalis* (MIC_{90}, ≤ 0.03 mg/L; >99.9 - 100.0% inhibited at ≤ 1 mg/L), see Table 1.
- Garenoxacin potency was 16- to 32-fold greater than ciprofloxacin or levofloxacin against *S. pneumoniae*, and two- to four-fold more active than moxifloxacin (Table 1).
- All tested fluoroquinolones were active against *H. influenzae* and *M. catarrhalis*, with moxifloxacin showing the highest MIC_{90} results for *M. catarrhalis* at 0.06 mg/L (Table 1).
- QRDR mutations produced elevated MIC values to currently available fluoroquinolones (Table 2), but many remained inhibited by garenoxacin at ≤ 1 mg/L; particularly the pneumococci (>90%).
- The most common QRDR mutations in *S. pneumoniae* associated with garenoxacin MIC results of ≥ 2 mg/L were:
  - For *gyrA*: Ser81Phe and Glu85Lys
  - For *gyrB*: Val432Asp or none
  - For *parC*: Gly77Glu, Ser79Phe, Asp83Tyr, and Lys137Asn
  - For *parE*: Ile460Val or none
- These QRDR multiple mutations (average ≥ 3) only elevated garenoxacin MIC values to the level of wild-type, non-mutant strain MICs of levofloxacin (1 - 2 mg/L; Table 2). These mutant strains remain susceptible to the bactericidal action of garenoxacin [Anderegg and Jones, 2004].

Table 1. Antimicrobial activity of garenoxacin and selected comparison agents tested against *S. pneumoniae* (18,887), *H. influenzae* (15,555) and *M. catarrhalis* (5,981) isolated in the SENTRY Program (1999-2005) worldwide.

	MIC (mg/L)		
Organism (no. tested)/ Antimicrobial Agent	50%	90%	% Susceptible/Resistant[a]
S. pneumoniae (18,887)			
Garenoxacin	0.06	0.06	>99.9/<0.1[a]
Amoxicillin/clavulanate	≤0.25	2	94.9/2.6
Cefepime	≤0.12	1	95.3/0.3
Ceftriaxone	≤0.25	1	96.3/0.8
Cefuroxime axetil	≤0.06	8	74.4/22.7
Chloramphenicol	≤2	4	92.0/8.0
Ciprofloxacin	1	2	0/3.4[b]
Clindamycin	≤0.25	4	85.3/14.2
Erythromycin	≤0.25	>8	70.9/28.4
Gatifloxacin	0.25	0.5	99.1/0.8
Levofloxacin	1	1	99.1/0.8
Linezolid	1	1	100.0/-
Moxifloxacin	0.12	0.25	99.2/0.4
Penicillin	≤0.03	2	66.7/18.1
Quinupristin/dalfopristin	≤0.5	≤0.5	99.8/<0.1
Rifampicin	≤1	≤1	99.3/0.4
Tetracycline	≤2	>8	81.5/12.8
Trimethoprim/sulfamethoxazole	≤0.5	4	65.2/23.9
Vancomycin	0.25	0.5	100.0/-
H. influenzae (15,555)			
Garenoxacin	≤0.03	≤0.03	>99.9/-[a]
Amoxicillin/clavulanate	≤2	≤2	99.7/0.3
Ampicillin	≤2	>4	77.3/21.4
Azithromycin	1	2	99.7/0.3
Cefepime	≤0.12	≤0.12	>99.9/-
Ceftriaxone	≤0.25	≤0.25	>99.9/-
Cefuroxime axetil	1	2	97.8/0.6
Chloramphenicol	≤2	≤2	97.9/1.8
Ciprofloxacin	≤0.03	≤0.03	>99.9/-
Clarithromycin	8	16	86.8/1.3
Gatifloxacin	≤0.03	≤0.03	>99.9/-
Levofloxacin	≤0.03	≤0.03	>99.9/-
Moxifloxacin	≤0.03	≤0.03	>99.9/-
Rifampicin	≤1	≤1	99.6/0.3
Tetracycline	≤2	≤2	97.9/2.1
Trimethoprim/sulfamethoxazole	≤0.5	>4	78.7/17.7
M. catarrhalis (5,981)			
Garenoxacin	≤0.03	≤0.03	100.0/-[a]
Amoxicillin/clavulanate	≤2	≤2	>99/<0.1
Azithromycin	≤0.5	≤0.5	100.0/-
Penicillin	>4	>4	4.7/95.3
Ceftriaxone	≤0.25	0.5	99.9/-
Cefuroxime axetil	1	2	98.6/0.2
Chloramphenicol	≤2	≤2	99.9/0.1
Ciprofloxacin	≤0.25	≤0.25	>99.9/-
Clarithromycin	≤0.25	≤0.25	100.0/0.0
Gatifloxacin	≤0.03	≤0.03	100.0/-
Levofloxacin	≤0.03	0.06	100.0/-
Moxifloxacin	0.06	0.06	>99.0/-
Rifampicin	≤1	≤1	>99.9/<0.1
Tetracycline	≤2	<2	98.8/1.2
Trimethoprim/sulfamethoxazole	≤0.5	≤0.5	96.8/0.3

a. Interpretive criteria of the CLSI were used, where available. For garenoxacin, the interpretive criteria of ≤ 1 mg/L (susceptible) was used; and for *M. catarrhalis*, the *H. influenzae* guidelines were utilized.
b. Resistant proportion indicates % of MICs at ≥ 4 mg/L = possible QRDR mutations for *S. pneumoniae*. The similar concentration for *H. influenzae* and *M. catarrhalis* may be as low as ≥ 0.25 mg/L, e.g. approximately 0.1 - 0.2% of strains.

Table 2. MIC distributions for garenoxacin tested against CA-RTI isolates with possible/confirmed QRDR mutations selected by elevated MIC values for ciprofloxacin or levofloxacin.

	Garenoxacin MIC		Cum. % inhibited at Garenoxacin MIC (mg/L):				
Organism/Subset (no. tested)	50%	90%	≤0.12	0.25	0.5	1	2
S. pneumoniae							
Ciprofloxacin MIC @≥4 mg/L (448)	0.06	1	76.6	80.5	88.5	97.5	99.2
Levofloxacin MIC @≥4 mg/L (171)	1	2	8.2	20.5	51.5	90.6	97.1
All strains (18,887)	0.06	0.06	98.9	99.2	99.5	>99.9	>99.9
H. influenzae							
Ciprofloxacin MIC @≥0.25 mg/L (19)	0.12	8	52.6	73.7	78.9	78.9	84.2
All strains (15,555)	≤0.03	≤0.03	>99.9	>99.9	>99.9	>99.9	>99.9
M. catarrhalis							
Ciprofloxacin MIC @≥0.25 mg/L (15)	0.12	0.5	60.0	86.7	100.0	-	-
All strains (5,981)	≤0.03	≤0.03	99.9	>99.9	100.0	-	-

Conclusions

- Garenoxacin is among the most potent fluoroquinolones (MIC_{90}, ≤ 0.03 - 0.06 mg/L) versus wild-type and mutant populations of *S. pneumoniae*, *H. influenzae* and *M. catarrhalis*.
- Garenoxacin remains active and inhibits more than 90% of ciprofloxacin- and levofloxacin-resistant *S. pneumoniae*, as well as the extremely rare ciprofloxacin-refractory *H. influenzae* and *M. catarrhalis* (MICs, ≥ 0.25 mg/L)
- Garenoxacin continues to exhibit high potency in 2004-2005 at a level unchanged since 1999, as documented in the SENTRY Program. Continued development of this novel des-F(6)-quinolone is warranted.

Selected References

Anderegg TR, Biedenbach DJ, Jones RN (2003). Re-evaluation of quality control guidelines for gatifloxacin and garenoxacin (BMS-284756) when susceptibility testing *Haemophilus influenzae* and *Streptococcus pneumoniae*. *Diagn Microbiol Infect Dis* 46: 147-150.

Anderegg TR, Jones RN (2004). Bactericidal activity of garenoxacin tested by kill-curve methodology against wild type and QRDR mutant strains of *Streptococcus pneumoniae*. *Diagn Microbiol Infect Dis* 50: 213-217.

Andes D, Craig WA (2003). Pharmacodynamics of the new des-f(6)-quinolone garenoxacin in a murine thigh infection model. *Antimicrob Agents Chemother* 47: 3935-3941.

Clinical and Laboratory Standards Institute. (2006). Approved Standard M7-A7: *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 7th ed*. Wayne, PA: CLSI.

Clinical and Laboratory Standards Institute. (2006). *Performance standard for antimicrobial susceptibility testing, 16th informational supplement M100-S16*. Wayne, PA: CLSI.

Fung-Tomc JC, Minassian B, Kolek B, Huczko E, Aleksunes L, Stickle T, Washo T, Gradelski E, Valera L, Bonner DP (2000). Antibacterial spectrum of a novel des-fluoro(6) quinolone, BMS-284756. *Antimicrob Agents Chemother* 44: 3351-3356.

Ince D, Zhang X, Silver LC, Hooper DC (2002). Dual targeting of DNA gyrase and topoisomerase IV: Target interactions of garenoxacin (BMS-284756, T-3811ME), a new desfluoroquinolone. *Antimicrob Agents Chemother* 46: 3370-3380.

Jones RN, Pfaller MA, Stilwell M (2001). Activity and spectrum of BMS-284756, a new des-F (6) quinolone, tested against strains of ciprofloxacin-resistant Gram-positive cocci. *Diagn Microbiol Infect Dis* 39: 133-135.

Jones RN, Biedenbach DJ (2003). Comparative activity of garenoxacin (BMS-284756), a novel desfluoroquinolone, tested against 8,331 isolates from community-acquired respiratory tract infections: North American results from the SENTRY Antimicrobial Surveillance Program (1999-2001). *Diagn Microbiol Infect Dis* 45: 273-278.

Lister PD (2003). Impact of AUC/MIC ratios on the pharmacodynamics of the des-F(6) quinolone garenoxacin (BMS-284756) is similar to other fluoroquinolones. *J Antimicrob Chemother* 51: 199-202.

Saravolatz L, Manzor O, Pawlak J, Belian B (2001). Antimicrobial activity of BMS-284756, a novel des-fluoro (6) quinolone and seven fluoroquinolones against *Streptococcus pneumoniae*. *Clin Microbiol Infect* 7: 572-573.

Schmitz FJ, Milatovic D, Boos M, Mayer S, Fluit AC (2002). In vitro activity of the novel des-F(6) quinolone BMS-284756 against genetically characterized clinical streptococcal isolates, including isolates with reduced quinolone susceptibility. *J Antimicrob Chemother* 49: 698-701.

Takahata M, Mitsuyama J, Yamashiro Y, Yonezawa M, Araki H, Todo Y, Minami S, Watanabe Y, Narita H (1999). In vitro and in vivo antimicrobial activities of T-3811ME, a novel des-F(6)-quinolone. *Antimicrob Agents Chemother* 43: 1077-1084.

Van Wart S, Phillips L, Ludwig EA, Russo R, Gajjar DA, Bello A, Ambrose PG, Costanzo C, Grasela TH, Echols R, Grasela DM (2004). Population pharmacokinetics and pharmacodynamics of garenoxacin in patients with community-acquired respiratory tract infections. *Antimicrob Agents Chemother* 48: 4766-4777.