P553

Definition of Wildtype MIC Distributions for Targeted Species for Determination of Optimal Dosing for Zabofloxacin, A Novel Fluoronaphthyridone

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ABSTRACT

Objectives: Zabofloxacin (formerly DW-224a) is a new quinolone-like agent with potent activity against pathogens responsible for respiratory tract and uncomplicated skin and skin structure infections (*S. pneumoniae* [SPN], *H. influenzae* [HI], *M. catarrhalis* [MCAT] and *S. aureus* [SA]). Zabofloxacin was evaluated by CLSI MIC methods for subsequent PK/PD target attainment (TA) analysis to optimize doses used in various clinical trials.

Methods: Recent (2006) wildtype clinical isolates from worldwide locations (Europe, Asia, North and South America) were susceptibility (S) tested by CLSI methods: SPN (225; 200 wildtype, 25 levofloxacin-resistant [LEVO-R]); SA (200 wildtype, 200 MRSA and LEVO-R; 25 CA-MRSA); HI (55 wildtype); MCAT (10 wildtype) and CoNS (40 wildtype with 20 LEVO-R). Zabofloxacin was compared to LEVO, gemifloxacin [GEMI] and moxifloxacin [MOXI]. PK/PD studies of TA were calculated by Monte Carlo simulation from this MIC distribution for doses 50-800 mg daily (protein binding at 77%).

Results: Wildtype SPN and SA (MIC_{90}, 0.03 mg/L); and HI and MCAT (MIC_{90}, 0.015 mg/L were very zabofloxacin-S. In contrast, LEVO-R SPN (MIC_{90}, 1 mg/L) and MRSA (MIC_{90} >8 mg/L) had higher zabofloxacin MIC values. CA-MRSA were zabofloxacin-S (also LEVO-S). Beta-lactamases and PBP-mediated resistances did not adversely influence zabofloxacin potency. Zabofloxacin (MIC_{50}, 0.015 mg/L) was two- and eight-fold more potent than GEMI and MOXI versus wildtype penicillin-R SPN. 90% TA was achieved against SPN, HI, and MCAT wildtype pathogens (MIC, ≤0.06 mg/L; ≥300 mg/day) and many LEVO-R SPN.

	Zabofloxacin MIC (mg/L)			MIC 90% (mg/L)		
Target pathogen (no.)[a]	50%	90%	Range	GEMI	LEVO	MOXI
SPN, WT (200)	0.015	0.03	≤0.0004-0.06	0.03	1	0.25
SPN, PCN-R (101)	0.015	0.03	0.008-0.06	0.03	1	0.25
SPN, LEVO-R (25)	0.12	1	0.06-8	1	> 8	8
HI, WT (55)	0.008	0.015	≤0.004-0.03	0.008	0.015	0.03
MCAT, WT (10)	0.015	0.015	0.008-0.015	0.015	0.03	0.06
MSSA, LEVO-S (200)	0.03	0.03	0.008-0.12	0.03	0.25	0.06
MSCoNS, LEVO-S (20)	0.03	0.03	0.008-0.03	0.03	0.25	0.12

a. PCN = penicillin, WT = wildtype.

Conclusions: Zabofloxacin was two- to 16- and two- to >64-fold more active than MOXI or LEVO, respectively; equal to GEMI versus RTI pathogens and wildtype MSSA. Optimal dosing appears to be achievable due to high zabofloxacin potency versus these 755 contemporary clinical strains. Clinical trial designs and PK/PD-based dosing regimens will be optimized for this promising, new orally administered agent.

INTRODUCTION

Compound zabofloxacin, a 6-F naphthyridine (Figure 1), has been studied in experiments reported at ICAAC in 2003-2006 as well as in cited publications. Generally, this so-called "fluoroquinolone" has been described as two- to 32-fold more potent than marketed agents in the same class against Gram-positive pathogens and toxicity analysis showed limited adverse effects (genetic toxicity, phototoxicity, convulsions).

Zabofloxacin has intracellular activity against *L. pneumophilia* and studies by United Kingdom-based investigators showed a superior potency against *M. hominis*, *C. pneumoniae* and *L. pneumophilia* when compared to moxifloxacin, gatifloxacin and levofloxacin. Appelbaum and colleagues established the $MIC_{50/90}$ of zabofloxacin against *S. pneumoniae* at 0.016/0.03 mg/L for levofloxacin-susceptible wildtype (WT) strains and for ciprofloxacin-resistant (MIC, ≥4 mg/L) strains the zabofloxacin $MIC_{50/90}$ was 0.12/0.25 mg/L (essentially the same as gemifloxacin). Resistance mutations were infrequent for zabofloxacin in passaging trials. Subsequent ICAAC abstracts showed zabofloxacin was bactericidal and did not alter blood glucose levels in diabetic rodent models. In 2006, Kwak et al. showed a dual targeting (DNA gyrase and DNA topoisomerase IV) feature against *S. pneumoniae*.

In this study, we report the results of two investigations to establish the wildtype (WT) distributions of zabofloxacin MICs when testing recent staphylococcal and *S. pneumoniae* isolates (also representative *H. influenzae* and *M. catarrhalis*) and to calculate the rates of target attainment (30, 60 and 120 AUC: MIC) for dosing levels ranging from 50 to 800 mg.

MATERIALS AND METHODS

Susceptibility testing: All MIC values were generated using the reference broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI) document M7-A7 (2006). Interpretive criteria for categorical interpretations were those from CLSI M100-S18 (2008) and quality control (QC) guidelines were derived from the same document. All QC results were within published ranges. Zabofloxacin QC strain results were as follows: *Staphylococcus aureus* ATCC 29213 (0.015-0.03 mg/L), *Enterococcus faecalis* ATCC 29212 (0.06 mg/L), *Escherichia coli* ATCC 25922 (0.03 mg/L) and *Pseudomonas aeruginosa* ATCC 27853 (1 mg/L). The tested antimicrobials were provided by Pacific Beach BioSciences (zabofloxacin) and their domestic manufacturers or Sigma Chemical Co. (St. Louis, MO, USA). Four quinolones were tested including Zabofloxacin, gemifloxacin, levofloxacin and moxifloxacin.

Organisms tested: A total of 225 *S. pneumoniae* (200 WT strains [50% penicillin-non-susceptible; 50 strains from each of four regions including North America, Latin America, Europe, Asia-Pacific] and 25 fluoroquinolone [levofloxacin]-resistant strains with documented QRDR mutations) were studied. Also, *S. aureus* (425 strains; 200 WT methicillin-susceptible, levofloxacin-susceptible strains and 200 WT methicillin-resistant, levofloxacin-resistant strains; 25 CA-MRSA that includes characterized SCC*mec*IVa, USA300-0114 or variants and PVL (+) isolates with all strains distributed across all four geographic regions) were processed. *H. influenzae* (50 strains; 20 ß-lactamase-negative, 10 BLNAR [MIC, ≥2 mg/L], and 20 ß-lactamase-positive); *M. catarrhalis* (10 strains; 8 ß-lactamase [+]), all USA strains; and coagulase-negative staphylococci (CoNS; 40 strains, 20 methicillin-susceptible, levofloxacin-susceptible and 20 methicillin-resistant, levofloxacin-resistant) were tested.

Monte Carlo simulations: Please examine the companion poster (P1261) by Ambrose et al.

RESULTS

- A summary table (Table 1) of all 755 strains tested against zabofloxacin generally demonstrates excellent potency against WT Gram-positive pathogens, *H. influenzae* and *M. catarrhalis*. Modal zabofloxacin MIC values were usually 0.016 or 0.03 mg/L. However, strains resistant to levofloxacin or other marketed fluoroquinolones routinely had Zabofloxacin modal MIC values ranging from 0.12 to ≥2 mg/L.
- Against all pneumococci (225), zabofloxacin was two-fold more potent than gemifloxacin (Table 2) and 64-fold more active than levofloxacin. The overall collection had 10.7% of strains resistant to levofloxacin. No effects of penicillin susceptibility patterns on any of the four tested fluoroquinolones was observed, but non-susceptibility to levofloxacin (MIC, ≥4 mg/L) markedly increased zabofloxacin MIC_{90} results from 0.03 mg/L (Figure 2) to 1 mg/L.
- *H. influenzae* and *M. catarrhalis* isolates were very susceptible to zabofloxacin and the three comparison agents (Table 2).
- Zabofloxacin and gemifloxacin were the most active agents (MIC_{50}, 0.03 mg/L; Table 2) against *S. aureus*, and while levofloxacin was the least active fluoroquinolone (MIC_{50}, 0.25 mg/L). Only 52.9% of the tested strains were considered fluoroquinolone-susceptible.
- Among WT MSSA with levofloxacin MIC results at ≤1 mg/L (susceptible), the zabofloxacin MIC_{90} was only 0.03 mg/L, equal to gemifloxacin and two-fold lower than moxifloxacin (data not shown). In contrast, levofloxacin-resistant MRSA had zabofloxacin MIC values 128-fold higher. Community-acquired MRSA (CA-MRSA) endemic strains (USA300-0114 or variants) are usually susceptible to fluoroquinolones and Zabofloxacin $MIC_{50/90}$ results were identical to those observed for levofloxacin-susceptible MSSA.
- Coagulase-negative staphylococci (10 species; Table 2) showed comparable susceptibility patterns to that of *S. aureus* versus the tested fluoroquinolones.

Table 1. Zabofloxacin MIC distributions for 755 key organisms tested by reference CLSI broth microdilution method.

Organism groups (no. tested)	Occurrences at MIC (mg/L): ≤0.004	0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	>8
S. pneumoniae													
All (225)	2	20	147	30	3	11	6	3	1	1	0	1	0
Penicillin-S (99)[a]	2	9	76	12	0	0	0	0	0	0	0	0	0
Penicillin-NS (101)[a]	0	11	71	18	1	0	0	0	0	0	0	0	0
Levofloxacin-NS (25)[a]	0	0	0	0	2	11	6	3	1	1	0	1	0
H. influenzae													
All (55)	1	35	16	3	0	0	0	0	0	0	0	0	0
ß-lactamase-positive (20)	1	11	5	3	0	0	0	0	0	0	0	0	0
ß-lactamase-negative (20)	0	16	4	0	0	0	0	0	0	0	0	0	0
BLNAR (15)[a]	0	8	7	0	0	0	0	0	0	0	0	0	0
M. catarrhalis													
All (10)[b]	0	2	8	0	0	0	0	0	0	0	0	0	0
S. aureus													
All (425)	0	9	76	131	8	1	1	4	33	47	48	20	47
MSSA, Levo-S (200)[a]	0	9	76	106	8	1	0	0	0	0	0	0	0
MRSA, Levo-NS (200)[a]	0	0	0	0	0	0	1	4	33	47	48	20	47
CA-MRSA (25)	0	0	0	25	0	0	0	0	0	0	0	0	0
Coagulase-negative staphylococci													
All (40)	0	3	6	11	0	0	1	2	3	3	3	1	7
Levo-S (20)[a]	0	3	6	11	0	0	0	0	0	0	0	0	0
Levo-NS (20)[a]	0	0	0	0	0	0	1	2	3	3	3	1	7

a. S = susceptible, NS = non-susceptible, BLNAR = ß-lactamase-negative ampicillin-resistant (MIC, ≥2 mg/L), MSSA = methicillin-S *S. aureus*, MRSA = methicillin-R *S. aureus*, Levo-S = levofloxacin-S and Levo-NS = levofloxacin-NS.
b. Includes 8 ß-lactamase-positive strains.

Table 2. Comprehensive activity of zabofloxacin and four other fluoroquinolones tested against 755 strains of recent clinical isolates.

Pathogen (no tested)/antimicrobials	MIC (mg/L) MIC_{50}	MIC_{90}	Range	% susceptible/ resistant[a]
S. pneumoniae (225)				
Zabofloxacin	0.015	0.12	≤0.004-8	- / -
Gemifloxacin	0.03	0.25	≤0.004-8	89.8 / 5.3
Moxifloxacin	0.12	1	0.008->8	90.2 / 4.9
Levofloxacin	1	8	0.25->8	88.9 / 10.7
H. influenzae (55)				
Zabofloxacin	0.008	0.015	≤0.004-0.03	- / -
Gemifloxacin	≤0.004	0.008	≤0.004-0.015	100.0 / -
Moxifloxacin	0.015	0.03	0.015-0.06	100.0 / -
Levofloxacin	0.015	0.015	≤0.008-0.03	100.0 / -
M. catarrhalis (10)				
Zabofloxacin	0.015	0.015	0.008-0.015	- / -
Gemifloxacin	0.008	0.015	0.008-0.015	- / -
Moxifloxacin	0.06	0.06	0.03-0.06	- / -
Levofloxacin	0.03	0.03	0.03	- / -
S. aureus (425)				
Zabofloxacin	0.03	>8	0.008->8	- / -
Gemifloxacin	0.03	>8	0.008->8	- / -
Moxifloxacin	0.06	8	0.015->8	52.9 / 46.6
Levofloxacin	0.25	>8	0.06->8	52.9 / 47.1
Coagulase-negative staphylococci (40)[b]				
Zabofloxacin	0.03	>8	0.008->8	- / -
Gemifloxacin	0.03	>8	0.015->8	- / -
Moxifloxacin	0.12	>8	0.03->8	50.0 / 50.0
Levofloxacin	0.25	>8	0.12->8	50.0 / 50.0

a. Criteria as published by the CLSI [2007], where available. - = no criteria have been published.
b. Includes: *Staphylococcus auricularis* (3 strains), *Staphylococcus capitis* (3 strains), *Staphylococcus epidermidis* (14 strains), *Staphylococcus haemolyticus* (6 strains), *Staphylococcus hominis* (7 strains), *Staphylococcus saprophyticus* (1 strain), *Staphylococcus schleiferi* (1 strain), *Staphylococcus simulans* (1 strain), *Staphylococcus warnerii* (3 strains), and *Staphylococcus xylosis* (1 strain).

- QRDR mutations for the *S. pneumoniae* (tabulated in Table 3) for levofloxacin-non-susceptible isolates (see Table 1) had a wide variety of patterns, most commonly in *GyrA* (mutations, S81F or Y) and in *ParC* (mutations, S79F or Y, N91D and D83N or Y). Multiple mutations are required to produce significant elevations in zabofloxacin MIC values.

Table 3. Listing of QRDR mutations found in levofloxacin-non-susceptible strains tabulated in Table 1.

Mutation pattern *GyrA*	*GyrB*	*ParC*	*ParE*	No. of strains
S81F	-	S79F	-	7
S81F	-	S79Y	-	3
S81F	-	S79F	I460V	2
S81F	-	S79F, N91D, K137N	I460V	2
S81F	-	K137N	D435N, I460V	1
S81Y	-	S52G, S79Y, N91D	I460V	1
S81F	-	D83N	-	1
S81F	-	N91D	-	1
S81F, E85K	-	S79F, D83Y	-	1
V71I	-	S79Y, N91D	-	1
E85K	-	D83N, N91D, K137N	-	1
-	D435I	S79F	-	1
-	V432D	G77E, S79F	I460V	1
-	-	S79F, K137N	-	1
-	-	S79Y	I460V	1

Figure 1. Zabofloxacin chemical structure.

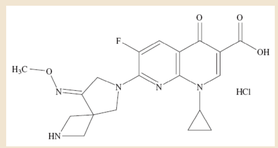
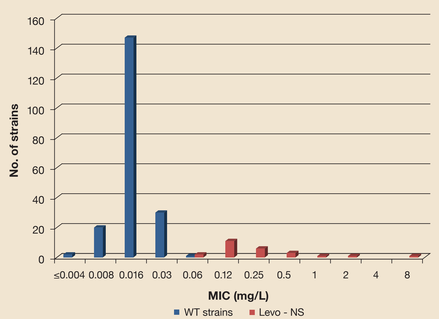


Figure 2. Zabofloxacin MIC results for *S. pneumoniae* isolates having wildtype (WT) fluoroquinolone MICs versus documented levofloxacin non-susceptibile (MIC, ≥4 mg/L) MIC values.



CONCLUSIONS

- Zabofloxacin activity against WT *S. pneumoniae* ($MIC_{50/90}$, 0.015/0.03 mg/L), *H. influenzae* (0.008/0.015 mg/L and *M. catarrhalis* ($MIC_{50/90}$ at 0.015/0.015 mg/L) was among the most potent ever reported and most similar to gemifloxacin.
- Zabofloxacin activity versus staphylococci was among the best when tested against levofloxacin-susceptible strains (MIC_{90}, 0.03 mg/L), but was less active against levofloxacin-resistant strains (MIC_{90}, >8 mg/L).
- **Table 1** lists the low zabofloxacin MIC values for WT organism populations that appear to be adequately covered by proposed dosing regimens of approximately 300 mg daily and significant target attainment against some levofloxacin-resistant, QRDR mutant pneumococci and levofloxacin-susceptible staphylococci would be predicted.

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