

Antibacterial Activity of NXL103 (linopristin/flopristin), *in vitro* Post-antibiotic Effect, and Spontaneous Frequency of Resistance

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ABSTRACT

Objectives: NXL103 (linopristin/flopristin) is an oral streptogramin which was recently evaluated in a Phase I clinical trial. Susceptibility of both community- (CA-MRSA) and hospital-associated (HA-MRSA) methicillin-resistant *Staphylococcus aureus* was evaluated against 160 clinical isolates and *in vitro* post-antibiotic effect (PAE) and spontaneous frequency of resistance have also been evaluated with selected strains.

Methods: Minimal inhibitory concentrations (MIC) were determined by CLSI broth microdilution method. *In vitro* post-antibiotic effect (PAE) was determined by incubating bacteria for 2 hours with antibiotic followed by dilution and incubation in antibiotic-free pre-warmed medium with bacterial enumeration on agar medium (37°C, 48 hours). PAE was defined as the difference in time required for antibiotic-treated bacteria to increase by 1-log₁₀ versus bacteria not exposed to antibiotic. Spontaneous frequency of resistance was determined by plating bacteria on brain-heart agar containing 2, 4, or 8x MIC of antibiotic (37°C, 48-72 hours). MICs of mutant and parent strains were subsequently confirmed.

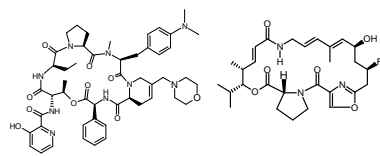
Results: NXL103 MICs ranged from 0.06-0.5 mg/L. NXL103 MIC₉₀ was 0.25 mg/L for CA-MRSA and 0.5 mg/L for HA-MRSA. NXL103 was active against erythromycin A-resistant bacteria with MICs 2-4 fold lower than for linezolid, daptomycin, vancomycin, and quinupristin/dalopristin. The PAE of NXL103 for *S. aureus* AS5155 and DEL4811 were 2 and 2.1 hours, respectively. First-step mutants of *S. aureus* ATCC 25923 were isolated at frequencies of 4.1 x 10⁻⁹ to 1.8 x 10⁻¹⁰ at concentrations of 2 and 4 x MIC (MIC increased from 0.25 to 2-4 mg/L). No mutants were isolated at 8x MIC (2 mg/L) (frequency <2.9 x 10⁻¹⁰). MICs of the mutants were similarly increased to pristinamycin and erythromycin A but not to pefloxacin, chloramphenicol or tetracycline. No mutants of *S. aureus* AS5155 were isolated at concentrations of 2, 4, or 8 x MIC (1, 2, and 4 mg/L, respectively) (frequency <3.1 x 10⁻¹⁰).

Conclusion: NXL103 exerts antibacterial activity against both community- and hospital-acquired MRSA. An *in vitro* post-antibiotic effect of about 2h is observed as well as a low spontaneous frequency of resistance, findings which support additional clinical evaluation of this compound for complicated skin and skin structure infections.

INTRODUCTION

Linopristin/flopristin (NXL103; formerly XRP2868) is an orally bioavailable streptogramin clinical candidate exhibiting substantial *in vitro* antibacterial activity against many pathogens responsible for respiratory tract (RTI) and complicated skin and skin structure infections (cSSSI).¹⁻⁶ It is composed of a combination of semi-synthetic streptogramin A and B analogs. The A or PI component primarily mediates the antibacterial activity but is synergized by the B or PII component particularly in regard to bactericidal activity.^{1,2,7-9} Flopristin, the streptogramin A component of NXL103, exhibits a lower MIC than the equivalent dalopristin component of Synercid® or the PII component of Pyostacine®, an orally-bioavailable streptogramin approved for use only in France and a few other countries.⁷⁻¹¹ The potential importance of NXL103 stems from its potent bactericidal activity and oral route of dosing such that it may be useful in the treatment of cSSSI caused by pathogens including community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) or *Streptococcus spp.*, or treatment of community-acquired pneumonia (CAP) due to *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, or atypical pathogens.¹⁻¹⁶ Remarkable evolution has continued to occur in regard to the clinical MRSA problem as evidenced by the advent of glycopeptide resistance in the hospital and the community-associated isolates with increased virulence. These epidemiological trends and the lack of effective oral antibiotics to aid in the therapy of cSSSI are important drivers for the development of antibacterials like linopristin/flopristin.

STRUCTURE



Linopristin (PI)

Flopristin (PII)

NXL103

METHODS

Bacterial strains were collected from hospitals in the Europe and the U.S. CA-MRSA were of SCCmec groups IV and V.

Reference strains including specific antibiotic resistant isolates were obtained from ATCC and NARSA.

Minimal inhibitory concentration was determined by broth microdilution method in cation-adjusted Mueller-Hinton broth according to CLSI guideline¹⁷. For *in vitro* susceptibility testing, linopristin/flopristin is combined in a 30:70 weight/weight ratio.

Post-antibiotic effect was determined following incubation at 4x MIC for two hours. Briefly, following antibiotic treatment bacterial cells were washed to remove antibiotic and then incubated in antibiotic-free medium at 37°C until one-log of regrowth was observed. The difference in time (hours) between return to log growth of antibiotic-treated versus untreated control bacteria was reported as the PAE.^{18,19}

Spontaneous mutational frequency was determined by plating of bacterial cells on Mueller-Hinton Agar containing 2x, 4x, or 8x MIC of antibiotic. Following incubation, MIC of surviving colonies was determined by the broth microdilution method. Spontaneous frequency is reported as the proportion of plated cells that survive and exhibit elevated MIC as compared to the bacterial inoculum.²⁰

REFERENCES

1. Antimicrob. Agents Chemother. 49:3034-3039.
2. Antimicrob. Agents Chemother. 50:237-242.
3. Antimicrob. Agents Chemother. 47:3270-3274.
4. Abstract. F1-365, 2008; ICAAC, Washington, DC.
5. Antimicrob. Agents Chemother. 49:408-413.
6. Antimicrob. Agents Chemother. 50:243-249.
7. Curr. Med. Chem. 4:185-217.
8. Expert Opin. Investigat. Drugs. 10:185-198.
9. Chem. Rev. 105:529-542.
10. J. Antimicrob. Chemother. 51:731-735.
11. Brit. Med. J. 325:864-868.
12. Curr. Opin. Infect. Dis. 16:103-124.
13. Clin Microbiol Infect. Suppl 4:8-15.
14. J. Antimicrob. Chemother. 44:19-23.
15. Dermatol. Clin. 16:509-525.
16. Dermatol. Clin. 15:341-349.
17. CLSI. 2006. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard- Seventh Edition. CLSI, Wayne, PA.
18. Antibiotics in Laboratory Medicine, p 296-329, 1996. V. Lorian (ed.). The Williams & Wilkins Co., Baltimore, MD.
19. Antimicrob. Agents Chemother. 44:1059-1061.
20. Biochem. Biophys. Res. Commun. 36:179-184.

RESULTS

Table 1. Susceptibility (MIC, mg/L) of community-associated MRSA (N=48) to linopristin/flopristin (NXL103) and comparators

	Linopristin/ flopristin	Quinupristin/ dalopristin	Linezolid	Vancomycin	Daptomycin	Tigecycline	Imipenem
MIC Range	0.06-0.5	0.25-2	0.5-4	0.25-2	0.25-2	0.06-1	0.125->32
MIC50	0.12	0.25	2	1	1	0.25	8
MIC90	0.25	1	2	1	1	0.5	32

Table 2. Susceptibility (MIC, mg/L) of hospital-associated MRSA (N=112) to linopristin/flopristin (NXL103) and comparators

	Linopristin/ flopristin	Quinupristin/ dalopristin	Linezolid	Erythromycin A	Vancomycin	Daptomycin	Tigecycline	Imipenem
MIC Range	0.06-0.5	0.12-1	1-16	0.5->16	1-4	0.5-32	0.12-0.5	≤0.06->32
MIC50	0.25	0.5	2	>16	1	1	0.25	8
MIC90	0.5	1	4	>16	2	2	0.25	>32

Table 3. Susceptibility (MIC, mg/L) of *S. aureus* reference and other strains to linopristin/flopristin (NXL103) and comparators

Strain	Antibiotics					
	Linopristin/flopristin	Linezolid	Vancomycin	Daptomycin	Tigecycline	Imipenem
ATCC 29213	≤0.03	2	0.5	0.5	0.06	≤0.03
ATCC25923	0.06	2	1	1	0.125	≤0.03
AS5155	0.25	2	1	1	0.125	8
DEL4811	0.25	2	1	1	0.125	8
IP8203	0.06	2	1	0.5	0.25	≤0.03
209P	≤0.03	2	1	0.5	≤0.03	≤0.03
NRS 77	≤0.03	2	1	1	0.06	≤0.03
NRS72	0.06	2	1	1	0.25	≤0.03
NRS100	0.06	2	2	1	2	>32
NRS123	≤0.03	2	1	1	0.5	1
NRS70	0.25	1	0.5	1	0.06	16
NRS71	0.5	2	0.5	1	0.25	>32
NRS1	0.25	1	8	8	1	32
NRS269	0.5	2	2	2	16	>32
NRS127	0.25	32	1	1	0.25	8
NRS119	0.125	32	1	2	0.06	>32
TAT2420	0.5	1	2	2	0.25	>32

NRS: NARSA

Table 4. *In vitro* post-antibiotic effect (PAE, hours) of linopristin/flopristin (NXL103) and comparators against *S. aureus*

	Linopristin/flopristin	Linezolid	Vancomycin
<i>S. aureus</i> ATCC 29213	6.5	1.3	2
<i>S. aureus</i> ATCC 25923	5.6	2.1	2
<i>S. aureus</i> AS5155	2	1.75	2
<i>S. aureus</i> DEL4811	2.1	0.96	0.75

Table 5. Spontaneous mutation frequency at 2-8 fold-MIC for *S. aureus* against linopristin/flopristin (NXL103) and vancomycin

	Linopristin/flopristin			Vancomycin		
	2x	4x	8x	2x	4x	8x
<i>S. aureus</i> ATCC 29213	1.5x10 ⁻¹⁰	1.0x10 ⁻¹⁰	<1.0x10 ⁻¹⁰	<1.0x10 ⁻¹⁰	<1.0x10 ⁻¹⁰	<1.0x10 ⁻¹⁰
<i>S. aureus</i> ATCC 25923	4.1x10 ⁻⁹	1.8x10 ⁻¹⁰	<2.9x10 ⁻¹⁰	<1.0x10 ⁻¹⁰	<1.0x10 ⁻¹⁰	<1.0x10 ⁻¹⁰
<i>S. aureus</i> AS5155	<3.1x10 ⁻¹⁰	<3.1x10 ⁻¹⁰	<3.1x10 ⁻¹⁰	<3.1x10 ⁻¹⁰	<3.1x10 ⁻¹⁰	<3.1x10 ⁻¹⁰
<i>S. aureus</i> DEL4811	<3.1x10 ⁻¹⁰	<3.1x10 ⁻¹⁰	<3.1x10 ⁻¹⁰	<3.1x10 ⁻¹⁰	<3.1x10 ⁻¹⁰	<3.1x10 ⁻¹⁰

CONCLUSION

1. MICs of the oral streptogramin clinical candidate linopristin/flopristin (NXL103) were 2-4 fold lower than for quinupristin/dalopristin against MRSA, and it was 4-8 fold more active than linezolid against the bacterial strains tested. No substantial differences were noted in susceptibility between community-associated and hospital associated MRSA.
2. Susceptibility to linopristin/flopristin (NXL103) was similar for strains susceptible or resistant to linezolid, daptomycin, and tigecycline, or intermediate susceptible to vancomycin.
3. PAE of linopristin/flopristin (NXL103) was similar to or longer than that of vancomycin, with a minimum PAE of 2 hours.
4. Spontaneous mutational frequency of *S. aureus* to linopristin/flopristin (NXL103) was very low and the few mutants selected exhibited MICs increased 4-16 fold; cross-resistance to pristinamycin and erythromycin A but not to other antibacterials was observed (data not shown).