

Antibacterial Activity of NXL103 (linopristin/flopristin), against Intracellular *Staphylococcus aureus* and Efficacy Following *per oral* Administration in Murine Models of Systemic Infection

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ABSTRACT

Objectives: NXL103 (linopristin/flopristin) is an oral streptogramin which was recently evaluated in a Phase II clinical trial. Activity has been observed against a wide variety of Gram-positive pathogens as well as *Haemophilus influenzae* and *Moraxella catarrhalis*. We here report *in vitro* antibacterial activity against intracellular *Staphylococcus aureus* (SA) and *in vivo* efficacy in murine infection models.

Methods: SA strains were opsonised in medium 199 containing 10% guinea-pig serum and incubated with J774 macrophages followed by inactivation of extracellular bacteria, and incubation with antibiotic (2-8x MIC; 1-4 h). Surviving intracellular bacteria were released by sonication and enumerated by plate counting. Septicemia was established in ICO:OF1 mice by intraperitoneal injection of approximately 7×10^5 CFU in 7.5% mucin suspension following growth in BHI broth. Efficacy in the murine thigh model was evaluated following injection of the right thigh muscle with 3×10^7 CFU of bacteria. Antibiotics were administered either orally (p.o.) or subcutaneously (s.c.) at 1 and 6 h post-infection, and the 50% effective dose (ED₅₀) was determined.

Results: NXL103 reduced susceptible SA counts from 1.25 to 1.96 log₁₀ within 4 h at concentrations from 1x - 8x MIC. Counts of two intracellular MLS₂-constitutively resistant SA strains were reduced by 1.41 to 1.58 log₁₀ when infected macrophages were treated with 8x MIC. This antibacterial effect of NXL103 was superior to that observed with clarithromycin or vancomycin. In the murine model of systemic bacterial infection, the NXL103 ED₅₀ (p.o.) ranged from 20 to 60 mg/kg for the methicillin-susceptible and methicillin-resistant MLS₂ strains tested. Oxacillin and clarithromycin activities (p.o.) were similar or inferior to NXL103 depending on the strain susceptibility, and the vancomycin ED₅₀ (s.c.) was 2.2-2.4 mg/kg for treatment of septicaemia, and 14-40 mg/kg in the thigh model.

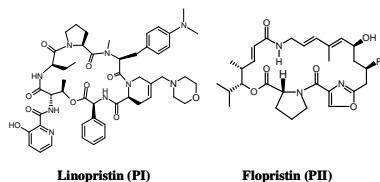
Conclusion: NXL103 kills intracellular bacteria in infected macrophages *in vitro* and is efficacious against *S. aureus*, including drug-resistant strains, following oral administration to infected mice.

INTRODUCTION

Linopristin/flopristin (NXL103; formerly XRP2868) is an orally bioavailable streptogramin clinical candidate exhibiting substantial *in vitro* antibacterial activity against many pathogens causative for complicated skin and skin structure (cSSSI) and respiratory tract infections (RTI).¹⁻⁶ The PI component flopristin, and the PI component linopristin act synergistically to mediate antibacterial activity against susceptible bacteria.^{1,2,7-9} Flopristin is perhaps the most active streptogramin A component that has been evaluated in human clinical trials.⁷⁻¹¹

Great concern has resulted from the continued evolution of virulence and antibiotic resistance among the MRSA.¹²⁻¹⁴ Further, owing to this resistance a limited selection of oral antibiotics is available to the physician either for parenteral-oral switch therapy, or for outpatient treatment of less severe infections. These conditions greatly elevate the potential importance of antibacterial agents like NXL103. Additionally, many pathogens including MRSA may reside at privileged intracellular sites in the host where many antibiotics may not readily penetrate to sufficient levels to result in eradication. Streptogramins appear to be able to access these sites, thus NXL103 might reduce the incidence of recurrence due to viable intracellular MRSA.¹⁶⁻¹⁸ In this study intracellular killing of MRSA in the J774 macrophage cell line, and *in vivo* efficacy in murine septicaemia and thigh abscess models were evaluated.

STRUCTURE



NXL103

METHODS

Minimal inhibitory concentrations (MIC) were determined by CLSI broth microdilution method.¹⁵ Linopristin/flopristin is in a 30:70 weight/weight ratio.

For evaluation of intracellular bacterial killing, *Staphylococcus aureus* cells were opsonised in Medium 199 containing 10% guinea-pig serum (1 h, 37°C), washed and then added to a suspension of J774 macrophage cells in Medium 199 (10⁶ to 10⁷ cells/mL).¹⁶⁻¹⁸ Following incubation to allow uptake (90 min, 37°C) extracellular bacteria were removed by lysostaphin treatment (5 IU/mL, incubation for 30 min, 37°C; followed by removal of lysostaphin with chymotrypsin), and treatment with antibiotic was initiated. Antibiotic was removed by washing, infected macrophages lysed, and surviving bacteria enumerated by plating on trypticase soy agar.

***In vivo* efficacy** was determined with ICO:OF1 mice. (1) In the septicaemia model, animals were injected with bacteria (0.5 ml i.p.; $1.3-1.5 \times 10^8$ CFU/mL) in a 7.5% mucin suspension. At six days post-infection the ED₅₀ was calculated as described by Miraglia in mg/kg/administration which protected 50% of the treated mice when 100% of the controls died.¹⁹ (2) In the case of the murine thigh model, bacteria (0.1 ml; approx. 3×10^8 CFU/mL) were injected into the right thigh of each mouse. Ten days after treatment, mice were euthanized by CO₂ inhalation and examined for septic abscess and viable bacteria in the muscle tissue according to Rolin et al.²⁰ For both models, NXL103 or clarithromycin were administered orally, and vancomycin subcutaneously at 1 and 6 hours post-infection.

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RESULTS

Table 1. Minimal inhibitory concentration (MIC, mg/L) for NXL103 (linopristin/flopristin) and comparator antibiotics

	Linopristin/flopristin	Clarithromycin	Vancomycin
<i>S. aureus</i> Rob	0.12	0.25	1
<i>S. aureus</i> IP8203	0.06	0.5	1
<i>S. aureus</i> AS5155	0.5	>128	1
<i>S. aureus</i> TAT2470	0.5	>128	2
<i>S. aureus</i> DEL4811	0.5	>128	1

NXL103 is 2-16 fold more active than vancomycin, and exhibits activity against macrolide-resistant strains of MRSA.

Table 2. *Survival of intracellular *S. aureus* in human macrophages

Fold MIC		Control			Linopristin/flopristin			Clarithromycin			Vancomycin		
		1h	2h	4h	1h	2h	4h	1h	2h	4h	1h	2h	4h
<i>S. aureus</i> Rob	2x MIC	0.25±0.08	0.72±0.13	0.95±0.1	-1.23±0.07	-1.48±0.07	-1.25±0.01	-0.43±0.04	-0.42±0.03	-0.47±0.02	-0.3±0.0	-0.49±0.03	-0.47±0.02
	4x MIC	ND	ND	ND	-1.94±0.05	-2.0±0.06	-1.96±0.06	-0.37±0.03	-0.51±0.01	-0.49±0.03	+0.06±0.01	+0.22±0.02	+0.42±0.06
	8x MIC	ND	ND	ND	-1.83±0.05	-2.06±0.03	-1.92±0.02	-0.45±0.05	-0.37±0.03	-0.55±0.02	-0.02±0.03	+0.01±0.02	+0.19±0.08
<i>S. aureus</i> AS5155	2x MIC	0.35±0.12	0.7±0.1	1.28±0.12	0.02±0.02	0.04±0.08	-0.21±0.11	ND	ND	ND	-0.02±0.04	0.01±0.01	0.06±0.03
	4x MIC	ND	ND	ND	-0.22±0.03	-0.15±0.01	0.06±0.03	ND	ND	ND	-0.02±0.04	0.01±0.01	0.06±0.03
	8x MIC	ND	ND	ND	-0.73±0.04	-0.97±0.02	-1.41±0.04	ND	ND	ND	-0.74±0.02	-0.68±0.05	-0.72±0.02
<i>S. aureus</i> TAT2470	2x MIC	0.16±0.12	0.35±0.12	1.0±0.12	-0.35±0.02	-0.31±0.05	-0.38±0.03	ND	ND	ND	-0.57±0.03	-0.67±0.02	-0.63±0.05
	8x MIC	ND	ND	ND	-1.58±0.09	-1.72±0.07	-1.55±0.08	ND	ND	ND	-0.57±0.03	-0.67±0.02	-0.63±0.05

*change in log bacterial number

Greater reduction in intracellular *S. aureus* is observed following NXL103 treatment than for clarithromycin or vancomycin.

Table 3. NXL103 (linopristin/flopristin) and comparator efficacy (ED₅₀ mg/kg) in the murine septicaemia model

	Linopristin/flopristin	Clarithromycin	Vancomycin
<i>S. aureus</i> IP8203	20	18	2.4
<i>S. aureus</i> AS5155	44	>300	2.2

NXL103 is active *in vivo* against macrolide resistant *S. aureus*.

Table 4. NXL103 (linopristin/flopristin) and comparator efficacy (ED₅₀ mg/kg) in the murine thigh model

	Linopristin/flopristin	Clarithromycin	Vancomycin
<i>S. aureus</i> Rob	24	6.5	14
<i>S. aureus</i> AS5155	60	>300	22
<i>S. aureus</i> TAT2470	60	>300	19
<i>S. aureus</i> DEL4811	50	>300	40

NXL103 exhibits similar activity in both septicaemia and thigh models.

CONCLUSION

1. Linopristin/flopristin (NXL103) reduced numbers of intracellular bacteria in a macrophage cell line *in vitro* more effectively than clarithromycin or vancomycin; linopristin/flopristin was active against intracellular bacteria resistant to clarithromycin.
2. In the mouse septicaemia model oral linopristin/flopristin was of similar activity to that of clarithromycin against susceptible bacteria, and active against clarithromycin-resistant strains, but less active than subcutaneously administered vancomycin.
3. In the mouse thigh model, oral linopristin/flopristin ED₅₀ ranged from 24 to 60 mg/kg while the activity of subcutaneously administered vancomycin ranged from 14 to 40 mg/kg; again linopristin/flopristin was efficacious against clarithromycin-resistant strains.