

Efficacy of Oral NXL103 against *Haemophilus influenzae* and *Streptococcus pneumoniae* in Mouse Pneumonia Models

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ABSTRACT

Background: NXL103 is a novel oral streptogramin antibiotic with potent activity against gram-positive bacteria, *H. influenzae*, *M. catarrhalis* as well as atypical and intracellular pathogens involved in respiratory tract infections (RTI). We report the activity of NXL103, in two different models of pulmonary infections in the mouse.

Methods: *H. influenzae*: C57Bl/6 aged-mice were intratracheally (IT) infected with 10^8 CFU of a serotype b and β -lactamase positive strain (Hib). Bacterial kinetics of NXL103 (MIC: 0.125 μ g/mL), erythromycin (ERY, MIC: 4 μ g/mL) and augmentin (Aug, MIC: >128 μ g/mL) were recorded for 24 hours following a single oral administration 15 h post-infection (p.i.). *S. pneumoniae*: Swiss OF1 mice were infected IT with 10^6 CFU of a fully susceptible virulent strain (Sp 4241 – EryS/PenS/FQS). Treatment with NXL103 (50 and 100 mg/kg) and Aug (25 mg/kg), b.i.d for 3 days was initiated per os 18 h p.i. in groups of 10-20 mice, when all animals were bacteremic. Survival and bacterial kinetics were recorded until 14 days p.i.

Results: NXL103 showed potent *in vitro* activity against both strains (MIC 0.125 μ g/mL). Against Hib, NXL103 and Aug exhibited similar activities at 24 h (mean log CFU \pm SD: 5.7 \pm 2 and 5.2 \pm 1.8) compared to untreated controls and Ery (mean log CFU \pm SD: 8 \pm 0.2 and 7.4 \pm 0.5) ($p < 0.05$), but initial bacterial clearance after 8 h, was more efficient with NXL103 (mean log CFU \pm SD: 5.9 \pm 1) compared to Aug and Ery (mean log CFU \pm SD: 8.5 \pm 0.1 and 8.1 \pm 1.5) ($p < 0.05$).

With the pneumococci, all untreated animals succumbed to the infection by day 4 p.i. Survival rates at 14 days p.i were comparable for NXL103 and Aug (75% and 73% respectively). However bactericidal activity of NXL103 was more efficient with NXL103 by about 1.5log CFU compared to Aug (mean log CFU \pm SD: 2.3 \pm 0.7 vs 4 \pm 1.2, $p < 0.05$).

Conclusion: The potent *in vitro* activity of NXL103 against *H. influenzae* and *S. pneumoniae* translated into good *per os* efficacy in the murine models of pneumonia and can be considered as the first-line antibiotic in RTI.

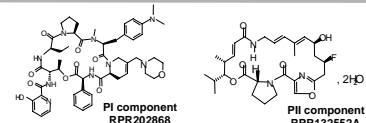
INTRODUCTION

Major bacterial pathogens responsible for community-acquired respiratory tract infections are *Streptococcus pneumoniae*, *Haemophilus influenzae*, and to a lesser extent *Moraxella catarrhalis*. Of existing oral compounds active against all three of these organisms, only amoxicillin/clavulanate and the broad-spectrum quinolone group are active against these three species. There is doubt as to the clinical activity of the macrolide-azalide-ketolide group against *H. influenzae* Of the streptogramin group, quinupristin/dalfopristin has MICs against *H. influenzae* which are above achievable serum levels (1, 2).

A new oral agent with a different mechanism of activity is needed against these organisms, especially in children where quinolones cannot be used. NXL103 (formerly XRP2868) is a novel semi-synthetic oral streptogramin which is a 70:30 combination of RPR132552 (pristinamycin IIB) and RPR202868 (pristinamycin IA). It has previously been shown *in vitro* to be potent against *H. influenzae*, *S. pneumoniae* and other Gram-positive organisms (3, 4, 5). NXL103 is being developed for the treatment of respiratory tract and skin and skin structure infections (6, 7).

The purpose of this study was to evaluate the *in vivo* activities of NXL103 in murine models of pneumonia caused by *H. influenzae* and *S. pneumoniae* and compare the potency of this drug with those of erythromycin or augmentin (amoxicillin/clavulanate).

STRUCTURE OF NXL103



NXL103 is 30% PI / 70% PII

METHODS

Bacterial strains:

S. pneumoniae (Sp 4241): is a capsulated serotype 3 virulent strain, originally isolated from blood culture of a patient. This strain is susceptible to penicillin, erythromycin and quinolones. *H. influenzae* (Hib 351RD7): This is a clinical isolate from Novoxel strain collection and is serotype b and β -lactamase positive strain.

Antibiotics:

Erythromycin (ERY) was provided by Aventis pharma; Augmentin iv (Amoxicillin/Clavulanate - 1 g / 200 mg) (AUG) was commercially available; NXL103 was from Novoxel.

in vitro:

Minimum inhibitory concentrations (MICs) were determined using CLSI (formerly the NCCLS) methods for antimicrobial susceptibility testing with cation-adjusted Mueller-Hinton broth (MH) supplemented with 5% red cell extract for *S. pneumoniae*. For *H. influenzae*, MH broth was supplemented with 15 μ g/mL of hemine, 5 mg/mL of yeast extract and 15 μ g/mL NAD. MIC was defined as the lowest concentration of antibiotic at which no turbidity was visible to the naked eye.

in vivo:

1. Pharmacokinetics studies in mice:

Drug concentrations in the epithelial lung fluid (ELF) and plasma ($n=8$ mice per time point) were determined by LC-MS/MS after administration of a single oral dose of NXL103 at 100 mg/kg in non infected and infected mice (Sp model: 18h p.i. and Hib model: 15h p.i.). The blood and ELF samples were obtained 0.25, 0.5, 1, 3 and 6 hours after drug administration. Drug concentrations in ELF were corrected for the ELF/Plasma ratio of urea.

2. Experimental *H. influenzae* pneumonia in mice:

Pneumonia was induced in C57Bl/6J mice aged 5-6 months by intratracheal inoculation with 50 μ L of a bacterial suspension of Hib (strain 351RD7) 10^8 CFU into anesthetized mice. Mice developed inflammatory bronchopulmonary disease that cured spontaneously. The NXL103, ERY or AUG were orally administered 15h after infection to each group ($n=10-20$ mice) with a single dose at 100mg/kg. A group of infected mice received only the vehicle.

Bacterial clearance: The bacterial kinetics in the lungs were performed 2, 5, 8 and 24 hours after the drug administration. ($n=4$ mice).

3. Experimental pneumococcal pneumonia in mice:

Pneumonia was induced in female Swiss mice (OF1; 20-22g, 7-8 weeks) by intratracheal inoculation with 50 μ L of a bacterial suspension of Sp (strain 4241) 10^6 CFU into immunocompetent mice. Mice developed bacteremic pneumonia and fatal disease within 2 to 4 days.

Survival study: Mice were treated 18h post infection *per os* with respectively 50 or 100 mg/kg of NXL103 and 25 mg/kg of AUG. Treatment consisted of six administrations at 10 to 12 hours intervals. Infected, non-treated mice received the same volume of vehicle. 12 animals were used per group. Death rates were recorded daily over a 14-days period, and cumulative survival rates were compared.

Bacterial clearance: Bacterial counts in the lungs ($n=5$ mice) were performed: 6 hours after the 1st, 3rd, 5th and 24 hours after the last drug administration.

RESULTS

in vitro activity:

Table 1. MICs (mg/L) of NXL103 and comparators

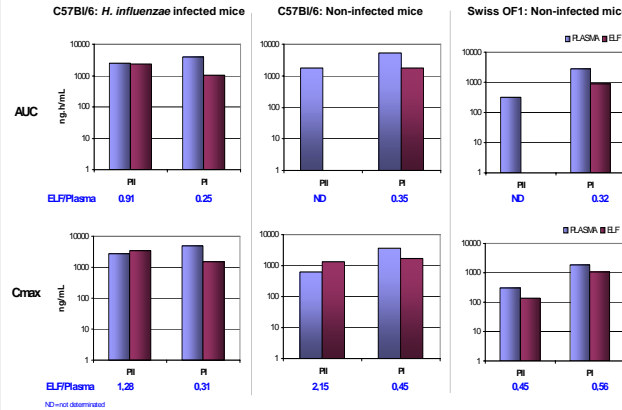
Strains	Lab ID	NXL103	AUG	ERY
<i>S. pneumoniae</i>	4241	0.125	≤ 0.03	0.06
<i>H. influenzae</i>	351RD7	0.125	>128	4

NXL103 showed potent *in vitro* antibacterial activity against *S. pneumoniae* and *H. influenzae*

RESULTS

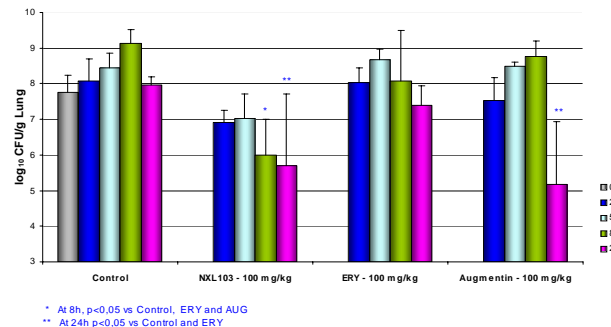
Pharmacokinetics of NXL103:

Figure 1. Mean Plasma and ELF pharmacokinetic parameters of NXL103 in mice



H. influenzae pneumonia:

Figure 2. Clearance of *H. influenzae* 351RD7 (serotype b and β -lactamase positive) from lungs of infected mice treated with NXL103, Erythromycin and Augmentin



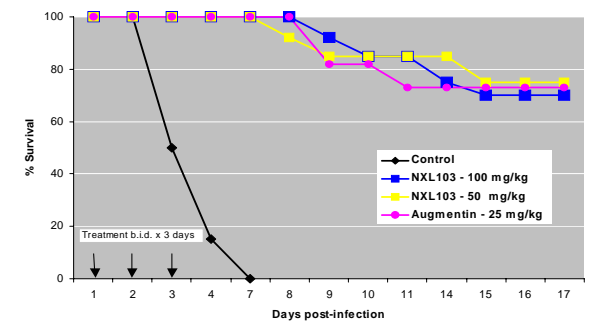
In the respiratory tract infection due to *H. influenzae* 351RD7, treatment with NXL103 led to significant reductions in viable bacterial counts in the lungs compared to untreated mice. NXL103 was more effective than erythromycin and augmentin at 8h.

CONCLUSION

- The potent *in vitro* activity of NXL103 against clinical isolates of *S. pneumoniae* and *H. influenzae* translated into good oral efficacy in mouse pneumonia models.
- In mice, NXL103 has a good distribution in the lung tissue and reduced the *H. influenzae* and pneumococcal lung burden, suggesting potential for this compound in the treatment of respiratory tract infections.

Pneumococcal pneumonia: Survival

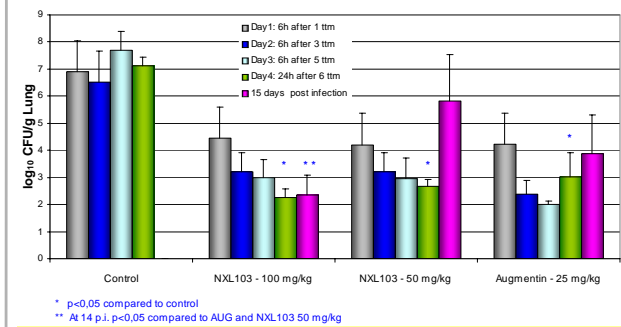
Figure 3. Survival of mice challenged intratracheally with *S. pneumoniae* Sp 4241 (penicillin- and macrolide- sensitive) and treated with NXL103 and Augmentin



In pneumococcal pneumonia model, all control mice died within 2- 4 days of infection. NXL103 and augmentin resulted in survival of 70% – 75% of animals 16 days post infection.

Bacterial clearance

Figure 4. Clearance of *S. pneumoniae* Sp 4241 (penicillin- and macrolide- sensitive) from lungs of infected mice treated with NXL103 and Augmentin



In the respiratory tract infection caused by *S. pneumoniae* SP4241, NXL103 reduced the number of viable cells in the lungs of mice treated with 50 and 100 mg/kg. However after 14 days postinfection, a 100 mg/kg of NXL103 showed better *in vivo* activity compared to 50 mg/kg NXL103 and 25 mg/kg augmentin. All pneumococci recovered after treatment remained susceptible to NXL103.

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