

ABSTRACT (amended)

Background: NXL104 is a novel non- β -lactam β -lactamase inhibitor able to inhibit both class A and class C β -lactamases *in vitro* and *in vivo*. The CAZ / NXL104 combination has shown clinical efficacy in murine models of infection induced by CAZ-resistant (CAZ^R) strains. We investigated NXL104 ability to cross inflamed meninges and to protect CAZ from β -lactamase hydrolysis.

Methods: Rabbits were intra-cisternally inoculated with an AmpC producing *K. pneumoniae* strain. MICs were: CAZ>128 mg/L, meropenem (MPN) 1 mg/L, and CAZ/NXL104 combination 1 mg/L. Intravenous (i.v) therapy was given 8 hours post-infection: CAZ 150 mg/kg, alone or in combination with NXL104, 37.5 mg/kg (4/1 ratio), or MPN 125 mg/kg. Cerebrospinal fluid (CSF) and blood were sampled during 8 hours and tested for CAZ, MPN and NXL104 concentrations; bacteria were enumerated in CSF for 8 hours.

Results: Kinetics of NXL104 in rabbit plasma and CSF paralleled those of CAZ : CAZ and NXL104 levels in the CSF increased slowly after i.v. dosing and peaked at 1-2 hours after injection. After a single dose, the mean penetration of NXL104 into CSF was 38%. CAZ monotherapy was unable to reduce the CSF bacterial load whilst MPN exhibited a mean killing rate of -4.23 log₁₀ over 8 hours. Co-administration of NXL104 restored significantly the CAZ antimicrobial efficacy since the mean killing rate was -5.66 log₁₀ /8h for the combination regimen (p<0.05).

Conclusion: NXL104 was shown to penetrate into rabbit inflamed meninges. CAZ / NXL104 combination demonstrated effective bacterial clearance in CSF of rabbits infected by a CAZ^R AmpC-producing *K. pneumoniae* strain. These data are indicative of a therapeutic potential for ceftazidime/NXL104 in the treatment of gram-negative meningitis including class C producing organisms.

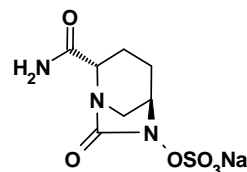
BACKGROUND

NXL104 is a novel non- β -lactam inhibitor of β -lactamases currently in clinical development. It displays a broad spectrum inhibition profile for both class A and class C enzymes; both types of enzymes are inactivated very efficiently at low IC₅₀ values, with low turn-over numbers and long covalent intermediate half-lives (1). NXL104 has virtually no intrinsic antibacterial activity, but efficiently protects β -lactams from hydrolysis in a variety of class A and class C producing strains, including ESBL producers. Protection against acute lethal infections has been demonstrated in murine models (2, 3).

The primary objective of this study was to investigate the ability of NXL104 to cross inflamed meninges, and to attain measurable concentrations in cerebrospinal fluid of rabbits. The second objective was to evaluate if cerebrospinal fluid concentrations of NXL104 achieved were sufficient to result in a successful therapy of Gram-negative meningitis, when given in combination with ceftazidime.

The pharmacokinetics of NXL104 and the bacteriological effect of ceftazidime / NXL104 combination were assessed in rabbits experimentally infected with a *Klebsiella pneumoniae* strain (283KB4 strain). This strain is resistant to ceftazidime due to production of a DHA-2 class C β -lactamase; in contrast, the strain is susceptible to the combination of ceftazidime / NXL104.

Figure 1: Chemical structure of NXL104



METHODS

Bacterial strain and susceptibility testing
MIC determination was performed using CLSI methods for antimicrobial susceptibility testing with cation adjusted Mueller-Hinton (MH) broth. MIC was defined as the lowest concentration which inhibited all visual growth. β -lactam / β -lactamase inhibitor combinations were tested at a 4/1 ratio w/w. The strain was adapted by three sequential passages in three animals. The Colony Forming Units (CFU) collected from the last passage were grown in MH broth, stored and used for all further experiments. MICs were not modified by passaging in animals.

Experimental meningitis model
The model described by Dacey (4) was used with pathogen-free New Zealand rabbits. The animals were inoculated with about 10⁵ CFU by direct injection into the subarachnoid space.

Antibiotic treatments given intravenously as a bolus were initiated (T0) approximately 8 hours following infection:
- meropenem at 125 mg/kg at hour 0 and at hour 4
- ceftazidime at 150 mg/kg at hour 0 and at hour 4
- ceftazidime at 150 mg/kg + NXL104 at 37.5 mg/kg (i.e. at antibiotic/inhibitor ratio of 4/1) at hour 0 ; then ceftazidime alone at 150 mg/kg at hour 4.
CSF (0.2 ml) was sampled at 0, 1, 2, 4, 6, and 8 hours after initiation of treatment. Blood was sampled at 0.25, 0.5, 1, 2, 3, 4, 4.25, 4.5, 5, 6, 7 and 8 hours after initiation of treatment.

Determination of antibiotic levels and CFU-titers
The meropenem concentrations in plasma and CSF were determined by diffusion microbioassays using agar plates containing *E. coli* ATCC 25922. In parallel, ceftazidime and NXL104 concentrations were determined in plasma and CSF using a LC-MS/MS method. The limits of quantitation were 0.05 and 0.01 mg/L for ceftazidime and NXL104, respectively.

AUC_t values were calculated as the area under the concentration time curve up to the last quantifiable datapoint.

CFUs were measured by serial dilution of CSF plated on MH agar plates and incubated overnight at 37°C.

Statistical methods
The Student t-Test and one-way analysis of variance (Newman-Keuls multiple comparisons test) were used for parametric data. Comparison of positive and negative cultures (CFU and killing rate values) were analyzed by the two-tailed Fisher exact test. A P-value of <0.05 was considered significant.

RESULTS

Table 1: Susceptibility testing

<i>K. pneumoniae</i> 283KB4 (DHA-2 class C)	
MIC values (mg/L)	
Ceftazidime	>256
Ceftazidime + clavulanic acid (4/1)	128
Ceftazidime + NXL104 (4/1)	4
Piperacillin + tazobactam (4/1)	128
Imipenem	1
Meropenem	2

NXL104 was able to protect ceftazidime from hydrolysis by the DHA-2 type class C β -lactamase expressed by 283KB4 strain; as a result, the strain was susceptible to ceftazidime / NXL104 combination. In contrast, clavulanic acid or tazobactam could not restore ceftazidime or piperacillin antibacterial activity.

RESULTS

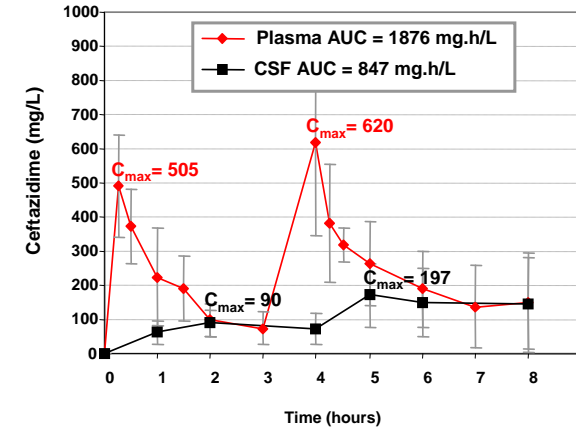


Figure 2: Mean concentration (mg/L) of ceftazidime in CSF and plasma sampled from animals treated with ceftazidime + NXL104.

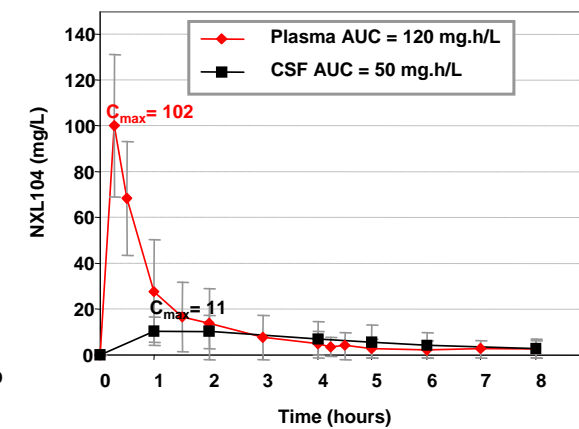


Figure 3: Mean concentration (mg/L) of NXL104 in CSF and plasma sampled from animals treated with ceftazidime + NXL104.

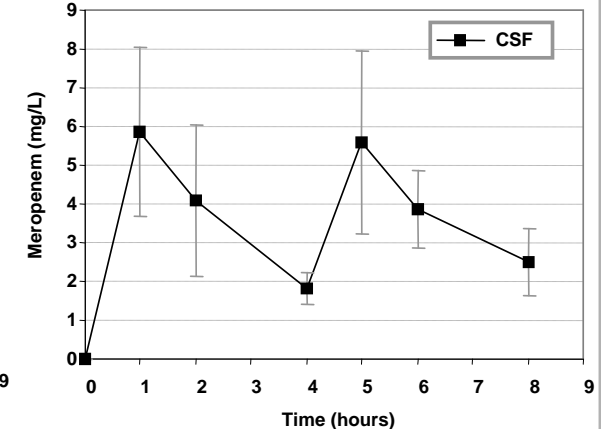


Figure 4: Mean concentration (mg/L) of meropenem in CSF sampled from animals treated with meropenem.

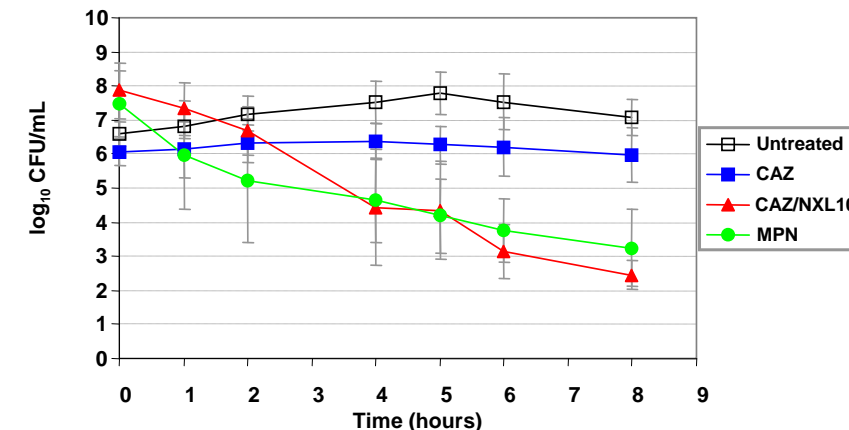


Figure 5: Mean bacterial titres \pm standard deviations in CSF samples.

Treatment	Number of animals	Initial bacterial titre (log ₁₀ CFU/mL) [Mean \pm SD]	Killing rate (log ₁₀ CFU/mL/8h) [Mean \pm SD]
None	5	6.54 \pm 0.36	+0.47 \pm 0.33
Ceftazidime	5	6.06 \pm 0.40	-0.10 \pm 0.45
Ceftazidime / NXL104	5	7.88 \pm 0.82 ^(1, 2)	-5.66 \pm 0.83 ^(1, 2)
Meropenem	5	7.46 \pm 0.97 ⁽¹⁾	-4.23 \pm 0.60 ⁽¹⁾

Table 2: Mean bacterial killing rates in CSF samples from infected rabbits.

(1) : significantly different from the value for ceftazidime treatment (p<0.05)
(2) : significantly different from the value for meropenem treatment (p<0.05)

CONCLUSIONS

The *Klebsiella pneumoniae* 283KB4 strain that expresses a class C β -lactamase was used to induce meningitis in the rabbit model.

1 – NXL104 levels in cerebrospinal fluid showed that it was able to penetrate well into rabbit inflamed meninges. After a single i.v. dosing at 37.5 mg/kg, it peaked around 11 mg/L one to two hours after injection, and almost remained stable, decreasing to 7 mg/L at hour 4. The penetration of NXL104 resulted in a cerebrospinal fluid AUC of approximately 38% of plasma AUC (43% for ceftazidime).

2 – Bacterial titres in cerebrospinal fluid decreased significantly following treatment with ceftazidime / NXL104 combination. The combination therapy demonstrated bactericidal activity (>3log reduction in bacterial count) in cerebrospinal fluid, that was achieved 5 hours following treatment initiation; at the end of the monitoring period (8 hours post therapy), >5 log reduction was observed. Meropenem was also found to be bactericidal, however it decreased the bacterial load to a lower extent than ceftazidime / NXL104 combination ; ceftazidime alone did not produce any significant bactericidal effect.

Overall, it was shown that NXL104 concentrations achieved in cerebrospinal fluid allowed for an effective protection of ceftazidime, that resulted in an efficient clearance of bacterial load.

REFERENCES

- Bonnefoy A et al. J Antimicrob Chemother (2004), 54:410-417
- Levasseur P et al. 45th Interscience Conference on Antimicrobial Agents and Chemotherapy (2005), Communication # F-1164
- Borgonovi M et al. 17th European Congress of Clinical Microbiology and Infectious Diseases (2007), P794
- Dacey RG et al. Antimicrob Agents Chemother (1974), 6:437-441
- Sakata Y et al. Antimicrob Agents Chemother (1983), 23:213-217