

Pharmacokinetics (PK) and Efficacy of Ceftazidime (CAZ) / NXL104 combination in a Murine Pneumonia Model Caused by an AmpC-Producing *Klebsiella pneumoniae*

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ABSTRACT

Background: NXL104 is a novel, non-β-lactam, β-lactamase inhibitor with a spectrum of activity encompassing both class A and class C β-lactamases. It is currently being tested in human phase 2 trials in combination with ceftazidime (CAZ). The aim of the study was to evaluate the efficacy of CAZ/NXL104 combination in a mouse model of lung infection, and to compare NXL104 PK parameters in healthy and infected animals.

Methods: Pneumonia was induced in immunosuppressed mice by intratracheal inoculation of an AmpC + SHV-11 *K. pneumoniae* producer. CAZ/NXL104 (150/37.5 mg/kg), or comparator treatments, were given subcutaneously 16 hr post-infection, q8h for two days. Efficacy was assessed by bacterial lung counts. CAZ, NXL104 and urea concentrations in plasma and bronchoalveolar lavages (BAL) were measured at 8 time-points from 5min to 8hr post-dosing in satellite animals. Drug concentrations in epithelial lining fluid (ELF) were calculated after correction for urea dilution.

Results: All untreated mice died within 48 hr of infection (mean log CFU ± SD = 11 ± 1.7; n = 10 mice). At 48 hr post-treatment, a significant 3-log reduction in lung bacterial counts was observed, as compared to CAZ alone (p < 0.05, mean log CFU ± SD 4.5 ± 1 and 7.9 ± 0.1 respectively). PK parameters of CAZ and NXL104 in plasma were similar in normal and infected mice. ELF exposure was less than plasma exposure: NXL104 ELF/plasma AUC ratios were 0.17 and 0.05 in normal and infected mice, respectively, and were 0.11 and 0.03 for CAZ.

Conclusions: In the pneumonia model, ELF exposure to CAZ/NXL104 combination resulted in therapeutic efficacy, as demonstrated by significant lung bacterial clearance. This β-lactam/β-lactamase combination is a promising new agent against difficult to treat gram-negative respiratory tract infections.

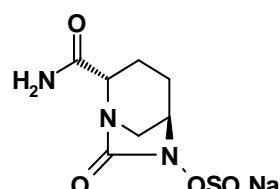
BACKGROUND AND OBJECTIVE

NXL104 is a novel, non-β-lactam, β-lactamase inhibitor with a spectrum of activity encompassing both class A and class C β-lactamases. It is currently being tested in human phase 2 trials in combination with ceftazidime (CAZ).

Study objective: To evaluate the efficacy of CAZ/NXL104 combination in a mouse model of lung infection, and to compare NXL104 PK parameters in healthy and infected animals.

CHEMICAL STRUCTURE

NXL104



MATERIALS AND METHODS

Bacterial isolate and susceptibility testing:

The *Klebsiella pneumoniae* 283KB5 known to harbor the LAT-4 class C and the class A SHV-11 β-lactamases was used in the study. Minimal Inhibitory Concentrations (MIC) for CAZ, imipenem, piperacillin and amoxicillin were determined using CLSI methods for antibiotic susceptibility testing with cation adjusted Mueller-Hinton (MH) broth. NXL104, clavulanate, and tazobactam β-lactamase inhibitors were used either at fixed weight ratios or at fixed concentrations of 4 μg/mL (Table 1).

Table 1 - MIC values (μg/mL) for 283KB5 strain

Treatment	MIC (μg/mL)
Imipenem	2
Aztreonam	32
Amoxicillin + Clavulanate 4 μg/mL	64
Piperacillin + Tazobactam 4 μg/mL	64
Ceftazidime	64
Ceftazidime + NXL104 4 μg/mL	0.5
Ceftazidime + NXL104 4/1	2
Ceftazidime + NXL104 2/1	1
Ceftazidime + Clavulanate 4/1	8

Study scheme: Female Swiss OF1 mice were divided into main and satellite groups for efficacy and PK assessment, respectively.

Main groups, associated treatments and efficacy assessments:

Mice were immunosuppressed with 150 mg/kg cyclophosphamide (i.p.) 3 days prior to infection. Anesthetized mice were intratracheally infected with ca. 10⁸ to 10⁹ cfu of the *K. pneumoniae* 283KB5. All treatments were initiated 16-18 h post-infection, and were given subcutaneously q8h for 2 days¹. Ceftazidime was combined with NXL104 or with clavulanate at 2/1 and 4/1 weight ratios.

- Group 1 (n=20): control, normal saline
- Group 2 (n=30): CAZ 150 mg/kg
- Group 3 (n=30): CAZ/NXL104 150/75 mg/kg (2/1 ratio)
- Group 4 (n=30): CAZ/NXL104 150/37.5 mg/kg (4/1)
- Group 5 (n=30): CAZ/clavulanate 150/75 mg/kg (2/1)
- Group 6 (n=30): CAZ/clavulanate 150/37.5 mg/kg (4/1)
- Group 7 (n=30): imipenem/cilastatin 150 mg/kg each

Efficacy was monitored as the bacterial burden in lung homogenates 24h and 48h after treatment initiation.

Satellite groups and associated treatments:

Treatments were administered as a single subcutaneous dose in groups 8 and 9:

- Group 8 (n=48): normal non-infected mice, CAZ/NXL104 150/37.5 mg/kg (4/1)
- Group 9 (n=48): immunosuppressed infected mice, CAZ/NXL104 150/37.5 mg/kg (4/1), 16hrs post-infection

PK assessments in satellite groups: Blood and BAL were sampled at 8 time-points from 5 min to 8h post-dosing, with 6 mice sampled at each time-point. Concentrations of NXL104 and CAZ in plasma and BAL were determined by LC-MS/MS. The lower limits of quantitation were 0.010 mg/L and 0.050 mg/L for NXL104 and CAZ, respectively. Urea was analyzed in plasma and BAL, using a colorimetric method. Concentrations of CAZ and NXL104 in ELF were calculated from BAL concentrations after correction for urea dilution².

PK analysis was non-compartmental. Standard errors on Cmax and AUC_{last} were computed by the Baile's method.

RESULTS

1. EFFICACY

Table 2: Change in Log10 CFU versus control at time 0

GROUP	TREATMENT	24 HOURS	48 HOURS
1	Control	+1.40	no survivors
2	Ceftazidime	+0.23	-0.70
3	Ceftazidime + NXL104 2/1	-4.96	-6.58
4	Ceftazidime + NXL104 4/1	-5.79	-6.60
5	Ceftazidime + clavulanate 2/1	-1.47	-1.27
6	Ceftazidime + clavulanate 4/1	-0.99	-0.02
7	Imipenem + cilastatin 1/1	-3.41	-6.74

2. PHARMACOKINETICS IN NORMAL NON-INFECTED SATELLITE ANIMALS

Table 3: PK parameters of CAZ and NXL104 in plasma and ELF

	PARAMETER	UNIT	CAZ	NXL104	CAZ / NXL104 RATIO
PLASMA	Cmax	mg/L	122.3 ± 7.1	46.2 ± 2.6	2.7
	AUC _{last}	h.mg/L	77.8 ± 4.4	23.1 ± 1.4	3.4
ELF	Cmax	mg/L	11.4 ± 5.0	8.0 ± 2.6	1.4
	AUC _{last}	h.mg/L	8.4 ± 1.6	3.9 ± 0.7	2.2
ELF / PLASMA RATIO	Cmax	-	0.09	0.17	-
	AUC _{last}	-	0.11	0.17	-

3. PHARMACOKINETICS IN IMMUNOSUPPRESSED INFECTED SATELLITE ANIMALS

Table 4: PK parameters of CAZ and NXL104 in plasma and ELF

	PARAMETER	UNIT	CAZ	NXL104	CAZ / NXL104 RATIO
PLASMA	Cmax	mg/L	113.2 ± 12.6	42.2 ± 6.0	2.7
	AUC _{last}	h.mg/L	84.6 ± 5.2	26.2 ± 2.1	3.2
ELF	Cmax	mg/L	7.8 ± 2.1	5.1 ± 1.6	1.6
	AUC _{last}	h.mg/L	2.5 ± 0.6	1.3 ± 0.3	1.9
ELF / PLASMA RATIO	Cmax	-	0.07	0.12	-
	AUC _{last}	-	0.03	0.05	-

CONCLUSION

PK parameters of CAZ and NXL104 in plasma were similar in normal and infected mice. ELF exposure to NXL104 was less than plasma exposure and was quantified by ELF to plasma AUC_{last} ratios of 0.17 and 0.05 in normal and infected mice, respectively. ELF exposure to CAZ was less pronounced, however demonstrated efficacy when combined with NXL104, with a significant 3-log reduction in lung bacterial counts compared to CAZ alone or CAZ/clavulanate.

REFERENCES

1. P. LEVASSEUR *et al.* 45th ICAAC meeting, Washington DC, 2005. Abstract F-1164.
2. S. KIEM and JJ. SCHENTAG. *Antimicrobial Agents and Chemotherapy*, 2008, 52(1):24–36.

Figure 1: Counts of *K. pneumoniae* 283KB5 (Amp C + Class A) in lung homogenates

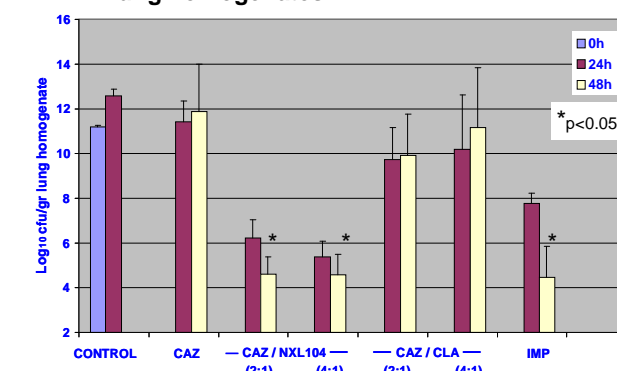


Figure 2: PK profiles of CAZ and NXL104 in plasma and ELF

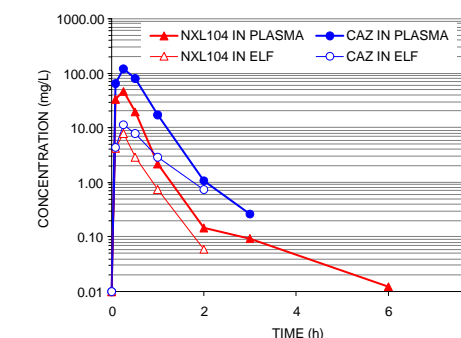


Figure 3: PK profiles of CAZ and NXL104 in plasma and ELF

