

# Efficacy of Ceftazidime (CAZ) /NXL104 Combination in Murine Septicaemia Caused by CTX-M-Producing *Enterobacteriaceae* species

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## AMENDED ABSTRACT

**Background:** NXL104 is a novel  $\beta$ -lactamase inhibitor that has been shown *in vitro* to inhibit both class A and class C enzymes. It is in phase 2 of clinical development in combination with CAZ. Within class A enzymes, CTX-M variants are currently the most prevalent extended spectrum  $\beta$ -lactamases. In this study, the efficacy of CAZ/NXL104 was evaluated against CAZ-resistant (CAZ<sup>R</sup>) *Enterobacteriaceae* bearing CTX-M  $\beta$ -lactamases in a murine septicaemia model.

**Methods:** MIC determination was performed using NXL104 at 4  $\mu$ g/mL fixed concentration, with variable concentrations of CAZ. Septicaemia: Groups of mice (n=10 to 20) were intraperitoneally infected with one of four CAZ<sup>R</sup> strains (MICs 32 - >128  $\mu$ g/mL) which expressed CTX-M  $\beta$ -lactamases; two strains of *Klebsiella pneumoniae* with phenotypes CTX-M-2 + SHV-2 + TEM-12 or CTX-M-2 + TEM-1B and two of *Escherichia coli* with phenotypes CTX-M-2 + TEM-1 or CTX-M-16 + TEM-1. Sub-cutaneous treatments were given one- and four- hours post-infection. CAZ/NXL104 was administered at 4/1 weight ratio; reference treatments were CAZ alone, cefotaxime (CTX) and piperacillin/tazobactam (PIP/TZB). The 50% effective dose (ED<sub>50</sub>) was calculated by the Probit method.

**Results:** MICs of CAZ/NXL104 for the four CTX-M producing strains were 0.5 - 2  $\mu$ g/mL. *In vivo*, CAZ/NXL104 was significantly more effective compared to CAZ, CTX, or PIP/TAZ against all strains (p<0.05). ED<sub>50</sub> ranges of CAZ/NXL104 were 11-27 mg/kg for these strains compared to >60mg/kg, >90 mg/kg, and >90 mg/kg respectively for CAZ, CTX, and TZP.

**Conclusion:** The potent *in vitro* activity of NXL104 when combined with CAZ, against CAZ<sup>R</sup> *Enterobacteriaceae* bearing CTX-M  $\beta$ -lactamases, translates into good parenteral efficacy in the mouse septicaemia model.

## BACKGROUND

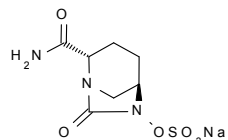
NXL104 is a new non- $\beta$ -lactam inhibitor of  $\beta$ -lactamases. It is currently being tested in human phase 2 trials in combination with ceftazidime (CAZ).

NXL104 has virtually no intrinsic antibacterial activity, but efficiently protects  $\beta$ -lactams from hydrolysis in a variety of class A and class C producing strains, including ESBL producers, both *in vitro* and *in vivo* (1, 2). The  $\beta$ -lactamase landscape is changing radically, with KPC and CTX-M types being the most-prevalent ESBLs worldwide. *In vitro*, combinations of oxymino-cephalosporins with NXL104 have been shown to overcome these resistances (3, 4).

The objective of the present study was to evaluate the *in vivo* activity of CAZ/NXL104 against CTX-M-producing strains of *Enterobacteriaceae* species.

## CHEMICAL STRUCTURE

FIGURE 1  
Chemical structure of NXL104



## MATERIALS AND METHODS

### Test compounds:

- ceftazidime pentahydrate (CAZ), cefotaxime (CTX), piperacillin/tazobactam (PIP/TZB);  
- the  $\beta$ -lactamase inhibitor NXL104

### Susceptibility testing:

MICs for CAZ and CTX alone and in combination with a 4 $\mu$ g/mL fixed concentration of the  $\beta$ -lactamase inhibitor NXL104 and PIP/TZB were determined using CLSI methods for antimicrobial susceptibility testing with cation adjusted Mueller-Hinton (MH) broth (5). MIC was defined as the lowest concentration that inhibited visual growth. The *in vitro* interpretive breakpoint criteria for CAZ and CTX were applied for determination of susceptibility/resistance to NXL104 inhibitor combinations (6).

### Murine septicaemia model:

**Test groups:** Male ICR (CD1) mice (6-7weeks)  
**Infecting strains:** *E. coli* strain Tunisie E4 (CTX-M16 + TEM1);  
*E. coli* strain TN06 (CTX-M2 + TEM1)  
*K. pneumoniae* strain 253 (CTX-M2 + SHV2 + TEM12)  
*K. pneumoniae* strain 465 (CTX-M2 + TEM1B)  
**Infecting inoculum:** 10<sup>8</sup> CFU/mouse  
**Infection:** Mice were infected intraperitoneally with the desired inocula in 0.5 mL hog gastric mucin  
**Treatment doses:** Groups of 10 – 20 infected mice were treated with either:  
CAZ: 3, 10, 30, 60 mg/kg  
CTX: 3, 10, 30, 60, 90 mg/kg  
PIP/TZB 4/1 ratio by weight (30/7.5; 60/15; 90/22.5 mg/kg)  
CAZ/NXL104 4/1 ratio by weight (3/0.75; 10/2.5; 30/7.5 mg/kg)  
A group of 10 – 15 infected mice received only saline at the  
**Control group:** indicated time  
**Treatment:** Subcutaneous (0.2 mL)  
**Administration:** Treatment was initiated 1- and 4- hours post-infection for one day

### Observations and measurements:

(1) In this model, infected mice developed septicaemia and became moribund within 2 days;  
(2) The *in vivo* efficacy was monitored using survival as the end point post-treatment. Survival was monitored for five days post-treatment.

### Evaluation of data:

50% Effective Dose (ED<sub>50</sub>) was evaluated by probit analysis.

## REFERENCES

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## RESULTS

Table 1. Efficacy of Ceftazidime/NXL104 (4/1 ratio) against CAZ- resistant strains of *E. coli*

Strains	$\beta$ -lactamase	Drug	MIC (mg/L)	ED50 (mg/kg/dose)
<i>E. coli</i> Tunisie E4	CTX-M16 + TEM1	CAZ	>128	74
		CAZ/NXL104	1	11
		CTX	>128	>90
		PIP/TZB	8	>90
<i>E. coli</i> TN06	CTX-M2 + TEM1	CAZ	32	>60
		CAZ/NXL104	0.5	27
		CTX	>128	>90
		PIP/TZB	16	>90

Table 2. Efficacy of Ceftazidime/NXL104 (4/1 ratio) against CAZ- resistant strains of *K. pneumoniae*

Strains	$\beta$ -lactamase	Drug	MIC (mg/L)	ED50 (mg/kg/dose)
<i>K. pneumoniae</i> 253	CTX-M2 + SHV2 + TEM12	CAZ	>128	>90
		CAZ/NXL104	2	27
		CTX	>128	>90
		PIP/TZB	>128	>90
<i>K. pneumoniae</i> 465	CTX-M2 + TEM1B	CAZ	128	>90
		CAZ/NXL104	2	18
		CTX	>128	>90
		PIP/TZB	>128	>90

## CONCLUSION

- NXL104 potentially restored the *in vitro* activity of ceftazidime against all four resistant isolates by protecting ceftazidime from hydrolysis by the ESBLs
- Against the *E. coli* and *K. pneumoniae* CTX-M-producing strains, the 4/1 ratio of ceftazidime/NXL104 demonstrated significant efficacy in the survival of mice, with an ED<sub>50</sub> dose of 11 – 27 mg/kg/dose compared to ceftazidime and cefotaxime alone which had ED<sub>50</sub>s of >74mg/kg/dose (p <0.05)
- Piperacillin/tazobactam efficacy was compromised in this septicaemia model, even with the two strains which showed low MICs
- Ceftazidime/NXL104 combinations may provide a therapeutic option in infections due to ESBLs, which are a growing worldwide problem