

Importance of NXL104 Pharmacokinetics (PK) in the Pharmacodynamics (PD) of Ceftazidime+NXL104 Combinations in an *in vitro* Hollow Fiber Infection Model

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A-023

ABSTRACT

Background: NXL104 is a novel β -lactamase inhibitor that restores ceftazidime (CAZ) activity against CAZ-resistant strains. CAZ pharmacodynamics is Time>MIC dependent, but little is known about β -lactam+ β -lactamase inhibitor PK/PD relationship. The aim of the study was to determine the importance of NXL104 PK in the PD of CAZ+NXL104 combinations.

Methods: An *in vitro* hollow fiber infection model was used to expose exponentially growing cultures of four *Enterobacteriaceae* strains to two different dosing regimens of the combination:

- 1) CAZ + NXL104 continuous infusion
- 2) CAZ+NXL104 human-like profile (mimicking a bi-exponential profile following a single 30 min intravenous administration in humans).

CAZ was set constant at 16 mg/L during the entire test period. NXL104 was given so as to have the same total exposure in the two regimens, but with different concentration-time curves. Samples were taken at different time points for determination of viable bacterial count and CAZ and NXL104 concentrations.

Results: The combination CAZ + NXL104 was rapidly cidal against *E. cloacae* 293HT96 (AmpC), *K. pneumoniae* (K.p.) Tunisie C4 (CTX-M-15), *K.p.* 181 and *K.p.* 236 (SHV-5, TEM-10), reducing the bacterial count by 3 log₁₀ within 4h. Growth of the four strains was fully suppressed throughout the test period following the continuous infusion regimen (Regimen 1), while antibacterial effect of the combination was lost when the concentration of NXL104 fell below a critical level, in these experimental conditions <0.5 μ g/ml, as seen after exposure to the human-like profile (Regimen 2).

Conclusions: These experiments have demonstrated qualitatively that one of the most important factors affecting the PD of the CAZ+NXL104 combination in this experimental setting is the maintenance of a critical concentration of inhibitor necessary to sufficiently suppress β -lactamase activity.

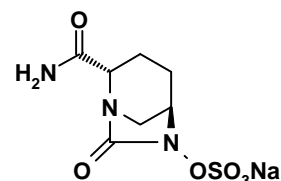
BACKGROUND

NXL104 is a β -lactamase inhibitor currently under development in combination with ceftazidime (CAZ); it potently restores CAZ susceptibility of isolates producing class A and class C β -lactamases.

CAZ PD are known to be Time>MIC dependent. Little is known about β -lactam+ β -lactamase inhibitor PK/PD relationship (Lister et al., AAC, 1997;41, 721-727), which is a pre-requisite to enable optimum design of dosage regimens.

The objective of this study was to determine the importance of NXL104 PK in the PD of CAZ+NXL104 combinations, using an *in vitro* hollow fiber infection model.

STRUCTURE OF NXL104



NXL104

(trans-7-oxo-6-(sulfooxy)-1,6-diazabicyclo[3.2.1]octan-2-carboxamide sodium salt)

METHODS

Test compounds: NXL104 (Novoxel) and Ceftazidime pentahydrate (Sandoz).

Strains: Four strains of *Enterobacteriaceae* from the Novoxel SA culture collection, originally collected from a number of clinical sources: *Enterobacter cloacae* 293HT96, *Klebsiella pneumoniae* (K.p.) Tunisie K4, K.p. 181 and K.p. 236.

MIC determination: MICs for CAZ and CAZ+NXL104 were determined using CLSI methods for antibiotic susceptibility testing with cation adjusted Mueller-Hinton (MH) broth. NXL104 was used at a constant concentration of 4 μ g/mL. MIC was defined as the lowest concentration that inhibited all visual growth. MICs of the CAZ+NXL104 combination are expressed in terms of CAZ concentrations.

Strain preparation: Overnight cultures of the strains were grown in MH at 37°C. Dilutions (1/5000) were made in pre-warmed MH broth and shaken at 37°C for 2h to allow exponential growth to approximately 1x10⁸ CFU/mL.

Hollow fiber infection model: The hollow fiber infection model permits the simulation of concentration-time profiles for any combination of antibiotic: an automated syringe pump delivers the antibiotics into a central reservoir (150mL of MH), in the required amounts at the desired schedule of administration.

Approximately 30mL of the strain culture were loaded into the peripheral compartments of Spectrum Labs Hollow Fiber Cellulose Bioreactors and kept in circulation by means of a peristaltic pump. Samples were taken from the peripheral compartment at different time points and viable bacterial count was determined by serial two-fold dilutions on MH agar plates.

When the strains were gas producers, leading to a reduction of the bacterial loop volume as the bacterial charge increased, sampling became meaningless, and was stopped. The samples were then centrifuged and supernatant separated and stored at -20°C for analysis by bioassay.

To avoid carry-over in low charged samples, the pellet was washed with 1ml of MH and plated for determination of viable bacterial count.

A 3-log₁₀ reduction in the original viable count was considered to be a bactericidal effect. The limit of detection was set at 20 CFU/ml.

Untreated growth control curves were obtained in the same conditions described above, but in the absence of CAZ and NXL104.

Dose regimens: For all experiments, CAZ was set constant at 16 μ g/mL (to be in excess of the MIC of all strains). Two different combinations of CAZ + NXL104 were used, so as to achieve similar NXL104 AUCs with two different regimens:

- Regimen 1: CAZ continuous infusion + NXL104 continuous infusion
- Regimen 2: CAZ continuous infusion + NXL104 human-like profile

The NXL104 human like profile was obtained by imposing a bi-exponential elimination using the half-lives observed in healthy volunteers following a 30-min infusion [T_{1/2}(1) = 0.16 h and T_{1/2}(2) = 2.0 h]. For each strain, the AUC_{inf} (area under the concentration-time curve to infinity) was calculated (WINNONLIN version 4.1).

Bioassay: CAZ and NXL104 were assayed in the samples by microbiological assays and the detection limits were 1 and 0.5 μ g/mL for CAZ and NXL104, respectively.

RESULTS AND DISCUSSION

The *in vitro* susceptibilities of the four strains are reported in Table 1. All strains were CAZ-R; the presence of 4 μ g/mL of NXL104 restored their susceptibility to ceftazidime.

Table 1: *in vitro* susceptibility (MIC μ g/mL) of *Enterobacteriaceae* species.

Strain	Phenotype	CAZ	CAZ + NXL104
<i>E. cloacae</i> 293HT96	AmpC	>128	4
<i>K. pneumoniae</i> Tunisie K4	CTX-M-15	>128	1
<i>K. pneumoniae</i> 181	SHV-5, TEM-10	>128	2
<i>K. pneumoniae</i> 236	SHV-5, TEM-10	>128	2

Typical concentration-time profiles obtained for CAZ and NXL104 are shown in Figure 1 (Regimen 1: CAZ continuous infusion + NXL104 continuous infusion) and Figure 2 (Regimen 2: CAZ continuous infusion + NXL104 human-like profile).

The actual total exposure to NXL104 in each experiment was equivalent for the two regimens or in favour of Regimen 2 (Table 2).

Table 2: AUC (μ g.h/mL) of NXL104 in Regimen 1 and Regimen 2.

Strain	Regimen 1	Regimen 2
<i>E. cloacae</i> 293HT96	55,3	125,5
<i>K. pneumoniae</i> Tunisie K4	120,5	126,7
<i>K. pneumoniae</i> 181	104,3	192,0
<i>K. pneumoniae</i> 236	101,9	122,0

With both regimens, the combination was rapidly cidal against these AmpC and ESBL producing strains (Figures 3-6).

When concentrations of NXL104 were maintained by continuous infusion (Regimen 1), a bactericidal effect was observed throughout the test period.

In contrast, some regrowth was observed when the concentration of NXL104 fell below a certain critical level, as seen after exposure to human-like profiles (Regimen 2). The critical NXL104 concentration was estimated below 0.5 μ g/mL in these experimental settings. It is most likely the concentration of inhibitor required to maintain continued suppression of β -lactamase in the presence of continuous enzyme production and inhibitor turnover. This critical NXL104 concentration is most probably strain-dependent.

Following Regimen 2, at the end of the study period a dense culture was obtained, comparable to that seen with untreated growth controls: strain re-growth in the absence of NXL104 was responsible for hydrolysis of CAZ, concentrations of which consequently decreased to undetectable levels, as seen in Figure 2.

The CAZ+NXL104 MICs of the colonies from the re-growth cultures were similar to that observed before the start of the experiment, thereby demonstrating that these colonies were not resistant mutants.

CONCLUSION

One of the most important factors affecting the pharmacodynamics of the CAZ+NXL104 combination is the maintenance (Time >) of a certain critical concentration of inhibitor necessary to sufficiently suppress β -lactamase activity. The critical NXL104 concentration, in these experimental settings estimated below 0.5 μ g/mL, is most likely the amount of inhibitor required to maintain continued suppression of β -lactamases.

The time dependence of the *in vitro* activity of NXL104 would imply that the goal for optimized dosing in the clinical setting is to maximize the time of exposure to NXL104.

Figure 1 - Concentration-time profiles of CAZ and NXL104 continuous infusion in a typical experiment

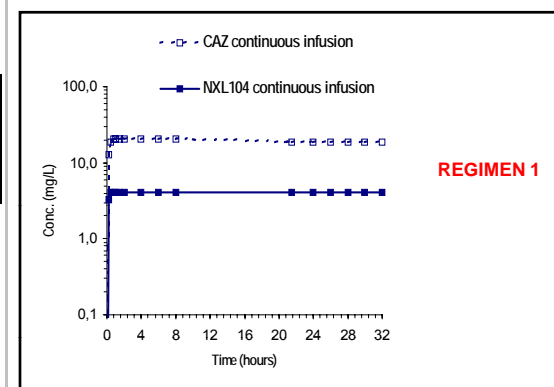


Figure 2 - Concentration-time profiles of CAZ continuous infusion and NXL104 human-like profile in a typical experiment

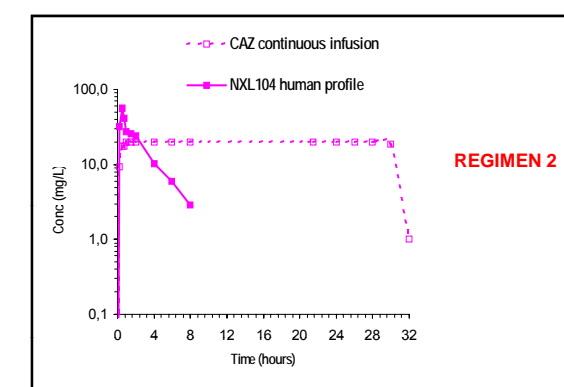


Figure 3 - Time-kill kinetics of two regimens of CAZ+NXL104 against *E. cloacae* 293HT96

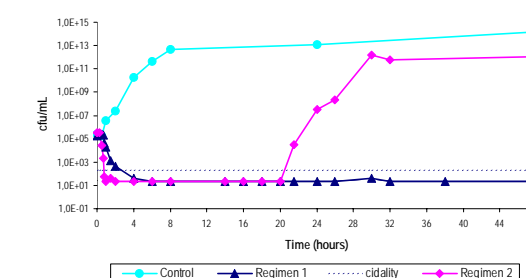


Figure 4 - Time-kill kinetics of two regimens of CAZ+NXL104 against *K. pneumoniae* Tunisie K4

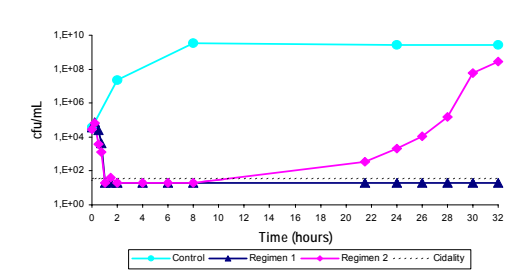


Figure 5 - Time-kill kinetics of two regimens of CAZ+NXL104 against *K. pneumoniae* 181

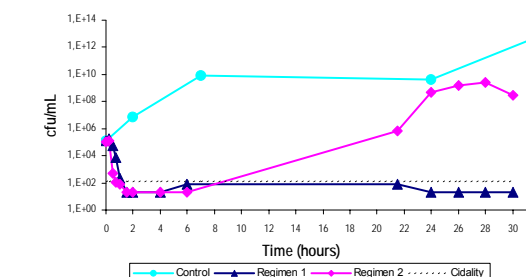


Figure 6 - Time-kill kinetics of two regimens of CAZ+NXL104 against *K. pneumoniae* 236

