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Efficacy of NXL104 in Combination with Ceftazidime in Murine Infection Models

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Abstract

Background: *K. pneumoniae* strains harboring bla_{KPC} (KPC-Kp) are rapidly emerging as pathogens of increasing medical importance. The blaKPC carbapenemase confers resistance to all beta-lactams and beta-lactamase inhibitor combinations. In addition, many KPC-Kp are present in strains that possess multiple beta-lactamases as well as other resistance determinants. Here, we tested the efficacy of NXL104 (NXL), a novel beta-lactamase inhibitor, in combination with ceftazidime (CAZ) in two murine infection models with a KPC-Kp strain. **Methods**: MICs were performed in MHA in accordance with CLSI standards. Female CD-1 mice were infected IP with 10^6 CFUs of the KPC-Kp followed 30 minutes later by a single subcutaneous (SC) treatment with CAZ alone or CAZ:NXL in ratios of 2:1, 4:1, 8:1 and 16:1. A census of survivors was taken for 5 days and ED50s calculated using Probit analysis. In the thigh infection model, mice were rendered neutropenic by IP injection of Cytoxan (150 / 100 mg/kg at days -4 / -1 pre-infection). Infection was established by injection of 10^5 CFUs into the right thigh of each animal. Mice were treated 1.5 hrs post-infection with either CAZ alone or CAZ:NXL ratios over a dose range. Thighs were removed and CFUs determined at 24 hrs post-infection. **Results:** The Kp KPC strain was resistant to CAZ (MIC> 256 mg/L). NXL added to CAZ (constant concentration of 4, 2, or 1 mg/L) lowered MICs to 0.25, 4, and 8 mg/L, respectively. In the septicemia model, the ED_{50} value for CAZ alone vs the KPC-Kp strain was 1578 mg/kg. When combined with NXL at 2:1, 4:1, 8:1 and 16:1, the CAZ ED_{50} was reduced to 8.1, 15.1, 16.9 and 29.5 mg/kg, respectively. The results of the thigh infection show a > 2-log CFU reduction with CAZ:NXL, as compared with untreated controls. CAZ alone exhibited only a 1.2 log reduction. **Conclusion:** Despite resistance to CAZ and possessing a complex beta-lactamase background, NXL104 combined with CAZ proved to be very effective in murine models of infection against this strain of Kp KPC.

Introduction

The emergence of KPC carbapenemases in strains of *Enterobacteriaceae* is attracting significant attention. In current surveys, *Klebsiella pneumoniae* is the most common pathogen harboring bla_{KPC} genes [1]. Additionally, bla_{KPC}-containing *K. pneumoniae* (KPC-Kp) isolates are becoming endemic in certain hospitals and are responsible for increasing numbers of outbreaks in several healthcare facilities located in the Eastern USA, Israel and Greece and sporadic detection in Central and South America, the Far East and Europe [1, 2].

KPC-Kp isolates demonstrate resistance or reduced susceptibility to most β-lactams as well as many other classes of antimicrobial agents (i.e. fluoroquinolones, aminoglycosides); resistance to colistin and tigecycline is also increasing [1, 2].

There are a relatively few novel compounds in development that promise to be active against these multiple drug-resistant pathogens. Recently, we have demonstrated the excellent in vitro activity of the new β-lactamases inhibitor NXL104 (Novexel) in combination with extended-spectrum β-lactams against a large collection of KPC-Kp isolates collected in Eastern USA [3].

Here we will attempt to evaluate and profile the *in vivo* antibacterial efficacy of ceftazidime with or without (w/wo) the β-lactamase inhibitor NXL104, in a mouse model of acute bacterial infection and the murine thigh infection model using well characterized KPC producing isolates.

Methods and Materials

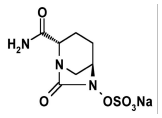
Minimum Inhibitory Concentrations (MICs) of β-lactams were determined using the agar dilution method on cation-adjusted Mueller–Hinton agar (BBL, Becton Dickinson) using a Steer's Replicator™ that delivers 10^4 CFU/spot [2, 3].

Analytical Isoelectric Focusing (aIEF) was performed to identify and determine the pIs of β-lactamases expressed by the KPC-Kp isolates. Crude cell lysates were prepared and enzyme extracts were loaded onto 5% polyacrylamide gels containing ampholines (pH range, 3.5–9.5) and electrophoresed using a Multiphor II apparatus. Gels were focused at 48C with 8 W for 150 min. The detection of β-lactamases was performed by the addition of 1 mM nitrocefin onto the gel [2].

Murine Acute Lethal Septicemia - Female CD-1 mice were infected with a pre-determined bacterial inoculum in 5% hog gastric mucin resulting in the death of untreated controls within 24-48 hr. A single subcutaneous treatment was initiated 30 min post-infection and survival ratios monitored for 7 days. Each test was repeated three times for Dose-Effect 50% (ED_{50}) determination by a computerized program of Probit analysis.

Murine Thigh Infection - Mice were rendered neutropenic by IP injection of Cytoxan (150 / 100 mg/kg at days -4 / -1 pre-infection). Infection was established by injection of 10^5 CFUs into the right thigh of each animal. Mice were treated 1.5 hrs post-infection with either ceftazidime alone or combined with NXL104 at a 4:1 ratio over the indicated dose range. Thighs were removed and CFUs determined at 24 hrs post-infection [4-9].

Figure 1: Chemical Structure of NXL104



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

Table 1: Sequencing of β-lactamase genes and aIEF results for *K. pneumoniae* VA-361 and VA-406 [ref #2]

	Isolate	
	VA-361[a]	VA-406[b]
KPC Type	KPC-2	KPC-2
TEM Type	TEM-1	TEM-1
SHV Type	SHV-11	SHV-11, SHV-12
IEF Bands (pI)	5.4, 6.7, 7.6, 8.2	5.4, 5.8, 6.7, 7.6

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Table 2. MICs of Selected Antimicrobials and Combinations vs. *K. pneumoniae* Isolates [ref #3]

	MIC (ug/mL)[a]	
	K. pneumoniae	*K. pneumoniae*
Compound	VA-361	VA-406
Ceftazidime	256	512
Caz + 4 ug/mL NXL[b]	0.25	≤ 0.06
Caz + 2 ug/mL NXL[b]	4	2
Caz + 1 ug/mL NXL[b]	8	8
Piperacillin	> 2048	2048
Pip + 4 ug/mL Tzb[c]	1024	1024
Cefotaxime	64	512
Imipenem	4	256
Meropenem	4	256
Ertapenem	16	512
Doripenem	4	256

[a] MIC listed for parent β-lactam
[b] MICs determined at a constant concentration of NXL104
[c] Tzb: Tazobactam

Table 3. Median Effective Dose (ED_{50}) Values for Ceftazidime + NXL104 Against KPC-producing *K. pneumoniae* Isolates

		ED_{50}; mg/kg (95% confidence limits)[a]					
Organism	Strain	Ceftazidime	Caz:NXL (2:1)[b]	Caz:NXL (4:1)	Caz:NXL (8:1)	Caz:NXL (16:1)	LD_{50} (CFU)
K. pneumoniae	VA-361	1578 (1244 - 2011)	8.1 (6.2 - 10.3)	15.1 (12.1 - 18.7)	16.9 (13.6 - 20.8)	29.5 (21.9 - 39.8)	3.6×10^5
K. pneumoniae	VA-406	709 (517 - 961)	3.5 (2.7 - 4.7)	3.8 (2.9 - 4.9)	7.2 (5.4 - 9.4)	12.1 (9.3 - 15.9)	3.31×10^5

[a] ED_{50} listed as ceftazidime component
[b] ceftazidime and NXL104 combined at the indicated ratio and administered as a single injection

Figure 2. Survival Curves for Ceftazidime +/- NXL104 against *K. pneumoniae* VA-361 in the Murine Septicemia Model

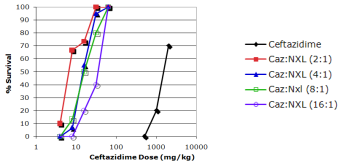
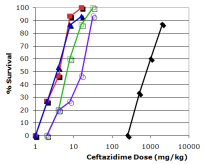
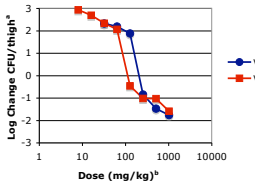


Figure 3. Survival Curves for Ceftazidime +/- NXL104 against *K. pneumoniae* VA-406 in the Murine Septicemia Model



- Ceftazidime alone required doses of 709 - 1578 mg/kg to protect 50% of the animals infected with these *Klebsiella pneumoniae* isolates.
- When combined with NXL104, a dose-dependent (by ratio) decrease of up to 200x in the ceftazidime ED_{50} was observed for both strains.
- In general, ratios of ≤ 8:1 (ceftazidime:NXL104) provided enhanced efficacy of the combination that was comparable to the observed *in vitro* activity.

Figure 4. Efficacy of Ceftazidime:NXL104 (4:1) Following Single Dose Administration in the Neutropenic Murine Thigh Infection Model



[a] Log change CFU/thigh relative to T_0 (1.5 hrs post-infection)
[b] Dose (mg/kg) listed for ceftazidime (in combination with NXL104 at 4:1)

- Ceftazidime doses of up to 2048 mg/kg did not effect a reduction in bacterial CFU/thigh as compared to untreated controls at T_0 (1.5 hrs) and only up to a 1.2 log reduction when compared to the 24 hr growth control counts.
- The addition of NXL104 to ceftazidime enhanced it's efficacy resulting in greater observed reductions in bacterial counts in thigh tissue.
- The ceftazidime:NXL104 combination (4:1) exhibited reductions of approximately 1 and 2 $\log_{10}$ CFU/thigh at doses of 256:64 and 1024:256 mg/kg, respectively.
- Static doses of 216 and 116 mg/kg ceftazidime (+ NXL104) were observed against VA-361 and VA-406, respectively.
- Combination doses of 256:64 mg/kg against VA-361 and 128:32 mg/kg against VA-406 resulted in reductions of > 2 log CFU/thigh as compared to the 24 hr untreated control counts.

Results and Conclusions

- Preclinical data [3] indicate that the spectrum of activity of NXL104 is broader than the commercially available β-lactamase inhibitors and that it inhibits both class A (including KPC carbapenemases) and class C β-lactamases.
- Both *Klebsiella pneumoniae* isolates were resistant to ceftazidime alone (≥ 256 mg/L), but the addition of constant amounts of NXL104 (1 - 4 mg/L) reduced the ceftazidime MICs to the susceptible level (i.e., ≤ 8 mg/L) [Table 2].
- ED_{50}s for ceftazidime alone in the murine septicemia model were 1578 and 709 mg/kg for VA-361 and VA-406, respectively [Table 3].
- Co-administration of ceftazidime:NXL104 in ratios of 2:1, 4:1, 8:1 and 16:1 significantly reduced the ceftazidime ED_{50} for both *Klebsiella pneumoniae* isolates.
- Ceftazidime:NXL104 ED_{50}s of 8.1, 15.1 and 16.9 mg/kg against VA-361 and 3.5, 3.8 and 7.2 mg/kg against VA-406 were achieved at ratios of 2:1, 4:1 and 8:1, respectively.
- In the neutropenic murine thigh infection model, ceftazidime alone exhibited poor efficacy at doses up to 2048 mg/kg.
- The efficacy of ceftazidime was enhanced in the thigh model when combined at a 4:1 ratio with NXL104 resulting in static dose measurements of 216 and 116 mg/kg for VA-316 and VA-406, respectively.
- The addition of NXL104 to ceftazidime results in the restoration of ceftazidime efficacy in these two murine infection models against *Klebsiella pneumoniae* isolates expressing the clinically relevant KPC β-lactamase.

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