

The Efficacy of Ceftazidime combined with NXL104, a novel β -lactamase inhibitor, in a mouse model of kidney infections induced by β -lactamase producing Enterobacteriaceae

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ABSTRACT

Objectives: NXL104 is a novel beta-lactamase inhibitor that has been shown *in vitro* and *in vivo* to inhibit both class A and class C beta-lactamases. The occurrence of Enterobacteriaceae producing extended-spectrum beta-lactamases and AmpC enzymes needs to be considered in the therapy of complicated urinary tract infections (UTI). The aim of the study was to demonstrate in a murine model that the combination of ceftazidime (CAZ) with NXL104 restored the bactericidal efficacy of CAZ against strains refractory to CAZ alone due to beta-lactamases.

Methods: Kidney infections were induced in immunodepressed, anaesthetized male CD1 mice by direct injection of 10^4 cells in 0.02 ml of exponentially growing culture by 25 gauge needle. Typically the kidney bacterial burden increased by 1.5 log₁₀ within 48 hours. Therapy commenced subcutaneously 4 hours after infection bid for 2 days. Bacteria were enumerated in the kidneys of treated and control mice 48 hours post infection.

Results: CAZ alone was ineffective against all 6 strains tested compared to the non-treated control group. The combination CAZ/NXL104 (4:1) was effective in a dose range 10-25 mg/kg in reducing the inoculum and preventing proliferation of *Escherichia coli* (one Class A and one AmpC), *Enterobacter cloacae* (AmpC), *Klebsiella pneumoniae* (Class A + AmpC), *Morganella morganii* (AmpC) and *Citrobacter freundii* (AmpC). In each case CAZ/NXL104 was significantly effective, reducing bacterial kidney burden by 2.6 to 4.5 log₁₀ compared to the CAZ treated group (p<0.05, Bonferroni).

Conclusion: The combination CAZ/NXL104 (4:1) was effective against representative strains of CAZ-resistant Enterobacteriaceae species in a murine kidney infection model. This combination could represent a useful therapeutic option for the treatment of infections due to beta-lactamase producing Enterobacteriaceae species, which are increasing in frequency in complicated UTI.

INTRODUCTION

Multi drug resistance in Enterobacteriaceae is a major therapeutic problem and, in particular, resistance to β -lactam antibiotics has been increasing worldwide. The spread of extended spectrum β -lactamases (ESBL) is largely responsible for the erosion in the numbers of Enterobacteriaceae susceptible to β -lactam antibiotics. In the North American SENTRY study (1998-2003) (1), of 65,746 clinical isolates collected from all infection sites, 22,680 were Enterobacteriaceae of which 4.5% were resistant, by Clinical and Laboratory Standard Institute (CLSI) criteria, to the third generation cephalosporin ceftazidime (CAZ). Furthermore, in some key species where ESBL production was confirmed, resistance rates to CAZ were much higher: *E. coli* 27.2% (n = 386) and *Klebsiella spp* 57% (n = 442). Today therefore, resistance due to ESBL in Enterobacteriaceae is already common and increasing in incidence.

ESBL enzymes are plasmid-mediated β -lactamases of predominantly Ambler class A. Chromosomally mediated β -lactamase (Ambler class C) production is mainly through expression of the *ampC* gene, which is either constitutively expressed or inducible and is found among the Enterobacteriaceae. *E. coli*, *Serratia*, *Morganella*, *Providencia*, *Enterobacter*, *Citrobacter freundii* have similar, although not identical, chromosomal *ampC* β -lactamase genes. Plasmid-encoded AmpC enzymes have been particularly reported from *Klebsiella spp.* and *E. coli* isolates.

NXL104 is a novel β -lactamase inhibitor currently in phase I of clinical development. It possesses a spectrum of activity encompassing both Class A and Class C β -lactamases, which include enzymes of profound clinical importance. None of the currently available β -lactamase inhibitors (clavulanate, tazobactam or sulbactam) are effective against Class C enzymes. The CAZ-NXL104 combination has been shown to be active against such strains (2), and therefore has the potential to be utilised in infections where Enterobacteriaceae are prevalent.

Urinary tract infections are one of the most common infections diagnosed in outpatients as well as in hospitalized patients. Almost all infections originate from faecal material where members of the Enterobacteriaceae predominate. Among urinary isolates, the prevalence of ESBLs varies between species and country to country. In western Japan (3), the rate of ESBL producing *E. coli* in in-patient urinary infection was 14%.

In southern Italy (4), 50 of 650 isolates of Enterobacteriaceae from UTIs were ESBL producers. In another SENTRY study in Latin America (5), >30% of *Klebsiella pneumoniae* were of ESBL phenotype. Increasing numbers of reports of community-acquired urinary tract infections caused by ESBL-producing organisms encourage physicians to reconsider initial antibiotic treatment strategies (6). Clearly ESBLs need to be taken into account when considering therapeutic options for UTI and NXL104 combined with ceftazidime could be indicated for such a role.

This study was conducted to examine whether the CAZ/NXL104 (4:1) combination was effective in the UTI setting *in vivo* against strains refractory to CAZ alone due to ESBL or cephalosporinase production. The combination was tested against representative clinical isolates of Enterobacteriaceae species in a mouse kidney infection model.

METHODS

Strains: *Escherichia coli* 250BE1 (SHV-4) and 250HT213 (AmpC), *Enterobacter cloacae* 293HT96 (AmpC), *Klebsiella pneumoniae* 283KB5 (SHV-11 + AmpC), *Morganella morganii* 313HT26 (AmpC) and *Citrobacter freundii* 261GR10 (AmpC) were cultured overnight in Mueller-Hinton (MH) medium and diluted 1:1000 in 0.9% saline to approximately 5×10^5 cells/ml. The inoculum size was verified by quantitative plating on agar MH plates. The compounds used for therapy were tested for *in vitro* activity against all strains according to the CLSI broth microdilution.

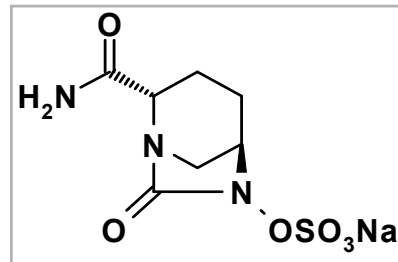
Animals: CD1 male mice (Charles River-France), 25g of mean body weight on the day of infection, were used. Experiments were performed after approval of the local ethics committee. The research complied with the national legislation and with Novexel Policy with regard to care and use of animals and related codes of practice. Animals were allowed free access to food and water throughout the experiment. Groups of 4 mice were immunosuppressed with two intraperitoneal injections of cyclophosphamide monohydrate (Sigma), 150 mg/kg the day before and the day of infection.

Infection: Mice were anaesthetized with an intramuscular injection (50 μ l/mouse) of a mixture of Imalgene 1000 (Meril) and Rompun 2% (Bayer) 1:1, diluted 1/2 with saline. The dorsal skin area over the left kidney was washed with an alcoholic solution and 0.02 ml of the bacterial culture were directly injected through the skin into the left kidney (which was located by palpation), by a small bore needle. Mice were infected with approximately 1×10^4 CFU/mouse.

Therapy: Ceftazidime (CAZ-Sandoz), Lithium Clavulanate (CLA-USP) and NXL104 were dissolved in saline. Imipenem (IPM-Tienam®-MSD) was diluted in saline. Mice were treated subcutaneously with CAZ or IPM alone (at 10 or 25 mg/kg), or with CAZ/CLA or CAZ/NXL104 combinations (4:1 ratio, w:w) at 4, 8, 24 and 32 h after infection. Control groups received saline.

Expression of results and statistical analysis: Mice were culled with CO₂ 4 hours (control) and 48 h (control and treated mice) post infection and bacteria were enumerated in the infected kidney by quantitative plating on agar MH plates. A contrast analysis was performed between treatment and control groups and P-values were corrected for multiplicity (p<0.05, Bonferroni).

Chemical structure of NXL104



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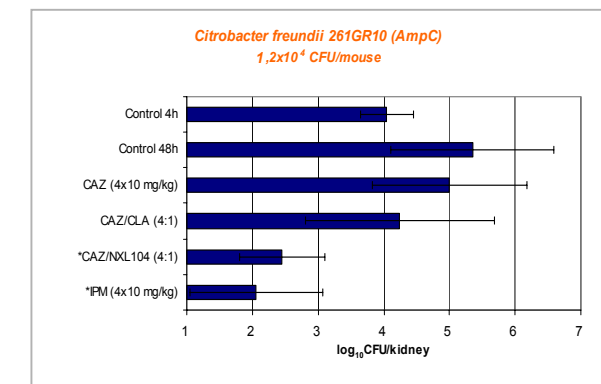
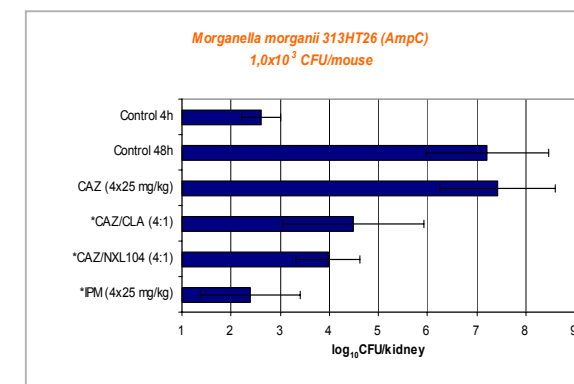
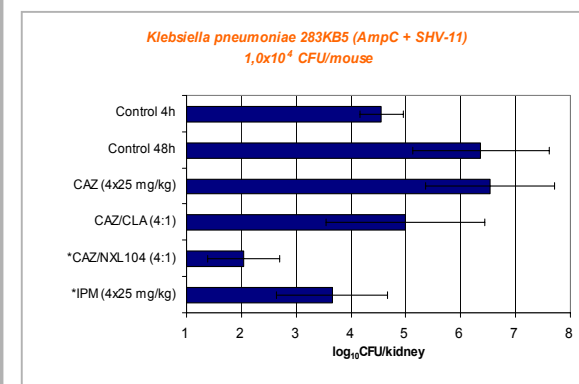
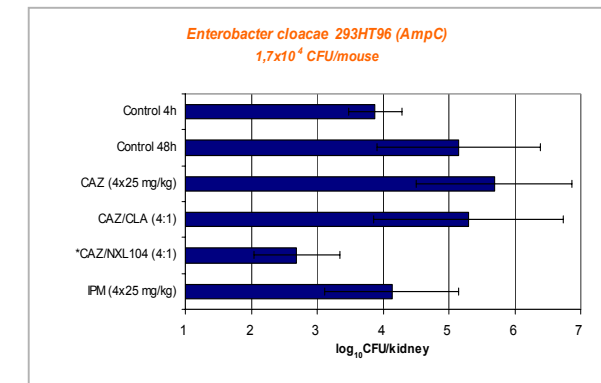
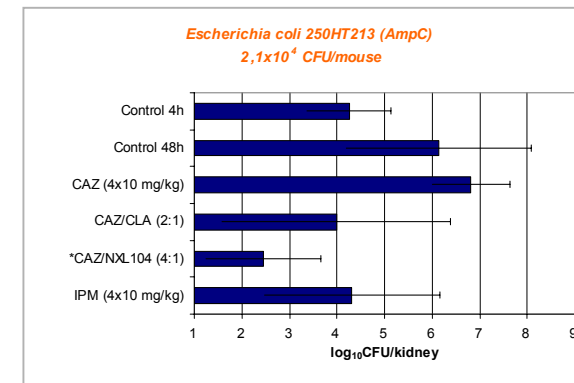
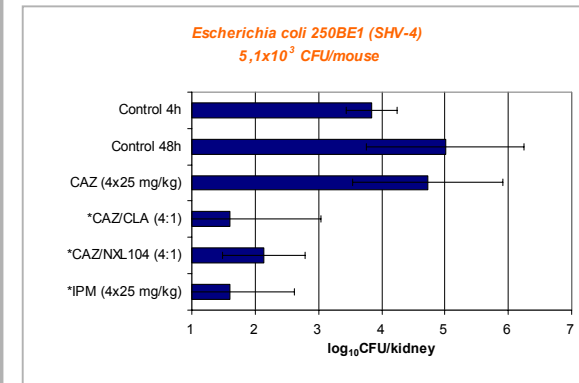
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RESULTS

In vitro susceptibility: All strains were resistant to CAZ *in vitro*, and the combination CAZ/CLA (4:1) was active only on *E. coli* 250BE1 that produces a Class A enzyme. NXL104 fully restored *in vivo* CAZ activity on all 6 strains expressing Class A and Class C β -lactamases. IPM was active against all strains.

In vivo efficacy: In the immunosuppressed mouse, all tested strains resulted in an infection evolving in time: 4 h after infection, the bacterial burden in the infected kidney was typically around 4.0 log₁₀ CFU/kidney, and increased by 1.5 log₁₀ within 48 hours in the non-treated control group. CAZ alone was ineffective against *E. coli* 250HT213 and *C. freundii* 261GR10 at 10 mg/kg and against *E. coli* 250BE1, *E. cloacae* 293HT96, *K. pneumoniae* 283KB5 and *M. morganii* 313HT26 at 25 mg/kg compared to the non-treated control group. The CAZ/NXL104 (4:1) combination was significantly effective in a dose range 10-25 mg/kg in reducing the inoculum and preventing proliferation of all 6 strains tested: CAZ/NXL104 (4:1) reduced bacterial kidney burden by 2.6 to 4.5 log₁₀ compared to the CAZ treated group (p<0.05, Bonferroni). The efficacy of IPM reached statistical significance against 4 strains out of 6 (*E. coli* 250BE1, *C. freundii* 261GR10, *K. pneumoniae* 283KB5 and *M. morganii* 313HT26). The association CAZ/CLA (4:1) had a significant effect against *E. coli* 250BE1 that correlated well with the *in vitro* susceptibility data, as this strain bears a Class A enzyme. Although CAZ/CLA (4:1) reached statistical significance against *M. morganii* 313HT26, this combination was not active against the other AmpC producing strains.

Figures 1-6: Efficacy of CAZ/NXL104 (4:1) in a murine model of kidney infection. Statistical significance is expressed as * (p<0.05).



CONCLUSION

- The murine model of kidney infection was established with 6 representatives of CAZ-resistant Enterobacteriaceae species.
- The CAZ/NXL104 (4:1) combination was effective against representative strains of Enterobacteriaceae species which were refractory to treatment with CAZ alone.
- NXL104 restored CAZ efficacy against strains producing both Ambler Class A and Class C β -lactamases.
- This CAZ/NXL104 (4:1) combination could represent a useful therapeutic alternative for the treatment of urinary tract infections due to β -lactamase producing Enterobacteriaceae species.