Poster # B-708

ED_{50} Determination of CXA-101 (CXA) Alone and in Combination with Tazobactam (TAZ) for Treating Experimental Peritonitis in Mice Due to ESBL-Producing *Klebsiella pneumoniae* strains: Comparison with Ceftazidime (CAZ) and Piperacillin/Tazobactam (TZP)

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REVISED ABSTRACT

Background: CXA-101 (CXA) is a novel parenteral cephalosporin with potent in vitro activity against *Klebsiella pneumoniae* (KP), and, in combination with tazobactam (TAZ), against extended-spectrum β-lactamase (ESBL) producers. The aim of this study was to determine the dose that is pharmacologically effective for 50% of the population exposed (ED50) of the new cephalosporin, CXA, alone or in combination with the β-lactamase inhibitor TAZ (in a fixed 2:1 ratio), in comparison with ceftazidime (CAZ) and piperacillin-tazobactam (TZP) against ESBL-producing KPs.

Methods: KP1 (ESBL), KP2 (ESBL+), and KP3 (ESBL+) strains were used in this study. MICs for CXA alone or combined with TAZ were 0.25/0.25, 16/0. 5, and 128/1, for KP1, KP2, and KP3 respectively. Mice were infected intraperitoneally with 0.6 mL of the infecting inoculum. Treatment was injected 2, 4, and 6h after the bacterial challenge using the subcutaneous route. After an observation period of 5 days, the Reed and Muench method was used for the determination of ED50.

Results: See Table 2.

Conclusions: 1. No impact of TAZ in addition to CXA was observed against KP1 strain as expected. 2. CXA, CXA/TAZ, and CAZ showed similar activity against KP1 strain. 3. TZP exhibited poor activity against KP1 and was not effective against ESBL isolates in this study (ED50>300 mg/kg). 4. Similar activity of CXA/TAZ and CAZ was observed against KP2 strain. 5. Against the high-resistant KP3 strain (CTX-M), CXA/TAZ was the only effective drug. Finally, the presence of ESBL doesn't have an impact on the in vivo activity of CXA/TAZ.

INTRODUCTION

•CXA-101 (Fig. 1), previously designated FR264205, is a novel parenteral cephalosporin (currently under clinical development) with potent anti-*Pseudomonas aeruginosa* activity and with an antibacterial spectrum against other organisms similar to that of ceftazidime (1,2). In combination with tazobactam, CXA exhibits potent *in vitro* activity against Enterobacteriaceae including ESBL-producers.
•Using an *K. pneumoniae* sepsis model in mice, the aim of this study was to assess the in vivo activity of CXA and CXA/TAZ (in a fixed 2:1 ratio) in comparison with ceftazidime and piperacillin/tazobactam (in a fixed 8:1 ratio, as found in Zosyn) against 3 *K. pneumoniae* strains (ESBL-negative and -positive strains).

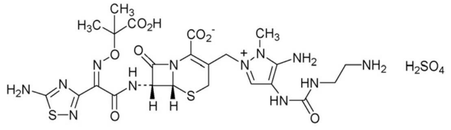
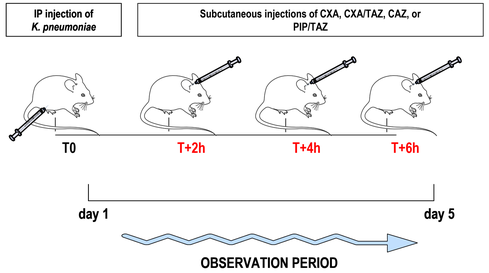


Figure 1. Structure of CXA-101

METHODS



•Six-week-old pathogen-free mice (weight, 20-24g) were used for this study (Janvier, France).

•Bacterial cells from overnight cultures were collected by centrifugation (2500 rpm, 10 min.), washed two times using sterile saline 0.9%, and then appropriately diluted suspensions of the infecting inoculum were prepared using 5% porcine gastric mucin (Sigma®, St. Louis, Mo, USA). Mice (20-24g) were infected intraperitoneally with 0.6 mL of appropriately diluted suspensions of the infecting inoculum. Half-log dilutions of studied drugs (CXA, CXA/TAZ in a fixed 2:1 ratio, CAZ, and TZP in a fixed 8:1 ratio) were prepared in sterile saline (0.9%) from 0.01 mg/kg to 300 mg/kg. Treatment was administered by subcutaneous injection at 2, 4, and 6 hours after bacterial challenge. Mice were observed for up to 5 days for mortality.

•The method of Reed and Muench (cumulative distribution function) was used for the determination of the ED50, as previously described (3).

RESULTS

•MICs of KP1, KP2, and KP3 strains are summarized in the Table 1 for CXA-101 (CXA), CXA-101/tazobactam (CXA/TAZ), ceftazidime (CAZ), and piperacillin-tazobactam (TZP).

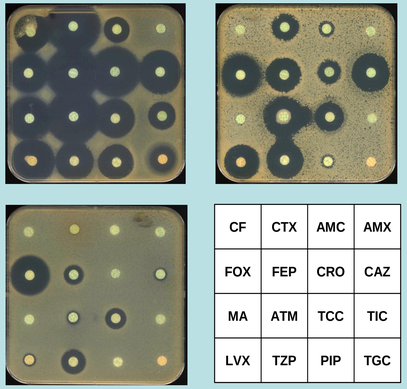
Table 1. MICs of CXA, CXA/TAZ, CAZ, and TZP tested isolates.

		MIC (mg/L)			
STRAIN	ESBL	CXA	CXA/TAZ	CAZ	TZP
KP1	-	0.25	0.25	0.25	2
KP2	+	16	0.50	1	2
KP3	+	128	1	32-64	16

RESULTS

•Studied drugs demonstrated similar in vivo activity against the non-ESBL producing strain (32.8, 30.0, and 17.6 mg/kg, for CXA, CXA/TAZ, and CAZ, respectively), except for TZP (ED_{50}=195.7 mg/kg). Indeed, the ED_{50} of TZP ranged from 195.7 mg/kg for KP1 to >300 mg/kg for KP2 and KP3 strains underlining the lack of efficacy of TZP in the mice sepsis experimental model, which is probably related to the very short half-life of TAZ in mice (approximately 3.6 min) and the relatively low ratio of piperacillin to tazobactam. Despite the presence of a CTX-M-enzyme, CAZ displayed a protective effect against the KP2 strain (ED_{50}= 20.8 mg/kg) probably due to the low CAZ MIC (i.e., 1 mg/L). Nevertheless, this drug showed no activity against the CAZ-resistant KP3 strain (CTX-M enzyme).
•Activity of CXA/TAZ was similar against both CTX-M positive strains in spite of the increased CAZ MIC (i.e., 32 mg/L) for KP3 strains.

Figure 2. Antibiograms of *K. pneumoniae* KP1 (ESBL -), KP2 (ESBL +), and KP3 (ESBL +) strains.



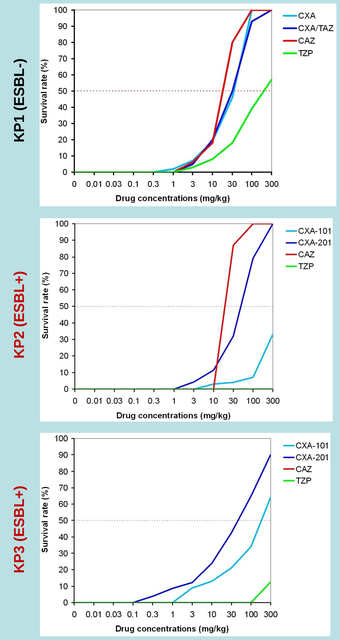
CF	CTX	AMC	AMX
FOX	FEP	CRO	CAZ
MA	ATM	TCC	TIC
LVX	TZP	PIP	TGC

AMX, amoxicillin; AMC, amoxicillin/clavulanic acid; ATM, aztreonam; CAZ, ceftazidime, CF, cefalotine; CRO, ceftriaxone; CTX, cefotaxime; FEP, cefepime; FOX, cefoxitin; LVX, levofloxacin; MA, cefamandole; PIP, piperacillin; TCC, ticarcillin/clavulanic acid; TGC, tigecycline; TIC, ticarcillin; TZP, piperacillin-tazobactam.

Table 2. 50% effective dose (mg/kg) of CXA, CXA/TAZ, CAZ, and TZP against KP1, KP2, and KP3 strains.

		ED_{50} (mg/kg)			
STRAIN	ESBL	CXA	CXA/TAZ	CAZ	TZP
KP1	-	32.8	30	17.6	195.7
KP2	+	>300	47.5	20.8	>300
KP3	+	183.3	44.9	>300	>300

Figure 3. Survival curves for CXA, CXA/TAZ, CAZ, and TZP against *K. pneumoniae* KP1, KP2, and KP3.



CONCLUSIONS

- A lack of efficacy of piperacillin/tazobactam was observed against ESBL-producing strains in this mice sepsis experimental model probably due to extremely rapid elimination of tazobactam in this animal species and relatively low tazobactam ratio in the Zosyn dosing regimen.
- Despite the unfavorable PK of tazobactam in mice (very short half-life), CXA-101/tazobactam showed similar activity as ceftazidime against KP1 and KP2 strains, and demonstrated better efficacy than ceftazidime against the KP3 strain, which produced a CTX-M enzyme (hydrolysing both cefotaxime and ceftazidime).
- Due to the poor PK of tazobactam in mice, the effect of combining tazobactam and CXA-101 is likely to have been greatly underestimated in this model.
- CXA-101/tazobactam was the only effective drug against the high-resistant KP3 strain (CAZ MIC = 32 mg/L).
- The presence of ESBL-enzyme doesn't seem to have an impact on the in vivo activity of CXA-101/tazobactam (30.0, 47.5, and 44.9 mg/kg for KP1, KP2, and KP3 strains, respectively.
- Although *E. coli* ranks higher in the number of infections occurrences than *K. pneumoniae*, the latter surpasses the number of multiresistant and ESBL phenotypes. In this context, it is of special interest to show that CXA-101 in combination with tazobactam is the only drug achieving efficacy against KP3 *K. pneumoniae* strain.

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