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Ceftriaxone-Sulbactam-EDTA vs. Meropenem: Analysis of Failed Patients With Assessment of MIC Increases and Changes in Genotypic Profile in PLEA (a Phase 3, Randomized, Double-Blind Clinical Trial in Adults With Complicated Urinary Tract Infections or Acute Pyelonephritis)

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Abstract

Background

Ceftriaxone-Sulbactam-EDTA (CSE) is a novel combination being developed to treat serious infections caused by gram-negative bacteria. In vitro molecular biology studies have shown that the addition of EDTA in the combination helps to prevent horizontal gene transfer during conjugation by chelating the divalent magnesium ions (Mg2+) required for the activity of DNA relaxases enzyme. An assessment of acquisition of resistant genes and a concomitant increase in MIC for patients that failed therapy in the Phase-3 clinical trial (NCT03477422) was conducted.

Methods

MICs were conducted on baseline and post-treatment isolates recovered during treatment period. MICs were determined using CLSI reference methods and MIC changes from baseline were further assessed. Bacterial DNA was extracted by the alkaline lysis method. β-lactamase (BL) genes were amplified in single PCRs using a panel of primers for detection of most beta-lactamase enzymes, including extended-spectrum beta-lactamases (ESBLs) (bla_{TEM}, bla_{SHV}, bla_{CTX-M}), metallo-beta-lactamases (MBLs) (bla_{VIM}, bla_{NDM}, bla_{IMP}), carbapenemases (bla_{OXA}, bla_{KPC}) and Class C cephalosporinases (bla_{AmpC}).

Results

9/143 [2/74 (2.7%) in CSE; 7/69 (10.1%) in MR (Meropenem)] patients had a microbiological failure at the TOC visit. Of these 9 patients (all *E. coli*), a variation in the post-treatment genotypic profile was noted for 4 patients (44.4%) in the MR group and 2 of these patients also reported a ≥4-fold increase in post-treatment MIC. Both patients harbored 4 distinct BL genes (bla_{TEM} + bla_{SHV} + bla_{CTX-M} + bla_{AmpC}) at baseline, and had acquired two additional genes (bla_{OXA}, bla_{KPC}), both carbapenemases, as a result of treatment failure (after 6 days and 8 days of IV therapy respectively) with MR. In the first case, MIC increased 16-fold (1 µg/mL to 16 µg/mL for MR and 2 µg/mL to 32 µg/mL for CSE), while in the second case, MIC increased 8-fold (1 µg/mL to 8 µg/mL) for MR and 32-fold (1 µg/mL to 32 µg/mL) for CSE. No such increase in MIC or acquisition of resistant genes was noted in patients that failed therapy with CSE.

Conclusion

These findings highlight the need for an effective choice of empirical therapy as failed treatments could lead to selection for resistant genes, rendering once susceptible drug non-susceptible.

Background

- CSE is a novel combination of Ceftriaxone (third generation beta-lactam cephalosporin), Sulbactam (beta-lactamase inhibitor) and Disodium EDTA (Class 1 Antibiotic Resistance Breaker), and it restores & enhances the in vitro activity of Ceftriaxone against ESBL/MBL producing gram- negative bacteria, including enzyme families that belong to Ambler class A (TEM, SHV, CTX-M), class B (NDM, VIM, IMP), class C (some variants of AmpC), and class D (OXA ESBLs), it do not work against serine carbapenamases (KPC and OXA)
- PLEA was a Phase III, prospective, randomized, multi-center, double-blind, double-dummy, parallel-group, comparative study to determine the efficacy, and safety of CSE versus Meropenem (MR) in the treatment of hospitalized patients with cUTIs, including Acute Pyelonephritis (AP).
    - A total of 230 patients were screened and randomized, of which, only 143 (62.2%) met criteria for the m-MITT population (74 in the CSE group and 69 in the Meropenem group).
    - Mean duration of IV therapy was 7 days for both treatment groups. IV to Oral switch was not permitted.
- The likelihood of resistance development during the course of therapy is an important parameter and can help improve our understanding of optimal therapy and drug exposures, allowing for better antimicrobial stewardship.
- To this end, we investigated any changes in the susceptibility of study drugs (and comparators) over the course of therapy in biological specimens contributed by patients that reported an adverse microbiological outcome in the trial.
- Further, we also analysed the impact of treatment failure on resistance selection by studying variations in the baseline genotypic profile

Methods

- Urine cultures were collected within 48 hrs prior to randomization and all biological specimens were checked for the growth of a gram-negative uropathogen (>10^5 CFU/mL) and susceptibility towards both study drugs.
    - MICs of study drugs were determined using Clinical and Laboratory Standards Institute (CLSI) reference methods at all visits where quantitative growth in the urine culture was found.
    - Quality Control (QC) was performed using *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603
- Susceptibility testing of the isolates was also performed for comparator drugs (Table 1) using disk diffusion test as per CLSI guidelines.
- Bacterial DNA was extracted by the alkaline lysis method. β-lactamase (BL) genes were amplified in single PCRs using a panel of primers for detection of most beta-lactamase enzymes.
- Beta-lactamase (BL) genes including bla_{TEM}, bla_{SHV}, bla_{AmpC}, bla_{CTX-M}, bla_{OXA}, bla_{KPC}, bla_{VIM}, bla_{NDM} and bla_{IMP} were amplified in single PCRs using gene-specific primers. PCR was performed with a final volume of 20µl reaction containing Taq buffer A Tris with 15 mM MgCl2, 0.5 mM of dNTPs, 1.25 µM of each primer, 1 µl of 3.0 µl/unit of Taq polymerase (HiMedia, Mumbai, India) and 200 ng of DNA. PCR products were analyzed by gel electrophoresis (Bangalore Genei, Bangalore, India) and 100-bp DNA ladder (HiMedia, Mumbai, India) was used as a marker.
- Criteria for evaluation of outcomes:
    - Clinical cure was defined as a complete resolution of signs/symptoms such that no further antimicrobial therapy was required.
    - Microbiological Eradication was defined as the reduction in the growth of the original uropathogen at <10^4 CFU/mL (FDA) and <10^3 CFU/mL (EMA) respectively.

Results

A total of 230 patients were randomized; 143 (62.2%) met all inclusion criteria and constituted the m-MITT population. Of the 143 patients, 10 patients (3 in CSE; 7 in MR) reported an adverse microbiological outcome (excluding indeterminate) at the TOC visit. The baseline characteristics and microbiological profile of these patients is presented in Table 1:

- Of the 10 failed patients, most had cUTI (9/10) and on average received 8 days of IV therapy. With the exception of one patient (102014; 85 years) who reported a superinfection at TOC visit, no other age or demographic related factors explain the associated treatment failures. In this patient however, age is a plausible factor that explains the increased likelihood of superinfection.
- Three patients (120043, 121015 and 121017) were clinically cured but reported microbiological failure at the TOC visit. These patients were categorised as having asymptomatic bacteriuria and no further antibiotic therapy was administered. These patients reported microbiological eradication at the follow-up visit (TOC + 7±2 days).

As can be seen from Table 1, the baseline MICs for both study drugs were in the susceptible range, however, many patients experienced a shift in the baseline MICs upon treatment failure, probably resulting from a change in the underlying genotypic profile.

- 5/10 (50%) patient' isolates reported a variation in gene profile as compared to baseline. Barring the patient that reported superinfection in CSE arm, all the other 4 patients received therapy with MR.
- In most cases, an upward trend of acquisition of genes, like SHV, CTX-M, OXA, KPC etc., that encode on plasmids was seen. This was accompanied by a concomitant change in the baseline MICs, usually an increase.
- 2 patients in particular (124010 and 124014) reported a ≥4-fold increase in baseline MICs. Both these patients had acquired Carbapenemase genes KPC and OXA-25, in addition to the TEM+SHV+CTX-M+AmpC genes that they were carrying at baseline, during the course of therapy with MR. As a result of this, not only did the MICs increase for MR, but the MIC for CSE also showed a >4-fold increase. It is also worth noting that in one of these patients (124010), neither the two study drugs, nor the four comparator drugs (A, G, CS, PT) retained their baseline susceptibility post the acquisition of the Carbapenemase genes.
- This is an important finding as it highlights how inadequate therapies and/or dosing regimens can increase the likelihood of selection for resistant genes during therapy and significantly worsen the prognosis of a patient.

Table 1. Overview of patients who reported unfavourable microbiological and/or clinical outcome(s) in either treatment group at TOC visit in the m-MITT population

	Patient ID	102006	102014*	120043	121015	121017	123018	124010	124014	125008	125013
	Demographic	72 yrs	85 yrs	37 yrs	28 yrs	23 yrs	42 yrs	32 yrs	34 yrs	28 yrs	68 yrs
	Infection Type	cUTI	cUTI	cUTI	cUTI	cUTI	cUTI	AP	cUTI	cUTI	cUTI
	Treatment Arm	MR	CSE	MR	CSE	MR	MR	MR	MR	MR	CSE
	Treatment Duration^	11 days	6 days	15 days	8 days	7 days	8 days	8 days	6 days	6 days	6 days
BASELINE	Pathogen	*E. coli*	*Klebsiella spp.*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*
	MIC CSE	2 µg/mL	2 µg/mL	4 µg/mL	0.5 µg/mL	0.5 µg/mL	2 µg/mL	2 µg/mL	1 µg/mL	2 µg/mL	4 µg/mL
	MIC MR	1 µg/mL	4 µg/mL	0.5 µg/mL	<0.25 µg/mL	0.25 µg/mL	1 µg/mL	1 µg/mL	1 µg/mL	1 µg/mL	0.5 µg/mL
	Gene Profile	TEM, SHV, AmpC, CTX-M	TEM	TEM, SHV, AmpC	TEM, SHV, CTX-M	TEM, SHV	TEM, SHV, AmpC, CTX-M	TEM, SHV, AmpC, CTX-M	TEM, SHV, AmpC, CTX-M	TEM, SHV, CTX-M, OXA	OXA
	Susceptible Drugs^^	A CS C Ci O	A G CS PT Ci O	A G CS PT	A CS PT	A G CS PT Ci O	G	A G CS PT	A G PT	A G CS PT	A CS PT
TOC	Pathogen	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*
	MIC CSE	2 µg/mL	16 µg/mL	2 µg/mL	<0.25 µg/mL	1 µg/mL	2 µg/mL	>32 µg/mL	>32 µg/mL	4 µg/mL	8 µg/mL
	MIC MR	4 µg/mL	8 µg/mL	1 µg/mL	<0.25 µg/mL	1 µg/mL	4 µg/mL	16 µg/mL	8 µg/mL	4 µg/mL	1 µg/mL
	Gene Profile	TEM, SHV, AmpC, CTX-M	TEM, SHV, AmpC, CTX-M	TEM, SHV, CTX-M, OXA	TEM, SHV, CTX-M	TEM, SHV, AmpC, CTX-M	TEM, SHV, AmpC, CTX-M	TEM, SHV, AmpC, CTX-M, OXA, KPC	TEM, SHV, AmpC, CTX-M, OXA, KPC	TEM, SHV, CTX-M, OXA	OXA
	Susceptible Drugs^^	CS	G	A G CS PT	A G PT C	A G PT	A		PT	A G CS PT Ci O	A G CS PT
	Clinical Cure	✗	✗	✓	✓	✓	✗	✗	✗	✗	✗
	Microbiological Eradication	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗

* This patient reported a superinfection at TOC visit and therefore, the variation in gene profile is due to colonisation by different bacterium and not due to gene acquisition; ^ Treatment duration was calculated for End of IV therapy; ^^ Amikacin (A), Gentamicin (G), Cefoperazone+Sulbactam (CS), Piperacillin+Tazobactam (PT), Ceftriaxone (C), Cefepime (Ce), Ciprofloxacin (Ci) and Ofloxacin (O).

Conclusions

- Failed treatment outcomes with MR were associated with genetic variations like acquisition of resistant Carbapenemase genes
    - Post-treatment MICs in such patients showed a ≥4-fold increase (in study drugs) and reduced susceptibility to comparator drugs.
- None of the patients treated with CSE reported any significant increase in the post-treatment MIC or acquisition of resistant genes.
- These results highlight the importance of effective empirical therapy, as inadequate treatments and/or dosage regimens can lead to selection for resistant mutants/genes, and can severely hamper the effectiveness of other drugs that may have otherwise proven effective.

Disclosures

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