Post β-Lactamase Inhibitor Effect of Tazobactam When Associated with Ceftolozane and Tested Against ESBL-Producing Strains

H.S. Sader, P.R. Rhomberg, R.N. Jones

JMI Laboratories, North Liberty, Iowa

Helio S. Sader, MD, PhD
JMI Laboratories
345 Beaver Kreek Ctr, Ste A
North Liberty, Iowa, 52317, USA
Phone: 319-665-3370
Fax: 319-665-3371
helio-sader@jmilabs.com

Poster #A-1030

ABSTRACT

OBJECTIVE: Ceftolozane/tazobactam is a novel antibacterial with potent activity against *P. aeruginosa* and other common Gram-negative pathogens. Tazobactam is an inhibitor of most common class A and some class C β-lactamases that protects ceftolozane from hydrolysis and broadens coverage to include most ESBL-producing Enterobacteriaceae. We evaluated the *in vitro* post β-lactamase inhibitor (BLI) effect of tazobactam when combined with ceftolozane using time-kill assays.

METHODS: Two recent clinical *E. coli* strains with ceftolozane/tazobactam MICs of 1 µg/ml were evaluated, one a CTX-M-15 (an ESBL) producing strain (#4643A; ceftolozane MIC, 128 µg/ml), and one a CTX-M-15 and TEM-1 producing strain (#5846A; ceftolozane MIC, 64 µg/ml). The organisms were exposed for 2 hours to 4x MIC of ceftolozane/tazobactam, ceftolozane alone (4x MIC for ceftolozane/tazobactam) and media containing no drug, washed and resuspended in media containing ceftolozane/tazobactam, ceftolozane, or media alone at the same concentration present during initial exposure. The post BLI effect was calculated as the time difference from when the number of viable organisms in the culture exposed to ceftolozane/tazobactam and resuspended with media containing ceftolozane alone increased 1 log_{10} compared to the time necessary for the culture exposed and resuspended with media containing ceftolozane alone to increase 1 log_{10} above the viable organism count observed immediately after washing.

RESULTS: Post BLI effect was calculated as 2.1 and 1.3 hours for strains #4643A (Figure 1) and #5846A, respectively. Ceftolozane/tazobactam post antibiotic effect (PAE) was also measured as 0.8 to 0.9 hours.

CONCLUSIONS: We calculated an *in vitro* post-BLI effect associated with ceftolozane/tazobactam when testing *E. coli* strains producing CTX-M-15 +/- TEM-1. A more complete understanding of post-BLI effect may lead to optimized modeling of β-lactam/BLI combinations.

INTRODUCTION

Ceftolozane/tazobactam (formerly CXA-201) is currently under clinical development for treatment of complicated intra-abominal infections (cIAI) and urinary tract infections (cUTI). Ceftolozane is a novel antipseudomonal cephalosporin with greater activity against *Pseudomonas aeruginosa* when compared to ceftazidime and cefepime. Ceftolozane has also demonstrated good potency against Enterobacteriaceae; however, like other structurally similar cephalosporins, ceftolozane activity can be adversely affected by bacterial production of some β-lactamases.

The production of β-lactamases is the main cause of cephalosporin resistance among members of the Enterobacteriaceae family, and $bla_{CTX\text{-}M\text{-}15}$ has been recognized as the most prevalent β-lactamase gene among Enterobacteriaceae, especially *Escherichia coli*. To decrease ceftolozane vulnerability to hydrolysis by β-lactamase, ceftolozane was combined with tazobactam, a β-lactamase inhibitor with established safety and efficacy when in combination with piperacillin.

The post-antibiotic effect (PAE) is the term used to describe the persistent inhibition of bacterial growth after antimicrobial exposure has ceased. It represents the time it takes for an organism to recover from the effects of exposure and resume normal growth. The post β-lactamase inhibitor effect (PBLIE) reflects the effect of the β-lactamase inhibitor component of the β-lactamase inhibitor combination on the persistent inhibition of bacterial growth. Thus,

INTRODUCTION (CONT'D)

in order to calculate the PAE, the organisms are exposed to an antimicrobial for a defined period of time and then washed and resuspended in drug-free media (no β-lactam and no β-lactamase inhibitor in the case of a β-lactam/β-lactamase inhibitor combination). To calculate the PBLIE, the organisms are resuspended in media containing the β-lactam but not the inhibitor. We evaluated the *in vitro* PBLIE of tazobactam when combined with ceftolozane using time-kill assays.

MATERIALS AND METHODS

Bacterial Isolates. Two contemporary clinical *E. coli* strains with ceftolozane/tazobactam MIC values of 1 µg/ml were evaluated:

- *E. coli* strain 136-4643A – a clinical strain with $bla_{CTX\text{-}M\text{-}15}$ (CTX-M-15 producing), with ceftolozane MIC of 128 µg/ml and ceftolozane/tazobactam MIC of 1 µg/ml.
- *E. coli* strain 107-5846A – a clinical strain with $bla_{CTX\text{-}M\text{-}15}$ and $bla_{TEM\text{-}1}$ (CTX-M-15 and TEM-1 producing), with ceftolozane MIC of 64 µg/ml and ceftolozane/tazobactam MIC of 1 µg/ml.

Methods. MIC and MBC values were determined in triplicate by the reference frozen-form broth microdilution method for ceftolozane alone and in combination with tazobactam at a fixed concentration of 4 µg/ml. In order to determine the PBLIE, five tubes were evaluated (see Table 1 and Figures 1 and 2):

- Growth control (**Tube C**): There was no drug exposure and the organisms were washed in drug free cation-adjusted Muller-Hinton broth (CAMHB) and resuspended at 1:1000 dilution (approximately 3 log_{10} dilution) in drug free CAMHB.
- Ceftolozane alone (**Tube Z**): The organisms were exposed for 2 hours to ceftolozane alone (4x to 16x the MIC for ceftolozane/tazobactam), the organisms were then washed in drug free CAMHB and resuspended at 1:1000 dilution (approximately 3 log_{10} dilution) in CAMHB containing ceftolozane alone at the same concentration present during exposure.
- PAE tube (**Tube F**): The organisms were exposed for 2 hours to ceftolozane/tazobactam, washed in drug free CAMHB and resuspended at 1:1000 dilution (approximately 3 log_{10} dilution) in drug free CAMHB.
- PBLIE tube (**Tube T**): The organisms were exposed for 2 hours to ceftolozane/tazobactam, washed in drug free CAMHB and resuspended at 1:1000 dilution (approximately 3 log_{10} dilution) in CAMHB containing ceftolozane alone at the same concentration present during exposure.
- Activity control (**Tube N**): The organisms were exposed for 2 hours to ceftolozane/tazobactam, washed in drug free CAMHB and resuspended at 1:1000 dilution (approximately 3 log_{10} dilution) in CAMHB containing both ceftolozane and tazobactam at the same concentration present during exposure.

MATERIALS AND METHODS

Colony counts of each of the study tubes were performed pre-antimicrobial exposure (T_0), 1 hour after antimicrobial exposure (T_1) and 2 hours after antimicrobial exposure (T_2). Colony counts were taken immediately after washing and resuspending the organisms, and each hour until visible turbidity was observed. The PAE for the ceftolozane/tazobactam combination was calculated as *F-C*, where *F* is the time for the number of viable organisms in the tube resuspended (after exposure to ceftolozane/tazobactam) with CAMHB containing no drugs (no ceftolozane or tazobactam) to increase 1 log_{10} above the number of viable organisms observed immediately after centrifugation (wash/resuspension), and *C* is the time for the number of viable organisms in the control tube (no exposure to ceftolozane/tazobactam) to increase by 1 log_{10} above the number observed immediately after antibiotic removal.

The PBLIE was calculated as *T-Z*, where *T* is the time for the number of viable organisms in the culture (after exposure to ceftolozane/tazobactam) resuspended with media containing ceftolozane (no tazobactam) to increase 1 log_{10} above the number observed immediately after centrifugation (wash/resuspension), and *Z* is the time for the number of viable organisms in the culture (after exposure to ceftolozane alone) resuspended with media containing ceftolozane alone (Tube Z) to increase 1 log_{10} above the number of viable organisms observed immediately after centrifugation.

RESULTS

E. coli strain 136-4643A (Table 1 and Figure 1)

- Ceftolozane/tazobactam MIC and MBC results for *E. coli* strain 136-4643A were identical at 1 µg/ml.
- Tubes C (growth control) and Z (ceftolozane alone) had very similar growth curves with rapid growth after wash/resuspension. It took 1.4 hours for the number of viable organisms in the growth control tube to increase 1 log_{10} after wash/resuspension.
  - The time interval for the number of viable organisms to increase 1 log_{10} after wash/resuspension was 2.2 hours in Tube F (both ceftolozane and tazobactam removed) and 3.5 hours in Tube T (only tazobactam removed) (Figure 1).
  - PAE for the combination compound ceftolozane/tazobactam was calculated as 2.2 – 1.4 = 0.8 hours (Tube F - Tube C; Figure 1).
  - PBLIE was calculated as 3.5 – 1.4 = 2.1 hours (Tube T - Tube Z; Figure 1).

RESULTS (CONT'D)

- Complete killing was observed 4 hours after wash/resuspension (T_6) in the activity control tube (N, organisms were resuspended in CAMHB containing ceftolozane/tazobactam at 4/4 µg/ml).

E. coli strain 107-5846A (Table 1 and Figure 2)

- Ceftolozane/tazobactam MIC and MBC results for *E. coli* strain 107-5846A were 1 and 1 µg/ml, respectively.
- Tubes C (growth control) and Z (ceftolozane alone) had very similar growth curves with rapid growth after wash/resuspension. It took 1.3 hours for the number of viable organisms in the growth control tube to increase 1 log_{10} after wash/resuspension.
- The time interval for the number of viable organisms to increase 1 log_{10} after wash/resuspension was 2.2 hours in Tube F (both ceftolozane and tazobactam removed) and 2.6 hours in Tube T (only tazobactam removed); (see Figure 2). Thus:
  - PAE for the combination compound ceftolozane/tazobactam was calculated as 2.2 – 1.3 = 0.9 hours (Tube F - Tube C; Figure 2).
  - PBLIE was calculated as 2.6 – 1.3 = 1.3 hours (Tube T - Tube Z; Figure 2).
- Complete killing was observed 1 hour after wash/resuspension (T_3) in the activity control tube (N, organisms were resuspended in CAMHB containing ceftolozane/tazobactam at 4 µg/ml).

Table 1. Summary of colony count results

	Colony counts (log_{10})				
Time (hour)	Tube C[a] Growth Control (no antibiotic)	Tube Z[b] Ceftolozane only (4 µg/ml)	Tube F[c] Ceftolozane and tazobactam removed	Tube T[d] Tazobactam removed	Tube N[e] Ceftolozane/ tazobactam at 4/4 µg/ml
E. coli strain 136-4643A					
0	6.3	6.3	6.3	6.3	6.3
1	6.8	6.7	6.3	6.2	6.1
2	7.9	7.7	4.5	4.4	4.4
Wash/resuspension					
2	4.6	4.6	2.3	2.2	1.8
3	5.3	5.1	2.4	2.2	1.6
4	6.6	6.5	3.3	2.3	1.5
5	7.2	7.0	4.3	2.9	1.3
6	7.9	7.9	5.2	4.0	0.0
7	8.7	8.7	6.1	4.7	0.0
8	10.0	9.0	7.0	5.6	0.0
9	-	-	7.7	6.5	0.0
10	-	-	8.3	7.3	0.0
E. coli strain 107-5846A					
0	6.4	6.4	6.4	6.4	6.4
1	6.8	6.3	6.0	5.5	5.9
2	7.8	7.3	4.1	3.8	4.0
Wash/resuspension					
2	4.6	4.1	1.7	1.5	1.3
3	5.3	4.9	1.5	1.0	0.0
4	6.2	5.8	2.6	2.0	0.0
5	7.2	6.7	3.2	3.1	0.0
6	8.1	7.7	4.1	3.8	0.0
7	8.5	8.4	4.9	4.7	0.0
8	-	-	5.7	5.5	0.0
9	-	-	6.9	6.6	0.0
10	-	-	7.7	7.7	0.0

a. Growth control: There was no drug exposure and the organisms were washed in drug free cation-adjusted Muller-Hinton broth (CAMHB) and resuspended at 1:1000 dilution (approximately 3 log_{10} dilution) in CAMHB.

b. Ceftolozane alone: The organisms were exposed for 2 hours to ceftolozane alone (4x to 16x the MIC for ceftolozane/tazobactam), the organisms were then washed in drug free CAMHB and resuspended at 1:1000 dilution (approximately 3 log_{10} dilution) in CAMHB containing ceftolozane alone at the same concentration present during exposure.

c. Post-antibiotic effect (PAE): The organisms were exposed for 2 hours to ceftolozane/tazobactam, washed in drug free CAMHB and resuspended at 1:1000 dilution (approximately 3 log_{10} dilution) in drug free CAMHB.

d. PBLIE tube: The organisms were exposed for 2 hours to ceftolozane/tazobactam, washed in drug free CAMHB and resuspended at 1:1000 dilution (approximately 3 log_{10} dilution) in CAMHB containing ceftolozane alone at the same concentration present during exposure.

e. Activity control: The organisms were exposed for 2 hours to ceftolozane/tazobactam, washed in drug free CAMHB and resuspended at 1:1000 dilution (approximately 3 log_{10} dilution) in CAMHB containing both ceftolozane and tazobactam at the same concentration present during exposure.

Figure 1. Post-β-lactamase inhibitor effect (PBLIE) of *E. coli* strain 136-4643A after 2-hr exposure to ceftolozane/tazobactam (4 µg/ml)

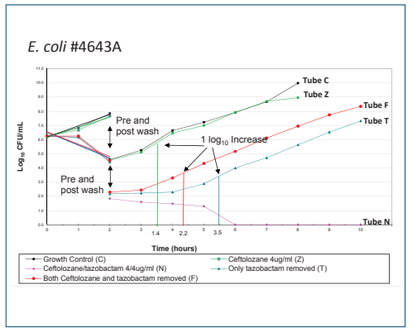
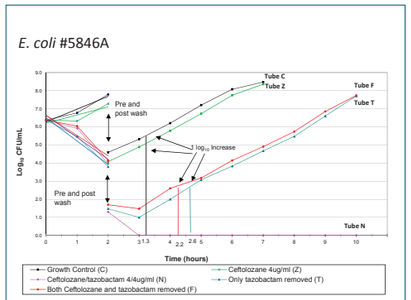


Figure 2. Post-β-lactamase inhibitor effect (PBLIE) of *E. coli* strain 107-5846A after 2-hr exposure to ceftolozane/tazobactam (4 µg/ml)



CONCLUSIONS

- Tazobactam efficiently protects ceftolozane from hydrolysis by CTX-M-15
- Ceftolozane/tazobactam PAE after 2 hours exposure at 4x MIC was 0.8 to 0.9 hours when testing *E. coli* clinical strains 136-4643A (CTX-M-15-producing) and 107-5846A (CTX-M-15- and TEM-1-producing)
- When only tazobactam is removed after 2 hours exposure to ceftolozane/tazobactam at 4x MIC, the time interval for the number of viable organisms to increase 1 log_{10}, ie. the PBLIE, was 1.3 to 2.1 hours.

REFERENCES

1. *Antimicrobial pharmacodynamics in theory and clinical practice*. (2002). Nightingale C. H., Murakawa T. and Ambrose P. G. New York, NY: Marcel Dekker, Inc.
2. Castanheira M, Farrell SE, Deshpande LM, Mendes RE, Jones RN (2013). Prevalence of β-lactamase encoding genes among Enterobacteriaceae bacteremia isolates collected in 26 USA hospitals: Report from the SENTRY Antimicrobial Surveillance Program (2010). *Antimicrob Agents Chemother* 57: 3012-3020.
3. Clinical and Laboratory Standards Institute (2006). *M26-A. Methods for determining bactercidal activity of antimicrobial agents; approved guideline*. Wayne, PA: CLSI.
4. Clinical and Laboratory Standards Institute (2012). *M07-A9. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard: ninth edition*. Wayne, PA: CLSI.
5. Clinical and Laboratory Standards Institute (2013). *M100-S23. Performance standards for antimicrobial susceptibility testing: 23rd informational supplement*. Wayne, PA: CLSI.
6. Lavigne JP, Bonnet R, Michaux-Charachon S, Jourdan J, Caillon J, Sotto A (2004). Post-antibiotic and post-beta-lactamase inhibitor effects of ceftazidime plus sulbactam on extended-spectrum beta-lactamase-producing Gram-negative bacteria. *J Antimicrob Chemother* 53: 616-619.
7. Miller B, Hershberger E, Benziger D, Trinh M, Friedland I (2012). Pharmacokinetics and safety of intravenous ceftolozane-tazobactam in healthy adult subjects following single and multiple ascending doses. *Antimicrob Agents Chemother* 56: 3086-3091.
8. Moody J, Knapp C (2004). *Tests to assess bactericidal activity. In Clinical Microbiology Procedures Handbook*. Washington, DC: ASM Press.
9. Thorburn CE, Molesworth SJ, Sutherland R, Rittenhouse S (1996). Postantibiotic and post-beta-lactamase inhibitor effects of amoxicillin plus clavulanate. *Antimicrob Agents Chemother* 40: 2796-2801.
10. VanScoy B, Mendes RE, Castanheira M, McCauley J, Bhavnani SM, Forrest A, Jones RN, Okusanya OO, Friedrich L, Steenbergen J, Ambrose PG (2013). Relationship between ceftolozane/tazobactam exposure and drug-resistance amplification in a hollow-fiber infection model. *Antimicrob Agents Chemother* in press.
11. VanScoy B, Mendes RE, Nicasio AM, Castanheira M, Bulik CC, Okusanya OO, Bhavnani SM, Forrest A, Jones RN, Friedrich LV, Steenbergen JN, Ambrose PG (2013). Pharmacokinetics-pharmacodynamics of tazobactam in combination with ceftolozane in an in vitro infection model. *Antimicrob Agents Chemother* 57: 2809-2814.

This study was funded by Cubist Pharmaceuticals