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A comparative study of Sulbactomax versus Ceftriaxone and betalactamase inhibitor and their effect on mutant prevention in ESBL producing organisms

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Abstract

Objective: In the present investigation, MPC of Sulbactomax was compared with ceftriaxone+sulbactam and ceftriaxone against ESBL producing *Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae, Proteus vulgaris, Escherichia coli and Citrobacter braakii.*

Methods: The MPC of each antibacterial agent was determined using agar dilution method with a final inoculum size of 10^{10} CFU/ml of each organism.

Result: The Sulbactomax MPC for ESBL organisms was in the range from 32 to 128 µg/ml and for ceftriaxone+sulbactam and ceftriaxone, it ranged from >256 and >512, respectively. The results suggest that Sulbactomax is highly efficacious *in-vitro* against selected ESBL producing organisms with lower MPC values, when compared with the ceftriaxone+sulbactam and ceftriaxone.

Conclusion: Sulbactomax is one of the best choice to treat the infections caused by the above said ESBL producing micro-organisms, indicating to be effective in the prevention of disease caused by these ESBL organisms.

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INTRODUCTION

Ceftriaxone is a broad spectrum third generation cephalosporin. It has activity against gram- negative as well as gram-positive organisms.[1,2] Widespread

use of third generation cephalosporins causes of the mutations in enzymes TEM-1, TEM-2 and SHV-I that has led to the emergence of the ESBLs (extended spectrum beta-lactamases).[3,4] In recent years there has been an increase in incidence and prevalence of ESBL producing microbial diseases. The worldwide prevalence of ESBL producing organisms varies from <1% to 74%.[5,6,7] ESBLs are more prevalent in outbreaks of infections caused by ESBL producing strains.[8]

Beta-lactamase producing organisms resistant to various beta-lactams, extended-spectrum cephalosporins, and even carbapenems are rising.[9] This emergence and spread of resistance is also threatening to create species resistant to all currently available agents. Approximately 20% of *K. pnenumoniae* infections and 31% of *Enterobacter* species infections in intensive care unit in the United States now involve strains that are not susceptible to 3rd-generation cephalosporins.[10] To overcome the problem of increasing antimicrobial resistance against ESBL producing organisms, one of the most promising methods is the development of new combinations to be effective against different ESBL producing organisms. A new combination of ceftriaxone and sulbactam with VRP1034 (EDTA) (Sulbactomax, by Venus Remedies Limited) is one such example.

Mutant prevention concentration (MPC) is a novel concept that has been employed in the evaluation of an antibiotic's ability to minimize or limit the development of resistant organisms [11,12]. The MPC has been defined as the MIC of the least susceptible single-step mutant. By definition, cell growth in the presence of antibiotic concentrations greater than the MPC requires an organism to have developed two or more resistance-causing spontaneous chromosomal point mutations[11,12].

Antibiotic activity can be measured in various ways. For susceptible organisms, activity is usually measured in terms of the minimum inhibitory concentration (MIC), the concentration of drug that prevents growth when 10^5 to 10^9 cells are used. But for resistant organisms, activity is measured in terms of mutant prevention concentration (MPC), the concentration of drug that prevents growth when at least 10^{10} cells are applied to agar plates. Experimentally, MPC has been taken as the drug concentration that allows no mutant to be recovered from a susceptible population of more than 10^{10} cells.[13,14]

MPC studies have been reported for fluoroquinolones, aminoglycosides, beta-lactam antibiotics including penicillins, cephalosporins and carbepenemsetc on ESBL producing *A. baumannii*, *Citrobacter freundii*, *Enterobacter cloacae, E. coli*, *K. pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Stenotrophomonas maltophilia*.[15,16,17] In view of increasing rates of resistance, MPC data may be expected to provide information other than that from MIC determinations or the values of pharmacokinetic/pharmacodynamic parameters on the antibacterial activities of antibacterial agents. To help halt further selection of resistant mutants, we have defined a drug concentration threshold above which bacterial cells require the presence of two or more resistance mutations for growth. The simultaneous occurrence of multiple mutations is a rare event relative to the number of cells present during infection; consequently, administration of antibiotic above the concentration threshold, which we call the mutant prevention concentration (MPC), should severely restrict selection of resistant mutants.

As far as we know, no MPC studies have been reportedon combination of beta-lactams antibiotics with beta lactamase inhibitors. Therefore, in the current study, effect of addition of beta lactamase inhibitor to cephalosporin antibiotic on MPC valueshas been studied. Also, we studied the effect of addition of VRP 1034 in combination of cephalosporin and beta-lactamase inhibitor on MPC

of ESBL producing *P.aeruginosa, A. Baumannii, K. pneumonia, P.vulgaris, E. coli, and C.braakii.*

MATERIALS AND METHODS

Bacterial strains

Following ESBL strains obtained from Post Graduate Institute of Medical Sciences, Lucknow, were used for this study- *P. aeruginosa, A. baumannii, K. Pneumoniae, P. Vulgaris, E. coli* and *C. Braakii*. All of the bacterial strains were obtained from clinical settings.

Antibiotics

Sulbactomax (ceftriaxone+sulbactam+EDTA), and ceftriaxone (Rocephin) used in the study were provided by Sponsor Venus Pharma GmbH, Germany and ceftriaxone+sulbactam (Oframax forte) was procured from Indian market on behalf of sponsor for the study.

Measurement of MPC

MPC was determined as described earlier with slight modifications.[14] Each strain was inoculated on SCDA (Soyabean casein digest agar) plate and incubated at 37°C overnight. 3 to 5 colony of each bacterial strain were collected from these plates, transferred to 50 ml SCDM (Soyabean casein digest medium) broth and incubated at 37°C overnight and bacterial cells were collected by centrifugation at 4°C. The cells were washed three times with broth medium and re-suspended in a small amount of broth, resulting in bacterial concentrations of about 10^{10} CFU/ml. 100-µl aliquots of suspension were plated onto an M-H (Mueller hinton) plate containing various concentrations of antibiotics. MPCs were determined to be the lowest antibacterial concentrations that completely inhibited bacterial growth after incubation at 37°C for 72 h.

RESULTS

To assess the potency of Sulbactomax, ceftriaxone+sulbactam and ceftriaxone against ESBL producing organisms, we measured MPC against various ESBL producing organisms. The MPC for Sulbactomax ranged from 32 to 128 µg/ml and for ceftriaxone+sulbactam and ceftriaxone, it ranged from >256 and >512, respectively. Thus, the values of Sulbactomax MPC are several folds less than ceftriaxone combined with sulbactam and ceftriaxone alone against different ESBL producing organisms used in the study.

DISCUSSION

Antimicrobial resistance has become a major health problem worldwide, affecting every country to some extent. The rate at which resistance is increasing among microbial populations is often driven by the overuse of antimicrobial agents in many clinical settings. Differences in susceptibility to antibiotics by microorganisms has become a major factor in drug choice and success of treatment. Great concerns have been raised regarding emerging antimicrobial resistance among bacteria that may result in unpredictable antimicrobial susceptibility and failure of therapy. [18,19,20]

Cephalosporins have significant and potential advantages over other broad-spectrum non-traditional beta lactam antibiotics.[21,22,23] However, some cephalosporins seem to have low affinity for major chromosomally mediated, beta lactamases and thus are less affected by the ß-lactamase producing organisms. ESBL producing organisms are a major problem in the area of infectious disease. The resistance by members of the enterobacteriaceae, especially *E. coli*, *Klebsiella* and *Salmonella* species have been increasing globally, such resistance has often been derived from TEM and SHV enzymes by mutation.[24,25,26] A combination of ß-lactam and ß-lactamase inhibitor has shown better bactericidal activities against ESBL organisms.[27] As antimicrobial resistance increases worldwide, there is a great need to develop methods to limit its further spread. The MPC is a concept that has been developed in the hope

of altering dosing regimes such that the growth of resistant organisms could be curtailed.

Concerning the susceptibility to antimicrobial agents all five isolates were found to be resistant to ceftriaxone (MPC >512) and ceftriaxone+sulbactam (MPC >256) in current study. There have been reports of cephalosporins and quinolones susceptibilities of ESBL producing organisms in Latin America declining from 2002 to 2008.[28] In another report, third-generation cephalosporins are poor choices for the treatment of serious infections due to ESBL-producing organisms.[10] In contrast, all five isolates were susceptible to Sulbactomax (32 to 128 µg/ml)in current study. It has also been reported that the ESBL producing organisms are found to be sensitive to the beta-lactam and beta lactamase inhibitor combinations.[7] In this study, Sulbactomax proved to be highly active against selected ESBL producing organisms and it can be used in the infections caused by ESBL organisms. It has been suggested that when infections are caused by these ESBL producing organisms, Sulbactomax can be dosed in such a concentration so that the relevant concentration (above MPC) can be maintained at the site of infection. MPC values, when considered with drug pharmacology, may allow prediction on the probability of resistance selection when bacteria are exposed to antimicrobial agents during therapy for infectious diseases. The above findings suggest that Sulbactomax is more effective as compared to ceftriaxone+sulbactam and ceftriaxone used alone due to presence of VRP1034. VRP 1034 possess chelating activity that might be one of the components interfering with DNA trascription or Plasmid transfer processes[29]. However, a detailed study is required to confirm the facts. The scope of current study was limited to comparative observation of MPC only.

The MPC concept can only be applied to situations in which the evaluated resistance mechanisms are the same as those observed in the clinical setting. Only then may the MPC be a tool by which the development of resistant organisms can be limited.

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Table 1: Comparative MPC values of various ESBL producing organisms against Sulbactomax, ceftriaxone+sulbactam, ceftriaxone

Name of ESBL Producing Microorganism	MPC (µg/ml)		
	Sulbactomax	Ceftriaxone + sulbactam	Ceftriaxone
P. aeruginosa	128	>256	>512
A. baumannii	128	>256	>512
K. pneumoniae	128	>256	>512
P. vulgaris	32	>256	>512
E. coli	128	>256	>512
C. braakii	64	>256	>512

REFERENCES

1) Nath SK, Foster GA, Mandell LA, Rotstein C. Antibacterial activity of Ceftriaxone versus cefotaxime: negative effect of serum albumin binding of Ceftriaxone. J Antimicrob Chemother. 1994;33:1239-1243.

2) Masood H, Naqvi SB, Aslam N. Cost effective analysis of different brands of ceftriaxone available in Karachi Pakistan. Pak J Pharmacol. 2008;25:13-19

3) Nathisuwan S, Burgess DS, Lewis II JS. ESBLs: Epidemiology, Detection and Treatment. Pharmacotherapy 2001;21:920-928.

4) Ayyagari A, Bhargava A. ß-lactamases and their clinical significance (A mini review). Hosp Today. 2001;6:1-6.

5) Phillipon A, Labia R, Jacoby G. Extended spectrum beta lactamases Antimicrobial Agents Chemother.1989;33:1131-136.

6) Thomson KS, Sanders CC, Moland ES. Use of microdilution panels with and without beta lactamase inhibitors as phenotypic test for β--lactamase production among *E. coli, Klebsiella spp, Enterobacter spp, citrobacter freundii* and *serratia marcescens*. Antimicrob Agents Chemother. 1994;43:1393-400.

7) Shriyan A, Sheetal R, Nayak N. Aerobic micro-organisms in post-operative wound infections and their antimicrobial susceptibility patterns. J Clin Diag Res. 2010;10:3392-3396.

8) Thokar MA, Fomda BA, Maroof P,Ahmed K, Bashir D, Bashir G. Proliferation of extended spectrum beta–Lactamase (ESBL) producing gram negative bacteria, diagnostic inputs and impact on selection of antimicrobial therapy. Physicians Academy 2010; 4:25-31.

9) Drawz SM, Bonomo RA. Three decades of ß--lactamase inhibitors. Clin Microbiol Rev. 2010; 23:160-201.

10) Paterson DL, Bonomo R A. Extended-spectrum β-lactamases: a clinical update. Clin Microbiol Rev. 2005;18:657-686.

11) Dong Y, Zhao X, Kreiswirth BN, Drlica K. Mutant prevention concentration as a measure of antibiotic potency: studies with clinical isolates of *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother. 2000;44, 2581-2584.*

12) *Blondeau JM, Zhao X, Hansen G, Drlica K. Mutant prevention concentrations of fluoroquinolones for clinical isolates of Streptococcus pneumoniae*. Antimicrob Agents Chemother. 2001;45, 433–438.

13) Dong Y, Zhao X, Domagala JM, Drlica K. Effect of fluoroquinolone concentration on selection of resistant mutants of Mycobacterium bovis BCG and Staphylococcus aureus. Antimicrob Agents Chemother. 1999; 43:1756-1758.

14) Zhao X, Eisner W, Perl-Rosenthal N, Kreiswirth B, Drlica K. Mutant Prevention Concentration of Garenoxacin (BMS-284756) for ciprofloxacin-susceptible or -Resistant *Staphylococcus aureus*. Antimicrob Agents Chemother. 2003;47:1023-1027.

15) Akins RL, Haase KK, Morris AJ. Comparison of various fluoroquinolones (FQs) and four other antibiotics by mutant prevention concentration (MPC) against multi-drug resistant gram-negatives utilizing kill curves based on MPC-derived doses. In Program and Abstracts of the Forty-second Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, CA, 2002. A-1211, p. 10. American Society for Microbiology, Washington, DC, USA.

16) Blondeau, JM. MPC: the mutant prevention concentration (MPC) as a predictor of antimicrobial resistance. In Program and Abstracts of the Forty-second Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, CA, 2002. A-1206, p. 468. American Society for Microbiology, Washington, DC, USA.

17) Zhao X, Drlica K. Restricting the selection of antibiotic-resistant mutant bacteria: measurement and potential use of the mutant selection window. J Infect Dis. 2002;185:561-565.

18) Huang , Lin TL, Wu CC. Antimicrobial susceptibility and resistance of chicken *Escherichia coli*, *Salmonella* spp., and *Pasteurella multocida* isolates. Avian Dis. 2009; 53:89-93.

19) Khameneh ZR, Afshar AT. Antimicrobial susceptibility pattern of urinary tract bacteria. Saudi J Kidney Dis Transpl. 2009;20:251-3.

20) Masood SH, Aslam N. *In-vitro* Susceptibility Test of Different Clinical Isolates against Ceftriaxone. OMJ 2010; 25:199-202.

21) Shrivastava SM, Saurabh S, Rai D, Chaudhary M. Comparative evaluation of microbial efficacy of Potentox, a fixed dose combination of cefepime amikacin with cefepime and amkacin alone. J Natcon. 2008;20:121-126.

22) Tsuji A, Maniatis A, Bertram MA, Young LS. *In-vitro* activity of BMY 28142 in comparison with those of other ß-lactam anti microbial agents. Antimicrob Agents Chemother. 1985;27:515-519.

23) Kessler RE, Bies M, Buck RE, et al. Comparison of a new cephalosporin, BMY 28142, with other broad-spectrum beta-lactam antibiotics. Antimicrob Agents Chemother. 1985;27:207- 216.

24) Bouchillon SK, Johnson BM, Hoban DJ, Johnson JL, Dowzicky MJ, Wu DH. Regional incidence of extended spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae*, *vancomycin resistant enterococcus faecium* (VREF), and *methicillin resistant staphylococcus aureus* (MRSA) from 31 Centers in 14 Countries: The Pan-european antimicrobial resistance using local surveillance (PEARLS). Antimicrob Agents Chemother. 2002; 42:27-30.

25) Kadar AA, Angamathu K. Outpatients and demonstrates their high resistance to various classes of antimicrobial agents. Saudi Med J. 2005;26:956-959.

26) Hasman H, Mevius D, Veldman K, Olesen I, Aarestrup FM. Beta-lactamases among extended-spectrum beta lactamase (ESBL)-resistant *Salmonella* from poultry, poultry products, and human patients in The Netherlands. J Antimicrob Chemother. 2005; 56:115- 121.

27) Phelps DJ, Carlton DD, Farrell CA, Kessler RE. Affinity of cephalosporins for ß-lactamase as a factor in antibacterial efficacy. Antimicrob Agents Chemother. 1986;29:845-884.

28) Villegas MV, Blanco MG, Sifuentes-Osornio J, Rossi F. Increasing prevalence of extended- spectrum betalactamase among gram-negative bacilli in Latin America - 2008 update from the study for monitoring antimicrobial resistance trends (SMART). Braz J Infect Dis. 2011;15:34-39.

29) Dwivedi V K, Payasi A, Ahmad A, Bhatnagar A, Siddiqui MR, Tariq A and Chaudhar M. In-vitro tissue penetration study of Sulbactomax:a novel fixed dose combination of ceftriaxone and sulbactam in rats.. Int J Drug Dev Res, 2010;2:141-147.