In Vitro Microbial Efficacy of Sulbactomax: A Novel Fixed Dose Combination of Ceftriaxone Sulbactam and Ceftriaxone Alone

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Abstract: Microorganism susceptible to beta lactam antibiotics are fastly becoming resistant because of production of beta lactamase by microorganisms. This study is aimed at evaluating microbial efficacy of Sulbactomax drug, a novel fixed dose combination of beta lactam antibiotic Ceftriaxone and beta lactamase inhibitor Sulbactam drugs. Efficacy was evaluated on the basis of Minimum Inhibitory Concentration (MIC) and time kill curve analysis in *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and *Klebsiella pneumoniae*. The MIC for Sulbactomax in *E. coli* was 0.0625 mg/l whereas Ceftriaxone alone showed MIC of 0.25 mg/l. In case of *S. aureus* and *P. aeruginosa* MIC were found to be 1 mg/l for Sulbactomax and 2 mg/l for Ceftriaxone. There was significant reduction of MIC values to 8 mg/l of Sulbactomax from 32mg/l of Ceftriaxone in *B. subtilis* and 2mg/l of Sulbactomax from 16mg/l of Ceftriaxone.

In all organisms under study, time-kill curve analysis demonstrated bacterial maximum killing at 6 hours. Sulbactomax demonstrated better bactericidal activity than Ceftriaxone alone.

In conclusion, Sulbactomax was found to have more bacterial inhibiting properties than Ceftriaxone alone in *in vitro* analysis.

Key Words: Sulbactomax, ceftriaxone, MIC, time kill curve.

INTRODUCTION

The third generation cephalosporins was introduced into clinical practice in the early 1980s, and since then they have served as efficacious and fairly safe agents for the management of many serious infections [1]. The recent appearance of Extended Spectrum Betalactamases (ESBLs) which are capable of conferring resistance to some Enterobacteriaceae, has compromised the effectiveness of the third generation cephalosporins in clinical practice [2, 3].

Many microorganisms initially susceptible to Ceftriaxone, a beta lactam antibiotic, have become resistant due to the formation of beta lactamases [4]. Beta lactamase was first identified in *Escherichia coli* in 1940 [5]. It is interesting to know that in certain pathogens, beta lactamase production was already widespread when semi synthetic penicillins first appeared [6]. Past attempts to counter beta lactam resistance centred on designing new cephalosporins that were more stable to enzymatic hydrolysis. However, experience has shown that bacteria soon produce new beta lactamase capable of destroying the more stable drugs. A more recent and perhaps more fundamental approach is to combine a beta lactam antibiotic with a familiar beta lactamase inhibitor in an attempt to restore full therapeutic potential. Indeed, suicide inhibitors such as clavulanic acid, sulbactam and tazobactam represent the current state of the art in sulbactam lactamase inhibition. In combination with penicillins or cephalosporins, they produce remarkably effective, broad spectrum of antimicrobial activity with the safety which is the characteristic of beta lactam antibiotics [6]. Sulbactam is a beta lactamase inhibitor similar in structure to clavulanic acid [7]. If sufficient inhibitor is present at the site of infection, the beta lactamase enzymes should be neutralized and thus the drug used in combination with inhibitor should have an opportunity to inhibit bacterial growth [8].

The objective of the present study was to see if combination of sulbactam with ceftriaxone potentiates the antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis* and *Klebsiella pneumoniae.*

MATERIALS AND METHODS

Bacterial Strains

Following strains obtained from Microbial Type Collection Centre of Institute of Microbial Technology, Chandigarh, India were used for this study – *Escherichia coli* (MTCC- 1687), *Pseudomonas aeruginosa* (MTCC- 1688), *Staphylococcus aureus* (MTCC- 737), *Bacillus subtilis* (MTCC – 736) and *Klebsiella pneumoniae* (MTCC - 109).

Antibiotic

Sulbactomax (FDC of ceftriaxone and sulbactam in the ratio of 2:1) and ceftriaxone used in the study were provided by manufacturer (Venus Remedies Limited, India) for the study.

Medium

Mueller-Hinton (MH) broth supplemented with calcium (25 mg/l) and magnesium (12.5 mg/l) was used for suscepti-

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1574-8855/09 $55.00+.00

Table 1. MIC of Sulbactomax and Ceftriaxone

S. No.	Microorganism	MIC of Sulbactomax (mg/L)	MIC of Ceftriaxone (mg/L)
1.	*E. coli*	0.0625	0.25
2.	*P. aeruginosa*	1	2
3.	*S. aureus*	1	2
4.	*B. subtilis*	8	32
5	*K. pneumoniae*	2	16

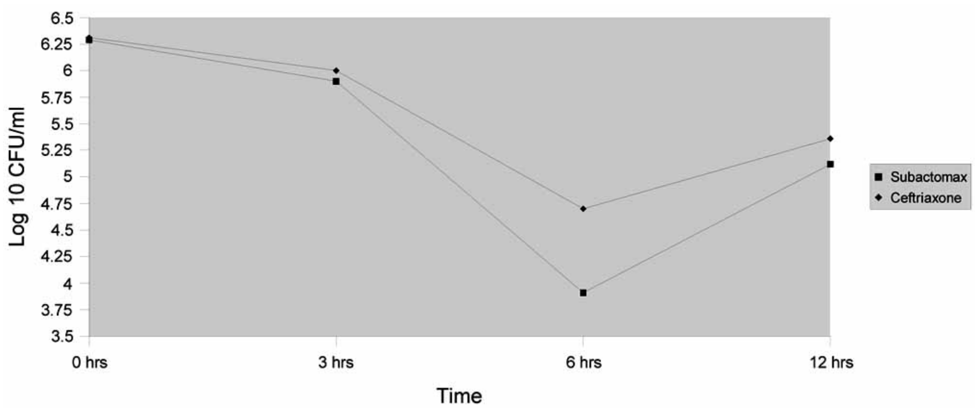

Fig. (1). Time kill curve of Sulbactomax in comparison with Ceftriaxone in *E. coli.*

bility tests and killing curve experiments. Colony counts were determined with MH agar plates.

Susceptibility Testing

The MICs of Sulbactomax and Ceftriaxone for the five strains were determined in cation-supplemented MH broth by the microdilution technique [9,10]. Overnight MH broth cultures were used to prepare inocula of 10^5 Colony Forming Unit (CFU)/ml. The Minimum Inhibitory Concentrations (MIC) were defined as the lowest concentration of antimicrobial agent that prevented turbidity after 24 h of incubation at 37^oC.

Time-Kill Curve Studies

For each strain, time-kill curve studies were performed in MH broth in glass flasks with an inoculum of 5 X 10^6 to 1 X 10^7 CFU/ml in the presence of a single ceftriaxone or Sulbactomax [11]. A flask of inoculated MH broth with no antibiotic served as a control. The surviving bacteria were counted after 0, 3, 6, and 12 h of incubation at 37^oC by subculturing 50-µl serial dilutions (in 0.9% sodium chloride) onto MH plates with a spiral platter.

RESULTS

Susceptibility studies: MIC of all microbial strains under study resulted in significant reduction in Sulbactomax when compared with Ceftriaxone alone (Table **1**).

The MIC for Sulbactomax in *E. coli* was 0.0625 mg/l whereas Ceftriaxone alone showed MIC of 0.25 mg/l. In case of *S. aureus* and *P. aeruginosa* MIC were found to be 1 mg/l for sulbactomax and 2 mg/l for Ceftriaxone. There was significant reduction of MIC values to 8 mg/l of Sulbactomax from 32mg/l of Ceftriaxone in *B. subtilis* and 2mg/l of Sulbactomax from 16mg/l of Ceftriaxone .

Time-kill curve analysis: Bactericidal effect, with 2X the MIC of Sulbactomax and ceftriaxone achieved the earliest killing at 2 hours. Bacterial killing rate in Sulbactomax was distinctly higher at 6 hours than Ceftriaxone.

In *E. coli*, time-kill curve analysis demonstrated bacterial killing from 6.31 to 4.7 $\log_{10}$ CFU/ml by 6 hours for ceftriaxone and killing from 6.29 to 3.91 $\log_{10}$ CFU/ml for Sulbactomax (Fig. **1**). Ceftriaxone has killing of 4.95 $\log_{10}$ CFU/ml, 4.9 $\log_{10}$ CFU/ml, 5.40 $\log_{10}$ CFU/ml and 5.43 $\log_{10}$ CFU/ml after 6 hours in *P. aeruginosa, S. aureus, B. subtilis* and *K. Pneumoniae,* respectively. When Sulbactomax was tested with these organisms bacterial killing of 4.85 $\log_{10}$ CFU/ml, 4.48 $\log_{10}$ CFU/ml, 5.08 $\log_{10}$ CFU/ml and 4.93 $\log_{10}$ CFU/ml after 6 hours was reported (Figs. **2**, **3**, **4**, **5**).

DISCUSSION

Ceftriaxone is a compound that has potent bactericidal activity against both gram-negative and gram-positive bacteria, particularly clinically relevant pathogens. Susceptibility

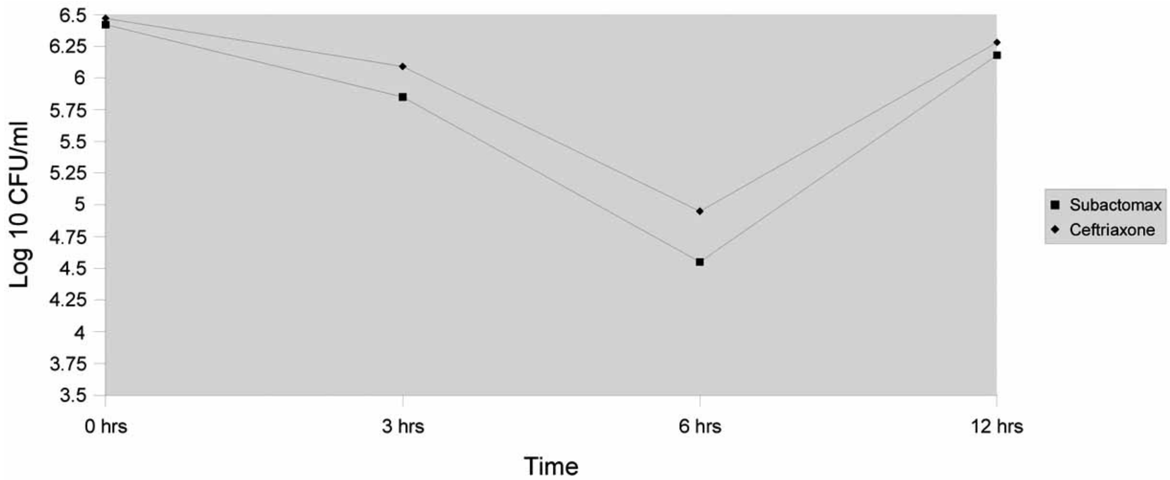

Fig. (2). Time kill curve of Sulbactomax in comparison with Ceftriaxone in *P. aeruginosa.*

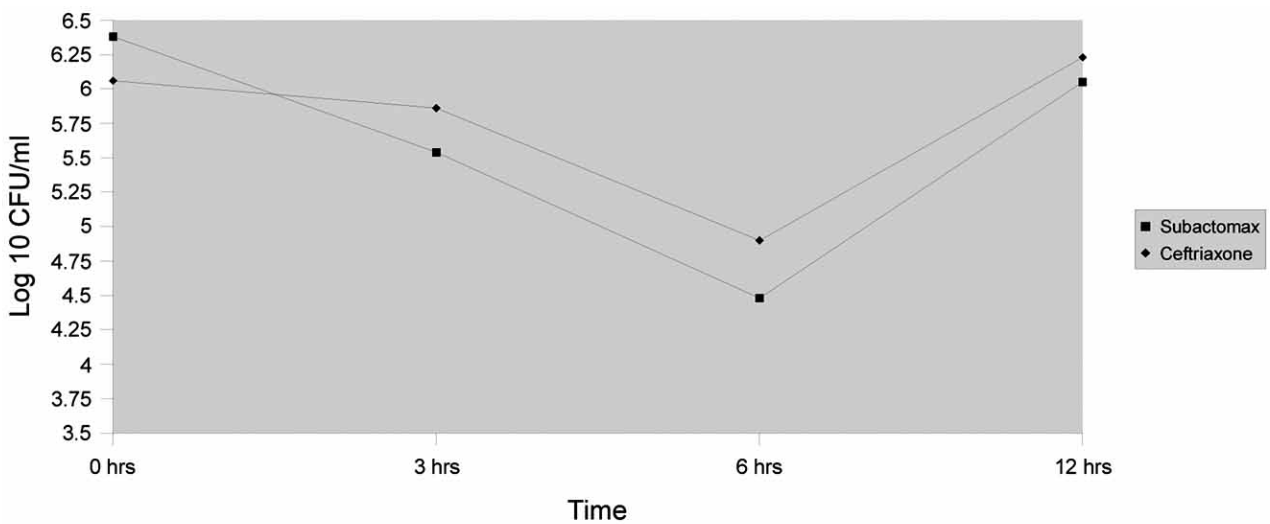

Fig. (3). Time kill curve of Sulbactomax in comparison with Ceftriaxone in *S. aureus.*

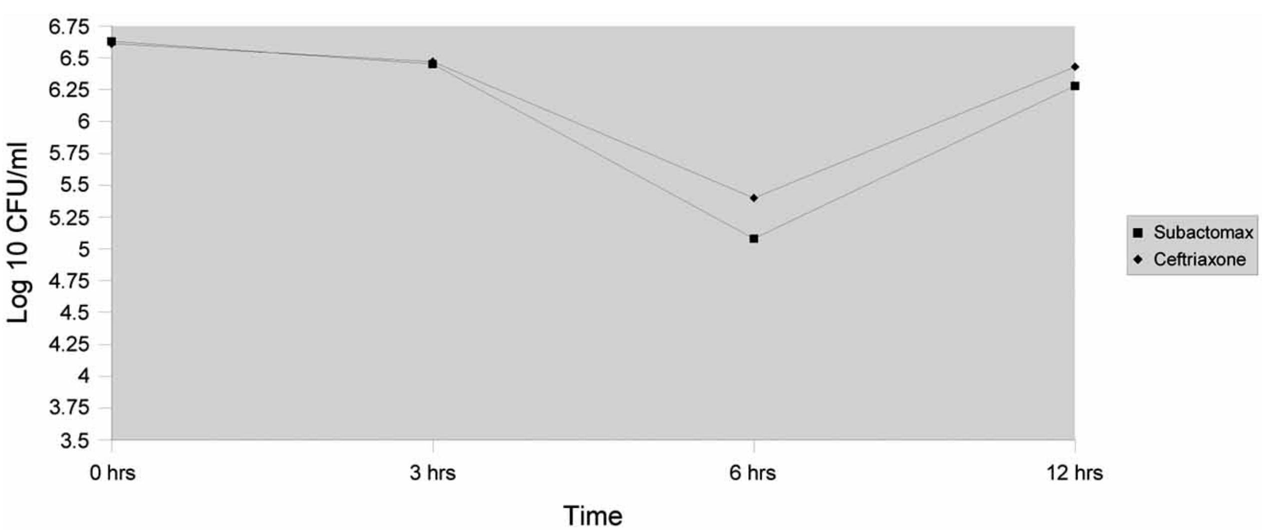

Fig. (4). Time kill curve of Sulbactomax in comparison with Ceftriaxone in *B. subtilis.*

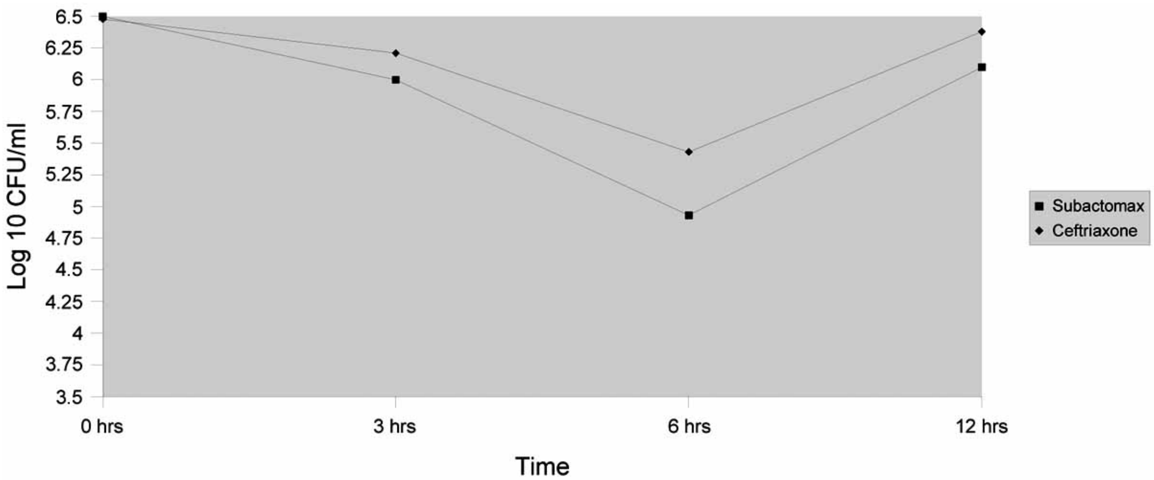

Fig. (5). Time kill curve of Sulbactomax in comparison with Ceftriaxone in *K. pneumoniae.*

data from our study demonstrated that ceftriaxone has higher MIC value than Sulbactomax, suggesting higher bactericidal activity in Sulbactomax. This was confirmed by time-kill analysis, which demonstrated that Sulbactomax has better bactericidal activity than Ceftriaxone even at a concentration of 2X the MIC after 12 hours. Indeed, in all organisms under study, Sulbactomax, demonstrated similar pattern of bactericidal activities when compared with Ceftriaxone.

Cephalosporins have significant and potential advantages over other broad-spectrum nontraditional beta lactam antibiotics [12-15]. In addition, some cephalosporins seem to have low affinity for major chromosomally mediated, beta lactamases and thus are less affected by the nonhydrolytic barrier mechanism of resistance in these bacteria. A combination of beta lactam and beta lactamase inhibitor has shown better bactericidal activities [16].

Sulbactam, being beta lactamase inhibitor also has minor therapeutic potential [17]. Since the activity of the combination of beta lactam antibiotic and beta lactamase inhibitor comes almost exclusively from the sulbactam component [18, 19]. The use of a combination of ceftriaxone and sulbactam in Sulbactomax may be able to provide better therapeutic effect than Ceftriaxone alone against infections of *E. coli, P. aeruginosa, S. aureus, B. subtilis* and *K. pneumoniae.*

In summary, the results of MIC and time kill studies are concordant for *E. coli, P. aeruginosa, S. aureus, B. subtilis* and *K. pneumoniae*. Sulbactomax has shown better bactericidal effect than Ceftriaxone alone in organisms under study.

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