Studies on Susceptibility, Minimum Inhibitory Concentration and Time Kill Curve of Cefpirome and Sulbactam in Combination and Cefpirome Alone in Proteus vulgaris, Pseudomonas aeruginosa, Escherichia coli and Klebsiella pneumoniae

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(Received: 08 September 2008; accepted: 21 November 2008)

Cefpirome is a fourth-generation cephalosporins with better broad spectrum activity against gram negative organisms. Sulbactam is a β -lactamase inhibitor similar in structure to clavulanic acid, if sufficient inhibitor is present at the site of infection, the β -lactamase enzymes should be neutralized and thus the drug used in combination with inhibitor should have an opportunity to inhibit bacterial growth. This study was aimed at evaluating microbial efficacy of combination in comparison with Cefpirome and Sulbactam alone. Efficacy was evaluated on the basis Susceptibility analysis, MIC (Minimum Inhibitory Concentration) and Time Kill Curve analysis in *Proteus vulgaris, Pseudomonas aeruginosa, Escherichia coli* and Klebsiella pneumoniae. In all organisms under study, time kill curve analysis demonstrated bacterial maximum killing at 3 hours. In conclusion, Combination of Cefpirome and Sulbactam was found to have more bacterial inhibiting properties than Cefpirome in *in - vitro* analysis.

Key words: Cefpirome, Sulbactam, MIC, Time Kill Curve, Anti-Susceptibility Test.

Cefpirome (CPR) is a fourth - generation cephalosporins group of antibiotics. It interfere in the cell wall formation leading to bacterial growth. It is a C-3' quaternary ammonium cephalosporin, which bears a 2,3cyclopentenopyridinium at the C-3 position of the cephem nucleus. It belongs to the parenteral 2-amino- 5 thiazolyl cephalosporins¹. It displays expanded antibacterial spectrum including against difficult-to-treat gram negative bacilli, such as members of the family *Enterobacteriaceae* producing class I β -lactamases.

Cefpirome has a low affinity for many β -lactamases of the periplasmic space. Cefpirome displays a well - balanced antibacterial spectrum including gram positive cocci such as methicillin - susceptible *Staphylococcus aureus* strains and *Streptococcus pneumoniae* isolates resistant to penicillin G².

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Sulbactam is a molecule which is given in combination with β-lactam antibiotics to inhibit β -lactamase, an enzyme produced by bacteria that destroys the antibiotics. Sulbactam is an irreversible inhibitor of β -lactamase, it binds the enzyme and does not allow it to interact with the antibiotic. Sulbactam is able to inhibit the most common forms of β -lactamase but is not able to interact with the ampC cephalosporinase . Thus, it confers little protection against bacteria such as Pseudomoans aeruginosa, Citrobacter, β -lactamases have proved to be extremely important in influencing therapy with penicillins and cephalosporins against gram - positive and gram - negative aerobic and anaerobic species. The β - lactam are small organic molecules with four - member strained lactam rings, were effective agents in nature and also became the first antibiotic in human medicine.

The β - lactamase proteins are special chemically because there is very little biochemical difficulty in slightly modifying a gene that codes for an endotranspeptidase so that the chemistry of the enzyme is changed and it becomes a hydrolase³. Many microorganisms initially susceptible to Sulbactam, a β - lactam antibiotic, have become resistant due to the formation of β -lactamases⁴. β - lactamase was first identified in Escherichia coli ⁵. It is interesting to know that in certain pathogens, â - lactamase production was already widespread when semi synthetic penicillins first appeared. Past attempts to counter β-lactam resistance centered on designing new cephalosporins that were more stable to enzymatic hydrolysis.

A more recent and perhaps more fundamental approach is to combine a β - lactam antibiotic with a familiar β - 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 antimicrobial activity with the safety which is characteristic of β - lactam antibiotics⁶. Sulbactam is a β - lactamase inhibitor similar in structure to clavulanic acid⁷. If sufficient inhibitor is present at the site of infection, the β - lactamase enzymes should be neutralized and thus the drug used in

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combination with inhibitor should have an opportunity to inhibit bacterial growth⁸. A 10 medical center study in India was initiated to benchmark prevailing resistance rates for a range of bacterial pathogens to β - lactams, and it found high rates of β – lactamase mediated resistance in *Escherichia coli and Klebsiella spp*. These rates included: cephalosporins (55.6 - 61.3% resistance), with extended-spectrum β -lactamase (ESBL) phetypes noted in over 60% of *E. coli* isolates and in *Salmonella spp*. (3.2-8.1%).

MATERIAL AND METHODS

Bacterial Strains

Following strains obtained from Microbial Type Collection Center of Institute of Microbial Technology, Chandigarh, India were used for the study-

Proteus vulgaris (MTCC NO - 426)

Pseudomonas aeruginosa (MTCC NO -1688) *Escherichia coli* (MTCC NO - 1687)

Klebsiella pneumoniae (MTCC NO - 109)

Antibiotic

Cefpirome and Sulbactam used in 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 (1.25 mg/l) was used for susceptibility tests and killing curve experiments. Colony counts were determined with MH agar plates.

Susceptibility Testing

The MIC of Cefpirome and Sulbactam for the four strain were determined in cation supplemented MH broth by the micro dilution technique (Amsterdam, 1996, NCCLS, 1997). Overnight MH broth cultures were used to prepare inocula of 10⁵ CFU/ml. The MIC was defined as the lowest concentration of antimicrobial agent that prevented turbidity after 24 h of incubation at 37 °C.

Time Kill Curve studies

For each strain, time kill curve studies were performed in MH broth in glass flasks with an inoculum of 5×10^6 to 1×10^7 CFU/ ml in the presence of a single Cefpirome and Sulbactam combination. A flask of inoculated MH broth with no antibiotic served as a control. The surviving

bacteria were counted after 0, 3 and 6 hrs of incubation at 37 $^{\circ}$ C by subculturing 50 μ l serial dilutions (in 0.9 % NaCl) in to MH plates with a spiral plater.

RESULTS

Susceptibility studies

The MIC of all microbial strains under study resulted in significant reduction in Cefpirome, Sulbactum and their combination. (Table-1)

 Table 1. Results of antibacterial susceptibility test

 of cefpirome, sulbactum and their combination

| | Zone diammeter (mm) | |
|----------------|-----------------------------|------------------------------------|
| Microorganisms | Cefpirome | Combination 30µg |
| | $20\mu g$, Avg. \pm S.D. | $(20\mu gC + 10\mu gS)Avg. \pm S.$ |
| P. vulgaris | 26.48±0.413 | 28.98±0.916 |
| P. aeruginosa | 25.20±0.732 | 28.21±0.811 |
| E. coli | 24.61±0.487 | 26.94±0.749 |
| K. pneumoniae | 23.15±0.645 | 27.73 ± 0.428 |

 Table 2. Results of minimal inhibitory concentration of cefpirome sulphate alone and in combination with sulbactam sodium

| Microorganisms | Cefpirome Sulphate (µg/Ml) | C-s Combination (µg/Ml) |
|----------------|-------------------------------|----------------------------|
| P. vulgaris | 2 | 1 |
| P. aeruginosa | 2 | 1 |
| E. coli | 4 | 2 |
| K. pneumoniae | 16 | 4 |









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MIC studies

In case of *Proteus vulgaris, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae* MIC were found to be 1 μ g/l, 1 μ g/l, 2 μ g/l and 4 μ g/l for Cefpirome and Sulbactam Combination respectively. In a Cefpirome alone the MIC was found to be 2 μ g/l, 2 μ g/l, 4 μ g/l and 16 μ g/l (Table 2).



Fig. 3. Time Kill Curve of Cefpirome and Cefpirome - Sulbactum Combination against *E. coli*



Fig. 4. Time Kill Curve of Cefpirome and Cefpirome - Sulbactum Combination against K. pneumoniae.

Time kill curve analysis

Bactericidal effect, with $2 \times$ the MIC of combination of Cefpirome and Sulbactam, Cefpirome and Sulbactam achieved the earliest killing at 3 hours. Bacterial killing rate in combination was distinctly higher at 6 hours than Cefpirome and Sulbactam alone.

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In a *P. vulgaris*, , time kill curve analysis demonstrated bacterial killing from 6.23 to 6.22 Log_{10} CFU /ML by zero hours for Cefpirome and Cefpirome - Sulbactam Combination.

DISCUSSION

There have been contrasting views regarding efficacy of Cefpirome, some authors reported it to be effective and other reported that there is increase in incidence of resistance to Cefpirome. Gupta et. al studied Imipenem, Piperacillin / Tozabactam, Cefoperazone / Sulbactam, Ticarcillin / Clavulanate, Cefdinir, Cefepime and Cefpirome for drug susceptibility pattern against 277 non-duplicate gram negative bacilli strains belonging to the Enterobacteriaceae family, Pseudomonas and Acinetobacter species, isolated from various clinical samples. Highest frequency of resistance (84.4 %) was observed with Cefpirome. They concluded that Ticarcillin / Clavulanate, Cefdinir, Cefepime and Cefpirome are relatively un effective in their environment. As the antimicrobial resistance is growing there is need to find more effective antimicrobial agents9.

Pathogens were isolated and identified from all the clinically evaluable patients. On 14th day complete eradication of pathogens was observed. Cefpirome has zwitter ionic structure, which allows rapid penetration through the outer membrane of gram negative bacilli and a high affinity for Penicillin – binding protein¹⁰. Moreover added Sulbactam inhibits β – lactamases activity enhancing the bactericidal activity of the combination .Goldstein and Citron studied MIC and MBC of Cefpirome in comparison with Ceftazidime and Cefotaxime against Pseudomonas aeruginosa, Enterococci, Staphylococcus epidermis and methiclin - resistant, susceptible and tolerant strains of Staphylococcus aureus. Coparatively, Cefpirome was the most active agent against all gram positive cocci, including enterococci and methicillin- resistant S. aureus and was as active as Ceftazidime against P. aeruginosa¹¹.

 $Chi - Tai Fang \ et. \ al$ studied safety and efficacy of Cefpirome and Ceftazidime in Chinese population suffering from sepsis and reported that both antibiotics had nearly similar safety and

efficacy profile¹². The bacteriological cure rate observed was only present study showed improved bacteriological cure rate of 100 % on day 14. It indicated that the combination for more effective than Cefpirome alone in various infections. Synergy between Sulbactam and β – lacatams has been established¹³. Cefpirome is also known to possess a greater antibacterial spectrum than third – generation cephalosporins¹⁴. Cefpirome has been reported to have better activity oxacillin against *Staphylococci aureus*¹⁵. Cefpirome reported to improve clinical signs and symptoms of infection and offers improved coverage against grampositive and gram-negative pathogens in patients with febrile neutropenia.

Thus, Cefpirome was suggested to be a valuable and cost - effective extended - spectrum agent for the empiric treatment of serve infection¹⁶. A multi centric, randomized comparative trial between Cefpirome (2g, iv, BD) and (2g, iv, TID) Ceftazidime showed clinical cure rate of 34 % and 36 % and bacteriological cure rate of 70 % and 71 %¹⁷, for lesser effective than the present study with combination of Cefpirome and Sulbactam. Thus the combination of Cefpirome - Sulbactam is found to be more effective and would be more cost effective as 1.5g BD dose was given to the patients. This study also demonstrated that Cefpirome -Sulbactam has an excellent safety profile with no adverse events related to drug therapy was observed

Our study indicated that the combination of Cefpirome with Sulbactam has more bactericidal properties than Cefpirome alone in bacteria under study.

ACKNOWLEDGMENTS

Authors are thankful to CGM, Domestic operations of Venus Remedies Limited for providing the samples of Cefpirome and Sulbactam for this study.

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