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Synergistically Active and Safe Fixed Dose Combination of Meropenem and Sulbactam

RESEARCH ARTICLE

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Abstract

Meropenem is a third generation broad spectrum antibiotic. Emergence of meropenem resistance has been reported due to development of mutant plasmid mediated metallo-β-lactamases (IMP-6) and AmpC β–lactamases. Sulbactam, a stable β-lactamase inhibitor, increase antimicrobial activity of meropenem by inhibiting the enzyme β-lactamase. Fixed dose combination of Meropenem-sulbactam in the proportion of 1:1, 1:2, 1:3, 2:1 and 3:1 were evaluated for the antimicrobial activity. Combination of meropenem and sulbactam in the ratio of 2:1 exhibited the synergistic activity. This combination was checked for the subchronic toxicity on wistar rats and no change in biochemical and physiological parameters was observed.

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INTRODUCTION

Since the advent of antibiotic these have been used to treat variety of bacterial infection. Prolonged and overuse of antibiotics have led to development of resistance against the several antibiotic in microorganism as a survival strategy. In human medicine the major problem of the emergence of resistant bacteria is due to misuse and overuse of antibiotics by doctors as well as patients. In addition to this, use of antibiotics in feed of livestock, household use of antibacterial in soaps and other products are some of reason which have contributed to development of antibiotic resistance. Antibiotic resistance can be a result of plasmid or transposons mediated horizontal gene transfer and also of unlinked point mutations in the pathogen's genome at a rate of about 1 in 10^8 per chromosomal replication (Yano et al., 2001). Microorganisms employ several strategies such as intracellular drug inactivation or modification, alteration of drug target site, changes in drug metabolic pathway and increase in drug efflux to increase the chances of survival under meropenem stress (Sinha and Srinivasan, 2010). Meropenem is one of the broad spectrum antibiotics among the carbapenem class therefore precautions should be taken to avoid development of resistant strain and strategies should be employed before the development of resistant strains for benefit of mankind. Several instance of appearance of meropenem resistant bacterial strains have been reported. The gene encoding IMP-6 MBL, a mutant β-lactamase active against the meropenem, was first identified in plasmid pKU501 from *Serratia marcescens* KU3838 (Yano et al., 2001). Since then it has been a major problem for meropenem resistant outbreak due to horizontal gene transfer (Masuda and Ohya 1992, Nordmann and Poirel 2002, Ryoo et al., 2009). Combination therapy has long been used to treat many infections to increase the efficacy of treatment and avoid the development of microbial resistance. Combination of antibiotic with β-lactamases enzyme inhibitor is another fruitful way to increase the spectrum of activity of β-lactam antibiotic and prevent the emergence of resistance strain. Sulbactam has been reported to extend spectrum of activity of several antibiotic such as ceftriaxone, carbenicillin, cefoperazone and ampicillin (Lim and Cheong 1995). Sulbactam has higher stability in the solution compared to its counterpart clavulanate. (Wise et al., 1980). Keeping in the view clinical significance of fixed dose combination of meropenem and sulbactam, we planned to study the antimicrobial activity of the combination and its safety profile.

MATERIALS AND METHODS

Bacterial culture, *Pseudomonas aeruginosa* ATCC 25619 and *Escherichia coli* ATCC 10536 were obtained from Hi-Media laboratories private limited, India. Lauria Bertani

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broth and Mueller Hilton broths were procured from Sisco Research Laboratories private limited (SRL) India. Meropenem carbonate and sulbactam were donated by Health Biotech Limited. Meropenem and sulbactam fixed dose combinations were prepared in the ratio of 1:1, 1:2, 1:3, 2:1 and 3:1 by mixing stock solutions of meropenem and sulbactam so that final concentration of meropenem in combination remained 100 μg/mL. These combinations were diluted to prepare the desired range of meropenem concentration from 0.2 to 25.0 μg/mL for minimum inhibitory concentration (MIC) and minimum bactericidal concentration analysis.

Antimicrobial Activity Analysis

All of the combinations were prepared in 5% sodium carbonate solution in deionized water. These stock solutions were diluted with deionized water to prepare the dilution for MIC and MBC analysis. Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), zone of inhibition and time Kill study were performed on bacterial strains *Pseudomonas aeruginosa* ATCC 25619 and *Escherichia coli* ATCC 10536. MIC and MBC were determined by double dilution technique as per NCCLS M7-A5 guidelines. Accordingly, 96-well microtiter plates containing 200 μL Muller Hinton (MH) broth (SRL, India) with meropenem-sulbactam combination (in the concentration range of 0.1-50.0 μg/mL) were inoculated with test culture (final cell density of 1 × 10^5 CFU/mL) and incubated at 37 °C for 24 h. The lowest concentration of meropenem in the meropenem-sulbactam combination showing growth inhibition (as seen visually) was considered as the minimum inhibitory concentration. The minimum bactericidal concentration was recorded as the lowest concentration of meropenem in meropenem-sulbactam combination that showed no growth on MH agar plates after spot inoculation and incubation for 24 h. Assay was performed in triplicate with appropriate controls (uninoculated medium, meropenem and sulbactam alone). The fractional inhibitory concentration index (FICi) for combination was determined using *Pseudomonas aeruginosa* ATCC 25619 and *Escherichia coli* ATCC 10536. MIC for each of the component was first estimated, and subsequently, the fractional inhibitory concentration (FIC) of a combination of meropenem and sulbactam was calculated (Bharadwaj et al., 2003).

The FIC was calculated as follows:

$FIC_A = MIC_A$ in combination / MIC_A

$FIC_B = MIC_B$ in combination / MIC_B

Where A = Meropenem, B = Sulbactam

$FICi = FIC_A + FIC_B$

The interaction was defined as synergistic if the FICi was ≤0.5, as partial synergy / additive if the FICi was >0.5 to 1.0, as indifferent if the FICi was >1.0 to 2.0, and as antagonistic if the FICi was >2.0.

Time kill study was performed on both bacterial strains. The cultures were inoculated in 2 mL of MH broth (final cell density of 1 × 10^5 CFU/mL) supplemented with meropenem-sulbactam combinations (at concentrations corresponding to 3 x MIC) and incubated for 8 h. Aliquots (0.1 mL) were removed at hourly intervals, serially diluted, and total viable counts on MH agar plates were determined after incubation at 37 °C for 24 h. Kill curves were constructed by plotting the log CFU against time.

Subchronic Toxicity Analysis

Subchronic toxicity study was performed with meropenem-sulbactam in 2:1 proportion. Healthy *Wistar* rats of either sex were divided into four groups and assigned as three treatment groups and one control group. All groups consist of 6 male and 6 female animals. Animals were provided with standard pellet diet and water was given *ad libitum*. They were housed in polyurethane cages (three in each) at controlled room temperature of 29 ± 2°C and a relative humidity of 50.5%, with a constant light-dark schedule (12 hours light and 12 hour dark cycle).

Animals were given freshly prepared intravenous injection of Meropenem-sulbactam for 28 days. The mixture of Meropenem-sulbactam was prepared in 0.9 % NaCl before administration and was injected at following dose levels; Group I –Control group, Group II 100 mg/kg, Group III 200 mg/kg and Group IV 400 mg/kg. Control group was injected 0.9 % NaCl only. Dosing was done approximately at the same time on each day. All the animals were observed for physical, biochemical and hematology alterations. Overnight fasted animals were sacrificed; blood and tissues samples were collected on 29th day. Hemogram was performed on Hematolgy Analyzer (Sysmax K 1000). For histopathological analysis liver, kidney, stomach, Heart and Lungs were removed from the sacrificed animals and were preserved in 10 % buffered formalin. Serum Gluatmic oxaloacetic transaminase (SGOT), Serum Gluatmic pyruvic transaminase activities (SGPT),

Alkaline phosphatase (ALP), Blood urea nitrogen (BUN) and plasma sugar levels were estimated on biochemistry analyzer using diagnostic kits (Robonik ASP-300). Dunnett's test was used for the evaluation of data and P <0.05 accepted as significant.

RESULTS AND DISCUSSIONS

Meropenem and sulbactam fixed dose combination exhibited greater antimicrobial activity compared to meropenem alone. Meropenem-sulbactam combination in the proportion of 2:1 showed bacterial growth inhibition for *E. coli and Pseudomonas aeruginosa* up to 35 hours effectively as compared to meropenem alone (Figure 1 & 2). Physical combination resulted in fast decrease in CFU per ml for first 6 hours, they maintained low CFU upto 24 hours and then slow increase in CFU per ml was seen as compared to meropenem alone. In case of meropenem alone CFU increased faster after 24 hours. There was decrease in MBC from 1.0 µg/mL to 0.8 µg/mL for *E.coli* and 4.0 µg/mL to 2.0 µg/mL for *Pseudomonas aeruginosa* for meropenem to sulbactam combination in proportion of 2:1. This combination has FICi value close to 0.5 and was found to behave synergistically as shown in Table-1.

Table 1. Nature of interaction of fix dose combination

Organism	MIC of meropenem	MIC sulbactam	MIC of combination in ratio of 2:1	FICi Value	Nature of interaction
E. coli	0.40	25	0.30	0.76	Partial synergy
P. aeruginosa	1.0	50	0.40	0.40	Synergistic

Meropenem-sulbactam combination of 2:1 was checked for subchronic toxicity in w*istar* rats at dosages of 100 mg/kg, 200 mg/kg and 400 mg/kg for 28 days. No behavioral changes were observed throughout the dosing period. No significant change group mean body weight was observed in all the groups as compared to control group on 29th day. In male and female rat groups, no significant change was observed in hemoglobin (Hb), red blood cell counts (RBC), Rt (Reticulocyte), hematocrit (HCT), mean corpuscular volume (MCV), mean cell haemoglobin (MCH), mean cell corpuscular hemoglobin concentration (MCHC) ,white blood cell (WBC) counts and platelet counts in all the treated groups as compared to respective control groups (Table 2 & 3).

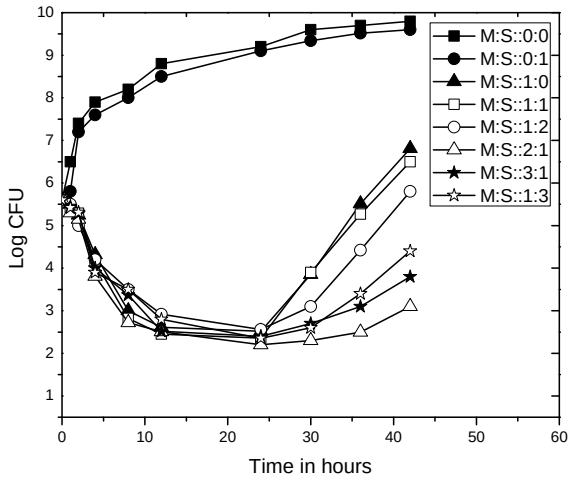

Figure 1. Time kill curve of meropenem-sulbactam combination and controls for *Pseudomonas aeruginosa* (M-Meropenem, S-Sulbactum)

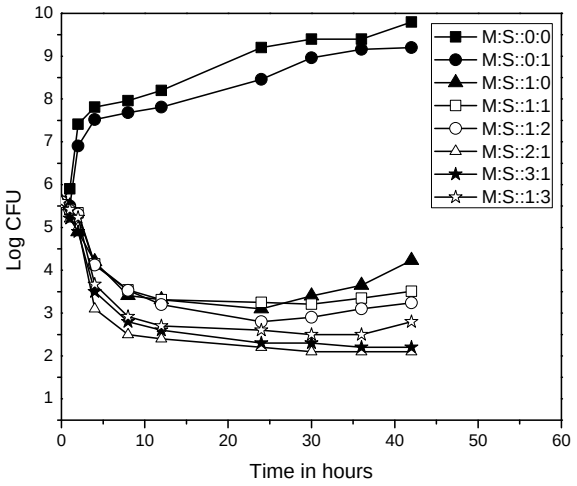

Figure 2. Time kill curve of meropenem-sulbactam combination and controls for *E. coli* (M-Meropenem, S-Sulbactum)

Further there was no significant change in SGOT, SGPT and SAP activities in all the treated groups as compared to respective control group. Serum proteins and Blood sugar levels were comparable treated and control groups (Table 4 & 5). This establishes the safety of meropenem sulbactam combination. SGOT, SGPT and SAP are critical indicator for hepatotoxicity in addition other hematological parameters.

Meropenem is used to treat several bacterial infections such as urinary tract infections, pneumonia, sepsis, intra-

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abdominal infections, skin and soft-tissue infections, meningitis and nosocomial infections (Huizinga et al., 1995, Mouton and Beuscart 1995, Hsu et al., 2001).

Table 2. Effect on hemogram in male rats

Gr. No.	Dose mg/kg	Hb (%)	Total RBC ($x10^6$/cmm)	Rt (%)	HCT (%)	MCV μm^3	MCH(pg)	MCHC (%)	Platelets (10^5/cmm)	Total WBC) $x10^3$/cmm
I	Control	15.22 ± 1.72	5.55±0.53	1.65±0.46	52.00±5.55	66.92±8.09	20.27±3.71	33.18±2.97	5.40±1.74	6.63±1.23
II	100	15.02 ±1.65	6.17±0.88	1.68±0.29	50.17±6.82	63.43±7.99	18.97±2.91	31.73±2.14	5.35±1.28	7.30±1.04
III	200	12.74±0.73	5.83±1.04	1.75±0.31	47.67±4.23	60.20±6.35	16.89±0.70	32.35±1.80	6.56±0.80	6.43±0.76
IV	400	12.47±0.43	6.31±0.48	1.82±0.45	46.50±3.94	59.90±3.17	16.19±0.92	33.55±3.09	5.93±0.72	5.97±0.67

Values are represented as Mean±SD, n=6.

Table 3. Effect on hemogram in female rats

Gr.No	Dose (mg/kg)	Hb (%)	Total RBC ($x10^6$/cmm)	Rt (%)	HCT (%)	MCV (μm^3)	MCH(pg)	MCHC (%)	Platelets ($/10^5$cmm)	Total WBC ($x10^3$/cmm)
I	Control	16.43±1.22	5.90±0.53	1.45±0.41	54.00±5.62	63.57±6.07	21.03±4.11	32.37±2.27	6.20±0.53	6.92±1.14
II	100	13.20±0.88	5.95±0.86	1.70±0.36	50.33±7.34	62.80±7.38	17.88±1.87	32.83±2.62	5.85±1.14	6.43±0.76
III	200	12.96±0.70	6.07±0.67	1.70±0.49	45.50±5.32	60.04±3.25	16.35±0.71	32.64±2.33	6.22±0.71	6.13±0.69
IV	400	12.47±0.41	6.02±0.58	1.70±0.42	42.42±2.38	59.70±2.16	16.03±0.93	34.66±3.37	5.89±0.74	6.06±0.19

Values are represented as Mean±SD, n=6

Table 4. Effect on biochemical parameters in male rats

Gr. No.	Dose (mg/kg)	TSP (g%)	BUN (mg%)	SGPT (IU/L)	SGOT (IU/L)	SAP (IU/L)	Blood Sugar (mg%)
I	Control	7.75±0.29	33.80±2.32	89.52±8.82	91.50±5.01	402.33±96.67	95.48±6.37
II	100	7.65±0.29	30.82±2.61	67.67±11.72	92.83±8.11	447.00±49.25	98.40±7.22
III	200	7.60±0.27	30.41±2.90	96.03±6.86	94.87±8.58	412.67±53.05	100.23±4.89
IV	400	7.48±0.40	40.43±6.3	80.17±12.64	92.50±9.7	428.33±40.01	98.67±5.77

Values are represented as Mean±SD, n=6

Table 5: Effect on Biochemical parameters in female rats

Gr. No.	Dose (mg/kg)	TSP (g%)	BUN (mg%)	SGPT (IU/L)	SGOT (IU/L)	SAP (IU/L)	Blood Sugar (mg%)
I	Control	7.74±0.42	32.78±3.83	71.17±15.42	89.67±8.55	420.33±37.73	97.98±3.91
II	100	7.59±0.43	30.78±4.12	66.83±12.54	92.00±6.26	413.17±35.10	94.77±5.96
III	200	7.53±0.46	30.48±1.98	86.00±14.56	90.53±6.43	428.17±48.44	97.90±3.27
IV	400	7.40±0.35	31.23±3.12	80.00±12.13	88.33±6.68	419.17±29.78	100.90±5.69

Values are represented as Mean±SD, n=6

It is also a promising antibiotic in the treatment of hospitalized infants and children with serious infections because of its broad spectrum antibacterial activity. Meropenem has been approved by United States Food and Drug Administration (US-FDA) for use in pediatric meningitis and severe infections in intensive care settings. Meropenem and sulbactam combination as well as Meropenem, sulbactam and colistin three drug

combinations has been reported to exhibit the synergistic effect on multidrug resistant *Acenetobacter baumannii* isolates. Lee CM *et al* have reported the role of sulbactam combination with carbapenem and second or third generation cephalosporins, antipseudomonas penicillins, or fluoroquinolones with aminoglycosides on Pan-drug resistant (PDRAB) *Acinetobacter baumannii*. They found that 30 % of bacteria turned sensitive to imipenem in presence of sulbactam (Lee et al., 2005). As per Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) report, Meropenem demonstrate good activity against *Enterobacteriaceae*, including strains producing ESBLs or AmpC (100% for *E coli*, 99% for other *Enterobacteriaceae*), meropenem usually being 2 to 4 fold more potent than imipenem (Pfaller and Ronald 2000, Laure et al., 2010, Hernández et al 2006) and susceptibility of *Acinetobacter* was close 94-98%.

The antibacterial activity of Meropenem results from inhibition of the bacterial cell synthesis. It readily penetrates through the cell wall of Gram positive as well as Gram negative bacteria to reach the penicillin binding protein target. Its greatest efficiency is for PBP 2 of *Escherichia coli*, PBP 2 and 3 of *Pseudomonas aeruginosa* and PBP 1, 2 and 4 of *Staphylococcus aureus*. Meropenem being susceptible to enzyme beta-lactamase and carbapenemases (Cécile et al., 2005) produced by the bacteria need to be protected from degradation by these enzymes. Sulbactam is an irreversible inhibitor of most of common beta-lactamase except amp C cephalosporinases. It binds the enzyme and does not allow it to interact with the antibiotic.

CONCLUSION

This study offers an unequivocal proof that meropenem and sulbactam combination act as a potent antimicrobial combination at ratio of 2:1 respectively. In vivo biochemical and hematological experiments established the safety of the combination. Hence this combination provides sustainable solution for antimicrobial chemotherapy.

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