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.

Keywords: Meropenem resistance; β-lactamase inhibitors; Meropenem toxicity

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 Ohyu 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 microbal 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...
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 × 10⁸ 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:

\[ \text{FIC}_{\text{A}} = \frac{\text{MIC}_{\text{A}}}{\text{MIC}_{\text{A}} + \text{MIC}_{\text{B}}} \]

where A = Meropenem, B = Sulbactam

FICI = FICA + FICB

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 × 10⁸ 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 Hematology 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 Glutamic oxaloacetic transaminase (SGOT), Serum Glutamic 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>
<thead>
<tr>
<th>Organism</th>
<th>MIC of meropenem</th>
<th>MIC of sulbactam</th>
<th>MIC of combination in ratio of 2:1</th>
<th>FICi</th>
<th>Nature of interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>0.40</td>
<td>25</td>
<td>0.30</td>
<td>0.76</td>
<td>Partial synergy</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>1.0</td>
<td>50</td>
<td>0.40</td>
<td>0.40</td>
<td>Synergistic</td>
</tr>
</tbody>
</table>

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-
Synergistic Effect of Meropenem and Sulbactam

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

Table 2. Effect on hemogram in male rats

<table>
<thead>
<tr>
<th>Gr. No.</th>
<th>Dose (mg/kg)</th>
<th>Hb (%)</th>
<th>Total RBC (x10^6/cmm)</th>
<th>Rt (%)</th>
<th>HCT (%)</th>
<th>MCV (µm³)</th>
<th>MCH(pg)</th>
<th>MCHC (%)</th>
<th>Platelets (x10^5/cmm)</th>
<th>Total WBC (x10³/cmm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>15.22 ± 1.72</td>
<td>5.55 ± 0.53</td>
<td>1.65 ± 0.46</td>
<td>52.00 ± 5.55</td>
<td>66.92 ± 8.09</td>
<td>20.27 ± 3.71</td>
<td>33.18 ± 2.97</td>
<td>5.40 ± 1.74</td>
<td>6.63 ± 1.23</td>
</tr>
<tr>
<td>II</td>
<td>100</td>
<td>15.02 ± 1.65</td>
<td>6.17 ± 0.88</td>
<td>1.68 ± 0.29</td>
<td>50.17 ± 6.82</td>
<td>63.43 ± 7.99</td>
<td>18.97 ± 2.91</td>
<td>31.73 ± 2.14</td>
<td>5.35 ± 1.28</td>
<td>7.30 ± 1.04</td>
</tr>
<tr>
<td>III</td>
<td>200</td>
<td>12.74 ± 0.73</td>
<td>5.83 ± 1.04</td>
<td>1.75 ± 0.31</td>
<td>47.67 ± 6.23</td>
<td>60.20 ± 6.35</td>
<td>16.89 ± 0.70</td>
<td>32.35 ± 1.80</td>
<td>5.61 ± 0.80</td>
<td>6.43 ± 0.76</td>
</tr>
<tr>
<td>IV</td>
<td>400</td>
<td>12.47 ± 0.43</td>
<td>6.31 ± 0.48</td>
<td>1.82 ± 0.45</td>
<td>46.50 ± 3.94</td>
<td>59.90 ± 3.17</td>
<td>16.19 ± 0.92</td>
<td>33.55 ± 3.09</td>
<td>5.93 ± 0.72</td>
<td>5.97 ± 0.67</td>
</tr>
</tbody>
</table>

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

Table 3. Effect on hemogram in female rats

<table>
<thead>
<tr>
<th>Gr. No.</th>
<th>Dose (mg/kg)</th>
<th>Hb (%)</th>
<th>Total RBC (x10^6/cmm)</th>
<th>Rt (%)</th>
<th>HCT (%)</th>
<th>MCV (µm³)</th>
<th>MCH(pg)</th>
<th>MCHC (%)</th>
<th>Platelets (x10^5/cmm)</th>
<th>Total WBC (x10³/cmm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>16.43 ± 1.22</td>
<td>5.90 ± 0.53</td>
<td>1.45 ± 0.41</td>
<td>54.00 ± 5.62</td>
<td>63.57 ± 6.07</td>
<td>21.03 ± 4.11</td>
<td>32.37 ± 2.27</td>
<td>6.20 ± 0.53</td>
<td>6.92 ± 1.14</td>
</tr>
<tr>
<td>II</td>
<td>100</td>
<td>13.20 ± 0.88</td>
<td>5.95 ± 0.86</td>
<td>1.70 ± 0.36</td>
<td>50.33 ± 7.34</td>
<td>62.80 ± 7.38</td>
<td>17.88 ± 1.87</td>
<td>32.83 ± 2.62</td>
<td>5.85 ± 1.14</td>
<td>6.43 ± 0.76</td>
</tr>
<tr>
<td>III</td>
<td>200</td>
<td>12.96 ± 0.70</td>
<td>6.07 ± 0.67</td>
<td>1.70 ± 0.49</td>
<td>45.50 ± 5.32</td>
<td>60.04 ± 5.23</td>
<td>16.35 ± 0.71</td>
<td>32.64 ± 2.33</td>
<td>6.22 ± 0.71</td>
<td>6.13 ± 0.69</td>
</tr>
<tr>
<td>IV</td>
<td>400</td>
<td>12.47 ± 0.41</td>
<td>6.02 ± 0.58</td>
<td>1.70 ± 0.42</td>
<td>42.42 ± 2.38</td>
<td>59.70 ± 2.16</td>
<td>16.03 ± 0.93</td>
<td>34.66 ± 3.37</td>
<td>5.89 ± 0.74</td>
<td>6.06 ± 0.19</td>
</tr>
</tbody>
</table>

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

Table 4. Effect on biochemical parameters in male rats

<table>
<thead>
<tr>
<th>Gr. No.</th>
<th>Dose (mg/kg)</th>
<th>TSP (g%)</th>
<th>BUN (mg%)</th>
<th>SGPT (IU/L)</th>
<th>SGOT (IU/L)</th>
<th>SAP (IU/L)</th>
<th>Blood Sugar (mg%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>7.75 ± 0.29</td>
<td>33.80 ± 2.32</td>
<td>89.52 ± 8.82</td>
<td>91.50 ± 5.01</td>
<td>402.33 ± 96.67</td>
<td>95.48 ± 6.37</td>
</tr>
<tr>
<td>II</td>
<td>100</td>
<td>7.65 ± 0.29</td>
<td>30.82 ± 2.61</td>
<td>67.67 ± 1.17</td>
<td>92.83 ± 8.11</td>
<td>447.00 ± 49.25</td>
<td>98.40 ± 7.22</td>
</tr>
<tr>
<td>III</td>
<td>200</td>
<td>7.60 ± 0.27</td>
<td>30.41 ± 2.90</td>
<td>96.03 ± 6.86</td>
<td>94.87 ± 8.58</td>
<td>412.67 ± 53.05</td>
<td>100.23 ± 4.89</td>
</tr>
<tr>
<td>IV</td>
<td>400</td>
<td>7.48 ± 0.40</td>
<td>40.43 ± 6.3</td>
<td>80.17 ± 12.64</td>
<td>92.50 ± 9.7</td>
<td>428.33 ± 40.01</td>
<td>98.67 ± 5.77</td>
</tr>
</tbody>
</table>

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

Table 5. Effect on Biochemical parameters in female rats

<table>
<thead>
<tr>
<th>Gr. No.</th>
<th>Dose (mg/kg)</th>
<th>TSP (g%)</th>
<th>BUN (mg%)</th>
<th>SGPT (IU/L)</th>
<th>SGOT (IU/L)</th>
<th>SAP (IU/L)</th>
<th>Blood Sugar (mg%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>7.74 ± 0.42</td>
<td>32.78 ± 3.8</td>
<td>71.17 ± 15.42</td>
<td>89.67 ± 8.55</td>
<td>420.33 ± 37.73</td>
<td>97.98 ± 3.91</td>
</tr>
<tr>
<td>II</td>
<td>100</td>
<td>7.59 ± 0.43</td>
<td>30.78 ± 1.12</td>
<td>66.83 ± 12.54</td>
<td>92.00 ± 6.26</td>
<td>413.17 ± 35.10</td>
<td>94.77 ± 5.96</td>
</tr>
<tr>
<td>III</td>
<td>200</td>
<td>7.53 ± 0.46</td>
<td>30.48 ± 1.98</td>
<td>86.00 ± 14.56</td>
<td>90.53 ± 6.43</td>
<td>428.17 ± 48.44</td>
<td>97.90 ± 3.27</td>
</tr>
<tr>
<td>IV</td>
<td>400</td>
<td>7.40 ± 0.35</td>
<td>31.23 ± 3.12</td>
<td>80.00 ± 12.13</td>
<td>88.33 ± 6.68</td>
<td>419.17 ± 29.78</td>
<td>100.90 ± 5.69</td>
</tr>
</tbody>
</table>

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 Acinetobacter baumannii
isolates. Lee CM et al have reported the role of sulbactam
combination with carbapenem and second or third
generation cephalosporins, antipseudomons 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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