INTRODUCTION

The third generation cephalosporins were 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 (Donowitz and Mandell, 1998). Cephalosporins are a class of beta-lactam antibiotics. Ceftriaxone is a broad-spectrum, semi-synthetic third-generation cephalosporin with a potent bactericidal activity against a wide range of gram-positive and gram-negative bacteria (Carmeli et al., 1999). The antibacterial activity of ceftriaxone is due to the inhibition of cell wall synthesis (Goldstein et al., 1995).

Sulbactam has recently been approved in many countries including India and is being combined with beta-lactam antibiotics (Levin, 2002). It is a molecule which inhibits beta-lactamase, an enzyme produced by bacteria that destroy antibiotics (Totir et al., 2007). It is a potent, highly specific inhibitor of a wide variety of beta-lactamases produced by common gram-negative and gram-positive aerobes and anaerobes (Bhattacharjee et al., 2008). By forming a protein complex with beta-lactamases, sulbactam irreversibly blocks their destructive hydrolytic activity (Betrosian et al., 2008). Thus, the full potential of ceftriaxone against enterobacter and pseudomonas species is restored by the addition of sulbactam (Corbella et al., 1998).

Third generation cephalosporins are potent antibiotic substances being used in the treatment of life-threatening infections. In the last few years, however, an increase in resistance, especially among Enterobacteriaceae, has been reported, resulting from a continuous spread of broad-spectrum beta-lactamases. Guerra-Romero et al. (1991) reported a combination of a penicillin-derivative drug (ampicillin) and sulbactam, for the treatment of experimental meningitis caused by a beta-lactamase producing strain of E. coli K-1.

Later, a combination of a cephalosporin (ceftriaxone) and a beta-lactamase inhibitor (sulbactam) is introduced to prevent the emergence of resistant bacteria (Caron et al., 1990; Chambers and Fournier, 1993).

A large number of studies available on pharmacokinetics of ceftriaxone and sulbactam alone in healthy volunteers and patients (Patel et al., 1981; Foulds et al., 1983;
Caine et al., 1984; Zhou et al., 1985; Sar et al., 2008). However, studies on pharmacokinetics of fixed dose combination (FDC) of ceftriaxone and sulbactam (sulbactomax) in healthy individuals are lacking. Therefore, the present investigation was planned to study the pharmacokinetics of sulbactomax, ceftriaxone and sulbactam alone in healthy individuals to determine changes in the pharmacokinetic profiles of FDC. Also, we have determined the minimum inhibitory concentration (MIC) of sulbactomax against certain gram positive and gram negative organisms to establish dose regimen of new FDC.

MATERIALS AND METHODS

Subjects
A total of eight healthy male volunteers meeting inclusion and exclusion criteria were enrolled for an open labeled, comparative pharmacokinetic study.

Inclusion criteria
Healthy volunteers, ranging from 22 to 32 years in age, and from 55 to 80 kg in weight, were selected. All volunteer's were non-smokers, nonalcoholic, with no evidence of underlying disease on physical examination, medical disorder or impairment. All selected volunteers have not been participated in any of the clinical trials since last 3 months and have been provided written informed consent.

Exclusion criteria
The volunteers were excluded if they had a history of hypersensitivity to the drug or related products, renal or liver function abnormalities, any clinically significant illness during the 4 weeks prior to this study or hospitalized during 3 months prior to the commencement of this study.

Dropouts and withdrawals
The volunteers are free to leave the study at any time without giving the reason. The volunteer's participation in the trial can be discontinued for any of the following reasons: adverse reaction, inter current illness, non-compliance and volunteer decision not to continue. If a subject does not follow pre-study directions regarding alcohol, drug use, fasting condition etc. can be removed from the study.

Concurrent medication
All volunteers were informed not to take any medications for 14 days prior to the study. In addition, non-concomitant medication was permitted during the study period. The trial was conducted in accordance with declaration of Helsinki and ethical approval obtained from the independent institutional ethics committee.

Physical examination
All the volunteers were evaluated physically and medically two days before the start of the study.

Study design

Dose and route of administration
The overnight fasten volunteers were given a single intravenous dose of sulbactomax (1.5 g) as a 30 min infusion in a 50 ml normal saline. The fast was continued for an additional 3 hr after drug administration. After 15 days of washing period volunteers were give ceftriaoxne (1 g) and after another 15 days of washing period sulbactam (500 mg) was administered to the volunteers.

Blood sample collection & sampling schedule
Blood samples were obtained at 15 min before the infusion and 0.5, 1, 1.5, 2, 4, 8, 12 and 24 hr after the infusion. Blood samples were collected in heparinized navy-blue vacutainer tubes and centrifuged immediately. Plasma was separated and stored at -20°C until use.

Hematological parameters
Blood samples were subjected for hematological parameters such as total leukocyte counts (TLC), erythrocyte sedimentation rate (ESR), hemoglobin (Hb).

Biochemical parameters
Biochemical parameters such as serum glutamic oxaaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP) activities, sugar, urea and creatinine were evaluated in plasma. All parameters were studied by Merck semi auto analyzer using Merck kits.

HPLC analysis of ceftriaxone and sulbactam
Ceftriaxone and sulbactam were detected in the plasma using high performance liquid chromatography (HPLC Agilant 1,200 series, Santa Clara, CA, USA) equipped with G1311A quaternary pump, Agilent variable UV/Visible detector and a G1329A auto injector.

Mobile phase
A buffer solution consisted of 50 ml of tetrabutyl ammonium hydroxide (TBAH) in 1,000 ml of distilled water was prepared and adjusted to pH 7.0 with orthophosphoric acid. The solvent used for mobile phase was a
mixture of buffer–acetonitrile (70:30). The mobile phase was passed through membrane filter (Millipore corp., Billerica, MA, USA), 0.45 μm pore size and degassed under reduced pressure.

**Ceftriaxone and sulbactam drug analysis**

Ceftriaxone and sulbactam drugs were analyzed by the method of Shrivastava et al. (2009a). For the analysis of ceftriaxone and sulbactam concentrations in plasma samples, 200 μl of sample was mixed with 150 μl of mobile phase and shaken vigorously. The chromatographic separation of ceftriaxone and sulbactam drugs was performed by HPLC with a mobile phase. A C-18 hypersil ODS (5 μ, 4.6 x 250 mm) column was used for the analysis. The flow rate and column temperature were maintained at 1.5 ml/min at 25°C respectively. After an equilibration of column with mobile phase for 2 hr, 20 μl of sample was injected and detection of ceftriaxone and sulbactam antibiotics was performed at 220 nm UV wavelength. Under the above mentioned chromatographic conditions, the retention time of ceftriaxone and sulbactam were found to be 5.2 and 3.3 min, respectively.

**Statistical analysis**

Pharmacokinetic parameters were analyzed statistically by two-way analysis of variance to determine the influence of dose. The dose effects were then compared by employing the two-tailed paired t-test.

**RESULTS**

No adverse effects or events were observed in any of the volunteers confirming the safety of the FDC of ceftriaxone-sulbactam.

The average plasma concentrations of ceftriaxone and sulbactam after 30 min intravenous administration are presented in (Table 1). The maximum plasma concentrations of ceftriaxone and sulbactam after administration of sulbactomax were 152.06 ± 6.55 μg/ml and 21.32 ± 1.79 μg/ml, respectively. The peak plasma concentrations of ceftriaxone and sulbactam when administered alone in the same volunteers after the washing period were 153.75 ± 6.43 μg/ml and 21.42 ± 1.28 μg/ml, respectively. After twenty four hours of drug administration, the mean plasma concentrations of ceftriaxone of sulbactomax and ceftriaxone alone were 8.38 ± 1.96 μg/ml and 6.18 ± 1.62 μg/ml, respectively. There were no significant changes observed in the plasma concentrations of ceftriaxone and sulbactam of sulbactomax as compared to the ceftriaxone and sulbactam when administered alone.

Half-life and AUC for ceftriaxone after administration of sulbactomax were 5.2 ± 0.35 hr and 760.16 ± 27.68 μg.hr/ml, respectively. Half life and AUC after administration of ceftriaxone alone were 5.6 ± 0.436 hr and 742 ± 29.56 μg.hr/ml, respectively. No significant changes were noted in the half-life and AUC of ceftriaxone of sulbactomax as compared to the ceftriaxone alone. Half life and AUC for sulbactam after administration of sulbactomax were 0.94 ± 0.038 hr and 20.74 ± 2.347 μg.hr/ml, respectively. Half life and AUC after administration of sulbactam alone were 0.985 ± 0.107 hr and 19.75 ± 1.876 μg.hr/ml, respectively. No significant changes were noted in the half-life and AUC of sulbactam of sulbactomax as compared to the sulbactam alone (Table 2).

Elimination rate constant for ceftriaxone after administration of sulbactomax was 0.133 ± 0.009 hr⁻¹. The elim-

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Ceftriaxone μg/ml</th>
<th>Sulbactam μg/ml</th>
<th>Sulbactam μg/ml</th>
<th>Ceftriaxone μg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>153.75 ± 6.43</td>
<td>21.42 ± 1.28</td>
<td>152.06 ± 6.55</td>
<td>21.32 ± 1.79</td>
</tr>
<tr>
<td>1</td>
<td>112.66 ± 6.88</td>
<td>9.8 ± 1.12</td>
<td>109.3 ± 5.09</td>
<td>11.01 ± 1.47</td>
</tr>
<tr>
<td>1.5</td>
<td>93.85 ± 3.35</td>
<td>6.08 ± 1.03</td>
<td>92.56 ± 3.79</td>
<td>7.05 ± 1.63</td>
</tr>
<tr>
<td>2</td>
<td>83.41 ± 2.61</td>
<td>3.77 ± 0.46</td>
<td>86.5 ± 2.22</td>
<td>3.93 ± 0.63</td>
</tr>
<tr>
<td>4</td>
<td>64.61 ± 2.99</td>
<td>1.57 ± 0.53</td>
<td>63.85 ± 5.29</td>
<td>1.47 ± 0.23</td>
</tr>
<tr>
<td>8</td>
<td>33.55 ± 1.13</td>
<td></td>
<td>39.88 ± 1.30</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>14.76 ± 1.97</td>
<td></td>
<td>15.66 ± 2.146</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>8.38 ± 1.96</td>
<td></td>
<td>6.18 ± 1.62</td>
<td></td>
</tr>
</tbody>
</table>
nation rate constant for ceftriaxone alone was $0.123 \pm 0.01$ hr$^{-1}$. No significant changes were noted in the elimination rate constant of ceftriaxone of sulbactomax as compared to the ceftriaxone alone. The elimination rate constant for sulbactam after administration of sulbactomax was $0.732 \pm 0.029$ hr$^{-1}$. No significant changes were noted in the elimination rate constant of sulbactam of sulbactomax as compared to the sulbactam alone.

Statistically no significant changes in the hematological and clinical biochemical parameters were observed after the administration of sulbactomax as compared to the start of the dosing indicating that the FDC of ceftriaxone and sulbactam is neither hepatotoxic nor nephrotoxic (Table 3). MIC of sulbactomax were also calculated against certain organisms to determine the dosing schedule of sulbactomax (Table 4).

## DISCUSSION

Ceftriaxone is a broad spectrum antibiotic that displays potent activity against gram-positive and gram-negative bacteria (Angehrn et al., 1980). The pharmacokinetic parameters of ceftriaxone and sulbactam alone has been reported in several literatures (Foulds et al., 1983; Patel et al., 1981; Meyers et al., 1983). The combination of sulbactam and ceftriaxone is active against all the organisms sensitive to ceftriaxone. In addition, it demonstrates synergistic activity (reduction in MIC for the combination versus those of each component) in a variety of organisms (Shrivastava et al., 2009b). There are no pharmacokinetic changes between the two drugs from the compound preparation, and there is no drug interaction between them (Yonghong et al., 2006).

The present study was conducted for pharmacokinetics of sulbactomax in comparison with ceftriaxone and sulbactam administered alone after intravenous infusion. The results of the study demonstrate that when 1.5 g of sulbactomax containing 1 g of ceftriaxone was administered, the plasma concentrations of ceftriaoxone at different time intervals were found to be similar as reported by the earlier researchers for 1 g of ceftriaxone (Patel et al., 1981; Meyers et al., 1983). The concentrations of sulbactam after intravenous infusion observed in our study

### Table 2. Pharmacokinetic parameters of ceftriaxone, sulbactam alone and sulbactomax

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Ceftriaxone</th>
<th>Sulbactam</th>
<th>Sulbactomax</th>
</tr>
</thead>
<tbody>
<tr>
<td>Half life t$_{1/2}$ (hr)</td>
<td>5.6 ± 0.436</td>
<td>0.985 ± 0.107</td>
<td>5.2 ± 0.35</td>
</tr>
<tr>
<td>Elimination rate constant $K_e$ (hr$^{-1}$)</td>
<td>0.123 ± 0.01</td>
<td>0.707 ± 0.07</td>
<td>0.133 ± 0.009</td>
</tr>
<tr>
<td>AUC0-24 (μg.hr/ml)</td>
<td>742 ± 29.56</td>
<td>19.75 ± 1.876</td>
<td>760.16 ± 27.68</td>
</tr>
<tr>
<td>Cmax (μg/ml)</td>
<td>153.75 ± 6.43</td>
<td>21.83 ± 1.41</td>
<td>152.06 ± 6.65</td>
</tr>
</tbody>
</table>

### Table 3. Hematological and biochemical parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before treatment</th>
<th>On completion of treatment</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLC (×/mm$^3$)</td>
<td>8500.25 ± 652.14</td>
<td>8181.25 ± 715.40</td>
<td>4000-10800</td>
</tr>
<tr>
<td>ESR (mm/hr)</td>
<td>6.0 ± 1.46</td>
<td>6.4 ± 1.13</td>
<td>Up to 10</td>
</tr>
<tr>
<td>Hb (g%)</td>
<td>14.4 ± 0.33</td>
<td>14.11 ± 0.34</td>
<td>14-18</td>
</tr>
<tr>
<td>Plasma sugar fasting (mg/dl)</td>
<td>90.0 ± 10.38</td>
<td>80.3 ± 14.39</td>
<td>70-110</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>28.09 ± 2.91</td>
<td>27.18 ± 2.30</td>
<td>Up to 40</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.78 ± 0.08</td>
<td>0.77 ± 0.09</td>
<td>Up to 1.0</td>
</tr>
<tr>
<td>SGOT (IU/l)</td>
<td>25.41 ± 4.86</td>
<td>24.68 ± 3.12</td>
<td>Up to 35</td>
</tr>
<tr>
<td>SGPT (IU/l)</td>
<td>28.2 ± 3.90</td>
<td>27.50 ± 2.90</td>
<td>Up to 35</td>
</tr>
<tr>
<td>ALP (IU/l)</td>
<td>160.63 ± 7.84</td>
<td>173.25 ± 12.84</td>
<td>65-306</td>
</tr>
</tbody>
</table>
Pharmacokinetic of sulbactomax

Table 4. MIC and dosing schedule of sulbactomax in organisms causing infections

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Microorganisms</th>
<th>MIC (μg/ml)</th>
<th>Dosing schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>E. coli</td>
<td>0.25</td>
<td>q24 h</td>
</tr>
<tr>
<td>2.</td>
<td>P. vulgaris</td>
<td>0.50</td>
<td>q24 h</td>
</tr>
<tr>
<td>3.</td>
<td>S. aureus</td>
<td>32</td>
<td>q8 h</td>
</tr>
<tr>
<td>4.</td>
<td>B. subtilis</td>
<td>4</td>
<td>q24 h</td>
</tr>
<tr>
<td>5.</td>
<td>A. baumannii</td>
<td>0.125</td>
<td>q24 h</td>
</tr>
<tr>
<td>6.</td>
<td>K. pneumoniae</td>
<td>0.625</td>
<td>q24 h</td>
</tr>
<tr>
<td>7.</td>
<td>C. braaki</td>
<td>2</td>
<td>q24 h</td>
</tr>
<tr>
<td>8.</td>
<td>P. aeruginosa</td>
<td>2</td>
<td>q24 h</td>
</tr>
<tr>
<td>9.</td>
<td>E. cloacae</td>
<td>4</td>
<td>q24 h</td>
</tr>
</tbody>
</table>

were agreed with the previous data (Foulds et al., 1983). The half life of ceftriaxone of sulbactomax and ceftriaxone alone that we obtained is comparable with that of reported by other researchers (Patel et al., 1981; Meyers et al., 1983). The half life of sulbactam of sulbactomax and sulbactam alone is also very close to that of reported by Foulds et al. (1983). The elimination rate constant of ceftriaxone of sulbactomax and ceftriaxone alone after intravenous administration that we obtained is very close to that of reported by Luderer et al. (1984). The elimination rate constant of sulbactam of sulbactomax and sulbactam alone after intravenous administration that we obtained is lower to that of reported by Foulds et al. (1983).

The area under curve of ceftriaxone of sulbactomax and ceftriaxone alone after intravenous administration that we obtained is slightly lower to reported by Meyers et al. (1983). The area under curve of sulbactam of sulbactomax and sulbactam alone after intravenous administration that we obtained is comparable to that of reported by Foulds et al. (1983). Our results clearly indicate that there are no pharmacokinetic interactions between two components (ceftriaxone + sulbactam) of the combination.

The concentrations of ceftriaxone of sulbactomax in the plasma remain maintained above the MIC for twenty four hours after intravenous administration of the sulbactomax suggesting that one dose of sulbactomax will be adequate to treat most of the gram-positive and gram-negative bacteria. These observations were agreement with the findings of other researchers (Shannon et al., 1980; Wise et al., 1980). However, MIC for S. aureus is 32 μg/ml because S. aureus expresses an additional penicillin binding protein 2 (PBP2) with reduced affinity for beta-lactam antibiotics, therefore it has little bit higher MIC value (Taylor, 2003).

There is no significant alteration in any of the hematological parameters before and after treatment with sulbactomax. Cephalosporin-induced hepatotoxicity is rarely observed. Common adverse effects are gallstones (cholelithiasis) or bile lumps. Despite the fact that only a few cases of elevated liver enzymes caused by ceftriaxone have been reported (Longo et al., 1998; Bell et al., 2005). Lee et al. (2009) also reported that ceftriaxone has less side effects. However, in this study, there were no significant changes in SGOT, SGPT and ALP before and after treatment with sulbactomax indicating that sulbactomax is not hepatotoxic. Nechifor et al. (1992) also reported that there is no treatment related changes in organs of any species.

Nephrotoxicity has never been reported with any of the broad spectrum cephalosporins (Fekety, 1990). Beauchamp et al. (1994) reported that ceftriaxone by itself had no detrimental effects on renal function, lysosomal enzymatic profile, or cellular regeneration. Our results also showed that there is no alteration in any of the parameters of kidney function test after administration of sulbactomax indicating that sulbactomax is safe and not causes nephrotoxicity.

In conclusion, our results indicate that there are no alterations in the pharmacokinetic parameters of the sulbactomax as compared to ceftriaxone and sulbactam administered alone. And based on the MIC and concentration of sulbactomax at different time interval, one dose of the sulbactomax at every twenty four hours is sufficient to treat the infections caused by these organisms.

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REFERENCES


