SYNERGISTIC ENHANCEMENT OF IN VITRO ANTIMICROBIAL ACTIVITY OF CEFMETAZOLE AND CEFAZOLIN, CEFOTIAM, CEFAMANDOLE OR CEFOPERAZONE IN COMBINATION AGAINST METHICILLIN-SENSITIVE AND -RESISTANT STAPHYLOCOCCUS AUREUS

I. EFFECT OF NaCl

TETSUO UETE and KIYOMITSU MATSUO
Department of Clinical Investigation, Kitano Hospital, Tazuke Kofukai Medical Research Institute, Kita-ku, Osaka
(Received for publication December 21, 1994)

The in vitro antimicrobial activity of cephamycin, e.g. cefmetazole and cephalosporin, such as cefazolin, cefotiam, cefamandole and cefoperazone, alone and in combination, was studied employing 9 strains of methicillin-sensitive Staphylococcus aureus (MSSA) and 30 strains of methicillin-resistant Staphylococcus aureus (MRSA). Using the checkerboard agar dilution method, strong synergism was demonstrable in a majority of MSSA and MRSA strains for cefmetazole combined with these cephalosporins, with the minimum fractional inhibitory concentration index \( \leq 0.5 \).

In the presence of a concentration \( \leq 6.25 \mu g/ml \) of these cephalosporins in Mueller-Hinton agar medium, the activity of cefmetazole against MRSA was most prominently potentiated by cefotiam, followed by cefamandole, cefazolin and cefoperazone. At a concentration of 12.5 \( \mu g/ml \), cefotiam and cefamandole showed a similar effect in potentiation of cefmetazole activity.

In hypertonic agar medium containing 4% NaCl, these synergistic combination effects were reduced. However, the activity of cefmetazole and cefamandole in combination under these conditions was influenced to a lesser extent and more potent than that of other combinations.

Cephamycin and some cephalosporin in combination has been reported to manifest the synergistic enhancement of their antimicrobial activities in vitro against methicillin- and multiple resistant Staphylococcus aureus (MRSA), such as latamoxef and cefamandole\(^1\) or cefazolin\(^2\), cefmetazole and cefazolin\(^3\) or cefotiam\(^4\), and cefotetan and cephramide\(^5\), and flomoxef and cefamandole\(^6\). It is, however, unknown as to which cephalosporin potentiates more the activity of cephamycin against S. aureus.

Staphylococcus in vitro are tolerant to hyperosmotic conditions\(^7,8\). MRSA has been known to increase their resistance against \( \beta \)-lactam antibiotics under such conditions in vitro\(^9\sim12\). In vivo varying osmolality occurs in various places. The ability of Staphylococci to survive and grow in hyperosmotic environment has been considered to be an important factor of their tendency to colonizes specific regions of the organism resulting in localized infections\(^13,14\). Staphylococci colonize the skin and tolerate the varying concentration of sweat-derived salt thus adjusting to the local conditions in hair follicles and sebaceous glands. Staphylococci can grow in media containing up to 20% NaCl\(^7,8\). Microorganism induce urinary tract infections under conditions of varying pH, osmolality and molecular composition. A question arises whether the synergistic enhancement of antimicrobial activity of cephamycin and cephalosporin in combination against MRSA is influenced by varying osmorality.

In this study, therefore, the in vitro interaction between cefmetazole and cefazolin, cefotiam, cefaman-
dox or cefoperazone was tested against MRSA and methicillin sensitive *Staphylococcus aureus* (MSSA) with respect to the antimicrobial activity by the means of checkerboard titration method in the presence of a low and high concentration of NaCl in the incubation media.

**Materials and Methods**

**Antibiotics**

Cefmetazole was obtained from Sankyo Co., cefotiam from Takeda Chem. Co., cefazolin from Fujisawa Pharm. Co., cefamandole from Shionogi Pharm. Co. and cefoperazone from Toyama Chem. Co.

**Bacterial Strains**

*Staphylococcus aureus* resistant to methicillin and multiple antibiotics (30 strains) and sensitive to these drugs (9 strains) were isolated from the clinical materials during 1991 in this hospital, propagated and used in this study.

**Susceptibility Tests**

The MICs for MSSA and MRSA of clinical isolates were determined by the agar plate dilution method\(^{15}\) using Mueller-Hinton agar (Difco) with or without 4% NaCl\(^{15}\) and an inoculum level of \(10^6\) CFU/ml\(^{15}\).

Checkerboard design studies were performed with combination of cefmetazole and cephalosporin by the agar plate dilution method. The fractional inhibitory concentration (FIC) obtained by dividing the minimum concentration of each of the two antibiotics that had an inhibitory effect when acting together by the MIC of each antibiotic alone, and the sum of the FICs of both antibiotics (FIC index) were evaluated\(^{16}\). The results were expressed as synergy, addition, and antagonism when the values of the FIC index were \(\leq 0.5\), \(>0.5<2.0\) and \(\geq 2.0\), respectively\(^{16}\).

**Results**

**Activity of Cefmetazole and Cefazolin, Cefotiam, Cefamandole or Cefoperazone Alone and in Combination Against MSSA**

Using Mueller-Hinton agar, the MICs of cefmetazole against 9 strains of MSSA were \(0.78\sim1.56\ \mu g/ml\), and these were \(1.56\sim3.13\ \mu g/ml\) with the addition of 4% NaCl. Similarly, the MICs of cefazolin, cefotiam, cefamandole and cefoperazone against these MSSA were \(0.39\sim0.78\), \(0.39\sim0.78\), \(0.39\sim0.78\) and \(3.13\ \mu g/ml\), respectively. With the addition of 4% NaCl the MICs of these drugs were \(0.39\sim0.78\), \(0.78\sim1.56\), \(0.78\sim1.56\), and \(3.13\sim6.25\ \mu g/ml\), respectively (Table 1).

In the combination of cefmetazole and cefazolin, cefotiam, cefamandole, or cefoperazone, the antimicrobial activity of these drugs against these MSSA was synergistically or additively enhanced under the conditions without or with 4% NaCl. No antagonistic interaction of these drugs in these combination studies was obtained with respect to the activity against MSSA strains studied (Table 1).

**Activity of Cefmetazole and Cefazolin, Cefotiam, Cefamandole or Cefoperazone Alone and in Combination Against MRSA**

Cefmetazole, cefazolin, cefotiam, cefamandole and cefoperazone alone: The MICs of these antibiotics against 30 strains of MRSA in the absence of 4% NaCl were \(12.5\sim100\), \(200\sim400\), \(50\sim800\), \(25\sim50\) and 800
Table 1. Effect of NaCl (4%) on in vitro antimicrobial activity of cefmetazole and cefotiam, cefamandole or cefoperazone alone and in combination against MSSA

<table>
<thead>
<tr>
<th>No. of strains</th>
<th>NaCl (-)</th>
<th>NaCl (+)</th>
<th>FIC indices ≤ 0.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSSA</td>
<td>MIC (FIC)</td>
<td>MIC (FIC)</td>
<td>NaCl (-)</td>
</tr>
<tr>
<td>Cefmetazole</td>
<td>0.78–1.56</td>
<td>1.56</td>
<td>1.56</td>
</tr>
<tr>
<td>(alone)</td>
<td>0.39–0.78</td>
<td>0.39</td>
<td>0.39</td>
</tr>
<tr>
<td>Cefotiam</td>
<td>0.10–0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>(in combination)</td>
<td>0.39–0.78</td>
<td>0.39</td>
<td>0.39</td>
</tr>
<tr>
<td>Cefamandole</td>
<td>0.10–0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>(in combination)</td>
<td>0.39–0.78</td>
<td>0.39</td>
<td>0.39</td>
</tr>
<tr>
<td>Cefoperazone</td>
<td>0.10–0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>(in combination)</td>
<td>0.39–0.78</td>
<td>0.39</td>
<td>0.39</td>
</tr>
</tbody>
</table>

Range: MIC (FIC)"
Table 2. Effect of NaCl (4%) on in vitro antimicrobial activity of cefmetazole and cefazolin, cefotiam, cefamandole or cefoperazone alone and in combination against MRSA

<table>
<thead>
<tr>
<th>No. of strains</th>
<th>MRSA (30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>12.5~100</td>
</tr>
<tr>
<td>NaCl (-)</td>
<td>50</td>
</tr>
<tr>
<td>MIC (FIC)</td>
<td>100</td>
</tr>
<tr>
<td>(FIC)</td>
<td>30</td>
</tr>
<tr>
<td>NaCl (+)</td>
<td>50</td>
</tr>
<tr>
<td>MIC (FIC)</td>
<td>100</td>
</tr>
<tr>
<td>(FIC)</td>
<td>30</td>
</tr>
<tr>
<td>NaCl indices ≤0.5</td>
<td>12.5~100</td>
</tr>
<tr>
<td>NaCl (+)</td>
<td>50</td>
</tr>
<tr>
<td>MIC (FIC)</td>
<td>100</td>
</tr>
<tr>
<td>(FIC)</td>
<td>30</td>
</tr>
</tbody>
</table>

556(116) THE JAPANESE JOURNAL OF ANTIBIOTICS 48-4 Apr. 1995
Table 3. *In vitro* antimicrobial activity of cefmetazole combined with cefazolin, cefotiam, cefamandole or cefoperazone against MRSA

<table>
<thead>
<tr>
<th>No. of strains (30)</th>
<th>MIC (µg/ml)</th>
<th>FIC index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 0.1 0.1 0.2 0.39 0.78 1.56 3.13 6.25 12.5 25 50 100 200 400 ≥800</td>
<td>≤0.5 1.0 ≥2.0</td>
</tr>
</tbody>
</table>

**Cefmetazole alone**
- (NaCl−): 5 14 11
- (NaCl+): 11 16 3

**Cefazolin alone**
- (−): 18 12
- (+): 3 27

**Cefotiam alone**
- (−): 1 3 11 12 3
- (+): 9 21

**Cefamandole alone**
- (−): 23 7
- (+): 9 18 3

**Cefoperazone alone**
- (−): 30
- (+): 30

**Cefmetazole with Cefazolin**
- 1.56 µg/ml (−):
  - 3 4 2 5 11 5
  - 6 24
  - (+):
    - 10 12 8
    - 0 30
- 3.13 (−):
  - 3 2 2 1 5 2 10 5
  - 8 22
  - (+):
    - 5 7 12 6
    - 4 26
- 6.25 (−):
  - 2 4 1 1 3 3 1 10 5
  - 11 19
  - (+):
    - 5 4 4 12 5
    - 9 21
- 12.50 (−):
  - 3 4 2 5 1 5 8 2
  - 15 15
  - (+):
    - 4 4 4 2 12 4
    - 13 17
- 25.00 (−):
  - 1 4 2 7 2 9 3 2
  - 17 13
  - (+):
    - 3 6 3 1 11 5 1
    - 20 10

**Cefotiam**
- 1.56 µg/ml (−):
  - 2 3 2 2 3 3 10 5
  - 0 30
  - (+):
    - 4 15 11
    - 9 21
- 3.13 (−):
  - 5 6 1 1 5 9 3
  - 12 18
  - (+):
    - 3 8 11 8
    - 2 28
- 6.25 (−):
  - 2 4 4 4 2 2 1 4 6 1
  - 19 11
  - (+):
    - 5 2 6 13 4
    - 7 23
- 12.50 (−):
  - 4 6 5 6 3 1 1 1 2 1
  - 26 4
  - (+):
    - 2 2 5 4 6 7 4
    - 18 12
- 25.00 (−):
  - 6 8 3 8 1 1 2 1
  - 27 3
  - (+):
    - 1 3 6 4 4 6 3 3
    - 22 8

**Cefamandole**
- 1.56 µg/ml (−):
  - 2 8 14 6
  - 1 29
  - (+):
    - 3 18 8 1
    - 0 30
- 3.13 (−):
  - 1 4 6 14 5
  - 2 28
  - (+):
    - 13 11 5 1
    - 3 27
- 6.25 (−):
  - 2 3 6 9 5 1 4
  - 22 8
  - (+):
    - 1 6 11 2 4 3 2 1
    - 25 5
Table 3. (Continued)

<table>
<thead>
<tr>
<th>No. of strains (30)</th>
<th>MIC (µg/ml)</th>
<th>FIC index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;0.1 0.1 0.2 0.39 0.78 1.56 3.13 6.25 12.5 25 50 100 200 400 ≥800</td>
<td>≤0.5 1.0 ≥2.0</td>
</tr>
<tr>
<td>Cefmetazole with Cefamandole</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.50 µg/ml (-)</td>
<td>1 4 7 10 1 1 1</td>
<td>2 2 1</td>
</tr>
<tr>
<td>(+)</td>
<td>6 8 6 5 1 1 1 1</td>
<td>1</td>
</tr>
<tr>
<td>25.00 (-)</td>
<td>23 2 3 1</td>
<td>1</td>
</tr>
<tr>
<td>(+)</td>
<td>9 17 3</td>
<td>1</td>
</tr>
<tr>
<td>Cefoperazone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.56 µg/ml (-)</td>
<td>7 13 10</td>
<td>0 30</td>
</tr>
<tr>
<td>(+)</td>
<td>14 14 2</td>
<td>0 30</td>
</tr>
<tr>
<td>3.13 (-)</td>
<td>2 1 2 4 12 9</td>
<td>3 27</td>
</tr>
<tr>
<td>(+)</td>
<td>4 11 14 1</td>
<td>0 30</td>
</tr>
<tr>
<td>6.25 (-)</td>
<td>1 5 1 4 2 8 8</td>
<td>12 18</td>
</tr>
<tr>
<td>(+)</td>
<td>2 8 6 14</td>
<td>2 28</td>
</tr>
<tr>
<td>12.50 (-)</td>
<td>5 4 2 2 1 10 5</td>
<td>14 16</td>
</tr>
<tr>
<td>(+)</td>
<td>1 2 4 3 3 10 7</td>
<td>12 18</td>
</tr>
<tr>
<td>25.00 (-)</td>
<td>2 5 5 2 2 1 9 4</td>
<td>16 14</td>
</tr>
<tr>
<td>(+)</td>
<td>6 4 2 2 12 4</td>
<td>14 16</td>
</tr>
</tbody>
</table>

~ > 800 µg/ml, respectively. Those in the presence of NaCl were 12.5~50, 200~400, 400~ > 800, 25~100, and 800~ > 800 µg/ml, respectively (Table 2).

In combination of cefmetazole and these cephalosporins, the MICs of these antibiotics against a majority of strains were reduced less than one fourth of the MICs of these antibiotics alone either without or with the addition of 4% NaCl (Table 2).

In combination study of cefmetazole and cefazolin, without the addition of 4% NaCl, when cefmetazole was combined with 3.13, 6.25 and 12.5 µg/ml of cefazolin, the strains with the MICs of cefmetazole reduced to less than 6.25 µg/ml were 8, 11, and 14 strains, respectively, being the FIC index ≤0.5 in all these strains. With 4% NaCl, the strains with the MICs less than 6.25 µg/ml of cefmetazole were 5, 9, and 12 strains, respectively, showing the FIC index ≤0.5 in 4, 9 and 13 strains (Table 3). Conversely, in the presence of 3.13, 6.25, and 12.5 µg/ml of cefmetazole, the strains with MICs of cefazolin reduced to less than 6.25 µg/ml were 8, 11, and 14 without 4% NaCl and 5, 9, and 13 with 4% NaCl, respectively, indicating the FIC index ≤0.5 in all these strains.

In the combination of cefmetazole and cefotiam, in the presence of 3.13, 6.25, and 12.5 µg/ml of cefotiam the strains with the MICs of cefmetazole reduced to less than 6.25 µg/ml were 12, 19, and 25 without the addition of NaCl, and 3, 7, and 13 with the addition of 4% NaCl, respectively (Table 3). Likewise, in the presence of cefmetazole 3.13, 6.25, and 12.5 µg/ml the strains with the MICs of cefotiam reduced to less than 6.25 µg/ml were 18, 19, and 19 without the addition of NaCl and 5, 7, and 13 with the addition of 4% NaCl, respectively (Table 3).

When cefmetazole was combined with 3.13, 6.25 and 12.5 µg/ml of cefamandole, the MRSA strains with the MICs of cefmetazole reduced to less than 6.25 µg/ml were 1, 11, and 25 without the addition of NaCl and 0, 20, and 28 with the addition of NaCl, respectively (Table 3). On the other hand, in the presence of cefmetazole 3.13, 6.25, and 12.5 µg/ml, the strains with the MIC of cefamandole less than 6.25 µg/ml were 6, 11, and 20 without the NaCl addition, and 18, 20 and 23, with the NaCl addition, respectively.
Similarly, if cefmetazole was combined with 3.13, 6.25, and 12.5 μg/ml of cefoperazone, the strains with the MICs of cefmetazole reduced to less than 6.25 μg/ml were 3, 8, and 13 without the addition of NaCl, and 0, 2, and 10 with the addition of 4% NaCl, respectively (Table 3). Conversely, in the presence of cefmetazole 3.13, 6.25 and 12.5 μg/ml, the strains with the MICs of cefoperazone reduced to less than 6.25 μg/ml were 7, 7, and 10 in the absence of 4% NaCl, and 2, 2, and 10 in the presence of 4% NaCl, respectively.

**Discussion**

In this study, synergistic enhancement of antimicrobial activity of cefmetazole and cefazolin, cefotiam, cefamandole or cefoperazone in combination against MSSA and MRSA was evident both in the absence and the presence of 4% NaCl in Mueller-Hinton agar media. The cefmetazole activity enhanced by cephalosporins against MRSA was stronger than that of cephalosporin by cefmetazole. The enhancing activity of cefmetazole by these cephalosporins generally reduced in the presence of 4% NaCl, except cefamandole.

In the absence of 4% NaCl in the Mueller-Hinton agar media the activity of cefmetazole against MRSA was enhanced most markedly by cefotiam at a dose level less than 6.25 μg/ml, followed by cefamandole, cefazolin and cefoperazone. Cefamandole required a greater amount such as 12.5 μg/ml to potentiate the activity of cefmetazole as did cefotiam. However, in the presence of 4% of NaCl the antimicrobial activity of cefmetazole against MRSA was most markedly increased by cefamandole, followed by cefotiam or cefazolin and cefoperazone. It remains to be clarified as to which combination is superior for clinical use cefmetazole and cefotiam or cefamandole.

Cefmetazole17–19) and cefamandole19–25) are one of the most active of β-lactam antibiotics against methicillin-resistant Staphylococci in vitro, showing a similar activity at a relatively low concentration 12.5 to 25 μg/ml. Clinical usefulness has been confirmed in treatment of patients with septicemia due to MRSA26), but not for deep-seated MRSA infection such as endocarditis26,27). Although the in vitro antimicrobial activity of these β-lactams in combination against MRSA was synergistically enhanced, the activity is sometimes not as great as that of vancomycin23–25). Therefore, until the limits of the anti-staphylococcal activity of these antibiotic combination are confirmed in vivo, these should not be used to treat established MRSA infections based on in vitro activity only.

However, a clinically useful advantage could be considered with regard to the treatment of MSSA infection and the prophylaxis of infections likely to be caused by MRSA.

Cephalosporins in clinical practice are used for prophylactic purposes in patients undergoing surgical operations, yet the most suitable cephalosporin still remains controversial. The present results together with previous findings1–6) suggest that the combination of cephamycin and cephalosporin, particularly cefmetazole and cefotiam or cefamandole may offer more effectiveness than these β-lactam alone.

The enhancement of the anti-staphylococcal activity by cefmetazole and cephalosporins in combination does not appear to be due to neither increased penetration of antibiotics to bacterial cells nor inhibition of β-lactamase. The mechanism of resistance may reside with penicillin-binding protein PBP-228,29), to which most β-lactam antibiotics do not possess an appreciable binding affinity. Since cephamycin have affinity for PBP-4 in S. aureus30), the combination of cefmetazole with cephalosporins which preferentially bind to PBPs other than PBP-4 appears to be essential for the synergy31).

**References**

1) YOSHIKAWA, O.; S. OHSHIMA, H. TAKATSUJI, M. OHTAKI & R. NAKAMURA: The effects of latamoxef and
560(120) THE JAPANESE JOURNAL OF ANTIBIOTICS 48—4 Apr. 1995

メチシリン感受性および耐性黄色ブドウ球菌へのセフェメタゾールとセファゾリン，セフォチアム，セファマンドールまたはセフォベラゾンの併用による相乗的 in vitro 抗菌力増強作用

I. NaCl の影響

植手鉄男・松尾清光

田附興風会医学研究所・北野病院臨床検査部

セファマイシン，例えばセフェメタゾール，フロモキセフなどとセファロスポリンの併用はメチシリン耐性黄色ブドウ球菌（MRSA）に対して相乗的に in vitro 抗菌力を増強することが知られている。しかし，セファマイシンとどのセファロスポリンとの併用がもっとも効果的かは不明である。ゆえに，本研究においてはメチシリン感受性黄色ブドウ球菌（MSSA）9 株，MRSA 30 株を用いて，セファメタゾールとセファゾリン，セフォチアム，セファマンドールまたはセフォベラゾンの併用抗菌効果を比較検討した。

Mueller-Hinton 寒天平板希釈法（日本化学療法学会法）により MIC を測定し，Checkerboard 法によりこれと薬剤の併用効果を評価した。2 薬剤併用時の FIC index ≤0.5 を相乗的抗菌力増強と見做した。

また，培地の NaCl 濃度が β-ラクタム剤の MRSA への抗菌力に影響することは良く知られている。また黄色ブドウ球菌は Salt-tolerant であり，Hyperosmotic の条件下で成長，増加する。生理的に生体内の NaCl 濃度，すなわち Osmolality は場所により差がある。ゆえに，食塩濃度の異なる条件下で抗菌力を測定した。すなわち，Mueller-Hinton 寒天培地に食塩の無添加の場合と 4% NaCl 添加した条件下でのセファマイシンとセファロスポリンの併用効果を比較検討した。

何れの条件下においても，セフェメタゾールとセファゾリン，セフォチアム，セファマンドールまたはセフォベラゾンの併用は MSSA および MRSA に対して相乗的抗菌力の増強を示した。NaCl 無添加の条件下において，6.25μg/ml 以下の低濃度のセファロスポリンによるセフェメタゾールの MRSA にたいする抗菌力の相乗的増強作用，検討したセファロスポリン中，セフォチアムが最も強力で，ついでセファマンドール，セファゾリン，セフォベラゾンなどであった。セファマンドールはセフォ
チアムと同様の増強効果を得るのに、より高濃度を必要とした。セファマンドール 12.5 μg/ml の濃度において、セフォチアム 12.5 μg/ml 存在時の場合と同様なセフメタゾールの抗菌力増強を示した。

4% NaCl を寒天培地に添加した場合、これらの相乗的併用効果は減少した。セフメタゾールとセファマンドールとの併用効果は、他のセファロスポリンとの併用効果に比し、NaCl の影響が少なかった。4% NaCl が存在する場合、セファマンドールは、6.25 ～ 12.5 μg/ml 濃度において、上記セファロスポリン中最も強力にセフメタゾールの MRSA への抗菌力を増強した。

セフメタゾールとセフォチアムあるいはセファマンドールの併用の MRSA への相乗的抗菌力増強は、セフメタゾールと他のセファロスポリンの併用より強力であった。しかし、仮の併用が臨床上もっとも有用かはさらに究明が必要である。