Effects of fosfomycin in combination against methicillin-resistant *Staphylococcus aureus*

*Nalinee Aswapaokee, *Surapee Tiengrim, **Busaba Charoensook

*Departments of *Medicine and **Preventive and Social Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand

Fosfomycin is a broad spectrum antimicrobial agent which inhibits cell wall formation at different step from beta-lactams. Fosfomycin is used for different conditions in different countries. In the USA, this agent is used as an oral therapy for uncomplicated lower urinary infections, while in European countries it is used in treating various infections. In Thailand, however, fosfomycin is widely used in treating infections caused by methicillin-resistant *S. aureus* (MRSA), due to its high activity against this pathogen.

Although fosfomycin resistance in clinical isolates is infrequently found, the use of fosfomycin as the sole agent in treating MRSA infections may readily promote mutation through several gene targets. Addition of second agent, therefore, may be mandatory in maintaining fosfomycin efficacy.

We undertook in vitro efficacy determination of fosfomycin in combination with several agents active against recent clinical isolates of MRSA.

**MATERIALS AND METHODS**

**Bacterial isolates**

A total of 113 clinical isolates of MRSA obtained from patients hospitalized at Siriraj Hospital during 2002 to 2004 was used. These were non-repetitive invasive strains.

**Antimicrobial agents**

Fosfomycin was combined with the following agents: oxacillin, clindamycin, sulfamethoxazole-trimethoprim, rifampin, fusidic acid and vancomycin. The above-mentioned agents were used as discs and were purchased from Oxoid Limited®.

**Susceptibility testing**

Standard disc diffusions (NCCLS, 2003) were performed for single agents. The following disc contents were used: fosfomycin, 50 μg; oxacillin, 1 μg; clindamycin, 2 μg; trimethoprim-sulfamethoxazole, 1.25/23.75 μg; rifampin, 5 μg; fusidic acid, 10 μg and vancomycin, 30 μg. Zone size determination for susceptibility was shown in table 1.

**Antimicrobial synergy testing**

Antimicrobial synergy was determined by double disk synergy method. Discs were placed approximately 20-40 mm apart. Synergy was defined as zone size enlargement of one agent towards the other agent. Antagonism was defined as reducing zone size of one or both agents, while indifferent effect was defined as unaltered zone size comparing to single disc diffusion test.
RESULTS

Activities of single agent

Fosfomycin, fusidic acid and vancomycin were highly active against these tested MRSA, as 96.5, 99.1 and 100 per cent, respectively of these organisms were susceptible. Susceptibility to rifampin was only 51.3%, an alarming rate of reduced susceptibility. Twenty two and 18.6% of these isolates were susceptible to clindamycin and trimethoprim-sulfamethoxazole. Table 1 summarizes single agent susceptibility.

Antimicrobial synergy results

Synergy with fosfomycin could not be determined when discs were placed at 20 mm apart. This was due to very large zone sizes of fosfomycin diffusion, of which the average size was 33.04 mm (range, 20-42 mm). When discs were placed 40 mm apart, synergy was shown with 41 out of 51 isolates (80.4%) of oxacillin-fosfomycin combination. Rifampin-fosfomycin combination showed 35% synergism and 65% indifferent. Most of rifampin-susceptible isolates showed synergism, while most rifampin-resistant isolates showed indifference. All other combination revealed indifferent effect, except for fosfomycin-vancomycin combination, in which 15 out of 20 isolates (75%) showed antagonism. Table 2 summarizes interaction effects with fosfomycin.

DISCUSSION

Fosfomycin has been shown to exert excellent activity against MRSA. In this study, it was shown that fosfomycin zones of inhibition were large, and the percentage susceptibility was high. Clinical trials, although not extensive, had repeatedly revealed high success rate in treating such infections. The problems of rapid emergence of resistance to fosfomycin was prevented by adding second agent to the regimen, thus maintaining fosfomycin activity was achieved.

Several studies determining effects of drug combination to fosfomycin were published. In our study, we have shown that fosfomycin acts synergistically with oxacillin, an agent inactive against MRSA. The combination with other agents such as clindamycin, trimethoprim-sulfamethoxazole and fusidic acid were indifferent. Antagonism was found, unfortunately, when fosfomycin was tested with vancomycin. Our study was both consistent or different from other studies.

Several groups of antimicrobial agents were chosen to combine with fosfomycin, based on different rationales.

Some authors chose agents with activities against MRSA and others based their choice on mechanism of action. Sieradski and Tomasz used agents which had potential of suppression of methicillin-resistant expression. They found that agents acting at early step of peptidoglycan synthesis, e.g. fosfomycin, bacitracin, vancomycin and teicoplanin, suppressed methicillin-resistant expression. This explained the synergic effect of fosfomycin and oxacillin, and antagonistic effect of fosfomycin and vancomycin.

Selection of synergy tests may also resulted in different effects. Determination with checkerboard technique almost always gave synergic effects, while time-kill studies invariably gave indifferent effect and / or antagonism. In this study, the use of double disc test gave different results with the above-mentioned techniques. In spite of these variations, however, effects of combination were likely to be explained by the mechanism of action of drugs employed in that combination.
In this study, we were not able to demonstrate prevention of resistance emergence to fosfomycin by a second agent.

The addition of second drug, however, may still be essential especially in clinical situation which inoculum size is large.

In conclusion, it is found that fosfomycin has excellent in vitro activity against MRSA. The percentage susceptibility to this agent also remains high during this recent period. The effects of combination with agents active against MRSA are indifferent.

Instead, the combined effects with agent acting on different stages of peptidoglycan synthesis is of interesting, while the effects caused by agents acting on same stage of cell wall inhibition resulted in antagonism. Selection of these combination in clinical situations could be based on in vitro activities plus basic knowledge of actions of the relevant compounds.

### Table 1: Zone size determination and susceptibility to single agent of MRSA

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>%Susceptible</th>
<th>Zone diameter (mm) for susceptible category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fosfomycin</td>
<td>96.5</td>
<td>≥ 20</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>22.0</td>
<td>≥ 21</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>18.6</td>
<td>≥ 16</td>
</tr>
<tr>
<td>Rifampin</td>
<td>51.3</td>
<td>≥ 20</td>
</tr>
<tr>
<td>Fusidic acid</td>
<td>99.1</td>
<td>≥ 22</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>100.0</td>
<td>≥ 15</td>
</tr>
</tbody>
</table>

### Table 2: Effects of fosfomycin in combination with other agents against MRSA

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>% of interaction</th>
<th>Synergism</th>
<th>Indifferent</th>
<th>Antagonism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxacillin</td>
<td></td>
<td>80.4</td>
<td>19.6</td>
<td>-</td>
</tr>
<tr>
<td>Clindamycin</td>
<td></td>
<td>-</td>
<td>100.0</td>
<td>-</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td></td>
<td>-</td>
<td>100.0</td>
<td>-</td>
</tr>
<tr>
<td>Rifampin</td>
<td></td>
<td>35.0</td>
<td>65.0</td>
<td>-</td>
</tr>
<tr>
<td>Fusidic acid</td>
<td></td>
<td>-</td>
<td>100.0</td>
<td>-</td>
</tr>
<tr>
<td>Vancomycin</td>
<td></td>
<td>-</td>
<td>25.0</td>
<td>75.0</td>
</tr>
</tbody>
</table>

Effects of fosfomycin in combination against methicillin-resistant *Staphylococcus aureus*
REFERENCES


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