Marked Reduction in the Minimum Inhibitory Concentration (MIC) of β-Lactams in Methicillin-Resistant Staphylococcus aureus Produced by Epicatechin Gallate, an Ingredient of Green Tea (Camellia sinensis)

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We found that epicatechin gallate, a constituent of an extract of tea leaves (green tea) markedly lowered the minimum inhibitory concentration (MIC) of oxacillin and other β-lactams, but not of other antibiotic agents tested, in strains of methicillin-resistant Staphylococcus aureus. The antibacterial action of epicatechin gallate plus oxacillin was a bactericidal one.

Key words epicatechin gallate; methicillin-resistant Staphylococcus aureus (MRSA); β-lactam; minimum inhibitory concentration (MIC) reduction

Methicillin-resistant Staphylococcus aureus (MRSA) is a serious problem in hospitals because it is a major cause of nosocomial infections. In addition, it is difficult to treat patients infected with MRSA using most common antibacterial agents. MRSA is resistant to not only methicillin and other β-lactams but also to many other antibiotic agents. Thus, MRSA exhibits multidrug resistance. Although the reasons for the multidrug resistance of MRSA are not completely clear yet, it seems that genes responsible for multidrug resistance are present mainly in the mec region of the MRSA chromosome and several other genes like fem, ltm and sigB are also involved. Several mechanisms are known by which microorganisms can overcome the toxicity of antimicrobial agents. These include inactivation of drugs, extrusion of drugs from cells, modification of targets for drugs, and production of drug-insensitive enzymes. It may be that most MRSA strains possess multidrug efflux pumps such as NorA and other drug resistance mechanisms.

It has been reported that an extract of tea leaves (green tea) shows antimicrobial activity. Catechins are major components of tea leaves and can be easily extracted with hot water. High concentrations of catechins are found in green tea. It has also been reported that catechins possess antimicrobial activity and an extract of tea leaves markedly lowered the minimum inhibitory concentration (MIC) of β-lactams. However, it was not clear which component was responsible for this effect.

Here we report that epicatechin gallate, a constituent of green tea, markedly reduces the MIC of β-lactams against MRSA. In other words, epicatechin gallate restores the effectiveness of β-lactams against MRSA.

MATERIALS AND METHODS

Bacterial Strains Methicillin-resistant strains of S. aureus, OM481, OM505, OMS84 and OM623 were clinical isolates from Okayama University hospital. Methicillin-sensitive S. aureus (MSSA) strain 209P was used as a control.

Cell Growth Cells of S. aureus were grown in Mueller-Hinton broth (Difco) at 32 °C under aerobic conditions. The MICs were determined by the liquid microdilution method. Serial two-fold dilutions of drugs were used. Cells were cultured at 32 °C without shaking in Mueller-Hinton broth. The MIC was determined as the lowest concentration of antimicrobial agent in which cells were not able to grow. Viable cell numbers were determined as reported elsewhere.

The fractional inhibitory concentration (FIC) index was calculated as reported elsewhere. The effects of the drugs were interpreted to be indicative of synergy or indifference when the index was ≦0.5 or ≧0.5, respectively.

Materials Epicatechin gallate, epigallocatechin gallate, epicatechin and epigallocatechin were prepared from leaves of Thea sinensis as described previously or purchased from Sigma Co. Antimicrobial agents were from commercial sources.

RESULTS AND DISCUSSION

Reduction in the MIC of β-Lactams against MRSA Caused by Tea Firstly, we confirmed that an extract of tea leaves (green tea) shows some antibacterial activity against MRSA. Thereafter, we measured the MICs of several antibacterial agents against 4 strains of MRSA and a control MSSA, 209P, in the absence or presence of the extract. Table 1 shows that the extract markedly lowered the MICs of all β-lactams tested against MRSA, but not against MSSA. No such marked effect of the extract on MRSA was observed when other antibacterial agents, such as fosfomycin, streptomycin, ofloxacin, erythromycin or tetracycline, were tested. However, some reduction in the MICs caused by the extract was observed with other antibacterial agents and strains (Table 1).

Epicatechin Gallate Markedly Reduced the MIC We attempted to determine which ingredient(s) of green tea reduced the β-lactam MICs in MRSA. It is known that green tea contains protein, catechins, caffeine and amino acids as major ingredients. We found that catechins, but not caffeine, were effective in reducing the MICs (data not shown). Next, we determined the MICs of each catechin against MRSA and MSSA. Although each catechin (epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG)) showed antibacterial activity against both MRSA and MSSA, the MICs were fairly high (128 μg/ml or above) (precise data are not shown). Thus, the an...
Table 1. MIC of Various Antibacterial Agents for MSSA and MRSA in the Absence or Presence of an Extract of Tea Leaves (Green Tea)

<table>
<thead>
<tr>
<th>Strains</th>
<th>MIC (µg/ml)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>MPIPC</td>
</tr>
<tr>
<td></td>
<td>a) 0.12, 0.06</td>
</tr>
<tr>
<td>OM481</td>
<td>512, 32</td>
</tr>
<tr>
<td>OM505</td>
<td>128, 32</td>
</tr>
<tr>
<td>OM584</td>
<td>256, 2</td>
</tr>
<tr>
<td>OM623</td>
<td>256, 2</td>
</tr>
</tbody>
</table>

a) MIC was determined by the microdilution method in the absence (–) or presence (+) of 150 µg/ml tea extract. Cell growth was assessed after incubation at 32°C for 24 h.

Table 2. Effect of Catechins on MIC of MPIPC (Oxacillin) for MRSA

<table>
<thead>
<tr>
<th>Strains</th>
<th>MIC (µg/ml)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Epicatechin (50 µg/ml)</td>
</tr>
<tr>
<td>209P</td>
<td>0.12</td>
</tr>
<tr>
<td>OM481</td>
<td>512, 512</td>
</tr>
<tr>
<td>OM505</td>
<td>128</td>
</tr>
<tr>
<td>OM584</td>
<td>128</td>
</tr>
<tr>
<td>OM623</td>
<td>256</td>
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</tbody>
</table>

b) MIC of oxacillin was determined in the absence (–) or presence of each catechin at indicated concentrations. Cell growth was assessed after incubation at 32°C for 24 h.

Fig. 1. Effect of Epicatechin Gallate and Oxacillin on Viable Cell Numbers of MRSA

Cells of S. aureus strain OM584 grown in Mueller-Hinton broth were diluted with fresh medium and incubated at 32°C under aerobic conditions in the absence (C), or presence of oxacillin (1 µg/ml) (B), epicatechin gallate (25 µg/ml) (△), or oxacillin (1 µg/ml) plus epicatechin gallate (25 µg/ml) (▲). Samples were taken at the indicated time points, diluted with saline and spread onto nutrient agar plates. Colonies, appearing on the plate after incubation at 32°C for 24 h were counted; viable cell numbers in the original samples were calculated.

The effects of these catechins are not strong. We tested the effect of each catechin on the reduction of MICs of β-lactams against MRSA at concentrations much lower than the MIC of each catechin itself. Of the four major catechins in green tea, epicatechin gallate showed the strongest activity followed by epigallocatechin gallate (Table 2). Epicatechin gallate (25 µg/ml) lowered the MIC of oxacillin against all four MRSA strains 250- to 500-fold, while epigallocatechin gallate (25 µg/ml) produced an 8- to 120-fold reduction. Both epicatechin and epigallocatechin lowered the MICs of β-lactams only slightly in the case of some MRSA strains. At present, we do not know the reason why these two catechins are not so effective. Similar levels of reduction of MICs by epicatechin gallate were observed with all other β-lactams tested, but not with other types of antibacterial agents tested, including fosfomycin, ofloxacin, erythromycin and tetracycline (data not shown).

We calculated the FIC index for the enhanced antibacterial effect of oxacillin plus catechin. The FIC index of oxacillin in combination with ECG ranged from 0.051 to 0.10 in MRSA, and that of oxacillin with EGCG ranged from 0.21 to 0.45. On the other hand, the index for oxacillin with ECG or EGCG ranged from 0.60 to 1.2 in MSSA. These results suggest that the combination of oxacillin and either ECG or EGCG is synergistic in MRSA, but not in MSSA.

**Bactericidal Effect of Epicatechin Gallate Plus β-Lactam**

We tested the effect of epicatechin gallate and oxacillin on the viability of MRSA cells (Fig. 1). Oxacillin or epicatechin gallate alone reduced the growth rate of MRSA, although viable cell numbers increased gradually. It seems that epicatechin gallate has some bacteriostatic action on MRSA, judging from the growth curve. After stopping the growth for a few hours in the presence of epicatechin gallate, cell growth was resumed. In contrast, simultaneous addition of epicatechin gallate and oxacillin greatly reduced viable cell numbers. Thus, we conclude that the action of epicatechin gallate plus oxacillin is bactericidal. Similar results were obtained with epigallocatechin gallate plus oxacillin (data not shown).

It is not clear yet how epicatechin gallate enhances the antibacterial activity of β-lactams against MRSA although it has been reported that bactericidal catechins damage the lipid bilayer. \(^{11}\) The concentrations of epigallocatechin gallate used in such experiments were 0.6 to 1.2 mM, corresponding to 275 and 550 µg/ml, respectively. A striking reduction in the MICs of β-lactams caused by epicatechin gallate or epi-
galloic acid was observed at only 25 to 50 \( \mu \text{g/ml} \). Thus, we believe that damage to the lipid bilayer of the cell membrane is not a major cause of the striking reduction in the MIC elicited by catechins. Although we do not know yet the mechanism by which epicatechin gallate markedly reduces the MIC of \( \beta \)-lactams against MRSA, it should be pointed out that catechins have no significant effect on the MIC of \( \beta \)-lactams in MSSA. Thus, it seems that penicillin-binding protein 2' (PBP2') \(^{14} \) or its gene expression is a target for catechin action.

It has been reported that a triazine dye (Cibacron blue F3GA) \(^ {15} \) and Triton X 100 \(^ {16} \) reduce oxacillin-resistance levels in MRSA. In addition, a synergistic effect has been reported for an MIC reduction for MRSA with a combination of polyoxotungstates and \( \beta \)-lactams. \(^ {10} \) Although the mechanism of the increased susceptibility to \( \beta \)-lactams is not yet very clear, Tajima et al. have suggested that polyoxotungstates inhibit both the synthesis of PBP and the activity of \( \beta \)-lactamase. \(^ {17} \)

After completion of this work, we found that Takahashi and coworkers had reported that catechins, especially epigallocatechin gallate, markedly lowered the MIC of \( \beta \)-lactams against MRSA, and that epicatechin gallate was ineffective (Takahashi Y., Toda M., T. Shimamura T., Hara Y.; abstract, 70th Annual Meeting of Japanese Society for Microbiology, 1997, p. 144). Since their results differ from ours with respect to the effect of epicathecin gallate and epigallocatechin gallate, we tested the effect of two types of catechins, one purified from an extract of tea leaves \(^ {21} \) and another obtained from a commercial source, on the action of \( \beta \)-lactams. Both types of catechins showed the same results: epicatechin gallate showed very high activity and epigallocatechin gallate showed weaker activity as far as reducing the MIC of \( \beta \)-lactams against MRSA was concerned.

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REFERENCES