Antibacterial Activity of Epigallocatechin Gallate against Methicillin-Resistant *Staphylococcus aureus*

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Abstract

The antibacterial activity of epigallocatechin gallate (EGCg), a catechin, against 53 clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) was evaluated and expressed as minimal inhibitory concentration (MIC). The MIC⁵₀ and MIC₉₀ of the strains were found to be 64 and 126 μg/ml, respectively by the microdilution method. A time-kill study using an isolate showed that EGCg appeared to be bacteriostatic at 1-2 × MIC and bactericidal at 6 × MIC against MRSA. In addition, the activity of EGCg was stable to various physical conditions including boiling or freezing. These findings suggest that EGCg could be a useful agent for treating MRSA infection.

Introduction

It is well known that Japanese green tea has antibacterial activity against various foodborne pathogenic bacteria including *Clostridium botulinum*¹,², *Staphylococcus aureus*³, *Vibrio parahaemolyticus*³, *Clostridium perfringens*³, *Bacillus cereus*³, *Salmonella typhi*, *Shigella dysenteriae*, *Campylobacter jejuni*, and *Vibrio cholerae*⁴. In addition, there are reports that catechin is effective against infections due to *Bordetella pertussis*⁵, *Mycoplasma pneumoniae*⁶, *Trichophyton* species⁷, rotavirus, enterovirus⁸, and influenza virus⁹. Moreover, the catechin in tea extract inhibits glucan synthesis by glucosyltransferase from *Streptococcus mutans*, thereby preventing dental caries¹⁰.

Recently, Toda et al. showed that epigallocatechin gallate (EGCg), one of the major classes of catechins, has antibacterial activity against methicillin-resistant *S. aureus* (MRSA)¹⁰. MRSA is a major nosocomial pathogen which causes problems worldwide. However, no clinical application of catechin in MRSA infection has yet been reported. The minimum inhibitory concentration (MIC) of catechin against MRSA likewise has not been determined.

In order to determine the possible clinical usefulness of catechin in MRSA infections, we quantitatively measured the activity of extracts of Japanese tea including EGCg against MRSA strains isolated in our university hospital. In addition, we examined the physical effects of conditions such as boiling and freezing, on catechin to decide on appropriate conditions for preservation of catechin for clinical use.
Materials and Methods

Bacteria

All strains of *S. aureus* that were recovered were identified in the Clinical Laboratory Service in our university hospital in 1993. Those strains of *S. aureus* that were found to be oxacillin-resistant by the Kirby-Bauer method were considered MRSA. Thus, 53 strains of MRSA were examined in this study. For the study of the time-kill curve and effect of physical treatments on catechin, MRSA strain YK that was isolated from a patient with sepsis was used.

Catechins

Purified epigallocatechin galate (EGCg) and Polyphenon-100 (crude extract of Japanese green tea, containing 57% EGCg) (Mitsui Norin Co., Ltd., Fujieda, Shizuoka, Japan) were kindly donated by the Central Laboratory, Mitsui Norin Co.

MICs

All isolates were tested for sensitivity to the antimicrobial agents by the microdilution method to determine the MIC. EGCg and Polyphenon-100 were diluted to concentrations of 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256, 512 µg/ml in Mueller-Hinton broth (Difco Laboratories, Detroit, Michigan, USA). MRSA strains were subcultured overnight, then suspended in saline solution. After transfer of the bacteria in an amount equal to 10⁶ to each well of multiwell culture plates, the plates were incubated at 35°C for 18 h. MIC was defined as the lowest concentration of catechin that allowed no visible growth.

Time-kill curve

Time-kill studies with catechin were performed with the YK strain of MRSA. The organism was cultured overnight and shaken. After incubation with shaking for 1 h, approximately 10⁶ CFU/ml of bacteria in the log phase were transferred to tubes containing 5 ml of Mueller-Hinton broth. The catechins were added to give final concentrations of 1, 2 and 6 × MIC. The MIC of the YK strain was 64 µg/ml. The tubes were incubated further at 37°C with shaking and samples were withdrawn at 0, 3, 6, and 24 h and spread on agar plates. The numbers of CFU were determined after overnight incubation. Control cultures without catechins were examined in parallel and each experiment was performed in duplicate.

Effects of physical conditions on antibacterial activity of catechin

Strain YK was stored in the cold for 40 days, boiled for 30 min in a capped tube to avoid evaporation, and autoclaved 120°C for 20 min to determine the effects of these conditions on the antibacterial activity of catechin against MRSA. Before, during, and after these treatments, catechins were tested to determine their MICs against the YK strain.

Results

Table 1 shows the sensitivity of the clinical isolates to each catechin. The MICs of EGCg and Polyphenon-100 were 32–64 µg/ml and 64–256 µg/ml respectively, against all strains. Fig. 1 shows the
time-kill curve of ECGg against MRSA. In concentrations 1- or 2 times the MIC, the number of bacteria only decreased to 1/10th of the original concentration, suggesting that the activity is bacteriostatic. On the other hand, concentrations 6-fold greater than MIC resulted in 3-log_{10} decrease in CFU/ml after 24 h, suggesting that the activity is bactericidal. As for the effects of physical conditions on the activity of the catechins (Fig. 2), the MIC of Polyphenon-100, 128 µg/ml, did not increase after refrigeration, freezing, boiling, or autoclaving. The MIC of EGCg was increased, but only from 64 to 128 µg/ml (this means one dilution higher by the microdilution method) after refrigeration, freezing, boiling, or autoclaving. In addition, the activity of EGCg and Polyphenon-100 was stable at room temperature for 2 weeks.

Discussion

In our country, Japanese green tea is a traditional, commonly consumed drink which is very familiar to the general population. Catechin is one of the major components of green tea (10-18%) and is a kind of polyphenol, the chemical structure of which has been determined. EGCg is the most important of the four classes of catechins (comprising about 50%), displays various antibacterial activities. The present study revealed the following: 1) Catechins are effective against clinical isolates of MRSA, although the MIC is somewhat higher than that of anti-MRSA agents such as vancomycin (MIC, 1-2 µg/ml). Since the activity of green tea is considered to be due to EGCg and since
Polyphenon-10 consists of 50% EGCg, the results seem to be reasonable. 2) The mechanism of action is killing of the bacteria at high concentrations, suggesting that EGCg has a concentration-dependent bactericidal activity. Toda et al. also showed that EGCg is bactericidal against MRSA. 3) These compounds are highly stable under various physical conditions including freezing and boiling. In addition, they are preserved well even at room temperature. Thus, catechins are so well preserved that they appear to be candidates for clinical use. However, the MICs of catechin are so high, unlike those of antibiotics, that it may be difficult to achieve catechin tissue levels that are high enough following intestinal absorption after oral administration. Catechins seem to be suitable for oral administration for the treatment of intestinal infections or colonization and for topical administration for the treatment of skin infections or colonization. Inhalation therapy with catechins could also be recommended for respiratory tract infections or colonization.

Catechins in solution can be well preserved in a refrigerator for up to a month. In addition, they are heatable, so that even hot water tea extracts (so-called tea drink), which contain antibacterial activity equivalent to 0.5-1.0 mg of polyphenon (data not shown), are also useful when catechin extract is not available, although the concentration does not appear to be high enough for clinical use.

In the future, we will investigate the mechanism of action of this EGCg against MRSA using in vitro and in vivo experiments. Furthermore, we may propose a clinical trial of this agent against MRSA infection and colonization.

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References

メチシリン耐性黄色プドウ球菌（MRSA）に対する
epigallocatechin gallate（EGCg）の抗菌活性の検討

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要 旨
メチシリン耐性黄色プドウ球菌（MRSA）の臨床分離53株に対するepigallocatechin gallate（EGCg）の抗菌活性を最小発育阻止濃度MICについて検討した。53株に対するEGCgの微量液体希釈法によるMIC50、MIC90はそれぞれ64、128μg/mlであった。分離株1株を使った時間殺菌曲線による検討では、EGCgはMRSAに対し1〜2MICでは静菌的、6MICでは殺菌的に作用すると思われた。また、EGCgは煮沸や凍結などの物理的処理に対しても安定であった。これらのことから、EGCgはMRSA感染症に対し有用性があると思われた。