

Additive, indifferent and antagonistic effects in combinations of epigallocatechin gallate with 12 non- β -lactam antibiotics against methicillin-resistant *Staphylococcus aureus*

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Additive, indifferent and antagonistic effects were observed in combinations of epigallocatechin gallate (EGCg, a main constituent of tea catechins) with 12 non- β -lactam antibiotics against methicillin-resistant *Staphylococcus aureus* (MRSA). The combinations of EGCg with the inhibitors of either protein or nucleic acid synthesis showed additive or indifferent effects. These antibiotics included tetracycline, minocycline, chloramphenicol, streptomycin, gentamicin, kanamycin, erythromycin, rifampicin and ofloxacin. In contrast, EGCg showed an antagonistic tendency against glycopeptide antibiotics (vancomycin, teicoplanin and polymyxin B). The common property of these antibiotics is the peptide backbone structure, suggesting a direct binding of EGCg with the antibiotics. The above results indicate that tea catechins may affect the activities of antibiotics both positively and negatively.

Introduction

Bacterial resistance to antibiotics is a serious global problem. In particular, methicillin-resistant *Staphylococcus aureus* (MRSA) has already made its way into the community and is not just limited to hospitalized patients and their care providers.¹ Therefore, novel antimicrobials and/or new approaches to combat the problem are urgently needed. Natural products and traditional medicines may be sources of new antimicrobial agents. With this in mind, our group has studied and demonstrated that tea (*Camellia sinensis*) and tea catechins such as catechin, epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate (EGCg) have bactericidal activity against various Gram-positive and -negative bacteria. Furthermore, we have found that low concentrations of EGCg reverse the resistance of MRSA to β -lactams.²⁻⁵ Using an aqueous extract of tea, two groups have also demonstrated synergy between tea and β -lactams.^{6,7} In contrast to the synergy between EGCg and β -lactams, we have noted only additive or indifferent effects between EGCg and other antibiotics including minocycline and ofloxacin.³

Vancomycin is an inhibitor of bacterial cell wall synthesis and is the first choice for MRSA treatment. Nevertheless, there are increasing reports of vancomycin-resistant MRSA.^{8,9} Here we present an extension of this work to look at the interaction between EGCg and vancomycin, as well as an extended range of other non-cell wall active antibiotics.

Materials and methods

Chemicals and antibiotics

EGCg was extracted from green tea and the purity was 98%, as confirmed by high-performance liquid chromatography. The following antibiotics were purchased from the sources indicated: minocycline, streptomycin, kanamycin, erythromycin, rifampicin, ofloxacin and vancomycin (Sigma, St Louis, MO, USA); tetracycline, gentamicin, chloramphenicol and polymyxin B (Wako Pure Chemical Industries, Tokyo, Japan); teicoplanin (Aventis Pharma Japan, Tokyo, Japan).

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Bacterial strains

Eight clinical isolates of MRSA were from specimens submitted for routine culture at the clinical microbiology laboratories of Showa University Hospital. All the strains were identified by PCR analysis of *mecA* gene expression as reported previously.³ *Escherichia coli* ATCC 25922 was used to test the EGCg–polymyxin B combination. The antimicrobial assay was carried out in Mueller–Hinton broth (MHB, Becton Dickinson, Cockeysville, MD, USA) supplemented with Ca²⁺ 25 mg/L and Mg²⁺ 12.5 mg/L.

MIC determination

MICs were determined using the broth microdilution method at a final inoculum of $>5 \times 10^5$ cfu/mL according to the guidelines of the NCCLS.¹⁰ After incubation at 35°C for 24 h, the lowest concentration of the two-fold serially diluted antibiotic(s) at which no visible growth occurred was defined as its MIC. The growth inhibition was also carried out in 3 mL of MHB with a start inoculum of 5×10^5 cfu/mL and the bacterial growth was detected using a spectrophotometer (OD at 600 nm). Combination effects were confirmed by the checkerboard method. Two-fold serial dilutions of an antibiotic were tested in combination with two-fold serial dilutions of EGCg.

Criteria to evaluate the combination effects between EGCg and antibiotics

The combination effects were evaluated by a fractional inhibitory concentration (FIC) index. FIC was calculated as the MIC of antibiotic and EGCg in combination, divided by the MIC of the antibiotic or EGCg alone, and the FIC index was obtained by adding the FICs. FIC indices were interpreted as synergic when values were ≤ 0.5 and as antagonistic when values were >4 . If a combination with EGCg resulted in a reduction in the antibacterial activity of an antibiotic, but the FIC index was still <4 , it was defined as an antagonistic tendency. The results between synergy and antagonistic tendency were defined as additive or indifferent.

Data presentation

The experiments were repeated three times for each strain. The combination effects between the antibiotics and EGCg for all eight strains of MRSA were determined and the eight strains did show the same tendencies. The data for strain F-74 only are presented as representative of the eight strains.

Results

Antagonistic tendency between EGCg and vancomycin, teicoplanin or polymyxin B

Figure 1i shows the chemical structure of EGCg. The MIC of EGCg alone for MRSA strains was 100 mg/L. EGCg–vanco-

mycin and EGCg–teicoplanin combinations showed an unexpected antagonistic tendency at certain concentrations and ratios. For example, in Figure 1b, EGCg at 12.5 mg/L antagonized the activity of teicoplanin at 0.5 mg/L, but EGCg at 6.25 and 25 mg/L showed an additive effect with teicoplanin at the same concentration. The antagonistic tendency was clearer when the bacteria were cultured at a fast-growing condition (shaking at 200 rpm) for 8 h or at an early phase of stationary culture. The antagonistic tendency was further confirmed by counting the cfu after plating the cells on agar plates (data not shown). Combination of EGCg with polymyxin B was also tested against *E. coli*. EGCg apparently antagonized the activity of polymyxin B (Figure 1c). The MIC of polymyxin B increased from 0.25 to 2 mg/L in the presence of 12.5 mg/L EGCg. The FIC index was >4 .

Additive and indifferent effects between EGCg and inhibitors of protein or nucleic acid synthesis against MRSA

Additive or indifferent effects were observed in combinations of EGCg with tetracycline, minocycline, chloramphenicol, gentamicin, streptomycin, kanamycin, erythromycin, ofloxacin and rifampicin. The partial data are shown in Figure 1(d–h).

Discussion

We have characterized *in vitro* that there are four combination effects between EGCg and antibiotics, i.e. synergic, additive, indifferent and antagonistic effects.

The additive or indifferent interactions were observed between EGCg and the inhibitors of either protein or nucleic acid synthesis. The increased permeability of the cell wall/membrane to antibiotics caused by the EGCg-induced damage of the cell wall might be the main factor for the additive effects.

The synergic effects between EGCg and β -lactams suggest a possible clinical use of EGCg to treat MRSA-infected patients, especially with topical or digestive tract infections. When we attempted to enhance the antibacterial activities of vancomycin and teicoplanin against MRSA through combinations with EGCg, however, an antagonistic tendency between EGCg and the two antibiotics was observed. According to the mechanism of synergy between EGCg and β -lactams against MRSA,³ the two antibiotics should have shown synergy with EGCg. Vancomycin and teicoplanin are glycopeptide antibiotics and EGCg may bind directly to the peptide structure of the antibiotics and then interfere with their activities. The balances between the synergic effect and the reduction of the antibacterial activities owing to their binding to each other may result in the antagonistic tendency at certain ratios of the antibiotics and EGCg but additive or

Effects of combinations between EGCg and antibiotics

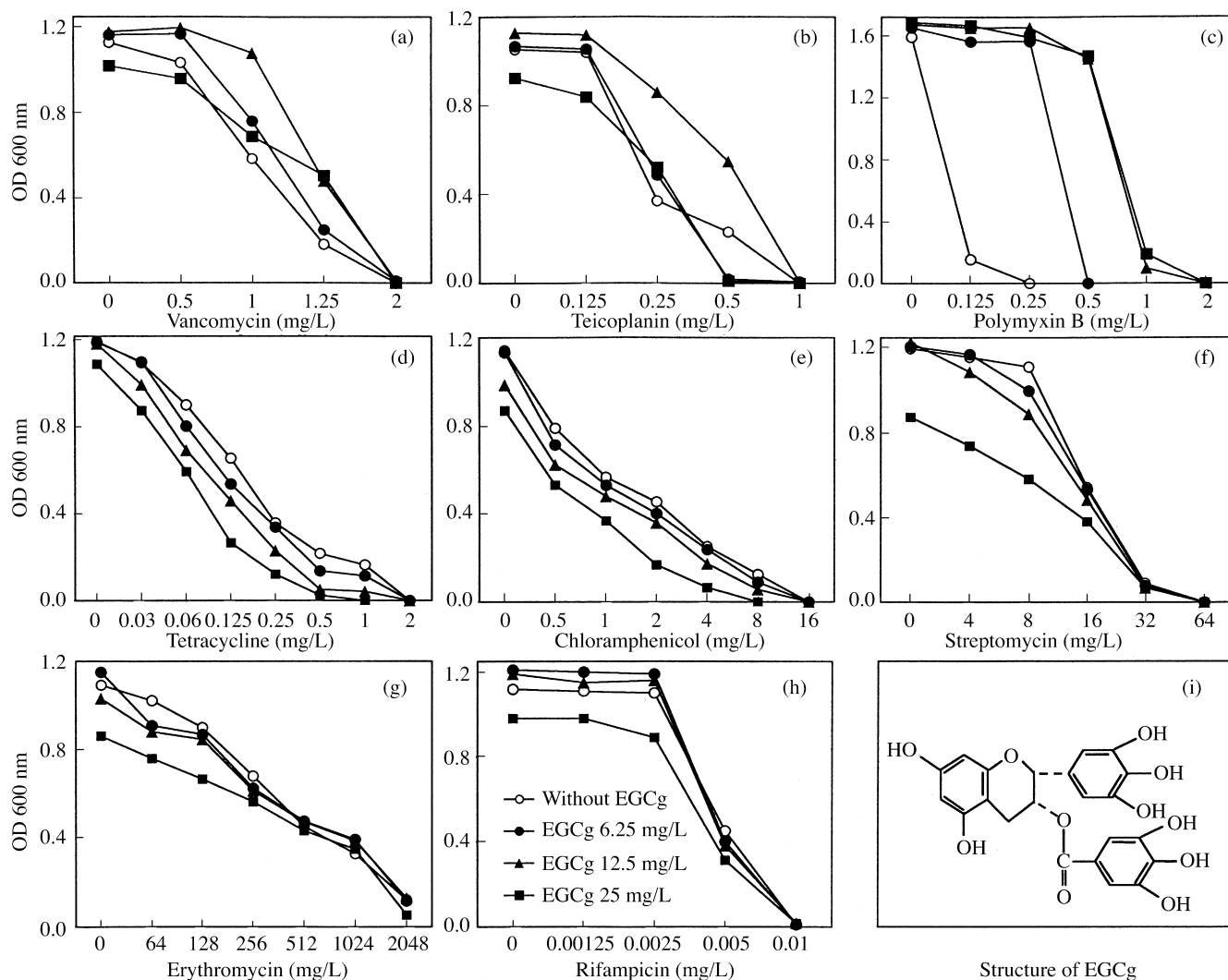


Figure 1. Antagonistic tendency between EGCg and vancomycin (a), teicoplanin (b) or polymyxin B (c); additive or indifferent interactions between EGCg and tetracycline (d), chloramphenicol (e), streptomycin (f), erythromycin (g) or rifampicin (h); and chemical structure of EGCg (i). *E. coli* ATCC 25922 was used as the target for polymyxin B in (c), but MRSA F-74 was used as the target for the remaining antibiotics. The key in (h) also applies to all the other panels.

indifferent effects at the other ratios (Figure 1b). To confirm this hypothesis, we detected the effect of an EGCg–polymyxin B combination against *E. coli*. Polymyxin B is also a glycopeptide antibiotic and highly active *in vitro* against most Gram-negative bacilli. EGCg clearly antagonized the activity of polymyxin B (Figure 1c, FIC index > 4), indicating direct binding between EGCg and glycopeptide antibiotics. Compared with the MIC of EGCg for *S. aureus* (100 mg/L), the MIC of EGCg for *E. coli* was >800 mg/L and there was no synergy between EGCg and ampicillin for *E. coli*.³ The lack of synergy between polymyxin B and EGCg for *E. coli* may explain the phenomenon that EGCg showed the strongest antagonism to the activity of polymyxin B.

Tea catechins, including EGCg, structurally belong to plant polyphenols and are generally known as tannins, as

reviewed previously.^{11,12} EGCg at concentrations >50 mg/L did precipitate proteins in broth, indicating a direct binding of EGCg with glycoproteins or polypeptides. The common property of tannins also strongly supports our explanation for the antagonistic tendency between EGCg and glycopeptide antibiotics.

It is hard to predict either synergic or antagonistic effects *in vivo* just according to the *in vitro* evidence presented. Usually, EGCg concentration in tea beverage is 2–3 g/L. Compared with that, 6.25, 12.5 or 25 mg/L EGCg are low concentrations. However, it is difficult to estimate the concentration of bio-available EGCg *in vivo* after drinking tea or taking EGCg capsules. EGCg is absorbed through the digestive tract and distributed to many organs of animals and humans. In rat blood plasma, EGCg at 5.6 mg/L was detected after being

orally administered with EGCg at 500 mg/kg body weight,¹³ and total catechins at 15–112 mg/L were detected 2 h after being orally administered with catechins at 5 g/kg body weight.¹⁴ In human blood plasma, EGCg at 2 mg/L was detected after 90 min of taking 525 mg EGCg capsules.¹⁵ Therefore, tea and EGCg may affect the activities of antibiotics not only *in vitro* but also *in vivo*.

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