Antimicrobial activity of Japanese green tea; present position and future prospects.

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Summary
It has been known for almost 100 years that aqueous extracts of tea show antibacterial activities. Detailed studies on Japanese green tea have been in progress since the late 1980s. Whole aqueous extracts and purified fractions have been shown to inhibit the growth of a wide variety of microorganisms (Gram-positive and -negative bacteria, viruses, mycoplasmas, fungi and protozoa), and/or to inactivate their toxins and enzymes. The main active principle is EGCG. Two particularly interesting aspects are the anti-cariogenic effects of tea drinking, and the bactericidal activity against methicillin-resistant *Staphylococcus aureus* (MRSA).

Our interest is in an entirely novel aspect, namely the potentiation of activity of β-lactam antibiotics against MRSA. We have shown this to be due to the action of ECG in preventing synthesis of PBP2', thereby negating the mechanism of methicillin resistance; thus, tea extracts and ECG act synergistically with methicillin or flucloxacillin against MRSA *in vitro*. ECG also markedly reduces the activity of staphylococcal β-lactamase activity. Unfortunately, ECG is labile *in vivo*: our present research activity is devoted to obtaining stable analogues of ECG that may have therapeutic potential .

Keywords:
Antimicrobial activity; EGCG; ECG; Methicillin-resistant *Staphylococcus aureus*.

Introduction

The drink made by infusing the dried leaves of *Camellia sinensis* in boiling water, called "tea", has for centuries been attributed, by anecdote, to have health-giving properties. Research over the past two decades has started to put these on a firm scientific footing (Hara, 2001).

Two main chemical classes have been found to be responsible for the pharmacological activities of tea:

* Caffeine (and other xanthine alkaloids) has a stimulatory effect on the central nervous system, due to inhibition of intracellular phosphodiesterase and blocking of adenosine receptors. Tea drinking causes a lift in mood, increased alertness and decreased fatigue. At higher concentrations, smooth muscle relaxes and the work capacity of striated muscle is increased. Excess caffeine may cause tremor, insomnia and ultimately an anxiety neurosis.

* Polyphenols: *epi*gallocatechin gallate (EGCG), *epi*gallocatechin (EGC) and *epi*catechin gallate (ECG) are powerful anti-oxidants, and may be anti-mutagenic and anti-carcinogenic. EGCG has also been reported to stimulate production of IL-1 from human leucocytes. Flavonoids such as querticin and kaempferol have beneficial effects on the cardiovascular system (eg atheroma formation is inhibited).

A recently appreciated health benefit of tea drinking is its very useful potential anti-cariogenic activity (Hamilton-Miller, 2001)

Our interest has been in the antimicrobial activity of tea and its chemical components, both in general and more specifically in the phenomenon of reversal of methicillin resistance in staphylococci.

The first documented report of an antibacterial action of tea was made in 1906, when McNaught, a British Army surgeon, showed that tea killed the causal organisms of typhoid fever (*Salmonella typhi*) and brucellosis (*Brucella melitensis*). However, no further work was done on this phenomenon for the next 50 years. Now it is known that tea has a very broad spectrum of antimicrobial action: viruses, bacteria, yeasts, fungi and various parasites have been reported by a variety of authors to be

susceptible *in vitro*. Further, certain bacterial virulence factors, such as adhesive ability and activity of toxins, may be prevented from acting by tea. The main active principle in green tea has been found to be EGCG; surprisingly little work has been done to determine its mechanism of action, which is assumed to be a consequence of its somewhat non-specific ability, in common with other polyphenols from tea, to denature proteins (astringency).

Antistaphylococcal activity of tea, and its components

Inhibition and killing of staphylococci by tea was first reported in 1962 by Das, using black tea against *Staphylococcus aureus*; Toda et al (1989) showed the same phenomenon with green tea for both *S.aureus* and *S.epidermidis*. Activity against methicillin-resistant *S.aureus* (MRSA) was confirmed by Toda et al (1991), using a "cup of tea" strength infusion. We have found *S.aureus* (including MRSA) and coagulase-negative staphylococci to be the species most susceptible to green tea, among a total of 32 tested (Yam et al 1997). EGCG appears to be chiefly responsible for this direct antibacterial activity, with theaflavins playing a part in black tea extracts. However, it is noteworthy that ECG, which has a very poor level of direct activity, causes severe disruption in the process of cell division in MRSA (Hamilton-Miller & Shah, 1999), suggesting that "sub MIC" effects should be carefully studied.

In 1998 Yam et al reported a completely novel antistaphylococcal activity in green tea ; this occurred at concentrations considerably lower than those causing a direct inhibitory effect on growth, and was separable from the latter by partition chromatography using Sephadex LH-20. We call this novel activity "activity II" to differentiate it from the direct inhibitory/bactericidal action described in the previous paragraph ("activity I"). Activity II manifests as a synergistic action with methicillin (or other penicillinase-resistant penicillin) against MRSA, but not against methicillin-sensitive *S.aureus* (MSSA) strains. During the course of these experiments, we also detected an anti-β-lactamase activity ("activity III") in green tea extracts, detectable by synergy for all *bla*$^+$ strains of *S.aureus*, whether MRSA or MSSA, with benzylpenicillin. We have not been able to separate activity III from activity II, and we concluded that they were properties of the same molecule.

An aqueous extract of Sencha tea was worked up to remove pigments and caffeine, concentrated and its catechin components separated by elution in chloroform 1: methanol 2: petrol 1 from a column of Sephadex LH-20. Five major peaks (D - H) were obtained; the fractions comprising these peaks were pooled, assayed for activities I, II and III, and freezedried. Activity I was spread across peaks E - H, but mainly in E, while activities II and III were at their maximum in peak G. Peak D was inactive. Chemical analysis of the active peaks was as follows: E - EGCG; F - 3"methylEGCG; G - ECG; H - EGCG and GCG.

We thus concluded that activity II was a property of ECG, a finding that we have published (Hamilton-Miller & Shah 2000).

Quantitative tests of synergy were carried out between crude extracts of tea and methicillin or flucloxacillin, using the chequerboard technique with 40 strains of MRSA. These showed that the presence of 200 μg/ml of crude tea extract rendered 89% of low-level MRSA and 36% of high level MRSA fully sensitive to methicillin. By formal microbiological assay, activity II was still detectable in such extracts at concentrations as low as 20 μg/ml, whereas activity I could not be detected below 200 μg/ml. A pure preparation of ECG assayed at about 2 μg/ml for activity II, while its MIC (activity I) was 512 μg/ml. Synergy was well demonstrated between ECG and flucloxacillin in growth curves, where the combination ECG 20 μg/ml + flucloxacillin 4 μg/ml inhibited growth of an MRSA for at least 24 h, while flucloxacillin 64 μg/ml alone allowed growth to occur to only a slightly lesser extent than found in the control (without antibiotic).

Several compounds chemically related to ECG were tested for activities I and II. While most did not show any activity II, EGCG, CG and theasinensin A were exceptions, with activity II potencies of about one-half, one-quarter and one-thirtieth, respectively, that of ECG. Activity I, on the other hand, was quite commonly found, being about equal to that of EGCG in the following compounds: *epi*afzelechin gallate, GC, EGC, theaflavin, theaflavin 3 gallate and theaflavin digallate.

The mechanism by which ECG exerts activity II has been shown to be the suppression of biosynthesis of PBP2', which is essential for the expression of methicillin resistance. This accounts for the finding that synergy between methicillin and tea extracts or ECG is selective for MRSA, as MSSA do not possess PBP2'. Activity III is caused by prevention of excretion of staphylococcal β-lactamase (normally an exo-enzyme), thereby reducing its overall ability to inactivate benzylpenicillin.

Future prospects

The ability to suppress the expression of methicillin resistance has obvious potential in the design of novel therapies for MRSA infections. Preventing synthesis of PBP2' *in vivo* should render MRSAs sensitive to flucloxacillin and cephalosporins. As ECG is labile *in vivo* (being broken down by the gut flora and liver enzymes to EC and gallate), analogues will have to be found that are both metabolically stable and retain activity II. Future research should be directed to this goal.

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