**In Vitro Anti-Adhesive Activity of Green Tea Extract against Pathogen Adhesion**

Ji-Hye Lee1*, Jin Sun Shim2*, Mi-Sook Chung2, Seung-Taik Lim1,8 and Kyung Hyun Kim3*

1Department of Food Technology, School of Life Sciences and Biotechnology, Korea University, Seoul 136-701, Korea
2Department of Food and Nutrition, Duksgung Women’s University, Seoul 132-714, Korea
3Department of Biotechnology & Bioinformatics, College of Science & Technology, Korea University, Chungnam 339-700, Korea

* Correspondence to: Seung-Taik Lim, Department of Food Technology, School of Life Sciences and Biotechnology, Korea University, Seoul 136-701, Korea. E-mail: stlim@korea.ac.kr

**Abstract**

*Camellia sinensis* polysaccharide has been reported to possess anti-adhesive activity against pathogens. The present study was designed to investigate whether hot water extracts obtained from green tea leaves might inhibit pathogen adhesion to human or mouse cell lines. Green tea extract 4 (CSI-4) with the maximum yield of 4% (w/v) is composed of a major proportion of carbohydrates containing 40% uronic acids, but lack of catechins. It showed strong inhibitory activities against hemagglutination mediated by pathogens *Helicobacter pylori*, *Propionibacterium acnes* and *Staphylococcus aureus* with the minimum inhibitory concentrations of 0.01–0.5 mg/mL. CSI-4 further demonstrated an inhibitory effect on the adhesion of these pathogens to host cell lines with the IC50 values (50% inhibition of adhesion) of 0.14–2.3 mg/mL. It exhibited the highest activity against *P. acnes*, but no inhibitory effects were observed against *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, *Escherichia coli*, or *Staphylococcus epidermidis*. Our results suggest that CSI-4 may exert a selective anti-adhesive effect against certain pathogenic bacteria with no adverse effects against beneficial or commensal bacteria. Copyright © 2008 John Wiley & Sons, Ltd.

**Keywords:** green tea; anti-adhesive; *Helicobacter pylori*; *Propionibacterium acnes*; *Staphylococcus aureus*.

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**INTRODUCTION**

Adhesion of pathogenic bacteria to host cell surface is a crucial event in colonization and infection (Hultgren et al., 1993; Sharon, 2006), and commensal bacteria protect by competing with pathogens for host binding sites (Tannock, 1995). Specificities of pathogen-host interactions have been the subject of many recent studies. In many cases, cell surface carbohydrates mediate the pathogen-host interaction via single or multiple interactions. Therefore, prevention of adhesion at an early stage should block the disease and carbohydrate analogs, as anti-adhesive agents would be effective in protecting hosts from infection and disease.

Green tea made from *Camellia sinensis* (Theaceae family) is a widely consumed beverage which provides a dietary source of biologically active compounds considered to be beneficial to human health. Green tea extract contains polyphenols, tannin and caffeine, and catechins have been most widely studied to prove the pharmacological action of green tea. They have demonstrated significant antioxidant, anticarcinogenic, anti-inflammatory, thermogenic, probiotic, and antimicrobial properties (Balentine et al., 1997; Roedig-Penman et al., 1997). Given that *C. sinensis* is consumed mainly as a hot water extract of fresh green shoots, however, other water-soluble components of *C. sinensis* have not been extensively investigated.

Water-soluble polysaccharides from green tea have been found to possess immunological, antiradiation, antiblood coagulation, anticancer, antiHIV, antioxidant, and hypoglycemic qualities (Dongfeng et al., 2001). Recently, an acidic polysaccharide isolated from *C. sinensis* leaves has shown remarkable inhibitory effects against the adherence of *Helicobacter pylori* to human gastric epithelial cells and *Propionibacterium acnes* and *Staphylococcus aureus* to NIH3T3 cells including erythrocytes (Lee et al., 2006). Since the purification yield of the polysaccharide was very low, less than 0.012% on average, the extracts were instead prepared from *C. sinensis* by hot water extraction, ethanol precipitation, enzyme treatment, and charcoal filtration. The objective of the present study was to evaluate the anti-adhesive role of the tea extracts against the pathogen adhesion to human or mouse cell lines, including beneficial or commensal bacteria.

**MATERIALS AND METHODS**

**Bacterial cell growth.** *H. pylori* (ATCC 43504 and 49503) cells were grown in Brucella broth (Difco, Sparks, MO, USA) containing 10% (v/v) fetal bovine serum (FBS), 0.2% (w/v) 2,6-di-o-methyl-β-cyclodextrin (CD). The cells were incubated in a 10% CO2 atmosphere at 37 °C for 2 days. The cultured bacteria were identified by urease and catalase reactions, and confirmed by polymerase chain reaction based on *VagA*, *CagI*, *Ic eA1*, *IceA2*, *BabA*, and *UreA* primers (Lee et al., 2004). *P. gingivalis* (ATCC 33277) and *A. actinomycetemcomitans*
(ATCC 29522) were grown for 2 days in trypticase soy broth (Difco) supplemented with 0.6% yeast extract at 37 °C under a condition of 80% N₂, 10% H₂ and 10% CO₂. In case of *P. gingivalis*, hemin (10 mL/L) and vitamin K3 (0.2 mL/L) were included. *P. acnes* (ATCC 6919) was grown for 2 days in brain-heart infusion (Difco) at 37 °C in an atmosphere of 80% N₂, 10% H₂ and 10% CO₂. *S. aureus* (ATCC 27217) and *Staphylococcus epidermidis* (ATCC 12228) were grown in brain-heart infusion (Difco) at 37 °C. *Escherichia coli* strains, BL21(DE3) and BL21 (DE3)pLysS from Novagen (Madison, U.S.A.), were cultured on Luria-Bertani (LB) agar plates. All bacterial cell stocks were maintained with 20% (w/v) glycerol in a liquid nitrogen tank. *Lactobacillus acidophilus* (ATCC 4356) and *Bifidobacterium bifidum* (ATCC 29521) were purchased from the Korean Culture Center of Microorganisms (Seoul, Korea).

**Mammalian cell growth.** AGS (ATCC CRL 1739), a human gastric adenocarcinoma epithelial cell line), KB (ATCC CRL 17; a human adenocarcinoma oral cell line) and NIH 3T3 cells (ATCC CRL-1658, a mouse embryo fibroblast epithelial cell line) were grown to confluence in tissue culture flasks (100 mm) in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% (v/v) FBS and 1% (v/v) antibiotic-antimycotics in a 5% CO₂ incubator at 37 °C for 2–3 days.

**Preparation of green tea extracts.** The tea leaves (50 g) were homogenized and extracted with hot distilled water (500 mL, 90 °C) for 3 h. After high-speed centrifugation and filtration for the removal of any insoluble materials, the supernatant (CSI-1) was precipitated using ethanol (final 70% concentration) at 4 °C. The precipitate (CSI-2) was dried and dissolved in PBS (100 mM sodium phosphate, pH 7.4, and 150 mM NaCl containing 10 mM MgCl₂ and 1 mM CaCl₂, and then incubated for 3 h with RNase and DNase at 37 °C. The enzyme reactions were stopped by heating at 100 °C for 10 min. After centrifugation, supernatant CSI-3 was concentrated by using rotary evaporator, and passed through the activated charcoal-packed column to remove the color substance of the extract. The pass-through eluent was precipitated by cold ethanol (final 70% concentration) and lyophilized (CSI-4).

**General methods.** Total carbohydrates, uronic acid, protein contents, and catechin were determined via the phenol-sulfuric acid (Dubois et al., 1956), carbazole (Chaplin, 1986), Bradford (Bradford, 1976), and vanillin spectrophotometric method (Moon et al., 2003), respectively, using glucose, galacturonic acid, bovine serum albumin, and commercial catechin as the respective standards. Carbohydrate compositions of CSI-4 were analyzed at the Carbohydrate Research Center of Sejong University (Seoul, Korea). Arabinose, altrose, galactose, glucose, fructose, xylose, mannose, rhamnose, tagatose, glucuronic acid, and galacturonic acid were used as reference sugars. In order to obtain monosaccharide, N-acetyl glycosyl glycerol was used for acid hydrolysis with 4 M trifluoroacetic acid at 100 °C for 4 h and the sample was washed three times with distilled water. After filtering with 0.45 μm filter, it was applied to high-performance anion exchange chromatography-pulsed amperometric detection (HPAEC-PAD) BioLC System (Dionex, Sunnyvale, CA, USA) using CarboPac PA1 column, and eluted with 18 mM NaOH for neutral sugars and 100 mM NaOH and 150 mM sodium acetate for uronic acids at a flow rate of 1 mL/min. The mole % was calculated for each of the samples.

**Hemagglutination assays.** Human erythrocytes in 10 mL PBS were incubated with trypsin (2 mg) at 37 °C for 3 h, washed three times, and suspended at 2% (w/v) in PBS. Equal volumes of two-fold dilutions of the bacterial suspensions with isolated extracts were mixed and incubated at room temperature for 30 min. Each mixture was then added to O-type blood samples in U-shaped 96-microwell plates. The extent of the inhibition of hemagglutination was visualized by microscopic inspection. Aspartic and glutamic acids were tested as non-specific reactions, and exhibited no inhibitory activities. Erythrocytes in the absence and presence of bacterial suspension were used as negative and positive controls, respectively (Molin, 2001).

**Anti-adhesion assay by urea phenol red method.** AGS cells (100 μL) were seeded in 96-microwell plates (Corning, Cambridge, MA, USA) at a density of 5 × 10⁴ cells/mL (Lee et al., 2006). After achieving confluence in 2 days, the confluent monolayers on the plates were washed three times with PBS. Non-specific binding was blocked by incubation with 0.5% BSA, prior to two rinses in PBS. 50 μL of *H. pylori* suspensions (OD₅₂₀nm = 1.0; 1 × 10⁶ cells/mL) were incubated with green tea extracts at various concentrations for 30 min. The bacteria-inhibitor mixture (100 μL) was added to the AGS cells and incubated for 1 h. 0.03% phenol red in 2% urea solution (pH 5.2) was added and absorbance was measured at 560 nm with a microplate reader. The percentage of attached *H. pylori* cells was calculated as follows: attached % = 100 − [(ODnegative)/(ODpositive − ODnegative) × 100]. The negative control contained only the AGS cells and the positive control contained the epithelial cells and bacteria, which were used to establish 100% attachment. For non-specific binding, FITC-labeled *E. coli* and AGS cells were incubated in the dark for 1 h. After washing three times with carbonate buffer (pH 6.0), adhesion was examined fluorometrically by monitoring the emission spectrum of FITC-labeled *E. coli* bound to AGS at 520 nm with excitation at 480 nm.

**Anti-adhesion assay by colony counting method.** KB and NIH3T3 cells were seeded at 1 × 10⁴ cells/mL in DMEM containing 10% FBS and 1% antibiotics in 96-microwell plates (Lee et al., 2006). After achieving confluence in 2 days, the confluent monolayers on the plates were washed three times with PBS. Various concentrations of tea extracts were incubated with bacteria (1 × 10⁴ cfu/mL) for 30 min. A 100 μL aliquot of each mixture was added to host cells, incubated at 37 °C for 30–60 min. After treatment of the cells with PBS containing 0.1% Triton X-100, the recovered cell extracts were serially diluted with PBS and plated on 5% sheep blood agar. After incubation for several days, colonies were counted visually or by using stereomicroscope. The activity to inhibit bacteria from binding to KB or NIH3T3 cells was estimated as follows: attached % = (cfu of bound bacteria in the presence of polysaccharide/cfu of bound bacteria in the absence of polysaccharide) × 100.
Hemolysis and cytotoxicity assays. Samples of blood suspension were treated with the selected extract concentrations in 96-well microtiter plates and centrifuged at 1000×g for 5 min. Hemoglobin release was monitored spectrophotometrically at 414 nm. Percent hemolysis was calculated as follow: % hemolysis = (Abs_{Sample} - Abs_{PBS})/(Abs_{GluconX-100} - Abs_{PBS}) × 100, where % 0% and 100% hemolysis were determined in PBS and 0.1% Triton X100, respectively. Cell viability was measured by quantitative colorimetric assay with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). AGS, KB, and NIH 3T3 cells were treated with green tea extracts for 24 h and MTT was added to each solution (final 0.5 mg/mL). After incubation for 2 h, a solubilization solution was added and the absorbance was measured at 595 nm (Maher and McClean, 2006).

Statistics. Statistical analysis was performed by a paired t test using the SPSS (v12.0) program. Differences were considered significant at p < 0.05.

RESULTS

Characterization of green tea extracts. Our new procedure provided green tea extracts from CSI-1 to CSI-4 with significantly high yields (Table 1) as a different procedure from the one reported previously (Lee et al., 2006). The recovery of CSI-4 from the tea leaves was found to be 4% which is more than 300-fold increase over that of anti-adhesive polysaccharide CS-F2 from green tea leaves (0.012%). Only a trace amount of proteins was found and catechin was absent in CSI-4, suggesting that both catechins and green tea pigments could be effectively and simultaneously removed by activated charcoal. Neither protein nor catechin was detected in the acidic polysaccharide CS-F2 (Lee et al., 2006). As the purification proceeds, the proportion of uronic acids in the extracts increased.

Carbohydrate analyses of CSI-4 indicated that it contains high amounts of arabinose, galactose and rhamnose as neutral sugars (Table 2). Interestingly, galacturonic acid was the highest (38.2%) in sugar composition, contributing to the high amounts of uronic acids, whereas glucuronic acid was found to be less than 2.0%. It was previously shown that the polysaccharides from the roots of Panax ginseng and the leaves of mugwort contain high amounts of galactose, arabinose and uronic acids (Lee et al., 2004), which are pectin-type polysaccharides.

Table 1. General characteristics of CSI from C. sinensis

<table>
<thead>
<tr>
<th>Green tea extracts</th>
<th>Purification yielda (Gram %)</th>
<th>Total sugara (µg/mg)</th>
<th>Uronic acidb (µg/mg)</th>
<th>Proteinb (µg/ml)</th>
<th>Catechinb (µg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSI-1</td>
<td>13.4 (26.7)</td>
<td>569.6</td>
<td>223.5</td>
<td>46.9</td>
<td>51.3</td>
</tr>
<tr>
<td>CSI-2</td>
<td>3.2 (6.3)</td>
<td>359.0</td>
<td>251.7</td>
<td>87.7</td>
<td>27.8</td>
</tr>
<tr>
<td>CSI-3</td>
<td>3.0 (6.0)</td>
<td>196.4</td>
<td>221.4</td>
<td>52.4</td>
<td>16.3</td>
</tr>
<tr>
<td>CSI-4</td>
<td>2.0 (4.0)</td>
<td>204.2</td>
<td>373.2</td>
<td>9.7</td>
<td>0</td>
</tr>
<tr>
<td>CS-F2</td>
<td>0.006 (0.012)</td>
<td>177.1</td>
<td>457.1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

a The purification yield was calculated from 50 g of green tea leaves.

b CSI-4 contains a trace amount of fucose, mannose, and xylose, which are less than 0.1%.

c CS-F2 is a polysaccharide purified from green tea leaves (Lee et al., 2006).

Table 2. Sugar compositions of CSI-4 from C. sinensis

<table>
<thead>
<tr>
<th>Sugar composition</th>
<th>mole %a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhamnose</td>
<td>9.6</td>
</tr>
<tr>
<td>Arabinose</td>
<td>25.3</td>
</tr>
<tr>
<td>Galactose</td>
<td>20.2</td>
</tr>
<tr>
<td>Glucose</td>
<td>5.2</td>
</tr>
<tr>
<td>Fucose, mannose, xyloseb</td>
<td>-</td>
</tr>
<tr>
<td>Galacturonic acid</td>
<td>38.2</td>
</tr>
<tr>
<td>Glucuronic acid</td>
<td>1.5</td>
</tr>
</tbody>
</table>

a Mole % of total carbohydrate content.
b CSI-4 contains a trace amount of fucose, mannose, and xylose, which are less than 0.1%.

Green tea extracts inhibit pathogen-mediated hemagglutination. CSI-4 exhibited strong inhibitory activity on hemagglutination mediated by H. pylori, P. gingivalis, P. acnes, and S. aureus, with minimum inhibitory concentration (MIC) of 0.01–0.5 mg/mL (Table 3 and Fig. 1), but no inhibitory activity against A. actinomycetemcomitans. It was previously reported that the polysaccharide CS-F2 inhibited the attachment of H. pylori, P. acnes, and S. aureus to erythrocytes, at concentrations of 0.01–0.1 mg/mL (Lee et al., 2006). Our data suggest that CSI-4 demonstrates strong anti-adhesive activities against these pathogens comparable to those of CS-F2, in addition to P. gingivalis (Table 3). Catechin was examined to show weak activity against the skin pathogens with MIC values of 1.0 mg/mL, and little activity against other pathogens (Fig. 1).

Notably, CSI-3 was found to have potent anti-adhesive activities, similar to those of CSI-4, but it showed the activity against E. coli with an MIC of 0.5 mg/mL (Table 3). CSI-1 and CSI-2 did not exhibit any activities against the pathogens even at concentrations of 2.0 mg/mL, and rather showed anti-adhesive activities against commensal bacteria E. coli, and S. epidermidis. In case of CSI-1, E. coli was blocked even at a very low concentration of 0.05 mg/mL.

CSI-4 inhibits pathogen adhesion to host cells. With regard to its ability to inhibit the adhesion of H. pylori to human AGS gastric epithelial cells, a significant inhibition by CSI-4 was observed with a 20–80% reduction of H. pylori attachment at concentrations between 0.25 and 2.0 mg/mL (Fig. 2A). Moreover, when CSI-4 was added to the AGS cell pre-incubated with H. pylori, it also revealed an inhibitory activity.
reaching a 10% reduction of the pathogen attachment at 2 mg/mL. Although it was less effective to detach the bacterial cell which had already attached to the host cell, our results demonstrate that CSI-4 could detach *H. pylori* from AGS cells and the effect was clearly dose-dependent at concentrations ranging from 0.125 to 2 mg/mL (Fig. 2B).

CSI-4 was found to reduce the attachment of *P. gingivalis* to human KB cells by ca. 20% at 1.0 mg/mL, confirming the result from the hemagglutination assay (Fig. 3A). However, CS-F2 was previously reported to show no activity against *P. gingivalis* (Lee et al., 2006). Both CSI-4 and CS-F2 did not show any inhibition against *A. actinomycetemcomitans* (data not shown).

50% inhibition by CSI-4 against the adhesion of *P. acnes* to NIH 3T3 fibroblast cells was observed at a concentration of 0.14 mg/mL (Table 3). The IC_{50} value (50% inhibition of adhesion) against *P. acnes* was the lowest among the pathogens examined by CSI-4 which further reduced the adhesion of the pathogen by ca. 80% at 0.25 mg/mL (Fig. 3B). The attachment of *S. aureus* to NIH3T3 cells was significantly reduced to ca. 40% at 1.0 mg/mL (Fig. 3C). Our results suggest that CSI-4 has very strong anti-adhesive activity on the adhesion of skin pathogens to host cells.

### Effects of CSI-4 on erythrocytes and host cells.

CSI-4 did not induce the hemolysis of erythrocytes at concentrations between 0.125 and 2 mg/mL (data not shown), and did not significantly influence on cell viability of AGS, KB, and NIH3T3 cells at concentrations between 0.125 and 2 mg/mL (Fig. 4). However, CSI-1 and CSI-2 caused the hemolysis up to 45% at 1–2 mg/mL (data not shown).

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**Figure 1.** Micrograph images of hemagglutination inhibition by CSI-4 and catechin (magnification × 100). A positive control (PC) for each bacterium is shown in left column. The anti-adhesive activities of CSI-4 and catechin are shown for each bacterium at their minimum inhibitory concentrations.
Table 3. Inhibition of bacteria-induced hemagglutination and adhesion to host cells by green tea extracts

<table>
<thead>
<tr>
<th>Inhibitors (Mw Da)</th>
<th>Minimum inhibitory concentration (mg/mL) a</th>
<th>Red blood cells</th>
<th>H. p b</th>
<th>P. g</th>
<th>A. a</th>
<th>P. a</th>
<th>S. a</th>
<th>S. e</th>
<th>E. c</th>
<th>L. a</th>
<th>B. b</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSI-1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.1</td>
<td>0.05</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CSI-2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.1</td>
<td>1.0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CSI-3</td>
<td>0.05</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.01</td>
<td>0.5</td>
<td>–</td>
<td>0.5</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CSI-4</td>
<td>0.01</td>
<td>0.05</td>
<td>–</td>
<td>–</td>
<td>0.05</td>
<td>0.5</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CS-F2c (80,000)</td>
<td>0.01</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.05</td>
<td>0.1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Catechin (442.4)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1.0</td>
<td>1.0</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>–</td>
</tr>
</tbody>
</table>

Inhibitors IC 50 values (50% inhibition of adhesion)

<table>
<thead>
<tr>
<th>Mammalian cells d</th>
<th>AGS</th>
<th>KB</th>
<th>KB</th>
<th>NIH3T3</th>
<th>NIH3T3</th>
<th>NIH3T3</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSI-4</td>
<td>1.26</td>
<td>2.3</td>
<td>–</td>
<td>0.14</td>
<td>0.83</td>
<td>–</td>
</tr>
<tr>
<td>CS-F2c</td>
<td>1.10</td>
<td>–</td>
<td>–</td>
<td>1.35</td>
<td>1.71</td>
<td>–</td>
</tr>
</tbody>
</table>

a The minimum inhibitory concentrations (MIC) in mg/mL were used in hemagglutination assays, while IC 50 values at which 50% inhibition of adhesion occurs were used in host cell adhesion assays. All the values correspond to the average in triplicates. ‘–’ represents no inhibition at high concentrations above 2.0 mg/mL. Aspartic and glutamic acids were used as non-carbohydrate acidic compounds, which did not cause non-specific reaction (data not shown). The green tea extracts have no influence on cell viability of erythrocytes and host cell lines below 2.0 mg/mL.


c The results of CS-F2 against bacteria are adopted from Lee et al. (2006).

d The results are obtained using the urea phenol red or visible colony-counting method.

**DISCUSSION**

An aqueous extract of green tea leaves has been a popular beverage in Asia for centuries and provides a dietary source of biologically active compounds considered beneficial to human health (Kohlmeier et al., 1997). It has a naturally high content of polyphenols including catechins that make up approximately 30–45% of the solid green tea leaves (Higdon and Frei, 2003). It was observed that the incidence of infection with H. pylori was lower in subjects who consumed tea regularly (Koo and Cho, 2004). Similar results were reported in animal studies confirming an anti-H. pylori effect of the tea and the active principles were demonstrated to be the tea catechins (Matsubara et al., 2003). Many studies have showed that the risk of stomach cancer decreases with the quantities of tea consumed (Koo and Cho, 2004). The mechanisms may involve the inhibition of the growth of H. pylori (Graham et al., 1992). We previously reported that the acidic polysaccharide CS-F2 from green tea leaves, with extremely low yield (merely 0.012%), has high inhibitory properties against the adherence of H. pylori to AGS gastric cells and of P. acnes, and S. aureus to NIH 3T3 fibroblast cells (Lee et al., 2006). An aqueous extract CSI-4 prepared by hot water extraction, ethanol precipitation, enzyme treatment, and charcoal filtration was found to make up approximately 4% of the green tea leaves and show strong anti-adhesive activities against the pathogens similar to those of CS-F2.

Notably, CSI-3 also has potent anti-adhesive activities, similar to that of CSI-4, but showed the inhibitory activity against a commensal bacterium E. coli. CSI-1 and CSI-2 showed anti-adhesive activities against more commensal or beneficial bacteria than CSI-3 or CSI-4. The anti-adhesive activities of the tea extracts demonstrated significantly more potent activities against the pathogens and became less effective against beneficial...
bacteria, as the purification process progresses, together with the increase in the proportion of acidic carbohydrates, such as uronic acids. Citrus pectin, a high uronic acid polysaccharide, exerts a strong anti-adhesive effect against P. gingivalis, P. acnes, and S. aureus without affecting commensal or beneficial bacteria (Lee et al., 2004). Negatively charged polysaccharides, such as dextran sulfate, also inhibit the adhesion of respiratory pathogens, including Pseudomonas aeruginosa, Burkholderia cenocepacia, B. pseudomallei, Legionella pneumophila, Bacillus anthracis, and Yersinia pestis (Thomas and Brooks, 2004).

Pathogens have a broad spectrum of different specificities in adhesion to host cells and a number of cell surface carbohydrates that mediate pathogen-cell interactions have been discovered (Sharon, 2006). In particular, the Lewis b blood group carbohydrate antigen has been demonstrated to mediate the adherence of H. pylori to human gastric mucosa, and the formation of biofilm from the fibrin fibers and glycocalyx is an important first step in the adhesion of S. aureus to the skin (Ilver et al., 1998; Krautgartner et al., 2005). The acidic polysaccharide CS-F2 inhibits the H. pylori-mediated hemagglutination (0.12 μM), much higher than sialyllactose (ca. 1.1 mM) (Lee et al., 2004). The tea extract CSI-4 was proved to inhibit the adhesion of H. pylori, P. acnes, and S. aureus to host cell lines as highly as CS-F2. In addition, CSI-4 can be a nontoxic substance without detrimental effects on cell viability of host cells and showed additional anti-adhesive activity against P. gingivalis, a major etiological pathogen in advanced adult periodontitis (Haffajee and Socransky, 2000), which was not observed in CS-F2. The basis for the difference in specificity to this oral pathogen is yet to be determined. Among all commensals and pathogens examined in this study, CSI-4 exhibited dramatically reduced adhesion of P. acnes to NIH3T3 fibroblast cells, reaching approximately 80–90% inhibition at concentrations of 0.25 mg/mL or above, indicating that the anti-adhesive activity of CSI-4 was the highest against P. acnes.

Bacteria that are attached to host cells are very difficult to dislodge. CSI-4 demonstrated to be able to detach H. pylori that had adhered to AGS human gastric epithelial cells in a concentration-dependent manner. In this context, CSI-4 could play a role as an analog of glycoconjugates on the cell surface and further dislodge the attached bacteria from the host cell. It was reported that sialyllactose not only inhibited H. pylori binding to duodenum-derived human cells, but also can detach bacteria that were already bound to cells via their surface lectin (Simon et al., 1997). Soluble carbohydrates can block adhesion of the bacteria to a variety of host cells, and ligand-coated bacteria would decrease the host cell adhesion. Several inhibitory oligosaccharides, which are common constituents of human milk, play a protective role against different infectious agents in breast-fed babies (Newburg et al., 2005). Our results are strongly suggestive that green
tea extract CSI-4 has excellent selective anti-adhesive activity against pathogens without adverse effect on beneficial bacteria. As anti-adhesion therapy may become feasible for treatment of infectious disease, herbal extracts as a promising therapeutic approach can be developed as potent inhibitors of bacterial adhesion to protect hosts against infection by pathogenic bacteria.

Acknowledgments

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