Suppression of Helicobacter pylori-induced gastritis by green tea extract in Mongolian gerbils

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Received 4 September 2003

Abstract

Since urease of Helicobacter pylori is essential for its colonization, we focused attention on foodstuffs which inhibit the activity of this enzyme. Among plant-derived 77 foodstuff samples tested, some tea and rosemary extracts were found to clearly inhibit Helicobacter pylori urease in vitro. In particular, green tea extract (GTE) showed the strongest inhibition of Helicobacter pylori urease, with an IC50 value of 13 μg/ml. Active principles were identified to be catechins, the hydroxyl group of 5-position appearing important for urease inhibition. Furthermore, when Helicobacter pylori-inoculated Mongolian gerbils were given GTE in drinking water at the concentrations of 500, 1000, and 2000 ppm for 6 weeks, gastritis and the prevalence of Helicobacter pylori-infected animals were suppressed in a dose-dependent manner. Since the acquisition by Helicobacter pylori of resistance to antibiotics has become a serious problem, tea and tea catechins may be very safe resources to control Helicobacter pylori-associated gastroduodenal diseases.

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Keywords: Helicobacter pylori; Green tea; Mongolian gerbil; Catechins; Gastritis; Urease inhibition

It is known that Helicobacter pylori infection is associated with upper gastrointestinal diseases, such as peptic and duodenal ulcers [1–3], as well as gastric cancer development [4–6]. Therefore the number of Helicobacter pylori carriers undergoing eradication therapy to prevent gastric disorders is increasing. A recent trend in therapeutic regimens for Helicobacter pylori eradication is adoption of a triple therapy with a proton pump inhibitor and two antimicrobials, amoxicillin, and clarithromycin [7]. However, the occurrence of strains resistant to clarithromycin has given rise to concern, and this problem might be of particular importance in areas where many people are infected with Helicobacter pylori, for example in Asian countries [8]. The current prevalence of clarithromycin-resistant Helicobacter pylori isolates in Japan is approximately 20% [9] and this would be expected to rapidly increase with wider use of eradication therapy. Therefore, it is important to search for non-antibiotic substances, which are both highly effective and safe in terms of gastrointestinal protection from Helicobacter pylori-derived diseases.

Urease, which constitutes 5–10% of the bacterial protein, is the most characteristic feature of Helicobacter pylori. This enzyme catalyzes the hydrolysis of urea to produce ammonia and carbon dioxide, and the most crucial role is to protect the bacteria in the acidic environment of the stomach [10]. It has been also reported that ammonia and monochloramine, which is a reaction product of ammonia and hypochlorous acid, exhibit potent toxicity in gastric epithelium [11,12]. Moreover, it has been demonstrated that Helicobacter pylori lacking urease activity are incapable of causing infection in animal models [13–15]. Thus, it is most likely that urease is essential for bacterial colonization and perhaps the pathogenesis of related disease in vivo.

Mongolian gerbils can be readily colonized by Helicobacter pylori, with associated development of chronic gastritis, gastric ulcers, and intestinal metaplasia after prolonged infection [16,17]. In this animal model, urease...
inhibitors, such as acetohydroxamic acid and fluoroac- 

In the present study, we examined the ability of plant- 

desalted through Sephadex G-25 column (PD-10 columns, Amersham–
to show the strongest effects, 

The influence of GTE on *H. pylori*-induced gastritis was also 

Materials and methods 

**Materials.** GTE (Polyphenon 70S, decaffeinated), containing 

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**Preparation of samples for screening.** The plant-derived foodstuff 

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<table>
<thead>
<tr>
<th>Foodstuffs</th>
<th>Foodstuffs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetables (28)</td>
<td>Asparagus, broccoli, burdock, cabbage, carrot, celery, Chinese yam, cucumber, eggplant, fang feng, Japanese turnip*, lettuce, magnolia bark, mitsuba leaf, onion, oriental celery, parsley, perilla, potato, radish, rava, shallot, spinach, sweet pepper, sweet potato, taro, tomato, watercress</td>
</tr>
<tr>
<td>Mushrooms (5)</td>
<td>Bunashimeji mushroom, cardoncello mushroom, enokitake mushroom, maitake mushroom, shitake mushroom</td>
</tr>
<tr>
<td>Seeds (6)</td>
<td>Almond, cashew, Chinese wolfberry, ginkgo, sesame, walnut</td>
</tr>
<tr>
<td>Beans (7)</td>
<td>Azuki bean, cassia, cowpea, Kentucky wonder bean, soybean, tora bean, wild lignonberry</td>
</tr>
<tr>
<td>Spices (26)</td>
<td>Angelica, Arabian jasmine, black mustard, caraway, chapli kabab, chilli pepper, cinnamon, clove, fennel, fenugreek, gambir, garden cress, gardenia, garlic, ginger, greater galangal, Japanese ginger, laurel, lemongrass, moringa, nutmeg, pepper, rosemary, rue, star anise, turmeric</td>
</tr>
<tr>
<td>Tea (5)</td>
<td>Black tea, green tea, jasmine tea, oolong tea, pu-erh tea</td>
</tr>
</tbody>
</table>

The foodstuffs samples for screening were extracted with 50% methanol at room temperature for 7 days. Figure in parentheses indicate the number of foodstuffs.

* Both leaves and root were tested.

**Urease inhibition assay.** Urease inhibition was assessed using jack 

Effects of GTE on *H. pylori*-induced gastritis in Mongolian gerbils. Specific pathogen-free male Mongolian gerbils (CLEA Japan, Tokyo, Japan), 6 weeks old, were housed in an air-conditioned biobehavioral room with a 12 h light-dark cycle. The animals were handled according to the guidelines of the Committee for Ethics of Animal Experimentation in National Cancer Center, Tokyo. They were fed a commercial diet (CE-2 powder; CLEA Japan) and water ad libitum until the start of the experiment. At 7-week-old, each animal was fasted for 24 h and then *H. pylori* culture was orally inoculated by gavage (0.5 ml, 1.7 × 10^8 CFU/animal, n = 25 for each group). Control animals without infection were given sterilized broth alone (n = 10 for each group). After inoculation, each animal was kept without food and drink for 24 h and then given a basal diet. Experimental groups received water supplemented with GTE (0, 500, 1000, or 2000 ppm for the *H. pylori*-inoculated groups and 0 or 2000 ppm for the uninoculated groups) from 3 days before *H. pylori* inoculation until the end of the experimental period. Body weights, and diet and drink intake were measured once, twice, and three times a week, respectively, and animals were monitored daily for their general health.

At 6 weeks after the inoculation of *H. pylori*, all animals were sacrificed under ether euthanasia and their stomachs were resected, opened along the greater curvature, and washed twice with saline. Then, macroscopically observed gastric lesions (edema and hemor-
To determine the level of gastritis, a microscopic score, varying from 0 to 7, was used as a measure of the level of gastritis. The significance of differences in quantitative data for gastric lesions and H. pylori infection was analyzed by Fisher’s exact test. Other data were examined using Dunnett’s multiple test.

**Results**

**Urease inhibitory activities of foodstuffs and constituents**

When samples of 50% methanol extracts prepared from 77 plant-derived foodstuffs were assayed for inhibitory activity using jack bean and H. pylori ureases, some tea samples (green tea, oolong tea, jasmine tea, and black tea) and rosemary extracts were shown to inhibit both enzymes. GTE showed the strongest inhibition of H. pylori urease with an IC50 value of 13 μg/ml. Corresponding values were 20 μg/ml for rosemary, 39 μg/ml for oolong tea and jasmine tea, and 56 μg/ml for black tea.

Since tea leaves contain various kinds of catechins, the urease inhibitory activities of individual forms were also assessed. Epigallocatechin gallate, gallocatechin gallate, gallocatechin, and epigallocatechin strongly inhibited H. pylori urease activity with IC50 values of 2.2–19.6 μM (Table 2). These catechins have a hydroxyl group at the 5′-position in the B-ring. Other catechins, lacking this feature, showed only weak inhibitory activity, with IC50 values of 226–861 μM.

**Suppression of H. pylori-induced gastritis by GTE in Mongolian gerbils**

In the in vivo experiment, GTE administration did not affect food intake and body weights, but water intake was reduced as follows (g/day/animal): from 12.0 for the H. pylori-inoculated control group to 11.0, 10.9, and 9.9 with 500, 1000, and 2000 ppm GTE groups, respectively.

The results for the effects of GTE on H. pylori-induced gastritis in Mongolian gerbils are shown in Table 3. In the control group given water alone, 56% of animals inoculated with H. pylori were infected and developed severe gastritis with edema and hemorrhage in the glandular stomach. Microscopic erosion with infiltration, featuring many polymorphonuclear leukocytes and lymphocytes, was also observed in all infected animals. Gastric changes were severe in the pyloric region, but moderate in the fundic region. The average microscopic score for gastritis of the H. pylori-inoculated control animals was 3.7, while that without H. pylori inoculation was 0. The average stomach weight of control gerbils inoculated with H. pylori was approximately 1.5-fold of that for animals without inoculation (Fig. 1).

Proportions of animals with bacterial infection were 32%, 36%, and 16% for the 500, 1000, and 2000 ppm GTE groups, and those with both edema and hemorrhage were reduced from 56% for the H. pylori-inoculated control group to 32%, 28%, and 16% for the 500, 1000, and 2000 ppm GTE groups, respectively. The reduction was statistically significant at 2000 ppm (p < 0.05). The microscopic scores for H. pylori-inoculated animals were also reduced by GTE treatment in a dose-dependent manner (Table 2) along with the average stomach weights (p < 0.05 and p < 0.01, respectively, of 500 and 2000 ppm) (Fig. 1). Regarding average numbers of viable bacteria (log [CFU/stomach]) obtained from H. pylori-infected stomach samples in the

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**Table 2**

<table>
<thead>
<tr>
<th>IC50 (μM)</th>
<th>(-)Epigallocatechin gallate</th>
<th>(+)Gallocatechin gallate</th>
<th>(+)Gallocatechin</th>
<th>(-)Epigallocatechin</th>
<th>(-)Epicatechin gallate</th>
<th>(+)Epicatechin</th>
<th>(+)Epicatechin gallate</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.2</td>
<td>9.8</td>
<td>8.7</td>
<td>19.6</td>
<td>226</td>
<td>689</td>
<td>452</td>
<td>861</td>
</tr>
</tbody>
</table>

IC50 values were calculated from the urease inhibition rate of eight doses, 0.128, 0.64, 3.2, 16, 80, 400, 2000, and 10,000 μg/ml in duplicate.

**Table 3**

<table>
<thead>
<tr>
<th>Green tea extract (ppm)</th>
<th>No. of animals</th>
<th>H. pylori infection (%)</th>
<th>Edema (%)</th>
<th>Hemorrhage (%)</th>
<th>Microscopic scorea</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>25</td>
<td>14/25 (56)</td>
<td>14/25 (56)</td>
<td>14/25 (56)</td>
<td>3.7 ± 3.3</td>
</tr>
<tr>
<td>500</td>
<td>25</td>
<td>8/25 (32)</td>
<td>8/25 (32)</td>
<td>8/25 (32)</td>
<td>2.0 ± 3.0</td>
</tr>
<tr>
<td>1000</td>
<td>25</td>
<td>9/25 (36)</td>
<td>7/25 (28)</td>
<td>7/25 (28)</td>
<td>1.9 ± 2.9</td>
</tr>
<tr>
<td>2000</td>
<td>25</td>
<td>4/25 (16)b</td>
<td>4/25 (16)b</td>
<td>4/25 (16)b</td>
<td>1.0 ± 2.5b</td>
</tr>
</tbody>
</table>

a Data are means ± SD values.

b Significantly different (p < 0.05) from the H. pylori-inoculated control value.
four groups, there were no significant intergroup differences, values being $5.5 \pm 0.5$ for the H. pylori-inoculated control group, and $5.1 \pm 0.8$, $5.4 \pm 0.7$, and $5.5 \pm 0.7$, for 500, 1000, and 2000 ppm GTE groups, respectively. GTE treatment at a dose of 2000 ppm in uninoculated animals did not affect the stomach weight or microscopic score.

Discussion

In the present study, we found green tea to have strong inhibitory activity (IC$_{50}$: $13 \mu g/ml$) on H. pylori urease in vitro. Tea catechins were considered to be the active components and GTE clearly suppressed the H. pylori-induced gastric lesions in vivo on administration at doses of 500–2000 ppm in drinking water throughout the experiment in Mongolian gerbils. It has been demonstrated that administration of GTE in the diet at doses of 5000–20,000 ppm, higher than those used in the present study, reduced H. pylori-induced gastric injury in the same model [20]. However, the suppression by GTE was not dose-dependent and the mechanisms were not fully clarified.

Among tea catechins, epigallocatechin gallate, gallo-
icatechin gallate, gallo-
catechin, and epigallocatechin were found to strongly inhibit urease activity. Based on the amounts of catechins in GTE, they could account for most of its urease inhibitory activity. This might also be true for the other tea samples, black tea, jasmine tea, and oolong tea, which similarly showed urease inhibitory activity. Epigallocatechin gallate, gallo-
catechin, and epigallocatechin, with a hydroxyl group at the 5'-position in the B-ring, showed 35–104-fold the urease inhibition compared with the catechins without the 5'-hydroxyl group. We considered the antibacterial [20], antioxidant potential [21,22], as well as hydrophobicity [23] of the tea catechins, but no good relationship between these three parameters and effects on urease was found. Thus, it is not known yet why the marked elevation of urease inhibitory activity is demonstrated by the presence of the 5'-hydroxyl group.

Helicobacter pylori is a unique bacteria that can sur-
vive in the acidic environment of the animal stomach because H. pylori can neutralize gastric acids with ammonia produced by urease. We reported previously that chemical urease inhibitors, such as acetohydroxamic acid and flurofamide, can suppress H. pylori-induced gastritis [18]. In the present study, epigallocatechin gallate, which is the main component of GTE, was shown to possess a lower IC$_{50}$ value for H. pylori urease than acetohydrox-
amic acid in vitro (20 $\mu M$). From our present observations, urease inhibition, leading to prevention of H. pylori infection or eradication, may be one of the mechanisms of suppression by GTE in H. pylori-induced gastritis in Mongolian gerbils. Other biological activities, including antioxidant activity, may also be involved.

Green tea is traditionally drunk in several Asian countries, including Japan and China. The general tea catechin concentration is estimated to be approximately 500–1500 ppm and such concentrations are similar to the doses which suppressed H. pylori-induced gastritis in Mongolian gerbils in the present study. It is well known that H. pylori infection causes chronic atrophic gastritis, a risk factor for stomach cancer, and green tea con-
sumption has been epidemiologically shown to reduce the risk of chronic atrophic gastritis [24,25]. Furthermore, some data from case-control studies indicate that stomach cancer risk is decreased by green tea con-
sumption [26,27], although a prospective study demon-
strated no benefit with consumption of up to five cups of green tea per day [28,29]. Thus, more studies are needed to obtain conclusive data regarding the relationship between green tea consumption and gastric cancer risk.

In conclusion, in the present study, GTE strongly in-
hibited H. pylori urease activity and suppressed H. pylori-
induced gastritis in Mongolian gerbils. Therefore, tea and
tea catechins may have the potential as very safe resources to control H. pylori-associated gastroduodenal disease.

Acknowledgments

We thank Naoki Uchiya, Mika Kawamura, and Yurika Teramoto for their expert technical assistance. This work was supported in part by Grants-in-Aid for Cancer Research and for the Second Term Comprehensive 10 Year Strategy for Cancer Control, from the Ministry of Health, Labor and Welfare, Japan, and also a grant from the Yakult Bio-science Foundation.
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