Leaf Extract of *Wasabia japonica* Relieved Oxidative Stress Induced by *Helicobacter pylori* Infection and Stress Loading in Mongolian Gerbils

Hirotaka Sekiguchi,1,3 Fumiyo Takabayashi,2 Yuya Deguchi,1,4 Hideki Masuda,1 Tomoyasu Toyoizumi,1,5 Shuichi Masuda,1,1 and Naohide Kinai1

1Department of Food and Nutritional Sciences, Graduate School of Nutritional and Environmental Sciences, and Global COE program, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan
2University of Shizuoka, Junior College, 2-2-1 Oshika, Suruga-ku, Shizuoka 422-8021, Japan
3Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan
4Faculty of Pharmaceutical Sciences, Nagasaki International University, 2825-7 Huis Ten Bosch-cho, Sasebo, Nagasaki 859-3298, Japan
5Laboratory of Genetics, Division of Toxicology, Hatano Research Institute, Food and Drug Safety Center, 729-5 Ochiai, Hadano, Kanagawa 257-8523, Japan

Received December 10, 2009; Accepted March 17, 2010; Online Publication, June 7, 2010 [doi:10.1271/bbb.90919]

Infection with *Helicobacter pylori* (*H. pylori*) can induce gastric disorders, and though its presence cannot explain disease pathogenesis and does not have associations with other factors, it is well known that *H. pylori* infection causes stomach inflammation following oxidative stress. We examined the suppressive effects of a leaf extract of *Wasabia japonica* on *H. pylori* infection and on stress loading in Mongolian gerbils. Following oral administration of wasabi extract of 50 and 200 mg/kg B.W./d for 10 d, the animals were exposed to restraint stress for 90 and 270 min. As for the results, the level of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) in the stomach and oxidative DNA damage in peripheral erythrocytes at 270 min significantly increased. That elevation was significantly suppressed by the addition of the leaf extract. We concluded that the simultaneous loading of *H. pylori* infection and physical stress loading might induce oxidative DNA damage additively, while a leaf extract attenuated this DNA damage in the stomach as well as the peripheral erythrocytes.

Key words: *Helicobacter pylori*; Mongolian gerbils; 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG); oxidative DNA damage; *Wasabia japonica*

*Helicobacter pylori* (*H. pylori*), which infects over half of all people in the world, is one of the most widespread human pathogens in diseases such as chronic gastritis and gastric and duodenal ulcers.1,2 In 1994, the organism was classified into group I, carcinogenic to humans, by the World Health Organization/International Agency for Research on Cancer (WHO/IARC).3 Although triple therapy using two antibiotics (amoxicillin and clarithromycin) and a proton pump inhibitor is widely employed in the treatment of *H. pylori*, antibiotic resistance to clarithromycin leads to treatment failure, especially in Asian countries.4,5

Adhesion of *H. pylori* to gastric epithelial cells is recognized as one of the essential steps in the development of gastritis, which leads to injection of the definitive virulence factor, cytotoxin-associated antigen A (CagA), through type-IV secretion systems.6,7 Several reports have suggested that *H. pylori* inulates CagA into gastric epithelial cells inducing phosphorylations of MEK, Src, and SHP-2, thereby promoting the production of IL-8, IL-1β, and tumor necrosis factor-α (TNF-α).8 These cytokines play many crucial roles in *H. pylori*-associated gastritis through recruitment, activation, and infiltration of neutrophils into the sites of infection as well as chronic inflammation and gastric injury.9-11 Reactive oxygen and nitrogen species generated by activated inflammatory cells upon infection can contribute to carcinogenesis through the formation of DNA base lesions, such as 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG), a marker of oxidative DNA damage, resulting from G:C-to-T:A transversion.12 In addition, 8-oxodG is remarkably increased in the gastric epithelium of patients infected with *H. pylori*.13

Since the development of *H. pylori*-associated diseases is influenced by a complicated cross talk among the bacteria, the infected host, and the environmental situation, the presence of *H. pylori* itself does not explain fully pathogenesis.14-18 This is reflected by the fact that many people do not develop *H. pylori* associated diseases, and that *H. pylori*-negative gastric ulcer patients are often found. Hence other factors, such as stress, diet, smoking, sanitation, and host genetic background might contribute to the pathogenesis of *H. pylori* associated diseases. Especially with respect to these factors, psychological stress has been found to trigger many diseases.19-24 A study done immediately after the great Hanshin Earthquake in 1995 in Kobe,
Japan, found that the recurrence rate of gastric ulcers in patients infected with *H. pylori* was much higher than that in patients in whom *H. pylori* had been eradicated.25,26 Hence we hypothesized that a synergic relationship between *H. pylori* infection and psychological stress on ulcer formation might exist.

Wasabi (*Wasabia japonica*) is used as a Japanese traditional spice to avoid both food poisoning and odor. Allyl isothiocyanate is a major pungent component of wasabi root, and is known to have strong anti-microbial activity.27,28 Recently, other compounds such as polyphenols in wasabi leaves have been identified that exhibit anti-oxidative and anti-*H. pylori* activities.29–31

It is widely accepted that the Mongolian gerbil is a good model for *H. pylori* infection, because *H. pylori* can easily be inoculated as compared with mice, rats, and others.32,33 In this study, we examined the effects of a leaf extract of *Wasabia japonica* (Wasabi extract) on oxidative DNA damage induced by *H. pylori* infection and on stress loading in Mongolian gerbils.

**Materials and Methods**

**Animals.** Male Mongolian gerbils (13 weeks old, MGS/Sea) infected with *H. pylori* (ATCC45504) were purchased from Seac Yoshitomi (Fukuoka, Japan).12,33 Thirteen week old male Mongolian gerbils (MON/Jms/Gbs) were purchased from Japan SLC (Shizuoka, Japan). The animals were housed in cages at a temperature 23 ± 2°C and humidity 55 ± 5% under a 12 h light and dark cycle, and were given Oriental MF9 sterilized with γ-rays from Oriental Yeast (Tokyo) and water ad libitum throughout the experiment. The animals were handled according to the guidelines of the Committee for Ethics in Animal Experimentation of the University of Shizuoka.

*Leaf extract of Wasabia japonica.** Leaves of *Wasabia japonica* were kindly provided by Tamaru-ya Honten (Shizuoka, Japan), and extracts were prepared by a method described previously.29,30 Briefly, fresh leaves (9.0 kg) were extracted with 20 liters MeOH 3 times at room temperature. After concentration of the solvent with a rotary evaporator in vacuo, the concentrate (273.2 g) was suspended in water and treated successively with hexane, EtOAc, and n-BuOH to divided it into hexane, EtOAc-, n-BuOH- and water-soluble layers. In this study, a mixture of EtOAc and n-BuOH-soluble layers, which exhibited antioxidative activity as previously described,29,30 suspended in distilled water was used as Wasabi extract.

**Experimental methods.** Following a pre-feeding period of 1 week, infected and non-infected Mongolian gerbils were divided into four experimental groups by Wasabi extract doses: untreated, 10, 50, and 200 mg/kg B.W./d. The Wasabi extract was administered orally following a 6-h fast every day continuously for 10 days. After the dosing period, the animals of all experimental groups, following a 24-h fast, were exposed to restraint stress for 0, 90, or 270 min. After treatment, the animals were sacrificed, and then the stomach and whole blood were obtained immediately. Then the stomach was opened along the greater curvature, and the intragastric contents were removed gently. To count colony forming units (CFU), half of each stomach was cut finely, homogenized in 7 ml of sterilized saline, followed by serial dilution with the same saline. Aliquots of the diluted homogenate (0.1 ml) were inoculated onto the *Helicobacter* agar plate (Nissui Pharmaceutical, Tokyo). The plates were incubated at 37°C under microaerophilic conditions for 5 d, and then the colonies were counted to detect the CFU. The degrees of gastric mucosal erosion and hemorrhage were determined by scoring the following parameters: gastric mucosal erosion (0, normal; 1, edematous; 2, erosion; 3, multiple erosion; 4, hemorrhage erosion and/or ulcers larger than 1 mm in diameter); hemorrhage (0, no bleeding; 1, one small bleeding spot; 2, multiple small bleeding spots; 3, one bleeding area; 4, multiple bleeding area).

---

**Table 1.** Experimental Groups

<table>
<thead>
<tr>
<th>H. pylori</th>
<th>Wasabi extract (mg/kg B.W./d)</th>
<th>Restraint time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>-</td>
<td>Group 1</td>
<td>Group 5</td>
</tr>
<tr>
<td>10</td>
<td>Group 2</td>
<td>—</td>
</tr>
<tr>
<td>50</td>
<td>Group 3</td>
<td>Group 6</td>
</tr>
<tr>
<td>100</td>
<td>Group 4</td>
<td>Group 7</td>
</tr>
<tr>
<td>0</td>
<td>Group H1</td>
<td>Group H5</td>
</tr>
<tr>
<td>10</td>
<td>Group H2</td>
<td>—</td>
</tr>
<tr>
<td>+</td>
<td>Group H3</td>
<td>Group H6</td>
</tr>
<tr>
<td>100</td>
<td>Group H4</td>
<td>Group H7</td>
</tr>
</tbody>
</table>

---

Quantification of the level of 8-oxodG. Nuclear DNA isolation was carried out as previously described.34 Each stomach was homogenized in an ice-cold 0.3 M Sarcosol solution. The crude pellets were incubated with proteinase K and 1% SDS/1 mM EDTA (pH 8.0) at 37°C for 90 min. The solution was mixed with 7 M NaI and isopropyl alcohol, and left to stand at −20°C for 10 min. The pellet DNA, was centrifuged and rinsed with 70% ethanol, Ribonucleases T1 and A were added to the withdrawn crude DNA, and the reaction mixture was incubated at 37°C for 1 h. Then chloroform: isomyl alcohol (24:1, v/v) was added, and the resulting aqueous phase was mixed with 13% PEG solution w/v containing 1.6 M NaCl. The DNA obtained was dissolved in water, and hydrolyzed with nuclease P1 at 37°C for 30 min, and then with alkaline phosphatase at 37°C for 1 h. The filtrated DNA was applied to a HPLC (LC-10 pump, Shimadzu, Kyoto, Japan) equipped with a Symmetry C18 column (particle size, 3.5 mm; 4.6 x 100 mm; Waters, Milford, MA). The mobile phase was 12.5 mM citrate buffer (pH 5.1) containing 6% methanol, and the flow rate was 0.8 ml/min. 8-oxodG was measured by electrochemical detection (ECD; ESA Coulochem II 5200, Bedford, MA) using an analytical cell model 5011 (Detector I, 150 mV; Detector II, 350 mV). Oxidative damage to DNA was expressed as the molar ratio of 8-oxodG to 102 g-deoxyguanosine (dG). The amount of dG was calculated from the absorption at 260 nm in the same sample as measured with a UV detector.

**Comet assay to detect Fpg-sensitive site.** Comet assay applied to detect formamidopyrimidine DNA glycosylase (Fpg)-sensitive sites was carried out basically as previously described, with some modification.35,36 Fpg has N-glycosylase and AP-lyase activities and repairs oxidative DNA damage by efficiently removing formamidopyrimidine lesions and 8-oxodG residues from DNA.37 The slide preparation was based on our previous studies.35,36 The slides were immersed in lysis solution (2.5 M NaCl, 0.2 M NaOH, 0.1 M EDTA, 0.01 M Tris base 1%, 10% DMSO and 1% Triton X-100, (pH 10)) at 4°C for 1 h. Treatment with Fpg (Sigma-Aldrich, St. Louis, MO) was carried out as follows: the cells, embedded in agarose, were overlaid with Fpg (1 mg/ml) or Fpg buffer (0.1 M NaCl, 1 mM EDTA, 0.01 M Tris base pH 7.5 and 100 mg/ml BSA) at 37°C for 15 min. The slides were placed in alkaline electrophoresis buffer (0.3 M NaOH and 1 mM EDTA). Following electrophoresis, they were washed with neutralizing Tris buffer (0.4 M Tris base pH 7.5) and stained with ethidium bromide solution. Comets in each slide were analyzed using a CCD camera and the Comet Analyzer (YOU WORKS, Tokyo). DNA strand breaks and Fpg-sensitive sites in the DNA of peripheral blood cells were represented by tail moment for 50 comets from one blood sample.

**Statistical analysis.** Data were expressed as mean ± SD or SE. Statistical significance at the same restraint stress time was evaluated by Tukey’s multiple comparison test after one-way ANOVA. Differences were considered significant at p < 0.05.

**Results**

**Improvement in lesions of the stomach induced by *H. pylori* infection and/or restraint stress by the leaf extract of *Wasabia japonica*.**

In the present study, administration of wasabi extract did not affect food intake, water intake, or body weight (data not shown). The effects of wasabi extract on...
stomach weight and CFU are shown in Table 2. After administration of wasabi extract for 10d, the average stomach weight markedly increased, by 2.0-fold, in group H1 (H. pylori infection) as compared with group 1 (no infection). The elevation was significantly less in groups H2 and H3 (10 and 50 mg/kg B.W./d during the experiment), although in group H3 (100 mg/kg) was not effective. On the other hand, the wasabi extract did not cause a reduction in H. pylori colonization. The macroscopic findings for gastric mucosa of the animals are shown in Table 3. The gastric mucosal erosion score significantly increased in group H1 (15-fold, H. pylori infection) and group 8 (9.0-fold, no infection, 270 min stress loading) as compared with group 1 (no infection). A decrease in the erosion score was observed only in group H9 (H. pylori infection, 270 min stress, 50 mg/kg) as compared to that of group H8 (H. pylori infection, 270 min stress). The hemorrhage score significantly increased in group H1 (H. pylori infection) and group 8 (270 min stress loading) as compared with group 1 (no infection). The score for group H9 (H. pylori infection, 270 min stress, 50 mg/kg) showed a 47% decrease as compared with group H8 (H. pylori infection, 270 min stress).

### Suppressive effects of leaf extract of Wasabia japonica on the level of 8-oxodG in the stomach

The concentrations of 8-oxodG in the stomach are shown in Table 4. Compared with group 1 (no infection), group H1 (H. pylori infection), and group 8 (270 min stress loading), they showed 2.4 and 2.7-fold increases in the level of 8-oxodG. Group H3 (H. pylori infection, 50 mg/kg) exhibited a 34% decrease in 8-oxodG as compared with group H1 (H. pylori infection). 50 and 100 mg/kg Wasabi extract (groups 9 and 10) decreased the level of 8-oxodG by 32–41% as compared with group 8 (no infection, 270 min stress). Group H8 (H. pylori infection, 270 min stress) showed a significant 1.4-fold increase as compared with group H1 (H. pylori infection), but the leaf extract decreased the levels of 8-oxodG by 41–48% (groups H9 and 10).

### Suppressive effects of the leaf extract of the Wasabia japonica on oxidative DNA damage in whole blood

The levels of the DNA strand break and the Fpg-sensitive site of the whole blood are shown in Table 5. Oxidative DNA damage in group H1 (H. pylori infection) showed a 4.6-fold increase as compared with group 1 (no infection), with statistical significance, but the leaf extract decreased by 27% the oxidative DNA damage (group H3 and H4). Oxidative damage significantly increased in group H8 (H. pylori infection, 270 min stress) as compared with group H1 (H. pylori infection). Furthermore, oxidative damage showed a 1.3-fold increase under H. pylori infection and 270 min stress loading (group H8) as compared with group H1 (H. pylori infection), but the leaf extract suppressed the damage by 26–33% (groups H9 and H10).

### Discussion

Previous reports have indicated the influence of H. pylori infection on the development of stress-induced gastric mucosal injury in animal models and humans.26,30 Despite exhibiting the close relationship between H. pylori infection and stress, as described...
above, few trials for prevention, especially in phytochemicals, has been yet to be reported. In this study, development of edema, erosions, ulcers, and hemorrhage spots in the gastric mucosa were observed after infection for several months (Table 2), but the mechanism and causal factor contributing to aggravation of gastric injury induced by physiological stress in a presence of H. pylori remain unknown. We suggested that oxidative stress was responsible for H. pylori and stress-associated gastric injury, since treatment by an antioxidant potently prevented H. pylori and stress-induced oxidative DNA damage.

In this study, we found no effect on H. pylori colonization under treatment with wasabi extract for 10d. Adhesion of H. pylori to gastric epithelial cells is one of the initial steps in gastric inflammation. Recent studies have provided evidence that chronic inflammation that follows oxidative stress caused by H. pylori infection plays a critical role in the development of gastric cancer.\(^{39}\) Ohshima \textit{et al.} reported that 8-oxodG and 8-nitroguanine as markers of DNA damage were stored in patients infected with H. pylori.\(^{33}\) Although in gastritis and gastric ulcer patients infected with H. pylori NADPH oxidase and inducible nitric oxide synthase (iNOS) expression as an effect of infiltrating inflammatory cells has been observed, eradication of H. pylori attributed to decrease these cells invasion and iNOS expression in the gastric mucosa.\(^{40,41}\) In the present study, wasabi extract did not effect H. pylori colonization, but oxidative DNA damage was decreased in the stomach and whole blood (Tables 4 and 5). In this context, a previous report suggested that antioxidant \(\alpha\)-tocopherol protects against gastric injury, although the presence of H. pylori caused significant deterioration of stress-induced gastric mucosal lesions as a result of increasing oxidative stress.\(^{42}\) In sum, antioxidant treatment decreased the risk of gastric injury due to oxidative stress.

Our previous study indicated that wasabi extract showed anti-oxidative and anti-H. pylori activities \textit{in vitro} and stronger activity than the root extract.\(^{31}\) In this study, we found wasabi extract to decrease the level of oxidative stress caused by both H. pylori infection and stress loading in Mongolian gerbils. Although major components of the root are isothiocyanates (allyl, 6-methylsulfinylhexyl, and other isothiocyanates), isothiocyanates in the leaf accounted for one-third as compared with the root.\(^{28}\) These data indicate that other components were associated with these activities. In this context, recently Hosoya \textit{et al.} reported that wasabi extract consisted of flavonoids and its glycosides, such as apigenine, luteolin, and isovitexin (the major component).\(^{29,30}\) Isovitexin is one of the antioxidants involved in natural products such as rice hulls and is known for various biological activities, for example, anti-oxidative and anti-inflammatory effects.\(^{33,44}\) These effects contributed to the scavenging superoxide anions, inhibited NO production, and freed of transcriptional control of cyclooxygenase (COX)-2, a mediator of inflammation. On the other hand, we have reported that

---

### Table 4. Effects of Leaf Extract of \textit{Wasabia japonica} on Amounts of 8-oxodG in Gastric Mucosa with and without H. pylori Infection

<table>
<thead>
<tr>
<th>H. pylori</th>
<th>Wasabi extract (mg/kg B.W./d)</th>
<th>Restraint time (min)</th>
<th>0</th>
<th>90</th>
<th>270</th>
<th>Two-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R, W, R × W</td>
</tr>
<tr>
<td>−</td>
<td>0</td>
<td>0.329 ± 0.038(^{b})</td>
<td></td>
<td></td>
<td>0.880 ± 0.142(^{b})</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.329 ± 0.020(^{a})</td>
<td></td>
<td>0.333 ± 0.019(^{b})</td>
<td>0.595 ± 0.157(^{b})</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.429 ± 0.058(^{a})</td>
<td></td>
<td>0.496 ± 0.102(^{a})</td>
<td>0.509 ± 0.148(^{b})</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>0</td>
<td>0.807 ± 0.051(^{b})</td>
<td></td>
<td>0.897 ± 0.004(^{b})</td>
<td>1.148 ± 0.029(^{b})</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.535 ± 0.051(^{b})</td>
<td></td>
<td>0.519 ± 0.031(^{b})</td>
<td>0.676 ± 0.029(^{b})</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.589 ± 0.038(^{b})</td>
<td></td>
<td>0.666 ± 0.021(^{b})</td>
<td>0.599 ± 0.091(^{b})</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD. Two-way ANOVA was separately done with and without H. pylori. Statistical significance at the same restraint stress time was evaluated by Tukey’s multiple comparison test after one-way ANOVA. Means without a common letter differ, \( p < 0.05\). R, restraint time; W, Wasabi extract.

### Table 5. Effects of Leaf Extract of \textit{Wasabia japonica} on Fpg-Sensitive Sites in DNA of Whole Blood with and without H. pylori Infection

<table>
<thead>
<tr>
<th>H. pylori</th>
<th>Wasabi extract (mg/kg B.W./d)</th>
<th>Restraint time (min)</th>
<th>0</th>
<th>90</th>
<th>270</th>
<th>Two-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R, W, R × W</td>
</tr>
<tr>
<td>Fpg (−)</td>
<td>−</td>
<td>1.02 ± 0.06(^{b})</td>
<td>0.86 ± 0.05(^{b})</td>
<td>1.45 ± 0.12(^{b})</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.70 ± 0.05(^{a})</td>
<td>0.85 ± 0.05(^{b})</td>
<td>0.88 ± 0.05(^{b})</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.70 ± 0.04(^{a})</td>
<td>0.70 ± 0.04(^{a})</td>
<td>1.16 ± 0.07(^{b})</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1.46 ± 0.20(^{a})</td>
<td>1.38 ± 0.10(^{a})</td>
<td>1.57 ± 0.16(^{a})</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>1.38 ± 0.16(^{a})</td>
<td>1.78 ± 0.09(^{a})</td>
<td>1.77 ± 0.11(^{a})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fpg (+)</td>
<td>−</td>
<td>1.40 ± 0.08(^{b})</td>
<td>1.63 ± 0.11(^{b})</td>
<td>2.98 ± 0.21(^{b})</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.97 ± 0.06(^{b})</td>
<td>1.30 ± 0.11(^{b})</td>
<td>1.85 ± 0.15(^{b})</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>1.27 ± 0.09(^{b})</td>
<td>1.46 ± 0.14(^{b})</td>
<td>1.94 ± 0.16(^{b})</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>6.38 ± 0.47(^{a})</td>
<td>6.90 ± 0.49(^{a})</td>
<td>8.18 ± 0.63(^{a})</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>4.68 ± 0.35(^{b})</td>
<td>5.45 ± 0.41(^{b})</td>
<td>6.01 ± 0.40(^{b})</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>4.58 ± 0.35(^{b})</td>
<td>5.30 ± 0.37(^{b})</td>
<td>5.44 ± 0.29(^{b})</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fifty cells were used per Mongolian gerbil. Mean values were obtained for 250 cells. The values are expressed as mean ± SEM. Two-way ANOVA was separately done with and without H. pylori. Statistical significance at the same restraint stress time was evaluated by Tukey’s multiple comparison test after one-way ANOVA. Means without a common letter differ, \( p < 0.05\). R, restraint time; W, Wasabi extract.
a leaf extract of *Wasabia japonica* inhibited the urease activity of *H. pylori*.\(^2\) Urease accounts for 5–10% of bacterial whole protein and is expressed in most if not all *H. pylori* strains.\(^3\) This enzyme catalyzes the hydrolysis of urea into ammonia and carbon dioxide, and its most important role is to protect the bacteria from the acidic conditions of the stomach by neutralization.\(^4\) Therefore, *H. pylori* urease is considered to be essential for bacterial colonization. Furthermore, ammonia produced by urease of *H. pylori* reacts with hypochlorous acid (HOCl) to generate monochloramine (NH₂Cl), stronger in DNA damage potency than HOCl.\(^4,5\) In other words, the leaf extract partly suppresses not only the stability of *H. pylori* colonization inhibiting urease activity, but also oxidative DNA damage.

Eradication therapy against *H. pylori* is fairly successful in many cases, although sometimes treatment failure occurs due to an antibiotic resistant strain.\(^6\) Therefore it is important to seek a non-antibiotic therapy that is not only highly effective but also non-harmful to humans, and to examine the suppression of chronic gastritis in high-risk areas including Japan. In conclusion, our data suggest that it is likely to be able to suppress oxidative DNA damage derived from reactive oxygen and nitrogen species induced by *H. pylori* infection and stress loading by treatment with antioxidants.

**Acknowledgments**

We are grateful to Professor Akira Kunugi and Dr. Takahiro Hosoya (School of Life Science, Tokyo University of Pharmacy and Life Sciences) for helpful suggestions, and to Dr. Satoko Akiyama (Graduate School of Agriculture, Kyoto University) for her advice on statistics. This work was supported by the Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (to S. M. and N. K.) and by the program of Innovative Technology and Advanced Research in the Environmental Area (CITY AREA) of the Ministry of Education, Culture, Sports, Science, and Technology of Japan (to F. T., S. M., and N. K.).

**References**