Antioxidant and Anti-inflammatory Activities of Several Commonly Used Spices

Tzung-Hsun Tsai, Po-Jung Tsai, and Su-Chen Ho

Abstract: Antioxidant and anti-inflammatory activities are extensively used to screen chemopreventive foods. Five well-known anticancer spices, ginger, red pepper, garlic, green onion, and leek, were selected and assessed in this study. Antioxidant function was determined based on the scavenging ability of the cation radical ABTS+. Moreover, anti-inflammatory activity was determined based on the inhibitory effect of nitric oxide (NO) production by lipopolysaccharide (LPS)-activated macrophages. The antioxidant activity of the 5 spices followed this order: garlic > ginger > red pepper > leek > green onion. All of these spices had a strict inhibitory effect on NO production. The anti-inflammatory activity could be ranked based on the IC50 of the spices, as garlic > ginger > green onion > leek and red pepper. Additionally, a significant correlation existed between antioxidant activity and total phenolics content. Obviously, total phenolics content was a crucial determinant of the antioxidant but not the anti-inflammatory activity of foods. The compounds responsible for the anti-inflammatory activity should differ from those responsible for the antioxidant activity.

Keywords: antioxidation, anti-inflammation, ginger, red pepper, Allium vegetables

Introduction

Several epidemiological studies reveal that certain dietary elements play a pivotal role in the etiology and prevention of several types of human cancers. People who consume much plant-derived foods such as fruits, vegetables, and soybeans were found to have a lower incidence of cancer (Steinmetz and Potter 1991; Ziegler 1991; Block and others 1992). Recently, evaluating plant foods and identifying phytochemicals with the capacity to interfere with carcinogenesis have received considerable attention. As a multi-stage process, carcinogenesis includes at least 3 processes: initiation, promotion, and progression. It is well established that oxidative insults to DNA can lead to mutations in crucial genes, which is involved in the initiation process of carcinogenesis. Furthermore, oxidation and inflammation are well recognized to be closely linked to the process of promotion. Consequently, determination of anti-oxidation and anti-inflammatory functions has been proposed to be a good indicator for screening or evaluating anticancer agents.

A well-balanced level of NO, produced by nitric oxide synthase (NOS), has been shown to be an important regulator of various physiological processes, such as vasodilation, neurotransmission, and host defense (Mayer and Hemmens 1997). Three NOS isoforms have been identified in cells. Both neural NOS and endothelial NOS are constitutive, whereas inducible NOS (iNOS) is inducible in response to various stimuli. Following induction, iNOS can be expressed quantitatively in various cells, such as macrophages, smooth muscle cells, and hepatocytes. Once NO is formed in the cell, it can react with superoxide anions and form peroxynitrite, the potent oxidizing and nitrating molecule. These reactive nitrogen species damage cellular macromolecules, such as proteins, DNA, and lipids, trigger several disadvantageous cellular responses, and cause diseases. Excess NO production by iNOS during chronic infection and inflammation has been implicated in cancer development. (Szabo 1995; MacMicking and others 1997; Wheeler and Bernard 1999). Hence, inducible nitric oxide synthase has been recognized as a molecular target of chemopreventive and anti-inflammatory agents.

To our knowledge, no putative method has been designed specifically for evaluating the anticancer potential of foods. Antioxidant activity is the assay most commonly used and widely accepted by researchers as an anticancer indicator. However, antioxidant activity cannot fully reflect the anticancer potential of foods. To precisely understand the anticancer potential of foods, it is important to obtain comprehensive and comparative information regarding various antioxidant abilities, such as antimutagenicity, anti-inflammation, and antiproliferation.

Owing to abundant phytochemicals and their culinary use, spices have become an important source of chemopreventive agents in the Orient. Consequently, in this study we selected 5 well-known cancer-protective (Caragay 1992) and commonly used spices in Taiwan: ginger (Zingiber officinale), red pepper (Capsicum annuum), garlic (Allium sativum), green onion (Allium fistulosum), and leek (Allium porrum); then we assessed their antioxidant and anti-inflammatory activities to obtain the comparative information regarding the 2 different anticancer abilities of these spices. Antioxidant function of the spices was evaluated based on their scavenging ability for the radical cation ABTS+. Moreover, we adopted LPS-induced iNOS expression in RAW 264.7 macrophages as an inflammatory model, and we determined the inhibitory activity of the tested sample on NO production to assess the anti-inflammatory ability.

Materials and Methods

Materials

RAW 264.7 cell line was obtained from the American Type Culture Collection (Manassas, Va., U.S.A.). The cells were cultured in DMEM medium supplemented with 10% heat-inactivated fetal
bovine serum (Gibco BRL Life Technologies Inc., Grand Island, N.Y., U.S.A.) and maintained at 37 °C in a humidified incubator containing 5% CO₂. Lipopolysaccharide (LPS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), and BCP/NBT liquid substrate system were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.).

**Extract preparation**

Fresh spices were purchased from a traditional market in Hsinchu City (Hsinchu, Taiwan). The edible portions of the spices were cut into small pieces and blended with a minimal amount of cold double-distilled water. After filtering through several layers of gauze, the filtrates were centrifuged at 12000 × g for 20 min to remove the solid debris. Supernatant then was removed and freeze-dried. Additionally, the residues of aqueous extracts were collected. For the cell culture experiment, weighed extract was dissolved in DMEM and filtered through 0.2-μm-pore-size membrane, and then diluted with DMEM to the target concentration.

**Determination of total phenolic compounds**

The total phenolics content was determined by the following procedure and expressed in terms of milligrams of gallic acid equivalents per gram of aqueous extract. Briefly, spice extract residue was dissolved in 2 mL double-distilled water to a final concentration of 0.5 mg/mL. Folin-Ciocalteu phenol reagent was then added to the sample and held for 3 min. Then 2 mL of 10% (w/v) aqueous sodium carbonate was added and allowed to stand at room temperature for 1 h. The absorbance of the developed color was measured by a spectrophotometer at 765 nm. The total content of phenolic compounds in each spice extract was then determined by a standard curve prepared with gallic acid.

**Evaluation of antioxidant activity**

The total antioxidant capacity of spice extracts was determined by a commercial kit (Randox Laboratories Ltd., Crumlin, U.K.) and expressed in terms of milligrams of Trolox equivalents per gram of aqueous extract. Briefly, ABTS⁺ radical was generated in a reaction buffer (0.5 mM ABTS, 0.7 mM potassium persulfate) and the absorbance was measured at 734 nm. The absorbance of the test sample was measured at the same time. The total antioxidant capacity of each spice extract was then determined following the instructions and calculated based on the suppressive effect related to a 1 mmol/L Trolox standard.

**Determination of NO production.**

To determine NO production, the cells were seeded at a density of 6 × 10⁴ cells per well in 96-well plastic culture dishes. Following a 24-h incubation, the adherent cells were washed 3 times with PBS. The cells were then incubated in 0.1 mL medium with aqueous extract from various spices, with or without 100 ng/mL LPS. Twenty-four hours after incubation, the medium was centrifuged at 1500 × g and 4 °C for 5 min and stored at −70 °C until assay. Moreover, cell viability was evaluated using the MTT method (Mosmann 1983). Finally, medium nitrite concentration was measured as an indicator of NO production by the Griess reaction (Kim and others 1995).

**Evaluation of iNOS enzyme activity**

The cells were cultured in a 10-cm culture dish and stimulated with LPS (100 ng/mL) for 12 h. Subsequently, the cells were harvested and plated in a 24-well culture dish, and then were treated with various spice extracts for a further 12 h. The medium was finally collected and assayed for nitrite.

**Evaluation of iNOS protein expression**

The cells were seeded at a density of 1 × 10⁵ cells per 6-cm culture dish and incubated for 24 h. After 3 washes, the adherent cells were incubated for 12 h in the presence or absence of 100 ng/mL LPS and the indicated concentration of spice aqueous extract. The adherent cells were then washed with PBS, scraped into cold PBS, and centrifuged at 500 × g for 10 min at 4 °C. The cell pellets were then suspended in lysis buffer (50 mM Tris, pH 7.6, 0.01% EDTA, 1% Triton X-100, 1 mM PMSE, and 1 μg/mL leupeptin) and centrifuged at 12000 × g for 20 min at 4 °C. Aliquots of the cell lysate were used for protein concentration assay by a BCA kit (Pierce Co., Rockford, Ill., U.S.A.). Additionally, supernatant iNOS protein levels were determined by Immunoblot analysis. Briefly, 50 μg of supernatant protein was denatured, separated on 10% SDS-polyacrylamide gel, and transferred to PVDF filters using a Holfer transblot cell. Meanwhile, filters were blocked at 4 °C overnight and then probed with primary antibodies (BD Transduction Laboratories, Josesan, Calif., U.S.A.). The filters were then incubated with anti-mouse IgG antibody conjugated to alkaline phosphatase and detected using a NBT/BCIP solution. Finally, the band intensities were quantified with a software-supported photoimager (ImageMaster VDS; Amersham Pharmacia Biotech Co., Piscataway, N.J., U.S.A.).

**Statistical analysis**

All data are presented as mean ± SD for 3 independent tests. The significance of the differences at each sample concentration was analyzed by ANOVA and Duncan's multiple range test using SPSS software. The paired t test was used to evaluate the differences between the treatments and the control, with the significant difference set at P < 0.05 (SPSS for Windows 10.0; SPSS Inc., Chicago, Ill., U.S.A.). The correlation between 2 variants was analyzed by the Pearson test.

**Results and Discussion**

**Total phenolics content and antioxidant activity**

Table 1 lists the total phenolics content and antioxidant capacity of the spice extracts. Aqueous extracts from ginger, red pepper, and leek appeared to contain more phenolic compounds than those from garlic and green onion. The antioxidant activities could be ranked as follows: ginger > red pepper > leek > green onion and garlic. Moreover, a significant correlation (r = 0.95, P < 0.05) was found between phenolics content and antioxidant capacity.

**NO production in unactivated and LPS-activated macrophages**

Analysis of NO production by measuring the nitrite with the Griess reaction revealed that placing unstimulated RAW 264.7 cells in culture medium for 24 h produced a basal amount of nitrite (Figure 1). When the cells were incubated with aqueous extracts from these spices in the absence of LPS, medium nitrite concentration was maintained at a background level similar to that in the unstimulated control (data not shown). After treatment with LPS for 24 h, the medium concentration of nitrite increased markedly compared with the control group (Figure 1). Significant concentration-dependent inhibition of NO production was detected when cells were cotreated with LPS and various concentrations of the 5 spice extracts (Figure 1). The inhibitory potency of spice extracts could be ranked based on their IC₅₀ as follows: garlic (66 μg/mL) > ginger (139 μg/mL) > green onion (213 μg/mL) > leek (254 μg/mL) and red pep-
per (637 μg/mL). To avoid possible cytotoxic effects of spices on NO production, cell viability was determined by MTT assay. The spices were not observed to have significant cytotoxicity under the experimental conditions.

**iNOS catalytic activity**

This study examined whether the inhibitory effects on inducible nitrite production resulted from a direct effect of these spices on intrinsic enzyme activity of iNOS. We selected a dose of 2000 μg/mL, at which the spices exerted the largest inhibitory action on NO production, to examine the effect of the spices on iNOS enzyme activity. At a treated concentration of 2000 μg/mL, all spices except ginger showed a significant inhibitory effect. Red pepper, garlic, red pepper, garlic, red pepper, garlic,

### Table 1—Total phenolics content and antioxidant activities of various spice extracts

<table>
<thead>
<tr>
<th>Spice</th>
<th>Total phenolics (mg GAE/g residue)</th>
<th>Total antioxidant capacity (mmol TE/g residue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ginger</td>
<td>22.5 ± 1.4a</td>
<td>117.1 ± 6.9a</td>
</tr>
<tr>
<td>Red pepper</td>
<td>19.7 ± 1.1b</td>
<td>92.5 ± 1.1b</td>
</tr>
<tr>
<td>Garlic</td>
<td>10.2 ± 0.6 c</td>
<td>4.4 ± 0.5d</td>
</tr>
<tr>
<td>Green onion</td>
<td>11.6 ± 1.2c</td>
<td>5.9 ± 0.6d</td>
</tr>
<tr>
<td>Leek</td>
<td>20.5 ± 1.3ab</td>
<td>63.4 ± 3.9c</td>
</tr>
</tbody>
</table>

Means not sharing a common letter were significantly different (P < 0.05) when analyzed by ANOVA and Duncan's multiple range test.

**Figure 1**—Effect of various spices on the NO production in LPS-activated RAW264.7 cells. The values are expressed as means ± SD of triplicate tests. Means not sharing a common letter were significantly different (P < 0.05) when analyzed by ANOVA and Duncan’s multiple range test.
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Table 2—Effect of various spices on the catalytic activity of iNOS enzyme in RAW 264.7 cells

<table>
<thead>
<tr>
<th>LPS induction</th>
<th>Spice</th>
<th>Nitrite production (μM)</th>
<th>Rate of inhibitiona (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>None</td>
<td>1.8 ± 0.3c</td>
<td></td>
</tr>
<tr>
<td>LPS</td>
<td>None</td>
<td>28.3 ± 1.9b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ginger</td>
<td>23.9 ± 3.5b</td>
<td>16.6</td>
</tr>
<tr>
<td></td>
<td>Red pepper</td>
<td>12.7 ± 2.1bc</td>
<td>58.9</td>
</tr>
<tr>
<td></td>
<td>Green onion</td>
<td>10.4 ± 1.6bc</td>
<td>67.5</td>
</tr>
<tr>
<td></td>
<td>Leek</td>
<td>11.8 ± 1.4bc</td>
<td>62.3</td>
</tr>
</tbody>
</table>

aThe rate of inhibition was calculated as: percentage inhibition = \[1 – \left( \frac{\text{NO sample} + \text{LPS}}{\text{NO control}} \right) / \left( \frac{\text{NO control}}{\text{NO control}} \right) \times 100\%.

As the anti-inflammatory analysis is based on the capacity to suppress NO production in LPS-activated RAW 264.7 cells. The antioxidant activities of the 5 tested spices can be ranked as follows: ginger > red pepper > leek > green onion and garlic. Furthermore, a significant correlation existed between antioxidant activities and phenolics content. This result was consistent with the report of Betancor-Fernandez and others (2003) that used the same antioxidant analytical method. Interestingly, a good correlation was also observed in the studies of Zheng and Wang’s (2001) and Velioğlu and others (1998), which applied the oxygen radical absorbance capacity and β-carotene bleaching method to analyze antioxidant activity, respectively. Phenolic compounds, such as curcumin and gingerol are considered to be the major phytochemicals present in ginger. Meanwhile, capsaicin is assumed to be the active constituent in red pepper (Surh 1999). However, the main bioactive substances present in Allium vegetables, such as garlic, onion, and leek, are organosulfur compounds (Kris-Etherton and others 2002). By determining the inhibitory effect of spice components on the oxidation of low-density lipoprotein, Naidu and Thippeswamy (2002) indicated that phenolic compounds such as curcumin, quercetin, and capsaicin are more effective antioxidants than nonphenolic compound such as allyl sulfide. Furthermore, it has been demonstrated that allicin, accounting for about 65 to 75% of the total organosulfur compounds in garlic, is not fully responsible for the antioxidant activity of Allium plants (Yin and Cheng 1998). Phenolic compounds in dietary plants appear to be the most significant contributor to antioxidant activity.

Using the method of ferric-reducing ability, Halvorsen and others (2002) conducted a large-scale determination of the total antioxidant capacity of dietary plants, and indicated that ginger, red pepper, leek, and garlic have 3.76, 2.46, 0.47, and 0.21 mmol per 100 g fresh weight of antioxidant activity, respectively. The antioxidant rank of the 4 spices tested in our study is accordant with their report.

The potent antioxidant activity of ginger and red pepper shown in this work is consistent with early reports (Halvorsen and others 2002; Chu and others 2002). Several phenolic compounds found in garlic including curcumin and a variety of gingerols, dihydroxyphenylalanoids, and shogaols have been identified to possess substantial antioxidant ability, even exceeding that of vitamin E (Kikuzaki and Nakatani 1993; Bang and others 2001). Besides capsaicin, the abundance of vitamin C, carotenoids, and other phenolic compounds also contribute to the antioxidant capacity of red pepper. Although levels of these phytochemicals are influenced by genetics, growth condition, and maturity (Marin and others 2004), phenolic compounds account for the largest portion of antioxidant activity in red pepper (Chu and others 2002). Recently, complete phenolic constituents, including 5 hydroxycinnamic derivatives and 23 flavonoids, in the pericarp of sweet pepper have been identified and quantified (Marin and others 2004).

Although garlic is a food with strong anticancer potency, fresh garlic extract showed poor antioxidant activity. Betancor-Fernandez and others (2003) applied the same analytical method to observe the same result. Indeed, fresh garlic has been demonstrated to possess little antioxidant activity, and even to have a prooxidant property (Imai and others 1994; Borek 2001). Although garlic lacks direct free radical scavenging ability, it cannot be denied that fresh garlic may exert its protective effects against oxidative stress by enhancing antioxidant enzyme activities and/or by interacting with other endogenous antioxidant molecules. In fact, several in vivo studies have demonstrated that garlic or some organosulfur compounds from Allium plants exert their antioxidantive protection by modulating antioxidant-related enzymes such as catalase, superoxide dismutase, glutathione reductase, and peroxidase (Helen and

Discussion

This investigation applied 2 functional tests as indicators of anticancer potential to evaluate 5 spices that have been identified as having cancer preventive properties. The antioxidant assessment is based on the ability to scavenge the cation radical ABTS+, where.
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The 5 herbs tested in this study showed significant, but varied, suppressive effects on NO production by LPS-activated macrophages. Ginger primarily exerted its inhibitory effect on iNOS expression. Furthermore, red pepper showed a suppressive action only on the catalytic activity of iNOS enzyme. However, Allium vegetables exhibited a suppressive action on the protein level and the catalytic activity of iNOS. Numerous phytochemicals have been identified in these spices as the main suppressors of iNOS expression. These phytochemicals include curcumin and [6]-gingerol in ginger (Chan and others 1995; Ippoushi and others 2003), capsaiacin in red pepper (Kim and others 2003), allin, ajene, and S-Allyl-cysteine in garlic (Dirsch and others 1998; Kim and others 2001) and widely distributed flavonoids, such as apigenin, quercetin, kaempferol, and genistein (Liang and others 1999; Chen and others 2001). However, molecules precipitated in the regulation of iNOS activity are unclear and further studies are needed to explore this issue. Despite the inhibitory mechanism, garlic was the spice with the strongest anti-inflammatory activity among the 5 spices tested. Moreover, this potent anti-inflammatory activity might provide garlic with some of its anticancer properties.

Besides antioxidant and anti-inflammatory, the subject spices have been demonstrated to have several other anticancer actions (Craig 1999; Surh 1999, 2002; Kris-Etherton and others 2002). For instance, garlic can inhibit nitrosamine formation and suppress DNA-adducts formation. Furthermore, ally-sulfides, organosulfur compounds derived from garlic, have been shown to exert their anticarcinogenic effect through inducing detoxification enzymes (Fukao and others 2004). Additionally, garlic is reported to destroy tumor cells by enhancing immune function (Craig 1999; Borek 2001; Kris-Etherton and others 2002). These protective actions provided by various active components interfere with different processes of carcinogenesis, provide different efficacies, and interact in a synergistic or additive fashion to interfere with cancer formation. Therefore, for developing anticancer foods, global evaluation, and promotion of properties of selected spices is needed. Further studies are needed to explore this area.

Conclusions

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