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Antioxidative effects of daikon sprout (*Raphanus sativus* L.) and ginger (*Zingiber officinale* Roscoe) in rats

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Abstract

The antioxidative effects of vegetables are expected to prevent carcinogenesis. The intake of daikon sprout (Japanese name ''kaiware-daikon'', *Raphanus sativus* L.) or ginger (*Zingiber officinale* Roscoe) significantly decreased the concentration of urinary thiobarbituric acid-reactive substances (TBARS) in rats as compared with those before the intake. Moreover, the intake of these vegetables reduced urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels in lipopolysaccharide (LPS)-treated rats as compared with those fed a basal diet only. These results show that these vegetables suppress lipid peroxidation and the formation of malonaldehyde, and protect DNA from LPS-induced oxidative damage in rats. The suppression of lipid peroxidation and oxidative DNA damage in rats by the intake of daikon sprout or ginger indicates that these vegetables have an antioxidative effect in vivo which could be related to the prevention of carcinogenesis.

Keywords: *Raphanus sativus* L.; *Zingiber officinale* Roscoe; Daikon sprout; Ginger; Antioxidative effect; Rat

1. Introduction

Daikon sprout (Japanese name ''kaiware-daikon'') is a widely eaten seedling of daikon (Japanese white radish; *Raphanus sativus* L., cruciferous vegetable) in Japan. A recent study (Takaya, Kondo, Furukawa, & Niwa, 2003) reported that the methanol extract of daikon sprout shows the highest hydroxyl radical scavenging potency using the bleomycin-Fe method among methanol extracts of 11 kinds of commonly available vegetables. Daikon sprout contains several kinds of sinapinic acid esters and flavonoids with high radical scavenging potency.

Isothiocyanates are pungent compounds characteristic of cruciferous vegetables and breakdown products of glucosinolates, an important and unique class of secondary plant compounds found in cruciferous vegetables, by plant and microbial (e.g. gut microflora) myrosinases (Fahey, Zalcmann, & Talalay, 2001). Isothiocyanates inhibit NADPH oxidase-derived superoxide (a precursor of reactive oxygen species) generation in the differentiated HL-60 cells (Miyoshi, Takabayashi, Osawa, & Nakamura, 2004), and suppress nitric oxide (a precursor of reactive nitrogen species) production in LPS-activated J774.1 macrophages (Ippoushi, Itou, Azuma, & Higashio, 2002). Radish (*R. sativus* L.) contains three main glucosinolates: 4-(methylsulfinyl)butyl glucosinolate (glucoraphanin) and 4-(methylsulfinyl)but-3-enyl glucosinolate (glucoraphenin), which are prevalent in the seed, and *trans*-4-(methylthio)-3-butenyl glucosinolate, which is the most important in the root (Visentin, Tava, Iori, & Palmieri, 1992). *Trans*-4-(methylthio)-3-butenyl (main) and phenethyl isothiocyanates derived from their correspondent glucosinolates are identified in daikon (Nakamura et al., 2001). Therefore, daikon sprout containing these glucosinolates may be expected to show an antioxidative effect through the inhibition of superoxide and nitric oxide generation by their isothiocyanates as mentioned above.

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The rhizome of ginger (*Zingiber officinale* Roscoe) is widely consumed as a common spice throughout the world and used in traditional oriental medicine (Lee & Surh, 1998). Its major pungent constituent, [6]-gingerol has been reported to exhibit antioxidative activity against linoleic acid autoxidation and peroxidation of phospholipid liposomes and to scavenge trichloromethylperoxyl- and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals (Aeschbach et al., 1994; Sekiwa, Kubota, & Kobayashi, 2000). In addition to these antioxidative effects, our recent study (Ippoushi, Azuma, Ito, Horie, & Higashio, 2003; Ippoushi, Ito, Horie, & Azuma, 2005) revealed that [6]-gingerol inhibits nitric oxide synthesis in activated J774.1 macrophages and prevents oxidation and nitration reactions induced by peroxynitrite (Radi, Peluffo, Alvarez, Naviliat, & Cayota, 2001), a strong reactive nitrogen species. Besides [6]-gingerol, ginger contains a homologous series of phenolic ketones expected to have an antioxidative effect, known as [4]-, [8]-, [10]- and [12]-gingerols (He, Bernart, Lian, & Lin, 1998).

In order to clarify the antioxidative effects of daikon sprout and ginger in vivo, this study was conducted to assay urinary TBARS (Botsoglou et al., 1994; Lee, Shoeman, & Csallany, 1992) and 8-OHdG (Abu-Qare & Abou-Donia, 2001) levels, oxidative stress markers, in normal or LPS-treated rats fed these vegetables or daikon as a control for daikon sprout in part of the experiment. Furthermore, the uptake of isothiocyanates in rats fed daikon sprout or daikon was evaluated using 1,3-benzodithiole-2-thione determined by cyclocondensation assay (Chung, Jiao, Getahun, & Yu, 1998; Zhang, Wade, Prestera, & Talalay, 1996).

2. Materials and methods

2.1. Materials

1,2-Benzenedithiol, LPS (*Escherichia coli*, O127:B8), propyl isothiocyanate, sodium tetrachloropalladate, and tetramethoxypropane were purchased from Sigma–Aldrich (MO, USA). Butylated hydroxytoluene (BHT), dinitrophenylhydrazine, L-ascorbic acid, and thiourea were purchased from Wako Pure Chemical Industries (Osaka, Japan). Dichlorophenol indophenol was purchased from Merck (Darmstadt, Germany). [6]-Gingerol was purchased from Nacalai Tesque (Kyoto, Japan) and Wako Pure Chemical Industries. Sinigrin was purchased from Extrasynthese (Genay, France). Thiobarbituric acid (TBA) was purchased from Sigma–Aldrich and Wako Pure Chemical Industries. Daikon, daikon sprout and ginger were purchased from a local supermarket. Edible parts of these plants were freeze-dried and milled into a fine powder. In order to identify the peak of 1,3-benzodithiole-2-thione on the HPLC chromatogram for total isothiocyanate determination, 1,3-benzodithiole-2-thione was synthesized and checked by NMR and MS analyses (Zhang, Cho, Posner, & Talalay, 1992).

2.2. Animals

Male Wistar rats were purchased from Japan SLC (Shizuoka, Japan), and kept in an environmentally controlled animal facility operated on a 12-h dark/light cycle at 23 ± 1°C and 55% humidity. They had free access to tap water and standard laboratory rat chow (MF, Oriental Yeast, Tokyo, Japan) prior to use. This study was carried out following the guideline for animal experiments according to Notification No. 6 of the Japanese government.

2.3. Total glucosinolate determination

Total glucosinolate content was determined by palladium test described by Thies (1982). Daikon powder in two tubes (500 mg × 2) or daikon sprout powder in one tube (200 mg) were extracted with 70% aqueous methanol (10 mL) for 20 min at 70 °C. After centrifugation, the pellets were reextracted with 70% aqueous methanol (10 mL) for 20 min at 70 °C, and this procedure was repeated once more. The total extracts were combined and the methanol was removed in vacuo. The residues of daikon or daikon sprout powders were dissolved and filled up to 5 or 10 mL with water, respectively. These solutions (15 μL) were mixed with 588 mg/L sodium tetrachloropalladate solution (300 μL). After 30 min at room temperature, the absorption was measured at 450 nm. The concentration was calculated by comparing the absorbance to a standard curve of sinigrin.

2.4. [6]-Gingerol determination

[6]-Gingerol content was determined by reversed-phase HPLC. Ginger powder (500 mg) was extracted with methanol (20 mL) for 1 h at room temperature. Aliquots of methanol extract were loaded onto a CAPCELL PAK, C18, 250 mm × 4.6 mm column (Shiseido, Tokyo, Japan) at 40 °C. The column was eluted at a flow rate of 1.0 mL/min with the following mobile phase composition: 0–8–17–32–40 min, linear gradient, 45:55–50:50–65:35–100:0–100:0 acetonitrile:water. The eluate was monitored by ultraviolet detection at 280 nm. The concentration was calculated by comparing the absorbance to a standard curve of [6]-gingerol.

2.5. Vitamin C determination

Vitamin C (a sum of L-ascorbic acid and dehydroascorbic acid) content was determined by HPLC after conversion to its dinitrophenylhydrazine derivative. Vegetable powders (250 mg) were extracted with 5% metaphosphoric acid solution, and the supernatants were collected after centrifugation. Then, 0.2% dichlorophenol indophenol solution (20–40 μL), 2% thiourea in 5% metaphosphoric acid solution (2 mL), and 2% dinitrophenylhydrazine in

9 N sulfuric acid solution (0.5 mL) were added to the extracts (2 mL), and the resulting mixtures were incubated for 90 min at 50 °C. After incubation, the reaction product was extracted into ethyl acetate. Aliquots of ethyl acetate extract were subjected to HPLC on a Develosil 60 silica column (250 mm × 4.6 mm; Nomura Chemical, Aichi, Japan) at 40 °C with isocratic mobile phase (40:50:10 *n*-hexane:ethyl acetate:acetic acid) at a flow rate of 1.3 mL/min, and the detection was carried out spectrophotometrically at 495 nm. The concentration was calculated by comparing the absorbance to a standard curve of L-ascorbic acid.

2.6. Evaluation of urinary TBARS level in rats

Six male Wistar rats at 10 weeks of age were fed an AIN-76 basal diet (15 g/day, CLEA Japan, Tokyo, Japan) or an AIN-76 basal diet supplemented with 10% (w/w) daikon (16.5 g/day), 5% daikon sprout (15.75 g/day) or 2% ginger (15.3 g/day) powders for 5 days. Urine from each rat was collected over 24 h before the intake of basal diet or basal diet supplemented with these vegetables and over the final 24 h of the intake. TBARS and creatinine concentrations of the urine samples were determined.

2.7. Evaluation of urinary TBARS and 8-OHdG levels in LPS-treated rats

Six or five male Wistar rats at 10 weeks of age were fed an AIN-76 basal diet (15 g/day) or an AIN-76 basal diet supplemented with 5% daikon sprout (15.75 g/day) or 2% ginger (15.3 g/day) powders for 5 days. Rats were injected intraperitoneally with LPS (10 μg/kg body weight) before the final 24 h of the intake, and the urine was collected over 24 h. TBARS, 8-OHdG and creatinine concentrations of the urine samples were determined.

2.8. Analysis of urinary total isothiocyanate in rats

Three male Wistar rats at 10 weeks of age were fed an AIN-76 basal diet (15 g/day) or an AIN-76 basal diet supplemented with 10% daikon (16.5 g/day) or 5% daikon sprout (15.75 g/day) powders for 2 days. Urine from each rat was collected over the final 24 h of the intake. Total isothiocyanate concentration of urine samples was determined.

2.9. TBARS determination

TBARS concentration was determined as follows. Urine samples (250 μL) were mixed with 11 mg/mL BHT in ethanol (7.6 μL) and TBA reagent (500 μL) consisting of 3.75 mg/mL TBA, 150 mg/mL trichloroacetic acid, 0.4 mg/mL BHT, 0.25 N HCl and 22% ethanol in water. The mixtures were heated at 100 °C for 15 min, cooled to room temperature, and centrifuged. The absorbance of the supernatant was read at 535 nm. The concentration was calculated by comparing the absorbance to a standard curve of tetramethoxypropane.

2.10. Creatinine determination

Creatinine concentration was determined by an assay kit (Sigma–Aldrich) based on modified Jaffe' reaction.

2.11. 8-OHdG determination

8-OHdG concentration was determined by an 8-OHdG ELISA kit (Japan Institute for the Control of Aging, Shizuoka, Japan).

2.12. Total isothiocyanate determination

Total isothiocyanate concentration was determined by the cyclocondensation assay (Chung et al., 1998; Zhang et al., 1996). Urine samples (100 μL) were mixed with 10 mM 1,2-benzenedithiol in 2-propanol (600 μL) and 0.1 M phosphate buffer (pH 8.5, 500 μL), and incubated for 2 h at 65 °C. To analyze 1,3-benzodithiole-2-thione formed from the cyclocondensation reaction, aliquots of these solutions were loaded onto a CAPCELL PAK, C18, 250 mm × 4.6 mm column at 40 °C. The column was eluted at a flow rate of 1.0 mL/min with isocratic mobile phase (70:30 methanol:water). The eluate was monitored by ultraviolet detection at 365 nm. The concentration was calculated by comparing the absorbance to a standard curve of propyl isothiocyanate.

2.13. Statistics

Data are presented as the means ± SD. Statistical analyses were performed by using a two-tailed *t* test.

3. Results

3.1. Total glucosinolate, [6]-gingerol and vitamin C contents

The levels of total glucosinolate, [6]-gingerol and vitamin C in freeze-dried vegetable powders used in this study are shown in Table 1. Total glucosinolate and vitamin C contents in daikon sprout were 23- and 2.4-fold higher than those in daikon, respectively.

Table 1
Total glucosinolate, [6]-gingerol and vitamin C contents in freeze-dried vegetable powders

Powder	Total glucosinolate (μmol/g powder)	[6]-Gingerol (μmol/g powder)	Vitamin C (mg/g powder)
Daikon	6.9 ± 0.15	Not analyzed	1.7 ± 0.15
Daikon sprout	160 ± 8.4	Not analyzed	4.0 ± 0.19
Ginger	Not analyzed	23 ± 0.45	0.29 ± 0.017

Table 2
Effects of daikon, daikon sprout and ginger on urinary TBARS level in rats

Diet[a]	TBARS (nmol/μmol creatinine)[b]		Change (%)	*P* value[c]
	One day before intake	Fifth day under intake		
Control	1.6 ± 0.72	1.6 ± 0.37	0	0.98
Daikon	1.6 ± 0.49	1.6 ± 0.16	0	0.90
Daikon sprout	1.9 ± 0.51	0.8 ± 0.34	−58	0.0069
Ginger	1.4 ± 0.45	1.0 ± 0.18	−29	0.049

[a] Six rats were fed basal diet (control) or basal diet with daikon, daikon sprout or ginger powders.
[b] TBARS concentration is normalized to creatinine concentration.
[c] Based on a paired *t* test.

3.2. Reduction of urinary TBARS level in rats

Urinary TBARS concentrations in rats were not affected by the intake of the AIN-76 basal diet (control) or the AIN-76 basal diet supplemented with 10% daikon for 5 days (Table 2). On the other hand, the intake of the basal diet with 5% daikon sprout or 2% ginger significantly ($P < 0.05$) reduced urinary TBARS concentrations (58% or 29% reduction, respectively).

3.3. Reduction of urinary TBARS and 8-OHdG levels in LPS-treated rats

Next, we investigated the effects of daikon sprout or ginger intake on urinary TBARS and 8-OHdG concentrations in rats intraperitoneally injected with LPS. Urinary TBARS levels of LPS-treated rats fed the basal diet only for 5 days (positive control) were 2.1 ± 0.59 (Fig. 1A) or 1.4 ± 0.28 (Fig. 1B) nmol/μmol creatinine, which showed no significant difference (unpaired *t* test, $P = 0.20$ or 0.82, respectively) from that (1.5 ± 0.98 nmol/μmol creatinine) of LPS-untreated rats fed the basal diet only (normal rats). As shown in Fig. 1A, the intake of the basal diet with 5% daikon sprout significantly decreased TBARS concentration (1.4 ± 0.51 nmol/μmol creatinine) as compared with the positive control. On the other hand, urinary 8-OHdG concentrations were elevated from 6.7 ± 1.4 (normal rats) to 11 ± 4.9 (Fig. 1A, positive control, $P = 0.083$) or 14 ± 2.9 (Fig. 1B, positive control, $P = 0.00053$) pmol/μmol creatinine, indicating that LPS administration induces oxidative DNA damage producing 8-OHdG in rats. The elevated 8-OHdG levels were suppressed by the intake of the basal diet with 5% daikon sprout (Fig. 1A, 6.8 ± 2.1 pmol/μmol creatinine) or 2% ginger (Fig. 1B, 9.3 ± 3.2 pmol/μmol creatinine).

3.4. Amount of urinary total isothiocyanate in rats

Finally, we measured the amount of urinary total isothiocyanate (isothiocyanates or their thiol conjugates) (Chung et al., 1998) in rats fed the basal diet (control) or the basal diet supplemented with 10% daikon or 5% daikon sprout for 2 days (Fig. 2). The amount of urinary total isothiocy-

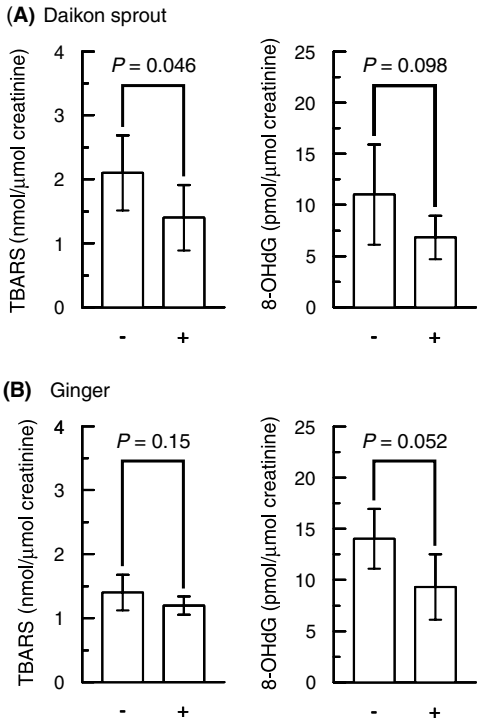

Fig. 1. Effects of daikon sprout (A) and ginger (B) on urinary TBARS and 8-OHdG levels in LPS-treated rats. Rats were fed basal diet (−) or basal diet with daikon sprout or ginger powders (+). TBARS and 8-OHdG concentrations are normalized to creatinine concentration. Data were obtained from six (A) or five (B) rats per group. All *P* values were obtained from unpaired *t* tests. TBARS and 8-OHdG concentrations of LPS-untreated rats ($n = 6$) fed basal diet were 1.5 ± 0.98 nmol/μmol creatinine and 6.7 ± 1.4 pmol/μmol creatinine, respectively.

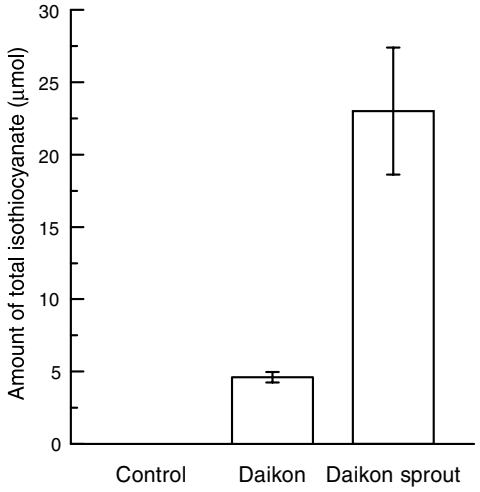

Fig. 2. Amount of urinary total isothiocyanate in rats. Three rats were fed basal diet (control) or basal diet with daikon or daikon sprout for 2 days. Urine from each rat was collected over the final 24 h of the intake. Total isothiocyanate level was determined as concentration of total isothiocyanate equivalent (isothiocyanates or their thiol conjugates) assayed by the cyclocondensation method.

anate in rats fed daikon sprout was 5.1-fold higher than that fed daikon. Total isothiocyanate of the control was not detected.

4. Discussion

Carcinogenesis is a multistage process that consists of at least three different but closely related processes: initiation, promotion and progression. Promotion is closely linked to oxidative and inflammatory tissue damage. Oppositely, a compound with strong antioxidant and anti-inflammatory effects is expected to act as an anti-tumor promoter. Epidemiological studies have proved an inverse correlation between the intake of vegetables and fruits and the incidence rate of diseases such as cancer and inflammation. Antioxidants present in vegetables and fruits are thought to be the effective compounds in the prevention of these oxidative stress related diseases (Surh, 2002; Huang, Ou, & Prior, 2005).

Malonaldehyde, one of the secondary products of lipid peroxidation, is of particular concern since it has been shown to be mutagenic and carcinogenic and implicated in other pathological processes (Botsoglou et al., 1994). Several reports have indicated that there is a positive relationship between in vivo lipid peroxidation and urinary malonaldehyde concentrations (Lee et al., 1992). Measurement of TBARS is a commonly used assay that measures malonaldehyde formed. In the present study, the intake of daikon sprout or ginger significantly decreased urinary TBARS concentrations in rats as compared with those before the intake (Table 2). This result shows that these vegetables suppress lipid peroxidation and the formation of malonaldehyde in rats. 8-OHdG is most commonly formed by the actions of reactive oxygen species on guanine (base modification) among the many forms of oxidative damage to DNA, and is an accurate marker of oxidative DNA damage (Lan, Henshall, Simon, & Chen, 2000). The intake of daikon sprout or ginger reduced urinary 8-OHdG levels in LPS-treated rats as compared with those fed the basal diet only (Fig. 1). This result indicates that these vegetables protect DNA from LPS-induced oxidative damage in rats.

The amount of urinary total isothiocyanate in rats fed daikon sprout was 5.1-fold higher than that fed daikon (Fig. 2), and this result shows that the amount of isothiocyanates absorbed from daikon sprout is greater than that from daikon. This phenomenon can be explained in that total glucosinolate content in the freeze-dried powder of daikon sprout was 23-fold higher than that of daikon (Table 1). Isothiocyanates inhibit NADPH oxidase-derived superoxide generation (Miyoshi et al., 2004) and nitric oxide production in LPS-activated macrophages (Ippoushi et al., 2002). Assuming that isothiocyanates derived from daikon sprout and daikon show an antioxidative effect in vivo through these inhibitions, the different effects between them on urinary TBARS levels (Table 2) are thought to be partly attributed to their difference in isothiocyanate absorption. Most attention has been focused on the inducing activity of isothiocyanates on Phase 2 enzymes of detoxication (Fahey et al., 2001). In the future, we will study the effect of daikon sprout on these enzymes in vivo. In addition, the amount of urinary total isothiocyanate of daikon sprout was lower than that estimated in consideration of the feeding condition (daikon sprout, 5% addition; daikon, 10% addition) and total glucosinolate contents in these vegetable powders. We speculate that this was due to lower productivity or absorptivity of isothiocyanates in the gut of rats in the case of daikon sprout as compared with daikon. Vitamin C content in the diet containing daikon sprout was slightly higher than that containing daikon (Table 1), and this also may be involved in the different effects between these vegetables on urinary TBARS levels (Table 2).

Ginger also showed an antioxidative effect in rats (Table 2, Fig. 1). To our knowledge, there are no experimental data on the absorption or metabolism of [6]-gingerol, a potent antioxidative compound contained in ginger, in humans. In laboratory animals, the pharmacological effect of [6]-gingerol has been reported to be maintained until 120 or 180 min after intravenous and oral administrations (Suekawa et al., 1984). Considering this report, [6]-gingerol present in ginger powder (Table 1) may be involved in the reduction of urinary TBARS (Table 2) and 8-OHdG (Fig. 1) levels in rats by the intake of ginger.

In summary, the reduction of lipid peroxidation and DNA base modification in rats by the intake of daikon sprout or ginger shows that these vegetables have an antioxidative effect which could be related to the inhibition of carcinogenesis.

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References

Abu-Qare, A. W., & Abou-Donia, M. B. (2001). Combined exposure to sarin and pyridostigmine bromide increased levels of rat urinary 3-nitrotyrosine and 8-hydroxy-2′-deoxyguanosine, biomarkers of oxidative stress. *Toxicology Letters, 123*, 51–58.

Aeschbach, R., Löliger, J., Scott, B. C., Murcia, A., Butler, J., Halliwell, B., et al. (1994). Antioxidant actions of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. *Food and Chemical Toxicology, 32*, 31–36.

Botsoglou, N. A., Fletouris, D. J., Papageorgiou, G. E., Vassilopoulos, V. N., Mantis, A. J., & Trakatellis, A. G. (1994). Rapid, sensitive, and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissue, food, and feedstuff samples. *Journal of Agricultural and Food Chemistry, 42*, 1931–1937.

Chung, F. L., Jiao, D., Getahun, S. M., & Yu, M. C. (1998). A urinary biomarker for uptake of dietary isothiocyanates in humans. *Cancer Epidemiology, Biomarkers and Prevention, 7*, 103–108.

Fahey, J. W., Zalcmann, A. T., & Talalay, P. (2001). The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry, 56*, 5–51.

He, X. G., Bernart, M. W., Lian, L. Z., & Lin, L. Z. (1998). High-performance liquid chromatography–electrospray mass spectrometric analysis of pungent constituents of ginger. *Journal of Chromatography A, 796*, 327–334.

Huang, D., Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry, 53*, 1841–1856.

Ippoushi, K., Azuma, K., Ito, H., Horie, H., & Higashio, H. (2003). [6]-Gingerol inhibits nitric oxide synthesis in activated J774.1 mouse macrophages and prevents peroxynitrite-induced oxidation and nitration reactions. *Life Sciences, 73*, 3427–3437.

Ippoushi, K., Ito, H., Horie, H., & Azuma, K. (2005). Mechanism of inhibition of peroxynitrite-induced oxidation and nitration by [6]-gingerol. *Planta Medica, 71*, 563–566.

Ippoushi, K., Itou, H., Azuma, K., & Higashio, H. (2002). Effect of naturally occurring organosulfur compounds on nitric oxide production in lipopolysaccharide-activated macrophages. *Life Sciences, 71*, 411–419.

Lan, J., Henshall, D. C., Simon, R. P., & Chen, J. (2000). Formation of the base modification 8-hydroxyl-2′-deoxyguanosine and DNA fragmentation following seizures induced by systemic kainic acid in the rat. *Journal of Neurochemistry, 74*, 302–309.

Lee, E., & Surh, Y. J. (1998). Induction of apoptosis in HL-60 cells by pungent vanilloids, [6]-gingerol and [6]-paradol. *Cancer Letters, 134*, 163–168.

Lee, H. S., Shoeman, D. W., & Csallany, A. S. (1992). Urinary response to in vivo lipid peroxidation induced by vitamin E deficiency. *Lipids, 27*, 124–128.

Miyoshi, N., Takabayashi, S., Osawa, T., & Nakamura, Y. (2004). Benzyl isothiocyanate inhibits excessive superoxide generation in inflammatory leukocytes: implication for prevention against inflammation-related carcinogenesis. *Carcinogenesis, 25*, 567–575.

Nakamura, Y., Iwahashi, T., Tanaka, A., Koutani, J., Matsuo, T., Okamoto, S., et al. (2001). 4-(Methylthio)-3-butenyl isothiocyanate, a principal antimutagen in daikon (*Raphanus sativus*; Japanese white radish). *Journal of Agricultural and Food Chemistry, 49*, 5755–5760.

Radi, R., Peluffo, G., Alvarez, M. N., Naviliat, M., & Cayota, A. (2001). Unraveling peroxynitrite formation in biological systems. *Free Radical Biology and Medicine, 30*, 463–488.

Sekiwa, Y., Kubota, K., & Kobayashi, A. (2000). Isolation of novel glucosides related to gingerdiol from ginger and their antioxidative activities. *Journal of Agricultural and Food Chemistry, 48*, 373–377.

Suekawa, M., Ishige, A., Yuasa, K., Sudo, K., Aburada, M., & Hosoya, E. (1984). Pharmacological studies on ginger. I. Pharmacological actions of pungent constituents, (6)-gingerol and (6)-shogaol. *Journal of Pharmacobio-Dynamics, 7*, 836–848.

Surh, Y. J. (2002). Anti-tumor promoting potential of selected spice ingredients with antioxidative and anti-inflammatory activities: a short review. *Food and Chemical Toxicology, 40*, 1091–1097.

Takaya, Y., Kondo, Y., Furukawa, T., & Niwa, M. (2003). Antioxidant constituents of radish sprout (kaiware-daikon), *Raphanus sativus* L.. *Journal of Agricultural and Food Chemistry, 51*, 8061–8066.

Thies, W. (1982). Complex-formation between glucosinolates and tetrachloropalladate(II) and its utilization in plant breeding. *Fette Seifen Anstrichmittel, 84*, 338–342.

Visentin, M., Tava, A., Iori, R., & Palmieri, S. (1992). Isolation and identification of *trans*-4-(methylthio)-3-butenyl glucosinolate from radish roots (*Raphanus sativus* L.). *Journal of Agricultural and Food Chemistry, 40*, 1687–1691.

Zhang, Y., Cho, C. G., Posner, G. H., & Talalay, P. (1992). Spectroscopic quantitation of organic isothiocyanates by cyclocondensation with vicinal dithiols. *Analytical Biochemistry, 205*, 100–107.

Zhang, Y., Wade, K. L., Prestera, T., & Talalay, P. (1996). Quantitative determination of isothiocyanates, dithiocarbamates, carbon disulfide, and related thiocarbonyl compounds by cyclocondensation with 1,2-benzenedithiol. *Analytical Biochemistry, 239*, 160–167.