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Inhibition of Malonaldehyde Formation in Oxidized Calf Thymus DNA with Synthetic and Natural Antioxidants

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Calf thymus DNA was oxidized by Fenton's reagent with or without synthetic antioxidants (Trolox and DMPO) and natural antioxidants—quercetin, apigenin, 2″-*O*-glycosylisovitexin (2″-*O*-GIV), (+)-catechin, cyanidin, pelargonidin, keracyanin, and callistephin. Malonaldehyde (MA) formed in oxidized DNA was analyzed using gas chromatography. MA formed from oxidized DNA without antioxidants was 4.0 ± 0.53 nmol/mg of DNA in buffer solution, 3.7 ± 0.34 nmol/mg of DNA in NaOH solution, and 4.6 ± 0.19 nmol/mg of DNA in HCl solution. MA formed from DNA with antioxidants (at the level of 0.1 μmol/mL) ranged from 1.90 ± 0.18 (catechin) to 4.10 ± 0.18 nmol/mg of DNA (cyanidin). Trolox and DMPO inhibited MA formation from DNA by 41.2% and 18.6%, respectively, at the level of 0.1 μmol/mL. Trolox (water-soluble vitamin E) exhibited dose-dependent inhibition. The decreasing order of inhibitory effect by flavonoids at the level of 0.1 μmol/mL was catechin (48.5%) > quercetin (47.1%) > 2″-*O*-GIV (40.5%) > apigenin (29.9%) and by the anthocyanins at the level of 0.1 μmol/mL was callistephin (45%) > keracyanin (33.2%) > pelargonidin (25.1%) > cyanidin (10.2%).

KEYWORDS: DNA; flavonoids; malonaldehyde; natural antioxidants; oxidative damage; Trolox

INTRODUCTION

Oxidative damage of protein, lipid, and DNA by reactive oxygen species (ROSs) has been known to play an important role in the pathogenesis of a number of human diseases, including cancer, atherosclerosis, cardiovascular diseases, Alzheimer's disease, arthritis, and diabetes (*1*−*3*). In particular, DNA base oxidation is considered to be a key event associated with disease initiation and progression in humans (*4*). It is becoming clear that endogenous generation of oxidants, such as hydroxyl radicals, leads to oxidation of DNA (*5*). Occurrence of cancer among individuals with no obvious exposure to cancer-causing agents may be due to these oxidative DNA damages promoted by ROSs. DNA is one of the target sites of ROSs, particularly the hydroxyl radical (•OH). It is well known that attacks on DNA cause several types of damage, including single- and double-strand breaks, release of free DNA bases, chemical changes to the bases, and sugar modifications (*6*, *7*). Therefore, determination of oxidized products, such as malonaldehyde (MA), from DNA will provide theoretical and practical information on the mechanisms of DNA oxidation following DNA damage. Furthermore, this information could help to develop a way to prevent DNA damage caused by oxidation.

Among the many products of oxidative damage, dicarbonyl compounds, such as MA, have received much attention as the chemicals implicated in various diseases (*8*, *9*). For example, a hydroxyl radical, which was induced by the Fenton reaction, produced malonaldehyde via the formation of base propenals upon degradation of the 2-deoxribose unit of isolated DNA (*10*). When 16 mmol of 2′-deoxyguanosine, 2′-deoxycytidine, 2′-deoxyadenosine, and thymidine were each oxidized by Fenton's reagent, 213, 130, 85, and 84 nmol of MA were formed (*11*).

The reactive carbonyl compounds, such as MA, formed from biological substances (lipid, protein, DNA, and sugar) by oxidation have been analyzed as a biomarker of oxidative damage. Among these chemicals, MA as thiobarbituric acid reactive substances (TBARs) has been most widely and commonly used as a marker of oxidative damage. However, methods that measure total products reacted with thiobarbituric acid always result in overestimating MA formation and are, moreover, not specific to MA (*12*). Recently, we developed a highly specific and sensitive gas chromatographic (GC) method for MA analysis in biological samples (*11*). This method involves derivatization of MA into more stable nitrogen-containing 1-methylpyrazole (1-MP) with *N*-methylhydrazine, after which 1-MP is analyzed by a gas chromatograph (GC) with a nitrogen−phosphorus detector (NPD).

In the present study MA formed from calf thymus DNA oxidized by Fenton's reagent with various antioxidants was analyzed by the above newly developed GC/NPD method to investigate oxidative damage of DNA caused by a hydroxyl radical.

MATERIALS AND METHODS

Chemicals and Materials. Calf thymus DNA, $FeCl_2$, H_2O_2, *N*-methylhydrazine, 2-methylpyrazine, and Chelex-100 were bought from Sigma-Aldrich (St. Louis, MO).

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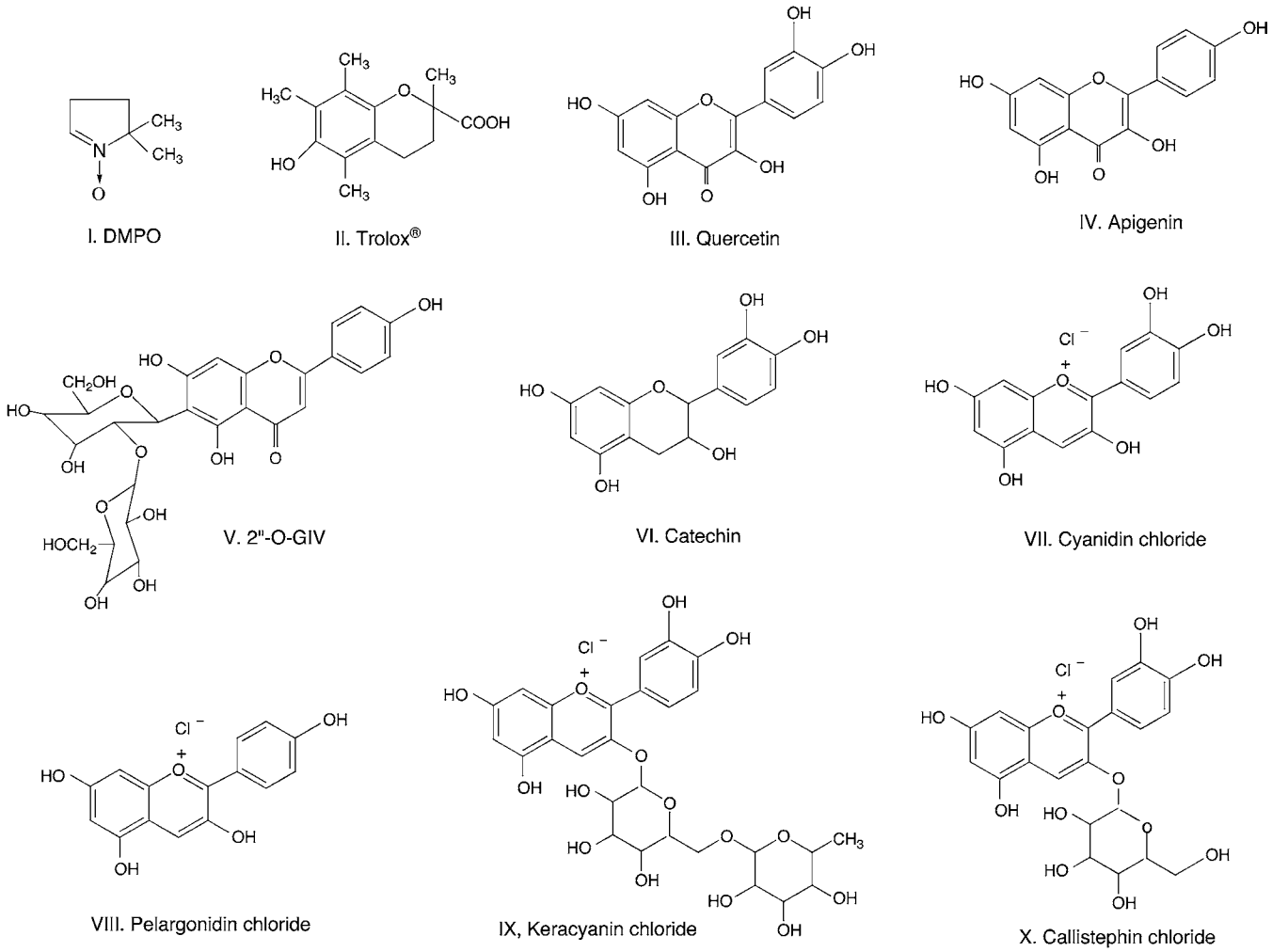

Figure 1. Structures of antioxidants tested in the present study.

Antioxidants: 2*H*-pyrrole, 3,4-dihydro-2,2-dimethyl-1-oxide (DMPO); 2*H*-1-benzopyran-2-carboxylic acid, 3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-(9Cl) (Trolox); 4*H*-1-benzopyran-4-one, 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-(9Cl) (quercetin); 4*H*-1-benzopyran-4-one, 5,7-dihydroxy-2-(4-hydroxyphenyl)-(9Cl) (apigenin); and 2*H*-1-benzopyran-3,5,7-triol, 2-(3,4-dihydroxyphenyl)-3,4-dihydro-(2*R*,3*S*)-(9Cl) [(+)-catechin] were purchased from Sigma-Aldrich (St. Louis, MO). 1-Benzopyrylium, 2-(3,4-dihydroxyphenyl)3,5,7-trihydroxy-, chloride (9Cl) (cyanidin chloride); 1-benzopyrylium, 3,5,7-trihydroxy-2-(4-hydroxyphenyl)-, chloride (9Cl) (pelargonidin chloride); 1-benzopyrylium, 3[{6-*O*-(6-deoxyl-α-L-mannopyranosyl)-*β*-D-glucopyranosyl}-oxyl]-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-, chloride (9Cl) (keracyanin chloride); and 1-benzopyrylium, 3-(*β*-D-glucopyranosyloxy)-5,7-dihydroxy-2-(4-hydroxyphenyl)-, chloride (9Cl) (callistephin chloride) were bought from Extrasynthese Co. (Genay Cedex, France). Chemical names are CA index names, and their structures are shown in **Figure 1**.

2″-*O*-Glycosylisovitexin (2″-*O*-GIV) was isolated from young green barley leaves (*Hordium vulgare L. var. nudum Hook*) harvested 2 weeks after germination by a previously reported method (*13*).

Authentic 1-methylpyrazole (1-MP) was synthesized according to a previously reported method (*14*).

A stock solution of DNA was prepared by dissolving 1 mg of DNA into 1 mL of phosphate buffer (pH 7.4). All reagents used in the oxidative reactions were prepared in Chelex-treated water. DNA, phosphate buffer, and H_2O_2 solutions were also treated with Chelex. $FeCl_2$ was used immediately after the preparation.

All other analytical-grade chemicals, reagents, and solvents were obtained from reliable sources.

Sample Preparation of Oxidized DNA with or without Antioxidant. Three types of reaction solutions were prepared.

(1) A 5 mL aqueous phosphate buffer solution (20 μmol/mL, pH 7.4) containing 0.5 mg/mL of DNA, 0.5 μmol/mL of H_2O_2, 1.0 μmol/mL of $FeCl_2$, and 0.1 μmol/mL of antioxidant (Trolox and DMPO) was incubated at 37 °C for 30 min. A solution containing exactly the same chemicals but with different amounts of Trolox (0.06, 0.1, 0.2, or 0.6 μmol/mL) was also incubated at 37 °C for 30 min for the dose-dependent study.

(2) A 5 mL aqueous NaOH solution (1 μmol/mL) containing 0.5 mg/mL of DNA, 0.5 μmol/mL of H_2O_2, 1.0 μmol/mL of $FeCl_2$, and 0.1 μmol/mL of antioxidant (quercetin, apigenin, 2″-*O*-GIV, or catechin) was incubated at 37 °C for 30 min.

(3) A 5 mL aqueous HCl solution (0.2 μmol/mL) containing 0.5 mg/mL of DNA, 0.5 μmol/mL of H_2O_2, 1.0 μmol/mL of $FeCl_2$, and 0.1 μmol/mL of antioxidant (pelargonidin, keracyanin, or callistephin) was incubated at 37 °C for 30 min. A buffer, NaOH, or HCl solution (5 mL) containing exactly the same chemicals as the above without antioxidants was incubated at 37 °C for 30 min and used as a control. After the reaction mixtures cooled to room temperature, oxidation of the samples was stopped by adding 2.5 mL of an aqueous solution containing 10 μmol/mL of thiourea, 30 μmol/mL of EDTA, and 0.6 μmol/mL of sodium acetate, after which MA was analyzed by gas chromatography.

Analysis of MA as 1-Methylpyrazole (1-MP). MA formed in oxidized DNA was analyzed as previously reported (*11*) with slight modification. A 100 μL amount of *N*-methylhydrazine solution (50%, v/v) was added to the above sample solutions. Immediately after the solutions were incubated at 37 °C for 30 min, they were placed into Bond Elut C18 cartridges (Varian, Inc., Harbor City, CA) connected to a vacuum manifold. After eluting the sample solutions, the cartridges were washed with 3 mL of deionized water twice, and then 1-MP was

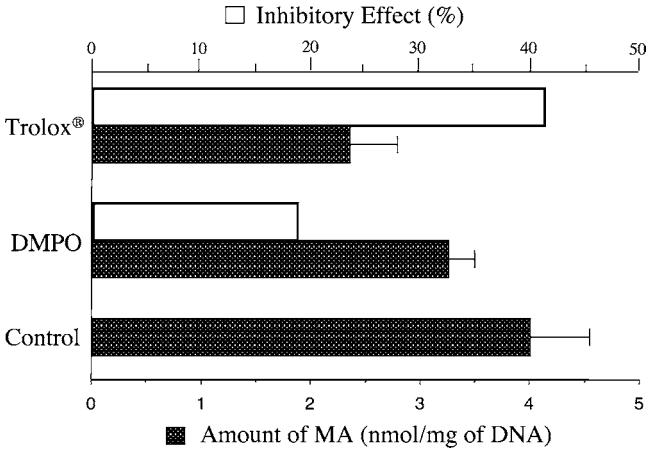

Figure 2. MA formed in the samples with synthetic antioxidants and their inhibitory effects.

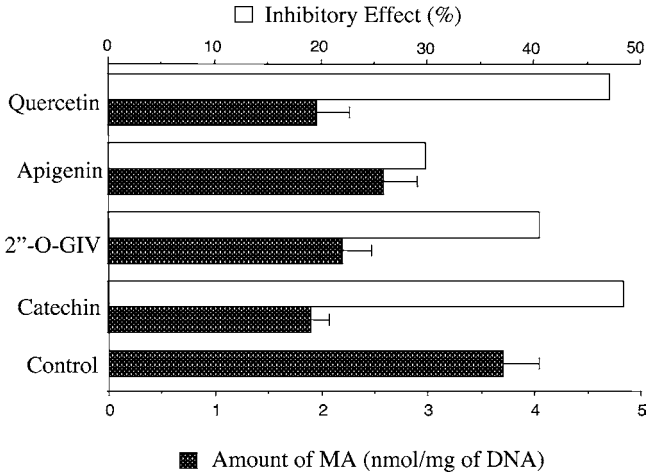

Figure 3. MA formed in the samples with flavonoids and their inhibitory effects.

eluted with 3 mL of ethyl acetate twice. The ethyl acetate solution was dried over anhydrous sodium sulfate for 30 min. After removal of the sodium sulfate, the solvent was evaporated under a purified nitrogen stream. The volume of the sample solution was then brought to 0.5 mL with ethyl acetate. A 20 μL amount of standard solution, 2-methylpyrazine (1 mg/mL), was added to the sample as an internal standard prior to GC analysis.

The resulting 1-MP was analyzed by a previously reported method (*11*). A Hewlett-Packard model 6890A GC equipped with an NPD and a 30 m × 0.25 mm i.d. (d_f = 1 μm) DB−Wax bonded-phased fused silica capillary column (J&W Scientific, Folsom, CA) was used. The detector and injector temperatures were 250 °C. The linear velocity of the helium carrier gas was 25 cm/s with a split ratio of 8:1. The oven temperature was programmed from 60 to 130 °C at 3 °C/min and 130 to 200 °C (5 min held) at 10 °C/min.

RESULTS AND DISCUSSION

A total of 10 antioxidants were examined for their inhibitory activity toward MA formation from DNA upon oxidation. The antioxidants were divided into three groups according to their solubility in water. The results of MA formed from DNA samples with 10 different antioxidants are shown: **Figure 2**, synthetic antioxidant group examined in buffer solutions, **Figure 3**, flavonoid group examined in NaOH, and **Figure 4**, anthocyanin group examined in HCl solutions. The values in the figures are mean ± standard deviation (n = 3). Addition of NaOH or HCl solution did not change the pH (7.4) of the reaction solutions.

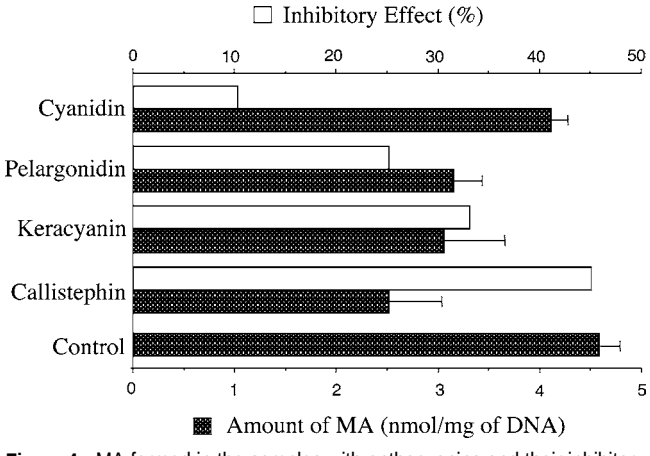

Figure 4. MA formed in the samples with anthocyanins and their inhibitory effects.

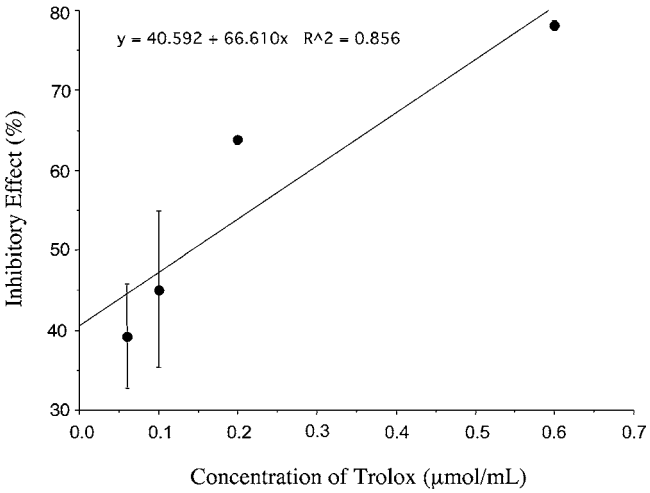

Figure 5. Inhibitory effect of Trolox at various levels.

MA formed in the control was 4.0 ± 0.53 nmol/mg in the buffer solution, 3.7 ± 0.34 nmol/mg in the NaOH solution, and 4.6 ± 0.19 nmol/mg in the HCl solution. Amounts of MA formed from DNA with the presence of antioxidants ranged from 1.90 ± 0.18 (catechin) to 4.10 ± 0.18 nmol/mg (cyanidin). The inhibitory effect (%) of antioxidants was calculated as follows

$$\text{effect} = \frac{\left(\begin{array}{c}\text{amount of MA formed in control} - \\ \text{amount of MA formed in sample}\end{array}\right)}{\text{amount of MA formed in control}} \times 100$$

Trolox (water-soluble vitamin E) inhibited MA formation more (41.2%) than DMPO (18.6%) did (see **Figure 2**) at a level of 0.1 μmol/mL. **Figure 5** shows the results of the dose-dependent study using Trolox. The values are mean ± standard deviation (n = 3). The effect of inhibition (%) and exponential Trolox concentration exhibited a linear relationship ($R2$ = 0.976). The highest inhibition of MA formation with Trolox was 78.2% at a level of 0.6 μmol/mL in the present study.

DMPO is one of the most widely used spin-trapping agents in biological systems. It forms an adduct with a hydroxyl radical (*15*). Trolox has been reported to function as an antioxidant which prevents oxidative DNA damage; for example, Trolox exhibited dose- and time-dependent inhibition of 8-hydroxydeoxyguanosine formation in calf thymus DNA (*16*), and oxidation induced by peroxynitrite in rat thymocytes was lowered by Trolox (*17*).

The decreasing order of inhibitory effect by flavonoids was (refer to **Figure 3**) catechin (48.5%) > quercetin (47.1%) > 2″-*O*-GIV (40.5%) > apigenin (29.9%). Flavonoid compounds, which are widely distributed in the plant kingdom, show various pharmacological activities, including an antioxidative effect. Among the flavonoids tested, catechin and quercetin inhibited MA formation nearly 50%. Catechin is one of the major components of green tea and is associated with the health benefits of green tea. Catechin has been known to possess various chemical biological functions, including eliminating free radicals, decreasing cholesterol levels in blood, preventing arterial sclerosis, and antibacterial and antivirus characteristics (*18*). Catechin protected in a dose-dependent manner against the cytotoxicity of high levels of reactive oxygen species (*19*). On the other hand, catechin induced metal-dependent H_2O_2 generation during the redox reactions and subsequent damage to cellular and isolated DNA (*20*). Quercetin inhibited hydrogen-peroxide-mediated DNA damage in human lymhocytes, suggesting that quercetin possesses some protective effect against oxidation of DNA (*21*), which is consistent with the results of the present study. However, quercetin showed carcinogenic activity in the kidney of the male rat (*22*). Therefore, catechin and quercetin may have both anticarcinogenic and carcinogenic potentials, even though the present study showed that they inhibited oxidation of DNA. 2″-*O*-GIV exhibited potent antioxidative activity against the oxidation of lipids, such as ethyl esters of fatty acids (*23*), phospholipids (*24*), and *ω*-3 polyunsaturated fatty acids (*25*). In addition, 2″-*O*-GIV inhibited MA formation from blood plasma at a level of 2 mmol/mL of plasma by 60% (26). In the present study, 2″-*O*-GIV inhibited MA formation from DNA at a level of 40 mmol/mg of DNA by 40.5%. The inhibitory activity of 2″-*O*-GIV against MA formation from DNA was comparable to those of catechin and quercetin. However, there is no report on the toxicity of 2″-*O*-GIV at the present time.

The decreasing order of inhibitory effect by the anthocyanin (see **Figure 4**) was callistephin (45.0%) > keracyanin (33.2%) > pelargonidin (25.1%) > cyanidin (10.2%). Recently, red wines have been reported to possess some heath benefit, which has been publicized as the French Paradox (*27*). Red wines were found to contain antioxidants, which have a strong association with health benefits, at considerably high levels. Later it was found that these antioxidants were anthocyanins. Anthocyanins give intense colors to many fruits and vegetables, such as grapes, berries, red cabbages, and purple sweet potatoes. There have been many reports on the bioactivities of anthocyanins (*28*), such as antioxidant activity in vitro and in vivo (*29*). Many anthocyanins (including cyanidin and pelargonidin) showed an activity comparable to the well-known antioxidants α-tocopherol, Trolox, catechin, and quercetin in previous reports (*30*), which is consistent with the results obtained in the present study.

There have been numerous reports on the structure−activity relationships of flavonoid antioxidants (*31*−*34*). Among the several factors affecting the antioxidative activity of flavonoids, hydroxyl groups on the B-ring seem to play the most important role (*35*, *36*). It is reported that among structurally homologous flavonoids, hydroxyl scavenging ability increases according to the total number of hydroxyl groups (*37*). In the present study, flavonoid quercetin containing two hydroxyl groups on the B-ring inhibited MA formation (47.1%, **Figure 3**) more than flavonoid apigenin containing one hydroxyl group on the B-ring (29.9%) did. On the other hand, in the case of anthocyanin (**Figure 4**), cyanidin containing two hydroxyl groups on the B-ring inhibited MA formation (10.2%) less than pelargonidin containing one hydroxyl group on the B-ring (25.1%) did. There was a slight difference in activity between quercetin (47.1%) and catechin (48.5%). There is no consistent correlation between 2 and 3 unsaturation (quercetin) and antioxidant activity (*36*).

Generally, aglycones exhibit higher antioxidative activity than their corresponding glycosides do (*38*, *39*). However, it was also reported that depending on the anthocyanidin, different glycosylation patterns either enhanced or diminished the antioxidative activity. When anthocyanins were tested in bulk oil, the glycosides were more effective than the aglycones (*30*). In the present study, aglycone (cyanidin, 10.2%) showed lower activity than its glycosides—keracyanin (33.2%) and callistephin (45.0%) (see **Figures 1** and **4**).

Flavonoids containing 5-OH and 4-oxa groups, such as quercetin and 2″-*O*-GIV, trapped Fe^{2+} by forming a chelate complex and consequently suppressed the activity of Fenton's reaction (*40*). In the present study, quercetin (47.1%) and 2″-*O*-GIV (40.5%) exhibited relatively higher antioxidative activity than other chemicals tested. The structure−antioxidative activity relationships of flavonoid or anthocyanins must involve many factors in addition to the ones described above. Therefore, it is difficult to explain activity differences among flavonoids (**Figure 1**, **III**−**VI**) and anthocyanins (**Figure 1**, **VII**−**X**) according to the structures of the chemicals tested in the present study.

It has been hypothesized that toxicity caused by oxidation is due to secondary products, such as MA, rather than reactive oxygen species (*41*, *42*) because some secondary products, including MA, exhibited mutagenicity (*43*). In addition, it was suggested that secondary products were associated with cancer formation (*46*) because they, including MA, formed an adduct with DNA (*44*, *45*). Therefore, investigation of MA formation and inhibition from DNA may be one avenue to clarify the damage caused by oxidation.

LITERATURE CITED

(1) Olinski, R.; Gackowski, D.; Rozaiski, R.; Foksinski, M.; Bialkowski, K. Oxidative DNA damage in cancer patients: a cause of a consequence of the disease development? *Mutat. Res.* **2003**, *531*, 177−190.

(2) Kang, D. Alterations of mitochondrial DNA: molecular basis of oxidative damage, aging, and diseases. *Rinsho Kagaku* **2003**, *32*, 49−52.

(3) Davydov, V.; Hansen, L. A. Is DNA repair compromised in Alzheimer's disease? *Neurobiol. Aging* **2003**, *24*, 953−968.

(4) Simandan, T.; Sun, J.; Dix, T. A. Oxidation of DNA bases, deoxyribonucleosides and homopolymers by peroxyl radicals. *Biochem. J.* **1998**, *335*, 233−240.

(5) Aust, A. E.; Eveleigh, J. F. Mechanisms of DNA oxidaion. *Proc. Soc. Exp. Biol. Med.* **1999**, *222*, 246−252.

(6) Marnett, L. J. Oxyradicals and DNA damage. *Carcinogenesis* **2000**, *21*, 361−370.

(7) Henle, E.; Linn, S. Formation, prevention, and repair of DNA damage by iron/hydrogen peroxide. *J. Biol. Chem.* **1997**, *272*, 19095−19098.

(8) Okado-Matsumoto, A.; Fridovich, I. The role of α,*β*-dicarbonyl compounds in the toxicity of short chain sugars. *J. Biol. Chem.* **2000**, *275*, 34853−34857.

(9) Siu, G. M.; Draper, H. H.; Valli, V. E. Oral toxicity of malonaldehyde: a 90-day study on mice. *J. Toxicol. Environ. Health* **1983**, *11*, 105−119.

(10) Frelon, S.; Douki, T.; Favier, A.; Cadet, J. Comparative study of base damage induced by gamma radiation and Fenton reaction in isolated DNA. *J. Chem. Soc., Perkin Trans. 1* **2000**, 2866−2870.

(11) Miyake, T.; Shibamoto, T. Formation of malonaldehyde and acetaldehyde from the oxidation of 2′-deoxyribonucleosides. *J. Agric. Food Chem.* **1999**, *47*, 2782−2785.

(12) Esterbauer, H.; Zollner, H. Methods for determination of aldehydic lipid peroxidation products. *Free Radical Biol. Med.* **1989**, *3*, 197−203.
(13) Osawa, T.; Katsuzaki, H.; Hagiwara, Y.; Hagiwara, H.; Shibamoto, T. A novel antioxidant isolated from young green barley leaves. *J. Agric. Food Chem.* **1992**, *40*, 1135−1138.
(14) Umano, K.; Dennis, K. J.; Shibamoto, T. Analysis of free malondialdehyde in photoirradiated corn oil and beef fat via a pyrazole derivative. *Lipids* **1988**, *23*, 811−814.
(15) Hawkins, C. L.; Davies, M. J. Comparison of 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) and a new cyclic nitrone spin trap-5-*tert*-butoxycarbonyl-5-methyl-1-pyrroline-*N*-oxide (BMPO) for the trapping of nitrogen-centered radicals. *Curr. Topics Biophys.* **2002**, *26*, 137−144.
(16) Qi, W.; Reiter, R. J.; Tan, D.-X.; Manchester, L. C.; Calvo, J. R. Melatonin prevents γ-amino levulinic acid-induced oxidative DNA damage in the presence of Fe2+. *Mol. Cell. Biochem.* **2001**, *218*, 87−92.
(17) Salgo, M. G.; Pryor, W. A. Trolox inhibits peroxynitrate-mediated oxidative stress and apoptosis in rat thymocytes. *Arch. Biochem. Biophys.* **1996**, *333*, 482−488.
(18) Higdon, J. V.; Frei, B. Tea catechins and polyphenoles: health effects, metabolism, and antioxidant functions. *Crit. Rev. Food Sci. Nutr.* **2003**, *43*, 89−143,
(19) Subirade, I.; Fernandez, Y.; Periquet, A.; Mitjavila, S. Catechin protection of 3T3 Swiss fibroblasts in culture under oxidative stress. *Biol. Trace Elem. Res.* **1995**, *47*, 313−319.
(20) Oikawa, S.; Furukawa, A.; Asada, H.; Hirakawa, K.; Kawanishi, S. Catechins induce oxidative damage to cellular and isolated DNA through the generation of reactive oxygen species. *Free Radical Res.* **2003**, *37*, 881−890.
(21) Duthie, S. J.; Collins, A. R.; Duthie, G. G.; Dobson, V. L. Quercetin and myricetin protect against hydrogen peroxide-induced DNA damage (strand breaks and oxidized pyrimidines) in human lymphocytes. *Mutat. Res.* **1997**, *393*, 223−231.
(22) Dunnick, J. K.; Hailey, J. R. Toxicity and carcinogenicity studies of quercetin, a natural component of foods. *Fundam. Appl Toxicol.* **1992**, *19*, 423−431.
(23) Nishiyama, T.; Hagiwara, Y.; Hagiwara, H.; Shibamoto, T. Inhibition of malonaldehyde formation from lipids by an isoflavonoid isolated from young green barley leaves. *J. Am. Oil Chem. Soc.* **1993**, *70*, 811−813.
(24) Nishiyama, T.; Hagiwara, Y.; Hagiwara, H.; Shibamoto, T. Formation and inhibition of genotoxic glyoxal and malonaldehyde from phospholipids and fish liver oil upon lipid peroxidation. *J. Agric. Food Chem.* **1994**, *42*, 1728−1731.
(25) Ogata, J.; Hagiwara, Y.; Hagiwara, H.; Shibamoto, T. Inhibition of malonaldehyde formation by antioxidants from ω-3 polyunsaturated fatty acids. *J. Am. Oil Chem. Soc.* **1996**, *73*, 653−656.
(26) Miyake, T.; Shibamoto, T. Inhibition of malonaldehyde and acetaldehyde formation from blood plasma oxidation by naturally occurring antioxidants. *J. Agric. Food Chem.* **1998**, *46*, 3694−3697.
(27) Formica, J. P.; Regelson, W. Review of the biology of quercetin and related bioflavonoids. *Food Chem. Toxicol.* **1995**, *33*, 1061−1080.
(28) Hou, D.-X. Potential mechanisms of cancer chemoprevention by anthocyanins. *Curr. Mol. Med.* **2003**, *3*, 149−159.
(29) Duthie, G.; Gardner, P.; Kyle, J.; McPhail, D. Antioxidant activity of anthocyanins in vitro and in vivo. In *Nutraceutical Beverages*; ACS Symposium Series 871; American Chemical Society: Washington, D.C., 2004; pp 90−102.
(30) Kahkonen, M. P.; Heinonen, M. Antioxidant activity of anthocyanins and their aglycones. *J. Agric. Food Chem.* **2003**, *51*, 628−633.
(31) Heijnen, C. G. M.; Haenen, G. R. M. M.; Oostveen, R. M.; Stalpers, E. M.; Bast, A. Protection of flavonoids against lipid peroxidation: The structure activity relationship revisited. *Free Radical Res.* **2002**, *36*, 575−581.
(32) Heim, K. E.; Tagliaferr, A. R.; Bobilya, D. J. Flavonoid antioxidants: chemistry, metabolism and structure−activity relationships. *J. Nutr. Biochem.* **2002**, *13*, 572−584.
(33) Peng, I.-W.; Kuo, S.-M. Flavonoid structure affects the inhibition of lipid peroxidation in Caco-2 intestinal cells at physiological concentrations. *J. Nutr.* **2003**, *113*, 2184−2187.
(34) Vaya, J.; Mahamood, S.; Goldblum, A.; Aviram, M.; Volkova, N.; Shaalan, A.; Musa, R.; Tamir, S. Inhibition of LDL oxidation by flavonoids in relation to their structure and calculated enthalpy. *Phytochemistry* **2003**, *62*, 89−99.
(35) Sekher Pannala, A.; Chan, T. S.; O'Brien, P. J.; Rice-Evans, C. A. Flavonoid B-ring chemistry and antioxidant activity: fast reaction kinetics. *Biochem. Biophys. Res. Commun.* **2001**, *282*, 1161−1168.
(36) Burda, S.; Oleszek, W. Antioxidant and antiradical activities of flavonoids. *J. Agric. Food Chem.* **2001**, *49*, 2774−2779.
(37) Cao, G.; Sofic, E.; Prior, R. L. Antioxidant and prooxidant behavior of flavonoids: structure−activity relationships. *Free Radical Biol. Med.* **1997**, *22*, 749−760.
(38) Ratty, A. K.; Das, N. P. Effects of flavonoids on nonenzymatic lipid peroxidation: structure−activity relationship. *Biochem. Med. Metab. Biol.* **1988**, *39*, 69−79.
(39) Gao, Z.; Huang, K.; Yang, X.; Xu, H. Free radical scavenging and antioxidant activities of flavonoids extracted from the radix of *Scutellaria baicalensis Georgi*. *Biochim. Biophys. Acta* **1999**, *472*, 643−650.
(40) Cheng, I. F.; Breen, K. On the ability of four flavonoids, baicilein, luteolin, naringenin, and quercetin, to suppress the Fenton reaction of the iron-ATP complex. *Biometals* **2000**, *13*, 77−83.
(41) Andrews, J. S.; Griffith, W. H.; Mead, J. F.; Stein, R. A. Toxicity of air-oxidized soybean oil. *J. Nutr.* **1960**, *70*, 199−210.
(42) Kubow, S. Toxicity of dietary lipid peroxidation products. *Trends Food Sci. Technol.* **1990**, *1*, 67−71.
(43) Mukai, F. H.; Goldstein, B. D. Mutagenicity of malonaldehyde, a decomposition product of peroxidized polyunsaturated fatty acids. *Science* **1976**, *191*, 868−869.
(44) Chaudhary, A. K.; Reddy, G. R.; Blair, I. A.; Marnett, L. J. Characterization of an N6-oxopropenyl-2′-deoxyadenosine adduct in malondialdehyde-modified DNA using liquid chromatography/electrospray ionization tandem mass spectrometry. *Carcinogenesis* **1996**, *17*, 1167−1170.
(45) Wang, M. Y.; Liehr, J. G. Induction by estrogens of lipid peroxidation and lipid peroxide-derived malonaldehyde-DNA adducts in male Syrian hamsters: role of lipid peroxidation in estrogen-induced kidney carcinogenesis. *Carcinogenesis* **1995**, *16*, 1941−1945.
(46) Kanazawa, K. Hepatotoxicity caused by dietary secondary products originating from lipid peroxidation. *Adv. Exp. Med. Biol.* **1991**, *289*, 237−253.

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