JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Free Radical Scavenging Activity of 4-(3,4-Dihydroxybenzoyloxymethyl)phenyl-*O*-*β*-D-glucopyranoside from *Origanum vulgare* and Its Protection against Oxidative Damage

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Supporting Information

ABSTRACT: 4-(3,4-Dihydroxybenzoyloxymethyl)phenyl-*O*-*β*-D-glucopyranoside (DBPG), a polyphenolic glycoside, isolated from *Origanum vulgare* has shown 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$)-scavenging capacity in previous work. This study demonstrated that DBPG exhibits antioxidant activity by a series of DPPH$^{\bullet}$, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS$^{\bullet+}$), and superoxide anion radical ($O_2^{\bullet-}$) radical-scavenging assays. The inhibition of lipid peroxidation (LP) by DBPG exceeded that by L-ascorbic acid (AA) in a liposome model system. Adding DBPG to mouse liver and brain tissue inhibited the formation of thiobarbituric acid reactive substances (TBARS) to a greater extent than did trolox. In the oxygen stress test, BNLCL2 and HaCaT cells pretreated with DBPG showed increased activities of glutathione peroxidase (GPx), perhaps as a result of reduction of the production of reactive oxygen species (ROS). These findings proved that DBPG had antioxidant activities and a cytoprotective effect in hepatocytes and keratinocytes, suggesting that DBPG may be a useful food and cosmetic additive.

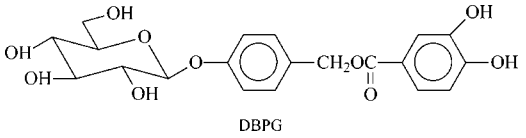
KEYWORDS: *4-(3,4-dihydroxybenzoyloxymethyl)phenyl-O-β-D-glucopyranoside, antioxidant, cytoprotective, Origanum vulgare, reactive oxygen species*

INTRODUCTION

Oxidative stress is a cellular imbalance between the production and elimination of reactive oxygen species (ROS) such as superoxide anion radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH$^{\bullet}$). It is also a pathophysiological reaction highly relevant to a variety of pathological processes, including ischemia−reperfusion injury, chronic progressive neurodegenerative disease, numerous inflammatory diseases, and cancer.[1] Oxidative stress results in skin aging and can adversely affect skin health. Therefore, antioxidants that are active in skin cells may support skin health.[2] To reduce oxidative damage by free radicals, cells are generally provided with enzymatic antioxidant factors such as catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD), as well as nonenzymatic ones such as glutathione (GSH), L-ascorbic acid (AA), and *α*-tocopherol.[3] Reducing oxidative stress is considered to be desirable for human health.

Herbs and spices have been shown to contain natural antioxidants that provide protections against harmful free radicals and antagonize the deleterious action of ROS on bioactive molecules.[4] Oregano (*Origanum vulgare* L.), a widely used Chinese herb and a common herb in the Western diet, has been found to exhibit antithrombin, anti-*Helicobacter pylori*, antimicrobial, antibiotic, and antihyperglycemia as well as antioxidation effects.[5,6] 4-(3,4-Dihydroxybenzoyloxymethyl)-phenyl-*O*-*β*-D-glucopyranoside (DBPG) (Figure 1), a polyphenolic glycoside, is a major constituent of oregano. Previous studies have demonstrated that when DBPG is orally administered, the serum metabolites of DBPG exhibit DPPH$^{\bullet}$ free radical scavenging activity.[7] However, their underlying antioxidant mechanisms and the mechanisms of their cytoprotective effects on liver and skin cells remain unknown.

Received: May 31, 2012
Revised: July 16, 2012
Accepted: July 16, 2012
Published: July 16, 2012



DBPG

Figure 1. Structure of DBPG.

Various methods have been developed to examine antioxidant activity, most of which have focused on evaluating the capacities of substances to scavenge free radicals and inhibit lipid peroxidation (LP).[8] Hence, this study examines the antioxidant properties of DBPG from *O. vulgare* by performing a series of chemical and biochemical assays. These assays for DBPG include DPPH$^{\bullet}$ radical scavenging activity, ABTS$^{\bullet+}$ radical scavenging activity, reducing power, metal chelating activity, inhibition of LP by liposomes, and inhibition of thiobarbituric acid reactive substances (TBARS) formation in mice liver and brain tissue. The cytoprotection ability of DBPG was examined by inhibition of cellular ROS production and increase of GPx activity following pretreatment of mouse hepatocytes (BNLCL2 cells) and human skin keratinocytes (HaCaT cells) with DBPG in H_2O_2-treated cells.

MATERIALS AND METHODS

Materials and Chemicals. 1,1-Diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS$^{\bullet+}$), 2,5,7,8-tetramethylchromancarboxylic acid (trolox), 2-thiobarbituric acid (TBA), ethylenediaminetetraacetic acid disodium salt dehydrate (EDTA), hydrogen peroxide (H_2O_2), L-ascorbic acid (AA), and trichloroacetic acid (TCA) were purchased form Sigma Chemical Co. (St. Louis, MO, USA). 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-4′,4″-disulfonic acid monosodium salt (ferrozine) was purchased from Fluka (Buchs, Switzerland).

Extraction and Isolation of Plant Material. DBPG was isolated from *O. vulgare* L. and identified by Hsiou-Yu Ding (Department of Cosmetic Science, Chia Nan University of Pharmacy and Science, Taiwan). The extraction and isolation of DBPG from *O. vulgare* L. were performed as described by Liang et al.:[9] colorless powder; mp 203−204 °C. DBPG exhibited spectroscopic data (IR, NMR, MS) similar to those in the literature.[10] DBPG was dissolved in dimethyl sulfoxide (DMSO) to a concentration of 100 mM.

Determination of Radical Scavenging Capability against DPPH$^{\bullet}$, ABTS$^{\bullet+}$, and $O_2^{\bullet-}$. The ability of DBPG to scavenge DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ free radicals was assessed as previously reported.[11−13] The inhibition percentage of ABTS$^{\bullet+}$ radical as determined by the extent of decolorization was taken to be proportional to the concentration of antioxidants. Trolox equivalent antioxidant capacity (TEAC) is calculated by comparison with the reactivity of the standard, trolox. The superoxide radical was generated by the PMS−NADH system as described by Fernandes et al.[14] with minor modifications. Briefly, DBPG (0, 5, 10, 50, 100, and 200 μM) (5 μL of solution) was added to a reaction mixture containing 45 μL of DMSO, 50 μL of phenazine methosulfate (240 μM), and 50 μL of β-NADH (1872 μM). After incubation at 25 °C for 10 min, the superoxide anion was measured in a total volume of 200 μL, which contained 50 μL of nitroblue tetrazolium (600 μM) in 0.1 M phosphate buffer containing 5 mM EDTA at pH 7.4. After 10 min of incubation at 25 °C, the absorbance was determined at 560 nm. Rutin and trolox were utilized as standards.

Detection of Reducing Power and Metal Chelating Activity. The reducing power of DBPG was determined by the $K_3Fe(CN)_6$−$FeCl_3$ method as reported before.[13,15] The chelating effect of DBPG was evaluated by following the method of Decker and Welch.[16]

Inhibition of Lipid Peroxidation. LP was determined by using the liposome as a model of biological membranes, as described previously.[13,17] Fe^{2+}/ascorbate-induced LP was used. A control with DMSO instead of sample was also analyzed and expressed no activity.

Inhibition of Thiobarbituric Acid-Reactive Substances Formation. Seven-week-old male Institute of Cancer Research (ICR) mice (National Animal Laboratory Center, Taipei, Taiwan), weighing 30−35 g, were used. The production of tissue LP was monitored by measuring concentrations of TBARS, using the method of Ding et al.[13] Briefly, liver and brain tissues were homogenized using a ploytron in ice-cold Tris-HCl buffer (40 mM, pH 7.4) to yield a 1:1 (w/v) homogenized tissue, which was centrifuged at 3000*g* for 10 min. An aliquot (0.1 mL) of the supernatant was incubated with DBPG (0.2 mL) in the presence of $FeSO_4$ (10 μM, 0.1 mL) and AA (0.1 mM, 0.1 mL) at 37 °C for 1 h. The reaction was terminated by adding TCA (28% w/v, 0.5 mL), followed by TBA (1% w/v, 0.38 mL), and the mixture was then heated at 80 °C for 20 min. Following centrifugation at 3000*g* for 10 min to remove the precipitated protein, the intensity of the color of the TBARS that formed in the supernatant was measured by its absorbance at 532 nm.

Cell Culture and Cell Viability Analysis. Murine normal embryonic liver BNLCL2 cells were purchased from the American Type Culture Collection (Rockville, MD, USA). Human premalignant keratinocytic HaCaT cells were kindly provided by Prof. Hamm-Ming Sheu (National Cheng Kung University Medical College, Tainan, Taiwan). The DMEM medium (GIBCO, USA) with 10% heat-inactivated fetal bovine serum (GIBICO, USA) and 1% penicillin/streptomycin was used to culture cells at 37 °C in 5% CO_2. Cells (1×10^4 cells/well) were seeded in 100 μL of 96-well plates and treated with H_2O_2 (0.01−1 mM) for 1 h or DBPG, AA, and trolox (0, 5, 10, 50, 100, and 200 μM) for 24 h. Cell viability was determined by using the MTT assay as reported.[13]

Cell Cycle Distribution Analysis. BNLCL2 and HaCaT cells (1×10^5 cells/mL) were seeded in 24-well plates and incubated with or without DBPG (200 μM) for 24 h. Cells were resuspended in 1 mL of PBS and 4 mL of 70% ice-cold ethanol. The cells were incubated overnight at 4 °C. The cells were washed with PBS and then resuspended in 250 μL of propidium iodide solution (50 μg/mL) containing 1 μL of 20 μg/mL RNase A. The cells were incubated in the dark setting for 15 min at room temperature and then analyzed by FACScan flow cytometer (Elite ESP, Beckman Coulter, Brea, CA, USA). A minimum of 10000 cells were collected, and the cell distribution in each phase of the cell cycle was determined using Windows Multiple Document Interface (WinMDI) software.

Measurement of Reactive Oxygen Species and Glutathione Production. BNLCL2 and HaCaT cells (1×10^5 cells/mL) were cultured in the presence of DBPG and AA (20, 50, and 100 μM) for 24 h and then with 0.1 mM H_2O_2 at 37 °C for 1 h. For cellular ROS content analysis, after replacement with new medium, cells were incubated in 100 μL of culture medium containing 10 μM 2′,7′-dichlorofluorescein diacetate (DCFH-DA) at 37 °C for 30 min. Fluorescence intensities of 2′,7′-dichlorofluorescien (DCF) were measured by excitation (*Ex*) at 504 nm and emission (*Em*) at 524 nm using a multidetection microplate reader (BioTek, Synergy2). For cellular GSH level analysis, cells were treated with new medium and treated with 50 μg/mL of *o*-phthalaldehyde (OPA) in 0.1 M Na_2HPO_4−5 mM EDTA buffer in the dark at 25 °C for 1 h. After two washing steps with phosphate buffer (pH 7.0), the GSH fluorescence was measured by *Ex* 355 nm and *Em* 460 nm.

Measurement of Glutathione Peroxidase and Catalase Activities. GPx and CAT activities were measured as described elsewhere,[18,19] but with the following modifications. BNLCL2 and HaCaT cells (1×10^5 cells/mL) were incubated with DBPG and AA (20, 50, and 100 μM) for 24 h and then with 0.1 mM H_2O_2 at 37 °C for 1 h. Cells were collected and centrifuged at 1000*g* for 10 min. The pellets were then resuspended in phosphate buffer (pH 7.0) and lysed by three cycles of freezing, thawing, and sonication, each for 10 s at 60 W. The cellular homogenates were centrifuged at 10000*g* for 30 min, and the supernatants were collected for the subsequent determination of GPx and CAT activities. The intracellular activities of GPx were measured by monitoring the decrease in the absorbance due to oxidation of nicotinamdie adenine dinucleotide phosphate (NADPH).[18] Briefly, to a reaction mixture that contained 1 mM GSH, 1 unit/mL glutathione reductase (GR), 1 mM NaN_3, 1 mM

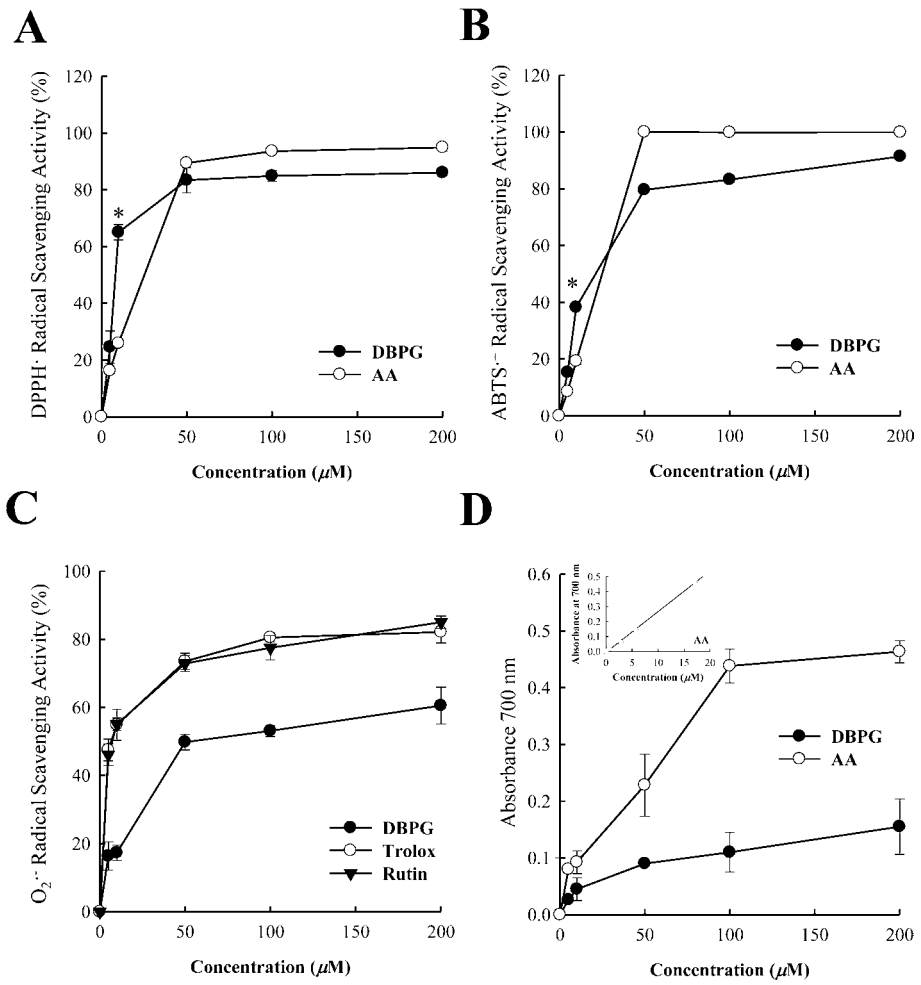


Figure 2. Free radical scavenging activities and reducing power of DBPG: (A) $DPPH^{\bullet}$ and (B) $ABTS^{\bullet+}$ radical scavenging capacities of several concentrations (0, 5, 10, 50, 100, and 200 μM) of DBPG and AA; (C) $O_2^{\bullet-}$ scavenging abilities of DBPG, trolox, and rutin (0, 5, 10, 50, 100, and 200 μM); (D) reducing power of DBPG and AA standard. Each value represents the mean ± SD from triplicate experiments. Statistical significance is determined by (*)$p < 0.05$ relative to results for AA group. ANOVA follows the Tukey–Kramer test.

EDTA, 0.2 mM NADPH, and 0.1 mL of cellular extract (40 μg) was added H_2O_2 (2.5 mM, 0.1 mL) 5 min after the formation of the mixture to determine GPx activity. The absorbance at 340 nm was measured at 3 min. The activity of CAT was measured as described elsewhere with minor modifications.[19] Cellular extracts (40 μg) were reacted with H_2O_2 (5 mM, 0.1 mL) in phosphate buffer (total volume of 1 mL), and the absorbance at 240 nm at 3 min was monitored.

Statistical Analysis. The results are expressed as the mean ± standard deviation (SD). Statistical differences were estimated by one-way analysis of variance (ANOVA) following Dunnett's test or the Tukey–Kramer test. A p value of 0.05 was regarded as significant. The data were analyzed and the figures plotted using SigmaPlot version 8.0 and SigmaStat version 2.03 software (Chicago, IL, USA).

■ RESULTS AND DISCUSSION

Free Radical Scavenging Activities of DBPG. The electron-donating abilities of DBPG and AA determined by examining the reduction of $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ with increasing concentrations of DBPG and AA (0, 5, 10, 50, 100, and 200 μM) are shown in Figure 2A,B. DBPG and AA exhibited significant free radical scavenging activity with EC_{50} (median scavenging concentration) values of 8.1 and 25.1 μM for $DPPH^{\bullet}$ and 21.3 and 25.2 μM for $ABTS^{\bullet+}$, respectively. In the $ABTS^{\bullet+}$ radical scavenging assay, the TEAC value derived from the dose–response curve for DBPG is 3.4 mM trolox/g. Although the free radical scavenging activity of DBPG was weaker than that of AA, DBPG and AA showed similar effects on the scavenging of free radicals at the high concentration (200 μM). The $O_2^{\bullet-}$ scavenging ability of DBPG evaluated using the PMS–NADH–NBT system[14] is presented in Figure 2C. DBPG inhibited the activity of $O_2^{\bullet-}$ in a dose-dependent manner. The $DPPH^{\bullet}$ assay was used to study the ability of the investigated DBPG to donate hydrogen atoms or electrons in the transformation of the $DPPH^{\bullet}$ into its reduced from, $DPPH^{\bullet}$–H. The performed radical monocation of $ABTS^{\bullet+}$ is generated by oxidation of ABTS with potassium persulfate and is reduced in the presence of the hydrogen-donating antioxidants. Moreover, $O_2^{\bullet-}$, molecular oxygen, just reduced to have one extra electron, is one of the most representative free radicals. Depending on the electron-transferring/hydrogen-donating ability, the potential multiple antioxidant activities of DBPG are demonstrated by these $DPPH^{\bullet}$, $ABTS^{\bullet+}$, and $O_2^{\bullet-}$ radical scavenging assays.

Reducing Power and Chelating Ability of DBPG. Previous studies have asserted that the reducing capacity of and chelation of metal ions by a compound may be significant indicators of its potential antioxidant activity.[20] As shown in Figure 2D, unlike AA, DBPG had almost no reducing power as calculated by comparison with the reactivity of the standard,

dx.doi.org/10.1021/jf302329m | J. Agric. Food Chem. 2012, 60, 7690–7696

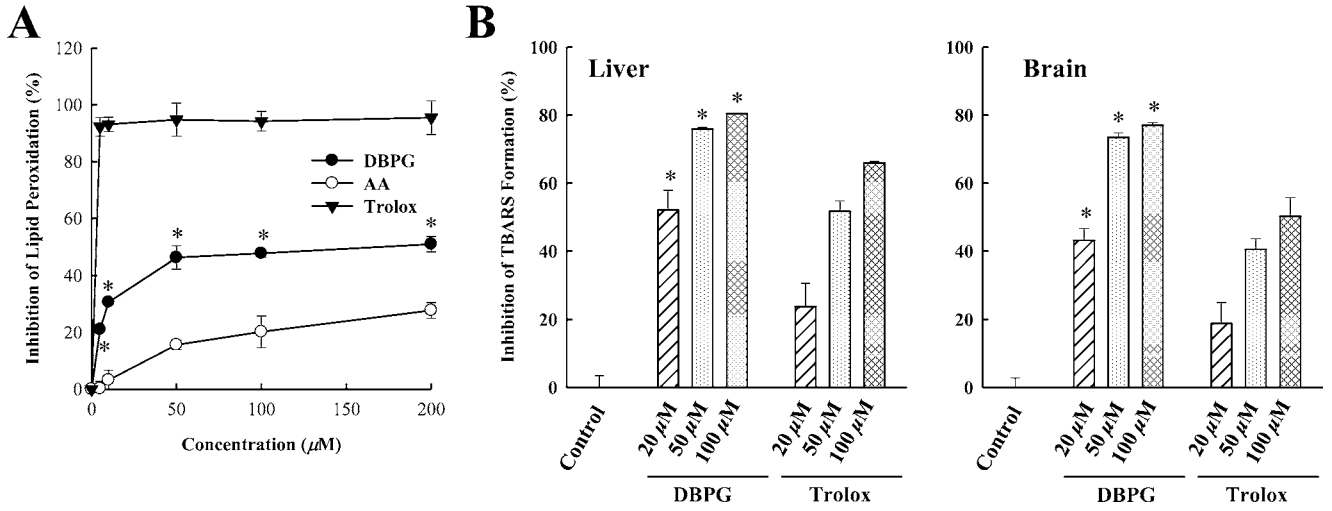


Figure 3. Inhibition of LP formation by DBPG: (A) inhibition of LP formation in Fe^{2+}/ascorbate system by increasing doses of DBPG, AA, and trolox (0, 5, 10, 50, 100, and 200 μM), using liposomes as an oxidizable substrate; (B) extent of formation of TBARS following incubation of DBPG and trolox (20, 50, and 100 μM) with mouse liver and brain homogenates. Each value represents the mean ± SD from triplicate experiments. Statistical significance is determined by (*) $p < 0.05$ compared with AA (A) or trolox (B). ANOVA follows the Tukey–Kramer test.

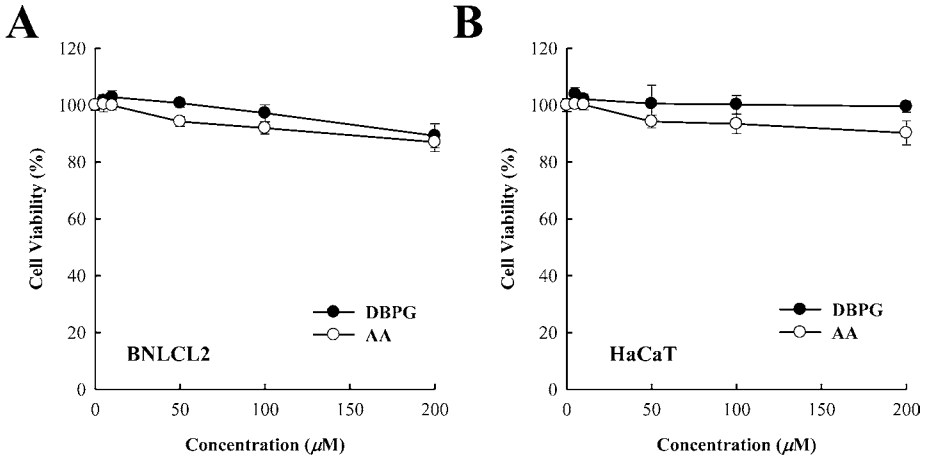


Figure 4. Cell viability of DBPG and AA in liver and skin cells. After treatment with DBPG and AA (0, 5, 10, 50, 100, and 200 μM) of (A) BNLCL2 and (B) HaCaT cells for 24 h, cell viability was investigated by MTT assay. Each value represents the mean ± SE of three individual experiments.

AA. The reducing power of 1 g of DBPG was equivalent to that of 1.3 mM AA, revealing that DBPG weakly reduced Fe^{3+} ions. By comparison with EDTA, DBPG exhibited much weaker Fe^{2+}-chelating activity (data not shown). These results suggest that the reducing power and chelating capacity of DBPG are not primary factors determining the antioxidative activity.

Inhibition of Lipid Peroxidation by DBPG. Lipid peroxidation is recognized as a potential mechanism of injury to liver mitochondria, microsomes, and splenic lymphocytes. In the presence of $OH^{\bullet}$, lipid radicals ($L^{\bullet}$) are believed to be formed from the reaction with polyunsaturated fatty acids (LH), and their subsequent reaction with oxygen forms the lipid peroxyl radical ($LOO^{\bullet}$), which damages cell and DNA architecture. Products of LP, such as malondialdehyde (MDA), form adducts with cellular DNA.[21] The capacity of DBPG to inhibit LP in a liposome model system was evaluated by measuring the MDA content. As presented in Figure 3A, the inhibition of LP by DBPG was much weaker than that by trolox, but the inhibition of LP by DBPG exceeded that by AA. TBARS, the final stable aldehyde products of peroxidation, are generated by the breakdown of peroxides and are critical indicators of cellular damage.[22] The inhibition of the formation of TBARS provides good evidence for the inhibition of LP by a compound. The reductions of TBARS content by DBPG of 20, 50, and 100 μM were 52.1, 75.9, and 80.4% in mouse liver tissue and were 43.2, 73.5, and 77.0% in mouse brain tissue, respectively (Figure 3B). Adding DBPG to mouse liver and brain tissue inhibited the formation of TBARS to a greater extent than did trolox. These results suggest that the antioxidant properties of DBPG may involve the inhibition of LP, one of the most important antioxidant characteristics of DBPG.

Scavenging of Reactive Oxygen Species by DBPG and Associated Cell Viability. Oxidative stress is a ubiquitous condition in which the generation of free radicals and ROS exceeds the capacity of endogenous antioxidant defense and repair mechanisms.[23] To understand the protective action of DBPG, oxidative damage in a cellular system was examined. To explore whether DBPG protects hepatocytes and keratinocytes against oxidative stress, the ROS and GSH contents of H_2O_2 on BNLCL2 and HaCaT cells in the presence and absence of DBPG were determined. First, after treatment of BNLCL2 and

dx.doi.org/10.1021/jf302329m | J. Agric. Food Chem. 2012, 60, 7690–7696

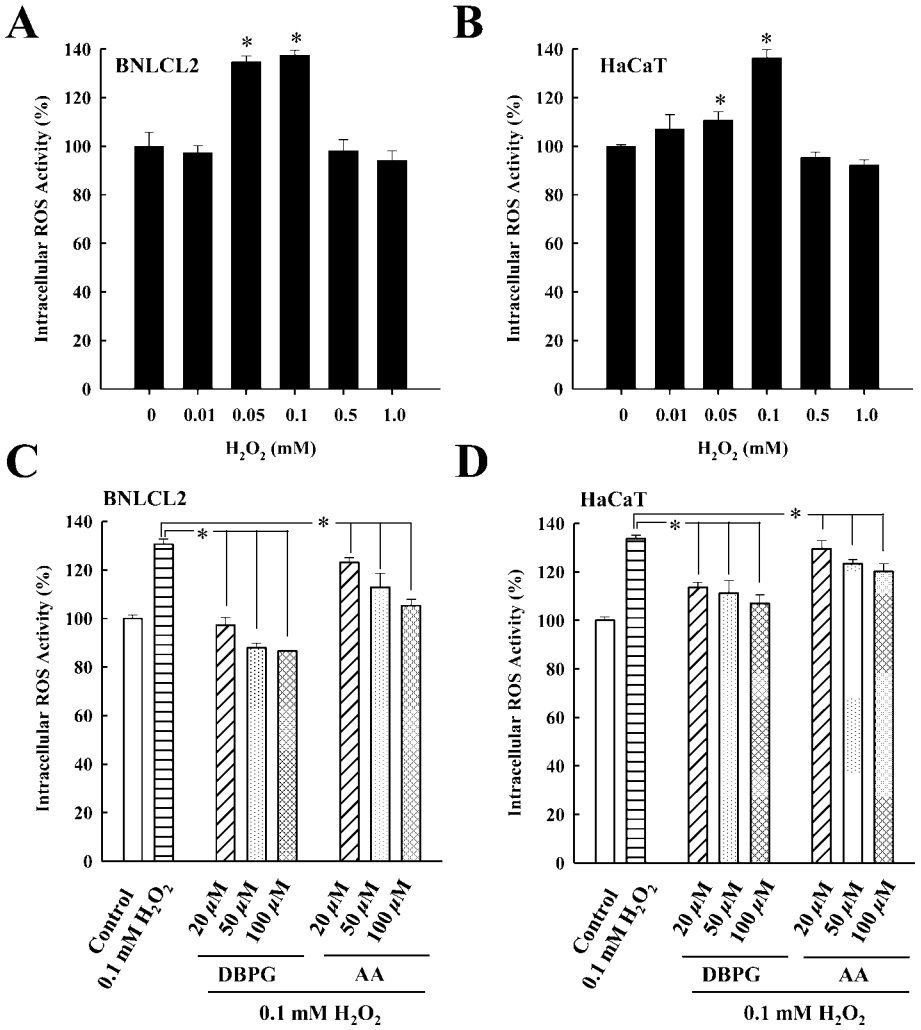


Figure 5. Changes in intracellular ROS level caused by DBPG in H_2O_2-treated BNLCL2 and HaCaT cells. After incubation in H_2O_2 (0, 0.01, 0.05, 0.1, 0.5, and 1 mM) of (A) BNLCL2 and (B) HaCaT cells for 1 h, ROS activity was determined by DCF fluorescence intensity. After pretreatment with DBPG and AA (20, 50, and 100 μM) of (C) BNLCL2 and (D) HaCaT cells for 24 h, the intracellular ROS level of 0.1 mM of H_2O_2-induced cell damage was measured. Each value represents the mean ± SD from triplicate experiments. Statistical significance is determined by (*) $p < 0.05$ relative to 0 mM H_2O_2 (A, B) or 0.1 mM H_2O_2 (C, D). ANOVA follows Dunnett's test.

HaCaT cells with DBPG and AA for 24 h, cell viability was assayed by an MTT method. No significant effect on cell viability was observed at test concentrations up to 200 μM DBPG (>90% cell survival) (Figure 4). Moreover, after treatment of BNLCL2 and HaCaT cells with DBPG (200 μM) for 24 h, cell cycle progression was assayed by flow cytometry. No significant change on cell cycle distribution was observed at 200 μM DBPG in BNLCL2 and HaCaT cells (Supplement data 1 in the Supporting Information). BNLCL2 and HaCaT cells were incubated with 0.01−1 mM H_2O_2 for 1 h, and no significant change in cell viability occurred at an H_2O_2 concentration of <0.5 mM (Supplement data 2 in the Supporting Information). Previous investigations have demonstrated that low regulatory H_2O_2 concentrations induce a large up-regulation of catalase, which is a fingerprint of the cellular oxidative stress response, but glyceraldehyde-3-phosphate dehydrogenase (GAPDH) oxidation and the consequent decrease in activity are observed only at high death-inducing H_2O_2 doses.[24] H_2O_2 generates aldehydic DNA lesions (ADLs) at low concentration (0.06 mM) in HeLa cells, and lower concentrations of H_2O_2 were much more efficient at inducing ADLs than higher concentrations.[25] In this study, in the treatment of BNLCL2 (Figure 5A) and HaCaT cells (Figure 5B) with serial concentrations (0, 0.01, 0.05, 0.1, 0.5, and 1 mM) of H_2O_2 for 1 h, the greatest ROS activity was obtained at 0.1 mM H_2O_2. Therefore, the inhibition of intracellular ROS by DBPG and AA in BNLCL2 and HaCaT cells was examined using 0.1 mM H_2O_2. In the treated BNLCL2 (Figure 5C) and HaCaT (Figure 5D) cells, the ROS levels were, respectively, 130.6 and 133.7% higher than that in the untreated cells. Pretreatment with DBPG at 20, 50, and 100 μM reduced H_2O_2-induced ROS formation in BNLCL2 cells from 97.2 to 86.5% and in HaCaT cells from 113.6 to 106.9%. DBPG-pretreated cells exhibited levels of ROS significantly and dose-dependently lower than cells treated with H_2O_2 only. GSH is modulated in disorders that are caused by free radical attack.[18,26] Measurement of the GSH level is useful in quantifying oxidative stress and can be used to monitor the effectiveness of antioxidant intervention strategies. In this paper, the effect of DBPG on GSH levels in H_2O_2-treated BNLCL2 and HaCaT cell was investigated. However, no significant effect on GSH level was observed (Supplement data 3 in the Supporting Information).

Antioxidant enzymes are the body's primary defense, protecting biological macromolecules against oxidative injury

dx.doi.org/10.1021/jf302329m | J. Agric. Food Chem. 2012, 60, 7690−7696

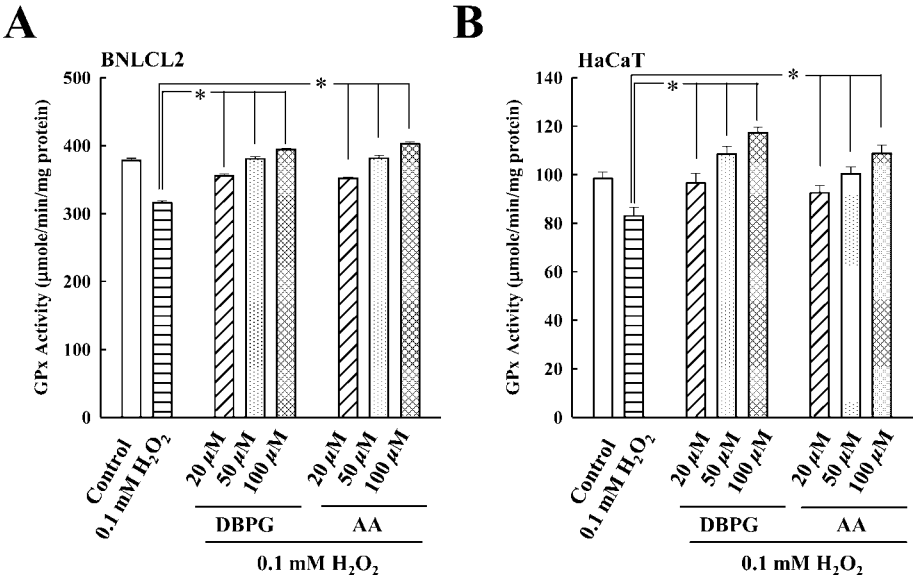


Figure 6. Up-regulation of GPx activities by DBPG in H_2O_2-treated BNLCL2 and HaCaT cells. After (A) BNLCL2 and (B) HaCaT cells were cultured in the presence of DBPG and AA (20, 50, and 100 μM) for 24 h and then with 0.1 mM H_2O_2 for 1 h, GPx activity was investigated. Each value is the mean ± SD from triplicate experiments. Statistical significance is determined by (*) $p < 0.05$ relative to 0.1 mM H_2O_2. ANOVA follows Dunnett's test.

and removing peroxides, free radicals, and superoxide anions that are generated within cells. GPx and CAT are the main enzymes that remove hydrogen peroxide generated by SOD in cytosol and mitochondria through oxidizing GSH to GSSG.[26] Figure 6 displays the effect of DBPG and AA on GPx activity. When H_2O_2 (0.1 mM)-incubated BNLCL2 (Figure 6A) and HaCaT (Figure 6B) cells were pretreated with various concentrations of **1** and AA (20, 50, and 100 μM), GPx activities were significantly increased in a concentration-dependent manner. At test concentrations of 100 μM DBPG, GPx activities in BNLCL2 and HaCaT were 1.3 and 1.4 times higher than in H_2O_2-treated cells. On the other hand, no change in the CAT activities in H_2O_2-induced BNLCL2 and HaCaT cells was observed upon exposure to DBPG (Supplement data 4 in the Supporting Information). The reduction of ROS production and up-regulation of GPx activity in BNLCL2 and HaCaT cells may provide clues regarding the antioxidant mechanism of DBPG in protecting cells against oxidative damages.

In conclusion, Lin et al.[7] has reported that DBPG showed scavenging activity against $DPPH^{\bullet}$ only. This study further used more free radical scavenging assays to realize the antioxidant characteristics of DBPG. It is likely because the phenolic groups in the structure of DBPG prevent chain initiations, inhibit the formation of lipid peroxyl radicals, and scavenge active oxygen species. To the best of our knowledge, this is the first confirmation of the protective effects of DBPG on liver and skin cells that are oxidatively damaged by H_2O_2. Down-regulation of ROS and up-regulation of GPx activities in hepatocytes and keratinocytes may play very important roles in the cytoprotective antioxidant mechanisms of DBPG. DBPG was found to be able to scavenge most free radicals, modulate antioxidant enzyme, and reduce oxidative damage. It is believed that DBPG may be a good food and cosmetic additive.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

‖These authors contributed equally to this work.

Funding

We thank the National Science Council of the Republic of China, Taiwan, for financially supporting this research under Contracts NSC 100-2622-E-041-001-CC3 and 100-2320-B-041-003-MY3.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Chung-Yi Chiang, Ph.D., a senior scientist at Unilever, for carefully revising the manuscript.

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