Food Chemistry 112 (2009) 369–373

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Biological functions of a synthetic compound, octadeca-9,12-dienyl-3,4,5-hydroxybenzoate, from gallic acid–linoleic acid ester

Aera Jang [a], Na-Young Lee [b], Bong-Deok Lee [c], Tae Hoon Kim [d], Jun Ho Son [e], Bong Jeun An [e], Cheorun Jo [c,*]

[a] Animal Products and Processing, National Institute of Animal Science, Suwon 441-706, Republic of Korea
[b] Department of Food Science and Technology, Chonbuk National University, Jeonju 561-756, Republic of Korea
[c] Department of Animal Science and Biotechnology, Chungnam National University, Daejeon 305-764, Republic of Korea
[d] Department of Herbal Medicinal Resources, Daegu Haany University, 712-715, Republic of Korea
[e] Department of Cosmeceutical Science, Daegu Haany University, 712-715, Republic of Korea

ARTICLE INFO

Article history:
Received 20 August 2007
Received in revised form 14 March 2008
Accepted 22 May 2008

Keywords:
Octadeca-9,12-dienyl-3,4,5-hydroxybenzoate
Gallic acid
Linoleic acid ester
Antioxidant
Cancer cell proliferation

ABSTRACT

Octadeca-9,12-dienyl-3,4,5-hydroxybenzoate (GA-LA) was synthesized chemically from gallic acid and linoleic acid ester and its biological function was investigated for further application purpose. This newly synthesized compound GA-LA possesses a strong and synergistic effect on cancer cell proliferation inhibition; however, no synergistic effect was found for 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, ferric reducing antioxidant power (FRAP), and inhibition effect against xanthine oxidase, hyaluronidase, and tyrosinase. Nevertheless, synthetic GA-LA possessed comparable biological activities when compared with gallic acid and tocopherol. With anti-mutagenic activity it can be concluded that this compound may be a useful functional material for the food, pharmaceutical, and cosmetic industry.

1. Introduction

Gallic acid is a phenolic compound and an important substrate for the synthesis of propyl gallate in the food industry and trimethoprim in the pharmaceutical industry (Wei, Zhang, Xu, & Ito, 2001). Since gallic acid is regarded as an antioxidant to prevent rancidity and spoilage in fats and oils, it has been used as an additive in cosmetics and foods such as shortnenings, baked goods, candy, and chewing gum (Lee, 2007). There are many reports that gallic acid possesses free radical scavenging effects, anti-allergic, anti-inflammatory, anti-mutagenic, and anti-carcinogenic activity (Fukumoto & Mazza, 2000; Lin, Lin, Liang, Lin-Shiau, & Juan, 1998; Negi et al., 2005). Also, some gallic acid esters are widely used as food additives to prevent food oxidation. Gallic acid derivatives, such as epicatechin, epicatechin gallate, and epigallocatechin gallate have been found in many phyto-medicines with various biological and pharmaceutical activities including free radical scavenging effect (Masaki, Atsumi, & Sakurai, 1995), inducing apoptosis of cancer cells, protecting cells from damage induced by UV or irradiation (Saeki et al., 2000). However, the cytotoxic and skin-trouble effects of gallic acid have also been reported, including a prooxidant effect caused by copper-dependent DNA damage (Yoshino et al., 2002).

Long chain fatty acids such as linoleic acid, α-linoleic acid, and docosahexaenoic acid have shown anti-inflammatory effects by decreasing the secretion of interleukin (IL)-6 and -1β, and the tumor necrosis factor α (Zhao et al., 2005). Wacher and Benet (2001) developed a new formulation for pharmaceutical compounds, gallic acid esters, to enhance the bioavailability of the active ingredient of the pharmaceutical compounds using not the long chain fatty acids such as linoleic acid (C18:2) but the medium chain fatty acids (C6–C14). From our previous study, octadeca-9,12-dienyl-3,4,5-hydroxybenzoate (GA-LA) synthesized from gallic acid and linoleic acid ester was reported to possess 90.9, 74.0, and 32.0% tyrosinase, cyclooxygenase (COX)-1, and COX-2 inhibition activity, respectively (Jo, Jeong, Lee, Kim, & Byun, 2006).

However, it is well known that high doses of gallic acid and many synthetic antioxidants possess carcinogenic activity (Ito et al., 1986). Therefore, the objective of this study was to evaluate further beneficially biological functions of the synthetic novel compound octadeca-9,12-dienyl-3,4,5-hydroxybenzoate (GA-LA) from gallic acid and linoleic acid ester for its potential use in the food, pharmaceutical, and cosmetic industries.

* Corresponding author. Address: Department of Animal Science and Biotechnology, Chungnam National University, 220, Gung-dong, Yuseong-gu, Daejeon 305-764, Republic of Korea. Tel.: +82 42 821 5774; fax: +82 42 825 9754.
E-mail address: cheorun@cnu.ac.kr (C. Jo).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.05.074

2. Materials and methods

2.1. Materials

Gallic acid, diisobutylaluminum hydride (DIBAL-H), tetrahydrofuran (THF), methyl-d_3 alcohol d (CD_4O), *N,N'*-dicyclohexylcarbodiimide (DCC), L-3,4-dihydroxyphenyl-alanine (L-DOPA), mushroom tyrosinase, xanthin, xanthin oxidase, hippury-histidyl-leusine (HHL), angiotensin converting enzyme, penicillin/streptomycin, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), tocopherol, 1,1-diphenyl-1-picrylhydrazyl (DPPH), 2-nitrofluorene (2-NF), 4-nitroquinoline-1-oxide (4-NQO), 2-aminoanthracene (2-AA), benzo[a]pyrene (B[a]P), sodium azide (SA), fetal bovine serum and dimethylsulfoxide (DMSO) were purchased from the Sigma–Aldrich Co. Ltd. (St. Louis, MO, USA). 2-Aminofluorene (2-AF) was purchased from the Fluka Chemical Co. Ltd. (Japan). Hyaluronidase, hyaluronic acid sodium salt, 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp), concanavalin A (ConA), 2,4,6-tripyridyl-2-triazine (TPTZ), $FeCl_3{\cdot}7H_2O$, CH_2Cl_2, $NaHCO_3$, sodium acetate, phosphomolybdic acid, ethyl acetate, methyl alcohol, ethyl alcohol, hexane, NaCl, $CHCl_3$, $CaCl_2$, NH_4NO_3, K_2HPO_3, K_2HPO_4, $MgSO_4$, nutrient broth (Difco, Sparks, MD, USA), tryptic soy agar (Difco, Sparks, MD, USA), peptone (Oxoid, Basingstoke, UK), total plate count agar (Difco, Sparks, MD, USA), eosin methylene blue agar (Oxoid, Basingstoke, UK), oxoid nutrient broth No 2. (Oxoid, Basingstoke, UK), RPMI 1640 (Gibco BRL Co., Grand Island, N.Y., USA), DMEM medium (Gibco BRL Co., Grand Island, N.Y., USA), cofactor (Wako Co., Lot No. 999902, Tokyo, Japan) were purchased for experimental study. S9 mix was purchased from Oriental yeast Co., Ltd. (Lot No. 0042101, Tokyo, Japan).

2.2. Synthesis

The synthesis was performed by the method of Jo et al. (2006) as described by the following steps.

2.2.1. Synthesis of octadeca 9,12-diene-ol

To a solution of linoleic acid methyl ester (3 mM, compound **1**) in CH_2Cl_2 (10 ml) cooled at −78 °C, diisobutylaluminum hydride (DIBAL-H, 6 mM) was slowly added under N_2 atmosphere. After the solution had been allowed to stir for 1 h, aqueous 5% $NaHCO_3$ was slowly added to interrupt the reaction and then the reaction mixture was diluted with methyl alcohol. The mixture was filtered, and the resulting filtrate was evaporated under reduced pressure and purified by column chromatography (SiO_2; elution with hexane:ethanol = 10:1). The purified compound was confirmed by spectroscopic methods (FR-IR and NMR, data not shown). Octadeca-9,12-diene-ol, compound **2**, was obtained at a 90% yield as a yellow oil.

2.2.2. Synthesis of octadeca-9,12-dienyl-3,4,5-hydroxybenzoate (GA-LA)

To a solution of gallic acid (5.5 mM, compound **3**) and compound **2** (5.5 mM) in tetrahydrofuran (THF, 15 ml) cooled at 0 °C, a solution of *N,N'*-dicyclohexylcarbodiimide (DCC, 16.5 mM) was added slowly. After the solution had been allowed to stir for 20 h at room temperature (ca. 23 °C) the reaction mixture was washed with ethyl acetate several times and filtered. The filtrate was washed successively with a diluted aqueous 5% citric acid solution, saturated aqueous $NaHCO_3$ solution, and water, dried by $MgSO_4$, filtered, and evaporated. The crude compound was purified by column chromatography (SiO_2; elution with $CHCl_3$:methanol, 95:2 (v:v)). Purified compound was confirmed by NMR spectroscopy and FT-IR (Tensor 37, Bruker, Germany). The GA-LA **4** was obtained at an 85% yield as a pale yellow powder and its characteristics were ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.91 (t, J = 6.8 Hz. 3H, H18), 1.31 (m, 16H, H3-7, H15-17), 1.72 (m, 2H, H2), 2.06 (m,4H, H8, H14), 2.79 (dd, J = 5.9, 5.9 Hz, 2H, H11), 4.26 (t, J = 6.4 Hz, 2H,-OCH2), 5.37 (m, 4H), 7.28(s, 1H, aromatic), 7.30 (s, 1H, aromatic); IR (CHCl3) 3356, 3008, 2928, 2855, 1686, 1613, 1465, 1395, 1242, 1184 cm^{-1}.

2.3. Biological functions

2.3.1. Anti-oxidative effects

2.3.1.1. 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. Free radical scavenging activity was estimated according to the method of Blois (1958) with some modification. The sample (10 ppm, 1 ml) was added into the 0.2 mM DPPH radical solution (1 ml) and vortexed. The mixture was reacted for 30 min at room temperature and the absorbance was measured at 517 nm with a spectrophotometer. Tocopherol (10 ppm) was used as a positive control. The scavenging activity of the DPPH radicals in percentage points was calculated by the following equation: scavenging activity (%) = $[1-(A_1/A_0)] \times 100$, where A_0 is the absorbance of the blank and A_1 is the absorbance of the sample.

2.3.1.2. Ferric reducing antioxidant power (FRAP) assay. The FRAP assay was estimated according to the procedure described by Benzie and Strain (1996). The FRAP reagent contained 2.5 ml of 10 mM TPTZ solution in 40 mM HCl plus 2.5 ml of 20 mM $FeCl_3$ and 25 ml of 0.3 M acetate buffer, pH 3.6, and it was freshly prepared. A 30 μl sample was added to 900 μl of FRAP reagent and 90 μl distilled water, and then they were mixed. The test samples and reagent blank were incubated for 30 min at 37 °C. The absorbance of the reaction mixture was measured at 595 nm. FRAP value of samples was calibrated with a ferrous sulfate ($Fe_2SO_4{\cdot}7H_2O$) curve within the range from 125 to 5000 μM. The reducing power was expressed as equivalent concentration.

2.3.2. Tyrosinase inhibition effect

The tyrosinase inhibition effect was measured spectrophotometrically by following the increase in absorbance by production of dopachrome from L-DOPA (Jimenez, Chazarra, Escribano, Cabanes, & Garcia-Carmona, 2001). A sample was added to a reaction mixture containing 10 mM L-3,4-dihydroxyphenyl-alanine (L-DOPA, Sigma Chemical Co., St. Louis, MO, USA) solution, 1/15 M sodium phosphate buffer (pH 6.8) and mushroom tyrosinase (100 unit/ml, Sigma). The reaction mixture was incubated at 25 °C for 15 min. The amount of dopachrome produced in the reaction mixture was determined at 475 nm by a spectrophotometer.

2.3.3. Hyaluronidase inhibition activity

Hyaluronidase inhibition activity was assayed by estimating the amount of *N*-acetylglucosamine released. Inhibition activity of the test compounds on activation of hyaluronidase was determined by the method as described by Reissing, Strominger, and Lelori (1955). Hyaluronidase was preincubated with the test compounds at 37 °C for 20 min in 0.1 M acetate buffer (pH 4.0). After preincubation for 20 min, calcium chloride (2.5 mM) was added to the above solution and incubated at 37 °C for 20 min. Then the reaction was started by adding hyaluronic acid sodium salt (0.4 mg/ml) to each reaction mixture by incubating at 37 °C for 40 min. As a control, test compounds were replaced by the buffer solution. Absorbance was measured at 585 nm. The inhibition activity was calculated as follows: inhibition (%) = $[(A-B)-(C-D)]/(A-B) \times 100$. A: Absorbance of control, B: Absorbance of blank (without hyaluronidase) for the control, C: Absorbance involving test compounds, D: Absorbance of test compounds alone.

2.3.4. Xanthine oxidase (XOase) inhibition effect

Xanthine oxidase (XOase) inhibition effect was determined spectrophotometically by measuring uric acid formation at 295 nm (Nam, Choi, Kang, Kozukue, & Friedman, 2005). The reaction mixture was prepared with 0.1 M sodium phosphate buffer (pH 7.4), 2 mM xanthine, and 0.2 unit xanthine oxidase. Xanthine oxidase activity was expressed as the percent inhibition of xanthine oxidase.

2.3.5. Cancer cell proliferation inhibition effect

The test cell lines of G361 (skin cancer, human origin), B16F10 (skin cancer, mouse origin), A549 (lung cancer), HT-29 (colon cancer), and SK MEL II (human melanoma) were purchased from the Korean Cell Line Bank (KCLB). The cells were adapted in a RPMI 1640 medium (Gibco BRL Co., Grand Island, N.Y., USA) and B16F10 was adapted in a DMEM medium with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (100 unit/ml) at 37 °C in a 5% CO_2 incubator and used for a proliferation. The determination of the optimum inoculation cell number for the growth inhibition of a cancer cell was performed by determining the time of the log phase. Inhibition effect of the proliferation of the cancer cells by the sample was determined by following the procedure of Carmichael, Degraff, Gazdar, Minna, and Michell (1987). Each cancer cell (5×10^4) was inoculated into a 96-well plate. After 24 h at room temperature, samples with different concentrations were added into the well. The control was prepared by adding the same volume of dimethyl sulfoxide (DMSO). After 72 h of incubation at 37 °C in a 5% CO_2 incubator, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution (5 mg/ml, 20 μl) was added and incubated for another 4 h. The growth medium was removed and 150 μl of DMSO:ethanol (1:1) was added into each well and vortexed for 30 min. The reaction mixture was read by an ELISA reader (Emax, Molecular Devices, Sunnyvale, USA) at 550 nm. The growth inhibition rate was calculated as follows: growth inhibition effect (%) = 100 × (absorbance of the sample/absorbance of the blank).

2.3.6. Anti-mutagenicity

Anti-mutagenicity of the samples was performed by a method of Lee, Jo, Sohn, Kim, and Byun (2006) which was originally modified by the Ames test (Maron & Ames, 1983). Strains of *Salmonella Typhimurium* TA98 were provided by the Korea Research Institute of Chemical Technology (KRICT, Daejeon, Korea). The strains were originally purchased from Molecular Toxicology Inc. (Boone, NC, U.S.A.) and cultured by the Korea Institute of Toxicology at KRICT. Each strain was tested for its genetic traits such as histidine requirement, deep rough (rfa) characteristic, UV sensitivity (uvrB mutation), and ampicillin- or tetracycline-resistance by the R-factor before use. The strains were inoculated on Oxoid nutrient broth No. 2 (Oxoid Co., Ltd., Hampshire, England) and cultured for 10 h at 37 °C with continuous agitation at 200 rpm (Vision Scientific Co., Incheon, Korea). The cell density was 2×10^9 CFU/ml. The S9 mixture (500 μl) was mixed when a metabolic activation was incorporated.

The inhibitions of the mutagenic activity of the 4-nitroquinoline-1-oxide (4-NQO), the 3-amino-1,4-dimethyl-5 H-pyrido[4,3-b]-indole (Trp), the 2-nitrofluorene (2-NF), the 2-aminoanthracene (2-AA), the Benzo[a]pyrene (B[a]P), and the 2-Aminofluorene (2-AF) by the tested samples were determined by using *S. Typhimurium* TA 98. The doses of the mutagens were selected from the dose-effect curves determined by preliminary experiments. The concentration of mutagens used, 4-NQO, Trp, 2-NF, 2-AA, B[a]P, and 2-AF were used 0.5, 1, 10, 0.4, 10, and 30 μg/plate, respectively. For each sample, three agar plates were used and the number of revertants per plate was counted with a colony counter.

2.4. Statistical analysis

All experiments were duplicated with three observation numbers adapted for each experiment. Analysis of the variance was performed using the raw data, and the mean values and standard deviation were calculated by the Statistical Analysis System (SAS Institute, 2000). Differences among the mean values were determined by the Duncan's multiple range test with a significance defined at $p < 0.05$.

3. Results and discussion

3.1. Anti-oxidative effect

Free radical scavenging activity of GA-LA at 10 ppm was superior to that of linoleic acid and tocopherol ($p < 0.05$), while lower than gallic acid (Table 1). This result suggested that gallic acid itself is a strong antioxidant but its synergistic effect with linoleic acid was not apparent at a concentration of 10 ppm. Jo et al. (2006) reported that 0.24 mM GA-LA showed identical DPPH radical scavenging activity as gallic acid and ascorbic acid, which was in the range of 95.2–96.2% ($p > 0.05$).

Antioxidants can be explained as reductants, and inactivation of oxidants by reductants can be described as redox reactions in which one reaction species (oxidant) is reduced at the expense of the oxidation of another antioxidant (Siddhuraju, 2007). Antioxidant power of the GA-LA was assayed from their ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II) complex (Table 1). Gallic acid had higher reducing power (30.17 mM kg^{-1}) than GA-LA (0.69 mM kg^{-1}) and tocopherol (2.29 mM kg^{-1}) at a concentration of 50 ppm ($p < 0.05$). Linoleic acid did not show any reducing activity at all. This result agrees with the DPPH radical scavenging activity test and also there was no synergistic effect found in this assay. However, the reducing power of GA-LA and tocopherol was not significantly different.

3.2. Tyrosinase inhibition effect

The inhibitory effect on tyrosinase was examined to investigate possibilities for its application in cosmetic materials (Table 2). Since tyrosinase is a key enzyme for melanin biosynthesis in plants and animals, inhibition of tyrosinase activity may be useful for the treatment of disorders associated with melanin hyperpigmentation (Masamoto et al., 2003).

GA-LA revealed a higher tyrosinase inhibition effect at 500 ppm than that of gallic acid, tocopherol, and linoleic acid ($p < 0.05$). According to Tables 1 and 2, linoleic acid did not possess any antioxidant effect, yet the melanin biosynthetic enzyme was inhibited by linoleic acid at 500 ppm. Neither gallic acid nor linoleic acid alone showed high inhibition effects, but the synergistic effect of synthetic gallic acid and linoleic acid to inhibit tyrosinase activity was observed as 51.51% at a 500 ppm level. Natural products such as green laver, Codiaceae, and the bundles showed 30, 11, and 24% of tyrosinase inhibition at a 1000 ppm level, respectively (Choi, Lee, Kang, Lee, & Lee, 1998) and a green tea polyphenol showed 60% at a 200 ppm level (An et al., 2005). Our findings suggest that the synthetic GA-LA could be more effective as a skin-whitening agent than gallic acid and tocopherol. Interestingly, tocopherol at 500 ppm showed two times higher inhibition effect against tyrosinase than gallic acid ($p < 0.05$). Jo et al. (2006) also reported that tyrosinase was inhibited up to 90.9% and 63.7% at 1.20 mM of GA-LA and ascorbic acid, respectively.

3.3. Hyaluronidase inhibition activity

Hyaluronidase is an enzyme degrading hyaluronic acid (hyaluronan), an important component of the extracellular matrix, which

Table 1
1,1-Diphenyl-2-hydrazyl (DPPH) radical scavenging activity (%) and ferric reducing antioxidant power (FRAP) value (mM g^{-1}) of synthetic octadeca-9,12-dienyl-3,4,5-hydroxybenzoate (GA-LA)[A]

Assay	Concentration (ppm)	GA-LA	Linoleic acid	Gallic acid	Tocopherol
DPPH	10	65.22 ± 0.069[b]	–[d]	96.73 ± 0.591[a]	24.77 ± 2.377[c]
FRAP	50	0.69 ± 0.758[b]	–[b,B]	30.17 ± 2.092[a]	2.29 ± 1.247[b]

[a–d]Means with the same letter in each row are significantly different ($p < 0.001$).
[A] Value are the mean ± S.D.
[B] Activity was not detected.

Table 2
Inhibition effect (%) of synthetic octadeca-9,12-dienyl-3,4,5-hydroxybenzoate (GA-LA) on tyrosinase, hyaluronidase, and xanthine oxidase[A]

Enzyme	Concentration (ppm)	GA-LA	Linoleic acid	Gallic acid	Tocopherol
Tyrosinase	500	51.51 ± 0.227[a]	5.93 ± 0.530[d]	19.02 ± 0.948[c]	37.58 ± 0.250[b]
Hyaluronidase	100	23.84 ± 4.904[a]	1.57 ± 1.415[c]	22.47 ± 4.609[a]	14.93 ± 2.203[b]
Xanthine oxidase	1000	20.03 ± 1.966[a]	–[c,B]	23.74 ± 4.250[a]	11.70 ± 1.295[b]

[a–d]Means with the same letter in each row are significantly different ($p < 0.0001$).
[A] Value are the mean ± S.D.
[B] Activity was not detected.

consists of 1→4-linked β-*D*-glucuronic acid-(1→3)-β-*N*-acetyl-*D*-glucosamine disaccharide units (Spickenreither, Braun, Bernhardt, Dove, & Buschauer, 2006). For this reason, inhibitors of bacterial and mammalian hyaluronidases might be useful agents in the treatment of various diseases, for example, bacterial infections (Hynes & Walton, 2000), cancer (Isoyama et al., 2006), or as contraceptives (Zaneveld et al., 2002).

The synthetic GA-LA at 100 ppm showed relatively higher hyaluronidase inhibition activity than those of linoleic acid and tocopherol, but no difference was found when compared with the activity of gallic acid (Table 2). Therefore, it can be suggested that GA-LA is a competitive functional agent with gallic acid in hyaluronidase inhibition activity for pharmaceutical and cosmetic industries.

3.4. Xanthine oxidase inhibition activity

Xanthine oxidase (XO) catalyzed the metabolism of hypoxanthine and xanthine to uric acid (Kong, Cai, Huang, Cheng, & Tan, 2000). Increase of uric acid leads to the incidence of hyperuricemia such as gout. Accordingly, one of the therapeutic approaches to treat gout is the use of XO inhibitors that prevent the production of uric acid. Table 2 shows the inhibition effect of GA-LA on xanthine oxidase compared to that of linoleic acid, gallic acid, and tocopherol at 1000 ppm. GA-LA showed similar inhibition effect to gallic acid but higher activities than those of linoleic acid and tocopherol. Dew, Day, and Morgan (2005) reported that theaflavin, theaflavin-3-gallate, and theaflavin-3,3-digallate were found to act as xanthine oxidase inhibitors, with IC_{50} values of 66 ± 4.2, 64 ± 4.4, and 49 ± 9.7 μM, respectively. The polyphenols separated from green tea showed 4.27% of activity at a 10 ppm level (An et al., 2004). From our findings it is suggested that GA-LA is not a very strong inhibitor against xanthine oxidase while it has a similar activity to gallic acid.

3.5. Inhibition of cancer cell proliferation

In recent studies, aging and related diseases, such as cancer and coronary heart disease and neurodegenerative disorders such as Alzheimer's disease were found to be related to oxidative and free-radical-mediated reactions (Weinbrenner et al., 2003). In Fig. 1, GA-LA showed very strong inhibition effect on various cancer cells on their proliferation compared to either linoleic acid or gallic acid even at relatively low concentration (10 ppm). In particular, human and mouse melanoma and lung cancer cell were highly susceptible to GA-LA. These results suggest that synthetic GA-LA has

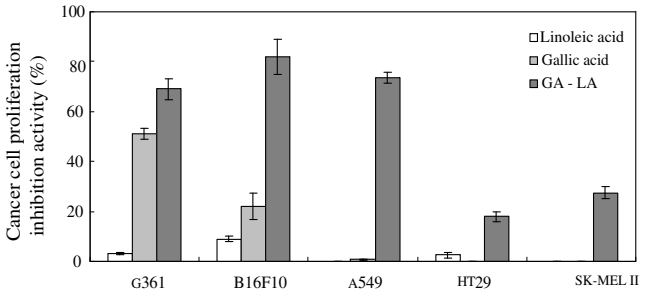

Fig. 1. Cancer cell proliferation inhibition effect by synthetic octadeca-9,12-dienyl-3,4,5-hydroxybenzoate (GA-LA) at 10 ppm (G361, human melanoma; B16F10, mouse melanoma; A549, lung cancer; HT-29, colon cancer; SK-MEL II, human melanoma).

very strong synergistic effect on inhibition of cancer cell proliferation compared with that of either gallic acid or linoleic acid alone at 10 ppm. Gallic acid showed higher inhibition effect on both human and mouse melanoma, however, but little inhibition effects on lung and colon cancer.

Endogenous or dietary factors play a major role in the anti-oxidative defenses of the organism against the ROS generated during normal cellular aerobic respiration (Kohen & Nyska, 2002). Therefore, it is generally assumed that increased intakes of dietary antioxidants may help to shift the balance towards an adequate antioxidant status (Halliwell et al., 1995). According to this, our findings in the present study indicate that our synthetic compound GA-LA may have strong potential to be used as a cytotoxic agent against cancer cells in food, pharmaceutical, and, under certain circumstances, cosmetic industries. Lee (2007) reported that a similar synthetic compound from gallic acid and conjugated linoleic acid inhibited the growth of human melanoma, mouse melanoma, lung and colon cancer cell by 69.9, 89.3, 62.3, and 6.9%, respectively. Also, Kawada et al. (2001) reported that gallic acid can induce apoptosis in four kinds of human lung cancer cell lines *in vitro* and dose dependently reduced cell viability of LL-2 mouse lung cancer cells with IC_{50} (50% inhibitory concentration) value at 200 μM.

3.6. Anti-mutagenic activity against S. Typhimurium TA 98

The anti-mutagenic activity of GA-LA was tested by modified Ames test method against six chemical mutagens including

Table 3
Anti-mutagenic activity of the synthetic octadeca-9,12-dienyl-3,4,5-hydroxybenzoate (GA-LA) against chemical mutagens using *S. Typhimurium* TA 98[a]

Sample	Mutagen[b]	S9[c]	Revertant colonies/plate	Inhibition rate (%)
Negative control	DMSO	–	21.0 ± 1.53	
	DMSO	+	25.0	
	4-NQO	–	440.0 ± 39.04	
	TRP	–	95.0 ± 6.25	
	2-NF	–	489.3 ± 33.53	
	2-AA	+	28.3 ± 6.43	
	B(a)P	+	119.0 ± 9.54	
	2-AF	+	276.7 ± 27.61	
625 μg/plate	4-NQO	–	92.0 ± 26.51	79.1
	TRP	–	23.7 ± 3.22	75.1
	2-NF	–	338.3 ± 27.14	30.9
	2-AA	+	10.0 ± 2.00	64.7
	B(a)P	+	27.3 ± 3.06	77.0
	2-AF	+	128.3 ± 5.13	53.6

[a] Value are the mean ± S.D.
[b] Abbreviations; Dimethyl sulfoxide, DMSO; 4-nitroquinoline-1-oxide, 4-NQO; 3-amino-1,4-dimethyl-5H-pyrido [4,3-b] indole, TRP; 2-nitrofluorene, 2-NF; 2-aminoanthracens, 2-AA; benzo(a)pyrene, B(a)P; 2-aminofluorene, 2-AF.
[c] S9 mix was added (+) or not (–).

4-nitroquinoline-1-oxide (4-NQO), 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (TRP), 2-nitrofluorene (2-NF), 2-aminoanthracenes (2-AA), bezo(a)pyrene (B(a)P), and 2-aminofluorene (2-AF). GA-LA showed a strong anti-mutagenicity on the tested mutagens and especially it had higher than 70% inhibition against chemical mutagens 4-NQO, TRP, and B(a)P (Table 3). Ueno, Kakmuri, Sayato, and Okada (1991) indicated that active oxygen scavengers reduce a mutation induced by various mutagens. It has been suggested that compounds that possess anti-oxidative activity can inhibit mutation and cancer because they can scavenge free radicals or induce antioxidant enzymes (Hochstein & Atallah, 1998).

These results indicate that a newly synthesized compound GA-LA from gallic acid and linoleic acid possesses a strong and synergistic effect on cancer cell proliferation inhibition. Whereas, no synergistic effect was shown on DPPH radical scavenging, FRAP, hyaluronidase inhibition, and xanthine oxidase inhibition but it had comparable activities when compared with gallic acid and tocopherol. The anti-mutagenic activity also found from GA-LA. Therefore, GA-LA can be a useful functional material in food, pharmaceutical, and cosmetic industries.

Acknowledgement

This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MOST) (No. R01-2007-000-10753-0).

References

An, B. J., Kwak, J. H., Son, J. H., Park, J. M., Lee, J. Y., Jo, C., et al. (2004). Biological and anti-microbial activity of irradiated green tea polyphenol. *Food Chemistry, 88*, 549–555.
An, B. J., Kwak, J. H., Son, J. H., Park, J. M., Lee, J. Y., Kim, Y. S., et al. (2005). Physiological activity of irradiated green tea polyphenol on the human skin. *American Journal of Chinese Medicine, 33*, 535–546.
Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma as a measure of antioxidant power: The FRAP assay. *Analytical Biochemistry, 239*, 70–76.
Blois, M. S. (1958). Antioxidant determination by the use of a stable free radical. *Nature, 181*, 1190–1200.
Carmichael, J., Degraff, W. G., Gazdar, A. F., Minna, J., & Michell, J. B. (1987). Evaluation of a tetrazolium based semiautomated colorimetric assay, assessment of chemosensitivity testing. *Cancer Research, 47*, 936–942.
Choi, B. W., Lee, B. H., Kang, K. J., Lee, E. S., & Lee, N. H. (1998). Screening of the tyrosinase inhibitors from marine algae and medicinal plants. *Korean Journal of Pharmacognosy, 29*, 237–242.
Dew, P., Day, A. J., & Morgan, M. R. (2005). Xanthine oxidase activity in vitro: Effects of food extracts and component. *Journal of Agricultural Food Chemistry, 53*, 6510–6515.
Fukumoto, L. R., & Mazza, G. (2000). Assessing antioxidant and prooxidant activities of phenolic compounds. *Journal of Agricultural Food Chemistry, 48*, 3597–3604.
Haliwell, B., Aeschbach, R., Loliger, J., & Aruoma, O. I. (1995). The characterization of antioxidants. *Food and Chemical Toxicology, 33*, 601–617.
Hochstein, P., & Atallah, A. S. (1998). The nature of oxidant and antioxidant systems in inhibition of mutation and cancer. *Mutation Research, 202*, 363–375.
Hynes, W. L., & Walton, S. L. (2000). Hyaluronidases of Gram-positive bacteria. *FEMS Microbiology Letters, 183*, 201–207.
Isoyama, T., Thwaites, D., Selzer, M. G., Carey, R. I., Barbucci, R., & Lokeshwar, V. B. (2006). Differential selectivity of hyaluronidase inhibitors toward acidic and basic hyaluronidases. *Glycobiology, 16*, 11–21.
Ito, N., Hirose, M., Fukushima, S., Tsuda, H., Shirai, H. T., & Tatematsu, M. (1986). Studies on auto oxidants their carcinogenic and modifying effects on chemical carcinogenesis. *Food and Chemical Toxicology, 24*, 1071–1076.
Jimenez, M., Chazarra, S., Escribano, J., Cabanes, J., & Garcia-Carmona, F. (2001). Competitive inhibition of mushroom tyrosinase by 4-substituted benzaldehydes. *Journal of Agricultural and Food Chemistry, 49*, 4060–4063.
Jo, C., Jeong, I. Y., Lee, N. Y., Kim, K. S., & Byun, M. W. (2006). Synthesis of a novel compound from gallic acid and linoleic acid and its biological functions. *Food Science and Biotechnology, 15*, 317–320.
Kawada, M., Ohno, Y., Ri, Y., Idoma, T., Yuugetu, H., Asai, T., et al. (2001). Anti-tumor effect of gallic acid on LL-2 lung cancer cells transplanted in mice. *Anticancer Drugs, 12*, 847–852.
Kohen, R., & Nyska, A. (2002). Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reaction, and methods for their quantification. *Toxicology and Pathology, 30*, 620–650.
Kong, L. D., Cai, Y., Huang, W. W., Cheng, C. H. K., & Tan, R. X. (2000). Inhibition of xanthine oxidase by some Chinese medicinal plants used to treat gout. *Journal of Ethnopharmacology, 73*, 199–207.
Lee, N.Y. (2007). Development of gallic acid-conjugated linoleic acid ester as novel functional materials and its biological activity. Thesis. Chonbuk National University, Korea. pp. 48.
Lee, N. Y., Jo, C., Sohn, S. H., Kim, J. K., & Byun, M. W. (2006). Effects of gamma irradiation on the biological activity of green tea byproducts and a comparison with green tea leaf extracts. *Journal of Food Science, 71*, C269–C274.
Lin, J. K., Lin, C. L., Liang, Y. C., Lin-Shiau, S. Y., & Juan, I. M. (1998). Survey of catechins, gallic acid, and methylxanthines in green, oolong, pu-erh, and black teas. *Journal of Agricultural Food Chemistry, 46*, 3635–3642.
Maron, D. M., & Ames, B. N. (1983). Revised methods for Salmonella mutagenicity test. *Mutation Research, 113*, 173–215.
Masaki, H., Atsumi, T., & Sakurai, H. (1995). Peroxyl radical scavenging activities of hamamelitammin in chemical and biological systems. *Free Radical Research, 22*, 419–430.
Masamoto, Y., Ando, H., Murata, Y., Shimoishi, Y., Tada, M., & Takahata, K. (2003). Mushroom tyrosinase inhibitory activity of esculetin isolated from seeds of Euphorbia lathyris L.. *Bioscience, Biotechnology and Biochemistry, 67*, 631–634.
Nam, S. H., Choi, S. P., Kang, M. Y., Kozukue, N., & Friedman, M. (2005). Antioxidative, antimutagenic, and anticarcinogenic activities of rice bran extracts in chemical and cell assays. *Journal of Agricultural and Food Chemisty, 53*, 816–822.
Negi, A. S., Drokar, M. P., Chattopadhyay, S. K., Grag, A., Bhattacharya, A. K., Srivastava, V., et al. (2005). Synthesis of a novel plant growth promoter from gallic acid. *Bioorganic Medicinal Chemistry Letters, 15*, 1243–1247.
Reissing, J. L., Strominger, J. L., & Lelori, L. F. (1955). A modified colorimetric method for the estimation of N-acetylamino sugars. *Journal of Biology and Chemistry, 217*, 959–966.
Saeki, K., You, A., Isemura, M., Abe, I., Seki, T., & Noguchi, H. (2000). Apoptosis inducing activity of lipid derivatives of gallic acid. *Biological and Pharmaceutical Bulletin, 23*, 1391–1394.
SAS Institute, Inc. (2000). SAS User's Guide. SAS Institute Inc., Cary, NC, USA.
Siddhuraju, P. (2007). Antioxidant activity of polyphenolic compounds extracted from defatted raw and dry heated *Tamarindus indica* seed coat. *Food Science and Technology LWT, 40*, 982–990.
Spickenreither, M., Braun, S., Bernhardt, G., Dove, S., & Buschauer, S. (2006). Novel 6-O-acylated vitamin C derivatives as hyaluronidase inhibitors with selectivity for bacterial lyases. *Bioorganic Medicinal Chemistry Letters, 16*, 5313–5316.
Ueno, H., Kakmuri, K., Sayato, Y., & Okada, S. (1991). Characteristics of mutagenesis by glyoxal in *Salmonella Typhimurium*: Contribution of singlet oxygen. *Mutation Research, 25*, 99–107.
Wacher, V. J., & Benet, L. Z. (2001). Use of gallic acid esters to increase bioavailability of orally administered pharmaceutical compounds. US Patent no. 6180666B1.
Wei, Y., Zhang, T.-Y., Xu, G.-Q., & Ito, Y. J. (2001). Application of analytical and preparative high-speed countercurrent chromatography for separation of lycopene from crude extract of tomato paste. *Chromatography, 929*, 169–173.
Weinbrenner, T., Cladellas, M., Isabel Covas, M., Fito, M., Tomas, M., Sent, M., et al. (2003). High oxidative stress in patients with stable coronary heart disease. *Atherosclerosis, 168*, 99–106.
Yoshino, M., Haneda, M., Naruse, M., Htay, H. H., Iwata, S., Tsubouchi, R., et al. (2002). Prooxidant action of gallic acid compound: copper-dependent strand breaks and the formation of 8-hydroxy-2'-deoxyguanosine in DNA. *Toxicology in Vitro, 16*, 705–709.
Zaneveld, L. J., Waller, D. P., Anderson, R. A., Chany, C., 2nd, Rencher, W. F., Feathergill, K., et al. (2002). Efficacy and safety of a new vaginal contraceptive antimicrobial formulation containing high molecular weight poly (sodium 4-styrenesulfonate). *Biological Reproduction, 66*, 886–894.
Zhao, G., Etherton, T. D., Martin, K. R., Vanden Heuvel, J. P., Gillies, P. J., West, S. G., et al. (2005). Anti-inflammatory effects of polyunsaturated fatty acids in THP-1 cell. *Biochemical and Biophysical Research Communications, 336*, 909–917.