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The Protective Effect of Chlorophyll a Against Oxidative Stress and Inflammatory Processes in LPS-stimulated Macrophages

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Abstract This study was designed to investigate the suppressive effect of chlorophyll a on nitric oxide (NO) production and intracellular oxidative stress. In addition, chlorophyll a regulation of nuclear factor (NF) κB activation and inducible NO synthase (iNOS) expression were explored as potential mechanisms of NO suppression in a lipopolysaccharide (LPS)-stimulated macrophage cell line. RAW 264.7 murine macrophages were preincubated with various concentrations (0-10 μg/mL) of chlorophyll a and stimulated with LPS to induce oxidative stress and inflammatory response. Treatment with chlorophyll a reduced the accumulation of thiobarbituric acid-reactive substances (TBARS), enhancing glutathione level and the activities of antioxidative enzymes including superoxide dismutase, catalase, glutathione peroxidase (GSH-px), and glutathione reductase in LPS-stimulated macrophages compared to LPS-only treated cells. NO production was significantly suppressed in a dose-dependent manner ($p<0.05$) with an IC_{50} of 12.8 μg/mL. Treatment with chlorophyll a suppressed the levels of iNOS protein and its mRNA expression. The specific DNA binding activities of NFkB on nuclear extracts from chlorophyll a treated cells were significantly suppressed in a dose-dependent manner with an IC_{50} of 10.7 μg/mL. Chlorophyll a ameliorates NO production and iNOS expression through the down-regulation of NFκB activity, which may be mediated by attenuated oxidative stress in RAW 264.7 macrophages.

Keywords: chlorophyll a, oxidative stress, inflammation, iNOS expression, NO, macrophage

Introduction

It has been suggested that oxidative stress and inflammation contribute to the etiology of cardiovascular and digestive tract diseases (1-3). Inflammation induces oxidative stress by the production of oxidants, such as superoxide anion and nitric oxide (NO) (4, 5), and NO produced by inducible NO synthase (iNOS) reacts with superoxide and yields peroxynitrite (6). High NO levels along with decreased superoxide dismutase (SOD) activity leads to peroxynitrite formation and thereby increases intracellular oxidative stress (2). Furthermore, it is known that NO interacts with thiol-containing glutathione (GSH). Depletion of GSH has been observed in cells exposed to oxidative stress which increase the sensitivity of cells towards the toxic effect of NO (7). Expression of iNOS is closely related to the up-regulation of nuclear factor (NF) κB (8). NFκB sites have been identified in the promoter region of the iNOS gene (9). NFκB, an inducible transcription factor, is activated in response to various extracellular stimuli, including cytokines (10-12), lipopolysaccharide (LPS) (9, 13), and oxidative stress (4, 10, 11, 13-15). Furthermore, antioxidants such as anthocyanins (16), quercetin (17-19), and genistein (20) from fruits and vegetables have been reported to suppress NO production in LPS-stimulated macrophages, and their inhibition mechanisms are based on their ability to inhibit the activation of NFκB. These observations suggest a role of oxidative stress in the intracellular signaling of LPS that leads to NFκB activation, thus enabling the inflammatory processes to continue.

Attenuation of oxidative stress and inflammatory processes by medicinal plants has been studied and explained by the existence of polyphenol and pigments (8, 16, 19, 21). Chlorophyll is the major pigment in green plants and chlorella, where chlorophyll a is the predominant form. Numerous studies have reported that chlorophyllin, the water-soluble sodium, copper salt derivative of chlorophyll, exhibits anti-inflammatory, antimutagenic, anticarcinogenic, radioprotective, and antioxidative properties in a variety of experimental systems (22-25). In contrast to these extensive studies on chlorophyllin, little is known about the antioxidative and anti-inflammatory actions of chlorophylls, which are more hydrophobic than chlorophyllin. Several studies have reported though that chlorophylls have anticarcinogenic, antimutagenic, and antigenotoxic activities (26-28). The objective of this paper is to study whether chlorophyll a has antioxidative and anti-inflammatory effects and to elucidate its possible mechanisms of action in LPS-stimulated macrophages.

Materials and Methods

Materials Dulbecco's modified Eagle Medium (DMEM), fetal bovine serum (FBS), and TRIzol® reagent were obtained from Gibco BRL (Gibco BRL, Gaithersburg, MD, USA). Chlorophyll a, LPS, dimethyl sulfoxide (DMSO), glutamine, protease inhibitor cocktail, nicotinamide adenine dinucleotide phosphate hydrogenase (NADPH), phenylmethylsulfonyl fluoride (PMSF), sodium dodecylsulfate (SDS), Nonidet® P40 substitute (NP-40), and Griess reagent were obtained from the Sigma Chemicals (Sigma Chemical Co., St. Louis, MO, USA). Maloney murine leukemia virus (MMLV) first strand

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cDNA synthesis kit (Invitrogen, Carlsbad, CA, USA), NFκB-specific oligonucleotide and T4 polynucleotide kinase (Promega, Madison, WI, USA) and microspin G-25 column (Amersham Bioscience, Piscataway, NJ, USA) were used for reverse transcription-polymerase chain reaction (RT-PCR) and electrophoretic mobility shift assay (EMSA). Bradford protein assay reagent and SDS-polyacrylamide gel electrophoresis (PAGE) standards were from Bio-Rad Laboratories. (Hercules, CA, USA). Anti-mouse iNOS antibody was from BD Transduction Laboratories (Lexington, KY, USA). Anti-mouse glyceraldehydes-3-phosphate dehydrogenase (GAPDH) antibody was from Abcam (Cambridge, UK). Alkaline phosphatase conjugated goat anti-mouse immunoglobulin G (IgG) secondary antibody was from Jackson Immunoresearch Laboratories (West Groove, PA, USA). Nitrocellulose membrane was from Schleicher and Schuell (Keene, NH, USA). The murine macrophage RAW 264.7 cell line was obtained from the American Type Culture Collection (TIB-71; Rockville, MD, USA). All other chemicals were of the highest commercial grade available.

Cell culture and treatment The murine macrophage RAW 264.7 cell line was obtained from American Type Culture Collection and cultured in DMEM supplemented with 10% FBS and 2 mM L-glutamine. Cells in 10 mm dishes (5×10^6 cells/dish) or 24 well plates (4×10^5 cells/well) were pre-incubated with and without indicated concentrations of chlorophyll a (2.5, 5, 10 μg/mL) for 2 hr, and then incubated with LPS (2 μg/mL) for 20 hr at 37°C in a humidified atmosphere containing 5% CO_2. Cells that were untreated provided the negative control without LPS (untreated), while cells treated with LPS alone provided the positive control (control).

Cell viability Cell viability was assessed by measuring the uptake of neutral red supravital dye by viable cells according to the procedure of Fautz *et al.* (29). After culturing the cells as described previously, the medium was removed and replaced with 0.5 mL fresh medium containing 1.14 mmol/L neutral red. After incubation for 3 hr, the medium was removed and the cells were washed twice with phosphate buffered saline solution (PBS, pH 7.4). The incorporated neutral red was released from the cells by incubation for 15 min at room temperature in the presence of 1 mL cell lysis buffer [50 mmol/L Tris-HCl (pH 7.4), 150 mmol/L NaCl, 5 mmol/L dithiothreitol (DTT), and Triton X-100 (1%, v/v)] containing acetic acid (1%, v/v), and ethanol (50%, v/v). To measure the dye taken up, the cell lysis products were centrifuged and absorbance of supernatant was measured spectrophotometrically at 540 nm.

Nitrite assay The nitrite accumulated in the culture medium is an indicator of NO production and was measured according to the Griess reaction (30). One hundred μL of each medium supernatant was mixed with 50 μL sulphanilamide (1% in 5% phosphoric acid) and 50 μL naphthylenediamine dihydrochloride (0.1%) and then incubated at room temperature for 10 min. The absorbance at 550 nm was measured against a $NaNO_2$ serial dilution standard curve and nitrite production was determined.

Lipid peroxidation Lipid peroxidation was measured by the production of thiobarbituric acid reactive substances (TBARS) as described by Fraga *et al.* (31). The absorbance at 532 nm was measured against a 1,1,3,3-tetraethoxypropane serial dilution standard curve and TBARS values were expressed as nmol of malondialdehyde equivalents.

GSH concentration GSH was measured by an enzymatic recycling procedure by Tietze (32) in which GSH is sequentially oxidized by 5,5"-dithiobis (2-nitrobenzoic acid) and reduced by NADPH in the presence of glutathione reductase.

Antioxidant enzyme assays Cell suspensions were sonicated three times for 5 sec on ice and then cell sonicates were centrifuged at 10,000×g for 20 min at 4°C. Cell supernatants were used for antioxidant enzyme activities. The protein concentration was measured using the Bradford assay (33) with bovine serum albumin as the standard. SOD activity was determined by monitoring the auto-oxidation of pyrogallol according to the method of Marklund and Marklund (34). A unit of SOD activity is defined as the amount of enzyme that inhibits 50% oxidation rate of 6 mM pyrogallol. Catalase activity was measured according to the method of Aebi (35) by following the decrease in absorbance of H_2O_2. The decrease in absorbance at 240 nm was measured for 2 min. Standards containing 0, 0.2, 0.5, 1, and 2 mM of H_2O_2 were used to construct a standard curve. GSH-peroxidase (GSH-px) activity was assayed by the method of Lawrence and Burk (36). A unit of GSH-px was defined as the amount of enzyme that oxidases one nmole of NADPH consumed per minute. GSH reductase activity was measured by following the oxidation of NADPH by Inger and Bengt (37). A unit of GSH reductase is defined as the amount of enzyme that catalyses the reduction of one nmol of NADPH per min.

Western blot analysis Western blot analysis was assayed by the method of Katsuyama *et al.* (38) with slight modifications. The cells were washed twice with PBS, scraped into 0.5 mL of ice-cold lysis buffer (50 mM Tris-HCL pH 8.0, 150 mM NaCl, 0.02% sodium azide, 0.1% SDS, 1% NP-40, 0.5% sodium deoxycholase, 1 mM PMSF) for 15 min and disrupted with a Handy Sonic Disrupter (Sonopuls UW 2070; Bandelin Electronic, Berlin, Germany). The lysis buffer containing the disrupted cells was centrifuged at 13,000×g and 4°C for 20 min. Protein content of the lysate supernatants was determined with the Bio-Rad protein assay reagent (Bio-Rad Laboratories). Protein samples (60 μg) from each lysate were separated on a 10% SDS-polyacrylamide gel and electrotransferred to nitrocellulose membranes (Schlecher and Schuell). Membranes were blocked for 1 hr at room temperature with 5% nonfat dry milk in a buffer that contained 10 mmol/L Tris, pH 8.0, 150 mmol/L NaCl, and 0.05% Tween 20. The reactions were then incubated at 4°C overnight with a 1:10,000 dilution of rabbit anti-mouse iNOS antibody and a 1:1,000 dilution of rabbit anti-mouse GAPDH antibody in blocking buffer. After the membranes were washed, they were further

incubated with a 1:10,000 dilution of alkaline phosphatase conjugated goat anti-mouse IgG secondary antibody for 1 hr at room temperature. The blots were finally developed with 5-bromo-4-chloro-3-indoyl phosphate (BCIP)/ nitroblue tetrazolium (NBT) color developing solution. Data were quantified using the Gel Doc EQ System (Bio-Rad Laboratories). All signals were normalized to protein levels of the housekeeping gene, GAPDH and expressed as a ratio.

RNA preparation and iNOS mRNA analysis by RT-PCR Total RNA was isolated using Trizol-reagent (Sigma Chemicals) according to the method of Chomczynski and Sacchi (39). Five μg total RNA was used to produce first strand cDNA using the MMLV first strand cDNA synthesis kit (Invitrogen). PCR (GeneAmp PCR System 2400; Perkin Elmer Life Sciences, Wellesley, MA, USA) was carried out in 50 μL reaction mixture containing the first strand cDNA, 10× PCR buffer, 2.5 mM deoxyribonucleoside triphosphate (dNTP), 20 pM of each primer and Taq DNA polymerase (Perkin Elmer Life Sciences). PCR primer sequences for iNOS and GAPDH were as follows: primers for iNOS were 5'-GCC TTC AAC ACC AAG GTT GTC TGC A-3'(sense) and 5'-TCA TTG TAC TCT GAG GGC TGA CAC A-3' (anti-sense). Primers for GAPDH were 5'-CAA TGC CAA GTA TGA TGA CAT-3' (sense) and 5'-CCT GTT ATT ATG GGG GTC TG-3' (anti-sense). The expected sizes of PCR products were 920bp for iNOS and 375bp for GAPDH. The amplification profile consisted of an initial denaturation at 94°C for 1 min followed by denaturation at 94°C for 2.5 min (for iNOS and GAPDH), annealing at 59°C for 2 min (iNOS), at 49°C for 2 min (GAPDH) and extension at 72°C for 2 min (for iNOS and GAPDH). Twenty seven cycles for iNOS and GAPDH resulted in the best amplification profiles to recognize differences among samples. Expression of the housekeeping gene, GAPDH, served as control. The PCR products specific for each cDNA were analyzed by electrophoresis on 2% agarose gels containing ethidium bromide (0.5 μg/mL) at 50 V for 70 min and were visualized with an ultra violet transilluminator. Data were quantified using the Gel Doc EQ System (Bio-Rad Laboratories). All signals were normalized to mRNA levels of the house keeping gene, GAPDH and expressed as ratios.

Electrophoretic mobility shift assay (EMSA) Nuclear protein was extracted using the slightly modified method of Dignam *et al.* (40). Cells in 10 mm dishes were lysed with buffer containing 0.6% igepal, 0.15 M NaCl, 10 mM Tris, pH 7.9, 1 mM EDTA, and 0.1% protease inhibitor cocktail, vortexed, kept on ice for 5 min, and centrifuged at 500×g for 5 min at 4°C. Pelleted nuclei were resuspended in 50 μL extraction buffer [10 mM Hepes, pH 7.9, 0.1 mM ethylene glycol tetraacetic acid (EGTA), 0.1 mM EDTA, 1.5 mM $MgCl_2$, 420 mM NaCl, 25% glycerol, 1 mM DTT, and 0.33% protease inhibitor cocktail]. Following gentle mixing and then keeping on ice for 20 min, samples were centrifuged at 500×g for 5 min at 4°C. The supernatant fraction was transferred to new tubes and stored at -70°C. Protein concentration was determined by Bradford assay (33). For the EMSA, NFκB-specific oligonucleotide was end-labeled with [γ-^{32}P]-ATP using T_4 polynucleotide kinase (Promega) and purified using microspin G-25 columns (Amersham Bioscience). An EMSA was performed according to the instruction manual of Promega. Five mg nuclear protein, 2 μL of 5× binding buffer, 1 mL ^{32}P-labeled NFκB, and 1 μL of 10× loading buffer were incubated for 30 min at room temperature. DNA-protein complexes were separated from unbound DNA probe by electrophoresis through a 4% polyacrylamide gel using 0.5× Tris-borate EDTA buffer as the running buffer. The gels were exposed to a phosphor screen (Packard Instrument Company, Inc., Meriden, CT, USA) for 2 hr at -20°C and the bands were quantitated by a phosphor image analyzer (Amersham Bioscience).

Statistical analysis All data are expressed as the means ±SD. The statistical analyses were performed using the SPSS program (SPSS 10.0). One-way ANOVA with Duncan's multiple range test was used to examine the difference between groups. Probability values <0.05 were considered significant, if not otherwise stated.

Results and Discussion

NO production Several studies have suggested that elevated NO production and oxidative stress in activated macrophages is closely related to inflammation, the development of atherosclerosis, and chronic diseases (1-3). The addition of LPS to cells significantly elevated the generation of nitrite, the oxidative product of NO, compared to LPS-untreated negative control, as has been documented by several researchers (9, 13). Chlorophyll a treatment was found to suppress NO production in a dose-dependent manner ($p<0.05$) with an IC_{50} of 12.8 μg/mL (Fig. 1). Cell viability was >95% at the concentrations treated as assessed by neutral red assay, which supports the idea that the suppressive effect of chlorophyll a on NO production is not due to cell death (data not shown).

Status of oxidative stress and antioxidative enzyme activities Markers of oxidative stress status, such as levels of TBARS, GSH, and antioxidative enzyme activities, were investigated in order to assess the defensive function of chlorophyll a against oxidative stress. As shown in Fig. 2, pre-incubation with chlorophyll a significantly decreased TBARS ($p<0.05$) in a dose-dependent manner, suggesting that lipid peroxidation was attenuated in LPS-stimulated macrophages by chlorophyll a treatment. Exposure of RAW 264.7 cells to LPS slightly decreased GSH levels. However, GSH levels were enhanced in LPS-treated RAW 264.7 cells pre-incubated with chlorophyll a compared to LPS-only treated cells (Fig. 3). The suppression of oxidative stress beyond the level of control by chlorophyll a might be explained by its action as an antioxidant or inducer of antioxidative enzymes. It has been suggested that the antioxidative ability of chlorophylls might derive from their metallo-porphyrin compound structure, which has more than 10 conjugated double bonds (23). Several researchers confirmed this hypothesis by reporting that porphyrins can decrease superoxide level (41), react with peroxynitrite and thereby

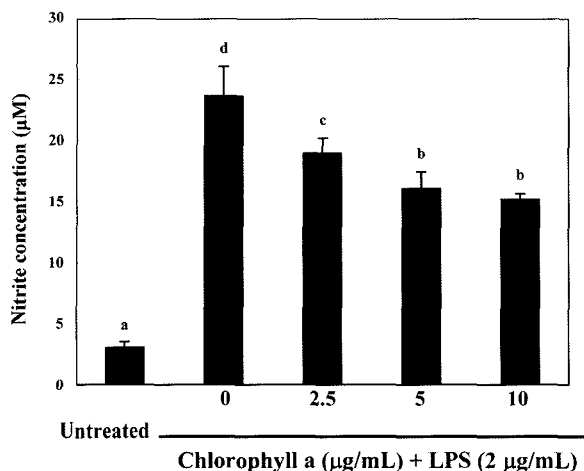

Fig. 1. Effects of chlorophyll a on NO production in LPS-stimulated RAW 264.7 macrophages. Data represent the means±SD of triplicate experiments. A value sharing the same superscript is not significantly different at $p<0.05$.

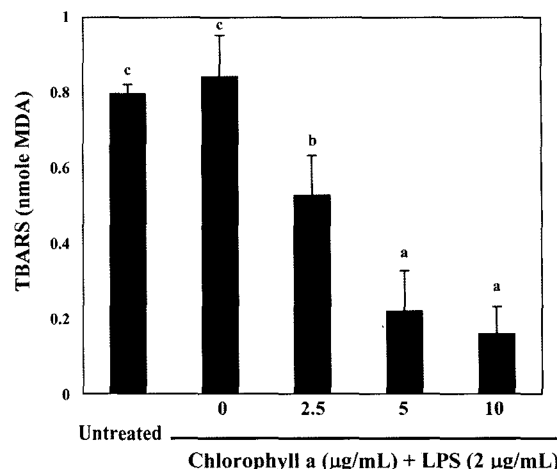

Fig. 2. Effects of chlorophyll a on TBARS generation in LPS-stimulated RAW 264.7 macrophages. Data represent the means±SD of triplicate experiments. A value sharing the same superscript is not signifi-cantly different at $p<0.05$.

decrease oxidative stress (42), scavenge free radicals and reduce oxidative DNA damage (25). These results might partially explain why green vegetables with chlorophyll can help in the prevention of reactive oxygen species (ROS)-related chronic diseases.

The effects of chlorophyll a on the specific activities of antioxidant enzymes in LPS-stimulated cells are shown in Table 1. Exposure of cells to LPS decreased the activities of all the enzymes except catalase, while exposure of cells to chlorophyll a (10 μg/mL) before LPS treatment significantly elevated all the enzyme activities compared to those of LPS-stimulated control ($p<0.05$). Previous findings of studies of the effect of LPS on antioxidative enzyme activities have not been consistent. Ben-Shaul *et al.* (43) reported that LPS injection to rats increased SOD and catalase but did not change GSH-px activity in the heart. Iqbal *et al.* (2) found GSH-px activity unaltered in myocardial disordered rat induced by LPS. However, Watson *et al.* (44) found that activities of GSH-px and SOD in the rat liver were decreased after LPS injection. Our study found that the antioxidant enzyme activity was decreased by LPS treatment. However, chlorophyll a preincubation before stimulating cells with LPS enhanced antioxidative enzyme systems that attenuate oxidative stress. There are a few studies that are in agreement with our result. Flavonoid treatment *in vitro* and *in vivo* increased catalase, mRNA expression (45), and GSH-px activities (46). These elevations of antioxidative enzyme activities by chlorophyll a may help attenuate the oxidative stress caused by inflammation.

Effects of chlorophyll a on iNOS protein and mRNA expressions and NFκB activity We investigated iNOS protein and mRNA expression in order to elucidate the mechanism of the suppressive effect of chlorophyll a on NO production. Although iNOS protein was hardly detected in unstimulated RAW 264.7 cells by Western blot analysis, it was highly elevated after stimulation with LPS, and the presence of chlorophyll a in LPS-stimulated cultures markedly decreased iNOS protein expression (Fig. 4). RT-PCR was performed to examine whether the inhibition of NO production by chlorophyll a was involved in iNOS mRNA expression. LPS-stimulated macrophages induced expression of iNOS mRNA, but not that of GAPDH. Chlorophyll a pretreatment suppressed the LPS-stimulated expression of iNOS mRNA ($p<0.05$). However, chlorophyll a did not affect the expression of the housekeeping gene GAPDH (Fig. 5). We also performed EMSA to see whether chlorophyll a interferes with the DNA binding of NFκB, a transcriptional factor regulating several genes including iNOS. Specific DNA binding of

Table 1. Effects of chlorophyll a on antioxidative enzyme activities in LPS-stimulated RAW 264.7 macrophages

	Untreated	Chlorophyll a (μg/mL) + LPS[5] (2 μg/mL)			
		0	2.5	5	10
Catalase (μmol/mg protein/min)	0.14±0.01[a1)]	0.13±0.01[a]	0.15±0.01[a]	0.20±0.03[b]	0.21±0.02[b]
SOD[2)] (unit/mg protein)	37.5±1.93[b]	31.6±5.58[a]	32.9±2.03[ab]	45.3±2.38[c]	46.7±1.90[c]
GSH-px[3)] (unit/mg protein)	1.10±0.19[b]	0.80±0.07[a]	1.00±0.11[ab]	1.04±0.10[b]	1.36±0.21[c]
GSH-reductase[4)] (unit/mg protein)	55.9±9.12[b]	34.4±11.8[a]	51.4±10.3[ab]	53.9±13.3[b]	76.7±13.2[c]

[1)]Data represent the means±SD of triplicate experiments. A value sharing the same superscript is not significantly different at $p<0.05$.
[2)]Superoxide dismutase. [3)]Glutathione peroxidase. [4)]Glutathione reductase. [5)]Lipopolysaccharide.

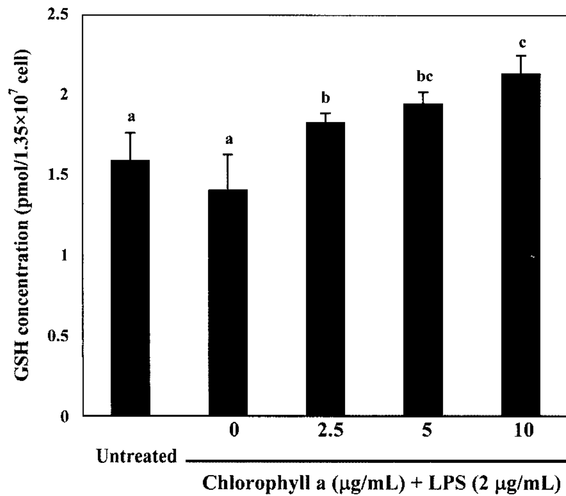

Fig. 3. Effects of chlorophyll a on GSH concentration in LPS-stimulated RAW 264.7 macrophages. Data represent the means±SD of triplicate experiments. A value sharing same the superscript is not significantly different at $p<0.05$.

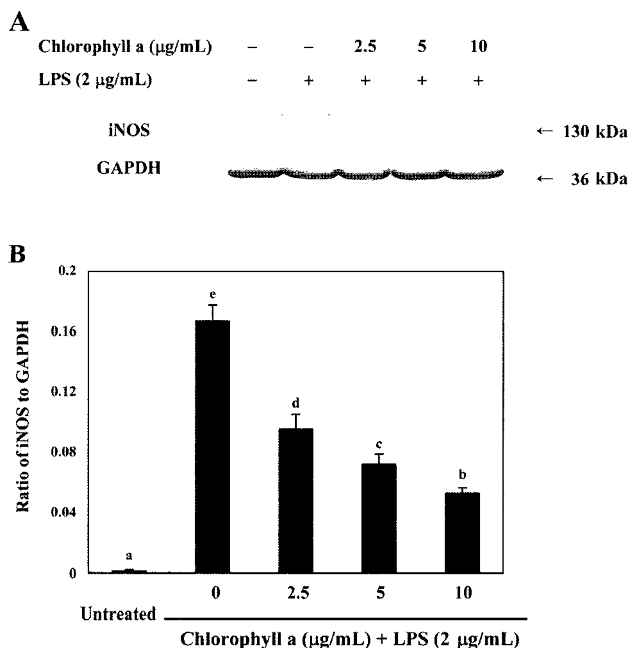

Fig. 4. Effects of chlorophyll a on iNOS protein expression in LPS-stimulated RAW 264.7 macrophages. (A) Levels of iNOS protein were measured by Western blot analysis using a monoclonal antibody for murine iNOS. The blot was rehybridized with antibody against GAPDH to verify equal loading of protein in each lane. GAPDH was used as an internal control. (B) All signals were normalized to protein levels of the house keeping gene, GAPDH and expressed as a ratio. Data represent the means±SD of triplicate experiments. Values sharing the same superscript are not significantly different at $p<0.05$.

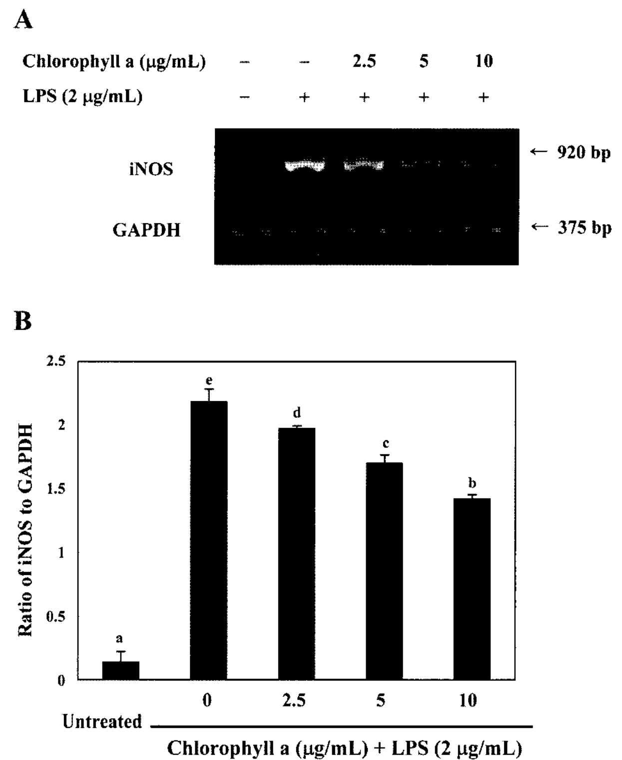

Fig. 5. Effects of chlorophyll a on iNOS mRNA expression in LPS-stimulated RAW 264.7 macrophages. (A) Levels of iNOS mRNA were determined by RT-PCR analysis. GAPDH was used as an internal control. (B) All signals were normalized to mRNA levels of the house keeping gene, GAPDH and expressed as a ratio. Data represent the means±SD of triplicate experiments. Values sharing the same superscript are not significantly different at $p<0.05$.

NFkB using EMSA showed that LPS treatment to RAW 264.7 cells enhanced NFκB activation (Fig. 6A). Pre-incubation of cells with chlorophyll a inhibited NFkB activity with an IC_{50} of 10.7 μg/mL in a dose-dependent manner (Fig. 6B).

Several researchers reported that the suppression of NO production by plant extracts may be attributed to direct free radical scavenging and the suppression of iNOS protein and mRNA expression (8, 18, 19, 47). In this study, Western blot and RT-PCR analysis revealed that chlorophyll a inhibited iNOS expression and lowered intercellular oxidative stress. Furthermore, this inhibition appeared to be mediated through NFκB, since the activation of NFκB is critical for the induction of iNOS gene expression in macrophages stimulated with LPS (4, 8). It is known that the promoter region of the iNOS gene contains several consensus sequences for the binding of NFκB (9). Based on the observations made in this study, the inhibitory effect of chlorophyll a on LPS-induced NFκB activation in RAW 264.7 cells can be explained by the attenuated intracellular oxidative stress in the LPS signaling pathway that leads to NFκB activation. Indeed, the suppressive effect of chlorophyll a on NFκB activation was concurrent with reduced NO, TBARS, elevated GSH generation, and enhanced antioxidative enzyme activities.

Activation of NFκB is related to the cellular redox state (1). The intracellular thiol level and antioxidant treatment attenuates oxidative stress and suppresses NFκB activation (14, 16). Indeed, it has been reported that carnosol (8), hydralazine (6), selenium (4), and dithiocarbamate (15) suppress oxidative stress and NFκB activation by LPS in

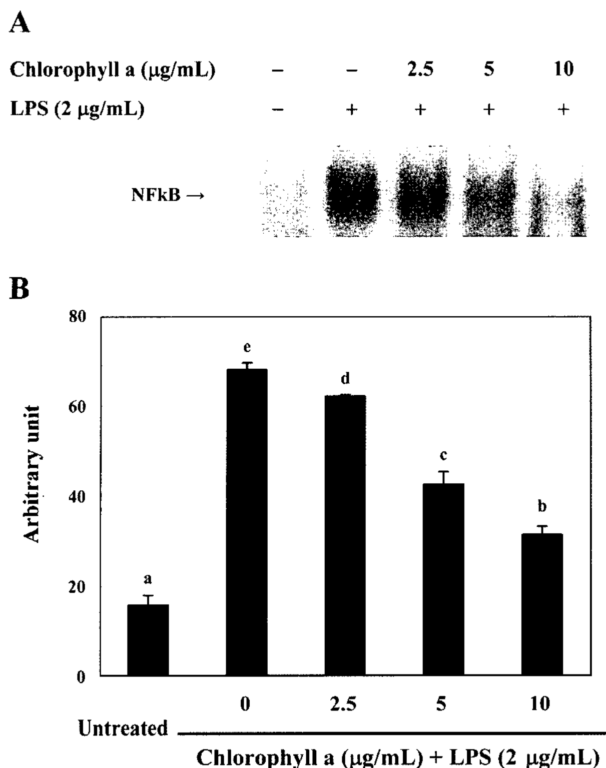

Fig. 6. Effects of chlorophyll a on NFkB activity in LPS-stimulated RAW 264.7 macrophages. (A) DNA binding activity of NFκB was performed by EMSA. (B) Values are expressed as relative intensity of radioactivity. Data represent the means±SD of triplicate experiments. A value sharing the same superscript is not significantly different at $p<0.05$.

various cell culture systems. Previous studies also observed antioxidative and anti-inflammatory effects of chlorophyllin (22) and chlorella extract (21) which contain high amounts of chlorophyll. This evidence suggests that the suppressed NO production and NFκB activation by chlorophyll a treatment might be attributed in part the to antioxidant properties of chlorophyll.

In summary, chlorophyll a may attenuate intracellular oxidative stress by delaying the consumption of cellular GSH and enhancing antioxidative enzyme activities, which result in the suppression of NFκB activation, iNOS protein and mRNA expression, and NO production by LPS. These results demonstrate that chlorophyll appears to be a potential nutraceutical for treating LPS-induced inflammatory processes. Taken together, these antioxidative and anti-inflammatory effects of chlorophyll partly explain the preventive effects of green vegetables on the development of chronic diseases by suppressing oxidative stress and inflammatory processes.

Acknowledgments

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