Photochemoprevention of UVB-induced skin carcinogenesis in SKH-1 mice by brown algae polyphenols

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Chronic exposure of the skin to ultraviolet B (UVB) radiation induces oxidative stress, which plays a crucial role in the induction of skin cancer. In this study, the effect of dietary feeding and topical application of brown algae polyphenols on UVB radiation-induced skin carcinogenesis in SKH-1 mice was investigated. SKH-1 hairless mice were randomly divided into 9 groups, including control, UVB control and treatment groups. They were treated orally (0.1% and 0.5% with AIN-76 diet, w/w) and topically (3 and 6 mg/0.2 ml of vehicle) with brown algae polyphenols and irradiated with UVB for 26 weeks. Dietary feeding (0.1% and 0.5%) of brown algae polyphenols significantly reduced tumor multiplicity (45% and 56%) and tumor volume (54% and 65%), and topical administration (3 and 6 mg) significantly decreased tumor multiplicity (60% and 46%) and tumor volume (66% and 57%), respectively, per tumor-bearing mouse. Dietary feeding and topical administration of the polyphenols also inhibited tumor incidence by 6% and 21%, respectively, but the results were not significant. Dietary and topical administration of the polyphenols markedly inhibited cyclooxygenase-2 activity and cell proliferation. These observations show that brown algae polyphenols have an antiphotocarcinogenic effect which may be associated with the prevention of UVB-induced oxidative stress, inflammation, and cell proliferation in the skin.
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Solar ultraviolet (UV) radiation is a major environmental carcinogen and primary cause of melanoma and nonmelanoma skin cancer.[1] Over 1,300,000 new skin cancers are diagnosed yearly in the United States, and approximately 90% of the new cases are attributable to UV light exposure.[2] The incidence of skin cancer has increased markedly in recent years and this increase is expected to continue as the mean population age increases and to enhanced exposure to UV radiation due to depletion of the ozone layer.[3] Ultraviolet radiation from the sunlight can be divided into 3 components depending on their wavelength: UVC (200–280 nm), UVB (280–320 nm) and UVA (320–400 nm). UVB is a major risk factor for the induction and development of nonmelanoma skin cancer; basal- and squamous-cell carcinomas; and many other skin disorders including sunburn, photoaging and actinic keratoses.[4]

Exposure to UVB radiation causes excessive generation of reactive oxygen species in the skin, which contributes to cancer induction, causing oxidative stress in the skin and ultimately damaging DNA.[5,6] The skin possesses an elaborate antioxidant defense system to protect it from oxidative stress,[7] but excessive exposure to reactive oxygen species can shift the prooxidant–antioxidant balance of the skin toward a more oxidative state. The resulting oxidative stress causes many adverse effects and pathological conditions, including cancer.[8–10] Under these circumstances, regular intake of dietary antioxidants or treatment of the skin with products containing antioxidant ingredients may be a useful strategy for preventing UVB-induced damage.[11–14] Several studies reported the chemopreventive effect of agents, both naturally-occurring and synthetic, against UVB-induced skin carcinogenesis.[15–21] Worldwide interest in the use of naturally occurring dietary supplements as chemopreventive agents has increased considerably. Developing effective and safe chemopreventive agents to inhibit the incidence of UVB-induced skin cancer in human populations is highly desirable.

Polyphenols are one of the most common classes of secondary metabolites in terrestrial and marine plants. Because polyphenolic compounds are relatively nontoxic, their use as chemopreventive agents has received much attention. Although terrestrial and marine polyphenols share a similar property in that both of them have radical scavenging activities, their different chemical structures lead to different physiological outcomes. Terrestrial polyphenols are polymers of flavonoids or gallic acid whereas, marine algae polyphenols, especially the phlorotannins, which are found only in brown algae (Phaeophyta), are restricted to polymers of phloroglucinol(1,3,5-trihydroxybenzene).[22] The phlorotannins have a wide range of molecular sizes (400–400,000 Da) and can occur in variable concentrations (0.5–20% of dry weight) in brown algae. They are known to be antiplasmin inhibitors,[23] bactericides,[24] glycosidase[25] and hyaluronidase inhibitors,[26] and antioxidants.[27] Recently, we demonstrated that the phlorotannins are very strong antioxidants capable of scavenging reactive oxidative species.[28]

In the present study, we assessed the chemopreventive efficacy of brown algae polyphenols against photocarcinogenesis in the SKH-1 hairless mouse skin model. This model of photocarcinogenesis is considered to mimic the development of skin carcinogenesis in humans.[29] UVB was used as a complete carcinogen. Although the precise mechanism of UVB-induced skin damage and development of skin cancer is not known, many studies have shown that exposure to UVB light results in inflammation and hyperproliferation of skin cells,[30–32] 2 processes involved in UVB-induced skin carcinogenesis.[33–35] Most photocarcinogenesis studies investigating the efficacy of dietary and topical administration of phlorotannins as chemopreventives for the skin have assessed their effects on inflammation and cell proliferation.

Material and methods

Materials

Dimethylsulfoxide (DMSO) was obtained from Sigma Chemical Company (St. Louis, MO). The prostaglandin E_2 (PGE_2) Enzyme Immunoassay Kit was purchased from Amersham (Piscataway, NJ) and the QuantiTect SYBR Green reverse transcription-polymerase chain reaction (RT-PCR) kit from Qiagen, Inc. (Valencia, CA).

A sample of brown algae polyphenols with >99% purity was kindly supplied by Livechem, Inc. (Taejeon, Korea). The sample was extracted from brown algae collected from Cheju Island, Korea. The composition of polyphenols in the sample was found to be: 2-*O*-(2,4,6-trihydroxyphenyl)-6,6′-bieckol (16.5%), 6,6′-bieckol (6.1%), 8,8′-bieckol (6.2%), 7-phloroeckol (8.1%), 2-phloroeckol

FIGURE 1 – Chemical structure of phlorotannins contained in brown algae polyphenols.

TABLE I – EXPERIMENTAL DESIGN

Group	Treatment	Treatment method	Diet	No. of mice
1	None	None	AIN-76A	20
2	Vehicle[1] alone	Topical	AIN-76A	20
3	BAP[2] (6 mg[3]) alone	Topical	AIN-76A	20
4	UVB alone	None	AIN-76A	20
5	UVB + vehicle[1]	Topical	AIN-76A	20
6	UVB + 0.1% BAP diet	Oral	AIN-76A + BAP (0.1%)	20
7	UVB + 0.5% BAP diet	Oral	AIN-76A + BAP (0.5%)	20
8	UVB + BAP (3 mg[3])	Topical	AIN-76A	20
9	UVB + BAP (6 mg[3])	Topical	AIN-76A	20

[1]Acetone + DMSO (1:1, v/v), vehicle for topical application of brown algae polyphenols.–[2]BAP, brown algae polyphenols.–[3]Dissolved in 200 μl of vehicle.

(2.4%), eckol (3.5%), dieckol (15.7%), phlorofurofukoeckol (3.7%), phlorotannin A (0.5%), fucofuroeckol A (0.1%) and various minor homologues (37.2%), as determined by HPLC [Waters, column: Spherisorb S_{10} ODS2 column (20 × 250 mm^2); eluent: 30% aqueous MeOH; flow rate: 3.5 ml/min]. Structures of these polyphenols are given in Figure 1.

Animals and diets

Female SKH-1 hairless mice (7–8 weeks of age) obtained from Charles River Laboratories (Wilmington, MA) were used in our study. The mice were housed in the vivarium at The Ohio State University according to the requirements established by the American Association for Accreditation of Laboratory Animal Care. All procedures were approved before the study by the institutional animal care utilization committee. After arrival in the animal facility, the mice were acclimatized for 2 weeks before the start of the experiments. They were housed 5 to a cage, fed AIN-76A synthetic diet (Dyets Inc., Bethlehem, PA) containing 20% casein, 0.3% D,L-methionine, 52% cornstarch, 13% dextrose, 5% cellulose, 5% corn oil, 3.5% American Institute of Nutrition salt mixture, 1% American Institute of Nutrition vitamin mixture, and 0.2% choline bitartrate. The synthetic diet and water were available *ad libitum*. Animal rooms were maintained at (20 ± 2)°C and (50 ± 10)% relative humidity with a 12-hr light/dark cycle throughout the study. Hygienic conditions were maintained by twice-weekly cage changes and routine cleaning of the animal rooms.

UVB source

Simulated solar ultraviolet (UV) radiation was provided by a bank of 6 fluorescent sunlamps (Phillips FS40) in a planar arrangement and filtered through 0.125 mm cellulose acetate that was changed weekly. Mice were irradiated unrestrained in cages custom designed for irradiation experiments; the distance from the light source to the target skin was 23 cm. The light source emitted about 80% radiation at 280–320 nm with peak emission at 314 nm as monitored with a PMA2100 meter/datalogger (Solar Light Company, Philadelphia, PA) equipped with a model PMA2101 biologically weighted UVB detector.

Carcinogenesis study

One hundred and eight mice were randomly divided into 9 experimental groups (20 mice per group). Mice were placed on AIN-76A diet alone or AIN-76A diets containing either 0.1% or 0.5% brown algae polyphenols (Table I) and maintained on these diets for the duration of the 26-week study. Oral treatment groups

included control (group 1), UVB control (group 4) and UVB + brown algae polyphenols (groups 6 and 7), and topical treatment groups included solvent vehicle control (group 2), brown algae polyphenol control (group 3), UVB + solvent vehicle control (group 5), and UVB + brown algae polyphenols (groups 8 and 9) (Table I). Mice in the topical treatment groups were treated with either 0.2 ml solvent alone or with 3 or 6 mg brown algae polyphenols in 0.2 ml solvent. The solvent for topical application of the brown algae polyphenols was composed of a mixture of DMSO and acetone (1:1, v/v). For the initial 2 weeks of the bioassay, the mice were treated orally or topically with brown algae polyphenols with no UVB irradiation.

Beginning in week 3, mice were irradiated 5 day/week. The initial dose of UVB was 18 mJ/cm^2 per day and the dose was gradually increased to 60 mJ/cm^2 per day by the end of week 4. From week 5 on, the mice were irradiated 3 day/week and the dose was increased weekly by 20% until week 10, giving a total daily dose of 160 mJ/cm^2 per day. From week 11, irradiation times and dose were held constant (3 day/week, 160 mJ/cm^2 per day) until the end of study. Topical treatments were performed 30 min before each exposure to UVB irradiation.

During the experimental protocols, mice were regularly monitored for food and water consumption and apparent signs of toxicity. The body weight and dietary intake of mice in each group was recorded weekly. The mice were euthanized by carbon dioxide asphyxiation after 26 weeks. The skin of each mouse was excised, tumor number was counted and tumor volume was calculated by the hemiellipsoid model formula: tumor volume = length × width × height × π/6. The whole skin section was separated from the tumors, snap-frozen in liquid nitrogen, pulverized in liquid nitrogen using mortar and pestle and stored in −80°C until analysis. No attempts were made to separate the epidermis from the dermis.

Real-time RT-PCR analysis

Total cellular RNA from dorsal skin that had been pulverized in liquid nitrogen was isolated using TRIzol reagent (Invitrogen Corp., Carlsbad, CA) according to the manufacturer's instructions. Ten skin samples from each experimental group were used for analysis. Each sample was extracted twice. Spectrophotometric analysis was used to determine the RNA concentration of the samples, then 1 μg RNA was electrophoresed on a 1.2% agarose-formaldehyde gel and stained with ethidium bromide to determine the integrity of the 18S and 28S ribosomal RNA. RT was performed with a PerkinElmer GeneAmp PCR system 9600 (PerkinElmer Corp., Norwalk, CT). RNA samples (2 μg) were incubated at 65°C for 5 min and then chilled to 4°C immediately before being reverse transcribed. cDNA was generated using a mixture of 5.0 mM MgCl$_2$; 1 U/μl RNAsin RNA inhibitor; 100 pmol random primers; 1.0 mM each of dATP, dGTP, dCTP, and dTTP and 2.5 U/μl SuperScript reverse transcriptase (Gibco-BRL) for 60 min at 37°C in 20 μl. An identical reaction without the reverse transcriptase was performed to verify the absence of genomic DNA. The cDNA was stored at −20°C until analyzed. Real-time PCR amplification was performed in a GeneAmp 5700 sequence detection system (PerkinElmer Corp.) using the QuantiTect SYBR Green PCR kit. The PCR reaction mix (10 ng template cDNA, 25 μl 2X QuantiTect SYBR Green PCR, 5 μM of forward and reverse primers, and water to the 50-μl reaction volume) was placed into MicroAmp 96-well plates capped with MicroAmp optical caps. Amplification was carried out at 95°C for 15 min (activation) followed by 40 cycles of 15 sec at 94°C (denaturation), 30 sec at 60°C (annealing) and 30 sec at 72°C (extension). The sequences of primers used were as follows: HPRT sense 5′-GCTTTCCCTGGTTAAGCAGTACA-3′ and antisense 5′-CAAACTTGTCTGGAATT TCAA-ATC-3′ and cyclooxygenase-2 (COX-2) sense 5′-AAGCGAGGACCTGGGGTTCA-3′ and antisense 5′-AAGGCGCAGTTTATGTTG-TCT GT-3′. All SYBR Green PCR data were collected using the SDS Sequence Detector Software (PE Applied Biosystems, Foster City, CA).

PGE$_2$ enzyme immunoassay

PGE$_2$ was isolated from dorsal skin that had been pulverized in liquid nitrogen using methanol. Ten skin samples from each experimental group were used for analysis. PGE$_2$ concentrations in the skin samples were determined using an ELISA kit according to the manufacturer's protocol (Amersham, Piscataway, NJ). A SpectraMaxM$_2$ microplate reader (Molecular Devices Corp., Sunnyvale, CA) was used to read the EIA plate.

Western blotting

For preparation of proteins, the skin tissues that had been pulverized in liquid nitrogen were homogenized in ice-cold lysis buffer consisting of 50 mM Tris-HCl, 150 mM NaCl, 1 mM EGTA, 1 mM EDTA, 20 mM NaF, 100 mM Na$_3$VO$_4$, 1 mM PMSF (pH 7.4), 0.5% NP-40 and 1% Triton X-100, with freshly added protease inhibitor cocktail (Protease Inhibitor Cocktail Set III; Calbiochem, LaJolla, CA). Ten skin samples from each experimental group were used for analysis. The homogenate was then centrifuged at 12,000g for 20 min at 4°C and the supernatant was collected, divided into aliquots, and stored at −80°C. The protein concentration was measured with the Bio-Rad protein assay kit (Bio-Rad, Richmond, CA) according to the manufacturer's protocol. Lysates (50 μg protein) were electrophoresed on 7%-Tris-glycine SDS-PAGE gels and transferred to a polyvinylidene difluoride membrane. Membranes were blocked using blocking agents in a chemiluminescence kit (Invitrogen Corp., Carlsbad, CA) overnight at 4°C and incubated with anti-COX-2 primary antibody (1:200; Cayman Chemical, Ann Arbor, MI) for 1 hr at room temperature. Membranes were subsequently incubated with a secondary antibody and visualized with the chemiluminescence kit according to the manufacturer's protocol.

Immunohistochemical detection of proliferating nuclear cell antigen

Immediately after the mice were euthanized, skin sections were placed in 10% neutral buffered formalin for 2 hr and washed with PBS. Sections were then embedded in paraffin blocks and sectioned onto slides. Paraffin was removed from the embedded tissue sections (4 μm); the sections were hydrated slowly with xylene and graded alcohol series and rinsed for 5 min in water. Tissues were antigen retrieved by microwaving at 70% power for 6 min in 10 mM citrate buffer; blocked with 3% H$_2$O$_2$ for 20 min, casein for 15 min, goat serum for 20 min and avidin/biotin for 30 min; incubated with the primary antibody, monoclonal mouse anti-PCNA (1:100; Biogenex, Inc., San Ramon, CA) for 30 min; then washed and incubated with biotinylated secondary antibody for 20 min and streptavidin-horseradish peroxidase label for 20 min. The color reaction was observed using 3,3′-diaminobenzidine for 1.5 min. Slides were counterstained with hematoxylin, dehydrated with alcohol and xylene and mounted using Permount (Sigma). Proliferating cell nuclear antigen (PCNA)-stained slides were viewed at 200× with a Nikon microscope mounted with a high-resolution camera. The camera was interfaced with a computer containing a matrox frame grabber board and image analysis software (Simple PCI Imaging Systems, Compix Inc., Cranberry Township, PA). The epidermal layer of each skin sample was examined, and a minimum of 15 fields (2000–2500 cells) were quantified to determine the mean labeling index (LI). The LI was calculated by dividing the PCNA-positive nuclear area by the total nuclear area in the epidermis and expressing the result as a percentage.

Statistical analysis

Body weight; food consumption; and tumor incidence, multiplicity, and volume data were determined for all control and experimental mice. Differences between groups were analyzed for statistical significance using one-way ANOVA followed by Bonferroni *t*-test for multiple comparisons. A 2-tailed Student's *t*-test was used to assess the statistical significance between the control,

TABLE II – EFFECT OF DIETARY FEEDING AND TOPICAL APPLICATION OF BROWN ALGAE POLYPHENOLS (BAP) ON UVB-INDUCED CARCINOGENESIS[1]

	Dietary feeding			Topical application		
	UVB alone	BAP (0.1%) + UVB	BAP (0.5%) + UVB	UVB alone	BAP (3 mg) + UVB	BAP (6 mg) + UVB
Tumor incidence (%)	100	79	94	100	93	89
Tumor multiplicity[2]	8.54 ± 0.75	$4.73 \pm 0.74 \ (45)^3$	$3.75 \pm 0.54 \ (56)^4$	8.45 ± 1.23	$3.42 \pm 0.56 \ (60)^4$	$4.56 \pm 0.56 \ (46)^3$
Tumor volume/tumor bearing mouse[2] (mm^3)	50.4 ± 7.5	$23.1 \pm 7.2 \ (54)^5$	$17.8 \pm 4.8 \ (65)^4$	69.4 ± 17.2	$23.7 \pm 4.8 \ (66)^5$	$29.7 \pm 8.5 \ (57)^5$
Tumor volume/ tumor[2] (mm^3)	6.43 ± 1.36	$4.23 \pm 0.81 \ (34)$	$3.86 \pm 0.79 \ (40)$	8.22 ± 1.93	$6.04 \pm 1.02 \ (27)$	$4.67 \pm 0.83 \ (43)$

[1]Total number of tumors and tumor volume in different treatment groups were recorded at 26 week. Percentage of inhibition is given in parentheses.–[2]Mean $\pm$ SE obtained from 20 animals in each group at the time of data recording.–[3]Highly significant versus UVB alone, $p < 0.005$.–[4]Highly significant versus UVB alone, $p < 0.001$.–[5]Significant versus UVB alone, $p < 0.05$.
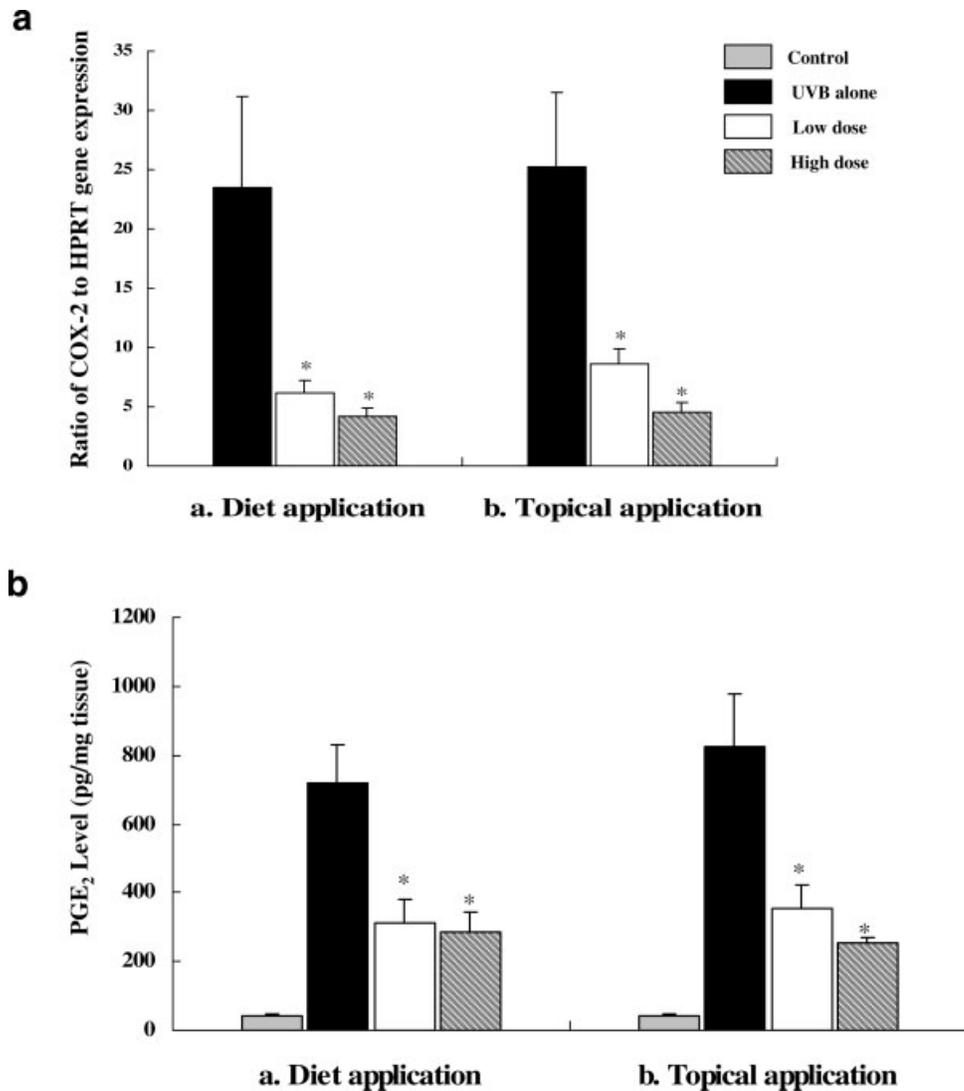
FIGURE 2 – Effect of brown algae polyphenols on UVB-induced COX-2 expression. COX-2 gene expression in skin was determined by (a) real-time RT-PCR and (b) ELISA of PGE_2. The values are expressed as mean $\pm$ SE obtained from 10 samples from each group. $*p < 0.05$ (versus UVB alone group).

UVB-exposed, and treatment groups. $p < 0.05$ was considered statistically significant.

Results

Protective effect of brown algae polyphenols on UVB-induced skin carcinogenesis

Mean body weights in treated and control groups did not differ significantly throughout the study. However, the body weights of animals in the UVB-alone group decreased slightly toward the end of the study (data not shown). Food consumption among the groups did not differ significantly throughout the study. These observations suggest that brown algae polyphenols are well accepted as a dietary component, are not toxic, and do not have adverse health effects in mice.

At the end of the experiment, no tumors were observed in groups 1–3, showing that long-term topical application of either the vehicle (DMSO and acetone) or brown algae polyphenols had no effect
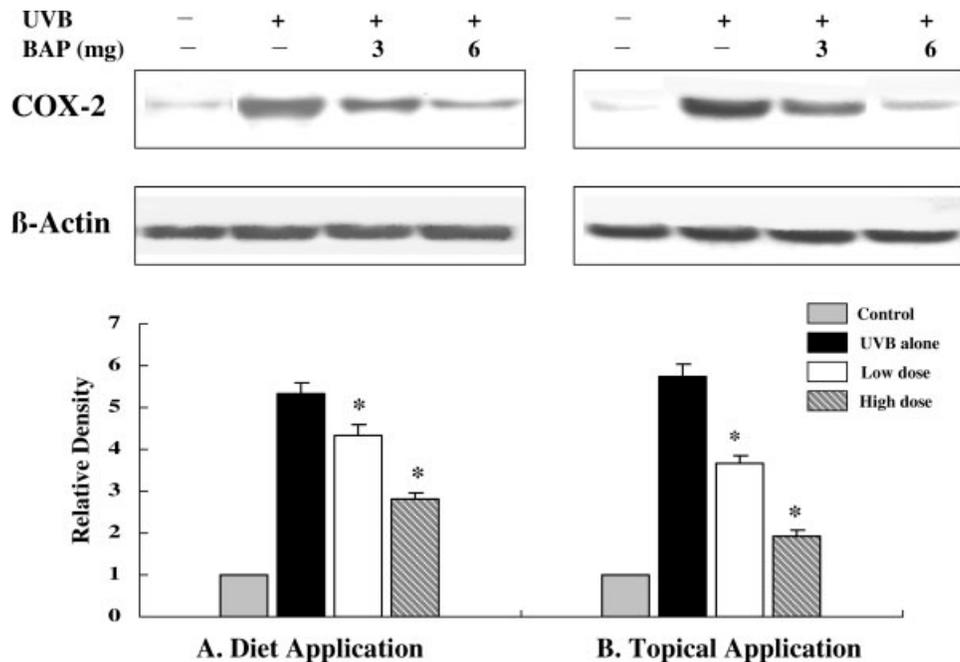
FIGURE 3 – Effect of brown algae polyphenols on UVB-induced increase in COX-2 protein. The mice were subjected to UVB and brown algae polyphenol treatment as described in Materials and Methods, and at 26 week, the animals were euthanized and the whole skin separated from the tumors and pulverized in liquid nitrogen. The pulverized skin section was homogenized in lysis buffer, and lysates were subjected to SDS-PAGE followed by Western blotting. The effects of treatment on COX-2 protein were assessed using immunoblot analysis (*a*) and followed by quantification with densitometry analysis of protein bands (*b*). Equal loading was confirmed by stripping the immunoblot and reprobing it for β-actin. The data (relative density normalized to β-actin) are expressed as mean ± SE obtained from 10 samples from each group. *$p < 0.05$ (versus UVB alone group) as determined by one-way ANOVA followed by a Bonferroni *t*-test for multiple comparisons.

on tumor development. Dietary and topical administration of brown algae polyphenols resulted in decreased tumor incidence between 79% and 94%, respectively, a moderate (21% and 6%; nonsignificant) protection (Table II). Although the effect on tumor incidence was moderate, the effect of brown algae polyphenols on tumor multiplicity and tumor volume was strong for both dietary and topical treatment. Thus, dietary treatment with 0.1% and 0.5% brown algae polyphenols reduced tumor multiplicity from 8.54 ± 0.75 to 4.73 ± 0.74 ($p < 0.005$) and 3.75 ± 0.54 ($p < 0.001$) tumors/tumor bearing mouse accounting for a 45% and 56% inhibition, respectively. Tumor multiplicity in animals topically administrated 3 and 6 mg brown algae polyphenols was reduced from 8.45 ± 1.23 to 3.42 ± 0.56 ($p < 0.001$) and 4.56 ± 0.56 ($p < 0.005$) tumors/tumor bearing mouse, respectively, accounting for a 60–46% inhibition. As shown in Table II, dietary feeding of 0.1% and 0.5% brown algae polyphenols significantly inhibited tumor volume by 54% ($p < 0.05$) and 65% ($p < 0.001$), respectively. Also, topical administration of 3 and 6 mg of brown algae polyphenols markedly prevented tumor volume by 66% ($p < 0.05$) and 57% ($p < 0.05$), respectively.

Inhibitory effect of brown algae polyphenols on UVB-induced COX-2 gene expression

Dietary feeding of 0.1% and 0.5% brown algae polyphenols significantly suppressed the gene expression of cyclooxygenase-2 (COX-2) in UVB-induced skin carcinogenesis by 74% ($p < 0.05$) and 82% ($p < 0.005$), respectively. Topical administration of 3 and 6 mg brown algae polyphenols resulted in a significant inhibition of UVB-induced gene expression of COX-2 by 66% ($p < 0.05$) and 82% ($p < 0.05$), respectively (Fig. 2*a*).

Inhibitory effect of brown algae polyphenols on UVB-induced PGE$_2$ production

Current data suggest that UV light exerts an initial stress on skin followed by up-regulation of COX-2 gene expression, which may

function to promote tumor development in various ways including enhancing PGE$_2$ levels in the skin. As shown in Figure 2*b*, PGE$_2$ levels in skin samples from UVB-alone groups was remarkably elevated when compared with unirradiated groups ($p < 0.0001$). Dietary feeding with 0.1% and 0.5% brown algae polyphenols significantly decreased PGE$_2$ levels in skin by 57% ($p < 0.01$) and 60% ($p < 0.01$), respectively. Topical application of 3 and 6 mg brown algae polyphenols also significantly reduced the PGE$_2$ levels by 57% ($p < 0.05$) and 70% ($p < 0.01$), respectively.
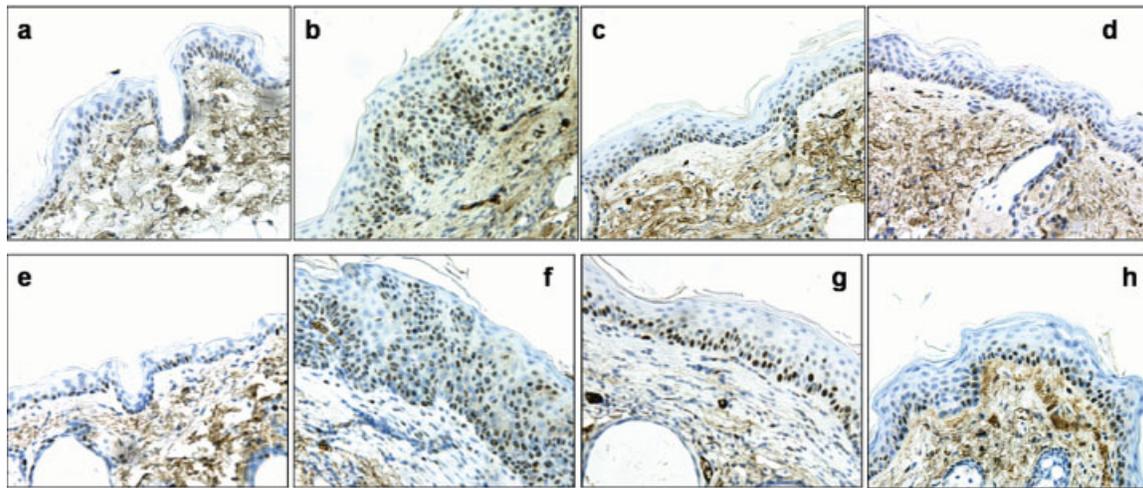
Inhibitory effect of brown algae polyphenols on COX-2 protein level in UVB-induced carcinogenesis

The inhibitory effect of brown algae polyphenols on UVB-induced COX-2 protein production in the skin was investigated using Western blot analysis (Fig. 3*a*) followed by densitometric analysis of the bands (Fig. 3*b*). Multiple UVB exposures resulted in significant increases in the COX-2 protein level in the skin (5- to 6-fold). However, dietary feeding of 0.1% and 0.5% brown algae polyphenols significantly inhibited the amount of COX-2 protein in the skin, accounting for 19% and 48% inhibition ($p < 0.05$), respectively. Topical application of 3 and 6 mg of brown algae polyphenols significantly reduced the level of COX-2 protein in the skin by 36% and 60%, respectively ($p < 0.05$).

Inhibitory effect of brown algae polyphenols on UVB-induced cell proliferation in the epidermis of mouse skin

PCNA is an excellent marker for cell proliferation. To determine whether dietary feeding and topical application of brown algae polyphenols affects UVB-induced cell proliferation, skin samples from all of the control and experimental groups were examined for PCNA immunostaining. Five samples per each experimental group were used for analysis, and 15 fields were randomly selected from the epidermis of each skin sample. As shown in Figure 4, UVB radiation without treatment with brown algae polyphenols resulted in a strong PCNA-positive staining in the
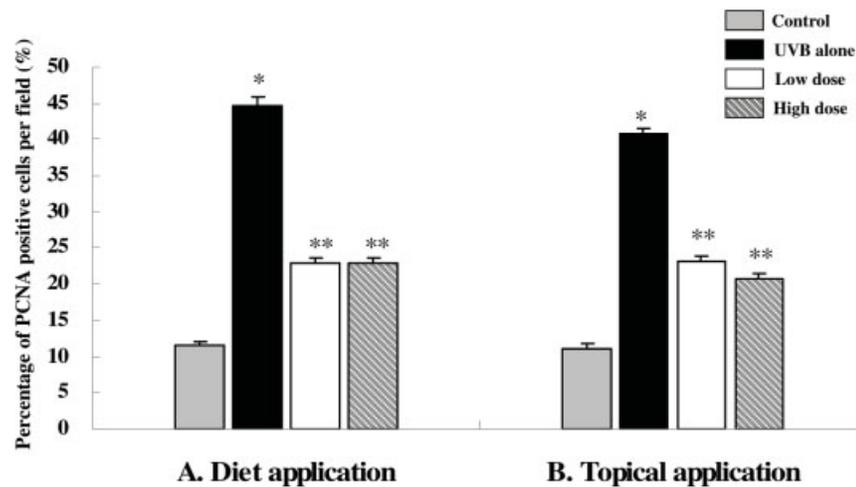
A.

B.

A. Diet application   B. Topical application

FIGURE 4 – Effect of brown algae polyphenols on UVB-induced cell proliferation in the epidermis of mouse skin. (*a*) Immunohistochemical localization of PCNA in the epidermis. PCNA-stained sections from (a) control group (b) UVB alone (c) UVB + dietary feeding of 0.1% brown algae polyphenols (d) UVB + dietary feeding of 0.5% brown algae polyphenols (e) topical treatment of brown algae polyphenols alone (f) UVB + topical application of solvent alone (g) UVB + topical application of brown algae polyphenols (3 mg) (h) UVB + topical application of brown algae polyphenols (6 mg) (400×). (*b*) Graphical representation of the immunohistochemical analysis of PCNA positive cells. The percentage of PCNA-positive epidermal cells was recorded in randomly selected fields (400×) from each tissue sample. Data shown are mean ± SE obtained from 15 fields randomly selected from each tissue sample. The statistical significance of difference between UVB-treated and all other groups was calculated by one-way ANOVA followed by a Bonferroni *t*-test for multiple comparisons. *$p < 0.001$ (versus control group), **$p < 0.01$ (versus UVB alone group).

epidermis compared with the unexposed control, accounting for $(44.7 \pm 1.3)\%$ and $(40.9 \pm 0.6)\%$ PCNA-positive cells ($p < 0.001$). However, dietary feeding of 0.1% and 0.5% brown algae polyphenols significantly reduced the percentage of proliferating cells in the epidermis to $(23.0 \pm 0.6)\%$ and $(22.9 \pm 0.9)\%$, accounting for 48% and 49% ($p < 0.01$) inhibition, respectively. Topical administration of 3 and 6 mg of brown algae polyphenols also significantly decreased the percentage of PCNA-positive cells in the epidermis to $(23.2 \pm 0.8)\%$ and $(20.9 \pm 0.5)\%$, accounting for 43% and 49% inhibition ($p < 0.01$), respectively.

Discussion

The purpose of this study was to evaluate the effectiveness of polyphenols extracted from brown algae in inhibiting UVB-induced skin carcinogenesis in mice. Our experimental data demonstrated that both dietary feeding and topical treatment of brown algae polyphenols decreased UVB-induced skin tumor development. We also demonstrated that these inhibitory effects were associated, in part, with suppression of COX-2 expression and cell proliferation. COX-2 is an enzyme induced by inflammatory stimuli, such as UVB light. Induction of COX-2 elevates levels of prostaglandin E_2 (PGE_2), which acts as a promoter in carcinogenesis, inflammatory cell infiltration and activation, and the development of skin tumors.[36–38] Numerous studies have shown that chronic inflammation is a major component of UVB-induced skin cancer development and that antiinflammatory agents can inhibit photocarcinogenesis.[39,40] Our study indicates that dietary feeding and topical application of brown algae polyphenols reduces inflam-

mation associated with chronic UVB exposure, at least in part, by suppression of COX-2 expression in the skin.

Brown algae grown in the sea are used as a foodstuff in some Asian countries, such as Korea and Japan. Several studies reported that fucoidan (a polysaccharide) and fucoxanthin (a carotenoid) extracted from brown algae possess antitumor activity.[41–43] However, only a few studies reported the cancer chemopreventive effect of seaweeds on carcinogenesis.[44–46] This is the first study demonstrating a chemopreventive effect of pure polyphenols from brown algae in a UVB-induced skin carcinogenesis model. As we previously reported, brown algae polyphenols exhibit excellent antioxidant activity,[28] with the potential to protect against chronic diseases that are, at least in part, due to oxidative stress.

Dietary administration of brown algae polyphenols imparted protection equal to that of topical application. Thus, the cancer chemoprotective effect of brown algae polyphenols is not via a simple sunscreen pathway but rather through absorption of polyphenol molecules through the skin to activate a protective signaling cascade. Absorption of the polyphenols through the skin is facilitated by their low molecular weight and significant lipophilicity. (Brown algae polyphenols used in this study are highly soluble in ethyl alcohol.)

In summary, our data show that the brown algae polyphenols exert highly protective effects against UVB-induced skin carcinogenesis. This result suggests the possibility for further development of brown algae polyphenols as cancer chemopreventive agents against photocarcinogenesis and other adverse effects of UVB exposure.

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