Cancer Chemopreventive Effects of Korean Seaweed Extracts

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Abstract Cancer chemopreventive effects can be exerted through the induction of phase II detoxification enzymes and the inhibition of inflammatory responses. In this study, the cancer chemopreventive effects and anti-inflammatory responses of 30 seaweed extracts were examined. The extracts of *Dictyota coriacea* and *Cutleria cylindrica* exhibited the high chemoprevention index, having 4.36 and 4.66, respectively. They also activated antioxidant response element at 100 μg/mL by about 3-fold while did not activate xenobiotic response element. Seven seaweed extracts, *Ishige okamurae*, *Desmarestia ligulata*, *Desmarestia viridis*, *Dictyopteris divaricata*, *D. coriacea*, *Sargassum horneri*, and *Sargassum yezoense*, showed significant inhibition on nitric oxide (NO) and prostaglandin E_2 (PGE_2) production in a dose-dependant manner in 5-20 μg/mL. These seaweed extracts could be used as food materials for cancer chemoprevention. *D. coriacea* could contain potential chemopreventive agents not only that regulate genes via an ARE-dependent mechanism but also prevent the inflammation through inhibition of NO and PGE_2 production.

Keywords: quinone reductase, chemoprevention index, detoxification enzyme, antioxidant response element, anti-inflammation

Introduction

Numerous epidemiological data have suggested that cancer is preventable disease. The factors causing various types of cancers share common pathogenic mechanisms such as DNA damage, oxidative stress, and chronic inflammation (1). Recently prevention of cancer is considered as a preferable option rather than a chemotherapy since cancer is a preventable disease by avoiding exposures to the risk factors. Cancer chemoprevention is defined as the pharmacological administration of synthetic or naturally occurring compounds that prevent, inhibit, or reverse carcinogenesis, or prevent the development of invasive cancer (2,3).

The potential chemopreventive agents regulating detoxi-fication enzymes are divided into two groups, designated monofunctional and bifunctional inducers. Monofunctional inducers upregulate a number of phase II detoxification enzymes, including quinone reductase (QR), which is also known as NAD(P)H: quinone oxidoreductase, NQO1 (4), and glutathione-S-transferases (GST) (5). Bifunctional inducers upregulate a similar array of phase II enzymes, in addition to a few phase I enzymes, including CYP1A1. Since phase I enzymes are involved in both bioactivation and detoxification of carcinogens, monofunctional inducers are closely related to chemoprevention, relative to bifunctional inducers (6). There are 2 regulatory elements, antioxidant response element (ARE) and xenobiotic response element (XRE) known for regulating detoxification enzyme by chemopreventive agents. The ARE is related

with induction of phase II detoxification enzymes (monofunctional induction), while the XRE functions in the induction of not only phase II but also some phase I cytochrome P450 enzymes (bifunctional induction). Both ARE and XRE are present in the regulatory region of *QR* and *GST* genes, (7,8) while only XRE is present in the regulatory region for cytochrome P450 1A (*CYP1A*) gene (9).

Anti-inflammatory agents can be also classified as cancer chemopreventive agents that can inhibit tumor promotion (1). Both nitric oxide (NO) and prostaglandins (PGs) which are synthesized by nitric oxide synthetase (NOS) and cyclo-oxygenases (COX), respectively, are known to be important mediators of acute and chronic inflammation (10-12). NO is a pleiotropic regulator, pivotal to numerous biological processes including vasodilation, neurotransmission, and macrophage-mediated immunity (13). There is a significant proof implicating NO in carcinogenesis as an endogenous mutagen, an enhancer of protooncogene expression, and an inhibitor of apoptosis (14-16). It appears that once the tumor is established and progressed, NO may also mediate pro-tumorigenic activities, including capillary leakage, angiogenesis, leukocyte adhesion, and infiltration, and eventually metastasis (17,18). Increased NOS expression and/or activity were also reported during tumorigenesis, suggesting common feature of many cancers (13). Thus, developing selective inhibitors of NO-releasing agents may lead to significant strategies for chemoprevention of cancer. PGs, other mediators of inflammation, belong to the class of prostanoid fatty acid derivatives of arachidonic acid, which is liberated from membrane phospholipids by action of phospholipases, are metabolized into prostagladin G_2 (PGG_2) and prostagladin H_2 (PGH_2) by COX-1 and COX-2, and are converted into prostagladin E_2 (PGE_2) by prostaglandin E systhetase (PGES). PGE_2 not only is

linked to the synthesis and release of several hormones (19,20), but also is important in normal joint physiology and is a principle mediator of the inflammatory response to tissue damage (21-23). PGE$_2$ also stimulates tumor cell proliferation and differentiation as well as tumor-associated neovascularization (24,25). Therefore, the agents which decrease the production of either NO or PGE$_2$ could be candidates of the chemopreventive agents.

Seaweeds, primary producers of the oceans, have served as human foodstuff, medicine, manure, animal fodder, and so on since ancient times. Korean people often had eaten either raw or cooked seaweeds which also have been proved as a rich source of structurally novel and biological active secondary metabolites (26). Chemopreventive effects of the seaweeds, however, have not been well-studied. The objectives of this study were to evaluate the chemopreventive potential of Korean seaweed extracts by measuring key chemopreventive effects including phase II detoxification enzyme induction and anti-inflammatory responses in animal cell culture system. Most putative chemopreventive agents, rather than having a single target, possess pleiotropic properties, and work via multiple mechanisms of action (1). Consequently, the agents that have abilities both detoxification of carcinogen and inhibition of inflammation could be highly effective for cancer chemoprevention.

Materials and Methods

Seaweed materials The 30 marine algae species used for this study were collected from December 2005 to May 2006 along the eastern and southern coast of South Korea. Samples collected were immediately transported to the laboratory and gently rinsed with filtered fresh water, dried under shade, and stored in a refrigerator until experiments were processed. Identification of seaweeds was performed by Professor Hyung-Seop Kim. The family, the scientific name, the local name, the collection time, and frequent uses in the traditional medicine systems for each tested species are summarized in Table 1 if they exist.

Preparation of 30 seaweed extracts Dried seaweed powder was extracted 3 times with 95% ethanol at room temperature. The ethanol extract was obtained after evaporation of solvent and the each weight was measured. The dry weight, the solvent used for extraction, and the weight of extract were listed in Table 1.

Cell culture HepG2 cells, Hepa1c1c7 cells, and RAW264.7 cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). These cells were maintained at subconfluence in 95% air and 5% CO$_2$ humidified atmosphere at 37°C. Dulbecco's modified Eagle medium (DMEM, Hyclone, Logan, UT, USA) was used for HepG2 cells and RAW264.7 cells cultivation and α-Minimum Essential medium (α-MEM, Hyclone) for Hepa1c1c7 cells cultivation. They were supplemented with 10% fetal bovine serum (FBS, Hyclone), penicillin (100 units/mL), and streptomycin (100 μg/mL).

Cell viability The cytotoxicity of seaweed extracts was evaluated using the Cell Counting kit (CCK-8; Dojindo Laboratories, Tokyo, Japan). In brief, 1×10^4 cells per well

were plated into 96-well plates, incubated at 37°C for 24 hr, and given a fresh change of medium. Cells were then treated with various concentrations of extracts and incubated at 37°C for an additional 24 hr. At that point, 10 μL of the CCK-8 solution was added to the wells and incubation was continued for another 1 hr. The absorbance at 450 nm was measured and the absorbance at 600 nm was subtracted using a PowerWave™ XS Microplate Spectrophotometer microplate reader (Bio-Tek Instruments, Winooski, VT, USA). Data were reported as percent cell growth relative to respective controls (cells treated with solvent only) for each sample concentration.

Quinone reductase assay The QR induction activities were determined by Prochaska modified bioassay with a little modification (7). Hepa1c1c7 cells (1×10^4 cells per well) were plated into 96-well plates (Techo Plastic Products AG, Trasadingen, Switzerland) and incubated for 24 hr prior to treatments. Growth media containing 2.5 μM sulforaphane were used as positive controls. The treated cells were rinsed with phosphate buffered saline (pH 7.4), lysed with 80 μL of 0.08% digitonin in 2 mM ethylenediamide tetraacetic acid (EDTA), incubated for 30 min, and subjected to QR assay. Protein content was measured in a 20 μL aliquot of the digitonin cell lysate in a separate 96-well plate. Total protein content was measured by Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA, USA). A 200 μL aliquot of mixed solution [49 mL of 25 mM Tris buffer; 34 mg of bovine serum albumin (BSA); 0.34 mL of 1.5% Tween-20 solution; 0.34 mL of thawed cofactor solution (150 mM glucose-6-phosphate, 4.5 mM nicotinamide adenine dinucleotide phosphate (NADP), 0.75 mM flavin adenine dinucleotide (FAD) in Tris buffer); 100 units of glucose-6-phosphate dehydrogenase; 15 mg of 3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide (MTT); and 50 μL of 50 mM menadione in acetonitrile] was added into a 50 μL aliquot of cell lysates. The absorbance at 610 nm was measured 5 times at intervals of 50 sec using a PowerWave™ XS Microplate Spectrophotometer microplate reader (Bio-Tek Instruments). Induction of the QR activity was calculated by comparing the QR specific activity of compound treated cells with that of control treated cells. Enzyme activity was expressed as quinone reductase activity (CD), concentration required to double QR activity. Chemoprevention index (CI) is obtained by dividing IC$_{50}$ values (concentration for 50% inhibition of cell viability) by CD values.

Transient transfection and ARE/XRE activation assay using CAT-ELISA HepG2 cells (1×10^5 cells/ mL) were cultured in 24-well tissue culture plates for 24 hr before transfection at 70-80% confluency. Cells were transiently co-transfected with 2.5 μg of one of two different reporter constructs containing either the antioxidant response element (ARE QR-CAT) or the xenobiotic response element (XRE QR-CAT) derived from the rat QR gene (24). All CAT reporter gene constructs were gifts from Dr. Cecil Pickett (Schering-Plough Research Institute, Kenilworth, NJ, USA). After 24 hr treatment, cells were lysed and assayed for CAT expression using a CAT-ELISA kit (Roche Biochemicals, Indianapolis, IN, USA), following the manufacturer's instructions. 3-Methylcholanthrene (Sigma-

Table 1. List of seaweeds evaluated in the experiment

Family	Scientific name	Dry weight (g)	solvent used (L)	extract weight (mg)	Location	Time collected	Traditional uses
	Enteromorpha intestinalis (Linnaeus) Nees	11.515	0.5	266.9	Gangneung Aninjin	06.05.13	Foodstuff (31-33), antimitotic, polysynoptic blocker (34), aphthae, back pain, paronychia, lymphatic swellings, and goiter (35)
Ulvaceae	*Enteromorpha linza* (Linnaeus) J. Agardh	89.308	2.0	1,021.0	Gangneung Aninjin	06.05.13	Foodstuff (31, 32, 36)
	Ulva armoricana P. Dion, B. de Reviers & G. Coat	55.729	1.0	890.3	Guryongpo Daebo	06.04.22	Unknown
Ishigeaceae	*Ishige okamurae* Yendo	38.789	1.0	338.6	Jeju Hado	05.12.16	Foodstuff (37)
Scytosiphonaceae	*Cutleria cylindrica* Okamura	20.000	0.5	879.9	Gangneung Geumjin	06.01.08	Unknown
	Scytosiphon lomentaria (Lyngbye) Link	34.098	1.0	446.8	Gangneung Jumunjin	06.05.04	Foodstuff (31, 32, 36, 37) dry coughs, laryngitis, lymphatic tuberculosis (35)
Desmarestiales	*Desmarestia ligulata* (Stackhouse) Lamouroux	14.130	0.2	1,104.2	Gangneung Aninjin	06.03.25	Agglutinin (38), animal fodder (36), and antimicrobial (39)
	Desmarestia viridis (Müller) Lamouroux	17.150	0.2	1,046.5	Gangneung Aninjin	06.03.25	Agglutinin (38)
Alariaceae	*Undaria pinnatifida* (Harvey) Suringar	15.507	0.5	1,070.2	Gangneung Aninjin	06.04.27	foodstuff, cultivation (31-33, 35, 37, 40, 41), nicotine poisoning cure, antihypertensives (42), stomach ailments, hemorrhoids, anal fistulas, leucorrhea, nocturnal enuresis, urinary diseases, and dropsy (43)
	Agarum cribrosum Bory	15.522	0.8	963.7	Gangneung Youngjin	06.04.11	Foodstuff (32) and alginates (31, 32)
	Costaria costata (C. Agardh) Saunders	50.103	1.0	1,224.4	Gangneung Youngjin	06.04.11	Foodstuff (31)
Laminariaceae	*Ecklonia cava* Kjellman in Kjellman et Petersen	65.000	0.5	665.6	Jeju Hado	05.12.16	Antihypertensives (42) and alginates (31, 32, 36)
	Laminaria japonica Areschoug	7.000	0.3	473.2	Gangneung Aninjin	06.03.07	Foodstuff (31-33, 36, 37, 41), dropsy (44), high blood pressure (35), anticoagulant, hypocholesterolemic (45), normalizing blood pressure, hyperthyroidism, goiter, dropsy, scrofula, stomach ailments, hemorrhoids, urinary problems, anal fistulas (31, 43, 46, 47), and alginates (31, 47)

Table 1. Continued

Family	Scientific name	Dry weight (g)	solvent used (L)	extract weight (mg)	Location	Time collected	Traditional uses
Dictyotaceae	Dictyopteris divaricata (Okamura) Okamura	14.265	0.5	1,477.7	Gangneung Sageunjin	06.04.27	Unknown
	Dictyopteris pacifica (Yendo) Hwang et al.	40.000	1.0	2,235.6	Gangneung Aninjin	05.11.04	Unknown
	Dictyota coriacea (Holmes) Hwang et Kim comb. Nov.	25.000	0.5	3,161.5	Kyungbuk Eubcheon	05.11.14	Unknown
	Pardina arborescens Holmes	25.000	0.5	2,223.1	Namhae	05.11.15	Unknown
Sargassaceae	Hizikia Fusiformis (Harvey) Okamura	64.401	1.0	1,887.7	Guryongpo Daebo	06.04.22	Foodstuff (31-33, 36, 41)
	Sargassum confusum C. Agardh	37.659	1.0	2,456.3	Gangneung Aninjin	06.03.25	Animal fodder and alginates (48)
	S. horneri (Turner) C. Agardh	48.738	1.0	4,460.4	Gangneung Aninjin	06.03.25	Foodstuff, goiter (47), animal fodder and alginates (31, 32, 37)
	S. miyabei Yendo	70.310	1.0	691.2	Gangneung Jumunjin	06.05.04	Drugs and alginates (37)
	S. yezoense (Yamada) Yoshida et T. Konno	62.838	1.0	6,395.7	Gangneung Aninjin	06.03.25	Antimicrobial (49) and alginates (31)
	S. thunbergii (Mertens ex Roth) Kuntze	40.000	0.3	357.3	Namhae Sachon	05.11.15	Foodstuff (32) vermifuge (31, 37), animal fodder, and manure (31)
Bonnemaisoniaceae	Bonnemaisonia hamifera Hariot	107.170*	0.4	984.0	Gangneung Sageunjin	06.05.15	Antimicrobial (39)
Halymeniaceae	Carpopeltis cornea (Okamura) Okamura	33.796	1.0	65.8	Gangneung Aninjin	06.05.13	Unknown
Phacelocarpaceae	Gracilaria textorii (Suringar) Hariot	23.085	0.8	1,029.5	Gangneung Sageunjin	06.04.27	Foodstuff and agar (31, 32)
	Gracilaria verrucosa (Hudson) Papenfuss	34.116	0.8	2,393.0	Anmyundo	06.05.23	Foodstuff, agar (31-33, 36, 37, 41, 50) antimicrobial (39), pulmonary tuberculosis, stomach disorders (51, 52), urinary diseases, dropsy, and goiter (43, 47)
Delesseriaceae	Delesseria serrulata Harvey	5.000	0.3	290.7	Gangneung Sacheon	06.01.03	Unknown
Rhodomelaceae	Laurencia nipponica Yamada	11.478	0.5	990.7	Gangneung Sageunjin	06.05.15	Unknown
	Polysiphonia morrowii Harvey	27.050	0.2	549.0	Gangneung Aninjin	06.03.25	Antimicrobial (39)

Aldrich, St. Louis, MO, USA), a typical XRE activator, was used as a positive control in this experimental system. CAT expression was normalized with respect to protein concentration, which was determined with the Bicinchoninic acid protein assay kit (Sigma-Aldrich) and presented as fold induction over the control.

Measurement of nitrite production using Griess reagent
For the assay of NO production, RAW264.7 cells were plated in 24-well plates at a density of 2×10^5 cells/well in 0.5 mL DMEM. After 24 hr incubation, culture media were replaced with fresh DMEM containing 10% FBS, and the samples were treated. After 4 hr incubation, the cells were stimulated with 10 µg/mL iipopolysaccharide (LPS) and 10 units/mL interferon-γ (IFN-γ), and incubated for 16 hr at 37°C. NO production in culture supernatant was spectrophotometrically evaluated by measuring nitrite, an oxidative product of NO. Nitrite was determined with the Griess reaction (8) by mixing 100 µL of culture supernatant with 100 µL of Griess reagent containing equal volumes of 1% sulphanilamide in 5% phosphoric acid and 0.1% H-(1-naphthyl)ethylenediamine solution. The absorbance at 540 nm was measured with a PowerWave™ XS (Bio-Tek Instruments). The value was calculated as percent NO production relative to respective controls (cells stimulated with 10 µg/mL LPS and 10 units/mL IFN-γ) for each sample concentration. Then, data were normalized for viable cell number percentage assessed by the cell viability assay.

Measurement of PGE$_2$ production by enzyme-linked immunosorbent assay (ELISA) PGE$_2$ production was measured in culture medium in order to determine COX-2 activity. For the assay of COX-2 induction, RAW264.7 cells were plated in 24-well plates at a density of 2×10^5 cells/well in 0.5 mL DMEM. After 24 hr incubation, the samples were treated. After 4 hr incubation, the cells were stimulated with 10 µg/mL LPS and 10 units/mL IFN-γ, and incubated for 16 hr at 37°C. After the treatment, the PGE$_2$ ELISA was performed according to the manufacturer's protocol (R&D Systems, Minneapolis, MN, USA). The absorbance in each well was measured at 450 nm and the absorbance at 540 nm was subtracted with a PowerWave™ XS (Bio-Tek Instruments).

Results and Discussion

Chemoprevention index (CI) of seaweed extracts in Hepa1c1c7 cells Many different methods for the determination of cancer chemopreventive effects have been developed and used to screen potential chemopreventive activity. The screening for the induction of phase II detoxification and for the inhibition of inflammatory mediators in our experiment was successfully used to systematically assess the cancer chemopreventive effects of the natural products. However, the cancer chemopreventive effect of seaweed extracts was not clarified yet, comparing it with a lot of biological activities of the seaweeds. A number of previous studies have suggested that induction of phase II detoxification enzymes including quinone reductase (NQO1), glutathione-s-transferase (GST), glutathione reductase, glucose-6-phosphate dehydrogenase, and epoxide hydrolase is a relevant mechanism for cancer chemoprevention

(5).

To determine the ability of seaweed extracts to induce the quinone reductase, dose-dependent experiments were performed in mouse hepatocarcinoma Hepa1c1c7 cells. Table 2 summarizes the quinone reductase activity (CD), cytotoxicity (IC$_{50}$), and CI of the seaweed extracts. QR specific activity in Hepa1c1c7 cells was measured after 24 hr treatment of the seaweed extracts in the broad range of concentrations (6.25-200 µg/mL). The CI is known to be a useful marker for the screening of potential chemopreventive agents showing a high QR activity with a low cytotoxicity. Among 30 seaweed extracts, *C. cylindrica* showed the highest CI value (4.7) resulted from that IC$_{50}$ value was larger than 200 µg/mL and a CD value was 42.9 µg/mL. Although *D. coreacea* showed a higher QR activity than *C. cylindrical*, CI value was relatively low (4.4) due to a high cytotoxicity (64.1 µg/mL). In addition, *Ulva armoricana* and *Undaria pinnatifida* showed a relatively high QR induction with a CD value less than 100 µg/mL and no cytotoxicity, and the CI value were 2.2 and 2.6, respectively (Table 2). Among the extracts, 4 seaweed extracts including *U. armoricana, C. cylindrical, U. pinnatifida,* and *D. coriacea* were used for searching potential monofunctional inducers.

Activation of ARE by 4 seaweed extracts in HepG2 cells The promoters of genes encoding phase II detoxification enzymes have 2 important response elements called ARE and XRE responding to various chemopreventive agents (8,27). The compounds that stimulate both XRE- and ARE-driven gene expression are designated as 'bifunctional inducers' (2). In contrast, the compounds that transcriptionally activate genes through ARE, but not XRE, are designated as 'monofunctional inducers' (2). The induction of phase I enzymes, such as cytochrome P450 isozymes which have XRE but not ARE, is required for metabolic disposal of xenobiotics (28) but is also considered as a risk factor due to the potential of activating procarcinogens (29). Therefore, the activation of ARE, not XRE, appears to be the common anticancer mechanism of detoxification enzyme.

To determine the ability of 4 seaweed extracts to induce ARE- or XRE-driven gene expression, we transiently transfected human hepatocarcinoma HepG2 cells with a CAT reporter construct containing either the ARE consensus (ARE QR-CAT) or XRE consensus (XRE QR-CAT). The CAT activity was measured after 24 hr treatment of extracts (1, 10, and 100 µg/mL). In *U. armoricana* treated cells, ARE was significantly activated and the maximum level was already reached at 1 µg/mL. In *D. coreacea* and *C. cylindrica* treated cells, ARE was significantly activated in a dose-dependent manner, while XRE was not activated at any of the concentrations tested (Fig. 1). From these results, *U. armoricana, D. coreacea,* and *C. cylindrica* extracts might be contained compounds which could be the monofunctional inducers. There results imply that the extracts may have the compounds exerting their chemopreventive effects through an ARE-dependent mechanism regulating anticancer-related genes encoding detoxification and antioxidant enzymes (Fig. 1). Those seaweeds have not been used for traditional medicine (Table 1), so that they could be promising candidates for developing as nutraceuticals for cancer chemoprevention.

Table 2. Cytotoxicity and chemoprevention index (CI) of seaweed extracts in Hepa1c1c7 cells and cytotoxicity in RAW264.7 cells

| Family | Scientific name | Hepa1c1c7 | | | RAW264.7 |
		CD (μg/mL)[1]	IC$_{50}$ (μg/mL)[2]	CI[3]	IC$_{50}$ (μg/mL)[2]
Ulvaceae	*Enteromorpha intestinalis* (Linnaeus) Nees	no induction	179.9	0.0	165.7
	Enteromorpha linza (Linnaeus) J. Agardh	112.6	200<[4]	1.8	200<
	Ulva armoricana P. Dion, B. de Reviers & G. Coat	91.4	200<	2.2	200<
Ishigeaceae	*Ishige okamurae* Yendo	no induction	200<	0.0	194.4
Scytosiphonaceae	*Cutleria cylindrica* Okamura	42.9	200<	4.7	200<
	Scytosiphon lomentaria (Lyngbye) Link	no induction	200<	0.0	200<
Desmarestiales	*Desmarestia ligulata* (Stackhouse) Lamouroux	no induction	68.3	0.0	200<
	Desmarestia viridis (Müller) Lamouroux	96.9	168.9	1.7	36.7
Alariaceae	*Undaria pinnatifida* (Harvey) Suringar	75.9	200<	2.6	200<
Laminariaceae	*Agarum cribrosum* Bory	no induction	200<	0.0	200<
	Costaria costata (C. Agardh) Saunders	no induction	200<	0.0	200<
	Ecklonia cava Kjellman in Kjellman et Petersen	561.1	200<	0.4	152.3
	Laminaria japonica Areschoug	no induction	76.73	0.0	114.2
Dictyotaceae	*Dictyopteris divaricata* (Okamura) Okamura	262.3	120.5	0.5	200<
	Dictyopteris pacifica (Yendo) Hwang et al.	no induction	122.8	0.0	200<
	Dictyota coriacea (Holmes) Hwang et Kim comb. Nov.	14.7	64.1	4.4	200<
	Pardina arborescens Holmes	no induction	200<	0.0	200<
Sargassaceae	*Hizikia Fusiformis* (Harvey) Okamura	no induction	200<	0.0	200<
	S. confusum C. Agardh	no induction	200<	0.0	189.5
	S. horneri (Turner) C. Agardh	no induction	128.0	0.0	200<
	S. miyabei Yendo	480.1	200<	1.0	200<
	S. yezoense (Yamada) Yoshida et T. Konno	no induction	39.2	0.0	200<
	S. thunbergii (Mertens ex Roth) Kuntze	no induction	115.1	0.0	134.7
Bonnemaisoniaceae	*Bonnemaisonia hamifera* Hariot	360.3	131.5	0.4	23.6
Halymeniaceae	*Carpopeltis cornea* (Okamura) Okamura	227.3	200<	0.9	200<
Phacelocarpaceae	*Gracilaria textorii* (Suringar) Hariot	no induction	200<	0.0	200<
	Gracilaria verrucosa (Hudson) Papenfuss	no induction	200<	0.0	200<
Delesseriaceae	*Delesseria serrulata* Harvey	no induction	174.3	0.0	200<
Rhodomelaceae	*Laurencia nipponica* Yamada	no induction	200<	0.0	200<
	Polysiphonia morrowii Harvey	no induction	69.5	0.0	200<

[1] Concentration required to double QR activity.
[2] Concentation required to inhibit cell growth by 50%.
[3] Chemoprevention index=IC$_{50}$/CD.
[4] The highest limit of test concentration was 200 μg/mL; if the extract has no toxicity, 200 μg/mL is used for CI value.

Inhibition of NO production in RAW264.7 cells In order to evaluate the anti-inflammatory capacity of seaweed extracts, NO accumulation was examined in culture medium of LPS/IFN-γ-stimulated RAW264.7 cells. We first performed the experiments to determine whether seaweed extracts affect NO production in RAW264.7 cells. Cells were stimulated with both LPS and IFN-γ in the presence or absence of 30 seaweed extracts for 16 hr, and the levels of NO were measured in the culture medium by Griess reagents. LPS/IFN-γ-stimulated cells increased the accumulation of nitrite, a stable oxidized product of NO in the culture medium while control cells did not (Fig. 2). Decreased NO production was observed in most of seaweed extracts-treated cells (20 μg/mL) compared to LPS/IFN-γ-stimulated cells (Fig. 2). Especially, *I. okamurae, Scytosiphon lomentaria, D. ligulata, D. viridis, D. divaricata, D. coriacea, S. horneri*, and *S. yezoense*-treated cells showed a marked decrease in NO production. Therefore, 8 seaweed extracts

were selected by inhibition ability for NO production. LPS/IFN-γ-stimulated NO productions were significantly decreased in a dose-dependant manner by co-treatment with 8 seaweed extracts at 5-20 μg/mL (Fig. 3).

Inhibition of PGE$_2$ production in RAW264.7 cells The effect of seaweed extracts on the level of PGE$_2$ in the LPS/IFN-γ-stimulated RAW264.7 cells was examined. PGE$_2$ concentration was measured under the same experimental conditions as NO production assay in the culture medium by using ELISA kit, and was normalized with total protein concentration by BCA protein assay. As shown in Fig. 4, the levels of PGE$_2$ increased in LPS/IFN-γ-treated cells while the control did not. Decreased PGE$_2$ production was observed in several seaweed extract-treated cells (50 μg/mL) (Fig. 4). Especially, *I. okamurae, D. ligulata, D. viridis, D. divaricata, D. coriacea, S. horneri*, and *S. yezoense*-treated cells showed a marked decrease in not only PGE$_2$
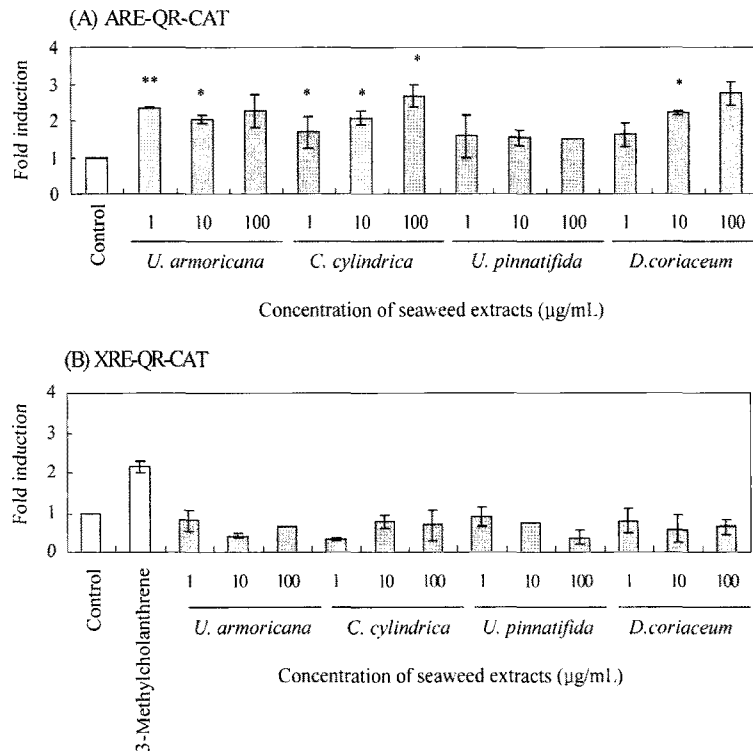
Fig. 1. Dose-dependent effects on ARE or XRE driven CAT expression by seaweed extracts in HepG2 cells. The cells were transfected with either ARE QR-CAT (A) or XRE QR-CAT (B) construct for 24 hr. CAT expression was normalized using the protein concentration present in each sample and expressed as fold-induction over the control. 3-Methylcholanthrene (0.1 μM) was used as a positive control for XRE activation. The bars marked with an asterisk are significantly different from control (*$p<0.05$; **$p<0.01$) using Student's t test, with $n=3$.
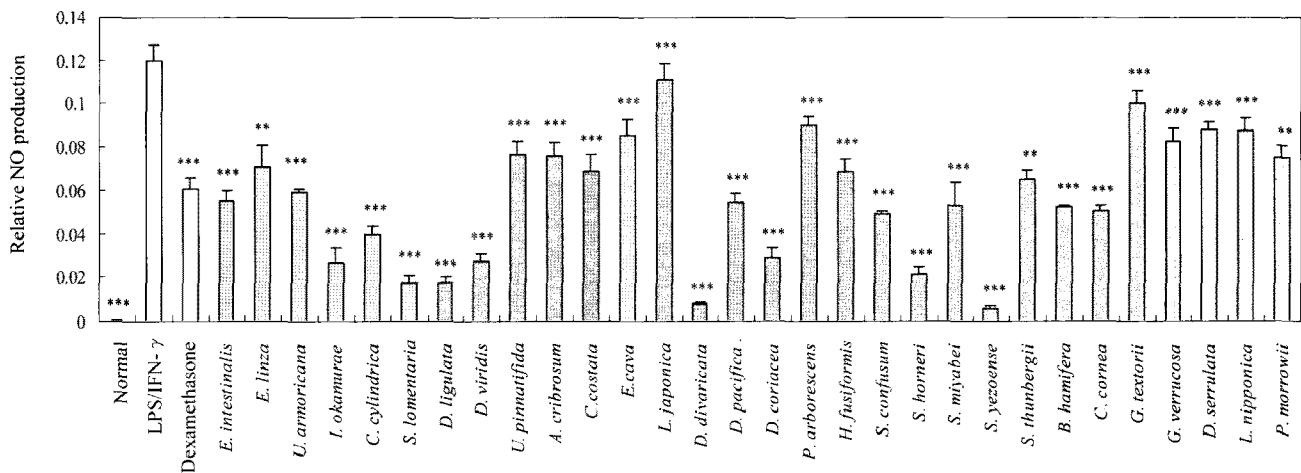
Fig. 2. Effects of 30 seaweed extracts on NO production in RAW264.7 cells stimulated with 10 μg/mL LPS and 10 units/mL IFN-γ. The concentration of seaweed extracts was 20 μg/mL (d.w./mL), and dexamethasone was treated with 20 μM. The asterisks are significantly different from LPS and IFN-γ stimulated RAW264.7 cells (**$p<0.01$; ***$p<0.005$) using Student's t test, with $n=3$.

but also NO production. In these seaweed extract-treated cells, PGE_2 production was significantly decreased in does-dependent manner at 5-20 μg/mL (Fig. 5).

Immune-activated macrophages up-regulate the expression of the inflammatory enzymes, such as iNOS and COX-2; these enzymes synthesize NO and PGE_2 from L-arginine and arachidonic acid, respectively. Therefore, decrease of both NO and PGE_2 in seaweed extracts-treated RAW264.7 cells could be an index of the anti-inflammatory ability. In addition, iNOS and COX-2 are regulated by nuclear factor-

κB (NF-κB) which is a transcriptional factor that acts as a central mediator of the human immune response and controls the expression of various genes involved in inflammation and proliferation (30). Seaweed extracts of *I. okamurae, D. ligulata, D. viridis, D. divaricata, D. coriacea, S. horneri,* and *S. yezoense* showed strong inhibition on both NO and PGE_2 production. Therefore, some bioactive components present in those seaweeds may inhibit NF-κB activation and decrease the level of iNOS and COX-2 expression in LPS/IFN-γ-stimulated cells. *D. coriacea* extract-treated
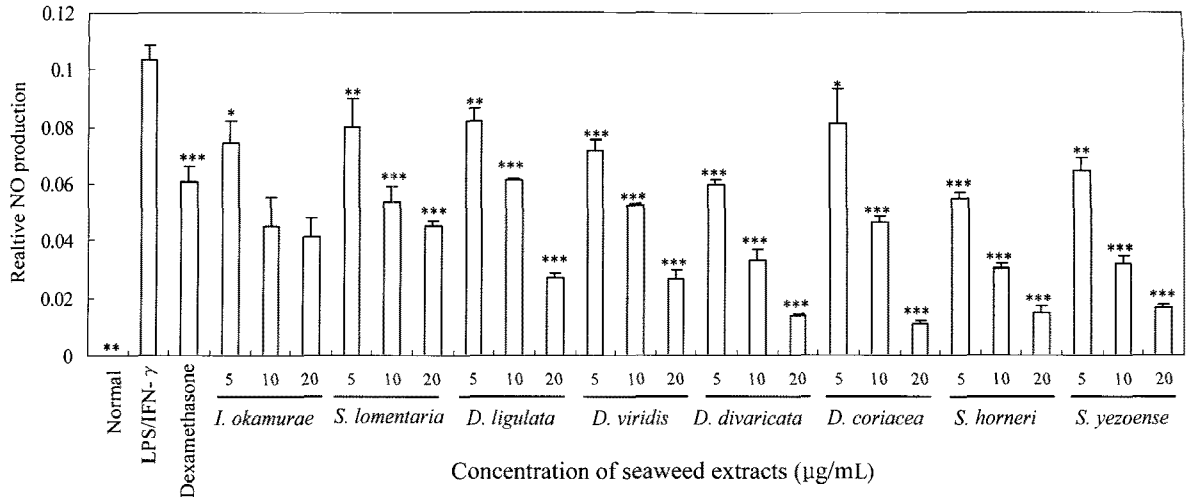
Fig. 3. Dose-dependent effects on NO production by 8 seaweed extracts in RAW264.7 cells stimulated with 10 µg/mL LPS and 10 units/mL IFN-γ. The concentration of seaweed extracts was 5, 10, and 20 µg/mL (d.w./mL) and dexamethasone was treated with 20 µM. The asterisks are significantly different from LPS and IFN-γ stimulated RAW264.7 cells (*$p<0.05$; **$p<0.01$; ***$p<0.005$) using Student's t test, with $n=3$.
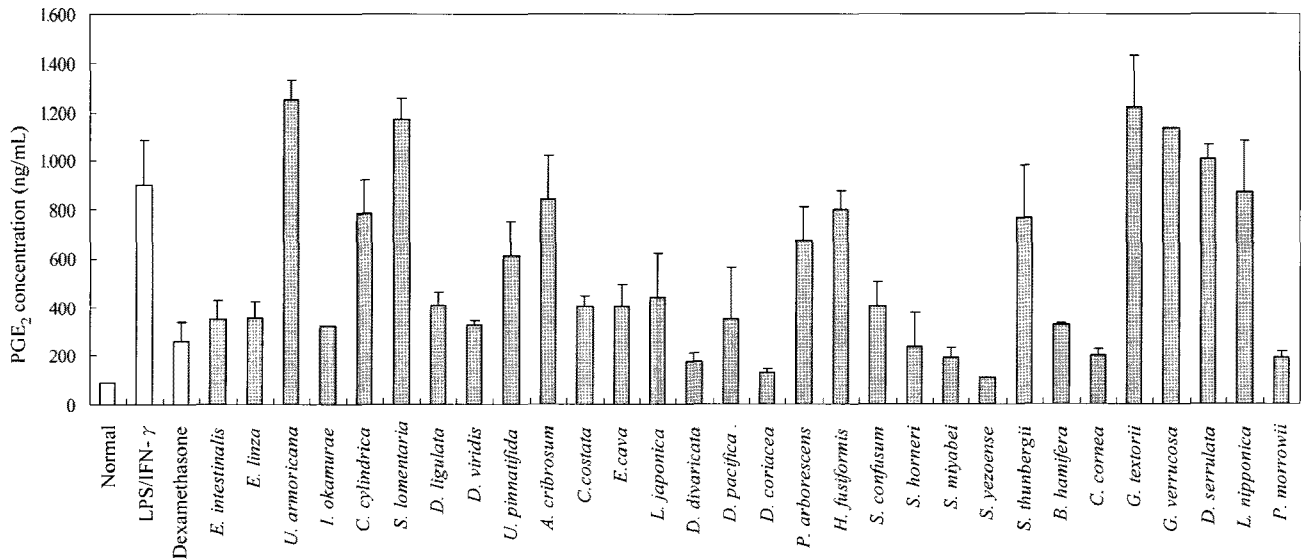

Fig. 4. Effects of 30 seaweed extracts on PGE$_2$ production in RAW264.7 cells stimulated with 10 µg/mL LPS and 10 units/mL IFN-γ. The concentration of seaweed extracts was 50 µg/mL (d.w./mL) and dexamethasone was treated with 50 µM.
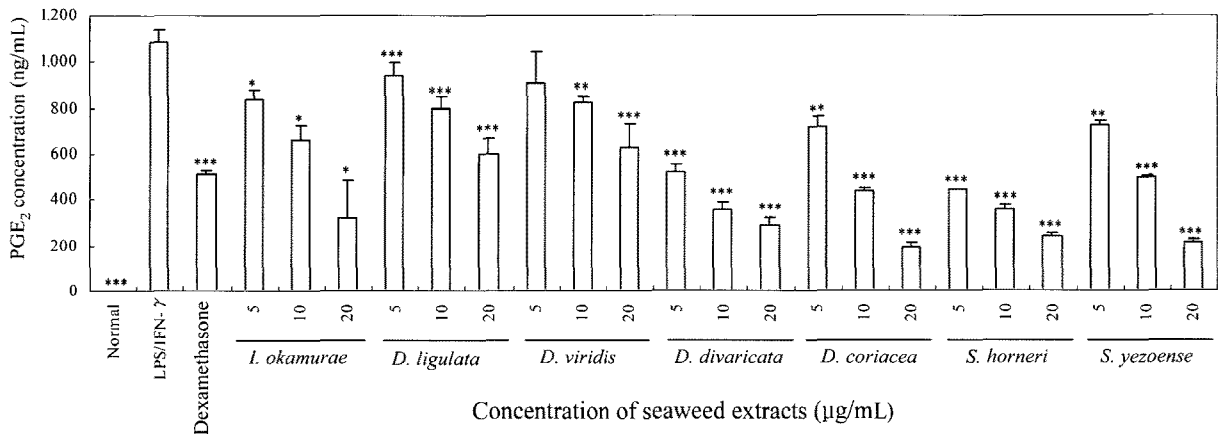

Fig. 5. Dose-dependent effect on PGE$_2$ production by 7 seaweed extracts in RAW264.7 cells stimulated with 10 µg/mL LPS and 10 units/mL IFN-γ. The concentration of seaweed extracts was 5, 10, and 20 µg/mL (d.w./mL) and dexamethasone was treated with 20 µM. The asterisks are significantly different from LPS and IFN-γ stimulated RAW264.7 cells (*$p<0.05$; **$p<0.01$; ***$p<0.005$) using Student's t test, with $n=3$.

cells showed the lowest CD value, 14.687 μg/mL, which is required to double QR activity, and also showed a significant ARE activation in transiently transfected HepG2 cells. In addition, *D. coriacea* extract significantly down-regulated the production either of NO or of PGE_2 in the murine macrophage cell line RAW264.7. These observations suggest that *D. coriacea* could be developed as nutraceuticals for cancer chemoprevention having dual functions which are induction of phase II detoxification enzyme and inhibition of inflammatory mediators.

In conclusion, the results clearly indicated that the 30 species of seaweeds tested in this study showed various degrees of induction of phase II detoxification enzyme and inhibition of the production of inflammation mediators, NO and PGE_2. Up to date, there has been no direct linkage established for seaweeds exerting cancer chemopreventive activities. Thus, the results presented in this report will provide useful guidelines that make it possible to identify the marine algal extracts in respect to their cancer chemopreventive effects. Further work is under way in our laboratory which is aimed at detailed investigation and characterization of the biologically active molecules that are responsible for the cancer chemopreventive effects found in this study.

Acknowledgments

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