

## Chemopreventive effects of polysaccharides extract from *Asterina pectinifera* on HT-29 human colon adenocarcinoma cells

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We examined the effects of polysaccharides extracted from *Asterina pectinifera* on the activities of quinone reductase (QR), glutathione S-transferase (GST), ornithine decarboxylase (ODC), cyclooxygenase (COX)-2 and glutathione (GSH) levels in HT-29 human colon adenocarcinoma cells. We found that the polysaccharides extract induced QR activity in a dose-dependent manner over a concentration range of 20~60 µg/ml and increased GST activity as much as 1.4-fold over controls. GSH levels were increased 1.3- and 1.5-fold with the extract at 40 and 60 µg/ml, respectively. The activity and protein expression of ODC in 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced colon cancer cells was inhibited by the extract. The polysaccharides suppressed TPA-induced prostaglandin (PG) production. These data indicate that polysaccharides from *A. pectinifera* increase phase II detoxification enzyme activity and inhibit ODC and COX-2 activities in HT-29 human colon adenocarcinoma cells. Consequently, this effect may contribute to the protective effect of polysaccharides from *A. pectinifera* against colon cancer. [BMB reports 2009; 42(5): 277-280]

### INTRODUCTION

Chemoprevention is regarded as one of the most promising approaches in the prevention of human cancer. Chemoprevention here refers to a means of cancer control in which the occurrence of the disease is prevented by the administration of chemicals or natural substances. Colorectal cancer is one of the most common forms of this malignancy in the industrialized world, contributing significantly to cancer mortality and morbidity (1).

Chemopreventive agents can function by a variety of mechanisms, directed at all major stages of carcinogenesis. Many inhibitors of carcinogenesis have been found to induce phase II detoxification enzymes, including members of the glutathione

S-transferase (GST) family and quinone reductase (QR) (2). Phase II enzymes detoxify electrophilic carcinogens by changing them into a form that is relatively inert and more easily excreted (3). GST detoxifies carcinogenic electrophiles by catalyzing their conjugation with reduced glutathione (GSH) (4, 5). Similarly, QR works by catalyzing two-electron reductions in free radicals and toxic oxygen metabolites; this reduction deactivates them and protects the surrounding tissues from mutagenesis and carcinogenesis.

Polyamines and enzymes responsible for their biosynthesis play a significant role in carcinogenesis, tumor promotion and cellular hyperplasia. Ornithine decarboxylase (ODC) is the first, and rate-limiting, enzyme in the polyamine biosynthetic pathway. ODC activity has been found to be significantly increased in carcinogenesis of the colon (6).

Cyclooxygenase (COX) is a key enzyme in the biosynthesis of prostaglandins (PG) from arachidonic acid (7), and is also considered important in colon carcinogenesis (8). Indeed, COX-2 inhibitors are effective in the prevention of colon cancer in several animal models (9).

Many marine flora and fauna contain substances that have anticancer, antiviral, antimicrobial, anticoagulant, cardioactive, or neurophysiological properties. These highly active compounds can serve as models in the development of new drugs and, over the past decade, about 3,000 new classes of anticancer agent from marine sources have been described with some entering into preclinical and clinical trials (10).

In the present study, we measured the effects of polysaccharides from *A. pectinifera* on phase II enzymes, ODC and COX-2 in HT-29 colon adenoma cells.

### RESULTS AND DISCUSSION

Table 1 shows the QR activity in HT-29 human colon cancer cells treated with polysaccharides extract from *A. pectinifera*. The polysaccharides increased QR activity in a dose-dependent manner within a concentration range of 20~60 µg/ml with a maximal 1.6-fold induction at the highest concentration level tested. The extract also significantly increased GST enzyme activity in cultured HT-29 cells (Table 1).

Detoxification enzymes play an important role in preventing carcinogen-induced colon cancer (11) and are surrogate biomarkers for chemopreventive potential. We observed that

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**Table 1.** Effect of our *A. pectinifera* polysaccharides extract on quinone reductase (QR) and glutathione S-transferase (GST) activities and glutathione (GSH) levels in HT-29 human colon adenocarcinoma cells

Sample	Concentration (mg/ml)	Ratio (treated/control)		
		QR	GST	GSH
Polysaccharides extract from	20	1.2 ± 0.1	1.1 ± 0.1	1.1 ± 0.1
	40	1.4 ± 0.1**	1.2 ± 0.1	1.3 ± 0.1*
<i>A. pectinifera</i>	60	1.6 ± 0.1***	1.4 ± 0.1**	1.5 ± 0.2**
Ellagic acid	15	1.8 ± 0.2***	1.4 ± 0.1**	1.6 ± 0.2**

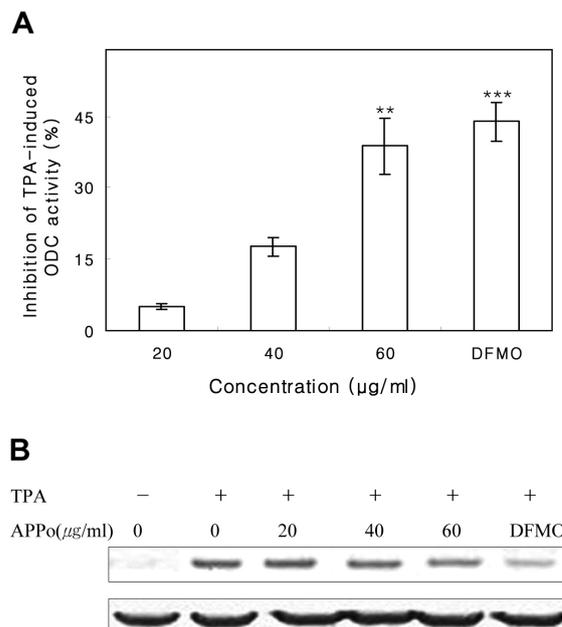
Data shown are mean values with bars indicating the SD of the mean (n=3). \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.005 as compared to the solvent control.

treatment with our *A. pectinifera* polysaccharides extract increased both QR and GST activities in human colon cancer HT-29 cells. Thus, some constituent/s in our extract may prevent the initiation of colon carcinogenesis by increasing the activity of enzymes responsible for detoxification.

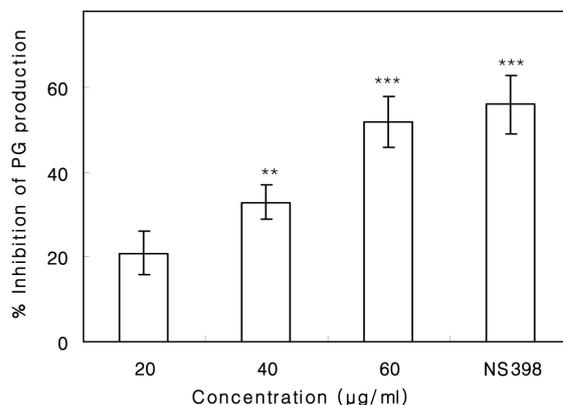
Table 1 shows the GSH levels in HT-29 human colon cancer cells treated with our polysaccharides extract. GSH levels were increased 1.3- and 1.5-fold with 40 (P < 0.05) and 60 µg/ml (P < 0.01) extract, respectively. Following its catalysis by GST, GSH is important in the detoxification of a variety of electrophilic compounds and peroxides, and induction of GSH is used to test potential chemopreventive agents (12). Therefore, the enhancement of GSH by our polysaccharides extract may play a role in carcinogenesis inhibition.

Fig. 1A shows that treatment of HT-29 cells with our polysaccharides extract inhibited TPA-induced ODC activity in a dose-dependent manner up to 60 µg/ml. At 60 µg/ml, treatment with the extract resulted in a 39% inhibition (P < 0.01) of TPA-induced ODC activity. A comparable effect was observed with difluoromethylornithine (DFMO), a suicide inhibitor of ODC, in the current study. To determine the relationship between ODC enzymatic activity and ODC expression, the protein levels of ODC were measured. Fig. 1B shows that HT-29 cells produced low basal levels of protein and that treatment with TPA produced a substantial increase after 6 h. Our polysaccharides extract inhibited TPA-induced expression of ODC protein (Fig. 1B). This result suggests that the decrease of ODC activity caused by the polysaccharides extract results from the decreased expression of ODC protein. ODC has been a major target for anticancer investigations. Excess induction of ODC activity stimulates tumor promotion. Studies have provided convincing evidence for the roles of polyamine and ODC in colon cancer cell growth and in the biological response of tumor promoters and growth factors (13, 14). Inhibition of ODC may be one of several anti-tumor-promoting effects of our *A. pectinifera* polysaccharides extract in colon cancer development.

Fig. 2 shows that our polysaccharides extract inhibited TPA-induced COX-2 activity in a dose-dependent manner. There is



**Fig. 1.** Effect of our *A. pectinifera* polysaccharides extract (APPo) on TPA-induced activity (A) and protein expression (B) of ODC in HT-29 human colon adenocarcinoma cells. The control ODC activity was 463 ± 59 pmol <sup>14</sup>C0<sub>2</sub>/h/mg protein. DFMO, 0.01 mM difluoromethylornithine. Data shown are mean values with bars indicating the SD of the mean (n=3). \*\*P < 0.01, \*\*\*P < 0.005 compared with the control.



**Fig. 2.** Effect of our *A. pectinifera* polysaccharides extract on TPA-induced enzymatic activity of COX-2 in HT-29 human colon adenocarcinoma cells. NS398, 40 µM NS398. Data shown are mean values with bars indicating the SD of the mean (n=3). \*\*P < 0.01, \*\*\*P < 0.005 compared with the control.

increasing evidence suggesting that inhibitors of COX-2 activity can be effective as anti-inflammatory agents and also in the prevention and treatment of colon cancer. This is because PGs are mediators of inflammation, and chronic inflammation pre-

disposes to carcinogenesis (15). Therefore, agents that can inhibit COX-2 activity might be useful for the inhibition of colon carcinogenesis.

The findings presented here suggest that our polysaccharides extract may act as a colon cancer chemopreventive agent. The reasoning behind this is two-fold: firstly, the extract has the ability to induce detoxification enzymes and, secondly, it inhibits the activities of ODC and COX-2. Additional studies should be undertaken to further characterize the protective effects of our polysaccharides extract from *A. pectinifera*. In this way a better understanding of its protective mechanisms against colon cancer may be gained.

## MATERIALS AND METHODS

### Chemicals

L-[1-<sup>14</sup>C]ornithine and PGE<sub>2</sub> Biotrak enzyme immunoassay kit were purchased from Amersham Biosciences (Piscataway, NJ, USA). Human anti-ODC antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, Calif, USA). 4-Nitro blue tetrazolium chloride/5-bromo-4-chloro-3-indolyl-phosphate substrate was purchased from Promega (Madison, WI, USA). All other necessary reagents of analytical grade were bought from Sigma-Aldrich Chemical Company (St. Louis, MO, USA).

### Animal material

The starfish *A. pectinifera* were collected along the coast of Pohang, Korea. The voucher specimen (A0014-3) of the starfish was deposited in the Intractable Disease Research Center, Dongguk University, Gyeongju, Korea. After collection, the material was washed with distilled water and stored at -20°C until used.

### Extraction of polysaccharides

*A. pectinifera* were cut into small pieces, and the extraction of the polysaccharides followed the procedure shown in Fig. 3 (16).

### Cell culture

Human colon adenocarcinoma cell line, HT-29, was obtained from the Korean Cell Line Bank (Seoul, Korea). It was cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum.

### QR Activity

The QR specific activity in HT-29 human colon adenoma cells grown in 96-well microtiter plates was measured according to the method of Shon *et al.* (17). The increase of QR activity was calculated from the ratio of the specific enzyme activity of sample-treated cells in comparison with a solvent-treated control. Ellagic acid was used as the reference compound in this series of experiments.

### GST activity

GST activity was measured using a modification of the procedure developed by Habig *et al.* (18) with 1-chloro-2,4-dinitrobenzene as the substrate. The protein content was measured

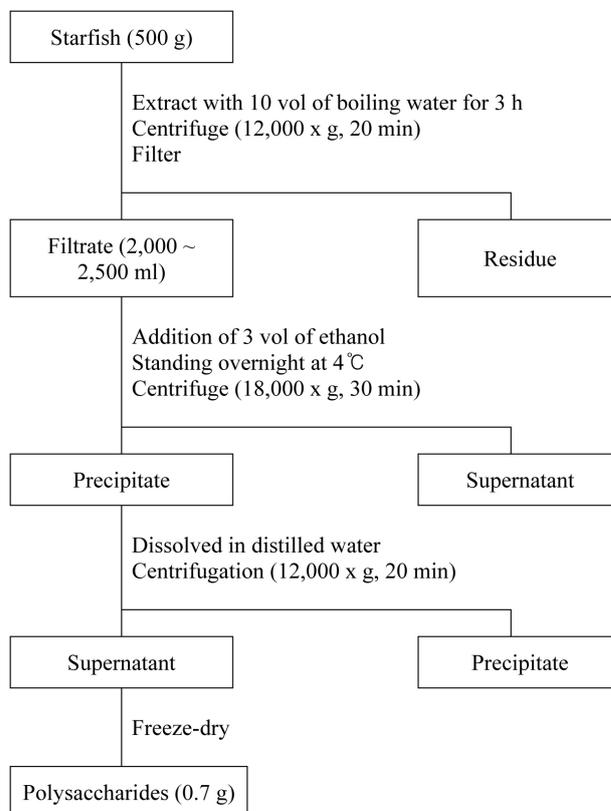


Fig. 3. Procedure used to extract polysaccharides from *A. pectinifera*.

in a duplicate plate using a bicinchoninic acid protein assay kit with bovine serum albumin as the standard. The GST activity was expressed as the slope/min/mg protein. The data derived from the sample-treated cells were compared to the values obtained for solvent-treated controls. Ellagic acid was used as the reference compound.

### GSH levels

GSH content in HT-29 cells were determined using the glutathione reductase-coupled 5,5'-dithiobis 2-nitrobenzoic acid enzyme-linked immunosorbent assay in a 96-well format (19).

### ODC activity

Human colon adenocarcinoma HT-29 cells were plated in 24-well tissue culture plates. After incubation at 37°C for 18 h in a 5% (v/v) CO<sub>2</sub> atmosphere, the media was removed and replaced with media containing 200 nM 12-O-tetradecanoylphorbol-13-acetate (TPA) alone or with TPA plus polysaccharides from *A. pectinifera* (20-60 µg/ml) or 0.01 mM difluoromethylornithine (DFMO) as a positive inhibitor of ODC. After an additional 6 h of incubation at 37°C, the cells were washed and subjected to three freeze-thaw cycles. ODC activity was assayed directly in the 24-well plates by measuring the release of <sup>14</sup>CO<sub>2</sub> from L-[1-<sup>14</sup>C]or-

nithine (20). The amount of radioactivity was measured by liquid scintillation counter (Beckman LS 6500, Fullerton, Calif, USA).

#### ODC expression (Western Blotting)

HT-29 cells were plated in 12-well plates and treated with various concentrations of the polysaccharides extract from *A. pectinifera* in the presence of 200 nM TPA. Confluent cells were washed, lysed in lysis buffer, and then clarified by centrifugation (12,000 rpm, 10 min) at 4°C. The cell lysate proteins (25 g) were electrophoresed on 9% SDS-polyacrylamide gels and then electrophoretically transferred to polyvinylidene difluoride membranes. To detect ODC, the membranes were incubated with human anti-ODC antibody (1:100) at room temperature. The membranes were then incubated with biotin-rabbit anti-mouse Ig GAM (H+L) and alkaline phosphate-conjugated streptavidin and visualized with the 4-nitro blue tetrazolium chloride/5-bromo-4-chloro-3-indolyl-phosphate substrate.

#### COX-2 enzymatic activity

Lysates of HT-29 cells were prepared as described above. COX-2 enzymatic activity was measured using a PGE<sub>2</sub> Biotrak enzyme immunoassay kit according to the manufacturer's protocol. The assay was based on competition between unlabelled PGE<sub>2</sub> and a fixed quantity of peroxidase-labelled PGE<sub>2</sub> for a limited number of binding sites on a PGE<sub>2</sub> specific antibody.

#### Statistical analysis

The data were analyzed for statistical significance using Student's t-test. P values less than 0.05 were considered significant.

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