KOREAN J. FOOD SCI. TECHNOL.
Vol. 21, No. 5, pp. 595~600 (1989)

Antitumor Activity of Seaweeds toward Sarcoma-180

Beung-Ho Ryu, Dong-Seuk Kim, Kyung-Ja Cho and Dong-Bun Sin*
Department of Food Science and Technology, Kyungsung University, Pusan
**Department of Nutrition, Hospital, Pusan National University, Pusan*

Abstract

Protein-polysaccharides(PP) extracted from four species of gulfweed, fusiforme, sea-mustard and sea-tangle were examined for antitumor activity toward sarcoma-180 implanted in mice, ICR.

Polysaccharides in protein-polysaccharides extracted from gulfweed, fusiforme, sea-mustard and sea-tangle were 61.48, 55.61, 34.06 and 30.28%, respectively. Monosaccharides of four seaweeds consisted of glucose, galactose, mannose, fructose and xylose, and major amino acids consisted of glutamic acid, aspartic acid, cystein, valine and glycine.

Antitumor activity of the protein-polysaccharides extracted from sea-mustard showed the highest inhibition ratio of 69.76% when PP was injected to the mice at the dose of 100 mg/Kg/day. PP of sea-tangle showed the maximum survival ratio of 25.22% when injected at the dose of 100 mg/Kg/day.

Key words: antitumor activity, protein-polysaccharides, sarcoma-180

Introduction

Seaweeds have been used for curing anticurare, anthelmintics, gout, eczema and gallstone as folk medicine[1]. In particular certain algae have been known and examined by researchers from the past[2,3,4]. *Corican mosa, Bull kelp, Dulse* and *Hypnea* have been used for squirrhe tumors[5]. Agasol prepared in Italy has been proved to be active against Walcher carcinoma, Erlich carcinoma and sarcoma-180[6]. *Dictyopteris, Codium* and *Sargassum* species possess substances which inhibit to different degrees, the growth of carcinoma and sarcoma transplanted to mice[1], though there is some difference of the inhibition. Marine organisms have been proved to be active antitumor compounds which contain polysaccharides[7,8,9].

In our recent investigations protein-polysaccharides were extracted with boiled water from the well known and most widely used seaweeds such as fusiforme, gulfweed, sea-mustard and sea-tangle, and were tested for antitumor activity toward sarcoma-180 implanted in mice, ICR.

Corresponding author: Beung-Ho Ryu, Department of Food Science and Technology, Kyungsung University, 110-1, Daeyeon dong, Namgu, Pusan 608-736

Materials and Methods

Materials

Seaweeds; fusiforme, gulfweed, sea-mustard and sea-tangle were obtained at Jakalchi market, Pusan area.

Animal and tumor

Female mice of ICR strain and sarcoma-180 were supplied by the College of Pharmacy, Seoul National University.

Extraction of protein-polysaccharide fraction (PPF)

The seaweeds were homogenized and boiled in water during 8 hours for extraction. After filtration, the residue was boiled during 8 hours for reextraction. The extracts were precipitated by adding equal volume of 95%-ethanol and allowed to stand at 4°C for 14 hours. The precipitates were collected by centrifugation at 10,000×g for 30 min and dissolved in distilled water. After filtration, PPF were dialyzed at 4°C for 48 hours using distilled water. The protein-polysaccharides

Tale 1. Measurement condition of monosaccharides by gas chromatography

Column	2% OV-17	
Temperature	column	100-220°C (rate : 6°C/min)
	injection	250°C
Flow rate	air	0.7 Kg/cm^2
	hydrogen	0.8 Kg/cm^2
	nitrogen	50 ml/min
Sensitivity	attenuate	3
	range	2
Detector	FID	
Model	Shimadzu GC-R1A	

fraction was lyophilized.

Analysis of polysaccharide and monosaccharide

Polysaccharide contents were quantitatively determined by anthrone test using D-glucose as a standard[10]. Gas chromatographic analysis of monosaccharide was performed according to the method of Mitruka[11]. Each lysophilized sample (20 mg) and each standard monosaccharide (10 mg) were dissolved in 3% HCl-methanol (4 m*l*) and methanolyzed at 80 ± 5°C for 20 hours in a cap tube filled with 0.2 m*l* of hexamethyldisilazane and 0.1 m*l* of trimethylchlorosilane. Condition of gas chromatography was given in Table 1.

Determination of protein and amino acid

Protein contents were determined using bovine serum albumin (BSA, Sigma Chem. Co.) as a standard protein by Lowry-Folin method[12] with absorbance at 750 nm.

Amino acid analysis of PPF was performed according to the method of Spackman[13]; sample (20 mg) was dissolved in 5 m*l* of 6N HCl and hydrolyzed at 110 ± 5°C for 24 hours in a cap tube filled with nitrogen gas. After filtration, the hydrolysate was evaporated and dissolved in 10 m*l* of 0.2N sodium citrate buffer (pH 2.2). Amino acids were analyzed with amino acid autoanalyzer (Hitachi Model, 835).

Inhibition test of solid tumor growth

Tumor cells ($1.0 \times 10^{(6)}$ cells/mouse) were inoculated subcutaneously into the left groin at 24 hours before the start of sample administration (7 mice to a group). Samples were administered once a day for ten days by interaperitoneal injection with each dose. On the day 26 after tumor implantation, the mice were killed and the tumors were extirpated and weighed. The inhibition ratio was calculated by the following formula;

$$\text{Inhibition ratio}(\%) = \frac{C_w - T_w}{C_w} \times 100$$

In which C_w is the average tumor weight of the control group and T_w is that of the treated group.

Survival test

Tumor cells (1.0×10^6 cells/mouse) were intraperitonealy implanted into ICR mice (7 mice to a group). On the first day sample was administered by intraperitoneal injection at a dose of 50 and 100 mg/Kg daily for 10 days. Saline was administered to all of the control groups. The number of survivors of each group were observed for 35 days and the prolongation ratio of life was calculated by the following formula;

$$\text{Prolongation ratio}(\%) = \frac{T-C}{C} \times 100$$

In which C is the average survival days of the control group and T is those of the treated group.

Results and Discussion

Composition of protein-polysaccharide fraction (PPF)

Polysaccharides of seaweeds were boiled with water. Polysaccharides of gulfweed, fusiforme, sea-mustard and sea-tangle were 61.14, 55.61, 34.06 and 30.28%, respectively (Table 2).

Gas-chromatogram of the water soluble polysaccharides showed the presence of at least five components. All of the seaweeds consisted of glucose, galactose, mannose, fructose and xylose. Seaweeds contained galactose most. The sea-mustard contained galactose the most among seaweeds, and its content was the highest level of 63.97%. It was reported that polysaccharides such

Table 2. Polysaccharide content and monosaccharide constituents in PPF of seaweeds

	Fusiforme	Gulfweed	Sea-mustard	Sea-tangle
Polysaccharide(%)	55.61	61.14	34.06	30.28
Monosaccharide(%)				
Glucose	9.83	20.17	18.25	14.18
Galactose	46.13	38.50	63.97	64.05
Mannose	12.50	9.06	0.77	1.62
Fructose	8.57	20.62	11.85	16.29
Xylose	22.97	11.65	5.16	3.86

as lentinan[2], tangle[14], *Sargassum horneri* and *Codium pugniformis*[15] gave possessed antitumor activities. They are composed of monosaccharides such as glucose, galactose, fructose, mannose and xylose. Another similar study was carried out by Ito and Sugimura[16] on an active substance extracted from *Sargassum thunbergii*. The active substance contained galactose, xylose, glucose and mannose in addition to a small amount of sulfate. Consistency with suggestion is the finding that the inhibition of tumor considerably depends upon polysaccharides[17].

On the other hand, proteins of fusiforme, gulfweed, sea-mustard and sea-tangle contained were 5.51, 2.84, 3.53 and 2.29%, respectively (Table 3). These proteins consisted of 12-15 kinds of amino acids. Fusiforme was composed of 12 amino acids which contained aspartic acid, glutamic acid, cysteine with 16.67, 12.32, 12.13 and 11.40 mg%, respectively. Sea-mustard consisted of glutamic acid, aspartic acid, glycine with 17.46, 14.45, 13.18 mg%, and sea-tangle also consisted of glutamic acid, aspartic acid, cysteine and valine with 15.13, 13.19, 11.54 and 11.61 mg%, respectively. Korean *Basidiomycetes*[18] consisted of 17 amino acids such as glutamic acid, aspartic acid, serine and cysteine, etc. Ryu, *et al.*[9] found 15 amino acids which contained glutamic acid, serine and aspartic acid, etc. *Codium pugniformis* is active against ascites tumor of sarcoma-180. The polysaccharides of *Codium pugniformis* consisted of galactose, arabinose and mannose but a small amount of amino acids[15]. PPF of seaweeds used

Table 3. Protein contents and amino acid composition in PPF of seaweeds

	Fusiforme	Gulfweed	Sea-mustard	Sea-tangle
Protein (%)	5.51	2.84	3.53	2.29
Amino acid(mg%)				
ASP	14.11	12.32	14.45	13.19
THR	10.53	0.79	3.78	4.56
SER	8.65	0.79	6.79	4.30
GLU	13.35	16.76	17.46	15.13
GLY	7.40	10.79	13.18	8.87
ALA	8.46	8.65	7.16	10.55
CYS	12.66	11.40	6.09	11.54
VAL	8.34	12.13	9.06	11.61
MET	—	—	—	—
ILE	4.76	7.43	5.78	6.57
LEU	5.08	7.86	5.15	6.91
TYR	4.70	—	—	—
PHE	—	3.22	3.08	2.50
LYS	0.31	5.85	3.48	4.28
HIS	—	2.01	1.10	—
ARG	—	—	1.70	—
PRO	—	—	1.76	—

in our experiment was composed of several monosaccharides and amino acids. The PPF of each seaweeds was employed for the inhibition of sarcoma-180 transplanted to mice.

Antitumor activities of PPF

Some examinations for the possible antitumor activity of algal extracts have recently been attempted. All of the PPF used in these experiments were extracted from seaweeds such as fusiforme, gulfweed and sea-tangle. The antitumor activity of PPF was indicated *in vivo* by the suppression of the tumor growth toward sarcoma-180 transplanted to mice. Antitumor activity of all of the seaweeds was recognized when they were administered the intraperitoneal injection once a day at the dose of 50 and 100 mg/kg/day of protein-polysaccharides (Table 4). The PPF of sea-mustard showed tumor inhibition at the dose of 100 mg/kg/day which showed the highest inhibition of 69.76%. However, fusiforme, gulfweed and sea-tangle demonstrated inhibition ratio of 56.53, 47.47 and 44.91%, respectively when injected

Table 4. Antitumor activity of the seaweeds in tumor bearing ICR mice with sarcoma-180

Sample	Tumor weight(g) (Mean ± S.D.)	Inhibition (ratio) (%)	Complete repression
Control	6.18 ± 1.07	—	—
Fusiforme			
50 mg/Kg	3.94 ± 0.37[b]	36.25	0/7
100 mg/Kg	2.68 ± 0.56[b]	56.63	1/7
Gulfweed			
50 mg/Kg	5.86 ± 0.60[a]	5.18	0/7
100 mg/Kg	3.25 ± 0.78[b]	47.47	0/7
Control	8.66 ± 0.83	—	—
Sea-mustard			
50 mg/Kg	3.97 ± 0.72[b]	54.16	0/7
100 mg/Kg	2.62 ± 0.56[c]	69.76	1/7
Control	12.76 ± 1.98	—	—
Sea-tangle			
50 mg/Kg	9.98 ± 2.31[a]	21.79	0/7
100 mg/Kg	7.03 ± 2.95[a]	44.91	0/7

a) N.S.: not significant
b) $p<0.05$
c) $p<0.01$

once a day at the dose of 100 mg/kg/day. Any group of mice has not been demonstrated the complete regression of tumor. Therefore, it was also caused the death of 1 out of 7 mice when fusiforme and sea-mustard were injected once a day at the dose of 100 mg/kg. Nakazawa, *et al.*[19] examined the antitumor activity of aqueous extracts from a variety of marine algae including green, brown and red algae by the intraperitoneal injection of various concentration. Furthermore, Yamamoto and Watanabe[20] reported a fraction of furanon from *Gloiopeltis furcata,* a sulfated galactose of carrageenan. Hot water extract from *Sargassum fulvellum* is curative for sarcoma-180 solid tumor. In similar studies[15], the range of doses from 125 to 750 mg of the polysaccharide fractions/mouse/day was found to be effective not only to the ascites tumor and Ehrlich ascites carcinoma, but also to the solid tumor of sarcoma-180. These results indicated that the PPF of almost all seaweeds had differences in the inhibitory effects.

The life span test was performed by observation of mice for 35 days after the injection of sarcoma-180. The mean survival period of the mice in the group treated with the PPF was compared with that of the control group. PPF of sea-tangle showed the maximum survival ratio of 19.86% when injected at the dose of 100 mg/kg/day (Table 5). The prolongation ratio of sea-tangle occurred 25.22% when injected at the dose of 100 mg/kg/day of the PPF. Ito and Sugimura[16] suggested using *Sargassum thunbergii* that each group of 10 mice was inoculated with Erlich ascites of 2×10^5 cells and raised with 20 or 10 mg of the polysaccharide/kg/day to measure the survival ratio of mice. All of the mice died on a 14th day after inoculation and the survival ratio of mice showed range from 40 to 100%. In our experiment, the species of seaweeds and the amounts of PPF influenced the prolongation of life span of mice.

Table 5. Effect of PPF in the seaweeds on life span of mice ICR

Sample	Average survival day(Mean ± S.D.)	Prolongation ratio(%)
Control	15.86 ± 1	—
Fusiforme		
50 mg/Kg	16.57 ± 1	4.48
100 mg/Kg	17.29 ± 1	9.02
Gulfweed		
50 mg/Kg	17.71 ± 1	11.66
100 mg/Kg	18.41 ± 2	14.38
Sea-mustard		
50 mg/Kg	17.42 ± 2	9.38
100 mg/Kg	18.86 ± 2	18.92
Sea-tangle		
50 mg/Kg	15.86 ± 1	0.00
100 mg/Kg	19.86 ± 2	25.22

References

1. Michanek, G.: Seaweed resources for pharmaceutical uses, *Marine algae in pharmaceutical science,* edited by Hoppe, H.A., Levring, T., Tanaka, Y. and Welter de Gruyter, Berlin, New Yok, 203-234 (1979)
2. Moor, R.E.: Toxins, anticancer agents and tumor promotors from marine procaryotes. *Pure & Appl. Chem.* **54**, 1919 (1982)

3. Ruggieri, G.D.: Drugs from the sea. *Sciences* **194,** 495 (1976)
4. Kaul, P.K.: Biomedical potential of the sea. *Pure & Appl. Chem.* **54,** 1963 (1982)
5. Erhart, J.P.: Contribution des algues a la medecine et a la biologie. *Rev. Intern. Oceanogr. Med.* **31,** 194 (1973)
6. Rositto, G.: Pharmacological and clinical study of a new seaweed extract registered in the Italian pharmacopeia as "Agasol T 331". Milano, p. 15 (1958)
7. Sasaki, T., Uchida, H., Uchida, N.A., Takacuka, N., Tachibana, Y., Nakamichi, K., Endo, Y. and Kamiya, H.: Antitumore activity and immunomodulatory effect of glycoprotein fraction from *Scallop Patinopecten Yessoensis. Nippon Suisan Gakkaishi* **53,** 267 (1987).
8. Okutani, K.: Antitumor activity of a polysaccharide preparation from marine bacteria. *Tech. Bull. Fac. Agr. Kagawa Univ.* **26,** 75 (1974)
9. Ryu, B.H., Chi, B.H., Kim, D.S., Jang, M.K., Kim, H.S. and Chung, S.J.: Antitumor activity of protein-polysaccharides produced from *Vibrio anguillarum. Kor. J. Food Hygiene* **3,** 111 (1988).
10. Herbert, D., Phipps, P.J. and Strange, R.E.: Chemical analysis of microbial cell, In *Methods in microbiology* (Ed. Norris, J.R. and Ribbons, D.W.) Vol. 5b, 265-301. Academic Press (1971).
11. Mitruka, B.M.: Gas Chromatographic Applications in Microbiology and Medicine. 158-164. John Willey & Sons (1971)
12. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193,** 265 (1951)
13. Spackman, D.H., Stein, W.H. and Moore, S.: Automatic recording apparatus for use in the chromatography of amino acids. *Anal. Chem.* **30,** 1190 (1958)
14. Yamamoto, I. and Nagumo, S.: Tumor-inhibiting effect of tangle and sargassum against sarcoma-180 solid tumor. Annual meeting of Jap. Soc. of cancer, *Abstracts,* p. 1225 (1973).
15. Nakazawa, S., Abe, F., Kuroda, H., Kohno, K., Higashi, T. and Umezaki, I.: Antitumor effect of water-extracts from marine algae (III), *Sargassum horneri, Codium pugniformis* Okamara. *Chemotheraphy* **24,** 448 (1976)
16. Ito, H. and Sugimura, M.: Antitumor polysaccharide fraction from *Sargassum thunbergii. Chem. Pharm. Bull.* **24,** 1114 (1976)
17. Nisizawa, K.: Pharmaceutical studies on marine algae in Japan, *Marine algae in pharmaceutical sciences,* edited by Hoppe, H.A., Levring, T., Tanaka, Y. and Welter de Gruyter, Berlin, New York, 244-264 (1979)
18. Kim, B.K., Chung, H.S., Chung, K.S. and Yang, M.S.: Studies on the Antineoplastic Components of Korean *Basidiomycetes. Kor. J. Mycol.* **8,** 107 (1980)
19. Nakazawa, S., Kuroda, H., Nishimo, T., Otsuki, M. and Umezaki, I.: Antitumor effect of water-extracts from marine algae(I).: Antitumor effect of water-extracts from marine algae(I). *Chemotheraphy* **22,** 1435 (1974)
20. Yamamoto, S. and Watanabe, K.: Fractionation and structural investigation of funoran, Proceedings of VIIth International seaweed symposium (ed, by Nisizawa, K. *et al.,*) pp. 451-454. University of Tokyo press, Tokyo, Japan (1971)

(Received Mar. 6, 1989)

해조류의 Sarcoma-180에 대한 항암효과

류병호·김동석·조경자·신동분*
경성대학교 공과대학 식품공학과, *부산대학교 병원 영양과

미역, 다시마, 톳 및 모자반 등의 해조류로부터 추출한 단백다당체의 조성과 항암효과를 알아보기 위하여 쥐에 sarcoma-180 세포를 주사한 다음 이들 단백다당체의 효과를 조사하였다.

끓는 물로 추출한 해조류의 단백다당체의 다당류는 모자반, 톳, 미역 및 다시마가 각각 61.14, 55.61, 34.06 및 30.28%였고 주요 단당류는 glucose, galactose, mannose, fructose 및 xylose 이었으며 주요 아미노산은 glutamic acid, aspartic acid, cysteine, valine 및 glycine 이었다. 항암효과를 나타내는 단백다당체의 종양성장저지율은 해조류 중 미역을 100 mg/kg/day로 10일간 투여했을 때 69.76%로 가장 높았고, 수명연장율은 다시마의 경우 100 mg/kg/day로 10일간 투여했을 때 25.22%로 가장 높은 효과를 나타내었다.