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Antitumor Effects of Glycoprotein Extracted from Sea Cucumber (*Stichopus japonicus*)

Jeung-Hye Moon, Hong-Soo Ryu† and Jae-Soo Suh*

Department of Food and Life science, Pukyong National University, Pusan 608-737, Korea
**Department of Food and Nutrition, Kosin University, Pusan 606-701, Korea*

Abstract

The antitumor and immunologic activities of the glycoproteins extracted from sea cucumber (*Stichopus japonicus*) on mice bearing sarcoma 180 cells were investigated. Maximum tumor suppression (64%) occurred at the dose of 100 mg glycoprotein/kg. The highest prolongation ratio was achieved at the level of 100 mg/kg and increased by 39% more than that of control. Glycoproteins from sea cucumber exhibited direct cytotoxic effect on the tumor cells. Dose-dependent increase of leucocyte, peritoneal exudate cell and weights of immunoorgans revealed the improvement of immunity. When the glycoprotein-administered group was compared with the control, a significant difference was not noted in the clinico-chemical values such as S-GOT, S-GPT, alkaline phosphatase activity, total protein, cholesterol, triglyceride, urea nitrogen and glucose levels in blood. These results suggest that the antitumor activity of sea cucumber glycoprotein is associated with activation of cells in the immune system.

Key words: sea cucumber, glycoprotein, antitumor activity, immunomodulatory effect

INTRODUCTION

Since current chemotherapeutic agents are limited in application due to their side effects, tests to develop new medicines against cancers from natural products in the hopes of finding substances with low side effects are in progress. In Korea, for example, it has been verified through clinical studies that polysaccharides extracted from plants (1-3) and seaweeds (4,5) are effective in reviving immunity and inhibiting a tumor. Sea cucumber has been used as a remedy for diabetes and asthma in Chinese medicine and folk remedies. The studies about sea cucumber until now include a content of inorganic substances and analysis on chemical composition such as chondroitin sulfate and glycosaminoglycan (6-12). Recently it has been known that a sea cucumber affects phagocytosis activities of marcophages and monocytes and activates immune system. There have been few studies about the physiological activity of polysaccharides extracted from sea cucumber. Therefore, the objective of our study was to investigate the antitumor and immunomodulatory activities of the glycoprotein from sea cucumbers against the mice bearing sarcoma 180 cells, and to evaluate the possibility of it as the natural antitumor agent with low side effects.

MATERIALS AND METHODS

Mice and tumor cells

Female Balb/c mice, weighing about 18~22 g, were purchased from Daehan Animal Research Center co., LTD. (Umsung, Korea). Sarcoma 180 cell from ascites fluid was supplied by Lab. of Microbiology, College of Medical, Kosin University (Pusan, Korea). 0.05 ml (about 2×10^6 cells) of 7 days old ascites tumors were transplanted subcutaneously into the right flank of the mice. After cervical dislocation, ascites were drawn from the Balb/c mice which were inoculated with sarcoma 180 tumor cells i. p. To isolate tumor cells, the ascites were adjusted with 0.83% NH_4Cl and set for 2 min. at room temperature to remove red blood cells. Broken cells were then removed by centrifugation for 3 min at $400\times g$. Pelleted sarcoma 180 tumor cells were washed once with sterile ice-cold PBS (phosphated buffered saline), and resuspended in the concentration of 1.0×10^6 cells/ml with PBS. The cell suspension above was used to inoculate into mouse.

Glycoprotein from sea cucumbers (13) was extracted using 20 mM sodium phosphate buffer (pH 7.0), salted out with 80% ammonium sulfate saturation and fractionated through the DEAE-cellulose ion exchange chromatography (14).

Solid tumor growth inhibition test

Tumor cells (1.0×10^6 cells/mouse) were inoculated subcutaneously into the left groin at 24 hours before the start of sample administration (7 mice distributed to a group). Samples were administered once a day for ten days by intraperitoneal 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 : Inhibition ratio = $(Cw-Tw/Cw)\times100$ where Cw is the average tumor weight of the control group and Tw is that of the treated group (15).

Survival test

The female Balb/c mice received i. p. 0.1 ml (1×10^6 cells/mouse) of ascites tumor from 7 day old sarcoma 180. Each dose of samples was injected i.p. once a day for 10 days after 24 hours from transplantation, and saline was adminis-

†Corresponding author. E-mail: hsryu@dolphin.pknu.ac.kr
Phone: 82-51-620-6333, Fax: 82-51-628-6670

tered to the control groups. The number of survivors of each group was observed for 40 days and the prolongation ratio of life was calculated by the following formula : Prolongation ratio (%) = (T-C/C) × 100 where C is the average survival days of the control group and T is those of the treated group (16).

Number of circulating leucocytes

The effect of glycoproteins on the number of circulating leucocytes was examined by following method (16). Each group consisted of 12 Balb/c mice was given intraperitoneal injections of the glycoproteins for ten days. Blood was collected from the hearts of mice, on the 1st, 4th, and 7th day after the last sample injection. Collected blood was diluted with citrate saline, stained by Turk's reagent and the total number of leucocyte was counted in hemocytometer chamber. Triple counting per sample was carried out and the mean value of results was calculated (17).

Number of peritoneal exudate cells (PEC)

Each group that consisted of 12 Balb/c mice was injected i. p. with 0.1 ml glycoprotein for three days. 4 mice in each group were sacrificed by cervical dislocation on 1st, 4th, and 7th day after the last sample injection. The peritoneal cavity was washed with 5 ml saline and the ascitic fluid was collected. The number of total PEC was counted in a hemocytometer chamber by using Turk's solution (18).

Lymphoid organ weight

Female Balb/c mice of test groups were treated i. p. with glycoproteins for ten days and those of control group with PBS. On the 1st, 4th, and 7th day after the last sample injection, mice were weighed and sacrificed by cervical dislocation. Liver and spleen were removed, weighed and compared with the results obtained from control mice (19).

Characteristics in serum

Female Balb/c mice of test groups were treated i. p. with glycoprotein for ten days and those of control group with PBS. On the 1st, 4th and 7th day after the last sample injection, blood was collected from the heart and centrifuged with at 12,000 × g for 10minutes. The clinico-chemical values, such as glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase, total serum protein levels, cholesterol, triglyceride, blood urea nitrogen (BUN) and glucose were determined on the serum of Balb/c mice administered with sea cucumber glycoproteins (13).

Statistical evaluation

Statistical significance of observed differences among means of experimental results was evaluated by student's *t*-test, using SigmaPlot® scientific graphing software ver. 4.0 (SPSS Inc., USA).

RESULTS AND DISCUSSION

Solid tumor growth inhibitory activity

The effect of glycoproteins extracted from sea cucumbers on sarcoma 180 solid tumor was presented in Table 1. Average tumor weight of control group was 5.41 g, and the maximum tumor suppression (64%) occurred at the dose of 100 mg/kg. When sea cucumber glycoprotein was administered at dose of 50 mg/kg, the tumor growth inhibition ratio was 40.80% ($p<0.05$) while a high dose of 150 mg/kg and 200 mg/kg resulted in 59.70% ($p<0.01$) and 59.33% ($p<0.05$) of tumor suppression, respectively. These are somewhat higher than the anti-cancer response of the glycoproteins extracted from *Vibrio anguillarum* (20). Other researchers reported that the higher anti-cancer effect could be obtained with glycoprotein from cell wall of *Aspergillus spp.* (21) and *Dodemum varians* (22).

Effect of sea cucumber glycoprotein on survival

To examine the effect of sea cucumber glycoprotein on the survival time of mice bearing sarcoma 180 ascites, life prolongation experiment was carried out through 40-day observation. As shown in Table 2, there was some life prolongation effect in the administered group. The highest life prolongation ratio was revealed at the dose of 100 mg/kg with 38.92% ($p<0.001$), and every prolongation ratio of ascites

Table 1. Antitumor activities of glycoproteins from sea cucumbers on sarcoma 180 solid tumor in Balb/c

Treatment	Dose (mg/kg)	Tumor weight (g/mouse)	Inhibition ratio[2] (%)	Complete regression[3)4)]
Control	-	5.41 ± 0.78[1]	-	0/10
Glycoprotein	50	2.77 ± 1.35*	40.80	2/10
	100	1.95 ± 1.13*	63.69	3/10
	150	2.18 ± 0.58**	59.70	1/10
	200	2.20 ± 0.94*	59.33	0/10

[1] Mean ± SE
[2] Inhibition ratio (%) = (Cw-Tw)/Cw × 100
Cw: Tumor weight of control group
Tw: Tumor weight of treated group
[3] The number of mice in the group which tumor was completely regressed
[4] The number of mice used
Balb/c mice were s.c implanted with 1×10^6 cells of sarcoma 180 into the left groin and tumors were resected on 35th day after tumor inoculation
Mice were i.p. administered with each samples for consecutive 10 days after tumor inoculation
*$p<0.05$ and **$p<0.01$ versus the control group

Table 2. Effect of glycoproteins from sea cucumbers on survival of Balb/c mice transplanted intraperitoneally with sarcoma 180 ascite tumor

Treatment	Dose (mg/kg)	Average survival days	Prolongation ratio (%)
Control	-	18.5	-
Glycoprotein	50	20.6	11.35
	100	25.7***	38.92
	150	23.5**	27.03
	200	20.8*	12.43

Balb/c mice were i.p. transplanted with 1×10^6 cells/ml of sarcoma 180 and administered with samples at 24 hrs after tumor inoculation
*$p<0.05$, **$p<0.01$ and ***$p<0.001$ versus the control group

tumor was lower than the inhibition ratio of solid tumor as presented in Table 1. Those results indicates that sea cucumber glycoprotein has better anticancer effect against solid tumor than against ascites tumor implanted in the abdominal cavity. Our results were somewhat higher than the reported prolongation ratio of marine algae (23), *Poria cocos* (24) and herb medicines (3). The anticancer effects of glycoprotein seems to be exerted mainly through a host-mediated reaction rather than direct action on tumorous cells.

Effect of glycoproteins on the number of circulating leucocytes

It has been known that the natural glycoproteins has not any direct cytotoxic action but a host-mediated reaction on tumorous cells. In order to determine the mechanism of the anticancer effect in detail, we studied the effect of sea cucumber glycoprotein on murine immune function (Table 3). The number of circulating leucocytes on 1th, 4th and 7th day after administration increased, compared to the control group. The number of leucocytes was increased maximally on 4th day through all doses, and after that it decreased gradually. Leucocytes are principal blood components and are composed of neutrophils, eosinophils, basophils, lymphocytes and monocytes. They protect organisms from bacterial contamination through phagocytosis, and neutrophilic leucocytes are involved in the leucocytosis in relation to inflammatory actions of organisms. Neurophilic leucocytes also protect organisms from inflammation through formation of immune bodies, and perform a very important function as the primary cell acting on the immune action (25).

Changes in the number of peritoneal exudate cells

To determine the immunomodulatory effect of sea cucumber glycoprotein, changes in the number of peritoneal exudate cells were observed. As shown in Table 4, the peak of peritoneal cell number reached on the 1st day. Cell numbers were almost same as those of control at 7th day. The peritoneal exudate cells are composed of polymorphonuclear leucocytes and monomorphonuclear leucocytes and marcophages. Phagocytosis by these cells contributes greatly to the protection of it from alien substances entering the body, and an increase in the peritoneal cells represents the improvement of immunocompetence. Similar immunity from strong cytotoxity on tumorous cells could be found in the results of glycan type *Lentinan* (26) and *Lyophyllum* A from *Lyophyllum decastes* (27).

Changes in the weight of immunoorgans

The changes of immunoorgans weight after 7 experimental days are presented in Table 5. The higher dose of glycoprotein administered, the heavier liver and spleen were gained. Changes in spleen weight gain were ranged from 23.08% (50 mg/kg) to 61.54% (200 mg/kg) of control. The percentage of the liver weight to the body weight showed a notable increase from 4.81% (control) to 6.04~6.54% (administered group). Spleen/body weight ratio was 0.73% in administered group while the control group was 0.58%. Because liver and spleen contain kuffer cells and splenic marcrophages respectively, they have the function of protecting body from alien substances entering the body.

Clinico-chemical characteristics

S-GOT, S-GPT and alkaline phosphatase activities were determined to know the hepatotoxicity by administered glycoprotein. As shown in Table 6, there was not measurable differences between the administered and control group. It

Table 3. Effect of glycoproteins from sea cucumbers on the number of circulating leucocytes in Balb/c mice (cell/mm^3)

Treatment	Dose (mg/kg)	1st day	4th day	7th day
Control	-	6,890±215[1)]	6,953±400	6,800±275
Glycoprotein	50	7,250±685	7,521±645	7,053±320
	100	8,450±458*	8,947±472*	8,545±516*
	150	9,650±586*	9,545±715*	9,720±751*
	200	12,000±751**	12,150±540**	10,800±873*

[1)]Mean±S.E.
*p<0.01 and **p<0.001 versus the control group

Table 4. Effect of glycoproteins from sea cucumbers on the number of peritoneal exudate cells in Balb/c mice (1×10^6 cell/ml)

Treatment	Dose (mg/kg)	1st day	4th day	7th day
Control	-	0.44±0.12[1)]	0.40±0.05	0.43±0.13
Glycoprotein	50	0.57±0.23	0.48±0.13	0.45±0.15
	100	2.09±0.23***	1.04±0.50	0.55±0.21
	150	1.46±0.41*	0.95±0.23*	0.56±0.38
	200	2.91±0.91**	0.97±0.13**	0.48±0.13

[1)]Mean±S.E.
*p<0.05, **p<0.01 and ***p<0.001 versus the control group

Table 5. Effect of glycoproteins from sea cucumbers on the weight of immunoorgans of Balb/c mice

Treatment	Dose (mg/kg)	Body weight (g)		Liver weight (g)	Spleen weight (g)
		1st day	17th day	17th day	17th day
Control	-	18.25±0.71[1)]	22.22±1.65	1.07±0.08 (4.81)[2)]	0.13±0.01 (0.58)
Glycoprotein	50	17.34±0.77	21.85±1.43	1.32±0.08 (6.04)	0.16±0.02 (0.73)
	100	18.14±1.21	21.91±1.51	1.36±0.05 (6.21)	0.18±0.01 (0.82)
	150	17.75±1.32	22.52±1.34	1.42±0.14 (6.31)	0.20±0.01 (0.89)
	200	18.42±1.31	22.02±1.32	1.44±0.12 (6.54)	0.21±0.02 (0.95)

[1)]Mean±SE
[2)]Data in parenthesis are means % of organ to body weight
*p<0.05 and **p<0.01 versus the control group

Table 6. Hematological indices of the Balb/c mice administered glycoproteins from sea cucumbers

Treatment	Dose (mg/kg)	S-GOT[1] (V/L)			S-GPT[2] (V/L)			Alkaline phosphatase (V/L)		
		1st	4th	7th	1st	4th	7th	1st	4th	7th
Control	-	210	235	227	54.5	52.5	56.0	9.49	10.95	11.04
Glycoprotein	50	214	217	228	53.0	53.5	52.7	10.82	10.74	11.52
	100	218	225	220	53.5	52.0	52.0	10.47	9.27	11.91
	150	217	215*	212	52.0	52.0	52.0	11.38	10.27	11.91
	200	205	230	217	56.0	56.5	52.0	10.87	10.47	10.63

Treatment	Dose (mg/kg)	Total protein (g/dl)			Cholesterol (mg/dl)			Triglyceride (mg/dl)		
		1st	4th	7th	1st	4th	7th	1st	4th	7th
Control	-	7.93	7.96	8.03	78.05	74.82	75.61	258.31	223.85	210.42
Glycoprotein	50	8.02	7.92	7.98	77.02	75.15	78.90	239.75	226.65	223.85
	100	7.95	7.84	8.15	76.45	77.32	75.12	239.72	254.77	251.46**
	150	7.83	7.81	8.20	75.59	80.24	75.40	220.59*	236.90	239.80
	200	8.18	8.91	8.03	68.56*	75.18	78.86	232.64	226.36	223.85

Treatment	Dose (mg/kg)	Blood urea nitrogen (mg/dl)			Glucose (mg/dl)		
		1st	4th	7th	1st	4th	7th
Control	-	31.28	23.47	28.26	156.16	168.77	162.98
Glycoprotein	50	25.11	21.78	22.38	159.21	163.71	160.25
	100	24.38	30.42	27.02	179.25*	160.64	164.73
	150	23.93	26.64	21.96	157.73	174.62	168.46
	200	23.06	27.85	22.38	156.45	169.80	164.73

[1]Glutamic oxaloacetic transminase
[2]Glutamic pyruvic transminase
*$p<0.05$ and **$p<0.01$ versus the control group

indicates that the administered glycoprotein could not damage the liver of mice at all. The contents of total proteins, cholesterol and triglyceride did not change significantly in all groups, indicating the administered glycoprotein did not involve in lipid metabolism. Sea cucumber glycoprotein administration also resulted in any difference in the contents of blood urea nitrogen and glucose.

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