Antitumor and Antimetastatic Activity of Fucoidan, a Sulfated Polysaccharide Isolated from the Okhotsk Sea *Fucus evanescens* Brown Alga

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Antitumor and antimetastatic activities of fucoidan, a sulfated polysaccharide isolated from *Fucus evanescens* (brown alga in Okhotsk sea), was studied in C57Bl/6 mice with transplanted Lewis lung adenocarcinoma. Fucoidan after single and repeated administration in a dose of 10 mg/kg produced moderate antitumor and antimetastatic effects and potentiated the antimetastatic, but not antitumor activities of cyclophosphamide. Fucoidan in a dose of 25 mg/kg potentiated the toxic effect of cyclophosphamide.

Key Words: *mice; Lewis lung adenocarcinoma; fucoidan; cyclophosphamide; cathepsins B, L, and D*

Sea algae are valuable perspective plant raw material containing a wide spectrum of compounds used for the creation of drugs and food additives: ω3-type polyunsaturated fatty acids, carotenoids, polysaccharides (sulfated galactanes, fucoidans, glucanes, *etc.*), vitamins, macro- and trace elements. Extracts of some brown and green algae are characterized by immunomodulating, antiinflammatory, antibacterial, antiviral, and antitumor effects [1,3, 4]. Antitumor activity was exhibited by multicomponent extracts of algae and by some terpene compounds, carotenoids, low molecular-weight peptides, *etc.*, isolated from algae [1,3].

We studied the antitumor and antimetastatic activities of fucoidan, a sulfated polysaccharide isolated from *Fucus evanescens* (brown alga in Okhotsk sea), on the model of experimental transplanted Lewis lung carcinoma (LLC).

MATERIALS AND METHODS

Experiments were carried out on 2-3-month-old C57BL/6 mice from Institute of Cytology and Genetics, Siberian Division of Russian Academy of Sciences. The animals were kept 8-10 per cage at natural illumination with free access to water and food (PK 120-1 granulated fodder for laboratory rats and mice, balanced by amino acid composition, minerals, and vitamins; Laboratorsnab Company). All experimental procedures were carried out in accordance with International Regulations on Handling Laboratory Animals. LLC cells were transplanted into the thigh in a dose of $(2\text{-}5)\times10^6$ cells/mouse.

Fucoidan (mol. weight 20-40 kDa) was isolated from *Fucus evanescens* brown alga by hot extraction [4].

In experimental series I, the animals were injected with fucoidan in a dose of 25 mg/kg alone or together with 100 mg/kg cyclophosphamide (CP; Biokhimik Company) 9 days after LLC transplantation.

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In experimental series II, one or three injections of fucoidan in a dose of 10 mg/kg were made 5, 8, and 12 days after LLC transplantation, while cyclophosphamide was injected in a single dose of 100 mg/kg on day 12 after tumor transplantation. The drugs were dissolved in saline and injected intraperitoneally (0.1 ml/10 g).

The animals were observed for 20 days, after which they were decapitated, the hind limbs were cut, and tumor weight was evaluated by the difference between the limb with the tumor and contralateral limb. Antitumor effect of the drugs was evaluated by tumor weight reduction in comparison with the control. The lungs were fixed in 10% formalin and lung metastases were counted under a binocular microscope.

Activities of cathepsins B and L were measured in liver and tumor homogenates using Z-L-Phe-L-Arg-MCA and Z-L-Arg-L-Arg-MCA as the substrates, respectively (Sigma). Cathepsin L activity was measured using CA-074 selective inhibitor of cathepsin B (kind gift from Prof. K. Hanada). Cathepsin D activity was measured spectrophotometrically with azocasein (Sigma) as the substrate.

The data were processed statistically using Statistica 6.0 software; the significance of differences was evaluated using Mann—Whitney nonparametric *U* test.

RESULTS

Single injection of fucoidan in a dose of 25 mg/kg did not inhibit tumor growth (Table 1). Moreover, fucoidan in this dose in combination with 100 mg/kg CP was toxic: by day 20 after transplantation 7 of 10 mice died. Of 10 mice injected with fucoidan alone (25 mg/kg) 3 died, while in the group of untreated animals and mice treated by CP alone no mortality was observed. Repeated injections of fucoidan in a dose of 10 mg/kg were well tolerated by the animals; the drug exhibited pronounced antitumor (33% tumor growth inhibition) and antimetastatic activities (29% reduction of the number of metastases) (Table 1). In experiments with combined administration of the drugs, fucoidan did not potentiate the effects of CP: inhibition of primary tumor growth was 51% after single injection of fucoidan and about 40% after 3 injections (Table 1). Combined use of fucoidan and CP produced a significant antimetastatic effect. The number of lung metastases in mice receiving CP alone was 4-fold lower, while in mice receiving CP in combination with one and three injections of fucoidan was 11- and 8-fold lower than in controls, respectively (Table 1).

For evaluation of the mechanisms underlying the effect of fucoidan, changes in activities of lysosomal cysteine proteases cathepsins B and L and aspartic protease cathepsin D in LLC tissue were evaluated. Lysosomal proteases, along with other cell proteases (serine proteases, plasminogen activators and inhibitors, matrix metalloproteases) play an important role in regulation of tumor growth and are involved in the realization of tumor cell apoptotic or necrotic death [2,5-8]. Three injections of fucoidan in a dose of 10 mg/kg alone or in combination with CP significantly reduced cathepsin L activity in tumor tissue, but did not modify

TABLE 1. Effects of Fucoidan and CP on Growth of Intramuscular LLC Transplants and Number of Lung Metastases in C57Bl/6 Mice (*M*±*m*)

Group	*n*	Tumor weight, g (% of control)	Mean number of metastases in the lungs (% of control)
Series I			
Control	10	4.20±0.41 (100)	—
Fucoidan, 25 mg/kg on day 9	9	5.2±0.5 (123.8)	—
Fucoidan, 25 mg/kg+CP, 100 mg/kg on day 9	3	4.2±0.4 (100)	—
Series II			
Intact	7	4.30±0.44 (100)	34.60±3.12 (100)
Fucoidan, 10 mg/kg on days 5, 8, and 12	9	2.9±0.2* (67.4)	24.60±3.33* (71.1)
Fucoidan, 10 mg/kg+CP, 100 mg/kg on day 12	6	2.10±0.29** (48.8)	3.20±1.54*** (9.2)
Fucoidan, 10 mg/kg on days 5, 8, and 12 +CP, 100 mg/kg, on day 12	9	2.60±0.25** (60.5)	4.10±0.92*** (11.6)
CP, 100 mg/kg on day 12	8	1.9±0.4** (44.2)	8.80±2.45*** (25.4)

Note. *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared to intact mice.

TABLE 2. Specific Activities of Cathepsins B, L, and D in Tumor Tissue of Mice with LLC Treated with Fucoidan and CP (*M*±*m*)

Group	Cathepsin D, E_{266}/min/g protein	Cathepsin B, μmol MCA/min/g protein	Cathepsin L, μmol MCA/min/g protein
LLC control	0.08±0.02	0.10±0.03	0.10±0.01
Fucoidan, 10 mg/kg on days 5, 8, and 12	0.10±0.01^{+}	0.08±0.01	0.030±0.004***
CP, 100 mg/kg	0.12±0.01	0.09±0.01	0.06±0.01**
CP, 100 mg/kg+fucoidan, 10 mg/kg on day 9	0.10±0.01	0.080±0.003	0.04±0.01***
CP, 100 mg/kg+fucoidan, 10 mg/kg on days 5, 8, and 12	0.17±0.01**$^{+}$	0.070±0.003	0.06±0.01**

Note. *p<0.05, **p<0.01, ***p<0.001 compared to control untreated animals with LLC; ^{+}p<0.01 compared to other experimental groups.

activity of cathepsin B. Cathepsin D activity was elevated in mice treated with CP in combination with three injections of fucoidan (Table 2). It remains unclear whether delayed (observed almost 10 days after treatment) changes in protease activities in tumor tissue are due to the antitumor effect of fucoidan.

Hence, it is shown for the first time on the model of transplanted LLC in mice that fucoidan is characterized by intrinsic antitumor and antimetastatic activities and potentiates the antimetastatic, but not antitumor effect of CP. Repeated use of the drug in moderate doses is more effective; in a high dose (25 mg/kg) fucoidan exhibited a toxic effect and caused death of some tumor-bearing animals. The mechanism underlying the effect of fucoidan is unknown; presumably, it is linked with stimulation of TNF production by macrophages and stimulation of phagocytic activities of macrophages and neutrophils [1,4,9].

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