Anticoagulant Activity of Fucoidan from Brown Algae *Fucus evanescens* of the Okhotsk Sea

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In vitro and *in vivo* experiments showed that anticoagulant activity of sulfated polysaccharide from *Fucus evanescens* (brown algae of the Okhotsk Sea) was similar to that of heparin. Anticoagulant properties of fucoidan are determined by thrombin inhibition mediated via plasma antithrombin III.

Key Words: *anticoagulants; fucoidans; sulfated polysaccharides; heparin*

The search for new substitutes of heparin among its structural analogues (available and harmless sulfated polysaccharides from plants) is an urgent problem. Published data show that extracts of various brown algae have anticoagulant activity determined by the presence of fucoidans [2,6,7]. The degree of anticoagulant activity correlates with structural and functional characteristics of fucoidans [4,10]. It should be emphasized that heparin in therapeutic concentrations produces no anticoagulant effect and inactivates thrombin (factor IIa) in the heparin-antithrombin III complex.

MATERIALS AND METHODS

Fucoidan was isolated from brown algae *Fucus evanescens* by hot extraction [11] and has a molecular weight of 20-40 kDa (protein-free form). Monosaccharide composition of fucoidan is presented by fucose, galactose, xylose, and glucose (71:9:10:8 ratio). The percentage of carbohydrates is 24%. The ratio between the amounts of fucose and sulfate residue is 1.0:0.9.

In vitro experiments were performed in platelet-poor plasma from healthy donors. Fucoidan and heparin were added to the plasma in doses of 1-1000 μg/ml and 1.0 U/ml, respectively. *In vivo* experiments were performed on BALB/c mice weighing 18-20 g and obtained from the Stolbovaya nursery. The mice were kept according to recommendations of the European Convention for the Protection of Experimental Animals. Fucoidan was injected intraperitoneally in a single dose of 5 mg/kg. The mixture of plasma samples was obtained from 10-15 animals 15, 30, 60, and 180 min after fucoidan administration.

We estimated various parameters of coagulogram. Activated partial thromboplastin time (APTT), prothrombin time (PT) and thrombin time (TT) characterize the internal mechanism of coagulation, external mechanism of coagulation, and final stage of coagulation, respectively [1].

The influence of fucoidan on coagulant properties of thrombin was studied in a thrombin-fibrinogen mixture. The test system with specific chromogenic substrate for thrombin was used to evaluate the mechanisms underlying the effect of fucoidan and determine the role of antithrombin III in the realization of anticoagulant activity. The inhibition of thrombin activity was studied in the test system containing fucoidan, heparin, or chromogenic substrate. The biochemical reaction was stopped by adding of 30% acetic acid 2 min after administration of the chromogenic substrate. Optical density of the solution was measured at a wavelength and optical path length of 405 nm and 1

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TABLE 1. *In Vivo* Effect of Fucoidan on APTT and TT in BALB/c Mice

Time after administration, min	Plasma coagulation time, sec			
	APTT	C, experiment/control	TT	C, experiment/control
Control	45.5±1.6		17.0±1.2	
15	150.0±54.3	3.3	80.0±14.5*	4.7
30	84.0±26.0	1.8	31.0±0.9*	1.8
60	46.8±2.4	1.0	17.3±1.5	1.0
180	43.2±1.6	0.95	17.5±1.2	1.0

Note. Here and in Table 2: *$p<0.01$ and **$p<0.05$ compared to the control.

cm, respectively. We used chromogenic substrate from KhromoTekh-Antitrombin diagnostic kit. The measurements were performed on a CD-4 coagulometer (Diamed) and RA-50 photometer (Bayer Diagnostics) using Tekhnologiya-Standart reagents. The results were analyzed by Student's *t* test.

RESULTS

Fifteen minutes after fucoidan injection the clotting time in APTT and TT tests increased by 3.3 and 4.7 times, respectively (Table 1). Thus, administration of fucoidan led to hypocoagulation in mice. The clotting time in these tests differed from the control level by 1.8 times 30 min after treatment, but returned to normal by the 60th minute.

We studied *in vitro* effects of fucoidan on APTT, PT, and TT in platelet-poor plasma from healthy donors. Fucoidan dose-dependently increased the clotting time in various coagulation tests (Table 2). Heparin possesses similar properties.

We studied the effect of fucoidan on the time of thrombin-induced coagulation of purified fibrinogen. Fucoidan and heparin added to the test system produced no anticoagulant effects.

We compared anticoagulant effects of fucoidan and heparin in the presence or absence of antithrombin III (Table 3). Optical density of the mixture of thrombin, fucoidan, and chromogenic substrate was similar to that observed after substitution of fucoidan for the buffer solution (control). Optical density of the mixture of thrombin, fucoidan, and chromogenic substrate was similar. Therefore, fucoidan possesses no intrinsic anticoagulant properties. The decrease in optical density of the mixture containing thrombin, fucoidan, chromogenic substrate, and antithrombin III was comparable to that observed in the mixture of thrombin, heparin, chromogenic substrate, and antithrombin III. It was probably related to inactivation of thrombin with the anticoagulant in the presence of antithrombin III. The decrease in optical density of the mixture containing thrombin, fucoidan, antithrombin III, and chromogenic substrate depended on fucoidan concentration.

Fucoidans belong to the family of highly sulfated homo- and heteropolysaccharides containing fructose as the major structural moiety. Much attention was given to anticoagulant activity of these compounds [4-11]. The anticoagulant effects of fucoidans from brown algae and heparin are realized via different

TABLE 2. *In Vitro* Effect of Fucoidan on APTT, TT, and PT in BALB/c Mice

Fucoidan dose, mg/ml	Plasma coagulation time, sec					
	APTT	C, experiment/control	TT	C, experiment/control	PT	C, experiment/control
Control	36.2±1.0		17.1±0.9		14.0±0.8	
1000	960±70*	26.5	390±35*	22.8	52.0±4.2*	3.7
100	940.0±207.3**	25.9	385.0±70.8**	22.4	42.0±3.5**	3.0
50	610.0±135.4**	16.9	—	—	—	—
10	210.0±39.2**	5.8	130.0±20.7**	7.6	33.0±4.8**	2.4
1	81.7±10.3**	2.3	42.8±8.5**	2.5	21.0±3.5**	1.5
Heparin	730±95*	20.2	421±86*	24.6	381±63*	27.2

Note. "—", not measured.

TABLE 3. Absorption in Samples (pH 8.3) at 405 nm

Samples	Tris-HCl buffer (control)	Fucoidan in Tris-HCl buffer		Heparin in Tris-HCl buffer, 5 U/ml
		1000 mg/ml	100 mg/ml	
Tris-HCl buffer without AT	0.455	0.395	0.444	0.468
Tris-HCl buffer with plasma and AT	0.449	0.142	0.289	0.085

mechanisms. Anticoagulant activity of fucoidans is related to the direct interaction with factors responsible for "internal" blood coagulation (XI, XII, and VIII), but not with antithrombin III [3]. The action of fucoidan is not realized via major effector sites of heparin. This can be explained by the absence of a specific polysaccharide sequence responsible for binding of fucoidans to antithrombin III, and by other features of the charged molecule.

Fucoidan was isolated from brown algae *Fucus evanescens* of the Okhotsk Sea. Fucoidan produces a dose-dependent anticoagulant effect, which is similar to that of heparin. Fucoidan has no anticoagulant activity in the coagulation test system with purified fibrinogen in the absence of antithrombin III.

Experiments with the chromogenic substrate for thrombin showed that anticoagulant activity of fucoidan was related to inactivation of thrombin and similar to that of heparin. It should be emphasized that inactivation of thrombin was not observed in the absence of antithrombin III. Comparative study with heparin used instead of fucoidan showed that it produced a cofactor effect.

Our results indicate that the anticoagulant effects of fucoidans from *Fucus evanescens* and other brown algae are realized via different mechanisms. This is related to peculiar chemical characteristics of fucoidan from *Fucus evanescens*, including low molecular weight, presence of fucose (major structural moiety) and other monosaccharide residues, and moderate degree of sulfation. Previous studies showed that anticoagulant activity of fucoidan is related to high content of fucose [10] and sulfates [4,8]. Complex relationships exist between the structure and anticoagulant properties of fucoidans [4,5,8,9].

REFERENCES

1. V. P. Baluda, Z. S. Barkagan, E. D. Gol'dberg, and B. I. Kuznik, *Laboratory Methods for Studying the System of Hemostasis* [in Russian], Tomsk (1980).
2. M. Ya. Rozkin, M. N. Levina, V. S. Efimov, and A. I. Usov, *Farmakol. Toksikol.*, **54**, No. 5, 40-42 (1991).
3. M. Ya. Rozkin, M. N. Levina, N. S. Kameneva, *et al*., *Ibid*., **52**, No. 3, 48-51 (1989).
4. F. Chanbet, L. Chevolot, J. Jozefonviez, *et al*., *Bioactive Carbohydratz Polymers*, Ed. B. S. Paulsen, Dordrecht, Boston (2000), pp. 59-84.
5. K. Katsuraya, H. Nakashima, N. Yamamoto, and T. Uryu, *Carbohydr. Res.*, **315**, 234-242 (1999).
6. J. Millet, S. C. Jouault, S. Mauray, *et al*., *Thromb. Haemost*., **81**, 391-395 (1999).
7. B. Mulloy, P. A. Mourao, and E. Gray, *J. Biotechnol*., **77**, 123-135 (2000).
8. T. Nagumo and T. Nishino, *Polysaccharides in Medicinae Applications*, Ed. S. Dumitriu, New York (1996), pp. 545-574 (1996).
9. T. Nishino and T. Nagumo, *Bot. Mar*., **34**, 387-389 (1991).
10. T. Nishino, T. Nagumo, H. Kiyohara, and H. Yamada, *Carbohydr. Res*., **320**, 108-119 (1991).
11. T. N. Zvyagintseva, N. M. Shevchenko, I. B. Popivnich, *et al*., *Ibid*., **322**, 32-39 (1999).