Fucoidan Extracted from *Fucus Evanescens* Brown Algae Corrects Immunity and Hemostasis Disorders in Experimental Endotoxemia

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Fucoidan extracted from brown algae (*Fucus evanescens*) was used for correction of immunity and hemostasis disorders in experimental endotoxemia induced by injection of LPS. Fucoidan reduced the elevated levels of proinflammatory cytokines (TNF-α, IL-1, IL-6) and partially arrested hypercoagulation phenomena, thus improving animal resistance to LPS.

Key Words: *fucoidans; hemostasis; immunity; cytokines; lipopolysaccharides*

Experimental endotoxemia, induced by injection of endotoxin (gram-negative bacterial LPS), serves as a model for studies of the mechanisms of inflammation, hemostasis abnormalities, endotoxin shock, and sepsis [7,8]. Penetrating into systemic bloodflow, LPS initiates a complex of pathological processes, including disorders of humoral and cellular immunity, development of disseminated intravascular coagulation (DIC syndrome), *etc.* [5,6,9,10,13, 14]. LPS interacts with macrophage monocytes, neutrophils, and endothelial cells stimulating hyperproduction of proinflammatory cytokines, damaging tissues and organs, and inducing endotoxin shock [2,9,10,13]. Hemostats disorders in endotoxin shock and sepsis are largely caused by the procoagulant activity of TNF-α, IL-1, and IL-6 [9, 10,13].

The search for drugs reducing the negative effects of endotoxin and increasing resistance to it is an important problem of endotoxemia therapy. We studied the possibility of using fucoidan extracted from *Fucus evanescens* algae characterized by immunostimulatory and anticoagulant activities [1,3] for correction of immunity and hemostasis disorders in experimental endotoxemia.

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MATERIALS AND METHODS

Fucoidan (sulfated polysaccharide) was isolated from brown algae (*F. evanescens*) by the hot extraction method [4]. The study was carried out on outbred mice and BALB/c mice (20-22 g) from Stolbovaya Breeding Center. The animals were handled in accordance with the European Convention for Protection of Animals Used in Experimental Studies.

The protective effect of fucoidan at different treatment protocols was evaluated by the percent of survivors and mean lifespan (MLS) of outbred mice injected with *Yersinia pseudotuberculosis* LPS in a dose of 6.25±0.5 mg/kg (LD_{100}). The effect of fucoidan on the cytokine levels and hemostasis values were studied in BALB/c mice over 24 h after LPS injection. Mice with endotoxemia induced by intraperitoneal injection of LPS in LD_{100} constituted group 1; group 2 animals were injected with fucoidan according to a therapeutic protocol (3 subcutaneous injections in a dose of 5 mg/kg after LPS injection); group 3 animals received fucoidan ac-

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cording to the preventive protocol (10 subcutaneous injections before LPS); and group 4 animals received fucoidan orally for 21 days before LPS; mice treated by 0.85% NaCl served as controls.

Serum levels of TNF-α, IL-1α, and IL-6 in BALB/c mice were measured by EIA using BD Biosciences kits (BD OptEIA™ Set Mouse TNF (mono)) according to manufacturer's instruction.

The coagulation component of hemostasis was evaluated by the activated partial thromboplastin time (APTT), prothrombin time (PT), and thrombin time (TT) of blood clotting; fibrinogen (FG) level was measured, fibrinolytic activity (FA) was evaluated in the clot spontaneous euglobin lysis test with reagents from Technology-Standard Firm.

The results were statistically processed using Biostat and Excel software; the significance of differences was evaluated using Student's *t* test, the critical significance level was assumed at 5%. The result ($M \pm m$) was the mean of 5-6 tests, each carried out with a blood pool from 15 animals.

RESULTS

All animals in group 1 died; their MLS was 27.1± 2.2 h. Fucoidan therapy (group 2) prolonged MLS to 43.2±3.4 h (p=0.012), after which all animals died. Preventive treatment with fucoidan (group 3) prolonged MLS to 52.8±4.3 h (p=0.01), the survival in this group was 18.9±1.2%; oral fucoidan (group 4) provided 24.4±4.3% survival at MLS of 57.6±3.6 h (p=0.002).

Evaluation of the dynamics of cytokines showed significantly (p=0.000) elevated serum concentrations of TNF-1 and IL-1α 2 h after LPS injection in group 1. After 4 h the concentrations of these cytokines decreased and by the term of 24 h gradually approached the values in the control group. In group 3 receiving preventive fucoidan treatment, the concentrations of TNF-1 and IL-1α were lower than in group 1 throughout the entire period of the study (Fig. 1). The dynamics of IL-6 exhibited a different pattern in this group: the peak was recorded after 8 h, after which the values gradually reduced, but the levels still surpassed those in intact controls. As a result of fucoidan treatment, IL-6 levels were lower than in group 1 (Fig. 1).

Studies of the hemostasis parameters showed that experimental endotoxemia led to the development of pronounced hypercoagulation early (3-4 h) after injection of LPS, which manifested by an increase in the total coagulant potential of the plasma (accelerated blood clotting in basic clotting tests) and to fibrinolysis inhibition (Table 1). These signs are characteristic of the initial stage of the DIC syndrome [11-14]. Treatment with fucoidan according to the therapeutic protocol (group 2) arrested hypercoagulation, which was seen by the APTT and TT and higher FA of the blood, presumably due to the anticlotting and fibrinolytic activities of fucoidan [1,3], but the blood clotting characteristics did not reach the control level (Table 1). Preventive fucoidan treatment (group 3) induced hypocoagulation shifts, manifesting by prolonged blood clotting time in comparison with group 1 and control (p=0.000). These processes were associated with higher FA in comparison with group 1 (p=0.001) and recovery of FG level (p=0.004), which approached the level in the control (Table 1).

Comparison of the time course of clotting parameters and serum cytokine (TNF-α and IL-1α) levels in mice injected with LPS showed that hyper-

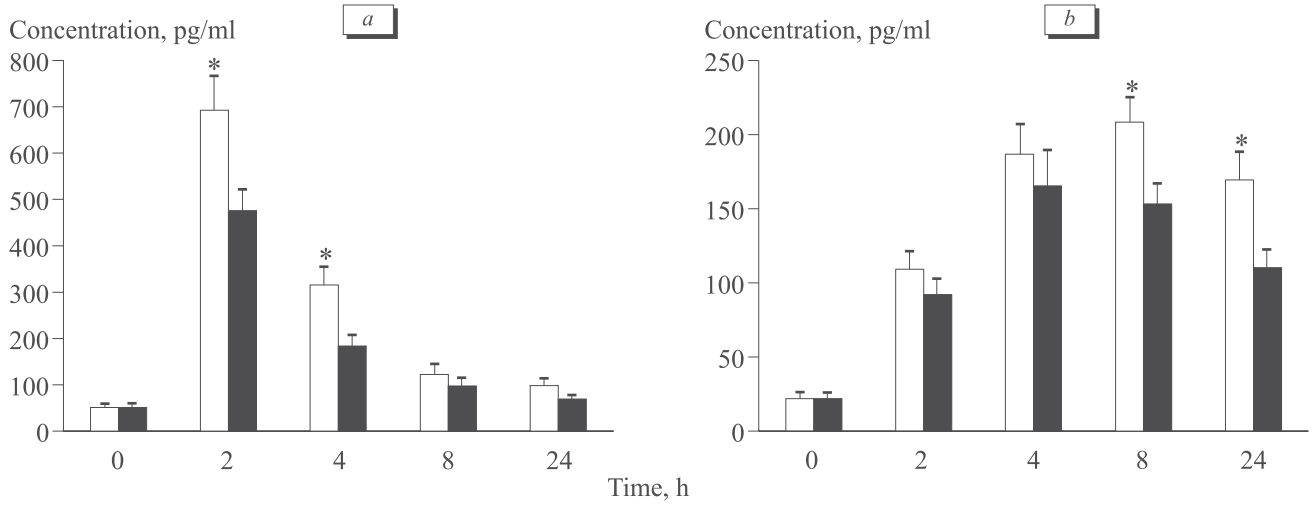

Fig. 1. Dynamics of TNF-α (*a*) and IL-6 concentrations (*b*) in the serum of BALB/c mice. Light bars: experimental endotoxemia; dark bars: endotoxemia+fucoidan treatment. n=6; *significant differences (p<0.05).

TABLE 1. Effects of Fucoidan on Hemostasis Values 4 h after LPS-Induced Endotoxemia (*M*±*m*)

Hemostasis parameter	Group of animals			
	control	1 (LPS)	2 (fucoidan therapy)	3 (fucoidan prevention)
APTT, sec	47.4±2.6	25.0±2.0	38.5±1.9	75.6±6.5*
	*p=0.000		*p=0.001	*p=0.000
			^{+}p=0.025	^{+}p=0.000
PT, sec	16.8±2.6	11.6±0.9	14.0±0.8	23.2±2.4
	*p=0.095		*p=0.081	*p=0.000
			^{+}p=0.331	^{+}p=0.108
TT, sec	18.6±0.6	12.8±0.4	15.7±1.0	67.8±4.7*
	*p=0.000		*p=0.027	*p=0.000
			^{+}p=0.028	^{+}p=0.000
FA, min	310±32	533±28	430±29	350±30*
	*p=0.001		*p=0.034	*p=0.001
			^{+}p=0.024	^{+}p=0.388
FG, g/liter	4.1±0.25	6.1±0.4	4.8±0.2	4.4±0.2*
	*p=0.003		*p=0.02	*p=0.004
			^{+}p=0.06	^{+}p=0.342

Note. n=5; *compared to group 1; $^{+}$compared to the control.

production of these cytokines preceded hypercoagulation increase. This is in line with the assumption on the key role of proinflammatory cytokines (specifically, their hyperproduction) in hypercoagulation and fibrinolysis inhibition in sepsis and endotoxin shock [9,10,13]. By the end of day 1 after LPS injection blood clotting time was prolonged, FA increased, and FG level decreased, reflecting the development of hypocoagulation changes as a result of blood clotting factors consumption. By this time, the concentrations of TNF-α and IL-1α decreased significantly, indicating attenuation of LPS-induced cytokine cascade. Anticoagulants (heparin, lepirudine, hyrudine, *etc.*) are effective during this stage of hypercoagulation, which was not once demonstrated in experimental studies [12-15]. Treatment with *F. evanescens* fucoidan promoted a reduction of hypercoagulation (due to the anticoagulant activity of this substance) and reduction of elevated TNF-α, IL-1α, and IL-6 levels. The latter fact is particularly important in the mechanisms of fucoidan action, because the therapeutic strategy in sepsis and endotoxin shock is directed against the hyperinflammatory cascade elements, specifically, against these cytokines. By realizing these mechanisms, fucoidan promotes attenuation of the clinical manifestations of endotoxicosis, reduction of hypercoagulation symptoms, and other signs of the DIC syndrome, and eventually promotes an increase of resistance to LPS toxicity.

Hence, fucoidan effectively regulates the immunity and hemostasis systems in experimental endotoxemia; preventive treatment with this substance attenuates the course of the DIC syndrome.

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