Antimetastatic Activity of Sea Buckthorn (*Hippophae rhamnoides*) Extracts

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Experiments on animals with transplanted tumor (Lewis lung carcinoma) demonstrated antimetastatic effects of extracts from shoots, bark, and complex extract from shoots and bark of sea buckthorn (*Hippophae rhamnoides*). Sea buckthorn extracts suppressed the metastatic process after removal of the tumor node. Sea buckthorn preparations reduce pain sensitivity in mice.

Key Words: *transplanted tumors; antimetastatic activity; analgesic effect*

The efficiency of antitumor therapy can be improved by supplementing classical methods (surgery, radiotherapy, chemotherapy, and their combinations) with administration of various modifiers of biological reactions, *i.e.* agents acting on both tumor cells and various regulatory systems of the organism [6]. These modifiers include natural and synthetic immunomodulators, cytokines and their inductors, monoclonal antibodies, differentiating agents, *etc*. Preparations from medicinal plants containing a variety of bioactive substances exhibiting a wide spectrum of pharmacological activities and modulating organism's homeostasis can serve as the source of these modifiers.

Sea buckthorn (*Hippophae rhamnoides*) perennial bushes are widely spread and are grown on plantations. This plant is a raw material for a number of drugs (sea buckthorn oil, sea buckthorn berry and leaf oil, Olazol aerosols, Oblekon sterile films). Regular felling and renewal of sea buckthorn plantations yield a waist amount of plant raw material (wood, bark, shoots), which is not utilized, but can be a source for new plant preparations. Previous studies showed that serotonin isolated from sea buckthorn bark exhibits antitumor activity, but high toxicity of this agent limits its application [7].

Here we studied the effects of extracts from sea buckthorn bark (SBBE) and sea buckthorn shoots (SBSE) on the development of tumor process and evaluated the possibility of improving the efficiency of treatment after removal of the primary tumor node.

MATERIALS AND METHODS

Sea buckthorn extracts were prepared by repercolation using 40% ethanol as the extragent; condensation and drying were carried out in a vacuum drying case [10]. The complex sea buckthorn preparation (liquid extract) was prepared by mixing shoots and bark in an 8:2 ratio (alkaloid content 0.35%).

The experiments were carried out on C57Bl/6, CBA, BALB/c and (CBA×C57Bl/6)F_1 mice (n=290). The animals were obtained from the Department of Experimental and Biological Models (Institute of Pharmacology, Tomsk Research Center) and were kept in accordance with European Convention on Protection of Vertebrate Animals Used for Experimental and Scientific Purposes (Strasbourg, 1986).

Lewis lung carcinoma (LLC) was transplanted to experimental animals and the test sea buckthorn

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extracts were administered. The tumor node was removed under ether narcosis.

The efficiency of treatment was evaluated by tumor weight, percent of growth inhibition (TGI), number of metastases in the lungs and their area, incidence of tumor metastasizing [12]. The weight of metastases was calculated by the difference between lung weight in experimental and control mice; inhibition of metastasizing was calculated as percent from the control. Index of metastasizing inhibition (IMI) was determined [1]. The degree of metastatic involvement was evaluated by a scale differentiating its severity depending on the number and size of metastases [13].

The analgesic effect was evaluated on the model of writhing induced by intraperitoneal injection of 3% acetic acid (0.1 ml per 10 g body weight) [11].

The data were processed statistically using nonparametric Mann—Whitney U test and φ coefficient (Fisher angular transformation) [5].

RESULTS

Analysis of the effect of SBSE in doses of 100-500 mg/kg on the development of LLC revealed its antitumor effect in doses of 200 and 300 mg/kg. The weight of the tumor in mice receiving SBSE in the specified doses was significantly lower (by 1.4 times) than in controls (Table 1). Higher doses (400 and 500 mg/kg) produced no TGI.

SBSE exhibited high antimetastatic activity (IMI> 50%) in all studied doses. The number of metastases in the experimental groups considerably decreased compared to the corresponding value in untreated mice (Table 1). After administration of SBSE in a dose of 100 mg/kg the area of metastatic involvement was minimum: 6.3-fold below the control. In mice receiving SBSE in a dose of 300 mg/kg, the incidence of metastases significantly decreased.

SBBE also produced the antimetastatic effect. In a dose of 100 mg/kg SBBE significantly reduced the number of metastases; the number of metastatic nodes decreased 1.3-fold compared to that in untreated controls (Table 1).

Administration of the complex extract from sea buckthorn shoots and bark (SBSBE) in doses of 1 and 5 ml/kg significantly reduced the incidence of metastasizing to 75%, whereas untreated animals had metastases in the lungs (Table 2). The number of metastatic nodes in treated mice also significantly decreased: by 2.1 and 4.1 times for SBSBE doses of 1 and 5 ml/kg, respectively. The area of metastases also significantly decreased. In mice receiving SBSBE in doses of 1 and 5 ml/kg this parameter was below the control by 2.1 and 8.6 times, respectively; IMI was also high (64 and 82%, respectively, Table 2).

These data suggest that sea buckthorn extracts exhibit antimetastatic activity. Therefore, at the next stage we evaluated their effects of the development of tumor process after removal of the main tumor node.

Removal of the tumor in mice with LLC transplanted into hind-paw pad significantly increased the area of metastatic involvement (by 6.5 times) despite minor decrease in the number of metastases (Table 3). Administration of SBBE in a dose of 100 mg/kg to operated mice decreased the area of metastases (by 4.8 times) compared to operated animals not receiving the extract (Table 3).

TABLE 1. Effect of SBSE and SBBE on Development of LLC in Female (CBA×C57Bl/6)F_1 Mice ($M\pm m$)

Preparation, dose, number of doses	n	Tumor weight, g	TGI or stimulation (+) of tumor growth, %	Incidence of metastasizing, %	Number of metastases	Area of metastases, mm²	IMI, %
SBSE							
before treatment	10	5.2±0.4	—	100	13.5±2.9	4.66±1.68	—
100 mg/kg, 15 doses	10	4.2±0.4	19	90	4.3±0.9**	0.74±0.32**	71
200 mg/kg, 15 doses	10	3.8±0.4*	27	100	5.1±1.1**	1.79±0.86*	62
300 mg/kg, 15 doses	10	3.6±0.5*	31	80**	4.8±1.6**	3.47±1.70	72
400 mg/kg, 15 doses	10	4.9±03	6	90	5.6±1.6*	7.36±5.05	63
500 mg/kg, 15 doses	10	5.2±0.4	0	90	4.1±1.1**	1.65±0.56	73
SBBE							
before treatment	8	4.1±0.3	—	100	8.9±2.6	1.72±0.69	—
100 mg/kg, 16 doses	9	4.6±0.6	+12	78*	6.6±2.3	4.19±2.19	42

Note. Tumor was transplanted in a dose of 1-2×10^6 cells in 0.1 ml physiological saline. SBSE and SBBE were administered starting from day 4 and day 3 after transplantation, respectively. Here and in Tables 2, 3: *p<0.05, **p<0.01 compared to values before treatment.

TABLE 2. Effect of SBSBE on the Development of LLC in Mice (*M*±*m*)

Object	SBSBE dose, number of doses	*n*	Tumor weight, g	TGI, %	Incidence of metastasizing, %	Number of metastases	Area of metastases, mm²	IMI, %
Male (CBA×C57Bl/6)F_1 mice	Before treatment	12	5.0±0.6	—	100	19.6±3.4	12.3±3.2	—
	1 ml/kg, 15 doses	8	4.5±0.3	10	75**	9.3±3.6*	5.8±2.8*	64
Female C57Bl/6 mice	Before treatment	15	4.4±0.3	—	100	14.7±1.3	3.17±0.60	—
	5 ml/kg, 9 doses	12	3.7±0.3	16	75**	3.6±0.7**	0.37±0.17*	82

Note. Tumor was transplanted in a dose of 1-2×10⁶ cells in 0.1 ml physiological saline. SBSBE was administered starting from day 5 after transplantation.

TABLE 3. Effect of SBBE and SBSE on Metastasizing of LLC in Mice after Removal of Tumor Node (*M*±*m*)

Object	LLC volume	Experimental conditions	*n*	Incidence of metastasizing, %	Number of metastases	Area of metastases, mm²	IMI, %
Female C57Bl/6 mice	3.75×10⁵ cells in 0.1 ml physiological saline into hind-paw pad	Control	9	100	13.2±3.8	3.82±1.16	—
		Surgery on day 20	5	100	5.2±1.2	24.86±9.98**	61
		Surgery+SBBE (100 mg/kg, 15 doses)	6	100	3.8±0.7	5.23±1.63⁺	71
Male (CBA×C57Bl/6)F_1 mice	4-6×10⁶ cells in 0.2 ml physiological saline subcutaneously on the back	Control	8	88	15.0±4.5	3.56±1.17	—
		Surgery on day 15	8	88	17.5±4.0	13.48±5.42	+17
		Surgery+SBSE (200 mg/kg, 9 doses)	9	78	6.4±2.2⁺	3.59±1.80⁺	62

Note. Extracts were administered starting from day 14 after transplantation. ⁺$p<0.05$ compared to values before surgery.

TABLE 4. Effect of SBSBE on Metastasizing of LLC in Female C57Bl/6 Mice after Removal of Tumor Node (*M*±*m*)

Experimental conditions	*n*	Number of mice with relapses, %	Lung weight, mg	Weight of metastases, mg	Incidence of metastasizing, %	Number of metastases	Area of metastases, mm²	IMI, %
Control	10	—	227.3±10.7	35.60±8.13	100	21.7±3.5	20.06±4.29	—
Surgery on day 22	8	38	274.1±58.9	78.50±58.85	100	25.6±5.3	39.35±15.74	+18
Surgery+SBSBE (1 ml/kg, 10 doses)	9	11	189.4±5.2⁺⁺	3.78±2.37⁺	89	6.3±2.3**	3.76±1.36⁺⁺	74

Note. Tumor was transplanted subcutaneously on the back in a dose of 4-6×10⁶ cells in 0.2 ml physiological saline. SBSBE was administered 1 h before surgery. Weight of the lungs in healthy animals was 196 mg. ⁺$p<0.05$ and ⁺⁺$p<0.01$ compared to values before surgery.

TABLE 5. Effect of SBSBE on Severity of Metastatic Involvement of the Lungs in Female C57Bl/6 Mice with LLC after Removal of Tumor Node (%)

Experimental conditions	*n*	Metastatic involvement					
		no metastases	I (>10)	II (10-30)	III (>30)	IV (>100)	V (>100)
Control	10	0	10	70	20	0	0
Surgery on day 22	8	0	12.5	75	12.5	0	0
Surgery+SBSBE (1 ml/kg, 10 doses)	9	11	78*+	11	—	0	0

Note. *$p<0.05$ compared to the control, +$p<0.01$ compared to values after surgery.

SBSE also considerably suppressed the metastasizing process after removals of the tumor node. For instance, in the experiment with subcutaneously transplanted LLC the number and area of metastases in operated animals receiving SBSE significantly decreased compared to the control (by 2.7 and 3.8 times, respectively; Table 3).

SBSBE also exhibited high antimetastatic activity after removal of the tumor node. Administration of SBSBE to operated animals considerably decreased the number of metastases and their area compared to those in operated untreated mice (by 4.1 and 10.5 times, respectively), IMI was 74% (Table 4).

In the group of operated mice treated with SBSBE, the weights of the lungs and metastases were below the corresponding parameters in operated untreated mice by 1.4 and 20.8 times, respectively (Table 4). Inhibition of metastasizing in this experimental group calculated by the weight of metastases was 89%, whereas in untreated mice stimulation of metastasizing attained 121% (Table 4). Grade I metastatic involvement of the lungs was found in the majority of animals receiving SBSBE (78%), whereas the majority of operated mice (75%) had grade II and 12.5% had grade III metastatic involvement of the lungs (Table 5).

Thus, SBSBE exhibited antimetastatic activity and considerably inhibited tumor dissemination after removal of the tumor node.

SBSBE also inhibited the growth of Ehrlich carcinoma (26-67%), B16 melanoma (30-48%), and suppressed the metastatic process in mice with B16 melanoma, rats with Pliss lymphosarcoma and Walker 256 carcinosarcoma [4].

The antitumor effect of preparations from sea buckthorn bark and shoots can be determined by alkaloids. Many substances belonging to this group are important drugs (atropine, platyphyllin, quinine, papaverine, morphine, *etc*.) or are the source for their synthesis [8]. Preparations created on the basis of alkaloids and exhibiting cytostatic activity are widely used in oncology (vinblastine, vincristine, etoposide, teniposide, colchicines, and colchamine). Serotonin isolated from sea buckthorn bark produces an antitumor effect, modulates oxygen regimen of normal and tumor tissues, and induces spasm and inflammatory changes in tumor vessels leading to inhibition of energy processes in the tumor tissue [7].

Moreover, sea buckthorn bark and shoots contain tannins producing astringent, bactericidal, anti-inflammatory, and antitumor effects and inhibiting free-radical reactions [8].

Of particular interest are the analgesic properties of sea buckthorn extracts. Experiments on mice showed that sea buckthorn extracts weaken nociceptive reaction to intraperitoneal injection of acetic acid. For instance, SBSE in doses of 100 and 200 mg/kg reduced pain sensitivity by 45 and 75%, respectively; writhing number decreased by 1.8 and 3.9 times, respectively (Table 6).

SBBE in doses of 100 and 200 mg/kg produced similar effects: pain sensitivity decreased by 47-51%, the effect was comparable to the analgesic activity of indomethacin.

SBSBE in doses of 0.5-5.0 ml/kg also considerably decreased pain sensitivity (by 33-43%; Table 6).

The presence of analgesic activity is very important, because the decrease in pain sensitivity can alleviate stress during surgical removal of the tumor. It is known that surgical removal of the tumor and emotional and pain stress are often accompanied by stimulation of metastasizing. The stimulating effect of stress of dissemination processes are realized via systems responsible for nonspecific antitumor resistance. Of particular importance is the effect of stress factors on functional activity of immunocompetent cells [2]. The stress-induced increase in corticosteroid level suppressed activity of natural killer cells (NKC) playing an important role in antitumor resistance of the organism. Pain hormone (substance P) released during stress reduces migration capacity of NKC [3]. In light of this, administration of preparations redu-

TABLE 6. Effects of Extracts from Sea Buckthorn Bark and Shoots on Pain Sensitivity on the Model of Writhing Induced by 3% Acetic Acid (*M*±*m*)

Object	Experimental conditions	*n*	Mean writhing number per 15 min	Decrease in pain sensitivity, %
Male BALB/c mice	Control	7	49.7±6.1	—
	SBSE, 100 mg/kg	7	27.1±5.0**	45
	SBSE, 200 mg/kg	7	12.6±4.4**	75
Male CBA mice	Control	7	35.3±3.4	—
	SBBE, 100 mg/kg	7	18.7±4.7*	47
	SBBE, 200 mg/kg	7	17.4±5.4**	51
	Indomethacin, 10 mg/kg	4	21.0±7.4**	41
Female (CBA×C57Bl/6)F_1 mice	Control (40% ethanol)	10	33.4±4.8	—
	SBSBE, 0.5 ml/kg	11	19.0±2.9**	43
	SBSBE, 1.0 ml/kg	10	19.7±3.3**	41
	SBSBE, 5.0 ml/kg	11	22.3±2.4**	33
	Indomethacin, 10 mg/kg, 7 doses	6	12.8±3.1**	62

Note. The preparations were administered daily for 7 days, the last dose was given 1 h before exposure. *$p<0.05$, **$p<0.01$ compared to the control.

cing the strength of stress factors and increasing NKC activity can be a promising approach to preventing stress-induced disturbances in the system of antitumor immunity.

Single administration of sea buckthorn extracts to healthy animals increased cytotoxic activity of splenic NKC [9]. Since cellularity of the spleen remained unchanged under these conditions, we can hypothesize that migration and proliferative processes were not crucial for the observed phenomenon, while the observed increase in NKC cytotoxicity was probably associated with accelerated maturation of a new generation of NKC from precursor cells. During the course treatment with the preparation, NKC cytotoxicity increased in parallel with the increase in spleen cellularity. In this case the increase in cytotoxicity can be associated with increased number of splenocytes, which can result from redistribution and migration of cells under the effect of the extract. Administration of the extract to tumor-bearing mice (LLC) prevented exhaustion of NKC cytotoxicity, decreased tumor-stimulating activity of peritoneal macrophages, and normalized the content of TNF [9].

The capacity of SBSBE to suppress the growth of transplanted tumors, to inhibit tumor metastasizing, and to increase NKC activity allowed us to refer SBSBE to the group of modifiers of biological reactions.

Thus, sea buckthorn preparations possessing antimetastatic and analgesic properties and improving functional activity of natural resistance system cells deserve investigation of the possibility of their practical application in oncology.

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