ONCOLOGY

Comparative Evaluation of the Efficiency of Various Alginate Forms under Conditions of an Oncological Experiment

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The effects of various alginate forms on the development of transplanted tumors in mice and efficiency of cytostatic therapy were studied. High-molecular-weight Ca and Na alginates, acid-soluble hydrolysate, and sodium alginate fraction inhibited the growth of Ehrlich adenocarcinoma. The use of acid-insoluble sodium alginate in chemotherapy protocol improved the treatment efficiency. All alginate forms inhibited metastasizing of Lewis pulmonary carcinoma; in combination with cyclophosphamide they potentiated its antimetastatic effect.

Key Words: *chemotherapy; alginate; transplanted tumors*

A trend to a higher incidence of oncological diseases is observed in the modern world, including Russia [4]. Chemotherapy remains one of the main methods for malignant tumor control [3]. However, antitumor drugs are not highly selective and their effects on rapidly regenerating cell systems are negative [2,6]. Therefore, a pressing problem of oncopharmacology is the search for new drugs with antitumor and antimetastatic activities potentiating the effect of cytostatics and/or reducing their toxicities. The data on the relationship between the polysaccharide structure and pharmacological characteristics, specifically antitumor activity are scanty. One of the products of enteric fermentation of polysaccharides – butyric acid – inhibits the proliferation of cancer cells in the colon, arresting the growth in the early G1 phase [12,14]. Antitumor activity of polysaccharides can be mediated through their immunostimulatory effects [14,16]. Polysaccharides bind proteins on cancer cell surface, responsible for adhesion of the host immunocompetent cells [7].

Alginates are natural polysaccharides, present in *Pseudomonas* and *Azotobacter* bacteria and in brown (*Pheophyta*) and red (*Corallinaceae*) algae cell walls [5,9,12,14]. They are used in food industry as solidifying agents and stabilizers. Sodium alginate is the active component of a recently released acidity-reducing drug Geviscon [12]. Two drugs containing sodium alginate and intended for local application as anti-inflammatory and hemostatic agents are recorded in the Federal Register of Drugs [12].

Alginates belong to the family of unbranched binary copolymers consisting of β-D-mannuric and α-L-guluronic acid residues bound via (1×4) bonds [5,9,12,14]. The proportion and sequence of acid residues depend on the source and method of their derivation [10]. This class of polysaccharides attracts much recent attention due to a wide spectrum of their pharmacological properties. Antiulcer effects of alginates in gastroduodenal disease have been detected [11-13]. In the gastrointestinal tract, these substances promote

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an increase of the content of bifidum and lactobacteria and in parallel reduce the level of pathogenic microflora, *i.e.* act as prebiotics [14]. Alginates eliminate toxins and radionuclides [8,9] and reduce blood cholesterol level [12]. Calcium alginate stimulates phagocytic activity of neutrophils in humans [8].

Here we compared the effects of various alginates on the development of transplanted tumors in mice and on the efficiency of cytostatic therapy.

MATERIALS AND METHODS

Experiments were carried out on 608 outbred and C57Bl/6 mice (19-26 g, 2-3 months, males and females) obtained from the Department of Experimental Biosimulation, Institute of Pharmacology (Quality Certificate No. 188-05). The animals were kept in accordance with regulations approved by the European Convention for Protection of Vertebrates Used for Experimental and Other Research Purposes. Experiments were carried out in accordance with the laboratory practice regulations (GLP), Order of the Ministry of Health of the Russian Federation No. 267, 19.06.2003 "On Laboratory Practice Regulations", Federal Law ("On Drugs" (article 36), and Manual of Experimental (Preclinical) Studies of New Drugs (Moscow, 2005). The experiment design was approved by the Ethics Committee of Institute of Pharmacology.

Experimental lot of various alginate forms was offered by Laboratory of Pharmacology of A. V. Zhirmunskii Institute of Marine Biology. High-molecular-weight calcium alginate and 4 specimens of sodium alginate were used. The physicochemical parameters of high-molecular-weight calcium alginate were as follows: molecular weight 403 kDa, alginic acid content 77.3%, calcium content 72.5 mg/kg, characteristic viscosity 1270 mg/g. Sodium alginate specimens differed by molecular weights, levels of sodium and uronic acids (Table 1).

Ehrlich adenocarcinoma (EAC) and Lewis lung carcinoma (3LL) were transplanted by routine methods. The studied preparations in doses of 50 and 100 mg/kg were dissolved in distilled water and were administered daily intragastrically through a metal tube for 7 days starting from the next day after EAC transplantation and for 12 days starting from day 7 after 3LL transplantation. Controls received an equivalent volume of distilled water. Cyclophosphamide (CP) was administered in a single dose of 150 mg/kg intramuscularly 72 h after EAC transplantation or intraperitoneally in a dose of 125 mg/kg on day 10 after 3LL transplantation. Controls received an equal volume of saline during the same periods.

The efficiency of treatment was evaluated by tumor volume (EAC) or weight (3LL), tumor growth inhibition (TGI) percentage, incidence of metastases, metastasis inhibition index [1], number of metastases and their area (3LL). The results were processed by nonparametric Mann–Whitney test (U) and Fisher angular transformation (φ).

RESULTS

Experiments on mice with EAC showed a significant inhibitory effect on tumor growth for the following specimens: high molecular calcium and sodium alginates in a dose of 100 mg/kg (TGI=20 and 46%), acid-soluble ethanol-precipitable sodium alginate hydrolysate in doses of 50 and 100 mg/kg (TGI=36%), acid-soluble ethanol-non-precipitable sodium alginate fraction in a dose of 50 mg/kg (TGI=42%; Table 2). The treatment efficiency was lower after CP in combination with high-molecular-weight calcium (50 and 100 mg/kg) and sodium (50 mg/kg; Table 2) alginates. On the other hand, addition of acid-insoluble sodium alginate in a dose of 50 mg/kg to chemotherapy protocol in cases without antitumor effect of the cytostatic resulted in a significant inhibition of EAC growth: the

TABLE 1. Physicochemical Parameters of Sodium Alginate Specimens

Specimens	Molecular weight, kDa	Content, %			
		uronic acids	sodium	ashes (sodium carbonate)	humidity
High-molecular-weight sodium alginate	403	77.3	8.45	28-31	≤8
Acid-insoluble sodium alginate hydrolysate	20-30	85.2	9.5-11.0	28-31	≤8
Acid-soluble ethanol-precipitable sodium alginate hydrolysate	1-10	85.2	10.0	28-31	≤8
Acid-soluble ethanol-non-precipitable sodium alginate fraction	1	85.2	10.0	28-31	≤8

TABLE 2. Effects of Various Alginate Specimens on EAC Development and Efficiency of CP Therapy in Outbred Mice ($X \pm m$)

Group, drug dose	Volume, ml		TGI, %
	ascites	tumor cells	
Series I (high-molecular-weight calcium alginate – specimen 1)			
Control (n=10)	3.48±0.16	1.03±0.06	—
Specimen 1, 50 mg/kg (n=10)	3.30±0.31	0.86±0.09	17
Specimen 1, 100 mg/kg (n=10)	3.38±0.41	0.82±0.08*	20
CP (n=10)	2.12±0.22	0.42±0.07*	59
CP+specimen 1, 50 mg/kg (n=10)	2.62±0.22	0.62±0.08^{o}	40
CP+specimen 1, 100 mg/kg (n=10)	3.42±0.32^{+}	0.79±0.07^{o}	23
Series II (high-molecular-weight sodium alginate – specimen 2)			
Control (n=12)	6.78±0.67	1.83±0.17	—
Specimen 2, 50 mg/kg (n=10)	6.77±0.27	1.97±0.04	-8
Specimen 2, 100 mg/kg (n=10)	4.19±0.56*	1.01±0.22*	45
CP (n=10)	6.02±0.51	1.40±0.22	23
CP+specimen 2, 50 mg/kg (n=10)	6.30±0.35	1.85±0.14^{o}	-1
CP+specimen 2, 100 mg/kg (n=10)	5.88±0.12	1.64±0.11	10
Series III (acid-insoluble high-molecular-weight sodium alginate – specimen 3)			
Control (n=11)	5.47±0.38	1.78±0.18	—
Specimen 3, 50 mg/kg (n=10)	6.79±0.45	2.13±0.11	-20
Specimen 3, 100 mg/kg (n=10)	6.15±0.36	1.75±0.08	2
CP (n=10)	5.10±0.41	1.47±0.13	17
CP+specimen 3, 50 mg/kg (n=10)	4.93±0.43	1.26±0.11*	29
CP+specimen 3, 100 mg/kg (n=10)	4.22±0.26	1.08±0.07^{+}	39
Series IV (acid-soluble ethanol-precipitable sodium alginate hydrolysate, specimen 4)			
Control (n=11)	6.16±0.36	1.69±0.13	—
Specimen 4, 50 mg/kg (n=10)	4.77±0.65*	1.08±0.11*	36
Specimen 4, 100 mg/kg (n=10)	5.18±0.52	1.08±0.13*	36
CP (n=10)	3.34±0.54*	0.75±0.11*	56
CP+specimen 4, 50 mg/kg (n=10)	3.40±0.30	0.56±0.11	67
CP+specimen 4, 100 mg/kg (n=9)	3.77±0.47	0.93±0.12	45
Series V (acid-soluble ethanol-non-precipitable sodium alginate fraction – specimen 5)			
Control (n=12)	6.25±0.38	1.92±0.14	—
Specimen 5, 50 mg/kg (n=12)	3.93±0.36*	1.12±0.13*	42
Specimen 5, 100 mg/kg (n=12)	4.53±0.62*	1.51±0.22	21
CP (n=12)	3.83±0.37*	0.84±0.12*	56
CP+specimen 5, 50 mg/kg (n=12)	3.18±0.43	0.57±0.06	70
CP+specimen 5, 100 mg/kg (n=12)	2.65±0.19^{+}	0.66±0.06	66

Note. Here and in Table 3: significant (p<0.05) inhibition *vs.* *control, $^{+}$CP; significant stimulation *vs.* ^{x}control, ^{o}CP.

TABLE 3. Effects of Various Alginate Specimens on 3LL Development and Efficiency of CP Therapy in C57BL/6 Females ($X \pm m$)

Group, drug dose	Tumor weight, g	TGI, %	Incidence of metastases, %	Number of metastases per mouse	Area of metastases per mouse, mm^2	Metastases inhibition index, %
Series I (high-molecular-weight calcium alginate – specimen 1)						
Control (n=10)	5.69±0.47	-	100	13.40±1.71	19.26±4.15	-
Specimen 1, 50 mg/kg (n=10)	5.83±0.31	-2	100	20.90±2.24^x	24.18±4.16	-56
Specimen 1, 100 mg/kg (n=10)	5.91±0.26	-4	100	24.50±2.49^x	53.03±13.85^x	-83
CP (n=9)	4.70±0.35	17	78*	3.78±1.33*	2.54±1.33*	78
CP+specimen 1, 50 mg/kg (n=9)	4.47±0.28	21*	56	1.67±0.87	0.44±0.23$^+$	93
CP+specimen 1, 100 mg/kg (n=10)	4.89±0.25	14	90	3.00±0.76	2.62±0.80	80
Series II (high-molecular-weight sodium alginate – specimen 2)						
Control (n=10)	7.09±0.28	-	100	27.80±2.01	60.84±19.51-	
Specimen 2, 50 mg/kg (n=10)	6.23±0.28*	12	100	27.80±2.58	67.28±21.64	0
Specimen 2, 100 mg/kg (n=10)	6.47±0.35	9	100	23.90±2.08	45.68±7.56	14
CP (n=10)	4.97±0.22*	30	80*	6.40±1.85*	1.67±0.68*	82
CP+specimen 2, 50 mg/kg (n=10)	4.15±0.29	41	60	1.60±0.69$^+$	0.67±0.58$^+$	97
CP+specimen 2, 100 mg/kg (n=10)	4.65±0.47	34	70	3.90±2.28	1.19±0.77	90
Series III (acid-insoluble high-molecular-weight sodium alginate – specimen 3)						
Control (n=10)	7.09±0.28	-	100	27.80±2.01	60.84±19.5	-
Specimen 3, 50 mg/kg (n=10)	7.29±0.22	-3	100	26.70±2.64	49.85±11.03	4
Specimen 3, 100 mg/kg (n=10)	7.15±0.20	-0.8	100	19,80±2.33*	26.60±3.56	29
CP (n=10)	4.97±0.22*	30	80*	6.40±1.85*	1.67±0.68*	82
CP+specimen 3, 50 mg/kg (n=10)	4.75±0.32	33	90	3.50±0.97	1.32±0.65	89
CP+specimen 3, 100 mg/kg (n=10)	5.17±0.29	27	70	1.60±0.43$^+$	0.11±0.03$^+$	96
Series IV (acid-soluble ethanol-precipitable sodium alginate hydrolysate, specimen 4)						
Control (n=10)	6.59±0.11	-	100	32.00±1.60	77.44±11.03	-
Specimen 4, 50 mg/kg (n=9)	6.09±0.30	8	100	28.63±1.20*	30.05±3.92*	10
Specimen 4, 100 mg/kg (n=9)	6.61±0.25	-0.3	100	24.60±2.10*	37.07±7.39*	23
CP (n=10)	5.20±0.18*	21	50*	3.70±1.57*	1.40±0.73*	94
CP+specimen 4, 50 mg/kg (n=10)	5.20±0.15	21	70	2.40±0.99	1.55±0.98	95
CP+specimen 4, 100 mg/kg (n=10)	5.53±0.26	16	60	1.30±0.50	0.45±0.25	98
Series V (acid-soluble ethanol non-precipitable sodium alginate fraction, specimen 5)						
Control (n=10)	6.59±0.11	-	100	32.00±1.60	77.44±11.03	-
Specimen 5, 50 mg/kg (n=9)	6.92±0.19	-5	100	34.56±4.41	50.76±11.79	-8
Specimen 5, 100 mg/kg (n=8)	6.75±0.27	-2	100	26.50±3.39*	49.42±7.99*	17
CP (n=10)	5.20±0.18*	21	50*	3.70±1.57*	1.40±0.73*	94
CP+specimen 5, 50 mg/kg (n=10)	5.19±0.08	21	70	2.20±0.80	0.72±0.44	95
CP+specimen 5, 100 mg/kg (n=10)	4.98±0.21	24	80	4.00±1.28	2.02±0.85	90

volume of tumor cells in mouse ascites decreased 1.4 times in comparison with that in untreated animals (Table 2). Use of this alginate specimen in a dose of 100 mg/kg in combination with CP more effectively inhibited tumor growth in comparison with CP alone (Table 2).

Experiments on the metastatic 3LL model showed a significant inhibition of primary tumor growth only under the effect of high-molecular-weight sodium alginate in a dose of 50 mg/kg (Table 3). Calcium alginate in this dose, added to chemotherapy protocol, exhibited a significant antitumor effect, while the cytostatic alone exhibited no statistically appreciable effect: the tumor node weight in mice of this group decreased by 1.3 times ($p<0.05$) in comparison with the control (Table 3).

Analysis of 3LL metastasizing showed that injections of acid-insoluble (100 mg/kg) and acid-soluble sodium alginate hydrolysate led to a significant reduction of the number of metastases in the lungs. Monotherapy with sodium alginate fraction in a dose of 100 mg/kg led to a significant reduction of the number of metastatic nodules in the lungs and of their area (1.2 and 1.6 times) in comparison with the control (Table 3). It is noteworthy that isolated high-molecular-weight calcium alginate stimulated 3LL metastases: the number of metastases increased by 1.6 (50 mg/kg) and 1.8 times (100 mg/kg), while their area increased by 2.8 times (100 mg/kg). By contrast, combined use of high-molecular-weight calcium alginate (50 mg/kg) and CP led to a reduction (at the level of a trend) of the number of metastases in the lungs and a significant shrinkage of their area (by 5.8 times) in comparison with mice receiving monochemotherapy. Addition of high-molecular-weight sodium alginate (50 mg/kg) also stimulated the antimetastatic effect of CP: the number of metastases in these animals decreased 4-fold and their area 2.5-fold in comparison with animals treated with the cytostatic alone (Table 3). Use of acid-insoluble sodium alginate hydrolysate (100 mg/kg) in chemotherapeutic protocol led to a 4-fold reduction in the number of metastases in the lungs and a manifold reduction of the area of metastatic involvement (Table 3). Injection of acid-soluble sodium alginate hydrolysate (50 and 100 mg/kg) in combination with CP caused a reduction (at the level of a trend) of the number of metastatic nodes in lung tissue (by 1.5 and 2.8 times, respectively; Table 3).

Analysis of the results indicated that high-molecular-weight calcium and sodium alginates, acid-soluble sodium alginate hydrolysate, and sodium alginate fraction used as monotherapies inhibited the growth of Ehrlich ascitic tumor. Only acid-insoluble sodium alginate hydrolysate stimulated the tumor inhibitory effect, while high-molecular-weight calcium and sodium alginates abolished the antitumor effect of the cytostatic. All the studied alginate forms positively inhibited the development of the metastatic process: their use led to reduction of the number and/or area of metastatic involvement in the lungs of mice with 3LL after monotherapy and combined therapy.

These results are in line with published data indicating that sodium alginate inhibits the growth of solid and ascitic sarcoma 180, ascitic EAC and JMC [15], stimulating the cytostatic and cytolytic activity of macrophages. Our experimental findings indicate that the tumor cell reaction seems to depend on molecular weight the alginates. Alginates with a molecular weight below 10 kDa (acid-soluble ethanol-precipitable sodium alginate hydrolysate and acid-soluble ethanol non-precipitable sodium alginate fraction) exhibited the highest efficiency.

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