PHARMACOLOGY AND TOXICOLOGY

Effect of Bioactive Additive Mammoleptin on Development of Transplanted Tumors in Mice

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Bioactive additive mammoleptin used for the treatment of fibrocystic breast disease did not stimulate the growth of primary tumors and metastases in mice with transplanted tumors. Mammoleptin in high doses inhibited the growth of Ehrlich adenocarcinoma and metastatic spreading of solid tumors.

Key words: *transplanted tumors; bioactive food additive; mammoleptin*

Malignant neoplasms frequently develop against the background of precancerous changes in normal tissues. Fibrocystic breast disease is a frequently occurring hormonal disorder in women of different age. Some forms of this disease are difficult to differentiate from cancer: moreover, it often transforms into malignant tumor and, therefore, can be referred to as precancerous diseases. Traditional Chinese medicine uses a biologically active compound certified in Russia as mammoleptin. This natural additive consists of deer antlers, *Sargassum palens*, *Laminaria japonica Aresch*, *Taraxacum mongolicus*, *Prunella vulgaris*, *Spatolobus erectus*, *Panax pseudoginseng*, *Paeonia suffruticosa* and *P. lactoflorum*, *Saussurea arctifolia*, *Echinops dahurica*, *Scrophularia marilandica*, *Thrichosantus polylobata*, *Forsythia suspensia*, *Crocus sativus L.*

Drug therapy in fibrocystic disease requires regular control of breast tissues to be sure that therapy does not increase the risk of cancer development. The aim of the present study was to examine the effect of mammoleptin on the growth and metastatic spreading of transplanted tumors in animals.

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MATERIALS AND METHODS

Acute toxicity of single peroral administration of mammoleptin in doses of 1, 2.5, 5, and 10 g/kg was studied on 24 random-bread female mice weighing 20 g. The animals were observed for 2 weeks. The effect of mammoleptin on tumor growth was also studied on outbread (n=31), C57Bl/6 (n=30), and (CBA×C57Bl/6)F_1 (n=88) female mice (Laboratory of Experimental Biomodelling, Tomsk Research Center). Solid tumors i. e., Lewis lung carcinoma (3LL), B-16 melanoma (B-16), and lung cancer-67 (LC-67) were transplanted intramuscularly ($4\text{-}6\times10^6$ cells in 0.1 ml physiological saline). Ehrlich adenocarcinoma (EAC) was injected intraperitoneally: 7.5×10^6 tumor cells in 0.2 physiological saline [2].

Mammoleptin (0.384 and 1.024 g/kg, aqueous solution) was administered daily via a probe to mice with EAC for 7 days starting from day 2 after inoculation. The animals with solid tumors received the same daily doses of the drug for 13-16 days starting from day 4-5 after transplantation. The doses used in the experiment corresponded to those recommended for women and were recalculated using Freireich's table [3]. In one experimental series, a group of 3LL mice received mammoleptin in increasing doses

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(0.384, 0.573, 0.789, and 1.024 g/kg for days 5-7, 8-10, 11-13, and 13-18 after transplantation, respectively), which corresponded to the treatment schedule for patients with fibrocystic breast disease.

The effect of treatment was evaluated on day 9 (EAC) or 17-20 (solid tumors) after transplantation by inhibition of tumor growth compared to the control (in %). Dissemination was evaluated by the incidence of metastases (number of animals with metastases to the total number of animals in the group), mean number of metastases, and area of metastatic spreading. The differences in metastatic spreading between the control and experimental groups were estimated by the index of inhibition of metastasizing (IIM):

$$IIM = \frac{(A_C \times B_C) - (A_E \times B_E)}{(A_C \times B_C)} \times 100\%,$$

where A_C and A_E are the incidence of metastatic spreading, B_C and B_E are the mean numbers of metastases in control and experimental animals, respectively.

After the experiment, the animals were sacrificed, autopsied, organs were revised, tumors and metastases were dissected.

The data were processed statistically using nonparametric Wilcoxon—Mann—Whitney U test and Fisher φ-angle transformation [1]. Differences were significant at $p<0.05$.

RESULTS

Studies of acute toxicity allowed to refer mammoleptin to relatively nontoxic compounds (harmless substances, class IV according to State Standards 12.1. 007-76). No lethality, pathology, behavioral disorders or changes in the general state were observed. The animals were active, had normal reflexes, smooth shining hair, and normal digestion.

Repeated administration of 1.024 g/kg mammoleptin to EAC female mice significantly inhibited tumor growth and 1.4-fold decreased tumor size compared to the control (Table 1). The effect of 0.384 g/kg mammoleptin on EAC was less pronounced: the tumor size decreased 1.2-fold (changes were insignificant, Table 1).

In (CBA×C57Bl/6)F_1 female mice with LC-67 treated with mammoleptin the weight of tumor node did not differ from the control. Mammoleptin in a dose of 1.024 g/kg decreased the number of metastases (1.3-fold), their area (2.9-fold) and the incidence of metastatic spreading (to 80%, Table 2).

Similar results were obtained for B-16 melanoma: mammoleptin did not stimulate tumor growth. Mammoleptin in doses 0.384 and 1.024 g/kg inhibited tumor growth by 11 and 9%, respectively ($p>0.05$). In all experimental groups, the mean number of metastases and their area did not differ from the control (Table 2). Moreover, mammoleptin decreased the incidence of metastases to 60% compared to 90% in the control ($p<0.053$, Table 2).

In C57Bl/6 female mice with 3LL, mammoleptin had no effect on tumor growth (the weight of primary node did not differ from that in controls), incidence of metastases, and the number of lung metastases (Table 3). In mice receiving low doses of mammoleptin the area of metastatic spreading in the lungs did not differ from the control, while kn a dose of 1.024 g/kg this drug 1.6-fold decreased this parameter compared to untreated animals ($p>0.05$).

The absence of stimulatory effect of mammoleptin on the growth of transplanted tumors was confirmed in a special experimental series on mice with 3LL. The sensitivity of this tumor to antineoplastic drugs is similar to that of human solid tumors and active experimental search for antimetastatic drugs became possible due to application of metastasizing 3LL tumor [4].

In animals with 3LL, increasing doses of mammoleptin had no effect on the growth of primary tumor, while 1.024 g/kg mammoleptin decreased the main tumor node (11% tumor growth inhibition, $p>0.05$).

Thus, mammoleptin had no effect on the number, incidence, and area of metastases. In a dose of 1.024 g/kg mammoleptin decreased the area of metastatic spreading (Table 3).

Thus, mammoleptin in the examined doses did not stimulate the growth of primary tumors and metastases. It should be noted, that in higher doses this drug significantly inhibited the growth of EAC and suppressed metastatic spreading of solid tumors.

TABLE 1. Effect of Mammoleptin on Growth of EAC in Outbread Female Mice

Parameter	Control (n=11)	Mammoleptin, g/kg	
		0.384 (n=10)	1.024 (n=10)
Volume of tumor cells, ml ($X \pm m$)	3.44±0.14	2.82±0.28	2.40±0.16*
Tumor growth inhibition, %	—	18	30

Note. *$p<0.01$ compared to the control.

TABLE 2. Effect of Mammoleptin on Metastatic Spreading of LC-67 and B-16 Melanoma in (CBA×C57Bl/6)F_1 Female Mice ($X \pm m$)

Parameter	LC-67			Melanoma B-16		
	control (n=10)	mammoleptin, g/kg		control (n=10)	mammoleptin, g/kg	
		0.384 (n=9)	1.024 (n=9)		0.384 (n=10)	1.024 (n=10)
Incidence of metastatic spreading, %	100	100	80	90	60	60
Number of metastases per mouse	3.20±0.57	4.56±0.88	2.56±0.77	5.10±1.73	5.1±2.2	5.20±0.82
Area of metastasis, mm^2	0.97±0.36	1.28±0.54	0.34±0.17	0.54±0.23	0.37±0.16	0.40±0.21
IIM, %	—	-43	36	—	33	33

TABLE 3. Effect of Mammoleptin on Metastatic Spreading of 3LL in C57Bl/6 and (CBA×C57Bl/6)F_1 Female Mice ($X \pm m$)

Parameter	C57Bl/6			(CBA×C57Bl/6)F_1		
	control (n=11)	mammoleptin, g/kg		control (n=11)	mammoleptin, g/kg	
		0.384 (n=10)	1.024 (n=9)		1.024 (n=9)	0.384 (n=10)
Incidence of metastatic spreading, %	100	100	100	100	100	100
Number of metastases per mouse	24.82±3.42	27.80±3.78	24.11±2.73	9.90±1.49	9.67±2.54	11.30±1.82
Area of metastasis, mm^2	21.42±5.14	22.5±7.1	13.61±1.78	2.17±0.66	1.80±0.41	2.87±0.95
IIM, %	—	-12	3	—	2.3	-14

Note. *See Methods.

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