Plant Polysaccharides in Combined Therapy of Transplanted Tumors

K. A. Lopatina, T. G. Razina, E. P. Zueva, S. G. Krylova, E. N. Amosova, and A. M. Guryev*

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Supplement 1, pp. 30-34, January, 2007
Original article submitted November 11, 2006

We studied the effects of polysaccharides from Siberian plants on the development of transplanted tumors under conditions of cytostatic treatment. Some mechanisms underlying the increase in the efficiency of antitumor therapy with flagroot (*Acorus calamus*) polysaccharides are shown.

Key Words: *transplanted tumors; cytostatic therapy; plant polysaccharides*

The central problem of oncopharmacology and experimental therapy, along with the search for new cytostatics with selective effect on tumor cells, is the search for drugs reducing side effects of antitumor agents, correcting hemostatic disorders caused by the tumor process and cytostatic therapy, and inhibiting tumor development through activation of natural resistance system [5].

Drugs created on the basis of medicinal plants occupy a special place among the agents potentiating the effects of cytostatics and reducing their toxicity; the spectrum of biological activity of these drugs is determined by the composition of these plants, including substances of different chemical classes [9]. Polysaccharides (PS) of plant origin are characterized by a wide spectrum of pharmacological effects, including regulation of the immune and endocrine functions, toxin adsorption, normalization of lipid metabolism, and antitumor activity [2,7,12]. We found no reports discussing the possibility of correcting side effects of cytostatic therapy with plant PS.

We studied the effects of water-soluble PS (WSPS) derived from Siberian plants on the development of tumors and metastases during monotherapy and in combination with cytostatics.

Institute of Pharmacology, Tomsk Research Center, Siberian Division of Russian Academy of Medical Sciences; *Siberian State Medical University, Tomsk

MATERIALS AND METHODS

Experiments were carried out on female C57Bl/6 mice and Wistar rats from Laboratory of Experimental Biological Modeling, Institute of Pharmacology. The animals were kept in accordance with the regulations of the European Convention for Protection of Vertebrates Used in Experimental and Other Scientific Purposes (Strasbourg, 1986). Lewis lung carcinoma (LLC) and B-16 melanoma were transplanted intramuscularly in a dose of $4\text{-}6\times10^6$ cells in 0.1 ml saline. Walker 256 carcinosarcoma was transplanted as 20% tumor cell suspension in 0.2 ml saline subcutaneously into the back [11].

Water soluble polysaccharides from *Acorus calamus*, *Ledum palustre*, *Tilia cordatum*, *Tussilago farfara*, *Calendula officinalis*, *Glycyrrhiza*, *Rhodiola rosea*, *Taraxacum officinale*, *Rhaponticum carthamoides*, *Plantago major*, *Echinacea purpura* were offered by Department of Pharmacognosia, Siberian State Medical University. Cyclophosphamide (CP) was injected to mice in a single intraperitoneal dose of 125 mg/kg on days 13-15 and 19 after transplantation of LLC and B-16, respectively, to rats with Walker 256 carcinosarcoma in a dose of 40 mg/kg intraperitoneally on day 10 of tumor development. 5-Fluorouracyl (5-FU) was intramuscularly injected to mice with LLC in a single dose of 80 mg/kg on day 14 after transplantation.

The treatment efficiency was evaluated by changes in the weight of the primary tumor node, incidence of lymphogenic and hematogenic metastases, their number, area, and metastasis inhibition index [1,8]. Functional activity of lymph node cells was evaluated by tumor cell neutralization test proposed by H. J. Winn [16]. Serum lipids in rats were measured by the phosphovanillin method using commercial kits (Pliva-Lachema a.s.) [6].

After the experiment the animals were sacrificed by cervical dislocation or ether overdosage with consideration for Regulations for Manipulations with Experimental Animals, approved by the Ministry of Health of the Russian Federation. The significance of results was evaluated using Wilcoxon—Mann—Whitney test and Fisher exact test [4].

RESULTS

By the results of screening of the effects of Siberian plant WSPS on the development of LLC in mice and the efficiency of cytostatic therapy the substances were tentatively divided into 4 groups (Table 1):

1) producing more pronounced inhibitory effect on the growth of primarily tumor node (*Rhaponticum carthamoides*, *Taraxacum officinale*, *Plantago major*);

2) predominantly modulating the metastatic process (*Rhodiola rosea* and *Glycyrrhiza*);

3) inhibiting the growth of primary tumor node and reducing the process of its dissemination into the lungs (*Acorus calamus*, *Tussilago farfara*, *Tilia cordatum*, *Ledum palustre*, and *Echinacea purpura*);

4) inert towards LLC (*Calendula officinalis*).

Acorus calamus PS proved to be the most perspective of the above substances as the basis for drugs for adjuvant therapy of malignant tumors. Injection of *Acorus calamus* PS in combination with CP 1.3 times reduced of the weight of primary node of LLC ($p<0.01$), the incidence of metastases (from 100 to 70%), decreased their number (3.1 times; $p<0.05$), area of metastatic involvement (13.1 times; $p<0.05$) in comparison with these parameters in animals treated with CP alone. The metastasis inhibition index in this group was maximum (90%).

Flagroot (*Acorus calamus*) is used as a local antiseptic, expectorant, tonic, sedative, anticonvulsant, antipyretic, soporific, analgesic, spasmolytic, and antitumor agent as monotherapy or a component of medicinal plant compositions in popular medicine of many countries. The officinal raw material is included in the Russian Pharmacopoeia (versions 1-11) and in Pharmacopoeias of many countries of the world. Decoction from the rhizome is used in modern medical practice as aromatic bitters improving the appetite and digestion [10]. The data of experimental studies of extracts from *Acorus calamus* rhizome and its bioactive substances in oncology are scanty and fragmented.

We studied the effect of *Acorus calamus* WSPS on the efficiency of 5-FU antimetabolite, growth and metastases of B-16 tumor in mice and evaluated activity of *Acorus calamus* WSPS administered via different routes on rats with Walker 256 carcinosarcoma.

Monotherapy with 5-FU decreased the tumor weight in mice with LLC by only 14% ($p<0.01$) in comparison with the control group. Addition of sweetflag PS to the cytostatic treatment protocol increased the percentage of tumor inhibition to 29% ($p<0.05$) in comparison with mice treated with 5-FU alone.

Experiments with B-16 melanoma showed that combination of *Acorus calamus* rhizome WSPS with CP produced more pronounced antimetastatic effect than CP alone: the area of metastases in mice receiving combined treatment with *Acorus calamus* WSPS in doses of 5, 10, 25, and 50 mg/kg decreased 1.3, 1.2, 2.6, and 2.0 times in comparison with the group receiving cytostatic monotherapy. Moreover, WSPS from *Acorus calamus* rhizome exhibited intrinsic antimetastatic effect towards this tumor. The treatment significantly decreased the number of metastatic colonies (3.9 times, $p<0.01$) and their area in the lungs of animals with B-16 melanoma ($p<0.05$). The metastasis inhibition index in tumor-bearing mice treated with *Acorus calamus* WSPS increased in a dose-dependent manner and was 82% at WSPS dose of 10 mg/kg, 83% at a dose of 25 mg/kg, and 94% at a dose of 50 mg/kg.

Intraperitoneal (25 mg/kg) and oral (75 mg/kg) treatment with WSPS from *Acorus calamus* potentiated the therapeutic effect of CP in male Wistar rats with Walker 256 carcinosarcoma. The weight of the lymph nodes in animals treated with PS (25 mg/kg) in combination with CP decreased in comparison with animals treated with CP alone: the axillary nodes decreased 1.2 times ($p<0.01$). The number of metastases and area of metastatic involvement of the lungs tended to decrease 2- and 5-fold, respectively, the number of metastases in the lymph nodes and their weight tended to decrease by 1.8 and 1.3 times, respectively, compared to the corresponding parameters in animals injected with CP alone. Combination of cytostatic therapy with a course of oral *Acorus calamus* WSPS (75 mg/kg) decreased the number and weight of lymphogenic metastases by 7.0 and 2.5 times, respectively ($p<0.05$); the number and area of hematogenic metastases in

the lungs of rats receiving combined therapy tended to decrease (by 3.1 and 1.7 times, respectively) in comparison with animals receiving CP monotherapy. The incidence of lymphogenic metastases was 20%, which was significantly lower than in animals treated with CP alone (75%). Total number of animals with metastases disseminating in the lymph and blood system decreased to 50% after combined therapy with CP and *Acorus calamus* WSPS (75 mg/kg) *vs.* 78% in the CP monotherapy group ($p<0.001$).

Acorus calamus WSPS in a dose of 25 mg/kg exhibited an independent antitumor effect, while in a dose of 75 mg/kg it inhibited dissemination of Walker 256 carcinosarcoma in rats, the incidence of lymphogenic metastases was 13% (*vs.* 67% in the control; $p<0.001$).

Hence, the results of experiments suggest the possibility of using *Acorus calamus* WSPS in combination with alkylating agents and with antimetabolites for potentiation of their antitumor effect. Different animal species (mice, rats) with tumors differing by growth rate, intensity and type of metastatic involvement (LLC, B-16 melanoma, Walker 256 carcinosarcoma) were sensitive to these bioactive substances.

Polysaccharides are the main structural components of cell membranes and energy material of any living cell from bacteria, protozoa, fungi, and plants to higher mammals. The interrelationships between the host and exogenous polymer of this class are, consequently, different. Recent studies proved that the main route of realization of biological activity of PS of different origin, including plant PS, is modulation of the immune response [12], which was shown in Winn's tumor cell neutralization test. One of the mechanisms of action of *Acorus calamus* WSPS used alone and in combination with CP providing an additional destructive effect on the tumor is activation of the lymph node

TABLE 1. Effects of WSPS (10 mg/kg) from Siberian Plants on the Development of LLC in C57Bl/6 Mice and Efficiency of CP Treatment

WSPS	Tumor weight	Incidence of metastases	Number of metastases	Area of metastases
Ledum palustre				↓; $p_1<0.05$
+CP	↓; $p_2<0.05$	↓	↓	↓
Tilia cordatum	—	—	—	—
+CP	↓; $p_2<0.01$	↓; $p_1<0.001$		
Glycyrrhiza glabra			↓; $p_1<0.05$	↓
+CP	↓			↑; $p_2<0.05$
Rhodiola rosea	—	—	—	—
+CP		↓; $p_2<0.001$		
Taraxacum officinale	—	—	—	—
+CP	↓; $p_2<0.05$		↓	↓
Plantago major	—	—	—	—
+CP	↓; $p_2<0.05$			
Rhaponticum carthamoides	—	—	—	—
+CP	↓; $p_2<0.05$			
Tussilago farfara	—	—	—	—
+CP	↓; $p_2<0.05$	↓; $p_2<0.001$	↓	↓
Acorus calamus	↓; $p_1<0.01$	—	—	—
+CP	↓; $p_2<0.01$	↓; $p_1<0.001$	↓; $p_2<0.05$	↓; $p_2<0.05$
Calendula officinalis	—	—	—	—
+CP	—	—	—	—
Echinacea purpura	—	—	—	—
+CP	↓; $p_2<0.01$			↓; $p_2<0.05$

Note. ↓ inhibitory effect; ↑ stimulatory effect. p_1 significant difference compared to untreated animals; p_2 significant difference compared to mice treated with CP alone.

cells; 70% of these cells are T lymphocytes involved in the development of specific antitumor immune response. CP reduced functional activity of the tested cells: the weight of the tumor node in mice transplanted lymph node cells from CP-treated animals with LLC was significantly (by 17%) higher than in mice transplanted a mixture of lymphoid and tumor cells from untreated mice. *Acorus calamus* WSPS injected in parallel with CP reduced its toxic effect on the immune system: after transplantation of the lymph node cells from mice treated with PS and CP together with LLC cells the resultant tumor weight was significantly lower (1.6 times; $p<0.01$), similarly as the area of metastatic involvement (3.3 times; $p<0.01$) in comparison with the respective control.

The development of a malignant tumor causes deep disorientation of homeostasis in the organism, neuroendocrine, hematological, vascular, biochemical shifts, and changes in carbohydrate, lipid, protein, and water-salt balance. Mobilization of lipids from fatty tissue, decreased utilization of alimentary triglycerides, and more intensive oxidation of free fatty acids underlie the disorders in lipid metabolism, paralleled by reduction of resistance to the tumor process [14]. Tumor development leads to an increase in serum levels of triglycerides and cholesterol, plastic elements for cell growth and division [17]. A correlation between high content of lipids in the blood of patients with malignant tumors and intensity of metastases in the lymph nodes was detected [13]. Hyperlipidemia is paralleled by suppression of immune reactivity and has a favorable impact on the neoangiogenesis process, while decreased blood concentration of lipids deteriorates the conditions for the development of new vessels [15]. PS is characterized by a hypolipidemic effect: pectins reduce the level of lipid at the expense of adsorption in the intestinal lumen, fructooligosaccharides inhibit *de novo* synthesis of fatty acids in the liver [3]. The level of serum lipids in rats with tumors during a course of intraperitoneal and oral treatment with *Acorus calamus* WSPS decreased 2.1 times ($p<0.05$) in comparison with the control. Single injection of CP led to a 1.7 times reduction of this parameter ($p<0.01$) in comparison with untreated rats. Addition of *Acorus calamus* PS in a dose of 25 mg/kg intraperitoneally to the cytostatic therapy protocol amplified the hypolipidemic effect, which manifested by a reduction of serum lipid concentration (1.7 times) in comparison with animals receiving CP monotherapy. A correlation between tumor weight and total serum lipid level was detected in untreated animals and in rats with Walker 256 carcinosarcoma after intraperitoneal course of *Acorus calamus* WSPS ($r=0.94$).

Hence, a principal possibility of using plant PS in combined therapy of cancer is proven, which suggests these compounds as candidates for creation of drugs for chemotherapy correction. New data on the pharmacological characteristics and mechanisms of *Acorus calamus* PS effect will in future extend the spectrum of this plant utilization.

REFERENCES

1. S. A. Arkhipov and V. M. Yunker, *Study of Tumor Induction and Metastases in Experimental Animals* [in Russian], Novosibirsk (1984), pp. 14-32.
2. N. N. Besednova, L. A. Ivanushko, T. N. Zvyagintseva, *et al.*, *Antibiot. Khimioter.*, No. 2, 37-44 (2000).
3. T. A. Vinogradova, B. N. Gazhev, V. M. Vinogradova, and V. K. Martynov, *Practical Phytotherapy* [in Russian], Moscow (2001).
4. E. V. Gubler, *Computation Methods for Analysis and Recognition of Pathological Processes* [in Russian], Leningrad (1978).
5. G. V. Yevtushenko and V. S. Svintsitskii, *Ukr. Khimioterapevtich. Zh.*, No. 4, 32-36 (2000).
6. V. S. Kamyshnikov, *Handbook of Clinical Biochemical Laboratory Diagnosis* [in Russian], in 2 vol., Minsk (2000), Vol. 2.
7. E. B. Lazareva and D. D. Men'shikov, *Antibiot. Khimioter.*, **44**, No. 2, 37-40 (1999).
8. L. F. Larionov, *Chemotherapy of Malignant Tumors* [in Russian], Moscow (1962).
9. T. G. Razina, E. P. Zueva, E. N. Amosova, *et al.*, *Byull. Eksp. Biol. Med.*, Suppl. 1, 35-41 (2005).
10. *Plant Resources of Russia and Adjacent Countries. Flowering Plants, Their Chemical Composition, and Use: Butomaceae-Typhaceae* [in Russian], St. Petersburg (1994), pp. 144-147.
11. Z. P. Sofyiana, A. B. Syrkin, A. Goldin, and A. Klein, *Experimental Evaluation of Antitumor Drugs in the USSR and USA* [in Russian], Moscow (1980).
12. C. Iguchi, Y. Nio, H. Takeda, *et al.*, *Anticancer Res.*, **21**, No. 2A, 1007-1013 (2001).
13. A. Sako, J. Kitayama, S. Kaisaki, and H. Nagawa, *Cancer Lett.*, **208**, No. 1, 43-49 (2004).
14. G. F. Torelli, A. Cascino, M. Muscaritoli, *et al.*, *Minerva Gastroenterol. Dietol.*, **43**, No. 4, 183-188 (1997).
15. E. Wiley and P. Meclain, *Nutr. Res.*, No. 8, 265-273 (1988).
16. H. J. Winn, *J. Immunol.*, **86**, No. 2, 228-239 (1961).
17. L. Wuermli, M. Joerger, S. Henz, *et al.*, *Prostate Cancer Prostatic Dis.*, **8**, No. 4, 316-30 (2005).