ONCOLOGY

Phytoadaptogen for Preventive Oncology: Immunobiological Criteria of Composition

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A system of *in vitro* immunobiological tests is developed for screening of phytopreparations intended for the use as immunomodulators in oncology. Proliferative activity of human tumor cells decreased after treatment with complex phytoadaptogen. The immunomodulatory effect of this phytoadaptogen on immunocompetent cells of cancer patients and its nonspecific interferonogenic effect were detected. The composition of adaptogenic complex for preventive oncology is determined.

Key Words: *phytoadaptogen; antiproliferative activity; immunomodulating effect*

Low efficiency of modern means for cancer control (surgery, chemotherapy, radiotherapy, and immunotherapy) determines limited possibilities of the armory of modern medicine. The general opinion now is that cancer is easier to prevent than to cure. In addition, it is now possible to carry out preventive treatment at different stages of tumor development: blastomogenesis, metastases, and relapses of tumors after surgery. Preventive measures can be aimed at regulation of the stress syndrome caused by modern methods for treating tumor diseases, at improvement of the quality of life and its prolongation after specific antitumor treatment [1]. We regard preventive oncology not only as prevention of tumors in risk groups, but also as prevention of complications of chemoradiotherapy and of metastases and relapses after specific treatment of cancer patients [2].

The results of experimental and clinical studies [3-5,8] were not introduced into wide clinical practice because the treatment with some adaptogens is to be paralleled by involved immunological control of individual sensitivity to monoadaptogens. That is why complex plant preparations, containing several adaptogens, and plants possessing combinations of protective and other regulating effects attract special attention [1,2].

This paper propose an experimental approach to the creation of a system for selection of adaptogenic drugs based on evaluation of the efficiency of their suppression of tumor cell proliferation and on characterization of the immunomodulating (including interferon-inducing) effects of phytopreparations.

MATERIALS AND METHODS

The development of complex plant preparation included three *in vitro* model systems.

The effects of individual plant extracts and their compositions on tumor cell growth were evaluated by the radiometric method. The antiproliferative effect was characterized using CE_{50} parameter (concentration of preparation causing >50% growth inhibition in cell culture).

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The effect of phytocompositions on immunological phenotype of lymphocytes in cancer patients was evaluated by the indirect immunofluorescence test. Whole blood was incubated with phytopreparations for 1 h.

Interferonogenic activity of lymphocytes under the effect of phytopreparations was evaluated by the biological method. The study included measurements of spontaneous IFN, comparison of the effects of different IFN preparations, their inductors, and plant complexes on the lymphocyte interferonogenesis *in vitro*, and evaluation of the number of patients responding by IFN production to the treatment with the above agents.

TABLE 1. PM Compositions in Volume Percents

PM No.	Percent content of group in a variant		
	A	B	C
1	5	25	70
2	25	5	70
3	33.3	33.3	33.3
4	5	5	90
5	5	70	25
6	70	5	25
7	15	15	70

RESULTS

The first stage of the study included screening of 46 plant extracts for their individual effects on the proliferative activity of cultured CaOv human ovarian carcinoma cells. An inverse correlation between preparation concentration in the incubation medium and ^{3}H-thymidine incorporation into tumor cell DNA was detected for 36 extracts: *Papaveraceae*, *Hypericaceae* grass extracts and *Crassulaceae*, *Araliaceae*, *etc.* root extracts. For 10 extracts (*Asteraceae* root extracts, *Plantaginaceae* leaf extracts, *etc.*) changes in the concentration of preparations had no effect on DNA synthesis in cultured CaOv cell.

Two types of effects of the test agents on CaOv culture were detected: inhibition of cell growth by 6 extracts (moderate antiproliferative effect at CE_{50}=70-100 μg/ml) and no effect for other extracts (CE_{50}>100 μg/ml).

Seven phytomixtures (PM) were composed of 40 extracts selected in the previous study. All ingredients were divided arbitrarily into 3 groups (Table 1). Group A included extracts from plants with adaptogenic effects (*Araliaceae*, *Crassulaceae*, *Compositae*, *etc.* roots and rhizomes), group B included plants serving as vitamin sources (*Rosaceae*, *Vacciniaceae*, *Saxifragaceae*, *etc.* fruits), and group C included plants with diuretic, cholagogue, and sedative effects (*Papaveraceae*, *Lamiaceae* grass, *Myrtaceae*, *Ericaceae* leaves, *etc.*).

The compositions were analyzed using the same test system.

PM 1, 2, 3, and 7 significantly decreased incorporation of ^{3}H-thymidine into tumor cell DNA. The maximum suppression of cell culture proliferation was observed with variants 1 and 7 (Fig. 1).

In order to select the most effective composition, the changes in the immunological phenotype of peripheral blood lymphocytes from cancer patients (n=31) under the effects of compositions 1 and 7 were studied *in vitro*. The incubation of lymphocytes with PM for 1-h did not impair expression of cell surface antigens. Moreover, positive changes in immunological values were observed (Table 2): increase in the helper/suppressor ratio (CD4/CD8), improvement of natural re-

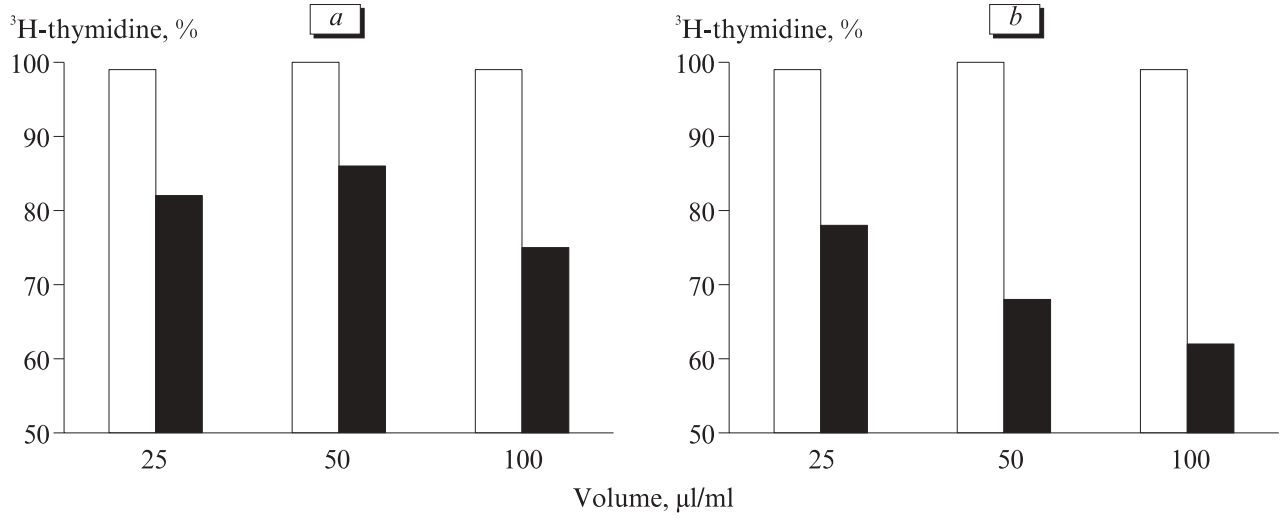

Fig. 1. Relationship between ^{3}H-thymidine incorporation in DNA of CaOv cell culture and phytomixture compositions 1 (*a*) and 7 (*b*) in incubation medium. Light bars: control; dark bars: phytomixture 1 (*a*), phytomixture 7 (*b*).

TABLE 2. Effects of PM 1 and PM 7 on Expression of Differentiation Antigens on Lymphocytes from Cancer Patients *In Vitro* (*M*±*m*)

Parameter	Normal level, %	Without incubation and ethanol	1-h incubation			
			without ethanol	with ethanol	with PM 1	with PM 7
CD3	60-75	59.4±2.2	58.6±1.9	58.7±2.3	64.2±2.2**	65.6±2.1*
CD4	35-46	32.0±1.5	34.0±1.9	34.7±1.9	40.2±2.0**	38.9±1.8**
CD8	25-30	25.3±1.7	24.7±1.8	23.2±1.6	24.3±1.2	22.2±1.3
CD20	5-15	6.1±0.5	6.0±0.8	6.1±0.7	7.7±0.7***	9.2±0.6*
CD16	10-20	12.1±0.9	10.5±1.1	11.5±1.1	13.9±1.1**	17.8±1.4
CD11b	15-20	17.8±1.2	17.6±1.3	15.9±1.0	17.9±1.4***	19.5±1.3*
CD18	56-64	55.9±1.9	56.6±2.1	58.5±2.9	60±2	62.0±1.9***
CD4\CD8	1.5-1.9	1.47±0.13	1.38±0.15	1.63±0.12	1.77±0.12	1.93±0.15*

Note. *p<0.01, **p<0.01, ***p<0.05 compared to parameters of immune status after lymphocyte incubation with ethanol.

sistance (CD16, CD11b) and B-cell immunity (CD20). It is noteworthy that PM did not modulate the number of suppressor cells. PM promoted the increase in the percentage of $CD11b^+$ and $CD18^+$ cells, which attested to proinflammatory stimulation and, hence, improved immunoreactivity of oncological patient [6,7]. The effects of PM 7 were more pronounced for all parameters.

The study of the effects of PM 1 and PM 7 on interferonogenesis in blood cells from cancer patients *in vitro* in comparison with known IFN inductors showed that IFN titers increased approximately 15-fold under the effects of IFN inductors, while PM increased the content of endogenous IFN about 30-fold. The mean titers of IFN induced by PM 7 were higher than those induced by PM 1.

Studies in patients whose blood cells responded by IFN production to injection of IFN-containing agents showed that 18% patients were sensitive to reaferon, 16.4% to realdirone, 19.7% to human leukocytic interferon (HLI), and 24.6% to Intron A; the sensitivity to Roferon A and gamma-feron was higher (31.1 and 40.9%, respectively). On the other hand, the sensitivity to inductors was higher: 64.6% to ridostine, 66 to neovir, 71.7 to cycloferon, and 73% to amixine. Injections PM 1 and PM 7 resulted in IFN production in 100% cases, which indicated the absence of individual sensitivity of patients to the compositions. As PM 1 and PM 7 are not IFN-containing preparations, they can be classified as nonspecific inductors of IFN.

TABLE 3. Drug-Induced Interferonogenesis (*M*±*m*)

Agent	IFN titer, U/ml
Spontaneously produced IFN	0.17±0.08
Ridostine	2.70±0.16
Amixine	3.11±0.20
Neovir	2.98±0.14
Cycloferon	3.04±0.13
PM 1	5.29±0.29
PM 7	5.50±0.29

Note. The values are presented as $\log_2$ (titer^{-1}).

Inductor agents and IFN-containing drugs stimulated lymphocyte interferonogenesis reaching the maximum of 71.7% for cycloferon and 73.1 for amixine (Table 3). Blood cells from patients produced IFN in 100% cases in response to PM 1 and PM 7, which indicates the absence of individual sensitivity of patients to the compositions. Since PM 1 and PM 7 are not IFN-containing preparations, they can be classified as nonspecific inductors of IFN.

PM 7 was chosen as the most promising composition for the base of Phytomix-40 preparation.

Hence, our study determined the system of immunobiological tests for screening of adaptogenic preparations intended for the use as nonspecific immunomodulators in oncology. The system includes *in vitro* study of the drug effect on proliferative activity of human tumor cells *in vitro*, on immunological phenotype and interferonogenic activity of lymphocytes of patients with tumors of different location (stages III-IV). Complex PM decreased proliferative activity of human ovarian carcinoma cell culture. Immunomodulating effect of PM is proven. The detected sensitivity of blood cells of all patients with complex PM indicates the absence of resistance to this preparation in all patients.

REFERENCES

1. E. D. Gol'dberg and E. P. Zueva, *Plant Preparations in Combined Therapy of Malignant Tumors* [in Russian], Tomsk (2000).

2. K. V. Yaremenko, *Adaptogens as Preventive Medicine Means* [in Russian], Tomsk (1990).
3. K. V. Yaremenko and V. G. Pashinskii, *Malignant Tumors: Treatment and Drug Prevention* [in Russian], St. Petersburg (2003).
4. V. Bespalov, V. Alexandrov, A. Limarenko, *et al.*, *J. Korean Med. Sci.*, Suppl. 16, S42-S53 (2001).
5. R. D. Medenica, S. Mukerjee, and T. Huschart, *Proc. Annu. Meet. Amer. Ass. Cancer Res.*, No. 35, 2865 (1994).
6. Y. Okamoto, T. Tsurunaga, M. Ueda, *et al.*, *Nippon Sanka Fujinka Gakkai Zasshi*, **45**, No. 6, 566-570 (1993).
7. A. Pierres, A. M. Benoliel, and P. Bongrand, *Rev. Med. Int.*, **20**, No. 12, 1099-1113 (1999).
8. Y. Taik-Koo, C. Soo-yong, L. Yun-Sil, *et al.*, *Functional Foods for Disease*, Washington (1997), pp. 162-176.