ESTIMATION OF PROPHYLACTIC AND IMMUNOSTIMULATING EFFECTS OF ELEUTHEROCOCCUS AND SCHIZANDRA CHINENSIS PREPARATIONS

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Protection against influenza is at present and will be in near future based on prophylactic vaccination, the efficiency of the measure depending upon preparation qualities and application conditions. While the quality of vaccines is determined by characteristics of influenza viruses and by technology of vaccine manufacturing, application conditions can vary. In particular, theoretically substantiated proposals have been advanced for the use of some synthetic and herbal drugs, e.g. extracts of certain far-eastern plants (Eleutherococcus senticosus Maxim. and Schizandra chinensis Baill), for stimulating post-vaccination immunity. There are literature data on the use of Eleutherococcus for prophylaxis of influenza and other acute respiratory diseases (ARD) (Brekhman, 1976; Barkan, 1978; Barkan et al., 1980; Primyagi, 1983). Several authors (Brekhman, 1976; Dardymov, 1976) associate the effect with the ability of Eleutherococcus to activate biosynthesis of proteins, in particular, to enhance formation of gamma-interferon (Wacker, 1978), thus providing a wide range of protective effects of the plant adaptogens mentioned above.

Considering the literature data, we studied the action of Schizandra chinensis upon immunogenesis after the introduction of live or inactivated influenza vaccines and evaluated the prophylactic effect of Eleutherococcus extract (EE) during influenza epidemics.

Materials and Methods

The Schizandra chinensis extract produced by Khabarovsk pharmaceutical plant was administered in a dose of 30 drops twice a day. The fluid EE, product of "Sanitas" (USSR), was used in a dose of 2 ml once a day. Both preparations were added to sweet-

ened drink, usually to tea.

Schizandra chinensis extract (SCE) was tested as an immunostimulator on 292 male students aged 18-24 years, in two collectives with equal labour, living and diet conditions (Vladivostok marine schools). Both collectives were preliminarily divided into two equal test groups, a unit of sampling was a school group. In the fall of 1981, all students of both collectives were vaccinated with commercial influenza preparations, one group with live intranasal monovaccine A(H1N1), the other with inactivated divaccine A(H1N1)+A(H3N2). Seven days before and 14 days post vaccination, each member of the test and the control groups received SCE or placebo (tea). It must be noted that SCE has a specific smell which changed the taste of the offered tea and several persons refused to drink it.

Three blood samples were taken at random from 100 members of each group: prior to vaccination, 3 weeks post vaccination and upon the cessation of the epidemic. Using the standard methods, blood sera were examined in hemagglutination-inhibition reaction with antigens of vaccinal strains of the influenza virus.

When analysing the frequencies of seroconversion to vaccinal viruses in both groups, standard indices were used with respect to the initial titer of antibodies (Zhovnirenko, 1981).

The prophylactic effect of EE was studied in Leningrad, again in two collectives of marine schools, the total number of persons under observation being 1376. From the beginning of and during the whole course of influenza epidemic in the city, both collectives, divided into two equal groups, received coded preparations: either EE or placebo, a solution of burnt sugar. The drinks looked and tasted alike.

In both studies (in Leningrad and Vladivostok), the number of influenza and ARD cases and the occurrence of most frequent complications (pneumonia, bronchitis, otitis and maxillary sinusitis) were carefully registered.

Statistical processing of results was made according to A.M. Markov (1963).

Results and Discussion

Upon vaccination with live intranasal vaccine, seroconversion to vaccinal virus A(Khabarovsk)1/77 (H1N1) was recorded in 66.3% of vaccinated students receiving SCE and in 61.4 % of vaccinated

students of the control group, where
characteristics). Introduction of ina
+A(H3N2) was accompanied by an increa
vaccinal viruses in 46.6 - 53.7 % of
ing SCE and in 57.6 - 48.1 % of vacci
Thus, administration of SCE during th
significantly the frequency of seroco
ses in humans vaccinated with live an
vaccines. The groups did not differ e
antibodies upon vaccine or the averag
the titer of antibodies in vaccinated
fect of SCE was found.

Frequencies of influenza and ARD c
SCE and EE did not differ from those
No difference was registered either d
preparations or during the following
ried.

Different results were obtained fo
during the analysis of frequencies of
cations. Pneumonia, bronchitis, maxil
were recorded in 3.7 cases per 100 me
in 7.8 cases per 100 members of the c
differences were statistically insigni

The occurence of these complicatio
was also lower (1.5 cases per 100 per
(3.2); the difference was found to be
($P<0.05$).

Thus, both preparations - Schizand
coccus sphaericus extracts - showed t
ased the occurrence of cases which of
and ARD (pneumonia, otitis, bronchiti
For EE this effect was statistically
show an immunostimulating effect in t

EE has an undoubted and important
not change the taste of drinks.

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of EE is need[illegible].

Table

Morbidity rate per 100 persons of test and control groups

Observation period	Diagnosis and group			
	Influenza and ARD		Pneumonia, bronchitis, maxillary sinusitis, otitis	
	Test	Control	Test	Control
Administration of *Schizandra chinensis*	5.3	5.8	1.1	0
Influenza epidemic	19.6	22.3	2.1	4.9
Post epidemic	13.8	15.5	0.8	2.9
Total	38.7	43.6	3.7	7.8
Prior to administration	3.5	4.8	0.2	0
Influenza epidemic + *Eleutherococcus senticosus* administration	18.6	15.7	1.1	1.8
Post epidemic	9.6	6.9	0.2*	1.4
Total	31.7	27.4	1.5*	3.2

*$P<0.05$

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INCREASED IMMUNOLOGIC REACTIVITY OF LYMPHOCYTES IN ONCOLOGIC PATIENTS TREATED WITH ELEUTHEROCOCCUS EXTRACT

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In the last decade, a number of studies have shown more or less decreased immunologic reactivity in almost all patients suffering from malignant tumors. In this connection, particular attention is paid to the use of various methods of immunotherapy and immunocorrection for complex treatment of malignant tumors. For this purpose, various immunostimulating preparations are prescribed. Studies on the immunostimulating ability of natural adaptogens and their potential use in clinical practice are now of great interest. Eleutherococcus extract (EE) is one of such promising preparations. Extensive research in the field of pharmacological action of natural biologically active substances, primarily of plant origin, in association with investigation of mechanisms of their action and the administration of such drugs for pharmacocorrection has been carried out by Prof. I.I. Brekhman.

Publications by Prof. I.I. Brekhman and his followers have shown the efficiency of EE as an agent that lowers toxicity and enhances the anti-tumor and anti-metastatic activities of some cytostatics used in conventional treatment. The immunostimulating ability of Eleutherococcus has not yet been studied.

Materials and Methods

Lyophylized EE was dissolved in medium 199 to concentrations of 0.01; 0.10 and 1.00 mg/ml. EE effect was examined in peripheral blood lymphocytes in 58 individuals (24 donors, 12 patients with skin melanoma, 10 patients with stomach carcinoma and 12 patients with mammary gland carcinoma). All tests were repeated in triplets. Lymphocytes were isolated from heparinized blood in the density gradient Lymphoprep 1077. Lymphocyte samples ($2 \cdot 10^6$ cells/ml) were incubated in medium 199 with aqueous solution of EE at 37° C for 30 min. The control was prepared in the same conditions, without adding the EE. Immunologic tests included determination of T-lymphocytes by the method of spontaneous "active" and total rosette-formation (TRa- and TRt-RFC, respectively). B-

lymphocytes were estimated by complementary rosette-formation (EAC-B-RFC). Taking into account the literature data on interferon-inducing activity of Eleutherococcus in mice (Wacker, Eilmes, 1978; Wacker, 1983), we have studied the ability of Eleutherococcus preparations to induce production of interferon by lymphocytes obtained from healthy humans. To this end, lymphocyte samples ($1 \cdot 10^6$) were incubated with Eleutherococcus preparation at the concentrations given above at 37° C for 72 hrs. After incubation, the samples were centrifuged and the supernatant was assayed for interferon.

Results

The results of immunologic tests in donors and in patients prior to Eleutherococcus administration are given in Table 1; a significant decrease in the value of TRa and TRt rosette-formation was recorded for all patients with stomach and mammary gland carcinoma and with skin melanoma.

Table 1

Immunologic reactivity indices for donors and patients with different forms of malignant tumors

Diagnosis, number of individuals examined	Immunologic indices RFC in %		
	TRa	TRt	EAC-B-rosette
Donors	50.2 ± 1.8	60.4 ± 1.3	16.4 ± 1.2
24	28 ÷ 71	46 ÷ 77	4 ÷ 32
Melanoma	43.1 ± 4.1	53.0 ± 2.5*	11.8 ± 4.1
12	19 ÷ 69	44 ÷ 74	1 ÷ 49
Stomach carcinoma	32.2 ± 2.2*	42.0 ± 3.2*	18.0 ± 1.9
10	25 ÷ 47	38 ÷ 74	11 ÷ 30
Mammary gland carcinoma	41.7 ± 3.4*	53.0 ± 3.6*	15.7 ± 1.8
12	26 ÷ 68	38 ÷ 74	8 ÷ 26

*Here and below $P < 0.05$

EE significantly enhanced the number of TEa-RFC lymphocytes in donors at all the concentrations studied. The values of TEt and EAC-B-RFC had the trend to increase (Table 2).

Table 2

Immunologic indices prior to and upon the administration of EE in various concentrations

Concentration, mg/ml	Immunologic indices, RFC in %		
	TEa	TEt	EAC-B-rosette
0.01	58.0 ± 1.3*	62.2 ± 1.5	19.9 ± 1.6
	42 ÷ 73	46 ÷ 83	3 ÷ 49
0.10	55.4 ± 2.1*	64.0 ± 1.0	18.9 ± 1.4
	26 ÷ 76	53 ÷ 79	1.7 ÷ 36
1.00	57.7 ± 1.6*	61.3 ± 1.2	18.1 ± 1.2
	40 ÷ 78	40 ÷ 77	2 ÷ 30
Background	50.2 ± 1.8	60.4 ± 1.3	18.4 ± 1.2
	28 ÷ 71	46 ÷ 77	4 ÷ 32

Table 3 shows an increase of immunologic indices in donors and in patients after administration of Kleutherococcus. The difference between the rates of increase of immunologic indices for donors and patients of different groups was not found to be statistically significant. However, almost all the immunological indices showed a higher increase in patients than in donors (Table 3).

This evidence was used as a basis for studying the immunostimulating ability of Kleutherococcus. To estimate an optimal dose of EE, we have conducted a study in vitro of the preparation in concentrations of 0.01, 0.1 and 1.0 mg/ml. The results were statistically processed using the number of individuals with the maximal increase of immunological indices in response to a studied EE concentration (Table 4).

It was found that 50 % donors had a maximal increase of TEa-RFC at EE concentration of 0.01 mg/ml. An increase of the concentration from 0.01 to 1.0 mg/ml resulted in a fall of the number of donors with the maximal response of this index (21 and

Table 3

Immunologic indices in patients and donors after administration of Kleutherococcus in vitro

Diagnosis, number of individuals examined	Immunologic indices, RFC in %		
	TEa	TEt	EAC-B-rosette
Donors, 24	29.9 ± 3.4	11.0 ± 1.2	25.5 ± 2.8
	5 ÷ 38	1 ÷ 20	1 ÷ 17
Melanoma, 12	39.3 ± 5.6	8.1 ± 2.3	72.7 ± 24.4
	7 ÷ 69	1 ÷ 28	6 ÷ 250
Stomach carcinoma, 10	44.1 ± 6.7	22.2 ± 4.4	32.3 ± 7.7
	1 ÷ 67	2 ÷ 46	5 ÷ 81
Mammary gland carcinoma, 12	28.7 ± 6.1	20.1 ± 4.8	36.3 ± 7.2
	1 ÷ 68	1 ÷ 53	1 ÷ 65

Table 4

Percentage of persons with a considerable increase of immunological indices in response to administration of Kleutherococcus in different concentrations and of persons insensitive to Kleutherococcus

Diagnosis	Kleutherococcus concentration, mg/ml									No effect		
	0.01			0.10			1.00					
	T-act.	T-tot.	B-comp.	T-act.	T-tot.	B-comp.	T-act.	T-tot.	B-comp.	T-act.	T-tot.	B-comp.
Donors	50	33	29	21	37	17	29	29	21	0	1	33
Melanoma	33	33	58	17	8	33	50	33	8	0	26	1
Stomach carcinoma	20	20	20	40	30	20	30	50	60	10	0	0
Mammary gland carcinoma	18	27	27	18	27	18	54	36	36	10	10	19

29 %). Accordingly, the TEa-RFC is an only reliable index for detecting the stimulation; its maximal value was recorded at EE concentration of 0.01 mg/ml. Thus, minimal EE concentrations can be recommended for healthy people. A group of patients suffering from skin melanoma had a lower percentage of persons sensitive to minimal doses of EE (33 % against 50 % in donors). The values of EAC-RFC proved to be most sensitive to small EE concentrations (58 % in comparison to 29 % in the control). An increase of EE concentration up to 0.1 and 1.0 mg/ml resulted in a fall of the percentage of persons with a minimal immunological response. Therefore, we can conclude that maximal EE concentrations can be recommended for skin melanoma patients.

Stomach carcinoma patients should be treated with medium and high EE concentrations (0.1 and 1.0 mg/ml), which induced stimulation of immunological indices: TEa in 30-40 %, TEt in 30-50 %, and EAC-E-RFC in 20-60 % patients.

For mammary gland carcinoma patients, a high EE concentration of 1.0 mg/ml appeared to be effective. It induced maximal immunological response in 54 % patients for TEa and in 36 % for TEt and EAC-E-RFC.

Therefore, it was found that the donors are more sensitive to EE in low concentrations, while melanoma, stomach carcinoma and mammary gland carcinoma patients are more sensitive to medium and high concentrations. This evidence allows us to conclude that the EE affects human lymphocytes in vitro and stimulates immunological indices in healthy man and in patients. Accordingly, we believe that general biological effect of EE is realized through immunological mechanisms, and Eleutherococcus can be included into the group of preparations with a pronounced immunostimulating effect.

Study of EE revealed its ability to induce production of γ-interferon by human peripheral blood lymphocytes (Table 5).

Under similar conditions Eleutherococcus did not induce production of interferon by splenocytes of mice. At the same time, Eleutherococcus showed some protective action against the infection of mice with encephalomyocarditis virus.

There are interesting clinical observations on Eleutherococcus administration to mammary gland carcinoma patients. Immunologic indices in Eleutherococcus-treated patients did not change after

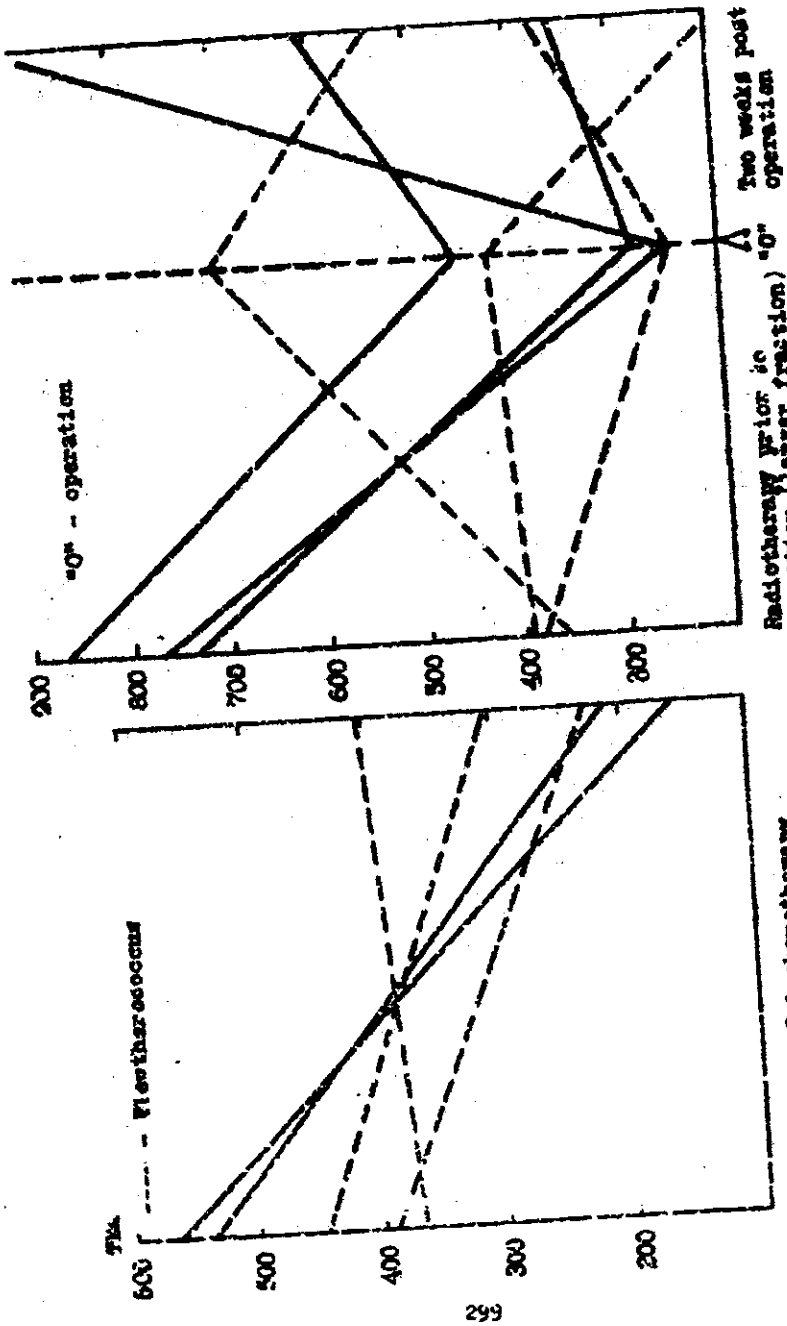

Fig. Changes in immunologic indices according to test for TEa-RFC in patients during the complex therapy of disseminated mammary gland carcinoma receiving Eleutherococcus extract.

0.0133 - 2	6(4 ÷ 8)
4	24(8 ÷ 64)

Note. Lymphocyte number in sample $1 \cdot 10^6$ cells/ml, after incubation for 72 hrs at 37° C.

radiotherapy or even somewhat improved. In contrast, in patients not given Eleutherococcus, immunologic indices sharply fell (c. Figure, left). In patients exposed to radiotherapy and to polychemotherapy, 2 trends were observed:

1. Patients not treated with Eleutherococcus could not endure more than one course of polychemotherapy, because of a sharp decline in the number of white blood cells. Immunologic indices in TEa also declined in absolute figures 2-4 times.

2. Patients treated with Eleutherococcus showed good tolerance to 2 courses of polychemotherapy. Leukogram of such patients did not suffer and immunologic indices changed slightly.

Thus, the studies on immunologic reactivity of oncologic patients treated with EE and the obtained results show a wide spectrum of immunostimulating activity (activation of TEa, TEt and EAC-B-RFC and induction of γ-interferon production).

Moreover, it is reasonable to use the preparation for treating patients exposed to radio- and chemotherapy.

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