Report on the study of immunomodulatory properties of drug Flaraxin.

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Goal of experimental study

Perspectives for wide use in oncological practice with therapeutic purpose the drug Flaraxin, and also application of it for the treatment of pathological conditions accompanied by appearance of secondary immunodeficiency (exposure to radionuclides, infectious factors, etc.) necessitated a more in-depth study of the immunomodulatory properties of Flaraxin.

The result in the report presents the study of different indicators of humoral and cellular immunity in cancer patients during appointment for therapeutic purposes the drug Flaraxin. Observations were made in patients with the following nosological forms of cancer: Lungs cancer (LC) 10 patients, Stomach cancer (SC) 9 patients, Mammary cancer (MC) 12 patients, Sigmoid and rectal cancer (SRC) 6 patients, Cancer of the female genital organs (CFGO) 10 patients.

In all patients, the cancer process was defined as stage III-IV of the disease. With aim of control, blood immunity indicators of 14 blood donors (practically healthy people aged 18-35) were studied for comparison with cancer patients.

Materials and methods

The object of the study was the blood of patients from which lymphocytes were extracted, and serum was also obtained. Lymphocytes were isolated from heparinized blood in a ficollurography density gradient(d=1,076 g/cm^3) and applied to further determination of their quantitative and functional characteristics. Blood serum was stored at t ° (- 4 °C) for further determination of humoral immunity factors in it.

The observed patients underwent a course of treatment with the use of the drug Flaraxin according to the scheme developed in the Cancer Curing Centre PHOENIX LLC: 16 infusions of Flaraxin were administered intravenously with a break of 7 days after 8 infusions (the drug was diluted in 20 ml of physiological solution NaCl 0,9% at the dose of 2 mg of Flaraxin per 1 kg of patient weight). Blood was collected prior to treatment and two weeks after the 16th infusion.

The determination of the quantity of immunocompetent cells in the blood was carried out by setting the rosette reaction with sheep erythrocytes in the following variants: total quantity of T-lymphocytes (E-RA Rosette Assay) (Jondal I. et al., 1972); the quantity of "active" T-lymphocytes (E_{act}-RA Rosette Assay) (Wybran, Fudenberg, 1973); the quantity of theophylline sensitive (suppressor) and theophylline resistant (helper) T-lymphocytes (E_{t4}-RA Rosette Assay) (Limanbul et. al., 1978). At the same time, quantitative determination of immune-competent cells subpopulations were carried out using a direct immune-fluorescent reaction with monoclonal antibodies of the “Becton Dickinson” company (USA). OCT – 4 - T-helpers / indicators; OCT-8 - T-suppressors / killers; OCT-11 is a common marker of T-lymphocytes and OCT-22 is a common marker of B-lymphocytes. Accordingly, the immunoregulatory index was determined - CD4 / CD8. The research results are summarized in table 1.

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Table 1. The content of immunocompetent cells in the blood of patients treated with Flaraxin.

Groups	E-RA	E_{act} - RA	E_{t4}-RA	E_{tr} - RA	E-0	OCT-11	OCT-4	OCT-8	OCT-22	CD_4/CD_8
Donors	60,3±2,1	38,9±22	45,6±1,2	19,1±0,9	23,4±1,8	57,1±2,1	42,9±2,4	24,6±1,9	27,4±1,2	1,8±0,1
Before treatment										
LC	38,1±3,4	21,7±3,4	41,0±2,4	13,8±0,6	18,2±1,2	39,6±2,4	33,2±3,1	20,5±1,5	22,5±2,3	1,5±0,7
SC	41,4±2,8	18,9±2,1	38,9±1,7	18,3±1,6	21,3±2,7	42,8±3,8	26,6±2,4	21,1±2,8	25,6±3,1	1,2±0,4
BC	38,4±1,9	22,4±1,6	32,4±2,3	15,0±2,3	24,7±2,3	37,4±2,9	22,8±3,5	19,0±3,1	18,1±2,9	1,2±0,2
IC	48,7±4,3	19,8±2,0	28,1±3,6	19,0±1,8	19,0±2,0	45,5±2,7	31,5±2,9	22,7±2,6	23,0±1,6	1,4±0,6
CFGO	41,4±3,9	18,1±2,5	35,0±2,9	13,2±1,6	18,4±2,9	37,8±2,0	23,5±4,2	17,3±2,0	22,4±0,9	1,3±0,4
After one course of Flaraxin treatment										
LC	43,8±2,8	22,4±2,0	42,1±3,1	16,7±2,7	19,6±1,4	42,8±3,4	37,9±2,1	21,6±1,8	23,8±3,2	1,8±0,6
SC	46,7±3,1	24,8±3,1	36,7±2,7	18,9±3,1	20,5±2,3	47,2±2,4	25,7±1,2	19,8±1,4	24,8±2,7	1,2±0,9
BC	42,0±2,5	25,6±2,3	34,6±1,9	17,6±2,0	17,1±1,8	40,2±1,9	21,8±2,8	14,7±2,2	21,9±1,8	1,5±0,7
IC	52,8±3,4	20,4±2,0	27,8±2,9	17,0±3,7	18,1±3,4	43,8±2,9	29,6±3,2	11,9±1,9	22,3±2,3	2,4±1,2
CFGO	43,7±2,1	28,3±3,0	39,2±3,1	16,8±2,2	19,5±2,3	44,5±1,9	22,8±1,9	11,6±2,7	26,9±1,9	2,0±1,6

Note: *The following conventions are adopted here and in the following tables: LC - lung cancer; SC - stomach cancer; BC - breast cancer, IC - intestinal cancer, CFGO - cancer of the female genital organs.*

The analysis of the quantitative indicators presented in the table for the content of immunocompetent cells in the blood indicates that in all the studied patients there was a pronounced secondary immunodeficiency. This was manifested in the reduction of T-lymphocytes and, to a lesser extent, B-lymphocytes and their subpopulation. A parallel formulation of the rosetting reaction, taking into account the sensitivity of lymphocytes to theophylline and their treatment with monoclonal antibodies, revealed more precise dynamic changes when using the latter technique. The most pronounced decrease in the quantitative indicators of lymphocytes was observed in patients with lung cancer and breast cancer. The presence of immunodeficiency testified and indicators of Immunoregulatory index.

After the course of treatment with the drug Flaraxin, all patients showed a positive dynamics of quantitative indicators of immunocompetent cells. A significant increase in the number of lymphocytes - helper / inducer was noted, which was reflected in the increase in the immunoregulatory index.

In the group of patients with intestinal cancer and patients with cancer of the female genital organs, the Immunoregulatory index exceeded this indicator in donors. The content of the total population of T-lymphocytes also statistically significantly increased in all groups of patients. Attention is drawn to a significant increase in the fraction of active T-lymphocytes, which indicates a gradual normalization of this important element of the immune system.

The functional activity of T-lymphocytes was assessed by their mitogenic effect (RBTL) on phytohemagglutinin (PHA) (“Difco” USA) by the radioisotope method using ZN-thymidine as a label (Adier et al., 1979) with recording the results on an “Inlertechnique scintillation counter IL-4000” (France) and the morphological method in the modification of E.F. Chernushenko and L.S. Kogosova (1978), which allows to obtain information about the nature and degree of cell transformation. Indicators of lymphocyte blast transformation on phytohemagglutinin (PHA) in the studied individuals are presented in table 2.

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Table 2. The Indicators of lymphocyte blast transformation on phytohemagglutinin (PHA) and patients in the course of treatment with Flaraxin.

Groups	Spontaneous inclusion of ZN-thymidine, imp./min	Stimulation of PHA imp./min.	Stimulation index	Morphological accounting,%
Donors	1385±360	92264±7217	67,99	72,14±2,1
Before treatment				
LC	984±235	40129±5100	40,78	42,41±4,7
SC	843±193	28524±2414	33,83	38,10±4,3
BC	860±211	32705±1138	38,02	41,64±3,9
IC	722±126	22629±1840	31,34	36,72±4,1
CFGO	81±107	28666±1732	35,34	39,40±2,6
After one course of treatment				
LC	1012±265	42504±2315	42,05	44,06±4,7
SC	890±159	31150±1165	35,64	42,90±4,3
BC	720±190	3528±1632	49,25	47,64±2,8
IC	750±175	27750±1575	37,18	42,90±2,6
CFGO	790±120	32390±1764	41,29	41,74±5,1

Note: *The following conventions are adopted here and in the following tables:*
LC - lung cancer; SC - stomach cancer; BC - breast cancer, IC - intestinal cancer, CFGO - cancer of the female genital organs.

The results of the study of mitogenic changes in T-lymphocytes under the influence of stimulation with phytohemaglutinin indicate that the initially decreased functional properties of lymphocytes observed in patients with different localization of neoplasms increased slightly. This was reflected in the increase in the stimulation index and the tendency for an increase in the percentage of lymphocyte blast transformation. However, their performance after 1 course of treatment with Flaraxin does not reach values in healthy individuals, which, apparently, requires continuation of the course of treatment with this drug.

An objective indicator of the functioning of the B-system of lymphocytes is the level of synthesis of immunoglobulins (Ig), in particular, classes IgA, IgM and IgG. The determination of these parameters was carried out in serum by the method of radial immunodiffusion by Manctnietal. (1965) using mono-specific anti-sera (II Mechnikov Research Institute of Vaccines and Serums, Moscow). The results of these studies are shown in table 3.

Table 3. The content of immunoglobulins in the serum of patients treated with Flaraxin.

Groups	Before treatment			After one course of treatment		
	Ig **A**	Ig **M**	Ig **G**	Ig **A**	Ig **M**	Ig **G**
Donors	2,1±0,24	1,61±0,24	15,7±1,7	-	-	-
LC	1,98±0,12	1,82±0,17	16,7±2,2	2,10±0,16	1,32±0,61	14,8±1,4
SC	1,81±0,18	1,56±0,12	13,4±1,3	1,76±0,32	1,64±0,28	15,1±2,1
BC	2,14±0,16	1,98±0,80	18,1±0,9	1,90±0,21	1,75±0,32	16,4±1,7
IC	2,02±0,14	1,63±0,12	14,4±1,4	1,85±0,30	1,50±0,34	15,3±2,0
CFGO	1,85±0,11	1,75±0,80	16,7±2,3	1,92±0,26	1,23±0,21	14,8±2,1

Note: *The following conventions are adopted here and in the following tables:*
LC - lung cancer; SC - stomach cancer; BC - breast cancer, IC - intestinal cancer, CFGO - cancer of the female genital organs.

The results of the determination of serum immunoglobulins in the examined patients indicate that the change in their content in different groups was ambiguous and did not have the nature of a certain pattern. Thus, the content of Ig class A was slightly different from that in the control group. After the use of the drug Flaraxin, some IgA decrease was observed, however, it was not statistically significant. The content of Ig class M in cases of breast cancer (BC) and lung cancer (LC) was elevated, and only after

the first course of treatment there was a tendency to decrease. The same pattern was observed at other tumour sites.

In cases of breast cancer (BC) and cancer of the female genital organs (CFGO), there is a tendency to increase the concentration of Ig class G. After treatment, a decrease in the content of Ig class G to control values was recorded.

Immune complexes (antigen-antibody complex) are an important element in the formation of an immune response. The content of immune complexes in the blood characterizes the severity of the clinical course of the disease, accompanied by chronic inflammatory changes and destructive processes. Observations on their dynamics and concentration can be used in oncological practice in diagnostics and for predictions, as well as to monitor their effectiveness of therapy.

The determination of circulating immune complexes (CIC) was performed according to the method of Haskova et at. (1978), using for their precipitation 3.5% solution of polyethylene-glycol - m.h. 6000 (“Loba Chemie”, Austria) based on the results on a “Specol-10” micro spectra photometer (Germany). The measurements were carried out at a wavelength of 450 nm (OD450) and expressed the results in arbitrary units of extinction.

The content of Circulating Immune Complexes (CIC) in the blood of practically healthy individuals (donors) averages (M ± m) - 49.92 ± 2.85 units. The CIC concentration in the serum of the examined patients was: gastric cancer (GC) - 152 ± 10.4 units, lung cancer (LC) - 181.0 ± 8.9 units, breast cancer (BC) - 174.1 ± 13 , 1 unit; intestinal cancer (IC) - 132.05 ± 10.2 units; cancer of the female genital organs (CFGO) - 139.08 ± 12.1 units.

After conducting 1 course of treatment with Flaraxin, the CIC content in the patients' blood was: gastric cancer (GC) —201 ± 16.3 units; lung cancer (LC) - 222 ± 14.9 units :, breast cancer (BC) - 189 ± 8.8 units; intestinal cancer (IC) - 156 ± 11.6 units; cancer of the female genital organs (CFGO) - 163 ± 16.5 units.

As can be seen from the above results, in all observed cases of diseases with different localization of the tumour, there was a significant increase in the content of circulating immune complexes (CIC). This phenomenon can be explained by the fact that under the influence of the drug Flaraxin, massive destruction of tumour tissue occurs with further resorption of decomposition products by the reticulo-endothelial system of the body. As a result of the binding of these products, which have a protein structure and carry antigenic information, the formation of corresponding antibodies occurs, which, in turn, form an additional amount of antigen-antibody complexes (CIC).

Perhaps the most significant in studying the state of the immune system in patients with malignant tumours is the determination of the quantitative composition and functional activity of the killer (cytotoxic) fraction of blood lymphocytes. These lymphocytes are a specialized population that has a cytotoxic effect on leukemic, tumour and virus-infected cells. In particular, Natural Killers (NK) are the "first line of defense" against the growth of tumour cells in the body and carry out antitumor surveillance, destroying malignantly transformed cells that appear in tissues and blood. The weakening of the activity of killer lymphocytes is one of the determining factors in reducing the resistance of the organism during the development of primary tumours. The literature data show that the development of the tumour process is accompanied by a significant inhibition of the killer link of immunological protection.

Natural killer lymphocytes were determined using monoclonal antibodies NKH-1 (“Coulter” USA) using the direct immunofluorescent method. (Peshkov A.L. and Poletaeva A.I.,1985). The cytotoxic activity of lymphocytes was assessed in reactions of natural and antibody-dependent cytotoxicity. (Gordienko S.M.,1983; Zheleznyakova G.F. and Gnilevskaya Z.U., 1988). Chicken erythrocytes were used as target cells. The ratio of effector cells and target cells in the study of antibody-dependent cytotoxicity was 25:1, and natural cellular cytotoxicity of 10:1. The research results are summarized in table 4.

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Table 4. The number of natural lymphocytes-killers (NK, %) , antibody-dependent cellular cytotoxicity (ADCC, %) and natural killer cytotoxicity (NKC, %) in the blood of patients before and after treatment with Flaraxin.

Groups	Before treatment			After treatment		
	NK %	ADCC %	NKC %	NK %	ADCC %	NKC %
Donors	13,15±1,43	22,42±2,59	34,11±3,01	-	-	-
LC	9,68±2,14	18,12±4,23	21,64±2,86	10,32±3,30	23,84±3,86	29,32±2,14
SC	5,03±1,08	10,70±2,74	19,17±3,72	7,48±2,17	15,38±2,84	23,02±3,07
BC	6,32±1,24	12,32±3,01	15,19±2,64	6,84±3,14	16,43±3,00	20,98±2,06
IC	8,70±1,96	17,14±2,72	17,32±3,12	8,95±2,16	19,08±2,83	23,74±2,23
CFGO	7,28±2,60	9,08±2,14	14,83±3,74	9,81±3,26	12,72±3,48	24,39±3,09

Note: *The following conventions are adopted here and in the following tables: LC - lung cancer; SC - stomach cancer; BC - breast cancer, IC - intestinal cancer, CFGO - cancer of the female genital organs.*

Based on the data in the table, we can conclude that the neoplasmic process in all studied patients is accompanied by a significant decrease in both quantitative and functional indicators of the killer system of lymphocytes. This defect was especially significant in the group of patients with gastric cancer and breast cancer. The first course of treatment with the drug Flaraxin led to a statistically significant increase in the activity of both the antibody-dependent and natural killer activity of lymphocytes.

However, by the time of observation indicators of activity did not reach normal values (control). Exceptions were indicators of antibody-dependent cellular cytolysis, and in the group of patients with lung cancer, where they even exceeded those of donors. The indicators of natural killer activity recovered partly more slowly.

The identified positive effect of the drug Flaraxin in the treatment of patients with neoplasms on the recovery of killer activity, as well as quantitative indicators of lymphocytes have a significant practical importance.

Chemotherapy courses for cancer patients lead to a significant inhibition of the functioning of the immune system and, above all, its cytotoxic factors. Given that a significant proportion in the antitumor defence of the body has a nonspecific immune response, mainly due to NK-activity, the restoration of this function correlates positively with the effect of chemotherapy.

Important meaning in the formation of antitumor resistance plays interconnection of cytological factors with interferon system. In the modern literature a lot of facts have been accumulated about the possible selective activity of the Natural killers (NK)-system, in particular, the influence of interferon and its inductors on their activation during tumour growth. It is assumed that all agents that enhance the Natural Killers activity can act through interferon, which can modulate the sensitivity of target cells and enhance NK-activity in animals and humans. (Klark E. et al., 1989; Hanna H.,1992 etc.). Many authors (Herberman R.B. et al., Ortaldo J.R. ,1991; Kadish A.S. et at., 1981 etc.) were shown that treatment of NK-cells with interferon significantly enhances the conjugation of NK-cells with various leukemic and tumour cells and contributes to the lysis of NK-cells.

An important role in enhancing the cytolytic effect of NK -cells is assigned to the inductors of interferon activity (Axberg I. et at., 1990; Herberman R.B.,1982 etc.). Taking into account the presence in the drug Flaraxin interferonogenic properties (look to the Report from Danylo Zabolotny Institute of Microbiology and Virology) it can be concluded that its use in antitumor therapy confirms the theory of the interaction of natural killer cells and target cells, which is justified by the results of our research.

Conclusions

1. Under the influence of Flaraxin, massive destruction of tumour tissue occurs with further resorption of decay products by the reticulo-endothelial system of the body.

   As a result of the binding of these products, having a protein structure and carrying antigenic information, the formation of the corresponding antibodies, which form an additional amount of antigen-antibody complexes (CIC), occurs.

2. The course of treatment with Flaraxin led to a significant increase in the activity of both the antibody-dependent and natural killer activity of lymphocytes.
3. Thus, a study of the effect of the drug Flaraxin on the performance of various parts of the immune system in patients with oncological pathology indicates that systemic immunodeficiency due to the tumor process is undergoes to a positive correction.
4. The restoration with the help of the Flaraxin drug of the functioning of the leading mechanisms of the immune process is accompanied by an improvement in the general clinical condition of patients, leading to a decrease, and in some cases, to the complete exclusion of the metastatic process, and to a significant reduction in tumour size.

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http://estetmed.org/?page_id=1892
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His famous articles and books

1. Immune response to Bacteroides fragilis in experimental local infection, // International Congress of Infection Diseases. Nairobi, 1992,
2. Immunoregulatory connections of the liquidators of the consequences of the Chernobyl accident on Chernobyl NPP.
   // International symposium on allergology and immunology. Almaty, 1994
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4. Immunocomplex mechanisms in the pathogenesis of lung diseases, // Problems of pulmonology. Moscow 1999.
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6. Immune-complex mechanisms of tissue damage in pulmonary pathology // Pulmonology: Coll. Proceedings of the Moscow Medical Academy named after IM. Sechenov. - M., 1999, etc.

He is a writer of more than 200 medical articles.

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His patents

1. The method for determining sensitization to epichlorohydrin. 1783441
2. The method of determining the T-and B-lymphocytes on the cytological preparation. 1712817
3. The method for the diagnosis of hepatitis. 1711076
4. The method of identification of t-helper and t-suppressor. 1709221
5. The method of correction of the cellular element of the immune system. 1659856
6. The method for predicting severe peritonitis. 1652915
7. The method for determining sensitization to allergens. 1569705
8. The method for the diagnosis of rheumatic myocarditis. 1561038
9. The method of determining organ-specific autoimmune processes. 1508162
10. The method of determining the phagocytic activity of leukocytes. 1446524
11. The method of modelling chronic allergic bronchopneumonitis. 1084859
12. The method of differentiation of antigens into t-dependent and t-independent. 1003813
13. The method of modelling hypertension in the pulmonary circulation. 875449
14. The method of modelling chronic pneumonia. 826401
15. The method of preparation of culture medium for cultivation. 659619

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