

"CONFIRM"  
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July\_\_\_\_, 2006

Report of the work on the subject of "Study of kinetics and pharmacodynamics of antibodies that are generated in the organism after introduction of the "Normogen" preparation" according to the Agreement No 02 with the Kazakh Oncology and Radiology Research Institute of the Ministry of Health of the RK, dated by May 26, 2006

Almaty - 2006

## Sections

1. Pharmacodynamics and pharmacokinetics of the "Normogen" preparation
2. Immunobiological methods of oncology diseases treatment
3. The "Normogen" preparation features
4. Some pharmacological effects of the "Normogen" preparation according to data of the patients' blood
5. Study of kinetics and pharmacodynamics of antibodies that are generated in the organism after introduction of the "Normogen" preparation
6. Conclusion

Pharmacokinetics and pharmacodynamics as integral parts of pharmacology

Pharmacology (from Greek *pharmakon* drug + *logos* science) - a science that studies the action of substances that are used for treatment, prophylaxes and diagnostics of human diseases. The integral parts of pharmacology are pharmacodynamics, pharmacokinetics and pharmacotherapy.

Pharmacodynamics (from Greek *pharmakon* drug + *dynamikos* powerful) - a part of pharmacology. Pharmacodynamics studies localization, mechanisms of action and pharmacological effects of medicinal substances.

The action of the medicinal substances on the physiological processes and vital activity of particular organs and tissues can be seen in the form of direct and indirect influence. Therefore, the studied effects will differ in complexity of their manifestation mechanisms. Direct interaction with substrate is often realized by binding of the medicinal substance particles with the specific receptors. Besides, some so-called unspecific receptors can bind the medicinal substance particles without any functional changes. Specific receptors have definite localization. Connection between medicinal substance particles and specific receptors can be realized by different chemical bonds that have different strengths.

Some specific receptors, which bind antitumor substances that belong to a group of alkylating agents, are known.

The nature of many specific receptors is not determined, although some different indirect data reveal their existence and, besides, there are some technical approaches, in particular, the method of radioisotopic tracer technique, are developed.

The covalent nature of interaction between drug molecules and receptors of organs gives sufficiently strong bonds that provides long and often irreversible action of substances (for example alkylating antitumor agents). But most of the medicinal substances have the reversible nature of connection with receptors.

Pharmacokinetics (from Greek *pharmakon* drug + *kinetikos* related to motion) is a second part of pharmacology.

Pharmacokinetics studies mechanisms of absorption, distribution, metabolism and excretion of the medicinal agents.

Study of these mechanisms are carried out also with use of mathematical modeling of abovementioned processes.

Determination of pharmacokinetic characteristics of new medicinal preparations are an important part of the preclinical and clinical trials.

Immunobiological methods of oncology diseases therapy

At present the fundamentally new methods and preparations for immunobiological treatment of the tumor are developed.

They are based on the series of the immunological status changes at oncological patients. Thus, now it is considered that, as a rule, during oncology diseases the activation of substances, which take part in the processes of accelerated growth and proliferation of the cells, has a place.

These oncogenes, as a matter of fact, are the normally blocked embryonic genes. Therefore, at tumors initiation the rise in number of embryonic forms of ferments, and other protein factors, which provide growth regulation and proliferation of the cells, is also observed.

These oncogenes include alfa-fetoprotein (AFP) - a protein of the embryonic blood serum that is usually not present in the blood of adult individuals, but appears at reparative (restorative) processes, liver and reproductive system tumors, and malignant tumors.

Activation of the AFP synthesis is also has place at physiological regeneration of the organism tissues.

But, usually, at this process, the greater part of AFP is quickly used in the tissues and doesn't accumulate in the blood. It is possible that AFP is synthesized and transformed in the liver, and thus, the level of AFP can increase in 10 or more times at the liver cirrhosis,

and hepatitis B and C. Hence, at some oncology diseases, AFP can appear in blood both as a component of the reparative mechanism, and, also, as a product of tumor cells expression. Thereafter, it is considered that, the growth of AFP synthesis at oncology diseases is an example of oncogene double action.

By the structure and physical-chemical properties AFP is very similar to serum albumin (this hampers detection of AFP level in blood). Another possible function of AFP is an immunosuppressor capability to suppress immune reaction to antigens of developing fetus.

The immunoglobulins (antibodies) that selectively interact with one or several elements (antigens, receptors) of tumor cell walls are considered as preparations for immunobiological impact against tumors.

Such immunoglobulins are included into such preparations as "Gerceptin", "Mabthera", "Panorex" and so on. These preparations selectively affect defined pathological cells. Their disadvantage is their impossibility to use them against antigens of the other kinds of tumors.

Some attempts to create universal antitumor preparation on the base of fetal protein - alfa-fetoprotein are known.

It is known that receptors, which bind alfa-fetoprotein, exist in cell membrane of all dividing cells, but the most large quantity of such antigens are localized on the surfaces of the transformed, tumor cells.

By binding of an alfa-fetoprotein molecule with highly toxic substance we can create a preparation that will selectively interact with tumor cells and induce destruction of the transformed cells due to the toxic component.

But we can also use for these purposes the produced polyclonal antibodies that are antiidiotypical to alfa-fetoprotein.

Such antibodies as native alfa-fetoprotein molecule have high affinity to alfa-fetoprotein receptors on the membranes of tumor cells and, besides, are capable to activate complement, that leads to selective, complement dependent lysis of tumor cells.

These principles were used to create the "Normogen" preparation - the set of polyclonal, native antibodies that are antiidiotypical to alfa-fetoprotein and are capable to selective interaction with corresponding receptors of the surfaces of any types of tumor cells, and to binding and activation of the complement components that induces, complement dependent lysis of tumor cells.

Antiidiotypical antibodies, antiidiotypes - antibodies that are specific to antigen determinants that are located on variable parts of other antibodies. Antiidiotypical antibodies chemically reproduce necessary configuration of the antigen and can be considered as imitators and analogs of the antigen.

Creation of such antiidiotypical antibodies is a fundamentally new approach to production of diagnostic, serum, and vaccine preparations. Methodical part of technology of antiidiotypical antibodies production is not sufficiently developed, in relation to human cell receptors.

Antiidiotypical antibodies take part in the system of humor and cell immune response regulation, because they are capable to both suppress and enhance antigen dependent production of antibodies, switching on selective, complement dependent lysis of cells that produce antibodies and cell receptors.

Idiotypical characteristics of antiidiotypical antibodies to the antigen that is produced in different people and animals are the same.

Oncological AFP provides growth and defense of the tumor from a reparative system of human organism, and physiological AFP is a component of this system.

The "Normogen" preparation features

The "Normogen" preparation consists of a set of natural immunoglobulins (lactoglobulins) that are supplemented with antiidiotypical antibodies to alfa-fetoprotein.

Alfa-fetoprotein is the only protein, which fulfills transport of polyunsaturated fatty acids to proliferating cells, by selective binding with the receptors specific to it.

The fundamental of the "Normogen" preparation from the other antitumor drug on the base of antibodies is the specificity of its immunoglobulins to normal receptors that bind alfa-fetoprotein, and not to pathological antigens, that are situated only on the surface of the transformed cells.

Efficiency of the "Normogen" preparation is estimated by the density of alfa-fetoprotein receptors, which exists in different quantity on the any cell surface. Normal cells have not got alfa-fetoprotein receptors or their density is scarce.

Dividing cells, including malignant tumor cells have the positive correlation between density of alfa-fetoprotein receptors and rate of cells division.

Selective binding of the antiidiotypical to alfa-fetoprotein antibodies on the surface of the transformed cells triggers following immunological mechanisms: complement-dependent cytotoxicity; antibody-dependent cell cytotoxicity; Ab<sub>2</sub> vaccine (induction of endogenic antibodies against tumor cells); blockade of the alfa-fetoprotein specific receptors; phagocytosis; intracellular effects.

The most important mechanisms are the first four ones.

During realization of the first mechanism binding of the antigen with antibody on the surface of the tumor cell leads to complement system activation. On the final stage of this activation the protein C9 is generated. This protein have the ability to perforate the membrane that leads to its destruction.

Antibody-dependent cytotoxicity is based on the fact that the antibodies activate the cytotoxic lymphocyte-"killers" by their hypervariable domain. The latter can synthesize and secrete proteins-perforines similar to protein C9 of complement system.

The third mechanism of antitumor action of antibodies is governed by production of Ab<sub>2</sub> vaccine. It realizes with usage of any antibodies, when the recipient organism produce its own antiidiotypical antibodies that are capable to connect with surface tumor antigen and additionally to carry out several more cycles of abovementioned cytotoxic mechanisms.

The forth mechanism of receptor binding by the "Normogen" drug immunoglobulins is connected with polyunsaturated fatty acids delivery process blockade with the help of antiidiotypical antibodies to alfa-fetoprotein. This significantly reduce the rate of cell division speed or stops it completely.

On the whole, antibodies of the "Normogen" preparation show two mechanism of influence on pathological cells: in the tumors with high rate of cell division the complement-dependent lysis of transformed cells is realized, in the tumors with low rate of cell division and with relatively small number of receptors to alfa-fetoprotein the blockade of such receptors has the place that makes their further division impossible.

Such system activity of the "Normogen" preparation repairs the antitumor defense of the organism that is not observed at exposure to many known immunobiological, chemiotherapeutical preparations during radiotherapy and surgical introduction.

Because the "Normogen" preparation shows its activity in presence of complement, realization of complement content analysis of the patients' blood and determination of its activity become necessary.

Presence of two mechanisms increases requirements to conditions of the "Normogen" preparation clinical usage.

The first requirement - to provide so high concentration of the "Normogen" preparation immunoglobulins in the patient's blood, that they will completely block all receptors to alfa-fetoprotein.

The second requirement - no suppress of the immune system with chemiopreparations and radioactive irradiation.

It is possible to create any concentration of the "Normogen" preparation in the blood of the patient with the maximal therapeutical effect at intravenous injection.

After introduction the preparation triggers the mechanism of tumor cell division suppress. But noticeable results of the therapy can be seen in 20-30 days. It is due to the fact that the reparation of the affected functions of the immune system and destruction of the pathological tumor cells takes time.

Remission time depends, in many respects, on presence of the preparation in blood. That is why the repeated courses of the preparation intake are carried out at the first signs of the patient's general condition aggravation, and also, at stop of dimensions reduction of the tumors and lymphatic nodes.

The indirect signs of the positive effect of the "Normogen" are - growth of number of erythrocytes and their hemoglobin content, reduction of erythrocyte sedimentation rate, ESR, rise of immune status of the organism (ratio CD 4 / CD8), because of the reduction of the number of suppressors and growth of number of leucocytes helpers etc.

Adverse reactions (rise of temperature, fever, chill) of the the "Normogen" preparation are induced by allergic and immunologic reactions of the organism to an alien protein.

These adverse reactions are also resident in other known antitumor drugs on the basis of antibodies (Gerceptin, Panorex etc.)

The proteins of the "Normogen" are more alien in comparison with aforesaid preparation, and thus, the manifestation of the adverse reactions is more expressed. Adverse reactions reduce with usage of antihistaminic medications and methods of desensibilization (decrease of the infusion rate etc.). Allergic reactions can develop after repeated introduction.

Since immunoglobulins raise the viscosity of blood at intramuscular injection, it is recommended to prescribe appropriate preparation (aspirin, trombo-acc and so on).

Some pharmacological effects of the "Normogen" preparation according to the data of patients' blood.

Since the process of the preparation influence on tumor cells, the complexes distribution in the tumor tissues, and other parameters of the therapeutical process can not be registered, the pharmacokinetics and pharmacodynamics of the "Normogen" preparation are measured by complexes presence in blood. And also by indirect signs of the positive effect of the "Normogen" - by the erythrocytes number growth rate and their hemoglobin content, reduction of the erythrocyte sedimentation rate, growth of immune status of organism (ratio CD 4 / CD8) due to reduction of suppressors number and growth of the leucocytes-helpers number etc.

Because of the above mentioned reasons a comparison of the patients' general blood analysis data was carried out before the beginning of the preparation application and after the course of therapy (tables 1 and 2).

Table 1

Data of the first (at the entrance before the beginning of the treatment) general blood analysis of the patients, who took the medicine.

	Patient	Sex	Age, years	Erithroc ytes	Hb	Leucoc ytes	Lympho cytes	Neutrop hils	Thromb ocytes	ESR
1.	A.D.E.	M	43	4,99	135,5	10,1	18	69	363	50
2.	B.A.	M	30	4,84	164	6,7	22,6	67,3	174	4
3.	E.G.	F	49	4,64	135,7	4,8	4	90	174	32
4.	Z.N.	F	53	4,8	147,3	6,6	7	88	254	46
5.	Z.V.V.	M	57	4,3	108,0	9,2	19	66	670	55
6.	M.A.	F	48	3,66	74	5,8	27,7	62,7	283	20
7.	S.L.	M	68	3,44	107	5	20	69	175	38
8.	W. S.	M	65	5	166	5,7	28,2	66	270	6
9.	In average		51,6	4,5	129,7	6,7	18,3	72,25	295,4	31,4

Table 2 Data of the patients' general blood analysis after the first course of therapy with the preparation

	Patient	Sex	Age, years	Erithroc ytes	Hb	Leucoc ytes	Lympho cytes	Neutrop hils	Thromb ocytes	ESR
1.	A.D.E.	M	43	4,99	135	10,6	20	69	261	42
2.	B.A.	M	30	4,24	141	13,1	14	83	171	7
3.	E.G.	F	49	4,78	120	5,8	22	74	173	36
4.	Z.N.	F	53	5,41	154	9,5	31	56	205	40
5.	Z.V.V.	M	57	4,51	111	16,3	17	72	589	48
6.	M.A.	F	48	3,77	77	7,3	21	75	226	18
7.	S.L.	M	68	3,55	110	5,6	22	69	181	26
8.	W. S.	M	65	5,04	162	9,5	22	70	195	20
9.	In average		51,6	4,54	126,25	9,71	21,13	71,00	250,13	29,63

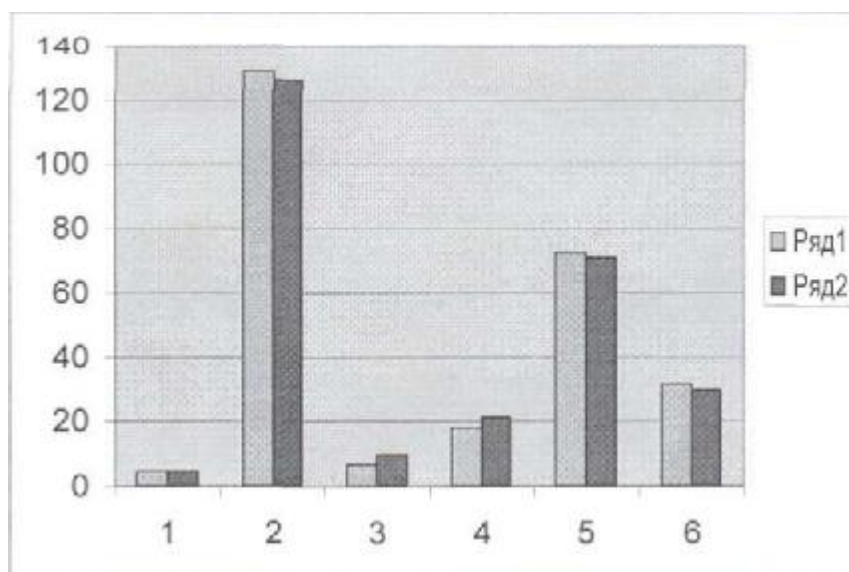


Figure 1. Parameters before (Row 1) and after (Row 2) the beginning of the "Normogen" introduction 1 - erythrocytes (millions/ $\mu$ l); 2 - hemoglobin (gr/l); 3 - leucocytes (thousands/ $\mu$ l); 4 - lymphocytes (% among leucocytes); 5 - neutrophils ((% among leucocytes); 6 - ESR.

It is seen in the figure 1 that after the first course of the "Normogen" preparation application the improvement of the basic general blood analysis parameters are observed.

Table 3

Biochemical parameters of the immunological status by the data of the first (at the entrance before the beginning of the treatment) general blood analysis

	Patient	Immuno globulin A	Immunoglobulin M	Immuno globulin G	Complement fraction 3	Complement fraction 4	Circulating immune complexes	Hemolytic activity of the complement system
1.	A.D.E.	409	101	1194	146	34	2,16	66,3
2.	B.A.	170	83	1097	155	46	1,8	66,2
3.	E.G.	241	111	1334	155	35	2,76	66,5
4.	Z.N.	542	134	1837	107	36	3	44,3
5.	Z.V.V.	283	122	1448	187	46	3,6	74,4
6.	M.A.	321	178	1007	90	18	2,64	53,6
7.	S.L.	177	121	911	ΠΟ	26	1,68	75,8
8.	W. S.	299	124	1008	125	35	2,16	66,4
9.	In average	305,3	121,8	1229,5	134,4	34,5	2,5	64,2

Table 4 Biochemical parameters of the immunological status by the data of the blood analysis after the first therapy course with "Normogen" preparation.

	Patient	Immuno globulin A	Immunoglobulin M	Immuno globulin G	Complement fraction 3	Complement fraction 4	Circulating immune complexes	Hemolytic activity of the complement system
1.	A.D.E.	423	120	1328	152	36	3	59
	B.A.	158	76	945	147	36	1,68	56,7
3.	E.G.	239	112	1372	140	28	2,4	64
4.	Z.N.	526	134	1842	168	35	2,4	87,1
5.	Z.V.V.	576	162	1675	196	52	5,4	59,3
6.	M.A.	354	258	1156	92	15,6	3,48	33,6
7.	S.L.	176	118	949	98	26	1,92	53
8.	W. S.	255	98	755	118	32	1,68	47,8
9.	In average	338,4	134,8	1252,8	138,9	32,6	2,7	57,6
10.	Difference with the first analysis	33,2	13,0	23,3	4,5	-1,9	0,3	-6,6

At comparison of the average data before and after the first course of treatment with the "Normogen" preparation, it is obvious that there is general tendency of immune status biochemical parameters improvement.

Table 5

Some cytological parameters of the immune status by the data of the first (at the entrance before the beginning of the treatment) patients' general blood analysis

	Patient	T-Lymphocytes	T-helpers	T-suppressors	Tx/Tc	B-Lymphocytes	D-Lymphocytes	O-Lymphocytes	Auto-lymphocytes
10.	A.D.E.	69/1,254	31/0,564	10/0,181	3,1	9/0,163	0	22/0,4	1/0,01
11.	B.A.	44/0,666	48/0,726	32/0,484	1,5	6/90,8	0	50/0,757	12/0,18
12.	E.G.	41/78,7	47/90,2	23/44,1	2	12/23	0	47/90,2	7/0,01
13.	Z.N.	57/0,263	40/0,184	21/97	1,9	13/60	0	30/0,138	5/0,02
14.	Z.V.V.	62/1,084	17/0,297	21/0,367	0,81	10/0,174	0	28/0,489	4/0,07
15.	M.A.	71/1,141	26/0,417	13/0,209	2	10/0,161	0	19/0,305	20/0,32
16.	S.L.	78/0,78	29/0,29	18/0,18	1,6	6/60	0	16/0,16	3/0,03
17.	W. S.	60/0,964	36/0,579	18/0,289	2	14/0,225	0	26/0,417	6/0,09
18.	In average						0		

Table 6

Some cytological parameters of the immune status by the data of the patients' general blood analysis after first course of treatment with the "Normogen" preparation

	patient	T-Lymphocytes	T-helpers	T-suppressors	Tx/Tc	B-Lymphocytes	D-Lymphocytes	O-Lymphocytes	Auto-lymphocytes
19.	A.D.E.	65/1,378	40/0,848	23/0,487	1,7	10/0,212	0	25/0,53	9/0,19
20.	B.A.	52/0,879	31/0,524	14/0,236	2,2	12/0,203	0	36/0,608	7/0,11
21.	E.G.	48/0,612	21/0,268	14/0,178	1,5	10/0,127	0	42/0,535	8/0,1
22.	Z.N.	67/1,715	31/0,793	22/0,563	1,4	9/0,23	0	24/0,614	9/0,23
23.	Z.V.V.	68/1,884	29/0,804	23/0,637	1,2	9/0,249	0	23/0,637	10/0,27
24.	M.A.	59/0,904	28/0,429	16/0,245	1,7	12/0,184	0	29/0,445	14/0,21
25.	S.L.	71/0,874	24/0,296	14/0,172	1,7	14/0,172	0	15/0,184	5/0,06
26.	W. S.	75/1,134	28/0,423	13/0,197	2,1	8/0,121	0	17/0,257	0
27.	In average	63,13/1,14	29/0,548		1,69		0		

At comparison of the average data before and after the first course of treatment with the "Normogen" preparation, it is obvious that there is general tendency of immune status cytological parameters improvement.

#### **Study of pharmacokinetics and pharmacodynamics of the antibodies that are generated in the organism after introduction of the "Normogen" preparation**

The "Normogen" preparation has no marks, which allow detecting its active substances in blood and tissues.

There is no reliable method of detection of the specific antibodies that are generated in the organism after introduction of the "Normogen" preparation. That is why pharmacokinetics and pharmacodynamics of such preparations are studied on the protein complexes, that are develop in the blood after binding of an antigen and the antibody.

Immune complexes are detected in blood plasma by nephelometric method. After a constant (in 1-2 days) analysis of these immune complexes we can "draw" a dynamics of their plasmatic content increase and decrease.

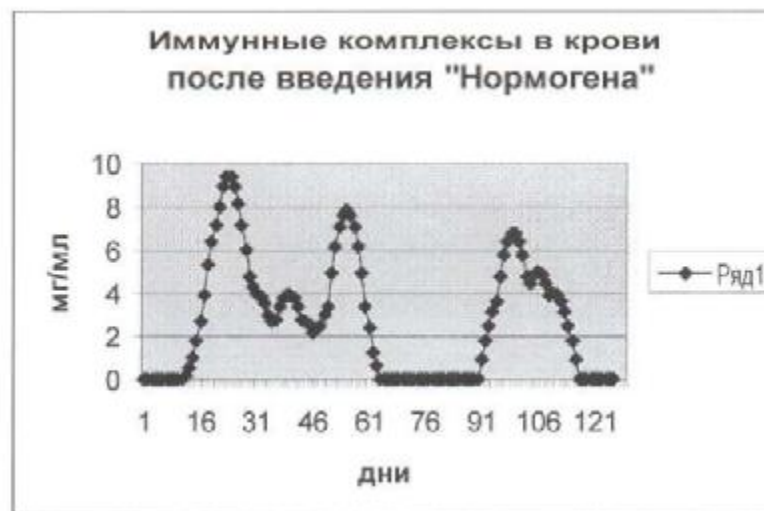
Usually the secondary antibodies are produced complementary to the complexes that, in the end, induce the mechanism of aggregate removal in the organs of reticuloendothelial system, RES (liver, kidneys, lymphatic nodes and so on) with participation of macrophages.

Although nephelometry allows detecting only the sum quantity of the aggregates (having therapeutical importance and other aggregate without specific therapeutical influence), the primary curve characterize predominantly the first group of aggregates, while the secondary wave of increase is a sum parameter (total index). Tables 3 and 4 have average of the immune complexes content that are circulating in the blood plasma before and after the first course of the treatment. After the treatment course their content moderately increases. But the average data do not reflect the dynamics of this parameter change immediately after introduction of the preparation. Dynamics is shown on the figures 2 and 3, timetable diagram characterize nephelometric curves of the "antigen-antibody" aggregate quantity increase and decrease in the blood of the patients, who were injected the "Normogen" preparation.

It is significant that protein aggregates influence upon rheology and hemocoagulation capacity of blood.

Hemolytic activity can change as an element of adaptation. So the probable adverse effect parameter should be monitored.

However, the hemolytic activity of the complement system is not directly connected with the therapeutical effect of the preparation that is introduced. That is why this parameter is not used at characterization of the pharmacodynamics and pharmacokinetics of the "Normogen" preparation.



mg/ml / days

Fig.3. Immune complexes in the blood plasma of the patient, who has three instances of "Normogen" introduction. The second introduction was carried out on the background of the first introduction.

It can be seen from the Figures 3 and 4 that it takes 8-12 days to achieve the maximum in the immune complexes quantity and the same number of days to their excretion from blood plasma. There are no significant differences in the characters of the "increase" curve and the "decrease" curve.



Fig.4. Immune complexes in the blood plasma of the patient, who has every next instance of the "Normogen" introduction only after removal of the complexes that were generated after previous instance of the "Normogen" introduction.

It can be seen from the Figures 3 and 4 that the immune complexes content in the blood plasma of different patients has the same general characteristics and differs only in detail. These general parameters of immune complexes removal from blood (and, respectively, from the organism) correlate with the data of other authors for similar preparations. (Clinical immunology and allergology. Under editorship of L. Yeger, In three volumes, 1990, Volume 1).

It is possible that after the first intravenous introduction the continuous "supporting influence" is necessary. Intraperitoneal introduction, introduction «per rectum» and indirect intralymphatic introduction can be such "supporting influence".

Secondary intravenous introductions of the preparation, should, possibly, be introduced with use of hipo- and desensibilization methods (one method - fractional, relatively continuous drop-by-drop introduction).

### Conclusion

Data of the patients' general blood analysis, parameters of biochemical and cellular immunology status, hemolytic activity of complement system, average and dynamical parameters of immune complexes content in the blood plasma show that pharmacokinetic and pharmacodynamic parameters of therapeutical substances, which are generated in organism after the "Normogen" preparation introduction, do not differ from the classical data, which are observed at people and laboratory animals after introduction of similar antigenic preparations.

The content of the circulating immune complexes only indirectly characterizes the therapeutical efficiency of the "Normogen" preparation.

The direct sign of the treatment efficiency is the reduction of malignant tumors dimensions, and also the immunology status improvement of blood parameters.

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