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(54) Titre : PROCEDE DE FABRICATION D'UN PRODUIT ANTIVIRAL IMMUNOTROPIQUE
(54) Title: THE METHOD OF OBTAINING IMMUNOTROPIC ANTIVIRAL PREPARATION

(57) Abrégé/Abstract:
The invention relates to chemical and pharmaceutical industry and medicine, namely, to the method of obtaining immunotropic, apyrogenic, non-toxic, non-resistant preparations possessing high antiviral activity towards RNA- and DNA-viruses and under multiple infection, these preparations being obtained from animal material (soft roe of sturgeon fish or salmon fish, calf thymus, chicken blood, pig spleen). To obtain immunotropic antiviral pharmaceutical preparations possessing high therapeutic activity towards RNA- and DNA-viruses defatted soft roe of sturgeon fish or salmon fish, or calf thymus, or chicken blood, or pig spleen are ground and homogenized in citrate-salt solution. Homogenate is incubated in presence of detergent; sodium chloride concentrated solution is added to the reaction mix under continuous stirring and high temperature. The reaction mix is cooled, sonicated under standardized conditions, then it is treated with sorbent and detergent, filtrated and concentrated. DNA-Na white amorphous precipitate is isolated from concentrate with ethanol, DNA-Na molecular mass is 270-500 kD, hyperchromic effect is not lower than 37%, protein content is less than 1%. DNA-Na precipitate is dried and dissolved in standardized transition metal solution to make 1.5% solution with hyperchromic effect not lower than 10%, the solution is sterilized by baro-membrane method and poured under sterile conditions in therapeutic doses (75 mg). Water-soluble salts of transition metals Zn, Co, Fe, Mn, Sb are used to obtain immunotropic antiviral preparations. DNA-Na to metal ratio in the preparation is within range 50-500:1-2. Immunotropic non-resistant apyrogenic preparations possess antiviral activity towards RNA- and DNA-viruses and their combined presence.

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ABSTRACT

The invention relates to chemical and pharmaceutical industry and medicine, namely, to the method of obtaining immunotropic, apyrogenic, non-toxic, non-resistant preparations possessing high antiviral activity towards RNA- and DNA-viruses and under multiple infection, these preparations being obtained from animal material (soft roe of sturgeon fish or salmon fish, calf thymus, chicken blood, pig spleen). To obtain immunotropic antiviral pharmaceutical preparations possessing high therapeutic activity towards RNA- and DNA-viruses defatted soft roe of sturgeon fish or salmon fish, or calf thymus, or chicken blood, or pig spleen are ground and homogenized in citrate-salt solution. Homogenate is incubated in presence of detergent; sodium chloride concentrated solution is added to the reaction mix under continuous stirring and high temperature. The reaction mix is cooled, sonicated under standardized conditions, then it is treated with sorbent and detergent, filtrated and concentrated. DNA-Na white amorphous precipitate is isolated from concentrate with ethanol, DNA-Na molecular mass is 270-500 kD, hyperchromic effect is not lower than 37%, protein content is less than 1 %. DNA-Na precipitate is dried and dissolved in standardized transition metal solution to make 1.5% solution with hyperchromic effect not lower than 10%, the solution is sterilized by baro-membrane method and poured under sterile conditions in therapeutic doses (75 mg). Water-soluble salts of transition metals Zn, Co, Fe, Mn, Sb are used to obtain immunotropic antiviral preparations. DNA-Na to metal ratio in the preparation is within range 50-500:1-2. Immunotropic non-resistant apyrogenic preparations possess antiviral activity towards RNA- and DNA-viruses and their combined presence.

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THE METHOD OF OBTAINING IMMUNOTROPIC ANTIVIRAL PREPARATION

FIELD OF THE INVENTION

This invention relates to technology of obtaining biologically active compounds, the invention may be used in biotechnology and chemical and pharmaceutical industry, and the product may be used in medicine, veterinary medicine and for scientific research. The invention relates to the method of obtaining preparations possessing high immunotropic and antiviral activity towards RNA- and DNA-viruses, these preparations being obtained from animal material (soft roe of sturgeon and salmon fish, calf thymus, chicken blood, pig spleen).

BACKGROUND

Within recent years many different compounds possessing antiviral properties were obtained from plant and animal materials. Recently in the process of searching for new bioactive materials investigations were aimed at the components of nuclear and cellular structures as well as to extraction and purification of biologically active materials from those structures. Different publications about extraction and purification of genetic material of plants and animals were identified.

The method of obtaining DNA-RNA hybrid possessing immunotropic antiviral activity is known. However the named property of this hybrid is weak because of related RNA-DNA activity (RU 2108796, 1998).

There is a known antiviral immunotropic agent (RU 2158592, 1999). But this agent is obtained from sodium salt of DNA which characteristics are not indicated and trivalent ferric oxide. The method of obtaining this agent is essentially omitted in the description. This agent possesses high pyrogenicity and cannot be used as a pharmaceutical preparation.

The agent described in RU 1833547, 1991, possessing antiviral activity is similar to the suggested invention.

However, the description of the method of obtaining antiviral complex is omitted in this patent and the essence of the process is not shown. Further the obtained agent with antiviral properties cannot be used as a therapeutic preparation because of its high pyrogenicity and toxicity.

The method of obtaining DNA-based antiviral preparation as described in RU 2108797, 1998, is the most related to the suggested invention considering operations in the production process.

According to this method such additives as peroxide, hydroperoxide, chlorates, permanganates, chromates, diazoamino-compounds etc. reducing pH and promoting DNA degradation are added to the reaction at the stage of sonicating highly diluted solution that leads to DNA destruction. Besides soluble proteins and insoluble protein mass as well as high-polymeric DNA react with additives. However practical quantitative content of ingredients in animal material always varies. That is why this process cannot lead to a pharmaceutical preparation of a stable standardized composition.

The main disadvantages of this method are:

- the nuclei lysis is realized at high dilution of reaction mass that leads to uncontrolled DNA degradation and denaturation;
- addition of the above mentioned compounds at the stage of sonication reduces pH of the reaction mass that leads to DNA denaturation;
- anions introduced by additives also lead to DNA degradation;
- metal salts added to the reaction destruct the DNA double helix (Nucleotide chemistry, A.Mickelson, MIR, Moscow, 1966).

Further, the subsequent product precipitation with 70% ethanol intensifies the process of DNA destruction and denaturation.

The product obtained according to this method consists of high-molecular polymer of DNA, RNA, protein with addition of metal, this product being characterized by high pyrogenicity and lack of biological activity (characterized by hyperchromic effect).

SUMMARY OF THE INVENTION

In one embodiment, the invention relates to immunotropic antiviral apyrogenic non-toxic preparations and uses thereof and methods for obtaining same. In one embodiment, the preparations can be used in the preparation of a medicament or a vaccine against RNA- and DNA- viruses.

In another embodiment the preparations can be used in the treatment of RNA- and DNA-viral infections and/or as a vaccine against RNA- and DNA-viruses.

In one embodiment, the invention provides a method of obtaining immunotropic antiviral apyrogenic non-toxic preparations based on sodium salt of native low-molecular deoxyribonucleic acid (DNA-Na) comprising grinding and homogenization of animal material in citrate-salt solution, homogenate incubation in the presence of detergent, adding of sodium chloride solution with obtaining of a reaction mass, sonication, subsequent purification and isolation of the desired product, wherein defatted soft roe of sturgeon fish or salmon fish, or calf thymus, or chicken blood, or pig spleen are used as the animal material, said reaction mass is sonicated for 80 minutes at 8 kHz-35 kHz, treated with sorbent, filtered and concentrated by baromembrane method, DNA-Na white amorphous precipitate is isolated with 96% ethanol, DNA-Na molecular mass is 270-500 kD, hyperchromic effect is not lower than 37%, protein content is less than 1 %, then DNA-Na precipitate is dried and dissolved in standardized solution of water-soluble salt of transition metal selected from Zn, Co, Fe, Mn or Sb to make 1.5% pharmaceutical immunotropic complex DNA-Na-Me with hyperchromic effect not lower than 10%, then said immunotropic complex DNA-Na-Me is sterilized and poured.

In one embodiment of the invention the DNA-Na to metal ratio in the preparation is within range 50-500 : 1-2.

In another embodiment, the invention provides an immunotropic preparation possesses antiviral activity towards RNA- and DNA-viruses and their combined presence.

In yet another embodiment, the invention provides an immunotropic antiviral apyrogenic preparation characterized in that the preparation is 1.5% complex DNA-Na-Me in the solution of water-soluble salt of transition metal selected from Zn, Co, Fe, Mn and Sb, and the said complex has hyperchromic effect not lower than 10% and ratio DNA-Na to metal is within 50-500 : 1-2, wherein the said preparation possesses antiviral activity towards RNA- and DNA-viruses including their combined presence.

In one embodiment the immunotropic antiviral apyrogenic preparation of the invention can be obtained by the methods described herein.

DETAILED DESCRIPTION OF THE INVENTION

In general the present invention relates to processes for obtaining new medicinal preparations based on native low-polymer DNA and using these preparations in the treatment of different complex diseases. This invention presents an improved method of industrial

production of biologically active materials possessing the complex of therapeutic properties and lacking toxicity, mutagenicity and carcinogenicity.

The technical result of this invention consists in obtaining immunotropic antiviral pharmaceutical preparations possessing high therapeutic effect towards RNA- and DNA-

viruses and their combined presence. These preparations are complex compounds possessing high immuno-modulating properties leading to increase in CD4 immunocompetent cells content after the course of treatment (table 5) at hyperchromic effect not lower than 10%, and high antiviral activity towards multiple infections and also under combined presence of the infections (decrease in viral loading by 2 log). These preparations are nontoxic, apyrogenic and cause no resistance (tables 7, 1, 5). Such products of native DNA sodium salt were not described in any previously known information sources.

The essence of the invention consists in the method of obtaining immunotropic antiviral apyrogenic non-toxic preparations based on native low-polymer desoxyribonucleic acid sodium salt (DNA-Na), these preparations being metal-complexes.

Defatted soft roe of sturgeon or salmon fish or calf thymus or chicken blood or pig spleen are ground and homogenized in citrate-salt solution, the homogenate is incubated in the presence of detergent, then under continuous stirring at high temperature concentrated sodium chloride solution is added. The resulting reaction mixture is cooled, sonicated, treated with sorbent and detergent, filtrated and concentrated. The amorphous white DNA-Na residue is then precipitated from the concentrate with ethanol. The molecular mass of the residue is 270-500 kDa, hyperchromic effect is higher than 37%, protein contents is less than 1 %. The DNA-Na residue is then dried in mild conditions (40°C-60°C) being the function of high purification level and distinct molecular mass fragmentation. The calculated amount of DNA-Na powder for obtaining 1.5% concentration is dissolved in an analytically prepared standardized solution of transition metal selected from Zn, Co, Mn, Fe, Sb, with DNA-Na to metal ratio of 50-500 : 1-2 that guarantees complete chemical reaction and creation of preserving effect of non-resistant pharmaceutical preparation, hyperchromic effect of the preparation must not be lower than 10%. The preparation is then sterilized by baromembrane method (d_{pore} 0.45μm and 0.22 μm) and poured in therapeutic doses of 75 mg under sterile conditions.

The conditions of interaction of DNA-Na and metal (of the aforesaid row) at the calculated ratio ensure long stability of the preparation because of the preserving effect appearing in the process of dissolving.

The above-mentioned properties of the preparations provide the opportunity to treat not only the main disease but concurrent diseases too even in the process of single-drug therapy. For example the high therapeutic effect was shown for HIV-infected and AIDS

patients with concurrent diseases of hepatitis C, herpes, cytomegalovirus infection. The preparations of the invention may be administered parenterally, through rectum or externally.

EMBODIMENT OF THE INVENTION

Defatted soft roe of sturgeon fish (1 kg) or defatted soft roe of salmon fish (1 kg) or calf thymus (1 kg) or chicken blood (1 L) or pig spleen (1 kg) are ground in mincer, homogenized in 2 L of citrate-salt solution (0.15M NaCl, 0.015M Na-citrate) and placed to the reactor with warmed citrate-salt solution (3 L), 10 L of 6% solution of sodium dodecylsulphate in 45% ethanol are added. The mixture is incubated at 60°C for 1.5 h under continuous stirring. Then the equal volume of 5M NaCl solution is added to the mixture and the process is continued under the same temperature for another 1.5 h. Then the mixture is cooled to room temperature and sonicated for 80 min at 8kHz, 2 W/cm^2 and bending moment of 0.3-0.5 μm. The sonicated mass is mixed with kieselguhr at the ratio of 5:1 (volume:mass) and the liquid phase is separated at nutch filter. After micro-filtration the liquid phase is concentrated by baro-membrane method (d_{pore} 0.45 μm). DNA-Na precipitate is isolated from the concentrate with 96% ethanol. The precipitate is thoroughly washed with 70% ethanol and dried at 40-60°C. The molecular mass of the native low-polymer DNA-Na is 270-500 kDa, hyperchromic effect is not lower than 37%, main substance contents are 90-100.5%, protein contents is less than 1 %. In order to get immunomodulating antiviral preparation the calculated amount of DNA-Na is weighed and dissolved in analytically prepared standardized solution of the calculated amount of transition metal selected from Zn, Mn, Fe, Co, Sb. The amounts of ingredients are calculated according to the necessary ratio. The dissolving is carried out under normal temperature (20°C-22°C) and pressure under continuous stirring and incubation during 6 hrs. The hyperchromic effect of the product is not lower than 10%. The further sterilization of the preparation is realized by the baro-membrane method with pore size of 0.45 and 0.22 μm and consequent pouring in therapeutic doses (75 mg) under sterile conditions.

As it is shown in the table 1 the preparations obtained according to the said method do not exhibit pyrogenicity and cytotoxicity at the high therapeutic activity. The hyperchromic effect of the preparations in all presented examples is not lower than 10% that confirms the immunotropic activity of the preparations.

EXAMPLES

Example 1.

1 kg of defatted soft roe of sturgeon fish is ground in mincer, homogenized in 2 L of citrate-salt solution (0.15M NaCl, 0.015M Na-citrate) and placed to the reactor with warmed citrate-salt solution (3 L), 10 L of 6% solution of sodium dodecylsulphate in 45% ethanol are added. The mixture is incubated at 60°C for 1.5 h under continuous stirring.

Then the equal volume of 5M NaCl solution is added to the mixture and the process is continued under the same temperature for another 1.5 h. Then the mixture is cooled to room temperature and sonicated for 80 min at 8kHz, 2 W/cm^2. The sonicated mass is mixed with kieselguhr at the ratio of 5:1 (volume:mass) and the liquid phase is separated at nutch filter. After micro-filtration the liquid phase is concentrated by baro-membrane method (d_{pore} 0.45 μm). DNA-Na precipitate is isolated from the concentrate with 96% ethanol. The precipitate is thoroughly washed with 70% ethanol and dried at 60°C. Then the calculated amount of metal selected from the said row is dissolved in apyrogenic water, the calculated amount of DNA-Na powder is added to the metal solution to obtain 1.5% concentration, then the solution was sterilized through membranes with pore size of 0.22 μm and poured in therapeutic doses of 75 mg in sterile conditions.

Example 2.

1 kg of defatted soft roe of salmon fish is ground in mincer, homogenized in 2 L of citrate-salt solution and then treated as described in Example 1. The preparation is obtained in the solution of one of the metals providing the ratio according to table 1.

Example 3.

1 kg of calf thymus is ground and homogenized in 2 L of citrate-salt solution. The process is realized as described in Example 1 up to the stage of barofiltration. Then the liquid phase is concentrated through the membrane with pore size of 0.8 μm and the process is carried out as described in Example 1. The preparation is obtained in the solution of one of the metals providing the ratio according to table 1.

Example 4.

1 L of chicken blood is homogenized in 2 L of citrate-salt solution. The process is realized as described in Example 1 but the time of sonication is 10 min at 2 W/cm^2 and 35

kHz, the ratio of kieselguhr and reaction mixture was 1:25. Then the process is carried out as described in Example 1. The preparation is obtained in the solution of one of the metals providing the ratio according to table 1.

Example 5.

1 kg of pig spleen is ground and homogenized in 2 L of citrate-salt solution. The process was realized as described in Example 1 up to the stage of barofiltration. Then the liquid phase is concentrated through the membrane with pore size of 0.8 μm. DNA-Na was precipitated with ethanol, alcohol to concentrate ratio is 1:2. The precipitate is dried at 50°C-60°C. The preparation is obtained in the solution of one of the metals providing the ratio according to table 1.

Example 6.

The process is realized as described in Example 1 up to the stage of sonication. Then the reaction mass is mixed with kieselguhr (5:1) and filtrated on nutch-filter. DNA-Na is isolated from the filtrate by twofold re-precipitation with ethanol without preliminary concentration, the powder precipitate is dried at 40°C. The preparation is obtained in the solution of one of the metals providing the ratio according to table 1.

The suggested accurate consequence of process operations and parameters of the process provide the technical result, also the result is achieved by subsequent using highly purified DNA-Na with strictly fragmented molecular mass and the main substance content of 90-105.0% and analytically prepared metal solution. These options were not achieved in processes described in any of the known information sources. The complex of operation parameters of the method provides stability of immunotropic and antiviral properties of obtained non-toxic apyrogenic preparations with hyperchromic effect not lower than 10% and that distinguishes the claimed invention from the known background.

Thus the suggested method of the invention allows to obtain the preparations providing the possibility of treating multiple infections, for example HIV+herpes+hepatitis C (results are shown in tables 5 and 6), in the course of monotherapy, these preparations possessing high immunomodulating properties and lacking pyrogenicity and resistance.

INDUSTRIAL APPLICABILITY

The stable human cell line MT-4 sensitive to RNA- and DNA-viruses was used to determine in vitro antiviral activity of obtained DNA-Na-Me preparations containing metal according to table 1. The cells were grown in RPM 1640 medium in concentration of (0.5-0.7)x10^6 cells in 1 ml of 20% fetal serum. Before the preparation was introduced into cell suspension the cells were infected with concentrate of cultural liquid of H9/IIIB cells producing HIV-1. The infection multiplicity was 0.01 CTD_{50} per cell. The cultures were incubated at 37°C under 5% CO_2 and 98% humidity atmosphere for 4-7 days, then nutrient medium was changed. The cytopathic effect of the virus in cell culture was determined on the 4-7th day of the experiment. The viability increase up to 70.7-85.2% was observed for the cells preliminary infected with the virus and treated with the preparations in concentrations of 600-1500 mg/ml correspondingly (control of non-infected cells – 89.6%, control of virus-infected cells – 29.3% on the 4th day post infection). The results of in vitro antiviral effect towards HIV-1 estimation for the obtained immunotropic preparations are shown in table 2.

The activity of the preparations presented in table 2 towards human cytomegalovirus infection was studied in vitro on the model of human diploid cells M-19 infected with cytomegalovirus. When the preparations were used in different concentrations the virus titres decreased by 1-2 lg that confirms the antiviral activity of the preparations of the invention.

The activity of the preparations towards human simplex herpes virus was studied on the model of mice herpes encephalitis caused by this virus. The study of protective effect against lethal herpetic infection was carried out in comparison with protective effect of interferon inducer redostin that is used now as an antiherpetic preparation. The preparations of the invention were introduced once intraperitoneally in the dose of 100 μg/kg 24 hrs before infection. The animals were infected intracerebrally in the dose of 3 lg of LD_{50}. Each group consisted of 30 mice. The results were estimated by survival (in %) of animals to the 15th day and by protective effect (in %) with respect to survived animals in control group. To the 15th day 96% of control mice died whereas 70% of mice survived in groups that were given preparation obtained according to the invention.

Considering that protective effect against lethal herpetic infection is observed under severe conditions of the experiment – intracerebral infection, high infective dose of the virus, single peripheral dosing of the preparation- it's possible to say that the preparation ob-

tained according to the invention possesses moderately expressed antiherpetic effect. The results of antiviral activity of the preparations in HIV-infected patients with hepatitis C are shown in table 6. The decrease in viral loading in HIV-infected patients under multiple viral infection is shown in table 7.

Immunotropic properties of the preparations of the invention were determined by morphometric method. The essence of the method is following. The immunodeficiency state was generated in mice by single intraperitoneal introduction of hydrocortisone acetate in the dose of 0.2 mg/mouse. 48 hrs after introduction single intraperitoneal injections of the DNA-Na preparations were made in super small doses during 3 days. The experimental study of the immunological activity of the preparations was carried out on 154 white mice and 98 hybrid CBA mice of both sex of 18-24 g in weight. The dosage was determined according to data from literature. Physiological solution was used as a control. The animals were slaughtered after 18, 36, 60 and 84 hours. Immunotropic properties appear in relative thymus mass decrease and as a rule in relative spleen mass increase.

The experiment results show that relative thymus mass decreases by 26% not depending on the preparation dose. No reliable difference in spleen mass was detected.

The effect of small doses of the preparation on thymus may be considered as the most characteristic factor of increase in mice immune system functional activity. The results of the experiment are shown in table 4. As it follows from the table DNA-Na-Fe preparation possesses evident immunotropic activity appearing in its ability to decrease mice relative thymus mass in small doses ($1*10^{-5}$ g/ml, $1*10^{-7}$g/ml, $1*10^{-9}$ g/ml), dose dependence being not detected. The preparation effect is marked after 18 hours (20.7%), maximum peak falls on 36 hours (28.6%), full normalization comes after 80 hours. DNA-Na-Sb, DNA-Na-Co, DNA-Na-Zn, DNA-Na-Mn possess the same immunotropic properties. Data of table 5 show the effect of the preparations on decrease in immunity CD-4 lymphocyte level in patient with multiple infection.

According to proposed method of obtaining immunotropic antiviral non-toxic apyrogenic preparations it is necessary to fulfill the process and operations under proposed parameters in stated order.

It guarantees long-term stability of immunomodulating and antiviral properties of the preparations obtained according to proposed method and long effective life of the preparations.

Table 1

Metal	Metal/DNA-Na ratio	Cytotoxicity results in vitro	
		Cells in 1 ml of culture x10^6	Cells viability, %
Zinc	1/50	0.89	0.86
	2/50	0.89	0.80
	1/150	0.89	0.83
	1/500	0.89	0.87
Iron	1/50	0.89	0.88
	2/50	0.89	0.86
	1/150	0.89	0.82
	1/500	0.89	0.87
Cobalt	1/50	0.89	0.81
	2/50	0.89	0.79
	1/150	0.89	0.81
	1/500	0.89	0.82
Manganese	1/50	0.89	0.86
	2/50	0.89	0.80
	1/150	0.89	0.82
	1/500	0.89	0.82
Stibium	1/50	0.89	0.84
	2/50	0.89	0.80
	1/150	0.89	0.82
	1/500	0.89	0.85

Table 2

DNA-Na-Me preparation 1:200 800 μg/ml	Cells viability, %				Syncytium formation. Cytopathic effect of the virus		Immune-enzyme analysis, optical density
	Non-infected		Infected				
	Day						
	4th	7th	4th	7th	4th	7th	
Cells control	89.6	82.3	-	-	-	-	0.104
Virus control	-	-	29.3	2.4	++++	++++	0.737
DNA-Na-Fe	87.5	84.6	78.6	35.4	+	++	0.248
DNA-Na-Zn	83.1	81.9	60.0	30.1	++	++	0.326
DNA-Na-Co	86.0	80.5	79.1	76.7	++	++	0.285
DNA-Na-Sb	83.5	75.3	68.0	29.7	++	++	0.487
DNA-Na-Mn	84.1	80.4	70.5	31.3	+	++	0.236
Azidothymidine 1.0	74.2	49.1	57.1	23.8	-	++	0.186

Table 3

Mice herpes encephalitis, type 1, L2 strain				
Preparation	Dose mg/kg	Scheme of intraperitoneal introduction	Survival %	Protective effect %
1	2	3	4	5
DNA-Na-Fe	100	24 hrs before infection	23.5	20
DNA-Na-Sb	100	The same	20	18
DNA-Na-Co	100	The same	25	22.3
DNA-Na-Zn	100	The same	18	15
DNA-Na-Mn	100	The same	15	12
Redostin	5	The same	30	28

Table 4

Time	Thymus (% of body weight)		Spleen (% of body weight)	
	Experiment DNA-Na-Fe	Control (physiological solution)	Experiment DNA-Na-Fe	Control (physiological solution)
18 hrs	0.230±0.009* n=18	0.29±0.021 n=18	0.501±0.027 n=18	0.490±0.026 n=18
36 hrs	0.253±0.03** n=20	0.353±0.014 n=20	0.591±0.045 n=20	0.506±0.036 n=20
60 hrs	0.28±0.018* n=20	0.35±0.023 n=20	0.52±0.05 n=20	0.54±0.038 n=20
84 hrs	0.30±0.019 n=19	0.304±0.015 n=19	0.61±0.053 n=19	0.53±0.038 n=19

* - $p<0.05$ for experiment in comparison with control

** - $p<0.01$ for experiment in comparison with control

Table 5

DNA-Na-Sb *

Patient	Diagnosis	Duration of disease	Infection ways	CD-4 count (% of abs)			
				Before therapy	After therapy	1.5 months after therapy	Repeated therapy
1	HIV A2	12 months	Parenteral	20%-312 0.28	25%-522 0.23	26%-574	28%-665
2	HIV B3 herpes candidiasis	15 years	Homosexual	19%-319 0.25	16%-359 0.30	21%-417	27%-588
3	HIV C3 herpes, cardiac ischemia	12 years	Homosexual	11%-146 0.20	10%-109 0.20	20%-258	23%-476
4	HIV A1 recurrence, hepatitis C	4 months	Parenteral	34%-909 0.77	24%-691 0.62	33%-882	36%-980
5	HIV A2 chronic viral hepatitis C	4 months	Parenteral	15%-386 0.22	16%-666 0.38	22%-580	25%-730

* - the preparation was introduced parenterally, 150 mg/day during 14 days

Table 6

AlAt dynamics in HIV-infected patients with chronic viral hepatitis C

DNA-Na-Fe

Periodicity of examination	Groups of patients	
	DNA-Na-Fe therapy	Basic therapy
Before therapy	285.6±30.5	266.4±28.6
1 month after therapy	84.4±9.2	248.5±26.2
3 months after therapy	36.2±4.2	127.6±18.4
6 months after therapy	34.8±3.6	88.6±11.2

20 patients were involved into experiment (in groups of 10)

Chronic viral hepatitis C was diagnosed on the basis of clinical data, anti-HCV-cor-M detection, hyper enzymia (increase in AlAt level 3-5 fold in comparison with standard during 2-5 months). PCR study for HCV RNA - positive result.

Table 7

Change in viral loading in HIV-infected patients under DNA-Na-Me therapy.

Factor	Weeks of study	
	4	8
Average of HIV RNA concentration change (log 10/ml)	-1.2	-2.0
P (0.05)	<	<

Claims

What is claimed is:

1. A method of obtaining an immunotropic antiviral apyrogenic non-toxic preparation based on sodium salt of native low-molecular weight deoxyribonucleic acid (DNA-Na) comprising:
(a) grinding and homogenization of animal material in citrate-salt solution to obtain an homogenate,
(b) incubating the homogenate in the presence of detergent,
(c) adding sodium chloride solution to obtain a reaction mass,
(d) sonicating the reaction mass, and subsequent purification and isolation of a DNA-Na white amorphous precipitate product,
wherein the animal material is selected from the group consisting of: defatted soft roe of sturgeon fish or salmon fish, calf thymus, chicken blood, and pig spleen,
said reaction mass is sonicated for 80 minutes at 8 kHz-35 kHz, treated with sorbent, filtered and concentrated by baromembrane method resulting in a product from which the DNA-Na white amorphous precipitate is isolated with 96% ethanol,
and wherein the DNA-Na molecular mass is 270-500 kD, having a hyperchromic effect not lower than 37%, and a protein content less than 1 %,
(e) drying the DNA-Na white amorphous precipitate and dissolving it in standardized solution of water-soluble salt of transition metal selected from Zn, Co, Fe, Mn or Sb to make 1.5% pharmaceutical immunotropic complex DNA-Na-Me with hyperchromic effect not lower than 10% and then,
(f) sterilizing and pouring said immunotropic complex DNA-Na-Me.

2. A method of claim 1, wherein DNA-Na to metal (Me) ratio in the preparation is within range 50-500 : 1-2.

3. A method of claim 1, wherein the immunotropic antiviral apyrogenic non-toxic preparation possesses antiviral activity towards RNA- and DNA-viruses and their combined presence.

4. An immunotropic antiviral apyrogenic preparation obtainable by a method of any one of claims 1 to 3, characterized in that the preparation is 1.5% complex DNA-Na-Me in the solution of water-soluble salt of transition metal selected from Zn, Co, Fe, Mn and Sb, and the said complex has hyperchromic effect not lower than 10% and ratio DNA-Na to metal is within 50-

500 : 1-2, wherein the said preparation possesses antiviral activity towards RNA- and DNA-viruses including their combined presence.

5. Use of a preparation of claim 4 for the preparation of a medicament or a vaccine against RNA- and DNA-viruses.