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Biotechnology For The Production Of Veterinary Drug Nukleinat Sodium From Microalgae.

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ABSTRACT

Prospects in the field of development and application of preparations on the basis of nucleic acids mainly for veterinary medicine are considered. The article presents the main stages of biotechnology preparation of sodium nucleinate. The activity and toxicity of the sodium nucleinate obtained from Chlorella vulgaris microalgae on the cells of the infus genus Stylonychia mytilus were screened and the prospects of further testing on laboratory and productive animals were presented.

Keywords: sodium nucleinate, biotechnology, DNA, RNA, veterinary medicine, Chlorella vulgaris, microalgae.

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INTRODUCTION

At the heart of any pathological process, as it is known, there is a violation of the protective functions of the animal body. Various immunocorrecting and immunostimulating biological agents are increasingly used to restore nonspecific and specific resistance in veterinary medicine.

At present, the list of immunomodulatory agents in both medicine and veterinary medicine is continuously increasing and supplemented with all possible synthetic drugs and drugs of natural origin. To a greater extent, preference should be given to drugs of natural origin [1]. Currently such drugs are preparations based on nucleic acids or their constituent components, purine and pyrimidine nucleotides [2].

Interest in the use of nucleic acids as drugs with bactericidal and immunological effects, as well as the ability to provide general resistance to external irritants, arose long time ago, as evidenced by the accumulated numerous data [3]. There are a lot of works, at the moment, which are devoted to the processes of directed action of nucleic drugs on the body, their selective effect on cell metabolism, as well as on the prevention and elimination of various pathologies and diseases [1-10]. It is known from history that nucleic acids were successfully used in the treatment of tuberculosis, cholera, diphtheria, anthrax, lupus and even many coccal infectious diseases [8, 9].

One of the first domestic preparations of nucleic acids with directed immunological action, which was obtained from microorganisms and milk of salmon fish, is sodium nucleinate. Basically, the drug of nucleic nature is represented by a small list of pharmaceutical drugs such as “Derinat”, “Biostim”, “Ridostin”, “Ferrovir”, etc.

Sodium deoxyribonucleate (or DNA-Na) is able to activate antimicrobial and antiviral immunity at the humoral and cellular levels, as it has interferogenic activity. The interaction of humoral and cellular immunity proceeds in such a way that at the initial stages of infection the cellular protection comes into effect first, but its activity has a limited period [22].

The drug “Derinat”, as indicated by manufacturers, regulates hematopoiesis, while normalizing the number of leukocytes, lymphocytes, phagocytes, platelets and granulocytes, as well as improves microcirculation in the heart muscle and myocardial contractility, which increases tolerance to physical activity, especially when walking, reducing pain in the calf muscles. As a preparation based on nucleic acids, “Derinat” has healing and regenerative effects, interacting at the molecular and cellular levels.

Biotechnology for the production of sodium nucleinate is carrying out various hydrolysis processes of cellular mass of biological material followed by purification of the hydrolysate by precipitation from it of the nucleic acid in the form of whisker of a white precipitate and then washing and drying the finished product[22].Usually, this drug is isolated from the milk of salmon fish and monoculture of baker’s yeast (Saccharomyces cerevisiae), as well as a number of other sources rich in nucleic acids [1]. The preparations obtained in the form of sodium salt of low molecular weight RNA or DNA from the above sources belong to different groups of preparations based on nucleic acids [2, 3].

Of course, the entire line of nucleic acid products, regardless of the type of biological material, is aimed at the treatment of immunopathological, metabolic disorders, restoration of impaired functions of the body with structural changes, elimination of disorders of nucleic metabolism, anemia, stimulation of hematopoiesis, interferon induction and resistance to radiation and infections, having an expressed antiviral activity in relation to immunodeficiency viruses. It is also believed that nucleic acids, having a powerful pharmacological effect, mainly stimulate leukopoiesis, increase the activity of T-killers and T-helpers, the activity of macrophages, neutrophils and all cells of the immune system, supporting the processes of regeneration and repair, have antioxidant properties.

As you can see, the scope of nucleic acid preparations is quite extensive. For the vast majority of known drugs (with DNA >90%), sturgeon milk is used as a raw material. The choice of raw materials is due to the fact that the sturgeon DNA is quite ancient in age, and the transmission of genetic features and possible infections with the drug is minimized, as well as the structural parameters of sturgeon DNA have a significant similarity with the DNA of leukocytes of higher mammals [5].

Purpose and objectives of the work

The purpose of our work was to develop a biotechnological process for obtaining a new therapeutic and prophylactic preparation of sodium nucleinate from green microalgae Chlorella vulgaris in an experimental form, which, we hope in the near future will be added to the list of drugs of nucleic nature, possibly having a new pharmacological effect.

To achieve the goal, a number of tasks were solved. The necessity was the screening tests of the obtained preparation for activity and toxicity, using the culture of single-celled microorganisms, such as infusorium of the Stylonychia mytilus genus for biotesting; the study of qualitative and quantitative features of the obtained preparation, which is the most important task to ensure high product performance in quality control.

MATERIALS AND METHODS

The biomaterial for the preparation of sodium nucleinate was the culture of microalgae cells Chlorella vulgaris (strain IGF № C-111), the biomass of which was obtained by precipitation using a laboratory centrifuge (CENTRIFUGECM-6M, for 10 minutes at 3500 rpm) from the suspension preparation “AlgaeVet”, the main component of which is microalgae [11-14]. Chlorella vulgaris is the most common and traditional representative of the genus of green unicellular, coccoid forms of algae, which reproduce by autospores [15].

Chlorella got its name because it contains, in a significant amount, useful green pigment chlorophyll (from Greek “chloros”– green, and Lat. “ella” – small). Microalgae were discovered by Danish microbiologist Beijerink M. in 1890, which was cultivated as a rich source of protein, amino acids, chlorophyll, vitamins, fats, carbohydrates, macro - and microelements, and a number of other useful components [16-18].

Deep study of these microalgae on morphological, biochemical, physiological, as well as molecular genetic principles, contributed to a significant enrichment of knowledge, both about the cell itself and its chemical composition, which is confirmed by a large number of scientific publications in this field [19-35].

The unique properties and no less unique biochemical composition of the green single-celled microalgae Chlorella vulgaris (Beyerink) put it in the category, one of the most interesting for scientific research, biological objects. The resulting cell biomass sediment was a thick paste-like concentrate, which was placed in a freezer at -20°C, followed by a double thawing stage to destroy the cell wall.

To obtain the preparation of sodium nucleinate from microalgae, the method developed by us was used. Using solutions of sodium chloride, sodium citrate, with certain concentrations, citrate-salt hydrolysis of microalgae biomass, previously freed from lipids and pigments, was carried out in a round-bottom reaction flask with three discharge tubes (for a paddle stirrer, a refrigerator and a mercury thermometer with a division of up to 300°C) for 3 hours at a temperature of 100°C (±2°C), and then using special methods of precipitation of nucleic acids in chilled ethyl alcohol (2 parts alcohol and 1 part hydrolysate), an experimental preparation of sodium salt of nucleic acid was received. Also, an option for deposition may be isopropyl alcohol (1:1) or acetone (1:1).The resulting reaction liquid was placed in a freezer at -20°C. As a result, the resulting flake sediment was collected by means of a centrifuge (3500 rpm for 5 min) in a plastic centrifuge tube, and the supernatant was discarded. The precipitate, which is a nucleic acid salt was dried in nitrogen current and ground into a dispersed powder. Quantitative and qualitative assessment of the obtained preparation was performed using conventional methods [36-38].

The drug was obtained in the laboratory on the basis of OBU “Kursk regional veterinary laboratory” in compliance with all the rules and regulations of the laboratory experiment. The experiments and studies used modern equipment with the right expertise timely passed the verification stages.

Equipment and reagents

The following basic laboratory equipment, instruments and reagents were used in the preparation of the veterinary drug sodium nucleinate from the green microalgae Chlorella vulgaris:

- laboratory centrifuge “CENTRIFUGECM-6M”, (ELMI, Latvia);
- centrifuge tubes with 50 ml volume caps, (Nunc, Denmark);
- freezer“Atlant”, (Russia);
- sodium chloride GOST 4233-77, (Russia);
- 5.5-water citric acid sodium GOST 22280-76, (Russia);
- ethyl alcohol GOST 18300-72, (Russia);
- isopropyl alcohol GOST 9805-84, (Russia);
- distilled water GOST R 58144-2018, (Russia);
- the cells of the microalgae Chlorella vulgaris, (“AlgaVet”, Russia);
- culture of ciliates of the Stylonychia mytilus genus, (Russia);
- laboratory fume hood “Lab-PRO-FH», (LOIP, Russia);
- three-neck round-bottom flask for hydrolysis 500 ml, (ACROS, Russia);
- Dimroth refrigerator “KHSV-400”, (Prime Chemicals Group, Russia);
- high temperature thermometer up to 300°C, (Russia);
- flask heater “LT-250”, (Labtech, Russia);
- mechanical dispensers (1-5 ml; 100-1000 µl) («Sartorius», «BioHit»);
- binocular MBS-9 with magnification 2×8 и 2×14, (LOMO, Russia);
- binocular microscope “MIKMED-1” (LOMO, Russia).

Preclinical testing of the drug (checking toxicity and activity)

Screening tests for the toxicity and activity of the nucleic acid preparation obtained by us by biotesting on cell culture Stylonychia mytilus were performed, guided by the requirements of GOST 31674-2012 and based on a number of publications on the determination of toxicity [39,40].The binocular microscope MBS-9 with magnification of 2×8 and 2×14 (LOMO, Russia) and a special well plate microaquarium were used in the calculation of the survivors of stylionihii.

The obtained and safety-tested nucleic acid preparation is an experimental model and is currently at the stage of preclinical tests, which provide for almost the entire range of biochemical and physiological parameters, according to the rules of good laboratory practice (GLP) and further clinical trials of the drug according to the rules of good clinical practice (GCP), first in laboratory, and then in productive farm animals [41].

RESULTS AND DISCUSSION

Thus, the preparation of the sodium salt of DNA from the microalgae Chlorella vulgaris was mastered and the author’s technique of the whole biotechnological process was developed.

In the course of scientific research, we developed a bioreactor and adapted it to the release of nucleic acids from Chlorella. This biomaterial was chosen by us due to the fact that the microalgae Chlorella vulgaris is the oldest representative of cellular biocenosis, and having a high nutritional value, which was also confirmed by our own studies [42].

According to numerous data, against the background of a rich biologically nutritious composition, Chlorella cell contains a significant amount of DNA and RNA, the genetic age of which is more than 2 billion years. It is believed that more ancient DNA of Chlorella can surpass the nucleic acid of sturgeon and salmon fishes in some cases. But this assumption can be resolved only in a comparative study.

Figure 1 shows a view of the green microalgae Chlorella vulgaris at 100 x magnifications through the binocular microscope “MIKMED-1” (LOMO, Russia).

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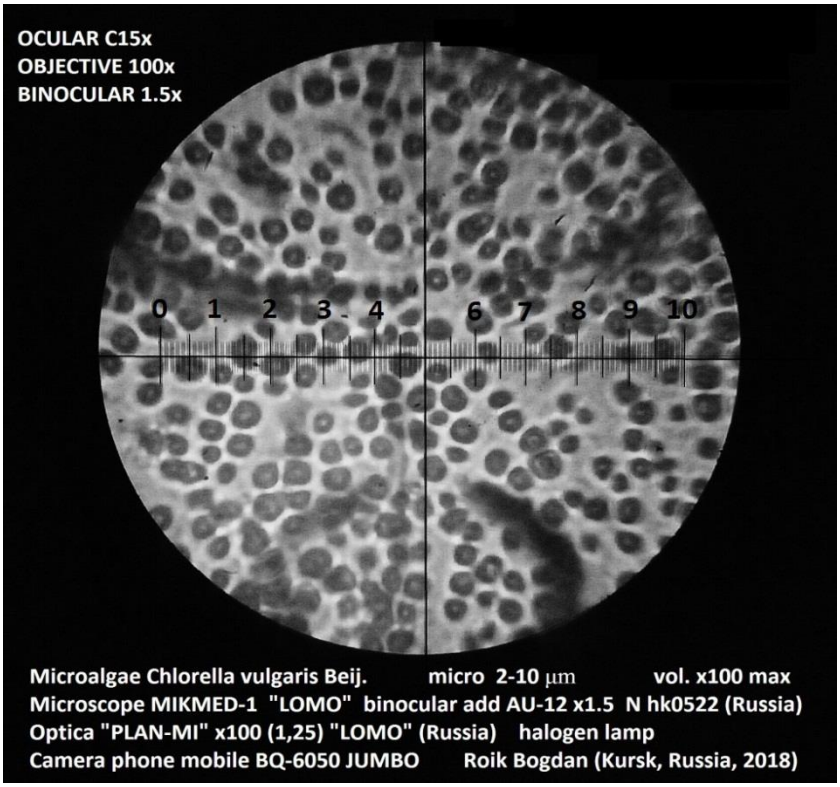

Fig 1: Microalgae Chlorella vulgaris Beijerink at 100x magnification

As it can be seen from the figure, the cells of Chlorella microalgae are very small and range from 2 to 10 microns. However, Chlorella cells contain quite a large amount of RNA and DNA.

Analysis of our drug on toxicity, using a biological test system in the form of infusory of Stylonychia mytilus genus, in comparison with the reference similar to the action of the drug “Derinat”, also showed us a clear absence of signs of negative effects on the cell[43].

It should be noted that the infusoria retained activity and survival even on the second day of screening studies, which confirms the active properties of the drug and its non-toxicity. The results of studies of the immunological drug “Derinat” are presented in table 1 [43].

Table 1: The results of the reaction of Stylonychia on the preparation “Derinat” after 3 and 24 hours of exposure

Name of a range of wells	The number of active Stylonychia in the wells of the microaquarium											
	in the beginning of the experiment						after 3 hours					
	№1	№2	№3	№4	№5	N_1	№1	№2	№3	№4	№5	N_2
Experimental	22	25	20	21	25	113	22	23	20	20	22	107
Control	24	20	19	23	18	104	23	19	19	20	18	99
Name of a range of holes	The number of active Stylonychia in the wells of the microaquarium											
	in the beginning of the experiment						after24hours					
	№1	№2	№3	№4	№5	N_1	№1	№2	№3	№4	№5	N_2
Experimental	22	25	20	21	25	113	21	20	19	20	21	101
Control	24	20	19	23	18	104	20	15	16	19	15	85

Table 2 presents the results of comparative studies of preparation sodium nucleinate made by us from microalgae Chlorella vulgaris.

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Table 2: The results of the reaction of Stylonychia on the nucleic preparation from microalgae Chlorella vulgaris after 3 and 24 hours of exposure

Name of a range of wells	The number of active Stylonychia in the wells of the microaquarium											
	in the beginning of the experiment						after 3 hours					
	№1	№2	№3	№4	№5	N_1	№1	№2	№3	№4	№5	N_2
Experimental	19	25	18	21	15	98	18	21	20	19	15	93
Control	20	25	20	21	17	103	20	23	20	20	15	98
Name of a range of holes	The number of active Stylonychia in the wells of the microaquarium											
	in the beginning of the experiment						after24hours					
	№1	№2	№3	№4	№5	N_1	№1	№2	№3	№4	№5	N_2
Experimental	19	25	18	21	15	98	16	17	15	17	12	77
Control	20	25	20	21	17	103	18	19	17	16	14	84

To date, we have obtained the necessary amount of experimental drug for preclinical testing on complex organisms (mice, Guinea pigs, rabbits) for the presence of signs of toxic effects (mutagenicity, allergy), as well as for a more complete physiological and biochemical justification. Thus, from the analysis of literary sources it should be noted the high value of nucleic acids in the creation of drugs of therapeutic and preventive orientation for veterinary medicine [3].

CONCLUSIONS

The safe experimental preparation of nucleic acids from Chlorella obtained by us, which has a positive result of screening tests and a rich nucleic composition, makes favorable prospects for the creation of an already experimental sample of the drug and its further testing on laboratory and then on productive animals**.**

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