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ALEUTIAN MINK DISEASE: THE EFFECTIVENESS OF IMMUNOCORRECTIVE THERAPY

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Abstract

Aleutian mink disease is one of the main problems of fur farming, since it causes colossal losses to the industry due to the mass death of animals and due to the lack of effective means of treatment and prevention. In the presented work the results of using an antiviral agent based on alloferon Allokin-alpha in Aleutian mink disease are reported for the first time, an effective scheme of drug use has been developed, differences in clinical signs, blood biochemical parameters, morphological changes in internal organs have been established for animals with and without the peptide. The objective of our work was to evaluate the effectiveness of the use of the drug Allokin-alpha in Aleut mink disease and to investigate the effects of the drug as a means of immune correction, ensuring the maintenance of the body condition of sick minks. The experiments were performed in compliance with the requirements of the European Convention for the Protection of Vertebrate Animals used for experiments or for other scientific purposes. The research was conducted from May to December 2019 in one of the fur farms of the Northwestern region of the Russian Federation on the mink (*Neovison vison* Schreber, 1777) of the sapphire breed of 30-day age. The control and experimental groups included 5 males and 25 female mink each. All individuals in the experiment were intraperitoneally injected with Aleutian Mink disease virus (Sapphire isolate) at a dose of 2 cm^3. The experimental minks were twice injected subcutaneously with the drug Allokin-alpha (0.5 mg per animal) with a 6-day interval. The control animals were injected subcutaneously with sterile saline solution (0.9 % NaCl) in the same dosage regimen. Clinical, laboratory and economic indicators were assessed in both groups over 6 months of the experiment. Minks' mobility, coordination, appetite, color of the mucous membrane, fur condition and response to external stimuli were recorded. Animal mortality in both groups was checked daily, and all minks were weighed at the beginning of each month. Biochemical blood parameters were determined after 1, 3 and 6 months. Macroscopic, microscopic and chemical studies of feces were performed 3 days after repeated use of the drug Allokin-alpha. At the end of the experiment, animals were subjected to diagnostic slaughter, the kidneys, spleen, ovaries of females and testes of males were collected. The biomaterial was fixed, dried, and poured into paraffin. Sections (5-7 microns thick) were stained with hematoxylin and eosin and examined under a microscope (LOMO Micromed-5, JSC LOMO, Russia). Our research data show that in the test group the minks were strong and had a proportional physique, the animals were very mobile and responded vividly to external stimuli compared to the control group, in which individuals exhibited reduced reactions, lethargy and drowsiness. The experimental minks had their digestion normalized. After receiving Allokin-alpha, the average bodyweight was 19.7 % higher in females and 15.6 % higher in males compared to control. The mortality in the test group was 0 % vs. 20 % in the control. The control animals had a high level of urea (84.05±4.22 mmol/l), creatinine (142.06±2.62 mmol/l), and transaminase activity (73.60±5.84 IU/l for AlAT and 286.60±3.36 IU/l for AsAT). In contrast, in the experimental minks, the indicators were significantly lower, the 9.88±3.88 mmol/l for urea, 97.71±1.47 mmol/l for creatinine, 130.73±4.43 IU/l for AlAT and 184.88±3.22 IU/l for AsAT. The use of Allokin-alpha caused a decrease in pH from 8.7±0.25 to 6.8±0.18 in feces. Intestinal epithelial cells, blood pigments, and soluble protein were not

found in the feces of the experimental minks, but they appeared in the control minks, which indicated the normalization of digestion. Internal organs' morphology showed the signs of glomerulonephritis and foci of lymphoplasmacytic infiltration of kidneys, spleen, liver and ovaries of female and testes of males. Nevertheless, these changes was much less pronounced in the test group than in the control group. Our findings indicate that the use of Allokin-alpha according to the developed scheme has a positive effect on the Aleutian mink disease symptoms, leads to a decrease in animals' death, an increase in bodyweight gain and significantly reduces economic in losses.

Keywords: Aleutian mink disease, viral plasmocytosis, Allokin-alpha, alloferon, immunocorrector

Aleutian mink disease is still a serious problem for commercial fur farming [1, 2]. As an epizooty, the disease causes significant economic damage due to the high mortality of minks, the deterioration of the quality of furs and the death of young animals [3]. An important factor is the lack of specific means of treating animals with this viral pathology, and, as a result, the disease has become widespread throughout the world [4, 5], including the Russian Federation [6]. Thus, from 1990 to 2000, approximately 50% of fur farms ceased to exist in our country, some of which due to 100% damage to the main mink population by Aleutian disease [7]. Now, the disease occurs in many areas in Russia, affecting up to 70% of the livestock in some fur farms [8].

The causative agent of the disease (*Aleutian disease virus*, *Amdoparvovirus*, *Parvoviridae* family, *Parvovirinae* subfamily*)* [9, 10], can persist asymptomatically in the body, causing chronic course of the disease due to antibody-dependent enhancement [11, 12]. This is explained by the fact that during phagocytosis of the antigen-antibody complex, the pathogen does not degrade. Phagocytic cells migrate from the entry site of infection to the liver, where it is destroyed. Intact virus infects hepatocytes and generalization of infection occurs. In addition, the virus inhibits the activity of dendritic cells and cytotoxic T-lymphocytes. B-lymphocytes, on the contrary, are highly active and during antigen-dependent blast transformation develop into plasma cells which produce antibodies in excess [13]. This leads to hypergammaglobulinemia, plasmacytosis and the lifelong presence of the virus in the infectious immune complexes [14-16].

Minks of all color variants at any age get sick, but the Aleutian blue and sapphire are the most vulnerable [17]. The source of infection is animals that have been ill and excrete the virus with urine, feces and saliva [18, 19]. Infection, as a rule, occurs during mating, less often by aerogenic or alimentary ways. At first, the disease is asymptomatic. With the accumulation of sick animals and the development of pathological changes, as well as the action of stress factors, the infection acquires an epizootic character. In this case, significant death of animals can occur, 70-80% of those diseased.

Pathoanatomic signs of the disease are pronounced. The kidneys significantly increase in size, acquire a light orange color, with multiple whitish areas, the capsule is easily separated, and there are a large number of stellate hemorrhages in the parenchyma [20]. Hemorrhages appear in the mucous membrane of the stomach and intestines, the liver becomes red, edematous, enlarged, splenomegaly is observed. Histologically, periarteritis is found in all organs, and the tissues of the organs are infiltrated with a large number of plasma cells [21].

Given the above features of pathogenesis, it can be assumed that successful treatment of viral plasmacytosis requires correction of the immune status in order to increase the efficiency of phagocytosis, cytotoxicity of T-lymphocytes, and activity of dendritic cells.

In recent years, many studies have been published showing that insect

and animal oligopeptides play an important role in the regulation of host innate immunity during invasion of pathogenic microorganisms, since they contribute to the production of cytokines that stimulate the action of T-cytotoxic cells and NK cells [22]. Expression of molecules of the major histocompatibility complex also occurs in infected cells to provide the presentation of viral peptides to other cells of the immune system [22]. It is for this reason that one of the options for solving the problem can be the use of the antiviral agent Allokin-alpha (RU N002829/01-210610) which is a linear oligopeptide histidyl-glycyl-valyl-seryl-glycyl-histidyl-glycyl-glutaminyl-histidyl-glycyl-valyl-histidyl-glycine (alloferon), originally isolated by Professor S.I. Chernysh from insects [23].

Alloferon induces the synthesis of endogenous interferons, mainly IFNG (interferon gamma) and activates cytotoxic CD3+HLA-DR+ T cells even under a decrease in the absolute number of CD3+CD8+ cells, which is important for the antiviral and antitumor response [24, 25].

After the administration of alloferon to mammals and humans, IFNG synthesis increases with an increase in its concentration in the cervical mucus by 37 times compared to the initial level and by 32 times compared to control [26]. IFNG activates the effector functions of neutrophils, macrophages, cytotoxic T-lymphocytes and natural killers, since these cells have receptors for this interferon. They have increased cytotoxicity, microbicidal activity, increased production of cytokines, nitrooxide radicals, superoxide radicals, which leads to the death of intracellular parasites, including viruses [27]. Along with this IFNG oppresses anti-inflammatory IL4 and B-cell response, but enhances the production of pro-inflammatory IL2 which stimulates the proliferation of killer T-cells [28]. IFNG increases the expression of major histocompatibility complex antigens of both classes, I and II in different cells and induces the expression of molecules even in those cells that do not constitutively express them. This leads to an increase in the efficiency of antigen presentation and antigen recognition by T-lymphocytes and natural killer cells.

When using alloferon, the concentration of IL1B increased by 24 times compared to the original [26]. This cytokine can induce NO synthases, thereby increasing the production of nitric oxide by phagocytes [29] which is directly involved in phagocytosis, in particular, in antigen degradation. There was also an increase in the concentration of nonspecific esterase by 3.4 times compared to the initial level and 2.7 times compared to control [26]. An increase in the activity of macrophages also illustrates an increase in the concentration of myeloperoxidase. Nonspecific esterase serves as a cytoplasmic enzyme of dendritic cells [30] and T-killers, so it can be concluded that their activity increases proportionally.

In the complex treatment of severe dysplasia of the cervical epithelium and cervical cancer, the use of alloferon leads to a significant decrease in the immunosuppressive proteins TGFB and FOXP3 which block the activation of lymphocytes and macrophages, and also enhance angiogenesis in the tumor [24]. It should be emphasized that these changes develop in the localization of the infectious agent or tumor tissue, and not systemically. The most important general immune effect from the use of Allokin-alpha is an increase in CD4 content from 32.8 to 50.54% by days 12-18 from the start of treatment and correction of the CD4/CD8 immunoregulatory index from 1.16 to 2.00 [30].

In Russia, Allokin-alpha was studied as an antiviral agent by modeling an experimental viral infection on the example of avian herpes virus where the drug showed high efficiency against the pathogen [32]. However, data on the use of synthetic oligopeptides, in particular the Allokin-alpha as a therapeutic or prophylactic

antiviral agent in fur farming, in particular, under Aleutian mink disease, could not be found.

Here, for the first time, we show the antiviral effectiveness of the alloferon Allokin-alpha-based agent against Aleutian mink disease. An effective scheme for the drug use was developed. When comparing groups that received and did not receive the peptide, differences in clinical signs, biochemical blood parameteres and morphological changes in the internal organs of animals were established.

The work aimed to evaluate the Allokina-alpha effectiveness under the Aleutian disease of minks and to reveal applicability of the drug as an immune correction agent that ensures the maintenance of the condition of diseased minks.

Materials and methods. For the experiment carried out from May to December 2019 at one of the fur farms of the North-West region of the Russian Federation, 60 sapphire minks (*Neovison vison* Schreber, 1777), including 50 females and 10 males at the age of 30 days were selected. Tests were performed in compliance with the requirements of the European Convention for the Protection of Vertebrate Animals used for experiments or for other scientific purposes [33]. All individuals were intraperitoneally injected with 2 cm^3 of the culture isolate Sapphire of the Aleutian Mink Disease virus. To confirm the development of viral plasmacytosis, animals were analyzed for specific antibodies in blood serum by immunoelectroosmophoresis (IEOF) test {34]. Glass plates with 0.7% agar gel and barbital acetate buffer solution (pH 8.6) were used. A virus-containing diagnosticum suspension (TOO IMGEN, Russia) was the antigen. Electrophoresis was carried out for 30 min at a voltage of 120 V in a device for immunoelectrophoresis PEF-3 (JSC Medlabortechnika, Russia). The results were evaluated visually by the appearance of precipitation bands.

Diagnostics were additionally performed using polymerase chain reaction (PCR) to exclude false positive results. DNA was isolated from faecal samples with a commercial kit AmpliPrime DNA-sorb-B reagents (OOO Next-Bio, Russia) according to the manufacturer's instructions. PCR was performed using a commercial kit of amplification reagents (ABN Test System, Central Research Institute of Epidemiology of Rospotrebnadzor, Russia). Amplification reaction (a Tertsik cycler, OOO NPO DNA Technology, Russia) was carried out in a 25 μl mix according to the following protocol: a hot start at 95 °C: 5 min at 95 °C (initial denaturation); 10 s at 95° °C (denaturation), 10 s at 63 °C (primer annealing), 10 s at 72 °C (polymerization) (42 cycles); 1 min at 72 °C (final polymerization). Amplification products were detected by electrophoresis in a 2% TopVision Agarose agarose gel (Thermo Fisher Scientific, USA) with the addition of GelRed (Biotium, USA) in 1× TAE buffer (ZAO Evrogen, Russia) for 35 min at a voltage of 85 B. DNA Ladder 100 bp (Thermo Fisher Scientific, USA) was a molecular weight marker. Electropherograms were visualized using a Cellmager transilluminator with Quantity One version 4.6.3 (Basic) software (Bio-Rad Laboratories, Inc., USA). The results of the PCR diagnostics revealed the presence of the virus in infected animals.

The minks selected for the study were divided into two groups (control and test) of 30 individuals (25 females and 5 males) each. All animals were kept in separate cages, in separate two-row sheds, equipped with running water and electric lighting, and were fed standard rations; the minks received feed once a day.

The minks of the experimental group were subcutaneously injected with the drug Allokin-alpha (RKNPK of the Ministry of Health of Russia, Moscow) 2 times with a 6-day interval at a dose of 0.5 mg per animal. To prepare the

solution, the contents of the drug ampoule (1 mg of alloferon) were diluted in 1 ml of sterile saline (0.9% NaCl). After dilution, the drug was injected into the skin fold at the withers. Minks of the control group were injected subcutaneously with sterile saline (0.9% NaCl) in the same dosing regimen. The condition of both groups of minks was assessed by clinical, laboratory and economic indicators for 6 months.

The functional state of the animals was assessed by clinical methods, including mobility, coordination of movements, appetite, color of mucous membranes, coat condition, response to external stimuli, body weight and mortality. The mucous membranes were examined once a week selectively in 3-5 minks from each group. For examination, animals were fixed by conventional methods with traps and thick gloves, the mouth cavity was opened with ribbons. The fur quality was also assessed, the position of the body in space and behavior were recorded, and after slaughter, the pelt size was calculated based on the body length and chest girth. In both groups, the mortality was checked daily, all minks were weighed at the beginning of each month.

Blood biochemical parameters were determined after 1, 3 and 6 months. To collect the material, the minks were fixed in traps, the hair was cut off at the tip of the tail, the skin was treated with an alcohol solution, and 2-3 mm from the tip of the tail were cut with scissors. Blood was collected drop by drop into Improvacuter plastic tubes (China) with a blood coagulation activator (SiO_2) sprayed onto the inner walls. The samples were delivered to the laboratory, observing the storage temperature regime (+4 °C). Biochemical studies were carried out using an automatic biochemical analyzer Idexx Catalist One (IDEXX Drive, USA). Total protein, albumin, globulin, urea, creatinine, alanine aminotransferase (AlAT) and aspartate aminotransferase (AsAT) were measured in the blood serum.

Macroscopic, microscopic and chemical examination of mink feces were carried out according to S.V. Written [35]. Fecal samples were taken 3 days after the repeated administration of Allokin-alpha. Freshly isolated feces were placed in clean disposable containers and delivered to the laboratory no later than in 8 hours. The color, texture, odor, presence of visible impurities were determined macroscopically; pH was determined with litmus indicator test strips. Microscopic examination of feces was carried out in wet native and stained preparations using Lugol's solution, Sudan-III, 0.5% methylene blue solution. Chemical studies included benzedine and sublimate tests.

Morphological study was carried out at the end of the experiment during the slaughter of the main stock. All animals were subjected to diagnostic slaughter, kidneys, spleen, ovaries from females and testes from males were taken. The selected organs were placed in glassware and fixed in a buffered 10% formalin solution. The fixative volume was 10-20 times the sample volume. The fixed material was dried and embedded in paraffin. Sections 5-7 μm thick were placed on glass slides and stained with hematoxylin and eosin. The resulting preparations were examined under a microscope (LOMO Mikromed-5, JSC LOMO, Russia). Microphotographs were made with an MS-3 camera (OOO LOMO-MA, Russia), image analysis was performed using the MCview program (https://www.lomo-microsystems.ru/doc/po-ru-ms.pdf).

The results were processed using Statistica 10.0 software (StatSoft, Inc., USA) and Microsoft Excel 2016. Data are presented as arithmetic means (M) and standard errors of the mean (±SEM). Groups were compared using Student's t-test. Differences were considered statistically significant at $p < 0.05$.

Results. The mucous membranes of the oral cavity in minks had a pale pink color. The color of the mucous membranes remained unchanged during

the experiment. All the minks of the experimental group were very mobile and responded vividly to external stimuli (the appearance of people near the cage, shouting, knocking, distributing food) compared to the animals of the control group, where there were individuals with a reduced response. The physique of the minks from the experimental group was strong and proportional in contrast to the animals from the control group. In general, individuals from the control group by the end of the study showed signs of malaise, the lethargy and drowsiness.

1. Bodyweigh of sapphire minks (*Neovison vison* Schreber, 1777) infected with Aleutian mink disease virus upon administration of Allokin-alpha injected subcutaneously (*M*±SEM, a fur farm of the North-West region of the Russian Federation, 2019)

Bodyweight, g	Age, days	Control		Test	
		females	males	females	males
At the beginning of the experiment	30	192±5.9 (n = 25)	207±5.7 (n =5)	190±6.1* (n = 25)	209±5.6* (n = 5)
6 months from the beginning of the experiment	210	1320±6.1 (n = 21)	2230±5.6 (n = 3)	1580±6.4* (n = 25)	2580±6.2* (n = 5)

N o t e. For a description of the groups, see the "Materials and methods" section.
* Differences with the control group are statistically significant at $p < 0.05$.

Animals in both groups ate feed completely. The average weight of females treated with the drug was 1580±6.4 g and was 19.7% higher ($p < 0.05$) than in the control group. In males, the average weight in the experimental group was 2580±6.2 g which was 15.6% higher than the control ($p < 0.05$) (Table 1).

By the end of the experiment, all animals of the test group were alive, while 20% of the control minks died. The pelt area in the experimental group from females averaged 1120.1±3.8 cm^2, from males 1586.2±4.1 cm^2, in the control group the values were 1069.2±4.4 and 1524.3±4.1 cm^2, respectively. The increase in live weight in minks treated with alloferon led to an increase in the pelt size by an average of 51 cm^2 in females and by 62 cm^2 in males. In animals that were injected with Allokin-alpha, an increase in fur density was visually recorded. When blowing through the hair, no free skin areas were found. The hairs were strong and elastic. The silkiness of the fur was tactilely noted. The color was uniform throughout the body with a pronounced brilliance.

Biochemical blood tests revealed changes characteristic of the Aleutian disease. In the minks of the control group, the concentration of globulins, urea, and creatinine was significantly higher than in the animals treated with Allokin-alpha (Table 2). In the test group, these changes also occurred, but their severity was significantly less.

2. Blood biochemical parameters of sapphire minks (*Neovison vison* Schreber, 1777) infected with the Aleutian disease of minks upon administration of Allokin-alpha injected subcutaneously (6 months after the injection *M*±SEM, a fur farm of the North-West region of the Russian Federation, 2019**)**

Parameter	Norm	Control (n = 24)	Test (n = 30)
Total protein, g/l	72.8	97.50±1.53	88.30±1.49*
Albumin, g/l	36.9	33.00±0.82	31.60±1.16
Globulins, g/l	31.0	65.00±0.94	55.43±0.87*
Urea, mmol/l	3.52	84.05±4.22	9.88±3.88*
Creatinine, rmol/l	50.9	142.06±2.62	97.71±1.47*
Alanine aminotransferase, IU/l	80.1	273.60±5.84	130.73±4.43*
Aspartate aminotransferase, IU/l	125.6	286.60±3.36	184.88±3.22*

N o t e. For a description of the groups, see the "Materials and methods" section. Normative indicators are given according to O.Yu. Bespyatykh et al. [37], C.Zh. Batoeva et al. [38], N.V. Mantatova et al. [39].
* Differences with the control group are statistically significant at $p < 0.05$.

Analysis of digestion products showed that the use of Allokin-alpha caused a decrease in pH from 8.7±0.25 to an almost neutral value (pH 6.8±0.18). In addition, in the feces of minks from the experimental group, we did not find intestinal epithelial cells, blood pigments, soluble protein while in the control these were detected which indicates the normalization of the overall digestion process when the drug was injected. Consistency, color, smell, presence of neutral fat, pus, plant cells, detritus were the same in both groups (Table 3).

3. Coprogram of sapphire minks (*Neovison vison* Schreber, 1777) infected with the Aleutian disease of minks upon administration of Allokin-alpha injected subcutaneously (*M*±SEM, a fur farm of the North-West region of the Russian Federation, 2019)

Parameter	Control (n = 30)	Test (n = 30)
Consistency	Solid	Solid
Color	Dark green with a brownish tint	Dark green with a brownish tint
Smell	Specific	Specific
pH	8.7±0.25	6.8±0.18*
Neutral fat	+	+
Fatty acid	–	–
Soaps	–	–
Starch	Minor amount	A very small amount
Bilirubin	Doubtful	Absent
Blood	–	–
Leftover undigested food	Present	Present
Plant cells	+	+
Detritus	+	+
Pus	+	+
Intestinal epithelial cells	+	–
Blood pigments	+	–
Soluble protein	+	–

N o t e. For a description of the groups, see the "Materials and methods" section. Normative indicators are given according to O.Yu. Bespyatykh et al. [37], C.Zh. Batoeva et al. [38], N.V. Mantatova et al. [39].
* Differences with the control group are statistically significant at $p < 0.05$.

When examining tissue morphology of kidneys in the control group, we found proliferation of mesangial cells, an increase in the thickness of the mesangial matrix of the renal bodies, narrowing of the lumen of the capillaries, edema of the glomeruli, glomerulonephritis and lymphoplasmacytic infiltration characteristic of interstitial nephritis (Fig. 1, A). In the medulla there were focal, in some places confluent hemorrhages. Microscopic areas of calcification were also observed. In the experimental group, similar signs were found in the kidneys, however, an increase in the mesangial matrix and obliteration of the capillary lumen were 2.5-3 times less, there was a plethora of blood vessels, while there were no foci of hemorrhage, and lymphoplasmic infiltration was 2 times less pronounced (see Fig. 1, B).

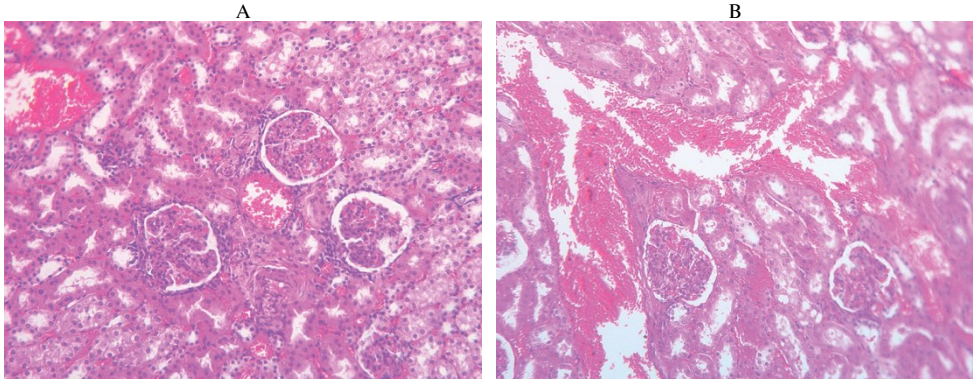

Fig. 1. The kidneys (histological sections) of sapphire minks (*Neovison vison* Schreber, 1777) infected with the Aleutian disease in the control (A) **and upon administration of Allokin-alpha injected subcu-**

taneously (B)**:** A — an increase in mesangial matrix of the renal glomeruli, capillary loops are poorly distinguishable, B — an increase in the mesangial matrix and a lower degree of obliteration of the capillaries of the renal bodies, less pronounced lymphoplasmacytic infiltration compared to the control (staining with hematoxylin and eosin, a microscope LOMO Micromed-5, JSC LOMO, Russia, magnification 10×). For a description of the groups, see the "Materials and methods" section.

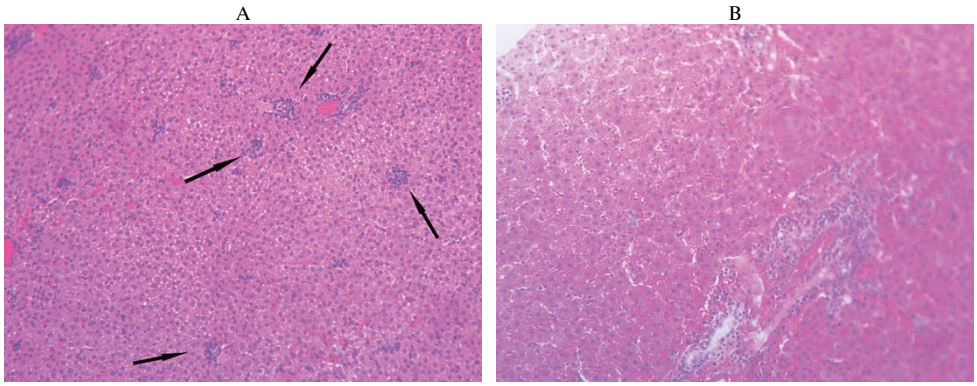

Fig. 2. The liver (histological sections) of sapphire minks (*Neovison vison* Schreber, 1777) infected with the Aleutian disease in the control (A) **and upon administration of Allokin-alpha injected subcutaneously** (B)**:** A — foci of lymphocytic plasma infiltration (indicated by arrows), B — lymphocytic plasma infiltration, no local foci (staining with hematoxylin and eosin, microscope LOMO Mikromed-5, JSC LOMO, Russia, magnification 10×). For a description of the groups, see the "Materials and methods" section.

Total hydropic dystrophy of hepatocytes occurred in the liver of control animals. Foci of lymphoplasmacytic infiltration of the stroma of the liver and bile ducts were revealed (Fig. 2, A). In the minks from the experimental group, the lymphoplasmic infiltration of the liver stroma was 40-50% less, and there were no local foci (see Fig. 2, B).

In the spleen of minks of the control group, we revealed hyperplasia (Fig. 3). Focal accumulations of a large number of plasma cells were observed around the blood vessels. Similar changes in the experimental group were 1.5 times less pronounced.

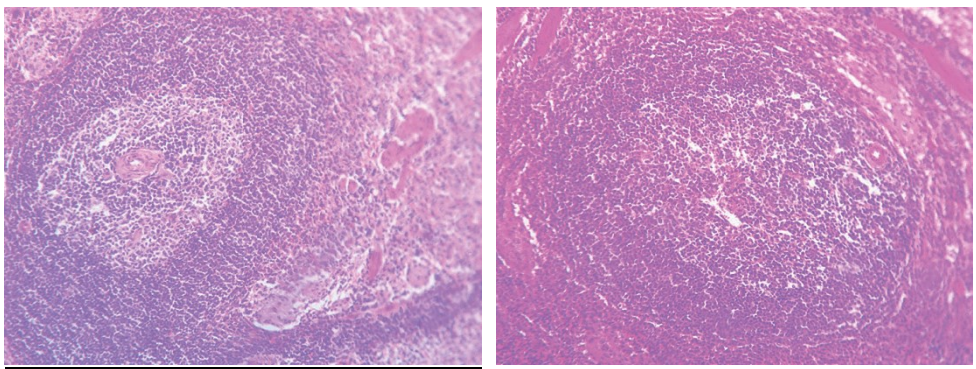

Fig. 3. Hyperplasia of the follicle in the spleen of sapphire minks (*Neovison vison* Schreber, 1777) infected with the Aleutian disease in the control (A) **and upon administration of Allokin-alpha injected subcutaneously** (B) (staining with hematoxylin and eosin, microscope LOMO Micromed-5, JSC LOMO, Russia, magnification 10×). For a description of the groups, see the "Materials and methods" section.

In the ovaries of control females, a large number of atretic bodies (dead oocytes), primordial, primary, secondary and tertiary follicles were found. In the experimental group, the number of primordial follicles in females increased while the number of atretic follicles decreased. Lymphocyte-plasmatic infiltration was expressed 2.5-3 times weaker (Fig. 4). In males, foci of lymphoplasmacytic infil-

tration also appeared in the convoluted tubules of the testes, however, these changes were 2 times less pronounced in individuals from the experimental group (Fig. 5).

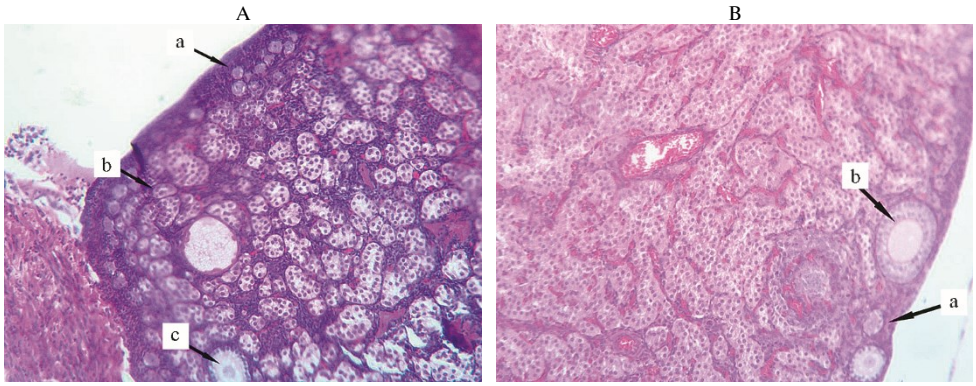

Fig. 4. Lymphoplasmacytic infiltration of the ovaries in female of sapphire minks (Neovison vison Schreber, 1777) infected with the Aleutian disease in the control (A) **and upon administration of Allokin-alpha injected subcutaneously** (B)**:** a — primordial, b — primary, c — secondary follicles (staining with hematoxylin and eosin, microscope LOMO Micromed-5, JSC LOMO, Russia, magnification 10×). For a description of the groups, see the "Materials and methods" section.

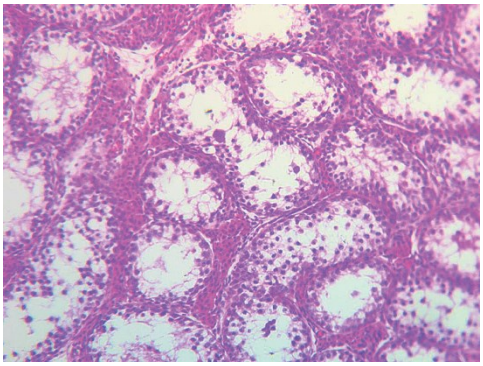

Fig. 5. Convoluted tubules of the testis in male of sapphire minks (Neovison vison Schreber, 1777) infected with the Aleutian disease in the control (A) **and upon administration of Allokin-alpha injected subcutaneously** (B) (staining with hematoxylin and eosin, microscope LOMO Micromed-5, JSC LOMO, Russia, magnification 10×). For a description of the groups, see the "Materials and methods" section.

A unified approach to the treatment and prevention of Aleutian disease of minks has not yet been developed [4, 5]. It is known that viral plasmacytosis can give a severe clinical signs characterized by anemia, cachexia, deterioration of fur quality, kidney failure and high mortality, which leads to enormous losses in mink farming [12, 36]. The use of the drug Allokina-alpha in our study prevented the development of such signs in the experimental animals, in contrast to the control group. The drug not only facilitated the course of the disease and mitigated its negative consequences, but also contributed to the restoration of body functions, as evidenced by increased activity and good quality of fur in experimental animals. In addition, the digestion processes normalized, the live weight indicators of females increased by 19.7%, of males by 15.6%, the physique of the minks treated with the drug remained stronger and more proportional. In addition, the use of Allokin-alpha ensure 0% mortality of the test animals vs. 20% of the control minks.

Many researches distinguish two stages in the pathogenesis of Aleutian mink disease: infectious and autoimmune. The infectious stage is characterized by stimulation of the proliferation of plasma cells that invade the spleen, liver, kidneys and other organs [18, 36, while the autoimmune phase is associated with the development of hypergammaglobulinemia [21]. The results of blood biochemical study obtained by us are consistent with the data of the authors who assert that at the autoimmune stage, the total protein in the blood of sick animals increases [36].

Thus, the total blood protein in the minks of the control group increased due to a sharp increase in the amount of globulins. This suggests that a significant amount of protein compounds in the blood are antibodies that form immune complexes which are subsequently fixed in the glomeruli of the kidneys, causing the development of glomerulonephritis. This is also confirmed by A. Prieto et al. [2], A.H. Farid et al. [10], O.Yu. Bespyatykh et al. [37]. In turn, the development of renal failure leads to an increase in the content of metabolic end products (urea and creatinine) which we observed in individuals of the control group. The morphological signs characteristic of glomerulonephritis and interstitial nephritis, i.e., proliferation of mesangial cells, an increased thickness of the mesangial matrix, narrowing of the capillary lumen, swelling of the glomeruli, hemorrhages, caused a significant decrease in the quality of the filtration capacity of the kidneys. All these changes, combined with a high-protein diet of minks, led to a higher urea content in the control animals and could indicate the transition of renal dysfunction to a chronic form. N.V. Mantatova et al. [39] also associated high urea content in minks (50.0 ± 0.58 mmol/l at $p \leq 0.05$) with kidney pathologies and a high-protein diet.

An increased amount of AsAT and AlAT indicated that during the phagocytosis of immune complexes by the reticuloendothelial system, the virus was released in the nuclei of liver macrophages and caused the destruction of hepatocytes. In the experimental group, on the contrary, the indicators for urea, creatinine and transaminase activity were much lower, which indirectly indicates a smaller scale of destructive changes in the kidneys and liver. Our data on organ morphology in the control animals are consistent with the reports of other researchers who claim that the Aleutian disease of minks is characterized by lymphoplasmacytic infiltration of internal organs, signs characteristic of the histological picture of glomerulonephritis, splenomegaly, and hydropic dystrophy of hepatocytes [17, 20]. It should be noted that in the test group, such changes were on average 2.5-3 times less pronounced. There were signs of intensive regeneration and functional restoration of damaged organs (e.g., plethora of blood vessels, absence of foci of hemorrhages, low the degree of oocyte apoptosis, low intensity of plasmacytic infiltration), which indicates an improvement in the general physiological state of sick animals treated with Allokin-alpha.

Thus, in mink infected with Aleutian disease, 2-fold subcutaneous injections of Allokina-alpha at a dose of 0.5 mg per animal with a 6-day interval had a positive effect on animal health, productive parameters and pelt quality. In individuals of the test group, the clinical condition improved markedly, activity and response to external stimuli increased. A decrease in the pH of feces from 8.7 ± 0.25 to 6.8 ± 0.18 indicates the restoration of digestion. The minks treated with the drug showed a noticeable increase in weight gain, their bodyweight was on average 19.7% higher in females and 15.6% in males. The pelts from test animals were larger, on average by 51 cm^2 for females and 62 cm^2 for males. Subcutaneous injections of Allokin-alpha significantly improved blood biochemical parameters. In the test animals, the urea content was 8.5 times less than in the control, which indicates a decrease in the development of renal failure. A significant decrease in the amount of alanine aminotransferase (by 2 times) and aspartate aminotransferase (by 1.5 times) indicates a low degree of destructive-inflammatory processes in the liver in animals from the test group. Morphological changes in the internal organs of the minks treated with the drug were 2.5-3 times less pronounced, while signs of restoration of damaged organs were observed. The positive effect of the antiviral agent Allokin-alpha on the biochemical mechanism of the disease development and the low degree of histo-

logical changes, contributed to avoiding mortality in the test group and an increase in the amount of pelts obtained, which significantly reduces economic damage. We can recommend Allokin-alpha for use at fur farms as an immunocorrector under viral plasmacytosis of minks, which improves the general physiological state of sick animals and fur maturation. This will minimize losses from the Aleutian disease of minks, and can also be considered as a means of nonspecific prevention and treatment. However, additional experiments are needed to clarify this possibility.

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