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COMPARATIVE STUDY OF EFFECTIVENESS OF USING THE MEDICINAL PRODUCTS VIFERON®, KIPFERON® AND ERGOFERON® IN AN EXPERIMENT ON RATS

D.I. Pozdnyakov[1, 2], V.E. Pogoreliy[1], I.V. Kukes[2]

[1] Pyatigorsk Medical and Pharmaceutical Institute, 11 Kalinin Ave., Pyatigorsk, 357502
[2] Autonomous non-profit organization International Association of Clinical Pharmacologists and Pharmacists, Scientific and Clinical Department,
2/1 M. Kalitnikovskaya St., Moscow, 109147

pozdniackow.dmitry@yandex.ru

Abstract

The study was focused on a comparative evaluation of the effectiveness of using the interferon-α-containing medicinal products Viferon® and Kipferon®, as well as the interferonogenic medicinal product Ergoferon®. The experiment was carried out on male Wistar rats in two phases. At the first phase, the change in the concentration of interferon-α in rats' blood serum was assessed after a single administration of these products, after which a respiratory infection was simulated. The products were administered for 14 days. The following values were studied: concentration of interferon-α (on the 14th day); interferon-γ (10th and 14th day); interleukin-6, C-reactive protein and leukocytes in the blood (3rd, 7th, 10th and 14th day). The number of viable microbial cells in pleural effusion, development of neurological impairment, and blood coagulation (on the 14th day) were also assessed.

As a result, it was found that the use of Kipferon® contributed to the increase in the concentration of interferon-α after a single administration to a greater extent than the use of Viferon® and Ergoferon®. It also restored the normal course of immune reactions amidst infectious respiratory pathology, reduced the number of viable microorganisms, which was associated with improvement in the animals' general condition, and no neurotoxic or hematotoxic effect that is characteristic of Viferon® was developed. Thus, the medicinal product Kipferon® had better therapeutic effectiveness compared to Viferon® and Ergoferon® in this experiment.

Keywords: interferons, respiratory tract infections, interferonogens, cytokines.

Introduction

Interferons (IFNs) are part of the larger family of class II cytokines, which also includes molecules associated with interleukin-10 (IL-10, IL-19, IL-20, IL-22, IL-24 and IL-26) [1]. These cytokines have a conservative structure consisting of six α-helices. Interferons are usually divided into two populations: type I and type III. In mammals, the type I IFN family includes several subtypes: IFN-α; IFN-β, IFN-ε, IFN-κ, IFN-ω (in primates) and IFN-ζ (in rodents). Intracellular signaling of all type I IFNs is mediated through the common heterodimeric IFNAR receptor consisting of two subunits IFNAR1 and IFNAR2, to which IFNs exhibit a greater affinity [2]. There are four subtypes of type III IFN in humans: IFN-λ1 (IL-29), IFN-λ2 (IL-28A), IFN-λ3 (IL-28B) and IFN-λ4. In rodents, the type III IFN family includes IFN-λ2 and IFN-λ3. The receptor for type III IFN is a heterodimeric IFNLR complex consisting of IFNLR1 (IL28Rα) and IL10Rβ. First, type III IFN interacts with IFNLR1, then IL10Rβ enters the cellular transduction. Table 1 shows a comparative analysis of type I and type III INFs [3, 4].

IFN-α belongs to type I IFN family; it is a pleiotropic IFN. The transduction of the intracellular IFN-α signal is associated with the activation of IFNAR2 receptors and the respective JAK/STAT pathway. In the case of direct interaction of IFN-α with a receptor's subunits, a heterodimeric ligand-receptor complex appears. This complex activates intracellular signal transduction through specific mutually phosphorylating kinases, Tyk2 and JAK1, that are associated with the receptor. The STAT1 and STAT2 molecules bind to the receptor complex and form a phosphorylated heterodimer. When it is released from receptors, the heterotrimeric transcription factor ISGF3 is formed, which moves into the nucleus and activates interferon-stimulated response elements (ISRE). The activated ISREs result in the expression of various antiviral proteins, such as Mx1, Mx2 and RNA-dependent protein kinase (PKR). This cascade of reactions induces several signaling cellular responses that promote inversely related regulation of genes stimulated by interferons, including the suppressors of cytokine signaling, pSTAT protein inhibitors and protein tyrosine phosphatase [5], which is the underlying mechanism for the antiviral effect of IFN-α [4].

In addition to its direct antiviral effect, IFN-α has an immunomodulatory effect and promotes the activation of adaptive immune responses. In this regard, *Crouse et al., 2015* found that IFN-α can stimulate or inhibit the priming of a T-population with the involvement of T-cell receptors (TCR) in the cellular response. At the same time, the activation of IFNAR and TCR promotes proliferation and differentiation of T cells. However, IFNAR activation without a concomitant increase in the TCR-cell response has a negative regulatory effect, suppressing the proliferation and stimulating T-cell apoptosis [6]. *Van Boxel-Dezaire et al., 2006* explain this effect by the involvement of various STAT transcription factors in the IFN-induced cellular response. Thus, STAT1 is a component of the signaling pathway that intensifies inflammation, proliferation, and programmed cell death, while STAT3, STAT4, and STAT5 promote survival and differentiation of T cells [7].

IFN-α also can indirectly modulate the T-cell response through several mechanisms [8]:

- increased expression of the major histocompatibility complex;
- increased synthesis of colony-stimulating factors;
- stimulation of migration of antigen-presenting cells by increasing the activity of CCR5 and CCR7;
- induction of natural killer cell regulators.

Considering their pronounced antiviral activity and the presence of pleiotropic effects, products based on IFN-α are widely used in practical medicine. Among them, the medicinal products Viferon® and Kipferon®, which are used to treat a variety of infectious diseases, stand out in particular [9, 10].

In addition to medicinal products based on interferon, the use of drugs that stimulate the production of endogenous interferon (interferonogens), including Ergoferon®, is of particular importance in the treatment and prevention of viral infections [11].

To date, it has been established that IFN-containing medicinal products with a comparable route of administration and concentration of the active

substance may differ in pharmacokinetic and pharmacodynamic characteristics, which makes the development of their pharmacological effect highly variable [12]. In this regard, comparative evaluation of some parameters characterizing pharmacokinetics and efficacy of IFN-containing medicinal products is particularly relevant. It is the specific purpose of this study.

Study objective: evaluating some parameters of pharmacokinetics in healthy animals and the level of pharmacological efficacy of the medicinal products *Viferon-rectal suppositories*, *Kipferon-rectal suppositories* and *Ergoferon* in animals with infectious respiratory pathology.

Materials and methods.

Experimental animals and their management

The study was carried out on 30 adult male Wistar rats with a body mass of 200 to 220 grams, obtained from the Rappolovo laboratory animal breeding center (Leningrad Region, Russia). Before they were included in the experiment, the rats were kept in a quarantine room for 14 days. At the time of the study, the animals were housed in standard macrolon cages with free access to water and food. The litter of granulated wood fraction was changed every 3 days. The conditions of housing included ambient temperature of 22 ± 20 °C, relative humidity of 60 ± 5%, and natural change in the diurnal cycle (12 hours of daytime/12 hours of nighttime). The animal management and manipulations with the animals corresponded to the generally accepted norms of experimental ethics (Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes, September 22, 2010). One experimental group included 6 animals.

Objects under study

This study was focused on the following medicinal products: Viferon® (LLC Feron, Russia), Kipferon® (JSC Binnopharm, Russia), and Ergoferon® (Research and production company LLC Materia Medica Holding, Russia). The administered doses of the medicinal products under study were calculated taking into account the interspecies dose conversion factor. The doses were as follows: 182,000 IU/day for Viferon® and Kipferon®, 0.009 g for Ergoferon® (in terms of affinity purified antibodies to interferon). Viferon® and Kipferon® were administered rectally using an atraumatic applicator. Ergoferon® was administered intragastrically through a metal probe with tin surfacing at the tip.

Study design

This work was performed in 2 phases. At the first phase, the change in the concentration of IFN-α in the blood serum of rats was assessed after a single administration of the products under study in the above-mentioned doses. At the second phase of the study, the effect of the medicinal products on the course of the respiratory infection development in the animals was assessed, and the following experimental groups were created: SO – sham-operated rats (the pathology was not reproduced); NC – a group of negative control animals (with the pathologic condition, but lacking pharmacological support); and three groups of rats treated with Viferon®, Kipferon® and Ergoferon®, respectively. The NC group of animals and the rats treated with the evaluated medicinal products were studied after the pharmacokinetic study. The medicinal products were administered immediately after modeling the infectious process in the respiratory tract and, afterwards, every day (once a day) for 14 days. On the 14th day of the study, the change in the concentration of IFN-α and the number of colony-forming units in pleural effusion were assessed, and the content of IFN-γ in the blood serum was determined on the 10th and 14th days of the experiment. On the 3rd, 7th, 10th and 14th day, the concentration of IL-6, C-reactive protein and leukocytes in the blood was assessed. On the 14th day, the functional condition of the rats' central nervous system was assessed in the 4 plates test, and the blood coagulation time was determined.

Biomaterial preparation

Blood was taken from the sublingual vein and collected into sterile Eppendorf tubes with citrate filling. The whole citrated blood was divided into 2 parts. In the first aliquot, the total content of leukocytes was measured. The second part was centrifuged for 15 minutes in a cold environment (4°C) at an acceleration of 1000g and removed for an ELISA test. The pleural effusion was collected

into a sterile syringe by a puncture of the pleural cavity.

Pharmacokinetic study

When determining the change in the concentration of IFN-α in the blood serum of the experimental animals after a single administration of the studied drugs, whole venous blood was collected from rats after 2, 4 and 6 hours from the moment of administration. Then the blood underwent the sample preparation procedure described above, after which the content of IFN-α was measured. The sampling points were selected taking into account the time of the peak concentration of IFN-α in rats' blood, which is 2 hours, according to *Kalra P et al. 2019* [13].

Experimental model of the infectious respiratory process

Respiratory pathology was simulated in the rats using the method described by *Cripps et al., 1994*. A bacterial culture of *S. pneumoniae* (collection strains) in phosphate-buffered saline was introduced endotracheally through a flexible silicone probe, the dose was 50 µl. In order to reach both lobes of the lung, the probe was introduced to a depth of 1 cm. After the introduction of the bacterial culture, air was inflated into the lungs three times, by pressing the plunger of a 1-ml syringe [14].

Assessment of the animals' overall health status

The animals' overall health status was assessed by evaluating the development of neurological impairment, which was determined using the mNSS (modified neurological severity score) psychometric scale. According to the mNSS assessment system, the severity of neurological impairment was evaluated based on these scores, with the total score ranging from 1 to 6 points indicating a slight neurological impairment, a score from 7 to 12 points indicating a moderately severe neurological impairment, and the total score of 13 to 18 indicating the development of a severe neurological impairment [15].

Assessment of the functional condition of the central nervous system in rats

The functional condition of the central nervous system in rats was assessed in a Four-plate test. The installation used was a square chamber divided into compartments, with current-carrying metal plates situated crosswise. The animals were allowed to walk freely around the chamber, but they got electric pain stimulation when passing from one sector to another. The test lasted 3 minutes, and the number of movements between sectors was recorded [16].

Determination of the concentration of viable microbial cells (colony forming units)

The test was performed according to GPM.1.7.2.0008.15.

The method consisted in plating a certain volume from a series of tenfold dilutions of the suspension of studied microorganisms on a solid nutrient medium (agar), incubation and counting of the colonies that appeared, considering that each colony resulted from the multiplication of one viable cell of the microorganism. The suspension was plated by the surface agar method.

When calculating the results, the average number of the colonies that grew after the plating of twofold dilutions was determined. To obtain reliable results, plates were selected where the number of bacterial colonies ranged from 30 to 300.

The average number of CFU (N) in 1 ml was calculated according to the formula:

$$N = \frac{c}{(n_1 + 0{,}1 \cdot n_2) \cdot d}$$

Where:

c was the sum total of the counted colonies on all plates;

n1 was the number of the plates with the first dilution;

n2 was the number of the plates with the second dilution;

d was the coefficient for the first dilution;

0.1 was the multiplicity factor of the first and second dilutions [17].

Assessment of the leukocyte content in the blood
Leukocyte counts were determined using the BC-2800vet Veterinary Automatic Hematology Analyzer (Mindray). The principle of determination was based on the impedance method, which consists in determining the number and size of cells depending on the change in electrical resistance when a particle (cell) passes through a small aperture in a current conductive liquid. During this procedure, each cell causes a change in the impedance of a current conducting suspension of blood cells. These changes are recorded as an increase in the voltage between the electrodes. The number of pulses determines the number of cells. The amplitude of the pulse is proportionate to the cell volume. Pulses are counted only in the boundaries between the preestablished lower and upper limits (discriminators).

Assessment of the blood coagulation time
The duration of blood coagulation was determined with a mechanical coagulation analyzer, N-334. Based on the obtained coagulation profiles, the duration of the blood coagulation process (in seconds) was determined [18].

ELISA study
In this study, the ELISA method was used to determine the concentration of IFN-α, IL-6, IFN-γ, and C-reactive protein (CRP). The study used reagent kits manufactured by *Cloudclone* (Houston, Texas, USA). The testing and preparation of the biological material were in keeping with the manufacturer's manual included in each kit. The results were read using an F50 semi-automatic ELISA reader (Tecan, Austria). The incoming signal was processed in a Magellan 50 system.

Statistical analysis
The obtained results were statistically processed using the statistical analysis package *STATISTICA 6.0* (StatSoft, USA). Data were expressed as M ± SEM. Further statistical analysis of the study results was performed by a method of one-way analysis of variance (ANOVA) with a Newman-Keuls post-hoc test.

Results
Results of the pharmacokinetic study
In the course of this experimental phase (Fig. 1), it was found that after a single administration of Viferon®, the peak concentration of IFN-α in the blood serum of the rats was observed in 2 hours after the beginning of the experiment, while in the rats treated with Kipferon® the peak content of IFN-α in the blood was observed after 4 hours. Two hours after the administration of the drugs, the concentration of IFN-α compared to the control group increased by 77.3% ($p < 0.05$) with the use of Viferon®, and by 156.2% ($p < 0.05$) with the use of Kipferon®. It should be noted that when Kipferon® was administered, the content of IFN-α at the 2nd hour of the study was 44.4% ($p < 0.05$) higher than in the rats treated with Viferon®. After 4 hours, the content of IFN-α in the rats treated with Viferon® and Kipferon® exceeded the same metric in the control group by 54.3% ($p < 0.05$) and 245.7% ($p < 0.05$), respectively. The use of Kipferon® in comparison with Viferon® caused a statistically significant increase in the content of IFN-α in the rats' blood serum at the 4th hour into the experiment – 2.2 times ($p < 0.05$). Six hours after the administration of the studied drugs, the concentration of IFN-α in the rats treated with Viferon® was 32.0% ($p < 0.05$) lower than that in the control group, while after the administration of Kipferon® this concentration was 99.1% higher ($p < 0.05$) and significantly (2.98 times ($p < 0.05$)) exceeded the content of IFN-α in the blood of the rats treated with Viferon®. It should be pointed out that the administration of Ergoferon® to the animals did not significantly affect the change in the IFN-α content (Fig. 1).

Impact of the study drugs on the change in the concentration of IFN-α and IFN-γ in the blood serum of the rats amidst respiratory infectious pathology
Amidst an experimental respiratory tract infection in rats, it was noted that the content of IFN-α (Fig. 2) in the NC animal group decreased 53.3% ($p < 0.05$) on the 14th day of the experiment, compared with the SO rats. After the administration of Viferon® and Kipferon®, the concentration of IFN-α exceeded that in the NC group of rats by 316.5% ($p < 0.05$) and 611.0% ($p < 0.05$), respectively, while the administration of Ergoferon® did not have a significant effect on the change in the content of

IFN-α in the blood serum of the rats with respiratory infectious pathology (Fig. 2).

The concentration of IFN-γ (Fig. 3) in the NC group of rats was lower than the same concentration in the SO rats – by 41.7% (p <0.05) on the 10th day of the study and by 53.6% (p <0.05) on the 14th day of the experiment. The use of Viferon® contributed to a significant increase (relative to the NC group of animals) in the content of IFN-γ – by 406.7% (p <0.05) on the 10th day and by 707.7% (p <0.05) by the 14th day of the study. The administration of Kipferon® to the animals also led to an increase in the content of IFN-γ in the rats' blood serum. Thus, compared to the NC group, the rats treated with Kipferon® showed a concentration of IFN-γ that was 180.0% (p <0.05) and 236.5% (p <0.05) higher, respectively, on the 10th and 14th day of the experiment (Fig. 3). It should be noted that the content of IFN-γ in the animals treated with Ergoferon® and in the NC group of rats did not differ in a statistically significant manner.

The impact of the study drugs on the change in the concentration of IL-6, C-reactive protein and leukocytes in the blood of the rats with respiratory infectious pathology

In this phase of the study, it was found that the NC group of rats tended to increase the concentration of IL-6 starting from the 3rd day of the experiment, with a peak on the 10th day of the study. Thus, on the 3rd, 7th and 10th days, the content of IL-6 (Fig. 4) in the NC group of animals was higher than this content in the SO rats by 28.1% (p <0.05); 65.1% (p <0.05); 104.2% (p <0.05), respectively. On the 14th day, a decrease in the concentration of IL-6 by 46.7% (p <0.05) was observed in the NC group of animals. The use of Viferon® promoted an increase in the IL-6 content on the 3rd day of the study by 30.1% (p <0.05), compared to the NC group. Subsequently, on the 7th and 10th day of the experiment, the content of IL-6 in the rats treated with Viferon® was lower than that in the NC group of rats by 22.9% (p <0.05) and 40.8% (p <0.05), respectively. On the 14th day, it increased by 163.7% (p <0.05). The administration of Kipferon® led to a decrease in the content of IL-6 compared to the NC group of animals by 42.6% (p <0.05), 44.3% (p <0.05) and 59.8% (p <0.05) on the 3rd, 7th and 10th day of the study, respectively, as well as to an increase in this content by 137.6% (p <0.05) on the 14th day. The use of Ergoferon® did not have a significant effect on the change in the concentration of IL-6 (Fig. 4).

The content of CRP (Fig. 5) in the NC group of rats on the 3rd, 7th and 10th days was higher than that in the SO animals by 8.1 (p <0.05), 7.9 (p <0, 05) and 2.8 (p <0.05) times, respectively. On the 14th day, it was 40.8% (p <0.05) lower. In the animals treated with Viferon®, there was a decrease in the concentration of CRP in relation to the NC group of rats on the 3rd, 7th, 10th day and an increase on the 14th day – by 62.4% (p <0, 05), 59.0% (p <0.05), 18.5% (p <0.05) and 245.2% (p <0.05), respectively. In the rats treated with Kipferon®, the content of CRP in the blood serum also tended to decrease on the 3rd, 7th and 10th days of the experiment (a decrease in the concentration of CRP compared to the NC group of rats by 68.9% (p <0.05), 72.3% (p <0.05) and 50.4% (p <0.05) was observed). On the 14th day of the study, the content of CRP in the animals treated with Kipferon® was 226.2% (p <0.05) higher than that in the NC group.

As a result of the assessment of the changes of the leukocyte content in the blood (Fig. 6), it was found that this content in the NC group of rats was higher than that in the SO animals – by 88.2% (p <0.05), 148.4% (p <0.05) and 29.2% (p <0.05) on the 3rd, 7th and 10th days of the study, respectively. On the 14th day, there was a sharp decline in the leukocyte concentration in the blood of the NC group of rats. The use of Viferon® reduced (compared with the NC group of animals) the content of leukocytes on the 3rd, 7th and 10th days, and increased this content on the 14th day of the experiment – by 20.3% (p <0.05), 45.5% (p <0.05); 16.7% (p <0.05) and 406.3% (p <0.05), respectively. In the rats treated with Kipferon®, there was a decrease in the content of leukocytes in the blood on the 3rd, 7th and 10th days compared to the NC group of rats – by 46.9% (p <0.05), 59.7% (p <0.05) and 25.0% (p <0.05), with an increase in the studied parameter by 306.3% (p <0.05) by the 14th day of the experiment. It should be noted that when using Ergoferon® no statistically significant changes in the concentration

of CRP and leukocytes compared to the NC group of animals were established.

Effect of the study drugs on the change in CFU the content in pleural effusion, the functional condition of the central nervous system, the animals' overall health status of animals, and coagulation in the rats with respiratory infectious pathology

On analyzing the change in CFU in pleural effusion of the rats, it was found that the use of Kipferon® contributed to a decrease in the CFU number by 46.4% ($p < 0.05$) compared with the NC group of animals, and with respect to the rats treated with Viferon® and Ergoferon® - by 39.7% ($p < 0.05$) and 36.4% ($p < 0.05$), respectively (Fig. 7).

The assessment of neurological impairment (Fig. 8) in the rats showed that in the NC group of animals the total score of neurological impairment was 19.2 times ($p < 0.05$) higher than that score in the SO rats. The Four-plate test (Fig. 9) showed a decrease in the number of movements by 82.6% ($p < 0.05$) in the NC group of animals in comparison with the SO rats. In the rats treated with Viferon® and Kipferon®, neurological impairment decreased, compared to the NC group of animals, by 20.8% ($p < 0.05$) and 45.8% ($p < 0.05$), respectively (Fig. 8), and the number of movements increased 13.3 ($p < 0.05$) and 4.5 ($p < 0.05$) times, respectively.

In the rats treated with Viferon®, the number of movements between sectors was significantly (2.9 ($p < 0.05$) and 7.2 ($p < 0.05$) times, respectively) higher than that in the rats treated with Kipferon® and Ergoferon® (Fig. 9). It should be noted that the use of Viferon® reduced the blood coagulation time (Fig. 10) in the rats by 25% ($p < 0.05$) compared to the NC group of animals, while the administration of Kipferon® normalized this indicator (no statistically significant differences were found in comparison with the SO group). The use of Ergoferon® did not have a significant effect on the change in the number of CFU, overall health status, the condition of the central nervous system and blood clotting in the rats.

Discussion

Today, medicinal products based on interferons are widely used. Due to their strong clinical effectiveness and multiple effects on human body, interferons can be used in various infectious processes. However, medicinal products containing the same active component often demonstrate different levels of efficacy, which may be due to both variability of the composition of dosage forms and difference in the properties of the pharmaceutical substance [19]. Therefore, this study had the purpose to compare the effectiveness of two IFN-α-containing drugs, Viferon® and Kipferon®, as well as the interferonogen product Ergoferon® with the routes of administration used in clinical practice: rectal administration for Viferon® and Kipferon®, oral administration for Ergoferon®.

Kipferon® is widely used in medicine as an immunomodulatory and antiviral drug: it is used for pneumonia, moderate flu, infectious mononucleosis, dysentery, and moderately severe community-acquired pneumonia. It should be noted that the use of Kipferon® in combination therapy for patients with infectious diseases helps to reduce the severity and duration of intoxication syndrome, treat the main clinical symptoms, bring back to normal the patients' general condition and laboratory values, and reduce the hospital stay [20]. Viferon® is an IFN-α containing drug with immunoregulatory properties. It is prescribed for bacterial and viral infections, including chlamydia, herpes, CMV infection, and enterovirus infection [21]. Ergoferon® is an interferonogen that regulates the functional activity, the formation and binding of endogenous IFN-gamma, CD4 and histamine molecules to receptors. Ergoferon® increases the virus-induced production of IFN-γ, IFN-α/β and interleukins conjugated with them (IL-2, IL-4, IL-10), improves the IFN ligand-receptor interactions, and restores the cytokine status. This medicinal product also promotes the activation of phagocytosis and NK cells, restoring the balance of T-helper activity [22].

The study showed that with a single administration of the study drugs to animals without a concomitant pathological background, Kipferon® showed a higher level of IFN-α in comparison with both the control group and with Viferon® and Ergoferon®. The peak concentration of IFN-α with the use of Kipferon® was observed 4 hours after the

administration, without a pronounced subsequent decline, while in the rats treated with Viferon® the maximum content of IFN-α was observed at the 2nd hour and had an abrupt character. Ergoferon® did not change the content of IFN-α in the rats' blood serum. In the animals with respiratory pathology, the administration of Kipferon® (to a greater extent) and Viferon® (to a lesser extent) led to an increase in the concentration of IFN-α and IFN-γ, and reduced the content of pro-inflammatory markers by the 10th day of the experiment. The immune status was preserved on the 14th day of the study, and the animals' overall health status improved. Meanwhile, during 2 weeks of the infectious process, fluctuations in blood levels of IL-6, CRP and leukocytes were observed in the NC group of rats. During the first seven days, an increase in the concentration of acute phase proteins, IL-6 and total pool of white blood cells was observed, which indicated the activation of the immune response.

However, on the 10th and 14th days, the NC group showed depletion of immune defenses, with a pronounced decrease in the concentration of leukocytes, IL-6 and CRP. It should be noted that on the 10th and 14th days of the study, the content of IFN-γ in the animals treated with Viferon® was significantly higher than this content in the NC group of animals, as well as in the groups treated with Kipferon® and Ergoferon®, which may be a predisposing factor for the development of neurotoxic and hematotoxic effects. This study found that a Viferon® therapy significantly increased the number of the rats' movements in the Four-plate test, which is a consequence of an increase in the level of anxiety and aggression. The use of Viferon® also reduced the time of blood coagulation. These data require additional research and evaluation, as they may have a clinically significant negative effect.

It is known that the neurotoxic effect of IFN-γ is associated with the activation of astrocytes that is accomplished through the JAK/STAT3 signaling pathway. Activated astrocytes release molecules, such as glutamate, cytotoxic forms of nitric oxide, and reactive oxygen species, that trigger neuronal apoptosis [23]. Hematological disorders mediated by IFN-γ are associated with a direct damaging effect on vascular endotheliocytes, a decrease in the activity of the antithrombin-III system, and the expression of chemoattractant proteins [24].

In addition to a positive effect on immunological processes, the use of Kipferon® reduced the number of viable microorganisms, which was not observed in the case of the use of Viferon® and Ergoferon®, and may be associated with normal human immunoglobulin included in the composition of Kipferon®. It should be noted that the use of Ergoferon® did not have a significant effect on the course of the respiratory infectious process in the rats.

Conclusion

A single rectal administration of Kipferon® increased the concentration of IFN-α in the rats' blood serum to a greater extent than the use of Viferon® (administered rectally in a dose equivalent to Kipferon®) and Ergoferon® (orally). Amidst an experimental infectious process in the respiratory system, the Kipferon® therapy contributed to preserving the immune response (which was not different from the immune response in the SO animals), with an improvement in the animals' overall health status, and a decrease in the number of viable microbial cells. Better clinical and laboratory results in the course of the infectious-inflammatory process were apparently associated with additional components of Kipferon® – normal human immunoglobulins [IgG + IgA + IgM] (KIP complex), which are not included in Viferon®.

Viferon® also showed negative neurotoxicity and hematotoxicity, which requires further study, as well as absence of significant changes in the course of the pathological process when using Ergoferon®.

Thus, based on the obtained results, it can be assumed that the IFN-α-containing medicinal product Kipferon® has a higher level of pharmacological effectiveness and a lower degree of toxicity compared to Viferon® and the interferonogen Ergoferon®.

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References

1. Krause CD, Pestka S.Cut, copy, move, delete: The study of human interferon genes reveal multiple mechanisms underlying their evolution in amniotes. Cytokine, 2015;76: 480–495.
2. Schreiber G. The molecular basis for differential type I interferon signaling. J. Biol. Chem, 2017; 292: 7285–7294.
3. Kotenko SV, Durbin JE Contribution of type III interferons to antiviral immunity: location, location, location. J. Biol. Chem, 2017; 292: 7295–7303.
4. Bamford CGG, Aranday-Cortes E, Filipe LC., Sukumar S, Mair D, Filipe ADS, Mendoza JL, García KC, Fan S, Tishkoff SA, McLauchlan J. A polymorphic residue that attenuates the antiviral potential of interferon lambda 4 in hominid lineages. PLoS Pathog, 2018;14: e1007307
5. Samuel CE. Antiviral Actions of Interferons. Clinical Microbiology Reviews. 2001;14(4):778–809. 10.1128/CMR.14.4.778-809.2001
6. Crouse J, Kalinke U, Oxenius A. Regulation of antiviral T cell responses by type I interferons. Nat. Rev. Immunol, 2015; 15: 231–242.
7. van Boxel-Dezaire AH, Rani MR, and Stark GR Complex modulation of cell type-specific signaling in response to type I interferons. Immunity, 2006; 25: 361–372.
8. Teijaro JR, Ng C, Lee AM, Sullivan BM, Sheehan KC, Welch M, Schreiber RD, de la Torre JC, and Oldstone MB. Persistent LCMV infection is controlled by blockade of type I interferon signaling. Science, 2013; 340: 207–211.
9. Afanasyev SS, Aleshkin VA, Ivanov SG Prospects for the use of Kipferon® suppositories in the treatment of infectious diseases. Kremlevskaya meditsina. Klinicheskiy vestnik, 2008; 1: 65-69 [in Russian].
10. Yanina MA, Osidak LV, Milkint KK. Adenovirus infection in children: the possibility of using the medicinal product Viferon® (gel and suppositories) in therapy. Farmateka, 2013; 11 (264): 64-69 [in Russian].
11. Gavryuchenkov DV. New antiviral drug ERGOFERON in the treatment of respiratory infections. Polyclinica, 2011;5-1: 78-81 [in Russian].
12. Tai DY. Pharmacologic treatment of SARS: current knowledge and recommendations. Ann Acad Med Singap. 2007; 36(6):438-43. PMID: 17597972.
13. Kalra P, Brandl J, Gaub T, et al. Quantitative systems pharmacology of interferon alpha administration: A multi-scale approach. PLoS One. 2019;14(2):e0209587. Published 2019 Feb 13. doi:10.1371/journal.pone.0209587
14. Cripps AW, Dunkley ML, Clancy RL. Mucosal and systemic immunizations with killed Pseudomonas aeruginosa protect against acute respiratory infection in rats. Infect Immun. 1994 r;62(4):1427-36. doi: 10.1128/IAI.62.4.1427-1436.1994..
15. Liu W, Chen Y, Meng J, et al. Ablation of caspase-1 protects against TBI-induced pyroptosis in vitro and in vivo. J Neuroinflammation. 2018;15(1):48. doi:10.1186/s12974-018-1083-y
16. Cryan JF, Sweeney FF The age of anxiety: role of animal models of anxiolytic action in drug discovery. Br J Pharmacol. 2011; 164(4): 1129- 1161.
17. State Pharmacopoeia of the Russian Federation XIV edition. OFS.1.7.2.0008.15 Determination of the concentration of microbial cells [electronic resource]. Access at: https://pharmacopoeia.ru/ofs-1-7-2-0008-15-opredelenie-kontsentratsii-mikrobnyh-kletok/
18. Voronkov AV, Pozdnyakov DI. Disorders of antithrombotic function of the vascular endothelium and some parameters of plasma hemostasis with underlying focal cerebral ischemia and their correction with 4-hydroxy-3,5-di-tert-butyl cinnamic acid. Tromboz, reostaz i reologia, 2017:2(70): 73-78. – DOI 10.25555/THR.2017.2.0788. [in Russian].
19. Bandurska K, Król I, Myga-Nowak M. Interferony: między strukturą a funkcją [Interferons: between structure and function]. Postepy Hig Med Dosw (Online). 2014;68:428-40. Polish. doi: 10.5604/17322693.1101229.

20. Korochkina OV, Sobchak DM Experience of using Kipferon® in clinical treatment of infectious diseases. Trudny patient, 2009; 7 (4-5), 32-35. [in Russian].

21. Malinovskaya VV, Chebotareva TA, Parfenov VV. Clinical effectiveness of Viferon® in the treatment of influenza and acute respiratory viral infections in adults. Almanakh klinicheskoy meditsiny. 2014;-35: 109-115. [in Russian].

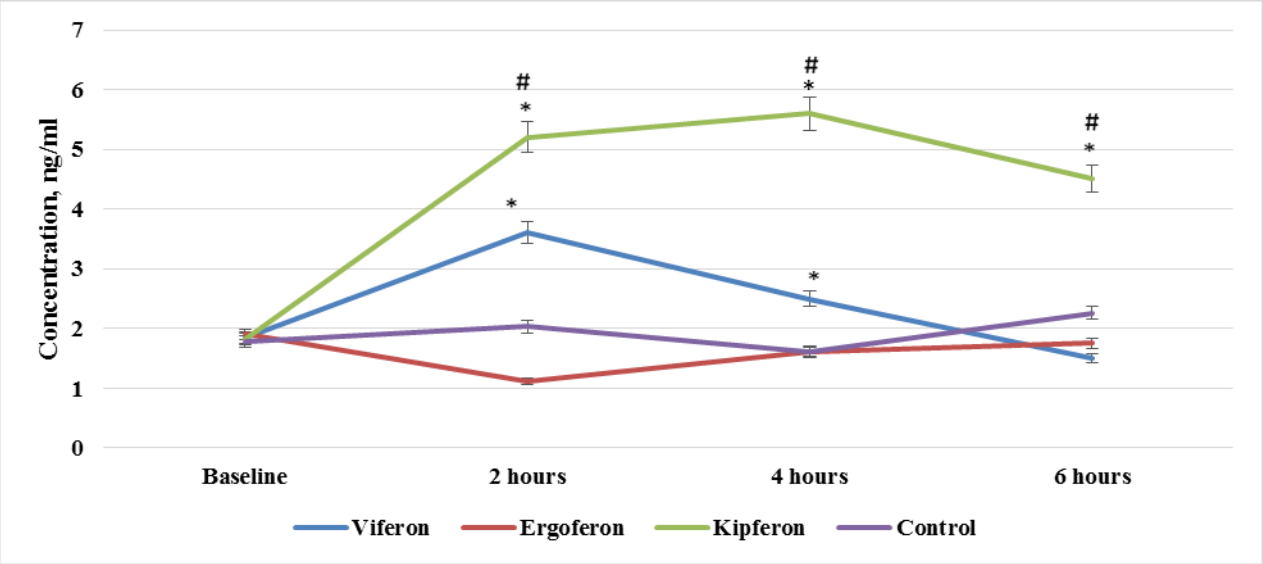
22. Usenko DV. Ergoferon: results of clinical studies in acute respiratory viral infections in children. Praktika pediatra. 2015; 4: 66-70. [in Russian].

23. Hashioka S, McGeer EG, Miyaoka T, Wake R, Horiguchi J, McGeer PL. Interferon-γ-induced neurotoxicity of human astrocytes. CNS Neurol Disord Drug Targets. 2015;14(2):251-6. doi: 10.2174/1871527314666150217122305.

24. Najem MY, Couturaud F, Lemarié CA. Cytokine and chemokine regulation of venous thromboembolism. J Thromb Haemost. 2020;18(5):1009-1019. doi: 10.1111/jth.14759. Epub 2020 Mar 5.

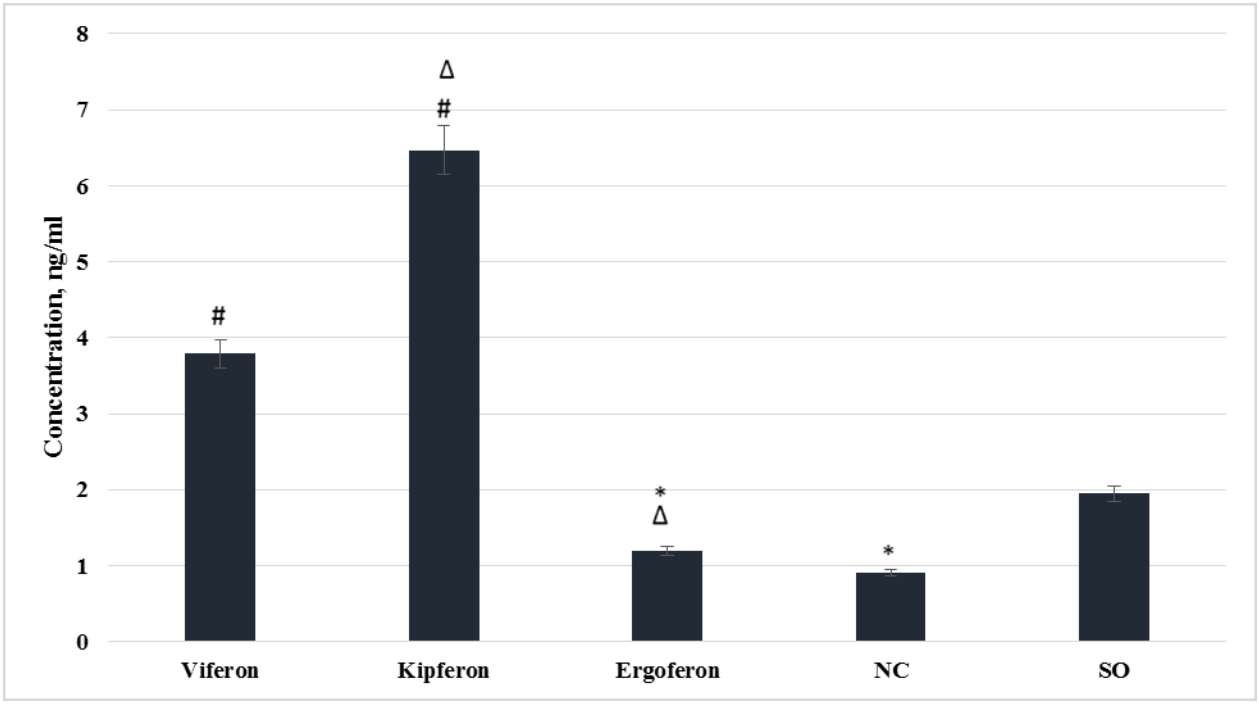
Table 1. Comparative analysis of type I and III IFN

IFN I	IFN III
Have a high affinity for IFNAR2 Do not interact with the cytokine site of the receptor Located in many organs and tissues Highly active Eliminated quickly Cause a system response Pro-inflammatory	Have a high affinity for IFNLR1 Have an affined site with cytokine receptors Located in epithelial cells, immune cells, and neutrophils Eliminated slowly Cause a local response Anti-inflammatory



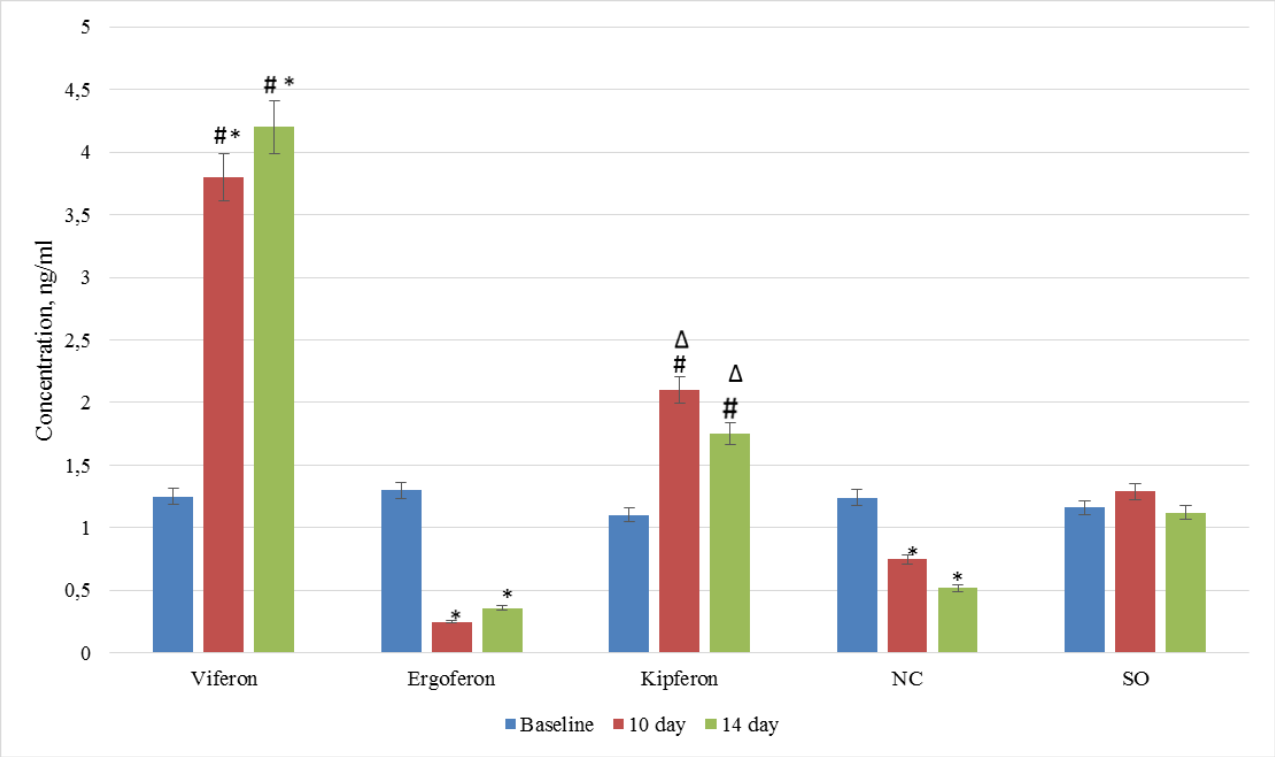
Note: * - statistically significant compared to the control group (Newman-Keuls test, p <0.05);
- statistically significant compared to the animals treated with Viferon® (Newman-Keuls test, p <0.05).

Figure 1. Changes in the concentration of IFN-α in the blood serum of the rats after a single administration of the study drugs to the animals without pathological background



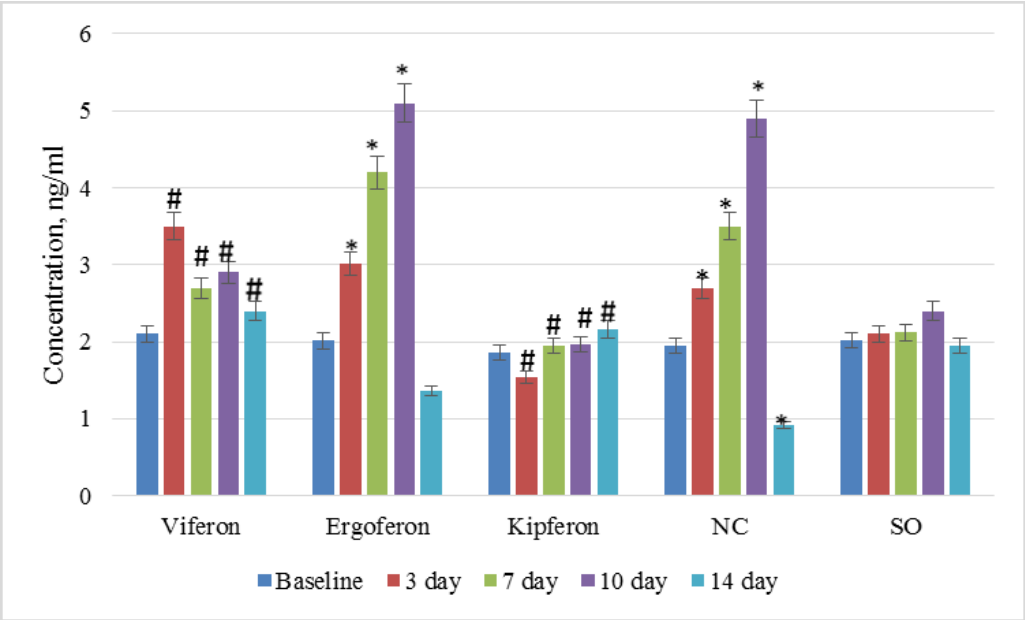
Note: * - statistically significant compared to the SO animals (Newman-Keuls test, p <0.05); # - statistically significant compared to the NC group of animals (Newman-Keuls test, p <0.05) Δ - statistically significant compared to the animals treated with Viferon® (Newman-Keuls test, p <0.05).

Figure 2. Changes in the concentration of IFN-α in the blood serum of the rats during course administration of the study drugs to the animals with respiratory infectious pathology



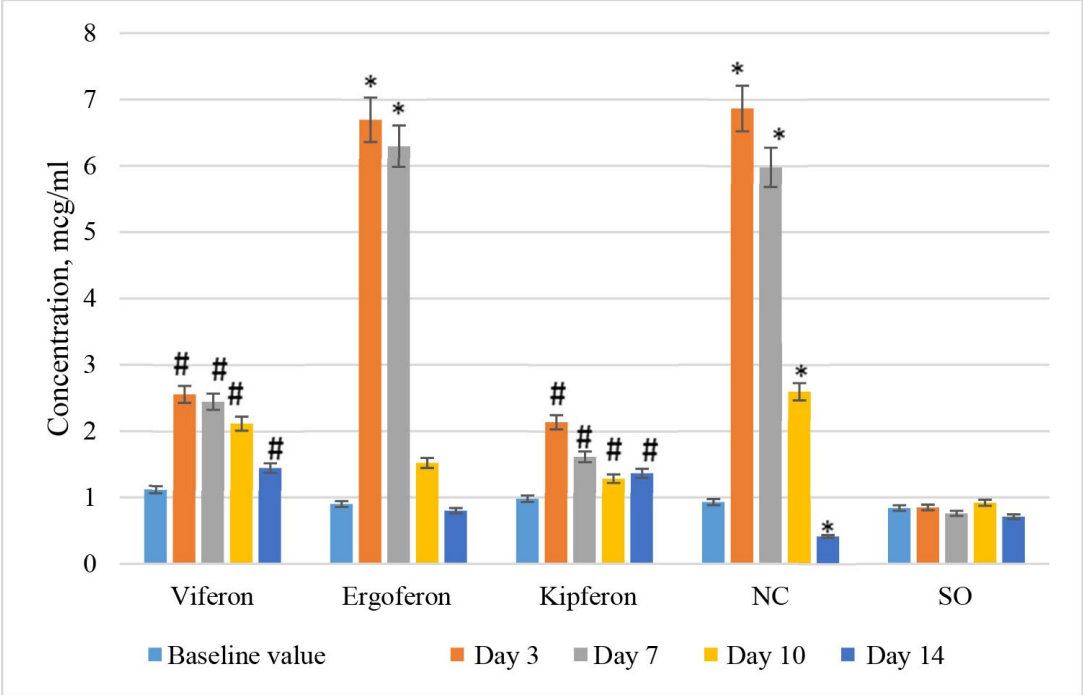
Note: * - statistically significant compared to the SO animals (Newman-Keuls test, $p < 0.05$); # - statistically significant compared to the NC group of animals (Newman-Keuls test, $p < 0.05$) Δ - statistically significant compared to the animals treated with Viferon® (Newman-Keuls test, $p < 0.05$).

Figure 3. Changes in the concentration of IFN-γ in the blood serum of the rats during course administration of the study drugs to the animals with respiratory infectious pathology



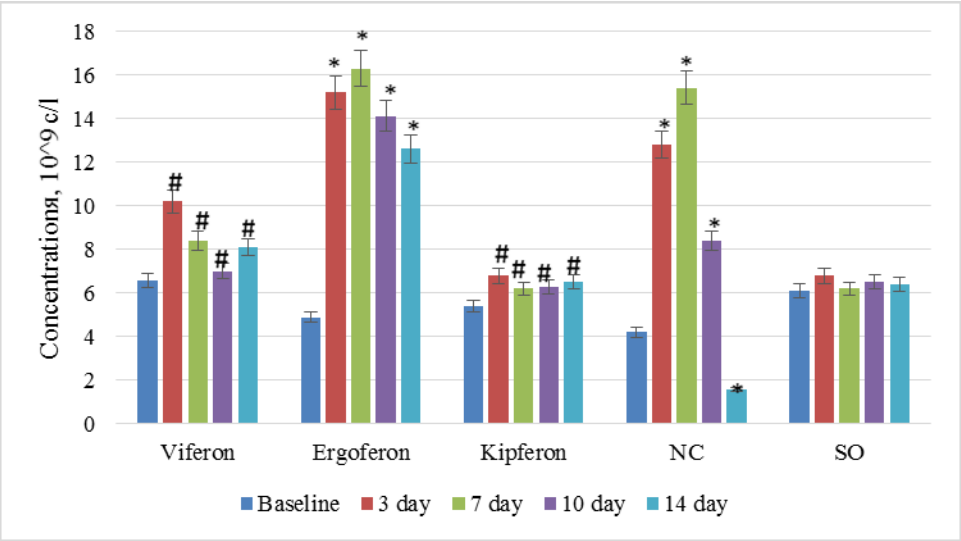
Note: * - statistically significant compared to the SO animals (Newman-Keuls test, p <0.05); # - statistically significant compared to the NC group of animals (Newman-Keuls test, p <0.05).

Figure 4. Changes in the concentration of IL-6 in the blood serum of the rats during course administration of the study drugs to the animals with respiratory infectious pathology



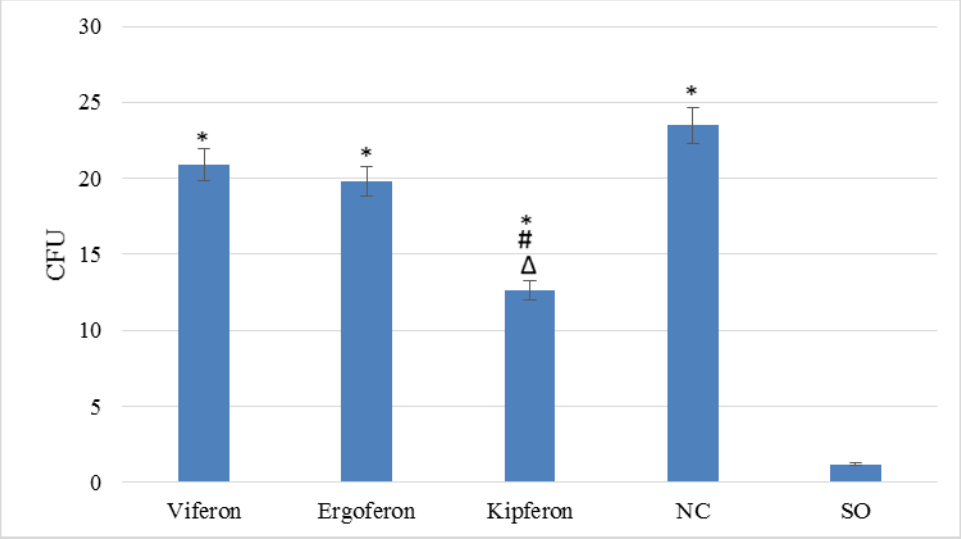
Note: * - statistically significant compared to the SO animals (Newman-Keuls test, p <0.05); # - statistically significant compared to the NC group of animals (Newman-Keuls test, p <0.05).

Figure 5. Changes in the concentration of C-reactive protein in the blood serum of the rats during course administration of the study drugs to the animals with respiratory infectious pathology



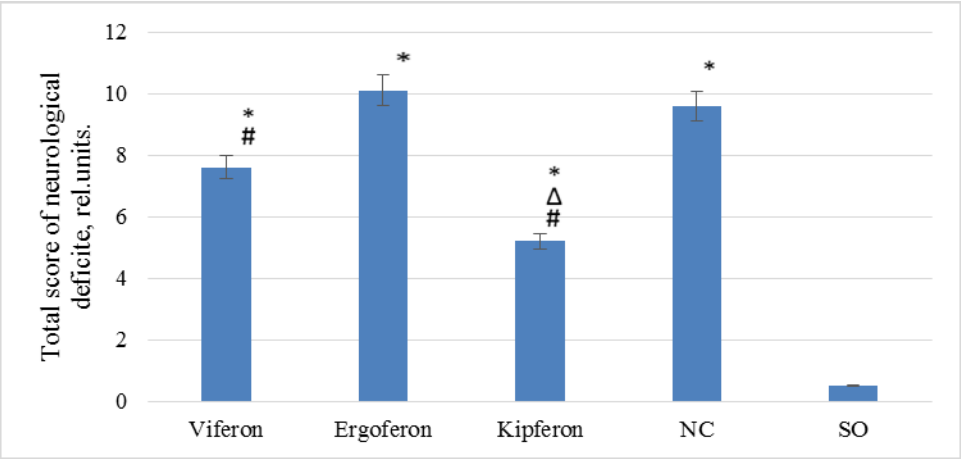
Note: * - statistically significant compared to the SO animals (Newman-Keuls test, p <0.05); # - statistically significant compared to the NC group of animals (Newman-Keuls test, p <0.05).

Figure 6. Changes in the concentration of leukocytes in the blood during course administration of the study drugs to the animals with respiratory infectious pathology



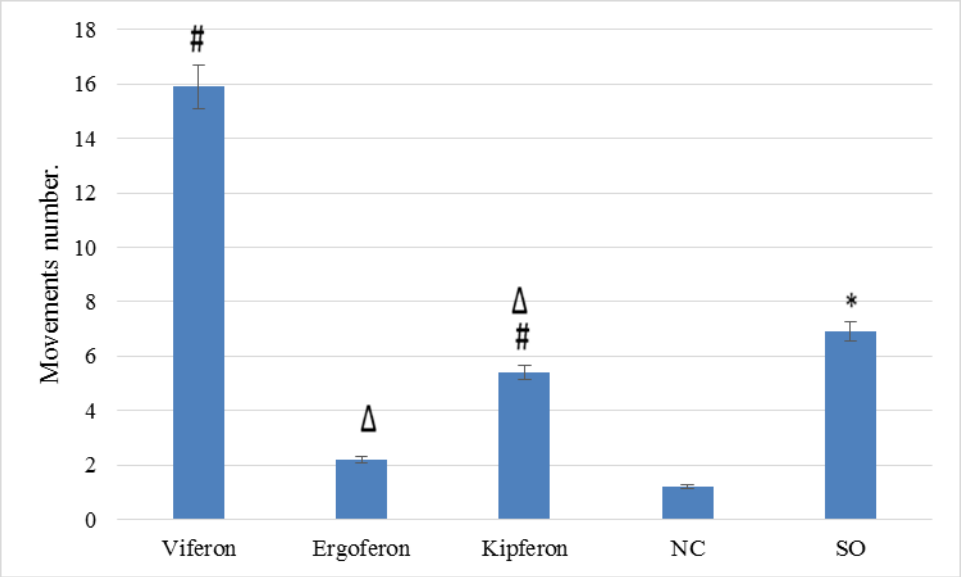
Note: * - statistically significant compared to the SO animals (Newman-Keuls test, p <0.05); # - statistically significant compared to the NC group of animals (Newman-Keuls test, p <0.05) Δ - statistically significant compared to the animals treated with Viferon® (Newman-Keuls test, p <0.05).

Figure 7. Changes in the concentration of colony-forming units in pleural effusion of the rats during course administration of the study drugs to the animals with respiratory infectious pathology



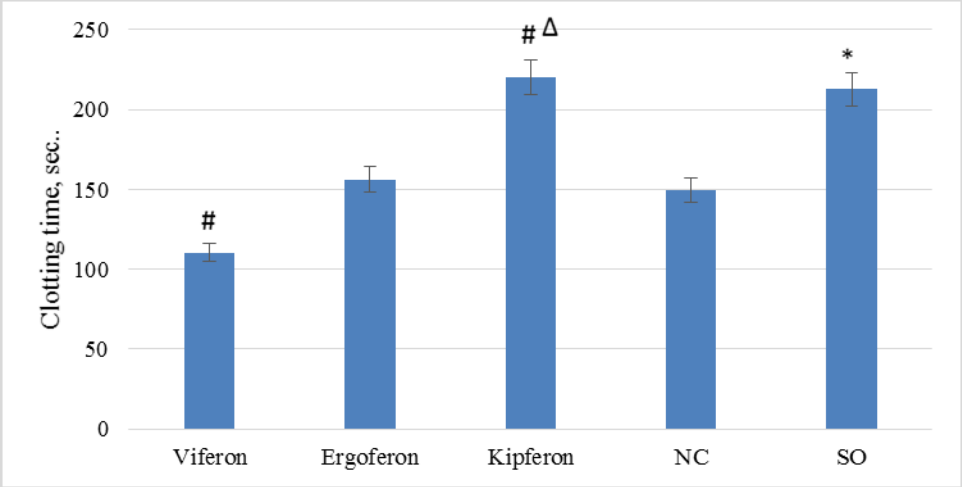
Note: * - statistically significant compared to the SO animals (Newman-Keuls test, p <0.05); # - statistically significant compared to the NC group of animals (Newman-Keuls test, p <0.05) Δ - statistically significant compared to the animals treated with Viferon® (Newman-Keuls test, p <0.05).

Figure 8. Changes in neurological impairment in the rats during course administration of the study drugs to the animals with respiratory infectious pathology



Note: * - statistically significant compared to the SO animals (Newman-Keuls test, p <0.05); # - statistically significant compared to the NC group of animals (Newman-Keuls test, p <0.05) Δ - statistically significant compared to the animals treated with Viferon® (Newman-Keuls test, p <0.05).

Figure 9. Changing the behavior of animals in the 4 plate test during course administration of the study drugs to the animals with respiratory infectious pathology



Note: * - statistically significant compared to the SO animals (Newman-Keuls test, p <0.05); # - statistically significant compared to the NC group of animals (Newman-Keuls test, p <0.05) Δ - statistically significant compared to the animals treated with Viferon® (Newman-Keuls test, p <0.05).

Figure 10. Change in the blood coagulation time in the rats during course administration of the study drugs to the animals with respiratory infectious pathology