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Results of the study of antiviral activity of ASD substance in experimental infection of mice with influenza viruses

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The aim. To evaluate the antiviral efficacy of ASD drug on a model of lethal influenza pneumonia in mice against influenza A viruses of subtypes H3N2, H5N2 and H5N8.

Materials and methods. Balb/c mice were infected with influenza viruses. The decrease of mortality and improvement in the weight dynamics of infected animals (15 animals per group) were evaluated, and the viral load in the lung tissue was determined on day 3 after infection (6 animals per group). Oseltamivir was used as a comparator medicine, and placebo was administered to negative control group animals. The medicines were administered according to a therapeutic-prophylactic or therapeutic regimen.

Results. The maximum weight loss with the H5N8 virus infected mice (placebo group) was 16.8% on day 12 (therapeutic-prophylactic regimen) and 14.8% on day 9 (therapeutic regimen). ASD worsened the dynamics of weight loss, up to 21% on day 12 (therapeutic-prophylactic regimen) and up to 28.4% on day 9 (therapeutic regimen). The maximum weight loss with the H5N2 virus infected mice was 34.6% on day 8 (therapeutic-prophylactic regimen) and 31.2% on day 7 (therapeutic regimen). The ASD medicine reduced the dynamics of weight loss: up to 29.9% on day 8 (therapeutic-prophylactic regimen) and up to 26.3% on day 7 (therapeutic regimen). The maximum weight loss with the the H3N2 virus was 38.4% on day 11 (therapeutic-prophylactic regimen) and 33.9% on day 9 (therapeutic regimen). The ASD medicine improved the dynamics of weight loss to 18.5% on day 11 (therapeutic-prophylactic regimen), but with the therapeutic regimen, it worsened the dynamics to 34.1% on day 9. A decrease of the survival rate of mice infected with the H5N8 virus using ASD (therapeutic regimen) was revealed. Mice receiving ASD and infected with the H3N2 virus (therapeutic-prophylactic regimen) or H5N2 (therapeutic regimen) showed a tendency towards a protective effect of the drug. No effect of the ASD medicine on the level of viral load was noted.

Conclusion. The ASD medicine exhibits antiviral properties when administered orally according to a therapeutic-prophylactic regimen against influenza A virus (subtypes H3N2 and H5N2).

Keywords: influenza virus; antiviral activity; in vivo testing; lethal influenza pneumonia

Abbreviations: ASD — antiseptic-stimulator Dorogova, fraction 2; ED50 — median effective dose — the dose of the drug that improves the survival rate of animals by 50%; PI — protection index — calculated indicator of drug effectiveness; MLD50 — 50% mouse lethal dose of the virus — the dose of the virus that causes the death of half of the mice in the group; OD — optical density; SOP — standard operating procedure; ALS — average life span; TCID50 — 50% tissue infectious dose — the dose of the virus that causes infection of 50% of cells; ANOVA — Analysis Of Variance; DPBS — Dulbecco's Phosphate-Buffered Saline; PBS — Phosphate-Buffered Saline.

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Результаты исследования противовирусной активности субстанции АСД при экспериментальном заражении мышей вирусами гриппа

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Цель. Оценка противовирусной эффективности препарата АСД на модели летальной гриппозной пневмонии у мышей в отношении вирусов гриппа А подтипов H3N2, H5N2 и H5N8.

Материалы и методы. Мышей линии Balb/c инфицировали вирусами гриппа. Оценивали снижение смертности и улучшение динамики веса зараженных животных (по 15 особей в группе), также определяли вирусную нагрузку в легочной ткани на 3 сут после заражения (по 6 особей в группе). Препаратом сравнения служил осельтамивир, животным из группы отрицательного контроля вводили плацебо. Препараты вводили по лечебно-профилактической или лечебной схеме.

Результаты. При инфицировании вирусом H5N8 максимальная потеря веса (группа плацебо) составила 16,8% на 12 сут (лечебно-профилактическая схема) и 14,8% на 9 сут (лечебная схема). АСД ухудшал динамику снижения веса, до 21% на 12 сут (лечебно-профилактическая схема) и до 28,4% на 9 сут (лечебная схема). При инфицировании вирусом H5N2 максимальная потеря веса составила 34,6% на 8 сут (лечебно-профилактическая схема) и 31,2% на 7 сут (лечебная схема). Препарат АСД уменьшал динамику снижения веса: до 29,9% на 8 сут (лечебно-профилактическая схема) и до 26,3% на 7 сут (лечебная схема). При инфицировании вирусом H3N2 максимальная потеря веса составила 38,4% на 11 сут (лечебно-профилактическая схема) и на 33,9% в на 9 сут (лечебная схема). Препарат АСД улучшал динамику снижения веса до 18,5% на 11 сут (лечебно-профилактическая схема), но при лечебной схеме ухудшал динамику до 34,1% на 9 сут. Обнаружили снижение выживаемости мышей, зараженных вирусом H5N8, при использовании АСД (лечебная схема). У мышей, получавших АСД, и зараженных вирусом H3N2 (лечебно-профилактическая схема) или H5N2 (лечебная схема) отметили тенденцию к протективному действию препарата. Влияния препарата АСД на уровень вирусной нагрузки не отметили.

Заключение. Препарат АСД проявляет противовирусные свойства при пероральном введении по лечебно-профилактической схеме в отношении вируса гриппа А (подтипы H3N2 и H5N2).

Ключевые слова: вирус гриппа; противовирусная активность; тестирование *in vivo*; летальная гриппозная пневмония

Список сокращений: АСД — антисептик-стимулятор Дорогова, фракция 2; ЕД50 — средняя эффективная доза — доза препарата, которая улучшает выживаемость животных на 50%; ИЗ — индекс защиты — расчетный показатель эффективности препарата; МЛД50 — 50% мышиная летальная доза вируса — доза вируса, вызывающая гибель половины мышей в группе; ОП — оптическая плотность; СОП — стандартная операционная процедура; СПЖ — средняя продолжительность жизни; ТИД50 — 50% тканевая инфекционная доза — доза вируса, вызывающая заражение 50% клеток; ANOVA — Analysis Of Variance, анализ достоверности различий между группами; DPBS — фосфатно-солевой буферный раствор по протоколу Дульбекко; PBS — фосфатно-солевой буферный раствор.

INTRODUCTION

The influenza virus causes a highly contagious respiratory disease, which is the cause of annual epidemics, affecting up to half of the world's population. Every year, about 1 billion cases of seasonal influenza are registered in the world, 3–5 million of which are severe. From 290 to 650 thousand people die annually from respiratory pathologies caused by influenza viruses [1]. Influenza is of particular importance due to the high virulence of the pathogen, the ability to pandemic spread, viral variability, and the development of complications. The most common complication of influenza is pneumonia, which accounts for 65% of all complications and often ends in death [2]. In addition, influenza can cause a number of non-respiratory complications, including febrile seizures, Reye's syndrome, and myocarditis [3–5].

According to a large meta-analysis conducted

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by K.E. Lafond et al. (2021), influenza viruses cause more than 5 million hospitalizations worldwide each year. They also found out that influenza accounts for an average of 14.1% of hospitalizations for acute respiratory diseases among the adult population, with no significant differences between age groups [6].

Despite the fact that severe influenza can be observed at any age, children are the most vulnerable. The incidence rate remains consistently high in the pediatric population [7, 8]. Every year, about 870,000 children under the age of 5 are hospitalized worldwide due to influenza [9]. A meta-analysis conducted by H. Nair et al. (2011) found that from 28,000 to 111,500 deaths among children under the age of 5 are associated with causes related to influenza morbidity, and the vast majority of them occur in developing countries [10]. P.J. Gill et al. (2015) assessed which children are at increased risk of developing complications from influenza. It was found out that prematurity, neurological disorders, sickle cell anemia, immunosuppression, diabetes and age under 2 years are risk factors for hospitalization. In addition, having more than one of these factors increased the risk of hospitalization from 52 to 74% [11].

Risk groups for severe influenza also include elderly people (aged ≥ 60 years), pregnant women, people with chronic diseases or congenital/acquired immunodeficiency [12].

This work is devoted to the study of the antiviral activity of the drug ASD-2 *in vivo* on a model of lethal influenza pneumonia in Balb/c mice.

In this work, a model of lethal influenza pneumonia was used, which is generally accepted in studies of the protective activity of chemotherapeutic agents intended for the treatment and prevention of influenza infection [13]. At the same time, the main indicators by which the effectiveness of drug samples was assessed were the dynamics of mortality of experimental animals and the dynamics of weight indicators. The viral load in the lung tissue was also assessed to confirm the specificity of the drug's action against the virus.

THE AIM. To evaluate the protective efficacy *in vivo* of the drug ASD on a model of lethal influenza pneumonia in Balb/c mice against influenza A viruses of subtypes H3N2, H5N2 and H5N8.

Research tasks:

1. Adaptation to Balb/c mice of influenza virus A/common duck/Uvs Nuur lake/26/2016 A (H5N8);
2. Adaptation to Balb/c mice of influenza virus A/Duck/Potsdam/1402-6/86 (H5N2);
3. Adaptation to Balb/c mice of influenza virus A/Aichi/2/68 (H3N2);
4. Determination of the dynamics of animal death when the test drug is administered according to a therapeutic and prophylactic regimen against influenza viruses A/common duck/Uvs Nuur lake/26/2016 (H5N8), A/Duck/Potsdam/1402-6/86(H5N2), A/Aichi/2/68(H3N2);
5. Determination of the dynamics of animal death when the test drug is administered according to a therapeutic regimen against influenza viruses A/common duck/Uvs Nuur lake/26/2016 (H5N8), A/Duck/Potsdam/1402-6/86(H5N2), A/Aichi/2/68(H3N2);
6. Determination of the dynamics of changes in body weight of animals when the test drugs are administered according to a therapeutic and prophylactic regimen against influenza virus A/common duck/Uvs Nuur lake/26/2016 (H5N8), A/Duck/Potsdam/1402-6/86(H5N2), A/Aichi/2/68(H3N2);
7. Determination of the dynamics of changes in body weight of animals when the test drugs are administered according to a therapeutic and prophylactic regimen against influenza virus A/common duck/Uvs Nuur lake/26/2016 (H5N8), A/Duck/Potsdam/1402-6/86(H5N2), A/Aichi/2/68(H3N2);
8. Determination of the viral load in the lung tissue of infected animals on the 3rd day after infection.

MATERIALS AND METHODS

Study design

The study was conducted on the basis of the Smorodintsev Research Institute of Influenza from December 2021 to June 2022.

The study drug ASD (antiseptic-stimulator Dorogova, fraction 2), manufacturer LLC "AVZ S-P", Sergiev Posad. The control drug is oseltamivir (Tamiflu®) LaRoche, Switzerland (positive control). Date of manufacture: 04.2016. Series: M1030. Expiry date: 04.2023. Dosage form: capsules of 75 mg. Storage conditions: in the refrigerator at a temperature of 2–8°C. Placebo (negative control) — saline solution.

The study was conducted on 423 female Balb/c mice aged 5–7 weeks weighing 19.2 8± 0.29. The choice of this line is fully consistent with the methodological documents used in the work[1, 2, 3]. When determining

1 Guidelines for conducting preclinical studies of drugs. Part one. Mironov AN, editor; Moscow: Grif and K; 2012. 944 p. Russian
2 GOST 33215-2014. Guidelines for the care and maintenance of laboratory animals. Rules of equipment of premises and organization of procedures; Introduced on 1.07.2016; Moscow: Standartinform; 2016. 12 p. Russian
3 Ivanov YuI, Pogorelyuk ON. Processing of the results of biomedical research on microcalculators according to programs. Moscow: Meditsina Publ.; 1990. 217 p. DOI: 10.19163/2307-9266-2025-13-5-385-402. Russian

the protective activity (reducing mortality and improving the weight dynamics of infected animals), 15 individuals were used in the group, to determine the viral load in the lung tissue — 6 individuals in the group. Mice were obtained from the "Stolbovaya", Moscow region. During the period of acclimatization and experiment, mice were placed in polycarbonate cages BENEX a.s., Czech Republic, type T3A, S = 1200 cm^2, in groups of 3 individuals (at the first stage) and 15 individuals (at the second stage), on bedding (wood granules); the cages are covered with steel lattice lids with a feeding recess. Granulated feed for keeping mice (LLC "Laboratorkorm", Moscow) was given ad libitum in the feeding recess of the steel lattice lid of the cage. The animals were given water purified by reverse osmosis on a MilliporeRiOs 30 water purification unit. Water in standard drinkers with steel lids-spouts was given ad libitum. Wood granules were used as bedding. The animals were kept in separate rooms of the vivarium of the Smorodintsev Research Institute of Influenza in controlled conditions (18–24°C and relative air humidity 50–80%). The photoperiod was 12 hours night / 12 hours day with artificial lighting with fluorescent lamps. Animal care and maintenance was carried out in accordance with SOP No. B/004/01, adopted at the Smorodintsev Research Institute of Influenza.

When determining the protective activity (reducing mortality and improving the weight dynamics of infected animals), 15 individuals were used in the group, to determine the viral load in the lung tissue — 6 individuals in the group.

Distribution by groups

Animals were distributed into groups by simple randomization according to body weight (individual body weight was within the range of variation ±10 % of the average value of the indicator). This range was chosen to ensure the homogeneity of experimental groups and exclude the influence of individual characteristics of animals on the outcome of the experiment.

For the purpose of identification, each animal in the group was marked with an individual number, which is recorded on the cage card. For marking, a biological dye (brilliant green "Lekker" BZ-3), safe for mice, was used. The following marking rules were followed: 1: left upper paw, 2: left side, 3: left lower paw, 4: head, 5: back, 6: tail, 7: right upper paw, 8: left side, 9: left lower paw, 10: no coloring, 11: left and right upper paws, 12: left and right side, 13: left and right lower paws, 14: head and tail, 15: strip from head to tail.

Laboratory animals were kept for 5 days before the start of the study for adaptation with group housing in cages. During this period, the clinical condition of the animals was monitored every day by visual examination; coat condition, presence of skin lesions, and mobility were assessed. Animals with abnormalities detected during the examination were not included in the experimental groups.

Euthanasia and ethics approval

At the end of the experiment, the animals were subjected to planned euthanasia by overdose of CO_2. Euthanasia was completed by displacement of the cervical vertebrae (cervical dislocation). All procedures with animals in the study were reviewed and approved by the Bioethics Commission of the Smorodintsev Research Institute of Influenza for compliance with regulatory acts (Minutes of the meeting of the Bioethics Commission of the Smorodintsev Research Institute of Influenza No. 49 dated March 04, 2022).

Infectious cultures

The following influenza virus strains were used for the work: A/common duck/Uvs Nuur lake/26/2016 (H5N8), A/Duck/Potsdam/1402-6/86(H5N2), A/Aichi/2/68(H3N2). All strains were obtained from the influenza virus collection of the Laboratory of Chemotherapy of Viral Infections of the Smorodintsev Research Institute of Influenza.

To determine the viral load in the lungs of animals, the MDCK (Madin-Darby canine kidney) cell culture of the spaniel kidney was used, as the most sensitive and permissive to various human influenza viruses [14]. The source of the cell culture is the MDCK London Line cell line (passage 8 / 8) was obtained from the Influenza Reagent Resource, CDC&P, Atlanta, Georgia, USA (cat. No. FR-58). The cells were cultured on a nutrient medium of the following composition: Alpha MEM medium (Biolot, Russia) with the addition of 5% fetal bovine serum (Biowest, USA) and a mixture of penicillin / streptomycin antibiotics at a concentration of 1% (Biolot, Russia).

Experimental design for adaptation of influenza viruses (stage 1)

To adapt the virus to animals, 5 passages were carried out through the lungs of mice. For each virus, the adaptation process was carried out as follows: three animals were infected with the corresponding strain in a dilution of 10:1, on the 3 day after infection, the animals were euthanized and the lungs were taken,

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homogenized, and the resulting suspension was used for subsequent infection.

The influenza virus after adaptation was propagated in the allantoic cavity of 10-day-old developing chicken embryos, after which the liquid was collected, clarified by centrifugation (Eppendorf 5424 centrifuge [Eppendorf, Germany], 5 min at 3000 rpm) and packaged into 1 ml aliquots. All aliquots were made from a single stock of allantoic fluid and frozen simultaneously at –80 °C. A preliminary titration of the virus on Balb/c mice was carried out to determine the 50 % mouse lethal dose of the virus (MLD50) (Tables 1 and 2).

Experimental design for determining antiviral activity (Stage 2)

For the experiment, the drug was diluted according to the following scheme: 0.1 mL is diluted in 39.9 mL of warm water. This dilution scheme allows to obtain the required concentration of the solution when using the minimum amount of the test. The dose was 0.8 mL of the resulting solution per mouse weighing 20 g. The specified volume corresponded to the calculated dosage of the drug, providing the required load of the active substance on the body weight of the animal and a sufficient level of exposure for the implementation of the pharmacological effect. The test drug, or placebo, (depending on the randomization group) was administered fractionally orally using a probe for oral administration 2 times/day with an interval of 6 hours in a volume of 0.4 mL.

All drugs were administered according to a *therapeutic and prophylactic regimen* (10 days before infection, then: for 10 days after infection) and according to a therapeutic regimen (for 10 days after infection) 2 times/day with an interval of 6 hours. These administration regimens were developed and used in the framework of this study to achieve the experimental objectives. Observation of the animals was carried out for 14 days after infection.

The oseltamivir was used to control the specificity of the pathological process. Oseltamivir is a neuraminidase inhibitor with proven clinical efficacy in the treatment and prevention of influenza A and B. The drug is recommended for use in influenza by international health organizations, including the World Health Organization (WHO)[4] and the US Centers for Disease Control and Prevention (CDC)[5]. In clinical guidelines for the treatment of influenza[6, 7], in the Russian Federation, oseltamavir is also recommended for the treatment of influenza in adults and children. It has been proven that oseltamivir shortens the duration of the disease, reduces the viral load and the risk of complications, especially with early initiation of therapy [15, 16]. The effectiveness of the drug in influenza has been confirmed in large systematic reviews and meta-analyses [17, 18].

Oseltamivir was administered to animals orally using a gastric probe (Fisherbrand, USA) in a volume of 0.2 mL at a dosage of 20 mg/kg/day, according to a similar scheme as the test drugs.

In the placebo groups, animals were administered sterile saline solution orally.

Mice under light ether anesthesia were infected intranasally with the virus in a volume of 50 µL at a dose of 2 MLD50. Mice were weighed daily and the death of animals was recorded. The number of animals was 45 individuals at the first stage and 378 individuals at the second stage. The number of animals in each group was 21 individuals, of which 15 individuals were intended to assess mortality and weight dynamics during infection, and 6 individuals — to assess the viral load in the lungs. The general scheme of the experiment is presented in Tables 1 and 2, the manipulations performed with the animals are indicated in Tables 3 and 4.

Collection of organs and preparation of homogenates

On the 3 day after infection, 6 animals from each group were euthanized, dissected, their lungs were isolated and weighed. Lung tissue samples were homogenized in Dulbecco's phosphate-buffered saline solution using a TissueLyserII device (Qiagen, USA).

Virus titration on MDCK cell culture

To assess the level of virus reproduction in animal lung tissue samples, its infectious activity was titrated in MDCK cell culture (4 wells of a 96-well plate for each dilution of the tissue sample). MDCK cells were seeded on 96-well plates in a volume of 100 µL of cell

[4] WHO Guidelines for Pharmacological Management of Pandemic Influenza A(H1N1) 2009 and Other Influenza Viruses. Geneva: World Health Organization; 2010. Available from: https://www.ncbi.nlm.nih.gov/books/NBK138515/

[5] CDC. Treating Flu with Antiviral Drugs. Available from: https://www.cdc.gov/flu/treatment/antiviral-drugs.html

[6] The flu. Clinical Guidelines. 2025. Ministry of Health of the Russian Federation. Available from: https://cr.minzdrav.gov.ru/view-cr/249_2. Russian

[7] Clinical recommendations. Flu in adults. 2022. Ministry of Health of the Russian Federation. Available from: https://cr.minzdrav.gov.ru/view-cr/749_1. Russian

suspension with a cell concentration of 1×105 / mL, i.e. the final cell concentration was 1×104 cells / well. Then MDCK cells were incubated for 24 hours in a CO_2 incubator at 37 °C in an atmosphere of 5% CO_2 until a monolayer was formed. After that, the cells were washed for 5 min with alpha-MEM medium with glutamine and used for virus cultivation. A series of 10-fold dilutions (from 10^{-1} to 10^{-7}) were prepared from the homogenate of tissue samples on alpha-MEM medium with glutamine with the addition of trypsin (1 μg/mL), which is necessary for the successful penetration of the influenza virus into cells, and 20 μg/mL of ciprofloxacin (a broad-spectrum antimicrobial agent of the fluoroquinolone group) and introduced them into the wells of the plate with MDCK cells. The plates were incubated for 72 hours at 37 °C in an atmosphere of 5% CO_2.

After the incubation period, 100 μL of culture fluid from each well of the plate was transferred to the wells of 96-well plates with a round bottom for immunological reactions and 100 μL of 1% suspension of chicken erythrocytes in saline solution was added to each well. The suspension of chicken erythrocytes was obtained according to SOP No. LHT/019/01 from whole blood of Leghorn chickens. The plates were incubated for 1 hour at room temperature, after which the presence or absence of hemagglutination in the wells was visually assessed. The virus titer was calculated by the Reed and Mench method [19] and expressed in 50% tissue infectious doses (TCID50) per 100 μL of volume.

Statistical processing

The main criterion for evaluating antiviral activity is a statistically significant difference in the survival rate of mice in the drug group compared to the placebo group.

Secondary criteria for evaluating antiviral activity:

— a statistically significant difference in the weight loss of animals in the placebo group compared to the drug group;

— a statistically significant decrease in the viral load in the lungs of mice in the drug group compared to the placebo group.

The criterion for the adequacy of the experiment is a statistically significant difference in the survival rate of mice in the comparison drug group compared to the placebo group.

The research materials were statistically processing with methods of parametric and non-parametric analysis. Accumulation, correction, systematization of initial information and visualization of the results were carried out in Microsoft Office Excel 2016 spreadsheets. Statistical analysis was performed with free software environment for calculations R v. 4.0.2 in the RStudio Version 1.3.1056 program[8].

Based on the data obtained from measuring the weight of mice after infection with influenza viruses, survival tables were constructed, then the Kaplan-Meier method was used to construct survival curves [20]. Comparison of survival functions by groups was carried out using the Gehan-Wilcoxon criterion and using the Cox-Mantel log-rank criterion [21][9].

The data obtained as a result of measurements were combined into variational series, in which the calculation of arithmetic mean values (M) and standard deviations (SD) was carried out. Quantitative indicators were assessed for compliance with the normal distribution (n = 10), for this purpose the Shapiro–Wilk criterion was used [22, 23]. To determine the significance of differences between group means in samples with a data distribution different from the normal one, the Mann-Whitney criterion was used [24].

The protection index was calculated using formula 1.

$$PI = (Mc — Me) / Mc \times 100\% \quad (1),$$

where Mc and Me are the percentages of animal deaths in the placebo group and the experimental group receiving the drug under study or the control drug, respectively, at the end of the experiment (14 days after infection). This formula is widely used to calculate the protection index in preclinical studies [25, 26].

For graphical representation of data on the relative decrease in body weight of animals, the relative value of body weight in % to the weight on the day of infection (day 0) was calculated for each animal. Then the arithmetic mean for the group and a curve of the dependence of the group average on the day after infection was constructed. To determine the significance of differences between groups on the day of greatest weight loss in the placebo group after infection, one-way ANOVA analysis of variance was used for group comparison, then the Dunnett's criterion for a posteriori pairwise comparisons with the placebo group. Differences between groups were considered statistically significant if the *p-value* did not exceed 0.05.

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RESULTS

Dynamics of body weight loss of experimental animals infected with influenza virus A/common duck/Uvs Nuur lake/26/2016 A (H5N8)

The study showed that infection with the influenza virus led to the development of a pathological process in laboratory mice. External signs of the disease were manifested in limiting the mobility of animals, increased breathing, as well as in reducing the consumption of food and water. All of these signs are typical for influenza pneumonia. The dynamics of changes in the body weight of animals with modeled influenza pneumonia is presented in Figure 1.

The maximum weight loss in the placebo group was 16.8 % on the 12 day after infection with therapeutic and prophylactic and 14.8 % on the 9 day with therapeutic regimens of administration.

The comparison drug oseltamivir reduced the weight loss in mice on the 12 day after infection compared to the Placebo group with the therapeutic and prophylactic regimen to 3.1 % and with the therapeutic regimen on the 9 day to 9.9 %.

The test drug ASD in both regimens of administration worsened the dynamics of weight loss in experimental animals, up to 21 % on the 12 day after infection and up to 28.4 % on the 9 day after infection in the therapeutic and prophylactic and therapeutic regimens of administration, respectively.

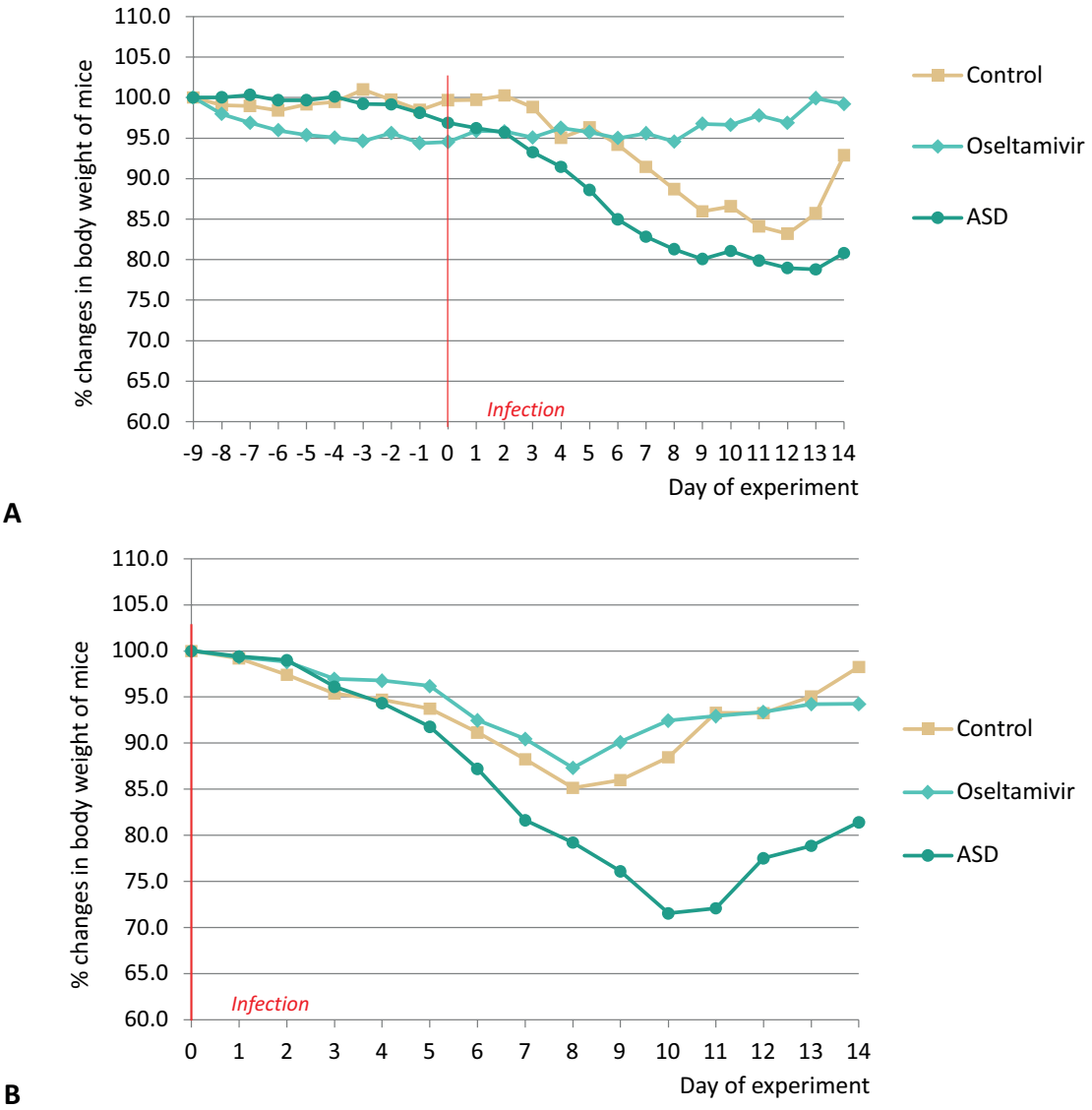

Figure 1 — Dynamics of changes in body weight of Balb/c mice with experimental influenza pneumonia caused by influenza virus A/common duck/Uvs Nuur lake/26/2016 A (H5N8) under conditions of using the test drugs according to a therapeutic and prophylactic regimen (n = 15).

Note: A — prophylactic regimen; B — therapeutic regimen.

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Dynamics of body weight loss of experimental animals infected with influenza virus A/Duck/Potsdam/1402-6/86 (H5N2)

The study showed that infection with the influenza virus led to the development of a pathological process in laboratory mice. External signs of the disease were manifested in limiting the mobility of animals, increased breathing, as well as in reducing the consumption of food and water. All of these signs are typical for influenza pneumonia [27, 28]. The dynamics of changes in the body weight of animals with modeled influenza pneumonia is presented in Figure 2.

The maximum weight loss in the placebo group was 34.6 % on the 8 day after infection with therapeutic and prophylactic and 31.2 % on the 7 day with therapeutic regimens of administration.

The comparison drug oseltamivir reduced the weight loss in mice on the 8 day after infection compared to the placebo group with the therapeutic and prophylactic regimen to 24 % and with the therapeutic regimen on the 7 day to 15.5 %.

The test drug ASD in both regimens of administration slightly reduced the dynamics of weight loss in experimental animals, up to 29.9 % on the 8 day after infection and up to 26.3 % on the 7 day after infection in the therapeutic and prophylactic and therapeutic regimens of administration, respectively.

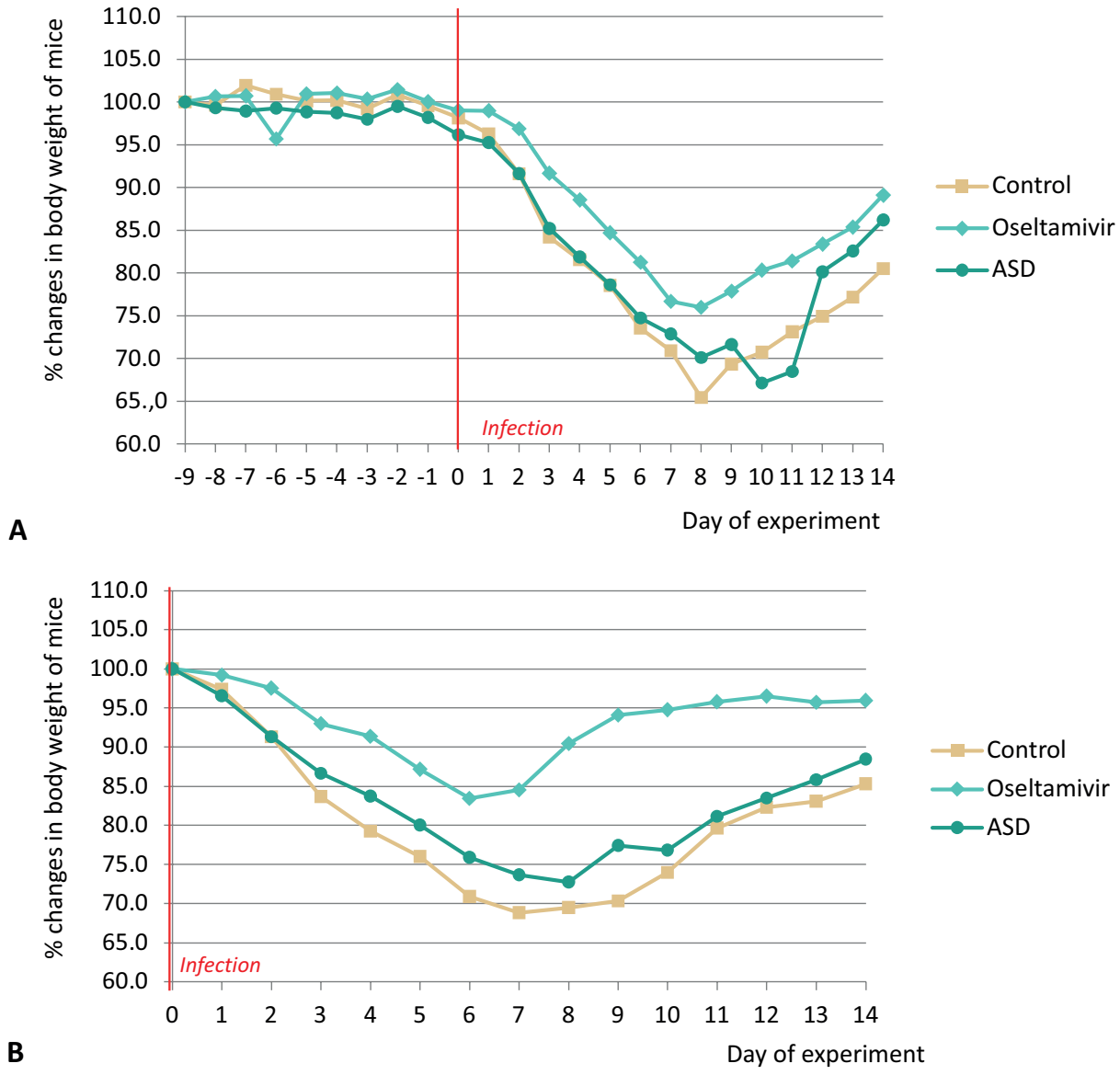

Figure 2 — Dynamics of changes in body weight of Balb/c mice with experimental influenza pneumonia caused by influenza virus A/mallard/Pennsylvania/10218/84(H5N2) under conditions of using the test drugs according to a therapeutic and prophylactic regimen (n = 15).

Note: A — prophylactic regimen; B — therapeutic regimen.

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Dynamics of body weight loss of experimental animals infected with influenza virus A/Aichi/2/68(H3N2)

The study showed that infection with the influenza virus led to the development of typical symptoms of the pathological process in laboratory mice. External signs of the disease were manifested in limiting the mobility of animals, increased breathing, as well as in reducing the consumption of food and water. All of these signs are typical for influenza pneumonia. The dynamics of changes in the body weight of animals with modeled influenza pneumonia is presented in Figure 3.

Infection with the influenza virus H3N2 led to weight loss in animals from all experimental groups. The maximum weight loss in the Placebo group was 38.4 % on the 11 day after infection with the therapeutic and prophylactic regimen and 33.9 % on the 9 day with the therapeutic regimen.

The comparison drug oseltamivir reduced the weight loss in mice on the 11 day after infection compared to the Placebo group with the therapeutic and prophylactic regimen to 9.6 % and with the therapeutic regimen on the 9 day to 14.9 %.

The test drug ASD administration improved the dynamics of weight loss in experimental animals, up to 18.5 % on the 11 day after infection with the therapeutic and prophylactic regimen of administration. With the therapeutic regimen of

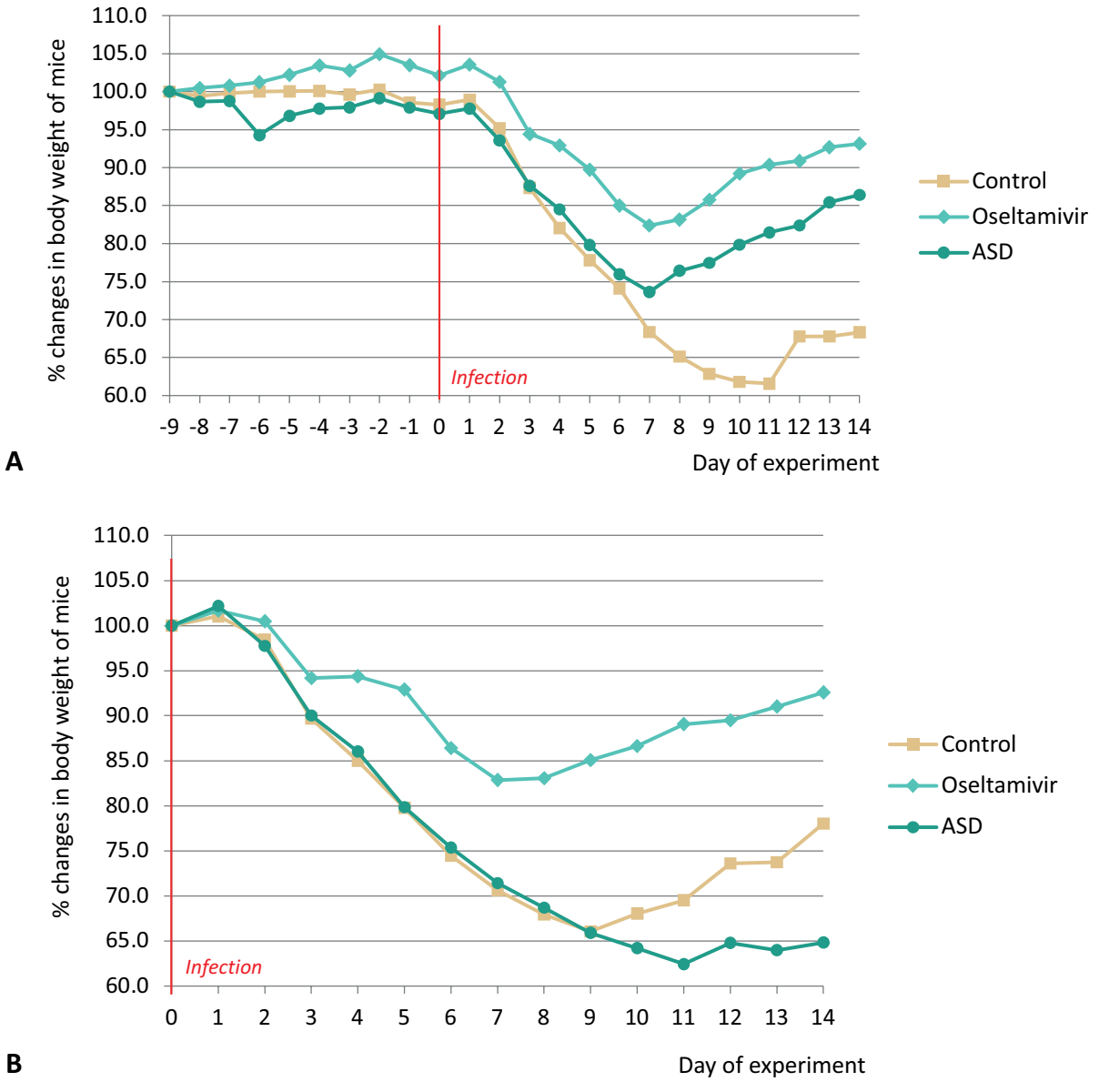

Figure 3 — Dynamics of changes in body weight of Balb/c mice with experimental influenza pneumonia caused by influenza virus A/Aichi/2/68 H3N2 under conditions of using the test drugs according to a therapeutic and prophylactic regimen (n = 15).

Note: A — prophylactic regimen; B — therapeutic regimen.

administration, the drug ASD worsened the dynamics of weight loss to 34.1 % on the 9 day after infection.

Statistical analysis of data obtained from the analysis of survival of mice after infection with influenza virus

In the course of survival analysis, fixed censoring was carried out: for each combination of "virus strain–treatment regimen–drug", a sample of 15 animals was observed for 14 days after infection. The identification of differences between the survival functions of mice receiving drugs and placebo in various experimental groups was carried out using the Gehan-Wilcoxon and Cox-Mantel tests. The proportions of surviving mice are presented in Tables 5–7. The results of comparing survival functions by groups are presented in Table 8, the dynamics of death are presented in Tables 9–11.

As it can be seen from Table 8, statistically significant differences in survival functions in a positive direction were found when compared with the placebo group of oseltamivir in the preventive and therapeutic regimen in the case of infection of mice with the H5N8 virus and in both regimens in the case of infection of mice with the H3N2 virus. In the case of the H5N2 virus, differences were also noted, although they were not statistically significant.

For the ASD, statistically significant differences from the placebo group were noted in the case of the H5N8 virus, when used according to the therapeutic regimen, and towards a worsening of the infectious process.

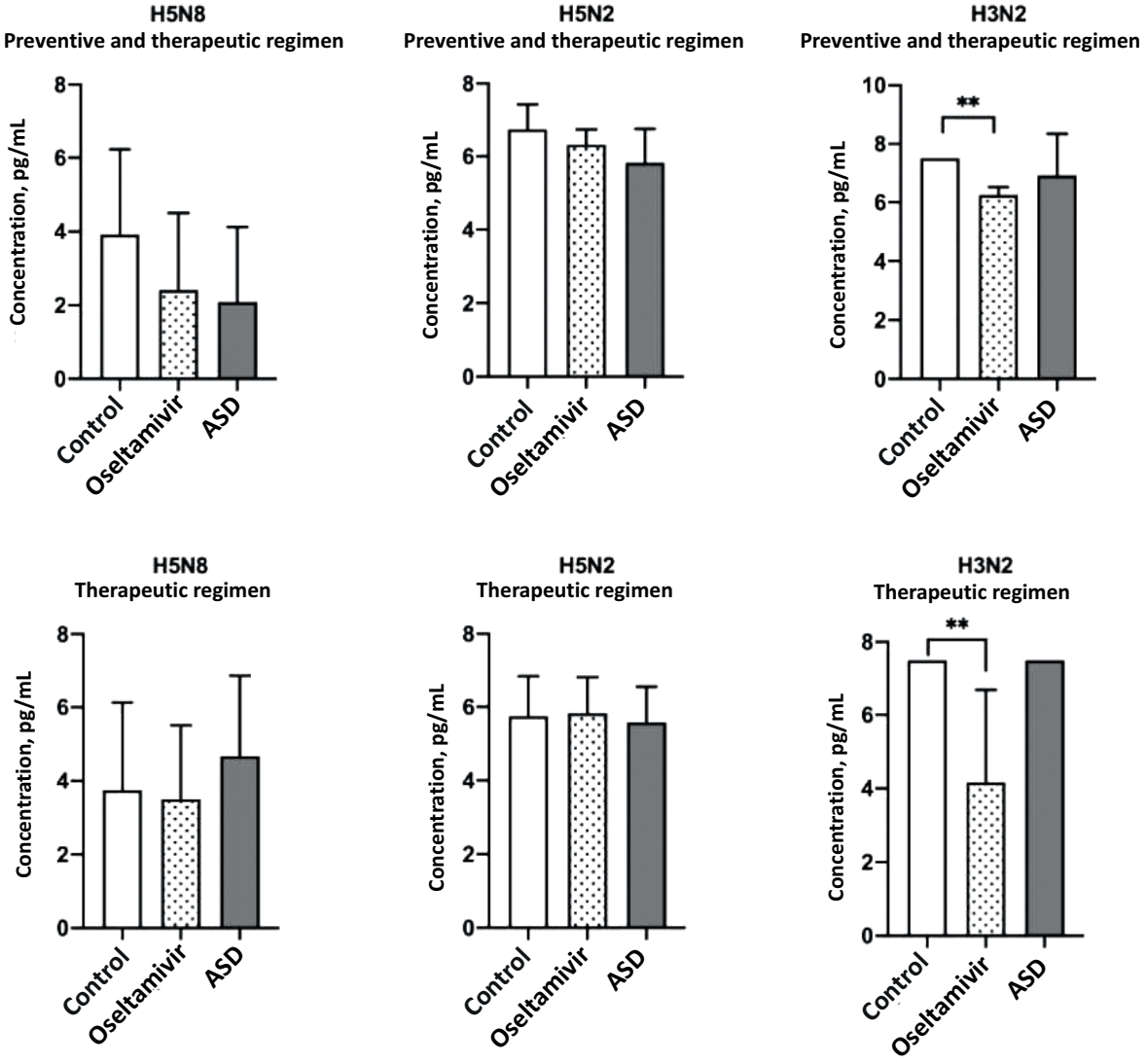

Figure 4 — Viral load in the lung tissue of animals infected with influenza virus (strains H5N8, H3N2, H5N2), determined by titration on a cell culture (n = 6 in each group).

Note: in the diagram, the columns with whiskers indicate the mean ± standard deviation (M ± SD). The P values are indicated above the brackets — the result of applying the one-sided Dunnett's test when compared with the Placebo group.

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Table 1 — Experimental design for adaptation of viruses to Balb/c mice (Stage 1), *n* = 45

Group No.	n of animals	Number of passages	Virus	Virus adaptation
1	15	5	H5N8	Infection with the virus, collection of organs — in 15 mice in the group
2	15	5	H5N2	
3	15	5	H3N2	

Table 2 — Manipulations performed with animals (Stage 1)

Group No.	Manipulations performed																
	-5	-4–-1	0	1–2	3	4	5–6	7	8	9–10	11	12	13–14	15	16	17–18	
1	A, Q	Q	I	E	C	I	E	C	I	E	C	I	E	C	I	E	C
2	A, Q	Q	I	E	C	I	E	C	I	E	C	I	E	C	I	E	C
3	A, Q	Q	I	E	C	I	E	C	I	E	C	I	E	C	I	E	C

Note: A — admission of animals; Q — quarantine; I — infection; C — collection of organs; E — conducting a clinical examination of animals.

Table 3 — Experimental design for determining the antiviral activity of samples on a model of lethal influenza infection in Balb/c mice with therapeutic and prophylactic and therapeutic regimens of administration (Stage 2), *n* = 378

Group No.	No. of animals	Drug	Administration regimen	Virus	Registered indicators
1	21	Placebo (Negative control)	Therapeutic and prophylactic. Administration of the substance for 10 days before infection and 10 days after infection.	H5N8	Lethality (2 times a day), body weight (1 time a day) — in 15 mice in the group, virus titers in lung tissue — in 6 mice in the group
2	21	Oseltamivir (Positive control)			
3	21	ASD substance			
4	21	Placebo (Negative control)		H5N2	
5	21	Oseltamivir (Positive control)			
6	21	ASD substance			
7	21	Placebo (Negative control)		H3N2	
8	21	Oseltamivir (Positive control)			
9	21	ASD substance			
10	21	Placebo (Negative control)	Therapeutic. Administration of the substance after infection for 10 days	H5N8	
11	21	Oseltamivir (Positive control)			
12	21	ASD substance			
13	21	Placebo (Negative control)		H5N2	
14	21	Oseltamivir (Positive control)			
15	21	ASD substance			
16	21	Placebo (Negative control)		H3N2	
17	21	Oseltamivir (Positive control)			
18	21	ASD substance			

Table 4 — Manipulations performed with animals (Stage 2)

Group No.	Manipulations performed								
	-15	-14 –-11	-10 –-1	0	1–2	3	4–10	11–13	14
1	A, Q	Q	B	B, I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F
2	A, Q	Q	B	B, I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F
3	A, Q	Q	B	B, I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F
4	A, Q	Q	B	B, I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F
5	A, Q	Q	B	B, I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F
6	A, Q	Q	B	B, I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F
7	A, Q	Q	B	B, I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F
8	A, Q	Q	B	B, I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F
9	A, Q	Q	B	B, I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F
10	A, Q	Q	Q	I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F
11	A, Q	Q	Q	I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F
12	A, Q	Q	Q	I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F
13	A, Q	Q	Q	I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F
14	A, Q	Q	Q	I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F
15	A, Q	Q	Q	I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F
16	A, Q	Q	Q	I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F
17	A, Q	Q	Q	I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F
18	A, Q	Q	Q	I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F

Notes: A — admission of animals; Q — quarantine; B — administration of the drug/placebo; E — collection of organs; C — weighing; D — recording of mortality; I — infection; F — euthanasia.

Table 5 — Proportions of surviving mice (in percent) infected with the H5N8 virus for two regimens of drug administration

Therapeutic and prophylactic regimen				Therapeutic regimen			
Day after infection	Placebo	Oseltamivir	ASD	Day after infection	Placebo	Oseltamivir	ASD
0	100	100	100	0	100	100	100
1	100	100	100	1	100	100	100
2	100	100	100	2	100	100	100
3	100	100	100	3	100	100	100
4	100	100	100	4	100	100	100
5	100	100	100	5	100	100	100
6	100	100	100	6	100	100	100
7	100	100	93.33	7	100	100	100
8	86.67	100	80	8	100	100	80
9	80	93.33	73.33	9	93.33	93.33	73.33
10	66.67	93.33	46.67	10	86.67	86.67	46.67
11	66.67	93.33	46.67	11	80	86.67	46.67
12	66.67	93.33	46.67	12	80	86.67	40
13	66.67	93.33	46.67	13	80	86.67	40
14	60	93.33	40	14	73.33	86.67	40

Table 6 — Proportions of surviving mice (in percent) infected with the H3N2 virus for two regimens of drug administration

Therapeutic and prophylactic regimen				Therapeutic regimen			
Day after infection	Placebo	Oseltamivir	ASD	Day after infection	Placebo	Oseltamivir	ASD
0	100	100	100	0	100	100	100
1	100	100	100	1	100	100	100
2	100	100	100	2	100	100	100
3	100	100	100	3	100	100	100
4	100	100	100	4	100	100	100
5	100	100	100	5	100	100	100
6	73.33	100	80	6	100	100	100
7	46.67	100	66.67	7	66.67	100	80
8	46.67	100	46.67	8	53.33	100	66.67
9	33.33	100	46.67	9	53.33	100	60
10	20	100	46.67	10	40	100	60
11	13.33	100	46.67	11	33.33	100	60
12	6.67	100	46.67	12	26.67	100	40
13	6.67	100	46.67	13	26.67	100	40
14	6.67	100	46.67	14	26.67	100	40

Table 7 — Proportions of surviving mice (in percent) infected with the H5N2 virus for two regimens of drug administration

Therapeutic and prophylactic regimen				Therapeutic regimen			
Day after infection	Placebo	Oseltamivir	ASD	Day after infection	Placebo	Oseltamivir	ASD
0	100	100	100	0	100	100	100
1	100	100	100	1	100	100	100
2	100	100	100	2	100	100	100
3	100	100	100	3	93.33	100	100
4	100	100	93.33	4	93.33	100	100
5	100	100	93.33	5	93.33	100	93.33
6	100	100	80	6	93.33	100	93.33
7	93.33	100	73.33	7	86.67	93.33	93.33
8	93.33	93.33	73.33	8	80	80	93.33
9	93.33	86.67	73.33	9	73.33	80	86.67
10	86.67	80	73.33	10	66.67	80	80
11	86.67	80	73.33	11	53.33	80	73.33
12	86.67	80	60	12	53.33	80	73.33
13	86.67	80	60	13	53.33	80	73.33
14	86.67	80	60	14	53.33	80	73.33

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Table 8 — Comparison of survival functions with the placebo group in experiments with two drug regimens after infection of mice with three strains of influenza virus

Preventive and therapeutic regimen				Therapeutic regimen			
Virus strain	Drug	Gehan-Wilcoxon criterion, *p-value*	Cox-Mantel criterion, *p-value*	Virus strain	Drug	Gehan-Wilcoxon criterion, *p-value*	Cox-Mantel criterion, *p-value*
H5N8	Placebo	–	–	H5N8	Placebo	–	–
	Oseltamivir	* 0.0380	* 0.0352		Oseltamivir	0.4407	0.3992
	ASD	0.2954	0.2865		ASD	* 0.0331	* 0.0478
H3N2	Placebo	–	–	H3N2	Placebo	–	–
	Oseltamivir	**** < 0.0001	**** < 0.0001		Oseltamivir	**** < 0.0001	**** < 0.0001
	ASD	0.1896	0.0560		ASD	0.3146	0.3269
H5N2	Placebo	–	–	H5N2	Placebo	–	–
	Oseltamivir	0.6549	0.6393		Oseltamivir	0.1989	0.1546
	ASD	0.0927	0.0992		ASD	0.2591	0.2580

Note: the presented P values are the result of applying the Gehan-Wilcoxon and Cox-Mantel tests; significance of differences: * — $p < 0.05$; **** — $p < 0.0001$.

Table 9 — Dynamics of death of Balb/c mice and indicators of protective activity of drugs during experimental lethal influenza pneumonia caused by influenza virus H5N8

Drug	Application scheme	Individuals in group	Mortality by days														Lethality, %	MLD, days	Protection index, %	p*
			1	2	3	4	5	6	7	8	9	10	11	12	13	14				
Placebo	P/T	15								2	1	2				1	40.0	12.9	n/a	n/a
Oseltamivir		15									1						6.6	14.6	83.5	**0.0035**
ASD		15							1	2	1	4				1	60.0	11.7	–	0.2865
Placebo	T	15								1	1	1				1	26.7	13.7	n/a	n/a
Oseltamivir		15								1	1						13.3	14.1	50.2	0.3931
ASD		15								3	1	4		1			60.0	11.7	–	0.0554

Note: * Log-rank (Mantel-Cox) test; MLD — mouse lethal dose; n/a — not available.

Table 10 — Dynamics of death of Balb/c mice and indicators of protective activity of drugs during experimental lethal influenza pneumonia caused by influenza virus H5N2

Drug	Application scheme	Individuals in group	Mortality by days														Lethality, %	MLD, days	Protection index, %	p*
			1	2	3	4	5	6	7	8	9	10	11	12	13	14				
Placebo	P/T	15							1		1						13,3	14,1	n/a	n/a
Oseltamivir		15								1	1	1					20,0	13,8	–	0,6393
ASD		15				1		2	1					2			40,0	12,1	–	0,0992
Placebo	T	15			1			1	1	1	1	2					46,7	11,5	n/a	n/a
Oseltamivir		15							1	2							20,0	13,5	57,2	0,2284
ASD		15					1				1	1	1				26,7	13,3	42,8	0,3607

Note: * Log-rank (Mantel-Cox) test; MLD — mouse lethal dose; n/a — not available.

Table 11 — Dynamics of death of Balb/c mice and indicators of protective activity of drugs during experimental lethal influenza pneumonia caused by influenza virus H3N2

Drug	Application scheme	Individuals in group	Mortality by days														Lethality, %	MLD, days	Protection index, %	p*
			1	2	3	4	5	6	7	8	9	10	11	12	13	14				
Placebo	P/T	15						4	4		2	2	1	1			93,3	8,5	n/a	n/a
Oseltamivir		15															0	15,0	100,0	**<0,0001**
ASD		15						3	2	3							53,3	10,7	42,9	0,0560
Placebo	T	15							5	2		2	1	1			73,3	10,3	n/a	n/a
Oseltamivir		15															0	15,0	100,0	**<0,0001**
ASD		15							3	2	1			3			60,0	11,5	35,7	0,3269

Note: * Log-rank (Mantel-Cox) test; MLD — mouse lethal dose; n/a — not available.

Table 12 — Values of influenza virus titers of strains H5N8, H3N2, H5N2 for two drug regimens (*n* = 6 for each group)

Preventive and therapeutic regimen					Therapeutic regimen				
Virus strain	Drug	M	SD	SEM	Virus strain	Drug	M	SD	SEM
H5N8	Placebo	3.917	2.311	0.943	H5N8	Placebo	3.750	2.382	0.972
	Oseltamivir	2.417	2.084	0.851		Oseltamivir	3.500	2.000	0.817
	ASD	2.083	2.035	0.831		ASD	4.667	2.206	0.901
H5N2	Placebo	6.750	0.689	0.281	H5N2	Placebo	5.750	1.084	0.442
	Oseltamivir	6.333	0.408	0.167		Oseltamivir	5.833	0.983	0.401
	ASD	5.833	0.931	0.380		ASD	5.583	0.971	0.396
H3N2	Placebo	7.500	0.000	0.000	H3N2	Placebo	7.500	0.000	0.000
	Oseltamivir	6.250	0.274	1.429		Oseltamivir	4.167	2.523	1.030
	ASD	6.917	0.112	0.583		ASD	7.500	0.000	0.000

Note: the mean values (M), standard deviations (SD) and standard errors of the mean (SEM) are presented

Table 13 – Values of influenza virus titers (strains H5N8, H3N2, H5N2), Shapiro-Wilk test

Preventive and therapeutic regimen				Therapeutic regimen			
Virus strain	Drug	*W-value*	*p-value*	Virus strain	Drug	*W-value*	*p-value*
H5N8	Placebo	0.9065	0.4140	H5N8	Placebo	0.9551	0.7815
	Oseltamivir	0.8129	0.0765		Oseltamivir	0.9780	0.9410
	ASD	0.7732	* 0.0333		ASD	0.9805	0.9538
H5N2	Placebo	0.8606	0.1912	H5N2	Placebo	0.8672	0.2151
	Oseltamivir	0.8216	0.0911		Oseltamivir	0.9241	0.5353
	ASD	0.8616	0.1948		ASD	0.9124	0.4522
H3N2	Placebo	–	–	H3N2	Placebo	–	–
	Oseltamivir	0.6827	** 0.0040		Oseltamivir	0.8381	0.1257
	ASD	0.4961	**** < 0.0001		ASD	–	–

Note: checking data for normality, W and P values are presented — the result of applying the Shapiro-Wilk test; significance of differences: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. Dash — calculation is impossible, since all values in the sample are the same.

Table 14 — Values of influenza virus titers (strains H5N8, H3N2, H5N2), Mann-Whitney test

Preventive and therapeutic regimen				Therapeutic regimen			
Virus strain	Drug	*W-value*	*p-value*	Virus strain	Drug	*W-value*	*p-value*
H5N8	Placebo	–	–	H5N8	Placebo	–	–
	Oseltamivir	10	0,2381		Oseltamivir	17,50	0,9827
	ASD	8	0,1190		ASD	13,50	0,5238
H5N2	Placebo	–	–	H5N2	Placebo	–	–
	Oseltamivir	11,50	0,3398		Oseltamivir	16,50	0,8658
	ASD	7	0,0887		ASD	16	0,8030
H3N2	Placebo	–	–	H3N2	Placebo	–	–
	Oseltamivir	0	** 0,0022		Oseltamivir	0	** 0,0022
	ASD	15	0,9999		ASD	18	0,9999

Note: the results of the Mann — Whitney test when comparing the drug and placebo for two drug regimens, *U*- and *p-values* are presented; significance of differences: ** $p < 0.01$.

It should also be noted that in the group infected with the H3N2 virus and receiving the ASD according to the preventive and therapeutic regimen, the *p-value* was borderline (0.056 *vs.* 0.05), which indicates the presence of a pronounced tendency towards a protective effect of the drug. For the H5N2 virus, there was also a tendency to reduce mortality in the group receiving the drug according to the therapeutic regimen, although it did not reach statistical significance.

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From the data presented in the tables, it can be seen that positive values of the PI for the ASD were noted in the groups infected with the H3N2 virus according to both regimens and in the group infected with the H5N2 virus according to the therapeutic regimen.

Statistical analysis of data obtained by measuring influenza virus titers

A study of viral load in lung tissue was carried out by titration on a cell culture. For each combination of "virus strain– treatment regimen–drug", measurements were performed in a sample of 6 animals. The average values of the measured titers of influenza virus (strains H5N8, H3N2, H5N2) for the therapeutic and preventive-therapeutic regimens are presented in Figure 4 and Table 12.

The data were checked for compliance with the normal distribution law in order to justify the choice of the test that identifies differences between measurement groups. The Shapiro-Wilk test was used to check the data for normality, the results of which are presented in Table 13.

As it can be seen from Table 13, with the selected significance level in 9 groups, deviations from the normal distribution are observed in the data. Thus, parametric tests are not applicable, and to identify differences between groups of drugs and the placebo group, we applied the Mann-Whitney U-test, the results of which are presented in Table 14.

As it can be seen from Table 14, with the selected significance level $\alpha = 0.05$, differences were found in the experiment between the oseltamivir and placebo groups in the case of infection with the H3N2 virus for both regimens. No statistically significant effect of the ASD on the level of viral load was noted for any of the studied viruses.

DISCUSSION

Influenza is one of the most significant infections in the world. According to WHO estimates, millions of cases are registered worldwide every year, and mortality from complications can reach up to 650 thousand people[10]. Seasonal influenza vaccination is the most effective tool for protection against infection [29]. However, influenza vaccination has limitations and is not always applicable in patients from risk groups, which include children, people over 60 years of age, as well as patients with chronic pathologies, immunodeficiency states and allergic manifestations [30]. Also, in these groups, vaccination may not be effective enough [31].

As an additional measure of protection in the interval between vaccination and the formation of an adequate post-vaccination immune response, as well as in persons with contraindications to vaccination, the use of antiviral drugs is indicated [32]. However, recently there has been a relative resistance to some traditional antiviral agents, including oseltamivir, zanamivir, amantadine and rimantadine. In this regard, it is critical to develop new strategies and drugs for effective control of viral infection [31].

In some cases, the effectiveness of treatment or prevention of influenza can be increased by using combined therapy consisting of existing and/or new antiviral agents with different mechanisms of action [31]. Ethnopharmacological drugs obtained from various plants or other natural resources that are potentially capable of reducing the severity of clinical symptoms and reducing the risk of complications of influenza and other respiratory infections can be used [33]. According to WHO data, about 21 thousand medicinal plants are known, most of which have an immunomodulatory effect and have the corresponding therapeutic potential [34].

In vivo studies have demonstrated the protective effects of colaviron, a bioflavonoid isolated from the plant *Garcinia kola* Heckel. Acting as a natural antioxidant and anti-inflammatory agent, colaviron is able to inhibit the activity of acetylcholinesterase in the hippocampus and striatum in rats [35]. In an experiment on mice, it was shown that colaviron can delay the appearance of clinical symptoms of influenza through a mechanism that differs from the mechanisms of action of existing drugs against influenza, but is closely related to the antioxidant and immunomodulatory action of this substance [36]. Similarly, it has been shown that an extract of the bark of the roots of the African baobab (*Adansonia digitata* L.; *Malvaceae*) has antiviral activity against avian respiratory virus and may be useful for relieving influenza symptoms [37].

In this study, the protective effect of the drug ASD on a model of lethal influenza pneumonia caused by influenza A viruses was studied. The drug of natural origin, antiseptic-stimulant Dorogova fraction-2 (ASD-F2) is a product of high-temperature dry distillation of meat and bone meal, which has found wide application in veterinary drug in the treatment

[10] Global influenza strategy 2019–2030. Geneva: World Health Organization; 2019. Available from: https://iris.who.int/bitstream/handle/10665/ 311184/9789241515320-eng.pdf?sequence=18

of animals and birds from numerous diseases of viral and microbial etiology, in the prevention of such diseases, as well as in increasing the productivity of cattle and birds. ASD-F2 has neither species nor organ specificity, because distillation ensures the gradual decomposition of organic substances to low-molecular components, which are similar in structure to cell metabolism metabolites. ASD-F2 has a multifaceted effect on the body. It intensifies metabolism, accelerates oxidative processes, increases the reserve alkalinity in the blood, which contributes to the normalization of metabolism in tissues, improves the processes of digestion, absorption of nutrients. The drug causes an improvement in the functional state of the mechanisms of natural resistance, enhances tissue regeneration processes, stimulates immunogenesis, as a result of which resistance to adverse effects, including pathogens of infectious diseases, increases [38].

Previously, the antiviral activity of drugs containing ASD-F2 was demonstrated in mice with infection caused by the influenza virus strain A/California/07/09 (H1N1), and in Syrian hamsters infected with the SARS-CoV-2 virus [39], and *in vitro* studies, its antimicrobial effect was recorded [40], including against Mycobacterium tuberculosis [41]. In this work, positive effects were also observed when using a drug containing ASD-2 in mice infected with influenza virus (strains H3N2 and H5N2). Despite the fact that the effect of the drug on the level of viral load was not revealed, it should be noted a tendency to improve the survival rate of mice infected with influenza (strains H3N2 and H5N2), as well as a decrease in body weight loss against the background of the use of the studied drug. The results obtained are consistent with previously published data indicating the presence of antiviral effects in the drug.

Study Limitations

In this study, the effectiveness of the drug was not studied against influenza virus strains H1N1. Also, the effectiveness of the drug was studied on one type of animal, which does not exclude the manifestation of other pharmacological manifestations.

CONCLUSION

A study of the protective activity of the drug ASD was carried out on a model of lethal influenza pneumonia caused by influenza A viruses A/common duck/Uvs Nuur lake/26/2016 (H5N8), A/Duck/Potsdam/1402-6/86 (H5N2), A/Aichi/2/68, in Balb/c mice with preventive and therapeutic and therapeutic regimens. During the experiments, the dynamics of death and changes in the weight indicators of animals during the pathological process, as well as the viral load in the lung tissue on the 3rd day after infection, were evaluated.

In relation to the influenza virus H3N2, an improvement in the dynamics of weight loss of infected animals was noted when used according to the preventive and therapeutic regimen. A decrease in mortality was observed in both regimens, but it was more pronounced for the preventive and therapeutic regimen.

For the influenza virus H5N2, the effectiveness of the drug was less pronounced, but a protective effect was also noted when used according to the therapeutic regimen.

Thus, based on the data obtained, it can be concluded that the drug ASD has a protective effect against the influenza virus in vivo, and the effect depends on the subtype of the virus and is most pronounced for the H3N2 strain.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

Sergei V. Engashev, Olga A. Dorogova, Ekaterina S. Engasheva — design, draft editing and final approval of the article; Irina Yu. Merkulova — data processing, writing the text of the article. All authors confirm that their authorship meets the international ICMJE criteria (all authors made a significant contribution to the development of the concept, conduct of the study and preparation of the article, read and approved of the final version before the publication).

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