



## ORIGINAL STUDY ARTICLE

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# Efficacy of the interferon inducer tilorone and its combination with antibacterial drugs in a murine model of secondary bacterial pneumonia caused by *S. aureus* after influenza infection

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## Abstract

**Introduction.** Secondary bacterial pneumonias are complications responsible for most fatalities following influenza infection, and antiviral drugs are used to prevent their development.

**The aim** of the study is to evaluate the efficacy of tilorone and its combination with antibacterial agents in a murine model of secondary bacterial pneumonia caused by *Staphylococcus aureus* after influenza infection.

**Materials and methods.** BALB/c mice were infected with influenza virus A/California/04/2009 (pdm H1N1 2009) virus, followed by *S. aureus* infection four days later. Treatments included the interferon inducer tilorone, comparator oseltamivir, antibacterial agents cefuroxime and amoxicillin, or combinations of antivirals with antibiotics. Efficacy was assessed by increased survival, reduced weight loss, and inhibition of pathogen proliferation in the lungs.

**Results.** The efficacy of tilorone, as indicated by increased survival, reduced weight loss, and decreased pathogen load in the lungs, in a mouse model of secondary bacterial pneumonia following influenza infection, increased with reducing viral dose, and intraperitoneal administration of the drug was more effective than oral administration. The combination of tilorone with the antibiotic cefuroxime, to which *S. aureus* was sensitive, was more effective than either agent alone and completely protected animals from death and from viral and bacterial proliferation in the lungs. In contrast, the combination with amoxicillin, to which *S. aureus* was resistant, had no effect on disease outcome.

**Conclusion.** The efficacy of tilorone in this model depended on viral dose and drug administration route. Combining tilorone with cefuroxime was more effective than monotherapy.

**Keywords:** *influenza virus; Staphylococcus aureus; secondary bacterial pneumonia following influenza infection; tiloron*

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**Conflict of interest.** The authors declare no conflict of interest.

**Ethics approval.** Authors confirm compliance with institutional and national standards for the use of laboratory animals in accordance with "Consensus author guidelines for animal use" (IAVES 23 July 2010). The study protocol (No. 15/2025 dated October 24, 2025) was approved by the Local Ethics Committee of the I. Mechnikov Research Institute of Vaccines and Sera.

ОРИГИНАЛЬНОЕ ИССЛЕДОВАНИЕ

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## Эффективность индуктора интерферонов тилорона и его комбинации с антибактериальными препаратами на модели вторичной бактериальной пневмонии мышей, вызванной *S. aureus* после гриппозной инфекции

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### Резюме

**Введение.** Вторичные бактериальные пневмонии – осложнения, вызывающие бóльшую часть смертельных исходов после гриппозной инфекции, для предотвращения развития которых применяют противовирусные препараты.

**Цель:** изучение эффективности тилорона и его комбинации с антибактериальными препаратами на модели вторичной бактериальной пневмонии мышей после гриппозной инфекции, вызванной *Staphylococcus aureus*.

**Материалы и методы.** Мышей BALB/c инфицировали вирусом гриппа А/Калифорния/04/2009 (пндм H1N1 2009), а через 4 сут – *S. aureus*. Лечение проводили индуктором интерферона тилороном, препаратом сравнения осельтамивиром, антибактериальными препаратами цефуроксимом и амоксициллином или комбинациями противовирусных препаратов с антибиотиками. Эффективность лечения оценивали по увеличению выживаемости, снижению потери массы тела, предотвращению размножения вируса и бактерий в легких.

**Результаты.** Эффективность тилорона увеличивалась с уменьшением дозы вируса, а внутрибрюшинное введение было эффективнее перорального. Комбинация тилорона с антибиотиком цефуроксимом, к которому был чувствителен *S. aureus*, была эффективнее, чем применение каждого препарата отдельно, и полностью защищала животных от гибели и размножения вируса и бактерий в легких, а комбинация с амоксициллином, к которому *S. aureus* устойчив, не влияла на исход заболевания.

**Заключение.** Эффективность индуктора интерферонов тилорона на модели вторичной бактериальной пневмонии мышей после гриппозной инфекции зависела от заражающей дозы вируса и способа введения препарата. Применение комбинации тилорона с цефуроксимом показало более выраженную эффективность, чем каждого препарата отдельно.

**Ключевые слова:** вирус гриппа; *Staphylococcus aureus*; вторичные бактериальные пневмонии после гриппозной инфекции; тилорон

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**Финансирование.** Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

**Конфликт интересов.** Авторы заявляют об отсутствии конфликта интересов.

**Этическое утверждение.** Авторы подтверждают соблюдение институциональных и национальных стандартов по использованию лабораторных животных в соответствии с “Consensus author guidelines for animal use” (IAVES 23 July 2010). Протокол исследования (№ 15/2025 от 24.10.2025) одобрен Локальным этическим комитетом НИИВС им. Мечникова.

### Introduction

Influenza is a respiratory disease whose annual epidemics, according to the World Health Organization, cause about 1 billion cases of infection worldwide [1]. Bacterial complications, primarily pneumonia, are the main cause of death after influenza [2]. The incidence of bacterial complications of influenza can reach 20% [1]; according to Russian epidemiological studies, it is 18% among outpatients from risk groups [3]. According to a meta-analysis of 135 studies (2010–2020), bacterial co-infection is diagnosed in an

average of 11% of hospitalized patients with influenza, significantly worsening the prognosis and increasing the risk of death by 3.4 times [4]. *Staphylococcus aureus* is one of the most common causative agents of secondary pneumonia [5, 6].

The mortality rate for secondary bacterial pneumonia associated with influenza infection remains high despite the use of antibacterial drugs [7], so limiting primary influenza virus infection is necessary to prevent secondary complications. In the clinical guidelines “Influenza in Adults,” approved by the Russian Ministry of Health on October 21, 2025, patients are recommended to use

antiviral drugs, including drugs with immunomodulatory properties, and in the development of viral-bacterial pneumonia, to supplement therapy with antibacterial drugs [8].

In Russia, interferon and its inducers are used for their antiviral and immunomodulatory effects. Interferon inducers cause the synthesis of endogenous interferon and ensure the prolonged circulation of interferons, whereas achieving similar concentrations with exogenous interferons requires multiple administrations of significant doses of the drugs [9].

Tilorone is a low-molecular-weight synthetic interferon inducer that stimulates the formation of alpha, beta, gamma, and lambda interferons in the body. The main structures that produce interferon in response to tilorone administration are intestinal epithelial cells, hepatocytes, T lymphocytes, neutrophils, and granulocytes. After oral administration of the drug, maximum interferon production is determined in the “intestine-liver-blood” sequence after 4-24 hours [10]. IFN- $\lambda$  is the first line of defense of the respiratory epithelium of both the upper and lower respiratory tract against viral infections [11, 12]. Tilorone has an affinity for the corresponding receptors on alveolar macrophages and induces the formation of interferons in the lungs [13, 14]. The mechanism of antiviral action is associated with the inhibition of the translation of virus-specific proteins in infected cells<sup>1</sup>.

We previously developed a model of secondary viral-bacterial pneumonia in mice [15] to evaluate the effectiveness of therapy with drugs that have different mechanisms of action.

**The aim** of the study was to investigate the efficacy of the interferon inducer tilorone and a combination of tilorone with the antibacterial drugs cefuroxime and amoxicillin in a model of secondary bacterial pneumonia in mice after influenza infection.

### Materials and methods

**Pathogens.** The influenza A/California/04/2009 (H1N1 2009) virus strain was obtained from the National Influenza Center at the A.A. Smorodintsev Research Institute of Influenza; strain *S. aureus* No. 1986 was obtained from the Microorganism Collection of the I.I. Mechnikov Research Institute of Virology and Immunology.

**Laboratory animals.** BALB/c female mice weighing 12–14 g were purchased from the Andreevka Branch of the National Center for Biomedical Technologies (NCBMT) of the Federal Medical Biological Agency (FMBA) of Russia and randomly divided into groups so that the individual body weight did not deviate from the average by more than  $\pm 10\%$ . Each group contained 9 to 15 animals. The study was approved by the Local Ethics Committee

of the Mechnikov Research Institute of Vaccines and Sera (meeting protocol No. 15/2025 dated October 24, 2025).

**Drugs.** The drugs were purchased from a pharmacy chain. Tilorone was crushed and dissolved in distilled water. The control drug oseltamivir (Tamiflu capsules containing 75 mg of oseltamivir; manufacturer Hoffmann-La Roche, Switzerland) was dissolved in distilled water. Cefuroxime (powder for preparation of a solution for intravenous and intramuscular administration Cefuroxime, containing 750 mg of cefuroxime; manufacturer PJSC Kraspharma, Russia) was dissolved in distilled water. Amoxicillin (Flemoxin Solutab dispersible tablets containing 125 mg of amoxicillin; manufacturer: Haupt Pharma Latina, Italy) was dissolved in distilled water.

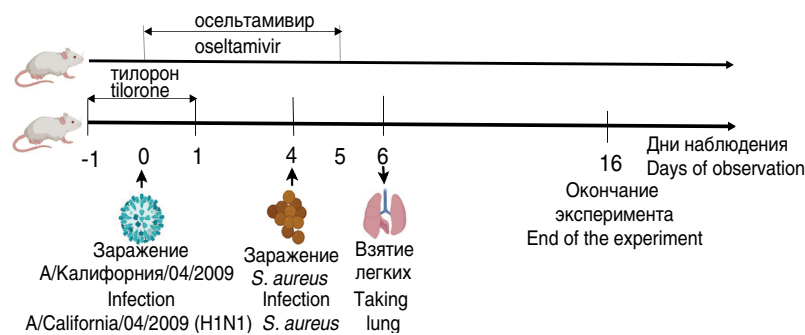
**Determination of the effectiveness of tilorone and its combination with antibacterial drugs in a model of secondary bacterial pneumonia in mice after influenza infection.** Mice were infected intranasally under general gas anesthesia with isoflurane with influenza virus at different doses (from  $10^3$  to  $10^{4.2}$  TCID<sub>50</sub>/0.1 mL), and after 4 days – with *S. aureus* at a dose of  $2 \times 10^9$  CFU/mL. Treatment of animals with tilorone at a dose of 30 mg/kg orally or 10 mg/kg intraperitoneally was started 24 and 4 hours before virus infection, then the drug was administered 24 hours after infection, with a total treatment duration of 3 days. Therapy with oseltamivir was started 4 hours before viral infection, then the drug was administered 4 hours after viral infection, then twice a day, with a total treatment duration of 5 days. Treatment with antibacterial drugs was started 3 hours after infection with *S. aureus* and continued for 3 days, once a day. The drugs were administered in a volume of 200  $\mu$ L, and their doses were calculated at 1 mg/kg of body weight per day. The mice were observed daily for 16 days (Figs. 1, 2). The control group of animals infected with influenza virus and bacteria received 200  $\mu$ L of distilled water as a placebo. Changes in body weight were recorded as described in the study [15]. Six days after infection with the influenza virus (two days after bacterial infection), three animals in each group were euthanized and their lungs were removed to determine the bacterial content using the Petri dish culture method with nutrient medium No. 10 (manufactured by the State Research Center for Applied Biotechnology and Microbiology) to identify *S. aureus* and the virus in MD-CK cell culture, as described earlier [15].

**The spectrum of sensitivity of the *S. aureus* 1986 strain to antibiotics** was determined by the disc diffusion method in accordance with MG 4.2.1890-04 “Determination of the sensitivity of microorganisms to antibacterial drugs.”

**Performance evaluation criteria:** clinical signs (survival rate and weight loss); viral characteristics (virus titer in the lungs); bacterial characteristics (bacterial content in the lungs).

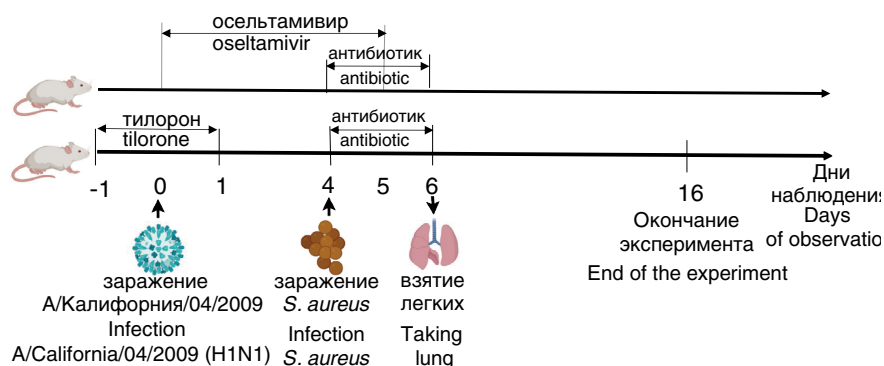
**Statistical data processing** was performed using Statistica 10 software. For intergroup comparisons of quantitative indicators presented as mean values with standard deviation, Dunn’s nonparametric test with Bonferroni correction was used, and preliminary analysis was performed using the Kruskal–Wallis criterion. Nonlinear four-parameter logistic regression was used to compare

<sup>1</sup>Instructions for medical use of the medicinal product Amixin® (tilorone, LP No. (004817) (RG RU)) [Electronic resource]. Moscow: Ministry of Health of the Russian Federation, State Register of Medicines / JSC Otisifarm Pro. Available at: <https://grls.pharm-portal.ru/grls/8a267191-4cf2-4d87-af75-64c2b971c787> (date of access: 16.01.2026).



**Fig. 1.** Study design for evaluating the efficacy of tilorone at different infectious doses in a secondary bacterial pneumonia model following influenza infection.

**Рис. 1.** Схема эксперимента по изучению эффективности тилорона при различных заражающих дозах на модели вторичной бактериальной пневмонии после гриппозной инфекции.



**Fig. 2.** Study design for evaluating the efficacy of tilorone and its combination with antibacterial agents in a secondary bacterial pneumonia model following influenza infection.

**Рис. 2.** Схема эксперимента по изучению эффективности тилорона и его комбинации с антибактериальными препаратами на модели вторичной бактериальной пневмонии после гриппозной инфекции.

changes in body weight and survival between groups. Survival curves were constructed using the Kaplan–Meier method. Differences were considered statistically significant at  $p \leq 0.05$ . The virus titer in the lung homogenate was calculated using the Reed and Metch limit serial dilution method [16], expressed in  $\lg \text{TCID}_{50}/\text{mL}$ , and a difference of more than 2  $\log_{10}$  was considered significant [17].

## Results

### *Study of the effectiveness of tilorone at different infectious doses of the influenza virus*

The efficacy of tilorone was studied in a mouse model of secondary bacterial pneumonia in three experiments (Fig. 1) with different infectious doses of the virus: high ( $10^{4.2} \text{TCID}_{50}/0.1 \text{ mL}$ ), medium ( $10^4 \text{TCID}_{50}/0.1 \text{ mL}$ ), and low ( $10^3 \text{TCID}_{50}/0.1 \text{ mL}$ ). The infecting dose of *S. aureus* for all experiments was  $2 \times 10^9 \text{CFU}/\text{mL}$ .

At a high infecting dose ( $10^{4.2} \text{TCID}_{50}/0.1 \text{ mL}$ ), combined sequential infection with pathogens resulted in the death of 93% of animals by the end of the experiment; body weight loss reached 15–18% by 9–11 days. Treatment with tilorone, both orally and intraperitoneally, as well as with oseltamivir orally, was ineffective; animal mortality and body weight loss were comparable to those in the control group.

Given the lack of effect of the drugs, in the next

experiment, the virus infection dose was reduced ( $10^4 \text{TCID}_{50}/0.1 \text{ mL}$ ). In the control group, 90% of the animals died on the 8th day after viral infection. The effectiveness of treatment with all drugs was higher when the infectious dose was reduced: oral administration of tilorone protected half of the animals in the group from death, and intraperitoneal administration completely protected all animals from death. Oral administration of oseltamivir increased survival to 80%. The results of the lung study corresponded to the survival data: Tilorone therapy reduced the virus titer by 3–4  $\log_{10} \text{TCID}_{50}/0.1 \text{ mL}$  and the bacterial density in the lung homogenate by 2  $\log_{10} \text{CFU}/\text{mL}$  with intraperitoneal administration compared to untreated control animals (**Table 1, Fig. 3**).

With a further reduction in the infectious dose to  $10^3 \text{TCID}_{50}/0.1 \text{ mL}$ , 67% of animals in the control group died. Oral administration of tilorone protected 89% of animals from death, while intraperitoneal administration of tilorone and oral administration of oseltamivir completely prevented the death of the study animals and the reproduction of pathogens in the lungs.

Thus, the interferon inducer tilorone was effective in a model of secondary pneumonia caused by infection with A/California/04/2009 followed by infection with *S. aureus*, both when administered intraperitoneally and orally. The effectiveness of treatment increased with a decrease in the infecting dose of the virus. Tilorone

therapy prevented the death of mice, reduced their weight loss, and also reduced viral replication in the lungs.

*Study of the effectiveness of combined use of tilorone and antibacterial drugs in a model of secondary pneumonia in mice after influenza infection*

To study the combined effect of tilorone with antibacterial drugs, an average infecting dose of  $10^4$  TCID<sub>50</sub>/0.1 mL was selected, since in a previous experiment, oral administration of tilorone at this dose was effective in killing 90% of the animals in the control group. Oseltamivir was administered at a dose of 10 mg/kg, and the dose of tilorone was reduced to 15 mg/kg.

One of the problems in combating bacterial complications is the increase in bacterial resistance to antibacterial drugs. In this regard, information about the sensitivity of the pathogen to different types of antibacterial agents is necessary to select the appropriate drug. Studies of the sensitivity spectrum of the *S. aureus* 1986 strain to antibacterial drugs have revealed

the resistance of bacteria to amoxicillin and azithromycin.

Thus, two antibacterial drugs were selected for the study: cefuroxime at a dose of 20 mg/kg as the antibiotic to which the studied *S. aureus* strain was found to be sensitive; as the antibiotic to which resistance was detected, amoxicillin at a dose of 20 mg/kg.

Complete death of animals in the control group was observed on the 9th day after viral infection (Table 2). The use of both antiviral and antibacterial agents increased survival by no more than 20%, did not prevent weight loss in animals, and did not reduce viral replication in the lungs. Antiviral drugs alone, as well as amoxicillin, to which *S. aureus* was found to be resistant, had no effect on the bacteria in the lungs. Furthermore, the combined use of tilorone and oseltamivir with amoxicillin did not affect the survival of mice or the replication of the virus in the lungs of mice.

The use of cefuroxime reduced the number of bacteria by 100 times, and its combination with tilorone or oseltamivir increased survival to 80–100%, completely

**Table 1.** Efficacy of tilorone in different routes of administration and different infection doses of influenza virus A/H1N1/California/04/2009 (2009 H1N1 pandemic strain) in a secondary bacterial pneumonia murine model

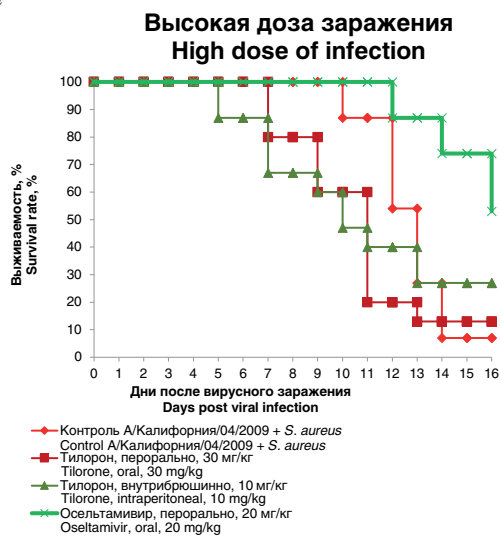
**Таблица 1.** Эффективность тилорона при разных способах введения и заражающих дозах вируса гриппа А/Калифорния/04/2009 (пндм H1N1 2009) на модели вторичной бактериальной пневмонии у мышей

Group Группа	Drug Препарат	Survival rate Выживаемость		Viral titer, log <sub>10</sub> TCID <sub>50</sub> /0.1 mL Титр вируса, lg ТЦИД <sub>50</sub> /0,1 мл	Bacterial count, log <sub>10</sub> CFU/mL Содержание бактерий, lg КОЕ/мл
		surviv./all жив./общ.	%		
High dose ( $10^{4.2}$ TCID <sub>50</sub> /0.1 mL) Высокая доза ( $10^{4.2}$ ТЦИД <sub>50</sub> /0,1 мл)	Tiloron, oral, 30 mg/kg Тилорон, перорально, 30 мг/кг	2/15 ( <i>p</i> = 0.55)	13.3	6.7 ± 0.6	5.5 ± 0.1
	Tiloron, intraperitoneal, 10 mg/kg Тилорон, внутривнутрибрюшинно, 10 мг/кг	4/15 ( <i>p</i> = 0.15)	26.7	6.3 ± 0.6*	5.29 ± 0.2*
	Oseltamivir, oral, 20 mg/kg Осельтамивир, перорально, 20 мг/кг	5/15 ( <i>p</i> = 0.07)	33.3	6.3 ± 0.6	5.51 ± 0.6
	Control A/California/04/2009 + <i>S. aureus</i> Контроль А/Калифорния/04/2009 + <i>S. aureus</i>	1/15	6.7	7 ± 0	5.6 ± 0.1
Medium dose ( $10^4$ TCID <sub>50</sub> /0.1 mL) Средняя доза ( $10^4$ ТЦИД <sub>50</sub> /0,1 мл)	Tiloron, oral, 30 mg/kg Тилорон, перорально, 30 мг/кг	5/10 ( <i>p</i> = 0.05)	50.0	4.0 ± 0.5**	5.15 ± 0.1*
	Tiloron, intraperitoneal, 10 mg/kg Тилорон, внутривнутрибрюшинно, 10 мг/кг	10/10 ( <i>p</i> = 0.001)	100.0	3.2 ± 0.3**	3.35 ± 0.04**
	Oseltamivir, oral, 20 mg/kg Осельтамивир, перорально, 20 мг/кг	8/10 ( <i>p</i> = 0.001)	80.0	3.2 ± 0**	3.49 ± 0.01**
	Control A/California/04/2009 + <i>S. aureus</i> Контроль А/Калифорния/04/2009 + <i>S. aureus</i>	1/10	10.0	6.7 ± 0.6	5.68 ± 0.05
Low dose ( $10^3$ TCID <sub>50</sub> /0.1 mL) Низкая доза ( $10^3$ ТЦИД <sub>50</sub> /0,1 мл)	Tiloron, oral, 30 mg/kg Тилорон, перорально, 30 мг/кг	8/9 ( <i>p</i> = 0.01)	89.0	Не выявлено Not detected	Не выявлено Not detected
	Tiloron, intraperitoneal, 10 mg/kg Тилорон, внутривнутрибрюшинно, 10 мг/кг	9/9 ( <i>p</i> = 0.001)	100.0	Не выявлено Not detected	Не выявлено Not detected
	Oseltamivir, oral, 20mg/kg Осельтамивир, перорально, 20 мг/кг	10/10 ( <i>p</i> = 0.001)	100	Не выявлено Not detected	Не выявлено Not detected
	Control A/California/04/2009 + <i>S. aureus</i> Контроль А/Калифорния/04/2009 + <i>S. aureus</i>	3/9	33	5.8 ± 0.3	4.5 ± 0.1

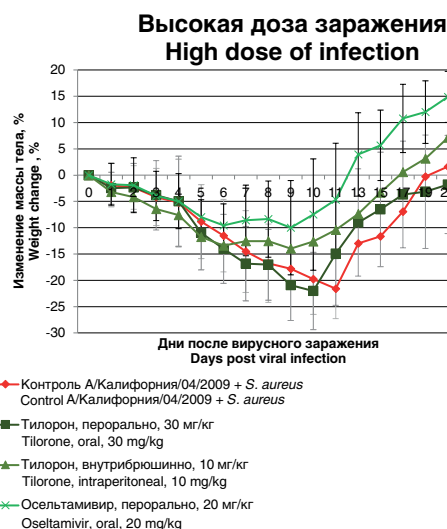
**Note.** Comparison of treatment groups with control, using the Mann–Whitney test with Bonferroni correction: \* – *p* < 0.05; \*\* – *p* < 0.01.

**Примечание.** Сравнение групп терапии с контролем, критерий Манна–Уитни с поправкой Бонферрони: \* – *p* < 0,05; \*\* – *p* < 0,01.

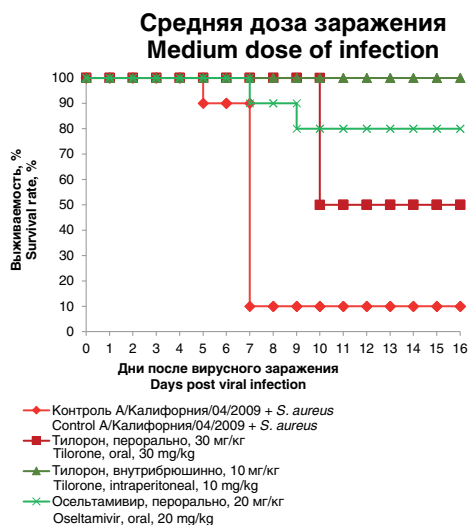
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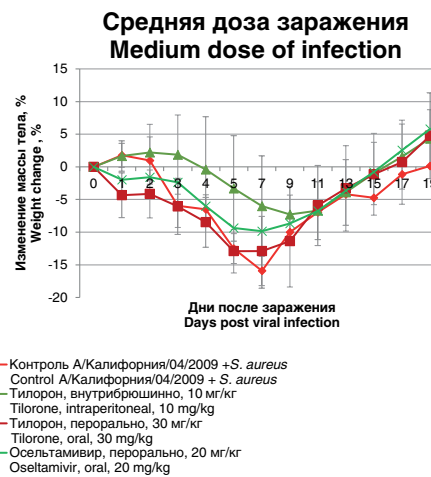
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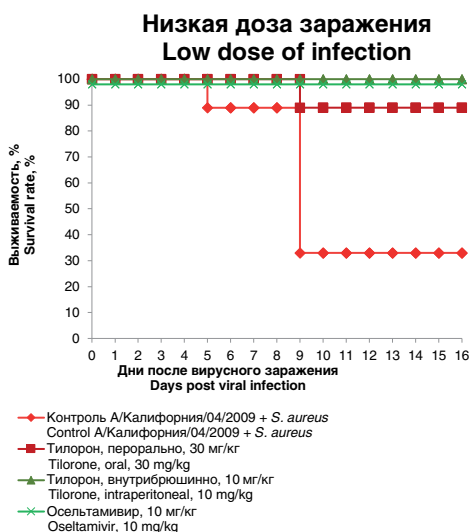
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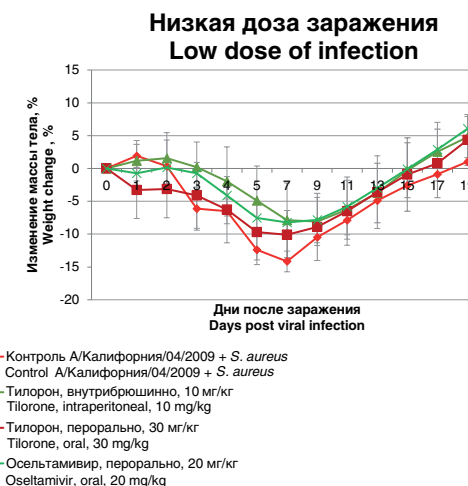
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b | б



**Fig. 3.** Survival rate (a) and weight change (b) during tilorone treatment at high ( $10^{4.2}$  TCID<sub>50</sub>/0.1 mL), medium ( $10^4$  TCID<sub>50</sub>/0.1 mL), and low ( $10^3$  TCID<sub>50</sub>/0.1 mL) infectious doses of Influenza A/California/04/2009 (pdm H1N1 2009) in a secondary bacterial pneumonia model following influenza infection.

**Рис. 3.** Выживаемость мышей (a) и изменение массы тела (б) при лечении тилороном при высокой ( $10^{4.2}$  ТЦИД<sub>50</sub>/0,1 мл), средней ( $10^4$  ТЦИД<sub>50</sub>/0,1 мл) и низкой ( $10^3$  ТЦИД<sub>50</sub>/0,1 мл) заражающей дозе вируса гриппа А/Калифорния/04/2009 (пндм Н1Н1 2009) на модели вторичной бактериальной пневмонии после гриппозной инфекции.

**Table 2.** Effect of combined antiviral and antibacterial therapy on survival, viral and bacterial titers in the lungs during treatment of secondary bacterial pneumonia caused by *S. aureus* following influenza infection**Таблица 2.** Влияние комбинации противовирусных и антибактериальных препаратов на выживаемость, титры вирусов и бактерий в легких при лечении вторичной бактериальной пневмонии, вызванной *S. aureus* после гриппозной инфекции

Group Группа	Survival Выживаемость		Viral titer, log <sub>10</sub> EID <sub>50</sub> /0.1 mL Титр вируса, lg ТЦИД <sub>50</sub> /0,1 мл	Bacterial count, log <sub>10</sub> CFU/mL Содержание бактерий, lg КОЕ/мл
	жив./общ. surviv./all	%		
Amoxicillin, oral, 20 mg/kg Амоксициллин, перорально, 20 мг/кг	0/10	0	6.5 ± 0.5	5.54 ± 0.06
Cefuroxime, oral, 20 mg/kg Цефуросим, перорально, 20 мг/кг	2/10 ( <i>p</i> = 0,150)	20	5.0 ± 0.5	3.37 ± 0.18*
Oseltamivir, oral, 10 mg/kg Осельтамивир, перорально, 10 мг/кг	2/10 ( <i>p</i> = 0,150)	20	5.7 ± 0.3	5.7 ± 0.14
Tiloron, oral, 15 mg/kg Тилорон, перорально, 15 мг/кг	2/10 ( <i>p</i> = 0,150)	10	6.3 ± 0.6	5.52 ± 0.11
Oseltamivir, oral, 10 mg/kg + Cefuroxime, oral, 10 mg/kg Осельтамивир, перорально, 10 мг/кг + цефуросим, перорально, 10 мг/кг	10/10	100	Не выявлено Not detected	Не выявлено Not detected
Oseltamivir, oral, 10 mg/kg + Amoxicillin, oral, 10 mg/kg Осельтамивир, перорально, 10 мг/кг + амоксициллин, перорально, 10 мг/кг	2/10 ( <i>p</i> = 0,150)	20	5.8 ± 0.3	5.62 ± 0.05
Tiloron, oral, 15 mg/kg + Cefuroxime, oral, 20 mg/kg Тилорон, перорально, 15 мг/кг + цефуросим, перорально, 20 мг/кг	8/10 ( <i>p</i> = 0,001)	80	Не выявлено Not detected	Не выявлено Not detected
Tiloron, oral, 15 mg/kg + Amoxicillin, oral, 20 mg/kg Тилорон, перорально, 15 мг/кг + амоксициллин, перорально, 20 мг/кг	2/10 ( <i>p</i> = 0,150)	20	6.2 ± 0.3	5.48 ± 0.11
Control A/California/04/2009 + <i>S. aureus</i> Контроль А/Калифорния/04/2009 + <i>S. aureus</i>	0/10	0	6.7 ± 0.6	5.43 ± 0.1

**Note.** Comparison of treatment groups with control, using the Mann–Whitney test with Bonferroni correction: \* – *p* < 0.05.**Примечание.** Сравнение групп терапии с контролем, критерий Манна–Уитни с поправкой Бонферрони: \* – *p* < 0,05.

prevented weight loss in animals, and completely suppressed the reproduction of the virus and bacteria in the lungs, indicating the additive nature of both combinations (Table 2, Fig. 4).

Thus, combined treatment with tilorone and the antibacterial drug cefuroxime showed greater efficacy than treatment with each drug separately.

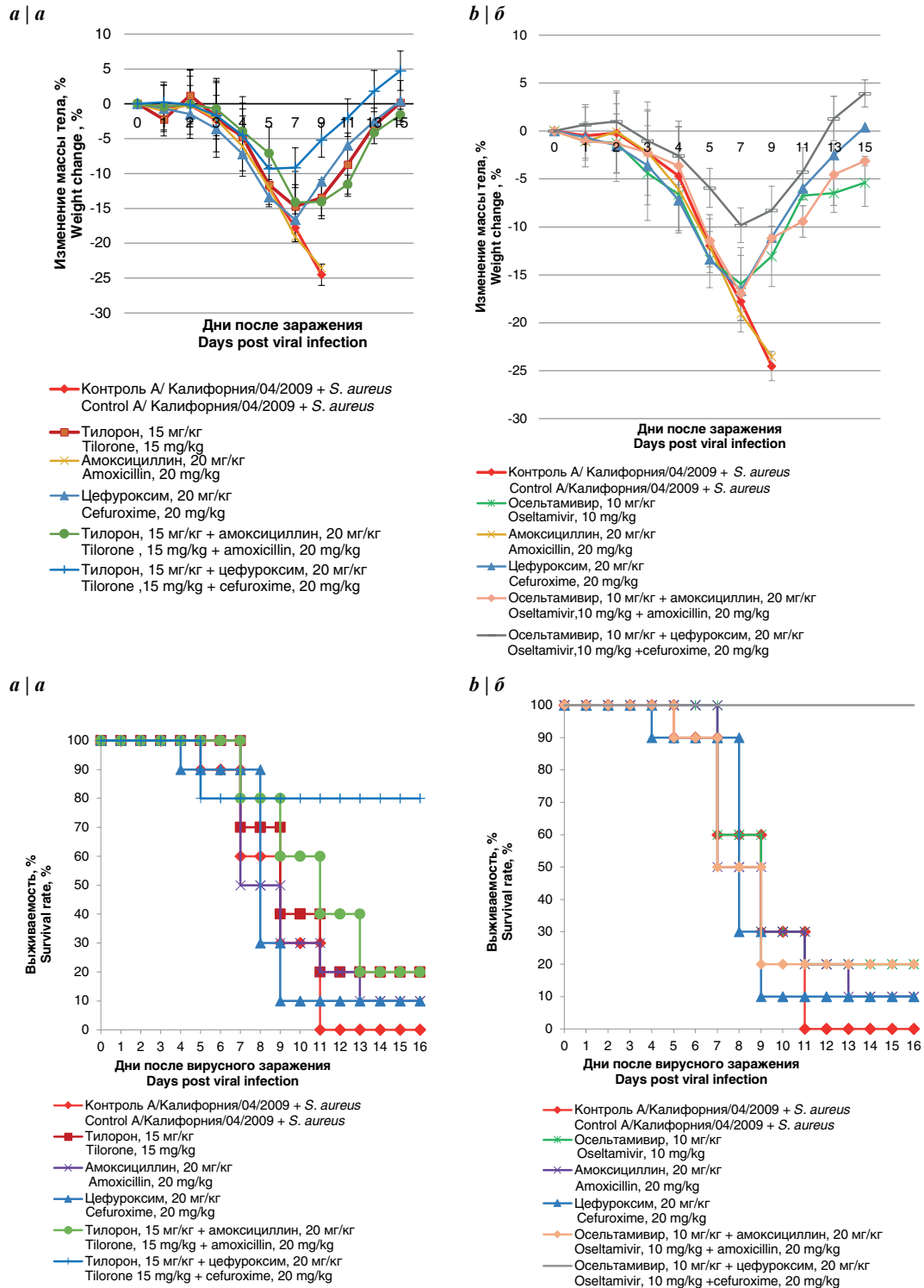
### Discussion

in a murine model of secondary bacterial pneumonia following influenza infection, it was shown that the efficacy of the interferon inducer tilorone, expressed as increased survival, reduced weight loss, and reduced pathogen content in the lungs, increased with reducing virus dose and intraperitoneal administration was more effective than oral administration.

The combination of tilorone with the antibacterial drug cefuroxime, to which the studied strain of *S. aureus* was sensitive, resulted in complete protection of animals from death, complete suppression of virus replication, and elimination of bacterial colonization of the lungs. In contrast, the combination of tilorone with amoxicillin, to which the pathogen was resistant, had no therapeutic effect. This highlights the importance of selecting an appropriate antibacterial agent based on the sensitivity of the pathogen and the promise of a strategy combining

antibiotics and antiviral drugs for post-influenza bacterial complications. A similar study previously showed that combination therapy with oseltamivir and ampicillin resulted in 100% survival in mice sequentially infected with *S. pneumoniae* after influenza A/Puerto Rico/8/34 (H1N1) virus [18]. Experimental results in a mouse model of secondary bacterial pneumonia were consistent with clinical observations: combination therapy of severe influenza with oseltamivir and antibiotics suppressed the inflammatory response, reduced the length of hospital stay, and decreased the frequency of admissions to the intensive care unit [1].

Tilorone stimulates the production of endogenous interferons of all major types ( $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\lambda$ ) and activates macrophages, NK cells, and T lymphocytes, which may explain its contribution to the increased effectiveness of combination therapy. Of particular importance is the induction of IFN- $\lambda$  in lung tissue, which has a pronounced antiviral and anti-inflammatory effect at the level of the respiratory epithelium [9, 11]. In the context of secondary viral-bacterial infection, such activation of immune mechanisms contributes to limiting virus replication, restoring the barrier function of the mucosa, and increasing the effectiveness of antibacterial therapy. Previously, in a similar model, it was shown that intranasal administration of IFN- $\lambda$  was associated with a decrease in the bacterial load in the lungs and a



**Fig. 4.** Survival rate (a) and weight change (b) during treatment with tilorone and oseltamivir at different infectious doses in a secondary bacterial pneumonia model following influenza infection in mice.

**Рис. 4.** Выживаемость мышей (a) и изменение массы тела (б) при лечении тилороном и осельтамивиром при различных заражающих дозах на модели вторичной бактериальной пневмонии после гриппозной инфекции у мышей.

reduction in the weight loss of animals, which corresponds to our data [11].

The experimental data obtained can serve as a basis for developing combined therapy regimens for influenza and its bacterial complications, aimed at improving treatment

efficacy and reducing the risk of developing antibiotic resistance.

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