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Miramistin effect on BHK-21 and PSGK-30 cell line proliferation and FMD virus reproduction in these cells

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SUMMARY
At present, Miramistin drug (benzyldimethyl [3-(myristoylamino) propyl] ammonium chloride monohydrate), which has a broad bactericidal effect, is used in veterinary practice. This antiseptic is active against most viruses, mycoplasmas, bacteria, fungi and protozoa. According to toxicometric parameters, Miramistin is classified as a low-hazard substance. Use of cell structures as test systems for assessing the toxicity of pharmacological substances instead of conventional tests on experimental animals allows us to better clarify the possible mechanism of the effect of the investigated substance. Since many types of mycoplasmas affect the genitourinary system organs, to assess the cytotoxicity of Miramistin kidney mammalian cells of various mammals can be used as test systems, in particular, the newborn Syrian hamster (VNK-21), Siberian mountain ibex (PSGK-30), and others. The possibility of using BHK-21 and PSGK-30 cell monolayer as test systems for assessing the baseline cytotoxicity of Miramistin was shown. When studying toxicity of the drug, the effect of its various concentrations on the cell morphology was studied, the number of viable cells and the total protein content were determined as an indicator of the cell mass increase. The results of a cytomorphological study indicate that the Miramistin maximum permissible concentration of for BHK-21 and PSGK-30 cell monolayer is 25 μg/cm^3. The use of this antibacterial drug in higher concentrations caused the appearance and increased signs of endogenous intoxication and degeneration. When evaluating the proliferative activity of cells under the influence of the Miramistin antibiotic in different concentrations, it was found that increasing the dose to 50, 75, 100, 125, 150 μg/cm^3 leads to decrease in the rate of cell growth compared to the control. The Miramistin content in the growth medium in an amount of up to 25 μg/cm^3 did not affect the intensity of protein synthesis. Presence of Miramistin in the culture medium at the maximum permissible concentration causes a slight decrease in the reproduction of foot and mouth disease virus in BHK-21 and PSGK-30 cell cultures by 4.5 and 4.0%, respectively, compared with the control without antibiotic. Since mycoplasmas are the most common contaminants of cell cultures, further studies will be aimed at exploring the possibility of using Miramistin for decontamination of BHK-21 and PSGK-30 cell monolayers.

Key words: Miramistin, BHK-21 and PSGK-30 cell monolayers, Mycoplasma.

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Влияние мирамистина на пролиферацию клеток линий ВНК-21 и ПСГК-30 и репродукцию вируса ящура в них

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РЕЗЮМЕ

В настоящее время в ветеринарной практике применяется препарат мирамистин (бензилдиметил[3-(миристоиламино)пропил] аммоний хлорид моногидрат), обладающий широким бактерицидным действием. Данный антисептик активен против большинства вирусов, микоплазм, бактерий, грибов и простейших. По параметрам токсикометрии мирамистин классифицируется как малоопасное вещество. Использование клеточных структур в качестве тест-систем для оценки токсичности фармакологических веществ вместо классических тестов на экспериментальных животных позволяет лучше прояснить возможный механизм действия исследуемого вещества. Поскольку многие виды микоплазм поражают органы мочеполовой системы, для оценки цитотоксичности мирамистина в качестве тест-систем можно использовать клетки почек разных млекопитающих, в частности новорожденного сирийского хомячка (ВНК-21), сибирского горного козерога (ПСГК-30) и другие. Показана возможность применения монослойных клеточных ВНК-21 и ПСГК-30 в качестве тест-систем для оценки базовой цитотоксичности мирамистина. В процессе исследования токсичности препарата изучали влияние его различных концентраций на морфологию клеток, определяли количество жизнеспособных клеток и содержание общего белка как показателя прироста клеточной массы. Результаты цитоморфологического исследования свидетельствуют о том, что предельной допустимой концентрацией мирамистина для монослойных клеточных линий ВНК-21 и ПСГК-30 является 25 мкг/см^3. Применение данного антибактериального препарата в бóльших концентрациях вызывало появление и нарастание признаков эндогенной интоксикации и дегенерации. При оценке пролиферативной активности клеток под влиянием антибиотика мирамистина в разных концентрациях выявлено, что увеличение дозы препарата до 50, 75, 100, 125, 150 мкг/см^3 приводит к снижению кратности прироста клеток по сравнению с контролем. Содержание в ростовой среде мирамистина в количестве до 25 мкг/см^3 не влияло на интенсивность синтеза протеинов. Наличие в культуральной среде мирамистина в предельно допустимой концентрации вызывает незначительное снижение репродукции вируса ящура в культурах клеток ВНК-21 и ПСГК-30 на 4,5 и 4,0% соответственно по сравнению с контролем без антибиотика. Поскольку микоплазмы являются наиболее распространенными контаминантами клеточных культур, дальнейшие исследования будут направлены на изучение возможности применения мирамистина для деконтаминации монослойных клеточных линий ВНК-21 и ПСГК-30.

Ключевые слова: мирамистин, монослойные клеточные линии ВНК-21, ПСГК-30, микоплазма.

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INTRODUCTION

Mycoplasmosis of animals is a group of infectious diseases caused by microorganisms such as *Tenericutes*, *Mollicutes* class, *Mycoplasmatacea*e family, *Mycoplasma* genus. Mycoplasmas are the most common cell culture contaminants. These prokaryotic microorganisms tightly adhere to the cell membrane, receive various nutrients and growth factors from it. Mycoplasma metabolites, including hydrogen peroxide and ammonia, accumulate in the culture medium and can have a pronounced cytopathic effect [1, 2, 3]. Mycoplasmas cause significant metabolic disorder in the cells, the consequence of which may be changes in the level of RNA and protein synthesis, the composition of the cell membrane, the appearance of chromosomal aberrations, induction or activation of cytokine expression, and a sharp decrease in proliferative activity [4]. Irreversible changes in the cells caused by the influence of mycoplasmas and their metabolic products lead to a distortion of the results of laboratory studies in which cell lines are used as test systems.

Mycoplasmas are resistant to sulfonamides, penicillins, streptomycin, but are sensitive to tetracyclines, macrolides, and fluoroquinolones [1, 3]. At present, Miramistin (benzyldimethyl[3-(myristoylamino)propyl] ammonium chloride monohydrate), which has a broad bactericidal effect, is used in veterinary practice. The drug belongs to the group of cationic surfactants, namely to quaternary ammonium compounds. According to the toxicometric parameters, Miramistin is classified as a low-hazard substance[1]. The drug is active against most viruses, bacteria, fungi and protozoa. The mechanism of Miramistin action is manifested in the process of active interaction of this compound with lipids and polysaccharides of the cytoplasmic membrane of mycoplasmas, leading to their death [2, 5].

Analysis of the microorganism ability to multiply and grow on media containing a decreasing concentration of a drug substance allows us to determine the minimum inhibitory concentration (MIC) of an antibiotic[2] that inhibits the life of mycoplasma *in vitro*. According to A. M. Dunaevsky et al., the MIC for Miramistin is 10–100 µg/cm^3 [6].

A study of the biological activity of antibiotics involves assessment of their cytotoxicity, which manifests itself in the occurrence of pathological changes in cells [1]. Depending on the concentration of the active substance and the sensitivity of cell receptors, a wide range of changes can be observed in the cell, which can manifest themselves in the form of a cytostatic effect or in the form of a cytocidal effect that causes its death [4].

To assess the toxicity of pharmacological substances, currently test systems using monolayer cell cultures are

[1] GOST 12.1.007-76. System of operational safety standards. Harmful substances. Classification and common safety requirements. M: Standartinform; 2007. 5 p.

[2] Arefyev V. A., Lisovenko L. A. English Russian dictionary of genetic terms. M.: VNIRO; 1995. 407 p.

widely used instead of classical tests on experimental animals [5, 7, 8]. Since a large number of mycoplasma species affect the organs of the genitourinary system, cells obtained from the kidneys of various mammals, in particular, the newborn Syrian hamster (BHK-21), Siberian mountain capricorn (PSGK-30), and others, are used as test systems to assess the cytotoxicity of Miramistin. A wide range of methods are used to study the cytotoxicity of antibiotics in cell culture, including determining the number of viable cells, colony formation, measuring the concentration of total cellular protein, assessing morphological changes in cells, and others [8].

The purpose of this study was to evaluate the effect of Miramistin on proliferation of BHK-21 and PSGK-30 cells, as well as on the reproduction of foot and mouth disease virus in them.

MATERIALS AND METHODS

Antibiotic. Miramistin antibiotic (LLC Scandia Eco, Russia) with concentrations of 5, 10, 25, 50, 75, 100, 125 and 150 μg/cm³ in the culture medium was used for analysis. Four controls were used in the study: No. 1 – tetracycline (5 μg/cm³); No. 2 – spiramycin (10 μg/cm³); No. 3 – ciprofloxacin (18 μg/cm³) [9]; No. 4 is a control, which is a cell suspension in a growth medium without antibiotics.

Test systems. To assess the baseline cytotoxicity of Miramistin, BHK-21 and PSGK-30 monolayer cell cultures, free of mycoplasma, were used as a test system.

A quantitative method for evaluating the effects of Miramistin on cell cultures. The effect of Miramistin on BHK-21 and PSGK-30 cell monolayer was evaluated by cell viability during three consecutive passages. The analysis consisted of counting the number of living and dead cells after dispersing the monolayer with a mixture of trypsin and versene (1:1) at a temperature of 37 °C. Proliferation intensity was estimated by the Proliferation Index (PI), using the following formula:

$$PI = a/b,$$

where a – live cell concentration in 48 hours after inoculation;

b – inoculation concentration.

When PI > 1, cell growth is observed, when PI = 1, no cell proliferation is observed, when PI < 1, cell death is observed.

Spectrophotometric method for assessing cell proliferation using a gentian violet. BHK-21 and PSGK-30 cells were seeded in 96-well culture plates in the amount of 250–300 cells per well. Miramistin in predetermined concentrations was added to the growth medium during the cell seeding. The cells without antibiotics as well as cells with tetracycline, spiramycin and ciprofloxacin in the specified above concentrations were used as the controls. After 48 hours of cultivation, the cells were fixed with a 70% ethanol solution and stained with a 0.1% gentian violet solution. To extract the dye from the cells, a 7% acetic acid solution in a volume of 200 μl per well was used. The proliferative activity of cells was determined by the optical density for a suspension of gentian violet associated with cellular proteins at a 570 nm wavelength.

Qualitative assessment of cell viability consisted in intravital visual observation under an inverted microscope of the cell morphology after 2 days of cultivation using an isotonic 0.4% aqueous solution of trypan blue.

Virus. The FMD Master seed virus (A/Zabaykalsky/2013 strain), obtained in the PSGK-30 cell culture at passage 5, was used as seed for infection of the specified cell monolayer at a dose of 0.05 TCD_{50}/cell. Virus reproduction was carried out for 15–17 hours until the appearance of a 95–100% cytopathic effect.

Determination of the FMDV 146S component. The amount of 146S particles of foot and mouth disease virus in inactivated material was determined using a quantitative variant of the complement fixation test [10].

Statistical data processing. The test was performed in triplicate. The data obtained were statistically processed, calculating the arithmetic mean values, the degree of reliability of the statistical difference between the average values determined by the Fisher's difference method. The differences were considered statistically significant at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

At the first stage of the study the Miromistin baseline cytotoxity in BHK-21 и PSGK-30 monolayers widely used as diagnostic test-systems in veterinary practice was determined. The choice of the antibiotic concentration was based on the data of its clinical tests [2, 3], as well as on the results of the Miramistin MIC analysis for mycoplasma, which falls within the range of 10–100 μg/cm³ [2, 3, 6]. On that basis, the following tested Miramistin concentrations were selected: 5, 10, 25, 50, 75, 100, 125, 150 μg/cm³. Cultivation of cells with the specified amount of the drug was performed during three consecutive passages.

Cytomorphological differences in the control and experimental samples. Cells without antibiotics, as well as grown in media with tetracycline (5 μg/m³), spiramycin (10 μg/cm³), ciprofloxacin (18 μg/cm³) had a distinct plasma membrane, a clear nuclear membrane, and transparent homogeneous cytoplasm (Fig. 1). In 48 hours after each passage in the control samples cell clones were dense and formed by more than 100 cells. Morphological characters of BHK-21 and PSGK-30 cells with Miramistin contents of 5, 10, and 25 μg/cm³ were most similar to the control. For these variants the clones were dense and were formed by 85–100 cells. Clones of the BHK-21 и PSGK-30 cell lines as a result of cultivation in the miramestin supplemented medium at concentrations 50, 75, 100, 125 μg/cm³ in comparison with the control without the antibiotic had a loose structure and contained 65–75, 45–60, 25–40, 15–25 cells respectively.

When using 150 μg/cm³ of Miramistin, clones were formed from single cells characterized by significant granularity and an increased number of lysosomes. The presented morphological changes were the result of local cytoplasmic degradation that develops in the cell under the influence of a toxic factor such as excessive Miramistin. In other words, the results of a cytomorphological study indicate that maximum permissible concentration of Miramistin for BHK-21 and PSGK-30 cell monolayer is 25 μg/cm³. The use of this antibacterial drug in higher concentrations resulted in the appearance and increase in signs of endogenous intoxication and degeneration.

A study was made of the effect of Miramistin in test concentrations on the proliferation index of BHK-21 and PSGK-30 cell lines basing on the results of three consecutive passages in comparison with the controls. The results are shown in the table and in Figure 2.

From the data presented in the table, it follows that the Proliferation Index of the BHK-21 cell line in the controls without antibiotic was 2.18 ± 0.16, and in the controls

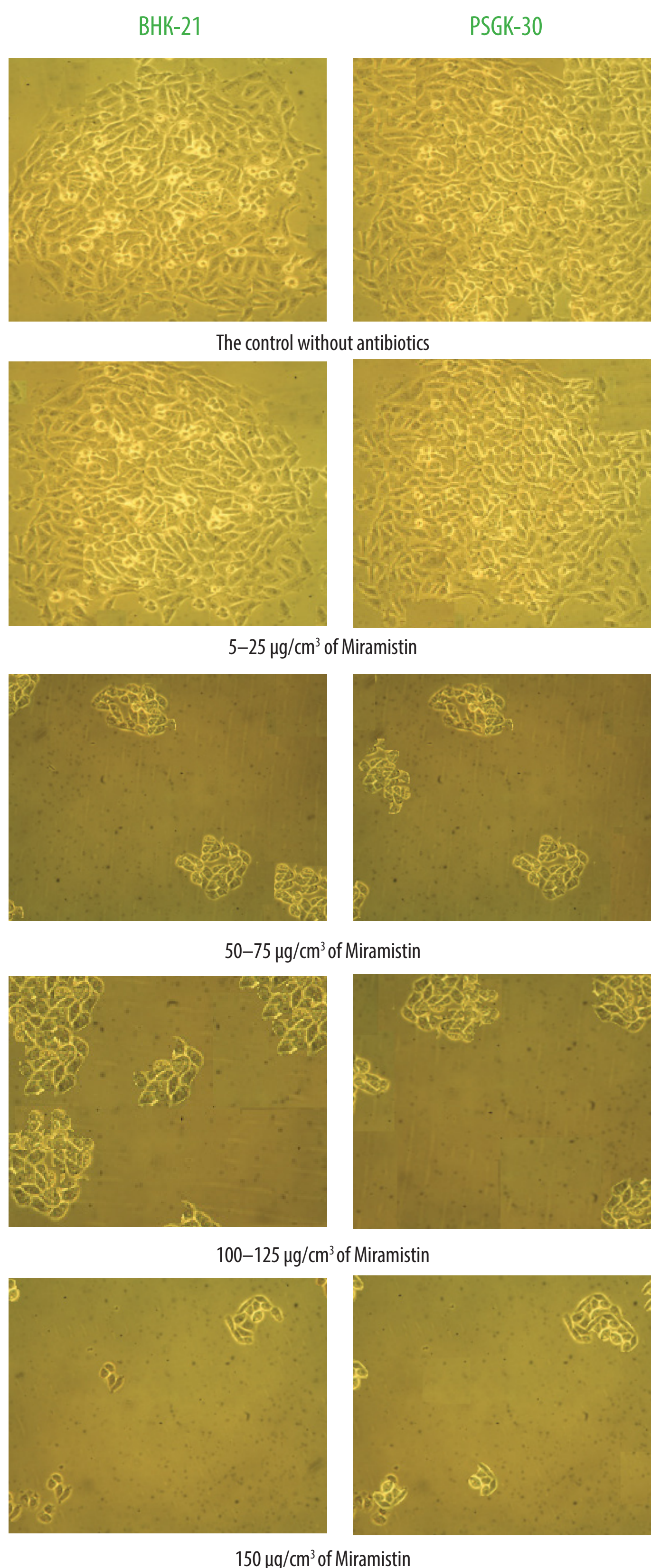

Fig. 1. Morphology of BHK-21 and PSGK-30 cell lines in clones, formed in 48 hours of cultivation in the medium supplemented with different Miramistin concentrations

Fig 1. Морфология клеток линий ВНК-21 и ПСГК-30 в клонах, образовавшихся через 48 ч культивирования в среде с мирамистином в различных концентрациях

with antibiotics – 2.17 ± 0.19 – 2.18 ± 0.17. When using Miramistin in amounts of 5–25 µg/cm^3, this parameter corresponded to 2.17 ± 0.19 – 2.18 ± 0.16. An increase in the dose of the drug to 50, 75, 100, 125, 150 µg/cm^3 led to a decrease in the cell proliferation index compared to the control by 4.6; 5.4; 7.5; 10.9 and 21.7 times, respectively. If the amount of antibiotic in the growth medium was 150 µg/cm^3, a visible cell death was observed. Thus, as a result of studying the proliferation of BHK-21 cells, it was determined that Miramistin in concentrations up to 25 µg/cm^3 does not induce the cytotoxic effect.

For PSGK-30 cells, the proliferation index in the control without antibiotic was 2.00 ± 0.15, in the medium with control antibiotics – 1.88 ± 0.15 – 1.97 ± 0.14. The proliferative potential of PSGK-30 cells when using Miramistin at concentrations of 5–25 µg/cm^3 was at the control level and was equal to 1.93 ± 0.18 – 1.97 ± 0.15. When the content of this antibiotic in the medium was 50, 75, 100, 125, 150 µg/cm^3, the cell proliferation index, compared to the control, decreased by 4.9; 5.3; 6.8; 10.0 and 21.1 times, respectively. When 150 µg/cm^3 of Miramistin was added into the growth medium, cell death was observed. Thus, when assessing the proliferation index in comparison with the control parameters, it was revealed that the growth of the PSGK-30 cell monolayer in a medium containing up to 25 µg/cm^3 of Miramistin did not have a cytotoxic effect.

Cell proliferation was also evaluated by the relative determination of the total cellular protein using spectrophotometry. The results of the study are presented in Figure 3, from which it follows that the protein content of BHK-21 cells grown in a medium with 5, 10, 25 µg/cm^3 of Miramistin corresponded to control parameters of optical density and amounted to 0.57–0.59. When the specified antibiotic was added into the medium at concentrations of 50, 75, 100, 125, 150 µg/cm^3, the amount of cellular proteins decreased by 4.3; 5.1; 8.0; 11.2 and 18.7 times, respectively, compared to the control (optical density was 0.13; 0.11; 0.07; 0.05; 0.03). Thus, results of total cellular protein determination compared to the control, revealed that cultivation of the BHK-21 cell monolayer in the medium with up to 25 µg/cm^3 of Miramistin did not reduce the translation intensity.

The amount of synthesized total protein of PSGK-30 cells grown in the medium with 5, 10, 25 µg/cm^3 of Miramistin was at the level of control parameters (optical density was 0.58–0.60). In the presence of the indicated antibiotic in the medium at concentrations of 50, 75, 100, 125, 150 µg/cm^3, the amount of cellular protein decreased by 4.8; 5.3; 7.3; 9.7 and 19.3 times, respectively, compared to the control (optical density was 0.12; 0.11; 0.08; 0.06; 0.03). In other words, results of total cellular protein determination compared to the control, revealed that that cultivation of the PSGK-30 cell monolayer in a medium containing up to 25 µg/cm^3 of Miramistin did not reduce the translation intensity.

According to the results of Miramistin cytotoxic potential assessmen, it was revealed that this antibacterial drug does not have any destructive effect on BHK-21 and PSGK-30 monolayers at a concentration of up to 25 µg/cm^3. At the same time, basing on the literature sources [2, 3, 6] it is know that the antibiotic concentrations presented are included in the MIC range for representatives of the *Mycoplasma* genus, and therefore, Miramistin in the indicated amounts allows the prevention of mycoplasma contamination of BHK-21 and PSGK-30 cell lines.

Table
Assessment of BHK-21 and PSGK-30 cell line proliferative activity influenced by different Miramistin concentrations ($n = 3$, $p < 0.05$)

Таблица
Оценка пролиферативной активности клеток линий ВНК-21 и ПСГК-30 под влиянием антибиотика мирамистина в разных концентрациях ($n = 3$, $p < 0,05$)

Cell line	Passage number	Cell Proliferation index											
		In the medium supplemented with different Miramistin concentrations (µg/cm³)								Control			
										With antibiotics			Without antibiotics
		5	10	25	50	75	100	125	150	No. 1	No. 2	No. 3	No. 4
BHK-21	1	2.21 ± 0.15	2.17 ± 0.17	2.22 ± 0.14	0.45 ± 0.15	0.40 ± 0.14	0.31 ± 0.13	0.22 ± 0.13	0.10 ± 0.15	2.22 ± 0.16	2.20 ± 0.17	2.22 ± 0.20	2.23 ± 0.19
	2	2.18 ± 0.15	2.20 ± 0.20	2.10 ± 0.15	0.47 ± 0.17	0.40 ± 0.14	0.29 ± 0.14	0.20 ± 0.12	0.09 ± 0.14	2.20 ± 0.20	2.22 ± 0.20	2.10 ± 0.13	2.10 ± 0.15
	3	2.15 ± 0.18	2.15 ± 0.21	2.20 ± 0.14	0.49 ± 0.11	0.40 ± 0.11	0.29 ± 0.11	0.18 ± 0.11	0.11 ± 0.11	2.12 ± 0.19	2.10 ± 0.19	2.20 ± 0.20	2.20 ± 0.14
	M ± *m*	2.18 ± 0.16	2.17 ± 0.19	2.17 ± 0.14	0.47 ± 0.14	0.40 ± 0.14	0.29 ± 0.13	0.20 ± 0.13	0.10 ± 0.12	2.18 ± 0.17	2.17 ± 0.19	2.17 ± 0.17	2.18 ± 0.16
PSGK-30	1	1.90 ± 0.14	2.01 ± 0.15	1.95 ± 0.12	0.37 ± 0.18	0.35 ± 0.18	0.30 ± 0.18	0.18 ± 0.18	0.10 ± 0.16	1.80 ± 0.17	2.03 ± 0.15	1.95 ± 0.14	1.95 ± 0.14
	2	1.98 ± 0.17	1.90 ± 0.21	2.00 ± 0.15	0.39 ± 0.21	0.34 ± 0.17	0.28 ± 0.16	0.19 ± 0.12	0.09 ± 0.17	2.00 ± 0.15	1.81 ± 0.20	2.06 ± 0.16	2.05 ± 0.15
	3	2.02 ± 0.17	1.88 ± 0.17	1.90 ± 0.16	0.40 ± 0.17	0.39 ± 0.17	0.27 ± 0.17	0.20 ± 0.12	0.09 ± 0.13	1.92 ± 0.14	1.80 ± 0.17	1.90 ± 0.14	2.00 ± 0.15
	M ± *m*	1.97 ± 0.15	1.93 ± 0.18	1.95 ± 0.15	0.39 ± 0.19	0.36 ± 0.16	0.28 ± 0.13	0.19 ± 0.11	0.09 ± 0.13	1.91 ± 0.15	1.88 ± 0.15	1.97 ± 0.14	2.00 ± 0.15

No. 1 – tetracycline (5 µg/cm³), No. 2 – spiramycin (10 µg/cm³), No. 3 – ciprofloxacin (18 µg/cm³), No. 4 – without antibiotic (w/a).

At the next stage, Miramistin influence on the sensitivity of BHK-21 PSGK-30 cell monolayers to the reproduction of foot and mouth disease virus was studied using the example of A/Zabaykalsky/2013 strain. Cells grown in a nutrient medium with 25 µg/cm³ of Miramistin were used for infection. The virus culture medium contained the same amounts of antibiotic that were added to the growth medium. The results of the test are shown in Figure 4.

From the data presented in Figure 4, it follows that as a result of the FMDV, A/Zabaykalsky/2013 strain cul-

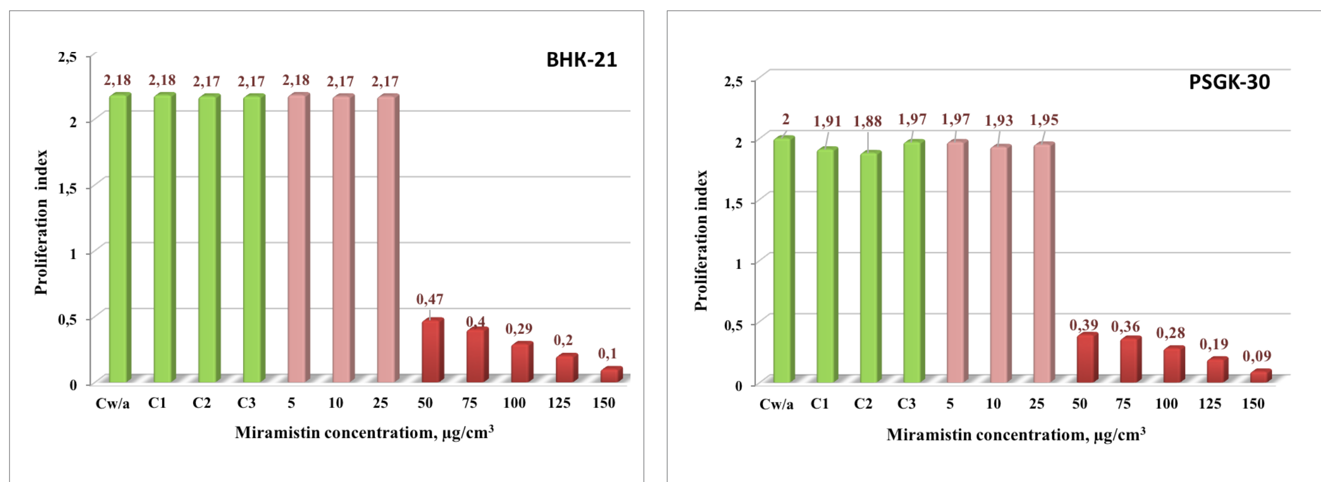

Fig. 2. Assessment of proliferation index of BHK-21 and PSGK-30 cell lines cultivated in the medium with different Miramistin concentrations (5, 10, 25, 50, 75, 100, 125, 150 µg/cm³)
Cw/a – control without antibiotics;
C1 – tetracycline-supplemented medium (5 µg/cm³);
C2 – spiramycin-supplemented medium (10 µg/cm³);
C3 – ciprofloxacin-supplemented medium (18 µg/cm³).

Рис. 2. Оценка кратности прироста клеток линий ВНК-21 и ПСГК-30, выращенных в среде с разным количеством мирамистина (5, 10, 25, 50, 75, 100, 125, 150 мкг/см³)
Cw/a – контроль без антибиотика;
C1 – среда с тетрациклином (5 мкг/см³);
C2 – среда со спирамицином (10 мкг/см³);
C3 – среда с ципрофлоксацином (18 мкг/см³).

tivation in BHK-21 cells grown with the addition of Miramistin, the concentration of the 146S component in the suspension was 0.64 ± 0.05 μg/cm³. The specified content of complete FMDV particles correlates with data for the control without antibiotics (0.67 ± 0.05 μg/cm³) and for the control with tetracycline (0.62 ± 0.05 μg/cm³), spiramycin (0.63 ± 0,06 μg/cm³) and ciprofloxacin (0.63 ± 0.05 μg/cm³). Therefore, the use of Miramistin at a concentration of 25 μg/cm³ does not reduce the sensitivity of the BHK-21 cell monolayer to infection and reproduction of foot and mouth disease virus.

During the reproduction of FMDV A/Zabaikalsky/2013 strain in PSGK-30 cells cultured in Miramistin-supplemented medium, the amount of 146S particles was 0.48 ± 0.05 μg/cm³. The obtained values correlate with the data for the control without antibiotic (0.50 ± 0.06 μg/cm³) and for the controls with tetracycline (0.46 ± 0.07 μg/cm³), spiramycin (0.48 ± 0.05 μg/cm³) and ciprofloxacin (0.46 ± 0.08 μg/cm³). Thus, Miramistin in an amount of 25 μg/cm³ does not reduce the sensitivity of the PSGK-30 monolayer to infection and reproduction of foot and mouth disease virus.

The results of the tests performed confirm that the presence of Miramistin antibiotic in the specified above maximum permissible concentrations in the growth and maintenance media does not cause a decrease in the concentration of the 146S component of the FMD virus compared to other drugs against mycoplasmas and the control without antibiotics.

CONCLUSION

The performed study showed the possibility of using BHK-21 and PSGK-30 cell monolayers as test systems for assessing Miramistin baseline cytotoxicity.

According to the results of assessing the cytotoxic potential of this antibacterial drug, when analyzing the morphological state and the cell proliferation index, as well as the amount of synthesized cell proteins, it was found that Miramistin did not have a toxic effect on BHK-21 and PSGK-30 cell monolayers at a maximum dose of 25 μg/cm³. The use of Miramistin in higher concentrations caused the appearance and growth of signs of endogenous intoxication, which manifested itself in a changed cell morphology, appearance of granularity and an increase in the number of lysosomes, as well as in a decrease in the cell proliferation index and proteins synthesized by them.

It was found that addition of 25 μg/cm³ of Miramistin in the growth and maintenance medium did not cause a decrease in the concentration of the 146S component during the reproduction of foot-and-mouth disease virus unlike addition of other antibiotics against mycoplasmas or no antibiotics at all. As the result of FMDV A/Zabaikalsky/2013 strain cultivation in BHK-21 cell line grown in a medium with 25 μg/cm³ of Miramistin, the concentration of the 146S component was 0.64 ± 0.05 μg/cm³, which is 4.5% lower than the control without an antibiotic; 3.2; 1.6 and 1.6% higher in comparison with tetracycline, spiramycin and ciprofloxacin. When foot-and-mouth disease virus was reproduced in PSGK-30 cells, which were also cultured in a medium with 25 μg/cm³ of Miramistin, the amount of 146S particles was 0.48 ± 0.05 μg/cm³, which is 4.0% lower compared to the control without antibiotic, 4.3% higher compared to tetracycline and spiramycin and equal for the medium with spiramycin.

Further research will be aimed at studying the possibility of using Miramistin for decontamination of BHK-21 and PSGK-30 cell monolayers from mycoplasmas.

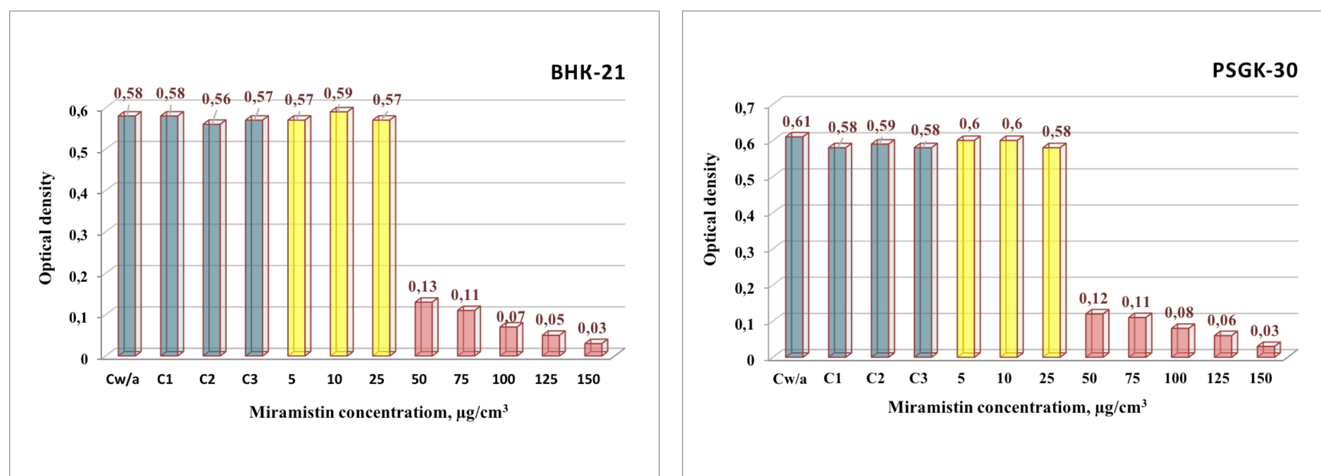

Fig. 3. Assessment of BHK-21 and PSGK-30 cell line proteins in 48 hours cultivation in the medium with different Miramistin concentrations (5, 10, 25, 50, 75, 100, 125, 150 μg/cm³)
Cw/a – control without antibiotics;
C1 – tetracycline-supplemented medium (5 μg/cm³);
C2 – spiramycin-supplemented medium (10 μg/cm³);
C3 – ciprofloxacin-supplemented medium (18 μg/cm³).

Рис. 3. Гистограммы оценки протеинов клеток линий ВНК-21 и ПСГК-30 через 48 ч культивирования в среде с разным количеством мирамистина (5, 10, 25, 50, 75, 100, 125, 150 мкг/см³)
Cw/a – контроль без антибиотика;
C1 – среда с тетрациклином (5 мкг/см³);
C2 – среда со спирамицином (10 мкг/см³);
C3 – среда с ципрофлоксацином (18 мкг/см³).

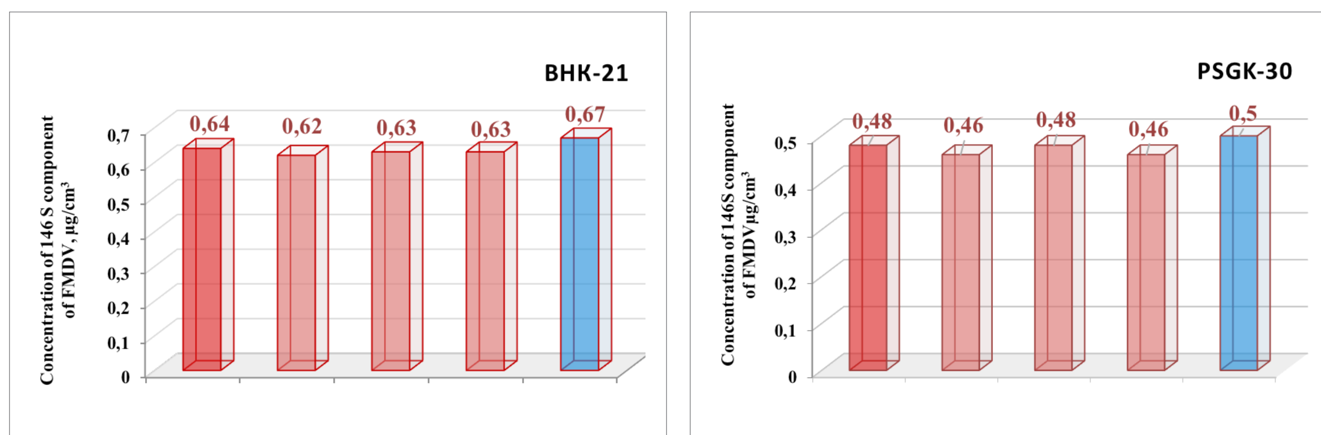

Fig. 4. Concentration of 146 S component of FMDV strain A/Zabaikalsky/2013 in inactivated suspensions after reproduction in BHK-21 and PSGK-30 cell lines cultivated in the Miramistin-supplemented medium compared with the controls

1 – Miramistin-supplemented medium;
2 – tetracycline-supplemented medium (5 μg/cm³);
3 – spiramycin-supplemented medium (10 μg/cm³);
4 – ciprofloxacin-supplemented medium (18 μg/cm³);
5 – control without antibiotics.

Рис. 4. Концентрация 146S компонента вируса ящура штамма А/Забайкальский/2013 в инактивированных суспензиях после репродукции в клетках линий ВНК-21 и ПСГК-30, культивируемых в среде с мирамистином, в сравнении с контролями

1 – среда с мирамистином;
2 – среда с тетрациклином (5 мкг/см³);
3 – среда со спирамицином (10 мкг/см³);
4 – среда с ципрофлоксацином (18 мкг/см³);
5 – контроль без антибиотика.

REFERENCES

1. Fischer O., Hájková M., Hořínová Z., Jurmanová K., Zendulková D. The use of ciprofloxacin for decontamination of LSCC-H 32 cell line of chicken embryonal fibroblasts contaminated by *Mycoplasma arginini*. *Acta Vet. Brno*. 1992; 61: 51–56. DOI: 10.2754/avb199261010051.

2. Mycoplasma – The Disaster of Cell Culture. Available at: https://www.capricorn-scientific.com/lp/mycoxpert-nl/ (date of access: 12.06.2019).

3. Waites K. B., Taylor-Robinson D. Mycoplasma and Ureaplasma. In *Manual of Clinical Microbiology*. Eds. P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, R. H. Yolken. 7th ed. Washington: ASM Press, 1999: 782–794.

4. Polyanskaya G. G.Patterns of karyotypic variability in cell cultures during prolonged cultivation in different lines [Zakonomernosti kariotipicheskoj izmenchivosti v kletochnyh kul'turah pri dlitel'nom kul'tivirovanii v raznyh liniyah]: Author's abstract. Dr. Biol. Sciences. SPb.; 2000.38 p. Available at: https://dlib.rsl.ru/viewer/01000255531#?page=1. (in Russian)

5. Yeropkin M. Yu., Yeropkina Ye. M. Cell culture as a model system for studying toxicity and screening of cytoprotectants [Kul'tura kletok kak model'naya sistema issledovaniya toksichnosti i skrininga citoprotektornyh preparatov]. Saint Petersburg: MORSAR AV; 2003. 239 p. (in Russian)

6. Dunayevsky A. M., Kirichenko I. M. Clinical trials of Miramistin use in treatment of infectious and inflammatory respiratory diseases. Literature review [Klinicheskoe obsledovanie ispol'zovaniya preparata Miramistin v terapii infekcionno-vospalitel'nyh zabolevanij respiratornoj sistemy. Obzor literatury]. *Poliklinika*. 2013; 5: 6–12. eLIBRARY ID: 23245010. (in Russian)

7. Romanova M. A., Dodonova A. Sh. Studying cytotoxicity of biologically active compounds in cell culture [Izuchenie citotoksichnosti biologicheski aktivnyh soedinenij na kul'ture kletok]. *Young Scientist*. 2016; 18 (122): 110–114. Available at:https://moluch.ru/archive/122/33642/. (in Russian)

8. Zurlo J. *In vitro* toxicological analyzes [Toksikologicheskie analizy *in vitro*]. Available at: http://base.safework.ru/iloenc?print&nd=857400333&spack=110LogLength%3D0%26LogNumDoc%3D857000927%26listid%3D010000000100%26listpos%3D2%26lsz%3D4%26nd%3D857000927%26nh%3D1%26. (in Russian)

9. Doronin M. I., Shishkova A. A., Klyukina N. D., Lozovoy D. A., Mikhalishin D. V. Use of ampicillin, ciprofloxacin, cefotaxime and polymyxin B for prevention of BHK-21/2-17 cell line microbial contamination. *Proceedings of the Federal Center for Animal Health*. 2016; 14: 159–176. eLIBRARY ID: 30095811. (in Russian)

10. Bondarenko A. F. Qualitative and quantitative immunochemical analysis of virus proteins [Kachestvennyj i kolichestvennyj immunohimicheskij analiz virusnyh belkov]. Suzdal; 1994. 92 p. (in Russian)

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