Part 2. Biosafety

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STUDY OF THE INFLUENCE OF BACTERICIDAL PREPARATION ON CELL CULTURES

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Summary. Pressing problem today is the existence of a large number of microorganisms — causative agents of viral diseases, highly resistant to bactericidal preparations. At the same time, an important direction of research is the study of the toxic effects of new disinfectants not only on the organism of laboratory animals, but also on cell cultures, which is more humane and meets the modern standards of bioethics. That's what determined the purpose of research — the study of the antiviral and toxic effects of the bactericidal preparation 'Barez' on the Aujeszky's disease virus strain 'Clone-B', tested and adapted to the continuous cell cultures SNEV and PTP. When conducting research there were studied methods for assessing toxic effects of antibacterial drug 'Barez' on the cell cultures of swine origin SNEV, PTP, and its effect on the titer of infectious activity of the vaccine strain 'Clone-B' of Aujeszky's disease virus from the collection of strains of the State Scientific Control Institute of Biotechnology and Strains of Microorganisms. When studying the antiviral properties of the bactericidal preparation on the Aujeszky's disease virus 'Clone-B' strain, tested and adapted for continuous cell cultures SNEV and PTP, there was determined that 0.2–0.5% solutions of the drug 'Barez' are not toxic when effecting cell cultures, but exhibit inactivating effect on the Aujeszky's disease virus. Therefore, it is possible to use it for preventive disinfection of objects of veterinary medicine in the presence of animals in recommended concentrations and exposures.

Keywords: bactericidal drug 'Barez', cell cultures, toxicity, Aujeszky's disease virus, silver nanoparticles, benzalkonium chloride, essential oils

Introduction. A serious problem in prevention and control of animal viral diseases is the resistance of pathogens to antiseptics and disinfectants. The species composition of antibiotic resistant microorganisms is highly varied; they are characterized by a higher resistance to disinfectants of various chemical groups and the ability not only to survive, but also to multiply in solutions of disinfectants and on various objects of the environment (Stadnytska et al., 2011; Ababutain, 2011).

Methods of studying the toxic effects of new disinfectants on laboratory animals are not humane, expensive and do not meet the global standards for biotic evaluation of experiments. Therefore, the application of the method for determining the toxic effect of disinfectants on cell cultures, as an alternative to experiments using animals, is very relevant in today's research practice (Ababutain, 2011; Pathmanathan et al., 2010; Kovalenko and Nedosiekov, 2011).

An important condition for the reliable biotesting of new disinfectants is the use of genetically homogeneous laboratory cultures, since they are tested for sensitivity, they are stored in special standard-determined laboratory conditions, which ensures the necessary reliability and reproducibility of the results of studies, as well as the maximum sensitivity to toxic substances.

The study of antiviral activity of disinfectants on viruses that can cause disease in animals and humans, is relevant in the practice of veterinary medicine as well as in many other areas where it is necessary to determine the action of disinfectants against specific infectious agents (Kovalenko et al., 2010; Deyneka, 1999).

The study of toxicity of bactericidal agents on cell cultures can be recommended for: an approximate accelerated assessment of the toxicity of bactericidal drugs; establishment of harmlessness of animal protection means during selective control; in order to obtain an indicative evaluation of the toxicity of active substance, solvent, filler, etc. at the stage of development or changes in the production technology; in cases where the amount of the preparation is too small to determine its toxicity on laboratory animals; ecological testing of bactericidal agents that may pose a threat to the environment (Pathmanathan et al., 2010; Kovalenko and Nedosiekov, 2011).

The aim of the study was to research the antiviral and toxic effects of bactericidal preparation 'Barez' on the

Aujeszky's disease virus strain 'Clone-B', tested and adapted for continuous cell cultures SNEV and PTP.

Materials and methods. In the course of the research, methods for assessing the toxic effects of disinfectants on the cell cultures of swine origin SNEV, PTP, as well as their influence on the titer of the infectious activity of Aujeszky's disease virus vaccine strain 'Clone-B' from the collection of strains of the State Scientific Control Institute of Biotechnology and Strains of Microorganisms.

The objects of the research were the solutions of bactericidal preparation 'Barez' based on silver nanoparticles, benzalkonium chloride and essential oils. In the course of the experiment there were used: a continuous cell culture of swine embryo kidney (SNEV) and a continuous cell culture of piglets testicular (PTP) grown on the surface of the well bottom of a 96-well microplate as a monolayer. To grow continuous cell cultures there were used following media: RPMI 1640, DMEM, lactalbumin hydrolysate medium, cattle blood serum series 07, phosphate-salt buffer. As a test object, a continuous cell culture with known characteristics and stable for at least three consecutive passages SNEV, PTP was used.

To comply with the standard cultivation conditions, cultural 96-well sterile microplates for cell cultures 'Sarstedt' were used in a quantity of two for each culture, and sterile 96-well microplates of the same firm for dilutions of the tested bactericidal agent. The criteria for toxic effects were: indicators of statistically reliable reduction of the cell culture proliferation index in comparison with control (weak toxicity); suppression for some time or complete loss of cell ability to multiply (strong toxicity); the effect of a bactericidal agent on a cell culture, accompanied by instantaneous visible destructive changes in the cells of the culture (extrusion of cells from the carrier surface), and its strong fixation to the surface of the carrier, or coagulation, can be described as super toxicity.

To determine the degree of antiviral action of the disinfectant on the vaccine strain 'Clone-B' of the Aujeszky's disease virus, its various concentrations were prepared in a microplate with U-shaped bottom. Dilutions of the drug were performed on the basis of phosphate-salt buffer.

After that, in the prepared dilution of the disinfectant, a virus suspension of Aujeszky's disease virus strain 'Clone-B' with activity (10^7 TCD_{50}/cm^3 — the dose of the virus, which caused in 24–28 hours without treatment cytopathic effect in the cell cultures SNEV and PTP) was added. The contact of virus with disinfectant was carried out in a titration microplate for 60 minutes. After the contact virus + drug prepared dilutions were placed onto the surface of the cell monolayer for possible adsorption of the virus, which was not inactivated by a disinfectant.

Identical consecutive dilutions of the preparation for each culture were introduced in two microplates with cell cultures SNEV and PTP, one plate for each culture. The inserted dilutions of the preparation were left for contact with 'Clone-B' strain virus suspension for 15 minutes.

At the end of the contact time, the microplates with the cell cultures were washed three times with phosphate-salt solution, the maintenance medium with cattle blood serum was introduced and placed for further incubation. At that, 16 wells with cell culture in each microplate were left as control, in which the preparation was not introduced. Microplates were observed two times a day by microscopy.

Results and discussion. The visual evaluation of the state of monolayer of taken in the experiments cell cultures, that came into direct contact with the consecutive dilutions of the drug, clearly reflected the result of the interaction culture-disinfectant compared with the control microplate wells. In the wells of a microplate with cell culture in which the drug was introduced at a concentration below the toxicity threshold, the monolayer remained intact and visually did not differ from the control wells with culture.

At the same time, during the visual evaluation, there were also found wells with culture of cells with signs of cytopathic action and degenerative changes of the monolayer in comparison with the control. The cause of degenerative changes in cell culture was the effect of the drug 'Barez' in the corresponding concentration, which was found to be toxic to this type of cell culture (Table 1).

Table 1 — Results of determination of the cytopathic effect of Aujeszky's disease virus treated with a bactericidal preparation 'Barez' on a cell cultures monolayer (n = 10)

Concentration, %	Cell culture, exposure 20 min		Manifestation of cytopathic action of the Aujeszky's disease virus treated with disinfectant, exposure 60 min			
	SNEV	PTP	Number of wells			
0.05	–	–	+	+	+	+
0.1	–	–	+	–	–	+
0.2	–	–	–	–	–	–
0.5	–	–	–	–	–	–
1.0	–	–	0	0	–	–
2.0	+	+	0	0	0	0

Notes: (–) — no cytopathic effect; (+) — reflects the cytopathic effect of the drug on the cell.

The results of the study were taken into account until the moment of degenerative changes in the microplate control wells. The 'Barez' drug in 1.0–2.0% concentrations on cell cultures SNEV and PTP showed cytopathic effects, so these concentrations were not evaluated concerning the effect of disinfectant on the Aujeszky's disease virus.

As a result of studies conducted to determine the toxic effect of the preparation after cell culture subcultivation under the influence of 0.2% solution of the drug 'Barez',

there was found that the drug at this concentration did not affect the change of the culture monolayer compared to the control (Table 1).

Subsequent studies have found that the bactericidal agent 'Barez' in concentrations of 0.2–0.5% exhibited inactivating effect on the Aujeszky's disease virus. Therefore, the drug in these concentrations can be recommended for disinfection in order to prevent viral diseases.

At the same time, the degree of cell proliferation was determined after the action of the investigated disinfectant on them. Observing the cell culture was carried out by microscopy from the 1st to the 6th day so far the completion of the experiment, and counting the number of cells in the microplate wells was carried out from the 4th to the 6th day inclusively. The time of formation of a monolayer in experimental carriers with culture or the absence of such a fact in general compared with the control culture is decisive in assessing the degree of toxic effects of a disinfectant.

For the statistical processing of the obtained research results, in addition to the visual evaluation of the cell culture monolayer, a method of counting cells from each carrier with cell culture, taken in the experiment separately, was used. After 6 days, the observations were discontinued due to degenerative changes in the control cell culture, which was due to the aging of the cell monolayer.

After re-seeding (removal of the cell culture monolayer for further reproduction), the following results were obtained. Under the influence of the drug 'Burez' in 0.3% concentration on the 4th day, the number of cells SNEV was 15,650.0 ± 34.1, on the 6th day — 16,320.0 ± 42.5 ($p \leq 0.05$). In the control experiment, 17,730.0 ± 53.4 SNEV cells were observed.

The same results were obtained with the cell culture PTP, where under the influence of the drug 'Barez' in 0.3% concentration on the 4th day the number of PTP cells was 18,320.0 ± 35.2 ($p \leq 0.05$), on the 6th day — 19,700.0 ± 41.3. In the control experiment, 19,630.0 ± 52.6 PTP cells were observed. In these cases, non-toxic effects of disinfectant were observed.

Conclusions. Bactericidal preparation 'Barez' in a concentration of 0.2–0.5% is non-toxic for exposure to cell culture, but exhibits inactivating effect on the Aujeszky's disease virus. Therefore, it is possible to use it for preventive disinfection of objects of veterinary medicine in the presence of animals in recommended concentrations and exposures.

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