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IN VIVO EVALUATION OF GENOTOXICITY OF "MULTIBOVISAN" VETERINARY VACCINE

In vivo vaccine genotoxicity estimation (assessment of DNA-damages) is informative and highly prognostic because of the possibility to predict the malignant degeneration of the eukaryotic cells as well as the level of risk for the posterity health in the case of essential changes in the DNA of animal reproductive cells. Comet assay based on the registration of differences in migration of lysed cells DNA and its fragments in the constant electric field. DNA breaks was found to interrupt the structural organization of chromatin, which leads to the relaxation of DNA and formation of its fragments. Alkaline gel-electrophoresis of isolated eukaryotic cells (Comet assay) in vivo has shown the absence of genotoxic influence of "Multibovisan" vaccines on the laboratory rabbits. According to the genotoxicity criteria, the studied vaccine is considered to be biosafe.

***Keywords**: "Multibovisan", genotoxicity, Comet assay, alkaline gel-electrophoresis, isolated eukaryotic cells, biosafety.*

Introduction. Vaccine genotoxicity estimation *in vivo* is highly prognostic because of the possibility to predict the malignant degeneration of the eukaryotic cells as well as the level of risk for the posterity health in the case of essential changes in the DNA of animal cells. At the present stage of investigations, *in vivo* Comet assay in alkaline conditions is a sensitive, quick and informative method for the estimation of genotoxicity of different chemical substances and physical agents [1, 2]. It is based on the registration of differences in migration of DNA and its fragments in the constant electric field wherein cells are lysed. DNA breaks interrupt the structural organization of chromatin, which leads to the relaxation of DNA and formation of its fragments. Alkaline treatment of lysed cell preparations stimulates untwisting of DNA duplex and allows individual threads to migrate in the electric field independently. Under such conditions, DNA migrates to the anode, forming the electrophoretic track that looks like the comet tail, whose parameters are depend on the level of DNA damage encountered [3, 4].

The goal of the work was the *in vivo* estimation of genotoxicity parameters for "Multibovisan" vaccine.

Materials and methods. For the *in vivo* estimation of genotoxicity of "Multibovisan" vaccine, rabbit males have been used.

All experiments with laboratory animals have been carried out in compliance with "Guide for the Care and Use of Laboratory Animals".

Rabbits were injected subcutaneously with 2 ml of "Multibovisan" vaccine. Vaccination was performed twice, according to the specification of the vaccine.

Experimental group consisted of 3 animals. The animals were kept in the vivarium in accordance with the appropriate sanitary regulations on a standard diet with 12-hour light regime and free access to food and water.

The level of DNA damage has been estimated by the Comet assay (alkaline gel-electrophoresis of isolated eukaryotic cells) [5, 6]. Cell isolation from liver, kidneys, spleen, bone marrow, heart, lungs, testicles and muscles of the hind limbs has been performed according to standard protocols [7].

Micropreparations were formed on the microscopic slides with agarose plate (1% agarose gel of normal molten agarose ($T_{melting}$ < 65ºC)), on which 60 μl of the treated cell suspension and 60 μl of 0.5% agarose gel have been spread. Then, slides were immersed in the freshly-prepared cold lysis solution (10 mM Tris-HCl (pH 10.0), 2.5 M NaCl, 100 mM EDTA-Na_2, 1% Triton X-100 and 10% DMSO) for 3 hours at 4 ^{0}C. After finishing the lysis procedure, slides were placed in a horizontal gel electrophoresis tank filled with the fresh cold electrophoresis solution (300 mM NaOH, 1 mM EDTA-Na_2, (pH>13.0) for the alkaline DNA denaturation (with the tank being switched off for 20 min). Distribution of the denatured DNA has been carried out by gel-electrophoresis during 20- 30 min at the field strength 1 V/cm and current intensity no more than 250 mA. After electrophoresis, preparations have been fixed by 70% ethanol solution during 15 min. Subsequently, they have been stained by an acridine orange fluorescent dye for 30 min. DNA comets have been visualized using a fluorescent microscope "LUMAM R8" (exciting filter 490 nm, dichroic mirror 510, reflective filter 530 nm, magnification X200–400).

For each micropreparation, 200 DNA comets without the tail overlays have been analyzed. DNA comets have been divided on 5 relative types with an appropriate number of 0 to 4 for each comet (Fig.1).

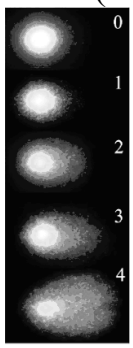

Fig. 1. DNA comets of different levels of DNA damage: 0 – electrophoretic tracks of the DNA comet of 0-type; 1 – electrophoretic tracks of the DNA comet of 1-type; 2 – electrophoretic tracks of the DNA comet of 2-type; 3 – electrophoretic tracks of the DNA comet of 3- type; 4 – electrophoretic tracks of the DNA comet of 4-type.

The level of DNA damage has been characterized by the DNA-comet index I_{DNA}, determined according to the formula:

$$\mathbf{I_{DNA} = (0n_0+1n_1+2n_2+3n_3+4n_4)/\Sigma,}$$ where

n_0–n_4 – number of DNA comets of each type, Σ – their total number.

Two parallel series of the experiments have been done. Statistical analysis of results obtained has been performed by comparing the indexes of DNA damage in experimental groups with those known from the experiments *in vitro* (positive and negative controls) [8]. The data of two replications have been combined and the average parameter for each group has been calculated. Statistically significant high indexes of DNA damage (data close to positive control) serve as criteria of a positive result. The probability differences of $p < 0.05$ were considered as significant.

Results of research and discussion. The *in vivo* evaluation of the "Multibovisan" veterinary vaccine genotoxic properties allowed to reveal the following effects. When using the Comet assay method in alkaline conditions for testing the "Multibovisan" genotoxicity, electrophoretic tracks of the DNA comet typical of the genotoxic influence on the eukariotic cell, as shown in Fig. 2, have not been observed.

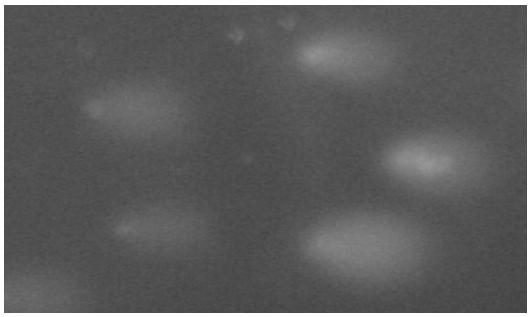

Fig. 2. Electrophoretic images of DNA-comets showing the genotoxic influence on the eukariotic cell. The longer tail, the greater level of DNA damage.

Electrophoretic images of Fig. 3 provide clear evidence that in all the samples of the target organs, such electrophoretic tracks were missing, indicating the absence the genotoxic influence on the eukariotic cell.

Parameters of the genotoxic influence of veterinary vaccine "Multibovisan" *in vivo* on the target organs of rabbits, determined by the Comet assay, are provided in table 1.

The obtained genotoxicity indexes I_{DNA} are close to those of the negative control (0.17±0.03 or 0.36±0.01), which have been determined under the conditions *in vitro* by the Comet assay, whereas the positive control gives the values of 1.94±0.11 or 2.28±0.18 [8].

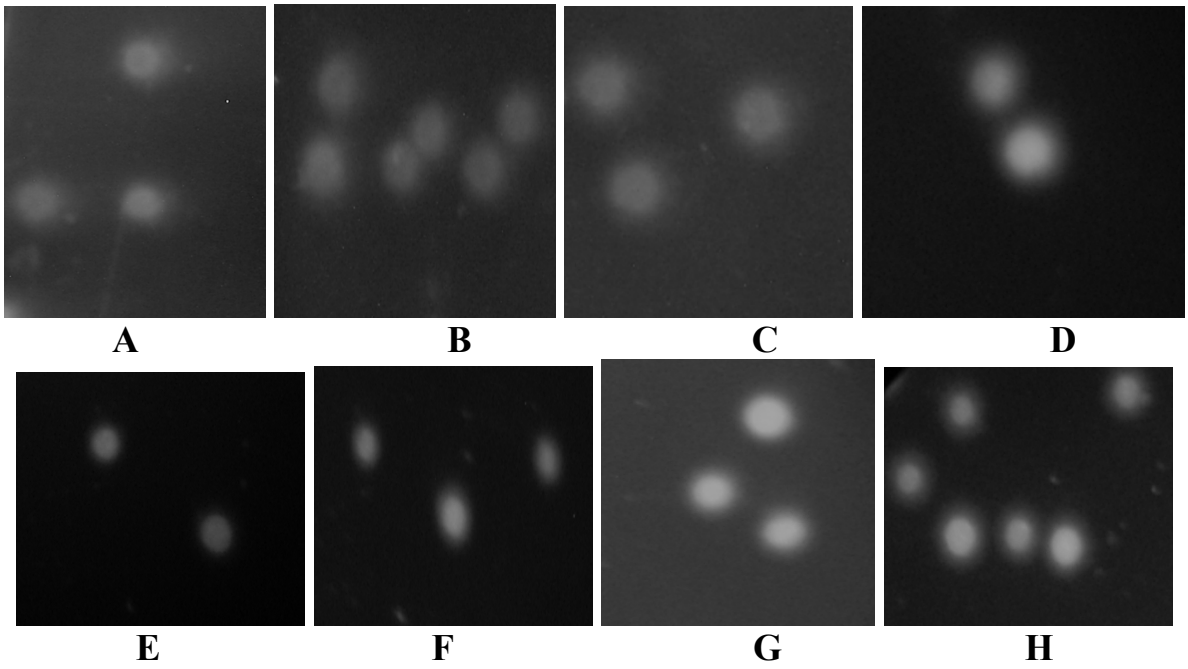

Fig. 3. Electrophoretic images of intact nuclei of cells: A – liver, B – spleen, C – kidneys, D – bone marrow, E – heart, F – lungs, G – testicles, H – muscles of the hind limbs.

Table 1

The genotoxicity indexes of "Multibovisan" veterinary vaccine

Target organ	Genotoxicity index «I_{DNA}»
Liver	0,21±0,02
Kidneys	0,41 ±0,03
Bone marrow	0,67±0,03
Heart	0,33±0,03
Spleen	0,44±0,01
Lungs	0,39±0,03
Testicles	0,43±0,04
Muscles of the hind limbs	0,29±0,03

Conclusions and prospects for the further research. By the Comet assay method *in vivo*, absence of the genotoxic effect of "Multibovisan" vaccine, has been shown.

According to its genotoxicity parameter, the investigated veterinary vaccines are considered to be biosafe.

The investigations fulfilled open wide perspectives for the future improvement of methods for the estimation of safety of immunobiological preparations for domestic animals, based on the parameters of their influence on the genetic apparatus.

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ОЦЕНКА ГЕНОТОКСИЧНОСТИ ВЕТЕРИНАРНОЙ ВАКЦИНЫ «МУЛЬТИБОВИСАН» *IN VIVO* / Дыбкова С.Н., Резниченко Л.С., Грузина Т.Г., Рыженко Г.Ф., Горбатюк О.И., Андриящук В.А., Жовнир А.М., Рудой А.В., Тютюн. С.Н.

Оценка генотоксичности вакцин in vivo (оцека уровня повреждений ДНК) является информативной и высокопрогностичной, поскольку позволяет предсказать злокачественное перерождение эукариотических клеток, а также предсказать уровень риска для здоровья потомства в случае существенных изменений в ДНК половых клеток животных. Метод ДНК комет основан на регистрации различий миграции нативной ДНК и ее фрагментов лизированных клеток в постоянном электрическом поле. Разрывы ДНК нарушают структурную организацию хроматина, приводят к релаксации ДНК и формированию фрагментов. Методом щелочного гель-электрофореза изолированных эукариотических клеток (ДНК комет) in vivo показно отсутствие генотоксического влияния вакцины «Мультибовисан» на организм лабораторных кроликов. В соответствии с критериями генотоксичности, изученная вакцина «Мультибовисан» – биобезопасна.

Ключевые слова: «Мультибовисан», генотоксичность, метод ДНК комет, щелочной гель-электрофорез, изолированные эукариотические клетки, биобезопасность.

ОЦІНКА ГЕНОТОКСИЧНОСТІ ВЕТЕРИНАРНОЇ ВАКЦИНИ «МУЛЬТИБОВІСАН» *IN VIVO* / Дибкова С.М., Рєзніченко Л.С., Грузіна Т.Г., Риженко Г.Ф., Горбатюк О.І., Андріящук В.А., Жовнір О.М., Рудой О.В., Тютюн С.М.

Генотоксичність (здатність викликати первинні ушкодження ДНК) ветеринарної вакцини – показник, який безпосередньо характеризує біобезпечність такого імунобіологічного препарату для генетичного матеріалу тварин. Тестування вакцин на генотоксичність in vivo є надзвичайно важливим для прогнозування потенційної мутагенної дії таких ветеринарних препаратів. Надзвичайно чутливим методом оцінки генотоксичності є метод ДНК комет в лужних умовах (лужний гель-електрофорез ізольованих еукаріотичних клітин), суть якого полягає у реєстрації відмінностей в електрофоретичній рухливості нативної і пошкодженої ДНК. При цьому формується

електрофоретичний слід, що нагадує «хвіст комети», параметри якого залежать від рівня пошкодження дослідної ДНК. Метою роботи була оцінка генотоксичності in vivo ветеринарної вакцини «Мультибовісан» методом ДНК комет в лужних умовах. В експерименті in vivo використовували самців лабораторних кролів, яким двократно підшкірно вводили вакцину «Мультибовісан» в об'ємі 2 мл. Із органів та тканин забитих кролів виділяли клітини та аналізували методом ДНК комет рівень пошкоджень генетичного апарату клітин тварин. Показано, що вакцина «Мультибовісан», не проявляла генотоксичної дії в клітинах печінки, нирок, серця, легень, селезінки, сіменників, кісткового мозку та м'язів задніх кінцівок. Отже, досліджена ветеринарна вакцина є біобезпечною за показниками генотоксичності in vivo.

Ключові слова: *«Мультибовісан», генотоксичність, метод ДНК комет, лужний гель-електрофорез, ізольовані еукаріотичні клітини, біобезпечність.*

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ANTIMICROBIAL ACTIVITY ESTIMATION OF EXPERIMENTAL COPPER NANOPARTICLES

The article presents results of physical and chemical characteristics and estimation of antimicrobial activity of experimental substance of spherical 20 nm copper nanoparticles. There were tested 9 pathogen strains of different genus for CuNP antimicrobial activity estimation. High