НЕЗАРАЗНА ПАТОЛОГІЯ

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DISINFECTANT TOXICITY STUDY IN VITRO

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It was investigated the cytotoxicity of disinfectant on cell cultures of epithelioid cells of lungs and large intestine.

Key words: disinfectant, cytotoxicity, cell cultures

In vitro models with the use of cell cultures and biochemical measurements at the cellular level lately have been applied more and more widespread in different areas of biotoxicological investigations and receive progressive recognition of regulatory organizations concerning replacement of biological tests on animals. At the same time, their application during disinfectants study is practically uninvestigated. In prospect similar studies should lead to the creation of optimal in vitro models, which would most adequately reflect the in vitro situation, as well as setting features of the correlation between structure and toxicity of the investigated materials.

In general, we investigated the basic toxicity which corresponds better with the acute toxicity on the level of microorganism.

There were developed many markers to asses the damage degree of cell cultures, but it is obvious that the violation of even one of the cellular functions will inevitably entail after a certain time the negative impact on the overall viability of the monolayer. This significantly facilitates the task of researchers as it provides at least on the first, assessment stage of in vitro toxicity study the application of limited set of cell cultures (most often standard, widespread lines) and a few simple indicators of cell viability [1, 4, 5].

All mentioned above is fully applied to disinfectants and their toxicity tests.

The purpose of research – determine the toxic effect of disinfectant which contains n-octadecyl dimethyl (3-trymetoksyselyl) propyl of ammonium chloride, benzylalkonium chloride and 10,0–15,0 % of isopropyl alcohol on the experimental cell culture in vitro.

Material and methods of research. Investigated disinfector contains n-octadecyldimethyl(3-trymetoksyselyl)propyl of ammonium chloride, benzylalkonium chloride and 10,0–15,0 % of isopropyl alcohol.

Cells A-549 (culture of human pulmonary adenocarcinoma epithelioid cells) and Colo-205 (epithelioid cells of large intestine adenocarcinoma) (received from the Bank of cell lines of the R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of the National Academy

of Sciences of Ukraine) were cultivated in full nutrient environment RPMI 1640 ("SIGMA", USA), which contained 4 mmol/dm^3 of L-glutamine, 10 % of fetal calf serum ("SIGMA", USA), 40 mkg/sm^3 of gentamicin in a humidified atmosphere with 5 % of CO_2 at 37 °C. Environment was replaced every two days. The subculture of cells was carried out with the help of versene solution during the formation of solid monolayer on the substrate by cells (4–5th day of growth).

The cells were planted in 96-well plates at a concentration of 1×10^5/sm^3 by 100 ml per well in full growth environment. Solution of investigated disinfectant in different concentrations was inserted after 24 hours. After 24 hours of cultivation the colorant MTT (3-[4,5-Dimetilthiazole-2-yl]-2,5-diphenylterazolium bromide; Thiazolyl blue)(SIGMA, USA) was inserted by 10 mcl/well in the concentration of 5 mg/sm^3 per 3 hours. After this the plate was centrifuged (1500 rounds per minute during 5 minutes), supernatant was removed and in the each well it was added 50 mcl of DMSO (dimethyl sulfoxide; SERVA) in order to dissolve the crystals of formazan. After 30 minutes of incubation at room temperature it was determined the optical density (OD) of wells' content at the wave length of 540 nm with the help of multi-well spectrometer Multiscan (Sweden). As a control were used empty wells and wells with cells, to which xenobiotics was not added, as well as control with the dissolvent – distilled, deionized water (2 %) in the environment nutrient for cells.

Painting by Sulphorhodamine B (Sulphorodamine) (SR test). After incubation with the investigated agent the cells were fixed by 50 % solution of trichloracetic acid (TCA) (final concentration – 10 %) during 1 hour at 4 °C and washed under running water. Cells fixed in the wells were painted by 0,4 % solution of Sulphorhodamine B (SIGMA, USA) during 30 minutes. On colorant removal, the wells were washed by 1 % solution of acetic acid and dissolved the colorant by adding 10 mM of Tris-base solution (holding 10 minutes on a shaker). The results of the investigation were registered with the help of multi-well spectrometer at the wave length of 540 nm.

Test with the neutral red colorant (NR test). After cultivation of cells with the investigated compound into each well was inserted an environment, which contained 2 % neutral red colorant and incubated during 3 hours in moistened atmosphere at 37 °C, then the supernatant was removed and the cells were washed with warm physiological solution. To fix the cells and elute the colorant with lysosome into each well was added the solution to dissolve the neutral red (1 % of glacial acetic acid, 50m % of ethanol and 49 % of distilled water). The results of the investigation were registered with the help of multi-well spectrometer at the wave length of 540 nm.

For the experiment was prepared a working solution of disinfectant. Distilled, deionized sterile water was used as the solvent. Further on, to obtain the necessary concentration which is inserted directly into the well, working solution was added into the environment nutrient for the cells in such a way that there were no more than 2% of the solvent.

Statistical processing of the results was carried out by the method of Prozorovskyi probit analysis, for which purpose the statistical processing program StatPlus was used.

Results. The culture A-549 was used to evaluate the toxicity and impact of disinfectant on the metabolism of the mammals' cells. The choice of this line is caused by the fact that the cells of pulmonary origin A-549 are highly sensitive to the quality of the components of nutrient environment and are commonly used to test its growth properties and toxicity [2].

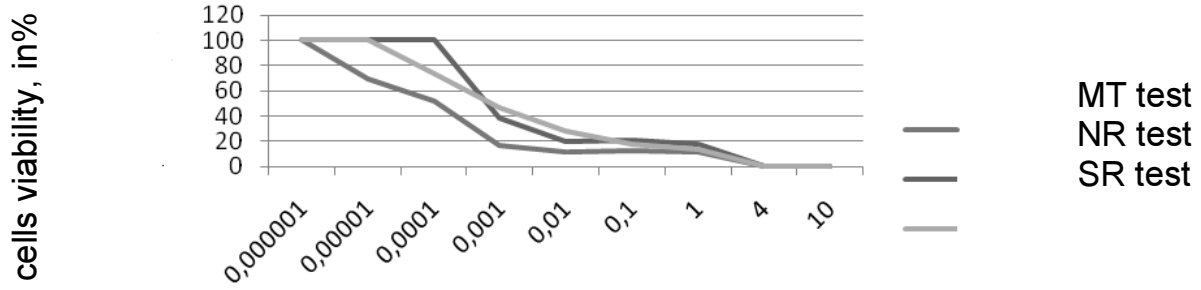
Selection of the length of exposure of the cells with xenobiotics was largely determined by the tasks of the test. That is, to determine the acute toxicity of disinfectors in vitro the duration of the experiment was 24 hours.

The results of the test showed that the preparation has a signified toxic effect and cause the dose-dependent response of cells in culture.

On comparing the indicators of cytotoxic impact of the investigated disinfectant according to the three different tests, there were received the following results (pictures 1, 2 and tables 1, 2).

1. Indicators comparison of the disinfectant cytotoxic impact on cells lines A-549 according to the results of three tests

Tests	Indicators of cells viability, in %						
MT test	51	16,2	12	14	12	0	0
NR test	100	40	22	24	19	0	0
SR test	77	45,8	26,7	19	16	0	0
Concentration, %	0,0001	0,001	0,01	0,1	1	4	10



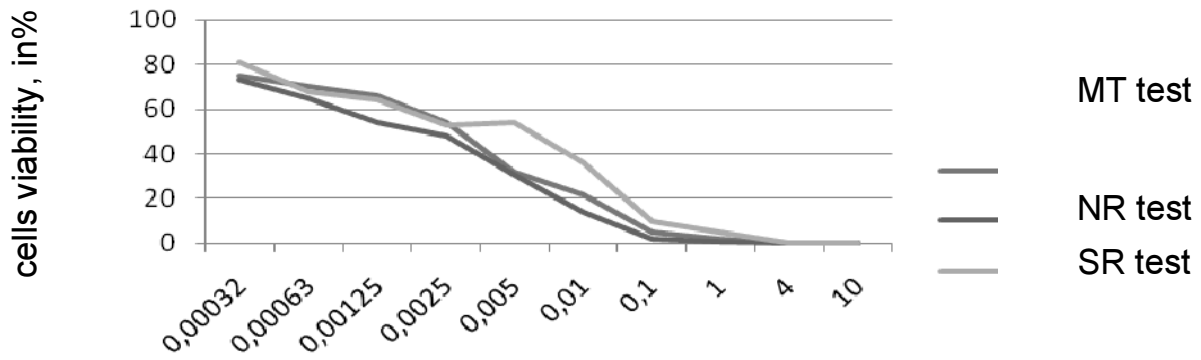
Picture 1 Concentration, %

2. Indicators comparison of the disinfectant cytotoxic impact on cells lines Colo-205 according to the results of three tests

Tests	Indicators of cells viability, in %						
MT test	56	33	24	7	2	0	0
NR test	50	30	16	2	1	0	0
SR test	54	55	38	11	4	0	0
Concentration, %	0,0025	0,005	0,01	0,1	1	4	10

Statistics presented above were matching for all three tests, confirming to the main indicators of disinfectant cytotoxic activity for organotypic cell cultures of lung and large intestine. Thus, for cell cultures A-549 and Colo-205 correlation coefficient indicators of cell viability in accordance with the percentage for the three preparation tests ranged from 0.9 to 0.99. A high correlation coefficient between statistics which have been found with the help

of various tests, proves that conducted investigations confirm general tendency with high reliability.



Picture 2 Concentration, %

Obtained indicators for two cell cultures were compared for the purpose of studying the possible effects of the preparation and specific definition of target organs.

On the base of these data we can conclude that the toxicity of disinfectant for the cultures of cells A-549 and Colo-205 is about the same. This can be explained by the fact that the cells are similar (epithelioid cells of lungs and large intestine). Toxic effects on experimental cell cultures is the result of the preparation's irritating action on the mucous membranes.

3. Comparison cytotoxic effect of the disinfectant on different cell cultures

Cell lines	Cells viability, %				
A-549	61	63	45	22	14
Colo-205	70	66	54	32	22
Concentration, %	0.00063	0,0013	0.0025	0.005	0.01

Because of the fact that the constituent parts of the disinfector are isopropyl alcohol, which is known for its catheresis, benzylalkonium chloride and 3-trymetoksyselylpropyl of ammonium chloride, which are surfactant and cationic detergents characterized by the fact that they can be integrated into the cell membrane, interact with membrane lipoproteins, thus damaging it and reducing the barrier functions of the cell, that is lead to its death [3].

Conclusions

1. It was established that the investigated disinfectant shows rather high toxicity towards the cell cultures (epithelioid cells of lungs and large intestine), in other words it is safe to predict irritating effect of the investigated disinfectant on the respiratory and digestive organs when applying.

2. In order to develop methods of using cultures of human cells for the toxicological evaluation disinfection preparations it is necessary to continue investigations with the use of other methods and test systems.

References

1. Еропкин М. Ю. Культуры клеток как модельная система исследования токсичности и скрининга цитопротекторных препаратов / М. Ю. Еропкин, Е. М. Еропкина. – СПб. : Морсар – 2003. – 239 с.
2. Трахтенберг І. М. Альтернативні методи і тест-системи. Лікарська токсикологія / І. М. Трахтенберг, В. М. Ковалеко, Н. В. Кокшарева, П. Г. Жмінько , В. Т. Чумак, О. П. Баула – К. : Авіцена, 2008 – 272 с.
3. Франклин Т. Биохимия антимикробного действия / Т. Франклин, Дж. Сноу. – М.: Мир, 1984. – 238 с.
4. Ekwall B. The basal cytotoxicity concept / B. Ekwall // Alternative Methods in Toxicology. V.11. / / Ed. By AMGoldberg, FM van Zutphen. – NY: Mary Ann Liebert, 1995. – P. 721–725.
5. Worth AP Alternative (non-animal) methods for chemical testing: current status and future prospects / A. Worth, M. Balls // ATLA. – 2002. – Vol. 30, Suppl. 1. – P. 1–125.

ДОСЛІДЖЕННЯ ТОКСИЧНОСТІ ДЕЗІНФЕКЦІЙНОГО ЗАСОБУ IN VITRO

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Досліджуваний дезінфекційний засіб містить у складі н-октодецилдиметил (3-триметоксиселіл) пропіламмоніюхлорид, бензалконію хлорид та ізопропіловий спирт.

Результати тестування показали, що засіб має виражену токсичну дію і викликає дозозалежну відповідь культури клітин. Токсичність дезінфекційного засобу для культур клітин А-549 та Colo-205 є приблизно однаковою. Це можна пояснити тим, що клітини є подібними у гістологічному відношенні (епітеліоподібні клітини легенів та товстого кишечника). Токсичний вплив на експериментальні культури клітин – це результат подразнюючої дії препарату на слизові оболонки.

Ключові слова: дезінфекційний засіб, цитотоксичність, культура клітин

ИССЛЕДОВАНИЕ ТОКСИЧНОСТИ ДЕЗИНФИЦИРУЮЩИХ СРЕДСТВ IN VITRO

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Исследуемое дезинфицирующее средство содержит в составе н-октодецилдиметил (3-триметоксиселил) пропиламмонияхлорид, бензалкония хлорид и изопропиловый спирт.

Результаты тестирования показали, что средство обладает выраженным токсическим действием и вызывает дозозависимый ответ культуры клеток. Токсичность дезинфицирующего средства для культур клеток А-549 и Colo-205 приблизительно одинаковая. Это можно объяснить тем, что клетки гистологически подобны (эпителиоидные клетки легких и толстого кишечника). Токсическое воздействие на

экспериментальные культуры клеток – это результат раздражающего действия препарата на слизистые оболочки.

Ключевые слова: дезинфекционное средство, цитотоксичность, культура клеток

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EUROPEAN CONCEPT OF ORGANIC DAIRY PRODUCTION IN THE SINGLE CHAIN "SOIL-PLANT-ANIMAL-CONSUMER"

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The work analyzes the European concept of organic dairy production in a single chain "soil-plant-animal-consumer".

The guarantee system of certification of organic production has been established to play the key role in organic production. All the stages of organic dairy production are carefully controlled by certification: the is analysed for contamination with pesticides, heavy metals and other toxic substances the conditions of animal maintenance and feeding are controlled as well as the conditions of milk production and storage, transportation and sale to the end consumer.

Key words: European concept, "organic" production, organic dairy products, soil, plant, animal, consumer

The organic production forms a complex system of management of agricultural enterprises and food production that combines best practices of environmental management, maintenance of high level of species diversity, protection of natural resources, application of high standards for the protection of animals and the production methods, that takes into account the fact, that certain consumers prefer products produced using natural substances and natural processes [4].

The purpose of research: to analyze the European concept of organic dairy production in a single chain "soil-plant-animal-consumer" and the state its implementation in Ukraine.

Material and methods of research. The analysis of the European concept of organic dairy production was performed by examining the EU