UDC 577.3:615.9:612.014

Some Aspects of the Polyhexamethyleneguanidine Salts Effect on Cell Cultures

M. S. Mandygra, A. V. Lysytsia

West Polissia Agriculture Institute NAAS
16/18, Kniazia Volodymyra Str., Rivne, Ukraine, 33028

e-mail: lysycya@ukr.net

Received on Feb 4, 2014

Aim. To investigate the effect of polyhexamethyleneguanidine (PHMG) to eukaryotic cell culture. **Methods**. The passaged bovine tracheal cells culture (TCC) and primary culture of chicken embryo fibroblasts (FCE) were used in the experiments. TCC and FCE monolayers were treated with aqueous solutions of PHMG chloride or succinate. The method of PHMG polycation adsorption to the cells' plasma membrane together with microscopy were applied. **Results**. The dependence of PHMG effect on the eukaryotic cells on the agent concentration, duration of exposure and the anion type has been fixed. The PHMG concentration of 10^{-5} per cent (0.1 μg/ml) never causes degradation of the previously formed cell monolayer, while the higher concentrations damage it. The conditions of the PHMG chloride and succinate's negative effect on cell proliferation and inhibition of monolayer formation were determined. The hypothesis that under certain conditions PHMG stimulates the proliferative activity of the cells has been confirmed. Stimulation may be associated with non-specific stress adaptation of cells. In this case, it is due to modifications of the cell membrane after PHMG adsorption to it. **Conclusions**. PHMG polycation binds with the membrane's phosphoglycerides firmly and irreversibly. A portion of the lipids are removed from participation in the normal cellular processes at that. At the same time, the synthesis of new lipids and membrane-bound enzymes is probably accelerated. The phospholipids' neogenesis acceleration can stimulate mitosis under certain conditions. The obtained results can be used in the biotechnologies.

Key words: polyhexamethyleneguanidine, cell culture, fibroblasts, proliferation, toxicity.

INTRODUCTION

Polymeric derivatives of guanidine are often used as the components of the disinfectants and antiseptics [1]. Polyhexamethyleneguanidine (PHMG) is the typical representative of this group and in the concentrations of 0.005−1.0 per cent possesses bactericidal, antiviral and fungicidal properties. Previously, PHMG biocidal properties and toxicity have been investigated [1, 2]. At the same time, widespread implementation of disinfectants on the basis of guanidine polymeric derivatives demands the more thorough analysis of all aspects of their effect on the biological systems.

Although, the precise mechanisms of the PHMG activity have not been finally explained yet, the substance is considered to make an effect on the membranes of cells predominantly [2−4]. At weak concentrations PHMG polycation, being adsorbed to cells' plasma membrane (CPM), change its "lipid pattern", permeability, phospholipids' lateral mobility, trans-membrane electric potential and so forth. Higher (biocidal) concentrations lead to CPM destruction and loss of cell. Since bacteria's CPM, as a rule, contains the higher percentage of acid lipids (phosphatidyl glycerol, phosphatidyl serine, cardiolipin and others) [5], their sensitivity to the PHMG agents must be higher than in eukaryotic cells, including the cells of the human-being [1].

At the same time, the disembodied data concerning the possible growth of PHMG enhancing properties began to appear. For example, treatment of wheat seeds with 0.1−1.0 per cent aqueous solutions of PHMG can not only protect these seeds from the defeat with molds, but also favorably affects their germination [6]. Furthermore, the study of the PHMG effect on the young individuals of the guppy fishes (*Poecilia reticulata*) showed that at concentrations of 10^{-7}−10^{-6} per cent the index of the specific growth rate increases through 7 days for more than 60 per cent in comparison with the control group [7]. The rates of increase (specific growth rate, fodder conversion efficiency, index of optimality) were somewhat reduced through 14 days, but still exceeded the control group. The authors consider that the

stimulating effect is connected to adaptation stress, and must be finally completed with the exhaustion phase (progressive toxicosis). It should be noted that in this case the agent constantly acted on the aquarium fishes – during entire time of experiment. It is also known that PHMG in concentration $1 \cdot 10^{-5}$ per cent can stimulate growth of the *Aspergillus niger* fungus, whereas $2 \cdot 10^{-5}$ per cent, on the contrary, suppress [8].

The PHMG inhibiting concentrations (IC_{50}) have been determined on the cell cultures of the human being [9]. For the cells of kidneys, skin and lungs at 24-h incubation they are within the limits of $0.5 \cdot 10^{-2}$–$1.3 \cdot 10^{-2}$ per cent, the kidney cells appeared to be more sensitive. Another guanidine polymeric derivative, polyhexamethylenebiguanidine (PHMB), favorably affects the keratocyte proliferation in the human body at concentrations $2 \cdot 10^{-5}$–$2 \cdot 10^{-4}$ per cent, whereas it already manifests toxic action at somewhat stronger doses [10]. Besides antiseptic effect, PHMB positively influences on the epithelization process during the treatment of burns [11]. Also, there are all reasons to assume the presence of the PHMB growth enhancing effect under specific conditions.

The aim of the present research is to detect the nature of PHMG salts influence on the eukaryotic cultures, and also to prove that the agent can make the stimulating effect on their proliferative activity.

MATERIALS AND METHODS

The primary culture of chicken embryo fibroblasts (FCE) and passaged bovine tracheal cells culture (TCC) were used as the objects of the current research. The cell cultures were prepared with application of the standard procedures in our modification [12]. Cell suspension in nutrient medium consisting of the mixture of medium 199 (45 per cent), minimal medium Igla or MEM (45 per cent) and livestock blood serum (10 per cent) was sown to the 96-well plastic plates, 0.1 ml per well. The TCC monolayer was formed using the suspension containing ≈ 800 thousand cells per 1 cm^3.

The influence of PHMG chloride (PHMG-chl) and PHMG di-basic succinate (PHMG-ds) (Termit, Ukraine), the molecular weight within the limits of 5–10 kDa, on the cells was studied. PHMG was dissolved in the sterile Hanks' balanced salt solution (pH 7.2–7.4) in volume ratio of constituents 1:9. For determining the inhibiting (toxic) and growth enhancing properties the PHMG-chl and PHMG-ds solutions were taken in the doses of 0.1–0.3 ml per well and in the concentrations from 10^{-9} per cent (0.01 ng/ml) to 10^{-2} per cent (0.1 mg/ml). On conversion to the molar concentration (throughout the monomer mass) it composes ≈ 10^{-10}–10^{-3} M. In the beginning of the test targeted on the study of the PHMG salts toxic properties, their solutions were single-time added to the already formed cells monolayer.

PHMG stimulating influence on cells' proliferative activity was studied by their suspension diluting (titration) in the growth medium in the wells from 1:2 to 1:128 for TCC and from 1:2 to 1:2,048 for FCE. After 24-h incubation of cells (t = 37 °C) the monolayer began to form in the wells. Depending on the cells initial titer, the degree of its formation was from 10 per cent to 50 per cent. The studied solutions of PHMG salts were added for 15 min in the dose of 0.1 ml to the forming monolayer of cells. After 15-min exposure the medium containing PHMG was carefully removed, then the cells were rinsed in the sterile buffered physiological solution (pH 7.0–7.2) and Hanks' solution, and the clean growth nutrient medium was added. In the control group, the Hanks' balanced salt solution was added to the cells for 15 min. The cell cultures in the microplates were placed into the incubator (t = 37 °C) and incubated during 4–6 days. The state of monolayer was estimated visually with the aid of the binocular microscope (×70), while the quantity of cells was calculated by means of Goryaev chamber after treatment with trypsin. Each test was reproduced into 3–5 repeats. The obtained data were statistically processed by common methods applying the Microsoft Excel software.

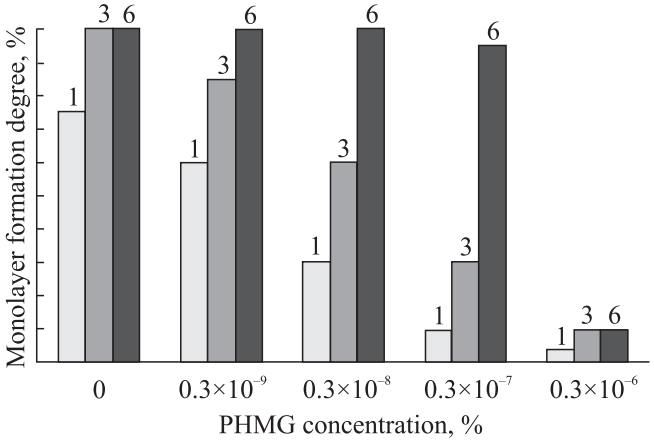
RESULTS AND DISCUSSION

The exploration of the PHMG-chl toxic action on the TCC formed monolayer fixed that the substance in concentrations starting from 10^{-3} per cent (10 μg/ml) and stronger, in doses of 0.1–0.3 ml per well causes the cells loss and monolayer destruction as early as during first 24 h. The concentration of 10^{-4} per cent, in doses of 0.1–0.3 ml per well leads to the loss approximately of half of cells during the first day and 100 per cent loss during following 1–2 days. The concentration of the agent in 10^{-5} per cent and in dose of 0.2 ml per well never causes noticeable changes of monolayer cells' state during the first day, while up to the sixth day of incubation about half of cells remained undamaged. The PHMGp-chl concentration of 10^{-5} per cent (or 0.1 μg/ml) in a dose of 0.1 ml per well is possible to be considered as nontoxic for TCC.

As for FCE, the summarized results of PHMG-chl toxic action exploration concerning this very culture in various concentrations in a dose of 0.1 ml per well are given in Table 1.

Table 1. PHMG-chl Toxic Action on the Chicken Embryo Fibroblasts in Various Concentrations

PHMG concentration, %	Fibroblasts monolayer state
10^{-3}	Monolayer destruction during the first 3–5 h
10^{-4}	Monolayer destruction during 1–3 days
10^{-5}	No changes during 5 days
10^{-6}	No changes during 5 days
10^{-7}	No changes during 5 days
Control group	No changes during 5 days



Dependence of the formation degree of the chicken embryo fibroblasts monolayer on the PHMG-chl concentration through 1, 3 and 6 days

Thus, the PHMG-chl concentrations of 10^{-5} per cent and weaker are possible to be considered as nontoxic for FCE as well. At least, they no longer exert a substantial influence on the formed monolayer of cells. In the course of tests it was also reported that if PHMG-chl is added to the monolayer for the short period (10–20 min) even in comparatively high concentrations (10^{-3}–10^{-2} per cent), and after the agent removal the monolayer is rinsed in normal saline, then no cells loss is observed. Short-term PHMG exposure in "biocidal" concentrations never damages the monolayer.

The research of the PHMG salts' effect on the cells monolayer forming rate proved the following: the adding of PHMG-chl in concentrations of 10^{-5}–10^{-6} per cent in the very beginning of the FCE monolayer formation leads to the complete inhibition of proliferative activity; whereas the concentrations of $0.3 \cdot 10^{-7}$–$0.3 \cdot 10^{-9}$ per cent at a constant effect partially inhibit the proliferative activity of the fibroblasts and also slow down the process of the cells monolayer formation (Figure). It appears that the FCE culture is considerably more sensitive to the PHMG action, than the cells of *Escherichia coli* to its analogue – PHMB [13].

Entirely different picture is observed at the short-term influence of the PHMG salts on the cells. As far as knowledge goes, in the titers from 1:2 to 1:64 the FCE monolayer is formed practically completely after the first day of incubation, the agents were added into the wells with the initial dilution of cells from 1:128 to 1:2,028. This made it possible to determine the dynamics of the monolayer forming process after their short exposure with the PHMG salts. The summarized results are given in Table 2.

Table 2. Dynamics of the Chicken Embryo Fibroblasts' Monolayer Shaping After 15-min Exposure With PHMG-chl and PHMG-ds

Substance concentration, %	Degree of the chicken embryo fibroblasts' monolayer formation, %														
	After 24 h (in the moment of the substance adding)					After 2 days					After 3 days				
	Cell culture titers														
	1:128	1:256	1:512	1:1,024	1:2,028	1:128	1:256	1:512	1:1,024	1:2,028	1:128	1:256	1:512	1:1,024	1:2,028
PHMG-chl															
10^{-5}	50	30	20	10	10	50	30	20	10	0	40	20	10	0	0
10^{-6}	50	30	20	10	10	60	30	30	10	10	40	70	40	30	20
10^{-7}	50	30	20	10	10	100	80	70	50	40	100	100	100	100	80
10^{-8}	50	30	20	10	10	100	80	80	50	40	100	100	100	100	100
10^{-9}	50	30	20	10	10	80	50	50	20	20	100	100	90	40	30
PHMG-ds															
10^{-5}	50	30	20	10	10	70	40	30	20	10	80	60	40	30	20
10^{-6}	50	30	20	10	10	80	50	50	20	20	100	100	80	40	30
10^{-7}	50	30	20	10	10	100	100	100	80	50	100	100	100	100	80
10^{-8}	50	30	20	10	10	100	100	100	90	80	100	100	100	100	100
10^{-9}	50	30	20	10	10	90	60	60	40	30	100	100	100	80	70
Control group	50	30	20	10	10	80	50	50	20	20	100	100	80	40	30

Note. $n = 3$, $P > 0.95$.

In the following concentrations: PHMG-chl – 10^{-6}–10^{-5} per cent, and PHMG-ds − 10^{-5} per cent inhibit the process of cells' monolayer shaping. PHMG-chl stimulates the fibroblasts' proliferative activity in concentrations of 10^{-8}–10^{-7} per cent, while PHMGsd – 10^{-9}–10^{-7} per cent. Why the same concentrations of PHMG-chl do slow down an increase in the monolayer at a constant presence in the growth medium (Figure)? Probably, it depends upon the time, necessary for the more complete PHMG polycation adsorption to CPM. In that case the stimulating concentrations of the substance could be 2−3 next-weaker orders providing its permanent effect. However, in such weak concentrations the substance remains in solutions for a short period of time. Therefore the stress appearing under the single-time short-term effect of the PHMG salts on the cells can be significant.

The TCC cells in 1:2 solution practically completely form the monolayer during the first 24 h of incubation. Therefore, the preparations were added to the wells containing the initial titer of cells from 1:4 to 1:128 in the second day. As early as 24 h the essential differences in the rate of the monolayer growth depending on the PHMG salt concentrations became noticeable. The summarized results are given in Table 3.

Thus, PHMG-chl in concentration of 10^{-7} per cent, and PHMG-ds – 10^{-8} per cent strengthen the proliferative activity of TCC. Although, it is important to note that the latter manifests the stimulating effect within the sufficiently wide range of concentrations – from 10^{-8} to 10^{-6} per cent. In concentration of 10^{-5} per cent PHMG-chl slows down the process of the monolayer formation.

One can see, the cells of the fibroblasts' primary culture form the monolayer considerably faster than the transformed trachea cells having been previously undergone more than thousand passages. Though, the stimulating effect of the PHMG salts manifests itself on both types of cells. At that, the fibroblasts are at least ten-times more sensitive.

The mechanism of the PHMG growth enhancing effect is not entirely clear. It can be caused, as in the case with the *P. reticulata* fishes [7], by non-specific (stress) adaptation of cells. Apparently, the changes, proceeding in CPM after polycation adsorption to it are the determining factor here. It is known that the surface and transmembrane potentials of a cell, the membrane permeability for both the water and low-molecular ions, also the mobility of phospholipids are changed with the adsorption, whereas the functioning of membrane-related membrane-bound enzymes of PHMG can inhibit the activity of such ferments, as catalase and superoxide dismutase. On some microorganisms, for example, *Candida tropicalis*, PHMG makes the similar effect in concentration of 10^{-3} per cent, while on *Salmonella typhimurium* – 10^{-5} per cent [14]. Since these ferments makes an antioxidant effect, the process of changes in the CPM properties (caused by PHMG

Table 3. Dynamics of the Bovine Tracheal Cells' Monolayer Shaping After 15-min Exposure With PHMG-chl and PHMG-ds

Substance concentration, %	Degree of the bovine tracheal cells' monolayer formation, %																	
	After 2 days						After 4 days						After 6 days					
	Cell culture titers																	
	1:4	1:8	1:16	1:32	1:64	1:128	1:4	1:8	1:16	1:32	1:64	1:128	1:4	1:8	1:16	1:32	1:64	1:128
PHMG-chl																		
10^{-5}	70	60	30	10	0	0	90	70	40	30	0	0	100	80	40	40	0	0
10^{-6}	70	70	50	10	10	0	100	80	70	50	50	20	100	100	80	60	60	40
10^{-7}	90	70	70	70	30	30	100	100	90	80	70	50	100	100	100	100	100	100
10^{-8}	90	70	70	50	20	10	100	90	90	70	50	40	100	100	90	90	80	70
10^{-9}	70	70	60	50	10	10	100	80	80	60	50	30	100	100	80	70	60	50
PHMG-ds																		
10^{-5}	70	50	30	40	10	0	100	70	60	50	50	30	100	100	80	70	50	40
10^{-6}	90	50	50	50	20	0	100	80	70	70	60	40	100	100	90	80	70	60
10^{-7}	90	50	70	50	20	30	100	100	90	80	70	40	100	100	100	90	80	70
10^{-8}	90	100	70	100	30	10	100	100	100	100	80	50	100	100	100	100	100	100
10^{-9}	70	70	60	30	20	10	100	80	70	70	50	30	100	100	80	80	70	50
Control group	75	70	40	30	10	10	100	75	70	60	50	30	100	100	80	70	60	50

Note. $n = 3$, $P > 0.95$.

adsorption) is aggravated with hydrogen peroxide and superoxide concentration increase. Aggressive H_2O_2 and $:O_2^-$ can interact with the remainders of the unsaturated fatty acids of diaphragm lipids and also strengthen the damaged membranes. At the short-term effect of the PHMG weak (stimulating) concentrations the inhibition of these ferments is reversible, whereas the cell's compensating mechanisms initiate new lipogenesis and synthesis of new ferments. It is known that PHMB can affect the expression of some genes in *E. coli*, the synthesis of ferments, and so forth [13]. PHMG included into the medicine Akacid can reduce the S-phase of the cancerous cells' cycle in the prostate, affect the D1, E, p27 cyclines and cycline-related kinases expression [15].

It should also be noted that PHMG polycation is quite steadily connected to phosphoglycerides, not necessarily with the acid ones only. For example, it is rapidly adsorbed, and so, changes the ionic permeability of a comparatively electrically neutral model bilayer lipid membrane from the phosphatidylcholine – cholesterol complex (volumetric ratio 2: 1) [16]. The removal of the part of the CPM phospholipids from active participation in the cell processes forces the initiation of the new lipids synthesis process. Probably, that particular influences on shortening of the cell cycle duration. Thus, at the PHMG stimulating effect on *A. niger* cell's adaptive mechanisms also start the synthesis of new polar lipids [8]. In this case lipids rich in saturated fatty acids are formed, hereupon CPM becomes more durable. Under certain conditions, the acceleration of the new phospholipids' synthesis stimulates mitosis. The obtained results can be extrapolated to the cells of the human being's tissues as well.

CONCLUSIONS

Thus, the performed tests fixed that the influence of polyhexamethyleneguanidine on the cell culture depends on the agent concentration, duration of exposure and the anion type of the salts. PHMG concentrations of $\leq 10^{-5}$ per cent (0.1 μg/ml) are possible to be considered as nontoxic for the already formed FCE or TCC monolayer.

The PHMG-chl concentrations of $\geq 10^{-6}$–10^{-5} per cent completely suppress the cells' proliferative activity in the growth medium, while concentrations $0.3 \cdot (10^{-9}$–$10^{-7})$ per cent partially inhibit fibroblast mitosis and slow down the rate of monolayer formation. Under preliminary 15-min exposure of fibroblasts with PHMG-chl at concentrations of 10^{-6}–10^{-5} per cent, or PHMG-ds − 10^{-5} per cent monolayer formation also slows down. On the contrary, PHMG-chl concentrations of 10^{-8}–10^{-7} per cent and PHMG-ds concentrations of 10^{-9}–10^{-7} per cent stimulate proliferative activity of fibroblasts. The short-term (during 15 min) PHMG-chl exposure on the trachea cells at concentration of 10^{-5} per cent inhibits mitosis, while at 10^{-7} per cent – stimulates. PHMG-ds manifests the stimulating effect on TCC within the concentration range of 10^{-8}–10^{-6} per cent.

The growth enhancing effect of the PHMG salts' weak concentrations is apparently connected to changes in the properties of the cell cytoplasmic membrane after the polycation adsorption to it and also expression of the genes corresponding to the synthesis of phospholipids.

ACKNOWLEDGEMENTS

The researchers are grateful to P. Yu. Kryvoshyia (West Polissia Agriculture Institute NAAS) for help in tests performance.

Деякі особливості дії солей полігексаметиленгуанідину на культури клітин

М. С. Мандигра, А. В. Лисиця

e-mail: lysycya@ukr.net

Інститут сільського господарства Західного Полісся НААН України

Вул. Князя Володимира, 16/18, Рівне, Україна, 33028

Мета. Дослідити дію полігексаметиленгуанідину (ПГМГ) на культури клітин евкаріотів. **Методи**. В експериментах використано перещеплювану культуру клітин трахеї теляти (КТТ) та первинну культуру фібробластів курячого ембріона (ФКЕ). Моношари клітин КТТ і ФКЕ обробляли водними розчинами солей хлориду ПГМГ або сукцинату ПГМГ. Застосовано метод адсорбції молекул полікатіону ПГМГ на плазматичну мембрану клітин, а також мікроскопія. **Результати**. Показано, що вплив ПГМГ на клітини евкаріотів залежить від концентрації препарату, тривалості експозиції і типу аніона. Встановлено, що концентрація ПГМГ 10^{-5} % (0,1 мкг/мл) не викликає деструкції вже сформованого моношару клітин, більш високі концентрації його пошкоджують. З'ясовано, за яких умов хлорид і сукцинат ПГМГ негативно впливають на проліферацію клітин і гальмують процес утворення моношару. Підтверджено припущення про те, що за певних умов ПГМГ стимулює проліферативну активність клітин. Стимуляція може бути пов'язана з неспецифічною стресовою адаптацією клітин. У цьому разі вона обумовлена змінами в плазматичній мембрані клітин після адсорбції на неї ПГМГ. **Висновки**. Полікатіон ПГМГ міцно і незворотно зв'язується з фосфогліцеринами мембрани. Частина ліпідів при цьому усувається від участі в нормальних клітинних процесах. Ймовірно, при цьому відбувається прискорення синтезу нових ліпідів і пов'язаних з мембраною ферментів. Прискорений неогенез фосфоліпідів за певних умов може стимулювати мітоз. Отримані результати можуть бути використані в біотехнології.

Ключові слова: полігексаметиленгуанідин, культура клітин, фібробласти, проліферація, токсичність.

Некоторые особенности действия солей полигексаметиленгуанидина на культуры клеток

Н. С. Мандыгра, А. В. Лисица

e-mail: lysycya@ukr.net

Институт сельского хозяйства Западного Полесья НААН Украины
Ул. Князя Владимира, 16/18, Ровно, Украина, 33028

Цель. Исследовать действие полигексаметиленгуанидина (ПГМГ) на культуры клеток эукариотов. **Методы**. В опытах использовали перевиваемую культуру клеток трахеи теленка (КТТ) и первичную культуру фибробластов куриного эмбриона (ФКЭ). Монослои клеток КТТ и ФКЭ обрабатывали водными растворами солей хлорида ПГМГ или сукцината ПГМГ. Применен метод адсорбции молекул поликатиона ПГМГ на плазматическую мембрану клеток, а также микроскопия. **Результаты**. Показано, что влияние ПГМГ на клетки эукариотов зависит от концентрации препарата, продолжительности экспозиции и типа аниона. Установлено, что концентрация ПГМГ 10^{-5} % (0,1 мкг/мл) не вызывает деструкции уже сформированного монослоя клеток, более высокие концентрации его повреждают. Выяснено, при каких условиях хлорид и сукцинат ПГМГ отрицательно действуют на пролиферацию клеток и тормозят процесс образования монослоя. Подтверждено предположение о том, что при определенных условиях ПГМГ стимулирует пролиферативную активность клеток. Стимуляция может быть связана с неспецифической стрессовой адаптацией клеток. В этом случае она обусловлена модификациями плазматической мембраны клеток после адсорбции на нее ПГМГ. **Выводы**. Поликатион ПГМГ прочно и необратимо связывается с фосфоглицеридами мембраны. Часть липидов при этом устраняется от участия в нормальных клеточных процессах. Вероятно, при этом ускоряется синтез новых липидов и связанных с мембраной ферментов. Ускоренный неогенез фосфолипидов при определенных условиях может стимулировать митоз. Полученные результаты могут быть использованы в биотехнологии.

Ключевые слова: полигексаметиленгуанидин, культура клеток, фибробласты, пролиферация, токсичность.

REFERENCES

1. Vointseva I., Gembitskyi P. Polyguanidines – disinfecting agents and multifunctional additives to composite materials. – Moscow : LKM, 2009. – 304 p.
2. Gilbert P., Moore L. E. Cationic antiseptics: diversity of action under a common epithet // J. Appl. Microbiol. – 2005. – **99**, N 4. – P. 703−715.
3. Lysytsia A. Mechanisms of bactericidal action of polyhexamethyleneguanidine // Scientific reports of the National University of Life and Environmental Sciences of Ukraine. – 2011. – **25**, N 3. e. publ.
4. Zhou Z., Zheng A., Zhong J. Interactions of biocidal guanidine hydrochloride polymer analogs with model membranes: a comparative biophysical study // Acta Biochim. Biophys. Sin. – 2011. – **43**, N 9. – P. 729−737.
5. Gabriel G. J., Som A., Madkour A. E., Eren T., Tew G. N. Infectious disease: connecting innate immunity to biocidal polymers // Mater. Sci. Eng. R Rep. – 2007. –**57**, N 1−6. – P. 28–64.
6. Gembitskyi P., Efimov K. The wheat protection from biodegradation // Bread products. – 1999. – N 11. – P. 26−27.
7. Ihnatiuk A. A., Havrikova V. S., Chumak V. L. The influence of polyhexamethylenegyanidine on bioproduction parameters of the young fish // Research Bulletin of NTUU «KPI». − 2010. – N 3. – P. 16−20.
8. Kuznetsova L. S. The mechanism of polyguanidine disinfectants action // Meat industry – 2001. – N 4. – P. 52.
9. Adamenko L. V. The toxicity of disinfectants in human cell culture in vitro // Animal biology. – 2010. – **12**, N 2.
10. Wiegand C., Abel M., Kramer A., Muller G., Ruth P., Hipler U.-C. Stimulation of proliferation and biocompatibility of polihexanide // GMS Krankenhaushyg. Interdiszip. – 2007. – **2**, N 2: Doc43
11. Daeschlein G., Assadian O., Bruck J. C., Meinl C., Kramer A., Koch S. Feasibility and clinical applicability of polihexanide for treatment of second-degree burn wounds // Skin. Pharmacol. Physiol. – 2007. – **20**, N 6. – P. 292−296.
12. Lysytsia A., Kryvoshyia P., Mandyhra M. Stepaniak I., Kot L., Zhygaliuk S., Andruschuk I., Dmitriev I. The complex action of polymeric guanidine derivatives to cell culture // Bull. of veterinary biotechnology. – 2013. – N 22. – P. 328−337.
13. Allen M. J., White G. F., Morby A. P. The response of *Escherichia coli* to exposure to the biocide polyhexamethylene biguanide // Microbiology. – 2006. – **152**, Pt 4. – P. 989–1000.
14. Marievskyi V. F., Danilenko I. I., Baranova A. I., Strikalenko T. V., Nizhnyk T. Y. Increasing of epidemic and chemical safety of water is a problem of the new reagent choice for disinfection // Preventive Medicine. – 2009. – **7**, N 3. – P. 53−62.
15. Neuwirt H., Muller H., Cavarretta I. T., Tiefenthaler M., Bartsch G., Konwalinka G., Culig Z. Akacid-medical-formulation, a novel biocidal oligoguanidine with antitumor activity reduces S-phase in prostate cancer cell lines through the Erk 1/2 mitogen-activated protein kinase pathway // Int. J. Oncol. – 2006. – **29**, N 2. – P. 503−512.
16. Lysytsia A. V., Kryvoshyia P. Yu., Shaturskyi O. Ya. Influence of polyhexamethyleneguanidine hydrochloride on the chicken embryos fibroblasts plasmatic membrane and artificial bylayer lipid membrane // Biotechnology. – 2010. – **3**, N 2. – P. 56−61.