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Original Research Article

HORMESIS EFFECT AND THE INFLUENCE OF ULTRA-LOW GLYCOSIDES DOSES ON THE BONE MARROW CELLS PROLIFERATIVE ACTIVITY IN CULTURE

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Abstract: : The influence of two glycosides (K 333 and K 322) at concentrations ranging from 10^{-4} M to 10^{-18} M on the changes in the number of rats bone marrow cells in primary culture, the preservation of the native state of the plasma membrane of cells and the change in morphophenotypic characteristics of these cells starting on the 1st ending on the 4th day of cultivation was studied. It is shown that the culture of bone marrow cells may be a good model to study the effect of xenobiotics in ultra-low doses.

It was found that the glycosides under study can help to increase the number of cells in culture on the first day, do not influence their content and reduce their number to the 4th day. A time dependence of the biological response on the glycosides action was presented.

A non-linear dosage-dependent bimodal effect of the bone marrow cells content changing at low and ultra-low doses of glycosides was revealed. Two similar in structure glycosides caused different by "power" responses and the various in the time of their appearance. Dose of 10^{-15} M K333 caused a greater effect of proliferation inhibiting, than the greater dose 10^{-13} M of the substance and for K 322 10^{-13} M dose caused greater inhibitory effect than the dose 10^{-8} M and 10^{-6} M. The identified bimodal effects of ultra-low doses of glycosides in culture of bone marrow cells may be due to the presence of several levels of interaction with the cellular components of xenobiotics: hydrated, molecular, supramolecular, and possibly field (electromagnetic fields). It is suggested that the biological response is the result of integrative interactions of different hierarchical levels of xenobiotics interactions with cellular components.

Keywords: Low and ultra-low doses, glycosides K 333 and K 322, xenobiotics, bone marrow cells.

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Introduction: The effect of stimulation of the biological response and the formation of toxic compounds in the subsequent resistance in these organisms to high doses of the same toxicant was firstly described in 1880 in the Hugo Schulz and Rudolf Arndt yeasts. Later, this phenomenon was called a hormesis effect.

Somewhat later, prof. V. Shrader in 1896 showed that the low-intensity of X-ray irradiation of guinea pigs provided them resistance to subsequent infection of diphtheria. Only the last 20 years the intensive study of possible mechanisms of hormesis effect was started.

It has been shown that the low doses of a variety of toxic compounds are resulting the increase of hundreds of genes expression, including genes of the stress response, repair systems and metabolic activation [1-4].

The various effects of low and ultra-low doses have been described in the mid-80s of the XX century [5, 6]. Mechanisms of this phenomenon are unknown and couldn't be explained by the standpoint of classical enzyme kinetics [8]. Most experts explain the rejection of the biological effects of ultra-low doses due to this phenomenon. Only a few molecules can get into a cell after the introduction into the organism 10^{-12}-10^{-13} M of a substance. Therefore, is the effect induced by individual molecules or do molecules have a direct impact on the activation processes of biological systems? This question is not yet resolved.

However, now we can talk about some of the general laws of the ultra-low doses action [9, 10]:

1 - they are characterized by: a non-monotonic, multimodal relationship between dose and effect. There is a "dead zone" in the dose dependence.

2 - degree and the "sign" of a biological effect depend on the initial characteristics of the object.

3 - action of low doses has an effect on the sensitivity of biological objects to a variety of other factors - a good analogy with the hormesis effect.

4 - small doses induce the expression of a variety of kinetic paradoxes.

The study of expression and mechanisms of action of low and ultra-low doses regularities is of a great fundamental importance as it allows: a) to complete fundamentally the existing paradigm in pharmacology and biology; b) to avoid the toxic effects and long-term consequences of the xenobiotics high doses; c) to explain the mechanism of hormesis effect and to evaluate its role in the Adaptatiology and Gerontology; d) to prove homeopathy and its application in a scientific way.

The study of the mechanism of action of low and ultra-low doses is an extremely complex task and it's successful solution would depend on the objects and methods. Currently, the effects of low doses are described for the enzyme systems *in vitro*, cell cultures, plant and animal facilities [11, 12]. However, the greatest interest in this respect is the cell culture, as it allows to study rapidly the effect of small doses over a wide range of concentrations and fix their "direct" effect on the molecular and morpho-functional levels, excluding the placebo effect and the effect of self-compensatory factors at the organism level.

One can get the "strengthening" effect of low doses relatively easily at the cellular level, as opposed to the whole organism to fix their "direct" expression at the molecular and morpho-functional levels.

According to this the study of low and ultra-low doses was carried out on the culture of bone marrow cells of 3 months old rats. Bone marrow cells are presented as the stem, and various types of differentiated cells [13]. This allows to determine the influence of low doses on their proliferative potential, viability and cell death in culture. It is known that the glycosides (synthetic glycosides) have a broad spectrum of biological activities [14]. It was shown previously that glucosides K 333 and K 322, that were synthesized in our laboratory, have a pronounced biological effect at the cellular level [15].

In this context the impact of two glucosides (333 K and 322 K) at concentrations ranging from $1x10^{-4}$ till $1x10^{-18}$ M on the change of the cells number in primary culture of rat bone marrow from the cultivation days 1 to 4, native plasma membranes of cells and in the same time morphophenotyic cells characteristics in culture.

Materials and Methods

Materials

The investigations were carried out in bone marrow cells of male 3 months old Wistar rats weighing 150-200 g, maintained under the standard vivarium conditions that matched "Sanitary Rules on the device, equipment and maintenance of the experimental-biological clinics". Totally 20 animals were used.

K 333 and K 322 glycosides were synthesized in our laboratory [15].

Methods

Preparation of bone marrow cells

Bone marrow cells were isolated from the diaphysis, after removing the epiphyses, of femoral and tibial bones of animals by the method [16]. Diaphysis were washed with the sodium phosphate buffer (pH = 7.4) chilled to 4 ° C under pressure. The resulting cell suspension with medullary tissue fragments was disaggregated mechanically by the resuspension. After that the suspension was filtered through a nylon filter with a pore diameter of 100 microns. The cell suspension was washed with sodium phosphate buffer by centrifugation for 10 minutes at 1500 g. Erythrocytes were removed from the cell suspension by a single treatment with a solution containing 154 mM of ammonium chloride, 10 mM of sodium bicarbonate, 0.082mM of ethylenediaminetetraacetate for 5 minutes at room temperature, then the medullary cell suspension was washed twice with cooled sodium phosphate buffer. The resulting suspension containing a single viable cell in an amount of 2-4 x 10^6 cells mL^{-1}.

The administration of K 322 and K 333 glucosides in the bone marrow cell culture

A suspension of cells derived from a single bone, in such a way that the initial cell concentration was always 2.0-2.3 million mL^{-1} was prepared, the suspension was transferred into a culture plate (35 cm^2) and cultures of glucosides under study by 20 µL at K 322 concentrations of 10^{-4}; 10^{-6}; 10^{-8}; 10^{-13}; 10^{-15} M and at K 333 concentrations of 10^{-4}; 10^{-8}; 10^{-13}; 10^{-15}; 10^{-18} M, and the sum of K322 + K333 glucosides at concentrations of 10^{-13} for each were added. 20 µL of 0.9% saline solution were added into the test samples.

Cultivation of bone marrow cells

In all experiments, the initial concentration of cells in the bone marrow was 2.3 ± 0,2x10^6 cells mL^{-1} of the animals. The incubation of the cells was carried out at 37 ° C in an atmosphere containing 5 % of CO_2 using a culture medium prepared on the basis of 199 medium supplemented with 20% inactivated fetal calf serum, 10 mM of HEPES-buffer, 2 mM of glutamine and antibiotics (100 U mL^{-1} of penicillin sodium salt, and 100 µg mL of gentamycin). Cultivation was carried out during 4 days without changing of the culture medium.

The evaluation of proliferative activity and native state of the plasma membrane of cells was carried out every day by counting the number of cells in a fixed field of view of the Goryaev's chamber.

The native state of plasma membrane of cells in the culture was determined using the Trypan blue method [17], the number of stained cells was counted in the Goryaev's chamber, assessing at least 500 cells, and data were presented as a percentage of the total number of cells. The experiment was repeated at least 5 times for every variant.

Morphological characteristic of cytological bone marrow preparations was carried out immediately after the suspension was obtained and on the 4th cultivation day. Thus the samples swabs were fixed for 5 min in methanol and transferred onto 25-40 minutes in the working solution of the prepared Romanovsky-Gimza dye (1:4). Thereafter, the samples were washed with distilled water and air dried. The evaluation of morphophenotypic characteristics of bone marrow cells of animals were carried out at the analysis of photomicrographs of randomly selected fields of view (but not less than 5-8), which was obtained using a phase-contrast light microscope (type microscope).

Statistical data processing

The average, standard deviation, standard error of the average, the sample size were used as characteristics of the obtained samples.

Statistical significance of the differences between two data groups were assessed using the nonparametric Mann-Whitney test. Statistical analysis was performed using the program "Excel" and "Statistica 7.0" for WinXP. The differences between the control variant and experiment data were considered significant if $p < 0.05$.

Results

K 333 glycosides influence on the growth rate and the preservation of the native state of the bone marrow cells plasma membrane in the primary culture

The KM cell suspension isolated from femurs of 3 months male rats were prepared in medium 199 with 20% of calf serum, so that 1 mL always contained 2.0 - 2.3 mL of cells. This number was taken as the initial cell concentration. The rate of culture growth obtained in the control animals for the 24 hours of cultivation was negligible, and the number of cells increased only by 25% compared with the initial concentration of cells (Fig.1). Two days later cell number has increased by 37% compared with the initial concentration at day 4 and their content was increased almost in 2 times (Figure 1).

So, the cell population capable to proliferate is presented in the isolated KM cells suspension in control animals. Such a culture can be used to estimate the proliferative activity of glucosides without changing the medium for 4 days. As it is known, the KM cell population is highly heterogeneous and the isolated bone marrow cells are represented by different cell types (Fig. 1).

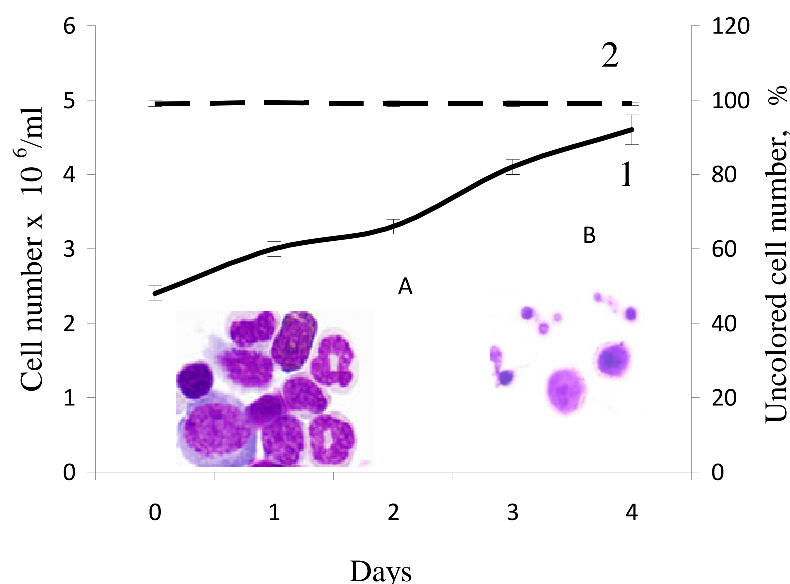

Fig. 1. The number of cells in the bone marrow in primary culture from the 1st to the 4th day of cultivation (1) and the the percentage of cells with native plasma membrane by Trypan blue exclusion method (2). The photo shows cells morphotypes in early culture (A) and on the 4th day (B).

It was found that the large amounts of myeloblasts, metamieloblasty, stab neutrophils, erythroblasts and other cell types were present in the resulting cell suspension on the first cultivation day (Figure 1). It is known that differentiated cells do not proliferate in culture but primarily stem cellare capable to proliferation [19]. The destiny of non-dividing cells in culture can be twofold. They may be subjected to degradation (necrosis and apoptosis), or maintained unchanged in culture for 4 days, i. e. to form a pool of surviving cells in primary culture.

The determination of morphological heterogeneity culture on the 4th day of the culture growth showed the presence of only

lymphocytes and erythrocyte precursors (Fig. 1).

Therefore, during the cultivation a "selection" and likely cell death of various degrees of differentiation during 1 - 4 days proceed.

Determination of plasmatic membranes in the native state in Trypan blue exclusion, which may indicate a failure on the necrosis type, showed that 99% of isolated cells in the control variant had a native membrane and it remained during 4 cultivation days (Table. 1, control variant).

Table 1:The number of bone marrow cells as a percentage of the native plasma membrane from the 1st to the 4th day of cultivation after the administration of the K 333 glucosides in different concentrations into the medium

Cultivation conditions	Cultivation time, days				
	0	1	2	3	4
Control	99±0.8	99.3±0.5	99±0.5	99±0.8	99±0.5
$1x10^{-4}$ M	98±2.0	99±1.0	95±4.0	94±0.05	60±0.5*
$1x10^{-8}$ M	98.7±0.5	99±0.8	99±0.8	99.6±0.5	99±0.8

It can be assumed that the destruction of the differentiated cells in the control cultures was carried out not by necrotic type, but as a result of apoptosis. Two-fold increase in cell number by the cultivation day 4 on the background of some part of cells elimination by apoptosis indicates that the proliferative capacity of bone marrow cells under these conditions is high enough.

The K 333 glucoside administration to the KM cells suspension in a concentration $1x10^{-4}$ M in 2.0 - 2.3 million cells per mL was accompanied by an increase in their number in the first 24 hours by 39% compared to the initial amount, and was even slightly higher than the control (Fig. 2). However, on the 2nd cultivation day the number decreased by 40% as compared to control and by 46% in comparison to the first day. Further, the number of cells in culture was reduced by 25 and 58% compared to the original amount on the 3rd and the 4th days, respectively (Fig. 2, curve 1).

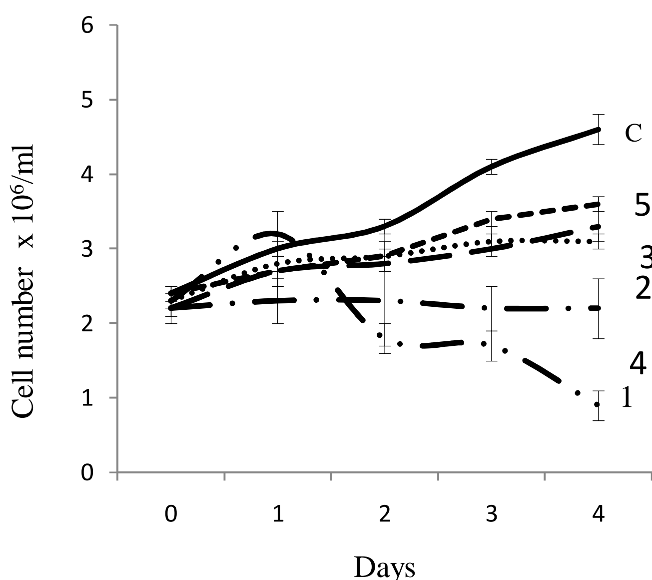

Fig. 2. The number of bone marrow cells in culture from the cultivation days 1 to 4 at K 333 administration at the beginning of cultivation at different concentrations: C – control; 10^{-4} (1); 10^{-8} (2); 10^{-13} (3); 10^{-15} (4); 10^{-18} M (5).

Therefore, K 333 glucoside at the concentration of $1x10^{-4}$ M has an inhibitory effect on the proliferative activity of KM cells in the culture. The inhibitory activity was expressed temporary; on the 1st day the culture growth stimulation occurred, the inhibition of the growth by the 2nd and the process was strengthened on the 4th day.

Determination of the number of KM cells with native plasma membrane in the culture showed that on the 4th day their number was 60% after the administration of K 322 at the concentration of 1.10^{-4} M (Table 1). At the same time, the decrease in the cell number “preceded” the necrotic process of native membrane disorders, which can be explained by the destruction of the cells as the type of apoptosis and necrosis. Thus, on the 3rd day, the number of cells was decreased by 25% and the number of cells with disrupted membranes at the same time was increased by not more than 5% (Table 1).

The addition of K 333 glucoside to the cell culture at lower concentrations $1x10^{-8}$ M and M $1x10^{-13}$ slowed the rate of growth of culture, much less than $1x10^{-4}$ M on the days 2-4 of cultivation.

Thus, if the number of cells in the initial suspension was 2.3 10^{6} mL^{-1}, it was 2.9 and 2.8 to the end of the day 1, and their number remained unchanged on the 2nd and the 3rd days, and it even slightly increased up to 3.1 to 3.5 10^{6} mL^{-1} on the 4th day, compared to the initial concentration, for $1x10^{-8}$ M and $1x10^{-13}$ M respectively. However, the number was on 33.4 and 24.1% less if compare to the control on the day 4 (Fig. 2, curves 2, 3). It should be noted that if the K 333 glucoside $1x10^{-4}$ M concentration stimulated the culture growth for the 1st day, then this was not observed for lower concentrations ($1x10^{-8}$ M и $1x10^{-13}$ M) (Fig. 2).

In this case, the plasma membrane of KM cells remained native, and all of the cells in culture remained viable throughout the period of cultivation, and in the control variant (Table. 1).

The K 333 concentration reducing up to $1x10^{-15}$ M in the medium was accompanied by appearance of the "stationary level" effect or dead zone, i.e. the number of cells in culture remained unchanged from the day 1 to day 4 (2.2 10^{6} mL^{-1}) (Fig. 2, curve 4).

This "stationary level" can be explained by two mechanisms: 1 - cells do not die and do not reproduce; 2 - number of dead cells and the number of appeared due to division one is equivalent.

Analysis of the cells morphological types on the cultivation day 1 and 4 showed that the population of cells in culture after K 333 glucoside addition stayed unchanged (Figure 1 (A, B).

This suggests that the second version of the software "stationary level" takes place. It is importantly to notice, the plasma membrane of cells in culture with K 333 concentration of $1x10^{-15}$ M preserved the nativity from the 1st to the 4th cultivation day (Table. 1).

The K 333 concentration reducing to $1x10^{-18}$ M in the cultivation medium was accompanied by an increase in the number of cells in culture on the 3rd and the 4th day (by 40 and 50% compared to the initial amount). However, their number was significantly decreased by 28 and 32%, respectively, at the same time as compared with the control (Fig. 2, curve 5).

Therefore, the administartion to the cultivation medium even $1x10^{-18}$ M of K 333 glucoside was associated with a significant inhibition of the cells in culture accumulation rate as compared to the control on the 3rd and the 4th day of growth.

If the number of cells contained in the culture on the 4th day of growth in the control and after application of various K 333 glucoside concentrations was presented, the dose-response curve has a pronounced bimodal character (Fig. 3), which was noticed by other authors for ultra-low doses of radiation, or biologically active compounds [1, 7, 8].

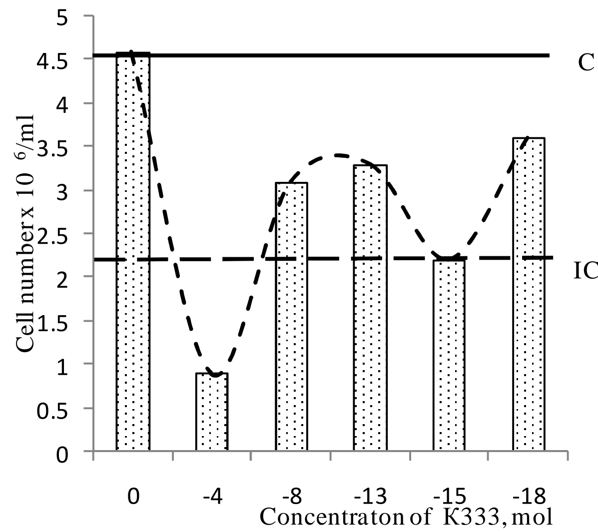

Fig. 3. The number of bone marrow cells on the 4th day of cultivation after the addition of K 333 different concentrations into the culture: 0 - saline. IK - marked the initial concentration of cells in the bone marrow; C is the number of cells in the control sample.

Influence of K 322 glucoside on the growth rate and preservation nativity of the plasma membrane of bone marrow cells in the primary culture

The administration of K 322 glucoside at the concentration of $1x10^{-4}$ M in the cultivation medium caused an expressed reduction in the lag period and after a day, the number of cells in the culture was increased compared with the initial number by 86% and 43% compared with the control, which is much greater than in the case with K 333 glucoside (Fig. 4).

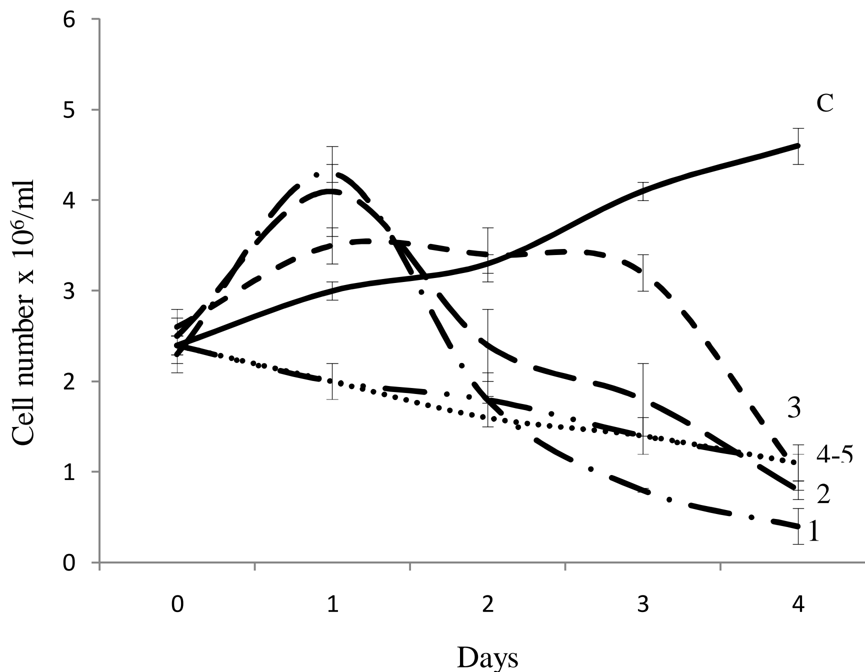

Fig. 4. The number of bone marrow cells in culture from the 1st to the 4th day of cultivation in the control variant (C), and after administration of K 322 into the medium at the concentrations of: 10 $^{-4}$M (1); 10 $^{-6}$M (2); 10 $^{-8}$M (3); 10 $^{-13}$M (4); 10 $^{-15}$M (5).

However, on the 2nd day cell number decreased in 2.3 times in comparison with the first day. The number of cells with native plasma membrane was only 18% (Fig. 5).

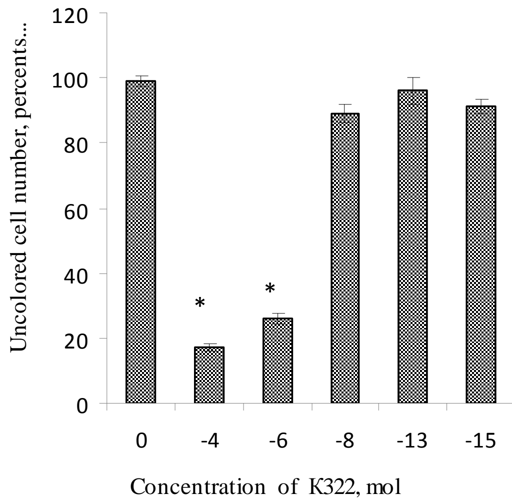

Fig. 5. The number of bone marrow cells with damaged plasma membrane in percentage in the test with Trypan blue on the 4th cultivation day after the addition of K 322 glucoside at various concentrations.

Therefore, K 322 glucoside differs from K 333 by both the degree of inhibition, the method of cells elimination and on the temporal dynamics response of cell culture after the glucoside introduction. And at the concentration of $1x10^{-4}$ M it induces the necrotic cell destruction.

Reducing the K 333 glucoside concentration up to $1x10^{-6}$ M on 2 million of cells in cultivation medium caused the same stimulation of proliferative activity on the day 1 and further inhibition of this indicator with the only difference that on the day 3 and 4 it was less expressed than at $1x10^{-4}$ M (Fig. 4, curve 2). The intensity of the disorder of the cells plasma membrane was also less expressed compared with the concentration of $1x10^{-4}$ M (Fig. 5).

The further reduction of the K 322 concentration to $1x10^{-8}$ M in 2 million cells in the medium also stimulated the increase in cell number in the culture compared with the control, although by a lesser degree. However further 2-3 days the number of cells did not change, and by the day 4 their number decreased in more than 3 times (Fig. 4, curve 3). At the same time the cells preserved native plasma membrane in 90% of the cells (Figure 5).

The decrease in the K 322 concentration to $1x10^{-13}$ M and $1x10^{-15}$ M caused the greatest inhibiting effect of the proliferative response and reduction of cells in the culture. Moreover, this effect appeared on the day 1 and to the 4th day 4 cell number was reduced by 55% compared with the initial and the number was less in 4 times as compared with the control at the concentrations both of $1x10^{-13}$ M and $1x10^{-15}$ M (Fig. 4, curves 4 and 5) . In this case, the cells preserved the native plasma membrane (Fig. 5)

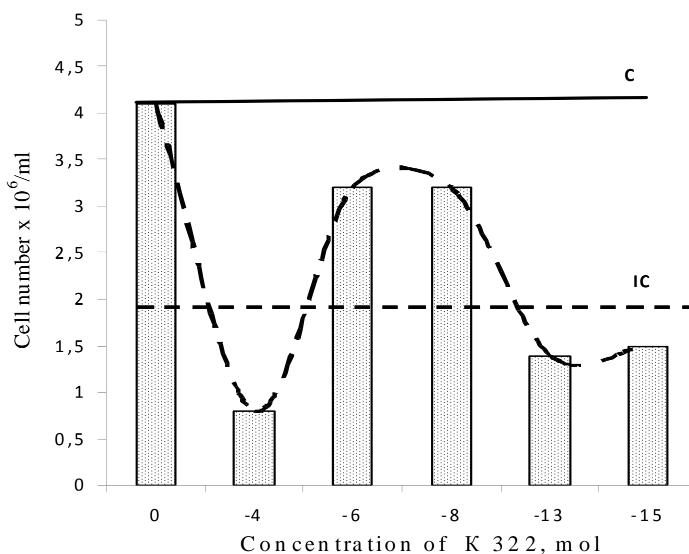

Fig. 6. The number of bone marrow cells on the 3rd cultivation day after the administration of K 322 in various concentrations into medium. C– conrol variant; IC - initial concentration of bone marrow cells.

If the number of cells in the culture on the growth day 3 of after the administration of K 322 glucoside in various concentrations is presented, then, as for K 333 bimodal dose dependence occurred (Fig. 6). It should be noted that K 322 glucoside concentrations of $1x10^{-4}$ M and $1x10^{-13}$ M on the cultivation day 3 had the similar inhibitory effects.

Therefore, for the two K 333 and K 322 glucosides under study the similarities in their proliferation effect of the KM cells and the difference in the time and the mechanism of the culture growth inhibitory effect were revealed. This could indicate that at different glucoside concentrations different response mechanisms are realized.

The combined action of K 333 and K 322 glucosides on the growth rate of bone marrow cells in the primary culture

Common in the biological response of the cell cultures to the glucosides introduction is that even in ultra-low doses of 10^{-15},10^{-18} M, they influenced the behavior of cells in culture. However, it was revealed that for different glucoside concentrations different dose-dependent characteristics were revealed. If the "strength" effect was evaluated, it was expressed to a greater degree for K 322 as compared to K 333. On this background K 333 and 322 K can be represented as two substances with different mechanisms of action. It is important to understand the similarities and differences between the possible targets for the K 333 and K 322. For this purpose, a separate series of experiments, the effect of these glucosides on the proliferative activity of KM cells in the culture, while administration of them to the culture was determined.

It was found that after the administration of K 333 to the culture in concentration of $1x10^{-13}$ M the number of cells did not decrease during the cultivation, and by the days 3 and 4 it even increased by 41 and 50% compared to the initial level, respectively (Fig. 7, curve 1).

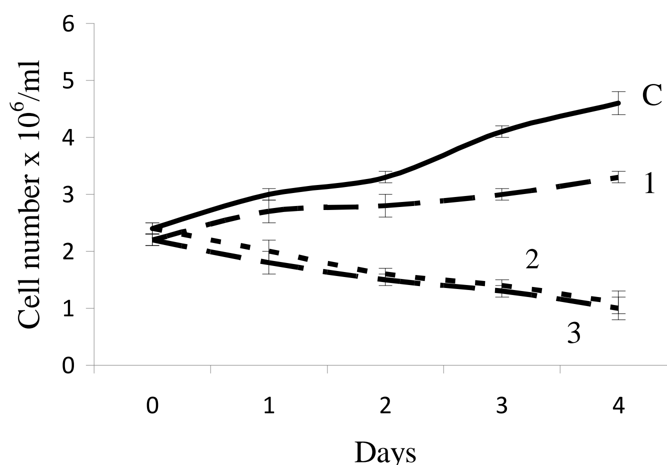

Fig. 7. The number of bone marrow cells in the culture from the 1st to the 4th day of cultivation in the control variant (C), after the administartion of K 333 glucoside at the concentration of 10^{-13} M (1) and K 322 glucoside at the concentration of 10^{-13} M (2) in the medium. Combined administartion of two glucosides at the concentrations of 10^{-13} M (3).

The administartion of K 322 glucoside at the same concentration caused an almost linear decrease in the number of cells in the culture (Fig. 7, curve 2). The same effect on the proliferative activity was caused by simultaneous administartion into the culture of K 333 and K 322 at the concentrations of $1x10^{-13}$ M (Fig. 7, curve 3). Thus 96-98% of the cells had the native plasma membrane.

Therefore, the combined administration of two glucosides in the cell culture was accompanied by the appearance of the activity only for K 322 glucoside, i.e. there has been the effect of complete domination of one of the glucosides.

Discussion

The results of the research can be deduced to a few statements: 1. K 333 and K 322 glucosides can lead to the increase of the concentration of cells in the culture, has no influence on this process and inhibit the proliferative activity of bone marrow cells in the culture that depends on their concentration and exposure. 2. For low and ultra-low doses of glucosides bimodal nonlinear dose-response bone marrow cell cultures is characteristic; 3. The effect of varying of the amount of cells in the culture for K 333 and K 322 glucosides was different both in the degree of inhibition, and in the dynamic effect; 4. At the simultaneous administration of two glucosides - K 333 and K 322 in cell culture activity was expressed only for K 322 glucoside, i.e., there is an effect of dominance.

These results agree well with the available data on the characteristics of the appearance of low and ultra-low doses of biologically active substances [5-8]. In addition, the expression of the glucosides biological action in primary culture of bone marrow cells can have their own characteristics and specificity, which is different from their effects on the organism. However, this model allows to obtain reproducible results in experiments and can be successful for understanding the biological mechanisms of action of ultra-low doses and hormesis effect. This is important, since one of the peculiarities of ultra-low doses action is experimental data poor reproducibility [8].

The K 322 glucoside administration to the culture in a wide concentration range from 10^{-4} M to 10^{-8} M provides an increase of the cells number in the culture, which exceeds the control variant. Such an expression can be defined as hormesis effect as confirmed by this study. However, the effect of stimulation was "short" and the cell number was decreased sharply on the 2nd day. This phenomenon can be due to the fact that K 322 glucoside stimulated the proliferation of the cells able to divide and, at this time differentiated cell types have not yet been eliminated, that in the amount provided a significant increase in the total number of cells in culture, i.e. stimulation occurred at the cellular level. On the second cultivation day, a major amount of differentiated cells in the culture was eliminated and only one or a few cell types that could be accompanied by rather "sharp" change of physico-chemical characteristics of the culture medium were left. And as the result of cell selection was performed. In addition, the "selected" in this way cells may be more resistant to following actions of the toxicant, i.e. they can have a hormesis effect in future.

Such a pronounced effect of cell proliferation in low doses suggests that glucosides especially K 322 glucoside can be considered as potential substances for the application in oncology.

Considering the possible mechanisms of action of low and ultra-low doses of glucosides, it is needless to answer a few key questions: first of all what is the mechanism of the enhancement of their action in the cell; and what is the mechanism of the bimodal nature of the dose response (why smaller doses can cause a greater quantitative effect than high doses).

Unfortunately, currently there is no generally accepted definition of "ultra-low" doses and different authors take different concentrations of substances from 10^{-10} M to 10^{-14} M as "ultra-low". Gurevich K. G. offers to accept ultra-low doses at such a concentration that by several degrees lower than the dissociation constants of biologically active substances at the place of their action [8]. For various receptor dissociation constant is the degree of 10^{-9} to 10^{-12}M. Dose of 10^{-9} M or less are appropriated to be referred to ultra-low doses.

As it was already noted, at the concentrations of 10^{-12}, 10^{-13}M 1-10 molecules can get into a cell. The following question arises whether a single molecule can cause a biological effect at the cellular level and even at the organism one and what is the mechanism of this phenomenon?

There is an experimental data that 36 pheromone molecules in 1 μL are able to

provide the specific biological effect [19]. Some authors believe that it can be a proof of the ability of individual molecules to induce a biological response.

There are mechanisms of receptor signal conduction and strengthening in the cell. In this respect, there is an example of consecution signal strengthening. For example, one active G-protein (conjugated receptor) can interact with other 10-100 G-proteins and activate them. In addition every of G-proteins can catalyze 1000 molecules cAMP. As a result, the receptor signal can be strengthened in 10^{-6} - 10^{-10} times [20].

Gurevich K.G. believes that the strengthening of the cell receptor signal from a single molecule can be explained by the action of ultra-low doses. The presence of so-called "dead zone", usually detected in the concentration range of 10^{-8} – 10^{-12}, which coincides the dissociation constants of ligand-receptor [8].

Currently, there is a quite a number of hypotheses to explain the possible mechanisms of biological action of ultra-low doses. Without details it can be noted that all of them can be divided onto two groups: various modifications of the classical kinetic concepts, which are based on the interaction - the ligand-receptor and non-contact activities that provide a biological action through a variety of field interactions.

To explain the biological effects at these concentrations, some authors propose to consider their actions through the features of the interaction of water molecules with macromolecules [21].

As the example of such non-contact interaction can be a Frolov Yu.P. experimental research. He showed that the benzoid compound influenced locomotor unicellular behavior, yeast metabolism and the rate of enzymatic reactions in the absence of chemical and optical contact, but the presence of the electromagnetic coupling, i.e. field communication [22].

Previously, it was hypothesized [23] that the molecular organization of the cell can be represented not only as a spatial-temporal compartmentalization of macromolecules, but also as a hierarchically organized water-molecular structured network (WMSN). Elements of this water- molecular network vary in size, power capacity, the ability to accumulate energy and spatial displacements. It has been shown that certain molecules may be a source of electromagnetic waves [24]. It can be assumed that these electromagnetic waves can be transmitted without contact to the water molecules, macromolecules and structured WMSN, causing their conformational changes, and as the result, changing their biological activity. In favor of this the large amount of data can be presented [23-25].

If we adopt this mechanism as a potential opportunity, it is not clear why different concentrations of the same xenobiotic cause different by direction and "power" biological responses.

In our opinion this can be explained in terms of hierarchy of WMSN. In a single hierarchical WMSN for the same substance exists a different number of targets or receptors. Which targets will be "used" depends not only on the properties of the active substances, but also on their concentration and the state of biological object. Thus, in the case of relatively large concentrations of the substance to be "used" various receptors on multiple levels of hierarchical organization of WMSN, in particular, at the intracellular membranes of individual enzymes and enzyme complexes, growth factors, and others. In such a situation there will be an appearance of the emergent properties, i.e. integral response to exposure can be formed. The mechanism of this integration, or emergency remains to be cleared. This level of interaction can be determined as a molecular, i.e. classical.

In the case of low concentrations only the change of the level of individual molecules and the active sites of enzymes can be achieved. This may be accompanied by conformational change of interactions at the level of hydration membrane of macromolecules. This level can be defined as the hydrate.

In the case of glucoside ultra-low concentrations the action of electromagnetic fields which are induced by them, and their receptors may be only those molecules which are capable to accept these electromagnetic waves at a given time can take place. Since such molecules in a cell may be a lot, it will reinforce the primary signal. This level of interaction can be defined as a field.

Thanks to WMSN hierarchy the signal can be strengthened many times at different levels of the system and integrated forming a biological response.

It should be noted that when dealing with high concentrations of xenobiotics that all the levels of hierarchy in WMSN can be involved, but in this case there may be the dominance of one of the levels. In favor of this data on the combined action of two glucosides can indicate indirectly.

Therefore, in the mechanisms of xenobiotics interaction or physical factors can be identified at least three different levels: molecular, hydrated and field. At ultra-low doses only field level of interactions occurs, at low doses - field and hydrated, at high doses - field, hydrated and molecular with the molecular dominating. The problem is in understanding the mechanisms of integration of different levels interaction of xenobiotics - cell components.

References

1. Sreenivasan. Y., Raghavendra. P.B. and Manna S.K. 2006. *Oleandrin-mediated expression of Fas potentiates apoptosis in tumor cells.* Journal of Clinical Immunology. 26: 308 – 322.
2. Mo. J., Xia. Y. and Wade. T.J. 2011. *Wade Altered Gene Expression by Low-Dose Arsenic Exposure in Humans and Cultured Cardiomyocytes: Assessment by Real-Time PCR Arrays.* Int. J. Environ. Res. Public Health. 8: 2090 – 2108.
3. Marmugi. S., Ducheix. F., Lasserre. et al. 2012. *Low Doses of Bisphenol A Induce Gene Expression Related to Lipid Synthesis and Trigger Triglyceride Accumulation in Adult Mouse liver.* Hepatology. 55, 2: 395 – 407.
4. Yang. C.P., Cherng. C.H. and Wu. C.T. 2013. *Intrathecal ultra-low dose naloxone enhances the antihyperalgesic effects of morphine and attenuates tumor necrosis factor-α and tumor necrosis factor-α receptor 1 expression in the dorsal horn of rats with partial sciatic nerve transaction.* Anesth Analg. 117, 6: 1493 – 1502.
5. Burlakova. E. B., Grechenko. T.N., Sokolov. E.N. and Terekhova S.F. 1986. *The Effect of Inhibitors of Radical Reactions of Lipid Oxidation on Electrical Activity of Isolated Neuron of the Edible Snail.* Biofizika. 31, 5: 921– 923 (in Russian).
6. Burlakova. E.B. and Khokhlov. A.N. 1985. *Changes in Structure and Composition of Lipid Phase of Biological Membranes under Action of Synthetic Antioxidants: Influence on Transmission of Information Signal at a Cellular Level.* Biologicheskie Membrany. 2: 557-565 (in Russian).
7. Burlakova. E.B., Konradov. A.A. and Maltseva E.L. 2003. *Action ultra-low doses of biologically active substances and low-intensity physical factors.* Khim. Physica. 22, 2: 21–40 (in Russian).
8. Gurevich. K.G. 2001. *Patterns and possible mechanisms of action of ultra-low doses of biologically active substances.* Vestn. Mos. Univ. Ser. 2, Khimiya. 2, 2: 131–134 (in Russian).
9. Bulatov. V.V., Khokhoev .T. Kh., Dikiy. V.V. et al. 2002. *The problem of small and ultra-low doses in toxicology. Fundamental and applied aspects*. Rus. khimicheskiy. zhurnal. XLVI, 6: 58–62 (in Russian).
10. Burlakova. E.B., Conradov. A.A. and Maltsev. E.L. 2006. *Action ultra-low doses of biologically active substances and low-intensity physical factors. Problems of regulation in biological systems* /ed. A.B. Rubin. - Moscow-Izhevsk: "Regular and Chaotic Dynamics." 480 p. (in Russian).
11. Senchina. D.S., McCann. D.A., Flinn. G.N. et al. 2009. *Echinacea tennesseensis ethanol tinctures harbor cytokine- and*

proliferationenhancing capacities. Cytokine. 46, 2: 267 – 272.

12. Tsai. R. Y., Shen. C.H., Feng Y.P. et al. 2012. *Ultra-low-dose naloxone enhances the antinociceptive effect of morphine in PTX-treated rats: regulation on global histone Methylation*. Acta Anaesthesiol Taiwan. 50, 3: 106 – 111.
13. Waldman. M., Hochhauser. E., Fishbein. M. et al. 2013. *An ultra-low dose of tetrahydrocannbinol provides cardioprotection*. Biochem. Pharmacol. 85, 11: 1626 – 33.
14. Kabachny. V.I. and Gorbunova. N.I. 2004. *Derivative glycosides – effective supplements to cryoprotective media.* Problemy Cryobiologii. 2: 28–35 (in Russian).
15. Kabachny. V.I. and Gorbunova. N.I. 2004. *The role of pro- and antioxidant properties of biologically active substances in enhancing cell resistance to hypothermia and cryopreservation.* Problemy Cryobiologii. 3: 9–17 (in Russian).
16. Javazon. E.H., Colter. D.C., Schwartz. E.J. and Prockop. D.J. 2001. *Rat marrow stromal cells are more sensitive to plating density and expand more rapidly from single-cell-derived colonies than human marrow stromal cells.* Stem Cells. 19: 219 – 225.
17. Tennant. J.R. 1964. *Evaluation of the trypan blue technique for determination of cell viability.* Transplantation. 2: 685–694.
18. Varfolomeev. S. D., and Gurevich. K. G. 1999. Biokinetica. Practicheskii kurs, M. FAIR-PRESS. 720 p. (in Russian).
19. Shahdadfar. A., Frønsdal1. K., Haug. T., Reinholt. F. P. and Brinchmann. J. E. 2005. *In Vitro Expansion of Human Mesenchymal Stem Cells: Choice of Serum Is a Determinant of Cell Proliferation, Differentiation, Gene Expression, and Transcriptome Stability* Stem Cells. 23(9):1357–1366, DOI: 10.1634/stemcells.2005-0094 2005.
20. Huch. M., Dorrell. C., Boj. S. F., and van Es. J. H. 2013. *In vitro expansion of single Lgr5+ liver stem cells induced by Wnt-driven regeneration.* Nature: 494, Pages: 247–250 Date published: (14 February 2013)DOI: doi:10.1038/nature11826
21. Zhang. Y. and Cremer. P. S. 2006. *Interactions between macromolecules and* ions: the *Hofmeister series*. Current opinion in chemical biology. 10, 6: 658–663.
22. Frolov. Yu.P. Noncontact action of benzoate compounds on biological systems / Ministerstvo obrazovaniya Rossiyskoy Federatsii; Samara State University. – 2000.-83 p. (in Russian).
23. Bozhkov. A. I., Menzyanova. N. G., Kovaleva. M. K. and Pyatak. N. I. 2014. *Growth of Dunaliella in "Ideal" Conditions Retains Annual Variability of Biochemical Features.* Universal Journal of Plant Science, 2: 31–39. doi: 10.13189/ujps.2014.020202.
24. Turton. D.A., Senn. H.M., Harwood. T., Lapthorn. A.J., Ellis. E.M. and Wynne. K. 2014. *Terahertz underdamped vibrational motion governs protein-ligand binding in solution.* Nat. Commun. 3, 5: 3999. doi: 10.1038/ncomms4999.
25. Acbas. G., Niessen. K.A., Snell. E.H. and Markelz. A.G. 2014. *Optical measurements of long-range protein vibrations.* Nat Commun. 5: 3076. doi: 10.1038/ncomms4076.