THE EFFECT OF PHOTOSENSITIVE PIPTIDOMYMETICS ON THE CONTENT OF PRODUCTS OF LIPID PERCEPTION OXIDATION IN CELL CULTURE

The purpose of the study was to evaluate the possibility of photosensitive peptidomimetik influencing the processes of lipid peroxidation in the culture of cells. The results have shown that the cells that were cultured with the addition of an open and closed forms of peptidomimetics to the predominant changes in the level of the formation of malonic dialdehyde were not observed.

Key words: peroxidation of lipid oxidation, peptidomimetik, lymphoblastoma.

Introduction. Increased formation of active forms of oxygen, increase of the level of products of free radical lipid peroxidation oxidation and decrease in the activity of antioxidant systems is observed in the pathogenesis of many diseases, in particular during the development of malignant neoplasms [1].

It is known that free radicals are one of the carcinogenic factors. They are present at all stages of the development of the tumor and have high reactivity and can cause damage to the lipid bilayer of cell membranes and directly to DNA molecules [1, 2].

Liperoxides are quite unstable, and are subjected to further oxidative degeneration. In this case, accumulate secondary oxidation products, the most important of which are malonic dialdehyde (MDA) [3].

Accumulation of lipid peroxidation (LPO) products in the body and the development of endotoxoidosis leads to stimulation of the monooxygenase system, changes in lipid, hormonal, immune, micronutrient, neurotransmitter status, number of binding sites and affinity of receptors to ligands, and depletion of the antioxidant system [4]. Previously, we showed a significant difference in the action of photosensitive peptidomimetics on tumor cell models in vitro in vivo [5]. However, in contrast to the traditional photo of dynamic therapy with irradiation in a narrow range of wavelengths, a photo-sensitive peptidomimetic is activated by visible light; so it is likely that the launch of cell death occurs without the formation of active forms of oxygen. To check this hypothesis, it was important to evaluate lipid peroxidation. Therefore, the purpose of our work was to determine the effect of photosensitive peptidomimetic (FPM or PM) on the content of lipid peroxidation products in the mouse lymphoblast cell line L1210. The tasks of the work were:

– Determine how the closed and open form of FPM affects the level of TBA-active products.
– Determine how the level of diene, triene conjugates and Schiff bases changes under the action of test substances.
– Determine the effect of FPM on the number of cells in a proliferative pool.

Materials and methods. The study used cell lines L1210 (suspension, mouse lymphoblastoma, lymphocytic lymphoma) cultured under standard conditions in an incubator at 37°C, 5 % CO2, DMEM medium. The number of cells counted in the hemocytometer. The protein level was determined using a set of reagents of the "File-Diagnostics" Ltd. The content of Schiff bases, diene conjugates, triene conjugates of unsaturated fatty acids of neutral lipids and phospholipids was determined according to standard techniques on a spectrophotometer at λ = 400, 232, 278, 220 nm, respectively [6]. The neutral lipids and phospholipids was separate by heptane and isopropyl fraction. The content of TBA-active products (MDA) was determined on a spectrophotometer at λ = 532 nm [7]. The antiproliferative effect was determined by flow cytometry. Experimental data was processed according to generally accepted statistical methods.

Results and discussion. We obtained the following results for the action of the open and closed forms of peptidomimetics: there are statistically insignificant fluctuations...
of the average value of the content of diene conjugates has not changed with respect to control in both phases and states of FPM (Fig. 1); the same thing was observed in the determination of trienic conjugates (Fig. 2); the content of TBA-active products in cells relative to control (Fig. 3); In the investigation of the content of Schiff bases, a statistical difference was observed only in the phospholipid phase with an open form of PM, in the neutral (open and closed forms of PM) and the closed form in the phospholipid phases have statistically insignificant changes (Fig. 4).

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**Fig. 1.** The content of diene conjugates in the fraction of neutral lipids (A) and phospholipids (B) of mouse lymphoblast cells of L1210 by the action of the test substances (M ± m, n = 5)

* – p < 0.05 in comparison with control.

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**Fig. 2.** The content of trienic conjugates in the fraction of neutral lipids (A) and phospholipids (B) of mouse lymphoblast cells of L1210 by the action of the test substances (M ± m, n = 5)
Fig. 3. Content of TBA-active products (malonic dialdehyde) in mouse lymphoblast cells of L1210 by the action of experimental substances ($M \pm m, n = 5$)

Fig. 4. Content of Schiff bases in the fraction of neutral lipids (A) and phospholipids (B) of mouse lymphoblast cells of L1210 on the action of experimental substances ($M \pm m, n = 5$)

* – $p < 0.05$ in comparison with control.

With all of the above, in the L1210 cells, a statistically significant increase in death through apoptosis was observed, both relative to the control group and the groups of cells that were incubated with open and closed forms of PM with each other (table 1).

**Table 1. Level of apoptosis in the cell line L1210 for the action of the test substances ($M \pm m, n = 5$)**

| Test substances | Level of apoptosis, % |
|-----------------|----------------------|
| Control         | 20.67 ± 1.1          |
| GS-DProSw (close) | 37.55* ± 0.9        |
| GS-DProSw (open) | 51.68* ± 0.8        |

* – $p < 0.05$ in comparison with control.

In the analysis of L1210 mouse lymphoblast cells in the cell cycle phases, the activity of the peptidomimete GS-DProSw was observed: statistical increase of cells in the G1 / G0 phase under the action of the open form of PM, relative to the control and the closed form; statistical increase of cells in the G2 / M phase under the action of the
Figure 5. Distribution of mouse lymphoblast cells of L1210 over the phases of the cell cycle by the action of the peptidomimetic GS-DProSw (M±m, n=5)

* – p <0.05 in comparison with control.

Conclusions. As a result of our studies, the content of lipid peroxidation oxidation products in the mouse lymphoblast cells of L1210 was determined by the action of a photosensitive peptidomimetic. It was found that in cells that were cultured with the addition of an open and closed form of peptidomimetic GS-DProSw, no preexisting changes in the level of malondialdehyde were observed. It was determined that the photosensitive peptidomimetics does not show an increase in the level of Schiff bases, diene and triene conjugates. It has been shown that the highest level of cell apoptosis was observed due to the exposure of the open form of the peptidomimetic GS-DProSw. The most prominent cytotoxic and cytostatic action is the open form of the photosensitive peptidomimetic GS-DProSw. Consequently, this allows us to conclude that the mechanism of action of substances does not involve active forms of oxygen to neutralize cells.

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ВПЛИВ ВРС₆₀ НА ШВІДКІСНО-СИЛОВІ ПАРАМЕТРИ ТЕТАНІЧНОГО СКОРОЧЕННЯ MUSCLE SOLEUS АЛКОГОЛОЗОВАНИХ ЩУРІВ ЗА УМОВИ ЕКСПЕРИМЕНТАЛЬНО-ІНДУКОВАНОЇ ІШЕМІЇ РІЗНОЇ ТРВАЛОСТІ

Висвітлено результати петанометричних досліджень впливу ВРС₆₀ (концентрація 0,15 мг/мл) у дозі 1 мг/кг на швидкісно-силові показники тетанічного скорочення muscle soleus за умови 1-годинної та 2-годинної ішемії у щурів із хронічною алкогольною інтоксикацією. Синергійний ефект ішемічного ушкодження та алкогольної інтоксикації, порівняно з нативним м'язом, проявляється у зниженні показників сили скорочення до 26,25±3,23 і 20,2±2.45 (p<0.01) та збільшенні часу досягнення її максимальних значень на 1,33±0,12 ч та 1,45±0,15 с (p<0.01) відповідно. Показано, що за умови внутрішньоче- ревинного введення розчину ВРС₆₀ ці показники зазнають значущих змін.

Ключові слова: фулерени, ішемія, хронічна алкогольна інтоксикація, muscle soleus.