ABSTRACT

Thesis of Yeshan Banu Gazizky for the degree of Doctor of Philosophy (PhD) in the specialty "6D060700-Biology" on the topic "**Studies of the molecular and physiological mechanisms of the action of the drug" Danazol** "on the energy exchange of breast cells Mcf10A"

General characteristics of the work. This work is devoted to the study of molecular and physiological mechanisms of action of the drug "Danazol" on the energy processes of breast cells Mcf10a (Michigan Cancer Foundation, benign tumor cell line) in fibrocystic mastopathy.

Relevance of the research topic. Fibrocystic mastopathy is one of the most serious and severe breast pathologies, occurring in almost 60% of women. The causes of cysts and the molecular and physiological mechanisms of fibrocystic mastopathy have not yet been fully investigated. in connection with the above, the current problem is to establish the molecular mechanisms of fibrocystic mastopathy and not to find new effective ways to treat them.

Due to the increase in the number of patients with fibrocystic mastopathy, multi-vector studies are being conducted to find effective drugs with effective therapeutic properties. Known drugs currently used for the treatment of fibrocystic mastopathy do not have high therapeutic efficacy.

It is established that this disease is getting younger in recent years. There are statistics indicating that fibrocystic mastopathy occurs after the age of 30, while there is a high risk of cystic mastopathy turning into a malignant breast tumor.

Given the variety of etiopathogenetic mechanisms of fibrocystic mastopathy and their impact on metabolic processes,it is necessary to establish cell exchange at certain stages of treatment of mastopathy.

It should be expected that positive results of treatment will be achieved only when the issues of the comprehensive influence of cell metabolism and the hormonal status of the body are taken into account.

There is also evidence of a high risk of developing this pathology in adolescents, while it is noted that pathological changes are manifested in the form of breast nodules.

A decrease in breast tissue hyperplasia in mastodinia was found during prolonged and regular use of Danazol. However, due to the obvious side effects of danazol, women refused to take It. The fact that The activity and effectiveness of danazol side effects have not been sufficiently studied at the molecular level reflects the relevance of this study.

Objective: to Identify the molecular and physiological mechanisms of action of the drug "Danazol" on the energy exchange of breast cells Mcf10a.

Research problem:

1. Determine the cytotoxicity of the drug Danazol;
2. Determination of cellular mechanisms of apoptosis in breast cells Msf10a under the influence of the drug Danazol.
3. Determination of the effect of the drug danazol on the membrane potential of the mitochondria of Msf10a cells and respiratory activity
4. Analysis of oxidative stress caused by danazol exposure to breast cells Msf10a
5. Study of the effect of the drug danazol on the level of cytosolic Ca2+ breast cells Msf10a

Objects of the study. The object of the study was the mcf10a breast cells isolated in women with fibrocystic mastopathy

Method of research. Cultured cells were used in vitro, flow cytometry (BD Accuri C6), high-precision respiometry (Oroboros Oxygraph-2k), fluorescence spectroscopy (BioTek Synergy 4), microscopy (Leica MZ16F, Motic AE2000), spectrophotometry (NanoDrop), and statistical analysis methods (GraphPad Prism).

Scientific novelty of the research:

- For the first time, systematic studies were conducted to control the actual effect of danazol on the energy exchange of Msf10a breast cells. the features of the cytotoxic effect of danazol on Msf10a breast cells were Revealed.
- For the first time, the phase of stopping the cycle of the Msf10a breast cell under the influence of the drug danazol was detected.
- Revealed changes in the mitochondrial metabolism of breast cells Msf10a, caused by the concentration of the drug danazol.
- It was revealed that the receptors of the progesterone hormone danazol act on the metabolism of the mitochondria of breast cells Msf10a.

Theoretical significance of the work. The effect of the concentration of danazol 25,3,10 and 30MKM on the mechanism of breast cells Msf10a on the cell membrane potential, oxidative stress, respiration and cell proliferation was established. The concentration of danazol 10 μm and 30 μm terminates cell cycle of cells Mcf10A at stages G0/S and a concentration of 30 μm caused a decrease of membrane potential of the cells Mcf10A to 77%.It was also found that danazol affects mitochondrial metabolism through progesterone receptors.

Practical significance of the work. The practical value of the study is related to the use of naturally occurring polyphenols curcumin and carnazolic acid and the treatment of prostate cancer cells. The results obtained were illustrated in the Kazakh research Institute of Oncology and radiology (implementation acts No.1, 27 of 2018-Appendix A). Form of implementation: a master class with a presentation and practical training on animals.

The results obtained are implemented in the educational process and are used when teaching the course "Fundamentals of animal physiology" (the act of implementing completed research work in the educational process in 2018-Appendix B).

The main provisions submitted for protection:

1.The cytotoxic effect of the drug danazol at cells in the breast Mcf10A related to the concentration.

2.The concentration of the drug Danazol trigger a process of apoptosis in breast cells Mcf10A.

3.It is established that the drug Danazol inhibits the proliferation of breast cells Mcf10A.

4.In the process of oxidative-phosphorylation of breast cells Msf10a, it was shown that the respiratory chain of the cell is affected by danazol.

5.It was found that Danazol affects mitochondrial metabolism through progesterone receptors.

6.For the treatment of fibrocystic mastopathy, danazol is recommended as a drug that can be used for medicinal purposes that give a local effect

Conclusions.

1. It is proved that fibrocystic mastopathy of breast cells Mcf10A helps the cyst to develop during cell growth is an important feature of the cells themselves.
2. Features of action of various concentrations of the drug "Danazol" on the cellular structure of the breast Mcf10A, so during the experiments was determined the drug concentration of 25 nm, 1 μm, 3 μm, 10 μm and 30 μm when the monitoring of the cytotoxic effect on the vital activity of cells. As a result, monitoring of cell apoptosis was observed after 24, 48, and 72 hours of cell activity. At a concentration of 25 nm of the drug "Danazol", its cytotoxic effect on cell activity was not detected, although it was found that at a concentration of 30 microns, Danazol had a significant effect on the vital activity of cells.
3. The peculiarities of the action of the drug danazol on fibrocystic mastopathy depending on its concentration and exposure were established. So, as the effect is obtained at a concentration of 30 microns.
4. Studies of the effect of Danazol on the mechanism of oxidative phosphorylation of Mcf10A cells, so in experiments it was observed that the potential of the mitochondrial membrane after 72 hours, Danazol showed its cytotoxic properties at a concentration of 30 microns.
5. It was determined that Danazol concentrations of 10 and 30 microns had their effects on the respiratory chain of the cell when controlling the respiratory ability of the Mcf10A cell line.

Personal contribution of the author. All results of the dissertation work were obtained in the presence and personal participation of the author. The author independently analyzed the literature data on the research topic, conducted experimental research, processed and analyzed the results of the research, and wrote and designed the dissertation manuscript.

Connection with the plan of the main scientific works. The dissertation work is part of a large-scale research and was supported by the Cornelius Beukenkamp Foundation(2013-2016) for the study of breast and prostate cancer (project Manager Professor Z. S. orynbayeva of Drexel University), aimed at establishing the molecular and physiological mechanisms of action of the drug "Danazol" on the energy metabolism of breast cells Mcf10a. The dissertation work was carried out in the framework of the scientific program of the Department of Biophysics and Biomedicine: "Actual problems of modern Biophysics and Biomedicine (2015-2018)".

Approbation of the work. The materials of the dissertation work were discussed and reported at international and national conferences:

- International scientific conference of students and young scientists "Farabi Alemi" (2017, Almaty, Kazakhstan);
- International scientific conference "Actual problems of ecological genetics and experimental biology" (2018, Almaty, Kazakhstan);
- International scientific and practical conference "Topical issues of medicine" (2018, Baku, Azerbaijan);
- XVIII international scientific and practical conference "Advances in Science and Technology" (2019, Russia, Moscow);
- International scientific Internet conference "Trends and prospects for the development of science and education in the context of globalization" (2019, Kiev, Ukraine).

Publications. The main content provided in the 13 published works, including 1 article in the international journal impactfactor cited Thomson Reuters and Scopus, 4 articles in national scientific magazines recommended by Committee for control in education sphere and science KR, 8 thesis in conference proceedings at national and international level.

The structure of the thesis. The dissertation is presented on 122 pages and consists of an introduction, review of literature, materials and research methods, results and discussion, conclusion, and list of sources used. Number of references 156. The work contains 21 figures and 5 tables.