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BIOENERGETIC CHARACTERISTICS OF THE MURINE NEMETH-KELLNER LYMPHOMA CELLS EXPOSED TO THIAZOLE DERIVATIVE IN COMPLEX WITH POLYMERIC NANOPARTICLES

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The development of a new anticancer drugs targeted at energy metabolism of tumor cells is a promising approach for cancer treatment. The aim of our study was to investigate the action of thiazole derivative N-(5-benzyl-1,3-thiazol-2-yl)-3,5-dimethyl-1-benzofuran-2-carboxamide (BF1) and its complex with PEG based polymeric nanoparticle (PEG-PN) on respiration and mitochondrial membrane potential in murine NK/Ly tumor cells. The rate of oxygen uptake in NK/Ly cells was recorded by a polarographic method using a Clark electrode. The mitochondrial potential relative values were registered using fluorescence TMRM dye. No changes in glucose-fuelled basal respiration or maximal FCCP-stimulated respiration was detected after 15-min incubation of cells with BF1 (10 μM), PEG-PN or BF1 + PEG-PN complex Fluorescent microscopy data showed that BF1 or PEG-PN separately had no effect on the value of mitochondrial membrane potential, while BF1 + PEG-PN complex caused a significant decrease in mitochondrial membrane potential, indicating on the decrease of NK/Ly cells viability.

K e y w o r d s: NK/Ly tumor cells, thiazole derivative, PEG, polymeric nanoparticles, cell respiration, mitochondrial membrane potential.

According to the American Cancer Society data 1,918,030 new cancer cases and 609,360 cancer deaths are projected to occur in the United States [1]. One of the main characteristics of tumor cells is their unlimited possibility to proliferation. Therefore, cancer cells need to adapt their energy metabolism to promote survival and multiplying [2]. Cancer cells preferentially use glycolysis, not oxidative phosphorylation, as a normal cell, for energy production, even in the presence of sufficient oxygen [3]. Furthermore, cancer cells that generate their energy using glycolysis are often resistant to most chemotherapeutic agents [2].

However, though cancer cells typically switch from “cellular respiration” to anaerobic glycolysis, recent studies indicate that mitochondrial functionality is important for carcinogenesis [4]. Mitochondria play an important role in cancer cells' survival through an indirect action mediated by reactive oxygen species (ROS) or directly through mitochondrial biogenesis because energy production also enables the synthesis of many molecules required for cellular biosynthesis, growth and proliferation [5]. There are a number of drugs that affect mitochondrial complexes, metabolism and ATP production and cause an intensive rise in oxygen radicals, leading to oxidative damage and tumors cells death [6].

Change in mitochondrial membrane potential is an important indicator of mitochondrial dysfunction [7]. Depolarization of membrane potential, mitochondrial swelling and the opening of mitochondrial permeability transition pore exert under the influence of anticancer agents [8].

Thus, studying the mechanisms of glycolysis, oxidative phosphorylation and changes in the mitochondrial membrane potential of tumor cells is

a perspective direction in design of effective next-generation anticancer drugs.

On the other hand, derivatives of thiazole belong to members of the great important antiproliferative agents. 2 Aminothiazole fragment is one of the significant pharmacophores in drug discovery process [9,10]. There are many substituted 2-aminothiazole derivatives covering a wide range of therapeutic targets, and they are privileged scaffolds for the discovery of anti-cancer drugs [11-13]. Therefore we are focusing on the synthesis and the bioactivity screening of 2-aminothiazole derivatives [14-17].

Previously we had found, that the synthesized derivative of 2-aminothiazole the N-(5-benzyl-1,3-thiazol-2-yl)-3,5-dimethyl-1-benzofuran-2-carboxamide (BF1) was cytotoxic to different of tumor cells, caused apoptosis and affected redox balance in cancer cells, but did not affect the membrane potential and oxidative phosphorylation of isolated mitochondria [17-19].

However, like most chemotherapeutics agents, this compound is poorly soluble in water and could potentially limit its effectiveness. The complexes of the investigated thiazole derivative with PEG-containing polymer nanoparticles (PEG-PNs) were formed to improve the solubility of BF1 and increase its penetration. It was shown, that influence of polyethylene glycol-containing surfactants changed cell respiration in different types of diseases [2, 20]. It was previously established, that the studied complexes were more cytotoxic to certain tumor cell lines compared to the control than unconjugated BF1 [21].

Thus, the aim of this work was to investigate the effect of BF1 and its complexes with PEG-containing polymer-carriers on respiration and mitochondrial membrane potential of murine NK/Ly tumor cells.

Materials and Methods

Murine NK/Ly lymphoma model. The study was performed on white wild-type male mice (20–30 g) with grafted NK/Ly lymphoma. Animals were kept in standard vivarium conditions at constant temperature on mixed ration. Manipulations with animals were carried out under the principles of the General Ethical Principles of Experimentation on Animals approved by the First National Congress on Bioethics (Kyiv, Ukraine, 2001) and European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, France, 1985).

Ascite tumor cells were passaged by intraperitoneal inoculation of $10–15\times10^6$ cells to mice. Ascites were drained from the abdominal cavity of anesthetized mice with sterile syringe 7–10 days after the inoculation.

Investigated compounds. The thiazole derivative BF1 (N-(5-benzyl-1,3-thiazol-2-yl)-3,5-dimethyl-1-benzofuran-2-carboxamide) was synthesized at the Department of Organic Chemistry of Ivan Franko National University of Lviv and used for preparing of initial 10 mM solution of BF1 in dimethyl sulfoxide [15, 18]. The PEG-PN Th1 (full name: poly(VEP-co-GMA)-*graft*-mPEG - k = 1.4% mol, l = 98.6% mol, M_n = 240 kDa) was synthesized at the Department of Organic Chemistry of the Lviv Polytechnic National University, as described earlier [21, 22].

Water dispersions of polymeric nanoparticles Th1 and its complexes with the BF1 were dissolved in dimethyl sulfoxide (DMSO) and the solutions were subsequently transferred in water (Th2). The BF1 concentration in the dispersed water solution was 0.3 mg/ml, and the Th1 concentration was 10 mg/ml.

Measurement of NK/Ly cells respiration. The cell suspension (2×10^6 intact cells/ml and 5×10^6 permeabilized cells/ml) was washed from the ascites fluid once with an extracellular saline solution containing (mM): NaCl – 140.0, KCl – 4.7, $CaCl_2$ – 1.3, $MgCl_2$ – 1.0, HEPES – 10.0, glucose – 10, pH 7.4. The investigation compounds in concentration 10 μM were added to lymphoma cells (the same volume of extracellular saline solution was added in control group) and incubated for 15 min. In one series of experiments, the cells were washed from the test compounds, in the other series cells were not washed.

Intact cells were sedimented at 1,000 rpm for 5 min and the supernatant was replaced with intracellular-like salt solution containing (mM): KCl – 90.0, NaCl – 15.0, KH_2PO_4 – 2.0, $MgCl_2$ – 1.0, HEPES – 10.0, EGTA – 0.5, pH 7.2. Then, NK/Ly cells suspended in the basic glucose-free solution were added into the polarography respiratory chamber. After one minute of incubation glucose was added and the cellular oxygen consumption rate (OCR) was measured. Then, every two minutes a protonophore carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP) was added in aliquots to increase its concentration by 0.25 μM (1^{st} and 2^{nd} doses) and 0.5 μM (3^{rd} - 5^{th} doses). The final cumulative concentration of FCCP was thus 2 μM.

Respiration rates of the lymphoma cells was measured using polarographic method and an RC650 6-cell Respirometer (Strathkelvin Instruments, Scotland). This method is based on the registration of the oxygen uptake using the Clark electrode in 1.5 ml glass chamber at 37 °C.

Mitochondrial potential measurement. Mitochondrial membrane potential Δψ was recorded by fluorescence microscopy. The method is based on recording differences in the fluorescence of cells treated with a specific dye. An Olympus IX73 inverted microscope was used for research, and a DP-74 digital camera was used for image acquisition. In order to register the relative values of the mitochondrial membrane potential, the fluorescent dye TMRM (tetramethylrhodamine methyl ester perchlorate) was used (the wavelength for the excitation filter was 540–585 nm, and for the beam splitter 595 nm. A barrier filter of 600 nm was also used). The cell incubation medium had the following composition (in mM NaCl – 140.0, KCl – 4.7, $CaCl_2$ – 1.3, $MgCl_2$ – 1.0, HEPES – 10.0, glucose – 10, pH 7.4). First, lymphoma ascites were washed and diluted 10 times. The tested substance BF1 was added to the cell suspension in concentrations of 10 and 50 μM, and DMSO in a final concentration of 5% was added to a separate sample and incubated for 15 min at 37°C. After incubation, the cells were washed again and rhodamine (10 μl) was added and incubated again for 15 min (temperature – 37°C). A few μl were taken from each sample with a dispenser and a drop was placed on a glass slide. It was covered with a cover glass and placed in a microscope (microscope magnification ×12.6). In the field of view, 4-5 different variants of cell images were selected, first in visible light, and then switched to the fluorescent light spectrum. The intensity of fluorescence was recorded, which reflected the changes in the value of the membrane potential of mitochondria Δψ and was evaluated using the ImageJ computer program.

Statistical analysis. Each experiment was repeated 5-6 times for each variant (control, substances) and the average value of mean was calculated. All results were shown as means ± SD. Fluorescence microscopy data were analysed using the ImageJ computer program. Mathematical and statistical processing of research results was carried out using the Microsoft Office Excel program. Statistical significance of difference between groups was determined with a one-way ANOVA following by t-test if significant effect was proven by ANOVA.

Results

Effect of BF1 in complex with polymeric nanoparticle on cells and mitochondria respiration. Fig. 1, (*A*) shows the respiration rates of intact NK/Ly cells upon the oxidation of glucose. The respiration rate of intact control (untreated) cells was 0.02 nmol O/(sec×10^6 cells) (n = 5). Thiazole derivative BF1 and PEG-based polymeric particle Th1 did not change the oxygen consumption in murine lymphoma cells. There was a tendency towards an increase of the rate of NL/Ly cell respiration under the action of complex Th2, but this difference was not proven with ANOVA (Fig. 1, *A*).

Protonophore FCCP was added step-wise into the respiratory chamber every two minutes (final concentration was 2 μM). The apparent increase of FCCP-stimulated respiration rates by complex Th2 when protonophore was added in the highest concentrations (1.5 μM) was not statistically confirmed with ANOVA.

To estimate the maximal oxidation capacity of NK/Ly cells, the FCCP-stimulated OCR was calculated. The respiration rate of untreated control NK/Ly cells was 0.086 ± 0.011 nmol O/(sec×10^6 cells). However, neither investigated compound significantly change the maximal FCCP-stimulated respiration compared to control.

Effect of BF1 in complex with polymeric carrier on mitochondrial potential. Although, BF1 or its complex with PEG-PN did not change the cellular respiration of NK/Ly cells, in order to further investigate the role of mitochondria under the effects of BF1 and complex with PC we measured the membrane potential of lymphoma cell mitochondria. Membrane potential is an important indicator of mitochondrial activity, which can be detected in particular, by fluorescence microscopy using potential-sensitive TMRM dye. An uncoupler of oxidative phosphorylation FCCP in high concentration (20 μM) was used to fully depolarize mitochondria of non-permeabilized NK/Ly cells and confirm that TMRM fluorescence depends on mitochondrial membrane potential. Since the investigated compounds were dissolved in the DMSO, the effect of this solvent on the membrane potential of mitochondria was also tested. It was found that FCCP reduced the fluorescence intensity by 63% ($P < 0.01$), confirming dissipation of mitochondrial membrane potential. At the same time, DMSO did not significantly change the membrane potential of mitochondria (Fig. 2).

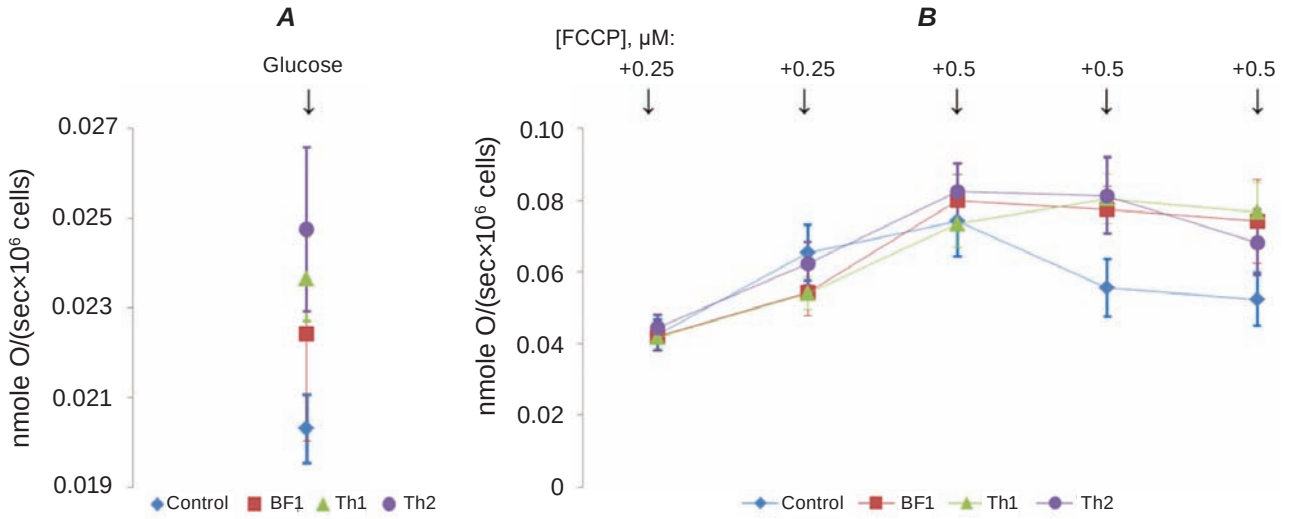

Fig. 1. Respiration rate of mitochondria in intact murine NK/Ly cells when: ***A*** *– glucose was used as substrate;* ***B*** *– and after FCCP in increasing concentrations was added under the action of thiazole derivative BF1, polymeric nanoparticle Th1 and complex of BF1 and Th1 – Th2. All data are shown as means ± SE, n = 5. The final cumulative FCCP concentration was 2 μM*

Fig. 3 shows the fluorescent images of NK/Ly lymphoma cells under the influence of the investigated compounds. Fluorescence was less intense under the action of BF1 (*b*) compared to the control (*a*). It is noticeable, that the fluorescence of lymphoma cells under the action of the complex Th2 (*d*) was lower than the fluorescence under the action of BF1 or control. PEG-PN Th1 (*c*) fluorescence of lymphoma cells was almost equal to control data.

BF1 at the concentration of 10 μM decreased mitochondrial membrane potential of NK/Ly cells by 12% but these changes were not statistically significant ($P = 0.15$). Th1 (unconjugated with BF1 carrier) did not affect the mitochondrial membrane potential of NK/Ly lymphoma cells. However, Th2 complex significantly decreased the mitochondria membrane potential of lymphoma cells by 23% ($P < 0.05$) (Fig. 4).

Discussion

The hypoxic microenvironment in the most type of cancer limits the ability of mitochondrial oxidative phosphorylation to generate ATP and increase anaerobic glycolytic intensity to compensate the energy deficiency [23, 24]. However, mitochondrial processes play an important role in tumor initiation and progression and may contribute to the development of cancer through the activation of glucose metabolism, the production of ROS and compromise of intrinsic apoptotic function [25].

In this study, mitochondrial membrane potential, the basal and uncoupled cell respiration of NK/Ly cells under the action of thiazole derivative BF1 and its complex with PEG-based polymeric nanoparticles were investigated. The only effect we found in this study was that complex Th2, unlike free compound FB1, significantly decreased the mitochondrial membrane potential. However, the mechanism of this is unclear. The complex might have directly or indirectly caused partial uncoupling of mitochondria. But this is usually accompanied by the increased respiration rate, as in case of FCCP titration. On the other hand, if the membrane potential drop

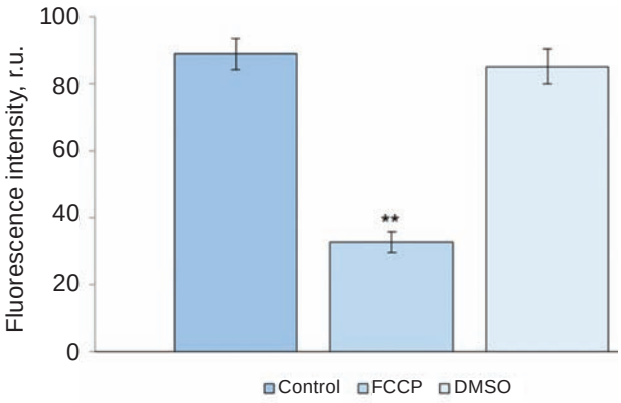

*Fig. 2 Mitochondrial fluorescence intensity in NK/Ly cells under the action of FCCP (20 μM) and DMSO (final concentration 5%). All data are shown as means ± SE, n = 5. **P < 0.01*

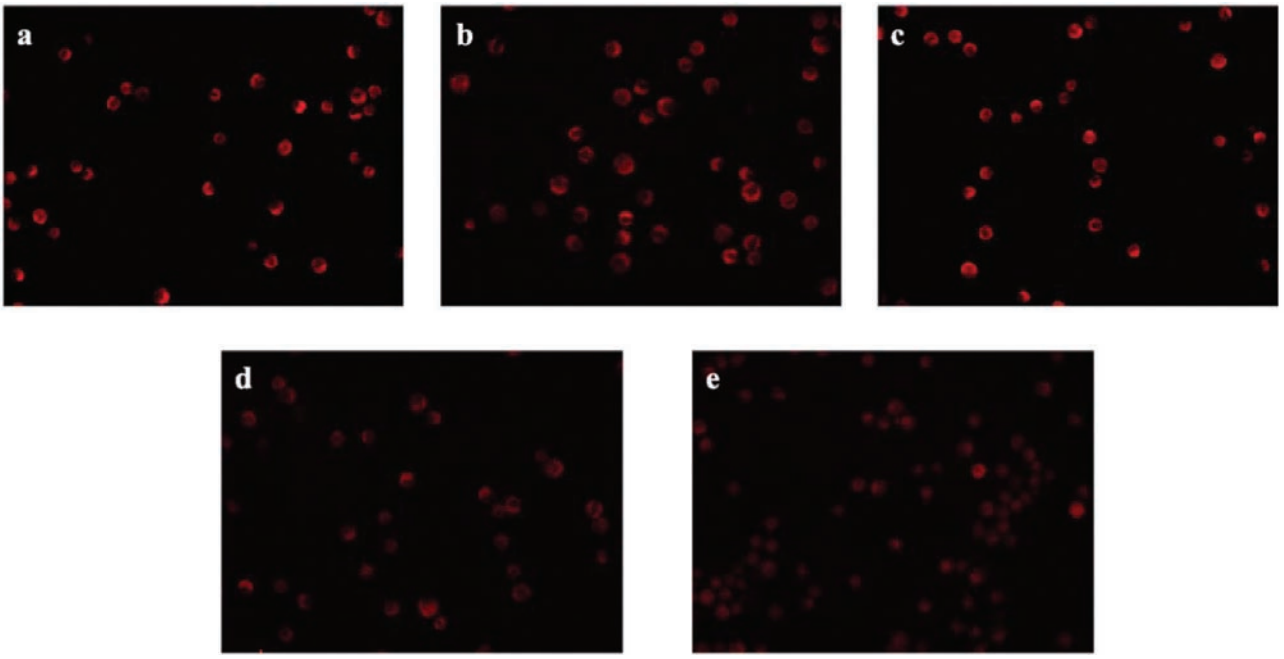

Fig. 3 Fluorescence of lymphoma cells with TMRM under the action of thiazole derivative BF1 in complex with PEG-based polymer nanoparticle: ***a*** *– control,* ***b*** *– unconjugated thiazole derivative BF1,* ***c*** *– polymeric nanoparticles based on poly(VEP-co-GMA)-graft-mPEG (Th1),* ***d*** *– complex of BF1 with Th1 (Th2),* ***e*** *– FCCP*

was associated with a decreased capacity of the electron transfer chain of the mitochondria, this would inevitably result in inhibition of uncoupled respiration rate. Yet, this was not observed in our study.

In was shown that the significant changes of mitochondrial endogenous and uncoupled respiration and ATP depletion were observed after only 48 h of DOX treatment [26]. Thus, a longer experiments are required to study the mechanism of Th2 action on mitochondria of NK/Ly cells.

It is well known that a drop in mitochondrial membrane potential can be associated with ROS production. Both parameters are prerequisite steps for the induction of mitochondrial permeability transition (MPT) and subsequent cell death by apoptosis or necrosis [27]. It was previously established, that both BF1 and Th2 increased the level of ROS in glioma cells and lead to apoptotic and necrotic changes in NK/Ly cells [17-19].

Overproduction of ROS can lead to mitochondrial damage, such as mitochondrial DNA mutations, damage to the mitochondrial respiratory chain and mitochondrial membrane permeability, and disruption to Ca^{2+} homeostasis [28]. In the previous investigation, the enhanced ROS production in lymphoma cells by unconjugated BF1 and its complex with PEG-PN was shown (paper is under review).

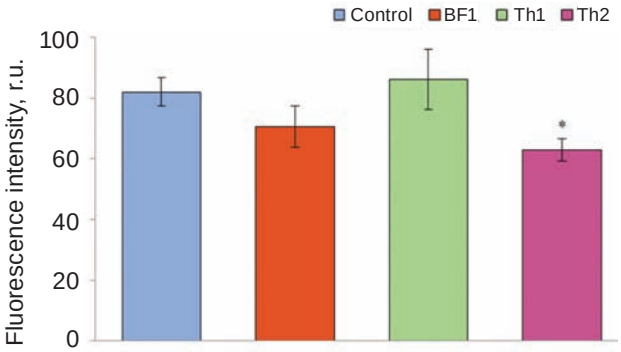

*Fig. 4. Mitochondrial membrane potential changes in NK/Ly cells under the action of thiazole derivative BF, polymeric nanoparticle Th1 and complex of BF1 and Th1 – Th2. All data are shown as means ± SE, n = 5. *P < 0.05*

Conclusion. The investigated complex of thiazole derivative BF1 with PEG-based polymeric nanoparticles may realize its cytotoxic effect by early depolarization of NK/Ly lymphoma cells mitochondria.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukr-biochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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БІОЕНЕРГЕТИЧНІ ХАРАКТЕРИСТИКИ МИШАЧОЇ ЛІМФОМИ НЕМЕТ-КЕЛНЕРА ЗА ВПЛИВУ ПОХІДНОГО ТІАЗОЛУ З ПОЛІМЕРНИМИ НАНОЧАСТИНКАМИ

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Розробка нових протипухлинних препаратів, спрямованих на зміну енергетичного метаболізму пухлинних клітин, є перспективним підходом до лікування раку. Метою нашого дослідження було дослідити дію похідного тіазолу N-(5-бензил-1,3-тіазол-2-іл)-3,5-диметил-1-бензофуран-2-карбоксаміду (BF1) та його комплексу. з полімерними наночастинками (PEG-PN) на основі PEG на процеси клітинного дихання та потенціал мітохондріальної мембрани в мишачих пухлинних клітинах NK/Ly. Швидкість поглинання кисню клітинами NK/Ly реєстрували полярографічним методом з використанням електрода Кларка. Відносні значення мітохондріального потенціалу реєстрували за допомогою флуоресцентного барвника TMRM. Після 15-хвилинної інкубації клітин з BF1 (10 мкМ), PEG-PN або комплексом BF1 + PEG-PN не було виявлено жодних змін у базальному диханні, що підживлюється глюкозою, або максимальному диханні, стимульованому FCCP. Дані флуоресцентної мікроскопії показали, що BF1 або PEG-PN окремо не впливали на величину мітохондріального мембранного потенціалу, тоді як комплекс BF1 + PEG-PN спричинював значне зниження мітохондріального мембранного потенціалу, що вказує на зниження життєздатності NK/Ly клітин.

Ключові слова: пухлинні клітини NK/Ly, похідне тіазолу, поліетиленгліколь, полімерні наночастинки, клітинне дихання, мембранний потенціал мітохондрій.

References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA Cancer J Clin*. 2022; 72(1): 7-33.
2. Chen XS, Li LY, Guan YD, Yang JM, Cheng Y. Anticancer strategies based on the metabolic profile of tumor cells: therapeutic targeting of the Warburg effect. *Acta Pharmacol Sin*. 2016; 37(8): 1013-1019.
3. Ghanbari Movahed Z, Rastegari-Pouyani M, Mohammadi MH, Mansouri K. Cancer cells change their glucose metabolism to overcome increased ROS: One step from cancer cell to cancer stem cell? *Biomed Pharmacother*. 2019; 112: 108690.
4. Fadaka A, Ajiboye B, Ojo O, Adewale O, Olayide I, Emuowhochere R. Biology of glucose metabolization in cancer cells. *J Oncol Sci*. 2017; 3(2): 45-51.
5. Liu Y, Shi Y. Mitochondria as a target in cancer treatment. *MedComm*. 2020; 1(2): 129-139.
6. Kapur A, Mehta P, Simmons AD, Ericksen SS, Mehta G, Palecek SP, Felder M, Stenerson Z, Nayak A, Dominguez JMA, Patankar M, Barroilhet LM. Atovaquone: An Inhibitor of Oxidative Phosphorylation as Studied in Gynecologic Cancers. *Cancers (Basel)*. 2022; 14(9): 2297.
7. Wen S, Zhu D, Huang P. Targeting cancer cell mitochondria as a therapeutic approach. *Future Med Chem*. 2013; 5(1): 53-67.
8. Li Q, Huang Y. Mitochondrial targeted strategies and their application for cancer and other diseases treatment. *J Pharm Investig*. 2020; 50: 271-293.
9. Alizadeh SR, Hashemi SM. Development and therapeutic potential of 2-aminothiazole derivatives in anticancer drug discovery. *Med Chem Res*. 2021; 30(4): 771-806.
10. Elsadek MF, Ahmed BM, Farahat MF. An Overview on Synthetic 2-Aminothiazole-Based Compounds Associated with Four Biological Activities. *Molecules*. 2021; 26(5): 1449.

11. Wan Y, Long J, Gao H, Tang Z. 2-Aminothiazole: A privileged scaffold for the discovery of anti-cancer agents. *Eur J Med Chem*. 2021; 210: 112953.
12. Bestgen B, Krimm I, Kufareva I, Kamal AAM, Seetoh WG, Abell C, Hartmann RW, Abagyan R, Cochet C, Le Borgne M, Engel M, Lomberget T. 2-Aminothiazole Derivatives as Selective Allosteric Modulators of the Protein Kinase CK2. 1. Identification of an Allosteric Binding Site. *J Med Chem*. 2019; 62(4): 1803-1816.
13. Ouyang B, Wang L, Qi J, Fan M, Wang H, Yao L. Synthesis and Evaluation of Biological Properties of 2-Amino-thiazole-4-carboxamides: Amide Linkage Analogues of Pretubulysin. *Biol Pharm Bull*. 2020; 43(8): 1154-1158.
14. Ostapiuk YV, Ostapiuk MY, Barabash OV, Kravets M, Herzberger C, Namyslo JC, Obushak MD, Schmidt A. One-pot syntheses of substituted 2 aminothiazoles and 2-aminoselenazoles via Meerwein arylation of alkyl vinyl ketones. *Synthesis*. 2022; 54(16): 3658-3666.
15. Ostapiuk YV, Barabash OV, Ostapiuk MY, Goreshnik E, Obushak MD, Schmidt A. Thiocyanatoarylation of Methyl Vinyl Ketone under Meerwein Conditions for the Synthesis of 2-Aminothiazole-Based Heterocyclic Systems. *Org Lett*. 2022; 24(25): 4575-4579.
16. Finiuk NS, Hreniuh VP, Ostapiuk YuV, Matiychuk VS, Frolov DA, Obushak MD, Stoika RS, Babsky AM. Antineoplastic activity of novel thiazole derivatives. *Biopolym Cell*. 2017; 33(2): 135-146.
17. Finiuk N, Klyuchivska O, Ivasechko I, Hreniukh V, Ostapiuk Yu, Shalai Ya, Panchuk R, Matiychuk V, Obushak M, Stoika R, Babsky A. Proapoptotic effects of novel thiazole derivative on human glioma cells. *Anticancer Drugs*. 2019; 30(1): 27-37.
18. Hreniukh VP, Finiuk NS, Shalai YaR, Manko BO, Manko BV, Ostapiuk YuV, Kulachkovskyy OR, Obushak MD, Stoika RS, Babsky AM. Effects of thiazole derivatives on intracellular structure and functions in murine lymphoma cells. *Ukr Biochem J*. 2020; 92(2): 121-130.
19. Shalai YaR, Popovych MV, Kulachkovskyy OR, Hreniukh VP, Mandzynets SM, Finiuk NS, Babsky AM. Effect of novel 2-amino-5-benzylthiazole derivative on cellular ultrastructure and activity of antioxidant system in lymphoma cells. *Studia Biologica*. 2019; 13(1): 51-60.
20. Kozak MR, Ostapiv DD, Mitina NY, Petruh IM, Volianiuk KA, Zaichenko AS, Vlizlo VV. An influence of complexes of therapeutic antisense oligodeoxynucleotides with cationic polymers on cell respiration. *Biopolym Cell*. 2021; 37(5): 357-368.
21. Finiuk NS, Popovych MV, Shalai YaR, Mandzynets' SM, Hreniuh VP, Ostapiuk YuV, Obushak MD, Mitina NE, Zaichenko OS, Stoika RS, Babsky AM. Antineoplastic activity *in vitro* of 2-amino-5-benzylthiasol derivative in the complex with nanoscale polymeric carriers. *Cytol Genet*. 2021; 55(1): 19-27.
22. Mitina NYe, Riabtseva AO, Garamus VM, Lesyk RB, Volyanyuk KA, Izhyk OM, Zaichenko OS. Morphology of the micelles formed by a comb-like PEG-containing copolymer loaded with antitumor substances with different water solubilities. *Ukr J Physics*. 2020; 65(8): 670.
23. Horbay RO, Manko BO, Manko VV, Lootsik MD, Stoika RS. Respiration characteristics of mitochondria in parental and giant transformed cells of the murine Nemeth-Kellner lymphoma. *Cell Biol Int*. 2012; 36(1): 71-77.
24. Moldogazieva NT, Mokhosoev IM, Terentiev AA. Metabolic Heterogeneity of Cancer Cells: An Interplay between HIF-1, GLUTs, and AMPK. *Cancers (Basel)*. 2020; 12(4): 862.
25. Fogg VC, Lanning NJ, Mackeigan JP. Mitochondria in cancer: at the crossroads of life and death. *Chin J Cancer*. 2011; 30(8): 526-539.
26. Kuznetsov AV, Margreiter R, Amberger A, Saks V, Grimm M. Changes in mitochondrial redox state, membrane potential and calcium precede mitochondrial dysfunction in doxorubicin-induced cell death. *Biochim Biophys Acta*. 2011; 1813(6): 1144-1152.
27. Galluzzi L, Larochette N, Zamzami N, Kroemer G. Mitochondria as therapeutic targets for cancer chemotherapy. *Oncogene*. 2006; 25(34): 4812-4830.
28. Zhao RZ, Jiang S, Zhang L, Yu ZB. Mitochondrial electron transport chain, ROS generation and uncoupling (Review). *Int J Mol Med*. 2019; 44(1): 3-15.