Ewelina Humeniuk

SUMMARY

Neoplastic diseases, including malignant melanoma, are one of the most serious challenges in the 21st century medicine. The greatest therapeutic problem in the case of malignant melanoma is the treatment of advanced disease with metastatic lesions. Despite the continuous development of new methods of treatment, including immunotherapy and targeted therapy, new solutions are sought that involve classic treatment methods such as X-rays and chemotherapeutic agents. The prospect of combining the classically used therapeutic methods with substances enhancing their pharmacological effect seems promising. However, the selection of the appropriate pairs of compounds must be based on the knowledge of the mechanisms taking place in the cell undergoing neoplastic transformation. The Warburg effect is one of the interesting changes taking place in a neoplastic cell and involves the activation of glycolysis, despite the presence of oxygen. There are several hypotheses concerning the causes of the Warburg effect, including damage to the mitochondrial function, as well as insufficient ATP production in the process of oxidative phosphorylation in relation to the energy demand of the tumor cell. It can be argued that compounds disrupting the functioning of mitochondria can sensitize cancer cells with properly functioning mitochondria to classically used treatments such as cytostatics and X-rays.

The aim of the study was to assess the possibility of sensitizing malignant melanoma cells to the classically used cytostatics from groups with different mechanisms of action and X-rays, through the use of model compounds disrupting the functioning of the mitochondria. In addition, we attempted to evaluate the metabolic phenotype profile of the malignant melanoma cell lines used in the study of malignant melanoma cell lines in the context of ATP extraction via mitochondrial respiration or glycolysis, and to assess whether any possible synergistic effect depends on the metabolic phenotype profile.

The experiments were performed with the use of the three malignant melanoma cell lines G-361, CRL-1619 and SK-MEL28. Three model compounds disrupting the functioning of mitochondria with different mechanisms of action were used in the research: 2,4-dinitrophenol (2,4-DNP), ethanol and potassium cyanide. 2,4-DNP in a concentration of 100 μM was selected for further studies of the combined effect with classical cytostatics and X-rays. The optimal concentrations of fifteen cytostatics for combined activity studies with 2,4-DNP were selected on the basis of the MTT assay, while the X-ray dose was selected based on cell cycle analysis. The incubation time of the cells with the test compounds was 48 hours. The evaluation of the metabolic phenotype profile was performed by measuring glucose concentration and lactate concentration in the culture medium at three time points during the 72-hour incubation of the tested malignant melanoma cells. Moreover, the mitochondrial

density was assessed by means of fluorescent staining and the ATP level in the tested lines under the influence of 2,4-DNP as a tool substance was assessed. Assessment of the effect of combined 2,4-DNP at a concentration of 100 μM and of classic cytostatics on malignant melanoma cells was performed using the MTT test. If a synergistic effect was observed, the cell cycle analysis was performed. The effect of combined 2,4-DNP and 10 Gy of X-rays was assessed on the basis of the MTT test, CVS test, and the analysis of cell morphology and the cell cycle.

Based on the research, the following conclusions have been drawn: (1) A differentiated response of the three examined malignant melanoma cell lines to the influence of several of the cytostatics used was observed. It cannot be excluded that the differential response is due to the metabolic phenotype; (2) Different types and activities of metabolic pathways were noted in the three studied lines of melanoma in terms of the dynamics of glucose consumption and lactate synthesis; the percentage share of substrates other than exogenous glucose in the synthesis of lactate, the use of exogenous glucose for purposes other than lactate synthesis, and oxidative phosphorylation. Such metabolic phenotypes may constitute an additional criterion for therapeutic decisions in the future; (3) The synergistic effect of 2,4-DNP was found with six out of fifteen cytostatics tested against the melanoma line G-361, one cytostatics against the CRL-1619 line, and no such synergy was demonstrated in any of the cytostatics tested for the SK-MEL28 line. Further research should focus on determining whether or not the demonstrated synergy is due to the metabolic phenotype; (4) It was demonstrated that 2,4-DNP did not increase the cytotoxic effect of X-rays in the three examined malignant melanoma lines.