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ASSESSMENT OF THE MECHANISMS OF CYTOTOXIC INTERACTION OF GLYCOLYSIS INHIBITORS AND HISTONE DEACETYLASE INHIBITORS (HDACI) IN AN IN VITRO MODEL OF GLIOBLASTOMA

Dissertation for the degree of doctor of medical sciences

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Summary

Assessment of the mechanisms of cytotoxic interaction of glycolysis inhibitors and histone deacetylase inhibitors (HDACi) in an in vitro model of glioblastoma

Tumor cells preferentially use the glycolysis process as the main pathway for glucose utilization and source of ATP regardless of the availability of oxygen (Warburg effect). GBM cells are mainly dependent on this process. An anti-cancer strategy is the inhibition of glucose metabolism by glycolysis inhibitors, e.g., 2-deoxy-D-glucose (2-DG). Unfortunately, therapeutic use of 2-DG is limited due to insufficient pharmacokinetic parameters of the compound. However, the acetylated derivative of 2-DG - WP1122 shows significantly better pharmacokinetic parameters.

Moreover, regulation of gene expression also plays an important role in tumor development - acetylation/deacetylation of histones, catalyzed by acetyltransferases (HAT) and histone deacetylases (HDAC). Overexpression of HDAC causes a decrease in histone acetylation and an abnormal silencing of the transcription of many genes. Therefore, HDAC inhibitors are considered potential anti-cancer drugs. The most frequently studied HDACis are sodium butyrate (NaBt) and sodium valproate (NaVPA).

This study aimed to analyze the effects and molecular mechanism of cooperation of glycolysis inhibitors (2-DG, WP1122) with histone deacetylases inhibitors (NaBt, NaVPA) in an in vitro glioblastoma model. The cytotoxic effect of the mentioned compounds was assessed based on the parameters of changes in viability (MTS test, IC50 determination), proliferation (BrdU test), and the intensity of protein biosynthesis (SRB test) of GBM cells (U-87 and U-251 cell lines). Obtained results showed that all tested compounds, depending on the dose and time, statistically significantly reduced the viability of both cell lines. In addition, combined treatment of GBM cells with compounds with distinct mechanisms of action enhances the cytotoxic effect. The sensitivity of cells to the cytotoxic effect of the tested compounds was not significantly different under normoxia and hypoxia-like conditions, which proves the strongly glycolytic metabolism of GBM cells.

The molecular mechanism of glycolysis inhibitors' action has been confirmed by evaluating lactate and ATP synthesis, which was significantly downregulated in response to 2-DG and WP1122 treatment compared to untreated cells. On the other hand, the specificity of HDACi confirmed the analysis of HDAC activity in GBM cells. Further investigation showed that cytotoxic action of analyzed compounds was mediated by apoptosis, but not autophagy process activation.

The combined treatment of GBM cells with glycolysis and histone deacetylases inhibitors synergistically potentiated their cytotoxic effects. On the other hand, the WP1234

derivative containing ethyl-butyrate substituent revealed its dual mechanism of cell death induction via glycolysis inhibition and concomitant modulation of histone acetylation and pro-/antiapoptotic proteins expression.

Summarizing the obtained results, it is evident that verified in the Ph.D. thesis strategy of simultaneous inhibition of glycolysis process and histone deacetylases activity appeared as an effective method of cancer cells elimination via physiological apoptosis process induction. Furthermore, GBM cells elimination was induced by combining 2-DG or WP1122 with NaBt or NaVPA, as well as, by bifunctional WP1234 derivative. Bearing in mind the ability of tested compounds to cross the blood-brain barrier and the limitations of currently available GBM therapies, the above-described therapeutic strategy should be further developed in preclinical and clinical studies as a potential therapy for GBM patients.