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COMPARATIVE ANALYSIS OF CYTOTOXIC ACTIVITY OF 2-DEOXY-D-GLUCOSE (2-DG) ANALOGS ON THE IN VITRO MODEL OF GLIOBLASTOMA MULTIFORME

Dissertation for a degree of doctor of medical sciences

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Studies were performed in: *Laboratory of Genetics and Molecular Biology, Military Institute of Hygiene and Epidemiology in Warsaw*

Warsaw, 2023

Summary

Despite the real breakthrough observed in the last 20 years in a field of anticancer drug development, glioblastoma (GBM) therapy is still ineffective and has not changed significantly since 1999. Brain tumors, especially gliomas, are one of the most aggressive and difficult cancers to treat due to its location, high degree of cell heterogeneity and drug resistance.

Cancer cells, especially GBM cells, preferentially use glycolysis process as a source of ATP, even under normoxic conditions (Warburg effect). Glycolysis inhibition leads to energy production inhibition, energy deficyt and in consequence to death and selective elimination of cancer cells and thus it is a promissing target in cancer therapy.

2-DG is the best known glycolysis inhibitor which crosses the blood-brain barrier and enters cells using the same transporters as glucose. However, due to its insufficient pharmacokinetic properties, this compound was not registered in clinic. New halogen 2-DG analogs (fluoro-, iodo-, chloro-, bromo-) and acetylated derivative, WP1122, are believed to have improved penetration and accumulation of compounds in cancer cells. In consequence lower treatment concentrations could become effective.

The aim of this study was to compare and determine molecular mechanism of the cytotoxic effect as well as to evaluate the influence of substituent of halogen 2-DG analogs (2-FG, 2-IG, 2-CG, 2-BG) and acetylated 2-DG analog, WP1122, on GBM U-251 and U-87 cell lines. Moreover, the aim of this research was to analyze the influence of 2-DG derivatives on HK II activity as well as to obtain recombinant human HK II protein in a bacterial system and to analize its interaction with halogen derivatives by X-ray crystallography.

Within the project, cellular viability in normoxia and simulated hypoxia conditions (MTS test), proliferaton (BrdU test) and protein synthesis (SRB test) upon 72 h of incubation with test compounds was analyzed in vitro on glioblastoma model. Obtained results showed that all compounds, depending on dose, statistically significantly reduced viability of both cell lines. Intensity of lactate production as well as hexokinase activity downregulation analyzes confirmed the mechanism of action by inhibiting glycolysis by all 2-DG analogs. Further analysis that cytotoxic action of analyzed compounds was mediated by apoptosis but not autophagy process activation, which had protective role for treated cells. Sensitivity of cells to the cytotoxic effect of tested compounds was not significantly different under normoxia and hypoxia-like conditions what proves the strongly glycolytic metabolism of GBM cells.

Additionally, activity assays of HK II upon extracellular interaction with tested compounds showed that fluorine analogs are the strongest HK II inhibitors. Crystallization and X-ray crystallographic analysis of 2-DG analogs were also performed. Furthermore, recombinant human HK II protein was obtained in *E. coli* bacterial system and then purified by IMAC and SEC. There were also attempts on crystallization of the obtained HK II.

Summarizing the results obtained as part of doctoral dissertation, it should be stated that although all tested compounds showed cytotoxic activity against GBM cells, WP1122 and fluorine derivatives turned out to be the strongest inhibitors of glycolysis and promising drug candidates for future therapy. Chloro- and bromo-analogs did not exert significant cytotoxic effect. Due to the high genetic variability of cancers, targeting the universal and basic cellular pathway of glucose utilization and ATP synthesis seems to offer the great opportunity to eliminate these cells. Taking into account the ability to penetrate the blood-brain barrier by 2-DG analogs and limited available GBM therapy, the development of 2-DG analogs should be continued in further preclinical and clinical stages, especially in the context of combined glioblastoma therapy.