Summary

Anaplastic astrocytoma and glioblastoma are central nervous system tumors with extremely poor prognosis. Current treatment allows only to prolong survival, and enhance the quality of patient's life. Due to their infiltrative nature, they are practically impossible to remove surgically, and resistance mechanisms are often observed during radio- and chemotherapy. For this reason, new compounds that can support the applied therapy are sought. Considering the widespread availability and wide spectrum of activity, a lot of attention is paid to natural coumarins. It has been shown many times that the conjugation of the action of these compounds with chemotherapy enhances the anti-cancer effect of cytostatics. The cytotoxicity of coumarins towards cancer cells depends on their chemical structure; therefore, understanding the effect of various substituents on the anti-tumor properties of these compounds will result in more effective planning of therapeutic strategies. Therefore, in this study an attempt was made to determine the influence of the presence and location of specific substituents on the antitumor activity of selected simple coumarins (osthole, 4-hydroxycoumarin, umbeliferon and esculin) and furanocoumarins (imperatorin, isoimperatoryin, bergapten and xanthotoxin).

At the molecular level, gliomas are characterized by the presence of mutations within genes, the products of which are involved in enhancement of intracellular signal transmission from the cell membrane to the nucleus. This applies in particular to the pro-survival pathways responsible for the regulation of cell proliferation and differentiation: RAS-RAF-MEK-ERK and PI3K-AKT/PKB-mTOR. It has been described that blocking the signal transmission would be beneficial in increasing glioma cell sensitivity. It is also known that combination therapy, especially with natural compounds, would increase the anticancer potential of clinically used pharmacotherapy. Therefore, the effect of the studied coumarins was coupled with temozolomide - a drug currently used in the treatment of glioblastomas and with inhibitors of the RAS-RAF-MEK-ERK (sorafenib) and PI3K-AKT / PKB-mTOR (LY294002) pathways.

The microscopic analyzes performed showed that all coumarins induced programmed death in human glioblastoma cells (MOGGCCM and T98G), and dominated type of death was apoptosis. It was also observed that the sensitivity of glioblastoma cells to the applied therapy largely depended on the cell line used and the structure of the tested compounds. The higher pro-apoptotic activity of both simple coumarins and furanocoumarins was due to the presence of isoprenyl group in the structure of osthole and imperatorin. On the other hand, the replacement of the methoxy substituent (present in the osthole molecule) with the furan ring

(present in the imperatorin structure) decreased the cytotoxicity of the compound in relation to both tested cell lines. The simultaneous application of temozolomide with tested coumarins significantly reduced the level of autophagy. As revealed by the immunoblotting technique, the pro-apoptotic activity of ostole and imperatorin, alone and in combination with temozolomide, was associated with the increased level and activity of caspase 3, and the reduce amount of the anti-apoptotic protein Bcl-2. Induction of autophagy, in turn, was accompanied by an increase in the level of beclin 1. Immunoprecipitation also showed that the presence of the Bcl-2: beclin 1 complexes played a key role in directing the lethal signal to the apoptotic pathway.

The conducted studies have shown that both osthole and imperatorin had the ability to reduce the level and activity of proteins involved in the transmission of the survival signal from the cell membrane to the nucleus, which are part of the RAS-RAF-MEK-ERK and PI3K-AKT / PKB-mTOR pathways. Moreover, combination therapy using inhibitors of these pathways: sorafenib (RAF inhibitor) and LY294002 (PI3K inhibitor) significantly decreased glioma cell resistance to induction of programmed death by osthole and imperatorin. Blocking the expression of these kinases with specific siRNAs further increased the sensitivity of glioblastoma cells to the induction of programmed death. Moreover, in addition to their pro-apoptotic properties, these compounds reduced the migration potential of glioblastoma cells.

Taking into account the widespread availability and high antitumor potential of the studied coumarin derivatives, the obtained results may constitute the basis for the development of new therapeutic strategies, sensitizing glioblastoma cells to the induction of programmed death. Understanding the function of particular substituents in the antitumor activity of coumarin derivatives will, in turn, facilitate the construction and prediction of the effects of their application.

Key words: furanocoumarins, simple coumarins, gliomas, programmed cell death, temozolomide, sorafenib, LY294002

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