SYNERGETIC EFFECT OF HETEROCYCLIC COMPOUND WITH ANTITUMOR DRUG

Tatsiana A. Gurinovich, Anastasia V. Kvachonak, Margarita O. Dosina, Svetlana G. Pashkevich

Institute of Physiology, National Academy of Sciences of Belarus, 28 Academicheskaya Str., 220072 Minsk, Belarus
gurinovich.tanya@gmail.com

Treatment of malignant neoplasms with cytotoxic drugs causes various side effects and fatal toxic complications. High resistance of malignant neoplasm cells to chemotherapy drugs, presence of multipotent chemo-and radio-resistant stem cells in neoplasm, metastasis and recurrence of tumors require the search for new approaches to increase the selective inhibition of tumor growth [1].

The purpose of the study was to assess the effectiveness of the combined application of heterocyclic compounds with chemotherapy drugs on the possibility of potentiating the effect of reduced dosage of a chemotherapy drug. Water soluble Li salts of comenic acid conjugates with isoxazole scaffold (4-oxo-5-((5-phenylisoxazol-3-yl)methoxy)-4H-pyran-2-carboxylate) were used for the study, which was synthesized in the Institute of Physical Organic Chemistry, the National Academy of Sciences of Belarus [2]. The use of water-soluble Li forms is due to preference for subsequent biotesting, as well as Li cations have a cytotoxic enhancing effect in contrast to other monovalent or divalent cations [3].

To evaluate the synergistic activity of the combined application of conjugate of the heterocyclic compounds with the antitumor drug Temobel® (Temozolomid), studies were carried out on C6 glioma cells in 96-well (in vitro) [2]. The initial concentration of C6 glioma cells was 5000 cells per well of the plate. The cells were cultured in F10 nutrient medium supplemented with 10% bovine fetal serum. The Vybrant MTT Cell Proliferation Assay Kit (Thermo Fisher Scientific, Lithuania) was used to determine the viability of cells [4]. The absorbance of the contents of the wells was measured on an automatic biochemical immuno-fermental analyzer ChemWell® 2910 (Combi) using ChemWell® software version 6.3 (Revision A), USA. An antitumor effect was evaluated for the recommended therapeutic dose of Temobel® 100 μg per 250 μl, as well as for a dose of 10 μg and 1,0 μg. Water soluble Li salts of comenic acid conjugates with isoxazole scaffold was used in the indifferent dosages which did not exhibited any cytotoxic effect.

With the application of Temobel® at a dose of 100 μg to C6 glioma cells, about 40% of the cells died within 28 h. While using a dose of 10 μg and 1.0 μg, there were no significant differences in the number of dead cells from their spontaneous death after 28 h (cell death of 5-8%). With a combined application of Temobel® at a dose of 10 μg and 1 μg with isoxazole conjugate at doses of 1.0 and 0.1 μg, a significant ($p < 0.05$) death of tumor cells (20 – 25%) was revealed, which indicates the manifestation conjugate of the synergistic effect. It is especially important that such an impressive cell death occurs only 28 h after the application of Temobel® in subthreshold concentrations with water soluble Li salts of comenic acid conjugates with isoxazole scaffold, which in the indifferent dosages used did not exhibited any cytotoxic effect.

The results obtained will be useful for further research in this area and for the development of new effective drugs and chemotherapy with reduced therapeutic doses.

[1] Li, Y. J. Autophagy and multidrug resistance in cancer / Y. J. Li, Y. H. Lei, N. Yao, C. R. Wang, N. Hu, W. C. Ye, D. M. Zhang, Z. S. Chen // Chinese Journal of Cancer. – 2017. – Vol.36, №1. – P.52-62.

[2] Kletskov, A. V.Synthesis and Biological Activity of Novel Comenic Acid Derivatives Containing Isoxazole and Isothiazole Moieties / A. V. Kletskov, V. I. Potkina, I. A. Kolesnika, S. K. Petkevicha, A. V. Kvachonakb, M. O. Dosinab, D. O. Loikob, M. V. Larchenkob, S. G. Pashkevich and Vladimir A. Kulchitsky// Natural Product Communications, Vol. 13 (11) 2018, 1507-1510

[3] R. Beyaert, C. De Potter, B. Vanhaesebroeck, F. Van Roy, and W. Fiers (1991) Induction of inflammatory cell infiltration and necrosis in normal mouse skin by the combined treatment of tumor necrosis factor and lithium chloride. The Americal Journal of Pathology. 1991 Mar; 138(3): 727–739.

[4] L. A. Quayle, P. D. Ottewell, I. Holen (2018) Chemotherapy resistance and stemness in mitotically quiescent human breast cancer cells identified by fluorescent dye retention. Clinical and Experemental Metastasis. 2018; 35(8): 831–846.