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Usnic acid derivative antitumor, antimetastatic, and hematoprotective effects in the Lewis carcinoma model

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Motivation and Aim: The search and development of inhibitors of DNA repair pathways is one of the main priorities of medical chemistry, since DNA repair inhibitors can ensure effective treatment of oncological diseases. Tyrosyl-DNA phosphodiesterase 1 (Tdp1) is a promising molecular target because it participates in the repair of several types of DNA damage caused by anticancer drugs widely used in clinic [1]. Thus, Tdp1 inhibitors can increase the effectiveness of treatment of various types of cancers. Olaparib is an effective clinically approved poly(ADP-ribose) polymerase 1 (PARP1) inhibitor. Its mechanism of action is based on preventing the synthesis of poly-ADP-ribose, thereby blocking the response to DNA damage. When used as monotherapy and in combination with conventional chemotherapy drugs, it inhibits the growth of certain tumor cell lines *in vitro* and tumor growth *in vivo*. Poly(ADP-ribose)polymerase 1 (PARP1) is one of the main regulators of the repair of single-stranded DNA breaks, thus playing an important role in maintaining genome stability and cell survival. It is known that PARylation of Tdp1 enhances its recruitment to sites of DNA damage without affecting the catalytic activity of Tdp1 [2]. PARP1 stimulates Tdp1 enzymatic activity at AP-sites. Thus, the combined use of PARP1 and Tdp1 inhibitors may be a promising therapeutic strategy for the treatment of various cancers. The development of new inhibitors of DNA repair enzymes based on natural compounds and their derivatives is particularly relevant, since such compounds often have complementarity to targets of biological origin.

A promising biologically active compound is usnic acid. It is a secondary metabolite of various lichen species with a wide range of biological activity (anti-inflammatory, antiviral, antitumor, etc.) [3]. By itself, usnic acid is quite toxic. Modifications of its structure, developed by our team, provide a reduction in toxicity and effective binding to the active center Tdp1. The derivative with chemical modifications of rings A and C was synthesized on the basis of usnic acid. The introduction of cyanogroups attached via long aliphatic linkers ensures binding to the enzyme, and the introduction of a pyrazole fragment into the C ring reduces the toxicity of the compound compared to the original usnic acid (toxicity is absent in the whole range of studied concentrations to 100 μM) [3]. According to the results of screening of the inhibitory activity of this compound with respect to purified recombinant Tdp1, it can be noted that it has a high inhibitory activity (IC_{50} = 2.9 ± 0.8 μM) [3, 4].

Methods and Algorithms: In the research, we used female mice of the C57BL/6 line (35 individuals) weighing 19–21 g. We transplanted an experimental Lewis lung carcinoma tumor model into mice intramuscularly. The animals were divided into five

groups: the control group without treatment; the group that was injected with vehicle (DMSO + Tween-80); the group that was injected with olaparib; the group that was injected with olaparib and Tdp1 inhibitor; the group that was injected with Tdp1 inhibitor only. The effect of the drugs was evaluated at the end of the experiment by the weight and size of the tumor, the quantity of metastases, as well as by the weight of the liver and spleen. Moreover, we calculated erythrocyte count, leukocyte count and leukocytic formulas.

Results: The derivative of usnic acid has a more pronounced antitumor and antimetastatic effect compared to olaparib and their combination. The Tdp1 inhibitor had no toxic effect on the internal organs of mice. The injection of the Tdp1 inhibitor leads to the normalization of hematopoiesis. At the end of the experiment blood levels were close to normal values.

Conclusion: This usnic acid derivative is promising prototype for anticancer drugs development. It is a non-toxic compound and has pronounced antitumor, antimetastatic and hematoprotective activity.

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