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Tyrosyl-DNA phosphodiesterase 1 Inhibitors as topotecan sensitizers

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Motivation and Aim: Topotecan is a cytostatic drug widely used in the treatment of cervical, ovarian [1] and small cell lung cancer [2]. This drug, getting into the cell, leads to DNA damage due to the ability to stabilize covalent complexes of topoisomerase 1 (Top1)-DNA [3], arising during the catalytic reaction of the enzyme. The accumulation of damage eventually leads to the death of tumor cells. However, there are mechanisms for removing such adducts in the cell, for example, the enzyme tyrosyl-DNA phosphodiesterase 1 (Tdp1) can hydrolyze the covalent bond of Top1-DNA [4], which reduces the effectiveness of Top1 inhibitors used in clinical practice. Therefore, the additional use of Tdp1 inhibitors in chemotherapeutic cocktails is a promising strategy for increasing the sensitivity of tumor cells to topotecan. Previously, it was shown that disaccharide nucleosides with lipophilic groups and similar monosaccharide derivatives inhibit Tdp1 in the range of submicromolar concentrations [5, 6]. Thus, the aim of the work was to study a series of new lipophilic nucleoside derivatives as Tdp1 inhibitors for the further development of drugs capable of sensitizing tumor cells to topotecan.
Methods and Algorithms: To evaluate the inhibiting effect of lipophilic nucleoside derivatives on the purified recombinant human Tdp1 enzyme, we conducted fluorescent screening and kinetic experiments, using a fluorescent method based on Tdp1's ability to remove the BHQ1 fluorescence quencher from the 3'-end of the oligonucleotide. To study the biological properties of the most active inhibitors, we identified their toxicity and the synergistic effect with the antitumor drug topotecan *in vitro* and *in vivo*.
Results: Effective Tdp1 inhibitors with IC50 from 0.18 to 6 μM were found among a series of lipophilic nucleoside derivatives. It was shown that the studied compounds are non-toxic in the concentration range up to 100 μM, and five of them increased the cytotoxic effect of topotecan on HeLa cells. Treatment of HeLa cells with a combination of topotecan and a leader compound resulted in an increase the level of DNA damage compared to the use only topotecan. Mice with ascitic carcinoma Krebs-2 treated with topotecan and the leader compound showed a significant decrease in the mass of ascites and the number of tumor cells in it compared with the control group of mice who did not get a therapy.
Conclusion: Lipophilic nucleoside derivatives are a promising class of Tdp1 inhibitors. A non-toxic compound capable to sensitize tumor cells towards the topotecan *in vitro* and *in vivo* was found in the investigated series. This leader compound is a promising candidate for the development of antitumor drugs that increase the sensitivity of tumors to the Top1 inhibitor used in clinical practice.

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