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Usnic acid derivative – Tdp1 inhibitor enhances the antitumor and antimetastatic effects of topotecan and normalizes hemopoiesis *in vivo*

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Motivation and Aim: Tyrosyl-DNA phosphodiesterase 1 (Tdp1) is an important DNA repair enzyme and one of the causes of tumor resistance to topoisomerase 1 inhibitors such as topotecan [1–4]. Inhibitors of this Tdp1 in combination with topotecan may improve the effectiveness of therapy. In this work, we synthesized a derivative of usnic acid, which is a hybrid of its known derivatives: tumor sensitizers to topotecan [5]. New compound inhibits Tdp1 in the submicromolar concentration range and enhances the effect of topotecan on the metabolic activity of cells of various lines according to the MTT test, while being non-toxic. *In vivo* experiments, this compound not only sensitizes tumors of mice to the action of topotecan, but also normalizes the state of the peripheral blood of mice [6].

Methods and Algorithms: The inhibitory properties of the compound (AF-185) on the purified Tdp1 were studied using a technique developed previously by our team [7]. A 16-mer single-stranded oligonucleotide carrying a fluorophore at the 5′-end and a quencher at the 3′-end was used as a biosensor. When within the Förster radius, the quencher suppressed the fluorescence of the fluorophore. The quencher was removed by Tdp1, resulting in fluorescence emission.

The cytotoxic/antiproliferative properties of the synthesized compound were studied using a standard MTT test on cell lines A-549, HCT-116, HeLa, MCF-7, T98G, MRC-5 and HEK293A. The ability of the usnic acid derivative to enhance the effect of topotecan was studied on the same panel of cell lines using a standard MTT-test.

In *in vivo* experiments, we studied the ability of AF-185 to enhance the effect of topotecan on murine Lewis lung carcinoma (LLC) and Krebs-2 carcinoma. LLC model is the only reproducible syngeneic murine model for lung cancer. This model is anaplastic, highly tumorigenic, and immunologically compatible with the murine system [8–10]. Subcutaneous transplantation leads to the development of a primary node at the injection site and the appearance of metastases in the lungs on days 17–21 [11]. The effect of the drugs was evaluated at the end of the experiment by the weight and size of the tumor, the quantity of lung metastases.

Krebs-2 carcinoma has a high degree of malignancy. The ascitic form of Krebs-2 leads to death on 14–18 days. The tumor is weakly sensitive or insensitive to the action of cytostatics [12]. The effect of the treatment was evaluated at the end of the experiment

by the weight of the tumor, the concentration of tumor cells in the ascitic fluid. In addition, we calculated the concentration of erythrocytes and leukocytes in the blood of mice.

Results: Half maximal inhibitory concentration (IC_{50}) of the usnic acid derivative in relation to Tdp1 was 0.12 ± 0.01 μM. Whereas, semitoxic concentration (CC_{50}) of the compound in relation to cell lines (CC_{50}, μM) was > 100 μM. Usnic acid derivative turned out to be promising sensitizer of the action of topotecan on cultured cell lines (data is presented in Table 1).

Table 1. CC_{50} values for topotecan in the presence of 5 μM Tdp1 inhibitor (AF-185), μM

	MRC-5	HeLa	HEK293A	HCT-116	A-549	MCF-7	T98G
Tpc+185	>10	4.6 *	0.033	0.21 *	0.25 *	0.63 *	1.3

* Differences in the presence and absence of the Tdp1 inhibitor are significant according to the Mann–Whitney test at a minimum of two (or three) concentrations of topotecan, $p < 0.05$; ** nd – not determined. A 50 % inhibition of cell survival was not achieved.

In the Lewis lung carcinoma model, it was shown that the administration of topotecan (1 mg/kg intraperitoneally, i/p) reduced primary node size; the tumor growth inhibition (TGI) value was 36.4 %, compared to intact control. The use of the combination of topotecan with 50 mg/kg AF-185 (intragastrical administration of AF-185, i/g) leads to a further reduction in tumor weight, TGI was 47.7 %. The administration of AF-185 i/g by itself did not lead to a decrease in tumor weight. The best results were obtained for the combination of topotecan with AF-185 (50 mg/kg, i/p), TGI was 57.2 %; the difference was significant both in comparison with both controls and with the topotecan group ($p < 0.05$). The effect of AF-185 on the antimetastatic properties of topotecan was less pronounced. At the dose used, topotecan had virtually no effect on the number of metastases in the lungs, and the number of metastases was not affected by the use of AF-185 both i/p and i/g, as well as the combination of topotecan with AF-185 i/g. The combination of topotecan with AF-185 i/p reduced the number of metastases, although the difference with the control and with the topotecan group was insignificant. Thus, the use of AF-185 in mono mode has no effect on either the growth of the primary node or the number of metastases in the lungs. In combination with topotecan, there was a tendency to reduce the weight of the primary tumor and the number of metastases, which was more pronounced with intraperitoneal administration of AF-185.

In the Krebs-2 ascitic carcinoma model, it was shown that the combination of topotecan and AF-185 i/p was more effective in terms of ascites weight and the number of tumor cells in ascitic fluid than the same combination with AF-185 i/g. Also, the combination of topotecan and AF-185 i/p had a more pronounced antitumor effect than topotecan, AF-185 i/p, and AF-185 i/g separately. Mice with Krebs-2 carcinoma (intact control) have an increased number of leukocytes and a decreased number of erythrocytes compared to healthy mice. An increase in white blood cell count in the peripheral blood is associated with the inflammatory process that develops during tumor formation and growth. The administration of solvent for AF-185 (DMSO + Tween-80), topotecan alone, as well as topotecan in combination with AF-185 i/g, slightly reduced the number of leukocytes. It should be noted that topotecan at the dose of 1 mg/kg was not hemotoxic and partly normalized the concentrations of leukocytes and erythrocytes, apparently due to its antitumor effect. Treatment of mice with only AF-185, regardless of the route of administration, reduced the number of leukocytes somewhat more effectively than the drugs listed above, but the decrease was significant only in comparison with the control

without treatment. Administration of topotecan in combination with AF-185 i/p significantly reduced the number of leukocytes compared to the control without treatment, the control with DMSO +Tween-80 and the group of mice receiving only topotecan.

Mice with Krebs-2 carcinoma have a reduced number of red blood cells compared to healthy mice, which may be caused by general intoxication of the body. The number of red blood cells practically did not change with the introduction of the solvent. At the same time, treatment with drugs and their combinations significantly increased the number of erythrocytes, regardless of the method of administration of AF-185. The largest effect was again exerted by the combination of topotecan and AF-185 i/p, which increased the number of erythrocytes to healthy control. Thus, the blood cell counts returned to normal values (red blood) or significantly improved (white blood) after treatment with combination Tpc + AF-185 i/p, i.e., hematopoiesis was normalized.

Conclusion: Thus, AF-185 has antitumor and antimetastatic effects, normalizes hemopoiesis and may be the prototype of a new class of additional therapy for cancer.

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