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COMPARATIVE INVESTIGATION OF THE EFFECT OF UKRAIN ON GROWTH OF ASCITE AND SOLID FORMS OF EHRLICH'S CARCINOMA

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Ukrain (NSC-631570) is a cytostatic and immunomodulating semisynthetic compound of thiophosphate-modified alkaloids of *Chelidonium majus L.* It has selective cytotoxicity against cancer cells without healthy cells damaging. Dosage range, methods of introduction and duration of administration of the drug vary. *Aim:* To carry out comparative investigation of effect of Ukrain on growth of different form of Ehrlich's carcinoma. *Methods:* Ehrlich's carcinoma cells were transplanted intraperitoneally and subcutaneously between the scapulas. Ukrain was administered intraperitoneally for 6 days (0.25 mg per mice of 20 g, it's 0.1 LD50). The effect of drug on tumor growth was evaluated by the indexes of tumor growth inhibition (in the case of solid form), total number of tumor cells in ascites, number of viable tumor cells (in the case of ascitic form) and average life span of experimental animals. Cell cycle distribution of cancer cells was determined by flow cytometry. The number of circulating phagocytes was determined by flow cytometry with use of FITC-labelled S. aureus Cowan. *Results:* Intraperitoneal Ukrain administration in mice with ascite form of Ehrlich's carcinoma resulted in moderate tumor growth retardation, but was accompanied by acute local inflammation and caused reduction of life span of experimental animals. In mice bearing solid form of Ehrlich's carcinoma treatment with Ukrain led to significant tumor growth inhibition and slight increase of life span. In mice bearing both of tumor variants treatment with drug caused restitution of the number of circulating phagocytes in peripheral blood, more expressed in mice bearing ascite tumor variant. *Conclusion:* Thus, anticancer activity of the Ukrain is more expressed in the case of solid variant of Ehrlich's carcinoma. This effect is mediated by direct apoptotic action of drug on Ehrlich's carcinoma cells and positive immunomodulating effect on tumor-bearing organism.
Key Words: **Ukrain, ascite and solid forms of Ehrlich's carcinoma.**

Ukrain is the semisynthetic compound of thiophosphate-modified alkaloids from Chelidonium majus L. It has cytotoxic and cytostatic effects on cancer cells due to ability to be selectively accumulated in tumor tissue and activate apoptosis of malignant cells but not in healthy cells [1–3]. Also Ukrain possesses an ability to increase total count of T-cell and T-helper lymphocytes along with decrease of T-suppressor cells [4]. Ukrain, like other preparation from C. majus L., has modulating effect on macrophage metabolism [5, 6]. Comparative assessement of intraperitoneal, intramuscular, peroral and intravenous administration of Ukrain demonstrated maximal therapeutic efficacy of the latest. However, in experimental studies intraperitoneal and intravenous introduction are often used for systemic administration of preparation [7]. Dosage range of Ukrain as an antitumoral and immunomodulating drug is rather wide, since data on its dosage are controversial [8, 9]. The aim of our work was to carry out comparative investigation of effect of intraperitoneally administered Ukrain on growth of different forms of Ehrlich's carcinoma.

Experimental animals and experimental tumor models. In the experiments, female white inbred mice 2–3 months old weighting 18–22 g bred in the vivarium of Biological Faculty of T.G. Shevchenko Kyiv National University (Kyiv, Ukraine) were used. All animal procedures were carried out according to the rules of local Ethic Committee.

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Abbreviations used: EACC – Ehrlich's ascites carcinoma cells.

The strain of Ehrlich's carcinoma was kindly supplied by the National Bank of Cell Cultures and Transplantable Experimental Tumors of R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology (Kyiv, Ukraine). Ehrlich's carcinoma is a transplantable, poorly differentiated malignant tumor which appeared originally as a spontaneous murine breast carcinoma. It arises in both solid and ascitic forms. To receive the solid form, tumor cells (1×10^6) were inoculated subcutaneously between the scapulas at the volume of 0.1 ml of physiologic solution. To get the ascite form, tumor cells (1×10^6) were injected intraperitoneally at the volume of 0.3 ml of physiologic solution.

Treatment regimen. Next day after tumor transplantation mice were randomly distributed according to their weight and divided into different groups including: (i) control set for solid form of Ehrlich's carcinoma (n = 12); (ii) mice bearing solid form of Ehrlich's carcinoma treated with Ukrain (n = 12); (iii) control set for ascite form of Ehrlich's carcinoma (n = 20); (iiii) mice bearing ascite form of Ehrlich's carcinoma treated with Ukrain (n = 20). For mice with ascite and solid tumors, starting from the 2nd day after tumor cells inoculation, Ukrain (Nowicky Pharma, Austria) was administered intraperitoneally (25 μg/g, it's 0.1 LD50) daily for a 6 days [7, 10]. The total course dose per animal was 3000 μg. The animals from control groups received intraperitoneal injection of distilled water. Mice were under the daily observation, and their weight was registered every day.

Anticancer effect evaluation. The effect of Ukrain on growth of solid form of Ehrlich's carcinoma

was evaluated by the indexes of tumor growth inhibition and average life span of experimental animals. The tumor size was recorded (with calliper) for a 31 days. Anticancer effect was characterized by growth inhibition indexes GII, which was calculated using formula:

$$GII = ((V_k - V_e) / V_k) \times 100\%,$$

where V_k and V_e — average tumor volumes for experimental and control group correspondingly.

To estimate effect of Ukrain on tumors growth dynamics in the case of ascite form of Ehrlich's carcinoma the animal weight (monitored every day during the study) and the number of Ehrlich's ascites carcinoma cells (EACC), evaluated on the 7-th day after tumor cells inoculation and at the moment of experiment cessation (15-th day after tumor cells inoculation), were used.

Isolation of EACC from mice peritoneal cavity. The EACC were isolated from the peritoneal cavity of tumor-bearing mice (control or treated). Two to three milliliters of sterile PBS was injected into the peritoneal cavity of the mice and the peritoneal fluid containing the tumor cells was withdrawn, collected in sterile Petri dishes and incubated at 37 °C for 2 h. The cells of macrophage lineage adhered to the bottom of the Petri dishes. The non-adherent population was aspirated out gently and washed repeatedly with PBS. The EACC viability was assessed by Trypan Blue dye exclusion [11].

Cell cycle distribution of cancer cells was determined by the method of flow cytometry.

The number of phagocyting leucocytes was determined by the method of flow cytometry with use of FITC-labelled S.aureus Cowan.

Statistical analysis was performed using Wilcoxon — Mann — Whitney test. p values < 0.05 were considered significant.

Ehrlich's carcinoma is a low immunogenic tumor due to the lack of antigenicity and could to grown in all mice without alloimmunity provocation. One of the promissing therapeutic approaches for the treatment of such tumor is an elevation of its immunogenicity with the use of adjuvant and/or immunomodulating substances. Use of Ukrain — cytostatic and immunomodulating preparation — is one of the example of such therapy.

In mice with solid form of Ehrlich's carcinoma, intraperitoneal administration of Ukrain simulates systemic action of the cytotoxic drug with selective accumulation in tumor tissue and targeted antineoplastic action. At the initial stage of tumor growth statistically significant difference in tumor volumes in control and treated tumor-bearing mice was absent (Fig. 1). However, some tumor growth impairment in treated mice was observed: palpable tumors in control tumor-bearing mice appeared on ninth day after tumor cells transplantation, while in treated tumor-bearing mice — on fourteenth day. Starting from twenty-first day, stable tumor growth inhibition (by 29% at average) in mice treated with Ukain has been observed. Maximal GII value (54.6) in treated animal was registered on the 25-th day after tumor cells transplantation. At the moment of the experiment cessation the volumes of primary tumors in mice that received Ukrain, were about 30% lower compared to the control ones (GII = 41.2).

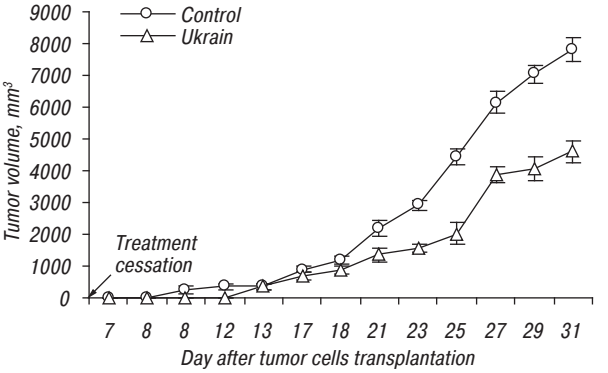

Fig. 1. Influence of intraperitoneal administration of Ukrain on the growth of solid form of Ehrlich's carcinoma (n = 12 per group)

Intraperitoneal administration of Ukrain has insignificant positive effect on survival of animals bearing solid form of Ehrlich's carcinoma (Fig. 2). The average life span in control tumor-bearing mice was 27.7 ± 0.7 days, in treated mice — 30.0 ± 0.6.

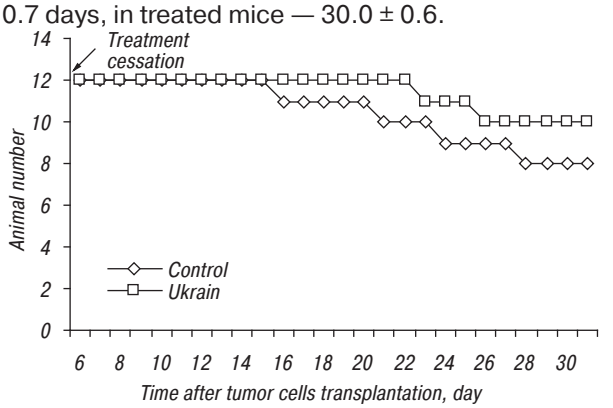

Fig. 2. Influence of intraperitoneal administration of Ukrain on life span of mice with solid form of Ehrlich's carcinoma (n = 12 per group)

Unlike, intraperitoneal administration of Ukrain has significant negative effect on survival of animals with ascite form of Ehrlich's carcinoma (Fig. 3). The average life span in treated mice was 9.2 ± 0.9 days (nearly 1.5-fold lower compared to the control group). Enhanced death rate in treated animals has been registered from the begining of treatment and till the end of experiment.

For mice bearing ascite tumor form intraperitoneal administration of Ukrain is local one. Introduction of drug at the dose of 25 μg/g to mice with ascite form of Ehrlich's carcinoma has been accompanied by moderate gain in animal weight (upon the average by 15%) throughout the treatment course (Fig. 4).

Table 1. Effect of Ukrain on cell fractions ratio in ascitic fluid of experimental animals bearing ascite form of Ehrlich's carcinoma

Animal groups	7-th day after tumor cell transplantation			15-th day after tumor cell transplantation		
	Total EACC number (x 10^6)	Percentage of viable EACC	Persentage of adherent cells	Total EACC number (x 10^6)	Percentage of viable EACC	Persentage of adherent cells
Control tumor-bearing mice (n = 20)	312.0 ± 35.4	92.9 ± 0.8	9.2 ± 3.6	482.1 ± 28.5	90.9 ± 2.5	11.5 ± 4.1
Tumor-bearing mice treated with Ukrain (n = 20)	324.1 ± 42.3	63.2 ± 2.1*	21.1 ± 2.9*	351.0 ± 41.1*	83.5 ± 2.7	19.4 ± 2.7

*In Tables 1–3: difference between control and experiment data is significant, $p < 0.05$.

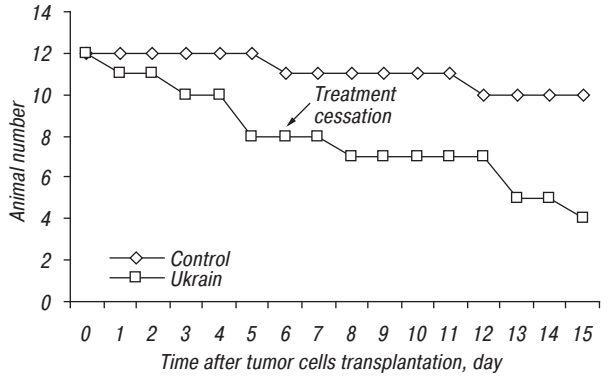

Fig. 3. Influence of intraperitoneal administration of Ukrain on life span of mice with ascite form of Ehrlich's carcinoma (n = 20 per group)

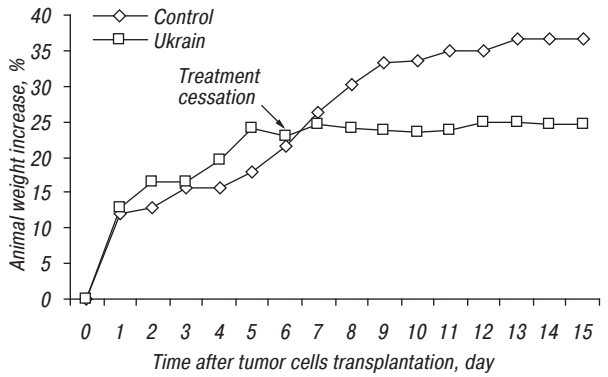

Fig. 4. Influence of intraperitoneal administration of Ukrain on the weight of animals bearing ascite form of Ehrlich's carcinoma (n = 20 per group)

Total number of EACC in tumor-bearing mice treated with Ukrain at the 7-th day after tumor cells inoculation was the same as that in control tumor-bearing mice (Table 1). However, the number of viable tumor cells was 32% lower than that in control animals. Meanwhile the number of adherent cells in ascitic fluid of treated animals was near 2 times higher in comparison with untreated tumor-bearing mice, that indicates the acute inflammation. Probably, just the acute inflammation caused accelerating of animals death. Treatment cessation resulted in gradual weight reduction in treated animals compared to control tumor-bearing mice.

Later on the weight reduction in treated animals became more significant and stable and at the moment of experiment cessation was about 40%. At this time point the total number of EACC in treated mice was 27% lower than that in untreated mice, but the number of viable tumor cells, as the number of adherent cells, did not differ significantly from the same indices in control tumor-bearing animals.

In addition, we have investigate the number of phagocyting leukocytes in peripheral blood of mice bearing solid and ascite form of Ehrlich carcinoma treated with Ukrain at the moment of experiment cessation. In mice with both solid and ascite form of tumor the number of circulating phagocytes was decreased as compared with intact animals at a lower degree in mice with solid form of Ehrlich's carcinoma (Table 2). It can be considered as a marker of tumor-mediated immunosupression.

Table 2. Phagocytic cell number in peripheral blood of mice with different forms of Ehrlich's carcinoma after the therapy with Ukrain

Animal groups	Phagocytic cell number, %
Intact animals	48.01 ± 1.45
Mice with solid form of Ehrlich's carcinoma, n = 12	40.01 ± 2.03
Mice with solid form of Ehrlich's carcinoma treated with Ukrain, n = 12	46.12 ± 2.08
Mice with ascite form of Ehrlich's carcinoma, n = 20	38.08 ± 2.09
Mice with ascite form of Ehrlich's carcinoma treated with Ukrain, n = 20	54.04 ± 1.11

Treatment with Ukrain was associated with restitution of the number of phagocyting leucocytes in peripheral blood, more expressed in mice bearing ascite tumor variant. It indicates the positive immunomodulating action of the drug.

It is known that the antitumoral effect of antineoplastic drugs using in targeted anticancer therapy can either modulate signaling pathways leading to cell cycle regulation or directly alter cell cycle regulatory molecules [12]. Ukrain inhibits cancer cell growth by induction of G_2/M or G_0/G_1 cell cycle arrest followed by apoptosis induction [13, 14]. Analysis of cell cycle distribution in ascite, performed at the moment of experiment cessation, confirmed of antitumor cell cycle targeted effect of Ukrain (Table 3). The number of apoptotic cells in ascite of treated mice was about 30% higher compared to the control ones (even nine days later after treatment cessation).

Table 3. Distribution of EACC over cell cycle phases, %

	Control tumor-bearing mice (n = 20)	Mice treated with Ukrain (n = 20)
Apoptotic cells	2.89 ± 0.4	4.72 ± 0.1*
Diploid cells	67.0 ± 2.7	55.7 ± 3.9
G_0/G_1 diploid cells	40.3 ± 2.9	41.0 ± 2.5
Diploid cells in		
G_2/M	28.8 ± 3.9	27.9 ± 4.2
S	31.3 ± 3.3	31.7 ± 4.5
G_2/M + S	59.9 ± 3.4	58.7 ± 2.9
Aneuploid cells	29.2 ± 2.1	39.1 ± 3.0*
G_0/G_1 aneuploid cells	59.8 ± 2.3	60 ± 5.3
Aneuploid cells in		
G_2/M	8.8 ± 0.9	13.7 ± 1.1*
S	31.3 ± 6.1	30.3 ± 2.2
G_2/M + S	42.2 ± 4.8	44.0 ± 3.4

Diploid cell number in ascites of control tumor-bearing animals and mice treated with Ukrain was the same. The number of cells in S-phase of cell cycle (characterizing tumor growth rate) was also similar in control and treated animals. At the same time significant increase in the fraction of G_2/M aneuploid cells in mice treated with Ukrain has been observed, influencing total number of aneuploid cells in treated animals (Fig. 5).

Thus, treatment with Ukrain resulted in significant inhibition of growth of solid form of Ehrlich carcinoma and retardation of growth of ascite tumor variant. It is mediated by direct apoptotic action of drug on EACC and positive immunomodulating effect on tumor-bearing organism. However it is necessary to point that in mice bearing ascite form of Ehrlich carcinoma intraperitoneal administration of Ukrain caused the death rate elevation, probably due to the acute local inflammation.

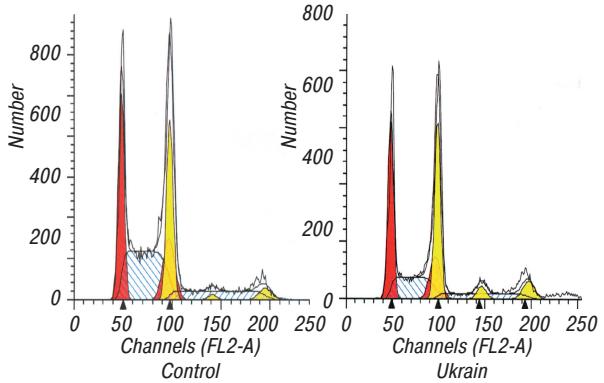

Fig. 5. Typical distribution of cells of ascite form of Ehrlich's carcinoma in accordance with cell cycle phases

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