НАЦІОНАЛЬНА АКАДЕМІЯ НАУК УКРАЇНИ
ІНСТИТУТ ЕКСПЕРИМЕНТАЛЬНОЇ ПАТОЛОГІЇ, ОНКОЛОГІЇ ТА РАДІОБІОЛОГІЇ ім. Р.Є. КАВЕЦЬКОГО
УКРАЇНСЬКА СЕКЦІЯ ЄВРОПЕЙСЬКОГО ІНСТИТУТУ ЕКОЛОГІЇ ТА РАКУ

НАЦИОНАЛЬНАЯ АКАДЕМИЯ НАУК УКРАИНЫ
ИНСТИТУТ ЭКСПЕРИМЕНТАЛЬНОЙ ПАТОЛОГИИ, ОНКОЛОГИИ И РАДИОБИОЛОГИИ им. Р.Е. КАВЕЦКОГО
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EDITORIAL

REVIEW

ORIGINAL PAPERS

ANTIMETASTATIC EFFECT OF UKRAIN AND ITS INFLUENCE ON THE OXYGEN AND ENERGY METABOLISM OF MICE WITH MELANOMA B-16

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АНТИБЛАСТИЧЕСКИЙ ЭФФЕКТ ПРЕПАРАТА УКРАИН И ЕГО ВЛИЯНИЕ НА КИСЛОРОДНЫЙ И ЭНЕРГЕТИЧЕСКИЙ ОБМЕН МЫШЕЙ С МЕЛАНОМОЙ В-16

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The influence of intravenous injections of Ukrain (1mg/kg) on the oxygen tension (pO_2) in muscle, the oxygen saturation rate and oxygen utilization rate as well as on the respiration and oxidative phosphorylation indices of the liver mitochondria in mice with melanoma B-16 was studied by polarographic method. Ukrain was shown to improve the oxygen delivery to the muscular tissue and to decrease a destructive effect of the malignant process on liver bioenergetics. The respiration rate of mitochondria during phosphorylation of exogenous ADP and the respiratory control coefficient were higher in the treated mice as compared to the untreated tumor-bearing animals. The drug significantly inhibited the growth of the primary tumor and its metastasis in lungs.

Key Words: **Ukrain, oxygen tension, mitochondria respiration, metastasis.**

На мышах линии C57Bl/6 с метастазирующей меланомой В-16 полярографическим методом изучали влияние 5 инъекций препарата Украин (1 мг/кг) на напряжение кислорода (pO_2) в мышце, скорость насыщения им ткани и скорость утилизации его тканью, а также на показатели дыхания и окислительного фосфорилирования митохондрий печени. Установлено, что препарат Украин улучшает доставку кислорода к мышечной ткани и замедляет деструктивное воздействие злокачественного процесса на биоэнергетику печени. Препарат достоверно угнетал рост первичной опухоли и ее метастазирование в легкие.

Ключевые слова: **Украин, напряжение кислорода, дыхание митохондрий, метастазирование.**

Modern oncology pays great attention to a quest for new antiblastic drugs. Ukrain preparation (manufacturer — firm Nowicky-Pharma) in which thiophosphoric acid is bound with alkaloids isolated from *Chelidonium majus L.* is one of such promising semisynthetic preparations. A number of experimental and clinical studies on Ukrain effect on various tumors *in vivo* and *in vitro* have been carried out. The data obtained are published in works [1–4]. The given publication presents the data not available in literature.The main goal of this work was to study the influence of Ukrain on the oxygen and energy metabolism in mice with B-16 metastasizing melanoma as well as to reveal its antiblastic properties in respect to the mentioned tumor.

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Abbreviations used: pO_2 — oxygen tension; V_2 — the rate of oxygen uptake by mytochondria in the presence of oxidation substrate (succinate or glutamate); V_3 — the rate of oxygen uptake by mytochondria during phosphorylation of ADP added to a cell; V_4 — the rate of oxygen uptake by mytochondria after conversion of the whole added ADP into ATP; CRC — the Chance respiratory control coefficient; ADP/O — the index indicating the quantity of ATP molecules synthesized on uptake of one oxygen atom; V_{dnp} — the rate of oxygen uptake by mitochondria in the uncoupled state.

Materials and Methods

The experiment has been carried out on 133 C57Bl/6 male-mice (weight 19–22 g) bred in the breeding farm of the R.E.Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology (Kyiv, Ukraine). Metastasizing melanoma B-16 was transplantated to the right shin muscle of each mouse (by 2×10^5 cells per animal in medium 199). On the 10th day after the tumor transplantation, that is when metastatic spreading into lungs occurs [5], the animals were divided into 2 groups. The first group of mice was given Ukrain to sinus venosus of the eye in a dose of 1mg/kg in the volume of 0.04 ml: 5 injections once in two days. The second (control) group of mice was given sterile physiological solution to sinus venosus of the eye in the same regime. A day after the first injection (that is on the 11th day after the tumor transplantation) and after the 5th injection (that is on the 19th day after transplantation) it was possible to determine *in vivo* in some animals from the experimental and control group the oxygen tension (pO_2) in the muscular tissue under different functional loadings (that is to say indices of the oxygen regime in the muscular tissue) and indices of respiration and oxidative phosphorylation of the liver mitochondria. On the 22th day after the tumor transplantation all the survived animals were killed. Then the antitumoural and antimetastatic effects of the preparation were determined.

Indices of the oxigen regime in the muscular tissue were determined by the polarographic method. Using the uncovered platinum electrode connected with the animal via salt solution. Special functional loadings: oxygen inhalation and application of a tourniquet on the extremety above electrode were used to study the oxygen saturation rate of the muscular tissue and utilization of the oxygen by the muscle [6, 7].

The indices of respiration and oxidative phosphorylation of the liver mitochondria were also determined by the polarographic method. To do this the liver isolated from the killed animals was homogenized at 3°C in the medium containing 0.25 M sucrose, 0.01 M Tris-HCl, 0.001 M EDTA and 0.5% bovine or human albumin (pH 7.4). Reagents of firms Serva (Germany), Reanal (Hungary) were employed in this work. The filtred 12% homogenate was subjected to centrifugation at 600 g for 3 min and then without stopping centrifuge K-24D (VEB MLW Medizintechnik, Germany) at 1,000 g for 7 min in addition. The obtained supernatant was subjected to centrifugation twice at 14,000 g for 10 min. Then the supernatant liquid was removed and the mitochondrial fraction was resuspended in 0.35 ml cooled isolation medium. Protein was determined by the method of Lowry [8]. To determine respiration of mitochondria we introduced 0.02 ml mitochondrial suspension, 266 nmoles of ADP and 0.005 ml solution of 2,4-dinitrophenol, the concentration of the latter in a cell being 80 μM, to the thermostated polarographic cell of 1 ml, in volume, which contained the incubation medium (0.15 M sucrose; 0.05 M KCl; 0.01 M KH_2PO_4; 0.003 M $MgCl_2$; 0.005 M Tris-HCl; 0.005 M succinate or glutamate, 0.0002 M EDTA, pH 7.4). The temperature of the incubation medium was 28°C. The oxygen uptake by mitochondria was determined by the covered combined platinum electrode of the Clark type. The following indices were studied: V_2 — the rate of oxygen uptake by mitochondria in the presence of the oxidation substrate (succinate or glutamate); V_3 — the rate of O_2 uptake by mitochondria in phosphorylation of ADP added to a cell; V_4 — the rate of O_2 uptake after conversion of the whole added ADP into ATP; CRC — the Chance respiratory control coefficient; ADP/O — the index indicating the quantity of ATP molecules synthesized on uptake of one oxygen atom; V_{dnp} — the rate of O_2 uptake by mitochondria in the uncoupled state. V_2, V_3, V_4 and V_{dnp} were expressed in O_2 nanoatoms per 1 mg of mitochondrial protein in minute.

Polarograph LP-60 (Laboratorni pristroje, Czechia) was employed in studies of the indices of the oxygen and energy metabolism. The data obtained were processed by the methods of variation statistics using Student's *t* test [9].

Results and Discussion

The experiments have established that a day after the first intravenous injection of Ukrain, that is on the 11th day after B-16 melanoma transplantation the indices of the oxygen regime in the muscular tissue noticeably improved. For example, the rate of the pO_2 level increased up to the maximum during the oxygen inhalation and the rate of pO_2 decrease from the maximal to the initial level after cessation of inhalation (Table 1). In this case there was a distinct tendency to an increase in the maximal pO_2 level during inhalation and rates of muscle utilization and saturation by oxygen when using the tourniquet probe.

In animals of the experimental group certain indices of the oxidative phosphorylation of the liver mitochondria also improved a day after the preparation administration (Table 2). In this case such an improvement was manifesteted rather notice-

Table 1. Change in indices of the oxygen regime of the muscular tissue in the C57Bl/6 mice with B-16 melanoma a day after the first intravenous injection of preparation Ukrain in a dose of 1 mg/kg (n = 10).

Type of the action	Initial level of pO_2, mm Hg	Maximal level of pO_2 during oxygen inhalation, mm Hg	The rate of pO_2 rise up to the maximal level during oxygen inhalation, mm Hg/s	The rate of pO_2 decrease from maximum to initial level after cessation of inhalation, mm Hg/s	The rate of pO_2 decrease from initial level to zero after tourniquet application, mm Hg/s	The rate of pO_2 restoration after tourniquet removal, mm Hg/s
Control	34.6 ± 2.5	104.7 ± 5.2	0.48 ± 0.03	0.72 ± 0.09	0.51 ± 0.07	0.40 ± 0.05
Ukrain	40.9 ± 5.2	128.8 ± 9.5	0.64 ± 0.04*	1.02 ± 0.06*	0.70 ± 0.06	0.57 ± 0.06

* $P < 0.05$ as compared to the control.

Table 2. Change in indices of respiration and oxidative phosphorylation of liver mitochondria in C57Bl/6 mice with B-16 melanoma a day after the first intravenous injection of preparation Ukrain in a dose of 1 mg/kg (n = 10).

Type of the action	Oxydation substrate	V_2	V_3	V_4	CRC	ADP/O	V_{dnp}
Control	succinate	20.4 ± 0.4	116.6 ± 7.5	25.2 ± 0.7	4.60 ± 0.18	1.64 ± 0.02	153.2 ± 12.8
	glutamate	12.9 ± 0.7	86.5 ± 4.5	17.4 ± 0.6	4.95 ± 0.22	2.33 ± 0.04	109.9 ± 6.8
Ukrain	succinate	20.7 ± 0.6	128.6 ± 6.0	26.0 ± 0.5	4.95 ± 0.29	1.70 ± 0.04	180.2 ± 6.0
	glutamate	14.7 ± 0.7	92.2 ± 3.1	17.2 ± 0.5	5.37 ± 0.13	2.43 ± 0.04	126.8 ± 2.0*

* $P < 0.05$ as compared to the control.

Table 3. Change in indices of the oxygen regime of the muscular tissue in the C57/Bl/6 mice with B-16 melanoma (the 19th day after the tumor transplantation) a day after the fifth intravenous injection of Ukrain in a dose of 1 mg/kg (n = 10).

Type of the action	Initial level of pO_2, mm Hg	Maximal level of pO_2 during oxygen inhalation, mm Hg	The rate of pO_2 rise up to the maximal level during oxygen inhalation, mm Hg/s	The rate of pO_2 decrease from maximum to initial level after cessation of inhalation, mm Hg/s	The rate of pO_2 decrease from initial level to zero after tourniquet application, mm Hg/s	The rate of pO_2 restoration after tourniquet removal, mm Hg/s
Control	20.6 ± 1.0	74.9 ± 3.1	0.27 ± 0.02	0.47 ± 0.03	0.32 ± 0.03	0.24 ± 0.02
Ukrain	29.5 ± 3.1*	89.6 ± 5.2	0.38 ± 0.03*	0.63 ± 0.07	0.41 ± 0.03	0.37 ± 0.03*

* $P < 0.05$ as compared to the control.

ably on oxidation of glutamate (NAD-dependent substrate). In the presence of this substrate the rate of oxygen uptake by mitochondria was reliably higher in the uncoupled state.

It is known that under progression of the malignant process the oxygen and energy metabolism is inhibited. This is confirmed by a comparison of the data from Tables 1 and 3, and also of 2 and 4. However, in mice which were given 5 injections of Ukrain such inhibition is less pronounced. In animals of the experimental group the level of oxygen tension in muscular tissue and the rate of O_2 delivery to it were statistically higher (Table 3). Besides, more reliable differences were revealed between mice of two groups in indices of respiration and oxidative phosphorylation of the liver mitochondria (Table 4). These defferences are pronounced to a greater extent on oxidation of glutamate than on oxidation of succinate (FAD-dependent substrate).

When studying the antiblastic effect of Ukrain it has been established that 5 injections of the preparation significantly inhibited the growth of the primary tumor of B-16 melanoma (Table 5). Moreover Ukrain inhibited its metastatic spreading as well. The mean volume of metastases per animal in mice of the experimental group was by 80% lower than in the control one. No metastases were revealed in three animals which were given the preparation.

Table 4. Change in indices of respiration and oxidative phosphorylation of liver mitochondria in the C57Bl/6 mice with B-16 melanoma (the 19th day after tumor transplantation) a day after the fifth intravenous injection of Ukrain in a dose of 1 mg/kg (n = 10).

Type of the action	Oxydation substrate	V_2	V_3	V_4	CRC	ADP/O	V_{dnp}
Control	succinate	17.1 ± 0.8	86.4 ± 6.4	27.2 ± 0.7	3.19 ± 0.17	1.55 ± 0.02	81.3 ± 7.0
	glutamate	10.2 ± 0.6	52.0 ± 4.2	18.4 ± 0.5	2.83 ± 0.20	2.15 ± 0.03	40.7 ± 6.0
Ukrain	succinate	19.0 ± 0.7	108.2 ± 7.0*	26.1 ± 0.6	4.12 ± 0.22*	1.60 ± 0.03	108.2 ± 7.2
	glutamate	12.8 ± 0.6*	70.1 ± 4.0*	18.0 ± 0.4*	3.90 ± 0.15*	2.30 ± 0.04*	67.4 ± 6.5*

* $P < 0.05$ as compared to the control.

Table 5. The antiblastic effect of 5 injections of Ukrain in the C57Bl/6 mice with B-16 melanoma (n =13).

Type of the action	Weight of primary tumour, g	Mean number of metastases per animal	Mean volume of metastases per animal, mm^3
Control	3.9 ± 0.2	7.3 ± 1.2	1.393 ± 0.378
Ukrain	3.1 ± 0.3*	4.9 ± 1.4	0.280 ± 0.090*

* $P < 0.05$ as compared to the control.

Thus, generalizing the data obtained it is possible to conclude that Ukrain in mice with B-16 melanoma improves the delivery of oxygen to tissues as well as inhibits the destructive effect of the malignant process on the organism bioenergetics.

References

1. **Nowicky JM, Greif M, Hamler F, Hiesmayer W, Staub W.** Biological activity of Ukrain *in vitro* and *in vivo*. Chemotherapia 1987; 6: 683–5.

2. **Liepins A.** Enhancement of cell mediated lysis of tumor cells by *Chelidonium majus L.* alkaloids (Ukrain). J Cancer Res Clin Oncol 1990; 116 (Suppl. 1): 436.

3. **Sotomayor EM, Rao K, Lopes DM, Liepins A.** Enhancement of macrophage tumouricidal activity by the alkaloid derivative Ukrain *in vitro* and *in vivo* studies. Drugs Exp Clin Res 1992; 18: 5–11.

4. **Musianowych J, Judmajer F, Manfreda D, Spangler P, Albrecht H, Hoffmann J, Meijer D.** Clinical studies of Ukrain in terminal cancer patients (phase II). Drugs Exp Clin Res 1992; 18: 45–50.

5. **Kozlov AM, Sof'ina ZP.** Frequency, terms and type of metastatic spreading of different transplanted tumours in mice. Bull Eksp Biol Med 1978; 86 (12): 715–8 (in Russian).

6. **Berezovsky VA, Rotar AYa.** Oxygen tension in musculus gastrocnemius on denervation. Fiziol Zhurn AN UkrSSR 1968; 14 (5): 625–31 (in Russian).

7. **Todor IN, Khasanova LT, Antonenko SG, Lyalyushko NM, Berdinskikh NK, Mosienko VS.** The effect of ceruloplasmin on oxygen tension in the muscular tissue of experimental animals. Fiziol Zhurn 1992; 38 (5): 76–80 (in Ukrainian).

8. **Lowry OH, Rosebrough NJ, Farr AL, Randall RJ.** Protein determination with the Folin phenol reagent. J Biol Chem 1951; 193 (1): 265–75.

9. **Lakin GF.** Biometry. Moscow: Vysshaya Shkola, 1980. 293 pp (in Russian).