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THE EFFECT OF THE ANTINEOPLASTIC DRUG UKRAIN ON THE ELECTROKINETIC POTENTIAL OF MALIGNANT AND NORMAL CELLS

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Summary: *The effect of Ukrain, a thiophosphamide derivative of alkaloids of* Chelidonium majus L., *on the electrokinetic potential of normal and tumor cells was studied. Initially the electrophoretic mobility of cells was determined using a purpose-built device. Subsequently their electrokinetic potential was calculated. Ukrain significantly decreased electrokinetic potential. Thiophosphamide and alkaloids of greater celandine also reduced electrokinetic potential, but only at much higher concentrations than Ukrain. In addition, the toxicity of Ukrain was lower than that of both thiophosphamide and greater celandine alkaloids.*

Introduction

The search for, and development of, effective anticancer drugs is a major challenge in modern oncology. Anticancer agents of plant origin may offer a solution, and a promising candidate in this area is the drug Ukrain, a semisynthetic product synthesized from thiophosphoric acid and alkaloids of greater celandine, *Chelidonium majus L.* (1-3). Both *in vitro* and *in vivo* studies have shown that Ukrain accumulates selectively in cancer cells (4) that are most sensitive to the effects of Ukrain in the G_1 and G_2 phases of the cell cycle (5). Ukrain inhibits RNA, DNA and protein synthesis (6, 7), induces apoptosis in malignant cells (8) and exerts an immunomodulatory action (9).

We had previously determined the balanced constant of the interaction between Ehrlich ascitic carcinoma cells and the drug Ukrain as being in the range of 10^{-5} mol/l (5). This is the evidence that Ukrain acts on malignant cells at the cellular membrane level, affecting the integrity and permeability of their plasmatic membranes. Other research groups have also noted the importance of cellular membranes in the modulation of the anticancer effects of Ukrain (8).

It is also known that even the most subtle changes in the state and properties of living cells, either

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changes in subpopulational characteristics, or plasmatic membrane or metabolic changes, have an immediate impact on the electrokinetic potential of a cell. This, in turn, controls its adhesive and aggregative properties, and plays an essential role in metastasis. As a consequence of electrokinetic potential alteration, changes occur in the linear motion speed of cells in an electrical field at constant pH, ionic strength and temperature values (10). It has also been shown that the electrical properties of cancer cells are linked with the expression of some specific genes. The relationship between the dielectric characteristics of cancer cells and human HER-2/neu (c-erbB-2) gene expression has previously been observed; HER-2/neu is amplified in many adenocarcinomas, overexpressed in about 30% of primary breast carcinomas, and considered an important prognostic factor (11). *In vitro* and *in vivo* experiments have demonstrated that overexpression of the normal HER-2/neu gene product, $p185^{neu}$, results in changes in cell morphology and manifestation of the tumorigenic phenotype in various cell lines (12, 13).

The effect of lymphokines on the electrophoretic mobility of macrophages was previously investigated (14). We were unable to find any information in the available literature concerning the effect of Ukrain on electrokinetic potential, an important membrane parameter. We therefore undertook this study to investigate the *in vitro* and *in vivo* effects of the drug Ukrain on the electrophoretic mobility and, therefore, on the electrokinetic potential of malignant and normal cells.

Materials and methods

The experiments were performed on C57BL/6 male mice weighing 20-25 g (Nursery of the A. Bogoolets Institute of Physiology, National Academy of Sciences of Ukraine, Kiev, Ukraine). The animals were on standard laboratory diet *ad libitum*. The Ehrlich ascitic carcinoma cell line (National Bank of Tumor Strains and Cell Lines, National Academy of Sciences of Ukraine) was chosen as a tumor model in view of the known resistance of its cells to many anticancer agents, including thiophosphamide (15). Ehrlich ascitic carcinoma were transplanted intraperitoneally at a dose of 5×10^6 cells per mouse. On day 5 after transplantation, *i.e.*, in the exponential phase of tumor growth, the ascitic fluid was aseptically washed out. Thereafter, the malignant cells were rinsed twice using Ringer solution by centrifugation at 3,000 rpm for 5 min at a temperature of 3 °C.

Lymphocytes obtained under sterile conditions in medium 199 by soft disintegration of the thymus using a preparation needle in the same strain of mice were used as non-malignant cells. Thus, lymphoid cells were represented by T-lymphocytes of various functional subtypes. The lymphocyte is a convenient cell model for studying membrane phenomena as a whole, *i.e.,* phenomena that go beyond the limits of immunology (16).

We performed *in vitro* and *in vivo* experiments to study the effect of Ukrain and its source materials, *i.e.,* thiophosphamide and alkaloids of *Chelidonium majus L.,* on the electrophoretic mobility of malignant and normal cells. Cell viability was estimated using trypan blue for vital staining. The *in vitro* experiments were performed three times with each compound. For the *in vivo* experiments, each group consisted of five animals for each compound. The *in vivo* experiments were repeated twice. In the first series of experiments, the Ehrlich carcinoma cells and thymocytes were incubated with Ukrain and either greater celandine alkaloids or thiophosphamide (Thiotepa Lederle, Wyeth-Lederle Pharma GmbH, Vienna, Austria). Incubation was performed in medium 199 for 40 min at 30 °C. Following incubation, the tumor cells or thymocytes were sedimented by centrifugation at 3,000 rpm and then resuspended in a sterile Na-K-phosphate buffer for cell electrophoresis at pH 7.4 (10).

In the *in vivo* experimental series, four groups, each of 10 mice, were treated with Ukrain (group I), thiophosphamide (group II), celandine alkaloids (group III) or normal saline (control). Ukrain, alkaloids or thiophosphamide was injected into the tail vein of the mice. The control animals were administered 0.1 ml of normal saline solution. Forty minutes after administration, we ablated the Ehrlich ascitic carcinoma cells or the thymocytes in anesthetized experimental and control mice. The cells were then rinsed, centrifuged and resuspended in the Na-K-phosphate buffer.

The cell linear movement rate in the electrical field was measured by a purpose-built device (Fig. 1). This was provided with a thermostated capillary along which cells move due to the voltage. Using Smolukhovsky's equation (10), the electrokinetic potential $\xi = 14 \times U$ was estimated, where U is the electrophoretic mobility expressed in extrasystemic units as a ratio of the linear movement rate in electrical field V (determined experimentally, μm/sec) to the E field voltage gradient (W/cm). In our case, the E value was 20 W/cm. Finally, the electrokinetic potential was calculated, and expressed in millivolts, using the formula:

$$\xi = 14 \times V/E \quad (10)$$

The results were statistically processed using Student's *t*-test (17).

Results

At an initial stage in our investigation, while determining the linear mobility in an electrical field, we studied the electrophoretic motion of malignant and normal cells after *in vitro* incubation for 40 min with the drug Ukrain. There were 2 - 4 × 10^6 cells in 1 ml of incubation medium. Since there is a direct proportional relationship between the cell linear movement

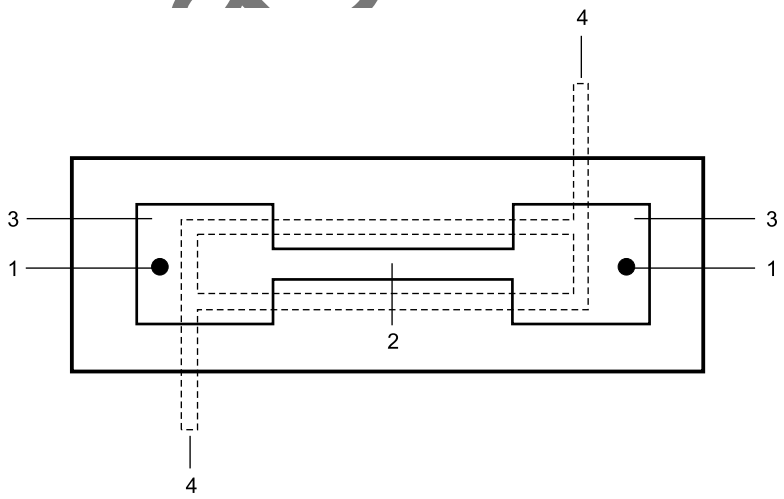

Fig. 1 Sketch of the device for cell electrophoresis. 1: electrodes; 2: thermostatic capillary; 3: cells in the buffer solution; 4: water flow from thermostat.

rate across an electrical field and the electrokinetic potential, we therefore determined only the value of the latter.

In our initial experiments we attempted to study the effects of Ukrain at various concentrations on the electrokinetic potential of malignant and normal cells. Incubation with Ukrain at a concentration of 10.2×10^{-6} M resulted in a slight reduction in the electrokinetic potential of Ehrlich ascitic carcinoma cells (Table I and Fig. 2). When the drug concentration was increased 2-fold (20.4×10^{-6} M), the studied parameter reduced significantly, by 1.9-fold, compared with control ($p < 0.05$). Further increases in concentration resulted in an even greater fall in the potential. Nevertheless, the curve of 1/electrokinetic potential (Ukrain), reflecting the inverse effect of Ukrain concentration, began to reach a plateau (Fig. 2). Ukrain at a concentration of 20.4×10^{-6} M reduced the electrokinetic potential of the malignant cells considerably, by 48% (Table I and Fig. 2), and we therefore used this drug concentration in our subsequent investigations.

Having studied electrokinetic potential changes in Ehrlich carcinoma cells following their incubation with Ukrain, we began to explore Ukrain's influence on this parameter in the thymocytes of intact C57BL/6 mice. It appeared that the electrokinetic potential of normal thymocytes, after incubation with Ukrain for 40 min, decreased only 1.5-fold in comparison with the corresponding control, whereas in malignant cells its value decreased 1.9-fold (Table II). The initial electrokinetic potential values of malignant cells and intact thymocytes differed only slightly from each other. Moreover, it was interesting to observe that 40 min incubation with Ukrain resulted in the death of 20.0% ± 2.8% of malignant cells, whereas only 12.0% ± 1.9% of thymocytes died within the same period. In contrast, when Ukrain was absent from the incubation medium, the death rates of cancer cells and thymocytes were similar (Table II). The findings of this experimental series are indicative of the differing reaction to Ukrain of malignant and normal cells, which is in accordance with the literature (3, 6).

As indicated above, Ukrain is a semisynthetic product synthesized from thiophosphoric acid and alkaloids from *Chelidonium majus L.* It was therefore of interest to perform a separate study on how cellular electrokinetic potential would be affected by each of Ukrain's two source materials. To this end, we first incubated malignant cells in the presence of thiophosphamide (Thiotepa), which was added to an incubation medium at various concentrations. It was only at a concentration of 80×10^{-5} M that thiophosphamide caused a statistically significant decrease in the electrokinetic potential of the Ehrlich carcinoma cells (Table III). For a better illustration, the data from Table III are presented in graphic format for coordinates 1/electrokinetic potential (thiophosphamid), where thiophos is the concentration of incubation medium agent (Fig. 3). Increase in the thiophosphamide con-

Table I *The electrokinetic potential of ascitic Ehrlich carcinoma cells after 40 min incubation with Ukrain (mean ± SD;* n = *17)*

Concentration of Ukrain x 10^{-6} M	Electrokinetic potential (mV)	Reduction of electrokinetic potential (%)*
–	14.9 ± 1.2	–
10.2	12.0 ± 0.9	19
20.4	7.7 ± 0.7**	48
40.8	6.8 ± 0.7**	54
81.6	5.8 ± 0.6**	61

*Compared with control; **$p < 0.05$ compared with control.

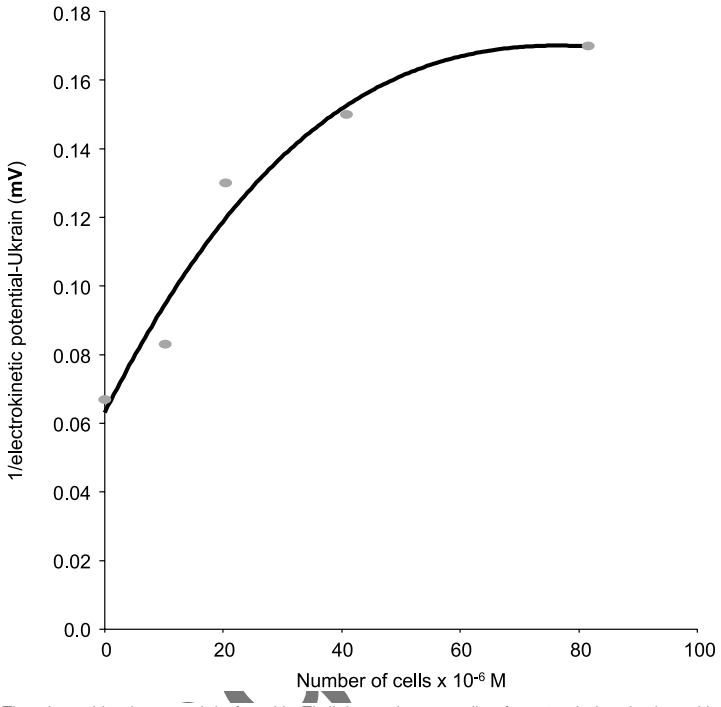

Fig. 2 The electrokinetic potential of ascitic Ehrlich carcinoma cells after 40 min incubation with Ukrain.

centration to 160 x 10^{-5} M brought about a 1.5-fold reduction in the electrokinetic potential. When the concentration was increased to 320 × 10^{-5} M, the electrokinetic potential value reduced only 1.6-fold, compared with control. It can be seen clearly that the effective concentrations of Ukrain are 80-100 times lower than the effective thiophosphamide concentrations (Tables I and III). In our further studies of normal thymocytes, we used a 160 × 10^{-5} M concentration of thiophosphamide. In intact mice, this concentration

Table II *The electrokinetic potential (EKP) and the number of dead Ehrlich ascitic carcinoma cells and thymocytes from intact mice after 40 min incubation with Ukrain (20.4 × 10^{-5} M)*

Type of cells	Form of exposure	EKP (mV) (n = 27)	Dead cells (%) (n = 7)
Thymocytes	Control	13.4 ± 1.2	3.3 ± 0.5
	Ukrain	8.9 ± 0.9*	12.0 ± 1.9*
Ehrlich carcinoma	Control	14.8 ± 1.0	3.5 ± 0.6
	Ukrain	7.8 ± 0.6*	20.0 ± 2.8**

*$p < 0.05$ compared with respective controls; **$p < 0.05$ compared with thymocytes incubated with Ukrain.

Table III *The electrokinetic potential (EKP) of Ehrlich carcinoma cells after 40 min incubation with Ukrain* (n = *16)*

Thiophosphamide concentration (cells $\times 10^{-5}$M)	Electrokinetic potential (mV)	Reduction of EKP (%)*
–	14.1 ± 1.0	–
40	13.0 ± 1.1	8
80	11.0 ± 1.0**	22
160	9.4 ± 0.9**	33
320	8.5 ± 0.8**	40

*Compared with control; **$p < 0.05$ compared with control in the absence of thiophosphamide.

led to a 1.5-fold decrease in electrokinetic potential in the Ehrlich carcinoma cells, and to a 1.2-fold decrease in the thymocytes (Table IV). At this thiophosphamide concentration, 15.0% ± 2.1% of the Ehrlich carcinoma cells and 9.8% ± 1.4% of thymocytes died within a 40-min incubation period (Table IV). The difference in death rates between malignant and normal cells in the presence of Ukrain is seen to be greater than that observed in the presence of thiophosphamide (Tables II and IV).

As a model of the second source material of Ukrain, we used greater celandine alkaloids. In the

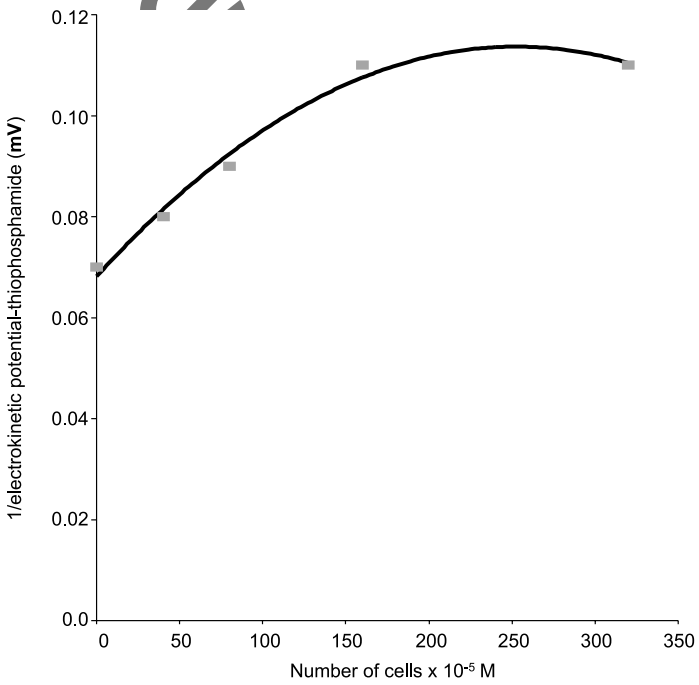

Fig. 3 The electrokinetic potential of Ehrlich carcinoma cells after a 40 min incubation with thiophosphamide.

Table IV *The electrokinetic potential (EKP) and the number of dead Ehrlich ascitic carcinoma cells and thymocytes from intact mice after 40 min incubation with thiophosphamide (160 × 10^{-5} M)*

Type of cells	Form of exposure	EKP (mV) (n = 24)	Dead cells (%) (n = 7)
Thymocytes	Control	13.6 ± 1.0	4.0 ± 0.5
	Ukrain	11.3 ± 0.9*	9.8 ± 1.4*
Ehrlich carcinoma	Control	14.3 ±0.9	3.0 ± 0.5
	Ukrain	9.5 ± 0.8*	15.0 ± 2.1**

*$p < 0.05$ compared with respective controls; **$p < 0.05$ compared with thymocytes incubated with thiophosphamide.

electrokinetic potential study, it appeared that these alkaloids, of which chelidonine accounts for nearly 50% (3), had very little influence on the parameter studied. Even at a concentration of 160 × 10^{-5} M, the greater celandine alkaloids reduced the electrokinetic potential of Ehrlich carcinoma cells by only 20% (Table V and Fig. 4). At 80 x 10^{-5} M concentration, the electrokinetic potential of the thymocytes of intact mice reduced 1.4-fold, while that of Ehrlich carcinoma cells reduced 1.2-fold, in comparison with the corresponding control values (Table VI).

Discussion

Having studied the electrokinetic potential of malignant and normal cells after incubation with Ukrain, thiophosphamide and the alkaloids of *Chelidonium majus L.,* it was of interest to observe this parameter following systemic, *i.e.,* intravenous, injection of each of the above agents. Measurements were taken 40 min after a single injection of Ukrain, thiophosphamide or greater celandine alkaloids, each at a dose of 100 mg/kg, into the tail vein of mice. Based on the data from previous pharmacokinetic investigations (18), it is clear that this time period is sufficient for the agents under study to accumulate in maximal amounts in most organs and in malignant cells, and for their action to manifest. The results of this study are presented in Table VII, and, as can be seen, under the given investigative conditions, none of the agents studied had a statistically significant influence on the electrokinetic potential of either the ascitic Ehrlich carcinoma cells or the thymocytes.

In conclusion, this study has shown that incubation of Ehrlich carcinoma cells with Ukrain leads to a significant fall in their electrokinetic potential. The electrokinetic potential value is associated with spe-

Table V *The electrokinetic potential (EKP) of Ehrlich ascitic carcinoma cells after 40 min incubation with the alkaloids of* Chelidonium majus L. *(n = 17)*

Alkaloid concentration (cells ×10^{-5} M)	Electrokinetic potential (mV)	Reduction of EKP (%)*
–	14.6 ± 0.8	–
20	14.2 ± 0.7	3
40	13.9 ± 0.8	5
80	12.1 ± 0.7*	17
160	11.6 ± 0.8*	20

*Compared with control; **$p < 0.05$ compared with control in the absence of alkaloids.

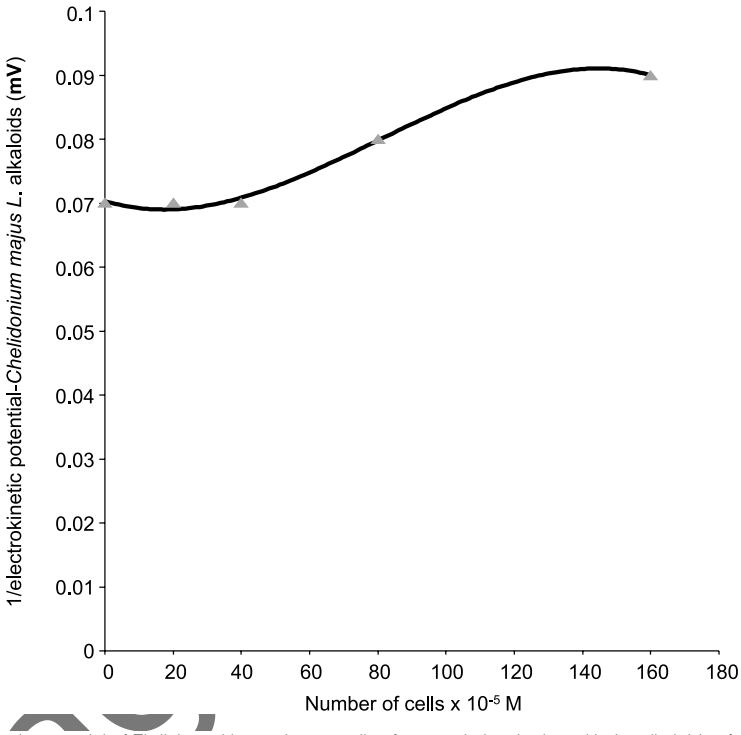

Fig. 4 The electrokinetic potential of Ehrlich ascitic carcinoma cells after 40 min incubation with the alkaloids of *Chelidonium majus L.*

cific changes in the cell membrane, such as the appearance or disappearance of certain charged groups, *e.g.,* amino acid residues in protein areas of the membrane. In addition, specific conformational changes in the cell membrane result in alterations of the membrane charge density, which may also influence the electrokinetic potential value. Likewise, Ukrain reduces the electrokinetic potential of thymocytes in intact mice, although this decrease is less marked than that observed in malignant cells. Since electrokinetic po-

Table VI *The electrokinetic potential (EKP) and the number of dead Ehrlich ascitic carcinoma cells and thymocytes from intact mice after 40 min incubation with greater celandine alkaloids (80 × 10^{-5} M)*

Type of cells	Form of exposure	EKP (mV) (n = 25)	Dead cells (%) (n = 7)
Thymocytes	Control	13.3 ± 1.0	4.1 ± 0.6
	Ukrain	9.5 ± 0.7*	11.4 ± 1.5*
Ehrlich carcinoma	Control	14.8 ± 0.8	3.3 ± 0.6
	Ukrain	12.0 ± 0.8*	13.6 ± 2.2*

*$p < 0.05$ compared with respective control.

Table VII *The electrokinetic potential of Ehrlich ascitic carcinoma cells and C57Bl/6 mice thymocytes 40 min after intravenous administration of Ukrain (100 mg/kg), thiophosphamide (100 mg/kg), or alkaloids of* Chelidonium majus *L. (100 mg/kg) (n = 15)*

	Electrokinetic potential (mV)	
Form of exposure	Ehrlich carcinoma	Thymocytes
Control	15.3 ± 1.1	13.9 ± 1.0
Ukrain	13.3 ± 0.8	11.8 ± 0.9
Thiophosphamid	16.6 ± 1.2	14.3 ± 1.1
Alkaloids	16.0 ± 1.0	13.6 ± 1.0

tential is one of the indicators of cell state, its unequal decrease in normal and malignant cells after their incubation with Ukrain would appear to be a consequence of the differing affinity of these cells for the drug. Similarly, thiophosphamide reduces the electrokinetic potential of normal and malignant cells, although at a dose concentration 80 to 160 times higher than Ukrain, and it should be noted that thiophosphamide toxicity is known to be much higher than that of Ukrain. The alkaloids of *Chelidonium majus L.* have only a slight influence on the electrokinetic potential of cells, and in concentrations much higher than the effective concentrations of Ukrain. Also, in the case of systemic administration, Ukrain was found to have a more profound effect on electrokinetic potential than either thiophosphamide or greater celandine alkaloids administered at the same dose.

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