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# SELECTIVE INHIBITION OF *IN VITRO* CELL GROWTH BY THE ANTI-TUMOUR DRUG UKRAIN

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**Summary:** *The inhibitory effect of Ukrain on malignant cells and on normal cells, in vitro, has been compared. To obtain a 50% inhibition of cell growth, a tenfold concentration had to be used with normal endothelial cells compared to a human osteosarcoma cell line. Hybrids of the two cell types showed nearly the same sensitivity as normal cells. A laser scanning microscope showed a high uptake of Ukrain in malignant cells, while the content in normal cells under the same experimental conditions was substantially lower.*

## Introduction

Ukrain is a semisynthetic compound derived from *Chelidonium majus L.* alkaloids and thiophosphoric acid triaziride. The following biological actions of Ukrain on normal and malignant cells have been demonstrated. *In vitro* it increases oxygen consumption in normal and malignant cells; $O_2$ consumption normalizes in normal cells within 15 min, whereas it decreases irreversibly to zero in cancer cells (1). In addition, Ukrain has been found to decrease DNA, RNA and protein synthesis in malignant cells (2, 3).

Sixty human cancer cell lines representing nine major types of cancers were tested by the National Cancer Institute, Bethesda, MD, USA. In all cell lines a 100% growth inhibition was found at 10/-4 µM. Single organ-specific inhibition was found especially in colon, stomach, ovary, kidney, small cell and non small cell lung cancers and melanoma. Cells from human tumour xenografts were tested by the European Organization for Research and Treatment of Cancer, Free University Hospital, Amsterdam, The Netherlands (EORTC). Ukrain showed inhibiting actions for ovary at 10 µg/ml. At 100 µg/ml it inhibited all cells tested: colorectal, gastric, mammary, melanoma and adenocarcinomas of the lung.

In human oestrogen dependent MCF-7 mammary carcinoma Ukrain showed an $LD_{50}$ of 4 µg/ml.

Ukrain was found to inhibit even strains of highly toxic Cis- Platinum resistant L 1210 cells. The $LD_{50}$ was between 4.0 and 7.0 µg/ml. Inhibition at 20 µg/ml ranged from 88 to 91%.

In contrast to its highly toxic action in cancer cells, Ukrain showed no toxic action against normal cells (human fibroblasts, umbilical venous endothelial and endothelial cells) at concentrations which are lethal to all cancer cells tested up to date. Therefore this property of Ukrain may be called "malignotoxic" (4).

Ukrain quickly accumulates after parenteral application to the site of the tumour or metastasis. This is easily demonstrated under UV light because of

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Ukrain's autofluorescence. Laser scanning microscopy shows that Ukrain concentrates quickly in the nuclei of malignant cells, a property which is not seen to this extent in normal cells (5, 6).

## Materials and methods

### *Cell culture*

Cell culture was performed in Roux bottles at 37°C in a humidified atmosphere containing 7% $CO_2$. Confluent cultures were detached by a solution of 0.01% trypsin and 0.2% EDTA in phosphate buffered saline (PBS) and split in a ratio ranging from 1:4 to 1:20.

Human endothelial cells were isolated from umbilical veins by collagenase treatment as described in detail (7). The culture medium for endothelial cells was M199 supplemented with 15% heat inactivated fetal calf serum (Biochrom, FRG), 200 µg ml-1 endothelial cell growth factor and 90 µg ml-1 heparin (8).

Human osteosarcoma and melanoma cell lines were received from the American Type Culture Collection (ATCC) and cultivated in DME medium supplemented with 5% FCS. Hybrid cell lines with human endothelial cells have been described (9).

### *Determination of the cell number in multiwell plates*

Growth inhibition was tested in 96-well plates. 5000 cells/well were seeded. On the next day different concentrations of Ukrain were added and incubated for 48 h. Cell numbers were estimated by measuring the activity of the acid phosphatase enzyme (10). The culture supernatant was aspirated, after washing once with PBS. A solution containing a phosphatase substrate (1mg/ml p- nitrophenylphosphate), 0.1% Triton x 100, 0.1 M sodium acetate. pH 5.5 was added. After 2 h incubation at 37°C the reaction was stopped with sodium hydroxide and colour development was determined using an ELISA plate reader (405 nm).

### *Fluorescence microscopy*

Cells were grown in 35 mm dishes and incubated with 100 µg/ml Ukrain for 30–60 min. The culture medium was aspirated, the cells were washed twice with PBS. Coverslips were mounted on the cells and fluorescence was excited using a confocal laser scanning microscope equipped with an argon laser source (MRC 600 from BioRad, 2000 Alfred Nobel Drive, Hercules, CA, 94547, USA). The emitted light was detected in a photomultiplier channel. The signals were imaged on a video monitor using the MRC 600 image processing software.

## Results and discussion

### *Growth inhibition*

The drug Ukrain is known to inhibit the growth of a variety of tumour cell lines. The authors have compared the inhibitory effect of Ukrain on malignant cells and normal cells *in vitro*. Cells were incubated with different concentrations of the drug for 48 h. In a range from 20–50 µg/ml Ukrain, about 50% inhibition of cell growth with endothelial cells was measured. This concentration killed the human osteosarcoma cell line (Fig. 1). Hybrids of the two cell types showed nearly the same sensitivity as normal cells.

### *Fluorescence microscopy*

Because of its autofluorescence Ukrain can be detected intracellularly. A laser scanning microscope showed a high uptake of Ukrain in malignant cells. The cytoplasm was stained weakly and

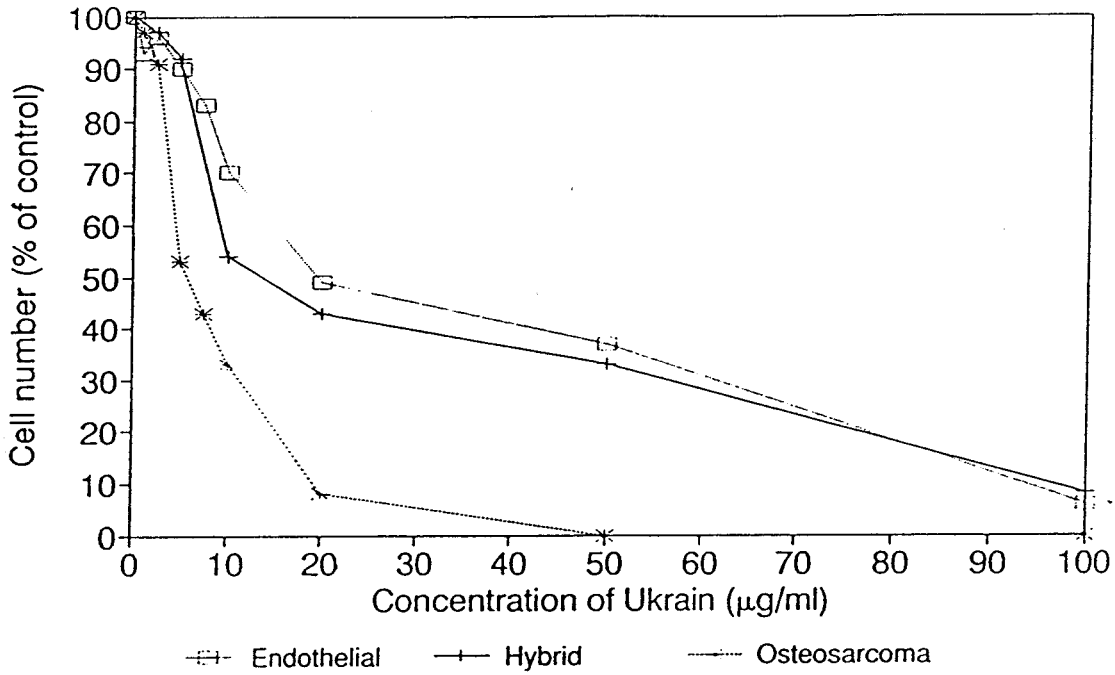


**Fig. 1** Inhibition of cell growth by Ukrain.

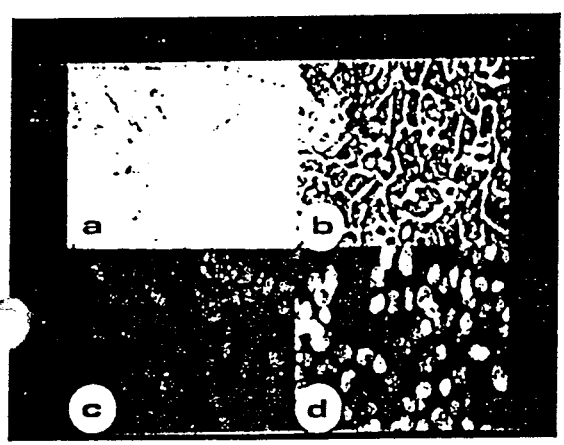


**Fig. 2** Ukrain uptake of melanoma cells compared to normal endothelial cells.
a: Phase contrast, endothelial cells
b: Phase contrast, melanoma cells
c: Fluorescence, endothelial cells
d: Fluorescence, melanoma cells


**Fig. 3** Detection of Ukrain in melanoma cells. Cells were treated with 100 µg/ml Ukrain for 30 min. Autofluorescence of the drug was excited by 488 nm laser light.
a: Fluorescence of cells shown in B.
Ukrain is accumulated in the cell nucleus and nucleolus.
b: Phase contrast photo micrograph.

showed granular structures. A large amount of fluorescence was located in the nucleus. Especially in the nucleoli an accumulation of Ukrain could be observed (Fig. 2 a, b). The content in normal cells under the same experimental conditions was substantially lower. In human endothelial cells fluorescence was weak and the difference between cytoplasm and nucleus was not as pronounced as in melanoma cells (Fig. 3 a–d).

The molecular mechanism of the cytotoxic action of Ukrain is not known. Of special interest is the selective inhibition of cell growth. The present results indicate that the different sensitivity of normal and transformed cells to Ukrain *in vivo* is in part due to different uptake rates. Different metabolic processing of the drug would be another possible explanation of these results.

## References

(1) Nowicky J.W. *New immuno-stimulating anti-cancer preparation Ukrain.* 13th Internat. Congress of Chemotherapy, Vienna, 28 August – 2 September, 1983.

(2) Nowicky J.W., Liepins A., Slesak B. et al. *Evaluation of clinical studies of Ukrain in cancer patients.* Proc. VII Mediterranean Congress of Chemotherapy, Barcelona, May 1990.

(3) Nowicky J.W., Hiesmayr W., Nowicky W., *Sensitization for specific lysis in target-effector system with derivatives of* Chelidonium majus *alkaloids, Ukrain.* Proc. 16th Internat. Congress of Chemotherapy. Israel, June 1989.

(4) Nowicky J.W., Staniszewski A., Zbroja-Sontag W., Slesak B., Nowicky W., Hiesmayr W. *Evaluation of thiophosphoric acid alkaloid derivatives from* Chelidonium majus L. *("Ukrain") as an immunostimulant in patients with various carcinomas.* Drugs Exptl. Clin. Res. **XVII**, 139–143, 1991.

(5) Nowicky J.W., Greif M., Hamler F., Hiesmayr W., Staub W. *Macroscopic UV-marking through affinity.* J. Tumor Marker Oncol., 3, 463–465, 1988.

(6) Nowicky J.W., Greif M., Hamler F., Hiesmayr W., Staub W. *Biological activity of Ukrain* In vitro *and* in vivo. Chemotherapia 6, (Suppl. 2), 683–685, 1987.

(7) Gimbrone M.A., Shefton E.J., Cruise S.A. *Isolation and primary culture of endothelial cells from human umbilical veins.* Tissue Culture Association Manual, 4, 813–817, 1978.

(8) Thornton S.C., Mueller S.N., Levine E.M. *Human endothelial cells: use of heparin in cloning and long-term serial cultivation.* Science, 222, 623–625, 1983.

(9) Hohenwarter O., Schmatz C., Katinger H. *Stability of von Willebrand Factor secretion in different human endothelial cell lines.* Cytotechnology, 8, 31–37, 1992.

(10) Connolly D.T., Knight M.B., Harakas, N.K., Wittwer A.J., Feder J. *Determination of the number of endothelial cells in culture using an acid phosphatase assay.* Anal. Biochem. **152**, 136–140, 1986.