Analysis of the short-term and long-term *in vitro* cytotoxic effects of the anticancer drug Ukrain in breast cancer models

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Abstract #5433

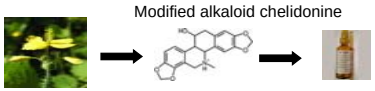
Ukrain is a semisynthetic compound derived from the extract of the plant *Chelidonium majus* L. Studies indicate that this anticancer drug exhibits cytostatic and cytolytic activity against several human cancer cell lines including colon, brain, ovarian, melanoma and lymphoma without adverse harmful side effects on healthy human cells. However, such activity has not been thoroughly investigated in a breast cancer model. In order to assess the *in vitro* cytotoxicity of Ukrain, we used mouse (**4T07 and TUBO**) and human (**SKBR-3**) breast cancer cell lines. 4T07 and TUBO are highly tumorigenic, nonmetastic tumor cell lines derived from spontaneous carcinomas in BALB/cfC3H and BALB-neuT mice, respectively. The TUBO and SKBR-3 cell lines constitutively express the HER-2/neu oncogene, which is overexpressed in 30% of human breast cancer patients. Tumor cells were trypsinized after reaching 90-95% confluency and plated with varying concentrations of Ukrain. Following 24, 48 and 72 hour treatment with Ukrain, cell number and viability was determined using the trypan blue exclusion method. To assess whether the tumor cells retained the capacity for unlimited proliferation, a clonogenic assay was conducted. Lastly, to determine whether the cytotoxic effects of Ukrain are due to the induction of apoptosis, we carried out an annexin V binding assay as well as intracellular staining for active caspase-3. We observed a dose-dependent and time-dependent inhibition of tumor cell growth. As low as 50 μg/mL of Ukrain led to a significant decrease in tumor cell viability when compared to untreated cells. The clonogenic assay showed the inability of treated tumor cells to form colonies and regain their proliferative capacity whereas the untreated cells formed multiple colonies. We observed a several fold increase in caspase 3 activation of Ukrain treated cells compared to untreated cells confirming apoptosis as the cytotoxic mechanism of action. In conclusion, our data suggest that Ukrain could be effective as an anticancer drug for breast cancer due to its short term and long term inhibitory effects on tumor cell viability and proliferation.

Introduction

Ukrain

Chemical structure:

❖A semi-synthetic compound derived from *Chelidonium majus* plant extract.



Clinical Efficacy:

❖Has shown cytostatic and cytolytic activity against several human cancer cell lines: colorectal, brain, ovarian, melanoma, lymphoma, and bladder
❖Not FDA approved as an anti-cancer drug; however, it is approved in parts of Europe, Asia, and Mexico and as an orphan drug for pancreatic cancer in the USA and Australia
❖Typically administered as a monotherapy for the treatment of cancer
❖No adverse side effects on healthy human cells

Mechanism of action:

❖Mechanism of action remains unknown
❖Possibly the result of alkaloids interfering with cancer cell metabolism which leads to programmed cell death

Experimental Design

Assessment of cytotoxic effects. Cells were cultured in 24 well plates at 5x10^4 cells/well and treated with several concentrations of Ukrain for 24, 48 and 72 hours. At the specified time point, cells were washed and total live cell count and viability was determined using the trypan blue exclusion method.

Detection of apoptosis induction using flow cytometry:

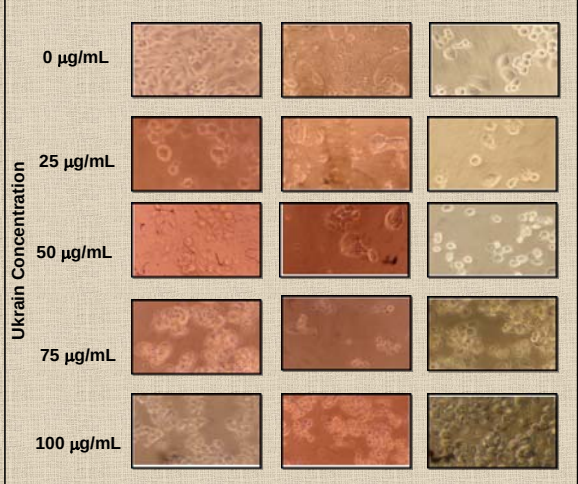
A)Intracellular staining for active caspase 3. Cells were washed, fixed and permeabilized for 20 min at 4^{o}C. Permeabilized cells were washed and stained for intracellular caspase 3 using the FITC anti-active caspase 3 antibody (clone C92-605) for 30 min at 4^{o}C in the dark.

B)Annexin V, PI staining. Cells were resuspended in 1X Binding Buffer. Annexin V-FITC (5uL) and Propidium Iodide-PE (10uL) was added to each cell suspension. Cells were incubated for 10 mins at RT in the dark.

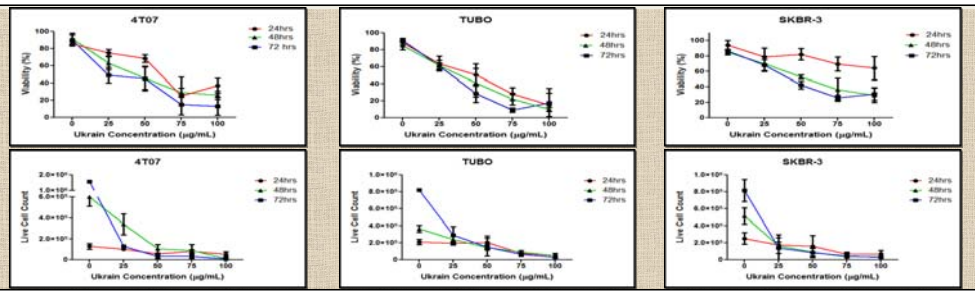
Clonogenic Assay. Cells were seeded out in appropriate dilutions to form colonies within 2-3 weeks. Colonies were fixed and stained with crystal violet (0.5% w/v) and counted by microscopy.

Results

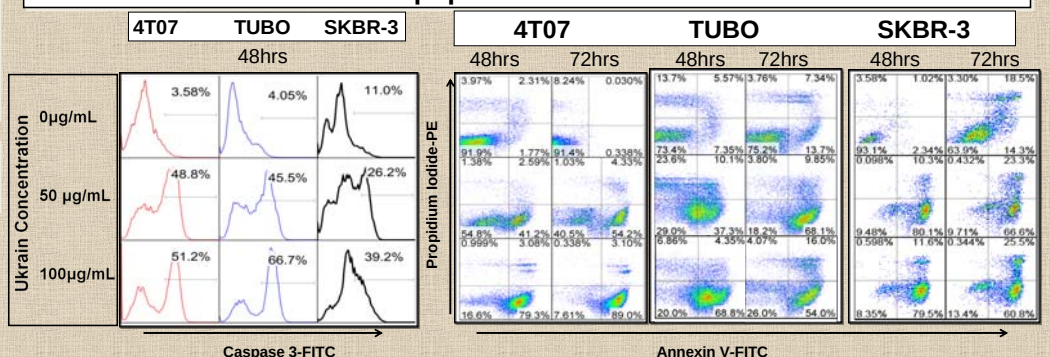
Morphological Changes (48 hrs)



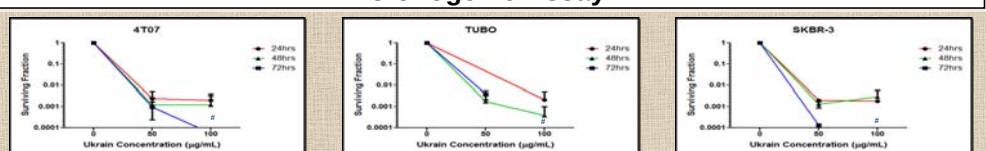
Kinetics of Cell Death



Apoptosis Induction



Clonogenic Assay



#: indicates that the surviving fraction was 0

Conclusions

The anticancer drug Ukrain exerts its cytotoxic effects on both mouse and human breast cancer cell lines in a dose and time dependent manner. Weeks following Ukrain treatment, cells maintained a reduced capacity to proliferate. Our data suggest that these cytotoxic effects are primarily mediated by a caspase-dependent mechanism of apoptosis. Taken together, our studies indicate the potential of Ukrain as an anticancer drug for the treatment of breast cancer.

Acknowledgments

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❖Ukrain was kindly provided by Stephen Karoly of Nowicky Pharma.