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Ukrain®, an alkaloid thiophosphoric acid derivative of *Chelidonium majus* L. protects human fibroblasts but not human tumour cells *in vitro* against ionizing radiation

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Abstract.
Purpose: Ukrain®, an alkaloid thiophosphoric acid derivative of *Chelidonium majus* L., has demonstrated a promising impact on chemotherapy in a variety of malignancies. The effects of the drug on cell survival, alteration of the cell cycle and induction of apoptosis were examined without and in combination with ionizing radiation (IR). The TP53 status of the cell lines used was also investigated.
Materials and methods: Exponentially growing human tumour cell lines MDA-MB-231 (breast), PA-TU-8902 (pancreas), CCL-221 (colorectal), U-138MG (glioblastoma), and human skin and lung fibroblastic cells, HSF1, HSF2 and CCD32-LU were studied by colony assay, flow cytometry (cell-cycle, annexin-V staining for apoptosis) and Western blotting. Ukrain was used in concentrations from 0.1 to 50 μg ml^{-1} for 1, 3 and 24 h and radiation as single doses of 1–10 Gy. Combined drug-radiation exposure employed 1 μg ml^{-1} Ukrain for 24 h plus 2–8 Gy.
Results: Ukrain cytotoxicity was time- and dose-dependent. The combination of Ukrain plus IR gave enhanced toxicity in CCL-221 and U-138MG cells, but not in MDA-MB-231 and PA-TU-8902 cells. Most strikingly, a radioprotective effect was found in normal human skin and lung fibroblasts. Flow-cytometry analyses supported the differential and cell line-specific cytotoxicity of Ukrain. CCL-221 and U-138MG cells accumulated in G_2 after 24-h Ukrain treatment, whereas no alterations were detected in the other tumour cells and normal fibroblasts tested. Western blotting of TP53 demonstrated non-functional overexpression in all tumour cell lines without affecting p21. HSF1 presented wild-type TP53 and a p21 response after IR. Flow-cytometric analyses of annexin-V staining showed no induction of apoptosis after Ukrain treatment in comparison with untreated controls.
Conclusions: Differential effects of Ukrain in modulating radiation toxicity of human cancer cell lines and its protective effect in normal human fibroblasts suggest that this alkaloid may have potential properties for clinical radiochemotherapy.

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1. Introduction

Ukrain® is the thio-TEPA conjugate of the alkaloid thiophosphoric acid derivative of *Chelidonium majus* L. It promises a new anti-tumour modality whose cytotoxic activity is based on a wide range of biological effects (Danilos *et al.* 1992). Based on its anti-tumour activity, Ukrain has been applied in clinical studies as monotherapy (Bondar *et al.* 1998, Nowicky *et al.* 1990, Jagiello-Wójtowicz *et al.* 1998, Uglianitsa *et al.* 1998). In addition to its anti-neoplastic potential, Boyko and Belskiy (1998) and Boyko and Zholus (1998) reported a radioprotective effect of Ukrain with respect to the survival of rat following high-dose radiation exposure. Furthermore, in a clinical study on colorectal cancers, monotherapy with Ukrain resulted in improved survival of patients as compared with combined modality of 5-fluorouracil plus irradiation (Bondar *et al.* 1998).

The National Cancer Institute Developmental Therapeutic Program performed a study to evaluate the effect of Ukrain on *in vitro* cultured human tumour cells of colon, lung, kidney, breast, brain, melanoma and leukaemia origin (Nowicky *et al.* 1993). The results indicated a strong anti-neoplastic activity of Ukrain and led to further clinical studies in breast, rectum and oesophagus carcinomas (Lohninger *et al.* 1993, Bondar *et al.* 1998, Uglianitsa *et al.* 1998). In these studies, the cytotoxicity of Ukrain was highly selective and, surprisingly, there was a concomitant protection of normal tissues. There was evidence of fast uptake and intracellular localization of Ukrain in tumour cells and a predominant accumulation of Ukrain in malignant as compared with normal tissue (Nowicky *et al.* 1988). The concentration-dependent cytotoxic effect of Ukrain on tumour-cell viability is most likely mediated through the inhibition of DNA, RNA and protein synthesis (Nowicky *et al.* 1996) and the induction of programmed cell death (Liepins *et al.* 1996, Roublevskaia *et al.* 2000). Ukrain-dependent cytotoxicity was also reported due to the effects of mitochondrial respiration (Brueller 1992, Zemskov *et al.* 1996). Furthermore, the drug has been described to alter the

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T-helper cell/T-suppressor cell ratio positively, thereby preventing haematotoxicity (Lohninger *et al.* 1993).

The aim of the present study was to investigate the radiobiological properties of Ukrain in modulating radiation toxicity of normal human fibroblasts and tumour cells *in vitro*.

2. Materials and methods

2.1. *Cell lines*

Breast carcinoma (MDA-MB-231), colorectal adenocarcinoma (CCL-221), glioblastoma (U-138MG) and pancreas adenocarcinoma (PA-TU-8902) cell lines were purchased from the American Type Culture Collection (ATCC; Rockville, MD, USA) or, in the case of PA-TU-8902, from the DSMZ (German Collection of Micro-organisms and Cell Cultures, Braunschweig, Germany). The normal human fibroblast cell strains HSF1 and HSF2 (skin) and CCD32-LU (lung) were also used, the former established and characterized at the Section of Radiobiology and Molecular Environmental Research (University of Tüebingen, Germany). The latter were purchased from the ATCC. Dulbecco's modified Eagle's medium (DMEM) including 4500 mg glucose l^{-1}, l-glutamine, pyridoxine, HCl and 3.3 mg l^{-1} NaHCO3 supplemented with 10% foetal bovine serum (Life Technologies, Germany), 1% sodium pyruvate (11.0 mg ml^{-1}) and 1% non-essential amino acids (Sigma, Germany) was applied to culture the MDA-MB-231, PA-TU-8902, U-138MG, HSF1, HSF2 and CCD32-LU cells. The CCL-221 cells were cultured using RPMI-1640 medium (Seromed Biochrom KG, Germany) including l-glutamine supplemented with 10% foetal bovine serum and 2 g l^{-1} NaHCO3 (both Life Technologies). Routinely, cells were incubated at 37°C in a humidified atmosphere containing 7% CO_2.

2.2. *Drug preparation*

Ukrain (Nowicky Pharma, A-1040 Vienna, Austria) was obtained as 5.0 mg *C. majus* L. alkaloid thiophosphoric acid derivative plus triethylene-thiophosphoric acid triamide (thio-TEPA)/5 ml H_2O. According to the manufacturers' advice, the stock solution was stored at room temperature. Working solutions contained 0.1–50.0 μg Ukrain ml^{-1} medium.

2.3. *Cellular response*

Colony assay was performed on single-cell suspensions of asynchronous exponentially growing cells. Cells were counted in fresh medium using a Coulter Counter (Casy 1, Schaerfe System, Germany). Cells were plated into six-well dishes (Falcon, Germany) and exposed to drug and/or radiation exposure 24 h after plating. After 8–10 days, colonies (>50 cells) were double stained with Coomassie blue and Giemsa–azur–eosin–methylene blue and counted. All experiments were repeated three times ($n = 18$).

2.4. *Drug exposure*

The effect of Ukrain on cell survival was tested at concentrations ranging from 0.1 to 50.0 μg ml^{-1}, which are in accordance with clinically achievable concentrations (Hohenwarter *et al.* 1992, Jagiello-Wójtowicz *et al.* 1998). The exposure times were 1, 3 and 24 h, after which cells were washed with phosphate-buffered saline (2×) and fresh medium was added.

2.5. *Radiation exposure*

Irradiation was delivered at room temperature using a linear accelerator (MEVATRON, 6 MeV, Siemens, Germany) at 2.0 Gy min^{-1}. Field size (30 × 30 cm^2) and source-surface distance (100 cm) were constant for all experiments (Hoffmann *et al.* 1999). To avoid sparing due to electron build-up, the culture plates were covered with a 0.5 cm slab of a tissue-equivalent material (RW3) at beam entrance. A 10 cm-thick block of the same material was used to compensate for losses due to backscatter. Dosimetry was performed with a 1 cm^3 thimble ionization chamber (type 23331, Dr Pychlau, Freiburg, Germany), which had been calibrated for absorbed doses to water in a ^{60}Co γ-ray beam. The applied doses ranged from 1.0 to 10.0 Gy.

2.6. *Combined drug plus radiation exposure*

After treatment with 1.0 μg ml^{-1} Ukrain for 24 h, cells were irradiated with single doses of 2.0, 4.0, 6.0 or 8.0 Gy.

2.7. *Flow cytometry*

After treating cells with 1.0 μg ml^{-1} Ukrain for 1, 4, 8, 24 h, 10^6 cells were detached using trypsin/EDTA, washed in PBS and fixed in 80% ethanol until use. Cells were then washed with PBS and prepared for analysis following the instructions of the CycleTEST™ PLUS DNA Reagent Kit (Becton Dickinson, Germany). After staining cells with propidium iodide solution for 30 min, acquisition of data for 10 000 events was performed using a Becton Dickinson Fluorescence-activated Cell Sorter. Distribution of cells in the different phases of the cell cycle were analysed from the DNA-histograms using CELLQuest software. Experiments were repeated three times.

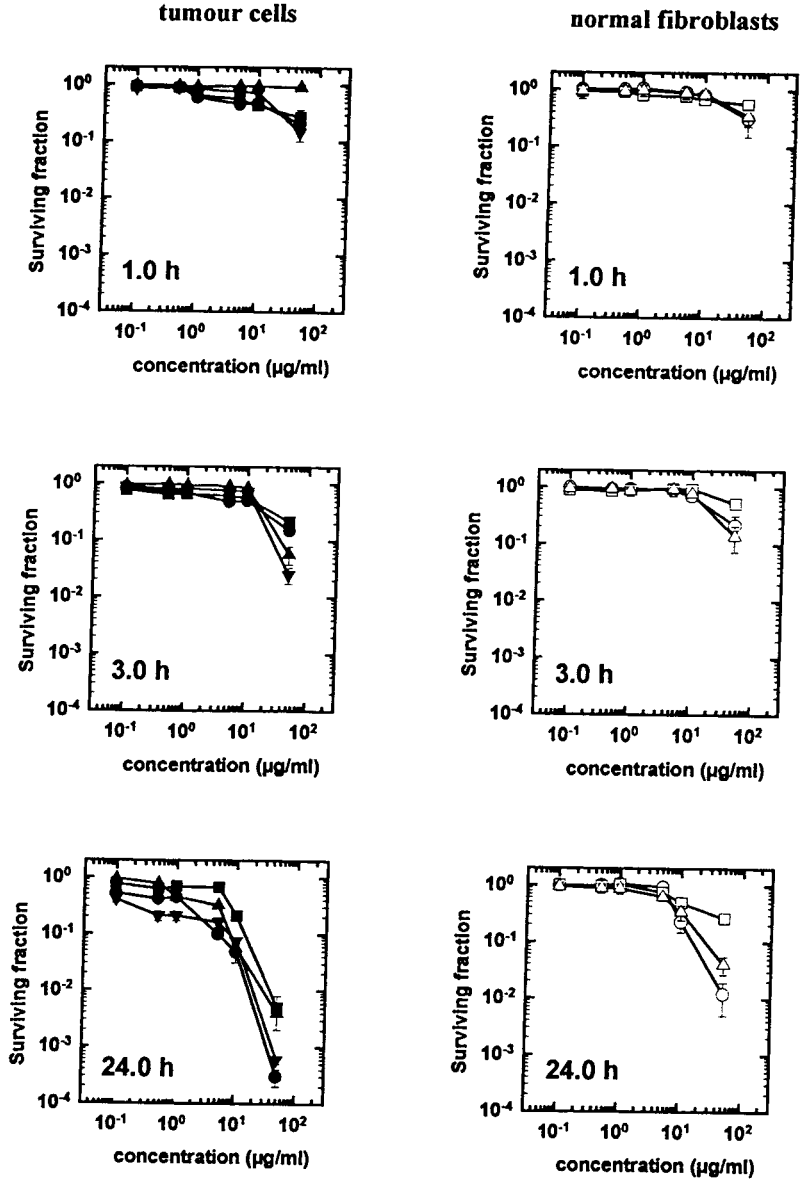


Figure 1. Treatment with increasing Ukrain concentrations and different incubation times led to dose-dependent tumour cell (left: ●, MDA-MB-231; ■, PA-TU-8902; ▲, CCL-221; ▼, U-138MG) and fibroblast cytotoxicity (right: ○, HSF1; □, HSF2; ▽, CCD32-LU). Data points show the mean ± SD of three independent experiments.

2.8. *Total protein extraction*

Cells were exposed to 5 Gy to analyse the function of the TP53 pathway. Two and 6 h after IR cells were harvested using trypsin/EDTA, resuspended in ice-cold medium and centrifuged at 4°C. After washing in ice-cold PBS and centrifugation, cells were lysed using 50 mM HEPES, 250 mM sucrose, 1 mM EDTA, 5 mM $MgSO_4$, 1 mM dithiothreitol, 900 $\mu l\ ml^{-1}$ complete (protease inhibitory Cocktail, Boehringen, Germany), 5 mM sodium vanadat, 5 mM sodium fluoride. Five-minute incubation on ice was followed by freezing three times in liquid nitrogen and thawing at 37°C. Total protein extracts were determined using a spectral-photometer (Helios Alpha, Unicam, Germany) and stored at −134°C.

2.9. *Western blot analysis*

A total of 25 µg total protein extracts was separated on a 12% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane (Schueller, Germany). After blocking the membrane with 5% non-fat dry milk powder in PBS, incubation of primary antibodies was accomplished for 1 h at room temperature. The following antibodies were used: anti-p53 (1:1000; Oncogene, Germany), anti-$p21^{CIP1/WAF1}$ (1:500;

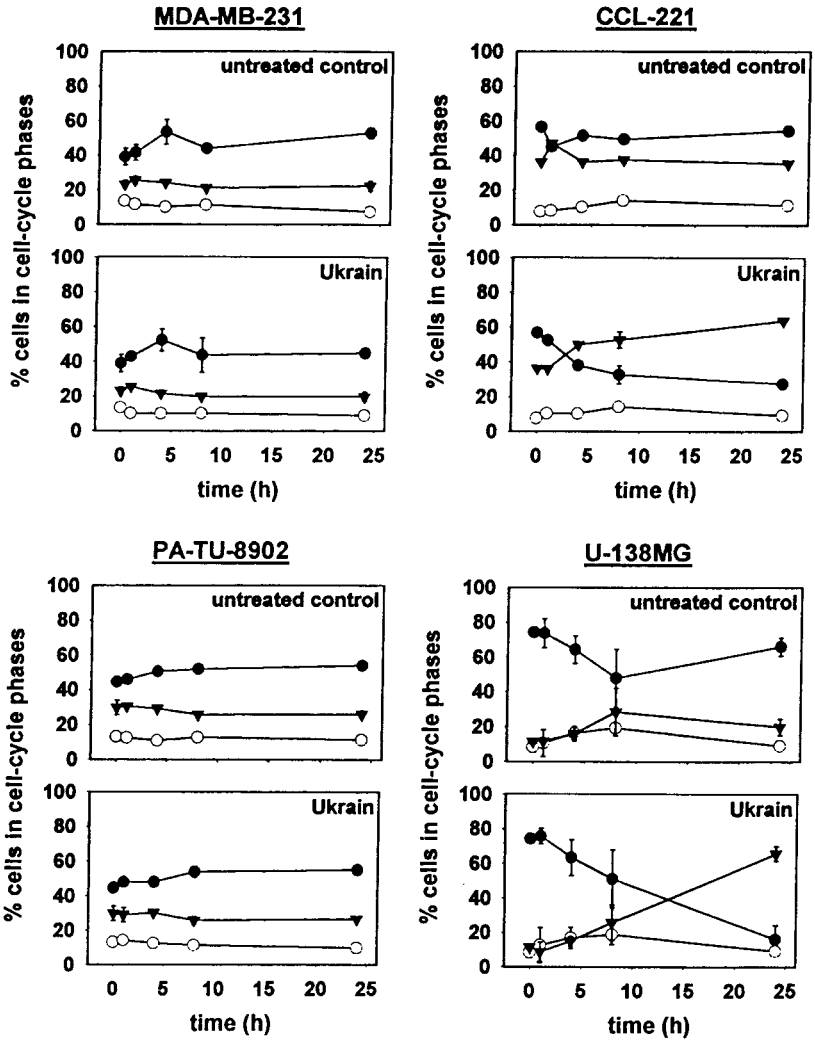


Figure 2. Cell-cycle distribution of tumour cells and normal fibroblasts exposed to 1.0 $\mu g\ ml^{-1}$ Ukrain for different time intervals. Results are plotted as percentage of cells in G_0/G_1 (●), S (○) and G_2/M (▼). Nuclei with a sub-G_1 DNA content (apoptotic) were ignored in these calculations. Each data point represents the mean ± SD of three independent experiments.

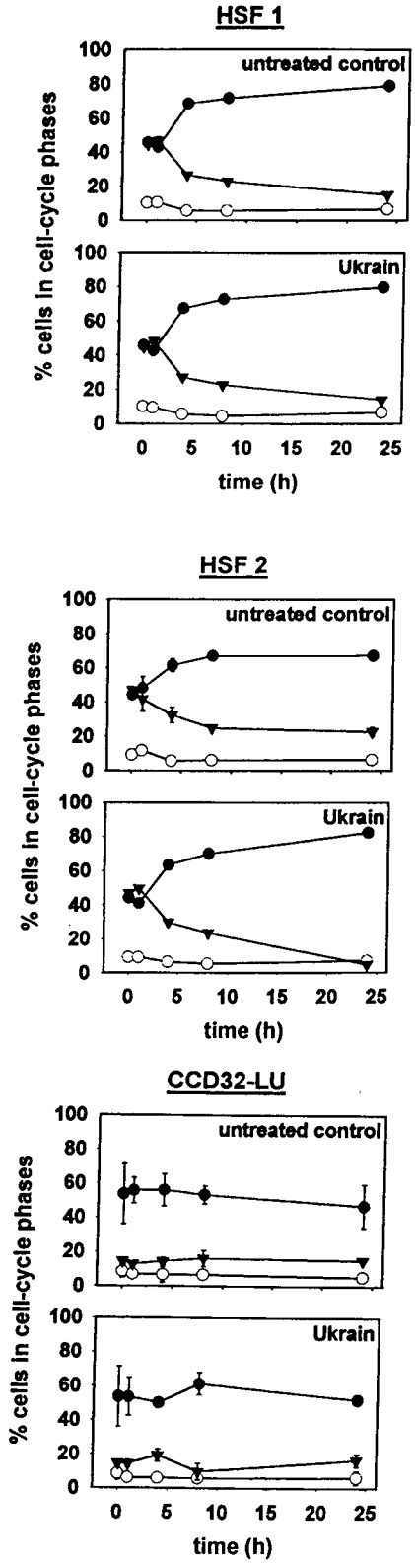
Pharmingen, Germany) and anti-β-actin (1:5000; Sigma-Aldrich, Germany). Protein detection employed specific horseradish peroxidase-conjugated secondary antibody anti-mouse (Santa Cruz, Germany) in combination with the enhanced chemiluminescence detection systems (ECL, Amersham, Life Sciences, Germany). Densitometric measurements of protein band density was carried out using ImageQuant v.5.0 software (Molecular Dynamics, Germany).

2.10. *Flow cytometric analysis of apoptosis by annexin-V staining*

The effect of Ukrain on the induction of apoptosis was analysed by flow cytometry. Treatment of 10^6 cells with 1.0 $\mu g\ ml^{-1}$ Ukrain for 0, 3, 6 and 24 h was followed by detachment of cells using trypsin/EDTA, washing with PBS and preparation for analysis following the instructions of the annexin-V-FLUOS staining kit (Roche, Germany). After simultaneous staining of cells with annexin-V-fluorescein plus propidium iodide solution for 15 min, acquisition of data for 10 000 events was performed using a Becton Dickinson Fluorescence-activated Cell Sorter. The distribution and differentiation of vital, apoptotic and necrotic cells was analysed from the dot plots using CELLQuest software. Experiments were repeated two times.

2.11. *Statistical analysis*

Means and SD of surviving fractions and cell cycle distributions were calculated with reference to untreated controls defined as 1.0 or in a percentage scale, respectively. To test statistical significance, analysis of variance was performed by means of



ANOVA with a software package (Statistica™) on IBM computer systems. Results were considered statistically significant if $p < 0.05$ was reached.

For further description of the dose–effect curves of single irradiation and combined drug-irradiation exposures, parameters of the linear–quadratic model ($\log S = -\alpha D - \beta D^2$) were calculated.

3. Results

3.1. *Drug exposure*

Ukrain treatment resulted in differential time- and dose-dependent reduction in clonogenic cell survival (figure 1). All four human tumour cell lines tested showed different sensitivities towards Ukrain with an up to a 100-fold higher reduction of clonogenic survival as compared with human fibroblasts for 24-h incubation. For all cells tested, 1.0 $\mu g\,ml^{-1}$ Ukrain produced non-significant effects on cell viability. Therefore, this concentration was tested further on to analyse the potential of Ukrain to modulate the cellular radiation response.

3.2. *Flow cytometric analysis*

As indicated by flow cytometry, Ukrain treatment exerted differential effects on the cell-cycle distribution of the analysed human tumour cells and normal human fibroblasts. A 24-h treatment of colorectal carcinoma (CCL-221) and brain tumour cells (U-138MG) resulted in a significant accumulation of G_2/M-phase cells and a concomitant decrease in G_1-phase cells, with no change in the fraction of S-phase cells (figure 2). Twenty-four hours after Ukrain treatment in these cell lines, the fraction of G_2/M-phase cells had increased from ~35% (CCL-221) and 10% (U-138MG) to ~65% (CCL-221) and ~70% (U-138MG), respectively. This increase in G_2/M phase cells was accompanied by a pronounced decrease in the fraction of G_0/G_1 cells to ~25% (CCL-221) and 15% (U-138MG). As a consequence of Ukrain treatment, no significant change was observable for the breast and pancreas carcinoma cell lines MDA-MB-231 and PA-TU-8902 as well as for the normal human fibroblast cell strains HSF1, HSF2, CCD32-LU (figure 2).

3.3. *Radiation exposure*

Cell survival studies after radiation exposure in the dose range 1.0–10.0 Gy showed the cell line PA-TU-8902 as the most radiosensitive ($SF_2 = 0.4 \pm 0.056$), followed by the U-138MG ($SF_2 = 0.63 \pm 0.06$) and MDA-MB-231 ($SF_2 = 0.8 \pm 0.055$) and CCL-221 ($SF_2 = 0.81 \pm 0.06$), respectively. Normal human fibroblasts showed similar

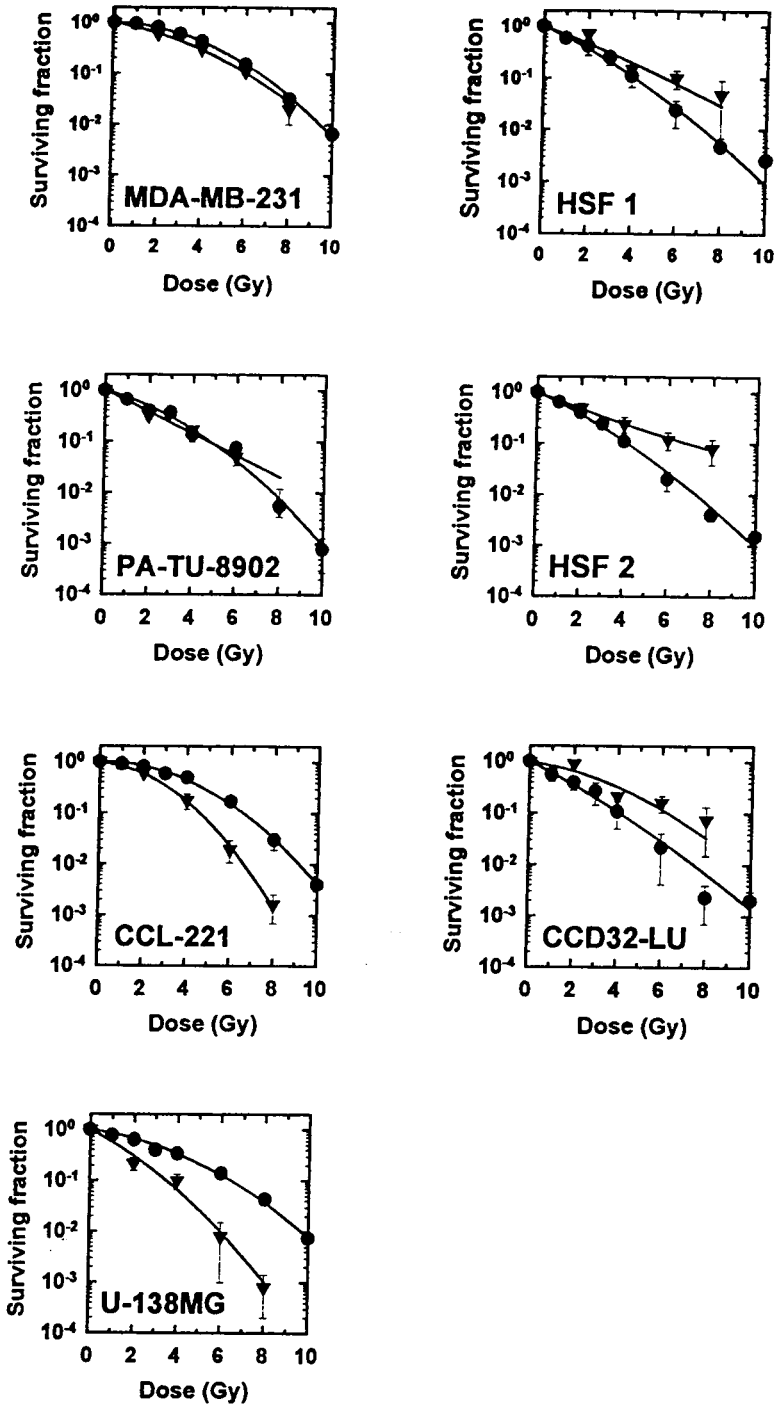


Figure 3. Comparison of the dose–effect curves for irradiation alone (●) and Ukrain plus irradiation (▼) demonstrated an enhancement only for the CCL-221 and U-138MG cells. All three normal fibroblastic strains showed a radioprotective effect upon Ukrain exposure. Data points show the mean ± SD of three independent experiments.

radiosensitivity to PA-TU-8902 cells with SF_2 from 0.4 to 0.41 (figure 3 and table 1).

3.3. *Combined drug-radiation exposure*

To investigate whether Ukrain in a concentration that does not affect cell survival to a significant degree can modulate the radiation response of human tumour cells and normal fibroblasts after single dose exposure, cells were pretreated with 1.0 $\mu g\,ml^{-1}$ Ukrain 24 h before irradiation. As shown in figure 3, a clear shift of the dose–response curve following Ukrain pretreatment was only apparent for the cell lines CCL-221 and U-138MG, although for all four

tumour cell lines a significant decrease in SF_2 upon Ukrain treatment was demonstrated (table 1). In contrast to the effect seen in tumour cell populations, treatment of normal human fibroblasts with Ukrain led to a shift of the dose–response curves, which can be interpreted as radioprotection (figure 3). SF_2 were significantly increased for HSF1 and CCD32-LU (table 1); HSF2 showed only borderline radioprotection (table 1).

Linear–quadratic fits to the radiation dose–response curves (figure 3) showed that the four human tumour cell lines analysed presented a low intrinsic α component (table 2). As a function of Ukrain treatment, a pronounced increase in the α term was only detected for CCL-221 and U-138MG. Most interestingly, however, although Ukrain treatment did not change the radiation response in MDA-MB-231 and PA-TU-8902 cells significantly, α was markedly increased, as was α/β ratios.

Table 1. Cell survival of irradiated cells with and without Ukrain treatment.

	Clonogenic survival	
	2 Gy	U^a + 2 Gy
MDA-MB-231	0.8 ±0.055	0.6 ±0.046**
PA-TU-8902	0.4 ±0.056	0.31 ±0.05**
CCL-221	0.81 ±0.06	0.6 ±0.07**
U-138MG	0.63 ±0.06	0.22 ±0.06**
HSF1	0.41 ±0.14	0.7 ±0.14**
HSF2	0.405 ±0.07	0.49 ±0.12*
CCD32-LU	0.4 ±0.11	0.91 ±0.12**

[a]Ukrain, 1.0 $\mu g/ml^{-1}$, 24 h.
*$p < 0.05$; **$p < 0.001$.

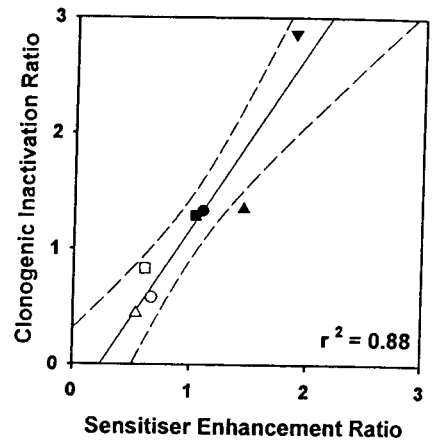
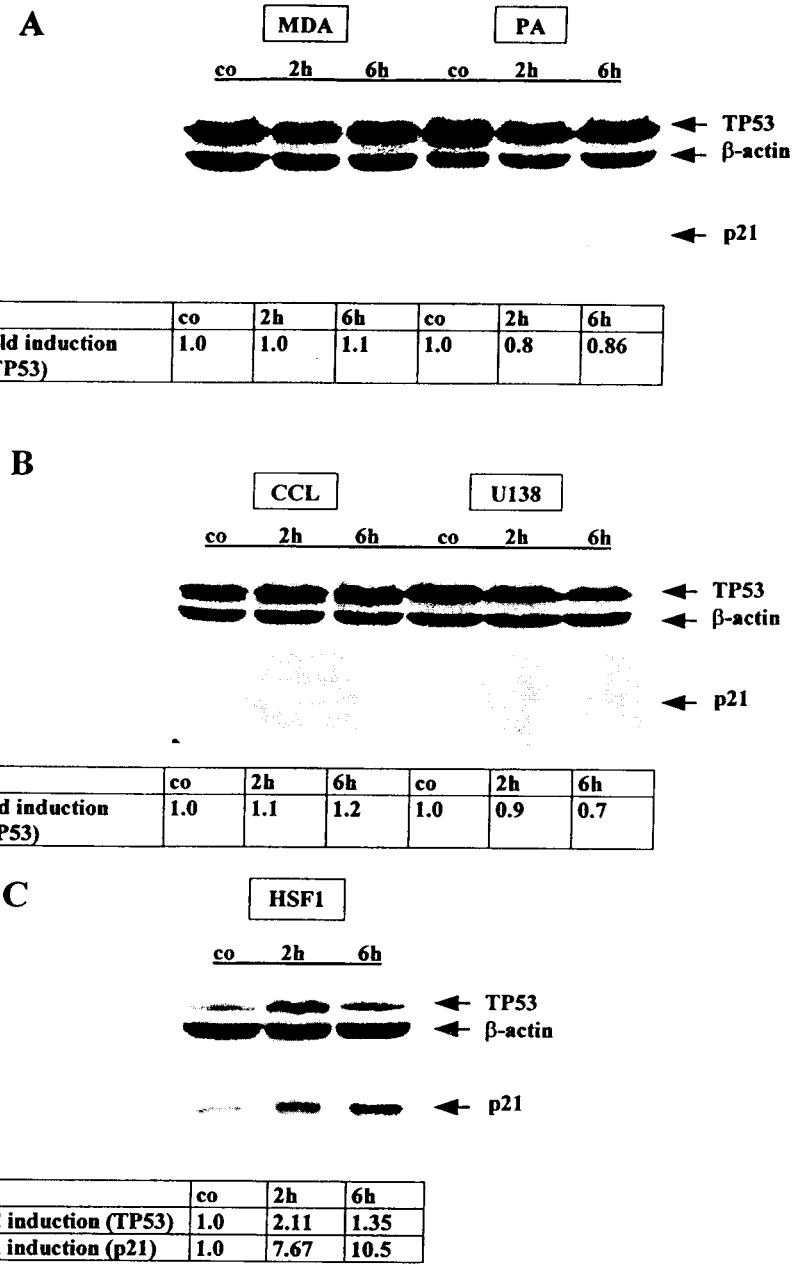


Figure 4. Correlation between clonogenic inactivation ratio and sensitizer enhancement ratio. The clonogenic inactivation ratio (CIR) was calculated according to $SF_{2untreated}/SF_{2treated}$. The sensitizer enhancement ratio (SER) was calculated by dividing the survival fraction of Ukrain-untreated cells by the survival fraction of Ukrain-treated cells at 10% cell survival. Values > 1.0 indicate enhanced drug-irradiation interaction and < 1.0 show protection. Each data point represents the mean of three independent experiments. ●, MDA-MB-231; ■, PA-TU-8902; ▲, CCL-221; ▼, U-138MG; ○, HSF1; □, HSF2; ▽, CCD32-LU.

Table 2. Effect of Ukrain on α and β.

Experiment	α (Gy^{-1})	β (Gy^{-2})	α/β
MDA-MB-231			
RT	2.09e-2 ± 9.67e-3	4.92e-2 ± 1.41e-3	0.42
Ukrain + RT	1.77e-1 ± 2.43e-2	3.35e-2 ± 4.80e-3	5.28
PA-TU-8902			
RT	2.69e-1 ± 6.66e-2	4.27e-2 ± 1.06e-2	6.3
Ukrain + RT	4.84e-1 ± 1.05e-1	3.36e-4 ± 2.26e-2	(1443)
CCL-221			
RT	2.57e-2 ± 1.89e-2	5.72e-2 ± 2.55e-3	0.45
Ukrain + RT	7.08e-2 ± 1.74e-2	9.32e-2 ± 3.35e-3	0.76
U-138MG			
RT	1.25e-1 ± 2.15e-2	3.59e-2 ± 2.56e-3	3.5
Ukrain + RT	4.94e-1 ± 1.43e-1	4.58e-2 ± 2.51e-2	10.8
HSF 1			
RT	4.50e-1 ± 6.07e-2	2.55e-2 ± 8.03e-3	17.67
Ukrain + RT	3.77e-1 ± 1.62e-1	8.22e-3 ± 3.31e-2	45.82
HSF 2			
RT	4.51e-1 ± 3.52e-2	2.44e-2 ± 5.36e-3	18.52
Ukrain + RT	3.88e-1 ± 1.89e-2	7.88e-3 ± 3.05e-3	49.26
CCD 32-LU			
RT	4.91e-1 ± 8.39e-2	1.65e-2 ± 1.01e-2	29.72
Ukrain + RT	1.41e-1 ± 1.78e-1	3.55e-2 ± 3.70e-2	3.96



	co	2h	6h	co	2h	6h
fold induction (TP53)	1.0	1.0	1.1	1.0	0.8	0.86

	co	2h	6h	co	2h	6h
fold induction (TP53)	1.0	1.1	1.2	1.0	0.9	0.7

	co	2h	6h
fold induction (TP53)	1.0	2.11	1.35
fold induction (p21)	1.0	7.67	10.5

Figure 5. TP53 and $p21^{CIP1/WAF1}$ expression. Protein extracts of irradiated cells were isolated 2 and 6 h post-radiation exposure. Detection of β-actin is shown to confirm equal loading of all lanes. Data give the ratio of the densitometric values of protein bands (TP53 or $p21^{CIP1/WAF1}$: β-actin) in relation to the untreated controls (co).

In contrast to the tumour cell lines the normal human fibroblast cell strains HSF1, HSF2 and CCD32-LU showed high intrinsic α (table 2). As expected from the radioprotective effect of Ukrain in these cells, the α components for HSF1 and CCD32-LU decreased markedly. However, for CCD32-LU the α component decreased upon Ukrain pretreatment and resulted consequently in a decrease of the α/β ratio.

Finally, all data sets obtained for the human tumour cells and normal fibroblasts tested were included in the calculation of the clonogenic inactivation ratio (CIR) and the radiation sensitizer enhancement ratio (SER) induced by Ukrain. The comparison of these two parameters revealed a strong correlation ($r^2 = 0.88$) (figure 4).

3.4. *Western blot analyses*

All four tumour cell lines and HSF1 were exposed to 5 Gy induction and stabilization of TP53 and the downstream cell-cycle inhibitor $p21^{CIP1/WAF1}$ were

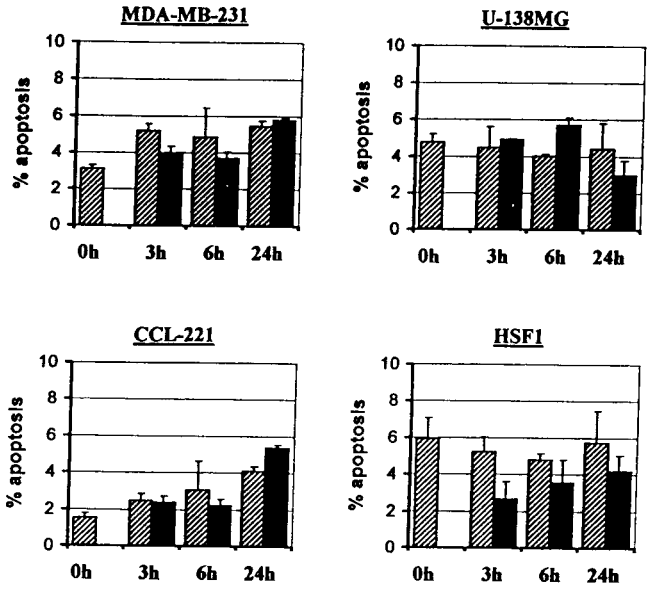


Figure 6. Apoptosis in MDA-MB-231, U-138MG, CCL-221 and HSF1 cells as determined by annexin-V staining after exposure to 1.0 $\mu g\ ml^{-1}$ Ukrain (black columns) in comparison with untreated controls (hatched columns). Cell viability >90% in all cases. Columns shown represent the means ± SD of two independent experiments.

tested using Western blotting. All four tumour cell lines showed a constitutive over-expression of TP53 protein (figure 5A, B). For MDA-MB-231 and CCL-221, these data are in agreement with reported data (Wang *et al.* 1999, Rodrigues *et al.* 1990). Concomitantly, a $p21^{CIP1/WAF1}$ response could not be detected in the tumour cell lines (figure 5A, B). The normal human fibroblasts (HSF1) showed both an induction and stabilization of TP53 and a $p21^{CIP1/WAF1}$ response upon delivery of 5 Gy (figure 5C).

3.5. *Flow cytometric analysis of apoptosis by annexin-V staining*

MDA-MB-231, U-138MG, CCL-221 and HSF1 showed no enhancement of apoptosis after Ukrain treatment with 1.0 $\mu g\ ml^{-1}$ at the time points indicated in comparison with untreated controls (figure 6).

4. Discussion

We have demonstrated for the first time a differential modulation of radiation toxicity in cultured human tumour cells and normal human fibroblasts by the *C. majus* L. alkaloid thiophosphoric acid derivative Ukrain. Both from a mechanistic and clinical point of view, it is most interesting that Ukrain treatment enhanced radiation toxicity in colorectal and brain tumour cells, that radiation toxicity of breast and pancreas cells was not affected by Ukrain, and that radiation toxicity of normal human fibroblasts was significantly *reduced* by Ukrain treatment. These data give the first strong indication for a differential effectiveness of Ukrain on tumour and normal cell survival when combined with radiation.

The anti-tumour activity of Ukrain with respect to clonogenic cell survival has been described by several reports during the last decade (Nowicky *et al.* 1993, Liepins *et al.* 1996) and was the basis for a National Cancer Institute Developmental Therapeutical Program study testing Ukrain's effectiveness in a variety of human tumour cells (Nowicky *et al.* 1993). We have confirmed the antiproliferative effects of Ukrain on tumour cells.

It has been reported that Ukrain treatment affects various cellular processes such as DNA, RNA, protein synthesis and apoptosis as well as cellular respiration (Brueller 1992, Lohninger *et al.* 1993, Liepins *et al.* 1996, Nowicky *et al.* 1996, Zemskov *et al.* 1996). Furthermore, alkaloids conjugated to alkylating agents such as Ukrain (Nowicky *et al.* 1993, Liepins *et al.* 1996) or alkylating agents themselves such as adriamycin (Momparler *et al.* 1976, Baral and Auer 1990) have been shown to affect nucleic and protein metabolism. Treatment of human K-562 leukaemia cells with Ukrain has been reported by Liepins *et al.* (1996) to inhibit topoisomerase-I and -II activity effectively. Since these enzymes are involved in DNA synthesis and repair, it has been suggested that inhibition of these activities results in a breakdown of cell proliferation and a stimulation of apoptosis, which ultimately led to an increased cytotoxicity in tumour cells. The data presented here could not confirm these findings concerning induction of apoptosis by Ukrain. Annexin-V staining of MDA-MB-231, U-138MG and CCL-221 tumour cells and HSF1 showed no Ukrain-induced increase of apoptosis as compared with untreated cells. On the basis of our flow cytometry studies, the cellular and molecular background of the reactivity profile of human tumour cells and normal fibroblasts to the combined treatment with Ukrain and ionizing radiation described appears to be influenced by two independent mechanisms. The accumulation of G_2/M-phase cells in colorectal (CCL-221) and brain tumour cells (U-138MG) treated with Ukrain for 24 h is induced at a concentration of Ukrain, which only leads to minor effects on clonogenic activity. Consequently, it can be argued that Ukrain treatment at low concentrations leads to enhanced cell-cycle activity of CCL-221 and U-138MG cells and their accumulation in the G_2/M-phase, the most radiosensitive phase of the cell-cycle at the time of radiation exposure (Sinclair 1968). Roublevskaia *et al.*

(2000) reported similar observations for human epidermoid carcinoma cell lines that were arrested in G_2/M after single Ukrain exposure.

The quite different radiation response observed for breast, pancreas human tumour cells as well as normal human fibroblasts cannot be explained by Ukrain-dependent changes in cell cycle distribution. Therefore, a different mechanism, especially with respect to the radioprotection observed in normal fibroblasts seems to be induced by Ukrain, perhaps connected with the TP53 status of the cells.

Wang *et al.* (1999) found that the breast carcinoma cell line MDA-MB-231 expressed mutant TP53 function and a mutation has also been described for the colorectal carcinoma cells CCL-221 (Rodrigues *et al.* 1989). Western-blot experiments from our laboratory analysing TP53 stabilization and $p21^{CIP1/WAF1}$ induction following radiation response confirmed these data. Likewise, for the pancreas carcinoma cell line PA-TU-8902 and the glioblastoma cell line U-138MG pronounced over-expression of TP53 without $p21^{CIP1/WAF1}$ response could be demonstrated.

In contrast, the three normal human skin fibroblasts used in the presented study have been described to present wild-type TP53 and a functioning radiation-inducible TP53 pathway, i.e. $p21^{CIP1/WAF1}$ induction (Dittmann *et al.* 1998a, b). To what extent this functional radiation response pathway can explain the observed radioprotection of normal fibroblasts by Ukrain needs further clarification. Differences in the TP53 pathway of normal fibroblasts and human tumour cells have been reported to be responsible for the radioprotective effect of Bowman–Birk protease inhibitor (BBI) in TP53-wild-type cells (Dittmann *et al.* 1998a, b) and it has been found that pretreatment with BBI leads to a stabilization of TP53 and subsequently to an induction of genes involved in DNA repair (Dittmann *et al.* 1998a, b, 2000). The TP53 pathway is of central importance for cell survival with respect to exogenous stress situations like exposure to DNA-damaging agents, e.g. Ukrain, or ionizing radiation (Kastan 1996). TP53-dependent and post-translational activated $p21^{CIP1/WAF1}$ specifically inhibits components of the cell-cycle machinery (Harper *et al.* 1993, Cox *et al.* 1994). Based on our Western-blot analyses (figure 5) and the flow cytometry data (figure 2), it seems likely that Ukrain mainly exerts cytotoxicity via a TP53-independent pathway. This is indicated by preliminary data from western blot analyses investigating the influence of 1.0 $\mu g\,ml^{-1}$ Ukrain on TP53 and p21 in wild-type TP53 A549 cells, TP53 over-expressing U-138MG cells and normal fibroblasts (Cordes *et al.* 2000, unpublished data). However, to what extent TP53 is involved in the radioprotective effects of Ukrain seen in normal fibroblasts expressing wild-type TP53 is not clear at present and needs further clarification. Detailed molecular biological studies are currently being performed to address this question.

The Ukrain-dependent changes in cell survival show that Ukrain acts as a radiosensitizer for human colorectal tumour and glioblastoma cells and as a radioprotector for normal human fibroblasts. Although from the present study the molecular mechanism of these differential effects cannot be identified, it seems that the radiosensitizing effect on colorectal carcinoma and glioblastoma cells is mediated by specific cell-cycle effects and the radioprotection observed for human fibroblasts seems to be independent of the TP53 pathway. Ukrain may have potential for clinical use in radiation oncology. Detailed molecular biological analyses are in progress addressing the specific function of Ukrain in tumour and normal cells with respect to the modulation of radiation response pathways.

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