In-vitro toxicity of Ukrain against human Ewing tumor cell lines

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Ukrain is advertised by the manufacturer as a drug for alternative cancer cures with high activity against progressive Ewing tumors. Using the MTT assay, we compared the cytotoxicity of Ukrain with the cytotoxicity of *N,N′,N″*-triethylenethiophosphoramide (thioTEPA), *Chelidonium majus* L. alkaloids, doxorubicin, cyclophosphamide and etoposide against four human Ewing tumor cell lines. In addition, we studied the cytotoxicity of thioTEPA combined with *C. majus* L. alkaloids after 48, 72 and 96 h. All compounds reduced the growth of Ewing tumor cell lines in a dose-dependent manner. The concentrations that reduced cell growth by 50% ranged between 6.2 and 31.1 μmol/l for Ukrain, 1.9 and 26.1 μmol/l for *C. majus* L. extract, and 1.7 and 448 μmol/l for thioTEPA. The sensitivity profile of Ukrain was comparable to that of the *C. majus* L. alkaloids, and different from that of thioTEPA, cyclophosphamide, etoposide and doxorubicin. Overall, doxorubicin was the most cytotoxic drug followed by cyclophosphamide. Ukrain and the *C. majus* L. alkaloids were slightly more cytotoxic than etoposide, while thioTEPA showed the lowest cytotoxicity. Co-exposure of thioTEPA with *C. majus* L. alkaloids resulted in additive but not in synergistic cytotoxicity. The in-vitro results indicate that the cytotoxicity of Ukrain against Ewing tumors is comparable to that of etoposide. While the latter can be used on the basis of broad clinical experience and known risk–benefit ratio, Ukrain for the present might be considered as a candidate for subsequent drug development by xenograft studies followed by systematic clinical trials. *Anti-Cancer Drugs* 17:1025–1030 © 2006 Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2006, **17:**1025–1030

Keywords: chelidonium alkaloids, cyclophosphamide, doxorubicin, etoposide, Ewing tumor, *N,N′,N″-triethylenethiophosphoramide, Ukrain*

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Sponsorship: This work was supported by the Friends of Children with Cancer, Viersen.

This work was part of the Doctoral Thesis of D.-M.N., J.K., A.S. and J.S.

Received 4 May 2006 **Accepted** 1 June 2006

Introduction

The use of alternative cancer cures is high and longitudinal data suggest that it is increasing. Ukrain is a drug that is advertised for alternative cancer cures by its manufacturer Nowicky Pharma (Vienna, Austria). Ukrain is a semi-synthetic product generated by thermal adduction between the *N,N′,N″*-triethylenethiophosphoramide (thioTEPA) and purified alkaloids from *Chelidonium majus* L. (Greater Celadine) (www.ukrin.com). It is licensed as a drug in Mexico and some of the countries of the former Soviet Union, such as the Ukraine and Georgia. It is not approved as a drug by the European Union, however.

The manufacturer Nowicky Pharma has produced a number of publications, which claim that Ukrain induces apoptosis, inhibits angiogenesis and metastasis, modulates immune function [1–5], selectively kills cancer cells without affecting normal healthy tissues, is devoid of mutagenicity, carcinogenicity and teratogenicity in animals [6–9], and was well tolerated by healthy human volunteers up to a daily dose of 50 mg without significant toxicity [5,10]. According to the manufacturer, Ukrain is active against colon, breast, bladder, prostate, ovarian, cervix, endometrial and bronchial carcinomas as well as testicular cancers, melanomas, leukemias, lymphomas and sarcomas [11–14]. So far, 203 cancer patients with advanced disease were reported, who received Ukrain over a mean period of 2.5 years. Overall, 20% of these patients, for whom no other therapies were available, achieved complete remission [15]. Partial remissions were observed in 60% of patients. Especially high rates of complete remission were reported for patients with neuroblastoma (60%) and with Ewing tumors (57%), indicating high activity of Ukrain against small, round, blue-cell tumors of childhood. These reports, however, were severely criticized for considerable methodological shortcomings and lack of rigorous independent replication [16]. Thus, a number of medical boards such as the German Society of Cancer, the German Society of Oncology, the German Society of Complementary Oncology of the German Alternative Practitioner and

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the Study Group on 'Methods of Unproven Efficacy in Oncology' of the Swiss Cancer League decidedly refuse the use of Ukrain for cancer treatment.

As to our knowledge preclinical data on the cytotoxicity of Ukrain against Ewing tumors have not been published so far, we compared the in-vitro toxicity of Ukrain, thioTEPA and *C. majus* L. alkaloids with the cytotoxicity of the standard anticancer drugs doxorubicin, cyclophosphamide and etoposide against four well-characterized human Ewing tumor cell lines.

Methods

Reagents

Ukrain was provided by Nowicky Pharma (Vienna, Austria) thioTEPA was purchased from Lederle (Wolfratshausen, Germany) and an ethanol extract from *C. majus* L., which contained chelidonine and other alkaloids, was obtained from Pascoe (Gießen, Germany). Doxorubicin was purchased from Pharmacia (Freiburg, Germany), vincristine from Hexal (Holzkirchen, Germany) and etoposide from Sigma-Aldrich (Deisenhofen, Germany). 4-Hydroxyperoxocyclophosphamide (4-OOH-CYC) was provided by ASTA medica (Frankfurt, Germany). The *C. majus* L. extract was standardized to a chelidonine content of 1.2 mg in 1 g extract. Stock solutions of Ukrain and thioTEPA were prepared by dissolution in sterile distilled water. Etoposide was dissolved in dimethylsulfoxide (Sigma-Aldrich). Stock solutions were diluted with complete cell culture medium. Controls consisted of complete cell culture medium.

Cell culture

VH-64, STA-ET-1 and STA-ET-2.1 were kindly provided by F. van Valen (Laboratory for Experimental Orthopaedic Research, Department of Orthopaedics, University Hospital, Munster, Germany). CADO-ES-1 was purchased from the German Collection of Microorganisms and Cell Culture (DMSZ, Braunschweig, Germany). CADO-ES-1 and VH-64 were derived from lung metastasis of typical Ewing tumors. STA-ET-1 and STA-ET-2.1 stem from primary peripheral neuroectodermal tumors.

All cell lines were grown in RPMI 1640 medium (Gibco/BRL cell culture, Invitrogen, Karlsruhe, Germany) supplemented with 200 mmol/l L-glutamine, 100 U/ml penicillin G, 100 µg/ml streptomycin, 25 µg/ml amphotericin B and 10% fetal calf serum on collagen-coated 7.5-cm^2 tissue culture flasks in a humidified atmosphere of 5% CO_2 at 37°C.

Cell viability assay

Chemosensitivity was evaluated by a modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) proliferation assay [17,18]. Cells were grown on collagen-coated 96-well flat-bottom microtiter plates (Becton Dickinson, Heidelberg, Germany). One hundred microliters of cell suspension containing 3×10^3 CADO-ES-1 or VH-64 cells, 6×10^3 STA-ET-1 cells, or 9×10^3 STA-ET-2.1 cells was seeded in each well. In order to allow adhesion to the collagen matrix and resumption of exponential growth, the cells were incubated in a humidified atmosphere with 5% CO_2 at 37°C for 72 h before 100 µl of medium containing the respective drugs at different concentrations was added. After 48, 72 and 96 h, 20 µl of MTT reagent (Sigma, Deisenhofen, Germany) was added to each well and the cells were incubated for another 4 h. The drugs were tested at the following concentrations: doxorubicin 0.004–40 µmol/l, *C. majus* L. extract standardized to chelidonine content 0.5–500 µmol/l, 4-OOH-CYC 0.5–500 µmol/l, etoposide 0.1–100 µmol/l, Ukrain 0.05–50 µmol/l and vincristine 0.05–50 µmol/l. The MTT reagent was dissolved in phosphate-buffered saline (pH 7.4) (Life Technologies, Karlsruhe, Germany) at a concentration of 5 mg/ml. In viable cells, mitochondrial dehydrogenases reduce the yellow soluble MTT to water-insoluble blue formazan crystals. An increase in the number of living cells resulted in an increase in total metabolic activity in the sample, which in turn correlated with the amount of purple formazan crystals formed. After 4 h, the supernatant was removed and the formazan crystals were dissolved in a solution of sodium dodecyl sulfate (20% w/v) in dimethylformamide and water (50% v/v). The absorbance of the dissolved formazan dye of each well was measured at 550 nm with a reference wavelength of 630 nm using an automated Dynatech MR 7000 microplate reader (Dynatech, Alexandrina, Virginia, USA).

Each drug concentration was tested in four replicates from which mean, standard deviation and coefficient of variation were calculated. Dose–response curves were plotted on a semi-logarithmic scale with the percentage of viable cells compared with untreated controls versus drug concentrations. The drug concentration capable of 50% growth inhibition relative to untreated controls (GI_{50}) at the respective time points after 48, 72 and 96 h was calculated with the equation ([percentage viable cells ($> 50\%$)] – 50)/([percentage viable cells ($> 50\%$)] – [percentage viable cells ($< 50\%$)]) × (drug concentration above 50% viable cells – drug concentration below 50% viable cells) + (drug concentration below 50% viable cells).

Statistics

Statistical analysis was carried out using the Statistical Software SigmaStat Version 3.1 (SPSS Software, Munich, Germany). The cytotoxicity of different drug combinations was compared by the one-way analysis of variance. For all pairwise multiple comparison procedures, the Holm–Sidak method was applied.

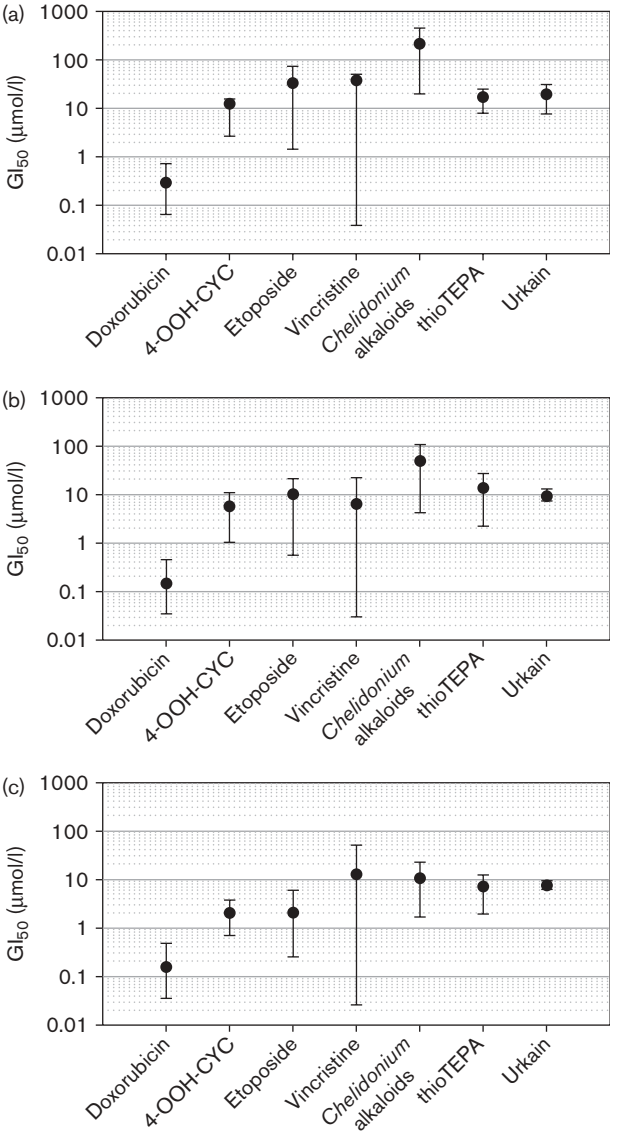
Results

All tested drugs inhibited the growth of all four Ewing tumor cell lines in a dose-dependent manner. The volumes of sterile distilled water, dimethylsulfoxide or ethanol, which were used for the highest drug concentrations, did not affect the growth of the four Ewing tumor cell lines CADO-ES-1, STA-ET-1, STAET-2.1 and VH-64. After 72 and 96 h, Ukrain significantly inhibited the growth of all cell lines treated with concentrations between 0.05 and 50 µmol/l. Figure 1 summarizes the GI_{50}s determined for Ukrain, *C. majus* L. extract, thioTEPA, etoposide, doxorubicin, vincristine and 4-OOH-CYC after 48, 72 and 96 h. The largest range between the most sensitive and the most resistant Ewing sarcoma cell line tested was observed for vincristine. The most sensitive cell line STA-ET-1 was more than 1000 times more sensitive to vincristine than the most resistant cell line CADO-ES-1 (Fig. 1). Among the four Ewing sarcoma cell lines, STA-ET-1 and VH-64 were sensitive to Ukrain and *C. majus* L. extracts, while CADO-ES-1 and STA-ET-2.1 were resistant. In addition, STA-ET-1 was most sensitive to doxorubicin, etoposide, vincristine, 4-OOH-CYC and thioTEPA. STA-ET-2.1 was most resistant to thioTEPA, 4-OOH-CYC and etoposide, while CADO-ES-1 was most resistant to doxorubicin and vincristine. For each drug, the cell lines are listed in Table 1 in ascending order from sensitive to resistant.

Overall, doxorubicin was the most cytotoxic drug against the four Ewing tumor cell lines followed by 4-OOH-CYC. With a mean GI_{50} (calculated from all cell lines and time points; $GI_{50\text{-mean}}$) of 0.2 µmol/l doxorubicin was about 30 times more cytotoxic than 4-OOH-CYC with a $GI_{50\text{-mean}}$ of 6.5 µmol/l. The $GI_{50\text{-mean}}$s of Ukrain and the *C. majus* L. extract were 11.9 and 12.3 µmol/l, respectively, which were about 60 times higher than the $GI_{50\text{-mean}}$ of doxorubicin. Etoposide ($GI_{50\text{-mean}}$: 14.9 µmol/l) was about 75 times and thioTEPA ($GI_{50\text{-mean}}$: 89.2 µmol/l) more than 450 times less cytotoxic than doxorubicin.

As our formula for the calculation of GI_{50}s differed from the formulas used by the National Cancer Institute (NCI), we also calculated growth inhibition of 50% ($GI_{50\text{-NCI}}$), total growth inhibition (TGI_{NCI}) and reduction of cell viability by 50% ($LC_{50\text{-NCI}}$) according to the NCI formulas (http://dtp.nci.nih.gov/branches/htb/ivclsp.html). For the Ewing tumor cell lines, we determined $GI_{50\text{-NCI}}$ concentrations between 4.32 and 11.8 µmol/l (mean: 7.57 µmol/l) after Ukrain exposure for 48 h. Concentrations of TGI_{NCI} were 11.5–34.2 µmol/l (mean: 27.0 µmol/l) and $LC_{50\text{-NCI}}$ concentrations ranged from 33.9 to above 50 µmol/l (mean: 40.5 µmol/l). The NCI determined for the 60 human tumor cell lines a mean $GI_{50\text{-NCI}}$ of 3.2 µmol/l, a mean TGI_{NCI} of 15.8 µmol/l and a mean $LC_{50\text{-NCI}}$ of 67.6 µmol/l.

Fig. 1



Mean GI_{50}s (50% growth inhibition), lowest and highest GI_{50}s determined after doxorubicin, 4-hydroxyperoxocyclophosphamide (4-OOH-CYC), etoposide, vincristine, *N,N′,N″*-triethylenethiophosphoramide (thioTEPA), *Chelidonium majus* L. extract and Ukrain exposure for 48 h (a), 72 h (b) and 96 h (c) in the four Ewing tumor cell lines studied. The dots represent the mean GI_{50}s for each tumor type. The range bars represent the lowest and the highest GI_{50}s determined after 48 h (a), 72 h (b) and 96h (c).

We further tested the cytotoxicity of thioTEPA combined with *C. majus* L. extracts without thermal adduction, and compared it with the cytotoxicity *C. majus* L. alkaloids and thioTEPA alone. The cell lines were incubated with either 5 or 50 µmol/l thioTEPA and with increasing concentrations of *C. majus* L. extract. Compared with untreated controls, the combinations of thioTEPA and *C. majus* L. extract were more cytotoxic than thioTEPA or the *C. majus* L. extract alone on all cell lines and at each

Table 1 Order of drug sensitivity ranking from sensitive to resistant for the four Ewing tumor cell lines STA-ET-1, STA-ET-2.1, CADO-ES-1 and VH-64

Drug	Order of cell lines
Urkain	STA-ET-1 = VH-64 < CADO-ES-1 = STA-ET-2.1
Chelidonium majus L. extract	STA-ET-1 = VH-64 < CADO-ES-1 = STA-ET-2.1
thioTEPA	STA-ET-1 < CADO-ES-1 < VH-64 < STA-ET-2.1
Etoposide	STA-ET-1 ≪ CADO-ES-1 = VH-64 < STA-ET-2.1
4-OOH-CYC	STA-ET-1 ≪ CADO-ES-1 = VH-64 < STA-ET-2.1
Doxorubicin	STA-ET-1 ≪ VH-64 = STA-ET-2.1 < CADO-ES-1
Vincristine	STA-ET-1 ≪ VH-64 = STA-ET-2.1 < CADO-ES-1

thioTEPA, *N,N′,N″*-triethylenethiophosphoramide; 4-OOH-CYC, 4-hydroxyperoxocyclophosphamide.

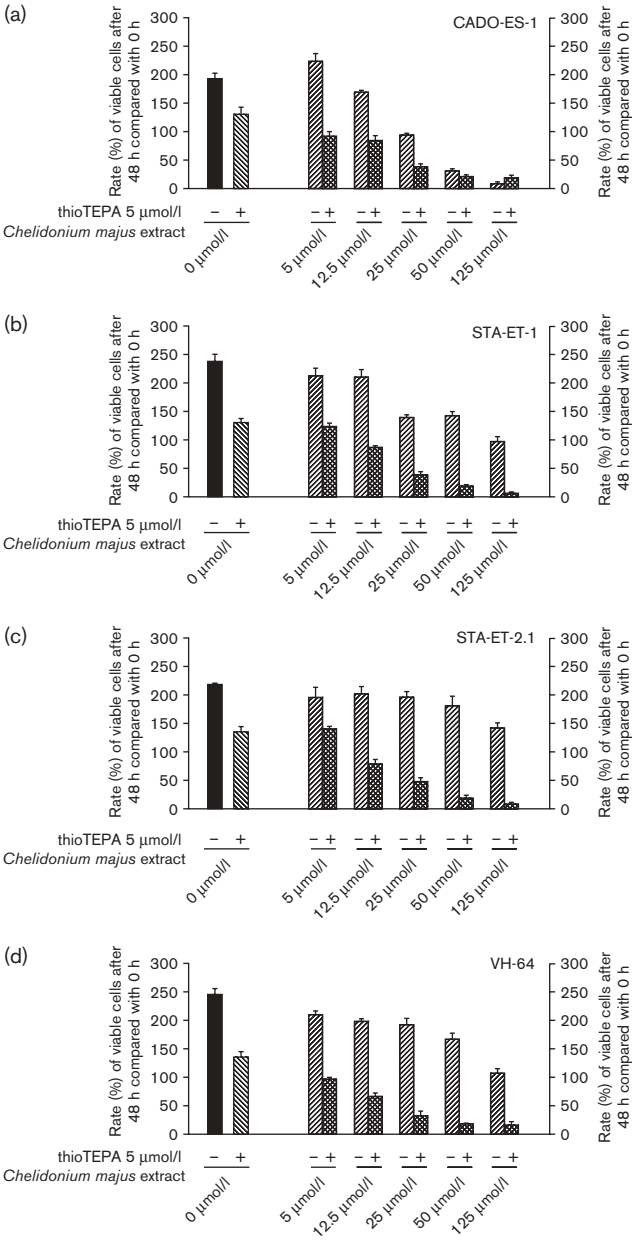
time point (Fig. 2). Comparing the viability of cells treated with 5 or 50 µmol/l thioTEPA combined with different concentrations of *C. majus* L. extract with the viability of cells treated with 5 or 50 µmol/l thioTEPA alone, however, the addition of *C. majus* L. extract to thioTEPA significantly increased the cytotoxicity after 48 h only for STA-ET-2.1 incubated with 5 µmol/l thioTEPA and 25 or 50 µmol/l *C. majus* L. extract and VH-64 incubated with 50 µmol/l thioTEPA and 25 µmol/l *C. majus* L. extract ($P < 0.05$; one-way analysis of variance, Holm–Sidak method) (Fig. 3). On the contrary, the addition of *C. majus* L. extract to 5 or 50 µmol/l thioTEPA consistently increased the rate of cell survival for STA-ET-1, which was the cell line most sensitive to thioTEPA and *C. majus* L. extract, compared with treatment with thioTEPA alone ($P < 0.05$; one-way analysis of variance, Holm–Sidak method). Thus, overall, the addition of *C. majus* L. extract to thioTEPA without the process of thermal adduction did not synergistically increase the cytotoxicity of the *C. majus* L. extract in Ewing tumor cell lines.

Discussion

The NCI screened the in-vitro toxicity of Ukrain on 60 human tumor cell lines as part of its Developmental Therapeutic Program (NSC 631570) (http://dtp.nci.nih.gov). As Ewing tumors were not among the tested tumors, we compared the cytotoxicity of Ukrain with the cytotoxicity of standard anticancer drugs against four human Ewing tumor cell lines *in vitro*.

In our experimental setting, Ukrain reduced the growth and viability of Ewing tumor cell lines in a dose-dependent manner. The effects of Ukrain were superior to that of thioTEPA and comparable to that of etoposide, which has been proven effective in the treatment of Ewing tumors *in vivo*. Ukrain, however, was significantly inferior to doxorubicin and the activated form of cyclophosphamide, which is one of the most active drugs in the treatment of Ewing tumors [19–21].

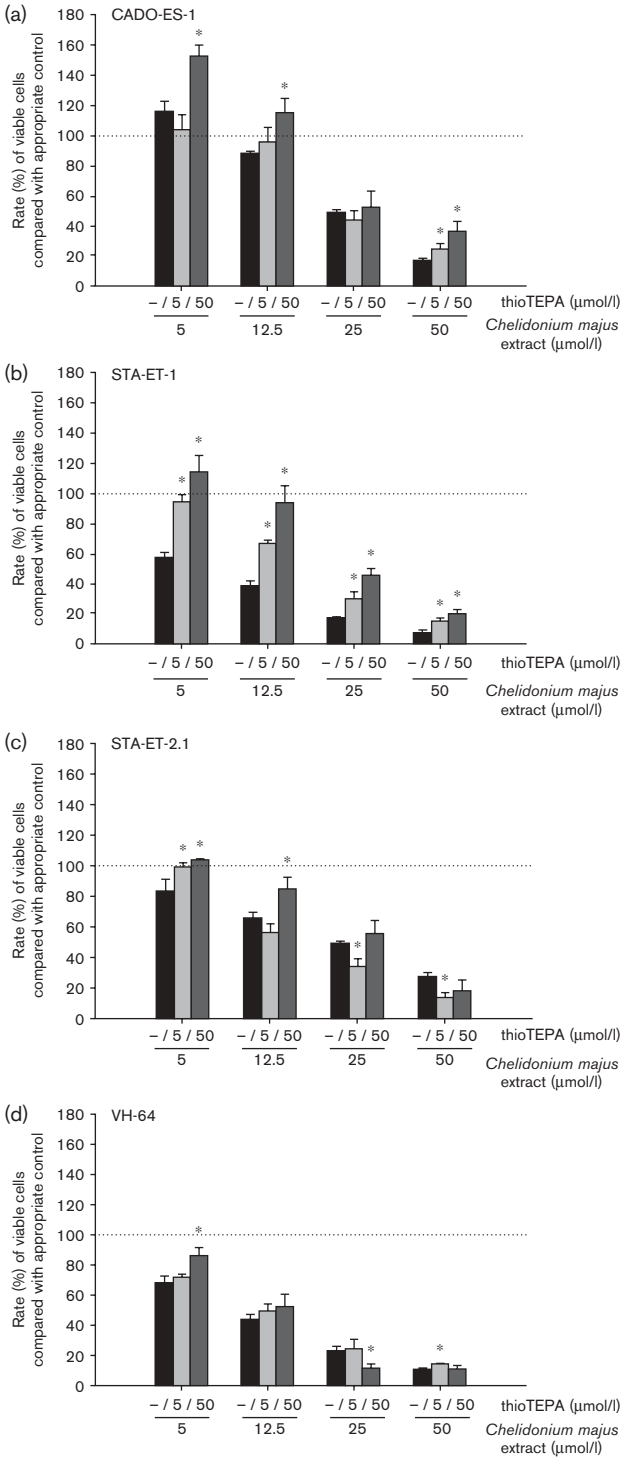
Fig. 2



Cell viability of (a) CADO-ES-1, (b) STA-ET-1, (c) STA-ET-2.1 and (d) VH-64 exposed to varying concentrations of *Chelidonium majus* L. extract with or without 5 µmol/l of *N,N′,N″*-triethylenethiophosphoramide (thioTEPA) for 48 h. The number of viable cells after 48 h was compared with the number of viable cells determined at the start of the experiment (0 h).

Regarding the $GI_{50\text{-}NCI}$ and the TGI_{NCI}, the Ewing tumor cell lines were in mean about two times less sensitive to Ukrain than the 60 human cell lines tested by the NCI Developmental Therapeutic Program. This difference might be explained by the different methods used for staining viable cells. The NCI uses the sulforhodamine B (SRB) protein assay to determine cell

Fig. 3



(a) CADO-ES-1, (b) STA-ET-1, (c) STA-ET-2.1 and (d) VH-64 exposed to varying concentrations of *Chelidonium majus* L. extract with or without 5 or 50 μmol/l of *N*,*N*′,*N*″-triethylenethiophosphoramide (thioTEPA) for 48 h. After 48 h, the viability of cells exposed to *C. majus* L. extract was compared with the cell viability of either untreated cells for the incubations with *C. majus* L. extract alone or cells exposed to 5 or 50 μmol/l thioTEPA over 48 h, respectively, for the coincubation experiments. The asterisks indicate significant difference, $P<0.05$ (one-way analysis of variance, Holm–Sidak method).

viability and the SRB protein assay has been shown to result in lower IC_{50} values than the MTT assay [22]. In addition, the cell lines used might have influenced the test results as well.

The resistance profile of Ukrain against the four Ewing tumor cell lines was comparable to that of *C. majus* L. alkaloids but not to that of thioTEPA. Similar observations were reported for HeLa, Hs27, Graham 293 and Vero cells [23,24]. The resistance profile of Ukrain also differed from those of doxorubicin, 4-OOH-CYC and etoposide (Table 1).

Panzer *et al.* [25] found *C. majus* L. alkaloids at least in a part of the commercial preparation. Habermehl *et al.* [26] did not detect the suggested trimeric structure of Ukrain in the commercial preparation, but found the *C. majus* L. alkaloids chelidonine, sanguinarine and chelerythrine, which like Ukrain effectively induced apoptosis of Jurkat cells through mitochondrial membrane depolarization and caspase activation. Regarding the sensitivity profile of Ukrain and *C. majus* L. extract against the four Ewing tumor cell lines, *C. majus* L. alkaloids might well have contributed to the cytotoxicity observed against the four Ewing tumor cell lines. The combination of thioTEPA and *C. majus* L. extract without thermal adduction increases the cytotoxicity because of the cytotoxicity of each compound. The combination of thioTEPA and *C. majus* L. extract, at least without thermal adduction, however, did not result in synergistic toxicity against the four Ewing tumor cell lines.

Oral preparations of *C. majus* L. extracts are used for spasmodic diseases of the biliary tract and spasmodic gastrointestinal diseases. Intravenous applications of *C. majus* L. are not available in the European market. Oral preparations of *C. majus* L. were repeatedly warned of because of severe side-effects such as hepatitis, cholestasis, necrosis of the liver parenchyma and lethal liver failure [27–29]. Thus, regarding the toxicities of oral *C. majus* L. preparations, the use of intravenously applied *C. majus* L. extracts or *C. majus* alkaloids should be considered with caution in general.

In our preclinical testing, Ukrain showed cytotoxicity against Ewing tumor cell lines. It was less cytotoxic than the anticancer drugs with the highest therapeutic activity in the treatment of Ewing tumors and it showed a different sensitivity profile in the Ewing tumor cell lines compared with the standard anticancer drugs, cyclophosphamide, etoposide and doxorubicin, used for the treatment of Ewing tumors. Before considering the clinical use of Ukrain in patients with Ewing sarcoma, however, further preclinical in-vivo studies and state of the art clinical trials, which address activity, tolerability and safety of Ukrain, are absolutely necessary.

Acknowledgements

The authors thank Tanja Möllers and Vera Eckernvoigt for their excellent technical assistance.

References

1 Cordes N, Plasswilm L, Bamberg M, Rodemann HP. Ukrain, an alkaloid thiophosphoric acid derivative of *Chelidonium majus* L. protects human fibroblasts but not human tumour cells *in vitro* against ionizing radiation. *Int J Radiat Biol* 2002; **78**:17–27.

2 Liepins A, Nowicky JW. Activation of spleen cell lytic activity by the alkaloid thiophosphoric acid derivative: Ukrain. *Int J Immunopharmacol* 1992; **14**:1437–1442.

3 Liepins A, Nowicky JW, Bustamante JO, Lam E. Induction of bimodal programmed cell death in malignant cells by the derivative Ukrain (NSC-631570). *Drugs Exp Clin Res* 1996; **22**:73–79.

4 Nowicky J, Greif M, Hamler F, Hiesmayr W, Staub W. Biological activity of Ukrain *in vitro* and *in vivo*. *Chemioterapia* 1987; **6**:683–685.

5 Nowicky JW. Ukrain antineoplastic immunostimulant. *Drugs Future* 1993; **18**:1011–1015.

6 Hohenwarter O, Strutzenberger K, Katinger H, Liepins A, Nowicky JW. Selective inhibition of *in vitro* cell growth by the anti-tumour drug Ukrain. *Drugs Exp Clin Res* 1992; **18** (**Suppl**):1–4.

7 Chlopkiewicz B, Marczewska J, Ejchart A, Anuszewska E, Koziorowska J. Evaluation of mutagenic, genotoxic and transforming properties of Ukrain. *Drugs Exp Clin Res* 1992; **18** (**Suppl**):31–34.

8 Kleinrok Z, Jagiell-Wojtowicz E, Nowicky JW, Chodkowska A, Feldo M, Matuszek B. Some pharmacological properties of prolonged administration of Ukrain in rodents. *Drugs Exp Clin Res* 1992; **18** (**Suppl**):93–96.

9 Kleinrok Z, Jagiello-Wojtowicz E, Matuszek B, Chodkowska A. Basic central pharmacological properties of thiophosphoric acid alkaloid derivatives from *Chelidonium majus* L. *Pol J Pharmacol Pharm* 1992; **44**: 227–239.

10 Danysz A, Kokoschinegg M, Hamler F. Clinical studies of Ukrain in healthy volunteers (phase 1). *Drugs Exp Clin Res* 1992; **18** (**Suppl**):39–43.

11 Aschhoff B. Ukrain treatment in a patient with stage IV neuroblastoma. A case report. *Drugs Exp Clin Res* 1998; **24**:243–245.

12 Aschhoff B. Ukrain and hyperthermia treatment in a patient with Ewing's sarcoma (case report). *Drugs Exp Clin Res* 1998; **24**:241–242.

13 Zemskov V, Prokopchuk O, Susak Y, Zemskov S, Tkachenko O, Hodysh Y, *et al.* Efficacy of Ukrain in the treatment of pancreatic cancer. *Langenbecks Arch Surg* 2002; **387**:84–89.

14 Gansauge F, Ramadani M, Pressmar J, Gansauge S, Muehling B, Stecker K, *et al.* NSC-631570 (Ukrain) in the palliative treatment of pancreatic cancer. Results of a phase II trial. *Langenbecks Arch Surg* 2002; **386**: 570–574.

15 Aschhoff B. Retrospective study of Ukrain treatment in 203 patients with advanced-stage tumors. *Drugs Exp Clin Res* 2000; **26**:249–252.

16 Ernst E, Schmidt K. Ukrain: a new cancer cure? A systematic review of randomised clinical trials. *BMC Cancer* 2005; **5**:69–75.

17 Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983; **65**:55–63.

18 Tada H, Shiho O, Kuroshima K, Koyama M, Tsukamoto K. An improved colorimetric assay for interleukin 2. *J Immunol Methods* 1986; **93**: 157–165.

19 Paulussen M, Frohlich B, Jurgens H. Ewing tumour: incidence, prognosis and treatment options. *Paediatr Drugs* 2001; **3**:899–913.

20 Kolb EA, Kushner BH, Gorlick R, Laverdiere C, Healey JH, LaQuaglia MP, *et al.* Long-term event-free survival after intensive chemotherapy for Ewing's family of tumors in children and young adults. *J Clin Oncol* 2003; **21**: 3423–3430.

21 Pinkerton CR, Bataillard A, Guillo S, Oberlin O, Fervers B, Philip T. Treatment strategies for metastatic Ewing's sarcoma. *Eur J Cancer* 2001; **37**:1338–1344.

22 Rubinstein LV, Shoemaker RH, Paull KD, Simon RM, Tosini S, Skehan P, *et al.* Comparison of *in vitro* anticancer-drug-screening data generated with a tetrazolium assay versus a protein assay against a diverse panel of human tumor cell lines. *J Natl Cancer Inst* 1990; **82**:1113–1118.

23 Panzer A, Joubert AM, Bianchi PC, Seegers JC. The antimitotic effects of Ukrain, a *Chelidonium majus* alkaloid derivative, are reversible *in vitro*. *Cancer Lett* 2000; **150**:85–92.

24 Panzer A, Hamel E, Joubert AM, Bianchi PC, Seegers JC. Ukrain™, a semisynthetic *Chelidonium majus* alkaloid derivative, acts by inhibition of tubulin polymerization in normal and malignant cell lines. *Cancer Lett* 2000; **160**:149–157.

25 Panzer A, Joubert AM, Eloff JN, Albrecht CF, Erasmus E, Seegers JC. Chemical analyses of Ukrain, a semi-synthetic *Chelidonium majus* alkaloid derivative, fail to confirm its trimeric structure. *Cancer Lett* 2000; **160**: 237–241.

26 Habermehl D, Kammerer B, Handrick R, Eldh T, Gruber C, Cordes N, *et al.* Proapoptotic activity of Ukrain is based on *Chelidonium majus* L. alkaloids and mediated via a mitochondrial death pathway. *BMC Cancer* 2006; **6**: 14–35.

27 Greving I, Meister V, Monnerjahn C, Muller KM, May B. *Chelidonium majus*: a rare reason for severe hepatotoxic reaction. *Pharmacoepidemiol Drug Saf* 1998; **7** (**Suppl 1**):S66–S69.

28 Stickel F, Poschl G, Seitz HK, Waldherr R, Hahn EG, Schuppan D. Acute hepatitis induced by Greater Celandine (*Chelidonium majus*). *Scand J Gastroenterol* 2003; **38**:565–568.

29 Benninger J, Schneider HT, Schuppan D, Kirchner T, Hahn EG. Acute hepatitis induced by greater celandine (*Chelidonium majus*). *Gastroenterology* 1999; **117**:1234–1237.