DRUGS EXPTL. CLIN. RES. XVIII (SUPPL.) 13–16 (1992)

STUDIES CONCERNING THE EFFECT OF UKRAIN *IN VIVO* AND *IN VITRO*

BRÜLLER W.*

Bundesstaatliche Anstalt für Experimentell-Pharmakologische und Balneologische Untersuchungen, Währinger Strasse 13a, 1090 Vienna, Austria.

Summary: *Ukrain, a reaction product of different alkaloids of* Chelidonium maius L. (*celandine*) *with Thio tepa has been investigated for possible use as an anticancer agent. A possible tumour inhibiting effect on the Ehrlich mouse ascites tumour is now tested* in vivo. *Moreover, possible changes in the oxygen consumption of mouse ascites tumour cells and a guinea pig liver homogenate are to be determined after the administration of Ukrain* in vitro *with the aid of an oxygen electrode.*

Introduction

Ukrain Tris {2-{[5bS-(5bα,6β,12bα)]-5b,6,7,12b,13,14-Hexahydro-13-methyl[1,3] benzodioxolo [5,6-c]-1,3-dioxolo[4, 5-i]phenanthridinium-6-0l}-Ethaneaminyl} Phosphinesulfide, 6HCl, is an alkaloid derivate from *Chelidonium majus* U.S. Patent No. 2, 670, 347. *In vitro* it has been shown to have toxic effects on malignant cells (1).

Materials and methods

In vivo *experiments in mice.* Ten control animals, (five animals in test group one and five test group two) were each injected with 50 µl of an Ehrlich mouse ascites tumour suspension i.p. which was 9 d old, freshly taken from a fully grown donor animal. The donor animal, as well as the animals participating in the experiment, originated from the Experimental Animal Breeding Institute, Himberg, Austria (Swiss albino/Himberg mice). The ascites tumour was originally inoculated from mice of the Cancer Research Institute of the University of Vienna. The control group was not further treated. Animal weight curve and mortality rate were followed. Test group one was injected with 10 mg Ukrain/kg animal weight in the abdominal area immediately after the tumour implantation. Equal doses were administered in 3, 6. 10, 13 and 17 d. Animal weight curve and mortality rate were followed up. Test group two received 10 mg Ukrain/kg T.G. s.c. immediately after the tumour implantation, and also 3, 6, 10, 13 and 17 d afterwards. The animal weight curve and the mortality rates were likewise determined.

In vitro *experiments, Ehrlich mouse ascites tumour.* The mouse ascites tumour cells were obtained according to the method indicated by Hornykievics and Satke-Eichler (2) from female Swiss albino/Himberg mice. As reported, there was a pH of 7.4 as a buffer for the *in vitro* measurements.

Guinea pig liver homogenate. A male guinea pig weighing about 270 g was sacrificed by nuchal rupture. The liver was removed immediately and cut

* Author to whom correspondence should be addressed at: Federal Ministry of Health, Sports and Consumer Protection, Department 111/2 (Toxicology), Radetzkystrasse 2, 1031 Vienna, Austria.

0378-6501/92/0000013 + 4 $02.00/0

into small pieces in an ice cold Ringer phosphate buffer. The fragments (without gall bladder) rinsed in the buffer were placed in a Potter Elvehjem homogenator and then homogenized by a single movement of the stamp up and down at 700 rpm. Thereafter the homogenate was centrifuged at 500 g for 10 min at 4°C. The homogenate was decanted and the sediment slightly suspended in ice cold buffer. The suspension obtained in this manner was used in further test steps.

Measurement of oxygen consumption. Both test vessels were filled with 3 ml buffer from the Biological Oxygen Monitor, Model 53 of the Yellow Springs Instrument Co., USA, with two Clark electrodes. That used for the actual isolation was allowed to temper at 37°C. An electrode was then inserted into each vessel and their equilibration followed by a recording device. After equilibrium adjustment and after both electrodes had been adjusted to each other, the tumour or liver cell suspension was added and the oxygen decrease was observed alternately in the measuring vessels and recorded by the recording device. Then, 0.2 ml aqueous Ukrain solution (5 mg/ml) was injected into one vessel and 0.2 ml distilled water injected into the other and the further oxygen decline was alternately measured. By switching over from one electrode to the other, an immediate, direct comparison of the action of Ukrain addition was possible on one hand, and the action of solvent addition to oxygen consumption was possible on the other hand.

Evaluation of test results. The percentage change of oxygen consumption immediately after drug administration was measured by a method developed by the authors. It indicates the extent of the effect of the drug. The numerical reproducibility of this measurement is independent of age, condition and number of the cells only in the optimal case where the conditions remain uniform. In the case of standardization to the greatest extent of the removal conditions and amount added, however, an at least semi-quantitative comparison is possible between the individual test series.

Results

In vivo *experiments in the mouse.* The results are given in the following Tables.

However, it is extremely noteworthy that, starting about 5 min after the addition of Ukrain, and after an initial increase of the oxygen consumption, in contrast to the ascites suspension in the parallel vessel to which only distilled water was added, a rapid decrease in oxygen consumption occurred in all cases.

Discussion

Ukrain, a derivative of *Chelidonium* alkaloids with Thio tepa, thought to be effective against cancer cells, was tested *in vivo* in an inhibition experiment with Ehrlich mouse ascites tumour and also *in vitro* by means of measuring the oxygen consumption of the same tumour type. A test was also made on a fraction of guinea pig liver homogenate consisting mainly of whole cells.

Mice implanted with the ascites tumour, either after intraperitoneal or after subcutaneous administration of Ukrain, showed a longer survival time than the implant animals which were not otherwise treated (Fig. 1). With respect to the animal weight curves, the Ukrain treated animals showed a trend towards increased weight gain compared with the control animals.

The measurement of oxygen consumption of an ascites tumour suspension by means of an oxygen electrode *in vitro* brought about a brief increase in consumption after the addition of Ukrain, followed however, by a rapid drop different from that of the control suspension not mixed with Ukrain.

An experiment carried out according to the same method with a fraction of guinea pig liver homogenate (Fig. 2) gave initially a somewhat higher increase of oxygen consumption after Ukrain addition, compared with the ascites tumour suspension and a subsequent reversal in the course, as would have been expected without pharmacon addition.

Table I In vitro *measurement of cell oxygen consumption, Ehrlich ascites tumour cells*

Injected amount cell suspension (ml)	Added amount of pharmacon (solvent) (ml)	Total vol measuring fluid (ml)	Tumour age* (days)
0.05	0.2	3.25	10

Concentration of Ukrain in the measuring fluid: 0.3125 mg/ml

Time after removal (min)		Percent change of O_2 consumption	Percent change of O_2 consumption with respect to uniform Ukrain concentration (% ml mg $^{-1}$)
19	Ukrain	+12.7	+40.6
19	H_2O	−8.9	
54	Ukrain	+17.4	+55.7
54	H_2O	−9.7	
90	Ukrain	+15.4	+49.3
90	H_2O	−6.4	

* Tumour cell age-time between inoculation of a cell suspension and its removal from the animal organism for measuring purposes. It is noteworthy that about 5 min after the addition of Ukrain (after initial increase of the oxygen consumption), in contrast to the ascites suspension in the parallel vessel to which only distilled water was added, a rapid decrease of the oxygen suspension occurred in all cases.

Table II *Guinea pig liver homogenate*

Injected amount suspension (ml)	Added amount of pharmacon (ml)	Total vol measuring fluid (ml)	Protein content of suspension (mg/ml)
0.4	0.2	3.6	97.5

Concentration of Ukrain in the measuring fluid: 0.3125 mg/ml

Time after removal (min)	Percent change of O_2-consumption	Percent change of O_2-consumption with respect to uniform Ukrain concentration (% ml mg $^{-1}$)
75	+36.6	-117.1
115	+41.3	-132.2
150	+58.1	-185.9

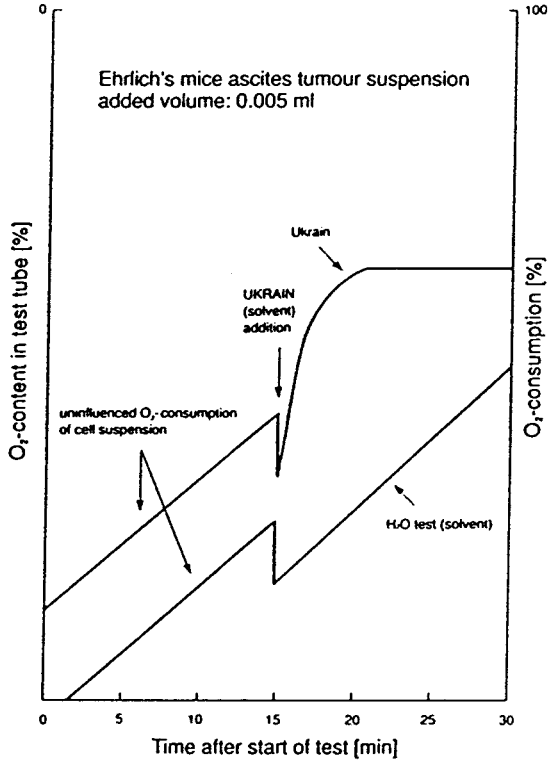

Fig. 1 Ukrain influence on oxygen consumption in malignant cells. (Ehrlich's mice ascites tumour suspension).

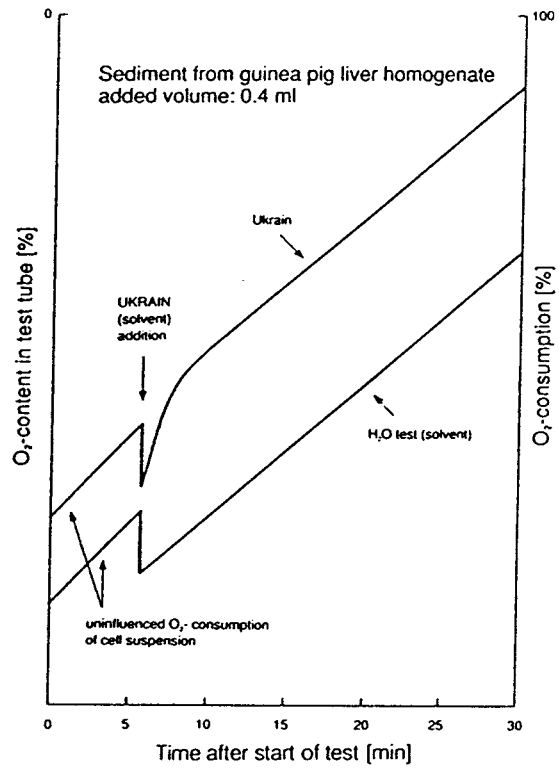

Fig. 2 Ukrain influence on oxygen consumption in normal cells. (Sediment from guinea pig liver homogenate).

However, there was no reduction in respiration compared with an untreated control suspension.

Further tests will be required to ascertain the effectiveness of Ukain against malignant cells without damage to normal cells. However, no toxic symptoms were to be found after administration of Ukrain directly on the normal cell at a dose approximately one thousand fold greater than a conventionally administered dose.

References

(1) Liepins A., Nowicky J.W. *Ukrain is selectively cytostatic and/or cytotoxic to human tumour and HIV-Infected cells but not to human normal cells.* (Abstract). Proc 17th Internat. Congr. of Chemotherapy, Berlin, 1991.

(2) Hornykievics O., Satke-Eichler I., *Influence of ergot alkaloids on the effect of 2, 4-dinitrophenol* in vitro. Arzneimittelforschung, 6, 117, 1956.