EVALUATION OF MUTAGENIC, GENOTOXIC AND TRANSFORMING PROPERTIES OF UKRAIN

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Summary: *Evaluation of mutagenic and genotoxic properties of Ukrain was on the basis of the Ames and micronucleus tests. Ukrain was investigated for its ability to induce morphological transformation of embryonic cells of the Syrian hamster. Under the experimental condition used in this study, Ukrain was found to be non-mutagenic and non-genotoxic, and futhermore did not induce morphological cell transformation.*

Introduction

Ukrain (UKSR-222) designates a thiotepa-*O*-aryl derivative fraction of alkaloids from *Chelidonium majus*. In traditional medicine *Chelidonium* sap is used for topical treatment of common warts and other skin lesions of viral and tumour origin. Alkaloids are thought to be responsible for this local effect as well as for systemic spasmolytic effect. The purpose of the present study was to evaluate mutagenic and genotoxic properties of Ukrain and its ability to induce morphological transformation in cultures of Syrian hamster embryo cells.

Materials and methods

Chemicals. 2-aminofluorene (2-AF) from Aldrich Chemical, Milwaukee, USA; Aroclor 1254 from Analabs Heidelberg, NY, USA; cyclophosphamide (CP) from Germed (Veb Jenapharm Ankerwerk, Rudolstadt); DMEM, mitomycinC and benzo(a)-pyren from Sigma Chemicals, St. Louis, MO, USA; fetal bovine serum from Gibco Laboratories, Grand Island, NY, USA. Ukrain preparation was obtained from Dr. J.W. Nowicky (Ukrainian Anti-Cancer Institute, Vienna, Austria).

Metabolic activation system. Liver homogenates (S-9) (9000 g supernatant) were obtained from Wistar Kyoto male rats weighing about 200 g each, induced with a polychlorinated biphenyl (PCP) (Aroclor 1254). A single i.p. injection of Aroclor 1254 diluted in corn oil to a concentration of 200 mg/ml at a dosage of 500 mg/kg was given to each rat five days before sacrifice. 1 ml of S-9 mix contained 100 µl of S-9, 5 µmol of glucose-6-phosphate, 4 µmol NADP, 5.6 µmol $MgSO_4$, and 100 µmol sodium phosphate, pH 7.4. The activity of S-9 was tested using a promutagen, 2-AF.

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Cell cultures. Primary cell cultures of Syrian hamster embryos at 13 days of gestation from Weterynarii Institute, Puławy, Poland were prepared and cryopreserved in liquid nitrogen as described by Pienta *et al* (1, 2). Ampoules with cryopreserved cells (SHE cells) were used as stock cultures in the transformation assay. Mass cultures were grown in Dulbecco's Modified Eagles Medium (DMEM) supplemented with 20% fetal bovine serum at 5% CO_2 atmosphere.

Ames test. Salmonella typhimurium strain TA100 was provided by Professor B.N. Ames, (Department of Biochemistry, University of California, Berkeley, CA, 94720 U.S.A.). The presence of the R-factor in this strain was checked by seeding bacteria on ampicillin-containing agar. The range of spontaneous mutation rates was 80–150. Assays were performed strictly as described by Maron and Ames (3). Ukrain was dissolved in water and added directly to the top agar at several concentrations with or without S-9 mix. After two days the colonies (revertants to histidine prototrophy) in test plates and controls were counted. In each experiment positive mutagenesis controls were included.

Micronucleus test. The micronucleus test was conducted in the manner described by Schmid (4). PZH SFISS male mice, weighing 30 g, were used in the experiments. Ukrain preparations as solutions in physiological saline were employed. Five animals were each injected with Ukrain at doses of 114 or 57 mg/kg. The doses selected ranged on both sides of the reported LD 50 in the mouse (60% and 30%). Animals injected with 50 mg/kg of cyclophosphamide served as positive controls. 24 h after the treatment the animals were sacrificed and bone-marrow smears were prepared and stained with Giemsa for evaluation. 500–1000 polychromatic erythrocytes per mouse were evaluated for the occurrence of micronuclei.

Transformation assay. SHE cells (ca 5 x 10^4) in 2 ml of DMEM were seeded on 30 mm petri dishes (Nunclon, Denmark). To obtain feeder layers 24 h cultures were exposed to mitomycin C (0.2 mg/ml/ for 18 h. 48 h later, 150 cells (target cells) in 1 ml medium were added. After 24 h Ukrain was added to obtain final concen-tration from 1:1000 to 1:10,000. The cultures were then incubated for 8 days at 5% CO_2 atmosphere, washed with phosphate-buffered saline, fixed with methanol and stained with Giemsa before counting and examination. The positive control was benzo(a)pyrene (0.25 µg/ml and 0.5 µg/ml of medium).

Quantification of transformation. Morphological transformation is defined as altered colony morphology consisting of criss-crossing and piling up of cells. To judge the substance positive in the assay, the test substance should give transformation frequencies higher than 1% in at least two independent experiments.

Results and discussion

The results of mutagenicity testing of Ukrain with strain TA100 (Ames test) are presented in Table I. The number of revertant colonies on the plates with Ukrain with or without S-9 mix is similar to the number of spontaneous revertant colonies irrespective of the concentration of Ukrain employed.

Table II summarizes the results of the effect of PC (positive control) and Ukrain on the incidence of micronuclei. The mean incidence of polychromatic erythrocytes with micronuclei in control mice was 0.46% and in cyclophosphamide-treated animals 4.45%. The mean incidence of micronuclei in polychromatic erythrocytes in Ukrain-treated animals was similar to that of controls.

The data presented in Table III indicate that Ukrain at the concentration 1:10,000 did not induce

Table I *Evaluation of mutagenic properties of Ukrain* (Salmonella [*Ames test*])

Concentration tested	his⁻ revertanta / plate*	
	without S9	with S9
0	2	22
1:2	67	76
1:4	101	78
1:8	99	85
1:16	102	97
1:32	89	101
1:64	78	120
1:128	109	127
1:250	61	95
1:500	54	92
1:1000	70	82
1:2000	70	99
1:4000	70	92

Table II *Evaluation of genotoxic properties of Ukrain* (*micronucleus test*)

Treatment	Polychromatic erythrocytes		
	scored	with micronuclei	%
Control	1500	7	0.46
Cyclophosphamide 50 mg/kg	2000	89	4.45
Ukrain 57 mg/kg	3400	15	0.44
Ukrain 114 mg/kg	4000	23	0.57

morphological transformation of SHE cells in the assay in which the transformation frequency in the cultures exposed to benzo(a)pyrene was on the average 6.7.

Under the experimental conditions used in this study, Ukrain was not mutagenic for TA100 strain and did not induce micronuclei in the PZH SFISS mice. Moreover, Ukrain did not induce morphological transformation of SHE cells. The method of transformation of mammalian cells in tissue culture by chemicals has been proposed as a rapid test to detect carcinogenic properties (5, 6) and to

Table III *Evaluation of transforming properties of Ukrain*

	Number* of colonies/dish	Cloning** frequency	Mean number of transformed colonies/dish	Transformation*** frequency
Control	36.5	24.3	-	-
Benzo(a)pyren				
0.25 µg/ml	25.0	16.6	1.0	4.0
0.50 µg/ml	16.0	10.6	1.5	9.3
Ukrain				
1:1000	-	-	-	-
1:2000	-	-		-
1:3000	-	-		-
1:4000	-	-		-
1:6000	-	-		-
1:8000	0.5	0.33		-
1:10000	26.3	17.6		-

* Mean number from 12 dishes
** Percent of target cells seeded
*** Percent of colonies

discriminate between carcinogens and non-carcinogens.

References

(1) Pienta R.J., Poiley J.A., Lebherz W.B. *Morphological transformation of early passage golden Syrian hamster embryo cells derived from cryopreserved primary cultures as reliable* in vitro *bioassay for identifying diverse carcinogens.* Int. J. Cancer., **19**, 642, 1977.

(2) Pienta R.J. *A hamster model system for indentifying carcinogens.* In: "Carcinogens: Identification and Mechanisms of Action". Griffin A.C., Shaw C.R., (eds). Raven Press, New York, 1979, pp. 121-141.

(3) Maron D.M., Ames B.N. *Revised methods for the* Salmonella *mutagenicity test.* Mutation Res., **113**, 173, 1983.

(4) Schmid W. *The micronucleus test.* Mutation Res., **31**, 9, 1975.

(5) Sanner T., Rivedal E., In: "Progress in Mutation Research", vol. 5. In conjunction with the World Health Organization, Geneva. Elsevier Science Publishers, Amsterdam, 1985, p. 665.

(6) Barrett J.C., Lamb P.W., In: "Progress in Mutation Research", vol. 5. In conjunction with the World Health Organization, Geneva. Elsevier Science Publishers, Amsterdam, 1985, p. 623.