DRUGS EXPTL. CLIN. RES. XVIII (SUPPL.) 93-96 (1992)

SOME PHARMACOLOGICAL PROPERTIES OF PROLONGED ADMINISTRATION OF UKRAIN IN RODENTS

KLEINROK Z.,[1*] JAGIEŁŁO-WÓJTOWICZ E.,[1] NOWICKY J.W.,[2] CHODKOWSKA A.,[1] FELDO M.,[1] MATUSZEK B.[1]

1) Department of Pharmacology, Medical Academy, Jaczewskiego 8, 20-090 Lublin, Poland.
2) Ukrainian Anti-Cancer Institute, Margaretenstrasse 7, 1040 Vienna, Austria.

Summary: *Some pharmacological properties of Ukrain administered intraperitoneally (i.p.) once daily for three months in mice and rats of both sexes were studied. A three month treatment with Ukrain significantly depressed the spontaneous locomotor activity and did not affect the motor coordination of mice and rats of both sexes. Ukrain did not affect the body weight gain as well as the mass of internal organs; the exception was an increase in the mass of the spleen in rats. Biochemical studies indicated that a three month treatment with Ukrain depressed the whole brain dopamine (DA) concentrations and did not affect the noradrenaline (NA) concentrations. Long-term administration of Ukrain showed no characteristic changes in the concentrations of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in the whole brain. The observed changes after three months' treatment with Ukrain are similar in mice and rats of both sexes.*

Introduction

In most studies it has been found that Ukrain may be considered as an alternative anticancer drug (1, 2, 3). After parenteral application, Ukrain quickly accumulates in the nucleus of malignant cells and causes a regression of tumours and metastases in many cancer patients (1, 4, 5). Furthermore, Ukrain can play a role in cancer control by its influence on some immunological parameters (2, 6, 7).

During therapy with Ukrain the patients tolerated the drug well. In some, negligible side effects were noted, i.e., fever and pain in certain organs and tissues. The changes may be due to the influence of Ukrain on the central nervous system. The central action of Ukrain has not yet been elucidated. Preliminary pharmacological studies (8) show that Ukrain produces significant central effects connected with an increase in the dopaminergic and an inhibition in the serotoninergic systems activities.

The aim of the present study was to investigate some of the pharmacological properties inherent in a three month administration of Ukrain to mice and rats of both sexes.

Materials and methods

Animals. The experiments were conducted on Albino Swiss mice (n = 80) of both sexes (28-30 g) and Wistar rats (n = 80) of both sexes (240-260 g). They were kept at an ambient temperature of 20-21°C on a natural day-night cycle, with free access to food and water throughout the experiment.

*Author to whom correspondence should be addressed

0378-6501/92:0000093 + 4 $02.00/0

Experimental and control groups consisted of 10 animals each, 40 in all.

Treatment. Ukrain was administered once daily for three months i.p. in doses of 4.75, 9.5 and 19.0 mg/kg to mice and of 7, 14 and 28 mg/kg to rats. The drug was injected as an aqueous solution in volumes of 0.1 ml/10 g for mice and 0.5 ml/100 g for rats. Control animals were given appriopriate amounts of the inert vehicle.

Experimental protocols

1. *Assessment of the dynamics of the body weight gain in mice and rats.* The animals were weighed before the first Ukrain application, then each week during three months of treatment and finally 24 h after the last dose of the drug.

2. *Investigation of the effect on the motor coordination.* The experiments were carried out according to Gross *et al.* (9). The mice or rats were placed on a rod rotating at 4 r.p.m. before treatment with Ukrain, then in the first, fourth and eighth week and finally 24 h after the last dose of the drug in the three month treatment. Motor coordination was considered to be undisturbed if an animal remained on the rod for two min or longer.

3. *Measurement of the spontaneous locomotor activity.* Locomotor activity of mice and rats was measured in circular photoresistor actometers (32 cm in diameter). Twenty four hours after the last dose of Ukrain the animals were placed in the actometers for one h. Each crossing of the light beam was recorded automatically.

4. *Measurement of the total body weight gain and the mass of internal organs.* Twenty four hours after administration of the last dose of Ukrain the mice and the rats were weighed and then decapitated. The brain, heart, stomach, liver, kidneys and spleen were weighed and their masses were expressed as percentage of the total body mass. Some organs were used for histological studies and the brains were collected for biochemical studies.

5. *Biochemical investigations.* The brains of mice and rats were removed and kept at −18°C until the spectrofluorometric assay. The NA and DA concentrations were determined using the method of Chang (10) as modified by Brodie *et al.* (11). The levels of 5-HT and 5-HIAA were assayed spectrofluorometrically according to Curzon and Green (12).

Statistical analysis. The results were compared with those obtained both in control and in Ukrain treated animals. The data were analysed by means of Student's t-test.

Drugs. Ukrain was obtained from the Ukrainian Anti-Cancer Institute, Vienna, Austria. All other chemicals were of analytical grade, purchased from Merck, Darmstadt, FRG.

Results

The present study demonstrated that a three month treatment with Ukrain in doses of 7, 14 and 28 mg/kg i.p. to rats of both sexes did not affect the dynamics of the body weight gain as compared with control groups. Similarly, no characteristic changes were observed in the dynamics of the body weight gain in the mice of both sexes. Between one and three weeks, slight laxation in both mice and rats was observed; it was higher in groups of female rats and mice than in male animals. However, during this period all the animals took food and water normally.

The results indicated that Ukrain treatment for three months practically did not affect the motor coordination in rats and mice of either sex.

Administration of Ukrain in doses of 14 and 28 mg/kg i.p. for three months significantly depressed the spontaneous locomotor activity of rats of both sexes (Table I). A dose of 7 mg/kg did not affect this. Similar effects were observed in mice. Ukrain in doses of 9.5 and 19 mg/kg i.p. significantly decreased the spontaneous locomotor activity of mice of both sexes (Table II). Administration of Ukrain in a dose of 4.75 mg/kg for three months gave no changes in the locomotor activity in mice

Table I *Locomotor activity of rats after three months treatment with Ukrain*

Treatment mg/kg i.p.	Sex	Time min 30	60
Control	male	168 ± 28.0	204 ± 21.6
Ukrain – 7	male	170 ± 30.0	189 ± 18.0
Ukrain – 14	male	101 ± 14.8*	120 ± 26.7*
Ukrain – 28	male	79 ± 26.4*	91 ± 23.0*
Control	female	156 ± 19.8	198 ± 23.4
Ukrain – 7	female	160 ± 23.2	186 ± 19.8
Ukrain – 14	female	111 ± 17.2*	132 ± 20.2*
Ukrain – 28	female	75 ± 20.6*	87 ± 24.7*

Results are mean ± s.e.
n = 10. *p < 0.001 as compared with the control group.

Table II *Locomotor activity of mice after three months treatment with Ukrain*

Treatment mg/kg i.p.	Sex	Time (min) 30	60
Control	male	186 ± 26.0	270 ± 31.2
Ukrain – 4.75	male	192 ± 30.0	258 ± 23.7
Ukrain – 9.5	male	109 ± 26.2*	163 ± 26.0*
Ukrain – 19.0	male	86 ± 12.0*	123 ± 21.4*
Control	female	174 ± 18.3	293 ± 28.1
Ukrain – 4.75	female	168 ± 23.0	287 ± 24.0
Ukrain – 9.5	female	111 ± 20.0*	174 ± 18.3*
Ukrain – 19.0	female	90 ± 26.0*	134 ± 26.0*

Results are mean ± s.e.
n = 10. *p < 0.001 as compared with the control group.

Table III *The effect of three months treatment with Ukrain on the size of spleen in rats*

Treatment mg/kg i.p.	Sex	Total body weight (g)	The mass of spleen
Control	male	345 ± 9.6	0.22 ± 0.02
Ukrain – 7.0	male	342 ± 10.2	0.34 ± 0.03*
Ukrain – 14.0	male	347 ± 5.6	0.40 ± 0.05*
Ukrain – 28.0	male	350 ± 10.1	0.47 ± 0.04*
Control	female	340 ± 11.0	0.21 ± 0.01
Ukrain – 7.0	female	344 ± 5.6	0.36 ± 0.02*
Ukrain – 14.0	female	350 ± 8.6	0.41 ± 0.02*
Ukrain – 28.0	female	342 ± 6.0	0.49 ± 0.04*

Results expressed as a mean percent of the total body ± s.e.
n = 10. *p < 0.001 as compared with the control group

Table IV *The effect of three months treatment with Ukrain on the levels of dopamine DA in the whole brain in rats*

Treatment mg/kg i.p.	Sex	DA in g fresh tissue
Control	male	1267 ± 36.0
Ukrain – 7.0	male	1284 ± 40.0
Ukrain – 14.0	male	1018 ± 26.0*
Ukrain – 28.0	male	963 ± 43.6*
Control	female	1311 ± 46.0
Ukrain – 7.0	female	1294 ± 29.0
Ukrain – 14.0	female	1052 ± 34.0*
Ukairn – 28.0	female	940 ± 38.0*

Each value is the mean ± s.e. from 10 determinations. *p < 0.001 as compared with the control group.

A previous study (8) indicated that after a single treatment with Ukrain similar effects were noted.

Results indicated that after three months' treatment with Ukrain the relative (and absolute) mass of the brain, heart, stomach, liver, kidneys and spleen did not differ from that of controls. The exception was an increase in the mass of the spleen in rats (three times more than in the control group). (Table III).

Biochemical studies demonstrated that in mice and rats of both sexes receiving Ukrain for three months, no characteristic changes were observed in the brain NA levels. Ukrain in doses of 14 and 28 mg/kg (for rats) and 9.5 and 19 mg/kg (for mice) significantly depressed the whole brain DA concentrations (Tables IV and V). A single administration of Ukrain showed no characteristic changes in the concentrations of NA and DA in the whole brain of rats but an increased utilization of both amines was noted (8). Administration of Ukrain for three months did not affect the 5-HT and 5-HIAA concentrations in whole

95

Table V *The effect of three months treatment with Ukrain on the levels of dopamine DA in the whole brain in mice*

Treatment mg/kg i.p.	Sex	DA /ng/g fresh tissue
Control	male	840 ± 32.0
Ukrain – 4.75	male	826 ± 18.0
Ukrain – 9.5	male	764 ± 22.0*
Ukrain – 19.0	male	703 ± 19.6*
Control	female	856 ± 27.0
Ukrain – 4.75	female	851 ± 30.0
Ukrain – 9.5	female	723 ± 21.2*
Ukarin – 19.0	female	700 ± 18.4*

Each value is the mean ± s.e. from 10 determinations. *p < 0.001 as compared with the control group.

brain of rats and mice of both sexes. Similar effects were observed after a single treatment with Ukrain (5).

Conclusion

The present results show that the prolonged administration of Ukrain to rats and mice of both sexes produces no toxic effects. During three months treatment with Ukrain only small changes in the behaviour of animals were observed, e.g., decrease of spontaneous locomotor activity. These changes are similar in rats and mice of both sexes.

The results indicate that the action of Ukrain during a three month treatment in animals is different from that obtained after a single administration of this drug. It seems that the three month administration of Ukrain causes the depression of dopaminergic transmission, which can be beneficial in clinical practice.

References

(1) Nowicky J.W., Greif M., Hamler F., Hiesmayr W., Staub W. *Biological activity of Ukrain* in vitro *and* in vivo. Chemioterapia, **6**, (Suppl. 2), 683, 1987.

(2) Nowicky J.W., Greif M., Hamler F., Staub W. *Regression of malignant tumours using the immuno-stimulation experimental drug Ukrain.* In: Proc. 6th Internat. Congr. of Immunology, Toronto, 1986, p. 445.

(3) Nowicky J.W., et al. *Evaluation of clinical studies of Ukrain in cancer patients.* J. Chemother. (Suppl. 4), 522, 1991.

(4) Nowicky J.W. *Inhibition of tumor progression and metastases by the immunomodulating agent Ukrain.* J. Cell. Biochem. (Suppl. 11D), 101, 1987.

(5) Nowicky J.W., Greif M., Hamler F., Hiesmayr W., Staub W. *Macroscopic UV-marking through affinity.* J. Tumor Marker Oncology **3**, 463, 1988.

(6) Nowicky J.W., Manolakis G., Meijer D., Vatanasapt V., Brzosko W.J. Ukrain both anticancer and immunoregulatory agent. Proc. 10th Interdisciplinary World Congress on Antimicrobiol and Anticancer Drugs. Geneva, April 1992.

(7) Nowicky J.W., Staniszewski A., Zbroja-Sontag W., Sęzak B., Nowicky W., Hiesmayr W. *Evaluation of thiophosphoric acid alkaloid derivatives from* Chelidonium majus L. ("Ukrain") *as an immunostimulant in patients with various carcinomas.* Drugs Exptl. Clin. Res. **XVII**, 139, 1991.

(8) Kleinrok Z., Jagiełło-Wójtowicz E., Matuszek B., Chodkowska A. *Basic pharmacological properties of thiophosphoric acid alkaloid derivatives from* Chelidonium majus L. Pol. J. Pharmacol. Pharm., **44**, 227, 1992

(9) Gross F., Tripod J., Meier R. *Zur pharmakologischen charakterisierung des Schlafmittels doriden.* Schweitz. Med. Wschr., **85**, 305, 1955.

(10) Chang C.C. *A sensitive method for spectrofluorometric assay of catecholamines.* Int. J. Neuropharmacol., **3**, 643, 1964.

(11) Brodie B.B., Comer M.S., Costa E., Dlabac A. *The role of brain serotonin in the mechanism of the central action of reserpine.* J. Pharmacol. Exp. Ther., **152**, 340, 1966.

(12) Curzon G., Green A.R. *Rapid method for the determination of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in small regions of rat brain.* Br. J. Pharmacol., 39, 653, 1970