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EFFECT OF THREE MONTHS TREATMENT WITH UKRAIN ON PERIPHERAL BLOOD MORPHOLOGY IN RODENTS

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Summary: *Studies on Albino Swiss mice and Wistar rats have demonstrated that Ukrain administered intraperitoneally (i.p.) for three months produces the following effects in the haematologic parameters: increased leucocytes and decreased thrombocytes. The haemoglobin and erythrocyte levels, as well as haematocrit value, were unchanged. In the leucogram changes were observed; i.e., a fall in the number of neutrophil segments and an increase in the lymphocyte count. The erythrocyte indices, P.C.V., M.C.V., M.C.H, and M.C.H.C. were not changed by a period of three months i.p. Ukrain administration. The observed changes were more marked in female than in male animals and were greater in mice than in rats.*

Introduction

Ukrain (thiophosphoric acid alkaloid derivatives from *Chelidonium majus L.*) is selectively cytostatic and cytotoxic to human malignant cells but not to human normal cells (1–4). Furthermore, this drug has immunomodulatory activity (2, 4, 5). The authors now present the results of three months treatment with Ukrain on peripheral blood morphology parameters in mice and rats of both sexes.

Materials and methods

Animals. The experiments were carried out on Albino Swiss mice of either sex weighing initially 25–27 g and on Wistar rats of either sex weighing initially 235–250 g. They were kept at room temperature (20°–21°C) on a natural day-night cycle, were housed in groups of ten per cage and had free access to food and water.

Treatment. Ukrain was given i.p. in doses equivalent to 0.025, 0.05 and 0.1 LD_{50} once daily for three months. The LD_{50} value for Ukrain in mice was 190 mg/kg i.p. and in rats 280 mg/kg i.p. (6). The drug was administered in an aqueous solution of 0.1 ml/10g to mice and 0.5 ml/100g to rats. Controls received the same volumes of the solution.

Experimental procedures. 24 h after injection of the last dose of Ukrain after 3-months treatment, the animals were decapitated and blood from the mice or rats was collected. Experimental groups consisted of ten animals each. The following parameters were determined:

a. Haemoglobin content was assayed colorimetrically (7). Erythrocytes were haemolysed with

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0.04% ammonia solution in which haemoglobin forms oxyhaemoglobin. The extinction of the solution was measured at 549 nm. A standard cyanohaemoglobin solution was used to prepare the calibration curve (7).

b. Erythrocytes: Blood was diluted 1:200 with Gowers' medium (7), and erythrocytes were counted in Bürker's chamber.

c. Leucocytes: Blood was diluted 1:20 with Türk's fluid and leucocytes were counted in Thoma's chamber.

d. The percentage composition of leucocytes was measured by the method of Schilling (8) after Pappenheim's staining (8).

e. The number of thrombocytes was assayed directly by the method of Dacie and Lewis (8). The blood was diluted 1:100 with a citrate-formol solution containing blue cresol. The thrombocytes were counted in Bürker's chamber (7, 8).

f. The haematocrit was estimated using a high-speed centrifuge and expressed as percentage (9).

g. Erythrocyte indices were calculated as follows (7, 8):

- colour index (P.C.V.) =
  = [haemoglobin /g/ 100mμ]: [Erythrocytes 3×/ millions/μl/]
- mean corpuscular volume (M.V.V.) =
  = [haematocrit/%/ ×10]: [erythrocytes/ millions /μl/]
- mean corpuscular haemoglobin (M.C.H.) =
  = [haemoglobin /g/ 100ml/×10]: [erythrocytes/ millions/μl]
- mean corpuscular haemoglobin concentration (M.C.H.C.) =
  = [haemoglobin/g/ 100ml/x10]: [haematocrit/%/]

Statistical analysis. The Student t-test was used for evaluation of the significance of results.

Chemicals. Ukrain (thiophosphoric acid alkaloid derivatives from *Chelidonium majus L.*, Ukrainian Anti-Cancer Institute, Vienna, Austria), cyanohaemoglobin (Biomed, Kraków, Poland). All other chemicals were of analytical grade and were purchased from Merck, Darmstadt, FRG.

Results

In male rats, administration of Ukrain in a dose of 7 mg/kg i.p. from 3 months scarcely affected the investigated parameters of peripheral blood, except for a decrease in the thrombocyte count (Table I). Ukrain in a dose of 14 mg/kg decreased the number of thrombocytes. The white cell pattern showed increased lymphocytes and decreased neutrophils.

Table I illustrates that the three month administration of Ukrain significantly increased the leucocyte count and decreased the thrombocyte count in male rats receiving 28 mg/kg of the drug. Changes in the leucogram pattern were also observed, i.e., an increase in the number of lymphocytes and a decrease of neutrophil segments. None of the doses affected either the haemoglobin and erythrocyte levels or the haematocrit value. The erythrocyte indices, P.C.V., M.V.V., M.C.H. and M.C.H.C. were not changed by three months Ukrain administration in male rats.

Table II shows that three months treatment with Ukrain to female rats in doses of 7, 14 and 28 mg/kg enhanced the leucocyte count and caused a depression in the number of thrombocytes (except for a dose of 7 mg/kg). The haemoglobin and erythrocyte levels, as well as the haematocrit value, were not changed. In female rats, administration of Ukrain in doses of 14 and 28 mg/kg caused changes in the white cell picture, i.e., a fall in the number of segments and an increase in the number of lymphocytes. The erythrocyte indices were not changed by three months i.p. Ukrain administration.

In male mice, administration of Ukrain in doses of 4.75, 9.5 and 19 mg/kg i.p. for three months caused an increase in the leucocyte count and a decrease in the thrombocyte count (except for a dose of 4.75 mg/kg) (Table III). The levels of erythrocytes and haemoglobin, as well as the haematocrit value, did not change. Ukrain in all doses enhanced the

Table I *Effect of a three month administration of Ukrain on peripheral blood morphology in male rats*

Treatment mg/kg i.p.	Haemoglobin g %	Erythrocytes 10^6/mm^3	Haematocrit %	Leukocytes 10^3/mm^3	Platelets 10^3/mm^3	Particular subtypes of white cells (%)				
						Neutrophils band	Neutrophils segments	Lympho-cytes	Mono-cytes	Eosino-phils
Control group	12.7 ± 0.12	4.23 ± 0.04	39 ± 0.36	6.10 ± 0.07	354.5 ± 2.7	1 ± 0.26	58 ± 0.6	40 ± 0.6	0.9 ± 0.3	0.5 ± 0.22
Ukrain 7.0	12.6 ± 0.12	4.19 ± 0.04	39 ± 0.38	6.18 ± 0.04	326.6 ± 4.8*	2.1 ± 0.3	60 ± 1.0	35.2 ± 1.16	1.5 ± 0.45	1.1 ± 0.27
Ukrain 14.0	12.7 ± 0.17	4.20 ± 0.04	39 ± 0.36	6.60 ± 0.1	335.7 ± 3.6*	4 ± 0.58*	22 ± 0.7*	73 ± 0.8*	0.7 ± 0.26	0.9 ± 0.27
Ukrain 28.0	12.6 ± 0.09	4.19 ± 0.03	39 ± 0.32	11.60 ± 0.24*	265.0 ± 3.3*	4 ± 0.4*	35 ± 1.3*	58 ± 0.8*	2 ± 0.65	0.7 ± 0.26

Male Wistar rats were treated wth Ukrain (7, 14 or 28 mg/kg i.p.) once daily for three months. Results are expressed as a mean ± s.d. (N = 10). * = significant difference ($p < 0.001$) with respect to control group, using Student's t-test.

Table II *Effect of a three month administration of Ukrain on peripheral blood morphology in female rats*

Treatment mg/kg i.p.	Haemoglobin g %	Erythrocytes 10^6/mm^3	Haematocrit %	Leukocytes 10^3/mm^3	Platelets 10^3/mm^3	Particular subtypes of white cells (%)				
						Neutrophils band	Neutrophils segments	Lympho-cytes	Mono-cytes	Eosino-phils
Control group	12.2 ± 0.13	4.09 ± 0.04	38 ± 0.42	6.30 ± 0.11	332.5 ± 4.13	2 ± 0.33	61 ± 0.56	36 ± 0.73	0.7 ± 0.21	0.6 ± 0.22
Ukrain 7.0	12.4 ± 0.1	4.15 ± 0.04	39 ± 0.32	7.81 ± 0.11*	325.7 ± 4.3	1 ± 0.23	58 ± 0.7	39 ± 0.7	1.1 ± 0.17	0.9 ± 0.23
Ukrain 14.0	12.4 ± 0.13	4.16 ± 0.05	39 ± 0.42	13.23 ± 0.18*	268.4 ± 4.12*	3 ± 0.3	26 ± 0.54*	69 ± 0.6*	1.3 ± 0.42	1 ± 0.25
Ukrain 28.0	12.5 ± 0.2	4.20 ± 0.06	39 ± 0.54	13.20 ± 0.22*	273.8 ± 3.03*	4 ± 0.4	23 ± 0.6*	72 ± 0.77*	0.8 ± 0.2	1 ± 0.23

Female rats were treated with Ukrain (7, 14 or 28 mg/kg i.p.) once daily for three months. Results are expressed as a mean ± s.d. (n = 10). * = significant difference ($p < 0.001$) with respect to control group, using Student's t-test.

number of lymphocytes, with a fall in the number of neutrophil segments. The erythrocyte indices were not changed by Ukrain administration for three months to male mice.

Table IV illustrates that in female mice administration of Ukrain in doses of 4.75, 9.5 and 19 mg/kg i.p. for three months caused an increase in the leucocyte count and a depression in the number of thrombocytes. The levels of erythrocytes and the haemoglobin, as well as the haematocrit value, did not change. Changes in the leucogram were observed, i.e., a fall in the number of segments and an increase in the number of lymphocytes. The erythrocyte indices, P.C.V., M.V.V., M.C.H. and M.C.H.C, were not changed by three months Ukrain administration.

Table III *Effect of a three month administration of Ukrain on peripheral blood morphology in male mice*

Treatment mg/kg i.p.	Haemoglobin g %	Erythrocytes $10^6/mm^3$	Haematocrit %	Leukocytes $10^3/mm^3$	Platelets $10^3/mm^3$	Particular subtypes of white cells (%)				
						Neutrophils band	Neutrophils segments	Lympho-cytes	Mono-cytes	Eosino-phils
Control group	10.9 ± 0.28	3.67 ± 0.1	34 ± 0.85	6.47 ± 0.08	281.5 ± 2.3	3 ± 0.5	57 ± 0.6	38 ± 0.86	2 ± 0.35	0.4 ± 0.16
Ukrain 4.75	10.9 ± 0.14	3.67 ± 0.04	34 ± 0.44	7.3 ± 0.06*	282.6 ± 0.73	1 ± 0.3	31 ± 0.8*	66 ± 1.13*	1 ± 0.26	0.2 ± 0.13
Ukrain 9.5	10.2 ± 0.21	3.56 ± 0.07	33 ± 0.65	9.7 ± 0.05*	266.3 ± 3.07*	4 ± 0.58	28 ± 0.56*	67 ± 0.8*	0.9 ± 0.31	0.3 ± 0.21
Ukrain 19.0	10.9 ± 0.23	3.64 ± 0.08	34 ± 0.73	11.7 ± 0.18*	259.5 ± 1.1*	8 ± 0.33*	24 ± 0.61*	65 ± 1.24*	0.2 ± 0.5	2 ± 0.43

Male mice were treated with Ukrain (4.75, 9.5 or 19 mg/kg i.p.) once daily for three months. Results are expressed as a mean ± s.d. (n = 10). * = significant difference ($p < 0.001$) with respect to control group, using Student's t-test.

Table IV *Effect of a three month administration of Ukrain on peripheral blood morphology in female mice*

Treatment mg/kg i.p.	Haemoglobin g %	Erythrocytes $10^6/mm^3$	Haematocrit %	Leukocytes $10^3/mm^3$	Platelets $10^3/mm^3$	Particular subtypes of white cells (%)				
						Neutrophils band	Neutrophils segments	Lympho-cytes	Mono-cytes	Eosino-phils
Control group	11.1 ± 0.3	3.47 ± 0.09	32 ± 0.85	6.6 ± 0.08	283.4 ± 2.0	3 ± 0.45	58 ± 0.105	39 ± 0.83	0.4 ± 0.22	0.3 ± 0.21
Ukrain 4.75	10.1 ± 0.13	3.35 ± 0.13	31 ± 0.4	7.2 ± 0.05*	271.6 ± 3.46*	0.8 ± 0.24*	28 ± 0.56*	70 ± 0.73*	0.5 ± 0.22	0.4 ± 0.22
Ukrain 9.5	9.9 ± 0.12	3.31 ± 0.04	30 ± 0.37	10.5 ± 0.84*	239.5 ± 2.58*	4 ± 0.5	22 ± 0.64*	72 ± 0.92*	1.1 ± 0.34	0.9 ± 0.27
Ukrain 19.0	10.1 ± 0.18	3.28 ± 0.07	30 ± 0.54	12.3 ± 0.16*	234.2 ± 3.23*	6.6 ± 0.47*	21 ± 0.62*	69 ± 0.71*	1.9 ± 0.48	0.9 ± 0.37

Female mice were treated with Ukrain (4.75, 9.5 or 19 mg/kg i.p.) once daily for three months. Results are expressed as a mean ± s.d. (n = 10). * = significant difference ($p < 0.001$) with respect to control group, using Student's t-test.

Discussion

The present study indicates that administration of Ukrain to mice and rats by intraperitoneal route for three months changes some investigated morphological parameters. The changes are more apparent in female than in male animals and are higher in mice than in rats.

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