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SIX-WEEK TREATMENT WITH UKRAIN IN RABBITS. PART I: MORPHOLOGICAL PARAMETERS

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Summary: *This study was performed to evaluate the effect of 6-week treatment with Ukrain in doses of 0.3, 1.5 and 3.0 mg/kg i.v. on the morphological and biochemical parameters of peripheral blood and morphology of body organs in rabbits. Ukrain application did not change the body organs and total body weight. The drug did not affect biochemical parameters of peripheral blood, except for a minor reduction in the total plasma level and increases in serum uric acid and urea indicating enhanced catabolism of proteins. The peripheral erythrocyte and leukocyte counts were not altered, whereas the percentages of lymphocytes, monocytes, and eozynophils were increased after higher doses of Ukrain. An increase in rod neutrophils and a decrease in the percentage of segmented neutrophils were also noted. These observations indicate a lack of organ toxicity following long-term treatment with Ukrain.*

Introduction

Ukrain, a semisynthetic compound consisting of alkaloids from *Chelidonium majus L.* conjugated to thiophosphoric acid, has been found to possess immunomodulatory and antineoplastic activity when studied *in vitro*, *in vivo,* and in clinical trials (1-3). We have demonstrated previously that single dose or chronic administration of Ukrain induces changes in morphological parameters of peripheral blood in rodents (4). The aim of the present study was a broader evaluation of morphological and biochemical parameters of peripheral blood following 6-week treatment with Ukrain in rabbits.

Materials and methods

Animals. The experiments were carried out on mongrel rabbits of both sexes weighing initially 3.0-3.5 kg. Rabbits were purchased from the Medical Academy breeding farm (Lublin, Poland). The animals were kept under standard laboratory conditions, at room temperature (20-21 °C) and 12 h day/12 h night cycle. Rabbits were housed in

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comprise direct cytostatic and/or cytotoxic activity *in vitro*. Studies have been performed on more than 60 human and animal cancer cell types, including brain, ovary, colon and kidney carcinomas, nonsmall and small cell lung carcinomas, melanomas, and leukemia/lymphoma (5). Malignotoxic effects are exerted at relatively low concentrations of the drug, ranging from $10^{-5.5}$ mol required to achieve 50% cell growth inhibition (GI_{50}), to $10^{-4.8}$ mol necessary for total growth inhibition (TGI), up to $10^{-4.17}$ mol required for 50% loss of the initial cell biomass (LC_{50}) (5). The cytotoxic activity of Ukrain is also observed in cisplatin-resistant malignant cell lines.

I.v. or i.m. application of Ukrain results in the selective uptake of the drug by human neoplastic cells (5, 14, 15). Fluoroscopic studies demonstrate high affinity of Ukrain to the nuclei of tumor cells in contrast to normal cells (14, 16). The subsequent inhibition of DNA, RNA and protein synthesis, as shown by the diminished incorporation of ^3H-labeled thymidine, uridine and leucine, occurs especially in malignant cell populations (17). The drug is reported to inhibit oxygen consumption *via* alterations in the mitochondrial respiratory chain and thus to induce irreversible damage to some carcinoma cell types (18). When used in therapeutic doses, Ukrain causes metabolic changes which are confined to malignant cells (18).

Ukrain potently augments (up to 48-fold) the lytic activity of splenic lymphocytes obtained from alloimmunized mice, probably due to direct activation of the effector cells' lytic mechanisms (3). Similarly, the lytic activity of interleukin (IL)-2-treated spleen cells and peritoneal exudate lymphocytes is significantly increased (3, 19). In addition, Ukrain enhances the tumoricidal function of peritoneal exudate macrophages in tumor-bearing mice, but not in normal mice (3) and increases macrophage lytic activity in murine adenocarcinoma (20).

Recent studies have revealed a new, fascinating property of Ukrain, which is able to evoke programmed cell death (apoptosis) with classical blebbing and shedding of membrane vesicles (2, 21). The process is probably mediated by quinidine-sensitive Ca^{2+}-dependent K^+-channels, as shown on human erythroleukemia cells (21). In addition, Ukrain is reported to cause another cell death program characterized by cell surface blister formation and probably resulting from inhibited microtubule formation (21).

When assessed in experimental tumor models Ukrain treatment is able to induce a significant reduction of tumor mass of up to 50% (2, 22). In mice the drug must be administered i.v. in order to exert its effects (2). When applied i.v., but not subcutaneously (s.c.) or intraperitoneally (i.p.) the drug delays the growth of syngenic mammary adenocarcinoma in Balb/c mice. Therapy was initiated 5 days after tumor implantation and consisted of five daily injections of the drug, followed by 3 days without treatment. This regimen was repeated three times (3). In other species Ukrain is effective through any parenteral route of administration (3, 4). As revealed in clinical studies, antineoplastic properties in humans are also displayed when the drug is applied parenterally, i.v. or i.m. (3, 4).

Toxicological analyses show a very high therapeutic index for Ukrain. Its LD_{50} (lethal dose necessary to induce deaths in 50% of studied animals) of about 190 mg/kg i.p. in mice and 280 mg/kg i.p. in rats, exceeds therapeutic doses by 1,000 times (23). Acute toxicity following enteral application of the drug is much smaller and LD_{50} reaches 460 mg/kg for mice and >2,000 mg/kg in rats (23). The maximum, well-tolerated dose of Ukrain is 3.5 mg/kg and 0.35 mg/kg i.v., in rats and rabbits, respectively (24). Neither cumulative toxicity nor mutagenic or teratogenic effects are observed following the application of Ukrain as indicated by

acute and chronic studies in rats, hamsters and Syrian hamster embryos (23, 25-28).

Our studies have shown that Ukrain may influence the function of the central nervous system, especially affecting serotoninergic and to a lesser extent GABA-ergic transmission (29, 30). We observed that Ukrain acts as a potent analgesic drug, able to antagonize competitively the action of morphine in animal models (31-33). We have recently attributed this to the interaction Ukrain with the opioid receptors (34). Indeed, Ukrain may interact with nonnarcotic analgesic drugs *in vivo* (35). The use of the drug does not evoke any signs of neurotoxicity (26).

Ukrain treatment does not induce any allergic response as evaluated in mice and guinea pigs (36).

Numerous clinical trials with Ukrain have been carried out on patients suffering from various types of malignant disease, usually in the advanced stage of the process, often following intense chemotherapy and/or irradiation. The majority of these reports reveal a prominent correction of patients' immune response, especially T-cell-mediated, after Ukrain treatment, as well as enhanced cell-mediated cytotoxicity with an increase in the total T-lymphocyte count and a normalization of the T-helper/T-suppressor cell ratio. No changes in serum immunoglobulin levels, complement components and acute phase proteins are observed (37). Improved functioning of the immune system is accompanied by a subjective feeling of better health, objectively better general condition of patients and regression of tumor and/or metastases (1, 8, 10-13, 37, 38). The optimal therapeutic regimen seems to be 10 injections of 5-20 mg Ukrain, every second day, in eight series separated by 10-15 days intervals. Therapy should continue for the first year with 2 months without the drug, during the second year with 3 months break, and in the third year of treatment with 4 months without Ukrain.

A study performed on a group of 70 patients suffering from different types of cancer (occurring at colon, breast, lungs, liver, *etc.*) was based on Ukrain application, either i.v. or i.m., at 2.5-25.0 mg per injection, every second, third, fourth or fifth day, for 10-90 days (10). The doses of the drug were either increasing, decreasing, or stable during the therapy. The analyses were performed on patient groups subjected to chemo- and/or radiotherapy alone, Ukrain together with chemotherapy and/or irradiation, and Ukrain as monotherapy. In some cases tumor regression was so prominent that it made surgical ablation possible. An improvement in patients' general condition, pain reduction, tumor regression, and prolongation of life were observed in several cases. In many patients reactions such as short-duration itching, feeling of warmth, and reddening or pain in the area of malignancy, were observed. This was considered to result from the degradation of tumor cells, especially when a patient responded to therapy.

The cytostatic effect of Ukrain was evaluated in 36 patients suffering from stage III cancer (rectal, breast, ovarian, liver) (11). The drug was injected every second day, at 10 mg per injection, for 30 days. The positive effect of therapy was monitored by means of ultrasonography, computerized tomography, and hematological parameters. A reduction in tumor size, and/or regression of metastases, diminished pain, and improved functioning of the immunological system were observed. Marked immunomodulatory effects in consequence of Ukrain application included an increase in the T-lymphocyte population, especially T2 and T4 subsets with subsequent correction of the T4/T8 ratio. Noteworthy in patients receiving Ukrain was that the levels of tumor markers CEA and CA-125 decreased by almost twofold, from 48-27 ng and from 3,200-1,100 U, respectively.

Potent immunomodulatory effects of Ukrain were observed in a study carried out on 103

Table IV *Effect of 6-week administration of Ukrain on blood cell count and morphological parameters of peripheral blood in female rabbits*

	Control	Ukrain (mg/kg)		
		0.3	1.5	3.0
Hemoglobin (g/dL)	12.3 ± 0.2	12.1 ± 0.4	12.6 ± 0.3	12.1 ± 0.3
Hematocrit (%)	36.7 ± 0.8	34.4 ± 3.4	35.2 ± 1.0	35.5 ± 1.2
MCV (fl)	67.7 ± 0.8	66.6 ± 0.9	67.4 ± 1.2	66.1 ± 0.9
RBC (10^6/mm^3)	5.4 ± 0.1	5.4 ± 0.3	5.2 ± 0.2	5.4 ± 0.2
Platelets (10^3/mm^3)	330.4 ± 0.9	259.1 ± 4.6*	183.1 ± 1.5*	218.8 ± 4.1*
WBC (10^3/mm^3)	5.2 ± 0.3	5.0 ± 0.7	5.9 ± 0.4	4.2 ± 0.5
including (in %):				
Rod neutrophils	2.6 ± 0.2	3.8 ± 0.3*	3.9 ± 0.5*	5.9 ± 0.8*
Segmented neutrophils	66.3 ± 0.5	55.4 ± 0.8*	42.9 ± 0.9*	37.4 ± 0.8*
Eozynophils	0.7 ± 0.2	0.9 ± 0.3	1.7 ± 0.3*	1.6 ± 0.3*
lymphocytes	26.6 ± 0.5	35.6 ± 0.5*	47.5 ± 0.6*	48.7 ± 1.6*
Monocytes	3.8 ± 0.3	4.3 ± 0.3	4.0 ± 0.3	6.4 ± 0.4

The results are expressed as a mean ±SE (n=10). * $p<0.05$ *vs.* control, Student's *t*-test
MCV = mean corpuscular volume of erythrocyte; RBC = red blood cells; WBC = white blood cells

Table V *Effect of 6-week administration of Ukrain on biochemical parameters of peripheral blood in male rabbits*

	Control	Ukrain (mg/kg)		
		0.3	1.5	3.0
Total protein (g/dL)	6.28 ± 0.20	5.70 ± 0.07*	5.61 ± 0.04*	5.73 ± 0.17*
Thymol turbidity test (U)	0.81 ± 0.04	0.99 ± 0.05	0.57 ± 0.05	0.87 ± 0.08
Bilirubin (mg/dL)	0.82 ± 0.10	0.68 ± 0.07	0.83 ± 0.09	0.61 ± 0.03
Uric acid (mg/dL)	3.20 ± 0.13	3.64 ± 0.11*	3.56 ± 0.08*	4.06 ± 0.05*
Urea (mg/dL)	14.80 ± 0.37	15.64 ± 0.52	17.34 ± 0.37*	15.90 ± 0.40*
Creatinine	0.46 ± 0.01	0.46 ± 0.02	0.40 ± 0.01	0.57 ± 0.02*
Na^+ (mEq/l)	141.30 ± 0.83	141.00 ± 0.74	140.90 ± 0.92	139.60 ± 0.48
K^+ (mEq/l)	4.07 ± 0.1	3.97 ± 0.03	3.95 ± 0.11	4.02 ± 0.04
AlAT (U)	22.70 ± 0.12	23.96 ± 0.37	22.87 ± 0.45	23.98 ± 0.16
AspAT (U)	20.10 ± 0.23	19.31 ± 0.18	19.76 ± 0.33	19.71 ± 0.15

The results are expressed as a mean ±SE (n=10). *$p<0.05$ *vs.* control, Student's *t*-test
AlAT = alanine; AspAT = aspartate aminotransferase

Table VI *Effect of 6-week administration of Ukrain on biochemical parameters of peripheral blood in female rabbits*

	Control	Ukrain (mg/kg)		
		0.3	1.5	3.0
Total protein (g/dL)	6.31 ± 0.16	5.95 ± 0.17	5.60 ± 0.21*	5.85 ± 0.13*
Thymol turbidity test (U)	0.77 ± 0.05	1.08 ± 0.06	0.69 ± 0.04	0.68 ± 0.10
Bilirubin (mg/dL)	0.76 ± 0.11	0.84 ± 0.13	0.63 ± 0.08	0.55 ± 0.07
Uric acid (mg/dL)	2.68 ± 0.11	3.63 ± 0.08*	3.53 ± 0.25*	3.90 ± 0.12*
Urea (mg/dL)	14.10 ± 0.27	16.10 ± 0.40*	16.60 ± 0.35*	16.60 ± 0.44*
Creatinine (mg/dL)	0.45 ± 0.01	0.43 ± 0.01	0.42 ± 0.01	0.57 ± 0.02*
Na^+ (mEq/l)	139.60 ± 1.13	139.80 ± 0.66	141.00 ± 0.57	142.60 ± 0.89
K^+ (mEq/l)	4.04 ± 0.05	3.91 ± 0.02	3.98 ± 0.03	4.12 ± 0.02
AlAT (U)	22.26 ± 0.59	23.80 ± 0.26	23.10 ± 0.64	23.20 ± 0.34
AspAT (U)	18.50 ± 0.17	18.80 ± 0.24	19.60 ± 0.16	20.05 ± 0.30

The results are expressed as a mean ±SE (n=10). *$p<0.05$ *vs.* control, Student's *t*-test
AlAT = alanine; AspAT = aspartate aminotransferase

Discussion

The data presented here indicate that Ukrain administered i.v. at different doses for 6 weeks did not induce changes in morphological parameters of body organs in rabbits. Ukrain, especially given at higher doses, evoked alterations in some morphological parameters of peripheral blood in rabbits of both sexes. The drug increased the percentage of rod neutrophils, eozynophils, lymphocytes, and monocytes, whereas the percentage of segmented neutrophils in peripheral blood decreased. The total WBC count was not changed after Ukrain application. Similar observations resulted from studies following acute and chronic administration of Ukrain in rodents (4, 5). Moreover, clinical trials reveal that Ukrain induces a prominent increase in the number of T-lymphocytes in cancer patients (6). The number and parameters of peripheral RBC remains unchanged in rabbits following Ukrain application.

Ukrain administration, especially at higher doses, evoked minor increases of peripheral uric acid and urea in rabbits of both sexes. This could suggest the increased metabolism of proteins due to Ukrain treatment. Indeed, prolonged application of Ukrain has also induced a reduction in plasma total protein content in rabbits. This effect may be attributed to the increase in serum thyroid hormone levels being a consequence of Ukrain administration (7) which could in turn enhance the catabolism of proteins. All other biochemical parameters of peripheral blood remained unchanged during 6-week treatment with Ukrain.

There were no signs of organ toxicity after Ukrain administration in rabbits. Other animals have also demonstrated a lack of toxicity following Ukrain application (8, 9). It might be concluded that prolonged treatment with Ukrain does not induce a toxic effect, whereas the modulatory action of the drug on peripheral immune system cells is clear-cut.

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