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SIX-WEEK TREATMENT WITH UKRAIN IN RABBITS. PART III: SERUM LEVELS OF THYROID HORMONES

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Summary: *The influence of 6-week treatment with Ukrain at doses of 0.3, 1.5 and 3.0 mg/kg i.v. on serum levels of thyroid hormones,* i.e., *thyroxine and triiodothyronine, was studied in rabbits of both sexes. Ukrain affected the serum levels of both hormones in males. An increase in the thyroxine level was found after Ukrain used at a dose of 1.5 and 3.0 mg/kg i.v., whereas increased triiodothyronine was noted when Ukrain was given at all studied doses. In females the thyroxine level was not altered by Ukrain administration, whereas the triiodothyronine concentration was increased following Ukrain at 0.3, 1.5 and 3.0 mg/kg i.v. It is probable that the altered thyroid hormone levels as a result of Ukrain application may contribute to the drug's potent immunomodulatory action.*

Introduction

Ukrain is a novel semisynthetic drug obtained from alkaloids of *Chelidonium majus L* conjugated to thiophosphoric acid. (1). Ukrain has been shown to inhibit potently the growth of cancer cells *in vivo* and *in vitro* and to improve the function of the host immune system (1-3). This drug also alters hormonal secretion in rats (4, 5). Prolonged treatment with Ukrain significantly increases the serum prolactin level in rats of both sexes (4). Furthermore, we have shown that Ukrain, applied for 6 months in ovariectomized rats, is able to normalize changes to the function of the endocrine system induced by ovariectomy (5). Six-week treatment with Ukrain induces minor changes in gonadal hormone levels as assessed in rabbits (6). This study was performed to investigate the effects of 6-week treatment with Ukrain on the serum levels of thyroid hormones in rabbits.

Materials and methods

Drugs. High-grade pure Ukrain (thiophosphoric acid alkaloid derivatives from *Chelidonium majus L.*) was obtained from the Ukrainian Anti-Cancer

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Institute (Vienna, Austria). The radioimmunoassay (RIA) kits for the determination of thyroxine and triiodothyronine levels were supplied by ORPiDI (Swierk, Poland). All other chemicals were of highest available purity and purchased from Merck (Darmstadt, Germany).

Animals. The experiments were performed on male and female mongrel rabbits weighing 3.0-3.5 kg (purchased from breeding farm of Lublin Medical Academy, Lublin, Poland). Animals were kept under standard laboratory conditions, and maintained on a 12 h day/12 h night cycle with free access to food and water. Rabbits were housed in groups of two animals per cage. Each experimental group consisted of at least seven animals.

Drug administration and blood sample collection. Ukrain was dissolved in physiological saline and injected into the rabbit ear vein (i.v.) in a volume of 0.2 ml/kg of body weight. The drug was administered at the doses of 0.3, 1.5 and 3.0 mg/kg i.v. once a day for 6 weeks. Control animals received injections of respective saline volumes. Blood specimens were collected from each animal 24 h after the last dose of Ukrain or placebo. The blood was allowed to clot and the serum fraction was separated and subsequently stored at -20 °C until RIA was performed.

RIA procedure. The levels of serum thyroxine and triiodothyronine were measured in duplicate by the RIA method according to the instructions supplied with the respective kits. The measurements were performed at room temperature using a γ-counter (Tesla Liberec, Czech). Serum hormone levels were expressed in ng/ml.

Statistical analysis. The results were evaluated using the unpaired, two-tailed Student's *t*-test. Data is presented as a mean ± standard error (SE).

Results

Six-week treatment with Ukrain at doses of 1.5 and 3.0 mg/kg i.v. daily induced a significant increase in serum thyroxine in male rabbits, from 95.7 ng/ml to 171.4 and 142.0 ng/ml, respectively (Table I). Triiodothyronine concentrations were also increased by Ukrain application at all studied doses, *i.e.,* 0.3, 1.5 and 3.0 mg/kg i.v., from 0.75 to 1.4, 1.92, and 1.09 ng/ml, respectively (Table I).

None of the studied doses of Ukrain evoked changes in serum thyroxine levels in female rabbits (Table II). Ukrain given at all studied doses (0.3, 1.5 and 3.0 mg/kg i.v.) for 6 weeks considerably increased triiodothyronine levels in female rabbits from 0.65 to 0.99, 1.85 and 0.95 ng/ml, respectively (Table II).

Table I *Effect of 6-week Ukrain administration on serum thyroid hormones level in male rabbits*

Group	Dose of Ukrain, mg/kg	Serum hormone level	
		Thyroxine, ng/ml	Triiodothyronine, ng/ml
Control	0.0	95.7 ± 6.70	0.75 ± 0.01
Ukrain	0.3	112.7 ± 6.72	1.40 ± 0.24*
	1.5	171.4 ± 19.21**	1.92 ± 0.18 ***
	3.0	142.0 ± 4.46***	1.09 ± 0.06**

The results are expressed as mean ±SE. * $p<0.05$; ** $p<0.01$; *** $p<0.001$ *vs.* control (Student's *t*-test). The blood was rapidly collected from the animals' ear vein 24 h after the injection of the last dose of the drug

Table II *Effect of 6-week Ukrain administration on serum thyroid hormones level in female rabbits*

Group	Dose of Ukrain, mg/kg	Serum hormone level	
		Thyroxine, ng/ml	Triiodothyronine, ng/ml
Control	0.0	125.9 ± 11.6	0.65 ± 0.02
Ukrain	0.3	113.7 ± 5.8	0.99 ± 0.04***
	1.5	118.3 ± 10.5	1.85 ± 0.24***
	3.0	126.1 ± 8.37	0.95 ± 0.10**

The results are expressed as a mean ±SE. ** $p<0.01$; *** $p<0.001$ *vs.* control (Student's *t*-test). The blood was rapidly collected from the animals' ear vein 24 h after the injection of the last dose of the drug

Discussion

In the present study we demonstrated that 6-week administration of Ukrain produces increases in serum triiodothyronine in rabbits of both sexes. In addition, thyroxine levels in males but not in females are also increased following the application of Ukrain.

Ukrain has been demonstrated to modulate immune system activity. Application of the drug induces an increase in the T-cell count and the correction of the T4/T8 lymphocyte ratio, with more numerous T-helper and less numerous T-suppressor cell subsets (3, 7). The drug also increases the number of natural killer (NK) cells and enhances the phagocytic activity of macrophages (8). The present report on increased thyroid hormones levels following the long-term application of Ukrain in rabbits brings new insights into the immunomodulatory action of the drug. It might be speculated that the effects of Ukrain on the function of the immune system are mediated *via* alterations in the thyroid hormone levels.

The cells of the immune system may produce and respond to hormones produced by the neuroendocrine system; moreover, both systems share a number of cellular receptors (9). Experimental studies have revealed that altered thyroxine levels may also modulate the immune function. In rodents high thyroxine levels increase the number of splenic and thymic T-cells and splenic NK-cells (10). Patients with hyperthyroid Graves' disease have a higher percentage of activated T-cells in peripheral blood (11) and an increased T4/T8 lymphocyte ratio (12, 13). Among patients with hyperthyroidism, an increased number of T8 cells has also been observed (14).

It is therefore possible that increased thyroxine and/or triiodothyronine levels following the long-term administration of Ukrain might evoke alterations in the number of peripheral lymphocytic cell subsets and contribute to the immunomodulatory action of the drug.

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