

SIX-WEEK TREATMENT WITH UKRAIN IN RABBITS. PART II: SERUM LEVELS OF GONADAL HORMONES

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Summary: *The effect of 6-week treatment with Ukrain at doses of 0.3, 1.5, and 3.0 mg/kg i.v. on the serum levels of steroid hormones, i.e., estradiol, testosterone, and progesterone, was studied in rabbits of both sexes. It is demonstrated that Ukrain treatment exerts minor changes in serum hormone levels. The level of estradiol was increased in the serum of male rabbits following Ukrain treatment only at the dose of 1.5 mg/kg i.v. Similarly, the estradiol serum level was increased after Ukrain given at 1.5 mg/kg i.v. in female rabbits. In male rabbits Ukrain application at 0.3 mg/kg i.v. increased the serum testosterone level. Serum testosterone levels were not altered following Ukrain administration up to 3.0 mg/kg i.v. in female rabbits. Ukrain raised the serum progesterone levels in male rabbits at the doses of 0.3 and 3.0 mg/kg i.v. In females, only the highest dose of Ukrain produced a significant increase of serum progesterone.*

Introduction

Ukrain, a semisynthetic drug derived from *Chelidonium majus L.* alkaloids conjugated to thio-phosphoric acid, has been demonstrated to exert antitumor effects and to induce partial and complete remissions in oncological patients (1-3). Previous reports indicate that either a single dose or long-term administration of Ukrain may cause changes in hormonal secretion in rats (4, 5).

Prolonged treatment with Ukrain significantly increases the serum prolactin level in both male and female rats (4). Furthermore, we have shown that Ukrain applied for 6 months in ovariectomized rats is able to normalize changes to the endocrine system function induced by ovariectomy (5). Clinical data have also suggested that Ukrain might affect the hormonal dynamics in breast cancer patients (6).

The aim of this study was to investigate the effects of 6-week treatment with Ukrain on the serum levels of gonadal hormones in rabbits.

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Materials and methods

Drugs. High-grade pure Ukrain (thiophosphoric acid derivatives of alkaloids from *Chelidonium majus* L.) was obtained from the Ukrainian Anti-Cancer Institute (Vienna, Austria). The radioimmunoassay (RIA) kits for the determination of estradiol, testosterone and progesterone levels were supplied by DRG Instruments GmbH (Marburg, USA). All other chemicals were purchased from Merck (Darmstadt, Germany) and were of the highest available purity.

Animals. The experiments were carried out on male and female mongrel rabbits weighing 3.0-3.5 kg, purchased from Warsaw Medical Academy breeding farm (Warsaw, Poland). Animals were kept under standard laboratory conditions and maintained on a 12 h day/12 h night cycle. Rabbits were housed in groups of two animals per cage and given food and water *ad libitum*. Each experimental group consisted of 10 animals.

Drug administration and blood sample collection. Ukrain dissolved in physiological saline was injected i.v. into the rabbit ear vein at a volume of 0.2 ml/kg of body weight. The drug was administered in doses of 0.3, 1.5, and 3.0 mg/kg i.v. once a day for 6 weeks. Control animals received i.v. 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 estradiol, testosterone and progesterone were measured in duplicate by the RIA method according to the instructions supplied with the respective kits. The measurements of the radioactivity of each tube were performed at room temperature using a γ -counter (Tesla Liberec, Czech). Serum gonadal hormone levels were expressed in the following units: estradiol, pg/ml; testosterone, ng/ml; and progesterone, ng/ml.

Statistical analysis. The results were evaluated using the unpaired, two-tailed Student's *t*-test. Data are presented as a mean \pm SE.

Results

Six-week treatment with Ukrain at 0.3 or 3.0 mg/kg i.v. did not induce significant changes in the serum estradiol in male rabbits. Ukrain at a dose of 1.5 mg/kg i.v. caused an increase in the serum hormone level from 19.8 to 33.1 pg/ml (Table I). Similarly, the serum estradiol level in females was

Table I Effect of 6-week Ukrain administration on serum gonadal hormones levels in male rabbits

Group	Dose of Ukrain, mg/kg	Serum hormone level		
		estradiol, pg/ml	testosterone, ng/ml	progesterone, ng/ml
Control	–	19.8 \pm 3.71	0.53 \pm 0.09	0.39 \pm 0.09
Ukrain	0.3	22.2 \pm 4.31	1.42 \pm 0.28**	0.89 \pm 0.06***
Ukrain	1.5	35.8 \pm 6.40*	0.55 \pm 0.18	0.57 \pm 0.11
Ukrain	3.0	29.5 \pm 5.20	0.73 \pm 0.23	0.69 \pm 0.12*

The results are expressed as a mean \pm 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 injection of the last dose of the drug.

Table II Effect of 6-week Ukrain administration on serum gonadal hormones levels in female rabbits

Group	Dose of Ukrain, mg/kg	Serum hormone level		
		estradiol, pg/ml	testosterone, ng/ml	progesterone, ng/ml
Control	–	18.8 ± 2.01	0.18 ± 0.02	0.68 ± 0.06
Ukrain	0.3	22.0 ± 3.81	0.18 ± 0.01	1.45 ± 0.42
Ukrain	1.5	33.0 ± 4.19**	0.18 ± 0.03	0.56 ± 0.14
Ukrain	3.0	19.3 ± 2.55	0.15 ± 0.09	1.23 ± 0.21**

The results are expressed as a mean ±SE (n=10). ** $p < 0.01$ vs. control (Student's *t*-test). The blood was rapidly collected from the animals' ear vein 24 h after injection of the last dose of the drug.

unchanged following Ukrain administration at 0.3 or 3.0 mg/kg i.v., whereas the dose of 1.5 mg/kg i.v. increased the level of hormone in the serum from 18.8 to 33.0 pg/ml (Table II).

Ukrain at a dose of 0.3 but not 1.5 or 3.0 mg/kg i.v. produced an increase in the serum testosterone level in male rabbits from 0.53 to 1.42 ng/ml (Table I). None of the doses of Ukrain studied influenced testosterone levels in female rabbits (Table II).

The treatment with Ukrain at doses of 0.3 and 3.0 mg/kg i.v. but not 1.5 mg/kg i.v. increased serum progesterone levels in male rabbits from 0.39 to 0.89 and 0.69 ng/ml, respectively (Table I). In female rabbits only the administration of Ukrain at 3.0 mg/kg i.v. significantly increased the serum progesterone level, from 0.68 to 1.23 ng/ml (Table II).

Discussion

Six-week treatment with Ukrain induced minor changes in gonadal hormone levels in rabbits of both sexes. In males the alterations in hormonal levels were observed following low doses of the drug, which brought about an increase in testosterone and progesterone concentrations. The estradiol level was altered by a medium dose of the drug in males. In females the testosterone level remained unchanged following Ukrain application.

The progesterone level was increased after administration of Ukrain at the lowest and highest doses, whereas only the moderate dose of the drug induced an increase in the estradiol level in female rabbits.

Ukrain has been shown to display antineoplastic and immunomodulatory properties and has already been used in oncological practise (1, 3). Our observations demonstrate that Ukrain does not depress gonadal function in experimental animals even following prolonged, 6-week application. This seems especially valuable in view of the fact that the majority of currently used antineoplastic agents may evoke profound alterations in gonadal hormone synthesis and reproductive functions (7, 8).

Presented data suggest that during Ukrain treatment, if anything, an increase in gonadal hormone levels is to be expected. An increase in testosterone and progesterone levels was found in male rabbits following the low dose (0.3 mg/kg i.v.) of the drug. There was also a tendency toward increased serum progesterone concentrations in females following the lowest dose of Ukrain; however, due to high variability, the data did not reach statistical significance. A significant increase in the progesterone level was observed only after the administration of the highest dose of the drug. Thus, the experimental data imply that therapy with Ukrain should not evoke any depression of gonadal function even during long-term administration.

References

- (1) Liepins A., Nowicky J.W. *Modulation of immune effector cell cytolytic activity and tumor growth inhibition in vivo by Ukrain (NSC 631570)*. *Drugs Exptl. Clin. Res.*, **XXII** (Suppl.), 31, 1996.
- (2) Hohenwarter O., Strutzenberger K., Katinger H., Liepins A., Nowicky J.W. *Selective inhibition of in vitro cell growth by the anti-tumor drug Ukrain*. *Drugs Exptl. Clin. Res.* **XVIII** (Suppl.), 1, 1992.
- (3) Nowicky J.W., Manolakis G., Meijer D., Vatanasapt V., Brzosko W.J., Lohninger A. *Ukrain both as anticancer and immunomodulatory agent*. *Drugs Exptl. Clin. Res.*, **XVIII** (Suppl.), 17, 1992.
- (4) Jagiello-Wójtowicz E., Kleinrok Z., Nowicky J.W., Matuszek B., Baran E., Surmaczyńska B. *Effect of single and prolonged administration of Ukrain on prolactin concentration in rats*. *Drugs Exptl. Clin. Res.*, **XVIII** (Suppl.), 89, 1992.
- (5) Jagiello-Wójtowicz E., Kleinrok Z., Nowicky J.W., Baran E. *Effect of six-month treatment with Ukrain on early osteoporosis induced by ovariectomy in rats. Part III: Preliminary studies of the levels of some hormones*. *Drugs Exptl. Clin. Res.*, **XXII** (Suppl.), 109, 1996.
- (6) Uglyanica K.N., Fomin K.A., Nefydov L.L., et al. *Influence of Ukrain on patients with surgically treated breast cancer. Part II: hormonal profile*. *Drugs Exptl. Clin. Res.*, **XXII** (Suppl.), 67, 1996.
- (7) Gradishar W.J., Schilsky R.L. *Effects of cancer treatment on the reproductive system*. *Crit. Rev. Oncol. Hematol.*, **8**(2), 153, 1988.
- (8) Apperley J.F., Reddy N. *Mechanisms and management of treatment-related gonadal failure in recipients of high dose chemoradiotherapy*. *Blood Rev.*, **9**(2), 93, 1995.