

## EFFECT OF SINGLE AND THREE MONTHS TREATMENT WITH UKRAIN ON AMINOTRANSFERASES (ALT AND AST) AND ON THE SERUM PROTEIN LEVEL IN RODENTS

JAGIELLO-WÓJTOWICZ E.,<sup>1\*</sup> KLEINROK Z.,<sup>1</sup> SURMACZYŃSKA B.,<sup>1</sup> BARAN E.,<sup>1</sup> FELDO M.,<sup>1</sup> NOWICKY J.W.<sup>2</sup>

1) Department of Pharmacology, Medical Academy, Jaczewskiego 8, 20-090 Lublin, Poland.

2) Ukrainian Anti-Cancer Institute, Margaretenstrasse 7, 1040 Vienna, Austria.

**Summary:** *The influence of Ukrain on the activity of aminotransferases (ALT and AST) and on the serum total protein content was estimated in mice and rats of both sexes receiving single or repeated doses of the drug. It was found that one hour after intraperitoneal (i.p.) administration of Ukrain no characteristic changes were recorded in the activity of the investigated enzymes, or in the serum protein content of animals of either sex. Similar effects were observed after three months treatment with Ukrain in rats of either sex. Only in mice receiving Ukrain for three months was a rise in ALT and AST activity found. No particular changes were observed in the total serum protein level, except for a small decrease in the sera of male mice.*

### Introduction

In many studies Ukrain was presented as a new drug with immunomodulating and anticancer properties (1-6). Preliminary clinical studies indicated that therapy with Ukrain in patients with various carcinomas was generally well tolerated (5, 7, 8). The therapeutic doses of Ukrain were some thousand times smaller than the toxic doses in rodents (3). During the treatment no significant changes in haematological parameters or in biochemical findings were observed in patients (5, 7).

Previous studies by the present investigators (9-11) have shown that, in experimental animals receiving single or repeated doses of Ukrain, there were negligible changes in biochemical and haematological parameters.

The object of the present work was to investigate the effects of a single and a three-months' treatment with Ukrain on the activity of aminotransferases (ALT and AST) and on the serum protein content in rodents.

### Material and methods

**Animals.** Two groups of Albino Swiss mice of either sex (18-20 g) and two groups of Wistar rats of either sex (180-220 g) were used in this study. They were kept in plastic cages in an air-conditioned room with normal day-night cycle, and received standard laboratory diet and water *ad libitum*.

**Drug administration and blood collection.** Ukrain was given i.p. in doses 4.75, 9.5 and 19 mg/kg to mice, and in doses of 7, 14 and 28 mg/kg to rats (i.e., equivalent to 0.025, 0.05 and 0.1 of their LD<sub>50</sub>). The

\* Author to whom correspondence should be addressed.

drug was administered in aqueous solution in the volume of 0.1 ml/10 g for mice and of 0.5 ml/100 g for rats. The control groups of animals received i.p. identical volumes of inert solution.

In long term experiments the animals received Ukrain in the above doses once daily for three months.

The mice ( $n = 80$ ) and the rats ( $n = 80$ ) of the first groups were sacrificed by decapitation one h after the injection of Ukrain or placebo, and blood was collected.

The mice ( $n = 80$ ) and the rats ( $n = 80$ ) of the second groups, which received Ukrain or placebo once daily for three months, were decapitated 24 h after the last dose of the drug and blood was collected.

**Experimental procedures.** The blood from each animal was allowed to clot and the serum was separated. The activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured by a colourimetric method according to Reitman and Frankel (12).

The total serum protein was assayed by biuret (13). The extinction was recorded by using a Specol colourimeter at 480 nm for the enzymes and at 550 nm for the protein.

**Drugs.** Ukrain, a thiophosphoric acid alkaloid derivative from *Chelidonium majus L.*, was received in its purest state from Ukrainian Anti-Cancer Institute (Vienna, Austria). All other chemicals were of analytical grade and were supplied by Merck (Darmstadt, FRG).

**Statistical analysis.** Student's t-test was used for evaluation of the significance of results.

## Results

The present investigation showed that a single treatment with Ukrain did not affect the activity of alanine and aspartate aminotransferases in the sera of mice and rats of either sex. No changes in the total serum protein were found.

Table I The effects of a three-month treatment with Ukrain on aminotransferase activity and serum protein levels in male mice

Treatment mg/kg i.p.	Unit activity of		Protein level (g %)
	ALT	AST	
Control	81.0 ± 0.60	63.0 ± 0.60	6.9 ± 0.06
Ukrain - 4.75	86.0 ± 0.63*	72.0 ± 0.82*	6.9 ± 0.05
Ukrain - 9.5	91.0 ± 0.75	69.0 ± 0.33*	6.0 ± 0.05*
Ukrain - 19.0	96.0 ± 0.56*	70.0 ± 0.60*	6.2 ± 0.04*

Each value is the mean ± s.d. from 10 determinations. \* $p < 0.001$  as compared to control group.

Table II The effects of a three-month treatment with Ukrain on aminotransferase activity and serum protein levels in female mice

Treatment mg/kg i.p.	Unit activity of		Protein level (g %)
	ALT	AST	
Control	82.0 ± 0.54	64.0 ± 0.70	6.98 ± 0.04
Ukrain - 4.75	83.0 ± 0.61	70.0 ± 0.52*	6.9 ± 0.03
Ukrain - 9.5	90.0 ± 0.62	71.0 ± 0.67*	6.9 ± 0.04
Ukrain - 19.0	91.0 ± 0.7*	69.0 ± 0.57*	6.70 ± 0.08

Each value is the mean ± s.d. from 10 determinations. \* $p < 0.001$  as compared to control group.

The administration of Ukrain once daily for three months affected neither the activity of the investigated enzymes nor the serum protein content in rats of either sex. As Tables I and II show, the changes in activity of the tested aminotransferases were observed in sera of male and female mice. A significant rise in the activity of ALT (except for a dose of 4.75 mg/kg) and of AST was noted after a three-month treatment with Ukrain. A small decrease in the total serum protein level was found after repeated administration of Ukrain in doses of 9.5 and 19 mg/kg in male mice (Table I), while in female mice this effect was observed only after a dose of 19 mg/kg (Table II).

## Discussion

On the basis of these results the authors suggest that either a single dose or long-term administration of Ukrain causes only negligible changes in mice, and none in rats, which means that Ukrain is safe for use. This seems to confirm previous pharmacological studies (9, 11, 14) and the preliminary clinical estimation of Ukrain concerning the non toxic activity of this drug (3, 7, 15).

## References

- (1) Liepins A. *Enhancement of cell mediated lysis of tumor cells by Chelidonium majus L. alkaloids (Ukrain)*. J. Cancer Res. Clin. Oncol. **116** (Suppl.), 436, 1990.
- (2) Nowicky J.W. *Inhibition of tumour progression and metastasis by the immunomodulating agent Ukrain*. J. Cell Biochem. (Suppl. 11D), 101, 1987.
- (3) Nowicky J.W., Greif M., Hamler F., Hiesmayr W., Staub W. *Biological activity of Ukrain in vitro and in vivo*. Proc. 5th Mediterranean Congr. of Chemotherapy. Chemioterapia, **6**, (Suppl. 2), 683, 1987.
- (4) Nowicky J.W., Greif M., Hamler F., Staub W. *Regression of malignant tumours using the immuno-stimulating experimental drug Ukrain*. Proc. 6th Internat. Congr. of Immunology, Toronto, 1986.
- (5) Nowicky J.W., et al. *Evaluation of clinical studies of Ukrain in cancer patients*. J. Chemother., **4**, (Suppl.), 522, 1991.
- (6) Nowicky J.W., Staniszewski A., Zbroja-Sontag W., Śliżak 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.
- (7) Liepins A., Nowicky J.W. *Ukrain is selectively cytostatic and/or cytotoxic to human tumour and HIV-infected cells but not to human normal cells*. Proc. 17th Internat. Congr. of Chemotherapy, Berlin, 1991.
- (8) Nowicky J.W., Manolakis G., Meijer D., Vatarasapt V., Brzosko W.J. *Ukrain: both anticancer and immunoregulatory agent*. 10th Interdisciplinary World Congress on Antimicrobial and Anti-cancer Drugs, Geneva, 30 March - 1 April, 1992.
- (9) Jagiełło-Wójciewicz E., et al. *Effect of a three-month treatment with Ukrain on peripheral blood morphology in rodents*. Drugs Exptl. Clin. Res., **XVIII**, (Suppl.), 79, 1992.
- (10) Kleinrok Z., Jagiełło-Wójciewicz E., Matuszek B., Chodkowska A. *Basic pharmacological properties of thiophosphoric acid alkaloid derivatives from Chelidonium majus L.* Pol. J. Pharmacol. Pharm., **44**, 227, 1992.
- (11) Kleinrok Z., Jagiełło-Wójciewicz E., Nowicky J.W., Chodkowska A., Feldo M., Matuszek B. *Some pharmacological properties of prolonged administration of Ukrain in rodents*. Drugs Exptl. Clin. Res., **XVIII**, (Suppl.), 93, 1992.
- (12) Reitman S., Frankel S. *A colorimetric method for determination of serum glutamic oxalacetic and glutamic pyruvic transaminases*. Am. J. Clin. Pathol., **28**, 56, 1957.
- (13) Tomaszewski L. "Mikrometody biochemiczne w laboratorium klinicznym". PZWL, Warszawa, 1970, p. 65.
- (14) Jagiełło-Wójciewicz E., Kleinrok Z., Nowicky J.W., Matuszek B., Baran E., Surmaczyńska B. *Effects of single dose and of prolonged administration of Ukrain on prolactin concentration in rats*. Drugs Exptl. Clin. Res., **XVIII**, (Suppl.), 89, 1992.
- (15) Malter M., Süss R. "Preliminary Report on Ukrain - Studies of Toxicity and Immunomodulation (The Effect on Cytotoxicity)". German Cancer Research Centre, Heidelberg, 1985, pp. 1-6.