ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XXII, N 4, 26 SECTIO DDD 2009

Department of Toxicology, Medical University of Lublin

MONIKA GAWROŃSKA-GRZYWACZ, AGNIESZKA ŻELAZOWSKA, ANNA OZIMEK, EWA JAGIEŁŁO-WÓJTOWICZ

Effect of combined administration of Ukrain and imipramine on selected biochemical serum parameters in rodents

Wpływ łącznego podawania leku Ukrain i imipraminy na wybrane parametry biochemiczne w surowicy krwi gryzoni

Semi-synthetic herbal drug Ukrain (i.e. tiophosphoric acid derivative of alkaloids *Chelidonium majus* L.) possesses a cytotoxic and/or cytostatic effect as well as immunomodulating properties [8, 13, 14]. It is applied before or after chemotherapy and/or radiotherapy because it reduces toxic effects of earlier administered cytostatics as well as prolongs and improves quality of patients' lives [2, 3]. The process of cancer diagnostics and treatment may cause depression which can be cured by tricyclic antidepressants. Unfortunately, these antidepressants are known for a lot of side effects especially liver dysfunction and interactions with other drugs [4, 11, 12]. On the contrary drug Ukrain exhibited a significant hepatoprotective effect in human and experimental animals hepatitis [5, 9, 10, 15]. These results open up the possibility of its use in the treatment of hepatitis induced as a side effect by different drugs. There is no literature data on the combined effect of Ukrain and tricyclic antidepressants. Therefore, the aim of the present study was evaluation whether, and in what degree, Ukrain with imipramine (IMI) influence the serum biochemical parameters which could indicate liver function in rodents.

MATERIAL AND METHODS

Drugs and chemicals. The following substances were used in the study: Ukrain – i.e. tiophosphoric acid derivative of alkaloids *Chelidonium majus* L. (aqueous high-purity concentrate 1:33, Ukrainian Anti-Cancer Institute, Vienna, Austria), imipramine hydrochloride (IMI) from Sigma-Aldrich GmbH (Germany) and also *aqua pro injectione* (Polfa Lublin, Poland). Ready-made diagnostic kits were used to determine: aspartate (AST) and alanine (ALT) aminotransferases activities, total protein level (Cormay Diagnostic S.A., Lublin, Poland) and α-fetoprotein (AFP) level (IBL, Hamburg and Dima GmbH, Goettingen, Germany).

Animals. The study was carried out on Albino-Swiss male mice (20-–5 g) and male Wistar rats (200–250 g) from a licensed breeder (Gorzkowska, Breeding of Laboratory Animals, Warsaw, Poland). The animals were kept at room temperature (20±1ºC) under a natural day-night cycle in constant environmental conditions (humidity, noise). They had access to food and water *ad libitum*. The experiments were approved by the Local Ethics Committee on Animal Experimentation of the Medical University of Lublin.

Treatments. Aqueous solutions of IMI (5 mg/kg for mice and 10 mg/kg for rats) and Ukrain (9.5 or 19 mg/kg for mice and 14 or 28 mg/kg for rats) were prepared *ex tempore* and administered intraperitoneally (ip) once daily for 10 days separated or combined in constant volumes 0.1 ml/10 g of mice body weight and 0.5 ml/100 g of rat body weight. The control groups received the same amounts of *aqua pro injectione*.

Experimental protocols. The experimental groups consisted of eight animals each. 24 hours after the last injection, the animals were decapitated and the blood was taken and centrifuged for 10 minutes at 3000 rpm. The serum was stored at -20ºC until biochemical determinations.

Statistical analysis. Results are expressed as mean ± SEM. Statistical significance among the groups was determined by Student's *t*-test and *p*-values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Drug polytherapy is commonly used in oncological treatment in order to achieve the best results. However, applying a lot of drugs, often of a different pharmacological profile, may result in toxic interactions. Adverse effects of combined drugs may lead to dysfunctions of organs, particularly the liver. Post-treatment liver damage is a common and serious clinical problem. The ALT and AST aminotransferases are basic diagnostic parameters of liver functioning. Each change in their activities, especially growth, supplies important information on the liver condition. Also, changes in total protein and AFP levels in blood serum point to the possibility of liver dysfunction.

The results indicated that10-day combined administration with Ukrain (9.5 or 19 mg/kg for mice and 14 or 28 mg/kg for rats) and imipramine (5 mg/kg for mice and 10 mg/kg for rats) caused changes in serum levels of total protein, AFP and activity of aminotransferases compared with those of Ukrain and imipramine groups of rodents. In mice the activities of both aminotransferases were reduced in serum (Fig. 1, 2) but in rats this combined treatment led to an increase of AST activity (Fig. 3). The examined proteins' concentrations (total protein and AFP) were significantly enhanced (Fig. 4, 5) in mice sera. However, in rats the above-mentioned combined administration led to an increase (Ukrain 14 mg/kg) or decrease (Ukrain 28 mg/kg) of total protein concentration (Fig. 6) while AFP concentration was unaffected.

It was found that imipramine administered at 5 mg/kg to mice and 10 mg/kg to rats for 10 days caused an increase of AST activity in mice serum (Fig. 1) and a decrease of ALT activity in rat serum (Fig. 7). However, the concentration of total protein after imipramine therapy was significantly enhanced in the serum samples of all examined rodent groups (Fig. 4, 6). The 10-day treatment with Ukrain 9.5 mg/kg and 19 mg/kg increased AST and ALT (only 19 mg/kg) activities in mice blood serum (Fig. 1, 2) and that with Ukrain 28 mg/kg contrarily decreased AST activity in rat blood serum (Fig. 3), which is in agreement with previous studies [6, 7]. The total protein levels were also affected in different ways in mice and rats. The 10-day administration of Ukrain in higher doses (19 mg/kg to mice and 28 mg/kg to rats) caused a reduction of this parameter in mice serum (Fig. 4) but a rise in the serum of rats (Fig. 6). The level of AFP in the sera of all rodents treated with Ukrain (9.5 or 19 mg/kg for mice and 14 or 28 mg/kg for rats) did not change compared with control groups (Fig. 5).

Literature data [4, 11, 12] give information on the potential damaging effect of IMI on liver cells characterized by enhanced activities of aminotransferases and total protein level. Ukrain, on the other hand, does not show hepatotoxic effect [1, 8, 14]. It can even decrease the number of side effects and improve the effectiveness of the human hepatitis treatment [15]. A hepatoprotective effect of Ukrain in experimental hepatitis in rodents was also observed [5, 9, 10].

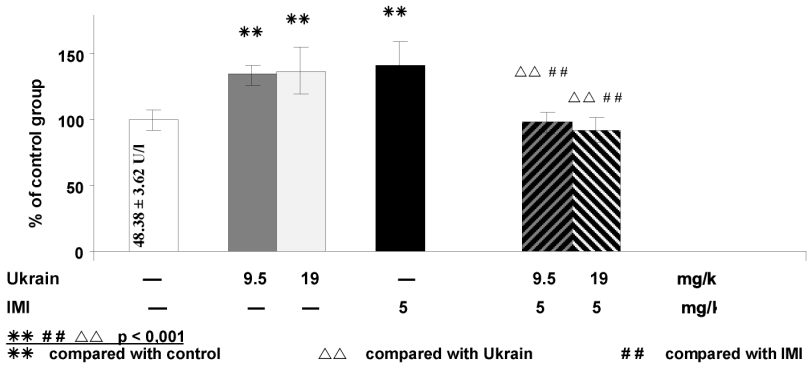

Fig. 1. Effect of 10-day combined administration of Ukrain with IMI on AST activity in mice serum

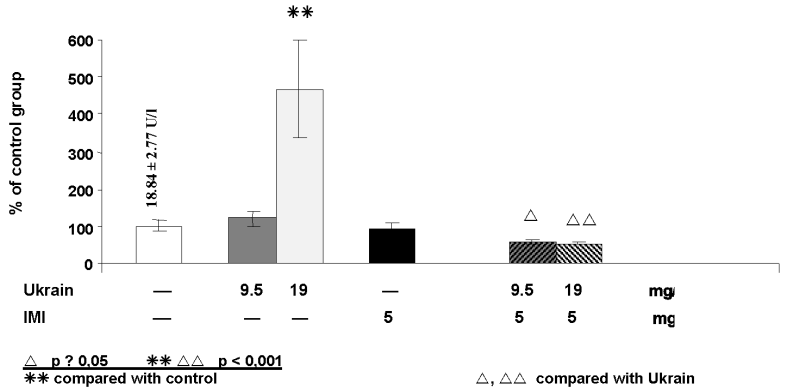

Fig. 2. Effect of 10-day combined administration of Ukrain with IMI on ALT activity in mice serum

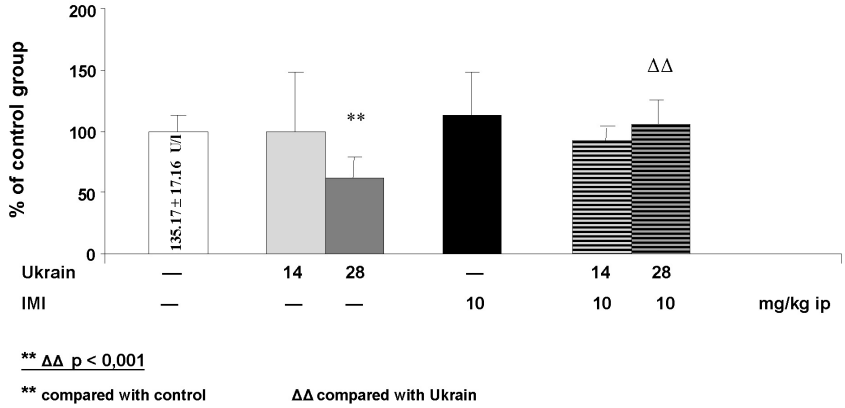

Fig. 3. Effect of 10-day combined administration of Ukrain with IMI on AST activity in rat serum

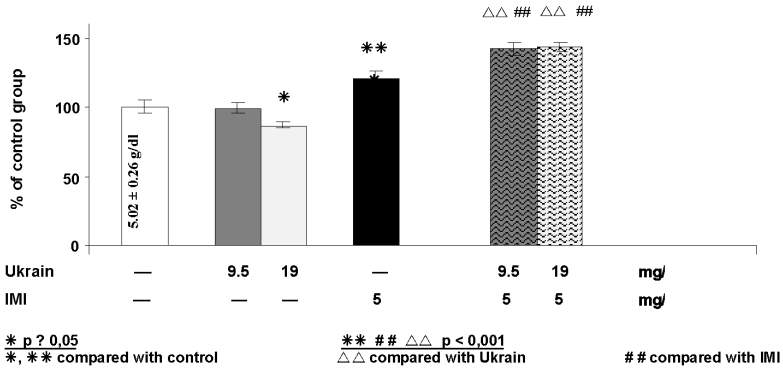

Fig. 4. Effect of 10-day combined administration of Ukrain with IMI on total protein level in mice serum

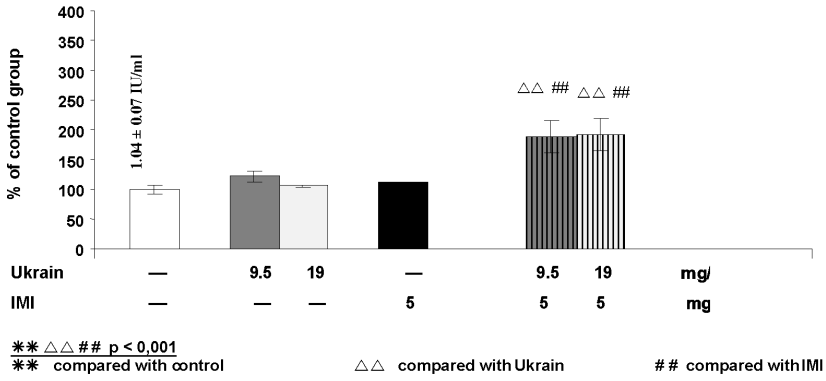

Fig. 5. Effect of 10-day combined administration of Ukrain with IMI on AFP level in mice serum

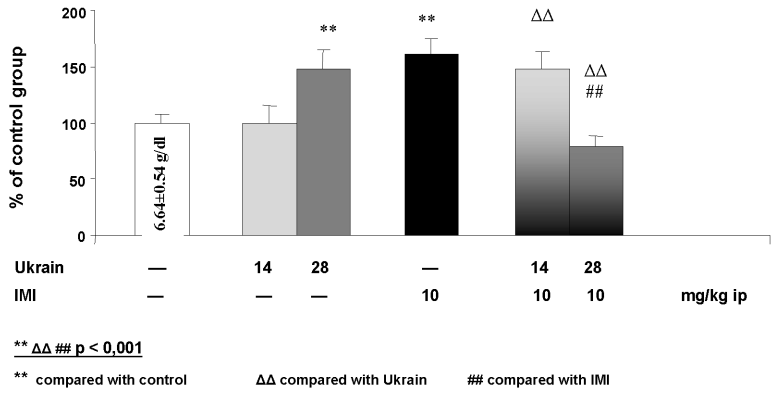

Fig. 6. Effect of 10-day combined administration of Ukrain with IMI on total protein level in rat serum

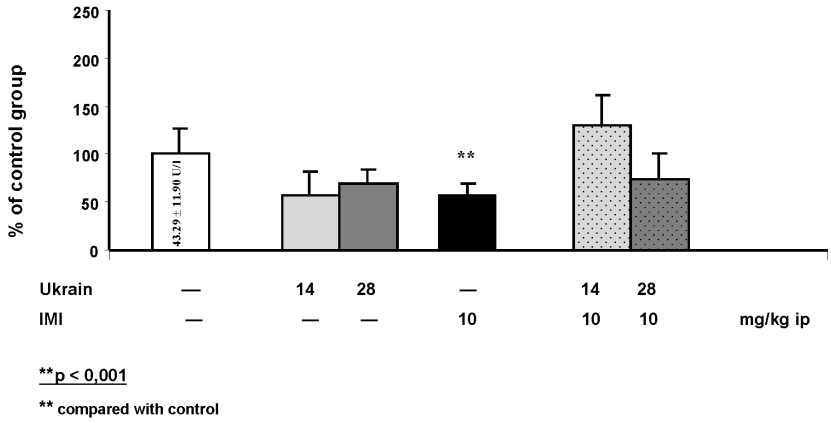

Fig. 7. Effect of 10-day combined administration of Ukrain with IMI on ALT activity in rat serum

The present study indicates that a combined 10-day treatment with Ukrain and imipramine causes a decrease of ALT and AST activities in blood serum of mice and an increase of the AST activity in rats (only for 28 mg/kg of Ukrain). The same treatment resulted in an increase of the AFP (only in mice) and total protein in all rodents. The observed changes in the examined biochemical parameters in animals treated with Ukrain and IMI together pointed out that Ukrain did not protect from adverse effect of imipramine on the hepatic functional state. Additionally, the results may suggest the possibility of hepatotoxic interaction between Ukrain and imipramine, but only when used for a long time.

Acknowledgements. The authors wish to express their gratitude to dr J.W. Nowicky (Ukrainian Anti-Cancer Institute, Vienna, Austria) for the generous gift of Ukrain.

REFERENCES

1. Danysz A. et al.: Clinical studies of Ukrain in healthy volunteers (phase 1). Drugs Exptl. Clin. Res., 18, 39, 1992.
2. Gansauge F et al.: The clinical efficacy of adjuvant systemic chemotherapy with gemcitabine and NSC-631570 in advanced pancreatic cancer. Hepatogastroenterology, 54 (75), 917, 2007.
3. Gansauge F. et al.: NSC-631570 (Ukrain) in the palliative treatment of pancreatic cancer. Results of a phase II trial. Langenbecks Arch. Surg., 386 (8), 570, 2002.
4. Horst D. A. et al.: Prolonged cholestasis and progressive hepatic fibrosis following imipramine therapy. Gastroenterology, 79 (3), 550, 1980.
5. Izdebska M., Jagiełło-Wójtowicz E.: Effect of single or 10-day treatment with Ukrain on some biochemical parameters in the serum of rats acutely intoxicated with alcohol. Toxicol. Lett., 158 (Suppl. 1), S6, 2005.
6. Jagiełło-Wójtowicz E. et al.: Effects of chelidonine and Ukrain on the activity of aminotransferases in the serum of rats intoxicated by tin chloride. Herba Pol., 48 (4), 278, 2002.
7. Jagiełło-Wójtowicz E. et al.: Effects of chelidonine and Ukrain on the activity of ALT and AST in rats intoxicated by copper chloride. Herba Pol., 48 (4), 274, 2002.

8. Jagiełło-Wójtowicz E. et al.: Ukrain (NSC-631570) in experimental and clinical studies: a review. Drugs Exptl. Clin. Res., 24 (5), 213, 1998.
9. Jagiełło-Wójtowicz E., Piątkowska-Chmiel I.: Protective effects of chelidonine and Ukrain in rats acutely intoxicated with tin chloride. 5th ISCNP, Abstracts, 112, 2006.
10. Levina O. A. et al.: Protective effect of Ukrain against acute acetaminophen-induced hepatitis in rats. Int. J. Immunotherap., 19 (2-4), 129, 2003.
11. Mitchell D. B., Acosta D.: Evaluation of the cytotoxicity of tricyclic antidepressants in primary cultures of rat hepatocytes. J. Toxicol. Environ. Health, 7 (1), 83, 1981.
12. Moskovitz R. et al.: Toxic hepatitis and single daily dosage imipramine therapy. J. Clin. Psychiatry, 43 (4), 165, 1982.
13. Nowicky J. W. et al.: Ukrain both as anticancer and immunoregulatory agent. Drugs Exptl. Clin. Res., 18, 51, 1992.
14. Uglyanitsa K. N. et al. : Ukrain: A novel antitumor drug. Drugs Exptl. Clin. Res., 26 (5), 341, 2000.
15. Voltchek I. et al.: Preliminary results of individual therapy of chronic hepatitis C by Ukrain and interferon-alpha. Drugs Exptl. Clin. Res., 26 (5-6), 261, 2000.

SUMMARY

The effects of 10-day treatment of rodents with Ukrain and imipramine (IMI) on selected biochemical parameters indicating liver functioning have been investigated. In blood serum of the animals AST and ALT activities, total protein and α-fetoprotein (AFP) levels were determined. The results show that10-day combined treatment with Ukrain and IMI significantly increased AFP (only in mice) and total protein in all rodents. The combined administration of Ukrain and IMI significantly decreased the activities of both AST and ALT only in mice serum. Therefore, a combined administration of Ukrain and IMI to rodents caused adverse changes in the examined biochemical parameters, which may indicate hepatotoxic interaction between these drugs.

STRESZCZENIE

Celem pracy była ocena wpływu łącznego 10-dniowego stosowania leku Ukrain oraz imipraminy (IMI) na wybrane parametry biochemiczne świadczące o funkcji wątroby gryzoni. W surowicy krwi zwierząt oznaczono aktywność AST i ALT oraz stężenie α-fetoproteiny (AFP) i białka całkowitego. Na podstawie uzyskanych wyników stwierdzono, że 10-dniowe stosowanie u gryzoni leku Ukrain w kombinacji z IMI powoduje zwiększenie stężenia AFP (tylko u myszy) oraz białka całkowitego (u wszystkich zwierząt) w surowicy krwi. Natomiast aktywność AST i ALT maleje tylko we krwi badanych myszy, u których zastosowana była łączona terapia Ukrainem oraz IMI. Nieprawidłowości w badanych parametrach biochemicznych w surowicy krwi gryzoni mogą wskazywać na hepatotoksyczną interakcję pomiędzy imipraminą a lekiem Ukrain.