INT. J. IMMUNOTHERAPY **XIX**(2-4) 121-124 (2003)

EFFECT OF UKRAIN ON HEPATIC DRUG–METABOLIZING ENZYMES IN RATS: PILOT STUDIES

ZVERINSKY I., MAXIMCHIK Y., MELNICHENKO N., ZALOGA I.

Institute of Biochemistry, Grodno, Belarus.

Summary: *The effect of intraperitoneal administration of Ukrain 1 and 2 mg/kg for a period of 6 days on the content of cytochrome P450 and b_5 and the activity of aminopyrine and ethylmorphine* N-*demethylases and glutathione S-transferase was investigated in cytosolic and microsomal fractions of rat liver. It was found that the activity of aminopyrine* N-*demethylase and microsomal glutathione S-transferase increased by 35% and 55%, respectively, (*$p < 0.03$*) following administration of Ukrain at a dose of 2 mg/kg. It was also shown that Ukrain had an inhibitory effect on the catalytic activity of cytosolic glutathione S-transferase. Possible mechanisms of Ukrain on the drug-metabolizing liver function are discussed.*

Introduction

Ukrain is a semisynthetic preparation of thiotepa and alkaloids from *Chelidonium majus L.* It has been shown to have anticancer and immunomodulating properties (1, 2).

It is well known that the liver plays the key role in the metabolism and excretion of xenobiotics, including drugs (3). Various liver enzymes are responsible for this function. One group, known as cytochrome P450-dependent monooxygenases, donates functional groups (-OH, -NH_2, -SH) that make molecules more hydrophilic. The other enzymes carry out the subsequent transformation of the metabolites, conjugating them to endogenous compounds (sugars, amino acids, glutathione, sulphates and acetates) and resulting in the formation of products that are easily excreted in the bile or urine (4).

Various biological tests relating to molecular, cellular or organ function are used to evaluate the activity and selectivity of new pharmaceutical compounds. The aim of each investigation determines the type and number of initial tests. Some of the necessary initial preclinical tests seek to detect the influence of the drug in question on the xenobiotic-metabolizing liver enzyme systems, and so reveal the possible action of the drug on the biotransformation of xenobiotics (5).

Address for correspondence: Dr. Igor Zverinsky, Institute of Biochemistry, 230017, BLK-50, Grodno, Belarus.
E-mail: zverinsky@rambler.ru

0255-9625/2003/2-4 00121+3 $02.50/0

The aim of this experiment was to evaluate the functional status of drug-metabolizing liver enzymes after administration of Ukrain.

Materials and methods

Chemicals. Ukrain (Tris{2-(5ba-6b,12ba)-5b,6,7,12b,13,14-hexahydro-13-methyl(1,3)-benzodioxolo-(5,6c)-1,3-dioxolo-(4,5-i)phenatridium-6-0l)-ethane-aminyl}-phosphinesulfide·6HCl) (Austrian Patent No. 354644) was obtained from the Ukrainian Anti-Cancer Institute (Vienna, Austria). Nicotinamide adenine dinucleotide phosphate (NADPH), dithionite, aminopyrine, and 1-chloro-2,4-dinitrobenzene (CDNB) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Other chemicals and solvents were of analytical grade and were purchased commercially.

Animals and microsome preparation. Male Wistar rats weighing 180-200 g (Institute of Medicine Center, Warsaw, Poland) were used in the experiment. Six to eight animals were housed per cage on a sawdust bedding; they were fed a standard diet and had free access to water. Room temperature was 20-25 °C and relative humidity was 40%-45%, with natural light only. Ukrain (Nowicky Pharma, Vienna, Austria) was injected intraperitoneally at doses of 1 and 2 mg/kg once daily for 6 days. The control group was injected intraperitoneally with 0.85% NaCl at doses of 1.5 ml/kg.

On day 7 the animals were killed by cervical dislocation, and the livers rinsed with ice-cold 1.15% KCl, removed and homogenized with 0.15% KCl 1:3 (w/v) in a glass Potter homogenizer (Analiz-X, Minsk, Belarus). The homogenates were centrifuged at 9,000 g for 20 min at 4 °C to obtain postmitochondrial supernatant. The supernatant was then centrifuged at 105,000 g for 60 min at 4 °C. The microsomal pellets were recovered and resuspended in 3-5 ml of 0.1 M HCl buffer, pH 7.4.

Enzyme assays. Cytochrome P450 and b_5 content were determined according to Omura and Sato (6). *N*-demethylation of ethylmorphine (cytochrome P450 3A-dependent reaction) and of aminopyrine (cytochrome P450 2C11-dependent reaction) by rat liver microsomes were determined by the methods previously described (7, 8). In the definitive assays, 0.1 ml reagent mixture was prepared by adding 0.05 mg microsomal protein to the following: 0.1 M tris-HCl buffer, pH 7.4; 5 mM $MgCl_2$; 2 mM NADPH; 2 mM ethylmorphine or amidopyrine. The combination of substrate, microsomes and cofactors was then incubated at 37 °C for 10 min. The reaction was stopped by adding 0.035 ml 15% $HClO_4$ and then chilled in ice for 10 min. The cold mixture was centrifuged at 1,000 g for 10 min. A sample of 0.1 ml was transferred to a new tube and 0.1 ml Nash reagent was added to each sample and to HCHO standards. The tubes were incubated at 37 °C for 45 min and the sample absorbance was recorded at 412 nm *versus* an H_2O blank. The final values were calculated from an HCHO standard curve and expressed as the protein content.

Activity of glutathione S-transferase was measured in cytosolic and microsomal fractions using CDNB as substrate (9). The reagent mixture contained 1 ml of enzyme preparation and 0.05 ml 50 mM glutathione (GSH), and was incubated for 5 min at 25 °C. Then 0.025 ml of 40 mM CDNB was added and absorbance at 340 hm recorded. The activity of glutathione S-transferase was expressed in nanomoles of CDNB conjugated/min/mg protein using 9.6 mM^{-1} x cm^{-1} for S-(2,4-dinitrophenyl)-glutathione.

Protein content was measured by the standard Lowry method (10).

Statistical analysis. Mean values were statistically evaluated using unpaired Student's *t*-test and were considered significant at $p < 0.05$ or $p < 0.01$.

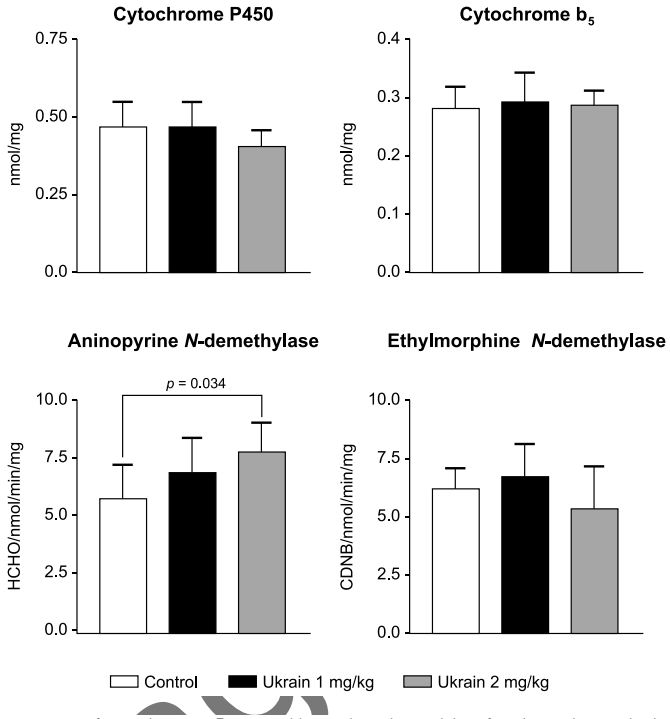

Fig. 1 Effect of Ukrain on the content of cytochromes P450 and b_5 and on the activity of aminopyrine and ethylmorphine *N*-demethylase in microsomal fraction of rat liver. Data are mean ± SD for eight rats. HCHO = formaldehyde; CDNB = 1-chloro-2,4-dinitrobenzene.

Results

It was shown that 2 mg/kg Ukrain by intraperitoneal administration led to a 35% increase in aminopyrine *N*-demethylase activity compared with the control group (5.76 ± 0.465 *versus* 7.78 ± 0.38 nmol/min/mg, respectively). Administration of Ukrain (1 and 2 mg/kg/day i.p. for 6 days) did not exert a significant effect on liver microsomal cytochrome P450 or b_5 content, nor on ethylmorphine *N*-demethylation rate (Fig. 1).

Administration of Ukrain had two contrasting effects on the enzymatic activity of glutathione S-transferase. It was shown that doses of 1 and 2 mg/kg decreased the activity of cytosolic glutathione S-tranferase to 51% and 41%, respectively. However, in the microsomal fraction the same doses increased the activity of glutathione S-transferase (Fig. 2).

Discussion

Taking into account the results of this investigation, we would like to discuss the effect of Ukrain on the activity of drug-metabolizing liver enzymes. It is well known that many alkaloids, *e.g.,* those of the isochinolonic group, are substrates of cytochrome P450. It is suggested that the activating influence of

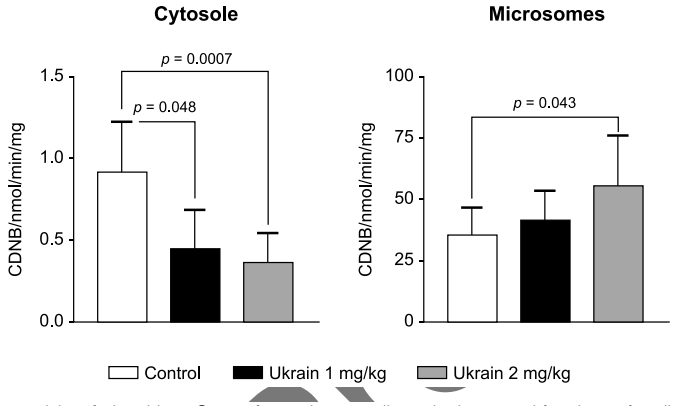

Fig. 2 Effect of Ukrain on the activity of glutathione S-transferase in cytosolic and microsomal fractions of rat liver. Data are mean ± SD for eight rats. CDNB = 1-chloro-2,4-dinitrobenzene.

Ukrain on the drug-metabolizing enzymes of rat liver is the effect of the nonconjugated (free) alkaloids fraction. Probably it is a post-transcriptive effect, as no increase in cytochrome P450 microsomal concentration was observed.

It is known that agents that cause SH-group oxidation are activators of microsomal glutathione S-transferase, but that cytosolic glutathione S-transferase can be inhibited by these agents (11, 12). This was confirmed by our investigations. It is proposed that free alkaloids and conjugated thiotepa can interact with SH groups.

In summary, investigation showed that Ukrain administered intraperitoneally at a dose of 2 mg/kg once a day for 6 days had a slight activating effect on the drug-metabolizing enzymes of rat liver.

References

(1) Kulik G.I. *Comparative in vitro study of the effects of the new antitumor drug Ukrain and several cytostatic agents on the thiol group in the tissue of Guerin carcinoma and its resistance to ciplatin variant.* Drug Exptl. Clin.Res., **24**(5/6), 277, 1998.

(2) Uglyanitsa K.N., Nefyodov L.I., Doroshenko Y.M., et al. Ukrain: A novel antitumor drug. Drugs Exptl. Clin. Res., **26**(5/6), 341, 2000.

(3) Guengerich F.P. Uncommon P450-catalyzed reactions. Curr. Drug Metab., **2**, 93, 2001.

(4) Guengerich F.P. Enzymatic oxidation of xenobiotic chemicals. Crit. Rev. Biochem. Mol. Biol., **25**(2), 97, 1990.

(5) Katzung B.G. (Ed.) "Basic and clinical pharmacology (sixth edition)." Prentice-Hall International Inc., New York, 1995.

(6) Omura T., Sato R., Cooper D.Y., et al. *Function of cytochrome P-450 of microsomes*. Fed. Proc., **24**, 1181, 1965.

(7) Amacher D.E., Schomaker S.J. *Ethylmorphine N-demethylase activity as a marker for cytochrome P450 CYP3A activity in rat hepatic microsomes.* Toxicol. Lett., **94**, 115, 1998.

(8) Guengerich F.P. *Analyses and characterization of enzymes.* In: Wallance Hayes A. (Ed.). "Principles and Methods of Toxicology (third edition)." Raven Press, Ltd., New York, 1994, pp. 1259-1308.

(9) Habig W.H., Pabst M.J., Jakoby W.B. *Glutathione S-transferases - the first enzymatic step mercapturic acid formation.* J. Biol. Chem., **249**(22), 7130, 1974.

(10) Lowry O.N., Rosebrough H.J., Farr A.L., Randall R.G. *Protein measurement with the folin phenol reagent.* J. Biol. Chem., **193**, 265, 1951.

(11) Morgenstern R., DePierre J.W., Ernstern L. *Activation of microsomal glutathione S-transferase activity by sulfhydryl reagents.* Biochem. Biophys. Res. Commun., **87**, 657, 1979.

(12) Sies H., Dafre A.L., Ji Y., Akerboom T.P.M. *Protein S-thiolation and redox regulation of membrane-bound glutathione transferase.* Chem. Biol. Interact., **111/112**, 177, 1998.