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ASSESSMENT OF THE FUNCTIONAL STATE AND MORPHOLOGICAL STRUCTURE OF THE LIVER OF RATS AFTER ADMINISTRATION OF THE DRUG UKRAIN

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Summary: *We studied the effect of the antitumor drug Ukrain on the function and morphological structure of intact rats. After administration of Ukrain at doses of 1 and 2 mg/kg body weight for 6 days, no significant changes were revealed in albumin and mucopolysaccharide concentration, aminotransferase activity, or thymol test value, or in the morphology of liver tissue, when comparing test and control groups. Administration of Ukrain significantly reduced the serum concentration of middle molecules.*

Introduction

Greater celandine extracts and tinctures have been used in folk medicine in western Europe and China as antiphlogistic, antimicrobial, antitumor and antiviral remedies (1). Currently, celandine preparations are used mainly in the treatment of liver and bile duct diseases, and as analgesics in the treatment of peptic ulcer disease (2).

Recently, some cases of acute liver toxicity following the use of celandine preparations have been described. Liver function recovered fully after celandine extract administration was discontinued. The pathogenesis of this phenomenon is so far unknown (3, 4).

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Ukrain is a semisynthetic compound from thiotepa and celandine alkaloids isolated from the roots of the plant (5). The aim of this study was to evaluate the effect of Ukrain on the morphological structure and function of the livers of intact rats.

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)-ethaneaminyl}phosphinesulfide · 6HCl) (Austrian patent No. 354644) was obtained from the Ukrainian Anticancer Institute (Vienna, Austria). Other chemicals and solvents were of analytical grade and purchased commercially.

Animals. Wistar male rats weighing 220-250 g were obtained from the Institute of Medicine Center (War-

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saw, Poland). The rats were divided into three groups: the first group was administered with normal saline at a dose of 2.0 ml/kg intraperitoneally; the second group was administered Ukrain at a dose of 1.0 mg/kg i.p.; and the third group received Ukrain at a dose of 2 mg/kg i.p. The treatment was performed as a single injection, once a day for 6 days. On day 7, all animals were sacrificed.

Investigations. Morphological investigation and biochemical tests were carried out to evaluate possible toxic action of Ukrain on the liver tissue. Damage to hepatocytes was evaluated using the activity of serum alanine aminotransferase (ALT) (Analiz-X, Minsk, Belarus) and aspartate aminotransferase (AST) (Analiz-X). Endotoxemia and inflammation intensity were assessed using the serum concentration of middle molecules and formation of globulin-thymol-lipid complex (thymol test) (Analiz-X).

The synthetic function of the liver was assessed using serum concentrations of albumin and mucopolysaccharides. All tests were performed using commercially purchased analytical kits from the firm Analiz-X and in accordance with the enclosed kit user instructions.

For morphologic evaluation, 0.6 cm liver tissue samples were fixed in 10% buffered formalin and then 5-7 μm thick sections were stained with hematoxylin eosin.

Each group consisted of eight to ten animals. Results are presented as mean ± standard deviation. Significance was assessed using Student's *t*-test.

Results

After administration of Ukrain at doses of 1 and 2 mg/kg body weight for 6 days, no significant differences between test and control groups were revealed in the concentrations of albumin and mucoplysaccharides, the activity of ALT and AST or in the thymol test value (Table I).

Administration of Ukrain, however, significantly and dose-dependently decreased concentration of serum

Table I *Concentrations of albumin, mucopolysaccharides and middle molecules, activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and thymol test value in the serum of rats after intraperitoneal administration of Ukrain at doses 1 mg/kg and 2 mg/kg for 6 days (n = 8; mean ± SD)*

	Groups		
Parameter	Control	Ukrain 1 mg/kg	Ukrain 2 mg/kg
ALT (μkat/l)	0.67 ± 0.03	0.65 ± 0.02 $p = 0.54$	0.68 0.04 $p = 0.97$
AST (μkat/l)	2.63 ± 0.09	2.28 ± 0.29 $p = 0.28$	2.43 ± 0.18 $p = 0.35$
Thymol test, unit of turbidity SH	0.41 ± 0.07	0.38 ± 0.07 $p = 0.81$	0.38 ± 0.08 $p = 0.82$
Albumin (g/l)	38.75 ± 2.50	41.59 ± 1.63 $p = 0.35$	39.92 ± 1.40 $p = 0.69$
Mucopolysaccharides (g/100 ml))	108.0 ± 4.75	103.9 ± 4.51 $p = 0.53$	115.6 ± 5.13 $p = 0.29$
Middle molecules (g/l)	0.40 ± 0.07	0.04 ± 0.002 $p = 0.0008$	0.025 ± 0.003 $p = 0.003$

P-values *versus* control.

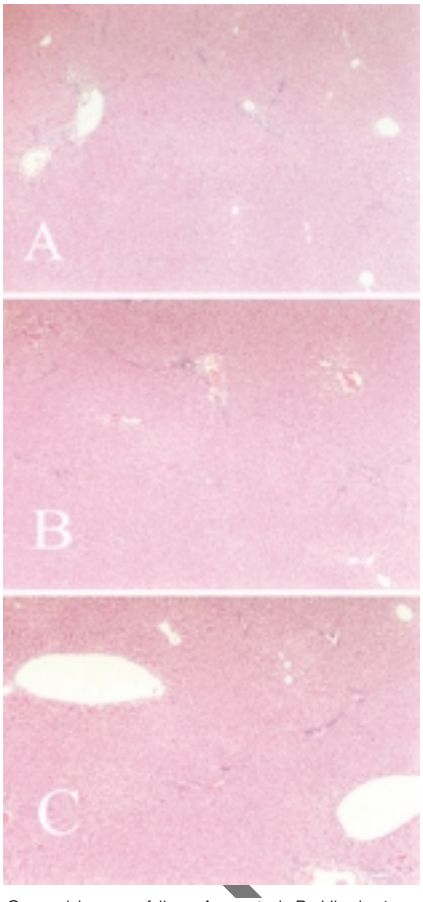

Fig. 1 General image of liver. A: control; B: Ukrain 1 mg/kg; C: Ukrain 2 mg/kg. Objective 3.2 × ocular 4.1.

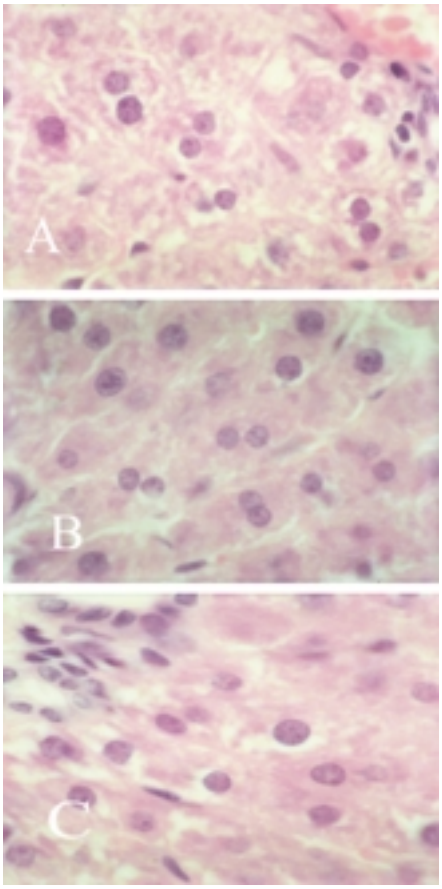

Fig. 2 Periportal zone. A: control; B: Ukrain 1 mg/kg; C: Ukrain 2 mg/kg. Objective 100 × ocular 3.2.

middle molecules: Control group: 0.40 ± 0.07 g/l; Ukrain 1 mg/kg group: 0.04 ± 0.002 g/l ($p = 0.0008$); and Ukrain 2 mg/kg group: 0.025 ± 0.003 mg/kg ($p = 0.003$).

Microphotographs of liver tissue samples at low magnification (× 36) were prepared (Fig. 1). Following administration of Ukrain, both tissue structure and microcirculation system of the liver were intact, there were no changes in the connective tissue system and there was no inflammatory infiltration.

Having considered zonal features of xenobiotic metabolism (including drugs), we examined the centrolobular and periportal liver cells separately. High-magnification (× 1,240) microphotographs of liver cells in the periportal area are shown in Figure 2. There are neither necrobiotic nor dystrophic changes nor infiltration following administration of Ukrain.

High-magnification (x 1,240) microphotographs of liver cells in the centrolobular area are shown in Fi-

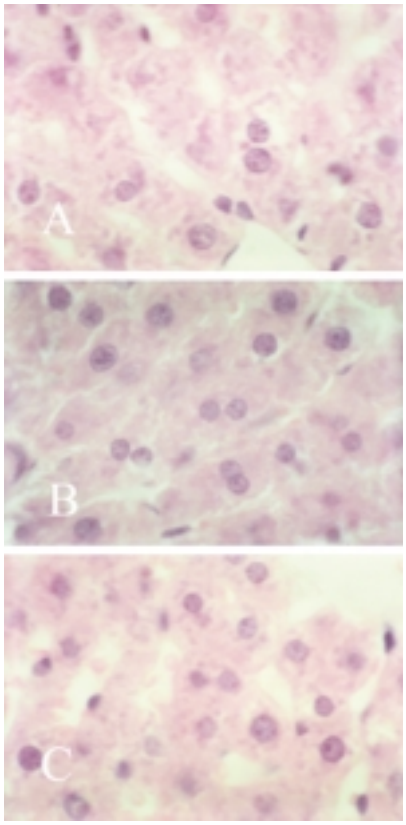

Fig. 3 Centrilobular zone. A: control; B: Ukrain 1 mg/kg; C: Ukrain 2 mg/kg. Objective 100 x ocular 4.1.

gure 3. Again, no pathological changes in hepatocytes in this area were revealed following administration of Ukrain.

Hence, morphological examination of histological samples did not reveal any differences between the control group and animals treated with Ukrain.

Discussion

Our results show that intraperitoneal administration of Ukrain to rats at doses 1 and 2 mg/kg body weight for 6 days affected neither the function nor the morphological structure of the liver. Moreover, administration of Ukrain exerted an antitoxic effect. In particular, the serum concentration of middle molecules decreased. It is known that middle molecule concentration increases in oncological diseases, chronic kidney failure, peptic ulcer, myocardial and liver diseases (including acute kidney failure), and in stress and immune-compromised conditions.

The pathogenetic role of middle molecules in these diseases is complex. They not only cause endogenous intoxication syndrome but also impair microcirculation and suppress the immune response. Dialysis has been established as therapy of choice for middle molecules intoxication.

The effect of Ukrain with regard to middle molecules is probably due to its SH-group alkylating properties. We have previously shown that administration of Ukrain *in vitro* and *in vivo* decreases concentration of reduced glutathione. Middle molecules are oligopeptides with a high content of bicarbonic amino acids cysteine, lysine and glycine, and a low content of aromatic amino acids. We suggest that the target of Ukrain is cysteine, a sulfur amino acid. Further studies are needed to evaluate this hypothesis.

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