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STUDY OF ACUTE TOXICITY OF UKRAIN IN RATS AFTER INTRAVENOUS INJECTION

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Summary: *The acute toxicity of i.v. Ukrain injection in rats was studied. The interrelation between toxicity (death of animals) and dosage was determined by nonlinear regression method. White blood count (WBC) in peripheral blood, weight of animals, and weight of major organs were determined in animals during all stages of investigation. Morphological studies of toxic changes in 40 different organs of rats were performed on macro- and microscopic levels.*

Introduction

In the search for new effective antitumor drugs, agents of herbal origin are being given special attention. One new antitumor drug is Ukrain (Nowicky Pharma, Austria), a semisynthetic product from the interaction of thiophosphoric acid and alkaloids of *Chelidonium majus L.* Despite many studies on Ukrain *in vitro* and *in vivo* (1-7), its mechanism of action has not been clarified completely. There have been studies of the teratogenic, mutagenic and genotoxic processes of Ukrain (8, 9). The therapeutic pathomorphosis of different tumors and their metastases under the influence of Ukrain have been described in detail (10-12). However, in medical literature we were unable to find a detailed description of the findings with respect to the morphology of acute Ukrain toxicity. Therefore, this investigation was dedicated to the detailed study of acute toxicity of Ukrain after i.v. administration.

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Materials and methods

Wistar rats (male and female) weighing 96-104 g were obtained from the breeding farm of R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology. The animals were kept

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under standard laboratory conditions. The room temperature was 20-22 °C, humidity 40-45%. The animals were given unrestricted granulated combined forage (certificate of quality AM-18 of 22.04.97 issued by Kiev Institute of Grain Products).

The rats were also fed fresh lettuce, spinach and chopped beets. Ukrain (Nowicky Pharma, Vienna, Austria) was injected i.v. into the tail vein over 5-7 min at doses of 120, 180, 240, 300, and 360 mg/kg. Each group consisted of 12 male and 12 female rats. The two control groups (male and female) were injected with normal saline into the tail vein.

We investigated the behavior of the animals (from the time of Ukrain injection to death); the interrelation between toxicity (death of animals) and dosage, which was determined by the nonlinear regression method with the help of the STATGRAF computer program; the white blood cell (WBC) count in peripheral blood, performed on the seventh day after injection; the weight of animals during the whole course of studies (on days 0, 1, 3, 5, 7, 10, 14, 17, and 21 after injection); and the weight of major organs at the time of killing the surviving animals (day 21 after injection). Autopsies were performed on animals that died or were killed on the 21st day after injection of the agent, including a description of macro- and microscopic changes in 40 organs. Tissue blocks were subject to standard dehydration followed by immersion in paraffin. Cuts up to 7 microns thick were stained with hematoxyline and eosin. Demonstration processes were subject to photography.

Results

Immediately after i.v. injection of Ukrain the animals sat still for 7-10 min. After that lacrimation started. Twenty min thereafter the animals became excited: they tried to move actively, but crawled and rolled to the side. Hind extremities did not function. Eye lids closed more often and twitched. Breathing became more rapid but in those rats that died it slowed down significantly immediately before death (as if the animals were choking). During this time the rats did not move. Then seizures in extremities appeared. After 40-60 min following the injection these animals died. However, in rats that survived, normal movement and breathing were restored 2-3 h after injection.

The interrelation between toxicity (following the death of animals) and the dosage of Ukrain was determined by the nonlinear regression method with the help of the STATGRAF computer program. The studies showed a likely positive correlation between death of animals N (%) and dosage of Ukrain D (mg/kg): the coefficient of correlation R constituted 0.9 (0.08 dependence of death N on dosage D was determined by the ratio:

$$N = 100 ((1 - e^{-a (D - Dt)}) \quad [1]$$

where the parameters in the ratio [1] equal:

$$a = 4.36 (10\text{-}3 (1{,}2 (10\text{-}3\ \text{kg/mg}))) \quad [2]$$

Dt (maximum dose that does not lead to death) = 160 ± 50 (mg/kg)

Table I shows dependence of death on dosage. Ratio [1] with calculated parameters [2] makes it possible to assess dosage D that causes 10%, 40%, 50%, and 100%.

The data obtained shows that the WBC in peripheral blood in rats on the seventh day after injection of Ukrain was the same as in the control group. Animals that survived gradually gained weight. The data presented point to no significant difference either between the different doses of Ukrain and control, or between the doses themselves.

Table I *Experimental data on mortality rate of animals as related to the dose received*

Dose of the agent (mg/kg)	Number of animals in groups prior to injection	Sex	Number of rats that died	Number of rats that survived
360	12	males	8	4
	12	females	8	4
300	12	males	6	6
	12	females	6	6
240	12	males	2	10
	12	females	2	10
180	12	males	0	12
	12	females	0	12
120	12	males	0	12
	12	females	0	12

mortality of animals: LD10 = 184 ± 45 mg/kg; LD40 = 277 ± 60 mg/kg; LD50 = 318 ± 70 mg/kg; LD100 = 783 ± 80 mg/kg.

Morphological changes in the experiments conducted were studied in groups depending on the dose of the injected agent. The organs of dead animals were taken for investigation immediately after death. Surviving animals were observed for 3 weeks before being killed. Thus, in the course of the experiment we were able to study not only the direct toxic effect of high doses of Ukrain and morphological changes that it caused (when animals died immediately), but also the morphology of those compensatory-adaptive processes observed in animals which tolerated the agent. As 3 weeks is sufficient time for the development of compensatory-adaptive and reparative processes, they were also studied in the group of animals that survived after injection of Ukrain.

Signs of disseminated intravascular coagulation (DIC) were seen in all the animals that died of intoxication. Maximum expression of the process was noted in parenchymal organs and tissues of large glands. Less expression of the process was noted in tubular organs and skin. Stasis and sludge were observed in microcirculatory vessels which showed paretic dilatation. Very often attention was drawn to central axial WBC disposition along precapillaries. These changes especially manifested themselves in the vessels of lungs (Fig. 1). In addition, intensive acute lung edema and sometimes even mass diapedesis of red blood cells (RBC) into alveoli were observed. The intensity of changes in all groups was the same and did not depend on the dosage, indicating that these changes were due to thanatogenesis.

Together with vascular changes, degenerative and necrobiotic processes, mainly in the kidneys, were observed. Nephrothelium of proximal tubules was in a condition of expressed hydropic degener-

Fig. 1 Morphological changes especially manifested themselves in the vessels of lungs.

ation, some nephroepitheliocytes underwent partial cellular necrosis. Nuclei were in the condition of picnosis and chromatin fragmented. The lumens of proximal tubules narrowed and sometimes could not be identified (Fig. 2). The complexity of the changes mentioned in the kidneys correlated to some extent to the dose of the agent, but was clearly observed in all groups. Glomerules and collecting ducts were minimally affected. Doses of 360 and 300 mg/kg Ukrain caused lesions to the nephrothelium of distal tubules, whereas with a dose of 240 mg/kg changes in the these areas were minimal. At the same time the last dose led to the hydropic, sometimes large vacuoles degeneration of hepatocytes, whereas lesions to the liver were insignificant in those animals that died of higher doses. What first appears as a paradox is explained by the later time of death of animals with lower doses of the agent and involvement of the liver parenchyma in the process of detoxification. No perivascular or pericellular swelling of the brain tissue was observed in any of the groups at any dose.

Due to blood circulation centralization that preceded DIC-syndrome, tissue of the spleen was relatively depleted of red pulp, which could be regarded as relative lymphoid and macrophagal stimulation of the spleen (Fig. 3). Maximum expression of the above-mentioned process was noted in minimal lethal doses and late death of the animals.

The morphology of adaptation-reparative processes in the organs and tissues of rats by the end of the third week following a single injection of a toxic dose of Ukrain was described, taking possible thanatogenesis artifacts due to killing by decapitation into consideration.

Changes to the lungs drew our initial attention. These were expressed in massive hemorrhages located basically paravasally and also at other sites of pulmonary parenchyma. Clear hemosiderosis, enlarged fixed macrophages and changes in tinctorial characteristics of RBC indicated the duration and extensiveness of the process (Fig. 4). Together with hemosiderosis, more intense proliferation in pulmonary parenchyma was observed (Fig. 5). Proliferation processes were more characteristic for high doses (360 and 300 mg/kg), as these are the result of massive lesions.

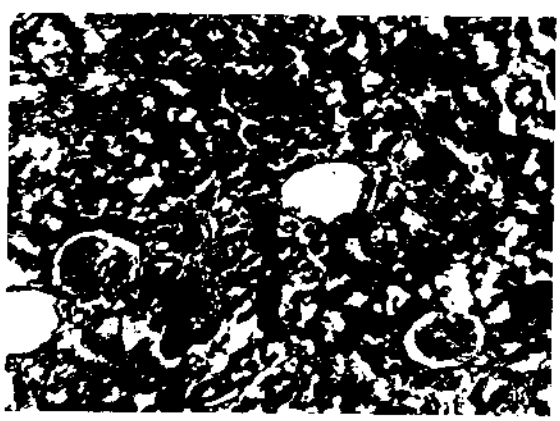

Fig. 2 The lumens of proximal tubules narrowed and sometimes could not be identified.

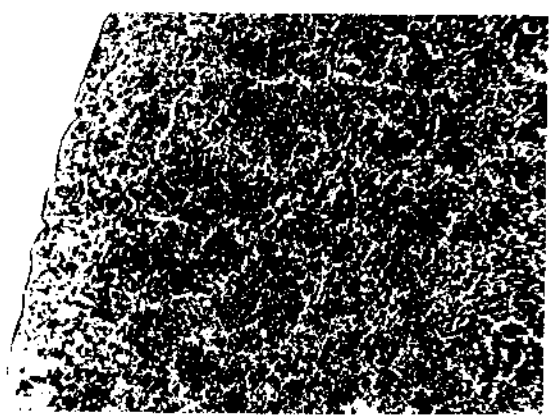

Fig. 3 Spleen tissue was depleted of red pulp, which could be regarded as lymphoid and macrophagal stimulation of the spleen.

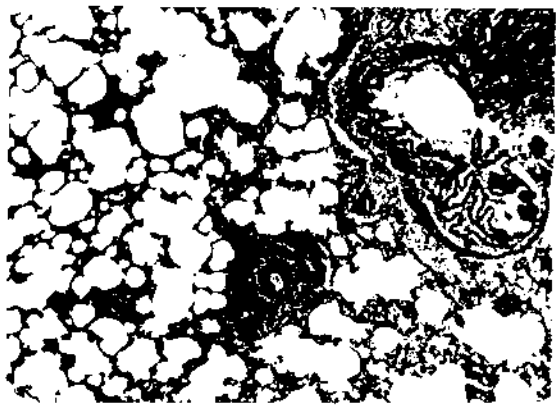

Fig. 4 Changes to the lungs were expressed in massive hemorrhages located basically paravasally and also at other sites.

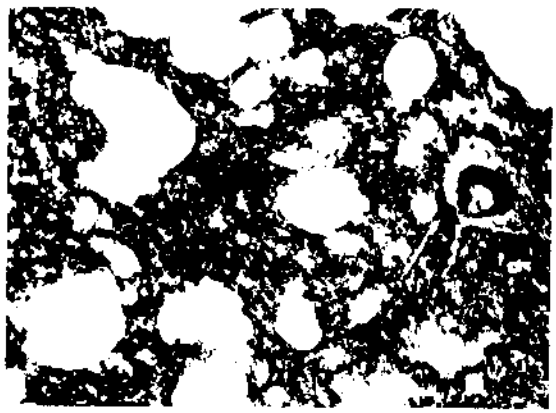

Fig. 5 Together with hemosiderosis, more intense proliferation in pulmonary parenchyma was observed.

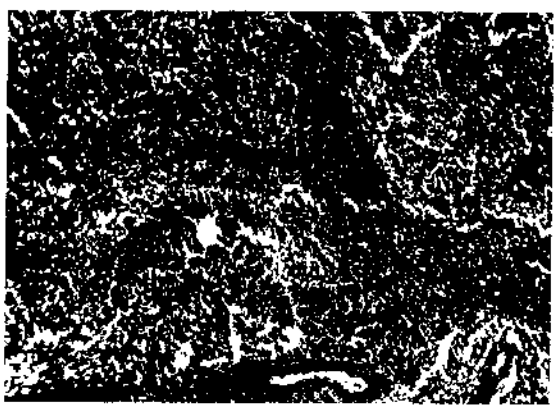

Fig. 6 Old hemorrhages and hemosiderosis were also observed in the spleen.

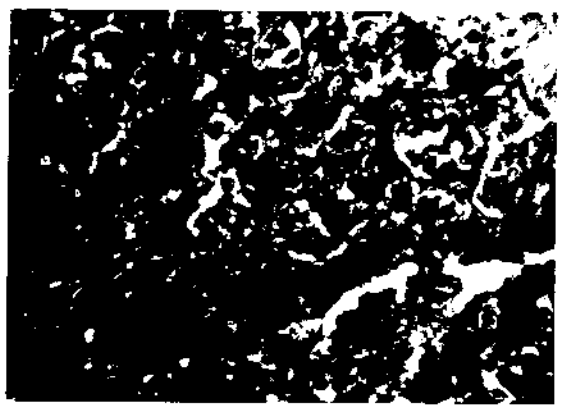

Fig. 7 Old hemorrhages and hemosiderosis were also observed in the medulla of adrenal glands.

Hemorrhages and hemosiderosis were more often seen at doses of 180 mg/kg as a result of vascular reaction. Old hemorrhages and hemosiderosis were also observed in the spleen (Fig. 6) and medulla of adrenal glands (Fig. 7). With regard to adrenal glands the above-mentioned phenomenon is characteristic for lower doses of the agent. In kidneys, together with destruction and desquamation of the nephrothelium of tubules, characteristic changes in glomerules were observed that expressed themselves as partial shrinking and relative enlargement of space of the capsule (Fig. 8). The intensity of the changes and their extensiveness was not dose-dependent.

The liver, as an organ that was actively involved in the process of detoxification, clearly demonstrated degenerative processes, the expression and extent of which correlated with the dose (Fig. 9). At

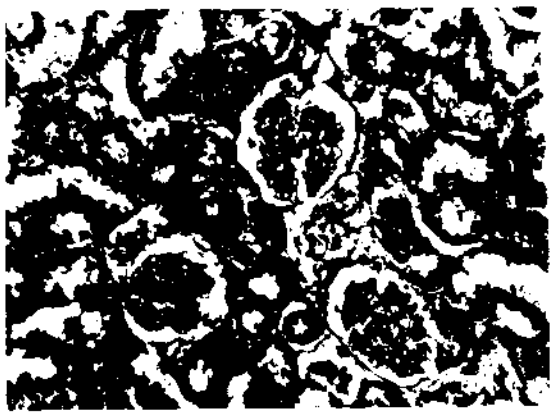

Fig. 8 In kidneys, changes in glomerules were observed, expressed as shrinking and enlargement of space of the capsule.

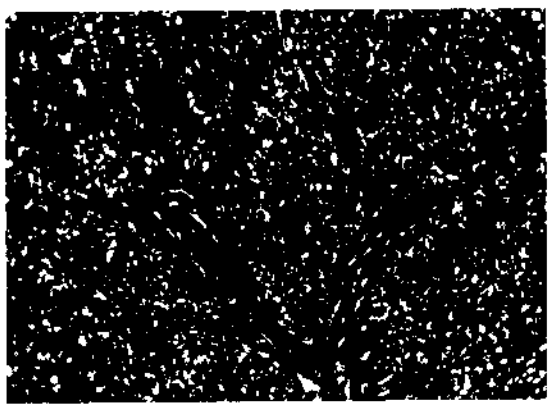

Fig. 9 The liver clearly demonstrated degenerative processes, the expression and extent of whichh correlated with the dose.

the same time, with high doses of the agent, single oxyphil hyaline-like granules in perisinusoidal spaces resembling Kaunsilman bodies were observed, which indicated coagulation necrosis of some groups of hepatocytes. Expression of changes in the brain was minimal and was basically seen as perivascular swelling. No lesions to the neurons or glia were noted (Fig. 10).

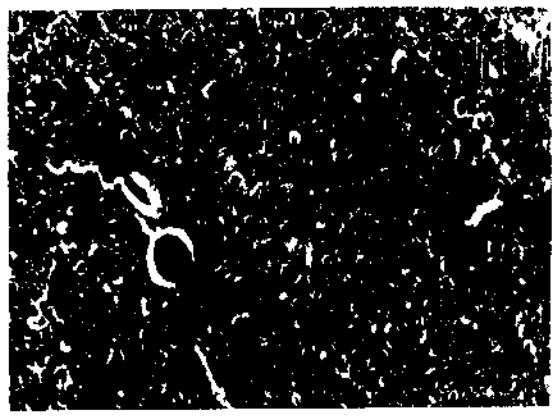

Fig. 10 No lesions to the neurons or glia were noted.

Discussion

Interrelations between acute toxicity (death of animals) and dosage, determined by the nonlinear regression method, coincide with data received empirically (13). The absence of statistically-proved changes in WBC formulas, in animal and organ weight both in experimental and control groups, indicates the mechanisms of toxic activity of Ukrain. Actually, the investigation points to processes which are not affected by Ukrain. We did not show an acute toxic influence of Ukrain on physiological growth and physiological regeneration of blood. This data is in agreement with the results of previous investigations (14).

Among thanatogenetic mechanisms in acute toxicity, most predominant were microcirculation dysfunction and lesions to the vital organs, primarily the lungs and kidneys, with development of their acute failure. Some morphological differences caused by the dose of the agent depended not only on the dose itself, but on the length of time from the moment of injection to death. This means that the cause of death of animals during the first day was

intoxication shock. Adaptive, compensatory and reparative processes in animals killed 3 weeks after injection of Ukrain were more specific. The changes described are actually a morphology of reparation after suffering intoxication shock. Together with such a specificity their dependence on the dose of Ukrain was also observed. Although the changes described above are relatively consistent with life, they should be regarded as irreversible.

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