Immediate and Remote Effects of Antitumor Drug on the Morphology of Rat Liver

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Experiments performed on rats have shown that a single intravenous injection of the antitumor preparations platidiam and pharmorubicine in the maximum tolerated dose cause morphological changes in the liver which are preserved for 3 months after platidiam and for 6 moths after pharmorubicine. Instability of reparative processes in the liver was revealed when the rats were poisoned with CCl_4 1, 3, and 6 months after administration of the cytostatics.

Key Words: *liver; platidiam; pharmorubicine; CCl_4-induced hepatitis*

Although hepatotoxicity in not the major complication of cytostatic therapy [6], there is pharmacokinetic evidence that antitumor cytostatics, as well as the majority of drugs, are metabolized in the liver with formation of toxic products that may damage hepatocytes [4,10]. Most cytostatics are excreted from the body with bile [11]. In oncological patients with liver disorders the clearance of cytostatics is impaired, which prolongs the circulation of toxic metabolites and aggravates the side effects of chemotherapy [9].

Bearing this in mind, we thought it reasonable to study morphological and functional changes in the liver, the dynamics of regeneratory processes, and the liver reserves providing an adequate response to repeated toxic influences of pharmacological preparations or to chemotherapy.

MATERIALS AND METHODS

Changes in the liver morphology caused by the platinum-containing antitumor drug platidiam (Lachema) and the anthracycline antibiotic pharmorubicine (Farmitalia) were studied in Wistar rats (n=285, 35 animals control) weighing 150-200 g. The rats were maintained under the standard vivarium conditions (20-22°C, 50% humidity, day-night cycle, PK120-3 fodder) in accordance with the regulations of the European Convention on Protection of Experimental Animals.

The cytostatics were administered as a single intravenous injections in the maximum tolerated dose calculated by probit-analysis [2]: 7.5 mg/kg for pharmorubicine and 4.5 mg/kg for platidiam. Acute toxic hepatitis was produced by intragastral administration of CCl_4 (50% solution in olive oil, 1.25 mg/kg for 4 days) 30, 90, and 180 days after administration of the cytostatics. Liver samples were collected on the 2nd, 5th, 10th, 15th, 30th, 90th, and 180th day after administration of the cytostatics and on the 2nd, 5th, 10th, and 15th day after administration of CCl_4. The rats were killed by decapitation. Liver samples were fixed in Carnoy's fluid. Deparaffinated sections were stained with hematoxylin and eosin, by the Ehneirson method for RNA, and by the McManus method for glycogen. The lipids were visualized with Sudan Black B on cryostat sections prepared from nonfixed material [8]. Cytophotometry was performed in a UNIVAR scanning cytophotometer (Reichert-Jung). The relative area of liver infiltration was determined on hematoxylin and eosin-stained sections using an

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TABLE 1. State of the Liver in Rats Treated with Antitumor Preparations ($X \pm m$)

Observation period, days	Infiltration area	Hepatocytes with pycnotic nuclei	Binuclear hepatocytes	Lipids	Glycogen	RNA	Alanine aminotransferase	Aspartate aminotransferase
	%			light absorbance, arb. units			mmol/liter	
Control	2.8±0.3	0.02±0.01	12.6±0.5	0.08±0.05	0.24±0.005	0.20±0.003	1.06±0.07	1.08±0.06
2	7.1±0.5* 5.7±0.4*	0.28±0.08* 0.51±0.02*	9.8±1.1 9.7±0.7*	0.12±0.004* 0.18±0.005*	0.20±0.006* 0.16±0.005*	0.16±0.004* 0.13±0.004*	1.11±0.05 1.11±0.12	1.06±0.06 1.24±0.04
5	9.0±0.5* 4.9±0.5*	0.42±0.02* 0.47±0.06*	7.9±1.2* 8.2±0.5*	0.16±0.003* 0.26±0.005*	0.17±0.003* 0.13±0.006*	0.07±0.001* 0.07±0.005*	1.11±0.12 0.99±0.10	1.11±0.10 1.35±0.11*
10	6.6±0.3* 6.4±0.5	0.47±0.02* 0.69±0.06*	6.8±0.6* 5.5±1.2*	0.20±0.006* 0.20±0.006	0.13±0.004* 0.18±0.005*	0.09±0.002* 0.08±0.004*	2.35±0.27* 1.35±0.05*	1.68±0.08* 1.62±0.11*
15	7.4±0.7* 4.2±0.3*	0.31±0.05* 0.41±0.05*	7.4±0.6* 7.0±1.5*	0.15±0.005* 0.12±0.006*	0.18±0.003* 0.17±0.006*	0.10±0.006* 0.08±0.004*	1.30±0.08* 1.51±0.14*	1.42±0.07* 1.46±0.06*
30	5.8±0.4* 3.5±0.5	0.10±0.04 0.25±0.02*	7.8±0.8* 8.5±0.9*	0.09±0.004 0.07±0.004	0.20±0.004* 0.17±0.007*	0.15±0.004* 0.11±0.008*	1.07±0.10 1.18±0.09	1.07±0.14 1.17±0.09
90	4.6±0.3* 3.9±0.7	0.11±0.02* 0.22±0.04*	11.5±0.7 8.2±0.4*	0.11±0.005 0.10±0.005	0.23±0.003 0.20±0.004*	0.19±0.004 0.14±0.006*	1.02±0.12 1.11±0.07	1.18±0.07 1.01±0.09
180	3.9±0.4 5.2±0.6*	0.16±0.02* 0.12±0.01*	11.2±1.2 11.6±1.6	0.08±0.003 0.09±0.004	0.24±0.006 0.23±0.003	0.18±0.005 0.16±0.005*	1.20±0.13 1.13±0.07	1.10±0.08 1.05±0.07

Note. Numerator: platidiam; denominator: pharmorubicine. *$p<0.05$ compared with the control.

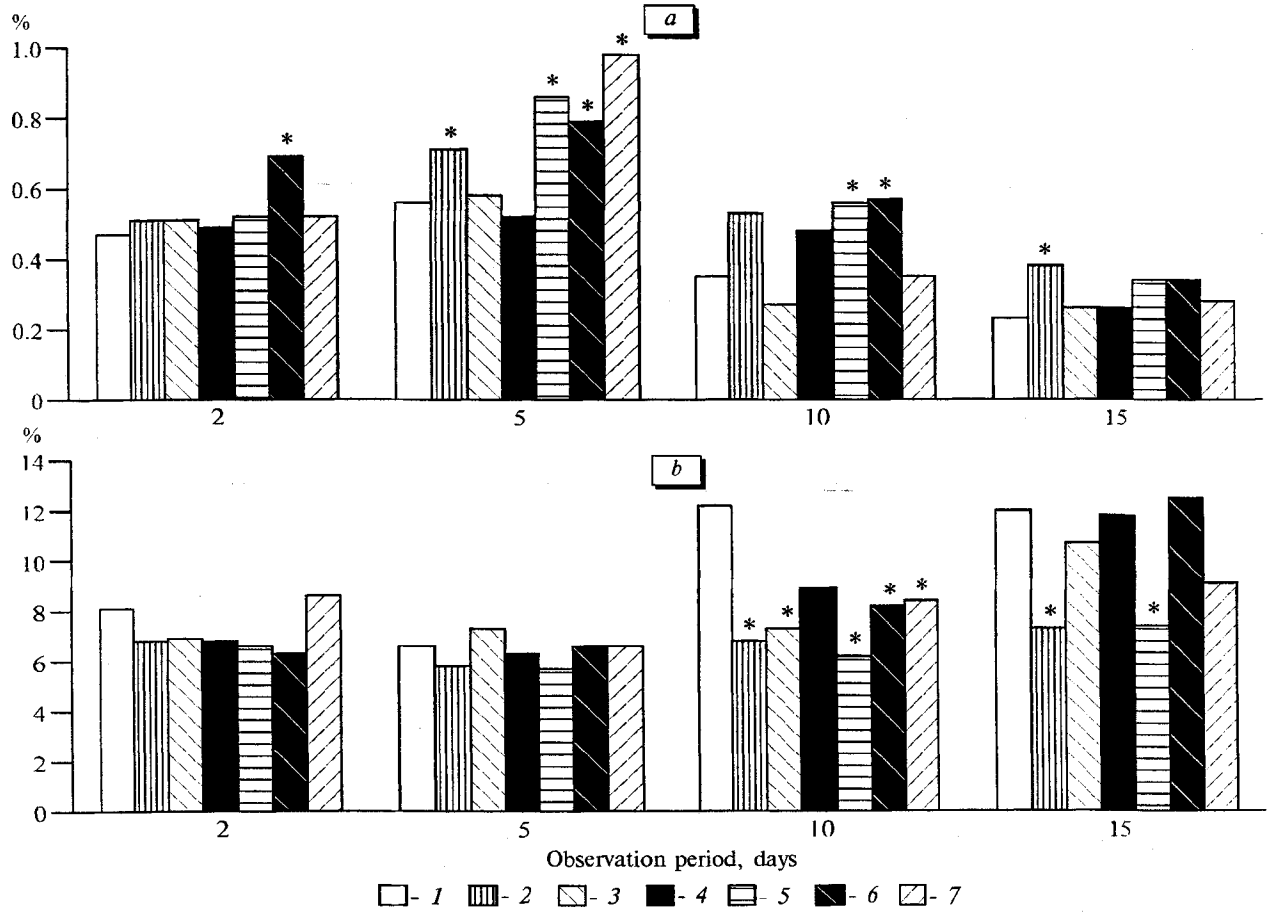

Fig. 1. Number of pycnotic *(a)* and binuclear *(b)* hepatocytes in the liver of rats with toxic hepatitis 1, 3, and 6 months after administration of antitumor preparations. Here and in Fig. 2: *1)* hepatitis in intact animals; hepatitis 1, 3, and 6 months after administration of platidiam *(2-4)* or pharmorubicine *(5-7)*. *$p<0.05$ compared with intact animals.

ocular grid [1]. These histological preparations were used to count pycnotic and binuclear hepatocytes. Serum activities of aspartate and alanine aminotransferase were determined as described elsewhere [7].

The results were analyzed by Student's *t* test.

RESULTS

Morphological changes (dystrophy and necrosis of hepatocytes, steatosis, and inflammatory reaction) induced in rat liver by platidiam and pharmorubicine can be classified as acute toxic hepatitis. Generally, monocellular or small-focus necrosis of hepatocytes was observed. On the 10th day after administration of the cytostatics (the most pronounced changes), the amount of pycnotic hepatocytes in comparison with the control was 35-fold higher after pharmorubicine and 24-fold higher after platidiam (Table 1). High serum level of liver enzymes at this period points to intense cytolytic processes in the liver. Steatosis of the liver (predominantly microvesicular) involved the central part of the lobule. Fatty infiltration of hepatocytes coincided with a decrease in the intracellular glycogen and RNA contents. The inflammatory reaction manifested itself in parenchymal infiltration by macrophages, lymphocytes, and neutrophilic leukocytes. The relative area of the infiltrate remained over the control level for 90 days after administration of platidiam and for 180 days after administration of pharmorubicine. Impairing the DNA synthesis, the cytostatics decrease the number of binuclear hepatocytes: platidiam up to the 30th day and pharmorubicine up to the 90th day after administration (Table 1).

Residual damage to the liver (necrosis, fatty dystrophy of hepatocytes, and lymphomacrophagal infiltration) with parallel reparative process persisted for at least 15 days after administration of the cytostatics. Hepatocytes with hypertrophied strongly basophilic nuclei appeared, the number of binuclear cells increased, steatosis and activity of liver enzymes decreased, the glycogen and RNA contents in the cytoplasm of hepatocytes increased (Table 1).

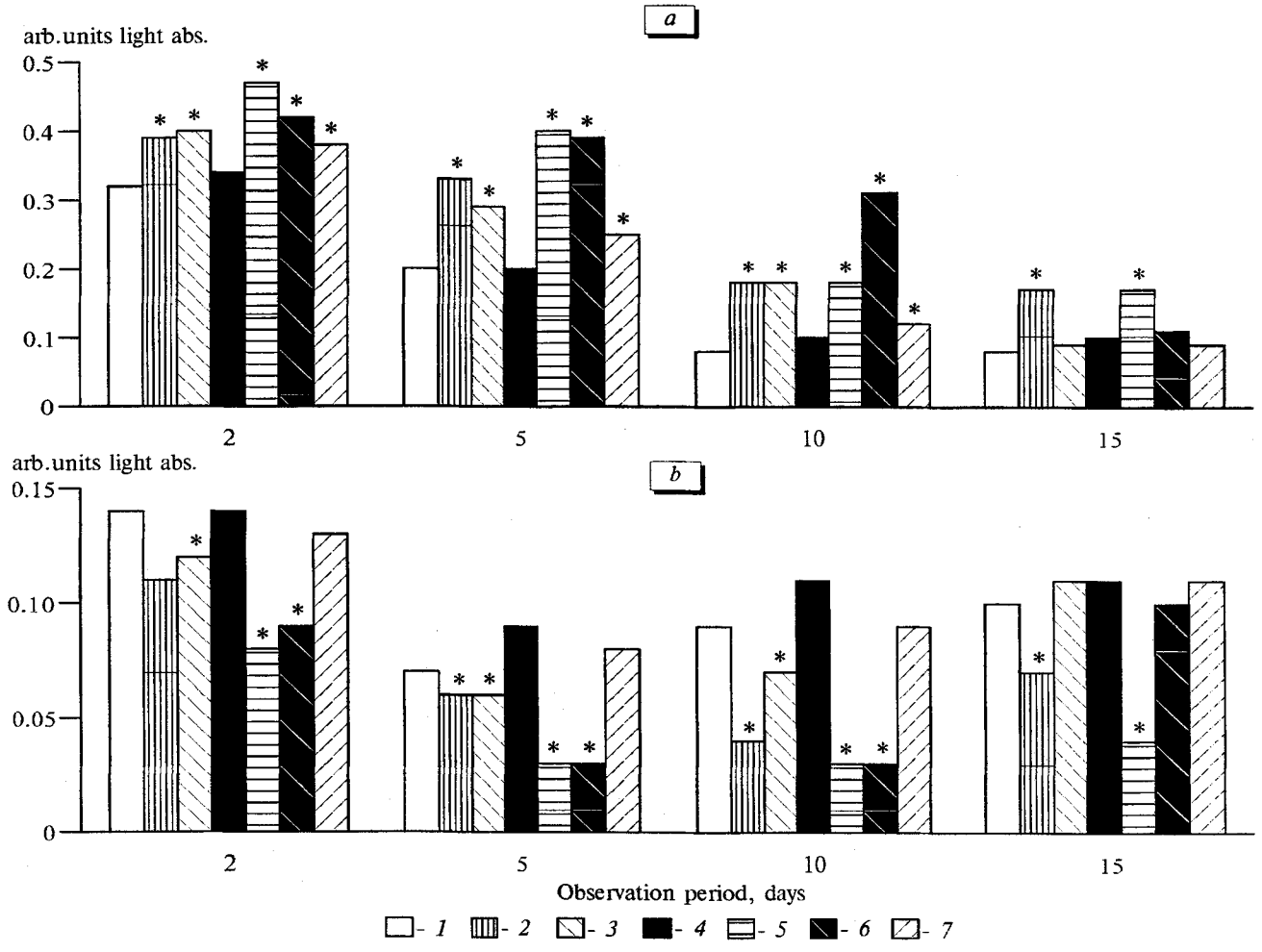

Fig. 2. Glycogen *(a)* and RNA *(b)* contents in hepatocytes of rats with toxic hepatitis induced 1, 3, and 6 months after administration of antitumor preparations.

In order to assess the intensity of reparative processes in the liver, the rats were poisoned with CCL_4 1, 3, and 6 months after administration of the cytostatics. Both platidiam- and pharmorubicine-treated rats have developed more severe and longer hepatitis in comparison with the control. The disease was characterized by expanded parenchymal necrosis, increased lipid accumulation, and decreased RNA and glycogen contents in hepatocytes, particularly in hepatitis developing against the background of pharmorubicine (Fig. 1, *a* and Fig. 2). The regeneration rate decreased (Fig. 1, *b*), as evidenced by the number of binuclear cells [3]. The severity of liver damage and reparation rate depend on the interval between administration of the cytostatics and the development of CCl_4-induced hepatitis as well as on the drug cytotoxicity. The most pronounced morphological changes in the liver were observed in CCl_4-induced hepatitis a month after administration of the cytostatics. After 6 months, the symptoms of toxic hepatitis in platidiam-treated rats did not differ from those in intact rats, while in pharmorubicine-treated rats they were more pronounced.

Induction of chromosome aberrations caused by platidiam and anthracycline antibiotics is a mechanisms underlying cell death [5]. Hepatocytes with chromosomal aberrations remain functionally active for a certain time period [2], after which they die and are eliminated. High content of pycnotic hepatocytes which is preserved for a long time after administration of cytostatic is probably associated with cell death. In addition, damaged hepatocytes are more sensitive to any toxic influence, which leads to severe liver disorders.

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