Structure of the Liver in Mice with Transplanted Lewis Carcinoma during Polychemotherapy and Correction with Betulonic Acid and Its Derivatives

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Morphological and morphometric studies of the liver with transplanted Lewis carcinoma were performed after polychemotherapy and correction with betulonic and [3-oxo-20(29)-lupene-28-oil]-3-aminopropionic acids and their methyl esters. It was demonstrated than betulonic and [3-oxo-20(29)-lupene-28-oil]-3-aminopropionic acids reduced the degree of degenerative changes and volume density of necrotic changes in hepatocytes after polychemotherapy. Methyl esters of these acids little changed the severity and spreading of destructive and necrotic changes in the liver caused by complex cytostatic therapy. It was also shown that all studied triterpenoids exhibited more pronounced antimetastatic effect (evaluated by the decrease in volume density of liver metastases) compared to polychemotherapy.

Key Words: *lung carcinoma; polychemotherapy; hepatotoxicity; betulonic acid and their derivatives; liver monphology*

The potential of tumor chemotherapy is often limited by hepatotoxicity of cytotoxic preparations undergoing biotransformation in the liver. The risk of toxic complications due to maintenance of high concentrations of cytostatic metabolites increases against the background of tumor intoxication and metastatic involvement of the liver [2]. Toxic reactions of the liver are realized via chemical and immunological mechanisms, often act in a cumulative manner, impede the course treatment, reduce the efficiency of cytostatic therapy, and require constant correction with hepatotropic preparations between the courses [3].

Betulonic acid (BA) and its derivatives, substances with high antioxidant, hepato-, nephro-, and cardioprotective activities and selective cytostatic effect on tumor cells, are promising correctors (and probably cytoprotectors) of toxic effects of cytostatics [4-7,11-14]. An important property of some triterpenoids is their capacity to inhibit metastatic activity of tumor cells.

Here we studied the effects of BA and [3-oxo-20(29)-lupene-28-oil]-3-aminopropionic acids (APA) and their methyl esters on liver morphology in mice with transplanted Lewis lung carcinoma (LLC) against the background of polychemotherapy.

MATERIALS AND METHODS

Experiments were carried out on C57Bl/6 mice (n=60) initially weighing 22-25 g and maintained under standard vivarium conditions. All animals received an injection of CLL cells (2×10^6 cells in 0.1 ml physiological saline) into thigh muscles. The cells were obtained from Bank of Tumor Cells, Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Medical Sciences. On day 10 after tumor transplantation, the mice were divided into 6 groups (10

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mice per group). Group 1 animals (controls) received no chemotherapy and no chemical agents after tumor transplantation. Chemotherapy performed in groups 2-6 consisted of single intraperitoneal injection of a complex of cytostatic preparations: 4 mg/kg doxorubicin (Lens-Farm), 50 mg/kg cyclophosphamide (Biokhimik), 0.1 mg/kg vincristine (Gedeon Richter), and 5 mg/kg prednisolone (Gedeon Richter). Group 2 animals receiving tumor transplantation and PCT course served as an additional reference group. The mice of groups 3-6 received different triterpenoids for correction of toxic effects of PCT: BA (group 3), its methyl ester metBA (group 4), APA (group 5), and its methyl ester metAPA (group 6). All compounds were synthesized at N. N. Vorozhtsov Institute of Organic Chemistry, Siberian Division of the Russian Academy of Sciences [10].

BA and its derivatives were administered in a dose of 50 mg/kg for 8 days through a gastric tube. Control mice received an equivalent volume of water with Tween. After 8 days, the mice were decapitated under ether narcosis, the liver was removed, fixed in 4% paraformaldehyde on 0.1 M Sorensen phosphate buffer (pH 7.4) for 4 days, and processed on a MICROM histological complex (Carl Zeiss). The sections were stained with hematoxylin and eosin, toluidine blue, and after van Gieson; PAS reaction with hematoxylin and orange G poststaining was also used. For preparing semithin sections, liver samples were poststained with 1% OsO_4 and embedded into epon and araldite Semithin sections stained with methylene blue and Schiff reagent were examined under an Axioskop 40 microscope at ×400 and ×1000.

Morphometry was carried out using an ocular grid consisting of 289 points [1]. Volume densities of cells with degenerative and necrotic changes and sinusoids with free lumen, lymphocytes, and metastases were determined. The data were processed by parametric methods; the results were significant at $p<0.05$.

RESULTS

In the liver of animals with transplanted LLC, both specific morphological changes related to general and local effects of the tumor and nonspecific alterations caused by chemotherapy and studied chemical compounds were observed.

In all groups, typical specific changes were presented by focal periportal and cell metastases in the sinusoidal lumens with active division of tumor cells leading to local discomplexation of hepatic cords.

Nonspecific changes in the liver of mice with transplanted LLC (group 1) manifested by regeneratory and plastic insufficiency of hepatocytes of different severity (degeneration and atrophy of some cells) and development of moderate intralobular sclerosis. Devastation of the cytoplasm associated with lysis and sequestration of glycogen granules was the leading type of hepatocyte damage (Fig. 1, *a*). At the same time, proliferation of hepatocytes and appearance of small binucleated cells with acidophilic cytoplasm were noted near the portal tracts (Fig. 1, *b*). The appearance of small binucleated hepatocytes under conditions of metastasizing attests to their preserved regeneratory potential. Hemodynamics disturbances manifested in inhomogeneous plethora and partial occlusion of sinusoids. Vascular walls were PAS-positive, toluidine blue yielded metachromatic staining, while van Gieson staining revealed no connective tissue in these structures. On semithin sections, stellate cells and polymorphonuclear leukocytes were seen in sinusoid lumens.

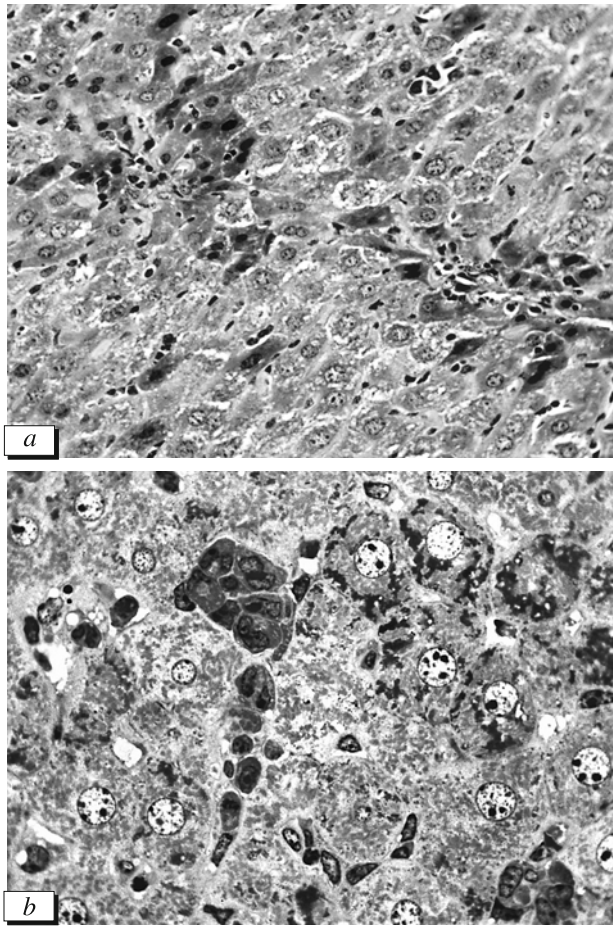

Fig. 1. Liver from mouse with transplanted LLC. *a*) focal devastation of hepatocyte cytoplasm, tumor cell metastases. Semithin section, PAS reaction – hematoxylin, ×1000; *b*) binuclear hepatocytes near the portal zones. Hematoxylin and eosin staining, ×400.

Complex cytostatic treatment (group 2) induced disturbances in hepatocyte metabolism. In many hepatocytes, large- and small-droplet lipid infiltration of the cytoplasm (the size of lipid droplets sometimes was comparable or even surpassed the size of cell nuclei;

Fig. 2, *a*). In central zones of the hepatic lobules, hepatocytes with focal degenerative changes were seen. Glycogen in the form of PAS-positive accumulations of different intensity of staining was primarily detected in periportal hepatocytes; in many hepatocytes, exhaustion of glycogen stores with the formation of optically empty spaces was observed. These morphological changes in hepatocytes can be also caused by other drugs and are considered as stereotypic changes [8,9]. The percent of monocellular necroses in the centrolobular zones increased by 88% compared to that in the control group (Table 1). The percent of sinusoids with free lumens decreased by 21%. On semithin sections, stellate cells (primarily of elongated oval shape) were seen in sinusoid lumens.

Administration of triterpenoids against the background of PCT modulated the structure of hepatocytes and sinusoids. After treatment with BA and APA, the percent of degeneratively changed hepatocytes did not considerably differ from the control and PCT alone, but the changes were less pronounced: devastation zones and lipid infiltration in hepatocytes were practically absent and minor subplasmalemmal vacuolation of the cytoplasm was noted (Fig. 2, *b*). The percent of monocellular necroses after administration of BA and APA decreased by 42 and 53%, respectively, compared to PCT, but not to the control group. Administration of metBA and metAPA against the background of PCT did not considerably reduce the volume density of necrotically changed hepatocytes compared to PCT alone.

Combined administration of all test compounds with PCT induced insignificant changes in the sinusoidal bed and pericellular spaces (Disse spaces). We observed scleroses of Disse spaces with growth of collagen bundles around hepatocytes leading to partial discomplexation of cells. In some cases, monocellular necrosis of hepatocytes and pronounced perisinusoidal collagenization led to considerable disturbances in liver architectonics: disappearance of cords (Fig. 2, *c*). Sclerotic processes were accompanied by the appearance of a great number of stellate cells with lipid inclusions in the cytoplasm in sinusoid lumens and around sinusoids (Fig. 2, *b*). Damage to hepatocytes and activation of stellate cells with inflammation mediators leading to activation of collagen synthesis are the main factors impairing architectonics and reducing (or blocking) transsinusoidal exchange [5,7-9].

Pronounced collagenization of Disse spaces and capillarization of sinusoids after PCT and triterpenoid treatment can be considered as a compensatory and adaptive remodeling of the liver in response to the action of toxic compounds. Impairment of transsinusoidal exchange under conditions of increased blood concentration of cytopathic agents protects hepatocytes from damage, but reduces the supply with plastic

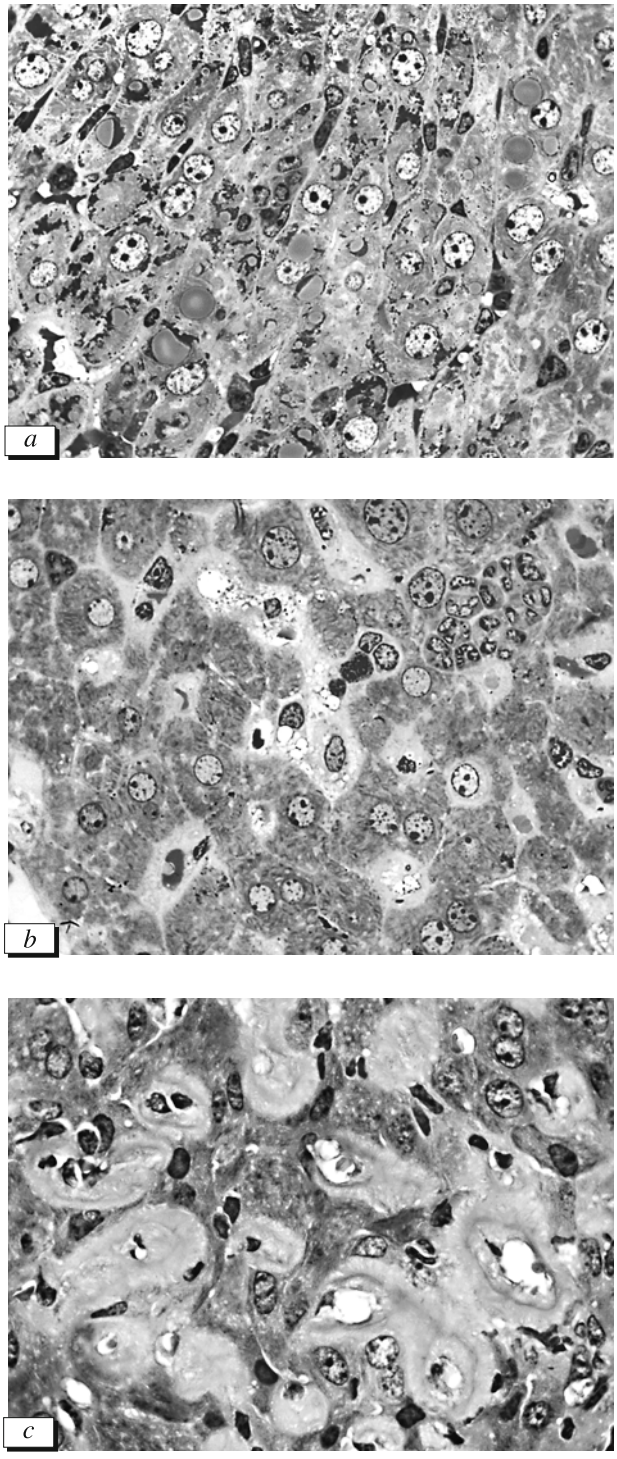

Fig. 2. Liver from mice with transplanted LLC after correction with BA and its derivatives, ×1000. *a*) after complex cytostatic treatment: large- and small-droplet lipid infiltration of hepatocytes and glycogen deposits. Metastases and stellate cells in sinusoids. Semithin section, PAS reaction – hematoxylin, ×1000; *b*) after treatment with cytostatics and APA: thickening of sinusoid walls with partial occlusion. Metastases and stellate cells in sinusoids. Semithin section, PAS reaction – hematoxylin; *c*) after treatment with cytostatics and triterpenoids: thickening of sinusoid walls with partial lumen occlusion, compression of hepatocytes. Metastases in sinusoids. Hematoxylin and eosin staining.

TABLE 1. Stereological Analysis of the Liver after Administration of BA and Its Derivatives to Mice with Transplanted LLC against the Background of PCT (*M*±*m*)

Group	Volume density (number)				
	hepatocytes		sinusoid lumens		
	with degenerative changes	with necrotic changes	free	with leukocytes	with tumor metastases
1 Transplanted LLC (control)	0.65±0.22	0.08±0.13	0.19±0.22	0.017±0.060	0.06±0.09
2 PCT (reference group)	0.620±0.002	0.150±0.007**	0.15±0.005*	0.028±0.005	0.046±0.009
3 PCT+BA	0.59±0.04	0.087±0.020^{+}	0.21±0.02^{+}	0.06±0.02	0.03±0.01*
4 PCT+metBA	0.62±0.01	0.12±0.01	0.210±0.009^{++}	0.024±0.005	0.034±0.008*
5 PCT+APA	0.62±0.26	0.07±0.01^{+}	0.20±0.02^{+}	0.029±0.011	0.043±0.006
6 PCT+metAPA	0.63±0.01	0.12±0.02	0.17±0.01	0.036±0.008*	0.034±0.008*

Note. *p<0.05, **p<0.1 compared to control group; ^{+}p<0.05, ^{++}p<0.01 compared to group 2.

substances and growth factors essential for intracellular and cell regeneration.

PCT and its combinations with the studied chemical agents considerably reduced the volume density of metastases in sinusoid lumens. After PCT in combination with triterpenoids, the volume density of metastases decreased most markedly: after treatment with BA and metBA by 50 and 43%, respectively, compared to the control (p<0.05). As a result, the volume density of sinusoids with free lumens after administration of BA, metBA, and APA against the background of PCT increased compared to the control (by 5-11%) and PCT alone (by 33-40%).

Thus, BA and APA reduce cytostatic hepatotoxicity, which manifested in reduced relative content of necrotically changed hepatocytes. Methyl esters of BA and APA were less effective in normalizing the hepatocyte structure and preventing their necrotic death. At the same time, all tested triterpenoids produced more pronounced antimetastatic effect than the complex of cytostatics. These findings suggest that BA and APA can be recommended for further comprehensive study as promising correctors of toxic effects of complex PCT of malignant neoplasms, while methyl esters of these acids as a promising antimetastatic agents for the therapy of metastasizing tumors.

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