Effect of Betulonic Acid and Its Derivative [3-Oxo-20(29)-Lupene-28-Oyl]-3-Aminopropionic Acid on Liver Structure in Mice with RLS Lymphoma

N. A. Zhukova, D. E. Semenov*, I. V. Sorokina, T. G. Tolstikova, S. V. Pozdnyakova, and O. R. Grek****

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Morphological study of the effects of semisynthetic derivatives of betulin (betulonic acid and [3-oxo-20(29)-lupene-28-oyl]-3-aminopropionic acid) on the liver of CBA/Lac mice with transplanted RLS lymphoma was studied in the control and after cytostatic polychemotherapy. The number of small focal necroses decreased, while the counts of hepatocytes in a state of slight hyaline droplet degeneration increased. Morphometry of the main elements of liver parenchyma showed that alanine amide derivative of betulonic acid decreases the severity of necrotic and degenerative changes in the liver parenchyma, induced by cytostatic polychemotherapy. Betulonic acid exhibited no appreciable hepatoprotective effect under these conditions.

Key Words: *polychemotherapy; liver; hepatoprotective effect; betulonic acid; [3-oxo-20(29)-lupene-28-oyl]-3-aminopropionic acid*

Cytostatic polychemotherapy is one of the main methods in the complex treatment of oncological diseases. However, high toxicity of antitumor drugs is a limiting factor in the therapy of neoplasms [3]. Application of these drugs is associated with severe disorders primarily in the hepatobiliary system and direct toxic effect on liver cells [2,8]. The development of new drugs for the correction of toxic effects of cytostatics remains a pressing problem. The attention of pharmacologists and medical chemists is now attracted to the natural lupane series triterpenoids, uniting high biological activity and availability. Betulin derivatives, triterpenoids from birch bark, characterized by antiinflammatory, immunomodulating, and antitumor effects, are perspective representatives of this class of substances [6,11].

Laboratory of Pharmacological Studies, N. N. Vorozhtsov Institute of Organic Chemistry, Siberian Division of Russian Academy of Sciences, Novosibirsk; *Institute of Regional Pathology and Pathomorphology, Siberian Division of Russian Academy of Medical Sciences, Novosibirsk; **Novosibirsk State Medical Academy. ***Address for correspondence***: sorokina@nioch.nsc.ru. I. V. Sorokina

Betulonic acid (BA) and its amide, containing β-alanine fragment in the lateral substitute: [3-oxo-20(29)-lupene-28-oyl]-3-aminopropionic acid (BA amide), were synthesized at Laboratory of Medical Chemistry, Novosibirsk Institute of Organic Chemistry [7]. These compounds exhibited antioxidant effect in mice and rats with toxic CCl_4-induced hepatitis [9,10]. The substances protected the liver of rats under conditions of cytostatic treatment (cyclophosphamide, vincristine, adriamycin, prednisolone), the severity of necrotic and degenerative injuries to hepatocytes decreased by 68-70% [10].

Here we studied the effects of BA and BA amide on morphological changes in the liver of mice with transplanted RLS lymphoma treated with cytostatics.

MATERIALS AND METHODS

Experiments were carried out on 60 male CBA/Lac mice with initial body weight 22-25 g, kept under standard vivarium conditions. All animals were transplanted RLS lymphoma (suspension of tumor cells in

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saline, 2×10^5 cells/ml) in the thigh. RLS lymphoma was obtained from the Tumor Bank, Institute of Cytology and Genetics. On day 5 after tumor transplantation the mice were divided into 6 groups (10 per group). Animals of groups 1, 2, and 3 received a single intraperitoneal injection of cytostatics, simulating polychemotherapy [4]: doxorubicin, 4 mg/kg (Lance-Pharm), cyclophosphamide, 50 mg/kg (Biokhimik), vincristine, 0.1 mg/kg (Gedeon Richter), and prednisolone, 5 mg/kg (Gedeon Richter). During the next 7 days the mice of groups 2 and 4 received intragastrically (through a tube) BA and mice of groups 3 and 5 received BA amide. The agents were administered as water-Twin suspension (0.2 ml/100 g). The animals of the reference group (group 1) and controls (group 6) received water-Twin mixture without drugs.

Eight days after drug treatment the mice were decapitated under ether narcosis, the liver was removed, fixed in 0.1 M phosphate buffer (pH 7.2-7.8) for 4 days, routinely processed on a MICROM histological complex (Karl Zeiss), including automated processing, embedding in paraffin blocks, and slicing (4-5 μ). The sections were stained with hematoxylin and eosin and by PAS/hematoxylin/Orange G method. The preparations were examined under an optic microscope in transmitted light.

Morphometric analysis of the sections was carried out using a 289-points ocular grid as described previously [1]. Volume density (V_v) of zones with degenerative and necrotic parenchymatous cells and volume density of sinusoids were evaluated. Changes in the volume density in experimental groups were expressed in percent of control. The data were statistically processed by parametrical statistical methods using Statistika 6.0 software. The results were considered significant at $p<0.05$.

RESULTS

RLS is a non-Hodgkin large-cell lymphoma characterized by aggressive course, formation of large extranodal infiltrations in the liver, and certain resistance to cyclophosphamide [5]. Extensive large and small foci of metastases developed in the livers of control and experimental animals after RLS transplantation; the metastases were located mainly periportally, disseminating beyond the terminal plate along sinusoids into the parenchyma. Small and large foci of fibrinoid necroses were seen in the center and at the periphery of metastatic foci. Hepatic cholestasis phenomena were seen in the parenchyma: diffuse impregnation of hepatocytes with bile, with "villous" degeneration of hepatocytes in some animals [8]. Multiple small focal necroses were seen in the centrolobular compartments. Fibrin and numerous PAS-positive macrophages were detected in sinusoids; sinusoidal cells were enlarged.

Injection of cytostatics induced severe hepatosis associated with increased number of necrotic zones and proportionate decrease in the number of degenerative hepatocytes (by 28% *vs.* control; Table 1). Pronounced hydropic and balloon degeneration of parenchymatous cells and absence of glycogen in cells indicated deep suppression of plastic processes (Fig. 1). Volume density of sinusoids decreased.

Treatment with triterpene compounds after polychemotherapy improved hepatocyte status. The number of necroses decreased in comparison with group 1 in the presence of a certain increase in the count of hepatocytes in the state of slight hyaline droplet degeneration. Glycogen was detected virtually in all hepatocytes, presenting as small grains. Intrahepatic cholestasis was slightly pronounced. Positive shifts were most pronounced in mice treated with BA amide. Treatment with BA alanine amide derivative led to a significant decrease in the volume density of necrotic zones (by 63%) and increase in the volume density of zones with degenerative hepatocytes (by 19%) compared to animals treated with cytostatics alone (Table 1). Analogous shifts were negligible in the BA group. A trend to increased volume density of sinusoids was

TABLE 1. Morphometric Analysis of the Liver in Mice with RLS Lymphoma (*M*±*m*)

Group	Volume density					
	zone of degenerative changes		necrotic zone		sinusoids	
	number	%	number	%	number	%
1	0.42±0.04*	72	0.370±0.017	128	0.095±0.020	90
2	0.490±0.017	84	0.310±0.026	122	0.110±0.004	100
3	0.53±0.01*	91	0.190±0.014+	65	0.130±0.011	118
4	0.450±0.016**	76	0.310±0.017	107	0.140±0.008	127
5	0.520±0.024	90	0.260±0.018	90	0.120±0.009	109
6 (control)	0.580±0.017	100	0.290±0.036	100	0.11±0.01	100

Note. *$p\le0.05$, **$p\le0.01$ compared to group 6; +$p\le0.001$ compared to group 1. Percent of control is shown.

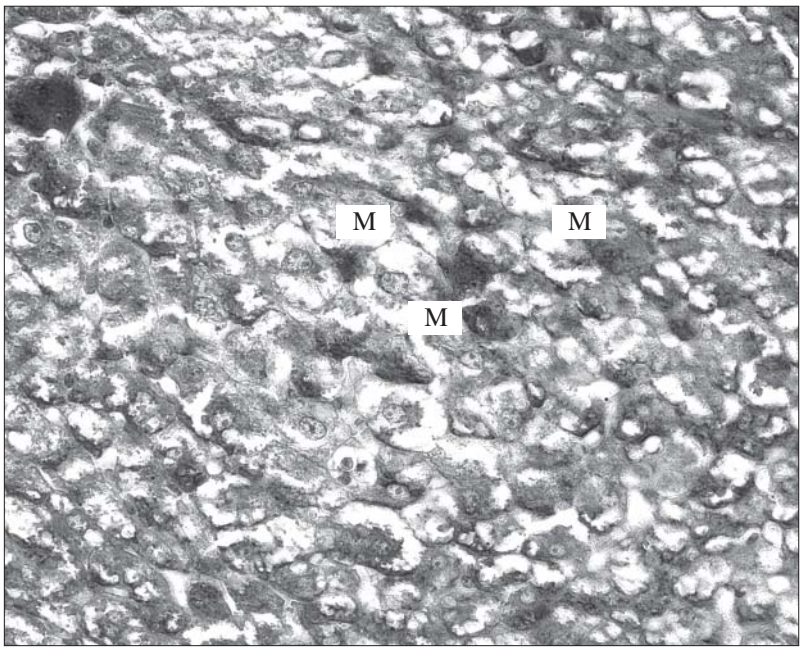

Fig. 1. Liver of a mouse with transplanted RLS lymphoma after a single injection of cytostatic complex. Hepatocytes: regeneratory plastic insufficiency of cytoplasmic structures, no glycogen in the entire section. PAS-positive macrophages (M) in sinusoidal lumen. PAS/hematoxylin/Orange G staining, ×400.

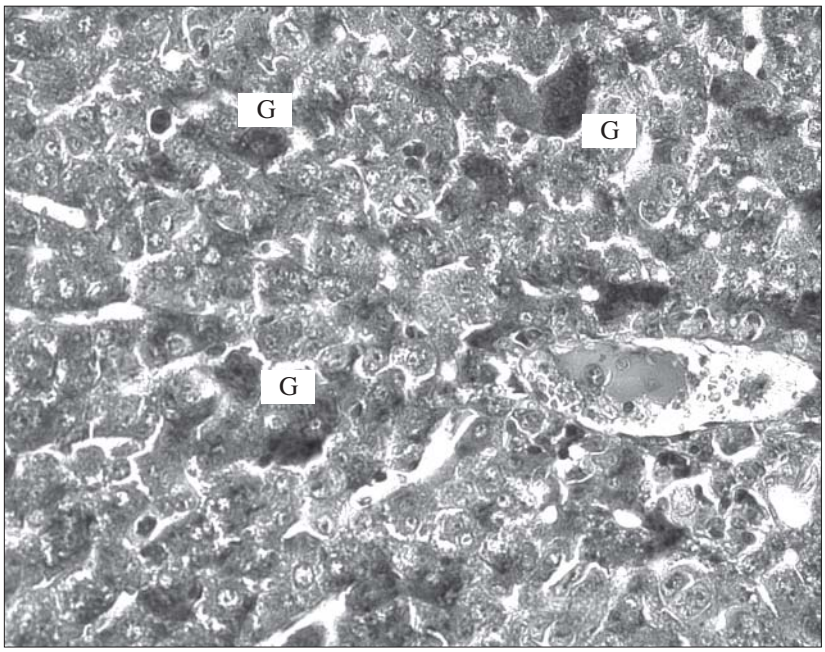

Fig. 2. Liver of a mouse with transplanted RLS lymphoma after injection of polychemotherapeutic complex and intragastric treatment with betulonic acid amide. Slight disorders in regeneratory processes in hepatocytes. Glycogen (G) is detected in hepatic cells in the entire section. PAS/hematoxylin/orange G staining, ×400.

observed in both groups (by 28% for BA amide and by 10% for BA).

BA amide did not change volume density of necrotic zones and areas of degenerative hepatocytes in mice receiving no cytostatics. Treatment with BA significantly reduced volume density of degenerative hepatocyte areas (by 24%) in comparison with the control, but did not influence the intensity of necrobiotic changes. Treatment with BA gave rise to a trend to an increase in the sinusoidal volume density. Triterpenoid treatment of animals in groups 3 and 4 led to slight hyaline droplet degeneration, glycogen was detected in the entire section (Fig 2), and cholestasis phenomena were less pronounced than in control animals with transplanted tumors.

These data indicate that BA alanine amide derivative is characterized by pronounced cytoprotective effect, which is seen in the morphofunctional status of the liver in mice with malignant lymphoma after cytostatic polychemotherapy. On the other hand, BA treatment after polychemotherapy had virtually no effect on the liver. These results correlate with previous data obtained on rats receiving experimental polychemotherapy, indicating decrease in the volume density of necrotic and degenerative hepatocyte zones under the effect of these compounds [10]. The appearance of

alanine amide fragment in BA molecule structure leads to a decrease in the hepatocyte cytolysis in experimental toxic hepatitis [9,10], and hence, the hepatoprotective effect of BA amide detected in this study can be realized through its stabilizing effect on cell membranes.

Thus, BA alanine amide derivative (BA amide) reduces the severity of necrotic and degenerative changes in hepatocytes caused by cytostatic polychemotherapy in mice with transplanted RLS. BA exhibited no appreciable hepatoprotective effect under these conditions.

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