Toxic Effect of Vepesid on Morphology and Function of the Rat Liver

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Intravenous injection of antitumor drug vepeside in the maximum tolerated dose (MTD) stimulated free radical oxidation processes in rats. Activities of blood aminotransferases and alkaline phosphatase changed. Morphological study of the liver revealed fatty degeneration, hepatocyte necrosis, and inflammatory infiltration.

Key Words: *vepeside; hepatotoxicity*

Clinical use of the majority of antitumor drugs is limited because of their toxic effects. Vepesid (etoposide), a derivative of podoxyphyllotoxins isolated from *Podophyllum peltatum* rhizome, is widely used for the treatment of small-cell cancer of the lungs, testicular tumors, acute non-lymphoblastic leukemia, Hodgkin's disease, stomach, ovarian, breast cancer, *etc.* [6,8]. Hepatotoxicity is one of the known side effects of vepesid [2]. The capacity of this drug to form intracellular free radicals suggests that the mechanism of the destructive effect of the cytostatic on liver cells can be associated with LPO intensification [4].

We studied the biochemical and morphological aspects of vepesid hepatotoxicity.

MATERIALS AND METHODS

Experiments were carried out on 36 Wistar rats (150-200 g). The animals were kept in accordance with regulations of the European Convention for Protection of Vertebrates Used in Experimental and Other Research Purposes (Strasbourg, 1986). Etoposide (vepesid, Bristol) was injected in a single intravenous dose of 30 mg/kg (MPD). The studies were carried out on days 2, 5, 7, 14, 21, and 28 after injection. The animals were decapitated.

Serum activities of AST, ALT, and alkaline phosphatase were measured using Lachema kits, the content β- and pre-β-lipoproteins was assayed by the methods of Burchtein and Samay, and MDA was measured as described previously [3].

The content of LPO products conjugated dienes (CD) and Schiff bases (SB) in the liver was measured as described previously [7,9]. Optical density was measured on a Hitachi-557 spectrophotometer at 232 nm. Schiff bases were evaluated by the fluorescence spectra of lipid extracts with maximum excitation at 360 nm and maximum emission at 420 nm on a Hitachi-650 spectrofluorometer.

Liver fragments for histological study were fixed in Carnoy fluid, deparaffinized sections were stained with hematoxylin and eosin. The area of infiltration on preparations stained with hematoxylin and eosin was evaluated using Avtandilov ocular grid [1]. Pyknotic and binuclear hepatocytes per 1000 hepatocytes were counted on the same preparations.

The data were processed statistically using Wilcoxon—Mann—Whitney nonparametric test and Student's *t* test.

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TABLE 1. Effect of Vepesid on the Content of LPO Products in the Serum and Liver Tissue ($X \pm m$)

Day of analysis	Liver		Blood	
	SB, U/mg lipids	CD, U/mg/lipids	MDA, μmol/mg protein	
			0 min	60 min
Control	0.29±0.02	0.27±0.04	8.78±1.16	9.74±1.00
2	0.18±0.02*	1.15±0.15*	16.96±0.98*	19.72±0.92*
5	0.25±0.02	2.15±0.23*	9.29±0.59	15.39±1.30*
7	0.44±0.01*	0.21±0.02	6.85±0.28	7.07±0.56
14	0.38±0.03*	0.26±0.05	10.28±1.18	10.66±0.95
21	0.57±0.04*	0.22±0.02	10.26±2.34	17.20±0.89*
28	0.29±0.02	0.32±0.05	10.08±1.42	5.53±0.59

Note. *$p<0.05$ compared to the control (Student's t test).

RESULTS

Injection of vepesid in MTD significantly activated LPO processes in rat liver and blood. Blood MDA content increased starting from day 2 of the experiment (2-fold *vs.* the control; Table 1). High level of MDA was also noted on days 5 and 21 of the experiment. Intensification of LPO was also seen from accumulation of CD and SB in the liver tissue. The content of SB decreased during the early period of the experiment (day 2), while on days 7, 14, and 21 this parameter significantly surpassed the control (by 51, 31, and 96%, respectively). The concentration of CD increased on days 2 and 5 after drug injection.

On day 7 after vepesid injection, serum levels of β- and pre-β-lipoproteins decreased virtually 4-fold compared to the control (7.67±1.87 *vs.* 29.0± 4.36 arb. units), which was presumably caused by their oxidative destruction and led to disorders in triglyceride transport from the liver into circulation.

The cytotoxic effect of the drug was linked to changes in activities of some enzymes. Serum ALT activity decreased on days 5-7. Activity of AST decreased significantly on day 5 (0.85±0.04 *vs.* 1.06±0.06 μmol/liter in the control), but increased on days 7 and 14. Blood level of alkaline phosphatase increased on days 14 and 21 after drug injection by 66 and 59%, respectively, compared to the initial level. It is known that the increase in activity of this enzyme reflects reactive changes in the hepatocyte plasma membranes and biliary duct epithelium [5]. These disorders in liver metabolism persisted until day 21 after vepesid injection. By the end of the experiment (day 28), the content of LPO products in the liver returned to normal.

Analysis of biochemical findings suggests that complex changes in the liver after vepesid injection were caused by LPO activation, which, in turn, disordered the structure of hepatocyte membrane and led to the release of aminotransferases and alkaline phosphatase into the blood.

Hence, vepesid injection caused morphological changes in rat liver characteristic of toxic hepatitis: fatty degeneration and hepatocyte necrosis, formation of cellular inflammatory infiltration. We observed small focal hepatocyte steatosis in the center of the lobule and monocellular hepatocyte

TABLE 2. Effect of Vepesid on Rat Liver Morphology and Function ($X \pm m$)

Day of analysis	Infiltration, %	Binuclear hepatocytes, %	Necrotic hepatocytes, %
Control	2.8±0.3	12.6±0.5	0.2±0.1
2	8.12±0.46**	6.66±0.64**	10.00±2.35*
5	11.88±0.46**	4.76±0.56**	12.6±1.21*
7	13.40±0.97**	6.96±0.83**	11.40±1.29*
14	13.02±1.41**	12.48±1.13	7.20±1.36*
21	10.42±0.84**	9.84±0.66**	6.40±1.21*
28	6.72±0.49**	9.50±1.22*	3.60±0.24*

Note. *$p<0.05$, **$p<0.01$ compared to the control (Wilcoxon—Mann—Whitney test).

necrosis. The increase (6-fold compared to the control) in the count of hepatocytes with pyknotic nuclei, detected on day 5 of the experiment, attested to high intensity of the destructive processes in the liver parenchyma, which was confirmed by increased activity of serum AST during this period. Accumulation of inflammatory cells (predominantly lymphocytes and macrophages) was observed in necrotic foci. Moderate infiltration was also seen in portal tracts. In some areas the inflammatory cells left the portal stroma and were located in the liver parenchyma among hepatocytes without necrosis. The severity of infiltration increased starting from day 2 after the cytostatic injection and peaked on day 7 of the study (the period of maximum changes) more than 4-fold surpassing the control level (Table 2). This indicates intensive inflammatory process in the liver parenchyma. The regeneratory processes were also disordered, which was seen from reduced count of binuclear hepatocytes on days 2-28 of the study.

Hence, single injection of antitumor drug vepesid in MPD caused significant metabolic disorders in rat liver associated with initiation of LPO processes, changes in enzyme activities (aminotransferases and alkaline phosphatase) persisting until day 21 of the experiment. Morphological studies showed disorders characteristic of toxic hepatitis, which were most pronounced on days 5 and 7 after drug injection and persisted until day 28.

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