MORPHOLOGY AND PATHOMORPHOLOGY

Toxic Effect of an Antitumor Drug Paclitaxel on Morphofunctional Characteristics of the Liver in Rats

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Single intravenous injection of paclitaxel to rats in a maximum tolerated dose of 4.6 mg/kg was accompanied by permanent structural and functional changes in the liver. The observed changes were typical of nonspecific reactive hepatitis: infiltration with lymphocytes and macrophages, pyknosis, and focal fatty degeneration of hepatocytes. Liver enzyme activity increased in blood plasma.

Key Words: *paclitaxel; hepatotoxicity; laboratory rats*

Until recently, liver dysfunction played little role among side effect of antitumor therapy. However, the incidence of these complications significantly increased with the development of new antitumor drugs and introduction of high-dosage therapy into oncological practice [3]. Paclitaxel is a promising plant-derived cytostatic drug, which belongs to the group of taxanes. Unique mechanism of action of this drug consists in inhibition of mitosis at the stage of microtubule formation [5,8-11, 14]. Taxanes suppress the growth of cancer cells, act synergistically with various chemotherapeutics, and *in vitro* potentiate the therapeutic effect of irradiation. Paclitaxel is effective in the therapy of non-small cell lung cancer, metastatic ovarian carcinoma, and breast carcinoma [6,8]. Similarly to other cytostatics, paclitaxel is metabolized in the liver and excreted with bile [8,11,12,15]. Side effects of paclitaxel therapy include changes in biochemical parameters such as increase in activity of aspartate transaminase (AST), alkaline phosphatase (AP), and bilirubin; moreover, cases of liver necrosis and hepatic encephalopathy were reported [3,4,8,11].

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Here we studied morphofunctional characteristics of the liver in paclitaxel-treated animals.

MATERIALS AND METHODS

Outbred female rats (150-200 g) received single intravenous injection of paclitaxel (Taxol, Bristol-Myers Squibb) in a maximum tolerated dose (MTD) of 4.6 mg/kg. The dose of paclitaxel was estimated by probit analysis. The study was performed on days 2, 5, 10, 15, 30, 90, and 180 after cytostatic treatment. For histological study, liver samples were fixed in Carnoy's fluid. The relative area of infiltration was calculated on preparations stained with hematoxylin and eosin using an Avtandilov ocular grid [1]. The number of pyknotic and binucleated hepatocytes (per 500 liver cells) were determined. Glycogen content in hepatocytes was determined on liver sections stained by the method of McManus. DNA concentration in hepatocyte nuclei was measured by means of computer graphic analysis after Felgen staining [7].

The effect of paclitaxel on hepatic metabolism ALT, AST, and AP was evaluated. The results were analyzed by nonparametric Mann—Whitney test.

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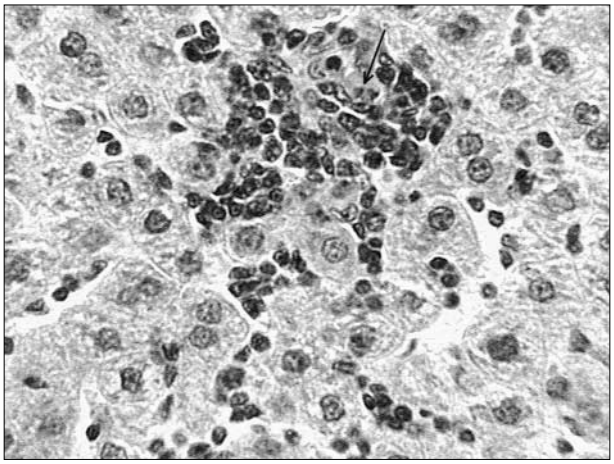

Fig. 1. Rat liver on day 10 after administration of paclitaxel in MTD. Lymphocyte and macrophage infiltration around dead hepatocytes. Arrow: Councilman body. Hematoxylin and eosin staining, ×600.

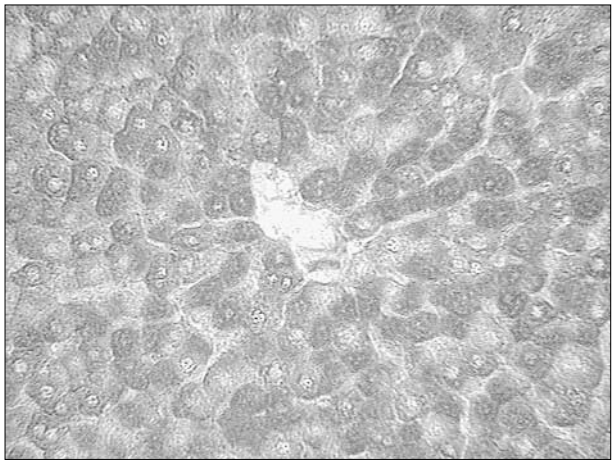

Fig. 2. Rat liver: high content of glycogen in hepatocyte cytoplasm under control conditions. Here and in Fig. 3: McManus staining, ×600.

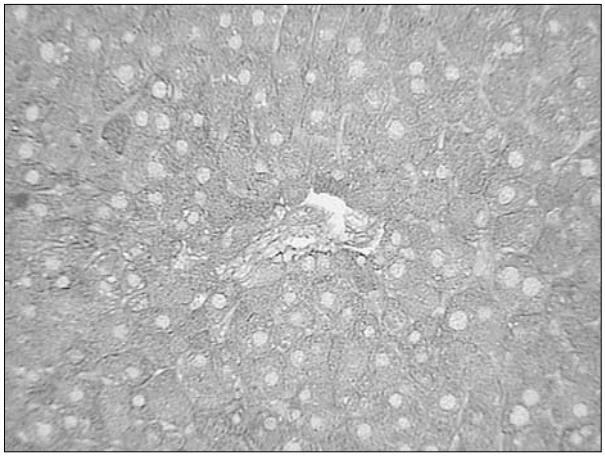

Fig. 3. Rat liver on day 10 after administration of paclitaxel in MTD: disappearance of glycogen granules from the hepatocyte cytoplasm.

RESULTS

Morphological study of liver samples from rats at various terms after paclitaxel injection revealed signs of nonspecific reactive hepatitis. They included changes in the structure of hepatic cords, widening of sinusoids, polymorphism of hepatocytes, focal fatty degeneration, and monocellular necrosis of liver cells. The portal tracts were moderately infiltrated with lymphocytes and macrophages. Moreover, these cells were diffusely localized between hepatocytes in the liver parenchyma (Fig. 1). Histological study of liver samples (McManus staining) revealed disappearance of glycogen granules from the hepatocyte cytoplasm. On day 5, glycogen granules were found only in the cytoplasm of hepatocytes at the periphery of the lobule, while on day 10 they disappeared in all liver cells (Figs. 2 and 3). Glycogen content in the hepatocyte cytoplasm progressively returned to normal from the 15th to the 30th day of study.

The number of hepatocytes with pyknotic nuclei increased on day 2 after cytostatic treatment, which reflects the cytotoxic effect of paclitaxel. The count of these cells reached maximum by the 10th day (1.28±0.23 *vs*. 0.10±0.05 in the control, $Pu<0.01$). This parameter slightly decreased by the 30th day, but remained above the control. The number of necrotic hepatocytes remained high on day 90, but did not differ from the control by the 180th day (Table 1).

The relative area of cellular infiltration in the liver parenchyma increased in all periods after paclitaxel injection.

Published data show that taxanes inhibit mitosis [5,8,11,14]. We showed that the cytostatic drug decreases proliferative activity of the liver. This conclusion was derived from the decrease in the number of binucleated hepatocytes and nucleic acid content in hepatocyte nuclei. The concentration of nucleic acids in hepatocyte nuclei decreased starting from day 5 after cytostatic treatment and was minimum on day 15 (2-fold below the control). These variations coincided with a decrease in the number of binucleated hepatocytes. Regenerative capacity of the liver significantly increased on the 30th day. The number of binucleated hepatocytes and nucleic acid content approached the control value. In the delayed period after treatment (days 90 and 180) these parameters did not differ from normal (Table 1).

Biochemical study revealed increased ALT and AST activities on days 10 and 15, respectively. AP activity increased on days 10 and 15.

No significant changes were found in the delayed period after paclitaxel injection. AST activity slightly decreased 3 months after treatment (Table 2).

TABLE 1. Effect of Paclitaxel on Morphological Characteristics of Rat Liver ($X \pm m$)

Period of study	Infiltration, %	Binucleated hepatocytes, %	Necrotic hepatocytes, %	DNA, arb. units
Control 1	5.78±0.65	13.23±0.47	0.10±0.05	0.96±0.01
24 h	8.20±0.52*	9.04±0.15**	0.44±0.07**	—
5 days	8.28±0.96*	7.18±0.22**	0.42±0.10**	0.85±0.05*
10 days	8.72±0.58*	9.84±0.47**	1.28±0.23**	0.73±0.04**
15 days	7.60±0.74	6.92±0.92**	0.86±0.24**	0.510±0.053**
30 days	8.88±0.38**	10.12±1.35*	0.76±0.13**	0.87±0.03*
Control 2	5.52±0.41	16.80±0.52	0.58±0.12	0.96±0.01
90 days	7.28±0.44*	18.00±1.86	1.72±0.26*	0.92±0.01
180 days	7.84±0.78*	15.80±1.63	1.44±0.49	0.94±0.05

Note. Control 1 and control 2: control values relative to days 2-30 and 90-180, respectively. *Pu<0.05, **Pu<0.01 compared to the control.

TABLE 2. Biochemical Markers in Blood Plasma from Rats at Various Periods after Paclitaxel Injection ($X \pm m$)

Parameter	ALT, μcat/liter	AST, μcat/liter	AP, U/liter
Control 1	0.50±0.07	0.56±0.03	373.50±21.57
2 days	0.66±0.01	0.62±0.03	440.36±32.62*
5 days	0.53±0.05	0.56±0.05	448.00±75.18
10 days	0.71±0.04*	0.63±0.02	570.30±45.35*
15 days	0.48±0.06	0.73±0.04*	483.52±30.62*
30 days	0.39±0.04	0.57±0.03	428.64±28.59
Control 2	0.40±0.04	0.52±0.03	246.90±51.17
90 days	0.36±0.02	0.43±0.02*	286.90±24.15
Control 3	0.48±0.06	0.47±0.02	258.62±50.12
180 days	0.62±0.04	0.53±0.04	321.80±49.75

Note. Control 1, control 2, and control 3: control values relative to days 2-30, 90, and 180, respectively; *Pu<0.05 compared to the corresponding control.

Our results indicate that single intravenous injection of antitumor drug paclitaxel in MTD causes structural and metabolic changes in the liver, which are typical of nonspecific reactive pancreatitis. The cytostatic drug as a nonspecific cytotoxic agent activates the general inflammatory response in the liver parenchyma, which manifested in the development of cellular infiltration [3,4]. The decrease in the number of binucleated hepatocytes and concentration of DNA in hepatocyte nuclei results from the antimitogenic effect of paclitaxel. Blockade of mitoses is followed by abnormal cell cycle and initiation of apoptosis [8]. These changes contribute to accumulation of hepatocytes with pyknotic nuclei and increase in the concentration of hepatic membrane markers in blood plasma on days 10-15 after paclitaxel injection. The toxic effect of paclitaxel on morphological and functional activity of the liver develops on day 2 and persists for 6 months after injection. These data attest to the necessity of using hepatoprotective drugs.

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