EFFECTS OF DOXORUBICIN AND RONCOLEUKINE ON LIVER MORPHOLOGY IN DIETHYLNITROSAMINE (DENA)-INDUCED HEPATOCARCINOGENESIS

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After experimental drug therapy for diethylnitrosamine (DENA)-induced carcinogenesis with doxorubicin, roncoleukin and their combination, mortalities by the end of experiment were 25; 25, and 16.7%, respectively, whereas in the untreated group was 30%. The most positive morphological changes in the liver tissue were identified after combined use of doxorubicin with roncoleukin, which was manifested, to some extent, by restoring of beam arrangement of hepatocytes, decrease of symptoms of tumor degeneration, hyperchromasia, and nuclear atypism.

Keywords: diethylnitrosamine-induced carcinogenesis, experiment, liver, doxorubicin, roncoleukin

The incidence of liver tumors trends to increase in recent years. Sherlock and Dooley (1999) reported that the incidence of malignant liver tumors is 2–3% among oncological diseases. Asriviadis et al. (1998) and Schoning-Hekele et al. (2001) found that primary malignant transformation develops in 86–90,2% of all liver tumors, and of them, hepatocellular carcinoma is about 90%. Increase of the number of primary liver cancer in last decade is, probably, because of increased frequency of liver cirrhosis and better detectability of cancer. Despite advances in the treatment of this disease, mortality remains high. A median survival in hepatocellular carcinoma is reported by Schoning-Hekele et al. (2001) to be less than 8 months.

Kiselevsky (2003) noted that cytotoxic lymphocytes and natural killer cells play the key role in the antitumor surveillance system and their killer activity is significantly increased under the influence of cytokines – interleukin-2 (IL-2) and interferon. Simbirtsev (2006) reported that these cytokines are characterized by participation in anti-tumor immunity. Study of the role of cytokines in the development of neoplastic processes allowed developing recombinant drugs of interleukins (IL).

The purpose of our study was to evaluate effects of doxorubicin and roncoleukine on liver structure in diethylnitrosamine (DENA)-induced carcinogenesis.

Materials and methods of research

The research was carried out in 70 male rats, weighing 100–120 g. The animals were kept in a vivarium on a normal diet without giving milk. We used a model of DAN-induced hepatocarcinogenesis by Evgrafov et al. (1966). To induce hepatocarcinogen, 60 rats were injected with carcinogen intraperitoneally 5 times a week at a dose of 10 mg/kg of body weight for 2 months. By the end of the 4th month, mortality was 23,3%. 46 surviving animals were divided into 4 groups:

1) control (10 rats) received saline solution 0,5 ml/100 g placebo;

2) 12 rats received doxorubicin at 0,6 mg/kg intraperitoneally in the tail vein daily within 3 days (Doxorubicin, «Pharmitalia»);

3) 12 rats received roncoleukin at 0,006 mg/kg intraperitoneally («Biotech», St. Petersburg);

4) 12 rats received a combination of these drugs in the same doses. Mortality by the end of experiment was 30, 25, 25 and 16,7%, respectively to groups. The animals were killed at 6, 7 and 8 months from the experiment onset. The comparison group consisted of 10 intact rats kept under the same conditions during the whole experiment. The animals were killed under light anesthesia after 7 months from the experiment onset in a cold room with an air temperature 0–+2°C. Part of the liver were taken for morphological and cytochemical studies.

Results of research and their discussion

Liver morphometry in DENA-induced carcinogenesis have shown that in the liver initially develop pathological changes in the form of dystrophic, dysregeneratory and dysplastic processes in liver parenchymatous elements. Later on, we observed discirculatory, dezorganizational, proliferative and sclerotic changes in liver stroma-vascular elements. Subsequently, they were aggravated; seal and homogenization of cytosol with impaired cell configuration were noted. In the nuclear structures we observed polymorphic changes as kariopicnosis, karyorrhexis and kariolisis of some hepatocytes, whereas in other cells was marked pathological hypertrophy of nuclear structures in the form of appearance of abnormal and dual-core structures (Fig. 1). In this case, sinusoids of hepatic tissue were significantly narrowed, twisted; in their lumen there were destructive and deformed blood elements. Subsequently, because of deformation and dysplasia of structural elements of the cytoplasm of hepatocytes, they turned into abnormal and polymorphic cells (Fig. 2).

In the cytosol of most cells we found vacuoles of different shape and size, lumps colored evenly basophilic with eosinophilic tint. The nuclei of these hepatocytes had different shapes and sizes, and their location and chromaphility were disrupted. Chromatin had no localization; due to it improper distribution in karyoplasms, abnormal hematoxylin nuclear structures were marked.

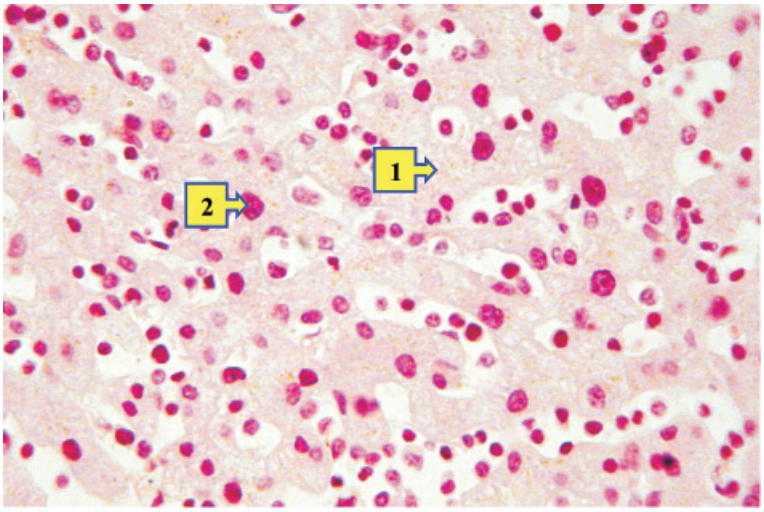

Fig. 1. Seal and homogenization of the matrix of the cytoplasm (1), the appearance of nuclear polymorphism (2) in DENA-induced carcinogenesis. Staining with hematoxylin and eosin. Magnification: ocx10, obx40

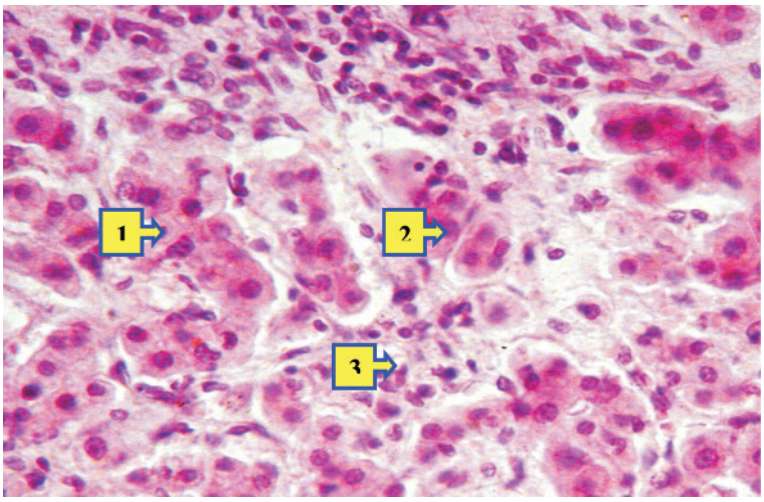

Fig. 2. Disorientation (1), appearance of abnormal tumor cells (2) and lymphoid infiltration (3) in DENA-induced carcinogenesis. Staining with hematoxylin and eosin. Magnification: ocx10, obx40

The appearance of many lymphoid cells between such dysplasia cells of hepatocellular origin corresponded to the development of anti-tumor immunity in liver tissue in the process of carcinogenesis. Histochemical study of the liver tissue during carcinogenesis showed that dysplasia cells degenerated in the tumor cells in PAS reaction had a significant increase in the number of pyronin-positive substance. Characteristic morphological changes in the cytoplasm of dysplasia hepatocytes for histochemical detection of NADPH-diaphorase were manifested by reduction of tetrosole granules in the form of small, vague inclusions of gray-brown or gray-violet colors. In the cytoplasm of hepatocytes with increased carcinogenic changes, their amount was increased and they evenly distributed throughout the entire area of the cytoplasm. In cells with pronounced signs of tumor growth, granules of NADPH-diaphorase were mostly concentrated in the perinuclear area of the cytoplasm and were fused with nucleus chromatin. Around carcinogenic dysplastic changes of the liver cells were determined accumulations of mononuclear hematohistiogenic as a defensive response to neoplastic degeneration of the liver parenchyma in induced carcinogenesis.

After pharmacotherapy with doxorubicin for hepatocarcinogenesis, in liver tissue pathological signs of tumor degeneration of hepatocytes are still remain. In this case, the liver

tissue had polymorphic structure with atypically localized elements of both parenchyma and stroma of the liver. Almost all of liver cells were vacuolated with loss of the typical structure of hepatocytes, localized as groups and atypically. Their nuclei were shifted to the periphery of cell, had polymorphic structure with hyperchromasia of chromatin. Separate groups of atypical hepatocytes contained formazan granules in the form of pulverized clusters with a concentration in the cell membrane. In other cells, where the cytoplasm was much vacuolated, formazan granules were located in the cell membrane. Between clusters of atypical hepatocytes we marked massive accumulation of histiocytic lymphoma cells. They had formazan granules in the cytoplasm of macrophages.

Pharmacotherapy with roncoleukin for hepatocarcinogenesis showed a significant effect in the form of normalization of the structure, shape and location of hepatocytes. Although the cells were arranged randomly, without beams orientation, and had no Disse spaces and sinusoids between them, in some places around the carcinogenic degenerated cells we determined small clusters of mononuclear cells from lymphocytes, macrophages, and young connective tissue cells. Hepatocyte nuclear structures had relatively uniform shape and size, and significant chromatin concentration with signs of mitosis and amitosis. Histochemical determination of NADPH-diaphorase marker in rat hepatocytes showed irregular arrangement of a significant number of small, sometimes medium formazan granules in the form of gray-purple to gray-blue granules. Also accumulation of formazan granules was found in the intercellular substance as gray-purple stripes.

The most positive morphological changes in the liver tissue were identified after use of combination of doxorubicin with roncoleukin. In this case, the beam location of hepatocytes was restored in only separate hepatocytes, were preserved signs of tumor degeneration in the form of an eccentric location, hyperchromasia, and nuclear atypism. In these cells, the cytoplasm was vacuolated, in matrix histochemically were determined some formazan granules in the form of small gray-brown clusters in the perinuclear region. While hepatocytes merged together, their small nucleus is divided into several parts; the cytoplasm is colored with grained formazan granules in the form of gray-brown inclusions.

We obtained positive results due to the fact that IL-2 has the ability to induce the activity of almost all clones of cytotoxic cells, as noted by Berezhnaya et al. (1992). Histochemical studies have shown that cytokines serve the protective role by providing recruitment of additional effector cells in pathological focus, stimulating their phagocytic activity and inducing antigen-run response, all of which contributes to the elimination of tumor cells (Jafarova, 2009). Studies by Chechina et al. (2011) demonstrated that recombinant IL-2 at a dose of 0.1 ng/mL has a pro-apoptotic activity against lymphocyte cells by changing the ratio of anti-(Bcl-2, Bcl-x1) and proapoptotic (Bad) family proteins Bcl-2 in favor of the latter.

Conclusion

Thus, pharmacotherapy with different preparations for liver induced tumors showed that the most positive morphological changes in liver tissue were detected by the action of doxorubicin in combination with roncoleukin.

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