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Morphological Studies of Hepatocellular Carcinomas in Male CBA Mice with High Liability to Cancer under the Effect of Phytoadaptogen

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Treatment of CBA mice predisposed to cancer with a complex phytoadaptogen in the therapeutic and preventive modes led to the appearance of moderate and low-differentiation hepatocellular carcinomas infiltrated by leukocytes. Destructive signs were detected in tumor tissue.

Key Words: *morphology of hepatomas in mice with high liability to cancer; hepatocarcinoma infiltration by leukocytes; phytoadaptogens (ginseng, rhodiola, eleutherococcus)*

A previous study on male CBA mice liable to spontaneous hepatocarcinogenesis has detected a reduction of the expression of leukocytic integrins LFA-1 and Mac-1 and increase of serum IL-6 and IL-10 levels. A short course of a complex phytoadaptogen (CP) Phytomix-40 during the early ontogeny (month 1 of life), involving the final period of differentiation of liver tissue, predisposed to tumor emergence, led to a lasting increase of expression of heterotypical adhesion molecules – leukocytic integrins LFA-1 (CD11/CD18) and Mac-1 (CD11b/CD18), maintaining contact interactions between the immune effectors and target cells, and to a reduction of serum IL-6 and IL-10 levels. On the other hand, a long course of treatment with the drug, starting from the period of tumor emergence until the natural death of animals, also maintained the correction of leukocytic integrin expression on immunity effectors and the serum concentrations of IL-6 and IL-10 [4]. This was paralleled by a reduction of the incidence of hereditary hepatomas, their number and size [7].

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Complex phytoadaptogen is a nontoxic drug Phytomix-40. Its components are extracts from 40 plants, included in the Pharmacopoeia of the Russian Federation: well-known adaptogens ginseng, rhodiola, eleutherococcus, Schizandra, aralia [3,5]. Methods for its biological and chemical standardization are developed [8,11]. Antioxidant, antimutagenic, antitumor, and immunomodulating effects of CP were demonstrated [1,2,6,9,10,13].

We studied the morphology of spontaneous hepatomas in CBA mice predisposed to cancer under conditions of CP treatment. Morphological changes in the spontaneous hepatomas of intact CBA mice and of males treated by CP in the preventive and therapeutic modes are studied.

MATERIALS AND METHODS

The study was carried out on male inbred CBA mice (n=370) with high liability to cancer (subline CBA/LacY) from Center of Biomedical Technologies, Federal Medical-Biological Agency of Russia, and from Department of Laboratory Animal Breeding, N. N. Blokhin Research Cancer Research Center. The

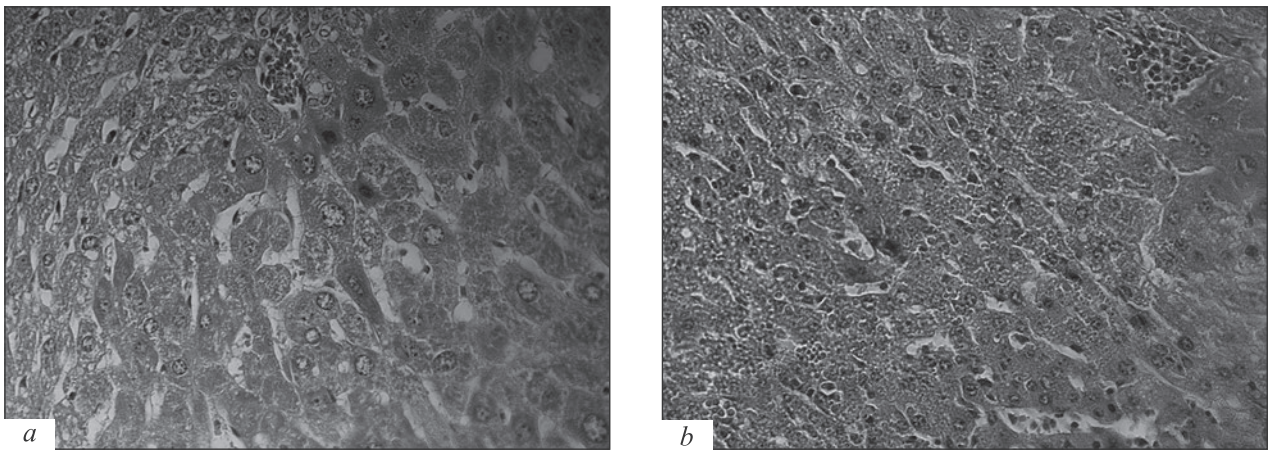

Fig. 1. Moderately differentiated trabecular hepatocellular carcinoma in male CBA mice aged 8 months. Hematoxylin and eosin staining, ×400. *a*) Control group; *b*) group 3 (CP in therapeutic mode): tumor infiltrated by leukocytes.

animals were kept under standard vivarium conditions in accordance with the International Ethical Standards.

The CBA line is the classical model of genetic liability to tumors of the liver with a high risk of their emergence. Distal chromosome 1 in the cells of these mice carries one and more genes responsible for liability to spontaneous and chemically-induced hepatocarcinogenesis [12]. The first spontaneous hepatomas emerge in male CBA mice from the age of 6 months and are 7-fold more incident than in females. Later, at the age of 18-22 months, hepatocarcinomas are detected in 100% males.

Control mice (n=90) received only water for drinking. Standard bowls were filled with water, the animals drank it *ad libitum*. As CP was a water-ethanol extract, ethanol in the relevant concentration (3%) was added to water for additional control groups for the preventive (n=60) and therapeutic (n=70) modes. The results were similar in all control groups, and hence, we pooled these data in one control group (group 1, n=220).

Preventive treatmnent was carried out in group 2 mice (n=80) as follows. Solution (10%) of CP was added to drinking water for females from late pregnancy until the end of feeding (1 month of postnatal development of newborn mice). Group 3 (therapy) mice (n=70) received CP similarly from the age of 6 months, by courses, until the natural death of animals. A course of treatment was 2 weeks long, at 2 week intervals.

The animals were sacrificed at the end of 4, 8, and 22 months, 13-15 animals from each group. The livers of control and experimental animals, in addition to macroscopic evaluation, were processed by the standard histological methods and stained by hematoxylin and eosin. Macroscopic revision of other organs showed no tumors.

RESULTS

Morphological studies of liver tissue of male CBA mice aged 4 months showed no tumors in control and experimental groups. The microscopic structure of hepatic tissue in animals of groups 2 and 3, aged 4 months, was normal and virtually did not differ from the control.

It is known that the first tumors emerge in the liver of male CBA mice, liable to cancer, from the age of 6 months. Macroscopic and microscopic studies in the control mice aged 8 months showed tumors in 10%. A tumor — trabecular hepatocellular carcinoma of moderate differentiation is presented in fragment *a*, Figure 1. Tumors of similar structure were detected at similar incidence in group 3. However, microscopic studies detected some specific features in these tumors. Studies of the hepatocarcinoma sections showed infiltration of the tumor by leukocytes, presumably, with

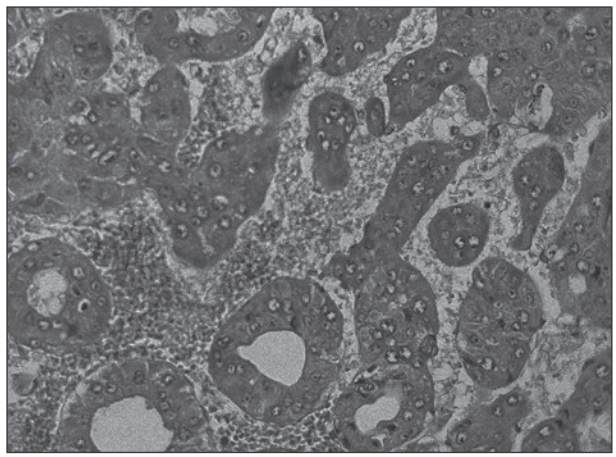

Fig. 2. Low-differentiated trabecular-acinar hepatocarcinoma. Liver section from a control animal aged 22 months. Hematoxylin and eosin staining, ×400.

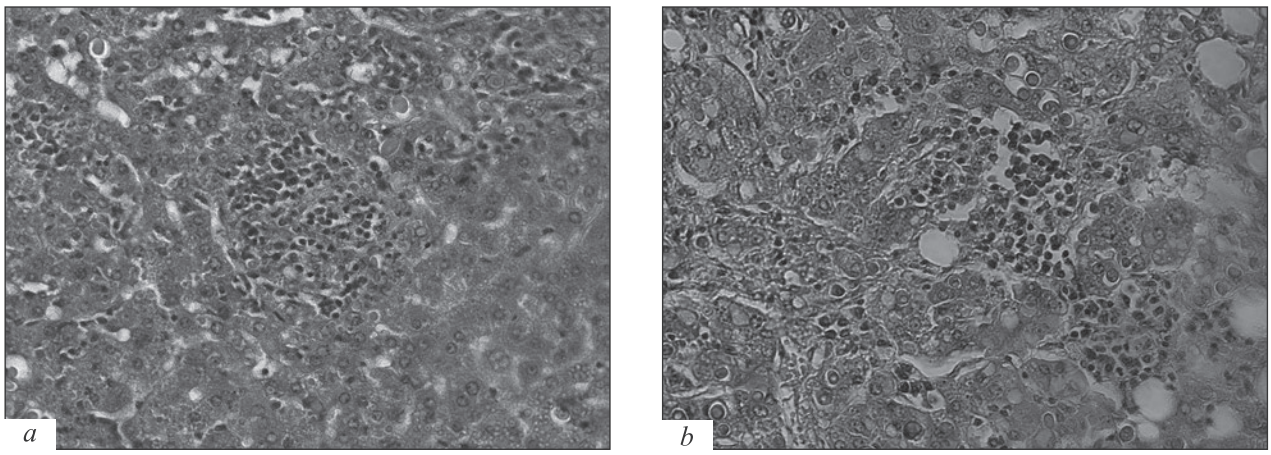

Fig. 3. Trabecular hepatocarcinoma infiltrated by leukocytes in male CBA mice aged 22 months. Hematoxylin and eosin staining, ×400. *a*) Group 2 (preventive CP); *b*) group 3 (CP therapy).

lymphocytes, forming foci (Fig. 1, *b*). The immune system cells migrated to the tumor in this case. On the other hand, no tumor process was observed in group 2 animals aged 8 months.

Microscopic studies in controls aged 22 months detected low-differentiated trabecular-acinar hepatocarcinomas (tumors of mixed structure; Fig. 2). In group 2 mice of the same age (preventive CP) leukocytic infiltration of tumors was detected in spontaneous hepatocarcinomas (Fig. 3, *a*). Manifest destructive signs were found in tumor tissue. Group 3 mice (CP therapy) also had trabecular-acinar hepatocarcinomas. A tumor site (Fig. 3, *b*) was infiltrated by leukocytes, presumably, mainly by lymphocytes forming groups and cords. Tumor tissue in these cases also had signs of destruction.

It is noteworthy that lymphocyte migration to the lymph nodes can be a positive prognostic factor for the tumor process [14]. Immunity effectors (activated lymphocytes) form contacts with target cells (perforating the tumor cell membranes and injecting lymphotoxins, oxygen intermediates, nitric oxide, and other toxic agents into these cells), which can become the critical factor eventually leading to tumor cell death [15]. As a result of CP treatment according to various protocols, the morphology of tumor nodules with signs of, most likely, lymphocytic infiltration and destruction was associated with higher expression of leukocytic integrins and suppression of hepatocarcinogenesis process in CBA mice: lesser incidence, number, and size of tumors. No leukocytic infiltration of the tumors was observed in spontaneous hepatocarcinogenesis (control group). This was associated with lower expression of leukocytic integrins, high incidence of tumors, their appreciable number and size.

The somatic status and life span of mice liable to cancer treated by CP remain to be studied.

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