METHODS

New Experimental Model of Multiple Myeloma

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 131, No. 6, pp. 717-720, June, 2001
Original article submitted January 12, 2001

NSO/1 (P3×63Ay 8Ut) and SP20 myeloma cells were inoculated to BALB/c OlaHsd mice. NSO/1 cells allowed adequate stage-by-stage monitoring of tumor development. The adequacy of this model was confirmed in experiments with conventional cytostatics: prospidium and cytarabine caused necrosis of tumor cells and reduced animal mortality.

Key Words: *multiple myeloma; experimental model; antineoplastic chemotherapy*

Antineoplastic chemotherapy is effectively used for many decades [3]. Many tumor models in experimental animals were described and studied: human tumors transplanted to immunodefficient mice, syngenic tumors, and spontaneous tumors. Etiology and pathogenesis of multiple myeloma were described in many publications. Most studies were carried out on murine syngenic tumors [8]. However, experimental data obtained on this model were not related to the stages of tumorigenesis, though this could provide useful information. The stage of tumorigenesis can be determined as a period of tumor development characterized by a number of specific clinical symptoms. Thus, elaboration of murine models of tumor process (in particular, syngeneic tumors) for the analysis of the efficiency of some antineoplastic drugs should be based on certain clinical stages of tumorigenesis. The stage of tumor process (in particular, of multiple myeloma) largely determines the results of correction. However, modern experimental system reproducing *in vitro* and *in vivo* myeloma growth and its clinical manifestations are imperfect, which decelerates the realization of new trends in chemotherapy.

A tendency to elaboration of more adequate animal models for the analysis various aspects of myeloma growth and effects of antineoplastic drugs on this process now predominates.

Myeloma cells transplanted to genetically identical mice are stable and retain their tumorigenicity and antibody producing capacity during many generations. Long-term culturing yields variants with impaired immunoglobulin production, which was used for selection of hybrid cell lines, in particular, P3× 63Ag8.653. Sp2/0-Ag14 and P3×63Ag8.NS0/1 lines. Sp2/0-Ag14 myeloma is a derivative of Sp2/HL myeloma produced by fusion of normal sheep erythrocyte-reactive splenocytes and P3×63Ag8 myeloma cells obtained from P3K cells. These cells do not synthesize immunoglobulines, are resistant to 8-azaguanine, and die in a selective medium containing hypoxanthine, aminopterin, and thymidine [7]. NS0/1 myeloma line is a subclone of P3/NS1/1-Ag4.1 (NS1) line also derived from P3×63Ag8 cells [4] and possessing selective characteristics of Sp2/-Ag14 line.

MATERIALS AND METHODS

Pathogen-free BALB/c OlaHsd mice weighing 18-20 g with transplanted NSO/1 (P3×63Ay 8Ut) and SP20 myeloma cells [4,9] were obtained from the vivarium of the Center of Preclinical Testing (Institute of Bioorganic Chemistry).

In experiments with inoculation of myeloma cells 46 mice were used, 21 and 18 animals were treated by

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0007-4888/01/1316-0609$25.00

prospidium and cytarabine, respectively, control group consisted of 20 animals. All manipulations were performed under aseptic conditions. The mice were sacrificed by cervical dislocation. Some animals died due to the development of neoplastic process.

NSO/1 and SP20 myeloma cells cultured in RPMI 1640 with 10% FCS at 37°C in the presence of 5% CO_2 were collected, washed in isotonic phosphate buffered saline (PBS), and inoculated to experimental mice (2.5×10^6 cells/mouse, in 0.5 ml PBS) [6]. Ten days before transplantation the animals received a single intraperitoneal injection of pristane (Sigma, 0.5 ml/mouse). The stage of the disease was determined according to TMN tumor classification elaborated by WHO for animals [1]. The stage of tumor process was determined during autopsy. The specimens were obtained on days 11 ($T_1N_0M_0$), 17 ($T_3N_2M_0$), 30 ($T_4N_3M_1$), and 50 (treatment by cytostatics) after implantation of tumor cell and analyzed using routine histological and histochemical methods [5].

Cytostatics cytarabine (3.6 mg/mouse, intraperitoneally) and prospidium (4 mg/mouse, into retroorbital sinus) were injected 3 h after tumor implantation.

RESULTS

The choice of myeloma cell line is a crucial conditions for examination of stage-by-stage development of multiple myeloma on a murine model. Comparative analysis of the development of tumor processes triggered by SP20 and NSO cells (Fig. 1) shows that NSO line is preferable by rate of neoplastic process and animals mortality. Our observations allow to refer hemoblastoma developed in mice to mixed tumors characterized by ascitic and predominantly solid growth. Primary tumor node was located in the site of myeloma cell injection between the muscular layer and mesothelium of the anterior abdominal wall. Tumor tissue infiltrated the muscular layer of the abdominal wall and penetrated into the abdominal cavity. Its parenchyma was presented by atypical lymphocyte-like cells with numerous mitoses (Fig. 2, *a*). Abnormal mitoses were often seen. The stroma consisted of fibrous connective tissue with few capillaries. The primary node grew and fused with the secondary nodes forming massive agglomerates. When the tumor attained a critical size, necrotic zones with signs of calcification and cyst formation appeared in the central part. The animals died from liver and kidney insufficiency at stage IV. In the liver, dilation of the portal veins and degeneration of hepatocytes were observed. These changes were most pronounced in the periportal zones. In the kidneys, pronounced degeneration of the parenchyma, necrosis of renal tubular epithelium, and focal amyloidosis of renal corpuscles typical for multiple myeloma were observed.

Cytostatic treatment inhibited the growth of hemoblastoma (NSO-myeloma) and considerably suppressed mitotic activity in tumor tissue in mice with transplanted myeloma. The effect of prospidium was more pronounced, while cytarabine was less effective (Fig. 3). It should be noted that single injection of these drugs 2-3-fold prolonged the lifespan in experimental mice (compared to controls), which agrees with published data [2]. The cells of the tumor node were more monomorphic. The central and peripheral zones of the tumor nodes contained large necrotic areas (Fig. 2, *b*) with cystic cavities filled with cell detritus.

Thus, our study presents a new version of multiple myeloma model on pathogen-free BALB/c OlaHsd mice

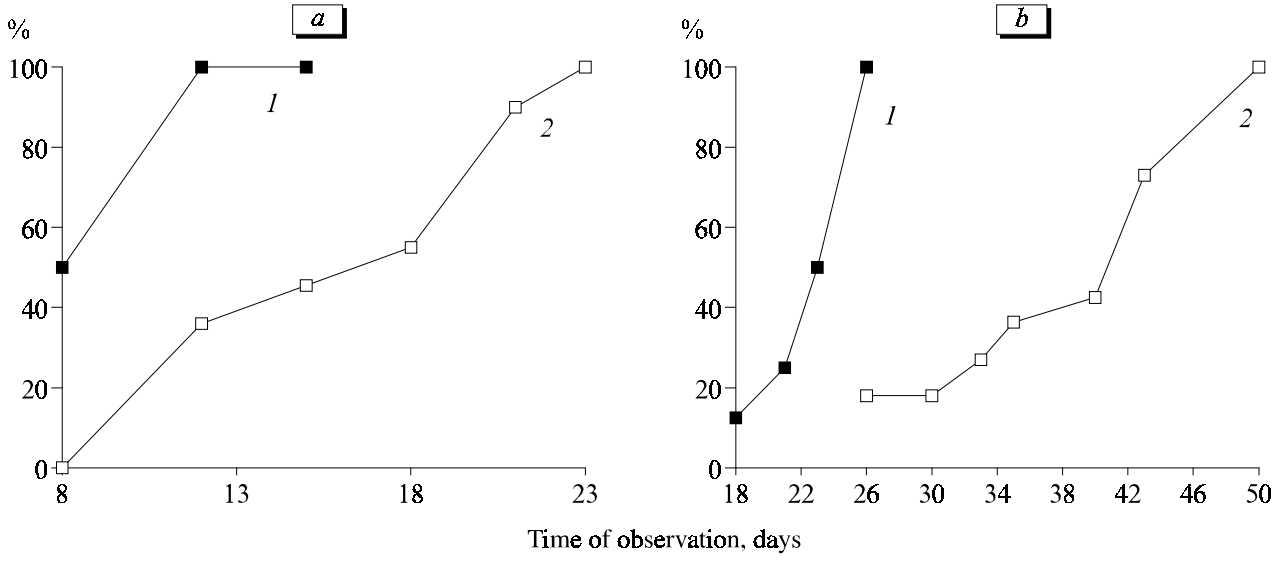

Fig. 1. Comparative analysis of tumor process, tumorigenicity (*a*) and mortality (*b*), induced by SP20 (*1*) and NSO (*2*) myeloma cell lines.

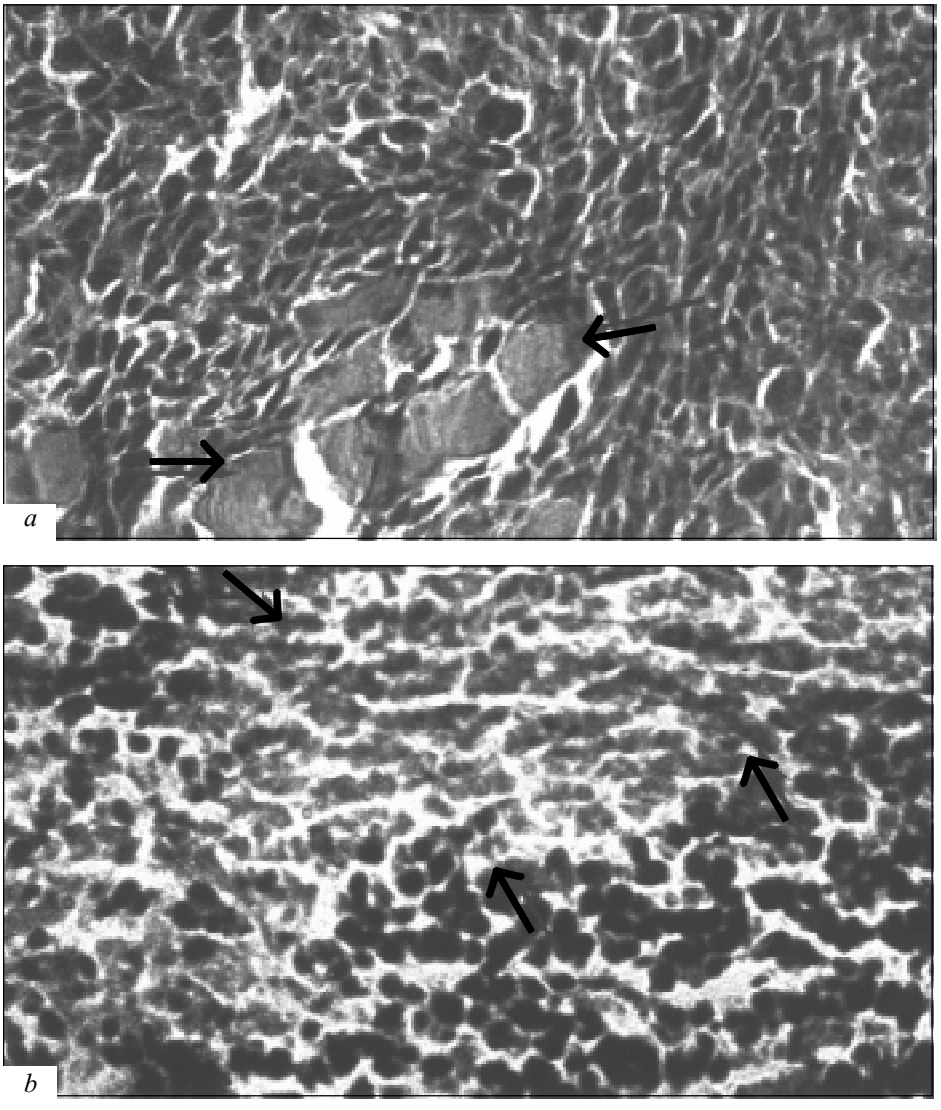

Fig. 2. Histological sections of experimental myeloma. Hematoxylin and eosin staining, ×350. *a*) penetration of tumor cells into the muscular layer (arrows) of the anterior abdominal wall; *b*) necrosis of tumor cells (arrows) in mice treated with cytostatics.

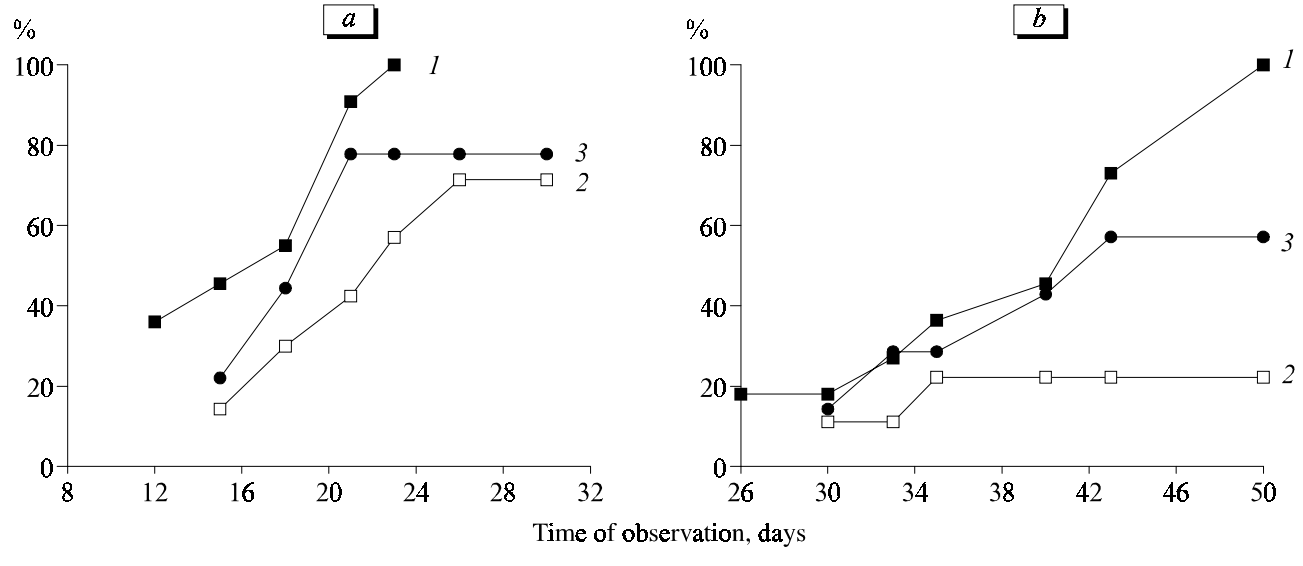

Fig. 3. Effect of cytoctatics on tumorigenicity (*a*) of NSO/1 myeloma and animal mortality (*b*). *1*) control, *2*) cytarabine, *3*) prospidium.

with transplanted NSO/1 (P3×63Ay 8Ut) myeloma. The advantage of this model is the possibility of studying certain clinical stages of tumor process. The experiments with routinely used cytostatics confirm the adequacy of this model as the test system for evaluation of antineoplastic activity of chemotherapeutic drugs.

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