EXPERIMENTAL STUDY OF THE EFFECT OF PROSPIDINE ON TUMOR CELL ULTRASTRUCTURE

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KEY WORDS: prospidine; tumor cells; experiment.

The original Soviet antitumor agent prospidine — N,N"'-di(γ-chloro-β-hydroxypropyl)-N',N" -dispirotripiperazine dichloride — researched at the S. Ordzhonikidze All-Union Pharmaceutical Chemical Research Institute, has been used in clinical oncology for the treatment of various malignant neoplasms [5]. Its molecule contains a γ-chloro-β-hydroxypropyl group, which can alkylate biological substrates. However, investigations have shown that prospidine differs from other known antitumor drugs belonging to the group of alkylating agents both in its pharmacological properties (low toxicity, wide therapeutic latitude, absence of inhibition of hematopoiesis) and also in its effect on many intracellular processes (cell cycle, DNA synthesis, glycolysis and respiration, effect on plasma membranes, and so on) [2-6]. It will be evident that prospidine has a unique mechanism of antitumor action, which is not yet sufficiently clear.

It was accordingly decided to study the effect of prospidine on tumor cell ultrastructure in order to determine the character and order of changes in the subcellular organelles, for this could make a useful contribution to our understanding of its mechanism of action.

EXPERIMENTAL METHOD

Transplantable rat sarcoma 45, with average sensitivity to prospidine, was used as the test object. The compound was injected intraperitoneally on the 6th-7th day after subcutaneous inoculation of a suspension of sarcoma 45 cells. The rats were decapitated at an appropriate time.

Two series of experiments were carried out, using the following doses of prospidine: 1) 1000 mg/kg once, the maximal tolerated dose (MTD), leading to inhibition of tumor growth by 50-60%; 2) 145 mg/kg daily for 10 days — the therapeutic dose causing inhibition of tumor growth by 60-70%.

When MTD was given, tissue for investigation was taken 3, 6, 12, 24, 38, 72, 96, and 120 h after injection, and when a repeated therapeutic dose was given, pieces of tumor were fixed 24 h after each injection and 48 h after the end of the course of treatment. At each time material was taken from at least four animals.

Pieces of tumor tissue 1 mm^3 in volume were taken from the periphery of the sarcomatous nodule in order to avoid choosing foci of spontaneous necrosis. The tissue was fixed in 2.5% glutaraldehyde solution and then postfixed in 1% OsO_4 solution in phosphate buffer. After dehydration in ethanol the pieces of tumor tissue were embedded in Araldite. Sections 0.5-1.0 μ thick were first cut from each embedded fragment, stained with methylene blue, and examined in the light microscope before ultrathin sections were cut. Ultrathin sections were obtained on an LKB ultramicrotome. Sections stained with uranyl acetate and lead citrate were examined in the IEM-7 or IEM-100B electron microscope.

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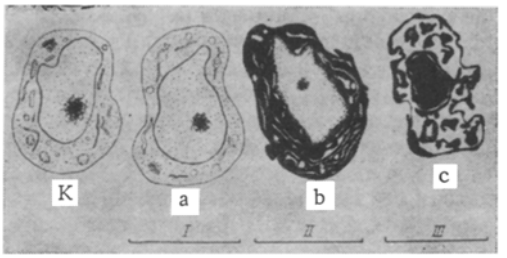

Fig. 1. Changes in tumor cell ultrastructure under the influence of prospidine. K) Intact tumor cell, a) reactive change in tumor cell, b) vacuolation of mitochondria and endoplasmic reticulum, osmiophilia, changes in nuclear structures of cell; c) destruction of tumor cell. I) Period from 12 to 48 h after injection of MTD and after 3-5 injections of therapeutic dose; II) period from 72 to 96 h after injection of MTD and after 6-8 injections of therapeutic dose; III) 120 h after injection of MTD and 48 h after end of course of therapeutic doses.

EXPERIMENTAL RESULTS

The electron-microscopic investigation showed that intact sarcoma 45 tissue is polymorphic in its cell composition. The tightly packed cells differ from one another in shape, electron density of their nucleo- and cytoplasmic matrix, and the distinctness of their intracellular organoids. Most cells in the peripheral zone of the tumor were characterized by the average electron density of the ground substance of their cytoplasm. The nucleus, with diffusely distributed chromatin and a well-developed nucleolus, occupied a large part of the cell. Slightly swollen mitochondria and many free ribosomes were present in the cytoplasm of the cells. The rough endoplasmic reticulum and intracellular lamellar complex were relatively normal in structure. Lipid inclusions and lysosomes, incidentally, were almost completely absent in intact sarcoma 45 cells.

In the early periods after injection of prospidine (3 and 6 h in the case of MTD and 24 h after the first two injections of a therapeutic dose) the cell ultrastructure was the same as in the control. In periods from 12 to 48 h after injection of MTD and after 3-5 injections of the repeated therapeutic doses, reactive changes were beginning in the cytoplasmic components of most cells in particular, swelling of the mitochondria, partial reduction of the cristae, and translucency of the matrix, as well as some dilation of the channels of the endoplasmic reticulum (Fig. 1a). Later these ultrastructural changes became more marked and sometimes progressed to advanced forms of cell damage. From 72 to 96 h after injection of MTD and after 6-8 injections of therapeutic doses, a process of vacuolation of the cytoplasm took place in most cells of the zone of the tumor studied, on account of channels of the rough endoplasmic reticulum. Vacuolation was combined with increased osmiophilia of the ground substance of the cytoplasm and destruction of the mitochondria. In the later stages vacuolation of the intracellular membranous structures of the cells became more advanced. The cavities of the endoplasmic reticulum became so dilated that at times they formed curious patterns composed of whole complexes of membranous structures (Figs. 1b and 2). By that time the nuclear structures of the cells also were modified (changes in the shape of the nuclei, widening of the perinuclear cistern, distribution of chromatin at the periphery of the nucleus, predominance of the fibrillary component over the granular in the nucleoli). Lipid inclusions and lysosomes appeared in the cytoplasm of the cells. Most cells in the peripheral zone of the tumor were disintegrating 120 h after injection of MTD and 48 h after the end of the course of therapeutic injections (Figs. 1c and 3).

The changes in cell ultrastructure described above thus reflect the ability of the compound to act both on the energy metabolism (effect on the mitochondria) and on the protein-synthesizing apparatus of the tumor cell (effect on the rough endoplasmic reticulum), with the ultimate loss of viability of most sarcoma 45 cells. Meanwhile changes in the cells ac-

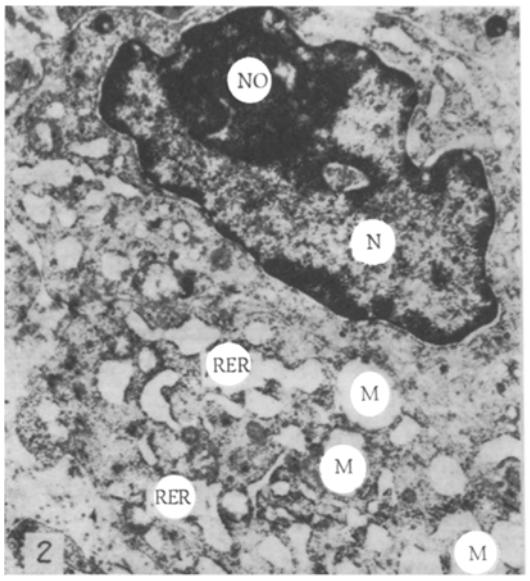

Fig. 2

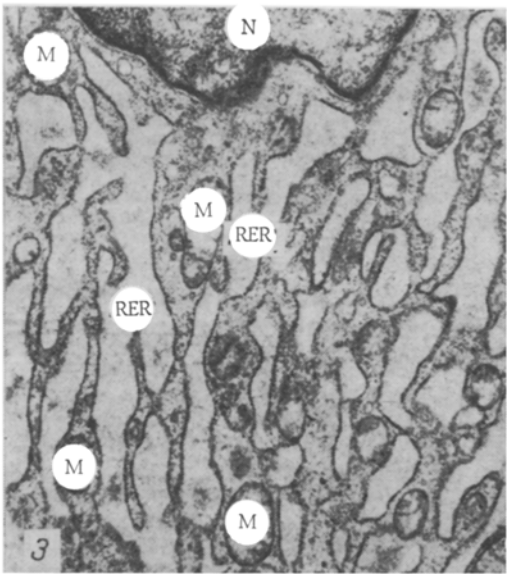

Fig. 3

Fig. 2. Changes in ultrastructure of sarcoma 45 cell 72 h after injection of prospidine in a dose of 1000 mg/kg. Magnification 10,500 ×. Here and in Fig. 3: N) nucleus, NO) nucleolus, M) mitochondrion, RER) rough endoplasmic reticulum.

Fig. 3. Changes in ultrastructure of sarcoma 45 cells after 10 injections of prospidine in a dose of 145 mg/kg. Magnification 16,500 ×.

companied by vacuolation of the rough endoplasmic reticulum, in our opinion, reflect the specificity of the action of prospidine on tumor cell ultrastructure. This type of change of submicroscopic morphology is not characteristic of any antitumor agent previously studied from this point of view, although similar changes in cells are found after exposure to certain other harmful factors [9, 10]. As we know from the literature [3, 8], prospidine selectively acts on the plasma membrane of tumor cells. Bearing in mind that the complex of intracellular membranes is a single system, including in its composition the rough and smooth reticulum, connected by anastomoses with the perinuclear cistern, and surrounding the cell with a membrane, a disturbance of the permeability or any other change in the properties of the plasma membrane must involve structural changes and deformation of the intracellular membrane systems performing vitally important functions in the cell. As a result of disturbance of these functions, general homeostasis of the cell is altered, and this leads to a decrease in the synthetic capacity of the nucleus. This is expressed morphologically as edema, a redistribution of chromatin, and reduction of the granular component of the nucleolus [1, 7]. The direct effect of prospidine on nuclear ultrastructures seems to be less likely, for they begin to be damaged in the late stages of exposure.

Data in the literature on unusual features of the mechanism of action of prospidine are thus reflected in the effect of this compound on tumor cell ultrastructure. The results of the present experiments confirm the view that prospidine has an original mechanism of action and, in particular, that membranous structures participate in it.

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CELL DIVISION AND CHANGES IN THE DURATION OF MITOSIS IN EHRLICH'S ASCITES MOUSE CARCINOMA AFTER SINGLE EXPOSURE TO CHALONE-CONTAINING EXTRACT FROM THIS TUMOR

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KEY WORDS: chalone-containing extract; Ehrlich's ascites carcinoma; mitosis.

Despite many investigations of the action of chalones on cell reproduction [2, 7, 11], the time course of the changes in mitotic activity of the cells under the influence of these substances has received little study. Yet their study is essential for a deeper understanding of the action of chalones on cell division.

The object of this investigation was to study the time course of cell division and the duration of mitosis in an Ehrlich's ascites carcinoma of mice after a single injection of chalone-containing extract from this tumor.

EXPERIMENTAL METHOD

Experiments were carried out on male noninbred albino mice weighing 18-20 g aged 1.5-2 months. A diploid strain of Ehrlich's ascites carcinoma (EAC), obtained at the Institute of Experimental and Clinical Oncology, Academy of Medical Sciences of the USSR, was inoculated by intraperitoneal injection of ascites fluid containing 10^7 cells every 7 days. Animals with a 5-day-old tumor were used in the experiments. The chalone-containing extract (CCE) from EAC was obtained from mice with a 13-day-old tumor by the method of Savchenko et al. [5] The dose of CCE injected was 10 mg per mouse. Colchicine was injected into the animals in a dose of 1 μg/g body weight. Altogether there were four experiments. The CCE was always injected at 1 p.m. Control animals were given an injection of physiological saline.

In the first two experiments the time course of mitotic activity (MA) of the EAC cells was studied after a single injection of CCE into the mice. In the experiments of series I the control and experimental animals were killed 5, 9, 13, 17, 21, and 25 h, and in series II 5, 8, 10, 12, 15, 17, 20, 22, and 24 h after injection of CCE. In the experiments of series III the animals were given an injection of CCE or physiological saline, and, before each animal was sacrificed, 5, 10, 15, 19, and 23 h after the injection, it was given an injection of colchicine in order to study the dynamics of colchicine (C) mitoses. In the experiments of series IV changes in the duration of mitosis were studied in the tumor cells after injection of CCE. The animals were divided into four groups: Groups 1 and 2 were controls, groups 3 and 4 consisted of experimental mice. The animals of groups 1 and 3 were given an injection of 0.2 ml of colchicine solution and the mice of groups 2 and 4 received an injection of the same volume of physiological saline 4 h before sacrifice. The mice were killed 5, 9, and 13 h after the beginning of the experiment. Films were made from the ascites fluid taken from the mice, fixed twice with methyl alcohol, and then stained with methylene blue. To estimate the inhibitory action of CCE on mitosis, the mitotic index (MI) and index of C mitoses were calculated per 5000 cells in each preparation and expressed in promille. The duration of mitosis was determined by the equation:

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