DETECTION OF TUMOR CELLS IN THE BLOOD STREAM

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UDC 616-006-033.2:611.018.5]-07:616.15-008.854.6-07

The effectiveness of trypan blue, acridine orange, tetracycline, and oxytetracycline for the detection of tumor cells injected into the bloodstream was investigated in experiments on rats. Cells were detected in the microvessels of the mesentery by intravital microscopy. Luminescence of the fluorochromed cells was observed in blue-violet (λ_{max} 400 nm) and ultraviolet (λ_{max} 365 nm) rays of a mercury vapor lamp and in laser radiation (λ 337 nm). The intensity of luminescence of cells stained with acridine orange was higher, and their structure could be distinguished better, than in cells containing tetracyclines. Identification of the cells by trypan blue was difficult. The luminescence method is sufficiently simple, and it can be used to discover neoplastic cells actually in the bloodstream.

KEY WORDS: tumor cells; luminescence; laser; tetracyclines; acridine orange.

An important place in the development of a metastasis is occupied by the stage of settling and survival of the tumor cells giving rise to growth of the new tumor node [5, 15, 16]. In the investigation of cancer cells circulating in the bloodstream, the problem of distinguishing them from normal blood cells remains acute [9-11]. Luminescence methods for the detection of tumor cells have been extensively used [2, 6, 13]. By means of acridine orange they can be detected by vital fluorochroming [4]. The property of luminescence in UV light is also possessed by a number of therapeutic substances (tetracyclines and certain carcinostatic agents [8]). The tetracyclines accumulate in tumor tissue and remain in it for a long time [12]. To excite luminescence in biological investigations mercury vapor lamps and lasers are used [14].

The object of this investigation was to assess the effectiveness of various dyes and sources of excitation of luminescence for the identification of tumor cells in microvessels of rats by intravital microscopy.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats weighing 180-200 g. The ML-2 luminescence microscope, equipped with a special heated stage for intravital microscopy of the rat mesentery, was used. Luminescence of the fluorochromed cells was studied in the blue-violet region of the spectrum (FS-1 filter, λ_{max} 400 nm) and in the ultraviolet region (UFS-3 filter, λ_{max} 365 nm), as well as in laser radiation. The LG1-21 molecular nitrogen laser (λ 337 nm, pulse duration 10 nsec), with a mean output power of 1.4 to 2 mW, emits pulses at frequencies of 10, 25, 50, and 100/sec.

Ascites fluid containing Zajdela's hepatoma cells was treated with heparin (50 i.u./ml) and the appropriate dye (to 1 ml fluid) – trypan blue (1 ml of 0.1% solution), tetracycline or oxytetracycline chloride (1000 units), or acridine orange (0.5 ml in a dilution of 1 : 10,000), and incubated for 30 min at 38° C, after which it was centrifuged for 1 min at 1000 rpm. Samples with acridine orange were centrifuged without incubation. The cell residue was diluted with Earle's solution to a volume corresponding to that of the ascites fluid taken originally. The viability of the cells in the suspension was tested by intraperitoneal injection of 0.5 ml of a suspension into control rats.

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Laboratory of General Pathology and Experimental Therapy, Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 80, No. 7, pp. 79-82, July, 1975. Original article submitted October 28, 1974.

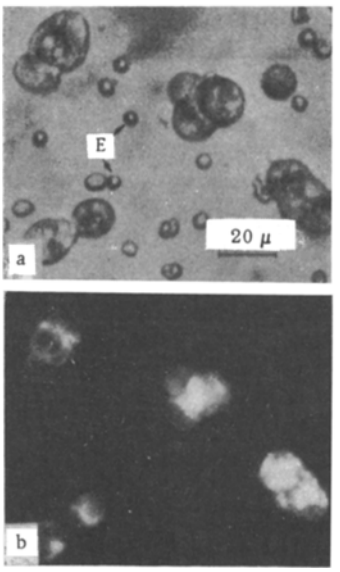

Fig. 1

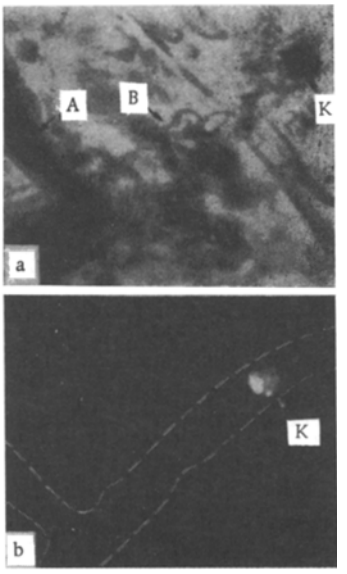

Fig. 2

Fig. 1. Zajdela's ascites hepatoma cell in a suspension (100 ×) a) ordinary light microscope; b) luminescence microscope (acridine orange); E) erythrocytes.

Fig. 2. Zajdela's ascites hepatoma cell (K) settling in arteriole of rat mesentery (100 ×): a) light microscope; b) luminescence microscope (acridine orange). Blood flow maintained in area A, stasis in area B. Broken lines in Fig. 2b indicate line of lumen of arteriole.

The anesthetized rat (urethane 1 g/kg body weight, intramuscularly) was placed on the heated stage of the microscope. A loop of small intestine was brought out through an incision in the abdominal wall so that the mesentery lay on the light guide. A suspension of stained tumor cells was injected from a microinjector into the carotid artery through a cannula. The dimensions of the cells and microvessels were determined by the image-splitting method [1]. The results were displayed by a video recorder [7].

EXPERIMENTAL RESULTS

Living tumor cells in the suspension, stained with trypan blue, had a bluish hue and their outlines and structural details were more clearly visible than in unstained cells (Fig. 1a). The cytoplasm of cells stained with acridine orange gave green luminescence in UV rays and orange-red inclusions could be seen in it. The nuclei of most tumor cells were bright green, but a few were yellow or orange. Leukocytes present in the suspension gave green fluorescence and their nuclei were clearly visible. The erythrocytes did not fluoresce. When the cells were irradiated with blue-violet light the pattern of luminescence was brighter (Fig. 1b). The cytoplasm of the tumor cells stained with tetracycline or oxytetracycline gave a diffuse bluish-green luminescence in UV and blue-violet light, against the background of which the nucleus appeared the same color but brighter. Granules similar in the character of their fluorescence to the substance of the nuclei could be seen in the cytoplasm of some cells. In cells in a state of mitosis bright fluorescence was observed in the zone of division. No difference was observed in the character of luminescence of tumor cells and leukocytes containing tetracyclines. Cells stained with the tetracyclines emitted luminescence faster than those stained with acridine orange. The intensity of luminescence after staining with tetracyclines was lower than when acridine orange was used, and the structural details were less clearly seen.

After the introduction of 0.1-0.3 ml of a suspension of fluorochromed tumor cells (about $5 \cdot 10^5$ cells) into the bloodstream of the rat, they appeared in the vessels of the mesentery. Under the luminescence microscope brightly shining cells could be seen against the dark background, moving rapidly along with the bloodstream. Clumps of three to five cells were held up in vessels 15-20 μ in dimaeter, causing stasis or to-and-fro movement of the blood. Some of these cell emboli (on the average one of five) were broken up by the thrust of the blood into single cells and the blood flow was restored, carrying them out of sight. Individual tumor cells were rarely held up (Fig. 1), even in small vessels, but changed their shape as they

passed along the capillaries or found a way out through the arteriolo–venular shunts. The behavior of tumor cells, leukocytes, and platelets settling in the blood vessel and the state of the surrounding tissues could be observed by changing to the ordinary light microscope.

During observation in transmitted light it was difficult to determine the outlines of the cell while in the blood vessel (and, consequently, to determine its size) exactly because of the identical optical densities of the cell and the surrounding tissues. By luminescence microscopy the outlines of a single cell could be clearly distinguished, changes in them could be studied, and measurements made. The diameters of cells settling in the microvessels of the mesentery were from 15 to 27 μ.

During exposure to the laser both fluorochromed and unstained tumor cells gave weak greenish-blue luminescence. The cytoplasmic inclusions detected during irradiation with blue-violet and UV light (λ_{max} 365 nm) acquired a green color. In the tissues of the mesentery the laser also excited a greenish-blue luminescence, which made it difficult to identify the injected cells among the surrounding tissues. This was evidently because of the considerable difference between the maxima of the absorption spectra of acridine orange (λ_{max} 475-504 nm [3]), tetracycline, and oxytetracycline (λ_{max} 390 nm [8]) and the wavelength of the radiation of the laser used. In both cases (stained and unstained cells) autofluorescence and not induced fluorescence was therefore observed. An increase in the output power of the laser or in the frequency of the pulses in order to obtain brighter luminescence led to destruction of the walls of the microvessels.

Of the dyes used to detect tumor cells and to reveal their structure, acridine orange was the most effective. Trypan blue did not give sufficient staining for identification of tumor cells in the mass of blood. The life spans of the animals after intraperitoneal injection of suspensions of stained hepatoma cells was 2-3 days longer than those of animals injected with intact ascites fluid. This points to preservation of the viability of the main mass of stained cells.

The luminescence method of detection of tumor cells is simple and can locate tumor cells directly in the bloodstream. This method can be used to study the role of disturbances of the microcirculation in metastasization, and in investigations of pathways of spread of cells and the mechanisms of their survival and destruction within the vessels.

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