EFFECT OF INJURY TO THE MICROVESSELS ON THE FREQUENCY OF TUMOR CELL LODGMENT IN THEM

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The action of local laser injury to the microvessels on the frequency of lodgment of tumor cells and on the ability of the cells to migrate from the lumen of the vessel was studied. A television microscope with video tape recorder and UV-laser were used. Cells of a Zajdela's ascites hepatoma were injected into the blood stream of a rat. Lodgment of tumor cells in the mesenteric vessels was observed infrequently (11%). The lodged cells adhered to the vessel wall and migrated from its lumen. Injury to the vessel wall by the laser beam led to the formation of microthrombi and to an increase in the frequency of lodging of the tumor cells in the vessels: 82% instead of 11%. The frequency of migration of cells from the lumen of the vessel under these circumstances, however, was reduced. The results confirm the role of microthrombus formation in metastasization; however, the role of microthrombus formation may be exerted in different directions for although the frequency of adhesion of the tumor cells is increased, their mobility is reduced for a few a few hours after lodging in the microvessels.

KEY WORDS: tumor cells; metastasization; microvessels; laser.

An important, but as yet inadequately studied stage of hematogenous tumor metastasization is the stage connected with lodgment of tumor cells in the microvessels and adhesion of these cells to the vessel wall.

Metastases are known to be formed more often through the lodgment of aggregates of cells rather than of single cells. Platelets participate in the aggregation of tumor cells [1]. Agglutination of platelets with each other and also their adhesion to the vessel wall lead to injury of the endothelial cells lining the walls of blood vessels [12, 13].

The fact that the frequency of lodgment of tumor cells is increased and the process of metastasization is intensified after various injuries to the tumor-bearing organism (mechanical and chemical trauma, stress, etc.) is well known [6, 8]. An important role in this state of affairs is played by microthrombus formation, a result of the inflicted injury [14].

In the search for data on the mechanism of adhesion of tumor cells to the wall of the microvessel, the writers studied the effect of local injury to the endothelium of these vessels on the process of lodgment of tumor cells in them and the participation of platelets in this process.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats weighing about 200 g. The anesthetized rat (urethane, 1 g/kg, intramuscularly) was placed on a thermostatically controlled stage of a microscope, a loop of small intestine was mobilized at operation and its mesentery was placed on the light guide of an intravital microscope. To prevent the mesentery from drying it was covered with a layer of PMS-500 (polymethylsiloxane) fluid. The mesenteric microvessels were observed under different magnifications (objective 10×, 58× UV, and 90×).

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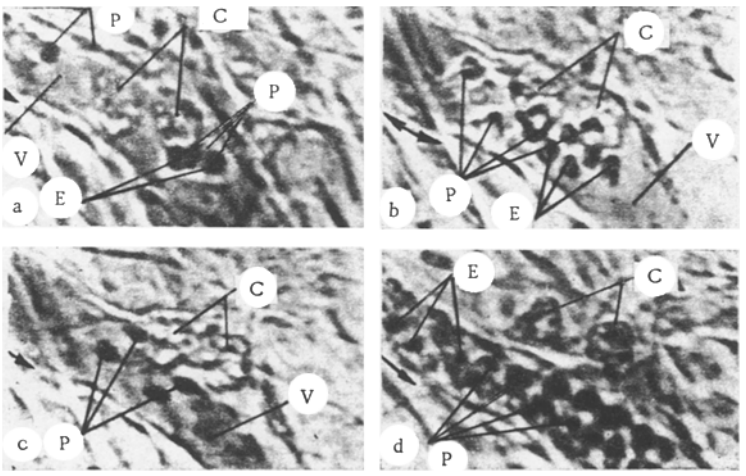

Fig. 1. Videogram of migration of tumor cells (C) (Zajdela's ascites hepatoma) from lumen of venule (V) of mesentery of rat small intestine. Photographs taken from monitor screen during reproduction of video recording: a) 1 h after lodging of tumor cells in vessel, stasis; b) 2 h after lodging, adhesion of tumor cells to venule wall, to-and-fro movement of blood in vessel; c) 3 h after lodging, half of previous number of cells lies outside lumen of venule; d) 4 h 30 min after lodging, tumor cells outside lumen of vessel; blood flow in venule restored. E) erythrocytes; P) platelets. Arrow marks direction of blood flow in vessel. Magnification on screen 3240 ×.

A combination of equipment for intravital microscopic investigation consisting of a television microscope with video tape recorder [4], and also a laser coupled to the microscope, was used. The LGI-21 molecular nitrogen laser with wavelength λ =337 nm and with a mean output power of 2 mW (pulsed output power 1600 W for a pulse duration of 10 nsec) was used. The diameter of the beam was controlled by a special diaphragm.

The test object consisted of cells of a Zajdela's ascites hepatoma,* stained with the luminescent dye Acridine Orange [5]. A suspension of cells was injected by means of a microinjector through a cannula into the rat's carotid artery. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

After injection of 0.1-0.3 ml of the suspension of tumor cells into the animal's blood stream the cells appeared in the mesenteric blood vessels. With luminescent illumination, the shining cells could be seen against the dark background, as they moved along with the blood flow. After a single injection of the suspension and the passage of labeled cells along the mesenteric vessels, as a rule they did not reappear in the field of vision of the microscope. Lodgment of the tumor cells was very rarely observed under these circumstances. Even relatively large cells (16-32 μ in diameter) passed through the capillaries, changing their shape or finding a way out through arteriolo-venular shunts. Cells held up in the microvessels most frequently were jerked along the capillaries by the blood flow into the venules, after which they were carried from the field of vision. Under these circumstances the diameter of the capillaries was increased by 1.5-2 times. Of 84 hepatoma cells which passed along the vessels examined in different experiments, only 9 cells lodged finally in them.

Lodgment of the cells took place in two main ways. The first was mechanical blocking of the microvessel by a cell which was much larger in diameter than the lumen of the vessel. This occurred either in the precapillary arterioles or in the capillaries. In the second method the cells lodged in vessels in which the blood flow had slowed for some reason or other (because of the periodic operation of arterio-venular shunts, closure of the entry into the capillary by the next tumor cell or leukocytes, and so on). This took place in the capillaries and postcapillary venules.

*The tumor strain was obtained from the Laboratory of Experimental Chemotherapy of Tumors, All-Union Pharmaceutical Chemical Research Institute.

TABLE 1. Effect of Injury on Lodging of Cells

Experimental conditions	Number of cells		
	entering vessels studied	lodging in vessels studied	migrating from lumen of vessel
Without injury to microvessels	84	9 (11%)	7 (78%)*
After injury to microvessels by laser beam	65	53 (82%)	3 (6%)*
P		<0,001	<0,001

*Relative to number of lodging cells.

Observations on the behavior of cells lodging in the vessels continued for 4-6 h. During this time 7 of the 9 tumor cells which lodged migrated with different degrees of activity. At the beginning they changed their position relative to the wall of the microvessel, but then they adhered to the wall and gradually migrated from its lumen (Fig. 1). The arresting of the tumor cells in the microvessels was accompanied by disturbances of the blood flow: slowing, to-and-fro motion of the blood, and stasis. After these cells had left the vessel to enter the extravascular space the blood flow in the vessel usually recovered slowly.

Soon after lodging of the tumor cells platelets were observed to settle on the vessel wall at the site of their lodging and also above and below it (relative to the blood flow). Platelets adherent to the walls gradually fused with the endothelium and became indistinguishable from it. Numerous platelets also adhered to erythrocytes in the region of lodging of the tumor cells (Fig. 1).

After laser injury to the wall of the microvessel the platelets moving along with the blood flow toward the site of injury settled above the injured area and some of them remained there to form a juxtamural thrombus, while others were carried away by the blood stream. Diapedesis of leukocytes was observed at the site of injury. The method of injuring the microvessels by the laser beam and the processes accompanying this phenomenon were described by the writers previously [2].

Injuries were inflicted simultaneously at several points of the mesenteric microvascular system. Next, a suspension of hepatoma cells was injected into the carotid artery of the rats and their appearance in the mesenteric blood flow was watched for. If the cells entered an injured vessel, in most cases they were arrested in the injured regions or slightly above them. The frequency of lodging of the tumor cells in the regions of microthrombus formation was high: 53 of 65 cells which passed through the damaged vessels.

A clot of platelets and a few leukocytes quickly formed around the lodged tumor cells. In that case the hepatoma cells either showed no activity whatsoever or their mobility was considerably less than that observed in the experiments without injury to the vessel wall. Of 53 tumor cells which lodged, only 3 migrated outside the vessel.

These experiments showed that injury to the wall of microvessels by laser radiation leads to microthombus formation and increases the frequency of lodging of tumor cells in these vessels: from 11% to 82% ($P < 0.001$) (Table 1).

This increase in the frequency of lodging of tumor cells may be associated with various factors: disturbance of the blood flow through thrombus formation at the site of injury, a local increase in the adhesive properties because of liberation of ADP from the platelets or erythrocytes damaged by the laser beam [3, 10, 13]. Injury to the wall of a blood vessel also causes changes in the electrical potential of the endothelial surface [11] and this contributes to increased aggregation of platelets, leukocytes, and other blood cells and to adhesion of the tumor cells to the vessel wall.

The ability of neoplastic cells to undergo active migration is one of their characteristic qualities [7, 15]. We observed high mobility of Zajdela's ascites hepatoma cells when lodging in unchanged vessels. If these cells lodged in areas of microvessels damaged by the laser radiation, this mobility was sharply reduced. The mobility of the cells may have been limited by a thrombus formed around them and also by the action of substances liberated during injury to the microvessel wall. The low frequency of emigration of the tumor cells in that case was evidently connected with the reduction in their mobility, for the permeability of the vessel wall for cells in the injured area was not reduced, as was shown by diapedesis of leukocytes observable in that situation.

Conversely, after inoculation of tumor cells into the carotid artery of the rats a marked increase in permeability of the mesenteric blood vessels was observed [10].

The increase in the adhesive properties of the platelets after injury to the vessel wall is connected with liberation of ADP. Electron-microscopic investigations have shown that after injury to the endothelium and its desquamation a "pseudoendothelium" consisting of a layer of platelets, temporarily covering the defect in the endothelial lining [9], is quickly formed. Under these circumstances contacts resembling ordinary contacts in

The results of these experiments confirm the role of microthrombus formation in metastasization. However, they also show that the role of microthrombus formation in this process is not always in the same direction, for although the frequency of lodging of the tumor cells increased, during the first few hours after settling both their mobility and the frequency of their immigration into the extravascular tissue decreased. On the other hand, a microthrombus may provide the microenvironment suitable for intravascular growth of tumor cells and the subsequent formation of the metastatic node. Chronic experiments are necessary in order to shed light on this process.

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