STUDY OF THE TISSUE–BLOOD BARRIER IN MALIGNANT BRAIN GLIOMAS

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UDC 616.831-006.484.04-008.6-07

KEY WORDS: brain; malignant glioma; tissue–blood barrier.

Knowledge of the particular features of the tissue–blood barrier in malignant gliomas of the brain is of great importance in connection with the choice of rational chemotherapy of these tumors. Some workers consider that the tissue–blood barrier in gliomas functions effectively, and they see in this one cause of the marked antitumor activity of nitrosoalkylureas, which pass through the blood–brain barrier, in the treatment of gliomas [6, 7, 10]. However, there are clinical observations [4, 8] and some experimental studies [1, 14] whose results do not agree with this view.

It was accordingly decided to study the permeability and ultrastructural features of vessels of malignant gliomas, with particular reference to gliomas of the rat brain.

EXPERIMENTAL METHOD

Experiments were carried out on 45 noninbred rats of both sexes weighing 60-70 g. Strains of transplantable gliomas obtained by adaptation of brain tumors induced by nitrosomethylurea [3] (three strains altogether), obtained by adaptation to intercerebral passage, and glioma C6 [11] were transplanted into the animals' brain. Before the beginning of the experiments the transplanted gliomas had undergone more than 20 passages. Vascular permeability in the course of development of the tumors (1 and 2 h, 1, 2, 5, 7, and 10 days after transplantation and in the terminal stages of development of the neoplasms) were studied by methods of intravenous, intraperitoneal, and intracarotid injection of vital dyes (0.5% trypan blue and 2% Evans' blue) and intracarotid injection of 1.5% horseradish peroxidase (HRP) solution. After injection of the vital dyes the brain was fixed in 10% formalin solution and frozen sections were cut and examined unstained and after staining with lithium carmine. After injection of Evans' blue, unstained sections were examined in the ML-2A luminescence microscope. Fixation and detection of HRP in the frozen sections were carried out by Karnovsky's method [2, 16].

For electron-microscopic study fragments of tumors measuring about 0.5 mm^3 were fixed by the usual method in 2.5% glutaraldehyde solution and 1% OsO_4 solution in 0.1 M cacodylate buffer, pH 7.4, dehydrated, and embedded in a mixture of Epon and Araldite. Sections cut on the LKB-3 untramicrotome were stained with uranyl acetate and lead citrate and examined in the EMV-100L electron microscope.

EXPERIMENTAL RESULTS

Investigation of the accumulation of vital dyes during the development of the gliomas revealed the general rules governing changes in the permeability of the blood vessels of these tumors and the consistency of these changes in different gliomas. Injection of the tumor cells itself did not cause any gross changes in permeability of the brain vessels in the region of injection, either immediately (after 1-2 h) or later (after 1-2 days). Microscopic accumulations of tumor cells, detected in the early stages of development of the tumors, likewise did not accumulate the markers. Changes in permeability of the tumor vessels took place stepwise and were observed after the formation of tumor nodules measuring over 1 mm^3 (Fig. 1a). Injected markers were found in all larger nodules. In the course of growth of the tumors, edema and swelling increased in the perifocal zone, and hydrocephalus

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(Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Romadanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 9, pp. 112-114, September, 1982. Original article submitted March 5, 1982.

0007-4888/82/9409-1295$07.50

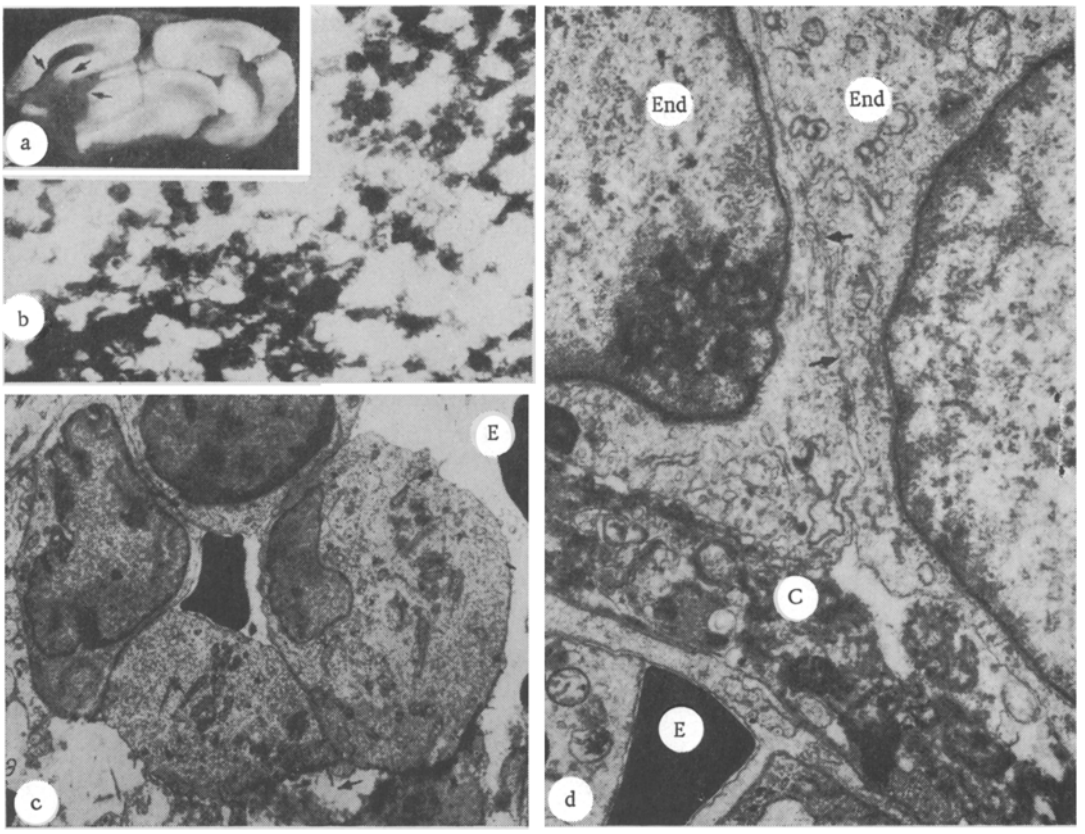

Fig. 1. Permeability and fine structure of microvessels in rat brain gliomas. a) Glioma (arrow) stained with trypan blue, b) injected HRP visible in glioma (peripheral area). Reaction with diaminobenzidine. 400 ×; c) transverse section through capillary of malignant glioma. Only a very small fragment of the loose basement membrane is present (arrow). Erythrocytes (E) can be seen outside the capillary. 3500 ×; d) fragment of capillary wall. Absence of specialized junctions between endotheliocytes (End). Erythrocytes (E) outside capillary (C) lumen between endothelial cells. 18,500 ×.

caused by disturbance of the CSF circulation also was frequently observed. However, despite these marked pathological changes, the brain vessels remained impermeable for the markers, both in areas adjacent to the tumors and some distance away from them, until the experimental animals died. Injection of trypan blue and Evans' blue enabled changes in vascular permeability to be observed macroscopically, but the concentration of these substances in the tumors was too low to allow microscopic study of their localization. The most suitable marker for microscopic investigation proved to be HRP. After the reaction with diaminobenzidine, the brain vessels could be distinguished on the preparations with clear outlines at a distance away from the tumor and also in the perifocal zone, whereas vessels of the tumor were surrounded by accumulations of enzyme and thus had indistinct outlines. Considerable quantities of enzyme were found in the central areas of the neoplasms, in intercellular space and in tumor cells. At the periphery of the tumors and in the zone of infiltrative growth no HRP could be seen in certain areas (Fig. 1b).

The ultrastructure of capillaries of the transplanted gliomas differed significantly from that of typical brain capillaries. The contacting membrane of the endotheliocytes forming the capillary wall in certain areas formed specialized junctions of the type known as tight junctions, but in some cases they were separated by a distance of 200-500 nm (Fig. 1d). Unique channels of communication thus were formed between the capillary lumen and the pericapillary space. The basement membrane in the plane of the ultrathin section was often absent or consisted of fragmented loose amorphous material (Fig. 1c). No structures homologous with the vascular pedicles of the brain gliocytes could be found in the tumors studied. These tumors were characterized by a loose and haphazard arrangement of their cells.

The experiments showed the absence of a complete tissue—blood barrier in malignant gliomas. The unrestricted penetration of high molecular weight substances (HRP and complexes

of dyes with albumin [5, 17]) into the tumor was not due to edema or swelling of the surrounding brain, for it was observed in the early stages of development of the neoplasms, when neither edema nor swelling was present, but instead it was due to the fine structural features of the capillary walls of these tumors. The sudden stepwise change in permeability of the vessels supplying the tumor makes it possible to extend to these neoplasms the hypothesis [12] of "avascular" and "vascular" phases of development of tumors, whereas the submicroscopic structural features of their capillaries confirmed the view that these vessels are formed de novo and are not pathologically changed brain vessels.

The similarity in principle between the submicroscopic structure of the capillaries of transplantable gliomas and of human malignant gliomas, for which the absence of tight junctions between endotheliocytes and changes in the structure of the basement membrane are also characteristic [9, 13, 15], must be emphasized. In this connection the results of this investigation can also be interpreted as evidence of increased permeability of the capillaries of human malignant gliomas compared with the capillaries of the surrounding brain. However, judging from the distribution of HRP in gliomas, the peripheral parts of the tumors, the zone of infiltrative growth, which play an important role in their growth, are supplied to a greater or lesser degree by vessels of the brain itself, which have preserved their barrier function. This must be taken into consideration when a potentially effective chemotherapeutic agent and the mode of its administration are chosen.

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