Quantitative autoradiographic measurements of blood-brain barrier permeability in the rat glioma model

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Quantitative autoradiographic technique was applied in measuring blood-brain barrier (BBB) permeability of autochthonous gliomas in rats. In small tumors (less than 2 mm in diameter), no increase in BBB permeability was noted. As the tumor grew and neovascularization occurred, BBB permeability increased in the center of the tumor, and it was suggested that the BBB was partly disrupted in the neovascularized vessels. In the fully grown tumors, BBB permeability was markedly increased in the viable part of the tumor to levels similar to the choroid plexus. Yet, the BBB was partly preserved at the periphery of the tumor and in the brain adjacent to the tumor. The heterogeneity of the BBB phenomenon according to the stage of tumor growth may be a major obstacle for uptake of chemotherapeutic drugs that do not cross the BBB easily.

The blood-brain barrier (BBB) has been reported to be disrupted in human and experimental brain tumors.1,4,5,7,9,16 Electron microscopic studies indicate that endothelial changes of capillary vessels occur to some extent in malignant brain tumors, and those changes have been related to an increase in BBB permeability. In experimental studies, several methods have been used to detect BBB permeability.7,13-15 Yet, none of these methods have quantified BBB permeability in relation to histological events.

In the present study, an autoradiographic technique was applied for measuring BBB permeability of experimental glioma in rats. This technique has enabled us to visualize BBB changes quantitatively and to correlate them with the histological findings. Since the tumor model used in this experiment was induced without any mechanical injury to the brain, the results may be applicable to clinical situations.

Materials and Methods

Ethynitrosourea (ENU, 50 mg/kg) was injected subcutaneously into newborn Sprague-Dawley rats on the 3rd day after birth. The rats were fed ad libitum and used for experimentation at 150 to 300 days of age.

Experimental Procedure

Five normal Sprague-Dawley rats and 20 ENU-treated rats were used for measurement of BBB permeability. Under ether anesthesia, animals were placed on a wooden plate with their extremities fixed by rubber bands. The right femoral artery and right jugular vein were cannulated with Silastic tubes.* The animals were allowed to recover from anesthesia for at least 1 hour before experiments were started.

The arterial catheter was attached to a transducer, and blood pressure was monitored. Rectal temperature was monitored and kept between 36°C and 37°C by cooling or warming. Arterial blood gases were measured periodically. After those parameters were found to be within normal range, 100 µCi of carbon-14 (¹⁴C alpha aminoisobutyric acid (AIB), 51.2 mCi/mmols)† per kilogram of body weight dissolved in 1

* Silastic tubes manufactured by Dow Corning Corp., Midland, Michigan.
† AIB supplied by New England Nuclear, Boston, Massachusetts.
ml of normal saline was injected at a constant rate for 1 minute through the catheter placed in the jugular vein. Blood samples were taken from the arterial catheter every minute for 10 minutes. Blood samples were placed in microhematocrit tubes, and plasma was separated. Plasma samples of 20 μl were placed in counting vials, suspended in 10 ml of scintillation phosphor, and radioassayed in a liquid scintillation counter. At 10 minutes after injection, animals were sacrificed by the rapid intravenous injection of saturated potassium chloride solution through the catheter in the jugular vein. The brain was rapidly removed, frozen in isopentane suspended in a bath of dry ice, and stored at -40°C.

Serial brain sections 40 μm thick were made by a cryostat at -20°C. The sections were placed on a cover glass and dried on a hot plate at 60°C within a few seconds. The cover glasses were attached to Kodak x-ray film (SB-5), with brain sections facing the emulsion of the film in the dark room. The 14C-methylmethacrylate standard plates were also attached to the x-ray film. These standards were pre-calibrated for their autoradiographic equivalency to the 14C concentrations of brain sections (40 μm thick). After 3 weeks' exposure, the x-ray films were developed. The densitometric measurements of the autoradiographs were made with a densitometer equipped with a 0.5 mm aperture. A calibration curve between optical density and tissue 14C concentration for each film was obtained by densitometric measurements of the standard plates. The representative brain sections were stained with hematoxylin and eosin for histological observation.

Calculation of BBB Permeability

Permeability of BBB was expressed as a unidirectional blood-to-brain transfer constant (ki), which can be calculated according to the following equation:

\[ k_i = \frac{C_b(T)}{\int_0^T C_p \, dt} \]

where \( C_b(T) \) is the concentration of the tracer in the tissue at the end of experimental period T, and \( C_p \) is the plasma concentration of the tracer. The equation indicated above can be applied only when the tracer fulfills the condition in which unidirectional transport of the tracer from brain to blood (back flux) must be negligible during the experimental period (0-T). For this reason, AIB was employed as reported by Blasberg et al., since this nonmetabolized amino acid is avidly taken up and trapped within brain cells by the small neutral amino acid transport mechanism in the cell membrane.

Results

Normal Brain Permeability

Regional BBB permeability in the normal brain tissue is listed in Table 1. Mean value of permeability in the cortex was about double that in the white matter. The choroid plexus, pineal body, tuber cinereum, and area postrema were shown to have high BBB permeability. However, considerable differences in the transfer constant were noted among those tissues (Table 1).

Tumor Capillary Permeability

A total of 45 intracerebral gliomas were identified in 20 ENU-treated rats. They were divided into groups of large, medium, and small tumors according to the size of the tumor. Permeability data are summarized in Table 2. In the small tumors (less than 2 mm in diameter), no increase in capillary permeability was noted. Therefore, autoradiographs could not identify the presence of the tumor (Fig. 1).

In the medium-sized tumors (2 to 4 mm in diameter), permeability began to increase in the center of the tumor. Mean value of the transfer constant in the tumor was significantly higher than that of normal cortex (Table 2). Typical autoradiographic and his-
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FIG. 1. Autoradiographic (left) and histological (right) picture of a small ethynitrosourea-induced tumor. There is no increase of capillary permeability in the tumor.

tological appearances of medium-sized tumor are shown in Fig. 2.

In large tumors (more than 4 mm in diameter), the center of the tumor became cystic or necrotic. Adjacent to the necrosis, an area with markedly increased permeability was identified. Histological examination revealed that numerous viable cells were located in this area. At the periphery, where tumor cells were infiltrating into surrounding brain tissue, there was also an increase in capillary permeability. Typical autoradiographic findings are shown in Fig. 3.

Adjacent Brain Permeability

Permeability of the BBB in the tissue adjacent to large and medium-sized tumors was significantly higher than in normal brain tissue. In the tissue adjacent to large tumors, BBB permeability was about 3.5 times higher than that of normal brain tissue (Table 3). The medium-sized tumors also induced a significant increase in BBB permeability in the adjacent brain tissue. Permeability of the BBB in areas of the brain distant from the tumors was similar to that found in normal rats, and no statistical differences were noted between them.

Discussion

It has been reported that capillary vessels in brain tumors undergo certain structural changes. Long reported that human gliomas and metastatic brain tumors showed changes in the endothelial tight junction of their blood vessels. He suggested that these changes might be the cause of increase in BBB permeability. Similar findings have been reported by Cox, et al., in the experimental glioma induced by ENU. They indicated that the larger the tumor was, the more marked were the vessel changes. These electron microscopic studies have revealed that ultrastructural defects in the capillary vessels may vary depending...
Blood-brain barrier in experimental tumors

FIG. 3. Autoradiographic (left) and histological (right) picture of a large ethylnitrosourea-induced tumor. The viable part of the tumor showed high permeability. At the periphery of the tumor and in brain tissue adjacent to the tumor there was also an increase in permeability. The choroid plexus and tuber cinereum have high permeability. Also note a small tumor (arrow) that exhibits no increase in permeability.

upon the malignancy of the tumor and location of vessels in the tumor. It has been postulated from those studies that the BBB is more complex rather than a simple all-or-none phenomenon. Therefore, the extent of increase in capillary permeability must be measured quantitatively.

In the present report, quantitative autoradiographic techniques are applied to measure BBB permeability in the tumor and adjacent brain tissue, with correlation to histological findings. An AIB autoradiographic technique was originally described by Blasberg, et al., for quantification of BBB permeability. This synthetic amino acid has slow transport across normal blood vessels, but is concentrated to a high level in the brain tissue when BBB permeability increases; in the latter instance, AIB is attracted rapidly to the cells by the same transport mechanism as alanine. However, since this synthetic amino acid is not further metabolized, it remains within the cells, and may be visualized autoradiographically.

The quantitative aspect of the present study correlated well with the results of electron microscopic study. In the small tumors with diameters of less than 2 mm (presumably the early stage of tumor growth), no increase in capillary permeability was noted and it was suggested that the BBB was completely preserved in the tumor. When the tumor became larger than 2 mm, permeability increased in the center of the tumor. Histological and blood flow studies indicated that neovascularization occurred in the tumor when it became larger than approximately 2 mm. This suggests that the BBB was partly disrupted in the newly formed vessels. Then, a marked increase in capillary permeability was evident when the tumor became larger and central necrosis occurred. Histological examination revealed that numerous capillaries were found in the viable part of large tumors. In this area, the BBB phenomenon seemed to be disrupted completely, since the permeability value was almost identical to that of the choroid plexus.

Permeability of brain tissues adjacent to the tumors started to increase when the tumor became larger than around 2 mm in diameter. In the tissue adjacent to large tumors, BBB permeability was about 3.5 times greater than that of normal brain tissue, but was about one-half to one-fifth of that in the viable part of large tumors. Electron microscopic studies revealed that ultrastructural changes in the capillaries were not prominent in the edematous brain tissue adjacent to the tumors. Consequently, it may be suggested that the source of edema fluid in the brain tissue is mainly derived from the tumor vessels where plasma is ultrafiltered. Then, fluid may extend to the surrounding brain tissue by simple diffusion or bulk flow.

In chemotherapy for brain tumors, anti-neoplastic drugs must reach the tumor cells by crossing capillar-

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<td>Regional capillary transfer constant in brain tissue in 20 rats</td>
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<td>Description of Region</td>
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<tr>
<td>adjacent brain tissue</td>
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<td>adjacent to large tumors</td>
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<td>distant &quot;normal&quot; brain tissue</td>
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* Significantly different (p < 0.01) from normal cortex.
† Significantly different (p < 0.01) from normal white matter.
ies in the tumor tissue. Therefore, uptake of chemotherapeutic agents in the tumor is largely dependent on the permeability of blood vessels in the tumor. This is especially so for water-soluble drugs that often have large molecular weights and have difficulty in crossing the BBB. In fully grown tumors, the BBB apparently becomes disrupted in the viable part of the tumor. However, it may be partly preserved at the periphery of the tumor and in the brain adjacent to the tumor. In those areas, there may be a certain limitation for uptake of water-soluble drugs. For this reason, chemotherapy of brain tumors with a single water-soluble agent is not effective. However, the lipid-soluble drugs, which readily cross the BBB, do not solve the problem of treatment of tumor cells infiltrating adjacent brain tissues. Since the uptake of lipid-soluble drugs is primarily dependent on the local blood flow, and as blood flow in the periphery of gliomas is low, lipophilic drugs may not be taken up in sufficient amounts at the periphery of the tumor, and this area might be the site of relapse. Several attempts have been made to increase drug delivery to these less permeable areas of low blood flow. Yet, no definite beneficial response could be obtained in the clinical trials. The present data may provide basic information for these therapeutic trials.

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References


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