Measurement of Cerebral Blood Flow, Cerebral Blood Volume, and Cerebral Capillary Permeability in Glioma-bearing Rats

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Abstract

Effective chemotherapy and radiation therapy of brain tumors require knowledge of the cerebral circulatory dynamics involved. In this study, regional cerebral blood flow (rCBF), regional cerebral blood volume (rCBV), and regional cerebral capillary permeability (rCP) were measured in Wistar King Aptekman rats bearing experimental KEG-1 gliomas. These parameters were assessed by autoradiography with 14C-iodoantipyrine, 14C-deoxyglucose-labeled red blood cells, and 14C-aminoisobutyric acid, respectively. rCBF within the tumor was approximately one third that in the contralateral cortex and was consistently higher in the periphery than in the center of the tumor. In the periphery of the tumor, rCBV was approximately twice that in the contralateral cortex, but it was very low in the center of the tumor. Throughout the tumor, rCP was sharply increased relative to that measured in the contralateral cortex, and the increase was especially pronounced in the central portion. Thus, rCBF, rCBV, and rCP each appeared to vary within the tumor, implying that the combined use of lipid- and water-soluble chemotherapeutic agents is reasonable. Measurement of these parameters may also provide indices of radiation sensitivity.

Key words: brain neoplasms, cerebral blood flow, cerebral blood volume, cerebral capillary permeability, cancer chemotherapy, radiation therapy

Introduction

Because of the limitations in the surgical removal of malignant glioma, radiation therapy, chemotherapy, and immunotherapy are commonly administered as well. Nonetheless, the outcome is unsatisfactory in most cases. This has been explained in terms of poor tumor penetration by antineoplastic agents and insensitivity to radiation due to the anoxia of gliomas. Assessment of the circulatory dynamics and capillary permeability within gliomas is therefore necessary if we are to solve these treatment problems and improve the prognosis.

In the study presented here, we measured regional cerebral blood flow (rCBF), regional cerebral blood volume (rCBV), and regional cerebral capillary permeability (rCP) in a rat glioma model.

Materials and Methods

KEG-1 cells were subcultured in vitro and, with the use of a stereotactic apparatus, injected at 5 × 10⁴ cells/10 µl into the right caudate nucleus of 20 male Wistar King Aptekman rats, weighing about 200 gm. rCBF, rCBV, and rCP were measured in six, three, and four animals, respectively. The remaining seven rats were used for observation of the natural survival course.

Fourteen to 16 days after the implantation of tumor cells, polyethylene catheters were inserted into the femoral artery and vein under anesthesia induced by 1.0-1.5% halothane. The lower half of the body was fixed in a plaster cast and the animal was allowed to recover from anesthesia. Blood pressure and pH were measured and blood gases analyzed immediately prior to autoradiographic assessments. Body temperature was maintained at 37°C with a heat lamp.

rCBF was measured by quantitative autoradiography, with 14C-iodoantipyrine (14C-IAP) as the

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tracer. According to a previously described procedure,27,28,39 25 μCi of 14C-IAP was administered at a constant rate over 1 minute via the femoral vein, and blood was simultaneously drawn from the femoral artery every 4-5 seconds to measure the plasma concentration of 14C-IAP. On completion of 14C-IAP administration, the rats were decapitated and the brains promptly removed, frozen at -40°C in liquid freon. Serial sections 20 μm in thickness were prepared in a -22°C cryostat (Model 855; American Optical Company, Buffalo, N.Y., U.S.A.) and placed on cover glasses. The specimens were fixed on a hot plate at 60°C and placed in an x-ray cassette with 14C-methyl methacrylate standards (Amersham Co., Buckinghamshire, England). They were then tightly attached to single-coated x-ray film (Kodak SB-5; Rochester, N.Y., U.S.A.) and placed in the dark for 5 days. After development, the x-ray film was subjected to densitometry (Sakura Microdensitometer PDM-5; Tokyo, Japan). Radioactivity was calculated from the optical density thus obtained. rCBF was calculated with the formula devised by Sakurada et al.30 The partition coefficient used was 0.8 for both normal and tumor tissues.

rCP was measured by quantitative autoradiography,5,36 with the use of 14C-aminoisobutyric acid (14C-AIB). Femoral arterial blood samples were obtained at predetermined intervals over a 10-minute period after bolus injection of 50 μCi of 14C-AIB into the femoral vein. The blood samples were immediately centrifuged and 20 μl of plasma was used to measure the 14C-AIB concentration. After the last blood sampling, the animals were immediately decapitated and the brains quickly removed. The remainder of the procedure was the same as that for 14C-IAP autoradiography.

Red blood cells labeled with 14C-deoxyglucose (14C-DG) were used to assess rCBV.17 Autologous blood (1.0-1.5 ml) was washed with a phosphate buffer solution (PBS) containing heparin, and the cells were incubated for 30 minutes at 37°C with PBS to which 14C-DG had been added. PBS was re-added, the mixture was centrifuged, and the supernatant was discarded. This washing procedure was repeated at least once. The red blood cells thus labeled with 14C-DG were injected into the rat femoral vein over a period of 1 minute. Ten minutes later, the rats were sacrificed by intravenous injection of a saturated KCl solution. The brain was removed during immersion of the skull in liquid freon at -40°C. rCBV was calculated as follows:

\[
rCBV = (C_b/C_a) \times \alpha
\]

Where Cb represents the radioactivity per 1 gm of brain tissue, Ca the radioactivity per 1 μl of arterial blood, and α the ratio of the cerebral-to-large vessel hematocrit (in this study, the value of 0.83 obtained by Everett et al.16 was used).

All brain specimens were embedded in paraffin after formalin fixation and sections were stained with HE. Sections used for autoradiography were also stained and examined.

Results

The mean survival period of the seven rats that were merely observed was 20.9 ± 2.8 (mean ± SD) days. The animals employed for the study of cerebral circulatory parameters were all alive 14-16 days after implantation, when the experiments were begun. The mean tumor diameter at that time was 5.2 ± 1.3 mm. The blood pressure and pH and blood gas data obtained just prior to the autoradiographic studies are summarized in Table 1.

The results of rCBF, rCBV, and rCP measurements are given in Table 2. Representative autoradiograms are shown in Fig. 1 upper, HE-stained sections of the specimen used for autoradiography are presented in Fig. 1 middle, and the densitometric profiles of the autoradiograms in Fig. 1 lower. The mean cortical rCBF and rCBV were somewhat

![Table 1 Physiological parameters](image)

<table>
<thead>
<tr>
<th>Study group</th>
<th>pH</th>
<th>PaCO₂ (mmHg)</th>
<th>PaO₂ (mmHg)</th>
<th>MABP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rCBF (n = 6)</td>
<td>7.35 ± 0.03</td>
<td>40.3 ± 3.4</td>
<td>84.8 ± 6.9</td>
<td>117 ± 8</td>
</tr>
<tr>
<td>rCBF (n = 3)</td>
<td>7.42 ± 0.02</td>
<td>35.3 ± 4.9</td>
<td>113.9 ± 20.2</td>
<td>120 ± 6</td>
</tr>
<tr>
<td>rCP (n = 4)</td>
<td>7.42 ± 0.08</td>
<td>31.7 ± 8.7</td>
<td>92.0 ± 14.8</td>
<td>113 ± 4</td>
</tr>
</tbody>
</table>

Values are means ± SD. MABP: mean arterial blood pressure.

![Table 2 rCBF, rCBV, and rCP in brain tumor and normal brain tissues](image)

<table>
<thead>
<tr>
<th>Region</th>
<th>rCBF (ml/min/gm) (n = 6)</th>
<th>rCBV (μl/gm) (n = 3)</th>
<th>rCP (ml/min/kg) (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contralateral cerebral cortex</td>
<td>1.44 ± 0.14</td>
<td>28.8 ± 5.6</td>
<td>5.0 ± 3.4</td>
</tr>
<tr>
<td>Ipsilateral cerebral cortex</td>
<td>1.17 ± 0.16</td>
<td>20.0 ± 4.0</td>
<td>4.8 ± 3.5</td>
</tr>
<tr>
<td>Contralateral white matter</td>
<td>0.35 ± 0.06</td>
<td>18.2 ± 4.6</td>
<td>4.5 ± 3.2</td>
</tr>
<tr>
<td>White matter adjacent to tumor</td>
<td>0.22 ± 0.06</td>
<td>12.4 ± 4.5</td>
<td>15.2 ± 10.2</td>
</tr>
<tr>
<td>Tumor:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>periphery</td>
<td>0.59 ± 0.09</td>
<td>53.9 ± 19.2</td>
<td>39.1 ± 19.3</td>
</tr>
<tr>
<td>center</td>
<td>0.42 ± 0.11</td>
<td>11.5 ± 1.5</td>
<td>53.1 ± 31.4</td>
</tr>
</tbody>
</table>

Values are means ± SD.
lower on the ipsilateral side than on the contra-
lateral side, whereas rCP was almost the same on
both sides. Relative to both cortices, rCBF was
markedly reduced within the tumor, somewhat
more so at the center (0.42 ml/min/gm) than at the
periphery (0.59 ml/min/gm). The difference in in-
tratumoral rCBV was more pronounced, being 11.5
μl/gm at the center and 53.9 μl/gm at the periphery.
The latter value was about twice that in the con-
tralateral cortex. rCP was markedly augmented
throughout the tumor, particularly in the central por-
tion of the tumor, where it was 10 times greater than
that in the contralateral cortex. Both rCBF and
rCBV in the white matter adjacent to the tumor
were substantially reduced. On the other hand, rCP
in the adjacent white matter was markedly increased
(15.2 ml/min/kg).

Discussion

The brain tumor model used in this study is an
established one, and our survival data (mean, 20.9
days) are in agreement with those reported by
Kaneko et al.\textsuperscript{18} One requirement of such a model is a
constant rate of tumor growth. In this study, at 14–
16 days after implantation, the tumor size averaged
5.2 mm, which suggests a relatively constant growth
rate.

The two types of experimental brain tumors are
those induced by carcinogenic agents (primary tu-
mors) and those induced by implantation of tumor
cells. For measurement of rCP, the former model, in
which mechanical damage is not inflicted, is thought
to be desirable. Those tumors, however, have the
disadvantage of being inconsistent in terms of loca-
tion, size, and histological type. Therefore, we used
a transplantation model. The mean diameter of the
tumors produced indicates that the influence of
mechanical injury at the time of implantation was
negligible.

There have been numerous reports on rCBF in
experimental rat brain tumors.\textsuperscript{1,3,4,6,12,14,16,19,24,33} The
rCBF values vary, probably because of differences
in the types of tumors and their ages at the time
of measurement. Whereas rCBF has been reported
as relatively constant in primary experimental tu-

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mors,1-12) that in transplanted tumors is reportedly low in the central portion and higher in the periphery of tumors that have reached a size of more than 2 mm² in cross-section 3,4,14,16,24) A similar tendency was noted in the present study.

In our study, the results of autoradiography indicated that the central portion of the tumor was viable (non-necrotic), but rCBF was low and there were fewer new blood vessels than in the periphery. In other words, tumor growth was faster in this transplantation model than in primary brain tumor models, so that vascular development lagged behind tumor growth. This suggests a close relationship between the heterogeneity of rCBF within the tumor and the rate of tumor growth (i.e., the degree of malignancy). rCBF in the ipsilateral cortex was decreased by 19% relative to that of the contralateral cortex, but was reduced by 37% in the white matter adjacent to the tumor. This may have been because the tumor had a more pronounced effect on the adjacent white matter through direct compression or edema. rCBV values paralleled those of rCBF.

rCBF in the contralateral (parietal) cortex was 84% of the value (1.72 ml/min/gm) we obtained in non-tumor-bearing rats previously.27) This reduction in rCBF on the contralateral side may be explained in terms of the decrease in perfusion pressure consequent to elevated intracranial pressure, and the impaired function on the ipsilateral side may eventually "spread" to the contralateral side via nerve fibers, constituting a fall of rCBF (diaschisis). However, this sequence of events is hypothetical at present.

rCP within the tumor was quite high, particularly in the central portion, which corroborates previously reported observations.2,3,7,13,15,22,23,33) According to Molnar et al.,23) the rate of transcapillary transfer of materials within the tumor becomes greater as the tumor enlarges, is low in necrotic or cystic regions, and is dependent on the properties of the blood vessels supplying the tumor. These authors added that the rate is generally higher in tumors fed by the blood vessels of the choroid plexus and meninges. In our study, the tumors were situated within the brain parenchyma and, with only minor variation, were consistent in size. Since the tumor tissue was heterogeneous, higher rCP was expected in the periphery of the tumor, where rCBF was abundant and blood vessels were dense. On the contrary, however, it tended to be higher in the central portion, which suggests a difference in the permeability of blood vessels among various sites within the tumor.

The results of this study may be relevant to the selection of treatment in the clinical setting. With such nitrosourea compounds as ACNU, distribution appears to depend on rCBF, since this agent is lipid-soluble.9,28,34) Therefore, in our tumor model, poor ACNU penetration would be expected in the central portion. Although the blood-brain barrier is an obstacle to water-soluble agents, the rCP is increased throughout the tumor, so that even at its center, an effective concentration may be achieved with a combination of a nitrosourea compound and a water-soluble agent. This problem requires further study, as the many reports concerning anti-cancer drug distribution pertain to tumors as a whole26,32) and do not describe local uptake by specific regions within tumors.

The relationship between radiation sensitivity and the oxygen concentration in tumor tissues has been known for many years.13) A few years ago, Pallavicini and Hill25) reported a decrease in the radiation sensitivity of a KHT sarcoma transplanted into mouse muscle in response to a reduction in intratumoral rCBF induced by anesthetics and several vasoactive agents. Like our experimental glioma, most tumors are poorly perfused with blood and hypoxic.20,21) The effects of radiation therapy have been evaluated clinically under hyperbaric oxygen conditions9) and in laboratory mice following the increase of oxygen transport by means of administration of a perfluorochemical emulsion.31) Oxygen transport is believed to occur through simple diffusion along the concentration gradient between capillary and brain tissue, and Tannock30) reported the arrival of oxygen up to 150 µm from the capillary. While the rCBF and capillary density may not be identical, they probably change in parallel. Therefore, estimates of the tissue oxygen concentration will probably be possible through measurement of rCBF and rCBV, and will serve as a useful index of radiation sensitivity.

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