Early changes measured by CT perfusion imaging in tumor microcirculation following radiosurgery in rat C6 brain gliomas

Laboratory investigation

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Object. In this paper, the authors’ aim was to use CT perfusion imaging to evaluate the early changes in tumor microcirculation following radiosurgery in rat C6 brain gliomas.

Methods. C6 glioma cells were inoculated into the right caudate nucleus of 25 Wistar rats using a stereotactic procedure. Tumor-bearing rats were randomly divided into 2 groups (tumor group and treatment group). Rats in the treatment group received maximal doses of 20 Gy delivered by the X-knife unit 16 days postimplantation. Computed tomography perfusion imaging was performed in all rats 3 weeks after tumor implantation prior to death and histopathological analysis.

Results. Hypocellular regions and tumor edema were increased in the treatment group compared with the tumor group. Parameters of CT perfusion imaging including cerebral blood volume (CBV) and mean transit time (MTT) of the tumors as well as the permeability surface area (PSA) product in the tumor-brain districts were decreased in the treatment group compared with the tumor group (p < 0.05). Although microvascular density (MVD) in the periphery of the tumors in the treatment group was higher than that in the normal contralateral brain (p < 0.05), MVD of the tumors in the treatment group was less than that in the tumor group (p < 0.01). There was a positive correlation between cerebral blood flow (CBF) and MVD as well as CBV and MVD in the center and periphery of tumors in both groups (p < 0.05).

Conclusions. A decrease in the perfusion volume of rat C6 brain gliomas was observed during the acute stage following X-knife treatment, and CBF and CBV were positively correlated with MVD of rat C6 brain gliomas. Thus, CT perfusion imaging can be used to evaluate the early changes in tumor microcirculation following radiosurgery. (DOI: 10.3171/2011.1.JNS101513)

KEY WORDS • glioma • rat • perfusion tomography • radiosurgery

GLIOMAS are one of the most prevalent types of primary brain tumors in adults. High-grade gliomas are aggressive, malignant tumors that are highly vascular and disrupt the BBB. Patients with these tumors have a poor outcome following diagnosis despite available treatment strategies. The tumors contain areas of necrosis and have a tendency to infiltrate. Since angiogenesis appears to play a central role in the growth and spread of tumors, quantifying tumor neoangiogenesis and neovascularity is important in predicting tumor grade, treatment options, treatment response, and prognosis. However, to date, it has been difficult to assess tumor angiogenesis primarily because of the complexity of the microvascular environments of the tumors as well as the limited resolution of available clinical imaging tools. Microvascular density has been used as the gold standard to assess tumor angiogenesis because of its direct association with angiogenic growth factor expression, tumor growth, postoperative survival, and occurrence of metastases.

The use of accurate noninvasive methods of assessing local angiogenesis in high-grade gliomas would improve prognostication and aid in the evaluation of the effectiveness of targeted molecular therapies as well as individual treatment responses. Tumor microcirculation can be studied during CT or MR imaging examinations with dynamic contrast enhancement. With dynamic contrast-enhanced MR imaging, however, the signal is a complex...
function of the contrast agent concentration and is influenced by many parameters such as relaxivity, magnetic susceptibility, inflow, and phase effects. In contrast, there is a direct relationship between CT attenuation variations and the iodine concentration in tissues and large vessels. In particular, CT perfusion imaging permits qualitative and quantitative evaluation of brain perfusion by mapping CBF, CBV, PSA product, and MTT. The increase in angiogenic activity and neovascularization in the neoplasm results in an increase in microvascular permeability and CBV, related to the presence of immature, disrupted, or absent vessels of the BBB. In recent studies, CT perfusion imaging of brain tumors has been shown to be useful for assessing preoperative tumor grade, differentiating between tumor enhancement and radiation-induced necrosis, and evaluating the response to antiangiogenic agents as well as guiding the biopsy procedure, in cases in which selection of the biopsy target is based on the identification of the hypervascularization area inside heterogeneous tumors.

Since the late 1970s, when adjuvant radiation therapy was found to improve survival, focused radiotherapy in the form of stereotactic radiosurgery or fractionated radiation therapy has become the primary or adjuvant treatment modality following resection or biopsy. Recent studies have examined tumor microcirculation during the middle and/or late phase of radiation using pathology and imaging techniques such as SPECT/PET, MR perfusion imaging, MR spectroscopy, and dynamic contrast-enhanced CT. However, during the acute or early phase after radiotherapy, tumor microcirculation has been rarely studied using contrast imaging along with pathological findings. Rat C6 brain gliomas, which resemble human Grade II gliomas, have been used predominantly in studies focusing on the changes induced by radiation rather than radiosurgery. In this study, our aim was to determine whether CT perfusion imaging could measure tumor perfusion and changes in tumor microcirculation in rat C6 brain gliomas at the early stage following X-knife treatment (20 Gy).

Methods

All experiments were performed after obtaining the approval of the animal care and use committee of the China Medical University. The protocols used in this study are depicted in brief in Fig. 1. We used a total of 25 female Wistar rats weighing 250–300 g. The rats were anesthetized with chloral hydrate (400 mg/kg intraperitoneally).

C6 Glioma Model

We obtained C6 glioma cells from the Department of Cell Biology, China Medical University. Cells were cultured in DMEM containing 10% calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 16.6 U/ml fungicide, and incubated at 37°C in an atmosphere consisting of 5% CO2. The medium was changed twice a week. During the logarithmic growth period, cells were pelleted by centrifugation and resuspended to a final concentration of 106 cells/ml (cells counted by a microscope phase micrometer). Cells were implanted into the rat host within 1–1.5 hours. The anesthetized rat was placed in a stereotactic headholder, and a small craniotomy (2 x 1 mm) was drilled 2–3 mm from the midline and 1 mm posterior to the coronal suture. The dura was not opened. A total of 106 C6 glioma cells (in a final volume of 5 μl) was implanted stereotactically into the right caudatum region, 5 mm below the craniotomy using a Hamilton syringe. The craniotomy was sealed with bone wax, and the scalp was closed with staples. As a control, 5 rats received stereotactic injection of 5 μl of phosphate-buffered saline without tumor cells in the same location.

Magnetic Resonance Imaging and Treatment Groups

All rat brains underwent imaging approximately 2 weeks after implantation of the C6 glioma cells to observe tumor growth and prepare for radiosurgery. Magnetic resonance imaging was performed using a 1.5-T magnet (Toshiba VISART 4.04), and the rat’s head was positioned over a 3-in surface coil. Each rat brain was imaged with precontrast multislice T1-weighted spin echo (TE 15 msec, TR 450 msec, FOV 7 cm, matrix 256 x 256, slice thickness 2.5 mm, gap 0.5 mm), T2-weighted fast spin echo (TE 100 msec, TR 420 msec, FOV 7 cm, matrix 256 x 256, slice thickness 2.5 mm, gap 0.5 mm). Contrast-enhanced T1-weighted spin echo images (TE 15 msec, TR 450 msec, FOV 7 cm, matrix 256 x 256, slice thickness 2.5 mm, gap 0.5 mm) were collected after intravenous administration of 0.2 mmol/kg Gd-DTPA. Images were acquired parallel to the anteroposterior direction (axially). The MR images showed that 18 rats (90%) had successfully grafted tumors. All tumor-bearing rats were assigned randomly to either the tumor (9 rats) or treatment (9 rats) group. The images of the latter were transferred to the BrainLAB workstation for designing the radiosurgery plan.

X-knife Treatment

Sixteen days after tumor implantation, rats in the treatment group were fixed using the Leksell Model G stereotactic frame equipped with a 5-mm collimator. Thread was used to fix the teeth of the rats. Computed tomography scanning was performed in these rats using spiral CT scanning (Toshiba EXPRESS/GX). First, unenhanced CT scans (FOV 40 cm, matrix 512 x 512, slice thickness 3 mm, 120 kV, 150 mA, spiral 1 second/scan) of each rat brain were obtained using a large field (FOV 40 cm) to identify spatial and anatomical markers for tumor location. Following unenhanced CT scanning, the contrast-enhanced scanning (FOV 18 cm; the other parameters were identical to the unenhanced CT scanning parameters) was performed after a 1-ml injection of contrast agent (Iohexol Injection, Beijing Beilu Pharmaceutical Co., Ltd.) was administered intravenously to identify the tumor. After reconstruction of the original data from these 2 scans, the images were transferred to the BrainLAB workstation to create the treatment plan for radiosurgery. The images were superimposed and then integrated with the contrast-enhanced T1-weighted images to define the target position (the radiosurgical area).
According to the plan, 9 rats each received maximal doses of 20 Gy using a single isocenter of irradiation delivered with the 5-mm collimator helmet and 5 converging arcs of the X-knife unit (BrainLAB) by using a 6-MV linear accelerator (Siemens Primus-M). The head of the rat was covered with the bolus material during treatment. The mechanical error was 0.2 mm.

**Computed Tomography Perfusion Imaging**

Twenty-one days after tumor implantation, CT perfusion imaging using multislice CT scanning (GE Lightspeed16) was performed in both groups. After the anesthetized rat was placed prone in a custom-made frame, the integrated catheter (BD Intima) was inserted into the tail vein and connected to an automated injector. An unenhanced CT scan (120 kV, 100 mA, FOV 9.6 cm, matrix 512 × 512, 2.5 mm section thickness, 8i, and 1 second/scan) was obtained first to localize the tumor. The back projection filter used in the reconstruction of CT images has a cutoff frequency of 10 line pairs per centimeter. Eight of the 16 slices were selected for the target section containing the whole brain. Then cine scanning with an overall interval of 60 seconds was performed using the same radiological parameters as those used for the localization scan. Computed tomography scanning was initiated 5 seconds prior to the intravenous injection (0.2 ml/second) of Iohexol Injection (a total volume of 1 ml) by means of the automated injector. Thus, each study was composed of 792 sequential images.

**Image Analysis**

The CT data were transferred to an image processing workstation (Advantage Windows 4.1, GE Medical Systems) and analyzed using CT Perfusion 3.0 software (GE Medical Systems). The modeling used by the software is based on compartmental analysis: a 2-compartment model (intravascular equivalent to blood and extravascular equivalent to tissue extracellular fluid) is used and assumes that the back flux of contrast medium from extravascular to intravascular compartments is negligible for the first 1–2 minutes (a technique known as Patlak analysis27). The basilar artery and superior sagittal sinus were selected as the input artery and vein (ROI of area range 18–25 mm²), respectively.

The images produced by the software are represented by 4 parametric maps: CBV, CBF, MTT (in seconds), and PSA product. Cerebral blood flow is the flow through a given vascular network in the brain (in ml/100 g/min). Cerebral blood volume is the volume of blood within the vessels (in ml/100 g). The MTT is the time of all blood elements entering the arterial input and leaving the venous output of the vascular network. Permeability surface area product is the capillary permeability–surface area product of the BBB to contrast material (ml/g/min).23

To acquire the mean values of the parameters, the following sets of tissue ROIs were drawn according to the different colors of the PSA product image with the maximum tumor diameter based on the histopathological data (Fig. 2): ROI 1 was placed in the red or yellow district and defined as the center of the tumor (ROI 1 was reflected across the midline into the left cerebral hemisphere to create the normal contralateral ROI [ROI 2]); ROI 3 was placed in the green or yellow-green district as the periphery of the tumor; and ROI 4 was placed in the green-blue region, where the tumor and brain tissue meet (referred to as the TBD). These ROIs were drawn so that no major blood vessels or necrotic area of the tumor was present within the regions. The values of the different parameters in the different areas were acquired automatically from the parameter images. All ROIs were drawn by 2 neuroradiologists with 2 years of experience with CT perfusion imaging. Representative parameter values from 2 radiologists were averaged.

**Histopathological Examination**

Immediately following CT perfusion imaging, all rats were killed and their brains were removed. The brains were fixed in 10% paraformaldehyde for at least 24 hours, paraffin-embedded, sectioned (4 μm), and exam-
CT perfusion imaging in tumor microcirculation after radiosurgery

Fig. 2. The sets of tissue ROIs shown on the transverse PSA product map (one of the reconstructed CT perfusion images) of a rat C6 glioma following radiosurgery (20 Gy). The ROIs are as follows: 1, center of the tumor; 2, contralateral normal tissue; 3, periphery of the tumor; 4, tumor-brain districts (TBD).

Histopathological Analysis

The tumor tissue in the tumor group was straw yellow and parenchymal with small areas of hemorrhage and necrosis. The tumors in the treatment group were paler than the tumors in the tumor group. Furthermore, the areas of necrosis in 2 of the treated rats were significantly larger. Microscopic analysis revealed that all tumors in both groups were Grade II and IV astrocytomas. As reported previously, the tumors in the tumor group were parenchymal, invasive, palisading, and compact malignant cells containing nuclear pleomorphism, the occasional formation of proteinaceous eosinophilic edema fluid, and neovascularization that was more evident at the periphery of the tumors, whereas the tumors in the treatment group showed increased cellular fragmentation and swelling (Fig. 3A and B). No response to the X-knife treatment was observed in the normal contralateral brain tissues. Four histological parameters were studied in the tumor and treatment groups by using light microscopy. The mean tumor diameter was less in the treatment group (0.58 ± 0.02 cm) than in the tumor group (0.60 ± 0.04 cm). There were slightly higher levels of intratumoral hemorrhage in the treatment group (4 [57%] of 7) than in the tumor group (3 [33%] of 9). No statistically significant differences between the tumor diameter and intratumoral hemorrhage were observed between the 2 groups (p > 0.05). Decreased cell density was present more in the treatment group (4 [57%] of 7) than in the tumor group (0 [0%] of 9). There were higher levels of tumor edema in the treatment group (5 [71%] of 7) than in the tumor group (1 [11%] of 9). There were significant differences of decreased cell density and tumor edema between the 2 groups (p < 0.05). As shown in Table 1, the areas of the hypoxic region in the tumors were greater in the treatment group (11,693.8 and 11,496.3 μm² at the center and periphery of the tumors) than in the tumor group (7575.7 and 7403.2 μm² at the center and periphery of the tumors) (p < 0.01). Moreover, the areas of edema region in the tumors were greater in the treatment group (12387.4 and 12079.6 μm² in the center and periphery of the tumors, respectively) than in the tumor group (8065.7 and 7923.4 μm² at the center and periphery of the tumors, respectively) (p < 0.01, Table 1). Immunohistochemical studies revealed that the tumor cells

Test were used to compare normally distributed quantitative data and numerical data, respectively. The Spearman product moment test was used to determine the correlation between CT measurements and the histopathological changes. Significance was declared at p < 0.05.

Results

Two rats in the treatment group died of intratumoral hemorrhage after treatment and were not included in the remainder of the study.

Statistical Analysis

Statistical analysis was performed using the SPSS 11.5 package. Standard descriptive statistics, such as mean ± SD, were calculated. The Student t-test and chi-square
were GFAP- and S100-positive, indicative of glial tumors. Tumor microvasculature stained positive for CD34+ (Fig. 3C and D). Microvessel densities in the tumor districts, except those in the center of the tumors in the treatment group, were greater than those in the contralateral brains (p < 0.05). Furthermore, MVDs at the periphery of the tumors were greater than those in the center in both groups (p < 0.01). Moreover, MVDs in the tumors were lower in the treatment group (17.29 and 48.29 at the center and periphery of the tumors, respectively) than in the tumor group (31.33 and 62.44 at the center and periphery of the tumors, respectively) (p < 0.01, Table 2).

**TABLE 1: Area changes after radiosurgery of C6 glioma in rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>CB (μm²)</th>
<th>TC (μm²)</th>
<th>p Value vs CB</th>
<th>TP (μm²)</th>
<th>p Value vs CB</th>
<th>p Value vs TC</th>
</tr>
</thead>
<tbody>
<tr>
<td>tumor (9 rats)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hypocellular region</td>
<td>7,289.4 ± 328.6</td>
<td>7,575.7 ± 529.4</td>
<td>&gt;0.05</td>
<td>7,403.2 ± 536.7</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>edema region</td>
<td>7,495.3 ± 412.1</td>
<td>8,065.7 ± 941.4</td>
<td>&gt;0.05</td>
<td>7,923.4 ± 978.5</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>treatment (7 rats)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hypocellular region</td>
<td>7,127.5 ± 290.2</td>
<td>11,693.8 ± 1,228.2</td>
<td>&lt;0.01</td>
<td>11,496.3 ± 1,148.5</td>
<td>&lt;0.01</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>edema region</td>
<td>7,648.6 ± 361.4</td>
<td>12,387.4 ± 1,422.8</td>
<td>&lt;0.01</td>
<td>12,079.6 ± 1,335.7</td>
<td>&lt;0.01</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>p value</td>
<td>&gt;0.05</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mean values are presented as the means ± SDs. Abbreviations: CB = contralateral brain; TC = tumor center; TP = tumor periphery.
Computed Tomography Perfusion Imaging

The CT perfusion imaging data were subjected to statistical analysis using the Student t-test (Tables 3 and 4). The CT perfusion images with T2- and contrast-enhanced T1-weighted MR images are shown in Fig. 4. A heterogeneous increase in the CBF, CBV, MTT, and PSA product in the tumors of both groups was observed compared with the normal contralateral brain regions and in areas adjacent to the tumor. The PSA product in the center of the tumor was higher than in the periphery of the tumor and in the TBDs in both the groups. A significant decrease in the CBV and MTT in the central and peripheral regions of the tumor as well as in the PSA product in the TBDs was observed in the treatment group compared with the tumor group (p < 0.05).

Computed Tomography Perfusion Correlated to the Histopathological Changes

There were no correlations between CBF, CBV, MTT, PSA, and the area of the hypocellular or edematous region both in the central and peripheral regions of the tumors in the treatment group (p > 0.05). However, there was a positive correlation between not only CBF and MVD but also CBV and MVD in the center of the tumors in the tumor group (r = 0.802 and 0.849, respectively; p < 0.01). In addition, a positive correlation between MVD and CBV, MVD and CBV, and MVD and PSA product was observed at the periphery of the tumors in the tumor group (r = 0.800, 0.793, and 0.714, respectively; p < 0.05). Furthermore, a positive correlation was observed between CBV and MVD in addition to CBV and MVD in the center and periphery of tumors in the treatment group (r = 0.899 and 0.900 [center]; r = 0.926 and 0.947 [periphery], respectively; p < 0.01).

Discussion

Radiosurgery is used to deliver a high dose of focused radiation at a defined target volume to elicit a desired radiobiological response while simultaneously limiting its effect on the surrounding normal tissue. The rat C6 brain glioma model has been used to study radiosurgery using a 4-mm collimator with the Leksell Gamma Knife Unit. For the radiosurgery to be successful, it is critical that the target position is sketched accurately. As we reported previously, projection of the target position depends on the imaging fusion function of the BrainLAB workstation for radiosurgery in combination with different CT scanning technologies. We proposed that this method could be widely applied to similar studies.

In this study, we observed intratumoral edema, reduction in cell density, and MVD at the early stage after X-knife treatment. Previous studies have reported that a reduction in tumor cell density may be due to cell death, intercellular edema, or a combination of the two. Tada et al. found a decrease in the cell density of the rat brain 1 week after X-knife treatment, whereas brain edema was observed 24 hours and 3 weeks after radiosurgery. In contrast, Kondziolka et al. showed varying levels of tumor edema and decreased cell density. It is well known that the effects of radiation on the C6 glioma model are critically dependent on the dose and time of exposure. In this study, we describe a higher level of tumor edema and a more intermediate level of decreased cell density than previously reported. These discrepancies could be due to differences in doses, time of exposure, animal models, and tissue samples. Furthermore, vascular injury was reported to be one of the major responses to radiosurgery. Ljubimova et al. reported that doses from 5 to 100 Gy caused a decrease in cell numbers of approximately 15% between 24 hours and 4 weeks after radiation therapy. Johansson et al. reported that MVD of rat brain gliomas was reduced after radiotherapy, while Acker et al. showed that decreases in vessel length density and blood flow increased with time after treatment. Another study demonstrated that the decrease in endothelial cell density

**TABLE 2: Microvascular density changes after radiosurgery of C6 gliomas in rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>CB</th>
<th>TC</th>
<th>p Value vs CB</th>
<th>TP</th>
<th>p Value vs CB</th>
<th>p Value vs TC</th>
</tr>
</thead>
<tbody>
<tr>
<td>tumor (9 rats)</td>
<td>21.56 ± 2.88</td>
<td>31.33 ± 3.46</td>
<td>&lt;0.05</td>
<td>62.44 ± 4.13</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>treatment (7 rats)</td>
<td>19.00 ± 1.91</td>
<td>17.29 ± 1.80</td>
<td>&gt;0.05</td>
<td>48.29 ± 5.38</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>p value</td>
<td>&gt;0.05</td>
<td>&lt;0.01</td>
<td></td>
<td>&lt;0.01</td>
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</tbody>
</table>

**TABLE 3: Computed tomography perfusion data of different regions in the tumor group**

<table>
<thead>
<tr>
<th>Region</th>
<th>CBF (ml/100 g/min)</th>
<th>CBV (ml/100 g)</th>
<th>MTT (sec)</th>
<th>PSA (ml/100 g/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB</td>
<td>432.00 ± 70.66</td>
<td>10.84 ± 1.79</td>
<td>1.51 ± 0.02</td>
<td>0.04 ± 0.06</td>
</tr>
<tr>
<td>TV</td>
<td>635.38 ± 158.32*</td>
<td>19.70 ± 6.32*</td>
<td>1.88 ± 0.23*</td>
<td>39.76 ± 11.39*</td>
</tr>
<tr>
<td>TP</td>
<td>630.21 ± 157.90</td>
<td>19.17 ± 5.13*</td>
<td>1.85 ± 0.24†</td>
<td>21.01 ± 8.10‡</td>
</tr>
<tr>
<td>TBD</td>
<td>510.19 ± 135.57</td>
<td>15.93 ± 5.51†</td>
<td>1.85 ± 0.22*</td>
<td>10.85 ± 4.65§</td>
</tr>
</tbody>
</table>

* p < 0.01 vs CB.  
† p < 0.05 vs CB.  
‡ p < 0.01 TP vs TC.  
§ p < 0.01 TBD vs TP.
in the rat spinal cord observed 24 hours after irradiation persisted for 7 days and was followed by some recovery of the endothelial density by Day 14.\textsuperscript{25} Here, we show that the MVD of rat C6 brain gliomas decreased significantly during the early stage after radiosurgery. The decrease in MVD may be due to increased endothelial cell death and/or decreased endothelial cell proliferation. In addition, an increase in leukocyte vessel wall interactions after irradiation may also play a role.\textsuperscript{12,16,19,25,26}

Our CT perfusion imaging data show the heterogeneous microcirculation in the tumor. Compared with the normal contralateral brain region, the changes in CBF, CBV, and PSA product were similar in the center and periphery of the tumors independent of radiotherapy. These findings were consistent with the histopathological analysis. Compared with untreated tumors, the CBV of the tumors was significantly decreased during the early stage following treatment. Furthermore, the permeability of the BBB increased while there was no reduction in perfusion in TBDs and normal contralateral brain tissues. These results were consistent with established responses to radiotherapy. The CBV and MVD in the tumor decreased during the early stage following radiotherapy, suggesting that there was close correlation between the reduction in CBV and inhibition of angiogenesis. Although increased perfusion volume in the center of the tumors was observed in treated tumors, there was no difference in the MVD between the center of the tumors and the normal contralateral brain tissues. These findings demonstrate that perfusion in the local brain region contributed to the observed changes in the vascular diameters and permeability. Moreover, compared with the center of the tumors, MVD in the periphery was significantly increased with no difference in perfusion volume, further demonstrating that multiple factors may affect regional perfusion. In this study, no statistically significant correlations were observed between the areas of hypocellularity and edema and the values of CT perfusion imaging (CBF, CBV, CBV, MTT).

<p>| TABLE 4: Computed tomography perfusion data of different regions in the treatment group |</p>
<table>
<thead>
<tr>
<th>Region</th>
<th>CBF (ml/100 g/min)</th>
<th>CBV (ml/100 g)</th>
<th>MTT (sec)</th>
<th>PSA (ml/100 g/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB</td>
<td>414.15 ± 69.76</td>
<td>10.43 ± 1.63</td>
<td>1.51 ± 0.04</td>
<td>0.45 ± 1.19</td>
</tr>
<tr>
<td>TC</td>
<td>539.19 ± 126.69*</td>
<td>13.69 ± 3.02*</td>
<td>1.54 ± 0.07</td>
<td>43.96 ± 5.94†</td>
</tr>
<tr>
<td>TP</td>
<td>523.18 ± 151.11</td>
<td>13.77 ± 3.72</td>
<td>1.59 ± 0.07*</td>
<td>27.24 ± 4.32†‡</td>
</tr>
<tr>
<td>TBD</td>
<td>439.61 ± 111.87</td>
<td>13.00 ± 4.52</td>
<td>1.74 ± 0.24*</td>
<td>5.50 ± 3.45†</td>
</tr>
</tbody>
</table>

* p < 0.05 vs CB.
† p < 0.01 vs CB.
‡ p < 0.01 TP vs TC.
§ p < 0.01 TBD vs TP.

\textbf{Fig. 4.} Images of a rat C6 glioma at the early stage after radiosurgery (20 Gy). \textbf{A} and \textbf{B}: Axial T2-weighted (\textbf{A}) and contrast-enhanced T1-weighted (\textbf{B}) MR images with Gd-DTPA showing a hyperintense tumor (arrows) with evident edema compared with the tissue surrounding the tumor. \textbf{C}–\textbf{F}: Reconstructed transverse images of perfusion CT scanning showing a heterogeneous increase in CBF (\textbf{C}), CBV (\textbf{D}), MTT (\textbf{E}), and PSA (\textbf{F}) (arrows) of the treated tumor compared with the surrounding tissue of the tumor and contralateral brain tissue.
MTT, and PSA) in the tumors of the treatment group, which seemed to demonstrate that the tumor microcirculation was slightly affected by the injured tumor cells. Furthermore, the smaller rat populations may be the reason for the results.

A few noninvasive methods have been used for measuring the perfusion volume of tumors during the early stage following radiotherapy. However, their findings were not consistent. Our data show that the perfusion volume of the tumors decreased during the early stage following radiosurgery. In this study, we found that the MTT in the treatment group decreased after radiotherapy, in contrast to a previous report. These conflicting results may be due to different radiation methods and doses as well as the use of different tumor models. The increased necrosis observed in the treated tumors may prolong the MTT. It is possible that the changes in the PSA product observed here may be due to endothelial cell damage. Thus, measurements of the CBV and PSA product before and after treatment may be useful for detection of early radiotherapeutic effects on the tumors. Diserbo et al. reported that the BBB was disrupted and permeability was increased after gamma whole-body irradiation at the early stage. These findings were verified further by our study showing that the PSA product of the tumors following radiosurgery increased during the early stage. Thus, the PSA product would be a sensitive indicator for evaluating the permeability of the BBB in vivo after radiosurgery.

In our study, we used CT perfusion imaging alone to examine the tumors 5 days after X-knife treatment. However, increased monitoring of tumor microcirculation after treatment could be performed by simultaneous use of several noninvasive methods such as CT perfusion imaging, perfusion MR imaging, and laser Doppler flowmetry. In addition to the study of permeability and the analysis of pathology, microcirculation levels and peritumoral edema need to be evaluated further. The rats in our study had shorter survival times than those in previous reports. This may be linked to increased tumor edema and brain herniation, which may lead to early animal death. Thus, corticosteroid administration will also be evaluated in future studies. In addition, the use of larger rat populations and the monitoring of different types of tumor entities with radiotherapy in future studies might lead to a better understanding of tissue behavior. In the future, measurement of PSA products and CBV values at more frequent time points may help determine their usefulness in the prognosis of tumor treatment outcome.

Conclusions

We have shown that CBF and CBV in tumors were positively correlated with relative MVD. These findings indicate that CT perfusion imaging could be an important noninvasive method for evaluating tumor angiogenesis and microcirculation in vivo after radiosurgery. At the early stage after radiosurgery, the therapeutic effects on the tumors may be reflected by CBV and PSA product values measured by CT perfusion imaging.