Pathological response of cavernous malformations following radiosurgery

Samuel S. Shin, MD, PhD, Geoffrey Murdoch, MD, PhD, Ronald L. Hamilton, MD, Amir H. Faraji, MD, PhD, Hideyuki Kano, MD, PhD, Nathan T. Zwagerman, MD, Paul A. Gardner, MD, L. Dade Lunsford, MD, and Robert M. Friedlander, MD

Departments of 1Neurological Surgery and 2Pathology, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania

OBJECT Stereotactic radiosurgery (SRS) is a therapeutic option for repeatedly hemorrhagic cavernous malformations (CMs) located in areas deemed to be high risk for resection. During the latency period of 2 or more years after SRS, recurrent hemorrhage remains a persistent risk until the obliterative process has finished. The pathological response to SRS has been studied in relatively few patients. The authors of the present study aimed to gain insight into the effect of SRS on CM and to propose possible mechanisms leading to recurrent hemorrhages following SRS.

METHODS During a 13-year interval between 2001 and 2013, bleeding recurred in 9 patients with CMs that had been treated using Gamma Knife surgery at the authors' institution. Microsurgical removal was subsequently performed in 5 of these patients, who had recurrent hemorrhages between 4 months and 7 years after SRS. Specimens from 4 patients were available for analysis and used for this report.

RESULTS Histopathological analysis demonstrated that vascular sclerosis develops as early as 4 months after SRS. In the samples from 2 to 7 years after SRS, sclerotic vessels were prominent, but there were also vessels with incomplete sclerosis as well as some foci of neovascularization.

CONCLUSIONS Recurrent bleeding after SRS for CM could be related to incomplete sclerosis of the vessels, but neovascularization may also play a role.

http://thejns.org/doi/abs/10.3171/2014.10.JNS14499

KEY WORDS cavernous malformation; stereotactic radiosurgery; brain hemorrhage

Cavernous malformations (CMs) are angiographically occult, low-flow vascular lesions that are found in 0.4%–0.8% of the general population.8,22,26 Familial CMs can be associated with mutations in the CCM and KRIT genes leading to vasculogenesis.3,16,17 Bleeding rates for clinically unruptured CMs range from 1% to 2% per year,12,14 although MRI always shows old blood deposition in the malformation. After an initial clinically recognized hemorrhage, the risk of additional hemorrhages increases to 4% per year and can be as high as 34% per year after 2 or more bleeds.14,15 When located in accessible brain regions, CMs can be microsurgically removed.7,9,28,29,35 However, hemorrhagic CMs located in deep-seated brain regions without a pial or ependymal access corridor are technically challenging and associated with significant complication rates.4,20,25,36 Stereotactic radiosurgery (SRS) can be a treatment option for such CMs.13,19,21 Although the recurrent hemorrhage rate of CMs following SRS has been reported,11,13,19 relatively few publications describe the histological response of CM after SRS.10,23,34 The studies that do exist are case reports and small case series describing obliteration and scarring of CM tissue after radiosurgery. Briefly, Nyáry et al. described the case of a CM treated with SRS resulting in fibrosis of the CM.23 Similarly, Tu et al. found hyalinization, collagen formation, and partial luminal occlusion of CMs after SRS in...
underwent subsequent resection of their CMs. One to 9 months, range 6–57 months). Patients in 5 of these cases experienced bleeding after Leksell Gamma Knife surgery (median 31 months, range 1–105 months). Among the 9 cases of CM with recurrent hemorrhage, the causes of repeated bleeding of CMs following SRS, according to previous studies, after SRS, between 8% and 17% of patients require delayed resection because of continued hemorrhagic events. To better understand the causes of repeated bleeding of CMs following SRS, we performed a histopathological analysis of the resected specimens in 4 patients who had undergone delayed microsurgery.

Methods

The University of Pittsburgh institutional review board approved this retrospective analysis. During the interval between 2001 and 2013, 9 cases of CM had recurrent bleeding after Leksell Gamma Knife surgery (median 31 months, range 6–57 months). Patients in 5 of these cases underwent subsequent resection of their CMs. One tissue sample obtained during microsurgical resection was small and insufficient for analysis. Thus, CMs from the remaining 4 patients were used to perform detailed histopathological analyses. The intervals between SRS and microsurgery were 4 months, 2.5 years, 3 years, and 7 years (Table 1).

Histology and Immunohistochemistry

Formalin-fixed paraffin-embedded 4- to 6-μm sections were stained with H & E. Gomori’s Modified Iron Stain and Masson’s trichrome stain were used to evaluate blood vessel walls and demonstrate hemosiderin. Immunohistochemistry for vascular endothelial growth factor (VEGF; mouse monoclonal antibody, 1:100, Millipore), basic fibroblast growth factor (bFGF; Biogenex ready-to-use kit), and matrix metalloproteinase 9 (MMP-9; 1:50, GenTex) was performed on 4- to 6-μm tissue sections. Pretreatment of sections for VEGF and MMP-9 consisted of antigen retrieval citrate buffer (1×, pH 6, Biogenex) in a steam chamber for 20 minutes and cooling for 20 minutes. No pretreatment was used for the bFGF. Each primary antibody was incubated for 45 minutes at room temperature in a humid slide chamber. Goat–mouse secondary antibody (Dako) was used on VEGF and MMP-9 (1:200) for 30 minutes. The EnVision system anti–mouse antibody (Dako) was used for 30 minutes for bFGF. The Vectastain ABC kit (Vector Labs) was applied to VEGF and MMP-9 for an additional 30 minutes for biotinylated enzyme activity. Sections were subsequently developed using the Vectastain ABC kit with the NovaRED peroxidase substrate kit (Vector Labs) and counterstained with Mayer’s hematoxylin and lithium carbonate solution.

Illustrative Cases

The 4 patients analyzed in this study had samples from resections performed at different periods following SRS. These samples were also obtained from 4 different anatomical locations: right thalamus/midbrain region, right pons, left mesial temporal lobe, and left pons.

Case 1

History and Examination

A 23-year-old female presented with acute-onset facial and oral numbness accompanied by mild frontal headache. Computed tomography studies of her brain demonstrated a 2 × 2–cm acute right thalamic hemorrhage that extended into the midbrain. Magnetic resonance imaging findings were compatible with a hemorrhagic CM. Given the lesion location and after discussing different options, observation was elected. The patient presented 6 months later with diplopia and left paresis, associated with further hemorrhage on MRI. She was discharged after stabilization and returned 2 months later for SRS.

Operation and Postoperative Course

The patient underwent Gamma Knife surgery: a 28.2-Gy maximum dose and 14-Gy margin dose delivered to a 2.33-cm³ malformation in a single session. Three months after SRS the patient reported worsening headaches, and repeat MRI demonstrated a new hemorrhage. She again improved over the course of 48 hours with no neurological deficits. One month later she experienced another acute onset headache, which progressed to left paresis, with an increase in the hemorrhage to 2.6 cm³ (Fig. 1A). She underwent microsurgical removal of the CM via a right subtemporal craniotomy, and the CM was resected en bloc.

Table 1. Clinical details and pathological findings in 4 cases

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sample Location</th>
<th>Resection Time</th>
<th>Clinical Presentation</th>
<th>Pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rt thalamus, midbrain</td>
<td>4 mos</td>
<td>Lt-sided paresis, Lt-sided anesthesia of face &amp; body, Lt blurry vision, frontal headache</td>
<td>Sclerosis &amp; partial luminal narrowing in a minority of vessels, large luminal diameter w/ thin walls in majority of vessels, small organizing vessels in areas of previous hemorrhage</td>
</tr>
<tr>
<td>2</td>
<td>Rt pons</td>
<td>2.5 yrs</td>
<td>Lt-sided paresis, Lt-sided anesthesia of face &amp; body, rt CN VI &amp; VII palsies</td>
<td>Sclerosis &amp; partial narrowing of most vessels; occasional sclerotic but large lumen vessels</td>
</tr>
<tr>
<td>3</td>
<td>Lt mesial temporal lobe</td>
<td>3 yrs</td>
<td>Medically intractable seizures, rt hemiparesis</td>
<td>Sclerosis &amp; obliteration of most vessels; minority of sclerotic but patent large lumen vessels</td>
</tr>
<tr>
<td>4</td>
<td>Lt pons</td>
<td>7 yrs</td>
<td>Rt facial numbness, Lt CN VII palsy, rt upper extremity paresis</td>
<td>Sclerosis &amp; obliteration of most vessels; enlarging new small vessels</td>
</tr>
</tbody>
</table>

CN = cranial nerve.
Six months postoperatively, she had persistent but markedly improved left paresis.

**Histology and Immunohistochemistry**

H & E–stained sections showed vascular sclerosis (Fig. 2A) and mineralization of vessels (Fig. 2B), but in some areas there were still numerous small vessels with thin fibrous walls according to trichrome stain (Fig. 2C). These vessels often stained positive for VEGF (Fig. 2D), whereas the sclerotic vessels did not. MMP-9 stained macrophages and showed diffuse staining in areas near these small vessels, but bFGF was negative.

**Case 2**

**History and Examination**

A 31-year-old male presented with 2 pontine hemorrhages during an 18-month period. The bleeds resulted in a progressive left paresis, right abducens paresis, and diffuse left sensory loss involving the face, arm, and leg. Magnetic resonance imaging demonstrated a hemorrhagic lesion in the right aspect of the pons (Fig. 1B).

**Operation and Postoperative Course**

Stereotactic radiosurgery was performed: 30-Gy maximum dose and 15-Gy margin dose delivered to a 2.8-cm³ volume. Two and one-half years after SRS, the patient had worsening diplopia and new right-sided facial nerve palsy. Magnetic resonance imaging showed recurrent and progressive hemorrhagic enlargement of the pontine CM. He underwent suboccipital craniectomy and had subtotal resection of the CM. Two months later, he continued to have persistent diplopia and facial weakness.

**Histology and Immunohistochemistry**

Examination of H & E and trichrome staining of the stereotactic biopsy specimen prior to SRS demonstrated ectatic back-to-back blood vessels characteristic of a CM (Fig. 3A). Resected CM tissue 2.5 years after SRS revealed incomplete vascular sclerosis and luminal narrowing (Fig. 3B). Vascular endothelial growth factor staining was weak, possibly because of cautery artifact. MMP-9 stained macrophages and showed diffuse staining in areas near these small vessels, but bFGF was negative.

**Case 3**

**History and Examination**

A 16-year-old epileptic female underwent partial resection of a left mesial temporal CM (Fig. 1C). Nine years later, when she was 25 years old, she presented with progressive right-sided weakness and uncontrolled partial seizures.

**Operation and Postoperative Course**

The residual CM was treated with Gamma Knife surgery: 30-Gy maximum dose and 15-Gy margin dose delivered to a 1-cm³ volume. Three years later, the frequency of seizures again increased, and she underwent repeat resection via a pterional craniotomy. Postoperative confusion and right-sided weakness gradually resolved within 5 days. One year after surgery her seizures were reduced in frequency while on antiepileptic medication.

**Histology and Immunohistochemistry**

H & E and trichrome stains of the resected CM 3 years after SRS revealed many sclerosed vessels, which were sometimes accompanied by increased numbers of smaller vessels (Fig. 4A). Immunostaining for VEGF was light in these vessels (Fig. 4B), but the tissue showed severe cautery artifact as well. The presence of erythrocytes adjacent to the residual patent vessels suggested persistent microhemorrhage in the malformation despite the absence of MRI evidence of interval hemorrhage. There were also MMP-9–stained macrophages and diffuse staining of VEGF in areas near the vessels, but bFGF was negative.

**Case 4**

**History and Examination**

A 51-year-old female presented with a 1-week history of facial numbness, dizziness, and blurred vision. A CT scan demonstrated a left pontine hemorrhage from a CM. Repeat MRI indicated further hemorrhage.

**Operation and Postoperative Course**

The patient underwent clot evacuation via a suboccipital craniotomy. Since postoperative imaging demonstrated...
a residual CM she underwent Gamma Knife surgery 4 months later: 28-Gy maximum dose and 14-Gy margin dose delivered to a 0.65-cm³ volume. She remained well until 7 years later, when she developed a new left-sided facial droop and right-sided facial numbness. Imaging studies demonstrated a new hemorrhage extending into the fourth ventricle (not shown in Fig. 1D). She underwent suboccipital craniotomy and resection of the entire CM.

**Histology and Immunohistochemistry**

H & E–stained sections showed prominent vascular sclerosis with fibrinoid necrosis and hyalinization (Fig. 5A and C). Trichrome stains showed that most vessels were completely sclerotic, but focally there were dilated thin-walled vessels with little sclerosis (Fig. 5B) as well as robust staining for VEGF (Fig. 5D).

**Discussion**

**Rationale for SRS in CMs**

Previous publications have indicated that the risk of additional bleeds from repeatedly hemorrhagic CMs declines following SRS from as high as 50% per year to 8%–10% in the first 2 years to 1%–1.4% thereafter. Although Karlsson et al. have remained skeptical about the eventual bleeding risk protection of SRS, they noted a trend for a decreasing hemorrhage rate 4 years after SRS. They also noted that higher radiosurgical doses reduce the

---

**Fig. 2.** Case 1. Photomicrographs obtained 4 months after SRS. Tissue sections show sclerotic vascular walls, mineralization, and areas with smaller vessels without sclerosis (A and B). Vessel walls (C) and immunostaining positive for VEGF (D) are visible. H & E (A and B) and trichrome (C). Original magnification ×100 (A) and ×200 (B–D). Figure is available in color online only.

**Fig. 3.** Case 2. A: Trichrome staining of the CM tissue from the stereotactic needle biopsy showed back-to-back thin-walled vessels consistent with a CM. Original magnification ×200. B: Two and a half years after SRS, the resected tissue showed some vessels with incomplete sclerosis (arrows), although many were partially or completely sclerosed. H & E, original magnification ×100. Figure is available in color online only.
risk of posttreatment hemorrhage. This slow reduction in hemorrhage risk years indicates that the time course of SRS obliteration took years to complete. During this latency interval, CM patients can have recurrent hemorrhages. Since most patients with CM who undergo SRS have deep-seated malformations that have repeatedly bled, it is not surprising that relatively few histological studies have been reported. To further understand the potential reasons for recurrent hemorrhages despite SRS, we studied the histological findings in 4 patients who required resection of a CM because of repeated bleeds.

**Histological Response of Vascular Malformation Blood Vessels**

Stereotactic radiosurgery for AVMs results in progressive luminal thrombosis in response to endothelial proliferation, creation of intraluminal myofibroblasts, and hyalinization and sclerosis of the treated blood vessel walls. However, reports detailing the histopathological analysis of CMs after SRS are sparse. Authors of a few studies have claimed that CMs treated by SRS have several similarities to AVMs treated with SRS. Nyáry et al. showed vasculature obliteration in both AVMs and CMs as well as marked fibrosis of stromal tissue by 1 year after SRS. Tu et al. found that there were proteinaceous thrombi and hyalinized vessel walls in both AVMs and CMs; however, a notable difference was the near complete or complete occlusion of the AVMs but the partial occlusion of the CMs after SRS. Cavernous malformations after irradiation commonly had fibrinoid necrosis but patent vascular channels.
We detected partially obliterated vessels and wall sclerosis as early as 4 months after SRS. However, as may be suspected this soon after SRS, most of the vessels were not sclerosed. Such patent vessels are prone to hemorrhage until further sclerosis and obliteration occurs. In agreement with this finding, there is a higher hemorrhage risk within the first 2 years following SRS, as demonstrated in our previous studies. Obstructed vessels are found more commonly in histological samples from patients who have undergone surgery at 2.5 and 3 years after SRS. However, there are many larger vessels with sclerotic vascular walls but open lumen even at these time points. The lack of extensive vessel obliteration indicates that the patients are still at risk for hemorrhage from these large vessels. This finding is in agreement with our prior analysis demonstrating an annual rehemorrhage risk of 8.8% in the first 2 years and a decrease to 1.1% thereafter.

By 7 years after SRS in the present study, most vessels were completely obliterated. However, these histological samples also demonstrated smaller patent vessels. These vessels had a wide range of diameters, and most showed no signs of sclerosis. Stereotactic radiosurgery leads to hyalinization of CM vessel walls, but these changes were not observed in small CM vessels with diameters on the order of capillaries and venules. Given that CMs proliferate via neoangiogenesis, we hypothesize that small diameter neovasculature developed after the original radiation (SRS) exposure. This initial neoangiogenesis may progress to become the more characteristic thin-walled large-lumen vessels seen in typical CMs. Despite the early effect of radiation in altering vessel morphology, neovascularization as well as continuing enlargement of thin-walled vessels should be considered as a possible mechanism underlying rehemorrhage even as late as 7 years after radiosurgery.

Prominent endothelial cells can be commonly found in either granulation tissue or larger vessels after SRS, similar to the organizing thrombi and neovascularization reported in nonirradiated CM. The endothelial cells participating in neoangiogenesis after irradiation have been previously studied in tumor xenograft models as well as ischemic injury models. Circulating endothelial progenitor cells from bone marrow have been shown to settle in areas of neovascularization and differentiate into endothelial cells. Although tumor and CM reactions to irradiation may be different, these studies suggest that a possible source of endothelial cells are adjacent or circulating progenitor cells. With newly recruited endothelial cell progenitors, neovascularization may give rise to new vessels prone to hemorrhage.

A limitation of this study is the small number of samples. Among the 9 patients who had recurrent bleeding, only 5 cases were deemed appropriate for resection (1 case was excluded from the analysis because of inadequate tissue sample). The small number of cases may provide a selection bias since they are more serious bleeds resulting in medically intractable symptoms. In addition, the timeline for the evolution of CM is also inadequate since only samples from 4 months and 2.5, 3, and 7 years were found. Moreover, these samples are from different regions of the brain. However, a cautious conclusion of the effect of SRS was made through an analysis of various sections as well as immunostains for markers of neovascularization in this study.

Conclusions

In this study, all 4 post-SRS CM cases had incomplete sclerosis of blood vessels, indicating a possible cause of rebleeding. In 3 cases there were vascular changes suspicious for neovascularization, suggesting that such changes could also play a role in some cases of rebleeding. It is possible that SRS results in vascular sclerosis, but stromal components continue to produce angiogenic factors that promote neovascularization. However, the number of cases in this study is too small to be definitive and warrants future study with a larger number of cases.

References

15. Kondziolka D, Monaco EA III, Lunsford LD: Cavernous

Author Contributions

Correspondence
Robert M. Friedlander, Department of Neurological Surgery, University of Pittsburgh Medical Center, UPMC Presbyterian, 200 Lothrop St., Ste. B–400, Pittsburgh, PA 15213. email: friedlanderr@upmc.edu.