Plasma Levels of Vascular Endothelial Growth Factor After Treatment for Cerebral Arteriovenous Malformations

Grace H. Kim, MD; David K. Hahn, BA; Christopher P. Kellner, BA; Zachary L. Hickman, MD; Ricardo J. Komotar, MD; Robert M. Starke, BA; William J. Mack, MD; J. Mocco, MD; Robert A. Solomon, MD; E. Sander Connolly, Jr, MD

Background and Purpose—The role of abnormal angiogenesis in the formation and progression of cerebral arteriovenous malformations (AVMs) is unclear. Previous studies have demonstrated increased local expression of vascular endothelial growth factor (VEGF) in AVM tissue and increased circulating levels of VEGF in AVM patients. We sought to further investigate the role of VEGF in AVM pathophysiology by examining changes in plasma VEGF levels in patients undergoing treatment for AVMs.

Methods—Three serial blood samples were obtained from 13 AVM patients undergoing treatment: (1) before any treatment, (2) 24 hours postresection, and (3) 30 days postresection. Plasma VEGF concentrations were measured via commercially available enzyme-linked immunosorbent assay (ELISA). For controls, blood samples were obtained from 29 lumbar laminectomy patients.

Results—The mean plasma VEGF level in AVM patients at baseline was $36.08 \pm 13.02$ pg/mL, significantly lower than that of the control group ($80.52 \pm 14.02$ pg/mL, $P = 0.028$). Twenty-four hours postresection, plasma VEGF levels dropped to $20.09 \pm 4.54$ pg/mL, then increased to $66.81 \pm 26.45$ pg/mL 30 days later ($P = 0.048$). The mean plasma VEGF concentration 30 days after resection was no longer significantly different from the control group ($P = 0.33$).

Conclusions—Plasma VEGF levels in 13 AVM patients were unexpectedly lower than controls, dropped early after AVM resection, then significantly increased 30 days later. These results support the key role of abnormal angiogenesis in AVM pathophysiology and suggest that a disruption in systemic VEGF expression may contribute to the natural history of these lesions. (Stroke. 2008;39:2274-2279.)

Key Words: angiogenesis ■ cerebral arteriovenous malformation ■ plasma levels ■ vascular endothelial growth factor
To further elucidate the role of abnormal angiogenesis in AVMs, we conducted a prospective case–control study to evaluate changes in plasma VEGF levels in patients undergoing surgical resection of AVMs. We aimed to assess VEGF as a potential systemic marker of local angiogenic activity and response to therapy. We hypothesized that increased levels of plasma VEGF in AVM patients would significantly decrease over time after definitive treatment of the lesion.

Methods

Patient Population
Thirteen patients with cerebral AVMs who were treated at Columbia University Medical Center between July 2005 and September 2006 were consented and prospectively enrolled in this IRB-approved study. Three serial blood samples were collected from each patient throughout their treatment process: before any treatment (baseline sample), 24 hours after surgical resection of the AVM, and 30 days after surgery during in-office follow-up. For controls, 29 patients undergoing lumbar laminectomy surgery during the same time period were enrolled. In these patients, a single plasma sample was obtained preoperatively. All control subjects were free of any known history of AVMs.

Cerebral angiography was performed on all AVM patients before their treatment according to standard protocol. All AVM patients were treated by surgical resection and all patients, except one in which the malformation had ruptured, underwent multi-staged endovascular embolization prior to resection. The number of embolization procedures received depended on the size, location, and morphology of the lesion. One (8%) patient did not undergo any embolization procedures. Five (38%) underwent 1, 3 (23%) underwent 2, and 4 (31%) underwent 3 embolizations. Measure-ments of platelet count, white blood cell count, and hemoglobin were within the normal range in all AVM and control subjects. Characteristics of the AVM study population are shown in Table 1.

Laboratory Analysis of Plasma VEGF
Peripheral arterial blood samples were obtained from indwelling arterial catheters or from venipuncture when arterial access was unavailable and collected in sterile heparin-coated tubes. All blood samples were centrifuged at 1957 g at a temperature of 4°C for 15 minutes within 30 minutes of collection. Plasma was separated, aliquoted, and stored at −80°C until analysis was performed.

Plasma VEGF levels were determined using a commercially available monoclonal antibody-based enzyme-linked immunoassay (ELISA) designed to measure VEGF levels (Quantikine, R&D Systems Inc, Catalog No. DVE00, Lot 240685) according to the manufacturer’s instructions. The assay exhibits no significant cross-reactivity with other angiogenic factors and has a sensitivity of 9.0 pg/mL. Optical density was measured at 450 nm using an automated microplate reader (Bio-Rad Laboratories, Model No. 680). VEGF concentrations were normalized to patient plasma protein levels and are reported as pg/mL.

Statistical Analysis
Data were tested for normality and were found to be normally distributed. Accordingly, data are presented as the mean±SEM unless otherwise noted. Statistical differences between groups were assessed using paired and unpaired Student t test where appropriate. Repeated measures analysis of variance (ANOVA) was used to compare differences in serial samples. Pearson’s correlation was used to evaluate correlations between continuous variables. A probability value of <0.05 was considered statistically significant. All analyses were performed using SPSS software (version 13.0, SPSS Inc).

Results
There were 13 patients in the AVM group and 29 in the control group. The mean age and standard deviation of AVM patients was 40±12 years, whereas the mean age of the control group was 61±12 years. In the AVM group, there were 8 females and 5 males, whereas in the control group there were 11 females and 18 males. Possible confounders were analyzed by comparing VEGF levels in patients without the confounder (AVM patients at baseline and control patients) to levels in patients with the confounder using a Student t test. Table 2 shows that age, gender, history of smoking, hypertension, and diabetes had no effect on plasma VEGF levels.

Plasma VEGF Levels in Controls
The mean plasma VEGF concentration in controls was 80.52±14.02 pg/mL. There was no significant difference in mean plasma VEGF levels between males and females (72.11±17.70 versus 92.44±24.20 pg/mL, respectively, P=0.49). No correlation was found between VEGF levels and age (r=−0.034; P=0.86).

Table 1. Demographic and Clinical Characteristics of 13 Cerebral Arteriovenous Malformation Patients

<table>
<thead>
<tr>
<th>No.</th>
<th>Age/Sex</th>
<th>Presentation</th>
<th>Size (cm)</th>
<th>Location</th>
<th>Drainage</th>
<th>Feeding Artery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60 M</td>
<td>Incidental</td>
<td>2</td>
<td>R Frontal</td>
<td>Superficial</td>
<td>MCA/ACA</td>
</tr>
<tr>
<td>2</td>
<td>35 F</td>
<td>Headaches, visual aura</td>
<td>2</td>
<td>R Parieto-occipital</td>
<td>Deep</td>
<td>MCA/PCA</td>
</tr>
<tr>
<td>3</td>
<td>28 F</td>
<td>Arm pain and weakness</td>
<td>1.4</td>
<td>R Temporo-occipital</td>
<td>Superficial</td>
<td>MCA/PCA</td>
</tr>
<tr>
<td>4</td>
<td>39 F</td>
<td>Headaches</td>
<td>3</td>
<td>L Parietal</td>
<td>Deep</td>
<td>ACA</td>
</tr>
<tr>
<td>5</td>
<td>67 M</td>
<td>Hearing loss</td>
<td>3.5</td>
<td>R Parietal</td>
<td>Superficial</td>
<td>MCA/ACA</td>
</tr>
<tr>
<td>6</td>
<td>36 F</td>
<td>Intracerebral hemorrhage</td>
<td>3</td>
<td>R Parietal</td>
<td>Superficial</td>
<td>MCA</td>
</tr>
<tr>
<td>7</td>
<td>32 F</td>
<td>Headaches, visual aura</td>
<td>3</td>
<td>R Frontal</td>
<td>Deep</td>
<td>MCA</td>
</tr>
<tr>
<td>8</td>
<td>52 M</td>
<td>GTC seizures, headaches</td>
<td>3</td>
<td>R Occipital</td>
<td>Superficial</td>
<td>MCA/PCA</td>
</tr>
<tr>
<td>9</td>
<td>41 F</td>
<td>Incidental</td>
<td>3.5</td>
<td>L Frontal</td>
<td>Superficial</td>
<td>MCA/ACA</td>
</tr>
<tr>
<td>10</td>
<td>36 M</td>
<td>Incidental</td>
<td>3.8</td>
<td>R Fronto-temporal</td>
<td>Superficial</td>
<td>MCA</td>
</tr>
<tr>
<td>11</td>
<td>33 M</td>
<td>Seizures</td>
<td>3.2</td>
<td>L Frontal</td>
<td>Superficial</td>
<td>MCA/ACA</td>
</tr>
<tr>
<td>12</td>
<td>27 F</td>
<td>Seizures</td>
<td>3</td>
<td>L Temporal</td>
<td>Deep</td>
<td>MCA/PCA</td>
</tr>
<tr>
<td>13</td>
<td>46 F</td>
<td>Headaches</td>
<td>2</td>
<td>R Parieto-occipital</td>
<td>Superficial</td>
<td>ACA</td>
</tr>
</tbody>
</table>

ACA indicates anterior cerebral artery; GTC, generalized tonic-clonic; MCA, middle cerebral artery; PCA, posterior cerebral artery.

Kim et al Plasma VEGF Levels in Patients Treated for Cerebral AVMs 2275
Plasma VEGF Levels in AVM Patients
The mean baseline plasma VEGF level in AVM patients was 36.08±13.02 pg/mL, significantly lower than the mean plasma VEGF level in the control group (P=0.028, Figure 1). There was no significant difference in VEGF levels between males and females (51.34±31.42 versus 26.55±9.28 pg/mL, respectively, P=0.38). No correlation was found between plasma VEGF level and patient age (r=−0.19; P=0.53) or AVM size (r=0.40, P=0.18). No significant differences were found in plasma VEGF levels according to patient clinical presentation (headache, 23.26±11.76, seizures, 34.17±15.25, asymptomatic, 74.40±50.87, P=0.45).

The Effect of Treatment on Plasma VEGF Levels
The mean plasma VEGF level 1 day after resection dropped to 20.09±4.54 pg/mL, with a mean decrease from baseline of 15.99±14.20 pg/mL, which then increased to 66.81±26.45 pg/mL 30 days later, with a mean increase from baseline of 30.73±17.02 pg/mL (P=0.048, Figure 2). The mean plasma VEGF concentration in AVM patients 30 days after surgery was no longer significantly different from the mean plasma VEGF concentration in the control population (66.81±26.45 versus 80.52±14.02 pg/mL, P=0.33, Figure 1).

Change in plasma VEGF levels in 13 AVM patients from baseline to 1 day and 1 month after resection.

Discussion
Angiogenesis is a tightly regulated process maintained by a delicate balance between proangiogenic and antiangiogenic factors and is vital to brain development and repair. Abnormal expression of angiogenic factors has been repeatedly observed to be involved in AVMs, making it increasingly more evident that a disruption in angiogenic signaling systems is involved in the formation and progression of cerebral AVMs.

The goal of the present study was to further our understanding of the angiogenic mechanisms regulating AVM formation and clinical behavior in vivo by studying changes in plasma VEGF levels in AVM patients undergoing definitive treatment for these lesions. A better understanding of the role of abnormal angiogenesis in AVMs may lead to improved patient management and novel therapeutic options. The results of our study were unexpected: plasma VEGF levels at baseline in patients harboring AVMs were significantly decreased when compared to our control population. Furthermore, plasma VEGF levels dropped 1 day after combined treatment with embolization and resection, then significantly increased to near control levels 1 month later (Figure 2).

The cause for these changes in circulating VEGF levels has been the source of much speculation. Since Robinson et al examined cellular adhesion molecules in AVMs in 1995, more than 20 studies have contributed to the investigation of AVM molecular biology. Enhanced VEGF expression in particular has been repeatedly demonstrated in endothelial and subendothelial layers of resected AVM specimens, whereas VEGF mRNA has been shown to be upregulated in cells adjacent to AVMs. Some authors, on the other hand, have reported associated VEGF receptors to be downregulated in AVM tissues. A recent study reported a significant increase in circulating VEGF levels in AVM patients compared to healthy controls. Increased expression of circulating VEGF has also been associated with other cerebrovascular disorders including intracranial aneurysms, dural arteriovenous fistulas, and vasospasm after subarachnoid hemorrhage.

VEGF expression is regulated by numerous factors, most significantly hypoxia. It is also upregulated by insulin and...
numerous growth factors and cytokines. The findings of the present study suggest the presence of a negative feedback system that may also help regulate systemic VEGF expression. In this model, elevated local VEGF production may cause the release of a molecular mediator which activates an inhibitory feedback mechanism that downregulates systemic VEGF production. Alternatively, VEGF itself may mediate its own inhibition via a negative feedback mechanism. This model may also explain our finding of significantly increased plasma VEGF levels after surgical resection of AVMs. Removal of the focus of elevated VEGF production would eliminate the stimulus for inhibition and thereby allow systemic production of VEGF to resume over time to normal levels as we found in our study. The cause for the drop in VEGF levels immediately after treatment is unclear but may represent an acute response to removal, in line with the half-life of VEGF (reported to be 30 to 45 minutes). Similarly, Kirsch et al found plasma VEGF levels in patients with dural arteriovenous fistulas to decrease following endovascular treatment.

Another possible explanation for our findings is the presence of an AVM “sink” in which circulating VEGF is trapped by the AVM, thereby leading to low systemic levels and high local levels of VEGF. After AVM resection, systemic VEGF levels normalize over time secondary to removal of the source of VEGF sequestration. In support of this theory, other authors have shown VEGF receptors to be elevated within AVM tissues. Although studies suggest that upregulated VEGF mRNA in cells adjacent to AVMs accounts for the elevated local VEGF protein levels, local production of VEGF does not necessarily preclude the additional presence of significant systemic sequestration.

Certainly, numerous other mechanisms could also account for our findings. Increased VEGF expression postsurgically may reflect local angiogenesis associated with recovery from surgery attributable to local VEGF production at the wound site. VEGF is a key component in wound healing and surgery attributable to local VEGF production at the wound site. VEGF expression has also been shown to be upregulated in endothelial cells, neurons, and astrocytes within brain tissue after ischemic insult, likely promoted by hypoxia, as well as after contusion. Furthermore, VEGF has been shown to have neuroprotective as well as neurotrophic properties in several animal models. Finally, Sure et al has demonstrated significantly higher expression of VEGF in the endothelium of partially obliterated (embolized) AVMs compared to nonembolized AVMs suggesting that resultant hypoxia or neoangiogenesis or both may stimulate increased expression of VEGF. However, it is difficult to determine which of, and to what degree, these phenomena account for our findings.

The findings of the present study contrast with the recent study by Sandalcioglu et al who found significantly elevated levels of plasma VEGF in patients with AVMs compared to healthy controls. This difference could be explained by a difference in patient populations examined. Eight of the 17 AVM patients examined in this previous study presented with hemorrhage of their AVM compared with only one patient in the present study. Platelets, a major source of VEGF stor-


Stein J, Neeman M, Shweiki D, Itin A, Keshet E. Vascular endothelial growth factor mRNA by hypoxia and hypoglycemia and


Plasma Levels of Vascular Endothelial Growth Factor After Treatment for Cerebral Arteriovenous Malformations

Stroke. 2008;39:2274-2279; originally published online June 5, 2008;
doi: 10.1161/STROKEAHA.107.512442
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/39/8/2274

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/