Blocking VEGF by Bevacizumab Attenuates VEGF-Induced Vasospasm After Experimental Subarachnoid Hemorrhage in Rabbits

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■ OBJECTIVE: **Vasospasm after subarachnoid hemorrhage (SAH) plays a vital role in the development of delayed cerebral ischemia. Anti- vascular endothelial growth factor (VEGF) antibodies, like bevacizumab (BEV), may attenuate VEGF-stimulated angiogenesis, reduced vascular cell proliferation, and improve vasospasm after SAH.**

■ METHODS: **Thirty-two adult male New Zealand white rabbits were randomly divided into 4 groups of 8 rabbits in each group: group 1 (control); group 2 (SAH); group 3 (SAH + vehicle); and group 4 (SAH + BEV). BEV (5 mg/kg, intraperitoneally) was administered 5 minutes after the intracisternal blood injection and continued for 72 hours once per day in the same dose for group 4. Animals were sacrificed 72 hours after SAH. Basilar artery cross-sectional areas, arterial wall thicknesses, and hippocampal degeneration scores were evaluated in all groups.**

■ RESULTS: **VEGF is associated with the narrowing of the basilar artery. Treatment with BEV statistically significantly increased the cross-sectional area of the basilar artery when compared with the SAH and the vehicle groups. Basilar artery wall thicknesses in the BEV group was statistically significant smaller than in the SAH and vehicle groups. The hippocampal degeneration scores for the BEV and control groups were similar and significantly lower than those for the SAH and vehicle groups.**

■ CONCLUSIONS: **Cellular proliferation and subsequent vessel wall thickening is a reason to delay cerebral ischemia and deterioration of the neurocognitive function. Intraperitoneal administration of BEV was found to attenuate cerebral vasospasm and prevent delayed cerebral ischemia and improve neurocognitive function after SAH in rabbits.**

INTRODUCTION

Subarachnoid hemorrhage (SAH) frequently leads to delayed cerebral ischemia, and infarction occurs in 30% of patients between the fifth and seventh day after SAH.[1,2] The leading cause of delayed cerebral ischemia is arterial vasospasm.[3,4] The treatment and prevention of vasospasm are the primary goals in the treatment of the patients surviving SAH. The specific etiologies underlying vasospasm remains unknown.

SAH typically occurs in the setting of a ruptured cerebral aneurysm or arteriovenous malformation, resulting in blood release into the subarachnoid space. Blood products in the

Keywords
- Bevacuzimab
- Cerebral arteries
- Delayed cerebral ischemia
- Hippocampus
- Rabbit
- Subarachnoid hemorrhage
- Vascular endothelial growth factor

Abbreviations and Acronyms
BBB: Blood brain barrier
BEV: Bevacizumab
CA: Cornu ammonis
PCNA: Proliferating cell nuclear antigen
PCO2: Partial pressure of carbon dioxide
PO2: Partial pressure of oxygen
SAH: Subarachnoid hemorrhage
VEGF: Vascular endothelial growth factor

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Table 1. Physiological Parameters of the Experimental Groups

Variables	Control	SAH	Vehicle	BEV	Statistics
pH	7.45 (0.02)	7.44 (0.03)	7.46 (0.01)	7.48 (0.01)	$\chi^2 = 3.983$, $P = 0.294$*
PCO2 (mm Hg)	35.6 ± 0.7	36,4 ± 0.8	36.1 ± 0.8	35.8 ± 0.6	F = 1.225, $P = 0.249$†
PO2 (mm Hg)	97.4 ± 0.9	94.7 ± 1.7	95.2 ± 2.0	95.6 ± 1.7	F = 2.125, $P = 0.410$†
MABP (mm Hg)	107 ± 7	103.4 ± 4.8	104.4 ± 6.4	106.3 ± 1.8	$\chi^2 = 2.148$, $P = 0.538$*
HR (ppm)	168 ± 8.2	164.5 ± 5.5	164.1 ± 6.1	165.2 ± 5.1	F = 1.301, $P = 0.269$†

Data are shown as the median (interquartile range) or mean ± standard deviation.
SAH, subarachnoid hemorrhage; PCO2, partial pressure of carbon dioxide; PO2, partial pressure of oxygen; MABP, mean arterial blood pressure; HR, heart rate; bpm, beats per minute.
*Kruskal–Wallis test.
†One-way ANOVA.

subarachnoid space activates platelets, which release potent growth factors for cells in the vascular wall.[5] Miller et al. hypothesized that cerebral vasculopathy is related to changes in vessel mechanics that occur as a consequence of vascular wall thickening caused by mitogen-induced proliferation on cerebral arteries.[2] Inflammatory cell and growth factors released from platelets which platelet-derived growth factor AB, transforming growth factor-beta, and vascular endothelial growth factor (VEGF) which release from platelets are increased in the cerebrospinal fluid and trigger cellular proliferation and matrix deposition in the walls of cerebral arteries.[2,3,6] VEGF is the main factor responsible for the proliferation of vascular endothelium and enhances vascular permeability.[7-9] The consequent proliferation of myofibroblast and smooth muscle along with extracellular matrix synthesis, as well as cellular necrosis and remodeling, are standard features at the vasospastic vessel wall.[10] Subsequently, these changes in the vasospastic segment may increase arterial wall thickness and decrease vessel compliance, which manifests as decreased vessel diameter at physiological pressures. Josko et al. demonstrated that the significant increase of VEGF expression in the cerebral hemisphere and different areas of the brainstem is an indicator of the brain ischemia caused by vasospasm.[1]

Bevacizumab (BEV) is a humanized monoclonal antibody that binds to human VEGF-A, neutralizing all isoforms and preventing VEGF interaction with its cell surface receptor.[8,9] Bevacizumab has been used extensively to inhibit angiogenesis in cancer and other diseases with pathological angiogenesis.[11-13]

Through previous studies, it had been shown that VEGF expression plays an essential role in the development of SAH-induced vasospasm. As a VEGF-inhibitor, we hypothesized that BEV might attenuate SAH-induced cerebral vasospasm.

METHODS

Experimental Groups

Animal care and all experiments were conducted following the European Communities Council Directive of November 24, 1986 (86/609/EEC) concerning the protection of animals for experimental use. All experimental procedures used in this investigation were reviewed and approved by the ethics committee of the Ministry of Health Ankara Education and Research Hospital Committee of Animal Ethics. Thirty-two male, New Zealand white rabbits, weighing 2,900–3,450 g were randomly divided into the following 4 groups of 8 rabbits:

CONTROL group (n = 8): A sham surgery group, in which SAH was not induced.

SAH group (n = 8): The SAH protocol was used to induce vasospasm as described below.

VEHICLE group (n = 8): Cerebral vasospasm was induced by SAH protocol as described below; in this group, after induction of anesthesia, the cisterna magna was punctured as described below, and 1 mL/kg of solvent solution of BEV (trehalose dehydrate, sodium phosphate, polysorbate, water) was slowly injected into the cisterna magna after removal of the same amount of cerebrospinal fluid.

Table 2. Scoring Table

	Score		
Function	0	1	2
Appetite	Finished meal	Left meal unfinished	No appetite
Behavioral changes	Active, alert or stand	Stand or walk with external stimulation	Inactive
Neurologic status	Impossible to walk or stand due to plegia	Paralytic gait due to paresis or ataxic gait	No deficits

This scoring table was previously modified and was used to evaluate the rabbit's daily neurologic function based on the results of 3 tests.[15,16]

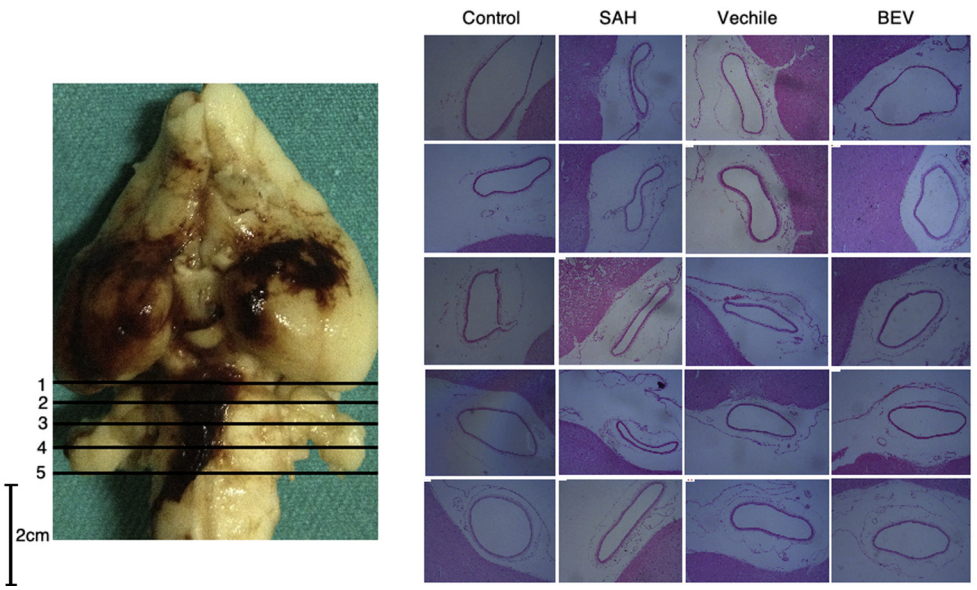

Figure 1. Macroscopic view of a basis of the rabbit brain from the subarachnoid hemorrhage group (**left**) and representative histological cross-sections of the basilar artery corresponding to all study groups (**right**, hematoxylin and eosin, 40X obj.). BEV, bevacizumab.

BEV group (n = 8): As for group 3, but rabbits received a single daily intraperitoneal dose of BEV 5 mg/kg (Avastin, B7106; Roche, Grenzach-Wyhlen, Germany). The treatment was started 5 minutes after the intracisternal blood injection and continued for 72 hours once per day in the same dosage. This dosage of BEV was selected based on results of past studies.[14]

Anesthesia and Surgical Procedure

The animals were kept at optimal (18–21°C) room temperature and fed with a standard diet where a 12-hour light-dark cycle was implemented. Free access to food and water was allowed. The animals were anesthetized by intramuscular administration of a 70 mg/kg ketamine (Ketalar, Parke Davis Eczacıbaşı, Turkey) and 5 mg/kg xylazine (Rompun, Bayer, Turkey) combination. All animals breathed spontaneously throughout the procedures. Arterial blood samples for partial pressure of oxygen (PO_2) and partial pressure of carbon dioxide (PCO_2) were taken from each animal from the catheterized ear arteries for blood gas analysis during the procedures, where only those animals with $PO_2 > 70$ mm Hg and $PCO_2 < 40$ mm Hg were included to the study. Heart rate and arterial blood pressure were measured with the use of an ear arterial catheter. The physiologic parameters of the experimental groups are summarized in Table 1. Core body temperature was monitored rectally and maintained at 37 ± 0.5°C with a heater.

Cerebral Vasospasm Model

The head of the rabbit was extended in the prone position. A midline nuchal incision was made, and dermal and subdermal tissues (fascia and paravertebral muscles) were dissected to expose

Table 3. Intra- and Interclass Correlation Coefficients for Cross-sectional Area and Wall Thickness Measurement

	CSA (μm²)		AWT (μm)	
	ICC	95% CI	ICC	95% CI
Intra-observer				
1st observer	0.838	0.753–0.923	0.785	0.645–0.925
2nd observer	0.879	0.823–0.935	0.863	0.856–0.900
Inter-observer				
1st measurement	0.630	0.378–0.882	0.680	0.429–0.931
2nd measurement	0.727	0.590–0.864	0.673	0.494–0.852

CSA, cross-sectional area; AWT, arterial wall thickness; ICC, intraclass correlation coefficient; CI, confidence interval.

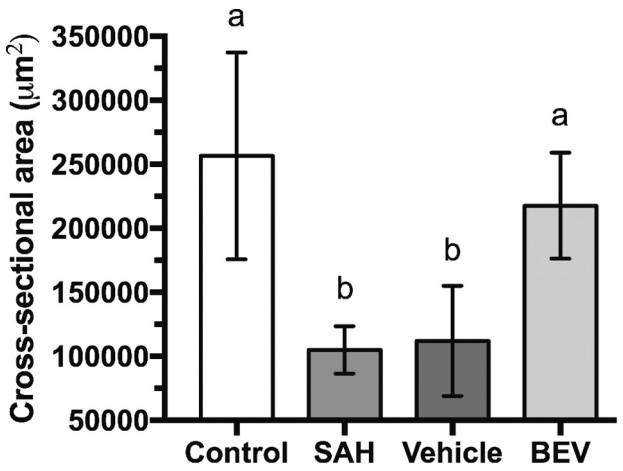

Figure 2. Mean basilar artery cross-sectional areas of the study groups. The horizontal lines in the middle of each box indicate the median, while the top and bottom borders of the box mark the 25th and 75th percentiles, respectively. The whiskers above and below the box mark indicate the maximum and minimum levels. BEV, bevacizumab; SAH, subarachnoid hemorrhage.

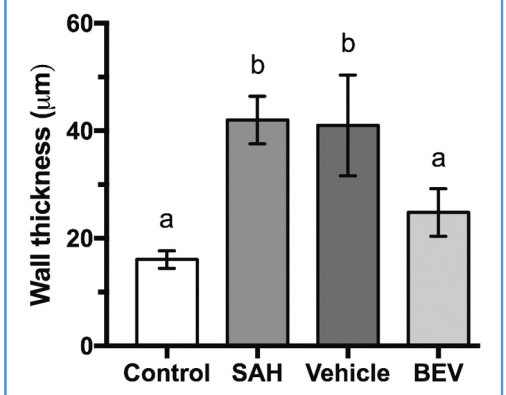

Figure 3. Mean basilar artery wall thickness of the study groups. The horizontal lines in the middle of each box indicate the median, while the top and bottom borders of the box mark the 25th and 75th percentiles, respectively. The whiskers above and below the box mark indicate the maximum and minimum levels. BEV, bevacizumab; SAH, subarachnoid hemorrhage.

the atlantooccipital membrane. A 25-gauge needle was inserted through the dura mater and the arachnoid membrane into the cisterna magna; 1 mL/kg of cerebrospinal fluid was withdrawn as well as an equal volume of fresh, non-heparinized autologous arterial blood, which was obtained from the ear artery injected into cisterna magna within 2 minutes. The animals were then placed in a head-down position at 30°C for 30 minutes to hold the blood in the basal cisterns. After the recovery from anesthesia and confirmation of vital signs, rabbits were left to their cages for the establishment of cerebral vasospasm.

Neurologic Examination

Daily neurologic examinations were performed, and clinical changes recorded by a blinded observer. The rabbits were monitored for behavioral changes, appetite, muscle weakness in any limb, paralysis, or ataxia. A scoring table that was previously modified was used to evaluate the rabbit's daily neurological function based on the results of 3 tests[15] (Table 2).

Perfusion-Fixation

All animals were euthanized by perfusion-fixation 72 hours after procedures. The animals were anesthetized as described above. The ear artery was catheterized for monitoring blood pressure and for blood gas analysis. When satisfactory respiratory parameters were obtained, a thoracotomy was performed, the left ventricle cannulated, the right atrium opened widely, and the descending thoracic aorta clamped. After perfusion with 300 ml of physiological saline, a fixative was perfused (10% formaldehyde, 200 mL). Perfusion was performed at a standard height of 100 cm from the chest. The brains were then removed and stored in formaldehyde solution at 4°C overnight.

Histologic Analysis of the Basilar Artery

Each brainstem specimen was embedded in paraffin. The entire basilar artery was sectioned into 5 segments of 2 mm in length (Figure 1), and stained with hematoxylin and eosin. The morphometric measurements on all 5 segments of the basilar were performed using BAB-Bs200ProP Image Processing and Analysis System (Ankara, Turkey). The luminal area was calculated as the area contained within the boundaries of the internal elastic lamina. The size of the cross-sectional area for each basilar artery was obtained by averaging these measurements. The mean ± SD value obtained from each artery was used as the final value for a particular vessel. The wall thickness between the lumen and external border of the muscle layer was measured at 4 quadrants of each segment of the basilar artery. If an undulating luminal border was encountered, a new measurement was performed from the internal elastic membrane to the external border of the muscle layer. The vessel wall thickness for each basilar artery segment was obtained by averaging these measurements. The mean ± SD value obtained from each artery was used as the final value for a particular vessel. All measurements were repeated 3 times for each artery in a blind fashion by 2 pathologists, and the absolute values were obtained by averaging these measurements. Inter- and intraobserver reliability levels are provided in Table 3.

Immunohistochemistry

Fixed brains were graded ethanol dehydrated and embedded in paraffin. Cross-sections (5 mm) of the basilar artery were taken to assess the vessel wall. Immunohistochemistry was performed to determine protein deposition and location. To determine whether cellular proliferation occurred, primary mouse antibodies directed against proliferating cell nuclear antigen (PCNA) (1:300, DAKO, St Louis, Missouri, USA) and antimouse immunoglobulin (Ig)G secondary antibodies conjugated with horseradish peroxidase were applied using a

Table 4. Mean Basilar Artery Cross-sectional Area and Wall Thickness Values

Group	CSA (μm^2)	AWT (μm)
Control	288,247.5 (15,692.3)*,†	28.6 (2.3)*,†,‡,¶
SAH	174,949.5 (31,810.93)*,§	39.3 (1.4)*,§
Vehicle	190,147.8 (16,578)†,‖	38.7 (2.3)†,‖
BEV	197,284.8 (3,546.2)§,‖	21.2 (1.1)§,‖,¶
Statistics	$\chi^2 = 29.656$, $P < 0.001$#	$\chi^2 = 16.980$, $P < 0.001$#

CSA, cross-sectional area; AWT, arterial wall thickness; SAH, subarachnoid hemorrhage; BEV, bevacizumab.
*Control vs. SAH ($P < 0.001$).
†Control vs. vehicle ($P < 0.001$).
‡Control vs. BEV ($P < 0.001$).
§SAH vs. BEV ($P < 0.001$).
‖Vehicle vs. BEV ($P < 0.001$).
¶Control vs. BEV ($P < 0.001$).
#Kruskal–Wallis test.

VectaStain ABC Elite kit mouse IgG and DAB kit (PK-6102 and SK-4100, respectively, Vector Laboratories, CA). Blinded observers counted numbers of positive nuclei.

Hippocampal Degeneration

Paraffin-embedded hippocampus slices were sectioned to 4- to 6-μm thicknesses and stained by hematoxylin and eosin. Under a light microscope, morphologic signs of neuronal degeneration, such as neuronal shrinkage, hyperchromasia, and nuclear pyknosis, were evaluated. The presence and extent of neuronal degeneration were scored semi-quantitatively in the Cornu Ammonis (CA) 1, CA3, and dentate gyrus regions as follows: 1 = normal appearance, 2 = few degenerated neurons among normal neurons, 3 = large number of degenerated neurons with scattered normal neurons, 4 = complete degeneration with no residual normal neuron.[16] Scoring was done for each of the th3ree regions of the hippocampus. The sum of these 3 scores was called the "degeneration score", and the means were used in statistical analysis.

Statistical Analysis

Data analyses were performed using SPSS for Windows, version 11.5 (SPSS Inc., Chicago, IL). Whether the distributions of continuous variables were normally distributed was determined by using the Shapiro-Wilk test. The Levene test was used for the evaluation of homogeneity of variances. The data are shown as mean ± SD, median (interquartile range), or median (min-max), where applicable. The mean differences among groups were compared by 1-way ANOVA, whereas the Kruskal-Wallis test was applied for comparisons of the median values. When the P values from Kruskal-Wallis test statistics were statistically significant, the Conover's nonparametric multiple comparison test was used to identify which group(s) differed. The intraclass correlation coefficient for area and wall thickness was calculated for determining both inter- and also intraobserver reliability levels. A P value < 0.05 was considered statistically significant.

RESULTS

No significant differences were seen when comparing physiological parameters among the groups or neurological scores between the BEV and control groups (1.6 [1–4] and 1.4 [1–3]; $P < 0.05$). Neurological scores were significantly lower (1.6 [1–4]) in the BEV group than in the SAH (2.9 [2–5]; $P < 0.05$), vehicle (2.6 [2–4]; $P < 0.05$), respectively.

Morphometric Analysis of the Basilar Artery

The mean basilar artery cross-sectional area in the control group was 288,247.5 ± 15,692.3 μm. In the SAH and vehicle groups, the mean basilar artery cross-sectional area decreased to 174,949.5 ± 31,810.93 and 190,147.8 ± 16,578 μm, respectively. These decreases were statistically significant when compared to the control group ($P < 0.001$ for both). There was no statistically significant difference between the SAH and the vehicle groups ($P = 0.578$). In the BEV group, the mean basilar artery cross-sectional area was 197,284.8 ± 3,546.2 μm. Treatment with BEV increased the cross-sectional area of the basilar artery when compared with the SAH and the vehicle groups ($P < 0.001$ for both) (Figure 2).

The mean value of the basilar artery wall thickness was 28.6 ± 2.3 μm in the control group, 39.3 ± 1.4 μm in the SAH group, and 38.7 ± 2. 3μm in the vehicle group. The BEV group had a mean value of 21.2 ± 1.1 μm for the basilar artery wall thickness. When the SAH and the vehicle groups were compared with the control group, following the induction of SAH, basilar artery wall thicknesses were increased ($P < 0.001$ for both). Basilar artery wall thicknesses in the BEV group was smaller than the SAH and vehicle groups ($P = 0.001$ and $P < 0.001$, respectively). There was no statistically significant difference between the SAH and the vehicle groups ($P = 0.356$) (Figure 3). Mean basilar artery cross-sectional areas and arterial wall thickness values are provided in Table 4.

Pathologic Examination of the Hippocampus

In the control group, light microscopic evaluation of the CA1, CA3, and the dentate gyrus samples from the hippocampus was typical.

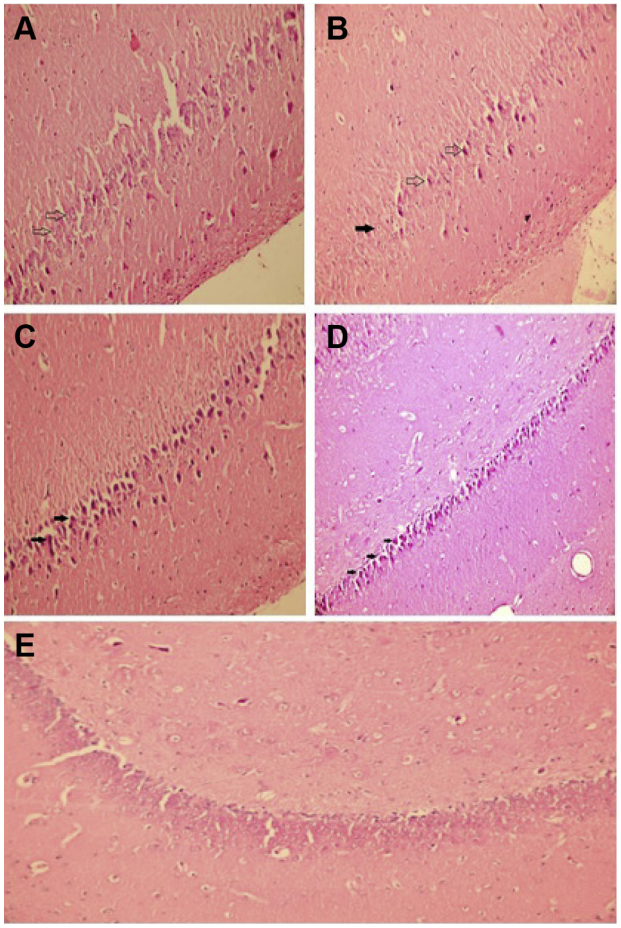

Figure 4. **(A)** Representative photomicrographs showing the standard neuronal view of the hippocampal CA3 region *(empty arrows)* in the control group (hematoxylin and eosin [H&E], x20). **(B)** Slide from the subarachnoid hemorrhage (SAH) group showing the regular neuronal *(empty arrows)* and mild neuronal degeneration *(dark arrows)* of the hippocampal CA3 region (H&E, x20). **(C)** Slide from the vehicle group showing severe neuronal degeneration *(dark arrows)* of the dentate gyrus (H&E, x20). **(D)** Slide from the SAH group showing the completely neuronal degeneration *(dark arrows)* of the dentate gyrus of the hippocampus (H&E, x20). **(E)** Slide from the BEV group showing only mild degeneration of the neurons in the CA 3 region (H&E, x20).

Complete degeneration of the CA1, CA3, and the dentate gyrus was observed in the vehicle and SAH groups, respectively. In the BEV group, the histopathological examination demonstrated less degeneration at the CA1, CA3, and the dentate gyrus, where few degenerated neurons with hyperchromasia and nuclear pyknosis were noticed (Figure 4). The mean degeneration score for the control group was 3.7 ± 0.8; the mean degeneration scores for the SAH and the vehicle groups were 11.2 ± 0.8 and 10.8 ± 0.8, respectively; and the mean degeneration score for the BEV group was 7.6 ± 0.7. The hippocampal degeneration scores for the BEV and control groups were similar ($P > 0.05$) and significantly lower than those for the SAH and vehicle groups ($P < 0.05$) (Figure 5).

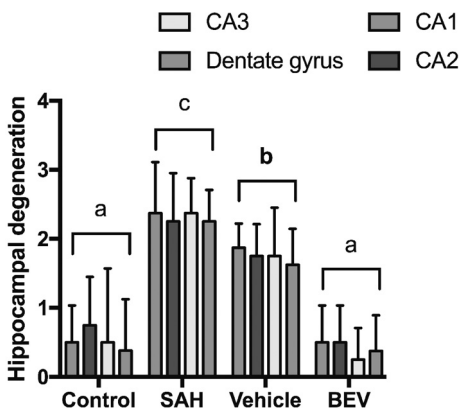

Figure 5. Pathological examinations of the hippocampus relevant to the study groups' hippocampal degeneration scores. Kruskal-Wallis test, (a) Control vs. bevacizumab (BEV) ($P < 0.05$); (b) Control vs. subarachnoid hemorrhage (SAH) ($P < 0.001$); (c) Control vs. Vehicle ($P < 0.001$); (a and c) SAH vs. BEV ($P < 0.01$); (a and b) Vehicle vs. BEV ($P < 0.05$).

Cellular Proliferation

We performed PCNA staining for determining of proliferating cells (Figure 6). PCNA positive nuclei counts were performed both for the adventitia and the media of the arterial wall. Proliferation was not statistically significant between the control group and the treatment groups ($P = 0.135$). In both regions, there was no significant difference between the vehicle and the BEV groups. There was no significant difference between the BEV and the SAH groups in both the adventitia and media. This situation can be explained as follows: The injection of any fluid into the subarachnoid space triggered inflammatory response and caused a proliferation of medial vascular smooth muscle cells and adventitial fibroblasts.

DISCUSSION

The potential mechanisms of prolonged SAH-induced vasospasm involve many neurogenic, local biochemical, and endothelial factors and cause high rates of morbidity and mortality. Intercourse between vasoconstrictive and vasodilatory substances after SAH is in favor of vasoconstriction. Additionally, the blood products directly cause constriction of vascular smooth muscle cells and indirectly induce vasospasm by activating ET-1 production. ET-1 induces the production of VEGF and inhibits synthesis of NO, eventually causes neural tissue hypoxia.[17,18] VEGF is a potent angiogenic factor and especially releases from ischemic areas.[19,20] SAH-induced hypoxia is a cause of increased expression of VEGF and directly stimulates vasospasm and brain angiogenesis.[21]

Animal studies have demonstrated an increase of VEGF expression as early as 3 hours after induction of hypoxia, with a peak density after 48 hours.[5] Mitogens and spasmogens are released from the clotted and diseased vessel in the first hours

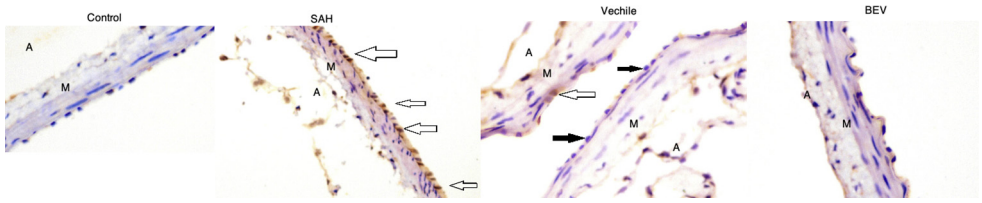

Figure 6. Rabbit basilar artery cross-sections. Representative samples of basilar arteries labeled with proliferating cell nuclear antigen (PCNA) to show cellular proliferation. At 3 days post-treatment, PCNA labeling shows some medial (M) smooth muscle proliferation for subarachnoid hemorrhage (SAH) and Vehicle groups. Adventitial (A) proliferation also occurred for SAH and Vehicle groups. Proliferation was not significant between the Control group and the bevacizumab (BEV) groups. *While empty arrows* indicate examples of positive nuclei, *dark arrows* indicate normal nuclei.

after SAH. Spasmogens cause temporary, early vasospasm, which improves over several hours to 1 day; in parallel, mitogens at the site stimulate vascular proliferation.[22] The accumulative effect reduces the elasticity of the local cerebral arteries, and these changes occur over 2–4 days after SAH in rabbits.[23] Miller et al. demonstrated that the degree of basilar artery narrowing 3 days after SAH was similar to the degree of narrowing caused by the cisternal injection of VEGF in a rabbit model of SAH.[2] Similar to these previous studies, we demonstrated that the basilar artery cross-sectional area was statistically significantly decreased in the SAH group when compared with the control group. In our study, PCNA immunostaining results revealed that vascular proliferation mediated by VEGF increased vascular wall thickness, arterial stiffness, and contributed to luminal narrowing. However, proliferation on both the adventitia and the media of the arterial wall was not statistically significant between the control group and the treatment groups due to inflammatory response.

It is interesting to ask whether the induction of vasospasm in brain vessels after SAH can be attenuated by blocking VEGF with a VEGF-specific antibody (BEV). BEV is able to cross the blood–brain barrier (BBB) in angiogenic foci after intraperitoneal injection into the brain parenchyma.[9,14] BBB permeability is increased by VEGF expression via induction of the secretion of NO and stimulation of cGMP activity.[24] Breaking of the BBB enables the transition of plasma compounds to the nervous tissue and the proliferation of microglia. The microglial cells release NO and superoxide free radicals, which are highly toxic for neurons.[3] Walker et al. indicated that BEV treatment in wild-type mice attenuated VEGF- stimulated angiogenesis, reduced vascular cell proliferation, and increased TUNEL-positive vascular cells.[14] Based on our study, we could comment that this vascular proliferation would decrease with intraperitoneal-injected BEV, the arterial diameter would be increased, and mechanical characteristics of the artery would be changed at physiological pressure; however, PCNA staining results were not statistically significant. Decreased elasticity due to cellular proliferation on arterial wall causes narrowing of the affected arteries at physiological pressures, and creates the clinical syndrome of "delayed cerebral ischemia." Although cellular proliferation after SAH is triggered immediately, cerebral vasculopathy takes several days to develop.[23,24] Collagen deposition in the arterial wall may account for the development of cerebral vasculopathy.[25] In our study, treatment with BEV increased the cross-sectional area of the basilar artery when compared with the SAH and the vehicle groups. Treatment with BEV as soon as possible after SAH decreases arterial wall thickness, increases the cross-sectional area, and may stop cerebral vasculopathy before starting.

Latzer et al. demonstrated for the first time that inhibition of the VEGF pathway profoundly affects hippocampal synaptic plasticity, neuronal responses, and spine integrity.[26] BEV weakened long-term hippocampal potentiation, and active membrane properties of CA1 neurons were affected in hippocampal slices.[26] Furthermore, BEV reduced hippocampal spine length and spine number in primary hippocampal neuronal cultures.[26] These alterations may constitute the cellular basis for the reported effects of BEV on cognitive function. In our study, we demonstrated less degeneration at the CA1, CA3, and the dentate gyrus in the treatment group with BEV, where few degenerated neurons with hyperchromasia and nuclear pyknosis were noticed.

CONCLUSIONS

Our data suggest that BEV treatment in rabbit attenuated VEGF-stimulated vasospasm and decreased subsequent vessel wall thickening after SAH. Furthermore, BEV diminished the degeneration of the hippocampal neurons and improved neurocognitive function. Bevacizumab is a promising treatment that deserves further investigation to determine the optimal dosage and clinical potential.

CRediT AUTHORSHIP CONTRIBUTION STATEMENT

Umut Dogu Akturk: Conceptualization, Data curation. **Cengiz Tuncer:** Investigation. **Huseyin Bozkurt:** Validation. **Omer Selcuk Sahin:** Writing - review & editing. **Husamettin Bulut:** Supervision. **Ata Arikok:** Visualization. **Cem Dinc:** Methodology, Software. **Bora Gurer:** Writing - original draft. **Erhan Turkoglu:** Supervision, Project administration.

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