VEGF Paradoxically Reduces Cerebral Blood Flow in Alzheimer's Disease Mice

Muhammad Ali[1] and Oliver Bracko[2]

[1]Department of Neurosurgery, Icahn School of Medicine at Mount Sinai, New York, NY, USA.
[2]Department of Biology, University of Miami, Coral Gables, FL, USA.

Neuroscience Insights
Volume 17: 1–4

DOI: 10.1177/26331055221109254
SAGE

ABSTRACT: Vascular dysfunction plays a critical role in the development of Alzheimer's disease. Cerebral blood flow reductions of 10% to 25% present early in disease pathogenesis. Vascular Endothelial Growth Factor-A (VEGF-A) drives angiogenesis, which typically addresses blood flow reductions and global hypoxia. However, recent evidence suggests aberrant VEGF-A signaling in Alzheimer's disease may undermine its physiological angiogenic function. Instead of improving cerebral blood flow, VEGF-A contributes to brain capillary stalls and blood flow reductions, likely accelerating cognitive decline. In this commentary, we explore the evidence for pathological VEGF signaling in Alzheimer's disease, and discuss its implications for disease therapy.

KEYWORDS: Alzheimer's disease, cerebral blood flow, VEGF-A signaling, blood-brain barrier

RECEIVED: June 7, 2022. **ACCEPTED:** June 8, 2022.

TYPE: Commentary

FUNDING: The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The authors disclosed receipt of the following support by the University of Miami Relief Funding Opportunity, University of Miami Institute for Data Science and Computing; Resources for Early Career Researchers Award and the Provost's Research Awards from the University of Miami to OB.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

CORRESPONDING AUTHOR: Muhammad Ali, Department of Neurosurgery, Icahn School of Medicine at Mount Sinai, 1 Gustave L. Levy Place, New York, NY 10024, USA.
Email: muhammad.ali@icahn.mssm.edu

Introduction

Vascular dysfunction plays a vital role in the pathogenesis of Alzheimer's disease. Not only are hypertension, diabetes, and atherosclerotic disease primary risk factors for Alzheimer's disease,[1] patient-level meta-analysis of multiple genome-wide association studies have highlighted the involvement of cerebrovascular disease-related pathways in Alzheimer's disease.[2] Alzheimer's disease-related vascular changes are characterized, in part, by impairment of autoregulation and reduced cerebral blood flow.[3-5] Importantly, these changes precede cognitive decline, making room for potential therapeutic interventions.[6]

Cerebral blood flow reductions of 10% to 25% present early in disease pathogenesis.[5] Mechanisms driving such drastic changes have largely remained unestablished. Recent studies employing in vivo microscopy have implicated cellular changes in mouse models of the disease, including pericyte constriction of capillaries,[7] vascular obstructions secondary to hypercoagubility,[8] and leukocyte capillary stalling.[9,10] Specifically, neutrophil adhesion to the cortical microvasculature leads to 17% reduced cerebral blood flow and cognitive deficits in the APP/PS1 and 5xFAD mouse models.[9] Detecting and addressing this phenomenon in humans may be critical in developing new treatment strategies.

Aberrant VEGF Signaling in Alzheimer's Disease

The vascular endothelial growth factor (VEGF-A) is involved in a broad array of signaling pathways contributing to angiogenesis,[11] neurogenesis,[12] and neuroprotection.[11] Physiologically, angiogenesis addresses local and global hypoxia. States of global cerebral hypoxia, like Alzheimer's disease, show evidence of increased VEGF-A levels[13-15] and capillary density.[16] However, vascular integrity is impaired, including the formation of vascular loops, glomeruloid structures, aberrant branching patterns, and irregular basement membranes, ultimately leading to insufficient oxygenation of brain tissue and neuronal dysfucntion.[17] In the end, though VEGF may induce neo-angiogenesis, it also contributes to vascular hyperpermeability and brain edema, which paradoxically contributes to diminished blood flow, reduced nutrient delivery, and entry of restricted molecules into the brain,[18] likely accelerating Alzheimer's progression.

We recently found that upregulated VEGF-A signaling contributes to cerebral blood flow reductions through capillary stalls in the APP/PS1 model of Alzheimer's disease (Figure 1A–C). Specifically, expression of the VEGF-A-associated tight junction protein occludin was downregulated in occluded capillaries.[19] The capillary stalling hypothesis suggests pathological VEGF-A/occludin- associated blood-brain barrier hyperpermeability activates local inflammatory markers in endothelial cells, recruiting leukocytes to the site of injury, increasing the incidence of stalled capillaries, and ultimately leading to cerebral blood flow reductions (Figure 1D). We targeted this pathway using an anti-VEGF-A antibody. Injection of the antibody immediately improved the integrity of the blood-brain barrier, leading to a reduction in stalled capillaries, and restoring cerebral blood flow. Longitudinally inhibiting VEGF-A through the vascular lumen specifically could also address the deleterious effects of pathological angiogenesis without compromising pathways of neuroprotection, such as neurogenesis, on the other side of the blood-brain barrier. Indeed, a recent perspective linked blood-brain barrier breakdown to cognitive impairment in Alzheimer's disease patients.[20] Overall, these publications indicate a critical role of a dysregulated blood-brain barrier in cognitive impairment and dementia.

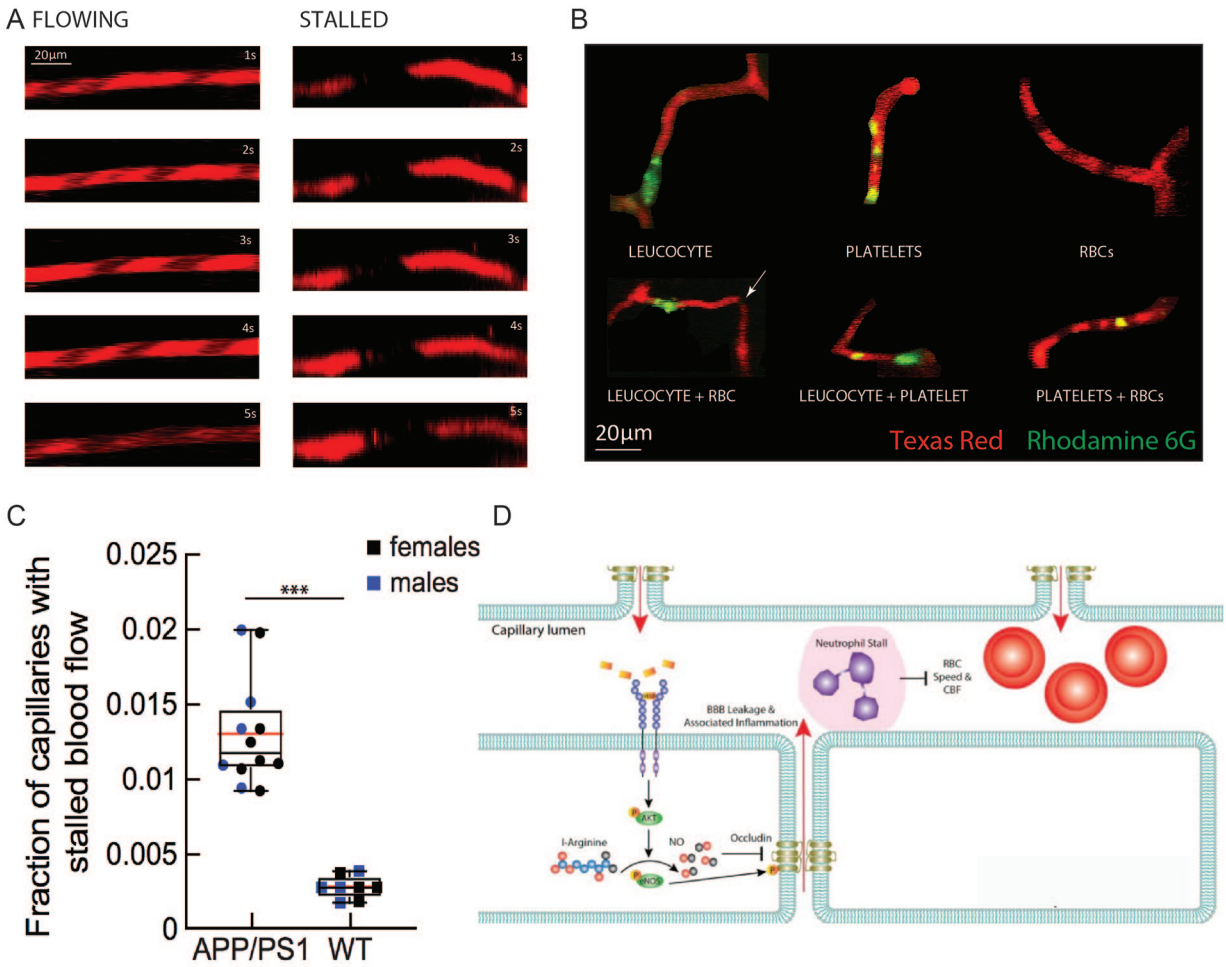

Figure 1. VEGF seeds leukocyte stalls in the brain microvasculature of APP/PS1 mice, leading to reduced cerebral blood flow. (A) Individual capillaries from in vivo 2-photon excited fluorescence microscopy image stacks. Capillaries were characterized as flowing or stalled based on the movement of unlabeled (black) red blood cells within the Texas Red labeled blood plasma (red) over the period of 5 seconds. (B) Z-projection of 2-photon excited fluorescence microscopy image stacks containing stalled capillaries labeled with Texas Red and Rhodamine 6G (green). Stalled capillaries contain a leukocyte (top left), platelet aggregates (top center), RBCs (top right), leukocytes and red blood cells (bottom left), leukocytes and platelets (bottom center), and platelets and red blood cells (bottom right). (C) Fraction of stalled capillaries in APP/PS1 (n = 12) and wild-type (WT) (n = 8) mice, ~23 000 capillaries; 2-tailed Mann-Whitney test, $P = .001$; box plot with red line representing median and black line representing mean. (D) In APP/PS1 mice amyloid-beta causes endothelial damage through reactive oxygen species, leading to increased angiogenic factor like VEGF-A. Increased VEGF-A levels leads to increased eNOS activity, downregulation of occludin, impairment of the blood-brain barrier, activation of local inflammatory markers, recruitment of leukocytes, stalling of capillary flow, and reduced cerebral blood flow. Images taken from Ali et al.[19]

Interestingly, recent evidence suggests mutations of VEGF-A protect against Alzheimer's disease.[21] Two epistatic interactions, each between VEGF-A related single nucleotide polymorphisms identified in large-scale GWAS studies (143 Alzheimer's disease cases and 180 controls), were the strongest protective factors against Alzheimer's disease in the absence of ε4 APOE allele, which remained the most significant genetic predisposition.[21] This study suggests that VEGF-A signaling may play a more significant role than previously indicated in the pathogenesis of Alzheimer's disease.

Models to Consider: Parkinson's Disease and Diabetic Retinopathy

The deleterious effects of VEGF-associated blood-brain barrier hyperpermeability seem not to be restricted to Alzheimer's disease. In post-mortem brain tissues of patients with Parkinson's disease, elevated VEGF-A and nitric oxide levels were detected at sites of astrocytic alpha-synuclein deposition.[22] Inhibition of VEGF-A blocked the deleterious effects of VEGF-A on the blood-brain barrier in a mouse model of Parkinson's disease, especially among younger mice, suggesting more beneficial effects early in disease pathogenesis.

Indeed VEGF-A inhibition is the most successful treatment for multiple ocular conditions, including diabetic retinopathy. Our hypothesis mirrors the proposed pathogenesis of diabetic retinopathy. The VEGF-A/eNOS/occludin pathway seems to drive leukocyte induced capillary stalling at the blood-retina barrier of patients with diabetic retinopathy (Figure 2A and B).[23-29] Inhibition of VEGF-A, by intravitreal injections, releases these pathognomonic obstructions, partially inhibiting the progression of diabetes-associated pathology. However, repetitive anti-VEGF-A injections are not without side effects.

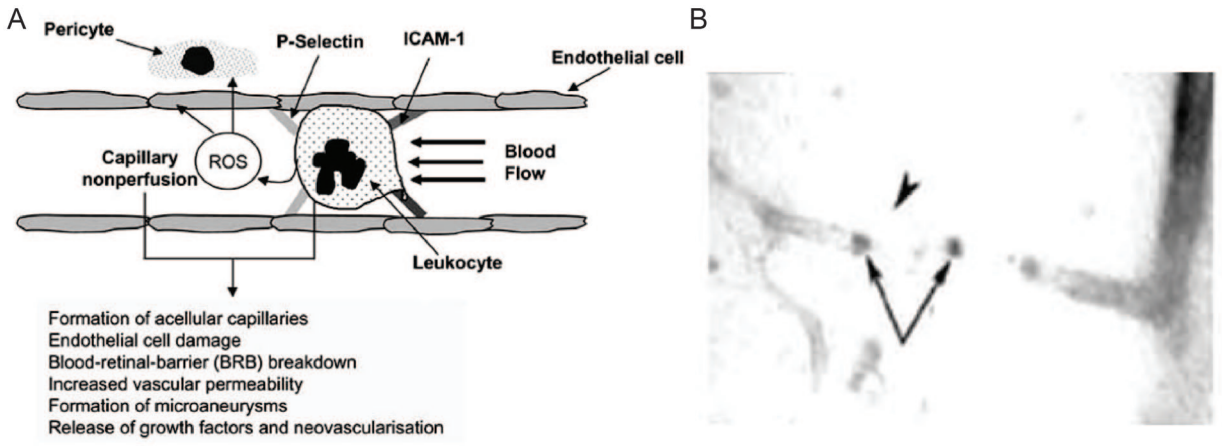

Figure 2. VEGF contributes to leukocyte stalls at the brain-retina barrier in diabetic retinopathy. (A) Schematic of increased endothelial cell adhesion of leukocytes in diabetic retinopathy. Diabetes-associated expression of adhesion molecules (ICAM-1 and P-selectin) causes increased leukocyte-endothelial interactions and stalled capillary blood flow. (B) Evidence of 2 neutrophils entrapped in a vascular segment of a diabetic monkey's retina. Images taken from Chibber et al.[24]

For example the repetitive treatment increases the risk for hypertension-induced brain hemorrhages[30] and maybe even cognitive decline.[31]

A study analyzed 175 patients undergoing anti-VEGF-A treatment for macular degeneration demonstrated those with 20 or more injections had a higher likelihood of mild cognitive impairment as calculated on an iPad-based brain health assessment.[31] In our study mice were administered at most 6 anti-VEGF-A injections over the course of 2 weeks.[19] Given the intraperitoneal mode of injection and size of the antibody used in our experiments, we largely saw effects on the vascular system rather than the brain parenchyma itself. Indeed, increased exposure to anti-VEGF-A injections could place patients at greater risk for impaired neuroprotection. Of note, those receiving 15 to 20 injections did not demonstrate a higher likelihood of cognitive impairment,[31] this could further support the need of a vascular specific VEGF-A inhibition.

One such option could be targeting the VEGF receptor-2 (VEGF-R2) specifically. VEGF-R2 is more specific for vascular VEGF signaling and VEGF-R1 seems to be more specific for neurological signaling.[32,33] In wild-type mice with microsphere-induced cortical capillary obstructions, VEGF-R2 inhibition reduced the pruning of obstructed capillaries and improved clearance of cortical capillary obstructions.[34] Indeed, in supplemental analysis of our study VEGF-R2 levels were increased in Alzheimer's mice as compared to their wild-type counterparts, whereas VEGF-R1 were decreased in the APP/PS1 mice,[19] further strengthening our hypothesis on the differential effects of VEGF-A on each side of the blood brain barrier, detrimental on one and therapeutic on the other.

Conclusion

We recently demonstrated that luminal VEGF-A signaling contributes to leukocyte stalling in brain capillaries and reduced cerebral blood flow in a mouse model of Alzheimer's disease. Targeting luminal VEGF-A signaling could address local inflammation, blood-brain barrier hyperpermeability, leukocyte recruitment, capillary stalling, and cerebral blood flow reductions. Though our findings may seem contradictory to evidence in the literature suggesting the beneficial effects of VEGF-A in Alzheimer's disease, VEGF-A may simply have a deleterious effect on the luminal side of the blood brain barrier and a therapeutic effect on the brain parenchyma. We recommend continued investigation in prospective and translational studies to further delineate a difference between luminal and parenchymal VEGF signaling in Alzheimer's disease.

Author Contributions

MA wrote the initial commentary and prepared the figures. OB guided the progress and contributed to the writing of this commentary.

ORCID iD

Oliver Bracko https://orcid.org/0000-0002-9414-6590

REFERENCES

1. Santos CY, Snyder PJ, Wu W, Zhang M, Echeverria A, Alber J. Pathophysiologic relationship between Alzheimer's disease, cerebrovascular disease, and cardiovascular risk: a review and synthesis. *Alzheimers Dement*. 2017;7:69-87.
2. Liu G, Yao L, Liu J, et al. Cardiovascular disease contributes to Alzheimer's disease: evidence from large-scale genome-wide association studies. *Neurobiol Aging*. 2014;35:786-792.
3. Dai W, Lopez OL, Carmichael OT, Becker JT, Kuller LH, Gach HM. Mild cognitive impairment and Alzheimer disease: patterns of altered cerebral blood flow at MR imaging. *Radiology*. 2009;250:856-866.
4. Wiesmann M, Zerbi V, Jansen D, et al. Hypertension, cerebrovascular impairment, and cognitive decline in aged AβPP/PS1 mice. *Theranostics*. 2017;7:1277-1289.
5. Bracko O, Cruz Hernández JC, Park L, Nishimura N, Schaffer CB. Causes and consequences of baseline cerebral blood flow reductions in Alzheimer's disease. *J Cereb Blood Flow Metab*. 2021;41:1501-1516.
6. Mokhber N, Shariatzadeh A, Avan A, et al. Cerebral blood flow changes during aging process and in cognitive disorders: a review. *Neuroradiol J*. 2021;34:300-307.
7. Nortley R, Korte N, Izquierdo P, et al. Amyloid β oligomers constrict human capillaries in Alzheimer's disease via signaling to pericytes. *Science*. 2019;365:eaav9518. doi:10.1126/science.aav9518

8. Cortes-Canteli M, Kruyer A, Fernandez-Nueda I, et al. Long-term dabigatran treatment delays Alzheimer's disease pathogenesis in the TgCRND8 mouse model. *J Am Coll Cardiol*. 2019;74:1910-1923.
9. Cruz Hernández JC, Bracko O, Kersbergen CJ, et al. Neutrophil adhesion in brain capillaries reduces cortical blood flow and impairs memory function in Alzheimer's disease mouse models. *Nat Neurosci*. 2019;22:413-420.
10. Yoon JH, Shin P, Joo J, Kim GS, Oh WY, Jeong Y. Increased capillary stalling is associated with endothelial glycocalyx loss in subcortical vascular dementia. *J Cereb Blood Flow Metab*. Published online February 9, 2022. doi:10.1177/0271678X221076568
11. Vezzani A. VEGF as a target for neuroprotection. *Epilepsy Curr*. 2008;8:135-137.
12. Gerhardt H, Golding M, Fruttiger M, et al. VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. *J Cell Biol*. 2003;161:1163-1177.
13. Tarkowski E, Issa R, Sjögren M, et al. Increased intrathecal levels of the angiogenic factors VEGF and TGF-beta in Alzheimer's disease and vascular dementia. *Neurobiol Aging*. 2002;23:237-243.
14. Thomas T, Miners S, Love S. Post-mortem assessment of hypoperfusion of cerebral cortex in Alzheimer's disease and vascular dementia. *Brain*. 2015;138:1059-1069.
15. Kim YN, Kim DH. Decreased serum angiogenin level in Alzheimer's disease. *Prog Neuropsychopharmacol Biol Psychiatry*. 2012;38:116-120.
16. Perlmutter LS, Chui HC, Saperia D, Athanikar J. Microangiopathy and the colocalization of heparan sulfate proteoglycan with amyloid in senile plaques of Alzheimer's disease. *Brain Res*. 1990;508:13-19.
17. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. *Nature*. 2000;407:249-257.
18. Kimura R, Nakase H, Tamaki R, Sakaki T. Vascular endothelial growth factor antagonist reduces brain edema formation and venous infarction. *Stroke*. 2005;36:1259-1263.
19. Ali M, Falkenhain K, Njiru BN, et al. VEGF signalling causes stalls in brain capillaries and reduces cerebral blood flow in Alzheimer's mice. *Brain*. 2022;145:1449-1463.
20. Barisano G, Montagne A, Kisler K, Schneider JA, Wardlaw JM, Zlokovic BV. Blood–brain barrier link to human cognitive impairment and Alzheimer's disease. *Nat Cardiovasc Res*. 2022;1:108-115.
21. Petrelis AM, Stathopoulou MG, Kafyra M, et al. VEGF-A-related genetic variants protect against Alzheimer's disease. *Aging*. 2022;14:2524-2536.
22. Lan G, Wang P, Chan RB, et al. Astrocytic VEGFA: an essential mediator in blood-brain-barrier disruption in Parkinson's disease. *Glia*. 2022;70:337-353.
23. Joussen AM, Poulaki V, Qin W, et al. Retinal vascular endothelial growth factor induces intercellular adhesion molecule-1 and endothelial nitric oxide synthase expression and initiates early diabetic retinal leukocyte adhesion in vivo. *Am J Pathol*. 2002;160:501-509.
24. Chibber R, Ben-Mahmud BM, Chibber S, Kohner EM. Leukocytes in diabetic retinopathy. *Curr Diabetes Rev*. 2007;3:3-14.
25. Miyamoto K, Khosrof S, Bursell SE, et al. Prevention of leukostasis and vascular leakage in streptozotocin-induced diabetic retinopathy via intercellular adhesion molecule-1 inhibition. *Proc Natl Acad Sci USA*. 1999;96:10836-10841.
26. Murakami T, Frey T, Lin C, Antonetti DA. Protein kinase cβ phosphorylates occludin regulating tight junction trafficking in vascular endothelial growth factor-induced permeability in vivo. *Diabetes*. 2012;61:1573-1583.
27. Jiang Y, Liu L, Steinle JJ. Compound 49b regulates ZO-1 and occludin levels in human retinal endothelial cells and in mouse retinal vasculature. *Investig Ophthalmol Vis Sci*. 2017;58:185-189.
28. Nakao S, Arima M, Ishikawa K, et al. Intravitreal anti-VEGF therapy blocks inflammatory cell infiltration and re-entry into the circulation in retinal angiogenesis. *Investig Ophthalmol Vis Sci*. 2012;53:4323-4328.
29. Liu X, Dreffs A, Díaz-Coránguez M, et al. Occludin S490 phosphorylation regulates vascular endothelial growth factor-induced retinal neovascularization. *Am J Pathol*. 2016;186:2486-2499.
30. Yoshimoto M, Takeda N, Yoshimoto T, Matsumoto S. Hypertensive cerebral hemorrhage with undetectable plasma vascular endothelial growth factor levels in a patient receiving intravitreal injection of aflibercept for bilateral diabetic macular edema: a case report. *J Med Case Rep*. 2021;15:403.
31. Ray SK, Manz SN. Brain health assessment in macular degeneration patients undergoing intravitreal anti–vascular endothelial growth factor injections (The Bham Study). *Retina*. 2021;41:1748-1753.
32. Harris R, Miners JS, Allen S, Love S. VEGFR1 and VEGFR2 in Alzheimer's disease. *J Alzheimers Dis*. 2018;61:741-752.
33. Zhang Z, Neiva KG, Lingen MW, Ellis LM, Nör JE. VEGF-dependent tumor angiogenesis requires inverse and reciprocal regulation of VEGFR1 and VEGFR2. *Cell Death Differ*. 2010;17:499-512.
34. Reeson P, Choi K, Brown CE. VEGF signaling regulates the fate of obstructed capillaries in mouse cortex. *eLife*. 2018;7:e33670. doi:10.7554/eLife.33670