

Hypothesis

Alzheimer's disease and angiogenesis

Anthony H Vagnucci Jr, William W Li

Despite enormous investigative efforts, the pathological basis for Alzheimer's disease remains unclear. Suggested mechanisms for the disorder include cerebral hypoperfusion, inflammation, gene polymorphisms, and molecular lesions in the brain. In this Hypothesis, we argue that the vascular endothelial cell has a central role in the progressive destruction of cortical neurons in Alzheimer's disease. In Alzheimer's disease, the brain endothelium secretes the precursor substrate for the β -amyloid plaque and a neurotoxic peptide that selectively kills cortical neurons. Large populations of endothelial cells are activated by angiogenesis due to brain hypoxia and inflammation. Results of epidemiological studies have shown that long-term use of non-steroidal anti-inflammatory drugs, statins, histamine H2-receptor blockers, or calcium-channel blockers seems to prevent Alzheimer's disease. We think this benefit is largely due to these drugs' ability to inhibit angiogenesis. If Alzheimer's disease is an angiogenesis-dependent disorder, then development of antiangiogenic drugs targeting the abnormal brain endothelial cell might be able to prevent and treat this disease. We suggest several laboratory and clinical approaches for testing our hypothesis.

Alzheimer's disease is one of the most common diseases of modern society. Affecting 10% of the world's population, this progressive neurodegenerative disorder causes untold human suffering and consumes more than US\$100 billion per year in health-care costs. Although the amyloid plaque, which contains among other elements, β -amyloid peptide fragments, has been identified as a primary pathological lesion of Alzheimer's disease, how these plaques form in the brain remains unclear. Heredity, gene polymorphisms, cerebral hypoperfusion, brain inflammation, and molecular lesions have all been suggested as potential mechanisms.¹ However, an integrated understanding of the disease with clear recommendations for interventions is lacking. Consequently, treatments are restricted to ameliorating the symptoms of dementia by increasing brain levels of acetylcholine with drugs such as tacrine, donepezil, rivastigmine, or galantamine.

Results of epidemiological studies suggest that chronic use of certain drugs significantly decreases the risk of Alzheimer's disease in high-risk populations (table 1).²⁻⁹ Such drugs include non-steroidal anti-inflammatory agents (NSAIDs), lipid-lowering statins, histamine H2-receptor blockers, and calcium-channel blockers. The clinical data are very persuasive. For example, results of a study of 6989 patients who did not have dementia at baseline showed that the relative risk of Alzheimer's disease fell to 0.20 with long-term (≥ 2 -year) NSAID use.² The results of at least 17 epidemiological studies from nine countries corroborate these results, including the original observations.^{3,6} Hence, brain inflammation has become a major focus for Alzheimer's disease research.

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Reference	Class of drug	Relative risk reduction
Breitner and colleagues ⁵	NSAIDs or steroids	0.24
Breitner and colleagues ³	H2 blockers	0.14
McGeer and colleagues ⁶	NSAIDs	0.50
Stewart and colleagues ⁷	Aspirin	0.74
Forette and colleagues ⁴	Calcium-channel blockers	0.50
Wolozin and colleagues ⁸	Lipid-lowering agents	0.60
Jick and colleagues ⁹	Lipid-lowering agents	0.29
in't Veld and colleagues ²	NSAIDs	0.20

Table 1: Evidence for drugs that reduce the risk of Alzheimer's disease with long-term use

Brain inflammation cannot, however, explain the risk reduction conferred by drugs that lack substantial anti-inflammatory activity. We have noted that putative Alzheimer's disease-preventive agents (table 2) inhibit angiogenesis, which led us to consider the role of the brain vascular endothelial cell. Endothelial cells respond to both hypoxia and inflammation by undergoing angiogenesis. Mediated by cytokine growth factors, this process involves the activation of endothelial cells from pre-existing venules to form tubular networks that augment the local microcirculation by bringing oxygen and nutrients to compromised tissue. The endothelium also exerts direct local effects by producing at least 20 paracrine factors that act on adjacent cells. Although many of these factors are antiapoptotic survival signals, microvessels in diseased tissues also secrete toxic substances including neurotoxins and amyloid precursors.^{10,11}

Agent	Anti-inflammatory activity	Antiangiogenic activity
Lovastatin	-	+
Simvastatin	-	+
Pravastatin	-	+
Sulindac	+	+
Diclofenac	+	+
Indometacin	+	+
Aspirin	+	+
H2 blocker	-	+
Nitrendipine	-	+
Nimodipine	-	+

+ and - denote the biological activity of each agent.

Table 2: Drugs associated with decreased risk of Alzheimer's disease or decreased formation of β -amyloid peptide

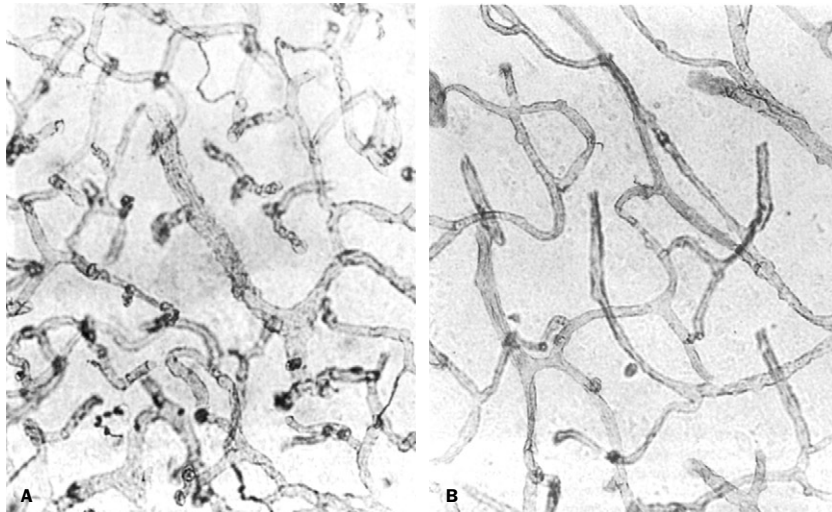


Figure 1: Microvascular density in brains in Alzheimer's disease (A) and in normal age-matched controls (B)

Vascular basement membranes are shown by immunohistochemical staining of heparan sulphate proteoglycan. Reprinted from reference 14. Copyright (1990), with permission from Elsevier Science.

Hypothesis

We propose that Alzheimer's disease is mediated by pathological angiogenesis. Neovascularisation in the brain in Alzheimer's disease occurs in response to impaired cerebral perfusion (oligaemia) and vascular injury (inflammation). Morphological and biochemical evidence for this process include regionally increased capillary density, vascular loop formation, glomeruloid vascular structure formation, and expression of angiogenic factors: vascular endothelial growth factor (VEGF), transforming growth factor β (TGF β), and tumour necrosis factor α (TNF α) (figure 1).¹²⁻¹⁵ We suggest that angiogenic activation of the brain endothelium in Alzheimer's disease leads to deposition of the β -amyloid plaque and secretion of a neurotoxic peptide that kills cortical neurons.

Alzheimer's disease is linked with the microcirculation. Ultrastructural studies have shown that brain microvessels are closely associated with β -amyloid plaques, and that Alzheimer's disease brain capillaries contain preamyloid deposits.¹⁶ The β -amyloid plaque generates reactive oxygen species that damage brain endothelium.¹⁷ A thrombogenic region develops in the vessel wall, leading to intravascular accumulation of thrombin. Thrombin activates vascular endothelial cells to secrete amyloid precursor protein via a receptor-mediated, protein kinase C-dependent pathway.^{11,18} Progressive deposition of amyloid precursor protein leads to accumulation of the β -amyloid plaque, which generates more reactive oxygen species and induces further endothelial damage. Thrombin accumulates and stimulates even more angiogenesis and production of amyloid precursor protein. We postulate that this cycle of endothelial-dependent events contributes to β -amyloid accumulation in the brain of people with Alzheimer's disease and to neuronal death (figure 2).

A second mechanism of angiogenic injury in Alzheimer's disease is the secretion of a soluble, neuroselective peptide toxin that kills primary cortical and cerebellar granular neurons. This neurotoxin is secreted in large quantities from microvessels taken from brains of Alzheimer's disease patients, by contrast with much smaller quantities secreted by vessels from brains of elderly people without dementia.¹⁰ Brain microvessels of young healthy patients do not secrete this toxin. Thus, compensatory attempts to neovascularise hypoxic regions in the brain in Alzheimer's disease promote deleterious endothelial-mediated neuronal killing, giving new meaning to the term pathological angiogenesis. The genetic or epigenetic factor or factors predisposing subgroups of elderly patients to this harmful vascular phenotype remain unknown.

How does angiogenesis occur in the brain in Alzheimer's disease? At least five overlapping mechanisms drive this process (figure 3).

- Hypoperfusion in the elderly brain leads to hypoxia, a stimulus that induces expression of vasoactive mediators such as nitric oxide, hypoxia-inducible-factor-1 α (HIF 1 α), and VEGF—one of the most potent angiogenic cytokines. Increased VEGF expression is seen in reactive astrocytes and perivascular deposits of Alzheimer's disease patients.¹⁹
- The neurofibrillary tangles of Alzheimer's disease, thought to be secondary to β -amyloid accumulation, contain heparan sulphate proteoglycans, a substrate that binds avidly to basic fibroblast growth factor (bFGF), another angiogenic cytokine.^{1,20}
- Thrombin itself directly stimulates angiogenesis in regions of injured vascular endothelium.¹⁸
- Inflammatory mediators found in brains in Alzheimer's disease, such as TNF α , interleukin 6, and monocyte chemoattractant protein-1, stimulate angiogenesis.²¹ In

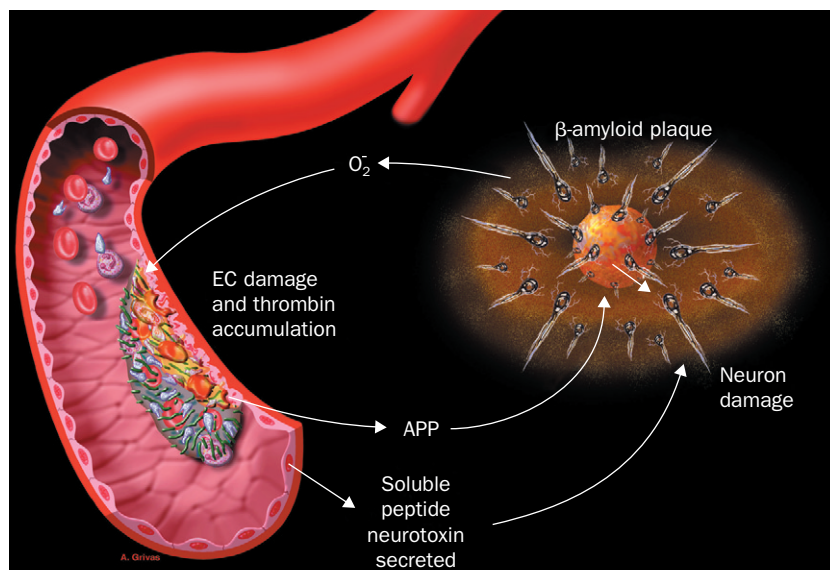


Figure 2: How the endothelium damages the brain in Alzheimer's disease
APP=amyloid precursor protein. EC=endothelial cell. Reprinted with permission from the Angiogenesis Foundation.

Alzheimer's disease, these factors may be induced by neuronal death, by β -amyloid binding of the C1q component of the complement cascade, and by peroxidative and free radical injury of microvessels, among other mechanisms. Invading macrophages and monocytes also release the angiogenic growth factors VEGF, bFGF, and platelet-derived growth factor (PDGF).

- The gene expression of an endogenous angiogenesis inhibitor, thrombospondin, is reduced near focal Alzheimer's disease lesions, leading to a proangiogenic state in those sites.²²

These redundant stimuli for neovascularisation bear remarkable similarity to the plethora of signals leading to tumour angiogenesis in cancer.²³

Why does angiogenesis lead to plaque formation in the brain in Alzheimer's disease but not with multi-infarct vascular dementia? We speculate that the brain endothelium in Alzheimer's disease possesses unique genotypic and phenotypic features not present in other brains. Such endothelial heterogeneity is seen in comparison of abnormal with normal tissues.²⁴ Therefore, whereas angiogenesis occurs in response to brain ischaemia and inflammation in both Alzheimer's disease and stroke patients, distinct pathological changes result in Alzheimer's disease.

Our hypothesis explains the puzzle posed by seemingly unrelated drugs that confer protection against Alzheimer's disease. Anti-inflammatory drugs, H₂-receptor blockers, antihypertensives, and statins can all inhibit angiogenesis.²⁵⁻²⁷ We propose that the substantial reduction in the risk of Alzheimer's disease noted in the results of population-based studies is predominantly due to the antiangiogenic actions of these drugs on the endothelial cell. This mechanism does not, of course, exclude other potential mechanisms of drugs, such as NSAIDs or statins, which might also directly suppress neuronal production of β -amyloid plaque. Many of these agents might possess relatively small antiangiogenic effects compared with the potent and specific angiogenesis inhibitors in clinical development for the treatment of cancer, retinopathies, and psoriasis.²³

Although Alzheimer's disease is without doubt a complex and multifactorial disorder, we think that brain angiogenesis should become a new focus for basic and clinical investigation. Even as researchers continue to unravel the mechanisms behind Alzheimer's disease, patients at high risk for the disease might benefit from the judicious use of commonly-used drugs which possess antiangiogenic activity.

Testing the hypothesis

The role of angiogenesis in Alzheimer's disease can be tested in laboratory and clinical studies. A transgenic mouse (presenilin/amyloid precursor protein) model for Alzheimer's disease induces amyloid deposition, microglia and astrocyte activation, and brain inflammation.²⁸ In this system, angiogenesis markers (such as VEGF or $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrins) can be studied by immunohistochemistry, and temporally and spatially correlated with β -amyloid deposition and neuronal death. Antiangiogenic agents can

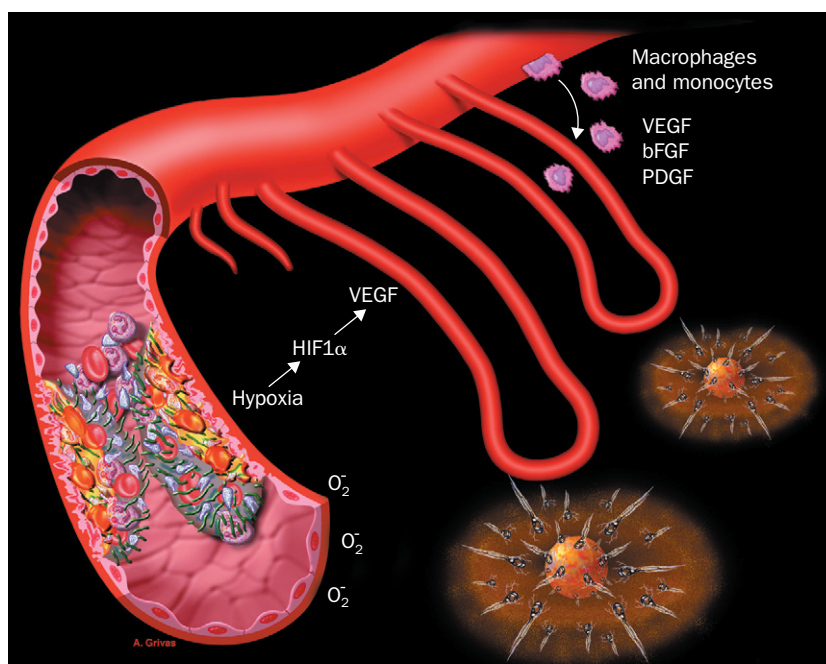


Figure 3: **Angiogenesis in the brain in Alzheimer's disease**

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be administered to investigate whether pathological features are ameliorated. Conversely, animals can be transfected with an adenovirus encoding the gene for VEGF to establish whether angiogenesis stimulation accelerates pathological changes related to Alzheimer's disease.

The microvessel neurotoxin can be studied by examination of endothelial cells grown in tissue culture that were derived from Alzheimer's disease brain tissue. Does the addition of angiogenic growth factors, such as bFGF or VEGF, increase neurotoxin secretion by endothelial cells? Can antiangiogenic drugs suppress neurotoxin production? Genomic studies of Alzheimer's disease-derived endothelium could identify genes that are uniquely expressed in Alzheimer's disease, which could be new molecular targets for therapy.²⁴

Clinical prevention studies can be done in patients at high risk for Alzheimer's disease by use of angiogenesis inhibitors. Many oral antiangiogenic agents are undergoing oncological trials, such as thalidomide, AE-941, PTK787, endostatin, and BMS275291.²³ A randomised, prospective, double-blinded trial of these agents can be done in an Alzheimer's disease prevention trial. Patients receiving an antiangiogenic drug would be expected to have a lower incidence of Alzheimer's disease, compared with a placebo control group. Intervention trials could be done with the expected endpoint of disease stabilisation. A drug could exist that provided protection against both Alzheimer's disease and cancer. Such trials would be lengthy and complex to manage, and therefore require strong supportive preclinical evidence from laboratory studies. The sheer magnitude of Alzheimer's disease in the ageing population, however, should provide incentive for researchers, clinical investigators, and industry.

Conflict of interest statement
None declared.

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