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VASCULAR OXIDATIVE STRESS IN ALZHEIMER DISEASE

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Abstract

Alzheimer disease and cerebrovascular dementia are two common causes of dementia and, by present diagnostic criteria, are mutually exclusive using vascular pathology as an arbitrary demarcation in differential diagnosis. However, evidence from epidemiological, neuropathological, clinical, pharmacological, and functional studies suggest considerable overlap in risk factors and pathological changes suggesting shared common pathogenic mechanisms between these two diseases such that vascular factors play a vital role in the pathogenesis of Alzheimer disease. A high energy demand and lack of an endogenous fuel reserve make the brain highly dependent upon a continuous blood supply where disruption of cerebral blood vessels and blood flow can have serious consequences on neural activities. Indeed, many studies implicate metabolic defects in Alzheimer disease, such a reduced brain metabolism is one of the best documented abnormalities in the disease. Notably, since endothelial reactive oxygen species such as nitric oxide act as vasodilators at low concentrations, increased production coupled with elevated reactive oxygen species scavenging of nitric oxide, can lead to reduced bioavailability of nitric oxide and increased oxidative stress that damage sensitive vascular cells. In this respect, we and others have demonstrated that oxidative stress is one of the earliest pathological changes in the brain of Alzheimer disease patients and plays a critical role in the vascular abnormalities underlying metabolic defects in Alzheimer disease. Here, we discuss vascular factors in relation to Alzheimer disease and review hypoperfusion as a potential cause by triggering mitochondrial dysfunction and increased oxidative stress initiating the pathogenic process.

Keywords

Alzheimer disease; hypoperfusion; mitochondria; nitric oxide; nitric oxide synthase; oxidative stress; vascular abnormalities

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Vascular Abnormalities in Alzheimer disease

Cerebrovascular function declines with aging as evidenced by declines in cerebral blood flow (CBF), loss of endothelial mitochondria, a thickening of the vascular basement membrane, and an increase in degenerative pericytes in the elderly [1]. A more pronounced decline is present in cases of Alzheimer disease (AD) with: reduction in number of cerebral microvessels, significant aberrations in capillary walls including decreased capillary diameters and increased capillary densities [2,3], agrin deposition in capillaries suggestive of the thinning and fragmentation of the basal lamina [4], atrophy of smooth muscle cells in cerebral vessels and attenuation of capillary endothelium resulting in the rupture of the vessel wall and intracerebral bleeding [5]. Resting CBF is reduced and the increase in CBF stimulated by neural activity is attenuated [6] and several factors that may ameliorate AD have either been associated with improved CBF or prevent CBF decline [7]. Several morphometric features of blood brain barrier (BBB) dysfunction in patient with pathologically confirmed AD have also been reported [8]. The association of serum amyloid P component, a protein not synthesized in the brain, with senile plaques and neurofibrillary tangles (NFT), supports the notion of BBB leakage in AD [9]. Amyloid-β (Aβ) deposits, one of the prominent features of AD, are found in cortical and subcortical gray matter and in meningeal and gray matter blood vessels [10,11], and the source of this Aβ is likely vascular endothelial cells and smooth muscle cells rather than neurons since endothelial cells and smooth muscle cells show abundant amyloid-β protein precursor (AβPP) immunoreactivity [12,13]. The frequent deposition of Aβ in arteriolar media and collagen deposition in the adventitia in AD may cause a narrowing of the lumen of cerebrovessels, even in the absence of overt endothelial injury, not mentioning the vasoconstriction effect of soluble Aβ [14]. Therefore, perivascular Aβ deposits may be a risk factor for reduced regional CBF [7]. Ultrastructural studies on blood vessels associated with Aβ deposits have shown their intermittent association with membrane abnormalities of smooth muscle cells [5]. Indeed, in AD cases with a clinical history of cerebral bleeding, the muscle layer is sometimes completely replaced by Aβ deposits, suggesting that the vascular system may be an initiator for the development of disease [12,13]. That said, the contribution of amyloid angiopathy in cases that do not progress to AD is less clear.

Vascular Oxidative Stress in Alzheimer Disease

Reactive oxygen species (ROS) are generated at sites of injury and/or inflammation. At low levels, ROS can function as signaling intermediates in the regulation of fundamental cell activities such as growth and adaptation responses, however, at higher concentrations, ROS cause cell injury and death. The vascular endothelium, neurons and glia are all able to synthesize, store and release ROS and vasoactive substances in response to certain stimuli, especially those by chronic hypoxia/hypoperfusion. The vascular endothelium, which regulates the passage of macromolecules and circulating cells from blood to tissue, is a major target of oxidant stress, playing a critical role in the pathophysiology of several vascular diseases [15, 16]. Specifically, accumulated oxidative stress: 1) interferes with nitric oxide (NO) function and endothelial relaxation; 2) increases vascular endothelial permeability and promotes leukocyte adhesions; 3) leads to alterations in endothelial signal transduction, redox-regulated transcription factors and reduced CBF [16].

It is well-characterized that there are increased regional levels of oxidative stress in the AD brain [17,18]. For example, recent studies have demonstrated a decline in polyunsaturated fatty acids (PUFA) [19,20], increased levels of lipid peroxidation markers [19,21,22], as well as protein oxidation [23,24], DNA oxidation [25–27] and RNA oxidation [28–31] during AD. Additionally, the presence of oxidative stress markers such as advanced glycation end products (AGE), glycoxidative end products, e.g., N^{ε}-carboxymethyllysine and lipid peroxidation adducts are detected in both NFT and senile plaques in AD [21–24,32–35]). Notably, similar

increases in oxidative stress also occur systemically in AD. Lower plasma antioxidant levels and alterations in antioxidant enzyme activities are reported in mild cognitive impairment (MCI) patients and patients at early AD stages [19,36–39] suggesting a systemic imbalance between ROS production and antioxidant defense systems in the plasma of AD patients and this is substantiated by increases in DNA, lipid, and protein oxidation products found in blood and cerebrospinal fluid (CSF) obtained from AD patients in comparison with controls [35, 40,41]. Reflecting such a systemic oxidative imbalance in AD, we also found oxidative damage in olfactory neurons and the surrounding epithelial cells from AD donors [42], and another group reported increased 8-hydroxy-deoxyguanosine (8OHdG) in the DNA of lymphocytes from AD donors [43], which inversely correlated with the plasma levels of several antioxidant carotenoids [44].

Interestingly, our recent finding demonstrated ultrastructural features of vascular lesions and mitochondria in brain vascular wall cells from human AD brain biopsy are also suggestive of oxidative damage [45,46]. *In situ* hybridization using mitochondrial DNA (mtDNA) probes for human wild type, 5kb deleted and mouse mtDNA and immunocytochemistry using antibodies against AβPP, 8-hydroxy-2′-guanosine (8OHG) and cytochrome C oxidase subunit 1 (COX) revealed similar ultrastructural localization [45,46]. As expected, there was a higher degree of amyloid deposition in the vascular walls in AD compared to aged-matched controls [45,46] and in addition, vessels with more severe lesions showed immunopositive staining for AβPP and contained large, lipid-laden vacuoles in the cytoplasm of endothelial cells. Significantly more mitochondria abnormalities were seen in microvessels where lesions occurred [45,46]. *In situ* hybridization using wild and chimera (5 kB) mtDNA probes revealed positive signals in severely damaged mitochondria from the vascular endothelium and in the perivascular cells of lesioned microvessels close to regions of large amyloid deposition. These features were absent in undamaged regions of human AD tissues or in age-matched control subjects. Importantly, vessels with atherosclerotic lesions revealed endothelium and perivascular cells possessing clusters of wild and deleted mtDNA-containing positive probes that were associated with increased amounts of immunoreactive AβPP, 8OHG and COX in the same cellular compartment [45,46]. Our observations demonstrate that vascular wall cells, especially their mitochondria, appear to be a central target for oxidative stress-induced damage before the development of AD pathology [45,46]. Notably, long-term ischemia/reperfusion leads to disintegration of mitochondria ultrastructure [47,48] and apoptosis of degenerating neurons occurs in association with the accumulation of perikaryal abnormal mitochondria and oxidative damage to the nucleus [49]. Likely not coincidentally, this same pattern of mitochondria lesions is observed in human AD brain biopsy samples [50].

Nitric Oxide Malfunction and Vascular Oxidative Stress in Alzheimer Disease

The vascular endothelial cells transduce circulatory stimuli to the arterial wall leading to the regulation of vascular tone, haemostasis, blood pressure and vascular remodeling through the synthesis and release of vasoactive NO [51,52]. NO is synthesized by the conversion of L-arginine to L-citrulline by the enzyme of nitric oxide synthase (NOS) which exists in three isoforms in brain: eNOS localized in endothelial cells; nNOS localized in neurons; and inducible NOS (iNOS) induced in neurons and glial cells after cytokine activation [51,52]. Due to its vascular effect, NO, at physiological level, improves tissue perfusion and exert a protective action and reduced bioavailability of NO is thought to be one of the central factors common to vascular diseases. However, overproduction, either by activation of nNOS by excitatory [53], or by induction of iNOS in glial, vascular or blood cells [54–56] might be deleterious, which is especially true if combined with concurrent increases in free radical production. For example, excess NO production is found during excitotoxicity, inflammation and ischemia reperfusion injury [57] and it is known that NO can react with superoxide and the reaction is approximately six-times faster than the dismutation of superoxide by superoxide

dismutase (SOD) [58]. Therefore, if the levels of NO or superoxide increase sufficiently, NO is able to outcompete SOD which leads to dual effects of scavenging NO and thereby actually reducing its bioavailability (ROS scavenging), but also of producing the potent oxidant peroxynitrite. Once formed, peroxynitrite can nitrate tyrosine residues to form nitrotyrosine. Additionally, substantial amounts of ONOO- can be protonated at physiological pH to form peroxynitrous acid, a strong oxidant itself, which in turn can yield the highly reactive OH•, a much more powerful oxidant that will readily react with any biological molecules it meets [51,52].

Increased nitrotyrosine is found in astrocytes, blood vessels and the neuronal cytoplasm of the cerebral cortex within regions of neurodegeneration in AD, yet it is undetectable in corresponding control regions [24,59,60]. There is also a significant two- to three-fold increase in the lipid nitration product, 5-nitro-γ-tocopherol, in affected regions of the brain in AD, suggesting NO is a significant contributor to lipid oxidation [61]. The widespread occurrence of nitrotyrosine immunoreactivity [24,59,60] suggests that chronic oxidative damage is not restricted to long-lived polymers such as NFTs, but instead, reflects a generalized oxidative stress contributing to the pathogenesis of AD. Consistent with these observations, aberrant expression of all isoforms of NOS and some related proteins is observed in AD. Dimethylargininase, primarily expressed in tissues containing the constitutive forms of NOS, like brain, kidney, and endothelium [62,63], regulates NO production by hydrolyzing free methylated arginine derivatives (effective endogenous inhibitors of NOS) [64]. The expression of dimethylargininase is dramatically increased in AD [65]. However, there are controversial reports regarding asymmetric dimethylarginine levels in AD [66,67]. The mRNA and protein levels of the enzyme argininosuccinate synthetase, the rate limiting enzyme in the metabolic pathway leading from L-citrulline to L-arginine (the physiological substrates of NOS), are significantly higher in glial cells of AD brain [68,69]. Metabolism of tetrahydrobiopterin (BH_4), an essential cofactor for NOS, is disturbed in AD patients [70,71], which will lead to the “uncoupling” of NOS favoring the production of superoxide anion and hydrogen peroxide [72]. Large and small mutipolar and pyramidal neurons demonstrate increased nNOS levels over the entire chronic AD evolution [72,73]. eNOS levels are also increased in AD brain and colocalize with nitrotyrosine [59,60]. The expression of iNOS is found in a variety of cells in response to lipopolysaccharides, certain cytokines and ROS generators [74]. Because iNOS produces much greater amounts of NO than either eNOS or nNOS [75], it is an important mediator of cytotoxicity in the brain. It is consistently reported by various groups that there is increased iNOS in glial cells and a subset of pyramidal neurons in AD [59,68,69,76–78]. Colocalization of argininosuccinate synthetase and iNOS is detectable in these cells, coupled with increased expression levels, suggesting high output of NO production [69] Genetic ablation of iNOS substantially protects AD-transgenic mice from premature mortality, cerebral plaque formation, amyloid-load, protein tyrosine nitration, astrocytosis and microgliosis [79]. Despite the consistent evidence of increased NOS expression in AD, the steady level of NO in the plasma of AD patient is actually decreased, suggestive of reduced bioavailability of NO underlying reduced cerebral blood flow [66]. Given that protein nitration is a non-crosslink-related oxidative modification which indicates more recent active modifications, the widespread nitrative modifications and decreased steady level of NO in AD actually highlights a critical pathogenic role of increased production coupled with elevated ROS scavenging of NO which leads to a net outcome of reduced bioavailability of NO and increased oxidative stress.

Vascular Risk Factors, Vascular Oxidative Stress and Alzheimer Disease

There are many common underlying risk factors that play key roles in the development of vascular diseases and AD including the presence of apolipoprotein E4 (ApoE) allele, hyperhomocysteinemia, diabetes mellitus, atherosclerosis and hypertention [80]. Almost all of

these factors are associated with vascular oxidative stress and/or vascular NO malfunction. For example, there is an isoform specific difference for ApoE in microglial NO production [81–83]: Mice expressing the ApoE4 protein isoform have a greater NO production than mice expressing the ApoE3 protein isoform and both neurons and microphages from ApoE4 transgenic mice exhibit a similar increase in the uptake of arginine, the sole substrate for NOS, over that from ApoE4 mice. Elevated cerebral oxidative stress has been observed in AD individuals carrying the ε4 alleles. Diabetes mellitus also appear to cause NO malfunction at least partly through enhancement of oxidative stress: advanced glycation products, accumulated in diabetic tissues [84], are toxic to endothelial cells which may lead to uncoupling of endothelial NO synthase such that it generates superoxide anion in addition to NO. Also, hyperglycemia and hyperinsulinemia increase both superoxide and hydrogen peroxide production [85,86] that enhances ROS scavenging of NO. Similarly, hyperhomocysteinemia can elicit mitochondrial damage that leads to increased ROS production as well as directly scavenge NO by forming S-nitrosohomocysteine [87].

Potential Sources of Vascular Oxidative Stress in Alzheimer Disease

The enzymatic origin of superoxide could potentially involve NAD(P)H oxidase, xanthine oxidase, lipoxygenase, NOS and also respiratory chain enzymes in the mitochondria, which all demonstrate alterations in AD. NAD(P)H oxidases are implicated in vascular oxidative stress associated with various vascular conditions such as hypertension and hyperhomocysteinemia and NADPH oxidase is significantly activated in AD brain [88]. Lipoxygenase enzymes by oxidizing polyunsaturated fatty acids synthesize hydroperoxyacids, which are potent pro-oxidant mediators [89,90]. Levels of 12/15 lipoxygenase as well as their metabolic products are significantly elevated in AD brain [88]. Mitochondrial dysfunction is a key step in AD progression. Damaged mitochondria are less efficient producers of ATP but more efficient producers of ROS. There is ample evidence suggesting mitochondrial abnormalities in AD brain as discussed earlier [91].

Hypoperfusion as a Potential Cause of Alzheimer Disease

Neuroimaging studies supports the notion that in MCI patients who later converted to AD, the presence of temporoparietal (including hippocampal) hypoperfusion, hippocampal-parahippocampal hypoperfusion, and posteriorcingulate hypoperfusion distinguish this population group from other groups suggesting that hypoperfusion is a very early feature during the development of AD [92–97]. Similarly, there are profound alterations in the regulation of the cerebral circulation in AβPP transgenic mice at a very early age which is well before other pathological changes [98]. It is likely that hypoperfusion plays a critical role in the pathogenesis of AD. de la Torre [99] proposed that advanced aging with a comorbid condition, such as a vascular risk factor, which further decreases cerebral perfusion, promotes a critically attained threshold of cerebral hypoperfusion (CATCH). Clearly, mitochondria in vulnerable cells almost always show signs of damage during ischemia [100]. Importantly, chronic reductions in cerebral flow of a magnitude thought to be harmless to neurons (i.e., reduced by 25~50%) induced disorganization of the CA1 sector where neurons demonstrate increased lipofuscin pigments, suggestive of mitochondrial abnormalities [101]. Therefore, it is likely that chronic hypoperfusion will trigger mitochondrial damage/dysfunction in vascular cells which, in turn, will enhance the production of ROS. The accumulation of ROS scavenges NO, impairs endothelial barrier function and promotes leukocyte adhesion, induces alterations in normal vascular function and results in further decreased CBF. Because glucose is the main fuel of brain cells, suboptimal delivery of energy substrates to neuronal tissue due to decreased CBF, together with a deficient delivery of oxygen, compromises neuronal stability because the supply for aerobic glycolysis fails to meet brain tissue demand and hypometabolism ensues. Clearance of Aβ through the vascular pathway is likely also impaired that may lead to its

deposition in the brain parenchyma. Sustained hypoperfusion, and then oxidative stress of brain tissues, could also stimulate secondary damage via the overexpression of inducible and neuronal specific nitric oxide synthase (NOS: iNOS and nNOS, respectively) in brain cells. It is possible that continuous accumulation of oxidative stress products, such as peroxynitrite accumulation (via the overexpression of the iNOS and/or nNOS), appear to be secondary and accelerating factors for damage and for compromising the blood brain barrier (BBB) in hypoxia/hypoperfusion or AD. All of these alterations probably contribute to the progressive cognitive decline characteristic of patients with AD, and regional anatomic pathology, consisting of synaptic loss, senile plaques, and NFT.

Conclusions

Vascular cells are sensitive to oxidative stress and it is likely that oxidative stress plays a critical role leading to vascular abnormalities in AD. We suspect that chronic vascular hypoperfusion is a central initiating factor for vascular alterations by inducing mitochondrial dysfunction, increasing ROS production, reducing NO bioavailability via ROS scavenging, and damaging vascular functions as well as severely affecting regional CBF which ultimately leading to cognition decline and the disease.

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Abbreviations

AGE
advanced glycation end products

AD
Alzheimer disease

Aβ
amyloid-β

AβPP
amyloid-β protein precursor

ApoE
apolipoprotein E

BBB
blood brain barrier

CBF
cerebral blood flow

CSF
cerebrospinal fluid

CATCH
a critically attained threshold of cerebral hypoperfusion

COX
cytochrome C oxidase subunit 1

8OHG
8-hydroxy-2β-guanosine

8OHdG
8-hydroxy-deoxyguanosine

MCI
mild cognitive impairment

mtDNA
mitochondrial DNA

NFT
neurofibrillary tangles

NO
nitric oxide

PUFA
polyunsaturated fatty acids

ROS
reactive oxygen species

SOD
superoxide dismutase