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Review

The Effects of Impaired Cerebral Circulation on Alzheimer's Disease Pathology: Evidence from Animal Studies

Alcibiades E. Villarreal[a,b,*], Rachel Barron[c], K.S. Rao[a] and Gabrielle B. Britton[a]
[a]*Center for Neuroscience, INDICASAT AIP, City of Knowledge, Republic of Panama*
[b]*Department of Biotechnology, Acharya Nagarjuna University, Guntur, India*
[c]*Department of Psychology and Program in Neuroscience, Lafayette College, Easton, PA, USA*

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Abstract. Persistent systemic hypoxia, a direct consequence of alterations in vascular function, can compromise the brain by increasing the risk of developing dementias such as Alzheimer's disease (AD). Vascular contributions to cognitive impairment and AD in aged individuals are common, and several vascular risk factors for AD are linked to hypoxia. Clinical evidence confirms that structural and functional changes characteristic of AD pathology also occur following hypoxic-ischemic events such as stroke and traumatic brain injury. Studies with transgenic and non-transgenic mouse models reliably show that hypoxia increases the levels of amyloid-β peptides that form the characteristic plaques in AD brains. Moreover, some studies suggest that vascular lesions also promote tau phosphorylation, modulate apolipoprotein E expression, and have more profound in effects in aged animals, but additional evidence is needed to establish these findings. Although the mechanisms underlying hypoxia-related effects remain unclear, controlled animal studies continue to reveal mechanistic aspects of the relationship between hypoxia and AD pathology that are necessary for therapeutic developments. The present review summarizes evidence from rodent studies regarding the effects of hypoxia on AD-related pathology and evaluates its impact on understanding human disease.

Keywords: Amyloid-β, apolipoprotein E, cerebral amyloid angiopathy, cerebral hypoxia, ischemia, tau protein

INTRODUCTION

Alzheimer's disease (AD) is one of the most prevalent neurodegenerative diseases associated with aging. The majority of AD cases manifest as a late onset sporadic form, accounting for more than 95% of cases, but genetically the disease is divided into familial and sporadic cases [1]. Familial AD is caused by mutations in the amyloid-β protein precursor (AβPP) and presenilin 1 and 2 genes [2]. Risk factors for sporadic AD include age, ApoE ε4 polymorphism, hypercholesterolemia, hypertension, diabetes mellitus, stroke, brain trauma, and obesity, among others [1]. The two main pathological hallmarks of AD are accumulation of amyloid-β (Aβ) plaques in brain tissue and in the walls of the small brain arteries and hyperphosphorylated tau filaments that aggregate as neurofibrillary tangles (NFTs). Aβ plaques and NFTs lead to cell and synaptic dysfunction and ultimately result in cognitive and functional deterioration. AD is frequently accompanied by vascular pathology, and various mouse models of AD have been employed in investigations of how alterations in vascular function impact AD-related processes, primarily those related

*Correspondence to: Alcibiades E. Villarreal, Center for Neuroscience and Clinical Research Unit, INDICASAT AIP, Apartado 0843-01103, Panamá, República de Panamá. Tel.: +507 517 0735; Fax: +507 507 0000; E-mail: avillarreal@indicasat.org.pa.

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to the expression of Aβ. According to the amyloid hypothesis, formation of Aβ plaques is one of the main influences on AD pathogenesis, and disease processes are believed to result from an imbalance between Aβ production and clearance [3]. Hypoxia, a direct consequence of cerebral hypoperfusion, increases Aβ production and reduces its clearance [4], and may trigger mechanisms that contribute to the cognitive impairment in AD patients. Moreover, hypoxia also induces microglia activation which results in the production of inflammatory cytokines and subsequent structural damage and neuroinflammation [5–7].

Currently, the proposed classification criteria for AD consist of core clinical features with evidence of pathophysiological processes, which include biomarkers of brain Aβ protein and downstream neuronal degeneration or injury [8]. Moreover, the term mild cognitive impairment has been coined to denote the early stages of cognitive decline that precede AD dementia [9]. The clinical features of vascular dementia, which are attributed to vascular-related brain lesions, are more variable than in AD dementia with respect to neuropsychological profiles, clinical phenotypes, and disease onset [10]. The diagnosis of vascular dementia is complicated further by the use of various clinical criteria [10–12]. Additionally, a wide range of vascular lesions produce cognitive impairment in vascular dementia [10]; thus, cognitive decline is not reliably associated with vascular pathology nor are there consensus criteria for pathological features of vascular dementia.

Vascular contributions to cognitive impairment and AD in aged individuals are common, and several vascular risk factors for AD are linked to hypoxia. Vascular pathology coexists in at least one-third of AD cases [13, 14], and a growing body of clinical-pathological research suggests that vascular factors play a role in the pathogenesis of AD [15]. Studies with transgenic and non-transgenic rodents provide supporting evidence that hypoxia promotes Aβ accumulation by enhancing Aβ production and reducing its clearance. Moreover, some studies suggest that vascular lesions also promote tau phosphorylation, but additional evidence is needed to establish this link. Currently the use of animal models to investigate the factors linking cerebral blood flow and AD pathology is the best approach for uncovering the mechanisms underlying the impact of neurovascular alterations on AD. In the present review, we summarize evidence from transgenic (Table 1) and non-transgenic (Table 2) rodent studies regarding the effects of hypoxia on AD-related pathology and evaluate its impact on understanding human disease.

CLINICAL-PATHOLOGICAL LINKS BETWEEN AD AND HYPOXIA

With aging, the human body becomes less efficient at delivering oxygen to cells and tissues, and therefore entire organs are compromised. The brain is particularly susceptible to hypoxia which can result in varying degrees of neural failure and structural damage [16]. Several cardiovascular and respiratory disorders are associated with neurodegenerative pathologies including AD, Parkinson's disease, and Huntington's disease [17]. The link between hypoxia and neurodegeneration is based on the oxygen supplies that are required for proper nervous system function. The brain consumes about 20% of the body's oxygen and receives up to 20% of the cardiac output [17, 18]. Under normal conditions oxygen is transported to brain tissue through microvessels by diffusion, and rapid localized delivery of oxygen occurs in response to increases in neuronal activity [19]. Imaging studies suggest that oxygen levels vary widely among different regions of the brain even in the resting state [18]. Further, although is it not clear whether cerebral hypoperfusion is a cause or a consequence of AD, various neuroimaging studies in AD individuals confirm a reduction in cerebral blood flow (CBF) from early to late stages of AD progression [20]. Vascular risk factors for hypoperfusion such as ischemic stroke, atherosclerosis, hypertension, diabetes and cardiac disease can lead to cognitive impairment by triggering hemodynamic changes in the brain microcirculature and impairing optimal delivery of oxygen and glucose to the brain [21]. Hypoperfusion also contributes to arterial stenosis by reducing CBF, preventing microemboli from being washed out of the arteries and restricting the transport of key nutrients [21].

The obstruction of blood resources to regions of the brain such as that which occurs following stroke represents one of the most damaging forms of hypoxia and can lead to severe pathological consequences [17]. Neuroimaging studies with positron emission tomography (PET) and single photon emission computerized tomography (SPECT) provide evidence that stroke produces region-specific hypoperfusion that results in the brain receiving just enough blood supply to support tissue viability but not enough to support cognitive or neurological function [22, 23]. In addition, the acute pathogenesis of stroke involves the activation of pro-inflammatory mediators that may exacerbate tissue damage in the long term [24, 25]. Various studies have confirmed that stroke victims are significantly more likely to develop AD in the years following stroke [26].

Table 1
Studies employing AD transgenic mouse models to examine links between low oxygen brain levels and AD pathology

Model	Characteristics (age, gender, strain)[1]	Treatment/Approach	Effects on AD-related pathology [ref]
Triple-transgenic mice (3xTg-AD)	15 months, male	Temporal occlusion of the bilateral common carotid arteries (12 min)	Decreased total tau and AT270; increased pAKT and GSK3β three months after injury [60].
	3 months, male	Temporal occlusion of the bilateral common carotid arteries (4 min)	Elevated $A\beta_{42}$ and oligemia for >3 weeks; robust increase in BACE1; reduced tau [58].
	5–7 months, both genders, homozygous	Experimental TBI with cortical impact by an electromagnetic device to produce mild, mild-moderate, and moderate injuries	Intra-axonal Aβ accumulation in the pericontusional fimbria; increased tau immunoreactivity in regions with moderate injury; increased total tau in contralateral CA1 [105].
Transgenic ArcAβ mice	4 and 24 months, both genders, expressing human AβPP 695 with both Swedish and Arctic mutation	CE-μMRA was used to assess cerebral artery and vein diameters	Reduction of functional intracortical microvessels; accumulation of Aβ and fibrinogen in small and medium sized vessels but not in large arteries in 24-month-old mice [68].
Transgenic mice (ApoE, AβPPsw and Tg2576)	15 months, AβPPsw mice expressing endogenous murine ApoE and AβPPsw, mice expressing human ApoE ε3 and ApoE ε4 isoforms (knock-in mice Tg2576)	Development of amyloid plaques and CAA	No Aβ deposition at 15 months after CAA with parenchyma plaque depositions in AβPPsw mice expressing ApoE ε4 and ApoE ε3; elevated levels of $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ and increased $A\beta_{40:42}$ ratios in young animals expressing ApoE ε4 [71].
	3–4 and 16–17 months, male, homozygous targeted replacement mice expressing human ApoE ε3 (TRE3) and ApoE ε4 (TRE4) genes	Intracerebral injections of human Aβ1-40	Increased Aβ deposits in hippocampus in TRE4 relative to TRE3 in both 3- and 6-month-olds [88].
Transgenic mouse models using AβPP and BACE genes	25–26 months, female heterozygous AβPP51/16 mice, female heterozygous AβPP23 mice, male heterozygous AβPP23	CAA association with alterations in microvascularisation	Severe CAA in thalamic vessels in AβPP23 mice compared to AβPP51/16 and wt; CAA-related capillary occlusion within the thalamus in AβPP23 but not in AβPP51/16 or wt mice [70].
	Two modified animal strains, AβPP −/− and BACE −/−	Global cerebral ischemia performed by bilateral clamping of the common carotid arteries (12 min)	AβPP −/− and BACE −/− mice presented greater risk of mortality and reduced CBF under hypoxic conditions; serum response factor and calsequestrin significantly altered in both strains [61].
	6, 11, and 20 months, male AβPP23 transgenic mice	Magnetic resonance angiography used to evidence cerebral arterial hemodynamics	AβPP23 mice of 11 and 20 months presented flow voids in the internal carotid arteries, with vessel elimination and deformation [67].
	2 months, mice overexpressing a mutant form of human AβPP, Swedish and Indiana ($A\beta PP_{sw/ind}$-Tg mice)	Chronic cerebral hypoperfusion with BCAS using microcoils	Impaired learning in BCAS-operated $A\beta PP_{sw/ind}$-Tg mice; reduced neural density correlated with low cognitive performance [65].
	Aged $A\beta PP^{sw/0}$ mice overexpressing human AβPP	Inhibition $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ with RAGE specific blocker, FPS-ZM1, and induced cellular stress	FPS-ZM1 inhibited RAGE mediated influx of circulating $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$; inhibited β-secretase activity and Aβ production; blocked RAGE activity at the BBB [50].

Table 1
(Continued)

Model	Characteristics (age, gender, strain)[1]	Treatment/Approach	Effects on AD-related pathology [ref]
	8 months, female, AβPP23 transgenic mice	Hypoxia produced in chamber at 8% O_2 for 16 h/day for 1 month	Upregulation of BACE1 promoter activity; increased AβPP processing and Aβ generation, β-secretase cleavage of AβPP and Aβ deposition; impaired memory [64].
	6 months, AβPP/PS1 double transgenic mice	Hypoxia produced by enclosure in airtight jar	Decreased memory and cognitive function; increased senile plaques and levels of tau phosphorylation [62].
	10 weeks, males AβPP/PS1 double transgenic mice	Hyperoxia produced in normobaric chamber at 40% O_2 for 8 h/day	Reversed deficits in spatial learning and memory; decreased Aβ deposition and neuritic plaque formation in cortex and hippocampus [66].
Transgenic CD-1 mice	6–8 weeks	Treatment with 3 intraperitoneal injections of LPS from *Salmonella typhimuriem*	Inhibitions of CSF bulk flow, impairment of central and peripheral clearance of Aβ, and increased vascular sequestration of Aβ [82].
Transgenic PDAβPP mice	4 months, mice containing the familial AD mutation V→ F at AβPP position 717 (PDAβPP)	Brain trauma induced by impacting a 3-mm diameter impounder onto the cortex through a 5-mm craniectomy	Increased Aβ, increased hippocampal neuronal death and memory impairment, but no increase in Aβ plaque formation [104].
Transgenic AβPP-YAC mice	Both genders, heterozygous	Brain injury by controlled cortical impact	Significant motor and memory deficits in WT and AβPP-YAC mice 7 days post brain injury [103].

AD, Alzheimer's disease; ApoE ε3, apolipoprotein E ε3; ApoE ε4, apolipoprotein E ε4; AβPP, amyloid-β protein precursor; $AβPP_{sw/ind}$-Tg mice, transgenic mice with AβPP and two mutations, Swedish and Indiana; AβPP 695, amyloid-β protein precursor 695; AT270, tau phosphorylated at Thr^{181}; BACE1, beta-site amyloid-β protein precursor cleaving enzyme 1; BCAS, bilateral common carotid artery stenosis; CAA, cerebral amyloid angiopathy; CBF, cerebral blood flow; CE-μMRA, contrast-enhanced magnetic resonance microangiography; CSF, cerebrospinal fluid; C99, membrane-bound peptide generated from AβPP; F, phenylalanine; FPS-ZM1, High affinity RAGE specific inhibitor; GSK3β, glycogen synthase kinase 3 beta; LPS, lipopolysaccharides; pAKT, serine/threonine-specific protein kinase; PS1, Presenilin 1; RAGE, receptor for advanced glycation end products; TBI, traumatic brain injury; wt, wildtype; V, valine; YAC, yeast artificial chromosome; 3xTg-AD, Triple-transgenic mice for AD. [1]Information provided when available.

Table 2
Studies employing non-transgenic animals to examine links between low oxygen brain levels and AD pathology

Animal model	Characteristics (age, gender)[1]	Treatment/Approach	Effects on AD-related pathology [ref]
Mongolian gerbils	Male	BCO, 10 min to produce global ischemia	Loss of 90% of the CA neurons 24 to 72 h after ischemia; decreased AβPP and Aβ immunostaining at 24 h after ischemia; increased AβPP and Aβ after 48 h that overlapped with increased ApoE expression and glial fibrillary acidic protein [80].
Sprague-Dawley rats	3–20 months, male	Reversible occlusion of the cerebral middle artery to produce focal cerebral ischemia	Upregulation of AβPP and Aβ fragments; presence of AβPP and Aβ in large round cells between macrophages from blood and/or brain in the infarct region (core and penumbra). Focal accumulation of AβPP and Aβ in adult rats [73].
	23 weeks, male	2VO	Deficits in memory after 30 days that worsened after 180 days in aged but not young adult rats; decreased cytochrome oxidase activity mostly in hippocampus and accumulation of Aβ oligomers in the CA1 area after 180 days 2VO [77].
Wistar rats	Male	4 groups: bilateral Aβ intracerebroventricular injection, BCCAo, sham, and Aβ toxicity and BCCAo	Impaired spatial memory in Aβ toxicity-BCCAo group compared to Aβ toxicity and BCCAo groups alone; exacerbated AD pathology in Aβ toxicity-BCCAo group compared to Aβ toxicity group [72].
	10 months, male	ME, occlusion of both external carotid arteries temporarily and then released 500 non-radioactive microspheres into the left common carotid artery	Brain injury associated with Aβ accumulation and tau pathology by microvessel injury; promoted neuropathology similar to NFTs and aberrant eNOS expression and protein tyrosine nitration in microvascular endothelial cells consistent with Aβ-amyloid accumulation [74].
	6–9 months, male	2VO to produce progressive neuronal damage and cholinergic dysfunction	Histologically observed infarction in the cortex of 28.6% and 42.9% in the striatum; neural loss 4 months after 2VO in CA1 hippocampus; rarefaction of white matter found 4 months after 2VO [78].
	10 months, male	2VO	Impaired learning and memory; downregulated synaptophysin in hippocampus; downregulated MAP-2 expression; upregulated GAP-43 mRNA [76].
	11 months, male	tMCAO	Maximal increase of ApoE expression in the core 7 days after tMCAO detection and in periischemic region at 7 and 21 days; increased ApoE mRNA in glial cells but not in neurons in periischemic region [89].

ApoE, apolipoprotein ε; AβPP, amyloid-β protein precursor; AVF, arteriovenous fistula; BCCAo: permanent occlusion of bilateral common carotid arteries; BCO, bilateral carotid occlusion; CBF, cerebral blood flow; GAP-43, growth associated protein 43; MAP-2, microtubule associated protein 2; ME, microsphere embolism; mRNA, messenger ribonucleic acid; NFTs, neurofibrillary tangles; NO, nitric oxide; tMCAO, transient middle cerebral artery occlusion; 2VO, Permanent occlusion of bilateral common carotid arteries. [1]Information provided when available.

In addition to stroke, a reduction in the levels of oxygen that reach the brain is often a consequence of microinfarcts. A recent study of postmortem brains showed that chronic microinfarcts and particularly multiple microinfarcts elevated the likelihood of dementia [27]. Microinfarcts located in cortical regions of the brain were associated with greater risk for dementia than those in subcortical regions [27]. Moreover, subjects with multiple microinfarcts exhibit greater overall cognitive impairment [27]. Clinical-pathological evidence shows that individuals with AD neuropathology and white matter or basal ganglia infarcts have a 20-fold increased risk of developing dementia compared to AD individuals without infarcts [28, 29].

In studies with AD patients, clinical evidence shows that hypoxia increases the levels of AβPP and Aβ in the vasculature of the brain [17]. Cardiac arrest, an extreme form of hypoxia, causes a massive increase in Aβ in blood [30]. Experimental studies support clinical observations showing that ischemia promotes the upregulation of AβPP resulting in an increase in Aβ accumulation and ultimately in the production of Aβ plaques [31–33]. Increases in Aβ are believed to produce neurotoxicity by causing perturbations in Ca^{2+} homeostasis, which can lead to a number of dysfunctions in cellular processes including neurotransmitter release and gene expression [34]. Chronic hypoxia has been shown to potentiate whole cell voltage-gated Ca^{2+} flows and produce overexpression of Aβ in various cell types [17]. There is a growing body of evidence that disturbances in calcium homeostasis provide a mechanistic link between hypoxia and AD pathology, although it remains to be established how calcium alterations account for AD pathogenesis [35].

Risk factors for cardiovascular disease have provided further insight into the relationship between hypoxia and AD pathogenesis. Hypertension is a risk factor for AD, and there are several reports that blood pressure increases in patients with AD years before the onset of the disease [36]. Chronic hypertension is often accompanied by additional vascular abnormalities that may threaten an optimal blood supply to the brain and increase the risk for dementia [36]. However, because hypertension is also associated with various risk factors for AD, including hypercholesterolemia, atherosclerosis, and obesity [37], a causal link between hypertension and AD pathology has not been established. Exploration of vascular risk factors in patients with AD is compulsory, and ongoing prospective studies should offer further evidence for developing preventive and therapeutic treatments. Even in the process of normal aging there are marked changes in the vascular system that are associated with changes in cognitive function [38]. Vascular structure and function are affected adversely over the course of aging by stiffening of the arteries and luminal dilatation [39]. An early study [40] showed that there are important substances in the microvasculature that play a major role in the interactions between the blood-brain barrier (BBB), astrocytes, and neurons (Fig. 1). Accordingly, structural changes related to microvascular pathology have been shown to be greater in demented compared to non-demented elderly subjects [40]. Thus, live evaluations of microvascular pathology offer a promising approach to the development of useful biomarkers for early detection and characterization of AD pathology [41]. Together, clinical-pathological evidence brings up several fundamental questions, namely, whether vascular risk factors are causally related to the development of dementia, and if so, whether early diagnosis and treatment of these pathologies could delay or prevent the progression of dementia. Currently, the best approach to these questions is by direct manipulations of oxygen supply and subsequent evaluations of behavioral and neuropathological hallmarks of AD using animal models.

MOLECULAR SIGNALING PATHWAYS LINKING AD AND HYPOXIA

The molecular signaling pathways related to the two main forms of neuropathology of AD, accumulation of Aβ and NFTs, have been characterized in numerous studies and will only be briefly discussed. Readers are referred to recent reviews [2, 42]. The Aβ peptide is released in brain by proteolytic processing of AβPP. Several insults such as hypoxia (Fig. 1A) can promote elevation of Aβ peptides, and genetic and environmental factors are believed to contribute to a chronic imbalance between Aβ production and clearance in AD. The Aβ peptide is the principal element in the extracellular plaques seen in AD brains, and insoluble forms of the peptide are produced via sequential cleavage of AβPP by two proteases, first by β-site AβPP cleavage enzyme 1 (BACE1) followed by γ-secretase and production of Aβ peptides. Hypoxia activates transcription factor hypoxia-inducible factor 1α (HIF-1α), which binds to and upregulates BACE1 (Fig. 1A), also promoting Aβ peptide production [43]. Toll-like receptor 4 (TLR4), a pattern recognition receptor mainly expressed in immune cells, is associated with hypoxic episodes in tissues like brain, heart, kidney, and lung [7, 44]. TLR4 is found to

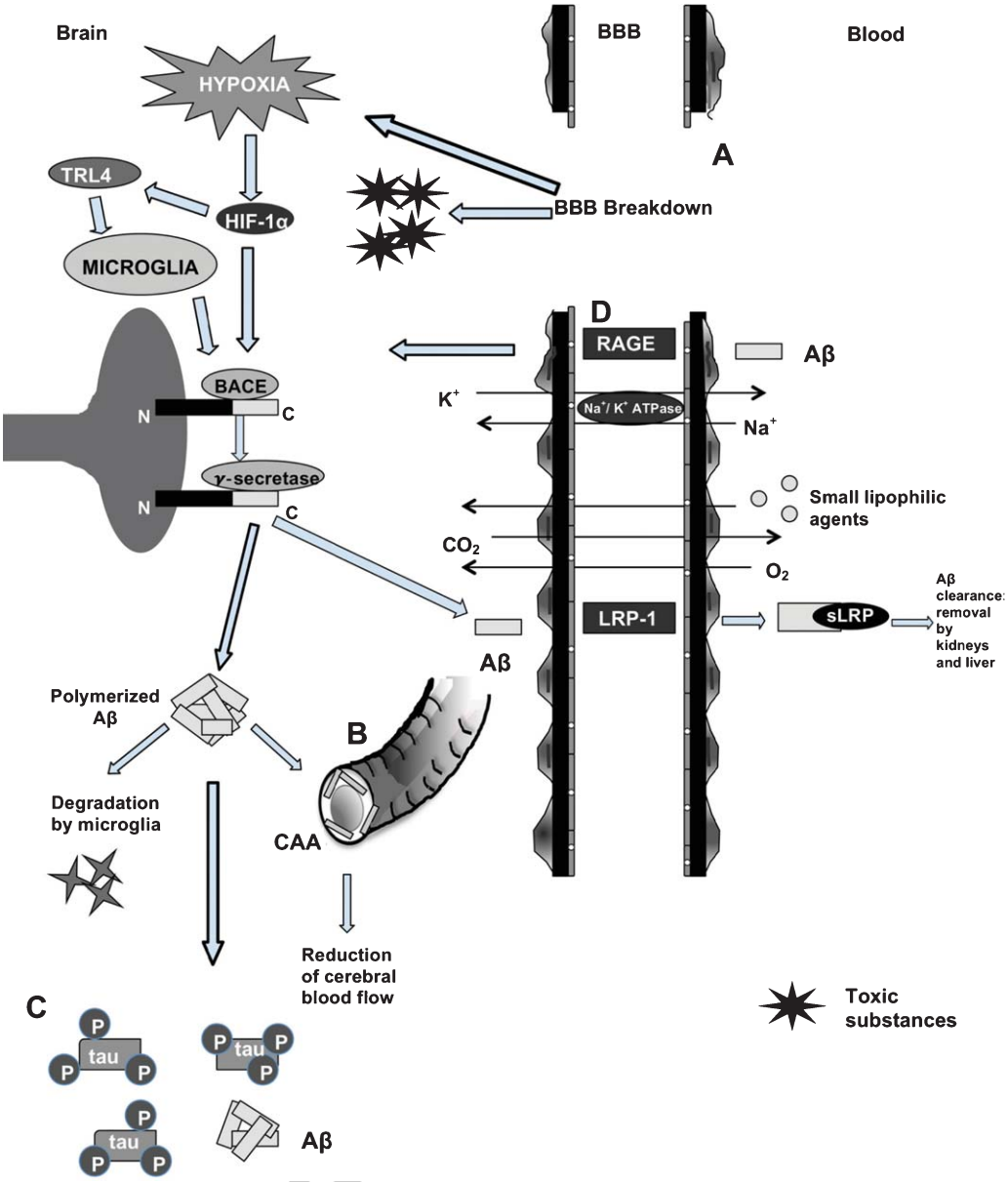

Fig. 1. A) Blood-brain barrier (BBB) breakdown caused by pericyte detachment leads to an accumulation of neurotoxic substances in the brain as well as a reduction in oxygen supply. This activates hypoxia-inducible factor (HIF-1α), which binds to and upregulates β amyloid cleaving enzyme-1 (BACE). HIF-1α promotes the expression of toll like receptor-4 (TLR4) and subsequent microglia activation with release of cytokines that upregulates BACE. Sequential cleavage of the amyloid-β protein precursor (AβPP) by BACE and y-secretase results in the amyloid-β (Aβ) peptide [44, 69]. Accumulation of Aβ results in the polymerization of Aβ into plaques that are one of the hallmarks of AD. The plaques may be degraded by microglia or accumulate in the brain parenchyma and walls of small brain arteries leading to a reduction of blood flow [69, 109, 110]. B) Cerebral amyloid angiopathy (CAA) also results from the accumulation of Aβ, which leads to capillary occlusion in the brain and a reduction of blood flow as well as local loss of neurons, microglial activation and microhemorrhage [69]. C) Aβ plaques may lead to hyperphosphorylation of tau protein, which contributes to neurofibrillary tangles and an increase in basement membrane thickness surrounding cortical microvessels [62]. D) Aβ is transported between the brain and blood through two main receptors: the receptor for advanced glycation end products (RAGE) and the low-density lipoprotein receptor-related protein 1 (LRP-1) [50]. RAGE, located on the luminal side of the endothelium mediates the influx of Aβ into the brain. LRP-1, located on the abluminal endothelial cell membrane mediates efflux of free Aβ from the brain interstitial fluid into the blood. Soluble LRP (sLRP) is formed in the liver by cleavage of LRP through β-secretase [87]. It binds to Aβ and is then removed by the liver and kidney. In this way, soluble LRP functions as a peripheral sink for Aβ [111]. LRP-1 also binds directly to AβPP affecting endoproteolytic processing of AβPP and increasing production of Aβ [87]. Small lipophilic agents as well as O_2 and CO_2 pass through the BBB by simple diffusion whereas ions require ATP-dependent transporters such as (NA^+ and K^+) ATPase [112].

be overexpressed in macrophages and microglia via HIF-1α under hypoxia, mediating brain inflammation and hypoxic-ischemic-related diseases [5, 44]. Activated microglial cells release several inflammatory mediators such as cytokines, reactive oxygen species, complement components, and nitric oxide that promote upregulation of BACE1 (Fig. 1A) and ultimately increase Aβ peptide production [7, 44, 45].

The brain uses several routes to clear Aβ from the brain. One of the principal routes is by the low density lipoprotein receptor-related protein (LRP), specifically LRP-1, a major cell surface Aβ clearance receptor located on vascular smooth muscles cells that allows the transport of Aβ peptides through the BBB [46] (Fig. 1D). LRP-1 is also found in neurons where it mediates Aβ-induced oxidative stress and intraneuronal transport, causing mitochondrial dysfunction [47, 48]. Aβ is also cleared from the brain by Aβ chaperones such as ApoE isoforms (ApoE2, ApoE3, or ApoE4), by microglia and perivascular brain macrophages, by direct enzymatic degradation of Aβ in the brain, and by passive drainage of Aβ into the perivascular space [46, 49]. The influx of peripheral Aβ to the brain is mediated by the receptor for advanced glycation end products (RAGE) (Fig. 1D). RAGE acts as a cell surface receptor that binds Aβ in BBB, neurons, and microglia [50]. Because of its diverse localization, RAGE contributes to various aspects of AD pathology, including Aβ-induced inflammatory response, oxidative stress, and intraneuronal mitochondrial dysfunction [50]. At later stages of AD progression, tau protein, a soluble microtubule-associated protein, becomes hyperphosphorylated and forms intracellular NFTs. In AD, NFTs compromise intracellular transport and the structural integrity of neurons [2, 51]. Hypoxia-induced alterations in AD metabolism may drive NFT formation (Fig. 1C). Both senile plaques and NFTs are used as markers for the definitive diagnosis of AD in postmortem brain.

Hypoxia increases Aβ production through its effects on Aβ signaling pathways

The bulk of experimental evidence linking hypoxia to AD pathology indicates that hypoxia exerts powerful modulatory effects on the Aβ signaling pathway. Studies of postmortem brain tissue have found that mild and severe ischemic episodes are associated with elevated levels of AβPP [52] and aggregation of $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ [53]. Similar studies using immunohistochemical evaluations of axonal pathology have shown an increase in AβPP and Aβ following severe head injury and cerebral ischemia [54–56]. Likewise, animal studies *in vivo* have demonstrated increased levels of AβPP and Aβ and upregulation of BACE1 under hypoxic conditions [57, 58]. Significantly, only a single, mild temporal occlusion (4 min) of the common carotid arteries in adult (3 months) 3xTg-AD mice was sufficient to produce acute elevations in Aβ levels by enhancing BACE1 that were sustained for at least 3 weeks [58]. Also, mild hypoperfusion produced a long-lasting reduction in tau, presumably through autophagy and ubiquitin-proteosomal pathway activation within the affected brain region [58]. Hypoperfusion altered phosphorylated tau proteins [58] that have been implicated in the long-term formation of NFTs in AD patients [58, 59]. In much older 3xTg-AD mice (15 months), a single but longer-lasting (12 min) occlusion of the bilateral common carotid arteries did not affect Aβ levels, but rather enhanced AβPP phosphorylation and insoluble tau levels at three months post-ischemia [60]. The same effects were produced in wild-type controls, suggesting that these parameters of global ischemia promote changes in AD-related pathways in this strain of aged mice regardless of genetic profile [60]. Other studies with knock-out transgenic mice confirm AβPP involvement in responses to vascular insults [61]. Global cerebral ischemia, performed by transient bilateral clamping of the common carotid arteries in mice lacking either AβPP or BACE1 genes (AβPP −/− and BACE −/−) increased the risk of mortality and reduced CBF compared to wild-type littermates [61]. Moreover, two molecules involved in vascular regulation, serum response factor and calsequestrin, were also significantly altered in both strains [61]. Thus, studies with AβPP and BACE1 knockouts suggest a beneficial role for AβPP and its cleavage fragments in the regulation of blood flow and the adaptation to ischemic insults. Taken together, results with transgenic mice confirm AβPP involvement in the brain's response to hypoperfusion, whereas hypoxic insults produce variable effects on Aβ and tau levels.

Chronic hypoxia appears to produce more pronounced and consistent effects on AD pathology. In a recent study AβPP/PS1 transgenic mice were exposed daily to hypoxia treatment for two months that produced numerous deficits associated with AD pathology, including worsened cognitive deficits, increased Aβ accumulation and subsequent formation of Aβ plaques, and increased levels of tau phosphorylation [62]. Similarly, long-term chronic hypoxia treatment was shown to produce more and larger Aβ plaques in two strains of transgenic mice (AβPPSwe + PS1A246E and AβPP23) [63]. Different

parameters for generating chronic hypoxia (8% O_2 for 16 h/day) in AβPP23 mice produced similar modulatory effects on Aβ pathways, namely upregulation of BACE1 promoter activity and increases in both BACE1 transcription and expression *in vivo* [64]. This is turn upregulated BACE1 cleavage of AβPP, increased Aβ production, deposits, and plaque formation, and worsened cognitive deficits in transgenic mice. Similar effects on cognitive function were observed in young adult mice (2 months) overexpressing a mutant form of human AβPP ($AβPP_{sw/ind}$-Tg mice). These mice exposed to chronic hypoperfusion via bilateral common carotid artery stenosis using microcoils exhibited greater cognitive deficits and hippocampal neuronal loss compared with controls [65]. Notably, insoluble Aβ was reduced, whereas soluble Aβ was increased, following six months of cerebral hypoperfusion, resulting in a reduction of Aβ deposition and plaque formation, suggesting that the cognitive impairment and neuronal loss associated with stenosis in this transgenic line may be a result of soluble Aβ species. In sum, chronic hypoperfusion produces effects on AD neuropathology that are more consistent with those observed in clinical cases, namely increases in Aβ plaques, tau phosphorylation, and cognitive impairments.

While chronic hypoxia has been shown to produce profound impairments in cognition and brain structure and function, hyperoxia treatment has been shown to have opposite effects. Chronic hyperoxia treatment (40% O_2; 8 h/day for 4 or 8 weeks) in young adult AβPP/PS1 transgenic mice produced significant improvements in spatial learning and memory and decreased Aβ deposition and plaque formation in cortex and hippocampus [66]. Biochemical analysis of brain tissue indicated that hyperoxia treatment reduced Aβ by inhibiting γ-secretase activity. The results of this study support the application of oxygen therapy as a useful way to reduce the neuropathological changes associated with AD progression, although this possibility requires further study.

Studies examining vascular profiles in AD transgenic mice have found that targeted mutations in these models not only produce forms of AD neuropathology but also various cerebrovascular pathologies. Imaging studies using magnetic resonance angiography to evidence cerebral arterial hemodynamics have shown that adult AβPP23 mice present flow voids in the internal carotid arteries that were observed as late as 20 months of age in large arteries in the circle of Willis. Vessel elimination and vessel deformation were also observed at the site of the flow voids [67]. Imaging techniques have also uncovered the deposition of Aβ peptides in intracortical vessels and its association with cerebral amyloidosis. A recent study using non-invasive high resolution contrast enhanced magnetic resonance angiography (CE-μMRA) in 4- and 24-month-old arcAβ mice showed an age-dependent reduction in the quantity of intracortical vessels in arcAβ mice compared to littermate controls [68]. Specifically, the number of functional intracortical microvessels was reduced in 24-month-old arcAβ mice compared to wild type controls, whereas no differences were found in four-month-old mice. Moreover, an accumulation of Aβ and fibrinogen, which is associated with vessel stenosis and a reduction in CBF [68], was found in small and medium sized vessels but not in large arteries in 24-month-old arcAβ mice. These results suggest that AβPP23 and arcAβ mice may be suitable models for examining links between AD neuropathology and neurovascular disease.

One of the principal vessel disorders associated with AD is cerebral amyloid angiopathy (CAA), which produces vascular deposits of Aβ similar to the senile plaques in AD (Fig. 1B). One outcome of CAA-related capillary occlusion is disruption of CBF, which leads to Aβ toxicity [69]. Aged AβPP23 mice exhibit CAA-related capillary occlusion in thalamic vessels that is not evident in control transgenic AβPP51/16 or wild type mice [70]. CAA has also been linked with ApoE expression. AβPPsw mice expressing endogenous murine ApoE or human ApoE3 and ApoE4 isoforms (knock-in mice Tg2576) develop amyloid plaques as well as CAA [71]. AβPPsw mice expressing ApoE4 at 15 months of age showed a change in Aβ deposition that lead to substantial CAA compared to age-matched mice expressing ApoE3 [71], providing evidence that links ApoE expression with Aβ retention in the brain by interfering with Aβ clearance mechanisms. Thus, AD transgenic mice provide evidence that capillary occlusion, which is present in human AD brains, is related also to CAA, pointing to these transgenic lines as useful models for gaining mechanistic insights into neurovascular and AD pathologies.

Additional evidence supporting the association between hypoxia and the development of Aβ pathology has been derived from studies with non-transgenic animals (Table 2). For instance, rats that received bilateral intracerebroventricular injections of Aβ fragments and permanent occlusion of bilateral common carotid arteries showed greater impairments in spatial memory and more extensive AD neuropathology relative to animals that received Aβ toxicity or occlusion alone [72]. Similarly, aged rats exposed to reversible occlusion of

the cerebral middle artery that produced focal cerebral ischemia showed an upregulation of AβPP and Aβ fragments [73]. The presence of AβPP and Aβ immunoreactivity in the infarct region indicated that concomitant reductions in CBF and cerebral ischemia provide the necessary elements for focal accumulation of AβPP and Aβ in adult rats [73]. Importantly, studies show that the effects of hypoxia on AD pathology can persist in time. A mild microsphere embolism in aged rats promoted eNOS expression and protein tyrosine nitration in microvascular endothelial cells, leading to Aβ accumulation in the lesioned area and hyperphosphorylation of tau protein in surrounding neurons [74, 75]. Both Aβ accumulation and hyperphosphorylated tau remained elevated for 12 weeks, indicating long-lasting effects of neurovascular injury on Aβ neurodegeneration [74, 75].

Chronic bilateral occlusion of the common carotid arteries in rats also reproduces several characteristics of human AD. Chronic hypoperfusion in adult rats has been shown to promote accumulation of oligomeric Aβ and impaired learning and memory that progressed as the period of hypoperfusion increased [76]. Significantly, hypoperfusion caused the down-regulation of various proteins important for synaptic plasticity and cognitive function including growth-associated protein-43 (GAP-43), synaptophysin, and microtubule-associated protein-2 (MAP-2) [76]. Similar effects were observed following double ligation of the carotid arteries in adult rats, namely profound deficits in spatial memory in aged but not young rats after 30 days that worsened after 180 days [77]. Hypoperfusion also caused an accumulation of Aβ oligomers in the CA1 region 180 days after surgery and synaptic changes in CA1 that correlated with the structural changes observed in AD progression [77]. Early studies demonstrated that permanent occlusion of bilateral common carotid arteries produced progressive neuronal damage in the hippocampus and white matter, evidenced by increased degeneration from one to four months after cerebral hypoperfusion [78]. Also, hypoperfusion produced long-lasting decreases in acetylcholinergic levels in cortex, striatum, and hippocampus after four months. These results suggest that progressive structural and functional changes in hippocampus and other brain areas play a role in the cognitive decline that occurs in aged persons following chronic hypoperfusion [78]. Moreover, other studies have reported that the observed hypoperfusion-induced deficits in spatial learning are a product of altered energy metabolism in various brain areas in addition to the hippocampus that are responsible for visuomotor integration [79]. On the other hand, there is evidence that the nature of structural changes varies post-ischemia. Global forebrain ischemia produced by bilateral carotid occlusion resulted in a loss of 90% of CA1 neurons 24 to 72 hours after ischemia and a decrease in AβPP and Aβ at 24 hours following ischemia in aged gerbils [80]. At 48 hours, there was an increase in AβPP and Aβ that overlapped with increased ApoE that may provide circumstances that are favorable for the formation of Aβ oligomers after ischemic insults [80]. Together, these results demonstrate that ischemia produces profound and long-lasting effects on brain tissue that are consistent with AD neuropathology, but that the spatial-temporal pattern of these effects varies among studies.

Evidence regarding hypoxia-ischemia effects on Aβ clearance mechanisms

Aβ is cleared from the brain through receptor-mediated endocytosis by cells in the parenchyma or through the BBB [81], but during a hypoxic episode the Aβ clearance mechanism is impaired. Systemic inflammation is one of the causes of impaired efflux of Aβ from the brain [82]. Young adult mice treated with lipopolysaccharides (LPS) showed several disturbances including inhibition of CSF bulk flow, impairment of central and peripheral clearance of Aβ, and increased vascular sequestration of Aβ [82], which together suggest that inflammatory responses disrupt Aβ transport and clearance that may exacerbate AD pathology. Other Aβ transport molecules that are capable of modulating cerebral blood flow responses and AD pathological processes include LRP1 and RAGE. Inhibition of RAGE, one of the receptors for Aβ in the BBB, has been shown to have positive effects on CBF and AD pathology. In one study, RAGE inhibition was shown to normalize CBF responses and cognitive performance in aged AβPP mice [50]. In mice that overexpress human AβPP ($A\beta PP^{sw/0}$), a high affinity RAGE specific blocker (FPS-ZM1) inhibited β-secretase activity and Aβ production, reduced $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ levels in the brain, and normalized cognitive performance and CBF in aged animals [50].

Modulation of LRP1 has also been shown to impact AD pathology and vascular processes in the brain. Evidence from studies with young and adult mice indicates that LRP-1 decreases with age [83]. Hepatic Aβ uptake, which accounts for 40–60% of total Aβ uptake, is also attenuated in aged rats, suggesting that Aβ levels increase during the normal aging process as a consequence of insufficient systemic clearance

[84]. Some proteins like receptor-associated protein (RAP)-chaperone facilitate the trafficking of LRP-1 by binding to multiple sites on LRP-1 and competitively blocking all known LRP ligands [83, 85]. It has been reported that increasing RAP concentrations decreases Aβ clearance [86]. Transgenic mice with decreased levels of LRP-1 exhibit greater Aβ accumulation than wild-type mice [86]. The increased levels of Aβ promote proteasome-dependent degradation of LRP-1 that lead to increased Aβ accumulation in a positive feedback loop, suggesting that Aβ peptides compete for the same LRP-mediated efflux system in order to exit the brain [86]. Animal experiments suggest that higher levels of Aβ may completely saturate LRP-1 leading to vascular accumulation of Aβ and subsequent development of cerebrovascular amyloid protein deposits [83]. As such, if the levels of extracellular Aβ exceed the transport capacity of LRP-1 or the transport systems are impaired by downregulation of LRP-1, Aβ could accumulate in brain tissue and vessels [83, 86, 87], producing toxicity and neuronal death.

Evidence linking hypoxia-induced pathology and genetic risk factors for AD

The ApoE4 polymorphism is a common risk factor in AD and CAA, and recent studies have revealed potential mechanisms linking ApoE4 to both diseases. Studies of postmortem brain tissue have found that mild and severe ischemic episodes are associated with increases in ApoE in the hippocampus [53]. Likewise, transgenic mice (Tg2576) expressing human ApoE4 showed substantial CAA and increased $A\beta_{40:42}$ ratios in brain [71]. A more recent study showed that animals expressing human ApoE4 genes that received intracerebral injections of human $A\beta_{1\text{-}40}$ presented significantly greater Aβ deposition in the hippocampus than those expressing ApoE3 at both 3 and 16 months of age, suggesting that ApoE4 disrupts Aβ clearance from the brain [88]. Further, the disruption in Aβ clearance was linked to morphological changes in the vasculature of aged mice [88]. Taken together, these studies suggest an age-dependent effect of ApoE4 expression on the elimination of Aβ from the brain along vascular basement membranes. Similar links between ApoE expression and cerebrovascular processes have been found in studies with non-transgenic animals. Aged Wistar rats exposed to transient middle cerebral artery occlusion showed long-term changes in ApoE immunoreactivity and mRNA expression [89]. After seven days of occlusion the maximal increase of ApoE expression was detected in the core, and at 21 days increased ApoE was detected in the peri-ischemic region in glial cells but not in neurons. Another source of ApoE expression was macrophages, which was attributed to necrotic tissue clearance after the ischemic insult. Together, studies with transgenic and non-transgenic animals link ischemic episodes with increased ApoE expression, which may disrupt Aβ clearance from the brain in a time-dependent manner post-ischemia.

Recently identified genetic risk factors for late onset AD can be present at low frequency but can represent almost the same risk as the common sporadic genetic risk factor ApoE. Some of these genetic variants such as TREM2, complement receptor-1 (CR1), and CD33 participate in microglia activation and the subsequent development of Aβ protein deposits [90, 91]. Expression of CD33, a transmembrane protein that encodes a myeloid cell-surface receptor, is a risk factor for AD and has been shown to inhibit the uptake and clearance of $A\beta_{42}$ in microglial cell cultures [92]. Further, studies in transgenic mice show that TREM2 expression is positively correlated with amyloid phagocytosis whereas TREM2 inhibition is related to accumulation of toxic products in brain [93]. Lastly, the presence of the CR1 risk allele or CR1 AD risk variant gene is related to impaired clearance and deposition of Aβ in brain and to an increased rate of cognitive decline [94–96]. Although these three genetic variants (TREM2, CD33, and CR1) occur less frequently than ApoE ε4 allele, they represent sporadic genetic risk factors for AD similar to ApoE ε4 and implicate microglial impairment as a factor in promoting AD neuropathological processes.

Hypoxia and head injury-produced Aβ pathology

Clinical studies have shown that head injury generates ischemic changes that induce tau-like pathology and AβPP cleavage [34, 97]. Aβ plaques have been seen in 30% of patients who die from traumatic brain injury (TBI) [98]. The accumulation of Aβ after TBI is believed to result from axonal damage [99, 100], which interrupts axonal transport and results in an accumulation of proteins in the axon, including AβPP [101, 102]. Several studies have used animal models to examine the pathology of Aβ accumulation following TBI [98, 103–105]. In one study, hippocampal damage and behavioral deficits were seen as a result of TBI in 3xTg-AD mice [105]. There was also an increase in Aβ accumulation in pericontusional white matter and an increase in total insoluble Aβ. Notably, the increase of Aβ in white matter was dependent on the severity of injury [105]. Similarly, a study conducted

in a swine model found an increase in AβPP and co-accumulation of Aβ in swollen axons and neuronal cell bodies, as well as formation of diffuse parenchymal Aβ plaques in both grey and white matter [106]. Further, adult 3xT-AD mice exposed to experimental TBI showed rapid intra-axonal Aβ accumulation in the area of the injury, increased tau immunoreactivity in brain regions following moderate injury, and no Aβ accumulation in areas without injury [105]. Together, the results of these studies suggest that trauma creates a unique situation in which all of the necessary enzymes for Aβ formation co-exist in axons. These results are consistent with those found in humans, where TBI produced long-term progressive axonal degeneration and intra-axonal Aβ accumulation that persisted for years following the initial trauma [54]. Evidence suggests that following hypoxia, eventual lysis and breakdown of damaged axons may be the underlying mechanism of Aβ release into the parenchyma where it aggregates and causes plaque formation [54]. In related studies using mice that overexpress normal human AβPP or a mutant form of AβPP brain injury produced an increase in Aβ in brain tissue but not an increase in plaque formation [103, 104]. In mice overexpressing mutant AβPP, the increases in Aβ led also to an increase in hippocampal neuronal death and memory impairment [104]. Clearly, brain injury produces alterations in Aβ pathways that promote pathologies similar to AD, although plaque formation is not reliably produced in some models.

CONCLUSION

Diagnostic criteria for dementias make a distinction between the impairment resulting from AD and that resulting from cerebrovascular insults despite evidence of extensive overlap between the two [17]. AD and vascular pathologies share several risk factors, and clinical and experimental research suggests that the burden of vascular and AD neuropathology may not be independent. The present review presented evidence from transgenic and non-transgenic rodent models linking AD and cerebrovascular neuropathology. Collectively, experimental studies with rodents provide strong evidence in favor of hypoxia-induced alterations in Aβ metabolism that may in turn drive the neurotoxicity marked by NFT formation and subsequent cell death. First, studies with transgenic mice confirm that oxygen deficiency facilitates AD pathogenesis by altering AβPP expression and driving Aβ overproduction [57, 58, 60–65]. Importantly, chronic hypoperfusion produces pronounced effects on AD neuropathology that include increases in Aβ plaques [63, 64] and tau hyperphosphorylation [62] and worsened cognitive deficits [64, 65], which are not consistently observed following mild hypoperfusion. Second, various AD transgenic manipulations produce vascular pathologies that provide compelling evidence of links between molecular pathways common to both AD and neurovascular disease [67, 68, 70, 71]. Third, studies with non-transgenic animals indicate that hypoperfusion produces pronounced effects on cognition, Aβ accumulation and tau hyperphosphorylation [72–78]. Fourth, various studies point to ischemia-induced time-dependent changes in brain structure and function [77–80] particularly in hippocampal areas afflicted in AD progression. Lastly, various studies suggest that hypoxia effects on Aβ accumulation may reflect disruptions in Aβ clearance mechanisms [50, 71, 88]. Taken together, hypoxia-induced effects may cause a significant shift toward increased Aβ deposition and reduced Aβ clearance that leads to the neurotoxic cascade and functional deterioration characteristic of AD neuropathology.

The evidence reviewed favors links between hypoxic insults and AD neuropathology, but variable findings among studies limits the degree to which rodent models can reliably reproduce human disease. Rodent species are amenable to genetic manipulations and large-scale studies, but differences between the human and rodent brain and limitations in rodents' behavioral repertoire represent challenges to translation. For instance, the evidence reviewed suggests that hypoxia-induced effects on Aβ pathology are more consistent across studies than effects on tau pathology, ApoE, or cognitive impairment. Accordingly, recent reviews have highlighted the limitations of animal models to produce effective neuroprotective agents for ischemic stroke in clinical trials and suggested that nonhuman primate models made be a more appropriate albeit ethically more challenging approach [107, 108]. Many biomarker-based studies have established that the neuropathology associated with AD, namely Aβ deposits and NFTs, progressively accumulates in the brain decades before behavioral symptoms appear [2]. Therefore, it remains a viable strategy to target the various vascular risk factors for AD during pre-symptomatic stages in AD development. In this regard, the use of rodent models continues to be the more cost-effective approach to explore the mechanistic factors linking reductions in CBF to AD-related neuropathologies and behavioral deficits, and the continued standardization and refinement of these approaches should be pursued.

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