New Therapeutic Targets in the Neurovascular Pathway in Alzheimer's Disease

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Summary: Recent findings indicate that neurovascular dysfunction is an integral part of Alzheimer's disease (AD). Changes in the vascular system of the brain may significantly contribute to the onset and progression of dementia and to the development of a chronic neurodegenerative process. In contrast to the neurocentric view, which proposes that changes in chronic neurodegenerative disorders, including AD, can be attributed solely to neuronal disorder and neuronal dysfunction, the neurovascular concept proposes that dysfunction of non-neuronal neighboring cells and disintegration of neurovascular unit function may contribute to the pathogenesis of dementias in the elderly population, and understanding these processes will be crucial for the development of new therapeutic approaches to normalize both vascular and neuronal dysfunction. In this review, I discuss briefly the role of vascular factors and vascular disorder in AD, the link between cerebrovascular disorder and AD, the clearance hypothesis for AD, the role of RAGE (receptor for advanced glycation end products) and LRP (low density lipoprotein receptor related protein 1) in maintaining the levels of amyloid β-peptide (Aβ) in the brain by controlling its transport across the blood–brain barrier (BBB), and the role of impaired vascular remodeling and cerebral blood flow dysregulation in the disease process. The therapeutic strategies based on new targets in the AD neurovascular pathway, such as RAGE and LRP receptors, and on a few selected genes implicated in AD neurovascular dysfunction (e.g., mesenchyme homeobox gene 2 and myocardin) are also discussed. **Key Words:** Blood–brain barrier, RAGE, LRP, amyloid β-peptide, ischemia, angiogenesis, dementia.

INTRODUCTION

Several epidemiologic studies, including the large population-based Rotterdam study,[1,2] have suggested that vascular risk factors might be responsible for cognitive decline in the elderly population. Old age, atherosclerosis, stroke, hypertension, transient ischemic attacks, cardiac disease, the ϵ4 allele of the apolipoprotein E gene, elevated homocysteine levels, hyperlipidemia, metabolic syndrome, obesity, and diabetes are among the risk factors for both vascular dementia and Alzheimer's disease (AD).[3–6] It remains unclear whether controlling vascular risk factors, vascular brain disorder, and metabolic syndrome in early-diagnosed AD individuals will prevent progressive cognitive decline and dementia.

Progressive cognitive decline in AD is associated with neurovascular dysfunction,[3,4] chronic neurodegenerative process, accumulation of neurotoxic amyloid β-peptide (Aβ) on blood vessels and in brain parenchyma,[7–9] intraneuronal lesions and neurofibrillary tangles (NFT),[10–12] and vascular deposition of amyloid resulting in cerebral amyloid angiopathy (CAA).[13,14] Whether neurovascular dysfunction and vascular lesions precede a chronic neurodegenerative process, as suggested by a number of recent studies reviewed elsewhere,[15] or if they develop in response to neurodegeneration is controversial and has been a subject of many debates. However, not knowing the exact relationship between vascular and neuronal lesions in AD makes it more difficult to develop new therapeutic approaches for treating cognitive decline, especially in AD cases with prominent cerebrovascular and metabolic comorbidity.

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ROLE OF BRAIN ISCHEMIA

Large cerebral arteries in AD do not develop CAA, but are frequently affected by atherosclerosis.[16,17] The Nun

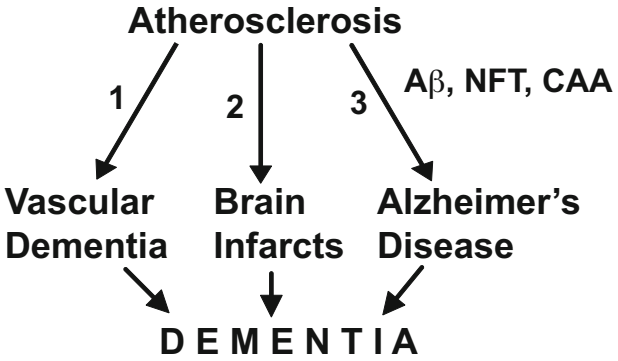

FIG. 1. Schematic illustrating different pathways by which atherosclerosis may lead to cognitive decline in the elderly. Pathway 1 leads directly to vascular dementia. Pathway 2 leads to brain infarcts increasing the risk for dementia. Pathway 3 leads to Alzheimer's disease pathology with accumulation of amyloid β-peptide (Aβ) and development of neurofibrillary tangles (NFT) and cerebral amyloid angiopathy (CAA). Molecular mechanisms in the cerebral vasculature and at the blood–brain barrier underlying pathways 1, 2, and 3 are largely unknown.

Study[18] found that cerebrovascular disease determines the presence and severity of AD, and that demented AD individuals with one or two lacunar infarcts have a much steeper drop in cognitive function than do individuals with no brain infarcts, irrespective of the number of NFT in neocortex. The Rotterdam Scan Study,[19] a prospective, population-based study with 1015 patients, demonstrated that silent brain infarcts detected on magnetic resonance imaging (MRI) doubled the risk for dementia in elderly people, with a hazard factor of 2.26. The Rotterdam Study, with 1730, subjects found that cerebral hypoperfusion preceded the onset of clinical dementia and that reductions in cerebral blood flow (CBF) velocity occurred before cognitive decline and before hippocampal and amygdalar atrophy were documented on MRI.[2] Nonetheless, the exact pathway or pathways by which atherosclerosis and arteriolosclerosis contribute to cognitive decline, and the relationship between vascular brain damage, white matter hyperintensities on MRI, and cognitive decline, are still not completely understood.[20] Figure 1 illustrates possible pathways by which atherosclerosis may lead to dementia. Whether progression of vascular disorder and dementia in AD could be controlled by a new class of agents exerting combined anticoagulant, anti-inflammatory and neuroprotective activities in the ischemic brain, such as activated protein C (APC),[21] remains to be explored.

In rodents, ischemia increases Aβ production,[3] accumulation of hyperphosphorylated tau in cortical neurons, and filament formation similar to that present in human neurodegenerative tauopathies and in AD,[22,23] which suggests that hypoperfusion may generate neuropathological changes similar to those seen in AD individuals. Amyloid β-peptide is a potent vasoconstrictor in brain.[24] In mice expressing Aβ-precursor protein (App), reductions in endothelium-dependent regulation of cortical microcirculation[25] and CBF dysregulation in response to brain activation have been reported prior to neuropathological changes.[26,27] Similarly, neurovascular uncoupling, reductions in CBF, and low brain uptake of glucose have been shown in sporadic AD prior to cognitive decline.[28–30]

CLEARANCE HYPOTHESIS FOR AD

Most AD cases (~99%) present with late onset (i.e., >65 years of age) and without clear evidence of genetic transmission.[31] Late-onset AD individuals do not normally have increased production of Aβ. Instead, Aβ likely accumulates in the brain of AD individuals because of deficient clearance from brain,[32–35] as illustrated in Figure 2. Recent evidence suggests that plaques are generated on blood vessels due to faulty Aβ clearance across the blood–brain barrier (BBB) or during its transport by passive diffusion across Virchow–Robin perivascular arterial spaces.[15]

Amyloid β-peptide is central to the development of brain pathology in AD.[7–9,12,33,36,37] Patients with sporadic AD and familial AD (FAD; <1% of all AD cases) typically develop focal increases in brain Aβ levels midway through the disease process or at a later stage. Elevated Aβ levels in brain generate neurotoxic Aβ oligomer species, which may disrupt normal brain function[37] or lead to Aβ deposits in the form of senile plaques. In murine models of AD, such as APP-overexpressing mice with Swedish mutation (*APPsw*$^{+/-}$) and transgenic *APP* mice harboring vasculotropic Dutch, Iowa, and Swedish triple *APP* mutations, dense plaques initially develop on blood vessels or as classical CAA.[38–40]

In more than 80% of AD cases , the pial and intracerebral arteries are affected by CAA.[14] Pathogenic levels of vasculotropic mutant forms of Aβ (e.g., Dutch, Iowa, Arctic, Flemish, Italian) accelerate degeneration of the vessel wall, thus contributing to hemorrhagic strokes, as in familial forms of AD.[13]

How we might prevent Aβ accumulation by accelerating its clearance from brain is an emerging area of research, one that holds promise for the development of new future therapies for AD.

RAGE AND LRP REGULATE Aβ BLOOD–BRAIN BARRIER TRANSPORT

RAGE

The receptor for advanced glycation end products (RAGE) is normally expressed at low levels in brain, except for the endothelium of brain capillaries and small arterioles (A. Sagare, R. Deane, R.D. Bell, and B.V. Zlokovic, unpublished observations). However, under pathophysiologi-

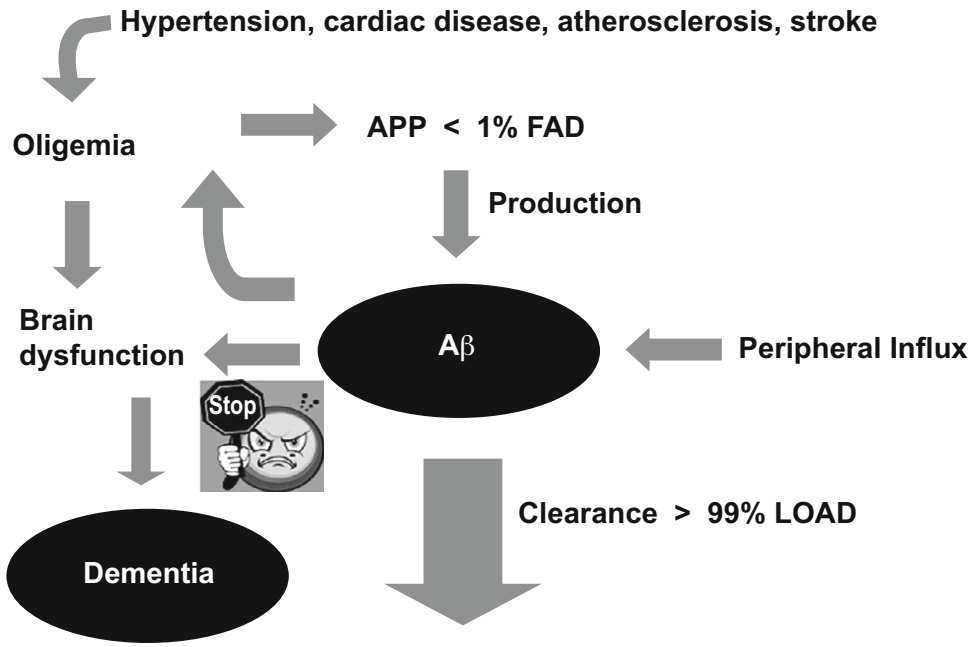

FIG. 2. The clearance hypothesis for Alzheimer's disease (AD). Amyloid β-peptide accumulates in late-onset AD (LOAD) (~99% of all AD cases), likely because of an imbalance in its production from the precursor APP protein, peripheral re-entry (influx) from blood, and clearance from brain. When the clearance mechanisms fail, Aβ accumulates, which affects brain function and reduces blood flow, resulting in oligemia. Oligemia increases Aβ production, which amplifies the positive feedback loop for Aβ accumulation. Vascular risk factors such as stroke, hypertension, cardiac disease, or atherosclerosis may all aggravate brain oligemia, which alters the balance between Aβ production and clearance in favor of production. In familial AD (FAD; <1% of all AD cases), an increased Aβ production outweighs the clearance capacity and Aβ accumulates in the brain, initiating the pathogenic cascade similar that found in LOAD.

cal conditions such as those associated with the accumulation of RAGE ligands on blood vessels (e.g., deposition of proteins modified by glycation and oxidation, whether AGE proteins or Aβ), the expression of cerebrovascular RAGE is increased. In AD and AD models, RAGE expression increases by several-fold in affected cerebral vessels and in microglia and neurons.[41–46]

RAGE binds different forms of Aβ, and its reaction with Aβ at the luminal membrane of the BBB (FIG. 3) mediates 1) re-entry of circulating Aβ into the brain across the BBB, followed by Aβ binding to neurons; 2) NF-κB-dependent activation of endothelium with expression of proinflammatory cytokines and adhesion molecules; and 3) secretion of endothelin-1 resulting in CBF reductions. In addition to the observations in mice and rats, transport of Aβ peptides from blood to brain across the BBB has been shown in other species, including guinea pigs and nonhuman primates.[47,48] Compared with other small peptides, such as vasopressin or enkephalins,[49,50] transport of Aβ40 across the mouse BBB was two- to threefold faster, although it was only a fraction of the rate determined for amino acid transport either across the BBB or the choroid plexus.[51,52]

RAGE is an important therapeutic target in AD. RAGE–Aβ interaction on neurons can kill neurons directly by producing oxidative damage or indirectly by activating microglia.[41] Inhibition of RAGE–Aβ interaction in the affected blood vessels blocks Aβ influx across the BBB and the associated oxidant stress and neuroinflammation.[43] RAGE and Aβ blockers are currently being tested in AD patients for safety and efficacy. A double-blind, placebo-controlled, randomized, multicenter study conducted under the Alzheimer's Disease Cooperative Study to evaluate the efficacy and safety of 18 months of treatment with PF-04494700 (TTP488) in participants with mild-to-moderate Alzheimer's disease began patient enrollment in December 2007.

LRP

Low density lipoprotein receptor related protein 1 (LRP), a member of the LDL receptor family, is a major clearance receptor for Aβ at the BBB.[53] Aβ binding to LRP is the first step in Aβ clearance from brain mediated by transvascular Aβ transport across the BBB[39,53–55] (FIG. 3). Substitution at codon 22 of LRP, as in Dutch type FAD, reduces clearance of Aβ from the CSF and brain into blood.[56] Reduced expression of LRP was found during normal aging in rodents, nonhuman primates, and in AD individuals associated with positive staining of cerebral vessels for Aβ40 and Aβ42.[39,45,53] Mice with severely depleted LRP levels at the BBB develop Aβ accumulations when crossed with APP-overexpressing mice.[57] Binding of Aβ to apolipoprotein J and E and α2-macroglobulin critically alters Aβ clearance rates from the brain and can influence vascular and

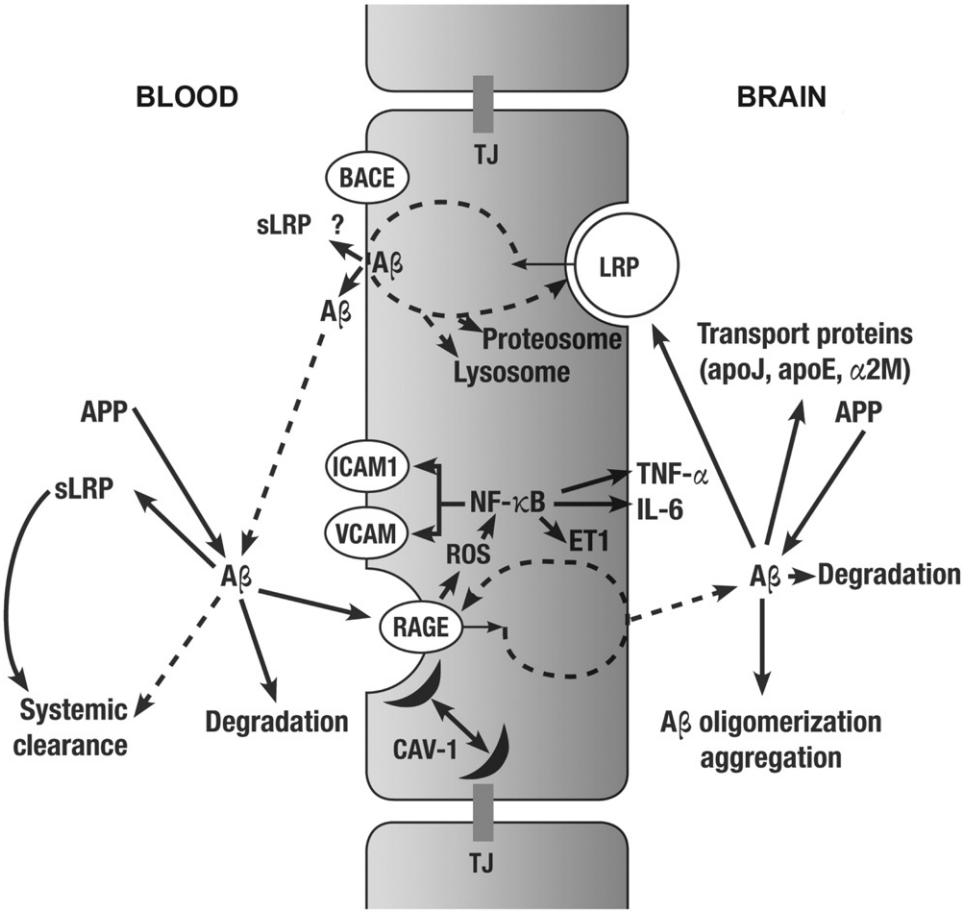

FIG. 3. Transport equilibrium for amyloid β-peptide (Aβ) at the blood–brain barrier (BBB). The cell surface LRP on the abluminal membrane binds different forms of Aβ (e.g., monomers, oligomers, aggregates) and initiates Aβ transcytosis across the BBB followed by its export into the circulation. In the case of Aβ overload, LRP (low density lipoprotein receptor related protein 1) loses its normal protein conformation and undergoes the accelerated proteosomal degradation. Aβ efflux is influenced by its transport binding proteins in the brain, such as apolipoproteins E and J (apoE, ApoJ), or α2-macroglobulin (α2M). β-Secretase (BACE) cleaves the N-terminus extracellular domain of LRP1, which generates a soluble form of LRP (sLRP). In human plasma, more than 70% of Aβ is normally bound to sLRP. Native plasma sLRP is a major endogenous peripheral sink agent for Aβ. The remaining Aβ in the plasma is bound to other Aβ-transporting proteins (e.g., apoJ). A small fraction of plasma Aβ is free. On the luminal membrane, free Aβ that escapes the sLRP surveillance in the blood interacts with the receptor for advanced glycation end products (RAGE). Aβ–RAGE interaction mediates re-entry of Aβ from blood to brain (influx), and activates the endothelium through reactive oxygen species (ROS)-induced nuclear translocation of NF-κB. This triggers secretion of proinflammatory cytokines, such as interleukin-6 (IL-6), tumor-necrosis factor-α (TNF-α), the expression of adhesion molecules at the BBB, such as intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion molecule (VCAM), and secretion of endothelin-1, a suppressor of blood flow. Modified from reference 15 (Zlokovic BV. The blood–brain barrier in health and chronic neurodegenerative disorders. Neuron 2008;57:178–201.), with permission.

parenchymal accumulation of Aβ.[35,55] Finally, LRP mediates Aβ systemic clearance from the liver.[58]

β-Secretase cleaves the N-terminal extracellular domain of LRP[59] and releases soluble LRP (sLRP) in plasma. Normally, sLRP binds 70% to 90% of Aβ in human plasma.[60] Aβ binding to sLRP is significantly reduced in late-stage cases of late-onset AD; this, in turn, may elevate brain Aβ, because of reductions in the endogenous peripheral sink activity of sLRP. Recombinant LRP clusters can effectively sequester Aβ in AD plasma and in *APPsw*$^{+/-}$ mice, promoting Aβ efflux from the mouse brain.[60] Thus, LRP fragments may have therapeutic potential as novel Aβ clearance agents or in sLRP replacement therapy for AD.

Mdr1a/Mdr1b null mice lack P-gp at the BBB and express lower levels of LRP in brain capillaries.[54] *Mdr1a/Mdr1b* null mice exhibit reduced clearance of Aβ from brain, whereas *APP* mice lacking P-gp have accelerated accumulation of Aβ and amyloid deposition. Thus, the *Mdr1a* and *Mdr1b* genes may influence Aβ clearance either directly through P-gp, indirectly through LRP, or through both.

IMPAIRED VASCULAR REMODELING

Reduced microvascular density, fragmented and atrophic string vessels, irregularity of capillary endothelial surfaces, reductions in the vessel diameter, thickening of

capillary basement membranes, and collagen accumulation in basement membranes have been described in AD.[61,62] It has been suggested that the degeneration of brain capillary endothelium seen in AD and AD models may reflect aberrant sprouting angiogenesis in response to chronic brain hypoxia.[15] A recent report found extremely low levels of expression of the mesenchyme homeobox gene 2 (MEOX2) in the BBB of AD individuals.[63] MEOX2 normally regulates vascular cell differentiation and repair, and its expression in the adult brain is restricted to the vascular system. Low levels of MEOX2 in AD endothelium mediate abnormal responses to angiogenic factors such as vascular endothelial growth factor, resulting in premature vessel regression, reduced resting CBF and improper formation of the BBB.[63] Low levels of MEOX2 also promote proteasomal degradation of LRP, which lowers the Aβ-clearing capability at the BBB, thus leading to Aβ accumulation on the blood vessels. Accumulation of Aβ on the abluminal membrane of the blood vessels is antiangiogenic, which amplifies the reductions in microcirculation in AD models[64,65] and possibly in AD. Thus, aberrant angiogenesis has an amyloidogenic effect at the BBB[66] And could represent an important novel target IN AD.

Recent studies have also shown that the expression of two transcription factors that control differentiation of vascular smooth muscle cells (VSMC), the serum response factor (SRF) and myocardin (MYOCD), is increased in AD, resulting in a hypercontractile phenotype in small cerebral arteries associated with brain hypoperfusion and diminished CBF responses to brain activation.[67] These events may also contribute to the hypoperfusion observed in AD brains. Thus, drugs that specifically disrupt SRF–MYOCD interaction in small brain vessels may hold potential to improve brain perfusion and CBF dysregulation in AD.

CONCLUSIONS

Recent clinical observations provide strong evidence for the link between cerebrovascular disease and AD, cognitive decline, or both. Here, we have briefly reviewed changes in the expression of key vascular receptors and genes in brain capillaries and small cerebral arteries in AD and in AD models that may lead to focal vascular and brain accumulations of Aβ, reductions in the resting CBF, attenuated CBF responses to brain activation, and focal neuroinflammatory response. The activation of the neurovascular pathogenic pathway may, in turn, compromise synaptic and neuronal functions, ultimately leading to neuronal damage with accumulation of intraneuronal tangles, neuronal loss, and dementia. The early molecular changes within the neurovascular pathway may offer new therapeutic targets for controlling progression of dementia in AD, including therapies based on the receptors RAGE and LRP or on genes implicated in the neurovascular AD model, such as *MEOX2*, or *SRF* and *MYOCD*.

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