Alzheimer’s disease and the blood–brain barrier: past, present and future

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Successful prevention and treatment of late-onset Alzheimer’s disease (AD) is a high priority for industrialized societies where the incidence is growing rapidly. Much of the underlying biology leading to AD is unknown, and the more knowledge we gain the more we appreciate the complexities involved. Popular etiologic hypotheses have largely ignored the blood–brain barrier (BBB) as an important factor contributing to the pathologic hallmarks of this most common form of dementia. Evidence identifying BBB dysfunction in AD or patients at risk (i.e., those with mild cognitive impairment) continue to escalate. This review highlights methodological issues facing investigators assessing BBB integrity in living patients while also discussing whether the BBB dysfunction is a cause, effect or epiphenomenon in AD. Rationale for future research pursuits aimed at describing the role of BBB function in AD pathogenesis is also presented.

Physiology of the blood–brain barrier

In 1885, Ehrlich reported that the brain, unlike other organs, failed to retain dyes that were injected systemically. Subsequent investigation by Goldman demonstrated that dyes will in fact stain the CNS if injected into the brain ventricular system, suggesting that Ehrlich’s observation was due to a barrier between blood and brain tissue [1,2]. Since blood vessels in the brain make intimate contacts with astrocytic foot processes, the astrocyte was presumed to represent the structural basis of the blood–brain barrier (BBB) until the 1960s when Brightman [3] and others [4,5] demonstrated that tight junctions between endothelial cells are the basis of the barrier function (Figure 1) [6].

An intact BBB restricts molecule transportation at the luminal (blood-facing) and abluminal (brain-facing) sides [7]. This is largely mediated by the molecular weight and lipid solubility of a given molecule, with lipid soluble agents able to cross the BBB more readily than water-soluble agents. For example, the intact BBB prevents the passage of water-soluble drugs with a molecular weight greater than 180 kDa [8]. This concept is also exemplified by albumin and immune globulin (IgG), which constitute the majority of high molecular weight components in peripheral circulation and are largely restricted from brain tissue by this BBB.

Selective pinocytosis and other transport mechanisms permit specific agents to cross an otherwise intact BBB, so some investigators have emphasized that the BBB has both ‘barrier’ and ‘carrier’ functions. At the luminal side of the BBB, transporters for specific classes of nutrients (e.g., glucose, amino acids and vitamins) and receptors (e.g., lipoproteins, regulatory peptides and proteins, hormones and metals) mediate transcellular influx of circulating substrates into the brain [9–11]. The transport systems at the abluminal side of the BBB eliminate potentially toxic molecules (e.g., amyloid β (Aβ), metabolic waste products and excitatory neurotransmitters) from brain interstitial fluid to the blood [12]. Over the past decade, a neurovascular hypothesis of Alzheimer’s disease (AD) has been proposed [7]. This hypothesis argues that the physiology of Aβ peptide clearance from the CNS across the BBB is disturbed by aberrant angiogenesis and cerebrovascular dysfunction that cripple receptors (e.g., LDL-receptor protein (LRP)-1 and the receptor for advanced glycation end products [RAGE]) that mediate CNS Aβ concentrations [11,13–15].

Methods for assessing blood–brain barrier integrity

Direct examination of brain tissue

Animal models of BBB function typically involve intra-arterial infusion of a marker normally excluded by a healthy BBB. Post-transfusion examination of the brain tissue identifies the presence of the markers, allowing some conclusion regarding BBB integrity. In the presence of BBB disruption the brain parenchyma will be stained with the marker. An analogous approach can be taken with post-mortem human tissue by probing in brain parenchyma for plasma markers normally excluded by a healthy BBB. These studies typically probe for IgG or albumin. Post-mortem studies of human subjects with AD have relied in part on these methods for evaluating BBB integrity in AD.

Keywords: albumin, Alzheimer’s disease, amyloid, blood–brain barrier, cerebral amyloid angiopathy, cholesterol, computed tomography, homocysteine, imaging, LRP-1, MRI, PET, RAGE
Clinical assessment of BBB integrity in living patients: the albumin index

Since cerebrospinal fluid (CSF) reflects the composition of extracellular fluid in the brain, CSF levels of large molecular weight blood-borne markers are dependent on the integrity of the BBB. Albumin is only synthesized in the liver, so any albumin in the brain and CSF is derived from the peripheral circulation. The assumption that albumin found in the CSF is derived from the periphery is supported by studies in different laboratories that all failed to prove its synthesis in the CNS [16–18]. Albumin has a molecular weight of approximately 67,000 kDa, a typical CSF concentration that is about 200-times lower than in serum and it is the most abundant protein component of the CSF in humans [19]. The ratio of albumin in the CSF compared with serum, the ‘CSF albumin index’ has been utilized as a marker of BBB integrity in living human subjects [20]. Although an elevated CSF albumin index is used as a marker of BBB ‘leakiness’, it is important to note that elevation of the CSF to serum albumin ratio may be due to either increased influx from the periphery (i.e., BBB disruption) or decreased efflux (i.e., albumin clearance from CSF).

Imaging techniques for the assessment of BBB integrity

The invasive nature of lumbar puncture limits the utility of the CSF albumin index, so non-invasive neuroimaging methods are attractive alternative methods for assessing BBB integrity. Routine clinical brain CT and MRI scans demonstrate regions of BBB impairment as focal areas of ‘contrast enhancement.’ Brain tumors, brain abscesses, subacute strokes and active multiple sclerosis lesions are all recognized by local contrast enhancement due to BBB impairment. In these instances the signal-to-noise ratio for the contrast agent is very high in the region of localized BBB impairment. Detection of subtler or more diffuse BBB dysfunction is not possible with routine clinical brain scans, and imaging modalities for examining BBB integrity have been sought for decades. PET with radiolabeled ethylenediaminetetraacetic acid (EDTA) was one method used in the past for tracking BBB integrity in living subjects [21], and CT scanning with the iodinated compound meglumine iothalamate [22,23] was another. These methods have been supplanted by an advanced MRI method known as dynamic susceptibility contrast imaging, which involves serial imaging of an intravenous bolus of gadolinium contrast. While this
method is most often used to determine rates of cerebral perfusion by tracking the appearance of contrast agent in parenchyma, it may also be used to determine BBB dysfunction. Prolonged retention of postbolus contrast material in brain parenchyma may reflect BBB dysfunction [24,25]. In addition to the advantage of noninvasiveness, imaging methods using tracers with a relatively low molecular weight (e.g., gadolinium ≤ 1 kDa) are potentially more sensitive to BBB dysfunction than biochemical methods dependent on a much larger tracer (e.g., albumin: ~67,000 kDa).

Pathophysiology of Alzheimer’s disease

Amyloid hypothesis

Approximately 100 years ago, Dr Alois Alzheimer reported his observation of fibrils that were coiled and twisted and a central core with a diffuse halo during pathologic examination of brain tissue from a patient with presenile dementia [26]. These neurofibrillary tangles and amyloid plaques have become the pathologic hallmarks of the disease, which now bears Alzheimer’s name. Nearly 80 years later the sticky Aβ peptide, which is the chief component of the amyloid plaque, was sequenced [27]. The gene that encodes the Aβ precursor protein (APP) was subsequently mapped to chromosome 21 [28–31].

The central premise of the amyloid hypothesis is that the neurodegenerative cascade in AD is due to a neurotoxic form of Aβ [32]. Since monomeric Aβ is synthesized and present in healthy brains, and since large plaques are immobile and somewhat inert, the current amyloid hypothesis argues that soluble oligomeric Aβ is the toxic form. However, this hypothesis remains unproven and hotly debated (Figure 2).

Perhaps the largest concern with the original amyloid hypothesis is that the number of Aβ deposits in the brain does not correlate well with the degree of cognitive deficits in AD [33]. The degree of dementia in AD correlates much better with Aβ assayed biochemically than with histologically determined plaque counts, and the concentration of soluble Aβ species (which are invisible to immunohistochemistry) appear to correlate with cognitive impairment [34–36]. Arguably the strongest evidence in favor of the amyloid hypothesis is that mutations in APP heighten self-aggregation of Aβ into amyloid fibrils, which are sufficient to produce clinical and pathological AD. The remarkably high incidence of AD pathology in Down’s syndrome, which involves a duplication of APP on chromosome 21, is another point in favor of the amyloid hypothesis. The plausibility of the hypothesis is illustrated by the many clinical trials aimed at reducing the production of, or promoting the clearance of, Aβ (e.g., secretase inhibitors, secretase modulators and anti-Aβ immunotherapy). Deficient Aβ clearance from the CNS to peripheral circulation for degradation and systemic removal may prove the most fruitful strategy for reducing Aβ given that only 1% of AD patients have demonstrated Aβ overproduction [32,37–40].

The amyloid hypothesis typically considers the neurotoxicity of only parenchymal Aβ, ignoring vascular Aβ, which is seen in most cases of AD. Vascular Aβ, or congophilic angiopathy is a well established cause of intracerebral hemorrhage [41]. Some investigators have suggested that silent microhemorrhages may contribute to the global cognitive decline in AD [42], and others have demonstrated neurotoxicity of endothelial cells exposed to Aβ [43], suggesting that Aβ may contribute to dementia by way of vascular mechanisms. Amyloid angiopathy is therefore one possible cause of BBB impairment in AD.

Nonamyloid cerebrovascular disease & Alzheimer’s disease

Atherosclerosis is another possible cause of BBB impairment in patients with a clinical diagnosis of AD. The diagnosis of AD is made on clinical

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**Figure 2. Amyloid cascade hypothesis.**

- Age-dependent changes in production or clearance, or missense mutations in APP, PS1 or PS2 genes
- Increased Aβ₄₂ production and accumulation
- Aβ₄₂ oligomerization and deposition as diffuse plaques
- Subtle effects of Aβ oligomers on synapses
- Progressive synaptic and neuritic injury
- Dementia

APP: Amyloid precursor protein; PS: Presenilin
Alzheimer's disease & the blood–brain barrier

Histological evidence of BBB disruption in post-mortem Alzheimer's disease brain tissue

Immune globulin, a high molecular weight protein that does not cross an intact BBB, has been repeatedly identified in brain tissue from AD patients, presumably due to extravasation from blood vessels [52,53]. In one study, the immune globulin was only present in the parenchyma in proximity to blood vessels that were damaged by arteriosclerosis. Subsequent studies have also demonstrated extravasated albumin in the AD brain, again primarily in the vicinity of damaged blood vessels, but in this case the blood vessels were involved by amyloid angiopathy or were surrounded by amyloid plaques [54,55]. Serum amyloid protein, another large molecular weight marker, has also been localized to amyloid plaques in AD [56], and, in the absence of any evidence that it is synthesized in the CNS, is also presumed to be derived from peripheral blood [56]. The morphology and ultrastructure of endothelial cells in AD brain tissue is also suggestive of BBB dysfunction [52,53,57,58].

BBB dysfunction in living Alzheimer’s disease patients: the CSF albumin index

Post-mortem studies, however, necessarily represent a late stage of AD when BBB dysfunction may represent an epiphenomenon in a badly damaged brain. If the pathogenesis of AD includes BBB dysfunction, then it should be detectable at earlier stages of the disease in living patients. Several studies have examined BBB integrity in AD subjects compared with controls using the CSF albumin index, with mixed results (Table 1). Of the ten studies reported, six found evidence of BBB dysfunction in AD [53–58,59–65] and four did not [66–69]. These and other studies consistently find an elevated CSF albumin index in subjects with vascular dementia, but only intermittently in subjects with AD. This implies that either AD populations in these studies included subjects with undiagnosed cerebrovascular disease, or that subpopulations of AD subjects exist with BBB damage due to AD per se.

Most of these studies have used the National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer’s Disease and Related Disorders Association criteria to diagnose probable AD (60,61,63–74), but the manner in which these studies controlled for vascular contribution to dementia has varied. We recently
added to this literature by describing the CSF albumin index in a population of AD subjects with rigorous exclusion of vascular disease and vascular risk factors [71]. This included a Hachinski ischemia score limitation and the absence of vascular disease on brain MRI.

In summary, the literature on CSF albumin index in AD indicates that a subpopulation of rigorously diagnosed AD subjects has BBB impairment. We will consider the etiology and consequence of BBB impairment in AD below, after reviewing other types of evidence of BBB impairment in living subjects with AD.

Imaging studies of BBB integrity in Alzheimer’s disease, mild cognitive impairment & nonimpaired elderly

The first imaging method applied to the question of BBB integrity in AD was PET imaging with radiolabeled EDTA (Table 2). A study of five AD and five control subjects with this method found ‘no evidence that intravascular tracer entered the brain in either the dementia or control group’ [74]. This small study was followed by two studies of dynamic CT imaging comparing AD patients and controls. In the first study, 26 AD patients and 15 control subjects were studied with a bolus injection of iodinated contrast, followed by serial CT images which were quantified in 16 regions of interest (ROI) [73]. In this method, delayed washout of contrast agent is considered evidence of BBB dysfunction. A nonsignificant trend toward delayed washout of contrast was seen in AD compared with control subjects in 11 out of 14 ROIs. However, a subsequent study with dynamic CT scanning that compared 14 AD and nine control subjects failed to find a difference between groups and concluded that there is no generalized abnormality of BBB in AD [72].

More recently, a small study of 11 subjects with MCI and 11 age- and sex-matched control subjects, examined BBB integrity with dynamic contrast-enhanced MRI [75]. One region typically involved by AD pathology (the hippocampus) and one region spared by AD pathology (the cerebellum) were studied. Contrast material was retained for a longer period of time in the hippocampi of AD subjects compared with controls, suggesting that BBB integrity is impaired in this area even at this very early ‘predementia’ stage of pathology. The absence of any difference in the cerebellum between MCI patients and controls suggests that the differences observed are specific to brain regions affected by AD pathology.

By contrast with the CSF albumin index studies described above, imaging studies of BBB integrity in AD has not included vascular dementia patients as a positive-control group. Imaging studies of vascular dementia have also not used dynamic contrast imaging per se, but have examined contrast enhancement at the site of white matter lesions at ‘optimal’ times after contrast administration. One study found increased contrast enhancement in diabetic patients with white matter lesions [76]; one study found no contrast enhancement in dementia patients with white matter lesions [77], and one study found increased contrast enhancement in patients with ‘Binswanger’s’ type of vascular dementia [78].
Blood–brain barrier dysfunction: cause, effect or epiphenomenon in Alzheimer’s disease?

Evidence for a pathogenic role of impaired barrier function

The literature reviewed above indicates that at least some patients diagnosed with AD have BBB impairment, but the literature is much less clear about the causes and consequences of BBB impairment in this setting. Some have suggested that BBB impairment could actually be an initiating event in the generation of AD pathology, with serum proteins such as plasma amyloid or serum amyloid protein providing a nidus for amyloid aggregation [7,41,46]. Others have argued that BBB impairment is simply a marker of a brain damaged by amyloid deposition, and others have argued that BBB impairment is independent of amyloid pathology altogether, and is instead a marker of arteriosclerotic vascular damage. Probably the most critical question is whether BBB impairment contributes to pathogenesis or rate of neurodegeneration in AD. If it does promote neurodegeneration, then it represents a potential therapeutic target, and discerning the cause of BBB dysfunction is important to those therapeutic efforts. If BBB dysfunction does not impact the course of the disease, then it probably represents an epiphenomenon that is largely irrelevant to therapeutic efforts. Imaging evidence of BBB dysfunction during the ‘preclinical’ MCI stage [75], and evidence that BBB dysfunction correlates with rate of disease progression [71], support the hypothesis that an impaired barrier function of the BBB may have a pathogenic role in AD.

Evidence for a pathogenic role of impaired carrier function

Transport systems along the BBB are important to maintaining brain Aβ homeostasis. There are a number of processes that regulate brain concentrations of Aβ [11,15,38-85]. LRP-1 is the main efflux transporter of Aβ across the BBB to the periphery and is expressed within the brain capillary endothelium [14,15]. LRP-1 has been genetically and biochemically linked to AD [86,87], and its expression is reduced in the brains of AD patients [14,15,88]. LRP-1-mediated transport of Aβ is initiated at the abluminal (brain-facing) side of the endothelium and, therefore, is directly responsible for Aβ clearance from the brain to the blood [10].

The receptor for advanced glycation end products is the main influx transporter of Aβ across the BBB to the brain. RAGE expression is regulated by the level of its ligands, where elevated ligand levels result in increased expression of RAGE [89-91]. In transgenic models of β-amyloidosis and in AD when pathogenic Aβ accumulates, RAGE expression is increased in affected cerebral vessels, neurons or microglia [11,92]. LRP-1 and RAGE play opposing roles in maintaining brain Aβ homeostasis at the BBB. Disturbance to the function of Aβ carrier systems at the BBB may be important to the pathogenesis of AD.

Future perspective

In order to determine if BBB impairment contributes to neurodegeneration in AD, prospective study of outcomes in patients with and at risk of AD will need to be performed. Imaging modalities for assessing BBB integrity are preferred to the CSF albumin index for this purpose, so further development and validation of these imaging modalities is a high priority for this area of research. If prospective studies then support our observation that the rate of disease progression is correlated with the degree of BBB dysfunction, then the next logical step would be to intervene with measures that improve BBB

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<th>Study</th>
<th>Method</th>
<th>Population</th>
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<tr>
<td>Schlageter (1987)</td>
<td>EDTA PET</td>
<td>5 AD; 5 control</td>
<td>No difference between groups, no evidence of BBB impairment in either group</td>
<td>[74]</td>
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<tr>
<td>Dysken (1990)</td>
<td>Dynamic contrast-</td>
<td>26 AD; 15 control</td>
<td>Nonsignificant trend to delayed washout (i.e., BBB dysfunction) in 11 of 14 ROI’s</td>
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<td>Caserta (1998)</td>
<td>enhanced CT</td>
<td>14 AD; 9 control</td>
<td>No difference between groups, no evidence of BBB dysfunction</td>
<td>[72]</td>
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<td>Wang (2006)</td>
<td>Dynamic contrast-</td>
<td>11 amnestic MCI; 11</td>
<td>Increased BBB permeability in MCI in hippocampus but not cerebellum</td>
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CT: Computed tomography; EDTA: Ethylenediaminetetraacetic acid; MCI: Mild cognitive impairment; ROI: Region of interest.
function, in order to definitively determine cause and effect \[71,93\]. However, since we have not yet identified such interventions, this will require further research as well.

One candidate strategy is lowering of serum homocysteine. Hyperhomocysteinemia precedes and increases the risk of AD and is associated with age-related cognitive decline \[94–96\], although no one is quite sure why or how. Since elevated homocysteine is known to promote vascular disease and endothelial toxicity \[97–102\], it is possible that homocysteine imparts an increased risk of dementia by promoting BBB dysfunction. A report demonstrating that a homocysteine-lowering intervention resulted in normalization of hyperhomocysteinemia and elevated CSF albumin index in MCI patients provides support for this hypothesis \[103\]. Similarly, hypercholesterolemia increases both vascular risk and risk of dementia. The effect of hypercholesterolemia, hyperlipidemia, dyslipidemia and strategies to improve lipid profile upon BBB function and neurologic outcomes is another potential area for investigation.

Defective transport (i.e., of glucose and vitamins) and receptor (i.e., lipoproteins and metals)-mediated transcellular flux and inflammatory aggravation may be consequences of BBB dysfunction that are significant to the pathogenesis of AD. There is very good clinical evidence that immunotherapy with monoclonal antibodies reduces the amyloid burden in blood vessels as well as in the brain. It is possible that amyloid-lowering strategies may act on the BBB by improving its function \[7,46,93\]. The success of these strategies now entering clinical trials may depend on their effects on the BBB.

**Executive summary**

**Physiology of the blood–brain barrier**

- An intact BBB restricts molecule transportation that is largely mediated by the molecular weight and lipid solubility of a given molecule, with lipid soluble agents able to cross the BBB more readily than water-soluble agents.
- Selective pinocytosis and other transport mechanisms permit specific agents to cross an otherwise intact BBB, so that some investigators have emphasized that the BBB has both ‘barrier’ and ‘carrier’ functions.

**Methods for assessing blood–brain barrier integrity**

- Direct brain tissue examination: probing for endogenous markers not usually found in brain tissue or infusion of an exogenous marker that stains brain tissue in the presence of BBB disturbance.
- Cerebrospinal fluid (CSF) albumin index: most commonly used marker of BBB integrity in living subjects. Quantifies CSF and serum albumin concentration and is reported as the CSF to serum albumin ratio or CSF albumin index. Albumin has a molecular weight of approximately 67,000 kDa and a concentration roughly 200-times greater in serum than in CSF. BBB disruption allows serum albumin greater access to CSF. This method requires that the patient undergo a lumbar puncture.
- Imaging: a tracer bolus is infused while MRI, CT scans or PET detects this tracer in regions of interest. The low molecular weight of these tracers (i.e., gadolinium) may allow detection of subtle changes in BBB integrity, which may be important to AD pathogenesis. The noninvasiveness is also appealing compared with the lumbar puncture required for the albumin index.

**Pathophysiology of Alzheimer’s disease**

- The central premise of the amyloid hypothesis is that the neurodegenerative cascade in Alzheimer’s disease (AD) is due to a neurotoxic form of amyloid β peptide. Amyloid angiopathy is a possible cause of BBB impairment in AD. Atherosclerosis is another possible cause of BBB impairment in patients with a clinical diagnosis of AD.

**Alzheimer’s disease & the blood–brain barrier**

- Histological evidence of BBB disruption in post-mortem AD brain tissue is available. Studies utilizing the CSF albumin index in living subjects with AD compared with controls are mixed, although a limited number carefully control for vascular factors. The development of imaging methods for assessing BBB permeability in AD is in its infancy. The most recent of the four reports discussed utilizes dynamic contrast-enhanced MRI. This technique may hold promise in detecting subtle changes in BBB physiology.

**Blood–brain barrier dysfunction: cause, effect or epiphenomenon in Alzheimer’s disease?**

- There is evidence to support dysfunction of the barrier and carrier functions at the BBB in AD.

**Priorities for future research in this area (in descending order)**

- Development and validation of imaging modalities for assessment of BBB.
- Prospective studies of the effect of BBB integrity upon cognitive outcomes in patients at risk of, or with, AD.
- Development and application of nutritional and antiamyloid interventions directed at ‘optimizing’ BBB function.
In summary, the priorities for future research in this area, in descending order, are:

- Development and validation of imaging modalities for assessment of the BBB;
- Prospective studies of the effect of BBB integrity upon cognitive outcomes in patients at risk, or with, AD;
- Development and application of nutritional and antiamyloid interventions directed at ‘optimizing’ BBB function.

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