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Drug Delivery to the Brain in Alzheimer's Disease: Consideration of the Blood-brain Barrier

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Abstract

The successful treatment of Alzheimer's disease (AD) will require drugs that can negotiate the blood-brain barrier (BBB). However, the BBB is not simply a physical barrier, but a complex interface that is in intimate communication with the rest of the central nervous system (CNS) and influenced by peripheral tissues. This review examines three aspects of the BBB in AD. First, it considers how the BBB may be contributing to the onset and progression of AD. In this regard, the BBB itself is a therapeutic target in the treatment of AD. Second, it examines how the BBB restricts drugs that might otherwise be useful in the treatment of AD and examines strategies being developed to deliver drugs to the CNS for the treatment of AD. Third, it considers how drug penetration across the AD BBB may differ from the BBB of normal aging. In this case, those differences can complicate the treatment of CNS diseases such as depression, delirium, psychoses, and pain control in the AD population.

Keywords

Blood-brain Barrier; Alzheimer's Disease; Drug Delivery; Cerebrospinal Fluid; Biologicals; Peptides; Regulatory Proteins; Transport; Transmembrane Diffusion; P-glycoprotein

1. Introduction: The Specter of Alzheimer's Disease

Alzheimer's disease (AD) is a neurodegenerative disease that is typically characterized by its histological findings of neurofibrillary tangles and amyloid plaque, by increased levels of oxidative stress and neuroinflammation, and by greatly reduced levels of acetylcholine. Chiefly characterized in its early stages by a decline in recent memory, its later stages are characterized by a cognitive decline so profound that its victims lose the abilities, interests, and skills to perform even simple activities of daily living such as bathing, dressing, eating, and toileting. Long before these final stages, a person with AD can no longer recognize children, spouses, and siblings and lose the personality traits that had characterized them as individuals. These losses coupled with the appearance of other behavioral problems that include violent behaviors, depression, delirium, various psychoses, and loss of judgment and social skills means that many persons with Alzheimer's disease spend their last years institutionalized and in a mental exile from others.

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AD is epidemic with an estimated 33.9 million people worldwide having the disease [1]. The incidence rate increases exponentially with aging so that at age 90 about 12% of people have AD, but about 40% of those over age 100 have it [2]. Several factors that put persons at increased risk of AD are a history of head injury, obesity, diabetes mellitus, hypertension, renal disease, and histories of smoking, traumatic brain injury, or depression [1, 3, 4]. As the occurrence of many of these factors is becoming more common, the incidence of AD may increase even more. Clearly, interventions that prevent, stabilize, remediate, or cure AD are desperately needed.

To some extent, a fundamental difference in the approach to therapeutic discovery has arisen between the clinical and basic sciences. Clinical work has shown that AD begins years prior to onset of symptoms as detected by commonly used cognitive instruments [5]. Given the assumption that cognitive decline and other symptoms in AD are primarily caused by irreplaceable neuronal loss, it is also assumed that AD drugs must be started as early as possible. This thinking has contributed to the recent NIH-supported revision in Alzheimer's diagnostic guidelines having a greater emphasis on preclinical diagnostic tests, imaging, and biomarkers (<http://www.nia.nih.gov/Alzheimers/Resources/diagnosticguidelines.htm>). Such earlier diagnosis would be needed in part to adequately test drugs at a time in the disease when brain function can be largely preserved. In contrast, work in animal models of AD shows that the histopathological hallmarks and cognitive impairments can be reversed even in animals with longstanding disease [6–13]. The animal work, then, suggests that a reversible neurotoxicity mediates the symptoms of AD to a significant degree. This, in turn, offers hope that even well-advanced cases of AD may be treatable and that preclinical diagnosis is not a prerequisite to effective therapy. In short, clinical research posits that treatment must be started early to be effective, whereas basic science research posits that treatments can be effective even in well-established disease.

Successful treatment of AD is likely to be pharmacologically based and will in almost every case target the central nervous system (CNS). Drugs targeted to the CNS must negotiate the blood-brain barrier (BBB). This review will examine various aspects of the BBB that are relevant to drug delivery. This includes strategies for getting drugs across or in some cases bypassing the BBB. It also includes ways in which alterations in the BBB can contribute to the onset and progression of AD; in such cases the BBB itself is a therapeutic target. Finally, the review will consider how alterations in the BBB of AD patients can alter uptake of CNS drugs in general and so complicate the treatment of depression, psychoses, and other CNS diseases in the AD patient.

2. Definitions of the BBB and Its General Relation to Drug Delivery

The term BBB has been used to mean many things in the scientific literature. Its roots began with experiments performed in the late 19th and early 20th centuries that found that some dyes could stain the brain after direct injection into the brain but not after peripheral administration [14]. A parallel line of study found that bile salts induced seizures when injected directly into the brain but not after peripheral administration. Explanation of the results of the dye study depends not only on a barrier separating the brain from the blood but also on the dye binding tightly to albumin in the blood. Thus, a conceptual definition of the BBB consistent with original observations is that of a composite of processes that control the exchange of substances between the blood and the fluids of the CNS (the brain interstitial fluid and the cerebrospinal fluid). This was elegantly stated by Roth and Barlow [15] over 50 years ago, a few years before the discovery of the ultrastructural basis of the BBB: “the blood-brain barrier is a complex anatomical, physiological and biochemical phenomenon, and no unitary hypothesis is adequate to embrace all the observed events.”

A much more restrictive definition of the BBB is an anatomically based one that identifies the BBB as the microvasculature of the brain. The arterioles, venules, and capillaries of the CNS are specially modified (intercellular tight junctions, decreased macropinocytosis, and greatly decreased number of intracellular fenestrae) so as to essentially eliminate the production of an ultrafiltrate [16]. This lack of production of an ultrafiltrate eliminates leakage, negates the need by the CNS for a well-defined lymphatic system, and largely accounts for many unique characteristics of the CNS, including the historical although not entirely accurate idea that it is an immunoprivileged space [17, 18].

However, the vascular BBB alone does not account for all of the aspects of the conceptual BBB. The choroid plexus, often termed the blood-cerebrospinal fluid barrier, is the major site of production for the cerebrospinal fluid (CSF) and is intimately involved in many aspects of blood-CNS exchange, including those related to drugs [19, 20]. The tanyctic barriers found at circumventricular organs [21], specialized barriers in the retina, and specialized barriers at the cranial/spinal nerves offer additional complexity [22]. Another level of complexity is added in that all barrier cells are in intimate communication with cells on both sides of the barrier. The communication provided by the resulting neurovascular unit acts to define the properties of the BBB under changing physiologic conditions and likely has important roles in disease [22–25].

Without the production of an ultrafiltrate, the CNS must be nourished by other mechanisms. The BBB, whether defined conceptually, restrictively, or broadly to include the choroid plexus and other barrier sites is intimately involved in CNS nutrition. The BBB is endowed with a host of specific and selective transporters that supply the CNS with glucose, free fatty acids, amino acids, vitamins, minerals, and electrolytes [26]. Brain-to-blood mechanisms such as reabsorption of CSF and efflux pumps such as p-glycoprotein (P-gp) aid the CNS in maintaining homeostasis [27]. The BBB also plays roles in communication by transporting between the blood and CNS informational molecules such as regulatory proteins and peptides [28–30]. Enzymatic and secretory functions of the cells that comprise the BBB contribute to the roles of nutrition, homeostasis, and communication. The transporters of the BBB are not static, but vary with development and aging in response to the changing needs of the CNS. This response is testimony to the cross-talk that is constantly going on between the cells which constitute the blood-brain barriers and the cells of the CNS including pericytes, microglia, astrocytes, and neurons [22, 31]. The BBB, then, is better conceived of as a regulatory interface between the CNS and blood than as a rigid barrier.

Finally, there are many aspects that impact on specific drug delivery strategies or that potentially affect disease progression in AD that should be considered in context with the BBB (Figure 1). These include CSF production and reabsorption, brain-CSF diffusion interactions, circulating binding proteins/soluble receptors, and neuroinflammatory mechanisms [17, 19, 31–35].

This complexity has several implications for AD:

1. Dysfunction of the BBB, either from lack of adaption to CNS demands or because of a primary defect in BBB dysfunction, can result in disease. Specific alterations in the BBB may affect the onset or progression of AD. In these cases, the BBB itself is a therapeutic target.
2. A myriad of strategies for delivering drugs to the AD brain have been proposed. This review will examine many of these strategies in the context of BBB function.
3. AD-related alterations in the BBB have implications for treatment of other CNS conditions in AD patients. For example, drugs used in the treatment of depression, delirium, pain control, and psychoses may access the brain differently in those with

AD than in those without AD. As a result, the dosages, efficacy, potency, therapeutic window, and side effect profiles of drugs may differ between AD and non-AD patients.

This review will consider drug delivery to the AD brain in these three contexts (Figure 2): 1) The BBB as causal to AD and therefore itself a therapeutic target; 2) selected strategies for delivering AD drugs across the BBB; 3) how a BBB altered by AD affects the delivery of drugs to the brain for the treatment of other (non-AD) CNS diseases.

3. The BBB as a cause of AD and therapeutic target

Over a dozen mechanisms have implicated the cerebrovasculature, choroid plexus, or CSF drainage as playing a role in the onset or progression of AD (Table I). These mechanisms are seldom mutually exclusive and many could be interrelated or aspects of an underlying process. This section will discuss the mechanisms that have been studied as therapeutic targets for the treatment of AD. Many of these mechanisms would also affect drug delivery to the brain in general and so are considered in that section as well.

3.1 BBB disruption

Some early observations reported that AD patients had increased levels of albumin in the CSF [36]. This was originally thought to represent evidence that the BBB was disrupted in AD. However, later studies have tended to find normal levels of albumin in the CSF [37, 38] or to attribute increased albumin to a slower reabsorption of CSF back into the blood stream, termed bulk flow [39]. Decreased bulk flow has also been observed in a mouse model of AD [40]. Measures of increased albumin in the CSF correlate with disease progression [41] and are inversely related to CSF/serum ratios of folate [42]. Although there is no evidence in humans or animal models for a massive disruption of the BBB like that seen in multiple sclerosis or even in stroke, there is evidence that microvascular leaks may be occurring. The “white matter changes” classically seen on AD imaging and thought to represent small vessel disease in the brain correlate with the degree of microalbuminuria caused by microvascular leakage at another very highly regulated interface: that of the kidney tubules [43]. Indeed, microalbuminuria, which is evidence of microvascular kidney disease, is a risk factor for cognitive decline [44]. This is consistent with ideas that AD has a systemic component and that it is part of a spectrum of vascular disease. On the one hand, shared risk factors with myocardial infarction and stroke support the idea of a spectrum of disease [45–47], whereas on the other hand a less robust protection from statins and nonsteroidal anti-inflammatory drugs argues against it [48–51]. Micropunctate lesions representing limited protein leakage at capillaries have been demonstrated in some animal models of AD [52]. Any BBB disruption in AD models seems to be too low to influence influx of drugs [53].

Repair of BBB integrity, were it to exist, would represent a very tempting therapeutic target. A great deal has been discovered in recent years about the construction and regulation of tight junctions and about their dysfunction in ischemia, hypoxia, neuroAIDS, epilepsy, and other conditions. Little attention has been paid to the other, perhaps dominant, mechanisms of BBB disruption involving macropinocytosis and other vesicular mechanisms. Interestingly, one of the few drugs available for the treatment of AD, the NMDA receptor antagonist memantine, protects against BBB disruption [54–56], probably by blocking NMDA-mediated oxidative stress at the brain endothelial cells (BEC) [56]. Activated protein C has been proposed to protect BBB integrity through several mechanisms including decreased apoptosis of BEC (and hence decreased BBB disruption), decreased neuroinflammation, and neuroprotection [57, 58]. Activated protein C binds to protease activated receptor-1 on BECs to protect them against apoptosis. It is also transported across the BBB by the endothelial protein C receptor to access other cells of the neurovascular unit.

The correlation between microproteinuria and white matter changes raises the question of whether treatment with drugs directed at the renin-angiotensin-aldosterone axis would be as effective in protecting the brain in AD as they have been in protecting renal function in diabetes mellitus. In this regard, angiotensin II has been shown to increase BBB permeability through both tight junction and vesicular mechanisms [59, 60]. Some activities of the brain's renin-angiotensin system have been reported to be increased in Alzheimer's disease [61] and treatment with angiotensin II blockers decreases brain inflammation [62]. Patients with Alzheimer's disease and microalbuminuria that were treated with agents that would either decrease angiotensin II levels or block its receptor were less likely to show mental status decline [44]. Several studies have found that angiotensin II receptor blockade is associated with a decreased incidence of Alzheimer's disease, including a recent case control analysis from the UK [63]. The angiotensin II blockers were more protective than other antihypertensive agents and more protective against Alzheimer's disease than against vascular dementias, suggesting that the effect was not attributable only to a decrease in blood pressure. These results are consistent with a therapeutic benefit from protection of the brain's microvasculature.

3.2 Decreased cerebrovascular blood flow and uptake of oxygen and glucose by the AD brain

For substances that cross the BBB extremely well, cerebral blood flow (CBF) dictates their rate of uptake by brain [64]. Glucose and oxygen are two substances vital to brain function with high extraction rates. CBF is not static but under the influence of the cells whose metabolic demands are dependent on that CBF [23]. Cerebral blood flow (CBF) both at rest and to activated areas is decreased in the preclinical stage of AD [5]. Whether the decreased CBF and decreased glucose and oxygen use by the AD [65] brain is because of a defect in flow mechanisms or in response to a decreased demand by the CNS is unclear, but is a vital question as to their role in the pathogenesis of AD and to therapeutic approaches [5]. If decreased CBF is in response to a lower metabolic demand by the brain, then altering CBF or increasing oxygen and glucose uptake would not be expected to result in a therapeutic benefit. If decreased CBF is a primary lesion, then its impairment would be slowly starving the brain and improved blood flow or improved oxygen and glucose delivery to the brain would be expected to preserve brain function. An increasingly tortuous capillary bed results in rheological alterations that impair the ability of nutrients to cross the BBB, thus magnifying the problem of decreased cerebral blood flow [66]. That "vascular starvation" can produce severe CNS disease is exemplified in a family described by De Vivo et al [67]. These individuals have a 50% decrease in GLUT-1, the BBB transporter for glucose, and have mental retardation and seizures.

Restoring blood flow or enhancing oxygen and glucose delivery is not likely to be easy. Simply increasing blood glucose will not greatly increase CNS levels of glucose because GLUT-1 is readily saturated; otherwise, diabetes mellitus would be expected to be a protective rather than a risk factor for AD. A brain-derived peptide preparation termed cerebrolysin increases GLUT-1 expression and glucose transport across the BBB by 60–90% in rats [68]. Transposition of the omentum as a means of improving CBF has been proposed and reported to reduce amyloid plaques although not neurofibrillary tangles [69]. A case series of six AD patients treated with omentum transposition showed significantly less decline than predicted [70]. However, fat, including omentum, has now long been known to be a source of neurotropic and neuroprotective agents such as leptin [71]. As discussed below, the gastrointestinal hormones represent a large class of potential therapeutics for the treatment of neurodegenerative diseases. It may be that the omentum is delivering neuroprotective gastrointestinal hormones to the brain.

3.3 Vascular injury and a stroke/multi-infarct dementia (MID)/AD spectrum

Consistent with this mechanism, hypertension and some of the other risk factors for stroke are also risk factors for AD; furthermore, treatment of hypertension and those risk factors is often associated with protection from AD. However, some anti-hypertensives may exert their protective effects against AD through mechanisms other than blood pressure control. For example, as discussed above, angiotensin II receptor blockers may protect against microvascular disease and, as discussed below, calcium channel inhibitors may improve brain-to-blood efflux of amyloid beta peptide (A-beta). Similarly, the risk for AD imposed by dyslipidemias is not reversed by lipid lowering drugs, suggesting that some other aspect of the metabolic syndrome is contributing to AD [51].

3.4 CSF drainage

Reabsorption of the CSF back into the blood stream is impaired in aging and even more so in patients with AD [39]. As a result, toxins may be cleared more slowly from the CNS. Most of the CSF is drained from the brain by way of the cribriform plate and is drained through the cervical lymphatics on the way to the blood [72, 73]. Such drainage likely modulates immune responses to CNS and peripheral antigens [17, 18]. Increased levels of CNS toxins and altered immune functions mean that decreased CSF drainage could have a number of negative effects on cognition.

One therapeutic approach has been to shunt CSF from the brain to blood in AD patients. Although a pilot study initially showed encouraging results for CSF shunting [74], a recent analysis of a randomized, double-blind, placebo-controlled trial of 215 patients failed to show benefit to shunting [75]. Treatment of a mouse model of AD with antisense directed at APP has been shown to reverse the defect in CSF reabsorption [40].

3.5 Endothelial function/secretions/BBB cell inflammatory responses

The cells that comprise the BBB, including BECs and the epithelial cells of the choroid plexus, have secretory capacity. Some studies have shown that BECs from AD patients secrete a neurotoxin [76]. Other studies have shown that they secrete cytokines, prostaglandins, nitric oxide and other substances both constitutively and in response to viral, bacterial, and hormonal substances [77–79]. BECs themselves are likely responding to various aspects of the AD environment. BECs respond to a wide range of substances in the blood and CNS. For example, insulin alters BEC alkaline phosphatase levels and the BBB transport rates of tryptophan and leptin [80–82].

One substance that BECs could be responding to is A-beta. BECs bind, internalize, and transport A-beta1–40 and A-beta1–42. A-beta1–40 taken up from the blood is not well transported across the BBB but mostly remains adhered to or internalized by the BEC, whereas A-beta1–42 is transported across the BBB [83, 84]. A-beta acts on BECs to induce chemokine secretion, monocyte trafficking, decreased proliferation, altered glycoprotein expression, altered permeability, and altered nitric oxide synthase activity [85–90]. Tau proteins also lead to disruption of the BBB through the release of cytokines from activated microglia [91]. These findings are consistent with the AD environment promoting barrier cell secretions that have detrimental effects on cognition by affecting neuronal, glial, and pericyte functions [92].

Treatments directed at disrupting the BEC/A-beta interactions could alter or control the toxic effects that result from BECs responding to A-beta. Because the BEC is exposed to peripherally generated A-beta on its luminal side and to CNS-generated A-beta on its abluminal side, treatments directed at A-beta within the brain may have different effects on BEC function than those directed towards peripheral sources of A-beta.

3.6 Abeta transport: RAGE, LRP-1, and P-gp

A-beta is transported bidirectionally across the BBB; that is, both in the brain-to-blood (efflux) and the blood-to-brain (influx) directions. Separate transporters are responsible with blood-to-brain transport being primarily mediated by RAGE and the brain-to-blood transport being primarily mediated by LRP-1 [93]. P-gp also seems to have an effect on the brain-to-blood transport of A-beta [94–96] as may other related transporters [97, 98]. Increased blood-to-brain transport by RAGE and decreased efflux by LRP-1 and P-gp all act to enhance the uptake or retention of A-beta in AD [94, 95, 99, 100].

The evidence for efflux being important in A-beta accumulation in brain and for cognitive impairment is especially convincing. Knockdown of LRP-1 with antisense results in decreased A-beta efflux, increased brain levels of A-beta_{1–42}, and cognitive impairment [101]. Mutations in LRP-1 result in decreased A-beta efflux [100] and P-gp knockout mice have accumulations in brain A-beta and cognitive deficits [102]. It is unclear how P-gp interacts with LRP-1 in the efflux of A-beta. One possibility is that P-gp primarily prevents the uptake by the BEC of A-beta from the circulation; another possibility is that LRP-1 and P-gp somehow interact in a two-step process to remove A-beta from the brain. Why A-beta has both influx and efflux transporters is not clear. However, A-beta has the ability to promote memory at lower concentrations [102] than that at which it impairs it [103]. As illustrated for potassium, the presence of influx and efflux transporters at the BBB allows for a very precise control of CNS levels [104]. It may be that efflux/influx transporters act in tandem to maintain the CNS levels of A-beta at its most optimal concentration.

The transporters are differentially regulated with regards to A-beta transport. For example, A-beta influx is altered by A-beta binding to apolipoproteins [105–107]. Transport in both directions is likely influenced by the primary structure of A-beta as exemplified by A-beta mutations being less well transported and by human A-beta being effluxed less well than murine A-beta in mice [108, 109]. Secondary structure is also likely important with the assumption that monomers are preferred ligands. Finally, inflammation induces an increased influx and decreased efflux of A-beta across the BBB [110], changes which may in part be mediated by inhibition of P-gp [111, 112].

Several therapeutic options are suggested by the alterations in A-beta transport; some of these have been tested and seem to have beneficial effects. Knocking down APP expression results in recovery of A-beta efflux, both suggesting that antisense directed against APP could be used therapeutically to correct efflux and also that A-beta somehow poisons its own efflux systems [101]. It could be that A-beta induces the oxidation of LRP-1 shown to occur in AD patients and thus impairs LRP-1 function [113]. In an AD mouse model with impaired A-beta efflux, treatment with APP-directed antisense reduces oxidative stress [114] and treatment with antioxidants lead to improved cognition [115]. Treatment with the nonsteroidal anti-inflammatory drug indomethacin restores the inflammation-induced inhibition of efflux, but not the enhancement of influx [110]. Indomethacin, but not necessarily other nonsteroidals, has been associated epidemiologically with protection against AD [116]. Vitamin D enhances A-beta efflux [117] and the calcium channel blocker nilvadipine increases A-beta efflux, reduces brain levels of A-beta, and improves cognition in an animal model of AD [118]; calcium channel blockers commonly used in the US such as amlodipine and nifedipine do not affect A-beta efflux. Thus, evidence exists that treatment with APP antisense, antioxidants, vitamin D, nonsteroidal anti-inflammatory drugs, and calcium channel blockers can restore the deficit in A-beta clearance from brain.

3.7 Other altered transporters

Transporters in addition to those for glucose and A-beta may be altered in AD or models of AD. Lower levels in brain, CSF, or CSF/blood ratios for insulin, vitamin B12, and folate suggest that transporters for these substances may be defective in AD [42, 119, 120]. In animal models of AD, regional transport of the cytokines tumor necrosis factor-alpha and interleukin-1 are altered [121, 122]. Examples of these have been provided in the SAMP8 mouse, a natural mutation that has an age-dependent accumulation of A-beta and age-dependent impairments in learning and memory [6, 12]. The SAMP8 mouse develops many of the findings of AD brains including cholinergic deficits, oxidative changes, impaired CSF reabsorption, and impaired A-beta efflux, all of which are reversed by treatment either with antibody directed against A-beta or antisense directed against amyloid precursor protein (APP) [8, 40, 115, 123–128]. IL-1 is not transported into the hippocampus, thalamus, hypothalamus, pons-medulla, or cerebellum of SAMP8 mice whereas it is transported into these regions of non-SAMP8 mice. How alterations in these transporters might affect the brain are largely unexplored.

4. Strategies used to deliver AD drugs to the brain

It is estimated that over 400 drugs for AD are being investigated in about 830 clinical trials (ClinicalTrials.gov). Many more substances are being investigated in animal models of AD. Drugs that must reach deep brain targets as is the case in AD must cross the BBB. However, many drug trials fail because of inadequate trial design with one of the chief flaws being a neglect regarding BBB penetration [129]. The BBB represents one of the greatest challenges for drug delivery to the CNS and many strategies have been devised to meet that challenge. Here, the mechanisms by which potential AD drugs cross the BBB are reviewed.

4.1 BBB disruption

At first it seems obvious that any disruption in the BBB would improve drug delivery to the brain. This has tempted many to propose disrupting the BBB for the purposes of drug delivery, despite the obvious problem that many of the endogenous substances that will then enter the brain from the blood are neurotoxic [130]. For this reason, disruption of the BBB in the delivery of therapeutics must be carefully controlled [131]. Studies in stroke models and with osmotic opening show that the resulting disruption of the BBB is sufficient to allow therapeutic levels of drug to accumulate in the disrupted region [131, 132]. However, other studies suggest that the increase in influx rate resulting from most approaches to BBB disruption is insignificant compared to the other dynamics that determine the equilibrium between brain and blood for a solute. For example, the efflux transporter for potassium is so robust that BBB disruption does not alter its concentration in the CSF [133]. The proposed micropunctate disruptions of the BBB proposed in AD and seen in some animal models may not be sufficient to allow drugs to reach therapeutic levels. This is because even a disrupted BBB is usually still very restrictive in comparison to peripheral tissue beds. Additionally, the poor diffusion within brain tissue would prevent drug from reaching areas of the brain more than a few hundred microns from the lesion. Recently, Cheng et al found that BBB disruptions in animal models of AD and multiple sclerosis were not sufficient to alter small molecule uptake by brain [53]. Thus, for chronic diseases like AD, current pharmacologic methods of BBB disruption do not offer an acceptable cost/benefit ratio for drug delivery.

4.2 Lipid Solubility

Most CNS drugs used in the clinic are small, lipid soluble molecules. As such, drug development in this area continues to be dynamic, as exemplified by the work on the cholinesterase inhibitors pheneserine and posiphen [134]. The presence of brain-to-blood transporters such as P-gp impede the ability of many small, lipid soluble planar molecules

from accumulating in the CNS to therapeutic levels [135]. However, P-gp activity is decreased in AD and so the AD brain may be exposed to much higher concentrations and to many more drugs than is the non-AD patient [94, 95]. A common misinterpretation of the literature [136] is that only molecules less than 400–500 Daltons can cross the BBB by lipid solubility/transmembrane diffusion, but in fact larger molecules can cross the BBB in amounts sufficient to affect the CNS in AD models. For example, the nonpeptide somatostatin agonist NNC 26–9100 (MW = 556 Daltons) exerts positive effects on cognition in an animal model of AD [137]. In particular, the rules derived from small molecules to predict non-saturable passage across cell membranes [138] do not apply very well to biologicals such as peptides and proteins. Breaker peptides, small peptides of 600–700 Daltons that attach to the termini of AB and so prevent or reverse fibrillation, cross the BBB sufficiently to reverse plaques and improve cognition in an AD mouse model [13, 139]. The 27 amino acid form of pituitary adenylate cyclase activating polypeptide (PACAP; MW = 3148 Daltons) crosses the BBB by transmembrane diffusion in sufficient amounts to improve cognition in an AD mouse model when its efflux from brain is inhibited [140].

4.3 Antibodies as Therapeutic Agents

Antibodies as therapeutics have been used in two main ways in AD: 1) as directly targeting pathologic agents and 2) as delivery vehicles. The strategies and the BBB interactions are very different for these two approaches. This section will consider the BBB aspects for antibodies directly targeting pathologic agents. The section on saturable transporters below will consider antibodies used as delivery vehicles.

The antibody target in AD has usually been A-beta. Animal studies show that active or passive immunization against A-beta can decrease plaque number and improve cognition. A significant number of patients actively immunized against A-beta developed problems related to the cerebrovasculature such as stroke and encephalitis. Phase 3 trials of passive immunization are ongoing.

The mechanisms proposed by which antibodies act all involve the BBB but in radically different ways. One proposal is that antibodies cross the BBB to directly interact with AB in the brain [141]. Antibodies given directly into the brain can indeed rapidly restore BBB deficits and reverse cognitive impairments in AD mouse models [52, 124, 125, 142, 143]. Antibodies cross the BBB slowly and in small amounts by the mechanism of the extracellular pathways [142, 144]. IgG molecules are transported out of the brain by a saturable efflux system as well as with the reabsorption of CSF [141], whereas IgM molecules return to the blood only with CSF reabsorption [105]. It was once thought that the saturable efflux system for IgG was mediated by FcRn [145], but recent work has shown that transport function occurs in FcRn knockout mice [146]. The lack of a saturable efflux component for IgM means that after a single intravenous injection the amount of IgM accumulating in the CNS is about twice that of IgG molecules [142]. Thus, the ability to cross the BBB, accumulate in brain, and to reverse disease means that it is possible that antibodies act in this manner.

Another proposal is that antibodies act by binding A-beta in the circulation [147]. This would prevent circulating A-beta from contributing to brain levels of A-beta. It is well established that A-beta is transported from blood into the brain by RAGE and that transport is enhanced in AD mouse models and with activation of the innate immune system [93, 110, 148]. Thus, circulating antibodies could prevent A-beta produced in peripheral tissues from entering the CNS as well as prevent A-beta that had been effluxed from the CNS by LRP-1 and P-gp from reentering the CNS. However, it is unclear whether circulating A-beta contributes to the A-beta load in brain. The evidence that blood levels of A-beta contribute

to brain levels is exemplified by the paper of Sutcliffe et al [149]. In that study, animals treated with STI571, a cancer therapeutic that decreases A-beta production, had a decrease in both their brain and blood levels of A-beta. Given that STI571 does not cross the BBB, it was argued that a reduction in A-beta production occurred at peripheral but not at CNS sites; therefore, brain levels of A-beta fell because of the decreased contribution of A-beta from blood. However, this paper did not rigorously show that STI571 does not cross the BBB. If it does cross the BBB, then it may have been decreasing A-beta production within the CNS. More convincing is the paper of Atwal et al who used a BACE1 inhibitor to decrease peripheral and brain production of A-beta [150]. They showed that when production of A-beta is decreased in the CNS, brain A-beta levels decrease rapidly. However, at a concentration that inhibited only peripheral production, brain levels of A-beta remained unchanged. This work strongly suggests that peripheral levels of A-beta contribute little or nothing to CNS levels of A-beta. Thus, a controversy exists in the literature as to whether circulating A-beta contributes to levels of A-beta in the brain; in turn, this means that it is unclear whether sequestration of A-beta in the blood is the mechanism by which antibodies affect AD.

4.4 Transport systems

Use of endogenous transport systems is the great, untapped strategy in drug delivery to the brain. The vascular BBB and blood-CSF barrier are both richly endowed with known transporters; yet it is estimated that the majority of BBB transporters have yet to be discovered [151]. Transporters for many of the peptides and regulatory proteins typically have the added complexity of having a heterogeneous distribution; this characteristic could be used to target drug delivery to specific areas of the brain. For example, the BBB transporter for interleukin-1 is especially concentrated at the posterior division of the septum, the leptin transporter at the arcuate nucleus of the hypothalamus, and the transporter for APP-directed antisense at the hippocampus [7, 121, 153, 154].

From clinical use to preclinical study, a few drugs used in AD are known to take advantage of transport systems. Donepezil and probably other cholinesterase inhibitors, one of only two classes of drugs approved for the treatment of AD, is transported across the BBB by an organic cation transporter, most likely that for choline [155, 156]. The antioxidants N-acetylcysteine and alpha-lipoic acid, the B vitamins, and to a large extent the vitamin E's have their CNS levels regulated by BBB transporters [157]. Caffeine, proposed to reduce amyloid burden in an AD mouse model [158], is transported across the BBB by the adenosine transporter [159]. Oligophosphorothioate antisense molecules cross the BBB by a saturable transporter [7]. An oligophosphorothioate antisense that targets APP is effective in reducing levels of APP in brain, stimulating A-beta efflux from brain, reducing oxidative stress, and improving cognition in the aged SAMP8 mouse [7, 8, 114, 160]. Another oligophosphorothioate antisense directed at an efflux component of peptide transport system-6 [140] decreases the brain-to-blood transport of the neuroprotectant pituitary adenylate cyclase activating polypeptide (PACAP). This, in turn, allows blood-borne PACAP to accumulate in brain and to improve cognition in an animal model of AD.

Many gastrointestinal hormones have effects on cognition and are being explored as treatments for AD. These include leptin [161–164], insulin [165, 166], ghrelin [167], glucagon-like peptide [168–171], vasoactive intestinal peptide and the closely related PACAP [172–174], and substance P [175, 176]. Most of these hormones cross the BBB by way of saturable transport mechanisms [154, 177–180] and likely have physiological roles in neural development, neuroprotection, and cognition. However, the short half-life in blood and peripheral effects of these hormones complicate their use for brain effects and so alternative strategies have been tried to improve brain delivery. For example, to overcome the very short circulation half-life of glucagon-like peptide-1 [178, 181], it and its longer

acting homolog exendin has been administered by the intranasal route [170]. Intranasal administration at the level of the cribriform plate has been shown to facilitate entry into the CNS for this and other peptides [182–186]. To overcome its hypoglycemic effect when given peripherally, insulin has also been administered by the intranasal route and shown to have positive effects in AD [187–189].

Trojan horse approaches attempt to harness transporters not to deliver their endogenous ligands, but to deliver attached therapeutic agents. A number of substances have been used as the endogenous ligand, including glucose. Antibodies with attached therapeutic cargos directed at BBB transporters such as transferrin and melanotransferrin have been widely investigated, including in the delivery of agents for the treatment of AD [190, 191]. The proposed mechanism is that an antibody is directed at a target, often a transporter, on the luminal surface of the brain endothelial cell. In theory, the antibody will be transported across the BEC by a vesicular mechanism so that it and any attached drug is delivered into the brain. This strategy has had some unforeseen complications, such as routing of the induced vesicles to the lysosomal compartment with subsequent return of the vesicle to the luminal (not the abluminal) membrane. Recently, Yu et al [192] have demonstrated that using high doses an antibody with a low affinity for the transferrin receptor can indeed deliver large amounts of drug to the CNS. Cognitive effects were not determined in that study.

5. Alterations in the AD BBB: Effects on drug delivery to the brain

The idea that drugs in common use may be taken up differently by the brains of AD patient than in the non-AD patient has rarely been considered. Yet many of the properties of the BBB that determine the extent to which currently used drugs are taken up by the brain are known to be altered in AD: CSF reabsorption, cerebral blood flow, and P-gp activity are the best studied examples [39, 94, 95]. Not all of these changes are relevant to all drugs and some of these effects will tend to increase and other effects will tend to decrease the equilibrium of drugs in the brain (Table 2). Therefore, it is hard to predict what the net effect of these changes will be on any particular drug. However, overall these differences could cause shifts in the efficacy, potency, therapeutic window, side-effect profiles, and dosage of commonly used drugs. These shifts could explain in part why AD patients seem at times at risk for certain side effects from CNS drugs such as drug-induced delirium. A better understanding of how brain pharmacokinetics differ in AD patients would allow appropriate adjustments in drug dosages and fewer CNS side effects.

5.1 BBB disruption

Micro-disruptions of the BBB could allow increased access of some drugs to the immediate environment around those disruptions. Diffusion within brain tissue is poor and so it is likely that drug would be limited to within a few 100 microns of the disruption.

Paradoxically, disruptions of the BBB could actually retard the brain retention of some drugs. Even modest disruptions of the BBB are pathologically significant and induce inflammatory responses. Tumor necrosis factor- α can increase Pgp activity leading to a further reduction of accumulation of Pgp substrates by brain [193–195].

5.2 Decreased CSF Reabsorption

This would be expected to increase the residence time of drugs within the CNS. This is true for drugs entering the CNS at the vascular barrier as well as at the choroid plexus.

5.3 Decreased Cerebral Blood Flow

Only drugs that rapidly enter the CNS are flow-dependent; drugs with lower rates of entry will not have their uptake by brain substantially altered by a decrease in CBF. Donepezil, for example, is likely a flow dependent drug, whereas the oligophosphorothioate antisense molecules are not. Therefore, a decreased cerebral blood flow would be expected to decrease donepezil uptake and its therapeutic effect, but not that of the oligophosphorothioate antisenses.

5.4 P-gp

P-gp function is decreased in AD [94, 95]. The decreased activity of P-gp may be mediated by the neuroinflammatory state of AD [111, 112]. A decrease in P-gp activity would have widespread implications for the use of CNS drugs in AD as so many commonly used drugs are P-gp substrates. Alternatively, tumor necrosis factor alpha can increase P-gp activity and so decrease drug access to the brain [193, 195, 196]. It may be that the increased access of these drugs to the brain or the sudden inhibition of drug transport into brain contributes to the increased vulnerability of AD patients to the development of delirium.

6. Conclusions

The BBB acts as a dynamic interface between the CNS and the peripheral tissues. Many aspects of the BBB are affected and these changes in turn have implications for the onset, progression, control, and treatment of AD. Changes in the BBB itself may contribute to the onset and progression of AD. In this case, the BBB itself becomes a therapeutic target. The BBB is also a formidable barrier for the delivery of drugs to brain tissue in the treatment of AD. Several strategies have been applied to drug delivery including development of lipid soluble drugs, BBB disruption, and use of transport systems. Drugs that normally cross the BBB may be taken up differently by the AD brain than by the normal BBB, thus complicating the treatment of CNS conditions such as pain, depression, psychoses, and delirium in the AD population.

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BRAIN

Figure 1.

The main functions of the BBB: Substances can enter the brain by extracellular, saturable influx, and lipid solubility (transmembrane diffusion) pathways. Cells enter by diapedesis. The physical barrier formed by the capillary wall, saturable efflux systems, enzymatic activity at the barrier cells, and CSF reabsorption limit uptake and retention of substances by the CNS. The barrier cells also secrete a number of substances into brain and blood.

Figure 2.

Three ways discussed in this review in which the BBB is relevant to Alzheimer's disease with examples. Endothelial cell #1: Dysfunctions of BBB can promote AD as exemplified here by impaired efflux of A-beta from brain. Endothelial cell #2: Drugs needed to treat AD must cross the BBB as exemplified here by the ability of donepezil to be transported across the BBB. Endothelial cell #3: Alterations in BBB means that delivery of drugs to the CNS for non-AD conditions (pain control, depression, delirium) is different than in non-AD patients as exemplified by impaired P-gp function.

Table 1

Proposed Mechanisms: Involvement of the Blood-brain Barrier and Cerebrovasculature in Alzheimer's Disease

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-	Leaky BBB: Toxins from the Circulation Enter the CNS
-	Tortuous Capillary Bed: Rheological Alterations Impair Nutrient Uptake by the CNS
-	Defective Glucose Transport
-	Brain Endothelium Induces Neuroinflammation
-	Brain Endothelium Releases Neurotoxins
-	Decreased Cerebral Blood Flow: Deficient Delivery Results in Hypoxia/Deficient Delivery of Nutrients
-	Atherogenesis: Stroke/MID/AD Lie on a Spectrum
-	A-beta Induces Ionophores in BBB Cell Membranes
-	Neurovascular Hypothesis: Defective Brain-to-blood Efflux Allows Accumulation of A-beta in Brain
-	Decreased CSF Reabsorption: Neurotoxic Substances Accumulate in CSF and Brain
-	Decreased P-gp Function: Accumulation of Xenobiotics, Endogenous Neurotoxins, and A-beta
-	Altered Expression/Function at BBB of Excitatory Amino Acid Transporters: Glutamate and other Neurotoxic Substances Accumulate in Brain
-	Oxidative Damage Induces BBB Dysfunction
-	Altered Metalloproteinase Activity Impairs BBB Integrity
-	Increased Blood-to-brain Transport of A-beta

Table 2

Effects of Changes in the AD BBB on Drug Uptake.

Alteration	Directional Effect	Drugs Affected
BBB Disruption	Increased Local Uptake	All Drugs [*]
Decreased CSF Reabsorption	Increased Residence Time in CSF and Brain Interstitial Fluid	All-to-Most Drugs [@]
Decreased Cerebral Blood Flow	Decreased Uptake	Flow-dependent Drugs
Decreased P-gp Activity [#]	Increased Uptake	P-gp substrates

Additional Considerations:

^{*} A molecular weight limit may occur depending on characteristics of disruption; induction of local events related to neuroinflammation may alter many aspects that impact on drug action such as degradation, sequestration, and receptor activity.

[@] Drugs entering the CNS at the vascular BBB that are small and highly lipid soluble, that have robust efflux systems, or that are avidly sequestered or degraded are less likely to be affected.

[#] Inflammatory conditions have also been proposed to result in increased P-gp activity.