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EXPERT REVIEW

Drug Access to the Central Nervous System in Alzheimer's Disease: Preclinical and Clinical Insights

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ABSTRACT Alzheimer's disease (AD) is a neurodegenerative disorder, characterized by β-amyloid plaques and hyperphosphorylated tau tangles in the brain. Alongside these pathological lesions, there have been multiple reports of physical and biochemical alterations to the blood-brain barrier (BBB) in people with AD, potentially impacting on the ability of systemically-administered drugs to reach the brain parenchyma. Though there has been much research into the identification of these BBB alterations during AD, there are very few studies that have assessed the impact of such BBB changes on the ability of therapeutic agents to traverse the BBB. Due to their increased age-associated risk of chronic disease, most people with AD are prescribed multiple concurrent medications. In people with AD, the altered nature of the BBB could impact upon the disposition and therefore pharmacological effects of a wide range of medicines. This review therefore evaluates the impact of BBB alterations in AD on CNS drug exposure, along with relevant examples of preclinical and clinical studies that address this current issue. This review highlights that the CNS exposure of drugs is likely to differ between people with AD and healthy individuals, warranting further clinical investigations and the consideration to tailor dosing regimens in people with this neurodegenerative disorder.

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INTRODUCTION

Alzheimer's disease (AD), a neurodegenerative disorder, is the leading cause of dementia amongst the elderly. Pathologically, it is characterized by two significant alterations (described by Alois Alzheimer in 1907), namely senile plaques (that consist of deposits of amyloid-β (Aβ) peptides) and neurofibrillary tangles (that consist of hyperphosphorylated tau protein) in the brain of people with AD [1]. AD starts with the loss of episodic memory, followed by a decline in cognitive and other intellectual functions, and eventually the execution of basic daily activities is hampered [2]. The prevalence of AD is growing rapidly in line with an increase in the ageing population. Currently, Alzheimer's Disease International estimates more than 36 million people worldwide are living with AD, and this number is expected to rise to 115 million by 2050 [3]. While there is currently no cure for AD, patients are frequently prescribed medicines to treat and manage their symptoms. The US-FDA approved drugs for treating AD symptoms include three cholinesterase inhibitors (galantamine, rivastigmine and donepezil) and the N-methyl-D-aspartate receptor antagonist, memantine [4]. In other countries, such as Australia, some antipsychotic drugs are also approved for the treatment of behavioural and psychological symptoms of dementia [5].

Multimorbidity is highly prevalent in old age, for example, amongst those aged ≥85 years in Scotland, 81.5% have multimorbidity and 30.8% have combined physical and mental health comorbidity [6]. Likewise, in Australia 83.2% of total surveyed patients in 2005 aged≥75 years had higher prevalence and complexity of multimorbiditiy and similar figures of increased multimorbidity with old age also holds

true for other countries [7, 8]. This results in a high prevalence of polypharmacy for treatment of a wide range of conditions in older people with AD [9]. For many of these drugs, central nervous system (CNS) side effects are common, and therefore, an understanding of their ability to traverse the blood-brain barrier (BBB) following oral or parenteral delivery would assist in predicting whether their disposition may alter during AD.

The BBB is the endothelial lining of cerebral capillaries separating the brain interstitial fluid from the peripheral circulation (blood). Under healthy conditions, the BBB maintains the homeostasis of the neuronal environment by regulating the free exchange of solutes between the blood and the brain, and by protecting the brain from xenobiotics, including drugs [10]. Unlike most other endothelial cells in the periphery, the endothelial cells forming the BBB have increased numbers of mitochondria [11], lack fenestrations [12], have markedly reduced pinocytic activity [13], and form characteristic tight junctions [14]. Therefore, the BBB normally prevents many systemically administered drugs from gaining access into the CNS and it has been suggested as a major barrier in minimizing the ability of drugs to exert their pharmacological effect in the CNS [15]. Besides the physical barrier formed by tight junctions, the BBB also presents a biochemical barrier to the transport of various endogenous and exogenous compounds through the presence of active efflux transport proteins such as P-glycoprotein (P-gp), breast cancer resistance protein (BCRP) and various isoform of multi-drug resistance associated protein (MRP1-6 in various species, with MRP1, MRP4 and MRP5 in humans) at the BBB [16–21]. In addition to these efflux transporters, there exist influx transporters that facilitate the transport of important nutrients and amino acids into the brain, such as the glucose transporter (GLUT-1), L-amino acid transporter-1 (LAT-1) and mono-carboxylate transporter-1 (MCT-1) [22–24] (Fig. 1).

However, it should be noted that endothelial cells lining the BBB form only one component of a complex biological unit termed the neurovascular unit. Besides endothelial cells, the neurovascular unit is comprised of pericytes, the basement membrane lining the endothelial cells, and astrocytes, microglia and neurons [25] (Fig. 2). While these additional components of the neurovascular unit ensure that the barrier function of brain endothelial cells is maintained, it is generally considered that tight junction proteins and influx/efflux transporters at the BBB are the prime determinants governing the CNS penetrating properties of a systemically administered drug. In addition, factors such as cerebral blood flow and the thickness of the capillary basement membrane (through which a drug molecule would have to diffuse to enter the brain interstitial fluid) may also play a role in overall CNS drug exposure [26–28]. Any changes in one or more of the above-mentioned factors during disease have the potenital to therefore impact on CNS exposure of systemically-administered drugs.

People with AD are often prescribed multiple medications due to concurrent co-morbidities related to their mental health and ageing [29, 30]. People with AD are frequently prescribed anti-psychotic drugs to treat symptoms of psychosis, agitation, and aggression that are generally associated with cognitive impairment [31, 32]. However the safety of anti-psychotic drugs in this patient cohort has not been completely evaluated clinically and their usage is of major concern as people with AD treated with these drugs have been observed to develop adverse effects including the risk of death and stroke and additional problems such as confusion, sedation and extra-pyramidal symptoms [31, 33]. In addition to CNS anomalies, older people with AD develop other physical illnesses related to ageing such as malnutrition, diarrhoea, gastro intestinal disorders, musculoskeletal disorders, hypertension, hypercholesterolaemia and diabetes [29]. People with AD are therefore likely to be prescribed a large variety of medicines for their co-morbidities, in addition to those medicines required to treat the symptoms of the underlying cognitive dysfunction. Therefore, older people with AD have a higher prevalence of polypharmacy and potentially inappropriate medication use than age matched controls [9, 34] and may subsequently be at an increased risk of drug-drug interactions and cognitive and physical adverse drug effects [35].

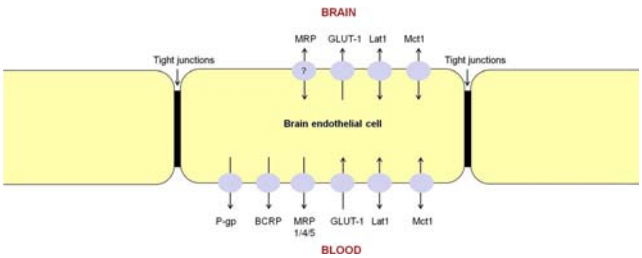

Fig. 1 A schematic representation of influx and efflux transporters that have been characterised in brain capillary endothelial cells. Data from rodents and humans are compiled together in one cell for illustrative purposes with transporters written in capitals referring to human proteins, and transporters in lower case referring to rodent proteins; P-gp (P-glycoprotein), GLUT-1 (Glucose transporter-1), BCRP (Breast cancer resistance protein), MRP1/4/5 (Multi-drug resistance associated protein 1/4/5), Lat1 (L-amino acid transporter 1), Mct1 (Monocarboxylate transporter 1) [16–21, 23, 24, 233].

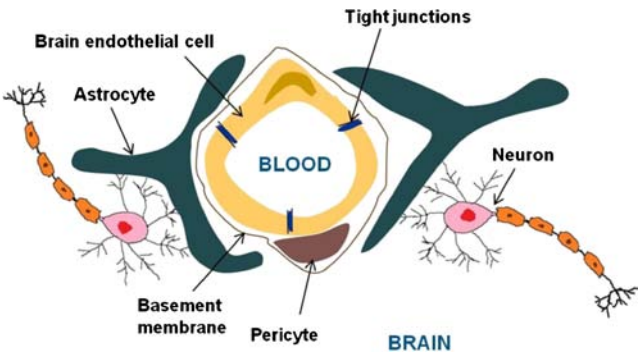

Fig. 2 The neurovascular unit comprised of brain endothelial cells in close proximity to other cell types: pericytes, astrocytes and neurons.

While it is desirable for CNS-acting medicines to cross the BBB and induce a central effect, the ability of non-CNS medicines to cross the BBB and induce adverse neurological effects and sudden behavioural disturbances may be overlooked in people with AD. Non-CNS acting medicines would normally exhibit minimal brain penetration due to the restrictive nature of the BBB. However, the BBB has been reported to be compromised and dysfunctional in AD. These BBB related alterations include physical and biochemical disturbances such as altered expression of efflux transporter proteins such as P-gp, disrupted tight junctions of the BBB, thickening of the cerebrovascular basement membrane and reduced cerebral blood flow [36–39]. As the BBB forms the major diffusion barrier for the entry of most drugs into the CNS, any such abnormalities may have an impact on the disposition of CNS, as well as non-CNS acting drugs, which may have clinical consequences. For example, if there is increased transport of non-CNS medicines into the brain due to increased permeability of the BBB in AD, this can lead to potential neurotoxicity. Conversely, decreased transport of drugs required to enter the brain to exert their pharmacological activity, such as anti-AD drugs, can plausibly lead to treatment failure.

The purpose of this review is to systematically discuss the physical and functional alterations of the neurovascular unit (with particular emphasis on the BBB) reported in AD and the likely outcome of such alterations on drug access into the CNS. Additionally, relevant preclinical and clinical studies assessing the impact of AD related BBB alterations on CNS drug exposure will be highlighted. Finally, recommendations for further clinical studies designed to improve the safety and therapeutic outcomes of medicines in the AD population will be provided.

HALLMARKS OF AD PATHOLOGY

The exact cause of AD has been under considerable debate and is constantly under review but still the amyloid cascade hypothesis appears to form the most widely-studied hypothesis. The hypothesis states that accumulation of Aβ in the brain is the primary factor that drives AD pathogenesis and the rest of the disease process, including the formation of neurofibrillary tangles (containing hyperphosphorylated tau species), and other complex clinical manifestations of the disease appear to be downstream of the initial Aβ accumulation [40, 41].

Role of Amyloid-β in AD

Aβ consists of a series of C- and N- terminally heterogenous proteolytic fragments, most of which are 40 or 42 amino acids in length, referred to as $A\beta_{40}$ and $A\beta_{42}$, respectively [1]. Aβ peptides are produced by the endoproteolysis of amyloid precursor protein (APP) [42]. The increased production of $A\beta_{42}$ leads to its progressive accumulation in brain interstitial fluid to form senile plaques, and these become widespread across the entire neocortex and other cortical regions [43]. Along with extracellular deposition, accumulation of $A\beta_{42}$ has also been shown intracellularly by immunoreactivity of $A\beta_{42}$ in neuronal cells in transgenic animals as well as in people with AD [44, 45]. With the advancing age in AD, the soluble monomeric form of Aβ readily aggregates to form multimeric complexes like low molecular weight oligomers, high molecular weight protofibrils and insoluble fibrils, and eventually forms dense-cord β-amyloid plaques [46, 47]. The formation of Aβ oligomers and fibrils inside the brain also leads to activation of microglia [48] and astrocytosis [49], that in turn may trigger a cascade of inflammatory responses such as the release of cytokines and interleukin-1β [50]. Furthermore, Aβ accumulation and related inflammatory events in the neurovascular unit give rise to excessive generation of free radicals and oxidative injury to neurons, proteins and other macromolecules [51]. The ultimate effects of these widespread, complex inflammatory, oxidative and ionic changes are neuritic dystrophy, synaptic loss and cell death across the hippocampus and cerebral cortex, leading to progressive cognitive decline. In addition, Aβ has been observed to aggregate on the vessel walls of meningeal arteries and capillaries, as well as intra-cortical vessels, causing cerebral amyloid angiopathy (CAA) [52]. Overproduction of Aβ, decrease of Aβ degradation and/or reduction of Aβ clearance pathways could lead to CAA. Severe CAA may lead to rupture of vessel walls, causing intracerebral bleeding and this has also been related to dementia and decreased cognitive function in people with AD [53–55]. Thus increased accumulation of Aβ has led to a number of cerebrovascular abnormalities such as accumulation of vascular amyloid in the walls of larger and smaller vessels forming the BBB, expression of inflammatory markers in the brain vasculature, and microvasculature degeneration [56].

Role of tau in AD

Tau proteins are microtubule-associated proteins that are abundant in neurons of the CNS. Abnormal hyperphosphorylation of tau and consequent neuronal death in AD has been related to an abnormal cyclin dependent kinase activity [57]. Hyperphosphorylated tau protein can result in the self-assembly/aggregation of paired helical filaments and straight filaments into larger entities known as neurofibrillary tangles [58, 59]. In addition, hyperphosphorylated tau can sequester normal tau and other microtubule associated proteins, resulting in destabilization and depolymerization of microtubules. This leads to impairment of axonal transport, decreased neurotransmission and the loss of synapses which has an immediate impact on

cognitive function [60]. Thus, the formation of neurofibrillary tangles confers an additional toxic lesion within the neuron, disrupting its structure, hampering the transport of important chemicals and eventually leading to neurodegeneration and associated memory loss. There is not much known about the direct connection between tau and cerebrovascular pathology in AD. However, interestingly, in a tau-only mouse model, an increase in the area of the capillary wall was observed without any massive distortion in BBB paracellular permeability [61].

CEREBROVASCULAR PATHOLOGY IN AD

Clinically, the progression of neuropathological events in AD begins several years prior to the cognitive decline and diagnosis of dementia [62]. Studies identifying the associated risk factors that contribute to the pathogenesis of sporadic, late-onset AD have indicated a strong connection between cognitive decline in AD and cerebrovascular disorders, and both of these have emerged as leading causes of dementias in the ageing population [63, 64]. Cerebrovascular dysfunction has been identified as an early event in AD [65] and it has been observed that cerebrovascular pathology appears to interact with the underlying AD pathology, affecting different facets of AD-associated neurodegeneration [56, 66]. Vascular anatomical defects observed in AD with regard to cerebral arteries, arterioles, capillaries and basement membrane of the capillaries further strengthen the hypothesis of a vascular component in the pathogenesis of AD [67, 68]. In addition, there are numerous reports describing significant reductions in cerebral blood flow in AD, and associated vascular metabolic dysfunction as a result of this hypoperfusion of the brain [69–74]. The following sections discuss in breadth the cerebrovascular alterations in AD that ultimately have the potential to affect drug exposure in the CNS.

Cerebral Blood Flow Changes in AD

Clinical evidence demonstrates reduced cerebral blood flow in people with AD, assessed using single photon excitation computed tomography, magnetic resonance imaging and ultrasonography measurements [74–76]. There are various factors that regulate normal cerebral blood flow in healthy conditions [62, 77] but reduced cerebral glucose utilization, abnormal or lost cholinergic innervations of intracerebral blood vessels, and upregulation of transcription factors in cerebral vascular smooth muscle cells are examples of specific factors that have been proposed to underlie the reduced cerebral blood flow observed in AD [78–80]. Moreover, a reduction in cerebral blood flow has been hypothesized to be important in causing vascular damage, capillary degeneration and CAA, dysfunctional synaptic plasticity and neuronal damage [81–85]. Since one of the major contributors to CNS drug delivery is the rate at which compounds enter the brain across brain endothelial cells, a reduction in cerebral blood flow may lead to reduced brain uptake of compounds whose entry into the brain is blood flow dependent such as diazepam. Until now, few preclinical or clinical publications have addressed the impact of reduced cerebral blood flow on CNS drug disposition in AD. One such approach to evaluate the impact of this important parameter would be to measure the brain uptake of drugs such as diazepam and propranolol (compounds for which brain uptake is blood flow dependent) after intravenous administration in AD mouse models and comparing the brain uptake to that measured in wild type (control) mice. Subsequently, clinical studies that evaluate the impact of reduced cerebral blood flow on CNS drug access will be useful.

Brain Metabolic Changes in AD

Glucose is an essential metabolic requirement for all mammalian cells. Hence the availability of glucose and its transport across the BBB and into individual brain cells plays a key role in maintaining normal brain physiological function [86]. A few reports that have assessed glucose transport using positron emission tomography (PET) have demonstrated reduced glucose transport in highly metabolically active brain regions such as the cortex and hippocampus of people with AD [71, 73, 87]. Similar reductions in glucose uptake were also observed in studies performed in animal models of AD [72, 88]. The significantly reduced cerebral blood flow in AD, as discussed earlier, has also been linked to depressed cerebral glucose metabolism, reflected by cerebral glucose utilization measurements, and vice versa (the affected areas exhibiting suboptimal metabolism coincided with those displaying a marked decrease in cerebral blood flow) [80, 89]. There are various hypotheses for the reduced glucose utilization observed in AD, but one which relates to changes in the functional activity of brain capillary endothelial cells could be particularly relevant to this review. In AD, there have been reports of reduced expression of the active glucose transporter proteins, GLUT-1 and GLUT-3, localized in the capillary endothelial membranes forming the BBB and at the plasma membranes of neuronal cells, respectively [90–92]. As glucose from the peripheral circulation has to enter the brain extracellular space and various cells using these specific glucose transport systems, lower expression of these transport proteins may account for the lower brain glucose utilization observed in AD.

There are various examples in the literature of approaches that have exploited glucose-mediated transport systems to deliver active components into the CNS for treating various brain disorders, including AD, Parkinson's disease, epilepsy and the neurocognitive decline related to human immunodeficiency virus (HIV) [93]. In this approach, glycosyl moieties are attached to an active drug and by the use of endogenous

glucose transporters such as GLUT-1, the targeted drug is ultimately transported into the brain parenchyma. Recently, glycosylated tetrahydrosalens were synthesized and evaluated *in vitro* for their potential use as AD therapeutics by chelating metal ions and reducing Aβ aggregation with metal ions [94]. A similar approach of synthesizing glycosylated ibuprofen was utilised by another laboratory for treating the neuroinflammation associated with AD [95]. While the approach to enhance CNS drug delivery using glycosylated drug derivatives for treating AD appears promising, synthetic medicinal chemists need to take into account the altered expression of endogenous glucose transporters in AD [96], and the potential impact this may have on pharmacological brain concentrations *in vivo* Moreover, some of these glycosylated drug derivatives have been reported to be substrates of another transporter, receptor for advanced glycation end products (RAGE), and may be using this transporter for entry into the brain [97]. The expression of RAGE at the BBB has been demonstrated to be increased in humans and rodent models of AD [98–100], and has been suggested by some to result in increased blood-to-brain trafficking of Aβ, given Aβ is also a substrate of RAGE [98, 101]. Therefore, the overall impact on the CNS exposure of specifically-glycosylated compounds may be decreased (if they are only substrates of GLUT-1), increased (if they are only substrates of RAGE), or potentially unaltered (if they are substrates of both GLUT-1 and RAGE; depending on their affinity for each transporter). Subsequently, a systematic evaluation of the impact of AD on the CNS exposure of glycosylated compounds needs to be addressed before any conclusions on their brain uptake characteristics in the AD phenotype can be drawn.

Cerebral Microvasculature Structural Anomalies in AD

Various anatomical defects in the cerebral microvasculature have been reported in AD [67, 68]. Under light microscopy, these microvascular abnormalities appear as thin vessels (known as atrophic or string vessels) and/or vessels with increased tortuosity or twisted vessels (known as glomerular loop formations) and/or fragmented microvessels (related to a decrease in the number of long microvessels and their branches) and there is reduced density of total overall microvasculature [102]. Furthermore, the endothelial cells of the capillaries demonstrate structural and functional deformities including atrophy, swelling or irregular nuclei [103], and an increased number of pinocytic vesicles. Genomic profiling of brain endothelial cells has revealed that people with AD may be exposed to aberrant angiogenesis and premature pruning of capillary networks resulting in reduced cerebral microcirculation [104]. Besides these microvascular morphological changes, there are also reports of compromised components of the neurovascular unit that normally maintain the stability of microvascular endothelial cells. Relevant alterations in AD include swollen astrocytic end feet [105], atrophic pericytes or a higher frequency of pericytes, and a robust thickening and local disruption of the capillary basement membrane [103, 106].

The effects of these reported cerebral microvascular anomalies on drug delivery to the CNS are quite complex. A thickening of the microvascular basement membrane has been noted in post mortem clinical reports and in an animal model of AD, and this pathology was mainly related to collagen fibrils forming fiber bundles, with a significantly increased content of collagen type IV in these fibrils [37, 107]. According to Fickian diffusion theory, a major factor affecting diffusion across a biological membrane is membrane thickness, and an increase in membrane thickness is likely to result in reduced permeability [28]. Although not traditionally considered to be an influential factor in drug transport across the BBB, it can be envisaged that major changes to the thickness of the basement membrane surrounding endothelial cells could impart a retarding effect on drug delivery into the CNS. This hypothesis is thought to be responsible for the results we recently obtained in *in situ* transport studies where radio-labelled transcellular markers, [^{3}H] diazepam and [^{3}H] propranolol, and an anti-AD drug, memantine, were transcardially perfused in wild type and triple transgenic (3×TG) AD mice at 18–20 months of age [108, 109]. We observed a marked and significant reduction in the brain uptake of these compounds in 3×TG AD mice when compared to wild type mice of the same age group (up to 57% in the cortex and up to 62% in the hippocampus). To clarify the potential mechanism responsible for the reduced transcellular transport in 3×TG AD mice, the thickness of the cerebrovascular basement membrane was measured by collagen-IV immunohistochemistry in cortical slices obtained from aged wild type and 3×TG AD mice. A significant increase in the thickness of the cerebrovascular basement membrane was observed in 3×TG AD mice relative to wild type mice, suggesting that the reduced uptake of the transcellular markers and memantine may have been due to a thickened cerebrovascular basement membrane and an increased path length through which these passively-diffusing molecules have to diffuse [108]. Given that the brain uptake of diazepam and propranolol are blood-flow limited, and given that reduced cerebral blood flow has been reported in AD, it could have been argued that this reduction in the brain uptake of diazepam and propranolol was due to decreased blood flow to the brain. However, the transcardiac perfusion rate was kept constant in wild type and 3×TG AD mice, and so this hypothesis was discounted. In addition to basement membrane thickening, other mechanisms may play a role in reducing the brain uptake of passively diffusing lipophilic compounds. For example, the reduced expression of adrenergic and GABA receptors observed in AD brain samples [110–112] may reduce the receptor binding of lipophilic ligands, such as propranolol and diazepam, respectively

leading to reduced brain uptake, but this mechanism is likely to be secondary to the impact of basement membrane thickening. Furthermore, the overall reduction in microvascular density and the related structural defects of capillaries that comprise the microcirculatory network of the brain may lead to impaired and/or reduced BBB transport of vital nutrients and of passively diffusing lipophilic drugs such as diazepam [113]. Overall, the transport studies in 3×TG AD mice demonstrate significantly reduced uptake of transcellular marker compounds, warranting further investigation in additional animal models of AD with a larger number of passively-diffusing compounds, in order to confirm whether this is a true phenomenon across all transcellular-diffusing molecules.

Correlating such reductions in BBB transport of lipophilic passively-diffusing compounds in humans with AD is difficult, given the lack of studies and complexities of such clinical studies. Once AD is established, there is population based evidence suggesting that exposure to medicines with sedative and anticholinergic effects, which are predominantly lipophilic drugs, plays a smaller role in increasing the risk of hospitalisation and mortality in people with AD than in matched controls [114]. While it has not been proven, one possible explanation for this could be reduced passive diffusion of such drugs across the BBB in people with AD.

While the above-mentioned modifications of the basement membrane and the reduced density of the cerebral microvasculature may retard passive transcellular transport across the BBB, other microvasculature aberrations related to altered endothelial structure (e.g. degeneration of endothelial cells, changes to tight junction proteins and increased pinocytic vesicles in the endothelium) are suggestive of a potentially impaired and leaky BBB, which would lead one to assume an increased permeability of xenobiotics across the BBB. In the next few sections of this review, alterations specifically related to the BBB and their potential impact on CNS drug access are therefore detailed.

BBB Integrity and Tight Junction Protein-related Changes in AD

Due to the presence of tight junctions between the endothelial cells forming the BBB, the paracellular route of transport is negligible under healthy conditions. For this reason, the brain parenchyma exhibits extremely low concentrations of plasma proteins such as albumin, which are not synthesized in the brain and are derived from serum [115]. Albumin, due to its high molecular weight, cannot cross the BBB in healthy conditions, with only small concentrations detectable in the cerebrospinal fluid (CSF) (around 200 times lower than serum) [116]. Therefore, the presence of albumin in brain parenchyma or CSF above a certain value is considered a sign of BBB or blood-CSF barrier breakdown, respectively [117]. Several studies have demonstrated increased concentrations of plasma albumin and/or other immunoglobulins in the CSF of people with AD relative to healthy subjects, which has been suggesting to be due to increased CNS access of plasma proteins and therefore BBB hyperpermeability in AD [118–121]. However, other research groups have reported no increase in the CSF levels of albumin in people with AD [122–126]. Indeed, the increased albumin in CSF may be due to a slower reabsorption of CSF back into the blood stream, as suggested by one study [127]. Regardless of these contrasting findings, results suggesting altered concentration of albumin in the CSF are more likely to be reflective of potential changes occurring at the blood-CSF barrier, rather than at the BBB. The blood-CSF barrier is quite different to the BBB in that it generally provides less paracellular resistance, due to more permeable tight junctions and a differential expression of drug transporters [128].

In order to better estimate the integrity of the BBB per se in AD, it would be more appropriate to detect albumin or other endogenous proteins in the brain parenchyma directly. One such previous study failed to detect plasma proteins in the brain parenchyma of people with AD when assessed immunohistochemically [129]. In a more recent study however, plasma-derived prothrombin was demonstrated in the walls of the cerebral microvasculature, in surrounding perivascular neuropils and in senile plaques of advanced stage human AD brain samples [39], indicating microvascular injury and leakage of this protein across the BBB in the advanced stages of AD. In contrast, clinical studies undertaken using neuroimaging approaches (such as computed tomography, magnetic resonance imaging and PET) have independently suggested no massive alterations and/or increases in BBB permeability in people with AD in comparison to elderly healthy controls using contrasting agents such as meglumine iothalamate (iodine), Gd-DTPA and [^{68}Ga] EDTA [130–132], although the sample sizes of these studies were small. From these conflicting clinical observations, it can be seen that the status of the BBB in AD patients remains ambiguous. However, given that studies assessing brain parenchymal accumulation of markers (rather than CSF accumulation of markers) suggest a lack of paracellular dysfunction, it is likely that tight junction dysfunction may not be a prominent component of the AD phenotype.

To further characterize the possibility of BBB paracellular dysfunction in AD, various *in vitro* and *in vivo* animal studies have been performed where the effect of various Aβ isoforms (e.g. 40 amino acid $A\beta_{40}$ or 42 amino acid $A\beta_{42}$) have been examined. *In vitro* studies using brain endothelial cells have shown reduced expression of tight junction proteins and increased permeability of high molecular weight compounds upon treatment with Aβ species [133–135]. For example, when primary and immortalized human brain endothelial cells were treated individually with soluble $A\beta_{40}$ aggregates, there was a decrease in the transendothelial electrical

resistance, and this was associated with a significant increase in the permeability of fluorescein isothiocyanate–bovine serum albumin and fluorescein isothiocyanate-dextran, respectively [133, 134]. Interestingly, in both these studies, there were changes with regards to tight junction protein levels and/or tight junction dynamics. In the first study, exposure to increasing concentrations of $A\beta_{40}$ aggregates resulted in a dose-dependent relocalization of the tight junction protein, zonula occludin-1, away from the cell membranes and into the cytoplasm [133], and in the second study there was a decrease in the expression of occludin at both the mRNA and protein level [134]. However, the concentrations of Aβ used in these studies (μM) far exceeded the pathophysiological concentrations of Aβ observed in people with AD (pM-nM range) [136] and therefore, such *in vitro* studies may not accurately reflect the status of the BBB paracellular route in people with AD.

In addition, a wide range of studies have evaluated the paracellular permeability of the BBB using animal models of AD. *In vivo* studies undertaken in healthy animals assessed the impact of exogenously-administered Aβ on BBB permeability. Upon intracarotid infusion of Aβ peptide into healthy rats, there was extravasation of Evan's blue dye and T-lymphocyte migration into the brain, suggestive of extensive BBB impairment and increased BBB permeability in these animals [137, 138]. However, it should again be noted that as observed in the previous section, the concentrations of Aβ used in these studies (μM range) were far beyond plasma $A\beta_{40}$ and $A\beta_{42}$ concentrations normally detected in people with AD (pM range) [136], therefore limiting the conclusions able to be drawn with regards to people with AD. Various animal models, from small invertebrates to mammals, have been created and explored to study the pathophysiology of AD but very few species spontaneously develop the cognitive, behavioral and neuropathological symptoms of AD as exhibited in humans [139]. Out of all these, transgenic mice genetically predisposed to develop AD-like pathology have been developed and used extensively by different researchers to study the etiopathology of AD [140]. *In vivo* studies have been conducted using transgenic mouse models of AD exhibiting either an over-accumulation of amyloid alone, or an over-accumulation of both amyloid and tau pathology, as observed in human AD. While some studies have revealed massive increases in BBB permeability (using high molecular weight protein compounds as markers of BBB integrity) [141–145], other examples of studies using large and small paracellular marker compounds (sucrose, inulin, and sodium fluorescein) have reported unaltered or reduced BBB permeability [146–149]. Similar to the limited clinical studies that have demonstrated no change in the paracellular route of BBB transport, recent studies in our laboratory using the 3×TG AD mouse model revealed no differences in the hippocampal and cortical uptake of the paracellular marker [^{14}C] sucrose between wild type and 3×TG AD mice at 18–20 months of age [108]. The key findings from *in vivo* mouse studies assessing BBB paracellular integrity are summarized in Table I, where it is clear that controversial results exist. The probable reasons for these conflicting results include differences in methodological techniques, the marker compounds employed, the particular animal models used and the stage of underlying AD pathology in these animal models (for example, some models exhibit amyloid pathology and some exhibit both amyloid and tau pathology), the duration of time allowed for the marker compound to diffuse into the brain and reliability of the detection method. While it would be expected that transient increases in the paracellular route of transport would lead to increased brain uptake of small molecular weight compounds (such as sodium fluorescein and [^{14}C] sucrose), and perhaps a less dramatic effect on larger molecular weight proteins, the opposite appears to have been reported in the literature. Based on the evidence generated to date from the available mouse models, it appears that for small drug molecules, no increase in paracellular transport would be anticipated in AD.

To clearly identify the impact of AD on BBB paracellular permeability, a suggested approach would be to complete a systematic assessment of BBB transport (particularly in AD-affected regions of brain; cortex and hippocampus) with a series of marker compounds with varying molecular weights in a valid animal model of AD. Furthermore, additional clinical investigations using sensitive neuroimaging techniques with a larger number of subjects would be beneficial, to confirm preliminary suggestions that while blood-CSF barrier permeability may be increased (as observed by the presence of albumin in AD CSF samples), BBB paracellular permeability is unlikely to be affected in AD. Based on these summarizing comments and one report of decreased transcellular permeability, it is possible that drug transport across the BBB may not be increased in AD; however, systematic clinical studies are definitely required for this conclusion to be drawn.

BBB Transporter Expression and Function in AD

Apart from the reported physical alterations in BBB integrity and the subsequent unclarified effect on drug permeability in AD, it should be recognized that the expression of various efflux and influx transporters located at the BBB have also been reported to be altered in AD. The modification of expression and function of transporters observed in AD in turn has been suggested to modify the transport processes of their respective substrates across the BBB.

According to the neurovascular hypothesis of AD, a dysfunction in transporter function at the BBB leads to the impaired clearance of Aβ in AD [150] (Fig. 3). The mechanism of Aβ trafficking across the BBB may involve various transporters including low-density lipoprotein receptor-related protein 1 (LRP-1), RAGE and transporters from the ATP binding cassette (ABC) superfamily such as P-gp, BCRP,

Table I A summary of the studies assessing BBB paracellular permeability in different mouse models of AD

Mouse model	Technique for assessing BBB permeability	Observations	Ref.
APP/PS1 (Swedish mutation in human amyloid precursor protein gene and mutation in presenilin 1 gene)	Brain extravasation of endogenous immunoglobulin G	↑ BBB permeability	[142]
	Brain uptake of systemically administered [^{131}I] albumin	↓ BBB permeability	[149]
	Brain uptake of systemically administered sodium fluorescein	No change in BBB permeability	[147]
Tg2576 (Swedish mutation in human amyloid precursor protein gene)	Brain extravasation of exogenous Texas-red conjugated bovine serum albumin	↑ BBB permeability	[143]
SAMP8 (senescence-accelerated mouse prone 8; a model of senescence with overproduction of amyloid precursor protein and Aβ)	Brain extravasation of immunoglobulin G	↑ BBB permeability	[141]
	Brain transfer of systemically administered [^{125}I] human serum albumin	↑ BBB permeability	[144, 145]
	Brain uptake of systemically administered [^{131}I] albumin	No change in BBB permeability	[148]
3 × TG (mutations in the human amyloid precursor protein, presenilin 1 and tau genes; exhibiting both the amyloid and tau pathologies of AD)	*In situ* perfusion of [^{14}C] sucrose and [^{14}C] inulin	↓ BBB permeability	[146]
	In situ perfusion of [^{14}C] sucrose	No change in BBB permeability	[108]

and MRP1 [151–153]. These transporters have shown to be altered in the AD brain microvasculature which may not only affect Aβ trafficking across the BBB, but may lead to altered BBB transport of compounds whose brain uptake is limited by these transporters. Therefore the roles of the above-mentioned transporters with respect to Aβ clearance, their expression profile in AD and the subsequent effect on altered BBB drug transport in AD are discussed in the following sections.

Low-density Lipoprotein Receptor-related Protein 1 (LRP-1)

LRP-1, a member of the low-density lipoprotein receptor family, is a large multifunctional scavenger and signalling receptor located at the abluminal side of the BBB. LRP-1 plays major role in 1) the transport and metabolism of cholesterol associated with apolipoprotein E containing lipoproteins [154], 2) the endocytosis of structurally-unrelated ligands (such as apolipoprotein E, α2-macroglobulin, tissue plasminogen activator, amyloid precursor protein, and Aβ wild type and mutant peptides) [154, 155], and 3) regulating the clearance of brain and systemic Aβ peptides which has been reviewed elsewhere [156]. The interaction of Aβ molecules with LRP-1 has been demonstrated *in vitro* and *in vivo* using surface plasmon resonance analysis and ELISA assays [155, 157]. Further, the involvement of LRP-1 in Aβ efflux across the BBB has been established by *in vivo* studies in mice, where in one particular example, LRP-1 expression was selectively decreased using antisense molecules that led to a decreased brain clearance of exogenously administered $A\beta_{42}$ and increased accumulation of endogenous $A\beta_{42}$, with subsequent cognitive decline [158]. LRP-1 expression at the BBB has been reported to be reduced in aged rodents and in people with AD and this reduction has been associated with positive staining of cerebral vessels for $A\beta_{40}$ and $A\beta_{42}$, suggesting that increased accumulation of Aβ in cerebral vessels may be due to decreased LRP-1 expression [155, 159, 160]. The exact cause of the initial impaired activity of LRP-1 is not known but recent studies in mice with systemic inflammation induced by lipopolysaccharide administration have suggested that systemic inflammation might be one of the factors that causes dysfunction of LRP-1 transport function and therefore reduced clearance of Aβ from the brain [161, 162]. Besides being an efflux transporter, LRP-1 is reportedly involved in the transcytosis of macromolecules from the blood into the brain parenchyma [163]. Two recent studies in the literature,

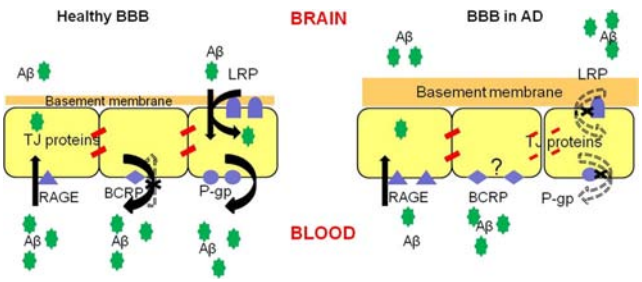

Fig. 3 Influence of AD pathology on BBB transporters and impact on Aβ clearance from the brain. In healthy controls, the efflux of brain parenchymal Aβ into the blood is suggested to be mediated by a 2 step mechanism; 1) transcytosis or endocytosis of Aβ peptide by LRP-1 located on the abluminal membrane and 2) luminal efflux by P-gp. Plasma derived Aβ is suggested to interact with RAGE at the luminal membrane followed by transcytosis into the brain parenchyma. In addition some studies have suggested that BCRP on the luminal surface limits the entry of peripheral circulating Aβ into brain parenchyma. In AD, the expression of these transporters has been reported to be altered. Reduced LRP-1 and P-gp expression at the BBB is suggested to result in reduced efflux of Aβ from the brain parenchyma into the blood; whereas increased expression of RAGE results in enhanced transport of Aβ from the blood into the brain. The expression of BCRP in AD is controversial as some studies have detected increased expression while others have found no change in its expression and therefore it is difficult to predict the outcome of BCRP transporter in AD. Another efflux transporter, MRP1 has been explored to investigate its role in Aβ pathology but the results are not confirmatory and further studies will be required, and therefore this transporter has not been shown in the figure [38, 153, 155, 160, 191, 192].

depicting interaction of LRP-1 with immunoglobulins and ANG1005 (paclitaxel conjugated with 19 aminoacid vector, Angiopep-2) have suggested a role of LRP-1 in transcytosis of these macromolecules across the BBB [164, 165]. This implies a potential role for LRP-1 in the influx of large molecules into the brain, however currently there is not a confirmed small drug molecule substrate of LRP-1. Therefore reduction of LRP-1 expression in AD could reduce the brain disposition of those macromolecules that interact with LRP-1, but is unlikely to have an impact on the transport of small drug like molecules across the BBB.

Receptor for Advanced Glycation End Products (RAGE)

RAGE is a multiligand receptor in the immunoglobulin superfamily expressed at the luminal membrane of brain microvascular endothelial cells that binds to an extensive number of ligands including products of non-enzymatic glycosylation, the S100/calgranulin family of proinflammatory cytokine-like mediators, the heparin-binding protein; amphoterin, and Aβ [101, 166, 167]. The influx of soluble forms of Aβ from the peripheral circulation (blood) into the brain parenchyma across the BBB has been suggested to be mediated by RAGE [98]. Interaction of RAGE and Aβ has been reported to produce several pathogenic neuro-vascular and cellular responses (oxidative stress, cell death and microglial activation, release of proinflammatory cytokines, and production of endothelin-1), leading to diminished cerebral blood flow [98, 101]. *In vivo* and clinical observations reveal that the expression of RAGE at the BBB is increased in AD [98, 99, 101], as well as in normal ageing [168], and this is expected to increase the transport of Aβ into the brain parenchyma. Therefore regulating RAGE activity using RAGE inhibitors and antibodies across the BBB seems to be a beneficial option for AD treatment [169]. There are not many studies in the literature that have explored RAGE as a transporter to deliver molecules into the brain. However, the enhanced BBB transport of glycosylated peptides (such as enkephalins) has been proposed to occur due to their interaction with RAGE [97]. Apart from this experimental context, there is no evidence of RAGE to be involved in the transport of drug-like molecules, and so it is unlikely that altered BBB expression of this protein would affect the transport of therapeutics into the CNS in people with AD.

P-glycoprotein (P-gp)

P-gp is a 170 kDa transmembrane protein which was first discovered in Chinese hamster ovary cells [170] and shortly afterwards was observed in human tumour cell lines [171]. In humans, P-gp is encoded by the ABCB1 gene (multi-drug resistance, *MDR1*), and in rodents by the abc1a (*Mdr1a*) and abc1b (*Mdr1b*) genes [172, 173]. P-gp is the most significant and clinically relevant transporters of the ABC family, located at a number of barriers and excretory tissues throughout the human body. It is expressed on hepatocytes, small intestinal epithelial cells, proximal renal epithelial cells, the blood-placental barrier (consisting of syncytiotrophoblasts), blood-testis barrier (formed by the tight junctions of adjacent Sertoli cells) and the BBB [16, 174]. In the CNS, the expression of P-gp is found to be relatively low in neurons, astrocytes, pericytes, microglia, and the epithelial cells of the choroid plexus, while it is found to be highest, and considered to be only expressed, in the capillary endothelial cells comprising the BBB [175, 176]. P-gp is located on the luminal side of the brain capillary membrane [177], albeit one study has suggested expression of this transporter on the abluminal membrane as well [178]. P-gp on the luminal side acts as a gatekeeper preventing the entry of a wide range of structurally unrelated compounds into the brain, while also expelling metabolic waste products from the brain [179]. The importance of its protective role at the BBB is demonstrated by various examples wherein chemical or genetic inhibition of P-gp leads to substantial increases in the transport of P-gp substrates into the brain [180–182]. While many exogenous therapeutics and toxins are known to be substrates of P-gp, a few endogenous compounds such as cytokines, steroids and bilirubin are also known to be substrates of P-gp [183–185]. In line with this, it has been shown that P-gp can effectively transport Aβ across the BBB, suggesting Aβ to be an endogenous substrate of P-gp [186]. Subsequently, the status of P-gp has become an important area of research in AD pathology.

Earlier *in vitro* studies using hamster P-gp enriched vesicles and *MDR1*-transfected proximal renal tubule epithelial cells suggested the involvement of P-gp in Aβ transport [186, 187]. While later studies by Cirrito demonstrated decreased BBB efflux of exogenously-administered Aβ, the expression levels of the other major Aβ-efflux transporter low density LRP-1 were also decreased in P-gp deficient mice, and therefore, the clear involvement of P-gp in these studies was not conclusive [36]. However, Cirrito *et al.* demonstrated a substantial increase in interstitial fluid concentrations of Aβ after chemical inhibition of P-gp with XR9576 (tariquidar) in a transgenic AD mouse model (Tg2576, mice with mutation in human APP gene), where LRP1 function was not altered, providing greater weight to the involvement of P-gp in Aβ efflux. This group also reported that P-gp deficient mice crossed with Tg2576 AD mice had a greater brain parenchymal Aβ load than that observed in P-gp expressing mice crossed with Tg2576 AD mice. This study provided an association between BBB P-gp activity and Aβ brain deposition and indicated that reduced Aβ efflux at the BBB in a mouse model of AD is, at least in part, governed by deficient P-gp activity at the BBB [36]. Moreover, studies carried out by Hartz *et al.* have reported a 60% reduction in the expression of P-gp in the isolated brain

microvessels of Tg2576 AD mice relative to wild type mice. This is similar to findings in our laboratory where we have demonstrated a 42% reduction in microvascular P-gp expression in 3×TG AD mice relative to wild type mice [108]. Hartz *et al* further demonstrated upregulation of P-gp expression in Tg2576 AD mice by using ligands that activated the pregnane X receptor (a nuclear hormone receptor regulating the expression of P-gp) led to significantly reduced Aβ accumulation in these AD mice [188]. Along with the *in vivo* demonstration of reduced P-gp expression in AD as shown by Hartz *et al.*, various clinical studies have demonstrated decreased expression of P-gp protein at the human BBB and have associated the reduced expression of P-gp with Aβ deposition in the surrounding brain tissue or in the cerebral vasculature [189–191]. An inverse correlation between vascular P-gp immunoreactivity and Aβ-positive plaques has been observed in brain samples from people with AD as compared with age matched non-demented patients, as reported by Volgelgesang *et al* [189]. In later studies by the same group, the authors determined P-gp expression in cerebral capillaries from non-demented elderly patients with CAA and observed that capillaries loaded with Aβ exhibited lower P-gp immunoreactivity compared to capillaries without Aβ deposition [191]. Recently, Wejisurya *et al.* reported significantly lower P-gp immunoreactivity in hippocampal brain vessel samples of post-mortem brain samples from people with AD compared to brain samples from age matched non-demented patients [192]. Similarly, another study by Jeynes has reported a negative correlation between densities of P-gp positive capillaries and neurofibrillary tangles and $A\beta_{40}$ positive plaques in AD brain samples as compared to control brain samples from non-demented elderly patients [190]. These studies suggest that Aβ pathology may influence the levels of P-gp in AD, though the mechanism by which P-gp is downregulated in AD is yet to be elucidated. A recent *in vivo* study in mice has shown that $A\beta_{42}$ itself downregulates P-gp expression at the mouse BBB [193] and this observation has been supported by a separate *in vitro* study where the authors have shown that Aβ may directly decrease P-gp expression via inhibition of Wnt/β-catenin signalling, albeit the concentrations of Aβ used far exceeded those observed pathologically [194]. In addition, P-gp expression at the BBB may be modulated in AD due to changes in the receptor levels regulating its expression. For example, lower levels of pregnane X receptor has been observed in AD mice [188] which may downregulate P-gp expression at the BBB. Additionally, environmental factors, ageing or genetics may play a role in amending P-gp expression. A decline in P-gp expression has been shown at the BBB as a function of age, one of the major risk factors which may contribute to AD and other neurodegenerative diseases [195]. Furthermore, the changes in P-gp function (measured by [^{11}C] verapamil binding potential with PET) at the BBB in people with AD have been reported to be associated with genetic polymorphisms in the ABCB1 gene (the gene that encodes P-gp) [196]. However, changes in cerebral blood flow as noted in AD should be accounted for when measuring P-gp activity in AD affected regions. It is observed that people with AD have reduced cerebral blood flow, especially in the cortical and hippocampal regions of the brain that is affected by AD [69]; and it may be assumed that the brain regions with reduced cerebral blood flow may have diminished binding potential of highly extracted P-gp substrates such as [^{11}C] verapamil, leading to what may appear as reduced P-gp activity. However, a recent PET study in people with AD demonstrated that reduced P-gp activity, expressed as a [^{11}C] verapamil radioactivity extraction ratio ([^{11}C] verapamil brain distribution clearance/regional cerebral blood flow), was independent of regional cerebral blood flow (measured by [^{15}O] water) [197]. This suggests that P-gp activity across the BBB is reduced in AD affected regions irrespective of changes in regional cerebral blood flow and any reduced binding potential of [^{11}C] verapamil is likely due to decreased P-gp function instead of reduced cerebral blood flow.

The reduced brain microvascular expression of P-gp in AD is likely to impair the protective function of the BBB. For many compounds whose access into the brain is restricted by the function of P-gp, it can be hypothesized that the reduced expression of this efflux pump in AD could lead to enhanced CNS exposure of P-gp substrates and undesired neurotoxicity. In line with this, recent clinical studies demonstrated that the binding potential of [^{11}C] verapamil, as assessed by PET, is significantly higher in several cortical regions and hippocampus of people with AD as compared to healthy controls [197, 198]. These studies provide direct evidence of reduced P-gp functionality at the BBB, and therefore, altered drug distribution in people with AD. In our recent transport studies in the 3×TG AD mouse model, we attempted to assess the functional impact of decreased P-gp expression on the brain uptake of well known P-gp substrates. The brain uptake of [^{3}H] digoxin, [^{3}H] loperamide and [^{3}H] verapamil was assessed in wild type and 3×TG AD mice at 18–20 months of age [108]. Though P-gp expression was reduced in these 3×TG AD mice by 42%, the brain uptake of the investigated P-gp substrates ([^{3}H] digoxin, [^{3}H] loperamide and [^{3}H] verapamil) was not different between the two genotypes [108]. The reduced expression of P-gp in AD may have been counteracted by other AD related BBB alterations observed in this mouse model of AD including increased thickness of the microvascular basement membrane [108], and increased expression of the other major efflux transporters such as BCRP [38], which may have complicated interpretation of the effects of AD on the overall brain uptake of various P-gp substrates. These preliminary studies call for further *in vivo* and clinical investigations with a greater number of P-gp substrates. Furthermore, as amyloid and tau pathology develop progressively in AD, it would be significant to investigate whether any

alteration in P-gp expression impacts upon the brain uptake of P-gp substrates in a manner paralleling the disease progression (from mild-to- moderate-to-severe AD pathology), both in relevant mouse models of AD and in a clinical setting.

Moreover, it has been observed clinically that digoxin, a P-gp substrate, is associated with delirium in older people, even at therapeutic serum concentrations [199]. While this association is thought to be pharmacodynamic, due to the possible anticholinergic activity of digoxin in the presence of reduced cholinergic transmission in old age and with AD, it may also be pharmacokinetic in nature and due to altered BBB transport. Tricyclic antidepressants are poorly tolerated by older people, with a high risk of delirium and postural hypotension, and as a class are consistently considered drugs to avoid in old age [200]. While their pharmacodynamic anticholinergic effects partly explain the poor tolerance of these drugs, tricyclic antidepressants are P-gp substrates and their effects may be exacerbated by P-gp dysfunction in old age. This is analogous to the exaggerated postural hypotension seen with P-gp polymorphisms [201]. The impact of interactions with P-gp inhibitors such as verapamil, amiodarone, macrolide antibiotics and non-dihydropyridine calcium channel antagonists on BBB transport must also be considered in older patients in whom polypharmacy is the norm.

Breast Cancer Resistance Protein (BCRP)

BCRP was first discovered in the human breast cancer cell line MCF-7/AdrVp and is another ABC efflux transporter, found in various organs/barriers of the body including the BBB [20, 202–204]. BCRP is highly expressed on the luminal side of the capillary endothelial cells comprising the BBB [205, 206]. As the tissue distribution and substrate spectrum of BCRP greatly overlaps with P-gp [207–209], there have been a number of recent studies addressing the involvement of BCRP in Aβ transport and exploring a role for this transporter in AD pathogenesis. Previous *in vitro* and *in vivo* studies using human brain endothelial cell lines and BCRP null and wild-type mice respectively have depicted BCRP mediated transport of Aβ across the BBB and have suggested that BCRP functions to prevent plasma-derived Aβ from entering the brain [38, 210]. However, other *in vivo* studies and a clinical report did not observe any interaction between $A\beta_{40}$ and the BCRP transporter [188, 211]. Similarly, there exists disparity between observations regarding the expression of BCRP in AD. Xiong *et al.* have shown an elevated BBB expression of BCRP in people with AD with CAA and in different AD mouse models when compared to their age matched controls. The authors observed that Aβ peptides alone or in combination with an hypoxic environment did not stimulate BCRP expression in human brain endothelial cells, but conditioned media from Aβ-activated microglia stimulated BCRP expression in these cells. This observation led to the suggestion that the upregulation of BCRP expression in human and mouse cerebral microvessels may be due to the release of paracrine factors from Aβ-activated microglia. However, another clinical study has reported no change in the expression of BCRP at the BBB in brain samples from people with AD [192]. Moreover, a recent study has demonstrated a significantly diminished BBB expression of BCRP in patients with severe CAA, and though not significant, the authors observed lower BCRP expression in people with AD without CAA [212]. These differences in results could be due to the differences in the techniques used for the detection of BCRP expression in these studies (such as microarray analysis by RNA extraction, immunohistochemistry and western blot) and in the brain regions used (such as whole brain, microvessels from the hippocampus and microvessels from cortical samples). Therefore, from the available literature, the exact involvement of BCRP in AD remains obscure and incomplete and future studies will be required to clarify the role of BCRP in Aβ clearance and its expression in early and later stage AD.

Regardless of the contribution of BCRP in AD pathogenesis, if the expression of BCRP is indeed enhanced or reduced in AD, this could affect the brain exposure of therapeutics that are substrates of BCRP. Accordingly, a reduced or enhanced CNS exposure of drugs that are expelled from the brain by the action of BCRP, for example mitoxantrone, imatinib and topotecan [213], may be expected. However, many of the substrates of BCRP have an overlapping affinity for P-gp [207–209], and as described earlier, P-gp expression is decreased in AD [188, 189]. Therefore, whether the BBB transport of a specific BCRP substrate is actually modified in the AD condition is difficult to predict, and a systematic assessment of the transport of each individual and combined substrate of P-gp and BCRP is therefore required.

From a clinical perspective, it is possible to estimate the expression of BCRP in AD from population data on cancer mortality. For example, on a population level, the first year after breast cancer diagnosis, the mortality rate of breast cancer patients with dementia exceeded that of breast cancer patients without dementia with a stage-adjusted Mortality Rate Ratio of 5.0 (95% CI: 3.6, 6.8) (95% CI: 3.6, 6.8) [214]. However, the role of drug disposition and response to treatment in this data is unclear and less aggressive treatment of cancer patients with dementia may explain the excess mortality rate amongst patients with dementia. However, it is clear that some clues with respect to drug disposition in AD may be obtained from findings obtained in other disease states.

Multi-drug Resistance Associated Protein 1 (MRP1)

MRP1, coded by the ABCC1 gene, was first discovered in the H69AR cancer cell line [215]. In brain, MRP1 expression has been detected in the luminal and abluminal membranes of the

capillary endothelial cells, along with other components of the neurovascular unit, including astrocytes, microglia, neurons and epithelial cells of the choroid plexus [21, 216–218]. Unlike P-gp, the substrates of MRP1 are not diverse in range, but are mainly organic anions including endogenous compounds like glutathione and sulphate conjugates, as well as exogenous compounds such as HIV protease inhibitors, antibiotics, chemotherapeutics and toxins [219, 220]. MRP1, like other ABC transporters, also acts as an efflux transporter at the BBB, protecting the brain against potentially harmful metabolites, xenobiotics and drugs [221]. The role of the MRP1 in Aβ elimination in AD has been explored recently. Krohn *et al.* have shown an age dependent increase in $A\beta_{40}$ and $A\beta_{42}$ along with enhanced Aβ plaque formation in the cerebral cortex of an AD mouse model deficient in MRP1, when compared against control AD mice (which did not lack ABCC1), suggesting a role for ABCC1 in Aβ accumulation and elimination across the BBB in AD. Secondly, this group have shown that dosing the control AD mice with thiethylperazine (identified as an inducer of ABCC1 *in vitro*) significantly reduced the amount of soluble $A\beta_{42}$ and also the number and size of Aβ plaques, when compared to ABCC1-deficient AD mice treated with thiethylperazine. The reduction of Aβ plaques was attributed to activation of the ABCC1 gene by thiethylperazine, though the exact molecular mechanism is unknown [211]. Likewise, in a later study by the same group, treatment with another activator of ABCC1 (a specific extract of St John's Wort) resulted in significant reductions in intracerebral $A\beta_{42}$ levels and a decrease in the number and size of amyloid plaques in an AD mouse model [222]. The authors of the above studies did not directly measure the expression of MRP1 at the BBB in the ABCC1 deficient AD mice but it can be hypothesized that MRP1 levels could have been reduced at the BBB, as activation of the gene encoding this transporter led to reduced Aβ accumulation in these mice. However, in contrast to this assumption, another study has shown a slightly higher expression of MRP1 in human AD hippocampal tissue compared with age-matched controls [223]. Since MRP1 is located in various other components of the neurovascular unit, along with the capillary endothelial cells, it is difficult to attribute this increased expression of MRP1 in brain homogenates only to changes at the protein level at the BBB.

With the limited information available concerning the role of MRP1 in AD pathogenesis, it is difficult to predict what impact or changes there may be with regards to the transport of MRP1 substrates across the BBB in AD. Altogether, more studies are required to confirm the existing data and to provide further insights into the role of this particular ABC transporter in AD pathology, its expression levels across the BBB in AD, and CNS exposure of MRP1 substrates in this condition.

SUMMARY AND FUTURE DIRECTIONS

This review reveals the multi-faceted role played by the cerebrovasculature in the pathogenesis of AD and its impact on CNS drug access. A summary of the major alterations and their anticipated outcome on drug access into the CNS is represented in Fig. 4.

Though the common factors of drug transport across the BBB (e.g. cerebral blood flow, P-gp transport, ultrastructure of the brain microvasculature) seem to be altered in AD, the proposal that the brain uptake of commonly used CNS drugs could be different between AD and non-AD patients has received very little attention. As reviewed in this article, there are multiple alterations occurring simultaneously in AD, particularly with regards to the BBB. The physical and functional changes to the brain microvasculature in AD could cause modifications in the efficacy, potency, therapeutic window, side-effect profile, and dosage of commonly used drugs in AD patients.

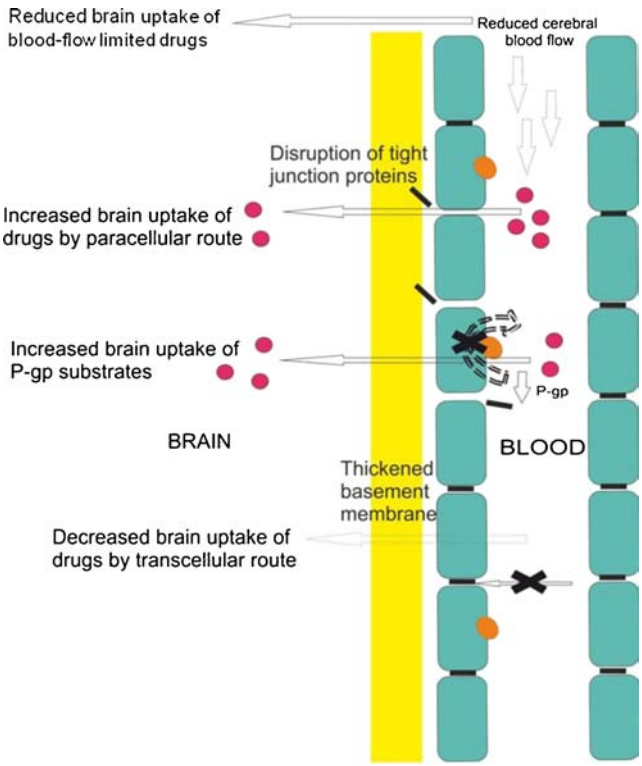

Fig. 4 Reported alterations at the BBB in AD and their potential influence on the brain uptake of small drug-like molecules that permeate the BBB by different mechanisms of transport. Reduced cerebral blood flow may decrease the brain uptake of compounds whose CNS disposition is blood-flow limited. Disruption of tight junction proteins in AD may increase drug permeability by the paracellular route that is otherwise restricted in healthy conditions. Basement membrane thickening in AD may potentially decrease the brain uptake of drugs entering the CNS by the transcellular mechanism, as the diffusion path length may increase when compared to healthy conditions. Reduced expression of P-gp in AD could hamper the efflux function of P-gp and this may increase the brain uptake of P-gp substrates whose entry is restricted in healthy conditions.

To date, most studies assessing BBB permeability in AD have focussed on the appearance of high molecular weight endogenous or exogenously administered protein-like compounds within the CNS [141–145, 148, 149], with less known about the disposition of smaller therapeutics in this disease. There is a general view that the permeability of the BBB is increased in AD, however, this may not be the case with the brain uptake of small drug-like molecules, particularly given our recent findings [108]. There exist few studies that have assessed the impact of AD on the BBB transport of small drug-like molecules as summarized in Table II. Limited changes to the brain exposure of diazepam, clioquinol, PBT2, GSK-A and GSK-B [146, 147, 224, 225] have been reported in AD mouse models. However, the brain uptake of each of these compounds was assessed in different animal models of AD, using different techniques for assessing brain uptake, and the brain uptake was not necessarily measured in AD-affected regions of the brain such as the cortex and hippocampus. In contrast to the above studies, a recent systematic and comparative study carried out in our laboratory that assessed the BBB transport of a series of small drug molecules (diazepam, propranolol, memantine, verapamil, loperamide, digoxin) in the 3×TG AD mouse model indicated that the transcellular mechanism of transport across the BBB was reduced in AD mice [108, 109]. Our findings clearly signify the need for further investigations in both the preclinical and clinical setting with a larger number of lipophilic drugs that traverse the BBB via the transcellular mechanism. Furthermore, as our studies revealed that the disposition of some therapeutics (including the anti-AD drug, memantine) into the brain might be significantly reduced in AD, potentially impacting upon clinical outcomes, it is important to consider whether this may lead to under-dosing of certain CNS drugs in people with AD. In addition, this raises significant concerns about the appropriateness of performing preclinical screening of AD drugs in healthy animal models as they may provide an overestimation of the brain uptake of clinical candidates compared with AD mice.

Preclinical studies in animals are crucial in the drug development process so that appropriate candidates are selected based on their safety and efficacy for further clinical trials. Likewise, most experimental data concerning BBB permeability are gained from animal studies. The BBB permeability and/or transport studies in transgenic AD mouse models are useful to screen/assess simultaneously a number of different probe compounds to understand the status of transport mechanisms across the BBB in AD and to investigate the molecular mechanisms related to BBB pathology. Though *in vivo* permeability studies may not completely correlate with the clinical studies (due to inter-species differences related to transporter expression, cerebral blood flow rate, brain lipid composition and complexity of pathological conditions/events in humans as compared to animal models) they offer insight into the status of BBB permeability in a particular mouse model and provide guidelines for the design of future clinical studies. To better correlate the results between *in vivo* and clinical studies, a greater number of nuclear medicine studies should be encouraged that provide an opportunity to examine BBB transport in both animal models and humans with and without AD, and other studies to compare BBB transport between species. For example, studies using 2-[^{18}F] fluoro-2-deoxy-D-glucose (FDG)-PET, which measures cerebral glucose transport across the BBB, have shown reductions in cerebral glucose uptake in individuals with mild cognitive impairment or probable and possible AD, prior to conversion to AD [226, 227]. Similarly, (FDG)-PET scans from the 3×TG AD mouse model observed brain regional changes in cerebral glucose uptake that were homologous to alterations seen in PET scans from humans with AD [72]. In addition, nuclear medicine studies across species have shown that compounds found to be P-gp substrates in rodents are likely also P-gp substrates in higher species, but sufficient BBB permeability may be retained in healthy humans to allow them to act at intracerebral targets [228]. These studies have important implications for translating findings on the BBB in animals to human studies.

The future hope in finding better therapeutic solutions for people with AD is in the discovery of early diagnostic biomarkers and new drugs that can prevent cognitive impairment or reduce AD pathology and aid in better management of AD symptoms, respectively. Drug discovery scientists and researchers are constantly investigating novel AD drug candidates and various targeting approaches for delivering drugs to AD affected target sites in the brain. These include therapeutics that act on cholinergic or glutamatergic receptors, drugs that modulate Aβ production and aggregation, immunotherapy against amyloid pathology and the associated inflammatory response, molecules targeting tau tangles and novel compounds targeting metabolic pathways such as glucose production and insulin receptors in the brain [229]. At present, there are approximately 185 clinical studies that have either completed investigating or going to investigate new drug candidates in people with AD [230]. These novel drug candidates that are investigated for AD are based on different BBB targeting strategies such as transmembrane diffusion of lipid soluble molecules, active or passive immunization wherein antibodies target Aβ pathology in the brain (by slowly crossing the BBB via extracellular pathway or interact with peripheral Aβ), drugs or antibodies embedded in liposomes and nanoparticles that can circumvent the BBB using endothelium receptors (e.g. transferrin) and through the use of endogenous transport systems (organic anion transporter and other saturable transport systems) [231]. However, the complexity of neuropathological events in AD has prevented many drug candidates in clinical trials to proceed into therapeutic development. This further highlights the significance of taking into consideration the BBB related alterations in AD when

Table II Summary of studies assessing small drug transport across the BBB in mouse models of AD disease

Drug	Mechanism of transport	Observation	Limitation	Ref.
Diazepam	Passive transcellular diffusion (limited by cerebral blood flow)	No difference in the cortical and hippocampal BBB transport of [^{3}H] diazepam in wild-type and 3 × TG AD mice at 11 months of age	The experiment was only performed over a 2 min period, which may not be sufficient time to allow for changes in BBB integrity to be identified	[146]
Clioquinol	Unknown	No difference in the brain-to-plasma ratio of [^{125}I] clioquinol between wild-type and Tg2576 AD mice at various time points (except at 120 min post-dose where AD mice exhibited a lower brain-to-plasma ratio)	Concentrations determined in whole brain, not specifically in AD-affected areas (e.g. cortex and hippocampus)	[224]
PBT2	Unknown	No difference in the brain-to-plasma ratio of PBT2 in wild-type and Tg2576 mice	Brain-to-plasma ratio only determined at one post-dose time point (120 min) and concentrations determined in whole brain, not specifically in AD-affected areas (e.g. cortex and hippocampus)	[225]
GSK-A and GSK-B	Passive transcellular diffusion	No difference in brain-to-plasma ratios between 'naive' and TASTPM AD mice	Concentrations determined in whole brain, not specifically in AD-affected regions (e.g. cortex and hippocampus)	[147]
Diazepam and propranolol	Passive transcellular diffusion	Significant reductions in cortex-to-perfusate and hippocampus-to-perfusate ratios in 3 × TG AD mice relative to wild-type mice at 18–20 months of age	The experiment was only performed over a 4 min period and steady state brain concentrations not assessed	[108]
Memantine	Passive transcellular and active influx	Significant reductions in brain-to-perfusate ratios in 3 × TG AD mice relative to wild-type mice at 18–20 months of age	The experiment was only performed over a 4 min period and steady state brain concentrations not asssessed	[109, 232]
Digoxin, lopermaide and verapamil	Active P-gp efflux	No significant difference in cortex-to-perfusate and hippocampus-to-perfusate ratios between wild-type and 3 × TG AD mice at 18–20 months of age	The experiment was only performed over a 4 min period and steady state brain concentrations not assessed	[108]

3 × TG: transgenic mice harboring mutations in three human transgenes of AD (amyloid precursor protein, presenilin 1 and tau); Tg2576: transgenic mice harboring Swedish mutation in human amyloid precursor protein gene; TASTPM: transgenic mice harboring mutations in amyloid precursor protein and presenilin 1

designing new drug candidates and targeting strategies for AD. Moreover, as pointed in this review, it is important for AD drug discovery scientists to consider the mechanism of transport of drug candidates across the BBB, and to assess the expression of relevant transport proteins if any transporter is likely to be involved in the movement of these drug molecules across the BBB, given the expression of transporters is altered in this disease.

AD is a debilitating disorder in the elderly population and its growing incidence is of concern. As for now, people with AD have to rely heavily on different classes of medications to manage their AD related symptoms as well as associated health problems due to ageing. Therefore, keeping in mind the simultaneous use of different classes of drugs in people with AD and observed physical and functional alterations of the BBB, it is of prime importance to evaluate the CNS access and safety profile of small therapeutics (when prescribed alone and in combination) in appropriate pre-clinical AD models followed with well-designed clinical trials. Additionally, it would be beneficial to investigate the role and expression of various other influx (e.g. MCT-1, organic cation transporters) and efflux transporters (e.g. OAT, MRP-1) in AD, and subsequently, to assess the transport of their relevant substrates if the expression of the transporter is found to be altered in AD. A better understanding of how CNS drug disposition differs in AD and, in particular, which mechanism/s of transport across the BBB are affected, would benefit drug discovery scientists when designing and evaluating newer molecules for the treatment of AD. More importantly, it will help clinicians select drugs and doses to optimize the safety and efficacy of all medications taken by older people with AD.

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