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Review

Alzheimer's disease and blood–brain barrier function—Why have anti-β-amyloid therapies failed to prevent dementia progression?

Jens Pahnke [a,*], Lary C. Walker [b], Katja Scheffler [a], Markus Krohn [a]

[a] University of Rostock, Department of Neurology, Neurodegeneration Research Laboratory (NRL), Gehlsheimer Str. 20, 18147 Rostock, Germany
[b] Yerkes National Primate Research Center and Department of Neurology, Emory University, Atlanta, GA 30322, USA

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ABSTRACT

Proteopathies of the brain are defined by abnormal, disease-inducing protein deposition that leads to functional abrogation and death of neurons. Immunization trials targeting the removal of amyloid-β plaques in Alzheimer's disease have so far failed to stop the progression of dementia, despite autopsy findings of reduced plaque load. Here, we summarize current knowledge of the relationship between AD pathology and blood–brain barrier function, and propose that the activation of the excretion function of the blood–brain barrier might help to achieve better results in trials targeting the dissolution of cerebral amyloid-β aggregates. We further discuss a possible role of oligomers in limiting the efficacy of immunotherapy.

Contents

1. Introduction

Alzheimer's disease (AD) is by far the most prevalent type of dementia, accounting for 60–80% of all patients diagnosed with

* Corresponding author. Tel.: +49 381 494 4700; fax: +49 381 494 4702.
E-mail address: jens.pahnke@med.uni-rostock.de (J. Pahnke).
URL: http://www.nrl.uni-rostock.de

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dementia (Alzheimer's Association, 2008). As a progressive neurologic disorder, AD is characterized by a significant loss of neurons and atrophy of the hippocampus and cerebral cortex. Its classical histopathological hallmarks, first clearly linked to dementia by Alois Alzheimer more than 100 years ago, are neurofibrillary tangles and senile plaques, which consist primarily of hyperphosphorylated tau protein and the amyloid-β peptides, respectively. According to the "amyloid hypothesis", the accumulation of Aβ peptides within the brain of Alzheimer's patients leads to the hyperphosphorylation of tau proteins and neuronal dysfunction/death, thereby impairing memory and other cognitive functions such as writing and reading (Hardy and Allsop, 1991; Greenberg and Murphy, 1994). In later stages of the disease, the ability to accomplish even basic activities of living (i.e. self-feeding) is reduced or lost; most patients die within 7 years of diagnosis, and less than 3% live more than 14 years (Molsa et al., 1986, 1995).

Aβ refers to a series of C- and N-terminally heterogeneous proteolytic fragments, most of which are 40 or 42 amino acids in length, that are produced during the normal processing of APP by different secretases. α-Secretase cuts APP within the Aβ peptide domain and thereby prevents Aβ generation, whilst sequential scissions by β-secretase and the γ-secretase complex yield Aβ (for a review see Selkoe and Wolfe, 2007). However, the reasons for the pathologic accumulation of Aβ in the brain are still not known. Only 496 families worldwide, accounting for less than 1% of all AD cases, display a hereditary (autosomal dominant) form of AD with an early onset of the disease (http://www.molgen.ua.ac.be/ADMutations). In these families, mutations in the APP gene, or in genes encoding proteins for the γ-secretase complex such as Presenilin 1 (PS1) or Presenilin 2 (PS2), lead to a dramatic increase of Aβ peptides production in the brain and thus to an early onset of disease, sometimes as early as 30–40 years of age.

The majority of AD cases belong to the sporadic (idiopathic) disease form, which tends to occur later in life, most often after the age of 65 years. The trigger for developing such late-onset AD (LOAD) is still unknown, but several risk factors are being discussed. An allelic variant of the apolipoprotein E (ApoE) gene is considered to be a susceptibility or risk factor for AD (Strittmatter et al., 1993a,b). ApoE is involved in the transport of cholesterol, lipoproteins and fat-soluble vitamins, and has been implicated as an anti-oxidant and immune response mediator (Cole et al., 1999). It exists as three major allelic variants, the protein products of which differ at amino acid positions 112 and 158. ApoE3 is the most commonly found variant, with a prevalence of 60–70%. ApoE4 has been found to increase the risk of developing AD. However, only 1–2% of the population carries two apoE4 alleles, and even in those instances there is no certainty of developing AD (Alzheimer's Association, 2008; Donahue and Johanson, 2008). Additional risk factors for AD include previous head injury and cardiovascular diseases, whereas people who remain intellectually active and/or have university degrees are less prone to develop AD. However, advancing age is still by far the strongest risk factor for AD (Alzheimer's Association, 2008).

1.1. Social impact of Alzheimer's disease—prevalence and costs

Due to many successes in the fight against deadly diseases like cardiovascular diseases, stroke and cancer during the past years, the average life expectancy in industrialized countries continues to rise (http://www.demogr.mpg.de/), and with it the risk of developing AD. In Germany, the number of citizens over 85 years of age will almost triple, from ~1.5 million in 2000 to about 4.2 million in 2050, and the number of people over the age of 65 will almost double (Bickel, 2001). According to the U.S. Census Bureau, this is not only true for Europe, but the increase will be even more dramatic for North America. Importantly, the number of Asians over 65 years will increase 5-fold by 2050, and those over 80 years will rise by a factor 10 (Table 1). In fact, the growth of the elderly population in Asia (>250 million people over 80) will account for more than 60% of the total increase worldwide. The rising number of elderly, and especially of the 'oldest old' (above 85 years), will result in a huge social and economic burden, since the prevalence of AD doubles every 5 years after the age of 65 (Bickel, 2001). In people who are 65–70 years old, only 1–3% are diagnosed with AD. Above 80 years of age, the prevalence increases to 30–50% (Bickel, 2001; Helmer et al., 2006; Alzheimer's Association, 2008). Thus, in 2050 the number of AD patients could reach 140 million worldwide (U.S. Census Bureau, 2008 http://www.census.gov/ipc/www/idb/index.html)!

Table 1
The elderly population in three different regions of the world according to the U.S. Census Bureau. The elderly population will increase 3- to 4-fold by 2050 in highly industrialized regions. Notably, over 60% of the worldwide increase in elderly individuals older than 80 years will be due to the growth of this age group in Asia.

	Year 2000		Year 2050	
	Age 65+	Age 80+	Age 65+	Age 80+
Western Europe	156,053,000	14,828,000	288,690,000	44,444,000
Northern America	97,004,000	10,333,000	253,529,000	36,863,000
Asia (excl. Near East)	470,003,000	27,997,000	2,376,391,000	281,051,000
Worldwide	986,509,000	70,112,000	3,967,164,000	470,254,000

2. Therapeutic strategies for Alzheimer's disease

At present, there is no preventive or cure for AD. Tremendous research efforts are currently underway to find a disease-modifying treatment for the disorder. In view of the emerging economic and social difficulties associated with AD, an effective therapy is a highly desirable objective (Table 2).

Only five compounds currently are approved by the FDA and EMEA for the treatment of AD. Four are acetylcholinesterase inhibitors (AChEI), and the fifth is an NMDA-antagonist. AChEIs are used to counteract the functional consequences of lost cholinergic neurons in AD brains. The cognitive function of patients can be stabilized for up to 1 year using AchEIs. The AChEIs Tacrine ("Cognex"), Galantamine ("Razadyne") and Rivastigmine ("Exelon") are approved for the treatment of mild-to-moderate stages of dementia. Only Donepezil ("Aricept") is approved for treatment of all stages of AD. Rivastigmine has been found to be beneficial in later stages of the disease (Raina et al., 2008). Regrettably, even if these pharmaceuticals can result in statistically significant improvements of cognitive and global assessment measurements of AD, their clinical benefits are marginal, and, more importantly, largely symptomatic.

Table 2
Summary of important ABC transporters at the blood–brain barrier. In total, 49 human ABC transporters have been described with variable locations in the tissues ant at barriers. At the BBB, ABC transporters are located either basolaterally or apically. According to the concerted function and different specificities of these transporters, the direction of transport may vary. Mouse models are a helpful tool to describe the transport specificities and developmental and maintenance functions.

Human transporter	Mouse transporter	Expression location at the BBB	Knockout animal model published by
ABCA2	ABCA2	No	Sakai et al. (2007)
ABCB1/P-gp/ MDR1-P-gp	Mdr1a and b	Lumenal	Schinkel et al. (1997)
ABCC1/MRP1	Mrp1	Lumenal a/o Basal	Schinkel et al. (1994)
ABCC2/MRP2	Mrp2	Lumenal a/o Basal	Wijnholds et al. (2000a)
ABCC4/MRP4	Mrp4	Lumenal a/o Basal	Vlaming et al. (2006)
ABCC5/MRP5	Mrp5	Lumenal	Leggas et al. (2004)
ABCG2/BCRP	Bcrp-1	Lumenal	Wijnholds et al. (2000b)

In recent years, experimental approaches have emerged to reduce Aβ levels by inducing the Aβ peptide degrading enzymes neprilysin (NEP), insulin-degrading enzyme (IDE), and endothelin-converting enzyme (ECE). Other researchers have sought to reduce Aβ content through the development of inhibitors of the APP-cleaving secretases that liberate monomeric Aβ from APP (reviewed in Ghosh et al., 2008; Wolfe, 2008). Further studies on the mechanism of action of these and other agents could disclose novel therapeutic approaches to AD.

2.1. Immunotherapy—cure or caveat?

Currently, the most advanced experimental AD therapy is the anti-Aβ immunotherapy, with several antibody-based treatments in phase 2 and phase 3 clinical trials.

The only Aβ immunization trial with postmortem follow-up data is the first trial of active immunization that was begun in 2000. 300 patients were treated with AN1792 (from Elan/Wyeth), a synthetic Aβ42 peptide along with QS21 as adjuvant. The clinical phase 2a trial was halted prematurely when 6% of the vaccinated patients developed subacute meningoencephalitis (Orgogozo et al., 2003). At the time the trial was halted, 274 patients had received two injections and 24 patients had received three injections of AN1792. Fifty-nine patients were confirmed as antibody responders, with specific IgG titers $\geq$1:2200.

Holmes et al. (2008) recently published an update based on autopsy investigations of a subgroup of the Elan/Wyeth patient cohort that received AN1792. Brains of eight immunized AD patients who had died and consented to postmortem examination were analyzed for Aβ and tau pathology. All of these cases met the criteria for Braak stage V to VI, and thus could be classified pathologically as end-stage AD. The authors found evidence that active anti-Aβ immunotherapy leads to a reduction of Aβ plaques in the brain. The Aβ load was 30% lower in immunized participants compared to the control group, although the Aβ load was highly variable within the immunized group. The degree of plaque removal correlated positively, and significantly, with the antibody response. An inverse correlation of plaque load with the antibody response during treatment was found, but this did not reach statistical significance. Therefore, in antibody responders this immunotherapeutic strategy appeared to be successful in the long-term reduction of Aβ plaque load in the examined patients.

In contrast, an analysis of the impact of immunotherapy on cerebral amyloid angiopathy (CAA) and microvascular lesions showed a significant *increase* in vascular Aβ42 and Aβ40 in cortical vessels (Nicoll et al., 2003; Ferrer et al., 2004; Masliah et al., 2005; Boche et al., 2008). After distinguishing between vessels that were only partially affected and those that were heavily affected (i.e. showing intense Aβ immunostaining through the full circumference of the vascular wall), Boche et al. (2008) found that the increase in overall CAA resulted mainly from an increase in the number of fully affected vessels. Furthermore, the number of microvascular lesions/microhemorrhages increased significantly in the immunized patients as well. The severity of CAA as well as microhemorrhages/lesions, unlike Aβ plaque load, did not correlate with the antibody response of the participants. The authors suggested the possibility that, given enough time after vaccination, Aβ might be cleared from the vasculature as well. However, the data provided little support for this possibility, since the longest-term survivor (who had the highest antibody response as well) had a significant amount of CAA and microvascular lesions. The second- and third-longest survivors also displayed either CAA or microvascular lesions or both. All examined patients reached AD end-stages according to the Braak scale, and disappointingly, no improvement in cognitive abilities or slowing of decline was found, although some studies have suggested positive effects on some measures (Gilman et al., 2005; Vellas et al., 2009).

More recently, Wyeth and Elan have disclosed that passive immunization with the humanized anti-Aβ monoclonal antibody bapineuzumab is, among other less prevalent side effects, associated with vasogenic edema (Grundman and Black, 2008), which has forced the companies to reduce the dose of bapineuzumab being tested in phase 3 clinical trials. Interestingly vasogenic edema was more prevalent in carriers of the ApoE4 allele. These findings suggest that vascular side effects are not restricted to active immunization, but are now also being observed with passive immunization.

Hence, it appears that Aβ immunization might result in the clearance of Aβ-plaques, but the solubilized Aβ appears to be translocated to the cerebrovascular walls, and the process of mental decline proceeds unimpeded in treated subjects. These data, though clinically disappointing, may be scientifically useful in that they support two recent hypotheses about the cause of (I) neuronal loss and (II) the pathologic accumulation of Aβ peptides in the brain. The reason why immunotherapy has not been successful to date may be that it is not targeting the right species (oligomers vs. plaques), and/or that plaque dissolution by immunotherapy is potentially deleterious due to the liberation of toxic oligomers or the redistribution of Aβ to the vasculature. Oligomers may be key to neuronal dysfunction and death in AD (Selkoe, 2008), but immunotherapy currently only targets senile plaques. Thus, the activation of the vascular clearance mechanisms involving BBB transport molecules might help to reduce the amount of soluble toxic Aβ oligomers resulting from plaque dissolution.

3. Oligomeric Aβ—the deadly moieties in Alzheimer's disease

The inability of the BBB to expel the Aβ liberated from plaques by immunotherapy may compromise the efficacy of the approach by trapping toxic species in the brain or in the vasculature. Senile plaques consist predominantly of more or less toxic Aβ peptides, mainly Aβ40 and Aβ42. The Aβ peptides are produced continuously in cells of the nervous system and systemic tissues by the cleavage of APP. Aβ42 is highly prone to aggregation, but there are many different forms of aggregates. Computational analyses have shown how these aggregates may form (Auer et al., 2008a,b). With age, soluble Aβ accumulates over time in the cerebral extracellular space, multimerizes into oligomers, protofibrils and insoluble fibrils, and eventually forms diffuse and dense-cored β-amyloid plaques. Since plaques are one of the main histopathological features of AD, and because familial forms of AD all result in an increase in the production of Aβ or its tendency to aggregate, it has long been assumed that Aβ is somehow at the root of the cognitive deficits in AD. However, the number of plaques correlates positively, but not very strongly, with the degree of dementia, casting doubt on a significant role of plaques per se. Evidence is rapidly accumulating, however, that small assemblies of Aβ (Aβ oligomers) of different sizes are the toxic moieties (Selkoe, 2008), rather than the fibrillar Aβ that comprises the bulk of senile plaques and CAA.

Oligomeric Aβ can be visualized with the oligomer-specific A11 antibody in AD brains in the form of small puncta as well as larger, more or less diffuse deposits that can be distinguished from Thioflavin S-positive plaques (Kayed et al., 2003). Lesné et al. (2006) found, in the brains of transgenic mice carrying a human APP transgene with the Swedish mutation (Tg2576), that monomers aggregate into trimers and higher molecular weight oligomeric aggregates. These oligomers were urea-resistant, with the trimers bound together most tightly, a finding consistent with computational simulations of Aβ oligomerization (Kelley et al.,

2008). The nonameric and, in particular, the dodecameric Aβ (so called Aβ*56—the 56 kDa heavy oligomer) emerged at an age when memory deficits occurred and remained stable during the subsequent months of aging. Furthermore, individual differences in Aβ*56 content significantly varied with performance on memory tasks such as the Morris water maze (MWM). In addition, purified nona- and dodecamers injected into the lateral ventricle of rats caused deficits in the MWM similar to those seen in Tg2576 mice. All animals showed no deficits during the acquisition phase of the new task but exhibited highly impaired long-term memory. Strikingly, Aβ oligomers of the same size had been found in AD brain samples and were shown to exhibit binding to cultured hippocampal neurons (Gong et al., 2003). More recently, Lesné et al. (2008) reported that some Tg2576 mice have markedly reduced levels of Aβ oligomers during a phase of ageing when the accumulation of plaques is accelerated and no Aβ*56 was found. These mice are considered to be plaque-bearing controls with non-oligomeric Aβ, and thus represent a model to examine the effects of the plaques themselves. The authors found no memory impairment if there are only plaques in the brain, in the absence of oligomers. Using transgenic mice bearing an additional "Arctic" mutation, Cheng et al. (2007) have shown that the burden of plaques per se is unlikely to account for memory deficits and neuronal loss. The Arctic mutation induces an acceleration of the Aβ aggregation process that leads to protofibrils and fibrils, but yields largely reduced amounts of Aβ*56. In accordance with the results of Lesné et al., the overall plaque load did not correlate with memory deficits, and "Arctic" mice behave like non-transgenic controls during behavioral testing (Lesné et al., 2006, 2008).

Further studies shed light on the possible impact of Aβ dimers and trimers on the progression of AD (Chen and Glabe, 2006; Hung et al., 2008). In 2000, it was shown that at least small molecular weight oligomers, namely dimers, originate from intracellular processes and are secreted into the extracellular space preferentially from neuronal cells (Walsh et al., 2000). Dimers of Aβ later were found to be present in the CSF of patients who had been diagnosed with AD, and were shown to potentially inhibit hippocampal LTP (Walsh et al., 2002). Very recently, an influence of small oligomers on the signaling of primary cultured microglia was reported (Sondag et al., 2009). Treatment with Aβ dimer/trimer-enriched preparations led to unique signaling of microglial cells that was distinct from cascades induced by fibrillar Aβ preparations. Moreover, oligomers led to the death of primary neurons, an effect that was augmented in neurons co-cultured with microglial cells. Both fibrillar and oligomeric Aβ-induced proinflammatory signaling, but again to different extents. Aβ oligomer incubation resulted in higher IL6 amounts and had a different specificity than did Aβ fibrils. Oligomers additionally elicited the release of the proinflammatory chemokine KC, a homolog of IL8. Both IL6 and IL8 have been found to be increased in the brains of AD patients. However, the authors could not exclude the possibility that higher molecular weight oligomers were (in part) responsible for some of their findings, since the Aβ dimer/trimer preparations further aggregated during the incubation periods. It is likely that there are different oligomeric Aβ species, each of which has a particular influence on diverse functions in the brain. The abundance of Aβ*56 correlates with spatial memory impairments, but appears at a much later age than does impaired long-term potentiation (LTP) and decreased spine density in the dentate gyrus (Jacobsen et al., 2006). Since lower-n oligomers are already apparent at younger ages, these may be responsible for the earlier functional effects (Lesné et al., 2006) (Fig. 1).

In conjunction with the immunization trial follow-up data by Holmes and Boche, investigations of transgenic mouse models support the finding that the burden of plaques within the brain is a weak predictor of the functional severity of the disease, and is not likely to account primarily for memory deficits and neuronal loss (Boche et al., 2008; Holmes et al., 2008). However, regardless of which Aβ species is the most toxic form, it is apparent that the multimerization of Aβ is critical to the pathogenesis of AD. Therefore, a key, unanswered question remains: what actually initiates the eventual pathologic accumulation of Aβ peptides within the brain? The large number of vessels that were found to be laden with aggregated Aβ in immunized patients suggests that a critically important route (besides degradation by proteases) by which the brain can expel Aβ peptides and lower

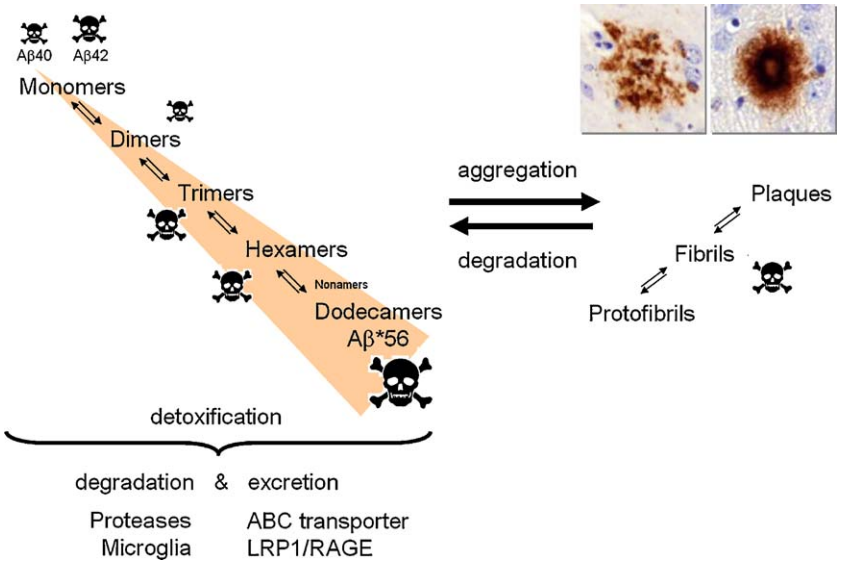

Fig. 1. Aggregation of Aβ monomers to higher MW aggregates generates toxic oligomers. Protofibrils, fibrils, and lastly the β-amyloid plaques exhibit less specific toxicity per mol of aggregated monomers than do oligomeric assemblies. Toxic oligomers form stable intermediates such as dimers, trimers, hexamers and dodecamers (12 mers). Plaques can be degraded, e.g. by immunotherapy, to lower MW Aβ fragments that might exert toxic effects. However, oligomers and monomers can be degraded or excreted via the BBB. Here, ABC transporters play an important role. Inhibition of ABC transporter function leads to temporal accumulation of Aβ within the brain. Conversely, ABC transporter activation helps to reduce the amount of monomers and toxic intermediates that can result from plaque-degrading approaches. Accordingly, we propose that, to be most effective, immunotherapy of AD patients must be accompanied by the activation of the brain's gateway – the BBB – to the peripheral sink – the blood – to sufficiently reduce toxic oligomers of Aβ.

MW Aβ aggregates is excretion via the BBB into the peripheral bloodstream.

4. The blood–brain barrier

The brain is by far the most complex organ of the human body, containing 10^{11} neurons with at least 1000 synapses and dendrites each, making enormous numbers of potential connectivity patterns possible (Lee and Bendayan, 2004). For optimal function, the brain needs an adequate and constant microenvironment, which is promoted on the one hand by the various types of glial cells that support, feed and modulate the action of neurons (Wolosker et al., 2008), and on the other hand by cerebral blood flow, which supplies nutrients, contributes to the maintenance of a constant pH, and is stringently adapted to the metabolic demands of cerebral tissue (Paulson, 2002). The blood–brain barrier (BBB) is a cellular and metabolic filter that regulates the exchange of materials between the blood and brain. The BBB consists of a monolayer of capillary endothelial cells surrounded by pericytes and astrocytic endfeet separated from the endothelium by the basal lamina. The endothelial cells are characterized by a high degree of polarization, a complete lack of fenestration, and interconnection with each other by tight junctions that prevent interstitial exchange. The BBB is largely impervious to hydrophilic substances, with the exception of certain substances such as glucose, which crosses the BBB by a mechanism of facilitated diffusion (Begley and Brightman, 2003). This design makes brain capillaries 50–100 times less permeable than are peripheral microvessels (Abbott et al., 2006). The influx and efflux of essential molecules such as nucleosides, amino acids, electrolytes and peptides, as well as protection against toxic waste products/environmental toxins is maintained by several membrane transporters expressed by the endothelial cells. This isolation and strictly regulated blood supply are prerequisites for the proper function of the brain, but the BBB also harbors potential sites of dysregulation that may contribute to neurodegenerative diseases (for more details information and a functional scheme see Pahnke et al., 2008).

4.1. ABC transporters

Eukaryotic ATP binding cassette (ABC) transporters are efflux pumps located in the plasma membrane, where they expel a wide variety of drugs, conjugates, metabolites and other mainly hydrophobic substances using the energy of ATP hydrolyzed at two conserved intracellular binding sites. ABC transporters are found in tissues and organs with secretory and barrier functions, including kidney, liver and gut. More importantly for neurodegenerative disorders, they are also found at the BBB, choroid plexus, and within the brain proper. At present, 49 human ABC transporters are known, and they are divided into seven subfamilies (ABCA to ABCG), with large and partially overlapping substrate specificities (Schinkel and Jonker, 2003). The asymmetric arrangement of ABC transporters within the brain capillary endothelial cells and other endothelia or epithelia constitutes a functionally effective barrier for potentially toxic molecules, inasmuch as most of the ABC transporters expressed face the luminal surface of vessels [for review see also (Schinkel and Jonker, 2003; Sun et al., 2003; Loscher and Potschka, 2005; Pahnke et al., 2008; Sharom, 2008). The most important transporters with regard to multidrug resistance are ABCB1 (P-glycoprotein, P-gp), ABCC1 (MRP1) and ABCG2 (BCRP), and these are also expressed at the BBB.

ABCB1 was first identified in 1976, in a Chinese hamster ovary cell line that was found to be drug resistant (Juliano and Ling, 1976). Later, it was the first ABC transporter to be detected at the BBB (Cordon-Cardo et al., 1989). ABCB1 is the product of a small group of related genes called multidrug resistance (MDR) genes encoding two gene products in humans (MDR1 and MDR2) and three isoforms in rodents (MDR1a, MDR1b, MDR2) (Chin et al., 1989; Hsu et al., 1990).

ABCG2 is structurally different, since it is essentially a half transporter with only 6 transmembrane domains, and is assumed to be functional as a homodimer. It was primarily isolated from human breast tumor cell lines displaying resistance against mitoxantrone, but which were negative for ABCB1 and ABCC1. ABCB1 and ABCG2 seem to have functional parallels, as ABCG2 mRNA was found to be upregulated 3-fold at the BBB in Mdr1a knockout mice (Cisternino et al., 2004). Furthermore, ABCB1 and ABCG2 are currently being regarded also as markers for neural stem/progenitor cells (Islam et al., 2005a,b; Lin et al., 2006; Mohan et al., 2006).

ABCC1 was identified in 1993 in a human tumor cell line (H69AR) and has substrate specificity for many drugs that are conjugated to glutathione, glucuronate or sulphate (Dallas et al., 2006). ABC transporters are the main reason for the apparent imperviousness of the BBB to molecules that, by their physicochemical properties, were predicted to enter the brain, abrogating the original thinking that transmembrane carriers are solely responsible for the efflux and influx of endogenous substances (Loscher and Potschka, 2005). This effect presents a considerable challenge in the development of pharmaceuticals for proteopathies of the brain with dementia and/or motor disturbances.

4.2. The BBB and brain disorders

ABC transporters at the BBB play direct and indirect roles in many neurological disorders. With respect to dementias, there is reasonable evidence that the BBB becomes increasingly permeable with increasing age, in the presence of AD, and especially in the presence of so-called vascular dementia (VD) (Farrall and Wardlaw, 2009). Limited evidence indicates increasing BBB permeability with increasing amounts of white matter lesions as a marker of VD. The evidence for BBB dysfunction is largely indirect (plasma:CSF albumin ratio on the whole), and it is not yet known what part of the BBB fails. In a recent study, patients with lacunar infarcts and without cognitive impairment showed evidence of BBB failure. MRI analyses detected increased permeability and enlarged perivascular spaces, further supporting the potential importance of interstitial clearance mechanisms in protecting the brain (Wardlaw et al., 2009).

Neoplasias of the brain are still among the tumors with the poorest prognosis. This is mainly due to the fact that 50–60% of all brain tumors are of neuroepithelial origin. These include astrocytomas, oligodendrogliomas, mixed gliomas, ependymal tumors and choroid plexus tumors (Bredel, 2001). All of the native cells that give rise to these tumors express, at a minimum, ABCB1 to protect the brain from potentially toxic substances. Therefore, tumors that arise from those cell types are almost always multidrug resistant from the outset. However, functional ABC transporters are not likely to be the cause of tumors, but rather a major key to protection because of their function as a barrier against cytotoxic molecules.

This barrier function also is discussed as a reason for intractable seizures in epileptic patients. Epileptics are treated with a range of anti-epileptic drugs (AEDs) to prevent seizures by reducing/controlling the excitability of neurons in a way that is still not fully understood. Most AEDs are substrates for different ABC transporters, especially ABCB1, and these AEDs directly or indirectly induce transporter expression (Hughes, 2008). Consequently, the dosage of AEDs must be increased continuously to achieve a constant concentration of drug within the brain. Furthermore, prolonged seizures themselves can contribute to the resistance against AEDs by inducing ABCB1 expression (Loscher, 2007).

4.3. ABC transporters in neurodegenerative diseases

The causal triggers of neurodegenerative diseases and continuous neuronal loss are still elusive. Age-related neurodegenerative disorders have in common the accumulation of insoluble neurotoxic proteins (Johnson, 2000; Walker and LeVine, 2000). One can suggest that proteins that become toxic within the brain are detoxified under normal conditions or cleared from the brain. The clearance of such proteins by ABC transporters has come into research focus only recently. For some types of Parkinsonism, it is thought that environmental neurotoxic substances that trespass the BBB result in neuronal loss in the midbrain. This hypothesis was bolstered by the finding that the neurotoxin MPTP causes a parkinsonian syndrome similar to Parkinson's disease (PD) (Calne and Langston, 1983). Furthermore, the exposure to pesticides is one risk factor for PD, and some pesticides have structures similar to that of MPTP (Rajput et al., 1986; Barbeau et al., 1987). Since a C3435T SNP of the abcb1 gene was shown to occur 5 times more often in pesticide-exposed PD patients (Furuno et al., 2002; Drozdzik et al., 2003), Kortekaas et al. hypothesized that a reduced function of ABCB1 may be a risk factor for PD (Kortekaas et al., 2005; Bartels et al., 2008a). These researchers injected PD patients and healthy individuals with [^{11}C]-verapamil, a specific substrate for ABCB1. Using positron emission tomography (PET) in PD patients, one restricted area of profoundly higher [^{11}C]-verapamil retention covered most of the midbrain, i.e. the substantia nigra pars compacta and part of the dorsal pons. These findings suggest that ABCB1 may be involved in the pathogenesis of Parkinsonism (Kortekaas et al., 2005).

Moreover, other ABC transporters may be involved; for instance, ABCC1 has a broad substrate spectrum, and is able to transport conjugates that are likely to be generated from pesticides and other toxins.

In the case of Alzheimer's disease, involvement of the BBB in the accumulation of Aβ peptides has been considered for a number of years. It was suggested that Aβ in plasma and cerebrospinal fluid exists at equilibrium, controlled by an as yet unknown mechanism that shifts the concentration toward the brain during plaque development (DeMattos et al., 2002a,b). Normally, the cerebral endothelium does not allow the free exchange of solutes such as Aβ between brain and blood due to the presence of the continuous monolayer of tightly junctioned endothelial cells (Begley, 2004). Thus, specialized transporters for Aβ must exist in the brain endothelium to expel the peptide from the CNS into the bloodstream, and/or to shuttle circulating Aβ into the CNS. It has been proposed that the Aβ-equilibrium between plasma and CSF is regulated at the BBB by an influx receptor (RAGE, receptor for advanced glycation end products) and an efflux receptor (LRP1, low-density lipoprotein receptor-related protein) (Zlokovic, 2004). Apolipoprotein E is considered to be involved in the RAGE/LRP1 shuffling mechanism (Shibata et al., 2000), and polymorphisms in apolipoprotein E (ApoE) are known to markedly influence the risk of late-onset AD (Saunders (Saunders et al., 1993; Schmechel et al., 1993; Strittmatter et al., 1993a,b; Warzok et al., 1998; Walker et al., 2000; Selkoe, 2001; Pahnke et al., 2003). Recently it has been found that ABCB1 is another important Aβ pump at the BBB (below).

4.4. AD and ABC transporters

Converging lines of evidence suggest that the ABC transporters, especially ABCB1, may have a substantial impact on the pathogenesis of AD. It is assumed that ABCB1 recognizes its substrate by attaching to it laterally while the substrate is located in the inner hemileaflet of the plasma membrane (Higgins, 1992; Higgins and Gottesman, 1992; Gottesman and Pastan, 1993). Thus, ABCB1 favors substrates that have at least partially hydrophobic characteristics. Aβ peptides feature 28 hydrophilic amino acids and 12 (Aβ40) or 14 (Aβ42) hydrophobic residues. The hydrophobic nature of Aβ is consistent with data indicating that the peptide has limited solubility in aqueous solutions and a preference for electrostatic binding to the membrane bilayer (Terzi et al., 1995, 1997).

The possibility of ABCB1-mediated Aβ efflux was first examined *in vitro* in transfected HEK293 cells (Lam et al., 2001). By applying the ABCB1 inhibitors RU486 and RU49953, Lam et al. showed that ABCB1 serves as an Aβ-efflux pump. Additionally, fluorescence quenching binding affinity determinations and transport competition experiments supported their data. The results were confirmed in cell culture experiments using MDR1-transfected polarized kidney epithelial cells (LLC-PK1) (Kuhnke et al., 2007). This latter group showed that Aβ peptides can be transported through a monolayer of highly polarized cells, quite similar to the situation *in vivo* at the BBB. Cirrito et al. (2005) added further evidence for a distinctive role of ABCB1 in the clearance of Aβ from the brain *in vivo*. Using mice deficient in both the abcb1a and abcb1b genes, they demonstrated significantly decreased elimination of [^{125}I]Aβ40 and [^{125}I] Aβ42 when compared to wild-type mice. The same was true for Tg2576 (APP-transgenic) mice treated intravenously with XR9576 (Tariquidar). Microdialysis revealed an almost 30% increase of soluble Aβ peptides starting 8 h after treatment. Tg2576 mice crossed with abcb1a/b-deficient mice displayed an increase in the area of Aβ42 immunopositivity within the hippocampus, but other areas only showed a trend toward more Aβ42 staining. For Aβ40, no significant increase in plaque load was detected. ELISA revealed an increase in insoluble Aβ42, but not in insoluble Aβ40 or in the soluble fractions of Aβ40 or Aβ42. It should be noted that the Tg2576 mice were crossed with abcb1a/b-deficient mice only to generation F1. Since Tg2576 mice are on a mixed C57BL/6-SJL background, and the transporter-deficient mice (as well as the wild-type controls) were on an FVB background, it is difficult to interpret these results. It has been shown that the background of APP-transgenic mice can have a major influence on Aβ deposition (Lehman et al., 2003). Soontornmalai et al. (2006) found that, of four examined mouse strains (including FVB and C57BL/6); only FVB mice have no endothelial ABCC2 expression in the brain. We have established several extensively backcrossed mouse models using the APPPS1 double-transgenic AD model (Radde et al., 2006) in concert with different ABC transporter knockout models. APPPS1 transgenic mice that are additionally deficient for abcb1a/b show only slight differences in Aβ deposition by histology but impressive differences in Aβ40 and Aβ42 levels by ELISA (data not published). The relevance of the above-mentioned results is confirmed by studies examining the abundance of Aβ and ABCB1 in humans (below).

In patients examined postmortem, differences in cerebrovascular ABCB1 expression have been found to correlate inversely with the degree of cerebral Aβ deposition (Vogelgesang et al., 2002, 2004). Interestingly, the inverse correlation between Aβ deposition and ABCB1 expression was most striking with regard to Aβ40 positivity. Investigations of the correlation between cerebrovascular β-amyloid angiopathy (CAA) and ABCB1 function indicated a loss of ABCB1 in vessels with abundant cerebrovascular Aβ in non-demented elderly humans (Vogelgesang et al., 2004). However, in that study, it was unclear whether the correlation was due to a primary loss of ABCB1 expression or a diminution of ABCB1 expression secondary to Aβ deposition. Two recently published works shed some light on this issue. At the International Conference on Alzheimer's Disease (ICAD 2008) in Chicago, van Berckel and colleagues presented data from a study of 14 healthy males. The subjects were divided into 3 groups of ~25, 46 and 62 years of age, all displaying a similar BMI of around

25 (van Berckel et al., 2008). The authors assessed ABCB1 function by volume distribution (VT) of the tracer (R)-[^{11}C]-verapamil using a 60 min dynamic 3D-PET scan. They found a significant, age-related decrease of ABCB1 function in all brain regions analyzed. Notably, the most prominent differences were within the thalamus, hippocampus and medial temporal lobe, the latter two regions being among the first and most severely affected structures in AD. Additionally, Bartels et al. (2008b) recruited seventeen healthy volunteers. Risk factors for vascular diseases such as diabetes, hypertension and high cholesterol were excluded, and the subjects did not use medication. In this study only two age groups of mean ages 24 and 60 years were examined using [^{11}C]-verapamil PET scans. Furthermore, subjects were checked for the abcb1 C3435T SNP, which has acknowledged importance for the transporter's activity. There was no incidence of altered ABCB1 function in subjects homozygous due to the TT or CC allele, respectively. However, the authors showed a significant decrease of ABCB1 function in the elderly brains. The most prominent decrease was found in white matter and orbitofrontal regions. Since damage to the white matter is a frequent occurrence in AD, the authors suggest that decreased ABCB1 function may cause myelin damage due to a higher Aβ load (Han et al., 2002; Song et al., 2004). Assuming that ABCB1 is an effective Aβ peptide efflux transporter, an age-related decrease in functionality is of importance, as it may help to explain why senescence is such an important risk factor for AD.

ABC transporters, in particular ABCB1, may also play a role in the intracerebral/intracellular distribution of Aβ, as they have been detected in neurons, astrocytes, microglia and within different intracellular compartments (Lee et al., 2001; Bendayan et al., 2002; Rajagopal and Simon, 2003; Schlachetzki and Pardridge, 2003; Volk et al., 2004, 2005; Donovan et al., 2006). These findings are supported by our own observation of early intracellular neuronal accumulations of Aβ in small vesicles in Mdr1a/b double knockout mice expressing the Dutch-type variant of APP (Pahnke et al., 2008).

Moreover, the transporters ABCA1, ABCA2 and ABCG2 have been suggested to play a role in the pathobiology of AD by their indirect influence on the processing of APP, or via a high chromosomal linkage with known AD loci (Pericak-Vance et al., 2000; Kim et al., 2007). ABCA1 and ABCG2 play a significant role in the regulation of brain cholesterol homeostasis. High cholesterol is a risk factor for AD, and the use of statins may be beneficial (reviewed in Pregelj, 2008). However, besides an enhanced amyloidogenic processing of APP through elevated cholesterol and altered ApoE levels, cholesterol might also influence Aβ clearance by ABCB1. It has been reported that cholesterol stimulates basal ATPase activity (Rothnie et al., 2001; Gayet et al., 2005), and the depletion of cholesterol reduces the transport activity of ABCB1, resulting in the intracellular accumulation of drugs (Troost et al., 2004; Gayet et al., 2005). However, Kimura et al. (2007) examined ATPase activity of ABCB1 using purified human ABCB1 reconstituted in liposomes containing 0–20% (w/w) cholesterol (Kimura et al., 2007). Interestingly, cholesterol affected not only basal ATPase activity but also the drug-stimulated ATPase activity of ABCB1. The effects of cholesterol on k_m were drug-specific. Cholesterol increased binding affinity to a variety of drugs (rhodamine 123, dexamethasone, verapamil, digoxin and others). Notably, there was a strong correlation between the molecular weight of the substrate and the cholesterol-induced shift of k_m such that small substrate binding was enhanced and binding was decreased with rising molecular weight. Thus, affinity to the heaviest substrate used (Valinomycin), with a MW of 1111 Da, was reduced in the presence of cholesterol. The cutoff MW between increasing and decreasing affinities due to cholesterol seems to be around 1000 Da. Therefore, one can suggest that the clearance of Aβ (with a MW of around 4 kDa) through ABCB1 may be reduced due to a reduced affinity when high cholesterol levels are present.

5. Conclusions and outlook

Environmental and genetic factors have been considered as potential causes of late-onset AD. A large number of polymorphisms in coding and non-coding regions of ABC transporter genes have been described that affect drug clearance by the transporter (Hoffmeyer et al., 2000; Schwab et al., 2003; Marzolini et al., 2004). To date, only one study has assessed the impact of three known SNPs of abcb1 on senile plaque prevalence, and this study did not detect a significant correlation (Vogelgesang et al., 2002). However, SNPs that are referred to as "silent", and thus not a focus of genetic association studies, can alter the functionality of proteins as well, and therefore further evaluation of this issue is recommended (Kimchi-Sarfaty et al., 2007).

Acute and long-term treatments for several diseases can influence ABC transporter function, as can diet. Assuming the clearance of Aβ peptides by ABC transporters as a mandatory mechanism, as well as the indirect modulatory effects on APP processing, the question arises as to the impact of this knowledge on various treatment regimes. For example, a large number of patients with high blood pressure, hypercholesterolemia, and obesity are treated with β-blockers, calcium antagonists, and anti-hyperlipidemic drugs. Treatment usually starts long before the onset of disorders such as PD or AD; hence, such drugs are circulating in the bloodstream for years, with the possible consequence of elevating cerebral Aβ levels. Some of these pharmaceuticals are inhibitors of transporter function; many are substrates of ABC transport proteins, thus competing with endogenous molecules for binding and transport. Furthermore, many dietary molecules were found to inhibit ABC transporter expression or function, including curcumin, piperine and ingredients of grapefruit juice (Bhardwaj et al., 2002; Zhou et al., 2004). Therefore, the influence of certain therapeutic drugs on the development of neurodegenerative disease must be examined, a goal currently sought through the use of systems biology approaches (Pahnke et al., 2008). A key question that needs to be answered is how much reduction of Aβ clearance is required to elevate the risk of AD—do we need 10%, 1%, or even less, and over how many years? Animal models can be helpful in addressing this issue.

In any case, knowledge of a potential role of membrane pumps in staving off neurodegenerative disease can be converted into new therapeutic or preventive strategies. The induction of ABC transporters may obviously be beneficial in preventing and treating AD. A candidate for this may be St. Johns Wort, which is known for its ABCB1-inducing properties, and today is widely used as an antidepressant (Perloff et al., 2001). Furthermore, compounds that interfere with the oligomerization of Aβ42 and favor either fibrillization or monomerization would be of interest, since the main neurotoxic Aβ species belong to the soluble oligomeric forms (Lesné et al., 2008; Selkoe, 2008).

The goal of such therapies will not necessarily be the extinction of AD, but rather a shift of the average age of onset to later ages. Because the prevalence of AD doubles every 5 years after the age of 60, and because the death rate from other causes rises markedly around this time, a treatment that results in a 5-year delay in the age of disease onset would substantially diminish the personal and societal costs of the disorder. We propose that, to be most effective, plaque dissolution treatments for AD patients must be accompanied by the activation of the BBB to sufficiently reduce the intracerebral load of toxic, oligomeric species of Aβ.

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