Bachelor Thesis

**The role of the Blood Brain Barrier in the development and treatment of Alzheimer’s Disease**

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**1. Introduction**

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder with an unknown etiology that accounts for 60 - 70% of all dementia cases (Burns and Iliffe, 2009; World Health Organization, 2016). The disease was first described by Alois Alzheimer in 1906 by examining a woman aged between 51 and 55 (Alzheimer, 1906). The blood brain barrier (BBB), which is the barrier between neuronal tissue and blood capillary cells, is suggested to be related in a certain way to AD pathogenesis and development (Yamazaki and Kanekiyo, 2017). The aim of this current study is to specify in which ways BBB’s functioning is related to AD pathogenesis and what this means for future therapeutic approaches in the treatment of AD.

*1.1 Alzheimer’s disease*

Neurofibrillary tangles and plaques are found in AD. Plaques are formed by cleaving at the β-amyloid precursor protein (APP). APP is a single-pass transmembrane protein found in many tissues and organs, but it is also highly expressed in the brain (O’Brien and Wong, 2011; Puig and Combs, 2013). Its primary function is not known, although it is suggested that the function of this protein in the brain is to help neurons develop synapses (Priller et al., 2006), to help neurons regulate their activity (Turner et al., 2003) and to regulate the iron export between neurons (Duce et al., 2010). APP has a high rate of metabolization: it is produced quickly but also broken down fast by α, β, and γ secretases (O’Brien and Wong, 2011). These different secretases cleave the protein at different sites (Figure 1).

In the non-amyloidogenic pathway, there is no amyloid beta formed because of the combination of the right secretases that are active. The α secretase cleaves first, APP followed by γ secretase. The α secretase cleaves in the middle of the amyloid beta fragment, which prevents the formation of free non-soluble amyloid beta. The γ secretase separates the extracellular domain from the intracellular domain. A 3 kDa soluble product (p3) is formed extracellular and the separated lower part of APP, called the APP intracellular domain (AICD), is formed intracellular. Furthermore, in the amyloidogenic pathway free non-soluble amyloid beta is formed. This occurs by β secretase cleaving APP followed by cleavage of γ secretase. B secretase separates the Aβ domain from the upper domain. The γ secretase separates the extracellular domain from the intracellular domain. In this way, AICD is formed intracellular and amyloid beta is formed extracellular. Amyloid beta monomers are sticky and

can form an aggregation of amyloid beta plaques (O’Brien and Wong, 2011). These plaques can block signal transmission of neurons and will eventually lead to neuronal death, due to neuronal dysfunction (Palop and Mucke, 2010). Amyloid beta plaques can also attach to blood vessels, which is called amyloid angiopathy (Smith and Greenberg, 2009). However, some other studies showed that under certain conditions amyloid beta may instead have a positive and even neuroprotective effect inside the brain. Amyloid beta belongs to a group of proteins that capture redox metal ions, such as Cu, Fe, and Zn. These metal ions participate in redox reactions, causing reactive oxygen species (ROS), which causes neuronal damage. Amyloid beta prevents redox metal ions from participating in redox reactions. Given that oxidative stress promotes amyloid beta generation, amyloid beta could be a compensatory response to prevent the increase of ROS production and oxidative stress (Atwood et al., 2003).



**Figure 1:** A schematic diagram of the cleavage of the amyloid precursor protein (APP), which occurs by two pathways. **A** | The non-amyloidogenic pathway involves the cleavage of α-secretase followed by γ-secretase. In this pathway, a 3 kDa product (p3) and the APP intracellular domain (AICD) are formed. **B** | The amyloidogenic pathway involves the cleavage of β secretase followed by γ secretase. Amyloid beta and AICD are formed in this pathway.

Secondly, neurofibrillary tangles are found in Alzheimer’s disease as well. Neurons have a cytoskeleton of microtubules. A protein called Tau prevents breaking down of microtubules in axons and helps the microtubules run in parallel and straight in a healthy situation (Avila et al., 2004). A current hypothesis is that the plaques outside the neuron initiate a response inside the neuron in which an enzyme phosphorylates microtubules and subsequently phosphorylates the tau protein. This phosphorylation stimulates a conformational change in the tau protein, causing the tau protein to dissociate itself from the microtubules. Afterwards, an accumulation of tau phosphorylated proteins will develop inside the neuronal soma. This accumulation of tau phosphorylated proteins is called neurofibrillary tangles. Due to this, microtubules are not able to function properly. This will disable the transfer a signal along the neuronal axon. The neuron will eventually undergo apoptosis (O’Brien and Wong, 2011).

The death of neurons and the disruption of signaling between neurons will cause memory loss and brain atrophy, which means that the brain shrinks. The structure of the brain changes, as well. The gyri will become smaller and the sulci will become bigger. While developing atrophy, the ventricles in the brain will become bigger as well (Pini et al., 2016). AD has a gradual start but turns more progressive during later phases. That’s why AD is often called a progressive neurodegenerative disease. The six stages of AD were described by Braak in 1991 (Braak and Braak, 1991). The spreading around of neurofibrillary tangles and amyloid beta plaques causes the different stages of Alzheimer’s disease. The first two stages are stage I-II, where symptoms are not yet clearly present. In stage III and IV the limbic regions such as the hippocampus become affected. From the hippocampus, the disease spreads towards the neocortex in stage V and VI (Braak and Braak, 1995).

The first symptoms consist of difficulties with short-term memory and difficulties with forming new memories, indicating affection of the hippocampus. Afterwards, regions responsible for language will be affected too. It becomes difficult to find the right words. As the disease continues with killing neurons in different regions, it becomes difficult to think logically and to perform daily activities, such as driving a car. Subsequently, all forms of memory become impaired amongst other difficulties such as problem solving and reasoning. Eventually, regions responsible for moods and feelings become impaired. The person can’t control his or her behavior anymore. Furthermore, in the progression of the disease, a person can’t determine what is real: hallucinations become common. In the last stages of the disease, a person can become mute, and the long-term memory will be affected. Afterwards, a person’s coordination of the body will be affected and someone becomes bedridden. Ultimately, the disease becomes fatal (Apostolova, 2016).

There are two different forms of Alzheimer’s disease: the sporadic and the familial form. Sporadic Alzheimer’s disease (SAD) accounts for 90 - 95 % of the disease cases and has a late onset. Environmental and genetic risk factors are important. Environmental risk factors include: lower social engagement, diabetes mellitus, and current smoking. Genetically, possessing the ApoE e4 gene forms a higher risk of developing AD. The expression of ApoE is decreased because of the e4 part of the gene. ApoE is a protein that helps to break down amyloid beta. When this expression is decreased, there is a higher risk of developing AD. When someone inherited this gene from both of the parents, the risk factor is even higher (Hersi et al., 2017).

Familial Alzheimer’s disease (FAD) accounts for 5 - 10% of all Alzheimer’s disease cases. FAD is also called early-onset Alzheimer’s disease, since it has an early onset. In FAD, several gene mutations can occur. For instance, mutations in PSEN-1 and PSEN-2 genes on respectively chromosome 14 and chromosome 1, changes the function of the γ secretase. PSEN-1 and PSEN-2 encode respectively for presenilin 1 and presenilin 2. Presenilin 1 and presenilin 2 are subunits of the γ secretase. When mutations in these subunits occur, the γ secretase can cut the APP protein at a different site, producing different lengths of Aβ molecules. The formation of plaques is in this way increased, and therefore the chance of developing Alzheimer’s disease is also increased (Sherrington, 1995; Matsumura, 2014; De Strooper, 1998; Wolfe, 1999; Veugelen, 2016).

FAD is also associated with another gene mutation; this gene mutation is in the APP gene. In Down syndrome, there is an extra chromosome 21. The gene responsible for expressing APP is located at chromosome 21. Due to this, APP is expressed at higher levels. With more APP present in neuronal membranes, more amyloid beta can be produced inside the brain. In this way, the chance of developing Alzheimer’s disease is increased (Goate, 1991).

The exact cause of Alzheimer’s disease is still unclear (Sharma, 2018). Next to these hypothesizes previously explained, here are a few more such as the cholinergic hypothesis, the oxidative stress hypothesis, the excitotoxic hypothesis, and the glycogen synthase kinase hypothesis. These will be discussed in the next sections.

The cholinergic hypothesis states that the production of ACh in the basal forebrain is lowered as a result of affected cholinergic neurons. ACh is an important neurotransmitter in memory, learning and cognitive functions (Blockland, 1995; Lane et al., 2006; Thompson et al., 2012). Because the synthesis of ACh is decreased, this results in cognitive dysfunctions. The cholinergic hypothesis dates back to 1970 when it started with observations of post-mortem brains of Alzheimer’s disease patients (Sharma, 2018). In these investigations, reduced levels of choline acetyltransferase (CAT) were found in the brain of patients that died of Alzheimer’s disease (Perry et al. 1977, 1978). CAT is responsible for ACh synthesis and was reported to be significantly decreased in the hippocampus and in the neocortex; sites where Alzheimer’s disease starts with neurodegeneration (Perry, 1986).

The excitotoxic hypothesis focuses at the excessive activation of NMDA type glutamate receptors in neurons. This causes calcium and sodium influx in neuronal cells. In a healthy situation, magnesium blocks the entry of calcium, but with bound glutamate, magnesium is released. The abundant influx of calcium ions inhibits the neuronal transmission and stimulates neurodegeneration and cell death (Olney et al., 1997). This process occurs mostly in the hippocampus and cortex regions where glutamate is present (Geddes et al., 1986).

The oxidative stress hypothesis or the mitochondrial dysfunction hypothesis focuses on the augmentation of free radicals caused by amyloid beta entering the mitochondria (Markesbery, 1997; Zhao and Zhao, 2013). This augmentation of free radicals causes oxidative stress. Oxidative stress is an imbalance between reactive oxygen species and the ability to detoxify these reactive oxygen species. Neuronal cells are more sensitive for reactive oxygen species than other tissues, because neuronal cells have less antioxidant enzymes and neuronal cells have a higher oxygen consumption (Coyle and Puttfarcken, 1993).

In the glycogen synthase kinase-3 hypothesis, the glycogen synthase kinase (GSK-3) is important. GSK-3 is produced in isoforms α and β. Both isoforms are abundantly present in the brain (Lovestone et al, 1994). GSK-3β plays chiefly an important role in AD. GSK-3β is involved in hyperphosphorylation of tau (Pei et al., 1997), Aβ aggregation, senile plaques (Phiel et al., 2003), oxidative stress (Rojo et al., 2008) and regulation of transcription factors responsible for neurodegeneration (Balaraman et al., 2006). In the GSK-3 hypothesis, it is believed that the overexpression of GSK-3 accounts for the development of AD (Hooper et al., 2008).

For the treatment of AD, there are currently two fronts at which treatments are aimed: Aβ aggregation and tau hyperphosphorylation. Only five drugs have been approved to improve APP processing via activation and/or activation of the machinery. But the benefits are small; there is some symptomatic improvement in AD, but nevertheless there is no effect on the disease progression. Furthermore, other strategies to halt the progression of AD are: immunotherapeutic approaches to existing Aβ aggregation, tau hyperphosphorylation therapies, therapies focused on oxidative stress and autophagy inducers to degrade the aggregation of Aβ plaques and tau tangles (Jan, 2017). Besides, potent biomarkers are important for the diagnose of AD. Aβ plaques disposition and tau hyperphosphorylation are core biomarkers of AD (Blennow et al., 2010). However, these biomarkers were not sensitive enough to be able to distinguish pathological changes between a plaque count of a healthy elderly person and an Alzheimer’s patient (Coart et al., 2015; Curtis et al., 2015). Therefore, research focuses also on other potent biomarkers as: Aβ oligomers (Overk and Masliah, 2014), Neurogranin, a dendritic protein (Díez-Guerra, 2010) and tau imaging (Jan, 2017).

In conclusion, there are different hypotheses explaining the development of AD. None of these fully explain the development of AD. Furthermore, diagnostic and therapeutic strategies targeting AD could improve as well, because biomarkers are not sensitive enough and current therapeutic strategies have still no effect on the disease progression of AD.

*1.2. The blood brain barrier*

The blood-brain barrier (BBB) is formed by brain capillary endothelial cells and is part of the neurovascular unit (NVU) (Figure 2). The neurovascular unit is composed of specialized endothelial cells surrounding brain capillaries, pericytes, astrocytes, neurons and extracellular matrix components (Chin and Goh, 2018; Muoio et al., 2014). Each of the component cell types are tightly packed together, so that cerebral blood flow can be precisely regulated. One endothelial cell forms a tube structure around a blood capillary. Endothelial cells in the central nervous system (CNS) have distinct properties which are different compared to endothelial cells in other tissues. CNS endothelial cells have BBB-specific transporter and receptor proteins, tight junctions (TJ’s), low levels of transcytotic vesicles and an absence of fenestrae, which are small pores normally present in peripheral endothelial cells. A high transendothelial electrical resistance (TEER) can be found in CNS endothelial cells, which limits movement between neighboring cells causing decreased paracellular and transcellular permeability (Yamazaki and Kanekiyo, 2017).

The membrane of endothelial cells consists of an apical side facing the lumen, which is also called the luminal side, and a basolateral side, or abluminal side, facing the basement membrane. The membranes of endothelial cells are polarized. They transport molecules in both directions: from the blood capillaries into the brain and from the brain into the blood capillaries (Figure 2). Therefore, the abluminal membrane could contain other transporters than the luminal membrane. Both membrane sides contain transporters, receptors, metabolite-degrading enzymes and ion channels. All these properties are important to maintain homeostasis within the CNS (Chin and Goh, 2018).

Tight junctions are multiprotein complexes consisting of a branching network. Their endings are embedded into plasma membranes of neighboring cells. At least 40 different proteins form a tight junction. These proteins can be divided into two groups: transmembrane proteins and cytosolic TJ-associated proteins. The three most important transmembrane proteins are occludin, claudins and junction adhesion molecules (JAMs) (Sandoval, 2008).

Occludin has a molecular weight of 60~65 kDA and consists of four transmembrane domains with both the N-terminus and the C-terminus located in the cytosol. Occludin has two extracellular domains and three intracellular domains (Wolburg, Lippoldt and Ebnet, 2006). The extracellular loops of occludins interlink in between neighboring cells. Balda et al. demonstrated that inserting occludin in a TJ region increases the TEER, which decreases movement between cells and thus a decrease in paracellular permeability. However, the C-terminus of occludin plays an important role: when the C-terminus is truncated, the paracellular permeability is still increased (Balda et al., 1996). The C-terminus of occludin is important in the barrier function of a TJ, because a certain part in the C-terminus forms an α-helix, called the coiled-coil domain. This domain binds the adapter protein zona occludens-1 (ZO-1) at the guanylate kinase (GUK) domain. This connection is important in maintaining the barrier function of TJ’s (Feldman, 2005). The function of occludin is also studied in occludin-deficient embryonic stem cells (Kniesel and Wolburg, 2000) and in occluding-deficient mice (Saitou et al., 2000). In these studies, it has been shown that occludin is not required to form TJ’s, when replaced with other junctional proteins (Saitou et al., 2000). However, in the same study in occludin-deficient mice, hyperplasia of the gastric epithelium, testicular atrophy and calcifications in the brain are present. Other studies have shown that occludin plays a role in the regulation of epithelial cell differentiation (Schulzke et al., 2005). To conclude, occludin is replaceable as a building block of TJ’s, TJ’sTJ’s, but is not replaceable when it comes to certain physiological functions (Zlokovic, 2008).

Claudins exist of a family of 24 proteins involved in the formation of TJ’s. Compared to occludin, the molecular weight of claudins is lower: ranging from 20 to 24 kDa. Like occludin, claudins have four transmembrane domains and two extracellular domains, but the tails with the N-terminus and the C-terminus are shorter (Gonzalez-Mariscal et al., 2003). The extracellular loops of claudins connect with each other in adjacent endothelial cells (Piontek et al., 2008). Claudins portray no homology in sequence compared to occludin. With their C-terminus, claudins can bind to the PDZ domains of ZO-1, ZO-2 or ZO-3 (Itoh et al., 1999; Ruffer and Gerke, 2004). The name PDZ comes from combining the first letters of three proteins that were discovered to bind this domain: post synaptic density protein (PSD95), Drosophila disc large tumor suppressor (DLg1) and ZO-1 (Kennedy, 1995). Regarding the claudins family, claudin -1, -2, -3, -5 and -12 are present in BBB endothelial cells (Huber, Egleton and Davis; 2001; Sandoval and Witt, 2008; Romanitan et al., 2010). Different studies have discovered claudin-5 to have an essential role in BBB regulation. In claudin-5 deficient mice, it has been shown that the BBB is permeable to molecules of less than 800 Da (Nitta et al, 2003). In zebrafish brains, claudin-5a appears to be the main component in the formation of a neuroepithelial barrier in the ventricular lumen expansion (Zhang et al., 2010). Furthermore, Honda et al. have shown that adrenomedullin, which increases the expression of claudin-5, increases TEER and decreases the BBB permeability (Honda et al., 2006).

JAMs are members of the immunoglobulin superfamily (Martìn-Padura et al., 1998) and have a molecular weight at about 40 kDA (Ballabh et al., 2004). There are different JAMs of which JAM-A, -B, and –C have been reported in endothelial cells, where especially JAM-A is highly expressed. They are part of the immunoglobin superfamily, because they contain two immunoglobin (Ig) loops in their extracellular domain (Said, 2012). They have a single transmembrane domain and a short cytoplasmic tail containing a PDZ domain. The Ig loops are formed with disulfide bonds. A molecular model has been proposed, where two JAMs form a homodimer in *cis* with their binding motifs on the Ig loops. This forms the shape of a ‘U’. Mandel et al. have shown that homodimer formation is important for JAM-A functioning. Two monoclonal antibodies which will be attached to the two JAMs, were successful in inhibiting barrier recovery, while one monoclonal antibody was not (Mandell, McCall and Parkos, 2004). These *cis*-homodimers bind in *trans* to other JAM homodimers from neighboring cells (Weber et al., 2007). In the cytosol, JAMs interact with ZO-1, multi-PDZ-protein-1 (MUPP-1), afadin (Af-6), and partitioning defective protein-3 (PAR-3) (Ebnet et al., 2003). Earlier it was already demonstrated that JAMs interact with tight junction components cingulin and occludin in hamster JAM-transfected ovary cells. In addition, an interaction with ZO-1 is established (Bazzoni et al., 2000). The interaction with other tight junction molecules suggests that JAMs are important in tight junction formation. Jam A and Jam C are important in maintaining the stability of a TJ, and JAM-B maintains the stability of a TJ indirectly by supporting JAM-C (Bradfield et al., 2007). In addition, in a BBB-breakdown model it is shown that JAM-A expression is decreased (Yeung et al., 2008).

Adherens junctions (AJs) are located basal to TJ’s to support them. AJs are involved in regulating BBB permeability (Dejana, Orsenigo and Lampugnani, 2008). Other functions of AJs are the regulation of actin cytoskeleton, transcriptional regulation and intracellular signaling (Hartsock and Nelson, 2008). AJs are formed by cadherins, which are part of the cadherin family of adhesion proteins. Cadherins are single-pass transmembrane glycoproteins with a molecular weight of approximately 120 kDA. The cadherin family consists of more than a hundred cadherins, divided into classical and non-classical cadherins (Porquet and Huot, 2011). Cadherins consist of a small intracellular component anchored to the cell membrane by a hydrophobic sequence, and five extracellular domains, which are linked by calcium ions. Binding of Ca2+ to each extracellular cadherin (EC) domain is important for the conformational organization to function (Pokkutta et al., 1994), which is why they are named ‘calcium-dependent adhesion’ molecules.

The endothelial cells are covered with the basement membrane. The basement membrane is also called the basal lamina and is composed of extracellular matrix (ECM), which include several structural proteins such as collagens, fibronectin, nidogen, perlecan, laminin and agrin (Morris et al., 2014; Yousif et al., 2013; Nitkin et al., 1987). The basement membrane is composed of two different types of membranes, each produced by different cell types. The endothelial basement membrane is composed of ECM secreted by endothelial cells and pericytes and the parenchymal basement membrane is secreted by astrocytes (Sixt et al., 2001; Owens, Bechmann and Engelhardt, 2008; Beaten and Akassoglou, 2011). There is also a difference in these two membranes regarding the isoforms of laminin: the endothelial basement membrane contains laminin α4 and α5 isoforms (Sorokin, 2010), while the parenchymal basement membrane is more enriched in the laminin isoforms α1 and α2 (Sixt et al., 2001; Owens, Bechmann and Engelhardt, 2008; van Horssen, 2005). The basement membrane appears to be a scaffold linking different molecules and cells of the NVU (Blanchette and Daneman, 2015), in this way crosstalk between the components of the NVU occurs (Keaney and Campbell, 2015). Yao et al. have recently shown that pericyte differentiation is influenced by the basement membrane component laminin, which is secreted by astrocytes. Disruption of laminin signaling changes pericytes from a resting phenotype into a contractile phenotype. This contractile phenotype of pericytes influences end-feet processes of astrocytes and decreases endothelial TJ expression. When the expression of TJ’s is decreased in the endothelial cells, the BBB properties are changed. Therefore, ECM components such as laminin are important in maintaining BBB properties (Yao et al., 2014). Besides, the ECM provides a structural support for blood vessels, is a scaffold for growth factors, and regulates BBB permeability. Moreover, it functions as a physical barrier against incoming molecules such as leukocytes (Blanchette and Daneman, 2015).

Pericytes are contractile cells surrounding the walls of capillaries on the abluminal surface (Chin and Goh, 2018). They are embedded in a thin layer of the basal lamina (Yamazaki and Kanekiyo, 2017). In the CNS the ratio of pericytes to endothelial cells is approximately 1:1, this is also the ratio in the retina. But the ratio of pericytes to endothelial cells is 1:10 in the lungs and only 1:100 in skeletal muscles. Therefore, pericytes are much more present in the CNS than in peripheral tissues (Shepro and Morel, 1993). Functions of pericytes are: the formation of extracellular matrix (ECM), regulating BBB functioning and angiogenesis (Winkler, et al., 2011; Daneman et al., 2009, 2010; Armulik et al., 2005, 2010; Bell et al., 2010). Most of the pericyte bodies do not have contact with the endothelial cells because of the basal lamina, but when the basal lamina is not present the pericyte can attach directly to the endothelial cell forming the peg-and-socket connections (Yamazaki and Kanekiyo, 2017). Through adherens junctions and gap junctions a pericyte can communicate with endothelial cells (Bonkowski, 2011; Winkler, 2011).

Smooth muscle cells are also present in the NVU surrounding larger blood vessels. They make changes in contractionsregulated by calcium and potassium channels. Astrocyte signals can influence these smooth muscle cell contractions (Haydon and Carmignoto, 2006).

Astrocytes are star-shaped glial cells attaching their end-feet processes on the abluminal surface of endothelial cells and on the synapses of neurons (Oberheim et al., 2009). In this way, neurovascular coupling is generated (Yamazaki and Kanekiyo, 2017). A single astrocyte can connect with thousands of capillaries and synapses (Haydon and Carmignoto, 2006). Astrocytes have different functions in the brain supporting neurons. For instance, they form a structure for neurons to grow with alongside during the development of CNS. They respond to CNS damage with gliosis, a process to repair neurons (Blanchette and Daneman, 2015). Astrocytes are important in the uptake of neurotransmitters and they regulate ion concentration, neuronal metabolism and immune reactions. Besides their role in supporting neurons, astrocytes are important in regulating vasodilation or vasoconstriction of cerebral capillaries in response to neuronal activity (Rodríguez-Arellano et al., 2016) and regulating brain water content (Zlokovic, 2008). Astrocytes also act in maintaining BBB properties and regulating polarization of transporters, thus maintaining the BBB phenotype (Yamazaki and Kanekiyo, 2017).

Microglia are technically not part of the NVU, but they play a major role in maintaining the function of the BBB (Chin and Goh, 2018). Microglia are the innate immune cells of the CNS. Microglia exist in multiple phenotypes (Sierra, 2016). They mainly have been categorized into 3 different phenotypes (Andjelkovic, 1998). These phenotypes are called: ramified, reactive and protective (Rock, 2004). Protective microglia are involved in tissue repair, phagocytosis of damaged neurons and releasing chemokines. Ramified or resting microglia constantly search the environment for intruders and disturbances (Aguzzi, 2013). Given that microglia are located close to the BBB and that microglia also interact with CNS capillaries, microglia must also play a role in regulating BBB properties (Fantin et al., 2010; Tammela et al., 2011). However, little is known about the function of resting microglia in forming and regulating homeostasis of the BBB when there is no injury, although more is known about the activated microglia in the brain (Keaney and Campbell, 2015). Reactive or activated microglia have an amoeboid cell body and can be detrimental in the brain because of the cytokines and chemokines they secrete. In this way, microglia can cause CNS inflammation, which affects the BBB. These chemokines and cytokines stimulate adhesion molecules on endothelial cells of the BBB, which allows the migration of immune cells, such as T cells, from the blood into the brain. Reactive microglia secrete TNF- α and IL-1β and activate NADPH-oxidase. NADPH-oxidase produces reactive oxygen species (ROS), which cause impairment of BBB function by altering the expression of certain TJ proteins such as zonula occludens-1, claudin-5 and occludines (da Fonseca et al., 2014). CNS disorders like AD are associated with neuroinflammation and BBB dysfunction. In these cases, microglial activation may be both a cause and a consequence of BBB dysfunction (Keaney and Campbell, 2015).

Neurons also regulate BBB properties. They are found close to capillaries and are in direct connection with endothelial cells and astrocyte end-feet processes. They have the ability to control BBB permeability and capillary blood flow through neurogenic signaling (Chin and Goh, 2018). Signals from neurons are processed with different neurotransmitters: noradrenaline (Ben-Menachem, Johansson and Svensson, 1982; Cohen, Molinatti and Hamel, 1997), acetylcholine (Tong and Hamel, 1999; Vaucher and Hamel, 1995), serotonin (Cohen, Bonvento, Lacombe and Hamel, 1996), and GABA (Vaucher, Tong, Cholet, Lantin and Hamel, 2000). Furthermore, glutamate signaling activates α-amino-3-hydroxy-5-methyl-4-isoxazol propionic acid (AMPA) and post-synaptic N-methyl-D-aspartate (NMDA) receptors, which leads to intracellular Ca2+ amplification and activation of cyclooxygenase 2 (COX-2) and neuronal NO synthase (nNOS). This process leads to vasodilation, because vasodilators such as NO and prostanoid are produced by the COX-2 and nNOS. Simultaneously, glutamate acts on metabotropic glutamate receptors in astrocytes, which causes Ca2+ accumulation in the astrocytes. This induces production of vasoconstrictive and vasodilative agents (Attwel et al., 2010; Lecrux and Hamel, 2016). Noradrenaline depletion has been shown to increase the BBB permeability (Ben-Menachem et al., 1982). In Alzheimer’s Disease, loss of cholinergic nerve fibers and an impaired connection between blood vessels and nerve fibers is present. Furthermore, the neurogenic blood flow regulation is impaired. These facts may act together in the overall progression of Alzheimer’s Disease (Tong and Hamel, 1999).

Although the movement of macromolecules in transcytotic vesicles is limited, there are still some molecules transported across the endothelial barrier. This occurs through different transport pathways (Figure 2).

Paracellular transport is the transfer of molecules through the intercellular space between cells. These molecules are water-soluble agents, including polar drugs. Nevertheless, this form of transport is severely restricted because of tight junctions (Wong et al., 2013; Abbott, Rönnbäck and Hansson, 2006).

The transcellular lipophilic pathway allows the influx of small lipid-soluble, nonpolar molecules into the brain, by transendothelial passive diffusion (Yamazaki and Kanekiyo, 2017). The majority of the small lipid-soluble, nonpolar molecules are transported back to the blood through ATP-dependent efflux transporters.

The carrier protein-mediated transport pathway facilitates the influx of glucose, amino acids, nucleotides and hormones. This pathway is susceptible to be affected by size, affinity and physiochemical properties, because the concentration gradient across the BBB is the main driving factor for carrier protein-mediated transport (Yamazaki and Kanekiyo, 2017). GLUT1 is an example of a glucose transporter found in brain endothelial cells. The presence of GLUT1 is very important for energy supply to the brain. Also, alternative energy metabolites such as lactate are transported with carrier protein-mediated transport (Sweeney et al., 2018).

Receptor mediated transcytosis is a specific process and enables the transport of several large molecules such as peptides, proteins (Yamazaki and Kanekiyo, 2017) and hormones (Pardridge, 2015; Zlokovic, 2008) across the BBB. For example, amyloid beta can cross the BBB through low density lipoprotein receptor-related protein-1 (LRP1), a multifunctional protein expressed in many different cell types in the body (Lillis et al., 2008). LRP1 is located at the abluminal surface and transports amyloid beta from the brain into the blood (Deane et al., 2004). Amyloid beta can also cross the BBB via the receptor for advanced glycation end products (RAGE) on RAGE-expressing endothelium from blood to brain. This happens particularly under pathological conditions (Deane et al., 2003, 2012).



**Figure 2:** A schematic diagram of the endothelial cells that form the BBB and their relation to neuronal synapses, microglia, pericytes and the end-feet processes of astrocytes. The main transport mechanisms for molecular transportation across the BBB are portrayed. **1** | Usually, tight junctions restrict the transportation of water-soluble agents, including polar drugs. **2** | However, for lipid-soluble nonpolar molecules transport is not restricted because of the lipid membrane present in the endothelium of endothelial cells. **3** | The membrane of endothelial cells contains transport proteins for glucose, amino acids, nucleotides, hormones and other substances. **4** | A couple of proteins, including insulin and amyloid beta can cross the BBB by specific receptors. This is called receptor-mediated transcytosis. **5** | Cationic proteins such as albumin and histone proteins can be absorbed by adsorptive-mediated transcytosis, because of charge differences resulting from cationization. **6** | ATP-binding cassette (ABC) transporters prevent drugs, toxins and therapeutic agents from crossing the BBB. These transporters are ATP dependent.

Adsorptive-mediated transcytosis is a nonspecific process, and is often mediated via a clathrin-mediated process, but it can also be a caveolae mediated process. Adsorptive-mediated transcytosis is a transport process that applies to cationic proteins such as albumin and histone proteins. These proteins have a positively charged moiety which interacts with the negatively charged membrane of endothelial cells. This interaction becomes electrostatic, causing a cationic net charge which can be recognized as an important determinant for uptake in endothelial cells of the BBB. Either caveolae or clathrin-coated pits mediate the pathway. Caveolae are non-coated membrane invaginations containing a high amount of receptors. Clathrin-coated pits are negatively charged and will attract positively charged molecules such as cationic proteins. Both caveolae and clathrin-coated pits are involved in adsorptive-mediated transcytosis, but they mediate different sets of molecules across the endothelial membrane (Hervé, Ghinea and Scherrmann, 2008).

Active efflux is mediated through ATP-binding cassette (ABC) proteins which are expressed on the luminal side of endothelial cells. ABC transporters are efflux pumps and are ATP-driven. They limit brain uptake of drugs (Begley, 2004), toxins and therapeutic agents (Miller, 2015). For example, P-glycoprotein (P-gp) is an ATP-dependent efflux transporter highly expressed in brain epithelial cells (Schinkel, 1999). P-gp is also called multidrug resistance protein 1 (MRP-1) because it ensures that many drugs cannot pass the BBB, leading to multidrug resistance (Régina et al., 2001). MRP-1 also has an effect on amyloid beta, as it is involved in amyloid beta clearance. Cirrito et al. has shown in mice that MRP-1 deficiency caused a decrease in amyloid beta clearance and an increase of amyloid beta aggregation (Cirrito et al., 2005).

In conclusion, TJs are important in the barrier function of the BBB, and each TJ has specific characteristics. In addition, the NVU of the BBB consist of different cells, and substances can be transported using different transport mechanisms across the endothelial membrane.

**2. The function of the blood brain barrier in Alzheimer’s Disease**

The BBB mediates the relation between the immune system and the CNS. The BBB mediates this relation through the transportation of cytokines, substances associated with immune cell activation and immune cells. Immune cells are transported by diapedesis, a highly regulated process between brain endothelial cells and immune cells (Erickson, Dohi and Banks, 2012). Diapedesis of immune cells across a non-inflamed BBB is dependent on the interaction of alfa4-integrin with vascular cell adhesion molecule-1 (VCAM-1), expressed on brain endothelial cells (Xu et al., 2003; Vajkoczy et al., 2001). Further communication between the immune cells and the endothelial cells (Greenwood et al., 2011) includes the recruitment of other factors necessary for diapedesis, including intercellular adhesion molecule-1 (ICAM-1) (Engelhardt and Coisne; 2011; Greenwood; 2002). This recruitment has an endurance of approximately 4-16h (Xu et al., 2003; Vajkoczy et al., 2001).

Cytokines are small proteins, peptides or glycoproteins with an immunological function (Stedman’s Medical Dictionary, 2006). Cytokines could be pro-inflammatory or anti-inflammatory. Chronic secretion of proinflammatory cytokines could cause neuroinflammation in AD (Becher et al., 2017). Many cytokines are able to cross the BBB (Banks, 2004; Pan and Kastin, 2008; Pan et al., 2011), such as interleukin (IL)-1α (Banks et al., 1989), IL-6 (Banks et al., 1994), tumor necrosis factor (TNF-α) (Gutierrez et al., 1993), and fibroblast growth factor (FGF) (Pedro cuevas et al., 1998). According to Mrak and Griffin, IL-1 is overexpressed in the brain of AD patients. This overexpression is directly related to plaque formation (Mrak and Griffin, 2001). Furthermore, overproduction of IL-6 is found in AD patients in another study as well (Cojocaru et al., 2011). Besides, TNF-α overexpression in AD enhances AD-associated pathology and neuronal impairment in AD-induced mice (Janelsins et al., 2008). On the other hand, FGF stimulates neurogenesis and protects the hippocampus from ischemic injury (Wagner et al., 1999; Pedro Cuevas, 1998). Brain endothelial cells can also secrete cytokines and substances with neuroinflammatory properties, such as nitric oxide (NO) and prostaglandins (Mándi et al., 1998; Fabry et al., 1993; Macvilay and Fabry, 1997; Reyes, Fabry and Coe, 1999). Proinflammatory cytokines increase the amyloid beta deposition and decrease the amyloid beta clearance, while anti-inflammatory cytokines such as IL-4, IL-10 and IL-13 decreases the amyloid beta deposition and increases the amyloid beta clearance (Cai, Hussain and Yan, 2014). Secretion of cytokines by brain endothelial cells is regulated by the polarity in their different membrane surfaces; the abluminal surface and the luminal surface. For example, adiponectin applied to the luminal surface reduces IL-6 release on the abluminal side (Spranger et al., 2006). In addition, lipopolysaccharide (LPS) applied to the abluminal surface increases the secretion of IL-6 from the luminal surface abundantly (Verma et al., 2006).

Furthermore, the BBB ensures a stable internal homeostasis and has to prevent dangerous substances, such as toxins and microorganisms, from entering the brain. This is important to prevent further neurodegeneration and helps the other cells such as microglia and astrocytes to function properly. Many ions cannot pass through the BBB, but glucose and other amino acids pass through the BBB more easily (Patchin, 2017; Tan et al., 2017). In AD, amyloid beta is produced because β secretase cleaves at the wrong place in APP. The BBB has to regulate entry of plasma-derived amyloid beta into the brain (Zlokovic et al., 1993; Maness et al., 1994; Martel et al., 1997; Poduslo et al., 1999; Wengenack et al., 2000), and clears amyloid beta that entered the brain (Shibata et al., 2000; Iwata et al., 2000; Bading et al., 2002; DeMattos et al., 2002). Cells at the neurovascular unit have the ability to endocytose amyloid beta, followed by lysosomal degradation (Kanekiyo and Bu, 2014). Amyloid beta transport across the BBB is mediated by several receptors and transporters. Through LRP-1 amyloid beta can cross the BBB from the brain to the blood, providing a clearance route (Shibata et al., 2000). Also, P-gp supplies a clearance route for amyloid beta. From the blood to the brain, amyloid beta can cross the BBB with RAGE, on RAGE-expressing endothelium (Deane et al., 2003, 2012).

Post-mortem studies of AD brains have shown that the pyruvate dehydrogenase complex (PDHC) and the alfa-ketoglutarate dehydrogenase complex (KGDHC) in the mitochondria, are impaired (Huang et al., 2003; Bubber et al., 2005), which leads to mitochondrial dysfunction. Mitochondrial dysfunction increases oxidative stress and the presence of ROS. In addition, the aggregation of amyloid beta causes oxidative stress by increasing H2O2 production (Readnower, Sauerbeck and Sullivan; 2011). In a study about liver failure, which is not a typical complication in AD, a relation between the expression of P-gp transporters at the BBB and the presence of ROS, accompanied with hyperammonemia, is found. In this study, they have shown that the ROS/ERK1/2 pathway activation leads to an upregulation of P-gp expression at the BBB. As described earlier, P-gp transporters can decrease the deposition of amyloid beta (Zhou et al., 2019). In this way, the BBB could possibly have a function in response to ROS, and reacts in maintaining brain homeostasis by amyloid beta clearance through the upregulation of P-gp transporters in AD.

To conclude, the function of the BBB in AD is to mediate the relation between the immune system and the CNS, and to ensure a stable homeostasis by regulating the influx en efflux of the CNS.

**3. The blood brain barrier alterations in Alzheimer’s Disease**

As described above, BBB integrity is strictly regulated by transporters, cell junctions, basement membranes, and different cells such as endothelial cells, pericytes, astrocytes and microglia. However, in AD, the BBB integrity is different compared to healthy conditions. In the following section, the BBB alterations of cell junctions, basement membranes, cells and transporters will be discussed.

*3.1 Transporters*

The functional activity and expression of ABC BBB transporters, such as LRP-1 and P-gp, are decreased in patients with AD (Zlokovic, 2011). Because LRP-1 and P-gp are responsible for amyloid beta clearance in a healthy situation, this leads to more amyloid beta accumulation in the brain (Cirrito et al., 2005). In addition, Deane et al., reported in 2003 that RAGE is upregulated in AD. These increased levels of RAGE caused upregulated levels of amyloid beta in the brain (Deane et al., 2003). Cai et al. explained that BBB dysfunction leads to changes in the function of different BBB transporters (Cai et al., 2018). Additionally, BBB dysfunction could activate the wrong secretases such as β-secretase and γ-secretase, which causes production of amyloid beta (Atwal et al., 2011 and Zhang et al., 2013).

*3.2 Tight junctions*

BBB dysfunction and damaged TJ’s cause a progression in numerous brain diseases, including AD (Yamazaki and Kanekiyo, 2017; Huber, Egleton and Davis, 2001; Bayonnis, 2015; Bednarczyk and Lukasjuk, 2011; Romanitan et al., 2010). A mechanism proposed in TJ disruption is the RAGE-mediated amyloid beta cytotoxicity, which contributes to the functional loss of brain endothelial cells, and TJ disruption via Ca2+ signaling and matrix metalloproteinase (MMP) signaling (Kook et al., 2012). MMP degrades the ECM and TJ’s between endothelial cells (Weekman and Wilcock, 2016). It has been shown that ApoE4 regulates TJ’s, because APOE4 knock-in-mice had disruption of BBB integrity via the CypA-MMP-9 pathway (Nishitsuji et al., 2011; Bell et al., 2012). Interestingly, apoE3 and apoE2, associated with a lower risk of AD, suppress the CypA-MMP-9 pathway through LRP1 on pericytes, which contributes to the maintenance of BBB integrity. TJ proteins identified as the most relevant in AD progression, are ZO-1, occludin and claudin-5. It has been shown that amyloid beta induced structural alteration and decreased the protein level. The depletion of these tight junctions enhanced the BBB permeability in culture (Kook et al., 2012). Furthermore, Kanoski et al. have shown that in the choroid plexus and the BBB of a rat, the deficiency of claudin-5 and claudin-12 led to an increased BBB permeability (Kanoski et al., 2010). When the BBB is more permeable, amyloid beta can cross the BBB more easily, therefore further progression of AD occurs (Pluta et al., 1996).

*3.3 Basement membrane*

As described in the previous section, the basement membrane is involved in the CypA-MMP-9 pathway, because MMP degrades the basement membrane. In this way, amyloid beta is also detrimental for the basement membrane. This is supported by a study of Donahu et al. that focused on specific components of the basement membrane, such as agrin and laminin. They showed in a post-mortem study of AD brains compared to normal brains, that agrin was thinned and fragmented in AD brains compared to the normal brains. In this study, they also conducted the immunoreactivity of agrin, in which they reported agrin immunoreactivity was concentrated around plaques, which were called ‘puncta’ (Donahue et al., 1999). In a subsequent study for laminin immunoreactivity, the same was found. They suggested that these abnormal agrin and laminin deposits would emerge from damaged basement membranes (Berzin et al., 2000).

*3.4 Endothelial cells*

In AD, there is a loss of endothelial cells (Kumar-Singh et al., 2005; Paul et al., 2007, Biron et al., 2011), and in human post mortem brain studies, brain endothelial degeneration is observed (Bailey et al., 2004; Baloyannis and Baloyannis, 2012; Halliday et al., 2016; Salloway et al., 2002; Sengillo et al., 2013; Wu et al., 2005). Besides, in the post mortem brain studies, reduced capillary length and microvascular degeneration is observed. The loss of brain endothelial cells cause leakage of blood-derived neurotoxic products. These include hemoglobin from red blood cells, generating free iron (Fe2+), which causes ROS, which in turn leads to oxidant stress to neurons. Also, potentially toxic plasma proteins could derive from the BBB, such as fibrinogen, plasminogen, thrombin and autoantibodies, leading to neuronal injury. Furthermore, the leakage of albumin causes edema and this causes ischemia hypoxia, which is also detrimental for neurons (Montagne, Zhao and Zlokovic, 2017).

*3.5 Pericytes*

Pericyte dysfunction is present in many neurological diseases, including in AD (Farkas and Luiten, 2001; Baloyannis and Baloyannis, 2012; Sengillo et al., 2013; Bell et al., 2010; Zlokovic, 2011; Bell et al., 2012; Sagare et al., 2013; Winkler et al., 2014; Montagne et al., 2015). The loss of pericytes in the cortex and hippocampus correlates with the severity of BBB dysfunction (Sengillo et al., 2013). Pericyte degeneration, accompanied with smooth muscle cell degeneration, causes amyloid beta deposition in cerebral vessels (Verbeek et al., 2000). Veszelka et al. have demonstrated that treatment of amyloid beta oligomers at hippocampal slices increases ROS production, which promotes pericyte loss. (Veszelka et al., 2013). Sagare et al. investigated whether pericyte loss can influence the natural course of AD progression. They crossed a transgenic mouse overexpressing the Swedish mutation of the human APP, with a pericyte-deficient, platelet-derived growth factor receptor beta mouse. The pericyte deficiency in these mice caused a faster deposition of amyloid beta plaques compared to the normal amyloid precursor protein mice. Furthermore, these mice would only develop amyloid plaques, but no tangles. Remarkably, these mice did exhibit a hyperphosphorylated tau deposition, resulting in tau tangles and neuronal loss during the early stages of the disease. This would suggest that pericyte deficiency could account for the development of tau pathophysiology (Sagare et al., 2013).

*3.6* *Astrocytes*

According to AD brain cortex biopsies, specific changes occur in astrocytes located close to amyloid beta plaques(Wisniewski et al., 1989). These specific changes could indicate that reactive astrocytes are present. Reactive astrocytes contain more neurotoxic properties and less neurotrophic properties than normal astrocytes. Postmortem studies have revealed that the number of reactive astrocytes increases as the disease progresses (Perez-Nievas and Serrano-Pozo, 2018). Furthermore, in transgenic mice characterized with strong CAA pathology, astrocytes surrounded with amyloid beta showed morphological changes, such as retraction and swelling, accompanied with the downregulation of GLUT1 and lactate transporters. These changes occur during an early stage of the disease progression. Neurovascular uncoupling is consistent with these morphological changes. These changes suggest that the dysfunction of astrocytes play a critical role in the development of early behavioral and cognitive impairments in AD (Merlini et al., 2011).

*3.7 Microglia*

Microglia initially play a protective role in maintaining BBB properties, by facilitating amyloid beta clearance in AD patients, but as the disease progresses, microglia can be detrimental. This will eventually lead to neuroinflammation and the accumulation of amyloid beta, since reactive microglia will secrete more pro-inflammatory cytokines, and will reduce their phagocytosis (El Khoury et al., 2007; Krabbe et al., 2013; Hickman et al., 2008; Heneka et al., 2015). As earlier described, reactive microglia secrete TNF-α and IL-1β, and activate NADPH-oxidase. NADPH-oxidase produces ROS (da Fonseca et al., 2014). ROS causes neurotoxicity and changes in the BBB function, such as reorganization of TJ’s and decreased TEER (Block, 2008, Sumi et al., 2010). Neuroinflammation and oxidative stress in the BBB will trigger β-secretases and γ-secretases, which causes amyloid beta aggregation, and decreases amyloid beta clearance (Tamagno et al., 2008; Cai, Hussain and Yan, 2014). Besides, IL-1β increases BBB permeability and suppresses the astrocytes to interfere with BBB integrity (Wang et al., 2014). Furthermore, studies on AD transgenic mice and AD patients revealed that cytokines such as IL-1β promote the migration of immune cells from the blood into the brain, providing a link between microglial activation and immune cell trafficking (Allen et al., 2012; Zenaro et al., 2015).

*3.8 Amyloid beta deposition*

In AD, there is a higher amount of amyloid beta deposition. Amyloid beta deposition in the brain could be detrimental for the different components of the BBB, and may lead to BBB breakdown. The TJ’s integrity has changed because of RAGE-mediated amyloid beta cytotoxity, which will induce a pathway were MMP and Ca2+ are involved (Kook et al., 2012). MMP degrades the basement membrane and TJ’s (Weekman and Wilcock, 2016). Furthermore, Veszelka et al. have demonstrated that amyloid beta oligomers increased the ROS production, which promotes pericyte loss (Veszelka et al., 2013). In another study from Merlini et al., it has been shown that astrocytes surrounded by amyloid beta show morphological changes that can lead to dysfunction of astrocytes (Merlini et al., 2011). Furthermore, amyloid beta causes neuroinflammation, which could lead to activated microglia and increased ROS production. Activated microglia will secrete proinflammatory cytokines and this, together with the elevated ROS production, will damage the BBB. However, as earlier described, under certain conditions, amyloid beta could instead have a positive effect on ROS production, because amyloid beta prevents redox metal ions from participating in redox reactions. Therefore, amyloid beta could be a compensatory response to prevent further increase of ROS production and oxidative stress. Besides, tau hyperphosphorylation seems to have a link in BBB dysfunction. It is suggested that pericyte deficiency could account for the development of tau pathophysiology (Sagare et al., 2013).

 In conclusion, all of the BBB components, such as tight junctions, adherence junctions, astrocytes, pericytes, endothelial cells, microglia and neurons become affected during AD. Amyloid beta plaques can cause neuroinflammation, the secretion of proinflammatory cytokines, and increased ROS production, which will all cause damage to the BBB. However, amyloid beta plaques could possibly prevent in a compensatory response the further increase of ROS production as well.

**4. Bypassing the blood brain barrier in the treatment of Alzheimer’s Disease**

The BBB prevents passage of a lot of drugs. Substances can cross the BBB mediated through paracellular transport, receptor mediated transcytosis, adsorptive-mediated transcytosis, and other transport mechanisms (Saeedi et al., 2019). Drug delivery can be done in three different ways. The first option is to inject the drug via intracerebral or intracerebroventricular injection, which is an invasive and very painful approach. This is usually the last option, and happens only when a patient is hospitalized. The second option is systemic administration via oral or intravenous ways. The drug needs to be transported across the BBB, unfortunately, this limits bioavailability, drug concentration, effectiveness of the drug, and increases the systemic side effects. The third option for drug delivery is intranasal administration, which is often used, because the substances do not have to pass the BBB. The drugs will be directly delivered to the brain via the olfactory region. This last option is very popular in the treatment of AD (Agrawal et al., 2017).

However, during AD, the permeability of the BBB is increased, indicating that more drugs can cross the BBB. This could be used in the treatment of AD (Dong, 2018). Nevertheless, the cerebrospinal fluid production rate (Silverberg et al., 2001), cerebral blood flow, and certain transporters, such as P-gp are involved in drug delivery as well (Banks, 2012). The cerebrospinal fluid production and the cerebral blood flow are reduced in AD (Roher et al., 2012; Silverberg et al., 2001). Banks et al. suggest that the cerebrospinal fluid reabsorption, the cerebral blood flow, and the P-gp activity influence drug delivery in drug-specific ways. Likewise, the cerebral blood flow does not always has the same effect on any particular drug (Banks, 2012). Drugs that enter the CNS rapidly, are flow-dependent; while drugs with lower rates of entry will not be influenced by a decrease in cerebral blood flow. For example, donepezil, a drug used to treat AD (Cacabelosm 2007), is likely flow-dependent, whereas antisense oligonucleotides (ASOs), also used in AD treatment (Wurster and Ludolph, 2018), are not flow-dependent (Banks, 2012). Overall, BBB alterations could cause shifts in the therapeutic window, side-effect profiles, efficacy, potency, and dosage of commonly used drugs (Banks, 2012). Furthermore, detailed knowledge about BBB permeability such as duration and size of BBB openings are currently not known (Dong et al., 2018). This detailed knowledge could help us to use the permeable BBB for drug delivery in a drug-specific way (Dong et al., 2018).

A new way of drug delivery is the development of nanoparticles with nanomaterials. A nanoparticle is any type of particle with the size between 1 and 100 nanometers. A nanomaterial is a material that, in one direction, has the length in the order of nanometers (Buzea, Pacheco and Robbie, 2007). When a nanomaterial is used to transport a nanoparticle, it is called a nanocarrier (Qian et al., 2012). Nanomaterials enhance drug property and pharmacokinetic behavior, because their translocation across the BBB is assisted by the nanocarrier (Agrawal et al., 2017). Nanomaterials are a solution to restricted drug delivery, and very important in future research (Saeedi et al., 2019). Different nanomaterials exist, such as: liposomes, polymeric micelles, dendrimers, nanogels, and carbon nanotubes (Agrawal et al., 2017; Saeedi et al., 2019). In the next section, each of these nanomaterials will be defined.

*4.1 Liposomes*

Liposomes consist of one or more lipid bilayers surrounding an aqueous compartment, and were discovered in the mid-1960s (Bangham, Standish and Watkins, 1965). Soon after this discovery, it has been recognized as a therapeutically active compound. Liposomes are able to incorporate lipophilic, hydrophilic, and hydrophobic therapeutic agents, because of their unique characteristics (Vieira and Gamarra, 2016). Depending on the targeting agents added to the liposomes, they penetrate the BBB by means of different transport mechanisms (Figure 2). The use of cationic liposomes will result in penetration by adsorptive-mediated transcytosis, due to the electrostatic interaction between the positive charge on liposomes, and the negatively charged membrane of BBB endothelial cells. Furthermore, the application of polyethylene glycol (PEG) on liposomes enables the additions of aptamers consisting of RNA nucleotides or antibodies to the PEG chains on liposomes. Specific receptors located at the BBB can bind the aptamers of antibodies on the liposome surface, resulting in receptor mediated transcytosis. Among the different nanomaterials liposomes have an advantage, since they can be easily modified at its surface. In this way, they can effectively target a specific site of interest and are a promising tool in drug delivery (Vieira and Gammara, 2016).

*4.2 Polymeric micelles*

In recent years, polymeric micelles have been developed for drug delivery (Zhang et al., 2014; Kedar et al., 2010). Micelles form spontaneously in amphiphilic copolymer solutions, where the concentration of amphiphilic copolymers is higher than the critical micelle concentration (CMC) (Zhang et al., 2014). An amphiphilic copolymer consists of a hydrophobic block and a hydrophilic block. The hydrophilic block will point towards water, and the hydrophobic block will point towards the other hydrophobic block. Together this forms a shell-core structure, with the hydrophobic blocks in the core and the hydrophilic blocks in the shell (Yokoyama et al., 1990). The particle size of polymeric micelles are approximately between 10 nm – 100 nm in size (Zhang et al., 2014). The core is able to incorporate water-insoluble drugs (Ahmad et al., 2014). The micelle keeps the drug from interacting with non-target cells, and crosses the BBB via passive diffusion (Mathot et al., 2007). After reaching the target cell, the drug is released through a diffusion mechanism (Ahmad et al., 2014). In conclusion, polymeric micelles are a promising drug delivery system to enhance bioavailability of water-insoluble drugs.

*4.3 Dendrimers*

Dendrimers, first discovered in 1978 by Buhleier et al., (Buhleier et al., 1978) are highly branched nanostructured polymers, well known for their applications in drug delivery. The name dendrimer comes from the branched tree-structure: the Greek word ‘dendron’ means ‘tree’ or ‘branch,’ and ‘meros’ means ‘part’ (Noriega-Luna et al., 2014). All dendrimers are taken up by fluid-phase endocytosis, but significant differences in uptake mechanisms exist. In A549 lung epithelial cells, anionic dendrimers are taken up by caveolae mediated endocytosis. Interestingly, cationic and neutral dendrimers seem to be taken up by a non-clathrin, non-caveolae mediated mechanisms that could be mediated by electrostatic interactions, or other non-specific fluid-phase endocytosis (Perumal et al., 2008). They consist of a hydrophobic core, several internal layers, and a high ratio of surface groups (Palmerston Mendes, Pan and Torchilin, 2017). The presence of these surface groups and the hydrophobic core makes it possible to load a high number of drugs and has made dendrimers a promising vector for drug delivery (Menjoge et al., 2010; Imae, 2012; Pardridge, 2005), including in AD (Klanjnert et al., 2006). Unfortunately, their use in biological systems is restricted because of the correlated toxicity issues (Madaan et al., 2014).

*4.4 Nanogels*

Nanogels are nano-sized drug delivery systems composed of cross-linked hydrophilic polymeric networks that form ionic or non-ionic bonds (Sultana et al., 2013). Nanogels can deliver oligonucleotides into the brain via the transcellular pathway. When the surface of nanogels is modified with transferrin or insulin, the transport efficacy is further increased (Vinogradov et al., 2004). Recent research has shown that nanogels can be delivered into the brain via intranasal administration. This is a by-pass mechanism of the BBB, because the compound doesn’t go from the blood to the brain. Instead, it has three options to depart from the nasal cavity: via the olfactory route to the cerebrospinal fluid into the brain, or directly from the olfactory route into the brain, or via the trigeminal route into the brain. In the trigeminal route, the trigeminal nerve enters the brain in rostral and caudal regions of the brain (Aderibigbe and Naki, 2018). Nanogels are used in brain diseases such as AD (Aderibigbe and Naki, 2018) and are also very useful in other diagnostics and therapeutics (Paul et al., 2017). They have a higher loading capacity than other nanomaterials (Kabanov and Gendelman, 2007). To conclude, nanogels are a promising tool in drug delivery and can use different mechanisms to by-pass the BBB. However, nanogels show limitations regarding their degradation mechanism and the optimization of biodistribution (Neamtu et al., 2017).

*4.5 Carbon nanotubes*

Carbon nanotubes (CNT) are discovered in 1991 by Sumio Lijima (Lijima, 1991). CNTs are cylinders of graphene sheets which are single-walled (SWNT) or multiwalled (MWNT) (Cellot et al., 2010). Graphene is an allotrope of carbon arranged in a hexagonal lattice. The cylinders are approximately 12 nm in diameter and when they are closed, they are closed with pentagonal rings (Singh et al., 2012). CNTs can cross the BBB by receptor mediated transcytosis, adsorptive-mediated transcytosis and passive mechanisms, depending on their surface characteristics (Costa et al., 2016). For example, MWNTs with NH3+ on its surface improves the cationic nature of nanotubes, which stimulates the uptake viaadsorptive-mediated endocytosis (Georgieva et al., 2011). CNTs are hollow and much smaller than blood cells, therefore they have a great potential to carry drugs (Singh et al., 2012). Furthermore, they have special electrical, magnetic, chemical and mechanical properties (Modi et al., 2010; Lee and Parpura, 2009; Komane et al., 2016; Singh et al., 2012). Besides, Cellot et al. have shown that the formation of nanotube-neural hybrid networks can possibly support the network communication, neuronal activity and synaptic formation of neurons (Cellot et al., 2009; DeAngelis, 2001). In conclusion, CNTs have interesting characteristics to be a future drug delivery system in the treatment of AD.

**5. Conclusion and discussion**

In conclusion, the BBB’s functioning is related to AD pathogenesis in different ways. All of the BBB components belonging to the NVU, such as tight junctions, adherence junctions, astrocytes, pericytes and endothelial cells are affected during AD. Because of impaired signaling between NVU components, neurovascular uncoupling occurs. Microglia and neurons become affected as well. Activated microglia will secrete pro-inflammatory cytokines and increase the neuroinflammation and ROS production. Secondly, BBB transporters become affected during AD. BBB dysfunction decreases the expression of transporters responsible for amyloid beta clearance, such as LRP-1 and P-gp, and increases the expression of RAGE transporters which are responsible for amyloid beta transport from the blood into the brain. Furthermore, BBB permeability is increased during AD because of dysfunction of tight junctions. Due to the change in activation of BBB transporters, and the increased permeability of the BBB, the amyloid beta deposition is enhanced during AD. However, some other studies showed that under certain conditions amyloid beta may instead have positive and even neuroprotective effects inside the brain.

Unfortunately, less is known about the relation of BBB dysfunction and tau hyperphosphorylation. It is believed that amyloid beta plaques outside the neuron initiate a response inside the neuron, in which an enzyme phosphorylates microtubules and subsequently phosphorylates the tau protein. In this way, BBB dysfunction could be indirectly related to tau hyperphosphorylation with amyloid beta as a key factor. Furthermore, it is shown that pericyte deficiency, accompanied with amyloid beta deposition, could account for the development of tau pathophysiology in pericyte deficient mice (Sagare et al., 2013).

On the other hand, some research results did not support the hypothesis that increased BBB permeability, as an occurrence in BBB dysfunction, is related to AD pathogenesis. Janelidze et al. have found that increased BBB permeability is associated with dementia and diabetes, instead of amyloid beta pathology or the APOE genotype (Janelidze et al., 2017). This points out that the relation between BBB dysfunction and AD pathogenesis is still not fully understood.

However, recent studies show that regulating BBB function is an effective measure to prevent and treat AD animals (Cai et al., 2018). Therefore, regulating BBB function could be a promising therapeutic target in the treatment of AD. Furthermore, growing efforts are made in the field of nanotechnology to develop medicine that can bypass the BBB. These are important efforts, because the BBB normally limits bioavailability, drug concentration, and effectiveness of the drug, and the BBB increases the systemic side effects. Promising applications in the treatment of AD are liposomes, polymeric micelles, dendrimers and carbon nanotubes, which are nanomaterials. Because of their unique structures these nanomaterials can make use of different transport mechanisms across the BBB and they can be targeted to a specific site of interest. These properties have made them a promising tool in delivering medicine across the BBB in the treatment of AD. However, use of nanomaterials is restricted in biological systems because of toxicity issues and other limitations.

In conclusion, despite growing evidence supporting the relation between BBB dysfunction and AD pathogenesis and development, it is still difficult to develop proper diagnostic and therapeutic strategies targeting the right disease aspects in AD and BBB dysfunction. A greater understanding towards the role of the BBB in the development of AD pathogenesis could lead to better diagnostic and therapeutic strategies. Therefore, further research should focus on the multiple aspects of BBB disruption and the related complexities in AD development.

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