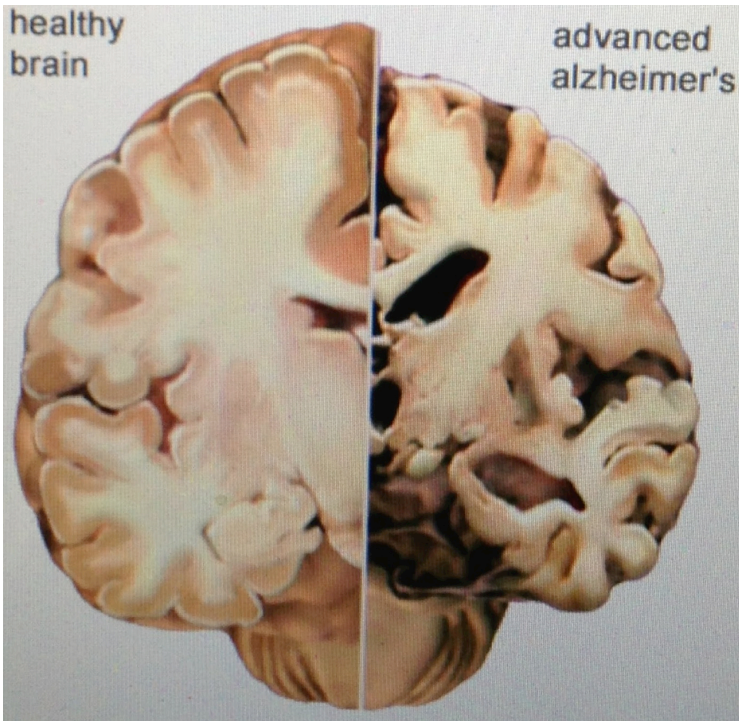
Biomolecular analysis of the blood-brain barrier in Alzheimer's disease



Healthy brain compared to Alzheimer's brain (picture taken from: www.alz.org).

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Master Thesis
Molecular and Cellular Life Sciences (MCLS)

July 2013

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Summary

The blood-brain barrier functions as a barrier between the brain and the circulating blood. It protects the brain from influences like blood composition and changes in blood pressure. It is also a protective barrier for the brain against pathogens like bacteria and viruses. The blood-brain barrier composition contains tight junctions and adherens junctions. These junctions form an impermeable barrier for most molecules, except small and lipophilic molecules. Large and hydrophilic molecules can still cross the blood-brain barrier when needed (for example nutrition of the brain) by means of active transport. Knowledge of the blood-brain barrier is of high essence, since its pathogenesis is involved in neurodegenerative diseases. This review will focus on the effects of Alzheimer's disease on the blood-brain barrier. Alzheimer's disease is one of the neurodegenerative diseases caused by neurotoxic amyloid proteins deposited in the brain, caused by a faulty amyloid beta clearance by the blood-brain barrier. Alzheimer's disease destroys this blood-brain barrier and neurotoxic amyloid beta fibrils or plaques gradually disable the brain functions. The neuronal functions of the brain are so severely diminished by pathogenesis, that they are completely lost in the end phase of the disease. This review explores the biomolecular processes behind Alzheimer's disease's destructive powers on the blood-brain barrier. The mechanism of attack by the disease and its effect on the blood-brain barrier are of major interest not only for Alzheimer's disease, but for other neurodegenerative diseases like Parkinson's disease and multiple sclerosis as well.

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1: Introduction

1.1: Properties of the blood-brain barrier: an overview

The blood brain barrier is part of a system of three barriers between the brain and circulating blood: the blood-brain barrier (proper), the blood CSF-barrier (BCSFB) and the arachnoid barrier (Abbott *et all.* 2009). The discovery of the blood-brain barrier predates from the 1880s from work of Paul Ehrlich, as discussed in a historical perspective on the blood-brain barrier (Engelhardt). The blood-brain barrier separates the central nervous system (CNS) from the circulating blood. The brain extracellular fluid (BECF) and cerebrospinal fluid (CSF) are separated from the circulating blood by this blood-brain barrier to create a neural microenvironment. The blood-brain barrier protects the central nervous system from changes of the blood composition and potential risks like pathogens. The blood-brain barrier is located in the endothelium and the main structure is composed of tight junctions (TJs). The structures that are part of the blood-brain barrier are: endothelial cells, astrocytes and pericytes (PCs). Pericytes surround the endothelium cells (Ballabh *et all.*). Tight junctions are multifunctional since they have distinct functions like barrier function, signaling function and fence (to establish an apical and a basolateral domain) function (Tsukita *et all.*). Tight junction molecules involved in the blood-brain barrier are: occludin, claudins (Cl-3, -5 and 12) and Ig-superfamily members JAM-A and ESAM. Adaptor proteins involved in the blood-brain barrier are: ZO-1, -2 and -3 and cingulin. Adherens junctions enable the primary contact between the endothelial cells (Wolburg and Lippoldt). Also involved in the blood-brain barrier is the regulatory protein Itch (Wolburg *et all*.). Rates of fluid phase endocytosis of the blood-brain barrier are low (Gloor *et al*.). The blood-brain barrier forms a blockade for transport from blood to brain for almost all molecules, except small and lipophilic molecules. However, small and large hydrophilic molecules can enter the brain by means of active transport. Transport of nutrients (like glucose, amino acids and related molecules such as L-DOPA) occurs through this active transport. Some macromolecules are receptor mediated as well. An example of a receptor involved in macromolecular transport to the brain is the transferrin receptor. The transporter P-glycoprotein has an opposed mechanism as it enables the transport of lipophilic molecules from the brain back into the blood (Rubin and Staddon).

1.2: Coupling of the capillary endothelial cells (ECs)

One of the distinct properties of the blood-brain barrier is the coupling of the brain capillary endothelial cells (ECs) by adherens and tight junctions (*Zonula occludens*). This coupling gives the blood-brain barrier a very distinct impermeable structure. Different other molecular components are involved in both structure and cellular interaction of the blood-brain barrier as well. These components will be discussed in more detail in the molecular components of the tight junctions section.

1.3: Development of the blood-brain barrier

The development of the blood-brain barrier starts during embryogenesis. The development of the blood-brain barrier does not take place at once, but different phases of development occur during embryogenesis. Two principal stages are involved in the development of the blood-brain barrier. The first stage is called the developmental basis and sets the foundations for the developing blood-brain barrier. The full mechanism of the developmental basis of the blood-brain barrier is still not fully understood. This is then followed by the differentiation, the phase in which the blood-brain barrier will achieve its final form. After differentiation the mature blood-brain barrier is completed (Engelhardt).

1.4: Regulation of the blood-brain barrier

Astrocytes are involved in the regulation of the blood-brain barrier (Abbott *et all.* 2006). Additionally, pericytes are involved in the regulation of the blood-brain barrier as well (Armulik *et all*.). Pericytes increase the expression of Cl-5 and strengthens the barrier function of the blood-brain barrier (Shimizu *et all.*). In short: pericytes increase the tightness of the blood-brain barrier and therefore the impermeability of the blood-brain barrier increases.

1.5: Highlighting the molecular components of tight junctions (TJs)

1.5.1: Occludin

Occludin (65kDa and consisting of 522 residues in humans) is the first molecular component discovered in the blood-brain barrier. Occludin contains four transmembrane domains. There is only one isoform known with different splice variants. It is involved in the modulation of the blood-brain barrier through intracellular signaling. Occludin is not required for the formation of tight junctions (Angelow *et all*.).

1.5.2: Claudins

The tight junction components involved in creating barrier properties are the claudins (20–27kDa). Claudere is Latin for "to close" and claudins were purified and identified first by Mikio Furose *et all*. Like occludin claudins contain four transmembrane domains, but there is no sequence homology between claudins and occludin. They have a short intracellular N-terminus of approximately 7 residues, a long first extracellular loop of 52 residues and a second extracellular loop varying in length from 16-33 residues. The cytoplasmic C-terminus shows big differences in length between the isoforms and varies from 21-63 residues. Claudins are essential for the formation of tight junctions. At least 24 different claudin isoforms are known, but only 3 of them (Cl-3, -5 and -12) are reported of being involved in the blood-brain barrier (Angelow *et all*.). Claudins are members of the PMP-22/EMP/MP20/Claudin superfamily. All members of this family have a large first extracellular loop with a conserved motif (W-LW-C-C). Also, members of this group are characterized by both short cytoplasmic loop and N-terminus (Suzuki *et all.*). Claudins also influence charge-selective small pores (radius 4Å) of tight junctions. Claudins are believed to be involved in other contexts than tight junctions as well, like the involvement in various types of cancer (Singh *et all.*).

1.5.3: Ig-superfamily members

Both JAM-A and endothelial cell-selective adhesion molecule (ESAM) are present in endothelial cells and are part of the blood-brain barrier. JAM-A is a member of the junctional adhesion molecules (JAM). They are both members of the immunogloblin superfamily. JAM-A and ESAM contain only one transmembrane, in contrast to the four transmembrane domains of both occludin and the claudins. For both JAM-A and ESAM 3 isoforms are known (Angelow *et all*.).

1.5.4: Adaptor proteins

The ZO-1, -2 and -3 and cingulin adaptor proteins are involved in the cellular interactions of the molecular components of the tight junctions. ZO-1 is a first order (direct) adaptor protein for occludin, claudins Cl-3, -5 and -12 and JAM-A. It also functions as a second order adaptor protein for ESAM (first order adaptor protein unknown). ZO-2 and -3 is a first order adaptor protein for both occludin and claudins Cl-3, -5 and -12. Cingulin functions as a second order adaptor protein for occludin and claudins Cl-3, -5 and -12. Itch is a regulatory protein of occludin (Wolburg *et all*.).

1.6: The pathogenesis of the blood-brain barrier

1.6.1: Disrupting the blood-brain barrier

Alterations in the tight junctions of the blood-brain barrier disrupt the blood-brain barrier and cause dysfunction of the blood-brain barrier. In case of deficiency of claudins Cl-3, -5 and -12 (especially Cl-5) the structure of the tight junctions and therefore the composition of the blood-brain barrier changes dramatically.

1.6.2: Blood-brain barrier leakage

Disruption of the blood-brain barrier results in blood-brain barrier leakage. Leakage hampers the protective mechanism of the blood-brain barrier and results in molecules entering the brain that should be shielded by the blood-brain barrier. In case of pathogens the consequences are severe, since they are not properly retained by the blood-brain barrier resulting in a high risk of brain contagion.

1.6.3: Neurodegenerative diseases caused by blood-brain barrier pathogenesis

In several neurodegenerative diseases the blood-brain barrier is severely affected. Alzheimer's disease (AD), Parkinson's disease (PD), Multiple Sclerosis (MS) and AIDS Dementia Complex (ADC) are all neurodegenerative diseases involving breakdown of the blood-brain barrier (Zlokovic). This review is focused on the biomolecular processes involved in Alzheimer' disease, but in the discussion there will be a brief overview of developments in Alzheimer's disease research that can be used for research in the other neurodegenerative diseases as well.

2: Biomolecular processes involving Alzheimer's disease

2.1: Amyloid proteins

Properties of amyloids – what are they? Amyloids are misfolded proteins that form aggregates. The amyloid protein involved in Alzheimer's disease and other neurodegenerative diseases is Amyloid beta (Aβ). Amyloid beta is related to neurodegenerative diseases due to its aggregation into oligomers to form neurotoxic amyloid fibrils. Amyloid beta fibrils are insoluble and can be distinguished from other cellular components under a polarized light microscope and the use of the dye Congo red. Amyloid beta fibrils are resistant to proteolysis, they alter or disrupt the structure and function of tissues and organs and they form neuritic plaques (Prasansuklab and Tencomnao). Gendoo and Harrison studied the principle of amyloid proteins extensively. They were the first to conduct a list of alpha helices and chameleon sequences with predictive powers about their amyloidogenicity. The structure of the amyloid beta peptide (residues 1-42) was solved with the use of solution-state nuclear magnetic resonance (NMR) by Crescenzi *et all.* They state that there are beta amyloid forms ranging from 39 to 43 residues in length, but this 42 residues peptide is the major form present in plaques. Amyloid beta peptides are cleavage products of a protein called amyloid precursor protein (APP). This glycoprotein is a common precursor and its length varies from 695-770 residues. This APP gene is located on chromosome 21 (Crescenzi *et all.*). People with chromosome 21 trisomy or Down Syndrome (DS) have a very high risk of developing Alzheimer's disease at a young age (clinical symptoms around 40), since almost everyone who is affected carries an extra copy of the APP gene (Prasansuklab and Tencomnao). Proteolytic cleavage takes place between residues 40 and 41 or 42 and 43. This cleavage is done by the proteases alpha, beta and gamma secretase (Crescenzi *et all.*). The sequential cleavage of beta and gamma secretase results in neurotoxic amyloid beta, whereas no beta amyloid is formed in the sequential cleavage of alpha and gamma secretase. The sequence of this peptide was deposited on the Protein Data Bank (or PDB) in 2003 by Crescenzi *et all.* (PDB entry: 1IYT) is DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA. In figure 1 the secondary structure of this peptide is shown containing two alpha helical regions connected by a beta turn. In table 1 this secondary structure is confirmed using the WHAT IF Web Interface* and using the following options: first "protein analysis" followed by "secondary structure, symmetry and accessibility".

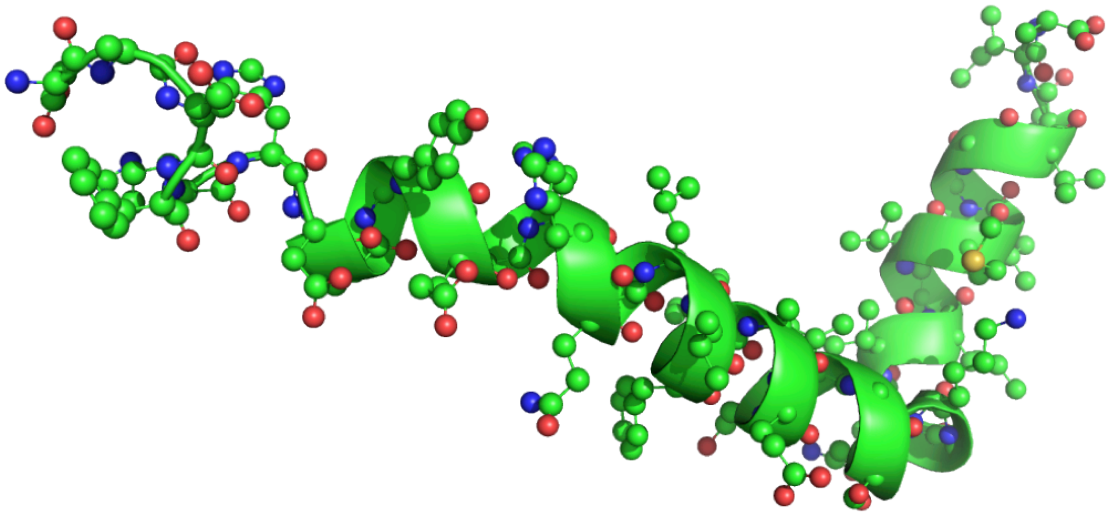

Figure 1. The secondary structure of the amyloid beta peptide (residues 1-42) in alpha helical conformation is shown as a cartoon. Two alpha helical regions are connected by a beta turn. In this conformation the amyloid beta peptide does not aggregate. Protein Data Bank (PDB) entry: 1IYT deposited by Crescenzi *et all*.

Secondary structure of amyloid beta residues 1-42
DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA
TTTTTHHHHHHHHHHHHHHHHHHTTHHHHHHHHHHHHT

Table 1. The secondary structure of the alpha helical amyloid beta peptide residues 1-42 by the WHAT IF Web Interface: http://swift.cmbi.ru.nl/servers/html/index.html. Legend: T=turn and H=alpha helix.

2.2: Role of amyloid beta in Alzheimer's disease

Cerebral amyloid angiopathy is one of the characteristics of Alzheimer's disease. In cerebral amyloid angiopathy there is Amyloid beta (Aβ) deposition in the brain. It destroys the cellular interactions of neurons in the brain by means of reduced blood flow and neurotoxicity. Neurotoxic amyloid beta accumulates in the brain. Both reduced blood flow and amyloid beta accumulation initiate neuronal injury (Zlokovic). Furthermore, Amyloid beta forms complexes with both acetylcholinesterase (AChE-Aβ) and the Receptor for Advanced Glycation end Products (RAGE-Aβ). Both increased AChE and RAGE expression are associated with neurotoxicity and Alzheimer's disease. Also, AChE-Aβ complexes are more toxic than Amyloid beta alone (Prasansuklab and Tencomnao). In figure 2 an example is shown of a possible secondary conformation of the amyloid beta peptide residues 1-42. The whole structure is shown as antiparallel beta strand for simplicity, whereas in reality it is also likely that other (intermediate) secondary conformations are present like beta turn, bend or a beta bridge.

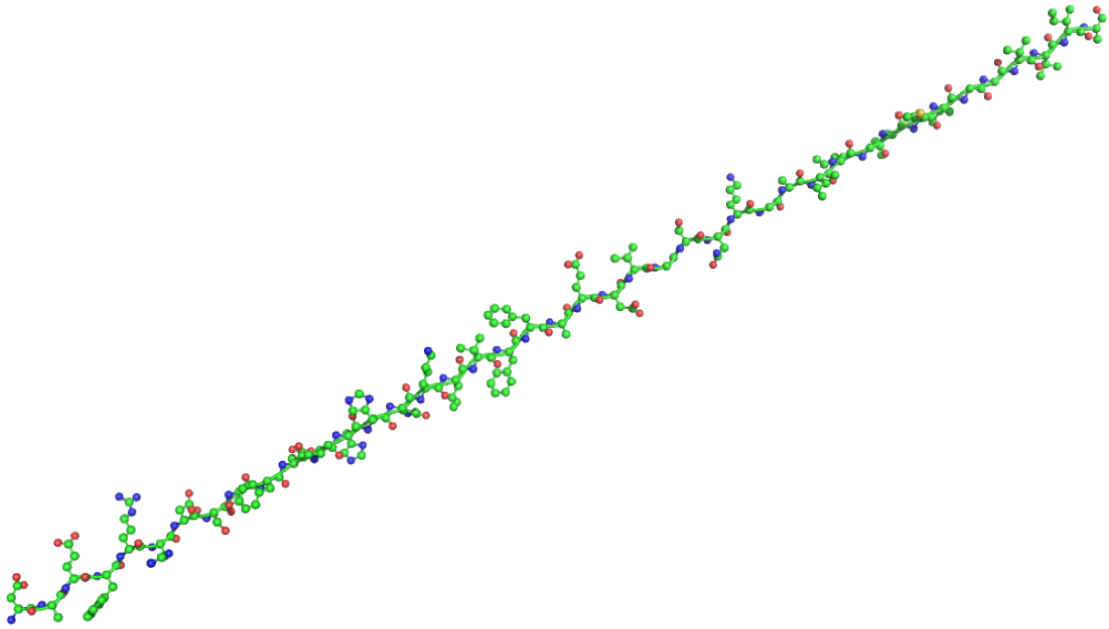

Figure 2. The amyloid beta peptide residues 1-42 in antiparallel beta strand secondary conformation. Beta sheets accumulate and form aggregates of amyloid beta in Alzheimer's disease and other neurodegenerative diseases. These plaques are neurotoxic and hamper neuronal function of the brain.

2.3: Loss of blood-brain barrier integrity caused by Alzheimer's disease

The loss of integrity of the blood-brain barrier is due to alterations in expression of its molecular components. In Alzheimer's disease the aggregation of amyloid proteins destroys the blood-brain barrier integrity and blood-brain barrier leakage. The loss of blood-brain barrier integrity dramatically influences the functioning of the blood-brain barrier. The blood-brain barrier becomes leaky and the aggregation of more amyloid protein material and further loss of blood-brain integrity becomes a vicious circle. The neuroinflammatory response mediated by activated endothelium secreting protoinflammatory cytokines and cerebral blood flow (CBF) suppressors causes further neuronal dysfunction. Activated astrocytes and microglia due to the neuroinflammatory reaction further disable the neurovascular pathway. In the end stage of the disease the capillary unit is completely buried under the amyloid deposition and the synaptic and neuronal functions are completely lost (Zlokovic). For clarity: this neurovascular unit that is described of being attacked by Alzheimer's disease contains neurons, the extracellular matrix, astrocytes, pericytes and the endothelium (Hawkins and Davis). Although the mechanism for leukocyte recruitment in neuroinflammation is subject of debate and is supposed to involve the induction of ectoenzymes like matrix metalloproteinases (MMPs) and others (Bechmann *et all.*), it is clear that neuroinflammation is a mediator for the destruction of the blood-brain barrier. Another cause of blood-brain barrier leakage is the thinning of the vascular basement membrane (VBM). Subsequently, this vascular basement membrane becomes discontinued. This results in the leakage of the plasma protein prothrombin. This leakage of prothrombin is associated with shrinkage of endothelial cells (Zipser *et all.*).

2.4: Health versus disease: molecular differences in the blood-brain barrier

The blood-brain barrier in Alzheimer's disease shows changes in key vascular genes. Also the receptors in brain capillaries and small cerebral arteries undergo slight changes in expression. The imbalance caused by these changes lowers cerebral blood flow and impair blood-brain barrier functions. Both the accumulation of amyloid beta and the triggered neuroinflammatory response result in the breakdown of the blood-brain barrier. Due to faulty clearance of amyloid beta, amyloid beta is deposited in the brain (Zlokovic). The neuroinflammatory reaction is involved in alterations in the expression of the tight junctions and Capillary Amyloid Angiopathy (CAA) in Alzheimer's disease (Carrano *et all.* 2012). The expression profile of claudin differs clearly from the normal aging brain due to all these alterations (Spulber *et all*). There is a severe loss of claudin-5 due to Capillary Amyloid Angiopathy. Not only claudin-5 is affected, there are also dramatic losses of ZO-1 and occludin (Carrano *et all.* 2011). Especially the loss of claudin is very problematic, since it is the most important factor in blood-brain barrier function maintenance (Shimizu *et all.*). Nevertheless, an extensive study using human samples of older people (>65 years) representing brain aging and Alzheimer's disease pathology did not show tight junction proteins to be down-regulated. Though, blood-brain barrier dysfunction was believed to be contributing to brain ageing and Alzheimer's disease (Viggars *et all.*).

2.5: Therapeutic approaches for Alzheimer's disease

In the current development and understanding in the therapeutics for the cure of Alzheimer's disease there are ways to use drugs to enter the brain and find a way for clearance of the amyloid protein material. Pathological changes in Alzheimer's disease can be measured by magnetic resonance imaging. This method can be used to determine the stage of the disease (Klohs *et all.*). Transporters of the blood-brain barrier can be used to distribute the drug in the brain, but still a lot needs to be investigated in understanding these transporters before drugs can be applied. The faulty clearance of amyloid protein material of the blood-brain barrier and the neuroinflammatory response of the central nervous system damaging the brain need to be explored further. One way to gain clearance of amyloid beta in the brain is cannabinoid treatment. This method has shown improvement of amyloid deposition in mice, increasing amyloid beta transport across the blood-brain barrier out of the brain (Bachmeier *et all.*). The use of peroxisome proliferator-activated receptor (PPAR) agonists have shown promising results as an Alzheimer's disease therapy as well, but still need to be further developed since they still fail in real patients (Zolezzi and Inestrosa). Other targets for Alzheimer's disease may be directed towards Low-Density Lipoprotein Receptor-Related Protein-1 (LPR1), also known as apolipoprotein (apoE) receptor. Capillary LPR1 is associated with Amyloid beta clearance, whereas its neuronal expression is implicated in amyloid beta accumulation. Alternatively, autophagy is necessary for the clearance of Amyloid beta as well. The mammalian target of rapamycin (mTOR) pathway plays an important role in the regulation of autophagy. Impaired autophagy can lead to the accumulation of Amyloid beta in the brain. Ser/Thr mTOR or other mTOR components can therefore be used as therapeutic targets (Prasansuklab and Tencomnao).

3: Discussion and conclusion

3.1: Prospects for a cure to Alzheimer's disease

Current research in Alzheimer's disease is still necessary to understand the biomolecular processes behind Alzheimer's disease. Even better understanding of the mechanisms behind Alzheimer's disease than there is now will give perspective of improving the strategy to find a cure for this neurodegenerative disease.

3.2: Slowing down Alzheimer's disease progression

There is no cure (yet), but are there means to slow down the progression of the disease? This is still difficult, but there are indications that there are possible targets to slow down the progression of the disease, although they are still in a preclinical phase. One way to slow down Alzheimer's disease is to improve the cerebral blood flow. Poor cerebral blood flow is highly associated with Alzheimer's disease, since it initiates stress responses responsible for the accumulation of Amyloid beta and a faulty Amyloid beta clearance by the brain. Ways to improve blood flow are exercise (both physical and intellectual) and a healthy diet. Some dietary supplements can be included as well. Also, herbal therapies are believed to be useful in several studies, but further proof needs to be presented still (Prasansuklab and Tencomnao).

3.3: Reflection on other neurodegenerative diseases

The blood-brain barrier in Alzheimer's disease is a very important topic in modern science, since it is also being covered in popular science magazines like Scientific American (Interlandi). This strengthens the general view that finding a cure for neurodegenerative diseases is of high public concern. The disruption of tight junctions of the blood-brain barrier is also a hallmark of some other types of pathology, for example: stroke, HIV encephalitis, multiple sclerosis (MS), bacterial meningitis (Huber *et all.*) and epilepsy (Bednarczyk and Lukasiuk). Since there is overlap in the pathogenesis of several neurodegenerative diseases, the research on Alzheimer's disease is beneficial for research on other neurodegenerative diseases as well. Research can be used for more protein misfolding diseases, like Parkinson's disease and type II diabetes mellitus (Cheng *et all.*). Inhibitors can be identified to block the aggregation of amyloid beta and other amyloidogenic proteins. So, of course there are possible outcomes of studies on Alzheimer's disease that improve understanding of other neurodegenerative diseases, and (obviously) vice versa. Furthermore, bioinformatics has proven to be a very useful tool for analyzing amyloid forming propensity in human islet amyloid polypeptide (hIAPP), associated with human type II diabetes (Chakraborty *et all.*). This study included the use of molecular dynamics simulations, which can implicate applications on other amyloids as well, like amyloid beta involved in neurodegenerative diseases.

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