Chapter

1

Introduction and outline

INTRODUCTION

Alzheimer's disease

Alzheimer's disease (AD) is a progressive neurodegenerative disorder accounting for 50-60% of all cases of dementia [1]. In 2009, worldwide, more than 35 million people suffered from dementia and this number is expected to increase up to 115 million people in 2040. As such, AD is a major public health problem [2].

Clinically, AD is manifested by deterioration of cognitive functions; progressive impairment of episodic and semantic memory, attention and executive abilities, language, visuospatial and perceptual skills and praxis causing progressive impairment of activities of daily living. Furthermore, a variety of neuropsychiatric symptoms and behavioral disturbances, such as apathy, delusions and hallucinations, is also seen in AD [3-4].

Neuropathologically AD is characterised by the accumulation of amyloid-beta (Aβ) in senile plaques and hyperphosphorylated tau in neurofibrillary tangles [5]. Accumulation of amyloid is thought to play a key role in AD [6]. Aβ pathology in the brain can be visualized and quantified *in vivo* using positron emission tomography (PET) and the amyloid ligand [^{11}C]PIB [7-8]. Apart from Aβ depositions in brain parenchyma, Aβ accumulation in cerebral blood vessel walls, known as cerebral amyloid angiopathy (CAA), is present in nearly all AD brains, although severity amongst cases varies strongly [9].

The mechanisms leading to intraparenchymal and intravascular Aβ depositions are still largely unknown. However, there is increasing concensus that the initiating event may lie in an imbalance between production and clearance of Aβ [10]. In Down's syndrome and familial AD there is a life-long increased production of Aβ due to mutations in the amyloid precursor protein, preseniline 1 or preseniline 2 gene [11-13]. These inherited autosomal dominant forms of AD, however, are rare and account for less than five percent of all cases [14-15]. Little evidence for increased production of Aβ in sporadic (non-genetic) AD exists, whilst an increasing body of evidence suggests that especially a failure of clearance of Aβ from the brain is crucial in the pathophysiology of sporadic AD [10, 16-17].

There are several pathways for clearance of Aβ, including degradation by proteolytic enzymes [18], perivascular drainage pathways [19], removal through the interstitial fluid bulk flow into the cerebrospinal fluid and from there into the bloodstream [20], and active transport over the blood-brain barrier [21-22]. Of these, the transport pathways are thought to be the most important routes [16].

Blood-brain barrier

The blood-brain barrier (BBB), a highly specialized structure composed of a monolayer of brain capillary endothelial cells, serves to maintain homeostasis in the central nervous system and protects the brain from toxic substances [23]. These BBB functions are realized

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via different mechanisms, such as tight junctions between adjacent endothelial cells to prevent entry of compounds into the brain and active efflux mechanisms to transport endogenous and exogenous substances from brain to blood [24]. Due to the presence of tight junctions between cerebrovascular endothelial cells, the transport of Aβ across BBB requires carrier-mediated or receptor-mediated transport systems [21]. The receptor for advanced glycation end products (RAGE) is thought to be a primary transporter of Aβ from the systemic circulation across the BBB into the brain, whereas the transporters low-density lipoprotein receptor-related protein-1 (LRP1) and P-glycoprotein (Pgp) are thought to mediate transport of Aβ out of the brain [25-28]. Pgp is thought to have an important role in the clearance of Aβ from brain [29].

P-glycoprotein

P-glycoprotein is a 170 kDa plasma membrane protein belonging to the ATP-binding cassette (ABC) transporter family, encoded in humans by the highly polymorphic ABCB1 gene. Pgp is located in several organs throughout the human body with an excretory and/or barrier function, such as the intestine, liver, kidney, testes and the BBB. At the endothelial cells of the BBB, Pgp functions as an efflux transporter, transporting substrates out of the brain into the bloodstream [30]. Pgp is considered to be one of the major efflux transporters at the BBB because of its high expression and its ability to transport a wide variety of structurally unrelated compounds from the brain [24]. It has been shown that Pgp transports Aβ *in vitro* and blocking Pgp function *in vitro* decreases transport of Aβ1-40 and Aβ1-42 [27,31]. Furthermore, Aβ depositions are inversely correlated with Pgp expression in the brain of elderly nondemented humans post mortem [32] and, in an AD mouse model, knocking out BBB Pgp expression increases Aβ depositions [28], while restoring BBB Pgp expression and transport activity reduces brain Aβ levels [33].

Measuring Pgp function with PET

Pgp function can be assessed *in vivo* using PET with the Pgp substrate (*R*)-[^{11}C]verapamil [34-37]. In general, PET enables *in vivo* visualisation and quantification of physiological and pathophysiological processes using positron emitting radionuclides [38]. Using PET, the time course of radioligand uptake in a target tissue can be measured accurately and subsequently these measurements can be translated into quantitative values of specific (patho)physiological parameters (e.g. blood flow, glucose metabolism, specific binding at a receptor site, volume of distribution of the radiotracer in the brain) using appropriate tracer kinetic models [39]. This analysis does require an input function describing delivery of the radiotracer to the tissue and the gold standard input function is a metabolite corrected arterial plasma curve. As it is not clear how many compartments are needed to describe (*R*)-[^{11}C]verapamil kinetics in terms of Pgp function, it is important that several alternative tracer kinetic models are investigated and characterised. To date, only one pilot study (six subjects)

has reported on reproducibility of (*R*)-[^{11}C]verapamil PET data [40]. Using (*R*)-)[^{11}C]verapamil and PET, it has been shown that BBB Pgp function decreases with normal aging [41-43] and that it is reduced in neurodegenerative diseases such as progressive supranuclear palsy and multiple system atrophy, with conflicting results in Parkinson's disease [44-45]. So far, there have been no PET studies reporting on *in vivo* BBB Pgp function in AD and, therefore, PET studies on BBB Pgp function in AD are needed to gain more insight into the role of Pgp function in the pathogenesis of AD.

OUTLINE

The general objective of this thesis was to explore several aspects of BBB Pgp function in healthy aging and AD, in order to gain further insight in the pathogenesis of AD. To this end, (*R*)-[^{11}C]verapamil PET was used to image and quantify BBB Pgp function.

First, in **chapter 2**, the optimal tracer kinetic model for (*R*)-[^{11}C]verapamil was revisited by assessing reproducibility of (*R*)-[^{11}C]verapamil parameters and outcome measures in healthy controls for several potential models. In **chapter 3**, the effect of age on BBB Pgp function was assessed, using a large group of healthy men and women, in order to establish whether healthy aging is associated with altered BBB Pgp function and whether there are gender differences. Next, in **chapter 4**, BBB Pgp function was assessed in [^{11}C]PIB positive AD patients and compared with age-matched healthy controls. Furthermore, in AD patients, the relation between BBB Pgp function and amyloid accumulation as measured with [^{11}C]PIB PET, was assessed. **Chapter 5** describes BBB Pgp function in AD patients with and without microbleeds. The purpose of this study was to assess whether there is additional Pgp dysfunction in AD patients with microbleeds. The effects of single nucleotide polymorhisms in the highly polymorphic ABCB1 gene on BBB Pgp function were investigated in healthy controls and AD patients in **chapter 6**.

Finally, in **chapter 7**, the main findings of this thesis are summarized, followed by a general discussion and recommendations for future research.

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