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## **No evidence for additional blood-brain barrier P-glycoprotein dysfunction in Alzheimer's disease patients with microbleeds**

Daniëlle M.E. van Assema<sup>1,2</sup>, Jeroen D.C. Goos<sup>2</sup>, Wiesje M. van der Flier<sup>2,3</sup>, Mark Lubberink<sup>1,4</sup>, Ronald Boellaard<sup>1</sup>, Albert D. Windhorst<sup>1</sup>, Philip Scheltens<sup>2</sup>, Adriaan A. Lammertsma<sup>1</sup>, Bart N.M. van Berckel<sup>1</sup>

<sup>1</sup> Department of Nuclear Medicine & PET Research, VU University Medical Center, Amsterdam, The Netherlands

<sup>2</sup> Department of Neurology & Alzheimer Center, VU University Medical Center, Amsterdam, The Netherlands

<sup>3</sup> Department of Epidemiology & Biostatistics, VU University Medical Center, Amsterdam, The Netherlands

<sup>4</sup> PET Centre, Uppsala University Hospital, Uppsala, Sweden

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## ABSTRACT

Decreased blood-brain barrier P-glycoprotein function has been shown in Alzheimer's disease patients using positron emission tomography with the radiotracer (*R*)-[<sup>11</sup>C]verapamil. Decreased P-glycoprotein function has also been hypothesized to promote cerebral amyloid angiopathy development. Here, we used positron emission tomography and (*R*)-[<sup>11</sup>C]verapamil to assess P-glycoprotein function in eighteen Alzheimer's disease patients, of which six had microbleeds, presumably reflecting underlying cerebral amyloid angiopathy. No differences were found in binding potential and non-specific volume of distribution of (*R*)-[<sup>11</sup>C]verapamil between patient groups. These results provide no evidence for additional P-glycoprotein dysfunction in Alzheimer's disease patients with microbleeds.

## INTRODUCTION

Pathological hallmarks of Alzheimer's disease (AD) are fibrillary amyloid-beta depositions in brain parenchyma and amyloid-beta accumulation in cerebral blood vessel walls, known as cerebral amyloid angiopathy (CAA) [1]. CAA is present in nearly all AD brains, although severity amongst individuals varies strongly [2]. Microbleeds (MBs), which can be observed using gradient echo weighted magnetic resonance imaging (MRI) supposedly reflect underlying CAA [3]. Prevalence of MBs in AD is reported to be around 23% [4].

The mechanisms behind intraparenchymal and intravascular amyloid-beta depositions in sporadic AD still are largely unknown. A widely held hypothesis states that impaired clearance of amyloid-beta from the brain is the main problem in AD [5-6]. There are several pathways for clearance of amyloid-beta, including degradation by proteolytic enzymes, perivascular drainage pathways and active transport over the blood-brain barrier (BBB) [7]. A major transporter at the BBB is P-glycoprotein (Pgp), a 170 kDa transmembrane protein, which is highly expressed at the endothelial cells that line the brain blood vessel walls. Pgp functions as an efflux transporter for a wide variety of substrates [8] and is involved in amyloid-beta transport [9]. Recently, decreased BBB Pgp function in sporadic AD patients was found using positron emission tomography (PET) with the radiolabelled Pgp substrate (*R*)-[<sup>11</sup>C]verapamil [10]. Decreased Pgp function has also been hypothesized to promote CAA development [11].

To date, no studies have been performed to assess differences in BBB Pgp function between AD patients with and without signs of advanced CAA in the brain. The purpose of the present study was to investigate global and regional associations between MBs and Pgp function in AD patients.

## MATERIALS AND METHODS

### Patients

Eighteen patients with probable AD in a mild to moderate disease stage (Mini-Mental State Examination (MMSE) scores  $\geq 20$ ) were included in this study. Six of these patients had MBs on brain MRI. Patients were recruited from the outpatient Memory Clinic of the Alzheimer Center of the VU University Medical Center (VUmc) in Amsterdam, where they received a standard dementia screening that included medical history, physical and neurological examinations, screening laboratory tests, and MRI. Diagnosis was established by consensus in a multidisciplinary meeting according to the criteria for probable AD as proposed by the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association. To confirm presence of AD pathology in the brain, increased cortical accumulation of [<sup>11</sup>C]PIB PET was required [12]. Parametric

[<sup>11</sup>C]PIB PET images were evaluated by an experienced nuclear medicine physician (BvB) and were rated as either being PIB positive (binding in more than one cortical (frontal, parietal, temporal or occipital) brain region) or PIB negative (predominantly white matter binding). All eighteen AD patients were classified as PIB positive. Exclusion criteria were major psychiatric or neurological disorders (other than AD), history of alcohol and/or drug abuse and use of medication that could possibly interfere with Pgp function [13]. The study was approved by the Medical Ethics Review Committee of the VUmc. Written informed consent was obtained from all patients after a complete written and verbal description of the study.

### **Magnetic resonance imaging**

Patients underwent structural MRI scanning using a 1.5 T Sonata scanner (Siemens Medical Solutions, Erlangen, Germany). The scan protocol included a coronal T1-weighted 3-D MPRAGE (magnetization prepared rapid acquisition gradient echo), which was used for co-registration and region of interest (ROI) definition. To assess presence of MBs and superficial siderosis, which is also a common MRI finding associated with CAA [14], a susceptibility weighted imaging (SWI) sequence was performed in all patients.

MBs were identified on the SWI sequence by an experienced neuroradiologist and defined as rounded, hypointense homogeneous foci up to 10 mm in size. Lesions in sulci probably representing flow voids from vessels and lesions in the globus pallidus, supposedly representing iron or calcium deposits, were not considered. Choroid plexus and pineal calcifications were also not considered, as were lesions suggestive of partial volume effects (PVE).

### **Positron emission tomography**

PET scans were acquired using an ECAT EXACT HR+ scanner (Siemens/CTI, Knoxville, USA) and data were corrected for attenuation, randoms, dead time, scatter and decay. (*R*)-[<sup>11</sup>C]verapamil and [<sup>11</sup>C]PIB PET data acquisition and analysis were performed as described previously [10,12]. In brief, after acquiring a 10 min transmission scan in 2D mode, a dynamic 60 min emission scan in 3D mode was started simultaneously with the intravenous injection of  $340 \pm 37$  MBq (*R*)-[<sup>11</sup>C]verapamil. During the scan, arterial blood was withdrawn continuously through a radial artery cannula and, at set times, manual samples were taken to obtain a metabolite corrected plasma input curve. Images were reconstructed using a standard filtered back projection (FBP) algorithm, applying a Hanning filter with a cut-off at 0.5 times the Nyquist frequency. A zoom factor of 2.123 and a matrix size of 256 x 256 x 63 were used, resulting in a voxel size of 1.2 x 1.2 x 2.4 mm and a spatial resolution of approximately 6.5 mm full width at half maximum at the centre of the field of view. Images were also reconstructed using a partial volume corrected ordered subset expectation maximization (PVC OSEM) reconstruction algorithm, reducing PVE [15]. Kinetic analysis

was performed using nonlinear regression of the standard two-tissue compartment model, including a blood volume component, and fixing the non-specific volume of distribution ( $= K_1/k_2$ ) to the mean whole brain grey matter value. Possible differences in  $K_1/k_2$  ratio and distribution volume  $V_T$  ( $K_1/k_2 \cdot (1+k_3/k_4)$ ) were investigated. The non-displaceable binding potential ( $BP_{ND}$ ) was used as outcome measure. These were obtained for the global cortical brain region, frontal, parietal, temporal and occipital regions, posterior and anterior cingulate cortices, medial temporal lobe and cerebellum [10].

### Statistical analysis

Data are presented as mean  $\pm$  standard deviation, unless otherwise stated. Statistical analysis was performed using SPSS version 15.0. Group differences were calculated using non-parametric Mann-Whitney U tests. A  $p$ -value  $<0.05$  was considered significant.

## RESULTS

Twelve of the 18 patients (of which five females) included did not have microbleeds or superficial siderosis on brain SWI MRI scan (AD MB- group), while six males had single or multiple MBs in the brain (AD MB+ group). In the AD MB+ group, three patients had a single lobar MB, the other three multiple lobar MBs (three, four and nine, respectively). The patient with nine MBs also had superficial siderosis. There were no significant differences between AD MB- and AD MB+ groups with respect to age, MMSE scores, injected dose and specific activity of (*R*)-[ $^{11}\text{C}$ ]verapamil (Table 5.1).

**Table 5.1** | Characteristics of patient groups.

	AD MB-	AD MB+	<i>p</i> value
n	12	6	
Male/female (% male)	7/5 (58%)	6/0 (100%)	0.07
Age (years)	63 $\pm$ 8	66 $\pm$ 2	0.12
MMSE	23 $\pm$ 3	23 $\pm$ 3	0.81
Injected dose (MBq)	348 $\pm$ 30	324 $\pm$ 48	0.35
Specific activity (GBq/ $\mu\text{mol}$ )	60 $\pm$ 36	51 $\pm$ 34	0.45

AD MB-: Alzheimer's disease patient group without microbleeds.

AD MB+: Alzheimer's disease patient group with microbleeds.

MMSE: Mini-Mental State Examination.

Injected dose: injected dose of (*R*)-[ $^{11}\text{C}$ ]verapamil.

Specific activity: specific activity of (*R*)-[ $^{11}\text{C}$ ]verapamil.

Group differences were calculated using Mann-Whitney U tests.

As shown in Table 5.2, there were no significant differences in  $BP_{ND}$  between AD MB- and AD MB+ groups for any of the regions investigated. Results were essentially the same after PVE correction (data not shown).

**Table 5.2** | Binding potential ( $BP_{ND}$ ) of (R)-[ $^{11}\text{C}$ ]verapamil for the several cortical brain regions.

	AD MB-	AD MB+	<i>p</i> value
Global	2.15 ± 0.34	2.20 ± 0.34	0.85
Frontal	2.10 ± 0.33	2.15 ± 0.42	0.85
Parietal	2.15 ± 0.36	2.16 ± 0.33	0.78
Temporal	2.23 ± 0.39	2.31 ± 0.35	0.78
Occipital	2.20 ± 0.33	2.23 ± 0.27	0.51
Posterior Cingulate	2.12 ± 0.44	2.17 ± 0.44	0.51
Anterior Cingulate	2.14 ± 0.36	1.95 ± 0.42	0.45
Medial Temporal Lobe	2.82 ± 0.48	3.12 ± 0.52	0.30
Cerebellum	2.03 ± 0.27	1.94 ± 0.28	0.51

AD MB-: Alzheimer's disease patient group without microbleeds.

AD MB+: Alzheimer's disease patient group with microbleeds.

$BP_{ND}$ : binding potential of (R)-[ $^{11}\text{C}$ ]verapamil.

Group differences were calculated using Mann-Whitney U tests.

Distribution volumes ( $V_T$ ) did not differ between the AD MB- and MB+ groups for any of the regions investigated (e.g. for the global cortical brain region,  $V_T = 0.84 \pm 0.16$  and  $0.89 \pm 0.17$  for MB- and MB+ groups, respectively;  $p$  0.35).

In addition, the non-specific volume of distribution ( $K_1/k_2$  ratio) did not differ (for the global cortical brain region: AD MB-  $0.27 \pm 0.08$ ; AD MB+  $0.29 \pm 0.07$ ,  $p$  0.71) between the AD MB- and AD MB+ group for any of the regions investigated.

## DISCUSSION

No differences were found in  $BP_{ND}$  of (R)-[ $^{11}\text{C}$ ]verapamil between AD patients with MBs and those without. These results indicate that there is no evidence of additional Pgp dysfunction at the BBB in support of the hypothesis of additionally impaired Pgp function in AD MB+ patients compared with AD MB- patients.

There may be multiple explanations for these findings. First, it is possible that decreased Pgp function in AD MB+ patients could not be demonstrated due to lack of statistical power (small group sizes). Second, it could be due to the inclusion criteria used in this study.

Autopsy studies have shown that nearly all AD patients show some degree of vascular amyloid-beta. Still, only a minority of AD patients shows signs of CAA on MRI such as microbleeds or superficial siderosis during life. It is possible that only patients with severe CAA show MBs on MRI, but conclusive evidence is missing. An alternative explanation would be that severity of CAA pathology is only weakly related to the presence and number of MBs on MRI. In addition, it is also possible that additional Pgp dysfunction does occur, but at a more locoregional level, e.g. directly around MB locations. This would, however, be beyond the spatial resolution of PET, given the relatively low target to background ratio of (R)-[<sup>11</sup>C]verapamil uptake.

It should be noted that it is also possible that AD MB+ and AD MB- patients really do not differ from each other in terms of Pgp function, since almost all AD patients do have some degree of CAA and all have amyloid-beta accumulation in the brain. Because both groups suffer from the same pathology, which very likely is present in the brain for several years or even decades before clinical symptoms occur, the possible destructive effects of these pathological processes will have taken place already. In a previous study, decreased Pgp function in AD patients compared with age-matched healthy controls was demonstrated [10]. The order of events regarding Pgp dysfunction and amyloid deposition has not been unravelled yet. BBB Pgp dysfunction could contribute to accumulation of intraparenchymal and intravascular amyloid, while, on the other hand, Pgp dysfunction could also be the result of these amyloid depositions having a destructive effect on the blood vessel walls.

Negative findings were not due to different patterns of atrophy between groups, as volumes of the various cortical brain regions, including the global cortical brain region, did not differ between groups (for the global cortical region: AD MB- 192 ± 24 mL; AD MB+ 193 ± 17 mL, *p* 0.85). Additionally, applying a PVE correction did not alter results.

A limitation of this study is the small sample size of the two groups. Strengths are the full dynamic scanning and quantitative kinetic modelling procedures. This is the first study that directly compares *in vivo* BBB Pgp function between AD patients with and without CAA characteristics, showing that Pgp function is comparable in AD patients with and without microbleeds.

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