Chapter 7

Summary and discussion

SUMMARY AND DISCUSSION

The aims of the studies described in this thesis were (1) to develop methods for quantifying P-glycoprotein (Pgp) function at the blood-brain barrier (BBB) using the Pgp substrate tracer (*R*)-[^{11}C]verapamil and positron emission tomography (PET), and (2) to evaluate BBB Pgp function in healthy aging and AD. These studies are important as they may provide further insight into the role of BBB Pgp function in both normal aging and the pathophysiology of AD.

To achieve these aims, several steps were performed. First, several methodological issues were investigated. Various tracer kinetic models for analyzing (*R*)-[^{11}C]verapamil data were developed and the reproducibility of these models was assessed. Next, (*R*)-[^{11}C]verapamil PET studies were performed in a relatively large group of healthy subjects, both males and females, in different age groups to investigate the effect of aging on BBB Pgp function. Effects of gender on BBB Pgp function were also assessed. Next, BBB Pgp function was investigated in AD patients and compared with healthy elderly subjects. In addition, (*R*)-[^{11}C] verapamil PET studies were performed in AD patients with and without characteristics of cerebral amyloid angiopathy (CAA), in order to evaluate the effect of CAA on Pgp function. Finally, effects of genetic variations in the Pgp-encoding ABCB1 gene on BBB Pgp function were assessed in both healthy subjects and patients with Alzheimer's disease.

In this chapter the main findings of these studies are briefly summarized and discussed. In addition, possibilities and directions for future research are discussed.

Main findings

First, methodological aspects of (*R*)-[^{11}C]verapamil PET studies were assessed. In **chapter 2**, test-retest (TRT) variability of (*R*)-[^{11}C]verapamil PET studies was evaluated for several tracer kinetic models used for analysis of (*R*)-[^{11}C]verapamil data [1-3]. In addition, the impact of corrections for partial volume effects (PVE) on reproducibility was assessed. All data were analyzed using single-tissue and two-tissue compartment models, and global and regional TRT variability was determined for various parameters and outcome measures. Analysis using the Akaike information criterion showed that a constrained two-tissue compartment model provided the best fits to the data. Global TRT variability of the volume of distribution (V_T) was comparable for single-tissue (6%) and constrained two-tissue (9%) compartment models. TRT variability of binding potential (BP_{ND}) derived from the constrained two-tissue compartment model was less robust, but still acceptable (22%). After applying PVE correction, there was essentially no change in TRT variability. It was concluded that the model of choice for analysing (*R*)-[^{11}C]verapamil data is a constrained two-tissue compartment model.

Next, in **chapter 3**, effects of age and gender on BBB Pgp function were assessed. Age is a risk factor for many neurodegenerative disorders, such as AD and Parkinsons's disease [4-5]. Loss of Pgp function with increasing age may be involved in the development of

those disorders. This may differ between males and females as, for AD, female gender is another risk factor [6-7]. Thirty-five healthy men and women in three different age groups were included and (*R*)-[^{11}C]verapamil PET data were obtained. In older men, increased V_T of (*R*)-[^{11}C]verapamil was found in several large brain regions, suggesting a decrease in Pgp function with age in men. Young and elderly women, however, showed comparable V_T values, suggesting no effect of age on BBB Pgp function in women. When compared with young men, young women had higher V_T values. Data in this study were assessed with and without PVE correction, again showing comparable results in V_T values. In conclusion, decreased BBB Pgp was found with aging, but effects of age on BBB Pgp function differed between men and women.

In **chapter 4**, BBB Pgp function in AD patients was compared with healthy age-matched control subjects. A major pathological hallmark of AD is the accumulation of amyloid-beta (Aβ) in the brain, which can be visualized using PET and the amyloid ligand [^{11}C]PIB [8-9]. In thirteen [^{11}C]PIB positive AD patients global (*R*)-[^{11}C]verapamil BP_{ND} values were increased significantly compared with fourteen healthy controls. Higher (*R*)-[^{11}C]verapamil BP_{ND} values were also found in AD for frontal, parietal, temporal and occipital cortices, and for posterior and anterior cingulate, suggestive of decreased BBB Pgp function in these regions. No differences were found in medial temporal lobe and cerebellum. No significant correlations were found between BP_{ND} of (*R*)-[^{11}C]verapamil and [^{11}C]PIB BP_{ND}, which may be due to a ceiling effect of amyloid pathology in AD patients. Nevertheless, findings indicate that Pgp function is decreased in AD patients, supporting the hypothesis that decreased Pgp function contributes to Aβ accumulation and may be involved in the pathogenesis of AD.

Next, as decreased BBB Pgp function was shown in AD patients using (*R*)-[^{11}C]verapamil PET, we investigated in **chapter 5**, eighteen [^{11}C]PIB positive AD patients, of which six had microbleeds (MBs) in the brain. MBs are thought to be an indication of CAA, a condition in which Aβ accumulates on brain blood vessel walls [10]. CAA is often found in AD brains, though with varying degree of severity [11-12]. Pgp dysfunction is thought to promote CAA development [13]. In this pilot study we found no differences in BP_{ND} of (*R*)-[^{11}C]verapamil between these relatively small patient groups, suggesting no evidence for additional Pgp dysfunction in AD patients with MBs.

Finally, in **chapter 6,** the effects of genetic variations in the highly polymorphic Pgp-encoding ABCB1 gene [14-15] on BBB Pgp function were assessed, both in healthy subjects and in AD patients. Three common single nucleotide polymorphisms (SNPs) were tested (C1236T, C2677A/T, C3435T) and correlated with BBB Pgp function as measured with (*R*)-[^{11}C]verapamil PET. In healthy subjects no effects of SNPs were found. In contrast, in AD patients with one or more T present in C1236T, G2677T and C3435T, (*R*)-[^{11}C]verapamil BP_{ND} was found to be significantly higher when compared to patients without a T. An effect of T dose in C1236T and G2677T on BP_{ND} was found, with higher BP_{ND} values as T dose increases, suggesting decreased BBB Pgp function in AD patients with these genetic variants.

GENERAL DISCUSSION

As the volume of distribution of (*R*)-[^{11}C]verapamil increased with age in several cortical brain regions, our data strongly suggest a progressive decrease in BBB Pgp function with age. However, the effects of age on BBB Pgp function were driven by men and in women, no main effect of age on BBB Pgp function was found. This suggests different aging patterns between men and women, which could implicate that women are exposed to higher concentrations of neurotoxins earlier in life and during a longer timeperiod, which in turn could possibly account for the increased risk of AD in women. Furthermore, decreased Pgp function with increasing age could account for increased drug toxicity and increased CNS side effects of drugs that are able to pass the BBB in the elderly. In addition, decreased BBB Pgp function could make the elderly more vulnerable to both exogenous as well ass endogenous neurotoxins that are transported by Pgp, such as Aβ. As such, progressive Pgp dysfunction with increasing age could account for the increasing risk of developing neurodegenerative diseases with age.

Decreased clearance of Aβ from the brain is thought to play a major role in the pathogenesis of AD [16-18]. There are several mechanisms involved in clearance of Aβ from the brain, including enzymatic degradation of Aβ by a variety of proteases [19], continuous slow removal of Aβ through the interstitial fluid bulk flow into cerebrospinal fluid and from there into the bloodstream [20], perivascular drainage of Aβ [21], and transport of Aβ across the BBB into the bloodstream [22-23].

At the BBB, several transporters are involved in regulating transport of Aβ and, as such, dysfunction of these transporters could be involved in the pathogenesis of AD. The receptor for advanced glycation end products (RAGE) is a primary transporter of Aβ from the systemic circulation across the BBB into the brain, whereas the low-density lipoprotein receptor-related protein-1 (LRP1) is one of the transporters involved in the transport of Aβ out of the brain [23-25]. Another transporter located at the BBB is breast cancer resistance protein (BCRP). A few studies suggest that BCRP might have a role as a transporter for Aβ [26-28], but at present the role of BCRP in Aβ clearance is still unclear [29]. On the other hand, Pgp is thought to have an important role in clearance of Aβ from the brain [30]. Evidence that Pgp plays an important role in Aβ transport from the brain is based on several studies. For example, it has been shown *in vitro* that Pgp transports Aβ and that blocking Pgp function decreases transport of Aβ [31-32]. Furthermore, Aβ depositions are inversely correlated with Pgp expression in the brain of elderly nondemented humans [33]. In addition, in an AD mouse model, knocking out BBB Pgp expression increased Aβ depositions [34], whilst restoring BBB Pgp expression and transport activity reduced brain Aβ levels [35]. Nevertheless, there was a clear need for *in vivo* studies on BBB Pgp function in AD. The results of this thesis are indicative that BBB Pgp function in AD is indeed decreased, further supporting the hypothesis that Pgp plays a role in the pathogenesis of AD.

If decreased Pgp function is involved in the pathogenesis of amyloid deposition in AD, this would implicate that Pgp may be a potential target for treatment, e.g. to modulate disease progression. In a broader sense, Pgp dysfunction in AD may be a (surrogate) marker for more widespread BBB dysfunction in AD, involving also other Aβ transporters, such as LRP1. However, further research into the role of these BBB transporters in AD is needed. It is conceivable that transporters involved in Aβ clearance act in concert with each other, and that disturbances in expression and/or function of one of these transporters has an effect (upregulation or downregulation) on expression and/or function of other transporters [36]. This is also likely to happen for other routes and mechanisms of Aβ clearance. For example, it appears that perivascular drainage compensates for both a blockade of the LRP-1 mechanism and reduced neprilysin, an Aβ degrading enzyme, levels in the brain [21, 37].

Although no significant correlations were found between amyloid load, as measured with [^{11}C]PIB PET, and BBB Pgp function, this does not mean that there is no relationship between them. There could be a ceiling effect for [^{11}C]PIB, as [^{11}C]PIB uptake appears to behave as an on/off phenomenon in AD patients, [^{11}C]PIB retention does not reflect disease severity [38] and [^{11}C]PIB binding does not increase substantially over time [39]. Furthermore, (*R*)-[^{11}C]verapamil and [^{11}C]PIB binding showed substantial spatial overlap, although also some inconsistencies (relatively high [^{11}C]PIB BP_{ND} in anterior cingulate and frontal cortex, while (*R*)-[^{11}C]verapamil BP_{ND} was only moderately increased) were observed. As such, there appears to be a regional distribution in the severity of Pgp dysfunction in AD and studies in larger samples are necessary to further address these regional differences and its relation to amyloid depositions.

Perhaps more importantly, Pgp is one of the major efflux pumps at the BBB involved in the transport out of the brain of various drugs and other toxic compounds [40-41]. Consequently, Pgp dysfunction could make AD patients more vulnerable to toxicity and central nervous system side effects due to compounds that enter the brain. For example, it has been shown that efflux of the pesticide endosulfan is mediated by Pgp [42]. Accumulation of environmental toxins could be an important mechanism underlying neurodegeneration associated with AD and other neurodegenerative diseases.

No differences were found in BBB Pgp function between AD patients with MBs and AD patients without. These results, albeit derived from small groups, 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 patients with, compared with AD patients, without MBs. Autopsy studies have shown that nearly all AD patients show some degree of vascular amyloid deposition. Still, only a minority of AD patients shows signs of CAA on MRI such as MBs. 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*)-[^{11}C]verapamil uptake.
Furthermore, in AD patients but not in healthy controls, SNPs (C1236T, G2677A/T and C3435T) in the Pgp-encoding ABCB1 gene were found to be related to changes in Pgp function at the BBB. As T dose in these SNPs increased, this lead to a decrease in BBB Pgp function in AD patients. As such, certain variations in the ABCB1 gene might contribute to the risk of developing AD and might influence disease progression once having amyloid depositions in the brain. These findings however, first need to be replicated in larger groups of patients.

Pgp radiotracers

Over the past years several PET tracers have been developed for quantifying Pgp function and expression *in vivo*, with most being based on Pgp substrates. Interpretation of measured tracer kinetic data in terms of Pgp transport function is still limited, as there are no independent measurements to provide physiological meaning to the various kinetic parameters. As there are marked species differences in BBB Pgp expression and function, here only tracers that have been used in humans are mentioned. These are racemic [^{11}C] verapamil, the pure enantiomer (*R*)-[^{11}C]verapamil and [^{11}C]-*N-desmethyl*-loperamide [43].
[^{11}C]verapamil, a calcium channel blocker, is both a substrate and an inhibitor of Pgp [44], but at the low (tracer) concentrations used in PET, it only acts as a substrate [45-46]. Both (*R*) and (*S*) enantiomers are substrates for Pgp, but a racemic tracer is not suitable for quantification. (*R*)-[^{11}C]verapamil is metabolized less and has lower affinity for calcium channels than (*S*)-[^{11}C]verapamil and it is therefore the preferred isomer for quantification of BBB Pgp function [1,47]. Limitations of (*R*)-[^{11}C]verapamil are its high lipophilicity (high affinity for plasma proteins, resulting in a low extraction fraction) and the formation of radiolabelled metabolites, some of which are lipophilic. Because of their lipophilicity, the latter metabolites are also able to cross the BBB and it has been shown that they are also Pgp substrates [48], making interpretation of measured signals more difficult. Despite these limitations, (*R*)-[^{11}C]verapamil has been used extensively as a tracer for assessing BBB Pgp function [43].
Loperamide, a potent over-the-counter opiate used to treat diarrhea, is also a substrate for Pgp at the BBB. A major metabolite of [^{11}C]loperamide is [^{11}C]-*N-desmethyl*-loperamide ([^{11}C]dLop), which is also a substrate for Pgp [43]. [^{11}C]dLop has been used to image BBB Pgp function *in vivo* in healthy humans [49-50]. The advantage of [^{11}C]dLop as a radiotracer is that it produces very few metabolites. On the other hand, a disadvantage is its very low brain uptake, which is even lower than that of (*R*)-[^{11}C]verapamil, making it more difficult to quantify the signal reliably [49-50].

Methodological considerations

An important methodological issue in PET imaging is the effect of subject motion. Patient motion may result in over- or underestimation of measured tracer uptake, and thereby affect accuracy of measurements. In the present studies data were analysed without applying motion corrections. However, during the 60 minutes in case of (*R*)-[^{11}C]verapamil and 90 minutes in case of [^{11}C]PIB PET scans, participants were continuously monitored and checked for motion using laser beams. In addition, all PET scans were checked for motion afterwards, and scans would have been excluded from analysis if severe motion had been present. However, this was not the case.

Furthermore, quantification of PET studies may be affected by PVE, resulting from the limited spatial resolution of a PET scanner. Unfortunately, many uncertainties may affect accuracy and precision of MRI based PVE corrections, with results depending on actual MRI scanner and sequence being used. To assess effects of PVE in this thesis, a partial volume corrected ordered subset expectation maximization (PVC OSEM) reconstruction algorithm was used in addition to the standard filtered back projection (FBP) reconstruction algorithm. PVC OSEM is a previously validated method that improves in-plane resolution of PET images by taking the point spread function of the scanner into account, resulting in improved image resolution and thereby reducing PVE [51-53]. PVC OSEM reconstructions were applied to all (*R*)-[^{11}C]verapamil scans in this thesis, although results were presented only in chapter 2 and 3. This choice was made, as differences between FBP and PVC OSEM reconstructed (*R*)-[^{11}C]verapamil data was only minor, probably because of the rather low (*R*)-[^{11}C]verapamil signal. In any case, atrophy would have resulted in lower BP_{ND} values of (*R*)-[^{11}C]verapamil in AD patients due to PVE. Therefore, actual increases in (*R*)-[^{11}C]verapamil accumulation in AD may even have been underestimated. This is, however, unlikely given the similar results for FBP and PVC OSEM reconstructed data.

Recommendations for future research

This thesis shows that BBB Pgp function, as measured with the Pgp substrate tracer (*R*)-[^{11}C]verapamil, decreases with healthy aging, but even more in AD patients, suggesting a possible role of Pgp in the pathogenesis of AD. At present, however, there is no ideal PET tracer for *in vivo* quantificantion of Pgp function. Despite its limitations, (*R*)-[^{11}C]verapamil still provides a useful tool to study Pgp function at the BBB *in vivo*. Unfortunately, using (*R*)-[^{11}C]verapamil and PET, it is not possible to differentiate between decreased Pgp function due to a decrease in BBB Pgp transporter expression and decreased transport functionality of Pgp with intact expression. The present results are indicative of decreased Pgp function in aging and results in AD could easily be a combination of both decreased expression and decreased function of Pgp at the BBB. Ultimately, the development of separate radiotracers, specific for either Pgp expression or function, is needed to further elucidate the role of Pgp in

health and disease. In addition, PET tracers should be developed for other BBB transporters that might be involved in Aβ transport across the BBB.

If such tracers would become available in the future, in case of AD, subjects should be scanned at a very early stage of the disease to determine the role of Pgp in accumulation of Aβ in the brain. In fact, this implies that subjects should be scanned even before any cognitive complaints occur. Ideally, a very large cohort of healthy controls should be included starting from age twenty and follow-up PET scans should be made every 5 years, preferably in combination with other techniques such as MRI and other PET tracers, especially amyloid tracers to measure early Aβ deposition. Finally, a large population based genetic study including both healthy subjects and AD patients (and preferably other types of dementia as well) should be performed to assess the prevalence of ABCB1 SNPs within these groups, and to further evaluate the potential role of these genetic variations in the risk of developing AD.

REFERENCES

1. Lubberink M, Luurtsema G, van Berckel BN, Boellaard R, Toornvliet R, Windhorst AD, Franssen EJ, Lammertsma AA. Evaluation of tracer kinetic models for quantification of P-glycoprotein function using (R)-[11C]verapamil and PET. *J Cereb Blood Flow Metab* 2007;27:424-433.
2. Muzi M, Mankoff DA, Link JM, Shoner S, Collier AC, Sasongko L, Unadkat JD. Imaging of cyclosporine inhibition of P-glycoprotein activity using 11C-verapamil in the brain: studies of healthy humans. *J Nucl Med* 2009;50:1267-1275.
3. van Assema DM, Lubberink M, Bauer M, van der Flier WM, Schuit RC, Windhorst AD, Comans EF, Hoetjes NJ, Tolboom N, Langer O, Muller M, Scheltens P, Lammertsma AA, van Berckel BN. Blood-brain barrier P-glycoprotein function in Alzheimer's disease. *Brain* 2012;135:181-189.
4. Blennow K, de Leon MJ, Zetterberg H. Alzheimer's disease. *Lancet* 2006;368:387-403.
5. Lees AJ, Hardy J, Revesz T. Parkinson's disease. *Lancet* 2009;373:2055-2066.
6. Candore G, Balistreri CR, Grimaldi MP, Vasto S, Listi F, Chiappelli M, Licastro F, Lio D, Caruso C. Age-related inflammatory diseases: role of genetics and gender in the pathophysiology of Alzheimer's disease. *Ann N Y Acad Sci* 2006;1089:472-486.
7. Launer LJ, Andersen K, Dewey ME, Letenneur L, Ott A, Amaducci LA, Brayne C, Copeland JR, Dartigues JF, Kragh-Sorensen P, Lobo A, Martinez-Lage JM, Stijnen T, Hofman A. Rates and risk factors for dementia and Alzheimer's disease: results from EURODEM pooled analyses. EURODEM Incidence Research Group and Work Groups. European Studies of Dementia. *Neurology* 1999;52:78-84.
8. Klunk WE, Engler H, Nordberg A, Wang Y, Blomqvist G, Holt DP, Bergstrom M, Savitcheva I, Huang GF, Estrada S, Ausen B, Debnath ML, Barletta J, Price JC, Sandell J, Lopresti BJ, Wall A, Koivisto P, Antoni G, Mathis CA, Langstrom B . Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. *Ann Neurol* 2004;55:306-319.
9. Tolboom N, Yaqub M, van der Flier WM, Boellaard R, Luurtsema G, Windhorst AD, Barkhof F, Scheltens P, Lammertsma AA, van Berckel BN. Detection of Alzheimer pathology in vivo using both 11C-PIB and 18F-FDDNP PET. *J Nucl Med* 2009;50:191-197.
10. Knudsen KA, Rosand J, Karluk D, Greenberg SM. Clinical diagnosis of cerebral amyloid angiopathy: validation of the Boston criteria. *Neurology* 2001;56:537-539.
11. Smith EE, Greenberg SM. Beta-amyloid, blood vessels, and brain function. *Stroke* 2009;40:2601-2606.
12. Viswanathan A, Greenberg SM. Cerebral amyloid angiopathy in the elderly. *Ann Neurol* 2011;70:871-880.
13. Vogelgesang S, Warzok RW, Cascorbi I, Kunert-Keil C, Schroeder E, Kroemer HK, Siegmund W, Walker LC, Pahnke J. The role of P-glycoprotein in cerebral amyloid angiopathy; implications for the early pathogenesis of Alzheimer's disease. *Curr Alzheimer Res* 2004;1:121-125.
14. Kerb R. Implications of genetic polymorphisms in drug transporters for pharmacotherapy. *Cancer Lett* 2006;234:4-33.
15. Cascorbi I. P-glycoprotein: tissue distribution, substrates, and functional consequences of genetic variations. *Handb Exp Pharmacol* 2011;261-283.
16. Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. *Science* 1992;256:184-185.
17. Hardy J, Selkoe DJ.The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* 2002;297:353-356.
18. Mawuenyega KG, Sigurdson W, Ovod V, Munsell L, Kasten T, Morris JC, Yarasheski KE, Bateman RJ. Decreased clearance of CNS beta-amyloid in Alzheimer's disease. *Science* 2010;330:1774.
19. Selkoe DJ. Clearing the brain's amyloid cobwebs. *Neuron* 2001;32:177-180.
20. Silverberg GD, Mayo M, Saul T, Rubenstein E, McGuire D. Alzheimer's disease, normal-pressure hydrocephalus, and senescent changes in CSF circulatory physiology: a hypothesis. *Lancet Neurol* 2003;2:506-511.
21. Weller RO, Subash M, Preston SD, Mazanti I, Carare RO. Perivascular drainage of amyloid-beta peptides from the brain and its failure in cerebral amyloid angiopathy and Alzheimer's disease. *Brain Pathol* 2008;18:253-266.
22. Deane R, Zlokovic BV. Role of the blood-brain barrier in the pathogenesis of Alzheimer's disease. *Curr Alzheimer Res* 2007;4:191-197.

23. Deane R, Bell RD, Sagare A, Zlokovic BV. Clearance of amyloid-beta peptide across the blood-brain barrier: implication for therapies in Alzheimer's disease. *CNS Neurol Disord Drug Targets* 2009;8:16-30.
24. Deane R, Wu Z, Zlokovic BV. RAGE (yin) versus LRP (yang) balance regulates alzheimer amyloid beta-peptide clearance through transport across the blood-brain barrier. *Stroke* 2004;35:2628-2631.
25. Donahue JE, Flaherty SL, Johanson CE, Duncan JA, III, Silverberg GD, Miller MC, Tavares R, Yang W, Wu Q, Sabo E, Hovanesian V, Stopa EG. RAGE, LRP-1, and amyloid-beta protein in Alzheimer's disease. *Acta Neuropathol* 2006;112:405-415.
26. Tai LM, Loughlin AJ, Male DK, Romero IA. P-glycoprotein and breast cancer resistance protein restrict apical-to-basolateral permeability of human brain endothelium to amyloid-beta. *J Cereb Blood Flow Metab* 2009;29:1079-1083.
27. Xiong H, Callaghan D, Jones A, Bai J, Rasquinha I, Smith C, Pei K, Walker D, Lue LF, Stanimirovic D, Zhang W. ABCG2 is upregulated in Alzheimer's brain with cerebral amyloid angiopathy and may act as a gatekeeper at the blood-brain barrier for Abeta(1-40) peptides. *J Neurosci* 2009;29:5463-5475.
28. Candela P, Gosselet F, Saint-Pol J, Sevin E, Boucau MC, Boulanger E, Cecchelli R, Fenart L. Apical-to-basolateral transport of amyloid-beta peptides through blood-brain barrier cells is mediated by the receptor for advanced glycation end-products and is restricted by P-glycoprotein. *J Alzheimers Dis* 2010;22:849-859.
29. Miller DS. Regulation of P-glycoprotein and other ABC drug transporters at the blood-brain barrier. *Trends Pharmacol Sci* 2010;31:246-254.
30. Vogelgesang S, Jedlitschky G, Brenn A, Walker LC. The Role of the ATP-Binding Cassette Transporter P-Glycoprotein in the Transport of beta-Amyloid Across the Blood-Brain Barrier. *Curr Pharm Des* 2011;17:2778-2786.
31. Lam FC, Liu R, Lu P, Shapiro AB, Renoir JM, Sharom FJ, Reiner PB. beta-Amyloid efflux mediated by p-glycoprotein. *J Neurochem* 2001;76:1121-1128.
32. Kuhnke D, Jedlitschky G, Grube M, Krohn M, Jucker M, Mosyagin I, Cascorbi I, Walker LC, Kroemer HK, Warzok RW, Vogelgesang S. MDR1-P-Glycoprotein (ABCB1) Mediates Transport of Alzheimer's amyloid-beta peptides--implications for the mechanisms of Abeta clearance at the blood-brain barrier. *Brain Pathol* 2007;17:347-353.
33. Vogelgesang S, Cascorbi I, Schroeder E, Pahnke J, Kroemer HK, Siegmund W, Kunert-Keil C, Walker LC, Warzok RW. Deposition of Alzheimer's beta-amyloid is inversely correlated with P-glycoprotein expression in the brains of elderly non-demented humans. *Pharmacogenetics* 2002;12:535-541.
34. Cirrito JR, Deane R, Fagan AM, Spinner ML, Parsadanian M, Finn MB, Jiang H, Prior JL, Sagare A, Bales KR, Paul SM, Zlokovic BV, Piwnica-Worms D, Holtzman DM. P-glycoprotein deficiency at the blood-brain barrier increases amyloid-beta deposition in an Alzheimer disease mouse model. *J Clin Invest* 2005;115:3285-3290.
35. Hartz AM, Miller DS, Bauer B. Restoring blood-brain barrier P-glycoprotein reduces brain amyloid-beta in a mouse model of Alzheimer's disease. *Mol Pharmacol* 2010;77:715-723.
36. Jeynes B, Provias J. An investigation into the role of P-glycoprotein in Alzheimer's disease lesion pathogenesis. *Neurosci Lett* 2011;487:389-393.
37. Shibata M, Yamada S, Kumar SR, Calero M, Bading J, Frangione B, Holtzman DM, Miller CA, Strickland DK, Ghiso J, Zlokovic BV. Clearance of Alzheimer's amyloid-ss(1-40) peptide from brain by LDL receptor-related protein-1 at the blood-brain barrier. *J Clin Invest* 2000;106:1489-1499.
38. Engler H, Forsberg A, Almkvist O, Blomquist G, Larsson E, Savitcheva I, Wall A, Ringheim A, Langstrom B, Nordberg A. Two-year follow-up of amyloid deposition in patients with Alzheimer's disease. *Brain* 2006;129:2856-2866.
39. Ossenkoppele R, Tolboom N, Foster-Dingley JC, Boellaard R, Yaqub M, Windhorst AD, Flier van der WM, Barkhof F, Lammertsma AA, Scheltens P, Berckel BNM. Time Course of Specific [11 C] PIB and [18 F]FDDNP Binding: Paired Studies in Patients with Alzheimer's Disease, Mild Cognitive Impairment and Healthy Controls. *Neuroimage* 2010;52:$52.
40. Schinkel AH. P-Glycoprotein, a gatekeeper in the blood-brain barrier. *Adv Drug Deliv Rev* 1999;36:179-194.
41. Fromm MF. Importance of P-glycoprotein at blood-tissue barriers. *Trends Pharmacol Sci* 2004;25:423-429.
42. Bain LJ, LeBlanc GA. Interaction of structurally diverse pesticides with the human MDR1 gene product P-glycoprotein. *Toxicol Appl Pharmacol* 1996;141:288-298.

43. Kannan P, John C, Zoghbi SS, Halldin C, Gottesman MM, Innis RB, Hall MD. Imaging the function of P-glycoprotein with radiotracers: pharmacokinetics and in vivo applications. *Clin Pharmacol Ther* 2009;86:368-377.

44. Elsinga PH, Hendrikse NH, Bart J, Vaalburg W, van WA. PET Studies on P-glycoprotein function in the blood-brain barrier: how it affects uptake and binding of drugs within the CNS. *Curr Pharm Des* 2004;10:1493-1503.

45. Hendrikse NH, Schinkel AH, de Vries EG, Fluks E, van der Graaf WT, Willemsen AT, Vaalburg W, Franssen EJ. Complete in vivo reversal of P-glycoprotein pump function in the blood-brain barrier visualized with positron emission tomography. *Br J Pharmacol* 1998;124:1413-1418.

46. Ambudkar SV, Dey S, Hrycyna CA, Ramachandra M, Pastan I, Gottesman MM. Biochemical, cellular, and pharmacological aspects of the multidrug transporter. *Annu Rev Pharmacol Toxicol* 1999;39:361-398.

47. Luurtsema G, Molthoff CF, Windhorst AD, Smit JW, Keizer H, Boellaard R, Lammertsma AA, Franssen EJ. (R)- and (S)-[11C]verapamil as PET-tracers for measuring P-glycoprotein function: in vitro and in vivo evaluation. *Nucl Med Biol* 2003;30:747-751.

48. Verbeek J, Syvanen S, Schuit RC, Eriksson J, de Lange EC, Windhorst AD, Luurtsema G, Lammertsma AA. Synthesis and preclinical evaluation of [(11)C]D617, a metabolite of (R)-[(11)C]verapamil. *Nucl Med Biol* 2012;39:530-9

49. Seneca N, Zoghbi SS, Liow JS, Kreisl W, Herscovitch P, Jenko K, Gladding RL, Taku A, Pike VW, Innis RB. Human brain imaging and radiation dosimetry of 11C-N-desmethyl-loperamide, a PET radiotracer to measure the function of P-glycoprotein. *J Nucl Med* 2009;50:807-813.

50. Kreisl WC, Liow JS, Kimura N, Seneca N, Zoghbi SS, Morse CL, Herscovitch P, Pike VW, Innis RB. P-glycoprotein function at the blood-brain barrier in humans can be quantified with the substrate radiotracer 11C-N-desmethyl-loperamide. *J Nucl Med* 2010;51:559-566.

51. 51. Mourik JE, Lubberink M, Klumpers UM, Comans EF, Lammertsma AA, Boellaard R. Partial volume corrected image derived input functions for dynamic PET brain studies: methodology and validation for [11C]flumazenil. *Neuroimage* 2008;39:1041-1050.

52. Brix G, Doll J, Bellemann ME, Trojan H, Haberkorn U, Schmidlin P, Ostertag H. Use of scanner characteristics in iterative image reconstruction for high-resolution positron emission tomography studies of small animals. *Eur J Nucl Med* 1997;24:779-786.

53. Mourik JE, Lubberink M, van Velden FH, Kloet RW, van Berckel BN, Lammertsma AA, Boellaard R. In vivo validation of reconstruction-based resolution recovery for human brain studies. *J Cereb Blood Flow Metab* 2010;30:381-389.