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ARTICLE

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Locally increased P-glycoprotein function in major depression: a PET study with [^{11}C]verapamil as a probe for P-glycoprotein function in the blood–brain barrier

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Abstract

The aetiology of depressive disorder remains unknown, although genetic susceptibility and exposure to neurotoxins are currently being discussed as possible contributors to this disorder. In normal circumstances, the brain is protected against bloodborne toxic influences by the blood–brain barrier, which includes the molecular efflux pump P-glycoprotein (P-gp) in the vessel wall of brain capillaries. We hypothesized that P-gp function in the blood–brain barrier is changed in patients with major depression. Positron emission tomography was used to measure brain uptake of [^{11}C]verapamil, which is normally expelled from the brain by P-gp. Cerebral volume of distribution (V_T) of [^{11}C]verapamil was used as a measure of P-gp function. Both region-of-interest (ROI) analysis and voxel analysis using statistical parametric mapping (SPM2) were performed to assess regional brain P-gp function. We found that patients with a major depressive episode, using antidepressants, compared to healthy controls showed a significant decrease of [^{11}C]verapamil uptake in different areas throughout the brain, in particular in frontal and temporal regions. The decreased [^{11}C]verapamil uptake correlates with an increased function of the P-gp protein and may be related to chronic use of psychotropic drugs. Our results may explain why treatment-resistant depression can develop.

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Key words: Depressive disorder, PET, P-glycoprotein, therapy resistant, [^{11}C]verapamil.

Introduction

A large body of evidence gathered during the past decades indicates that brain monoaminergic systems play a key role in the pathogenesis of affective disorders. However, not all symptoms of depression can be related to dysfunctions in monoaminergic systems. For instance, it is now known that stressful events paving the way to affective disorders, lead to changes in neuroplasticity, impair neurogenesis and may lead to a neuroinflammatory response in the brain (Trentani *et al*. 2003). As such, it has been suggested that a dysfunction of the blood–brain barrier (BBB) and blood–cerebrospinal fluid (CSF) barrier may contribute to the pathophysiology of major depressive disorder (MDD). Hampel and colleagues reported that serum/CSF ratios of several inflammatory proteins (used as an indirect measure of BBB function) were altered in depression (Hampel *et al*. 1995, 1997). Several endogenous substances, such as cortisol, are found in the brain parenchyma in high concentrations in subjects with a MDD. In normal circumstances the BBB limits the access of cortisol to the brain (Karssen *et al*. 2001).

The BBB is formed by the brain capillary non-fenestrated polarized endothelial cells that have high-resistanct tight junctions. Besides low passive

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permeability, the brain is protected from potentially harmful endogenous and exogenous substances by efflux transporter proteins, located in the brain capillary wall.

P-glycoprotein (P-gp), a product of the *MDR1* gene in humans and the *MDR-1a* and *MDR-1b* genes in mice, is a major drug efflux transporter, involved in the efflux of a wide variety of lipophilic drugs and endogenous substances (Schinkel *et al*. 1994). Attenuation of P-gp function, for example through use of pharmacological inhibitors, results in substantial changes in the pharmacokinetics and pharmacodynamics of various substrates (Cordon-Cardo *et al*. 1990; Loscher & Potschka, 2005; Schinkel *et al*. 1996).

Nuclear medicine imaging techniques such as positron emission tomography (PET) have been evaluated as non-invasive techniques for the evaluation of the BBB, allowing study of the P-gp pump (Elsinga *et al*. 2004). Using several experimental designs, the applicability of [^{11}C]verapamil as a PET tracer has been extensively studied (Elsinga *et al*. 1996; Hendrikse *et al*. 1998, 1999) and has been found to be a suitable methodology for the *in-vivo* assessment of P-gp functionality in humans (Sasongko *et al*. 2005) and allows the quantification of BBB P-gp function in neurodegenerative diseases (Elsinga *et al*. 2004; Hendrikse *et al*. 1998, 1999; Kortekaas *et al*. 2005).

The protective role of P-gp may be negatively influenced in neurodegenerative diseases. Animal models of neuroinflammation have demonstrated that P-gp is down-regulated by pro-inflammatory cytokines (Bauer *et al*. 2005; Fernandez *et al*. 2004; McRae *et al*. 2003).

In a recent PET study using [^{11}C]verapamil it was found that the function of BBB P-gp was diminished in later stages of Parkinson's disease, whereas *de-novo* patients with Parkinson's showed a regional up-regulation of P-gp in frontal regions (Bartels *et al*. 2008*a*). In another [^{11}C]verapamil PET study in patients with schizophrenia it was shown that P-gp function was locally increased (O. L. de Klerk *et al*. unpublished observations). The authors stated that P-gp induction may be critically involved in the development of drug resistance in schizophrenia. P-gp function is possibly under the influence of genetic polymorphisms (Hoffmeyer *et al*. 2000).

To date, no detailed evidence is available showing dysfunction of the BBB through P-glycoprotein modulation in MDD. It is not known whether P-gp function or expression is altered during a depressive episode.

In this exploratory study we hypothesized that P-gp function would be altered in limbic areas (hippocampus, amygdala) as well in frontotemporal areas (including anterior cingulate cortex) since these areas are known to play a role in depression (Drevets, 1999; Mayberg, 2002). We further hypothesized that this altered functional activity of P-gp could be connected to a genetic polymorphism of the *MDR1* gene. To assess these hypothesized changes in P-gp function, PET brain imaging with [^{11}C]verapamil as radiotracer was performed in depressed patients and healthy controls. The distribution volume of [^{11}C]verapamil was used as a measure of total P-gp function.

Methods

Subjects

Fourteen patients suffering from depression were recruited and participated in the study. All underwent a Mini International Neuropsychiatric Interview (M.I.N.I. plus 5.0.0., Dutch version, 2000) for DSM-IV and fulfilled the criteria for a major depressive episode (Sheehan *et al*. 1998). Inclusion criteria for subjects with a major depressive episode were (1) age 40–80 yr; (2) fulfilment of DSM-IV criteria for major depressive episode; and (3) capacity to give informed consent. The age range 40–80 yr was chosen, because we expected to find larger differences at a later age in particular, since P-gp function declines with ageing (Bartels *et al*. 2008*b*). Moreover, the control group was also used in another study. Exclusion criteria were (1) use of known P-gp modulating agents (cardiovascular drugs, antimalarial drugs, cyclosporine A, phenothiazines, hormones (e.g. tamoxifen), certain antibiotics such as cefoperazone, ceftriaxone, erythromycin) (Matheny *et al*. 2001); (2) any somatic disease of kidney, liver, heart or brain; (3) history of traumatic brain damage; (4) electroconvulsive treatment in the past 3 months; (5) abnormalities at clinical (including neurological) and laboratory examination; (6) pregnancy. Antidepressant medication was allowed. This research was approved by the Ethics Committee of the University Medical Centre Groningen, and all subjects gave written informed consent according to the Declaration of Helsinki. All patients had a minimum score of at least 19 on the 17-item Hamilton Depression Rating Scale (HAMD) at the time of the PET study (Hamilton, 1960). All patients had a physical examination and laboratory evaluation. All were in good physical health and none had meaningful laboratory abnormalities.

The healthy controls as well as their first-degree relatives were required to have no history of any psychiatric disease. The other inclusion and exclusion criteria for healthy controls were similar to the

patients. Before the scan, blood was drawn from the venous cannula for genotyping. Three common MDR1 single nucleotide polymorphisms (SNPs) were detected using a PCR analysis.

Radiochemistry

Racemic [^{11}C]verapamil was produced as previously described (Wegman *et al*. 2002). The injected radioactivity of [^{11}C]verapamil was comparable for the control group and subjects with a major depressive episode (see Results section). Specific activity for all subjects was at least 16 GBq/μmol. Following radiotracer injection, subjects underwent a dynamic PET acquisition protocol as described previously (Kortekaas *et al*. 2005).

PET procedure

All scans were performed with the use of an ECAT EXACT HR+ positron camera (Siemens/CTI, USA). After the radiotracer injection of [^{11}C]verapamil serial dynamic PET scanning was done at escalating timeframes and serial arterial blood sampling for [^{11}C]verapamil took place during the scan in order to define the input function. The samples of all subjects were collected with an automated sampling system, together with six manually drawn samples per subject. These samples (collected at 10-min intervals) were further processed to measure the radioactivity in plasma and blood. In this way the contribution of the injected activity to the PET signal could be calculated. No metabolite analysis was performed. Images were reconstructed in brain mode using an iterative reconstruction (ordered subsets – expectation maximization) with four iterations and 16 subsets and a Gaussian filter of 4 mm. The scans were performed in 3D mode.

Data analysis

The PET data were analysed with both a voxel-wise group analysis using statistical parametric mapping (SPM2, The Mathworks Inc., USA) and a ROI (regions of interest)-based approach. Results from both methods were used and compared.

First, all images were stereotaxically normalized to MNI space using SPM2 and a [^{11}C]verapamil template image that could be used from earlier studies of our group (Bartels *et al*. 2008*a*; Kortekaas *et al*. 2005). The resulting images were analysed with SPM2 as described below. For the ROI analysis the following ROIs, based on the literature as cited above, were selected for analysis: prefrontal cortex, anterior cingulate cortex, temporal lobes, amygdala and hippocampus. Therefore, predefined ROIs from the Anatomical Automated Labeling package (Tzourio-Mazoyer *et al*. 2002) were used to select the appropriate voxels and calculate the corresponding time–activity curves using in-house developed software. In addition, a whole brain ROI was manually drawn using Clinical Applications Programming Package software (CAPP; CTI/Siemens PET Systems, USA).

A graphical analysis according to Logan for quantification of the dynamic PET data was done with plasma data as input. The Logan plot was started at 5 min. With this method the distribution volume (V_T) was estimated. Because the slope (i.e. V_T effect) obtained in the graphical approach may be biased in the presence of noisy data (Abi-Dargham *et al*. 2000), we verified the V_T in a kinetic analysis (i.e. single tissue compartment model). The influx rate constant (K_1) and efflux k_2 were derived from this model and on all parameters (i.e. V_T, K_1 and k_2) the group means (patient group *vs*. control group) were compared with each other, using parametric tests. Analysis of covariance was performed in order to find relevant (clinical) predictors (age, length of present episode, number of previous episodes, severity of symptoms) of V_T or K_1. To exclude a possible confounding effect of subjects without a strict DSM-IV diagnosis of MDD, a subanalysis was also done for the group with MDD only ($n=10$).

Data were then analysed with SPM2. To adjust for differences in individual neuroanatomy and to improve the signal-to-noise ratio, a 12-mm full-width at half-maximum Gaussian smoothing filter was applied to all images. We first compared the groups by looking at absolute differences in V_T, using t test and ANCOVA with cofactors (MDR1 allelic variation, age, length of present episode, number of previous trials, severity of symptoms, injected activity of [^{11}C]verapamil). Clusters of $\geqslant 8$ voxels at a threshold of $p_{FDR}<0.05$ (false discovery rate) were considered to be significant. Coordinates were transformed into Talairach space (Talairach & Tournoux, 1988) using the mni2tal-transformation (http://www.mrc-cbu.cam.ac.uk/Imaging/).

Results

The mean injected [^{11}C]verapamil dose was not different for the two groups (control group: mean = 317 MBq, S.D. = 119; group with depression: mean = 279 MBq, S.D. = 179; t test: $t=-0.344$; d.f. = 24; $p=0.734$). Table 1 shows the demographic characteristics and medication use of the patient group. Twelve

Table 1. Characteristics of subjects with major depressive episode

Subject no.	Treatment setting	Gender	Age (yr)	Age of onset (yr)	Length of PE (wk)	DSM-IV	HAMD	Medication
1	PC	M	47	22	20	BP-I, D, P	22	Valproic acid 2000 mg/d (level 79 mg/l); venlafaxine 225 mg/d, risperidone 2 mg/d
2	O	F	72	67	12	MDD	20	Mirtazapine 30 mg/d, temazepam 20 mg 1 dd 1, cetirizine 10 mg/d
3	PC	F	71	45	104	MDD	25	Imipramine 100 mg/d; lactulose 30 ml/d; esomeprazole 20 mg/d; quetiapine 200 mg/d
4	DT	F	51	27	26	BP-I, D	22	Lithium carbonate 900 mg/d (level 0.88 mg/l); diclofenac 150 mg/d
5	PC	F	52	33	26	MDD, P	37	Tranylcypromine 60 mg/d; olanzapine 15 mg/d
6	PC	M	60	60	8	MDD, P	26	Citalopram 20 mg/d
7	PC	M	56	51	52	MDD, PTSD	31	Lithium carbonate 1000 mg/d (level 0.90 mg/l); nortriptyline 100 mg/d
8	PC	M	55	23	26	MDD, PD	25	Nortriptyline 100 mg/d
9	PC	F	56	31	104	MDD	27	Tolderodine 4 mg/d; temazepam 20 mg/d; cisordinol 2 mg/d; mirtazapine 30 mg/d
10	O	M	41	39	6	MDD	24	Citalopram 40 mg/d; mirtazapine 30 mg/d; oxazepam 50 mg/d
11	PC	F	52	38	12	BP-I	29	Valproic acid 1500 mg/d; temazepam 20 mg/d; asacol; thyrax 75 μg/d
12	PC	M	46	46	20	MDD	24	Venlafaxine 150 mg/d
13	PC	M	52	44	6	MDD	30	Venlafaxine 150 mg/d
14	O	F	57	56	12	MDD	19	Mirtazapine 30 mg/d; citalopram 20 mg/d

BP-I, bipolar disorder type I; D (current phase) depressive; DT, day treatment; HAMD, Hamilton Depression Rating Scale; MDD, major depressive disorder, O, Outpatient; P, psychotic features; PC, psychiatric clinic; PD, panic disorder; PE, present episode; PTSD, post-traumatic stress disorder.

patients had a previous depressive episode, three patients had a bipolar type I disorder, the current episode being depressive. Three subjects had a depressive episode with psychotic features. One patient was excluded from the analysis because the V_T was considered an outlier, since the difference to the group mean was 4.6 times the standard deviation of the group for unknown reasons. The patient group [6 female/7 males, mean ($\pm$S.D.) age 54.1$\pm$7.6 yr] was compared to a sex-matched comparison group (6 female and 7 male subjects; mean age 56.3$\pm$14.3 yr). Patients had a mean score of 25.8 ($\pm$4.8) on the 17-item HAMD (range 19–37). Nine patients were currently depressed for >12 wk. Interestingly, seven of this group could be considered to be treatment resistant, using the criteria of Dunner (2006). Comorbid disorders included post-traumatic stress disorder (PTSD) (n=1) and panic disorder (n=1). All subjects used an antidepressant or a mood stabilizer at the time of scanning.

We determined three common MDR1 SNPs (−129 T>C (exon 1), 2677 G>T/A (exon 21) and 3435 C>T (exon 26)) in our patient group. For the SNP 3435 on exon 26 the allele frequencies in the patient group were CC 23%, CT 38% and TT 39%. The allele frequencies of SNP 3435 in our subjects were not different from the frequency in the population (Tan *et al*. 2004).

The mean brain time–activity curves (after correction for weight and injected dose) for the whole brain of patient and control groups were compared and the area under the curve (AUC) was calculated. No differences in the AUC were found. Both curves overlapped (data not shown). The V_T values of the ROI (whole brain) were calculated with both the Logan analysis and the 'single tissue compartment model', in order to verify that the V_T effect measured by the Logan method was concordant with another method. Pearson's correlation coefficient (r) was very good, 1.000 (p=0.000). The Logan curve gave an excellent fit

Table 2. Characteristics of significant clusters in SPM2

Cluster size (cm^3)	$p_{FDR\text{-}corr}$	*T* value (max)	MNI coordinates	Location
52.8	0.028	3.66	−38, −2, −10	Left temporal lobe, grey matter
	0.028	3.62	−12, −6, 8	Left thalamus, ventral anterior nucleus
	0.028	3.40	28, 12, −24	Right temporal lobe, superior temporal gyrus
35.6	0.028	3.64	44, 26, 22	Right frontal lobe middle frontal gyrus
		3.37	−20, 46, 22	Left frontal lobe, superior frontal gyrus
8.0	0.028	3.37	−42, −52, −18	Left cerebellum
1.5	0.028	2.83	−4, −62, 4	Left occipital lobe, lingual gyrus
4.3	0.028	2.81	−6, −2,6 48	Left frontal lobe, paracentral lobule

in all cases. The V_T in ROI whole brain in the group of patients ($n=13$) with depression was lower at a nearly significant level ($Z=-2.008$, $p=0.055$). K_1 (whole brain ROI) showed no significant difference between the groups (values given are mean ± S.D.) [0.48 ± 0.15 (controls) *vs.* 0.41 ± 0.10 (patients), $p=0.15$]. Neither did the k_2 values [0.71 ± 0.10 (controls) *vs.* 0.75 ± 0.15 (patients), $p=0.46$]. In the ANCOVA none of the co-factors (age, length of present episode, number of previous episodes, severity of symptoms) showed a significant effect on the results.

Both groups were compared with a Student's *t* test for the selected ROIs (prefrontal cortex, anterior cingulate cortex, temporal lobes, hippocampus, amygdala). Here we found a significantly lower V_T in the patient group for the prefrontal cortex [0.64 ± 0.20 (controls) *vs.* 0.44 ± 0.15 (patients), $p=0.009$], the temporal lobes [0.66 ± 0.21 (controls) *vs.* 0.44 ± 0.19 (patients), $p=0.01$], the anterior cingulate cortex [0.53 ± 0.20 (controls) *vs.* 0.34 ± 0.18 (patients), $p=0.016$], and for amygdala [0.72 ± 0.30 (controls) *vs.* 0.49 ± 0.24 (patients), $p=0.045$] but not for hippocampus [0.55 ± 0.32 (controls) *vs.* 0.41 ± 0.13 (patients), $p=0.146$]. V_T differences in prefrontal cortex and temporal lobes were significant after Bonferroni correction for multiple tests. K_1 and k_2 showed no differences between the groups for any of the ROIs. The mean ± S.D. of V_T for each group are shown for each ROI in Fig. 1.

A pixel × pixel *t* test (without scaling) comparing both groups (13 *vs.* 13) in SPM2, showed several clusters, mainly located in temporal and frontal regions, in which the tracer uptake (V_T) was lower in the patient group, $p_{FDR}=0.028$ (see Table 2). The largest cluster measured 52 cm^3, including predominantly temporal lobes, reaching from the precentral gyrus to cerebellum and to the parietal lobes. The other large cluster (36 cm^3) included the prefrontal cortex (see Fig. 2). The clusters that reached statistical significance overlapped

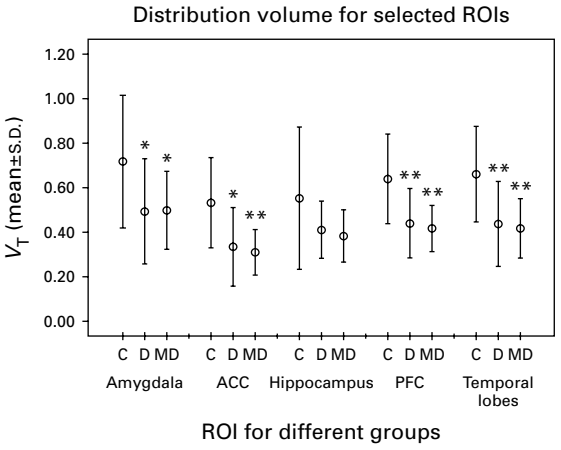

Fig. 1. *t* test comparison of the two groups at selected regions of interest (ROI). Distribution volume (V_T) (○, mean; I, standard deviation) is shown in regions relevant for depression. ACC, anterior cingulate cortex; PFC, prefrontal cortex; C, control group; D, patient group with a depressive episode ($n=13$); MD, subgroup of patients with a major depressive disorder ($n=10$). Both patient groups showed a significant decrease in V_T in all regions except the hippocampus (* $p<0.05$, ** $p<0.05$ after Bonferroni correction).

to a great extent with the areas found in the ROI analysis. Length of present episode (in weeks), HAMD scores, number of previous episodes, allelic variation of MDR-1 polymorphisms 3435C > T and 2677G > T, diagnosis and administered verapamil activity as covariates in ANCOVA had no significant effect as confounder on the results.

A subanalysis of the 10 patients with MDD was also performed. The subset was compared to a matched control group both at ROI level and in a voxel-wise analysis in SPM2. The results in the voxel-based approach indicated a somewhat stronger decrease in

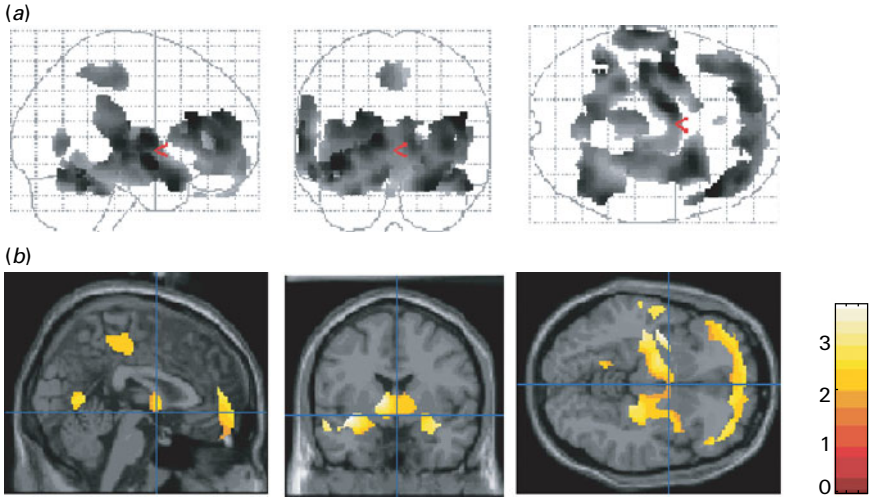

Fig. 2. T-map showing a diminished distribution volume of [^{11}C]verapamil in 13 patients with a depressive episode compared to the healthy control group ($n=13$). The clusters that reached statistical significance in a voxel-wise t test ($p_{FDR}<0.05$) (in SPM2) are shown. (*a*) Significant projections on a glass brain. (*b*) MRI overlay: a large cluster located in frontal cortex, left and right temporal lobes, and subcortical nuclei is shown.

V_T compared to the control group. A large cluster (240 cm^3, $p_{FDR}=0.016$, $t_{max}=4.83$) was found, comprising mainly temporal lobes and frontal cortex (data not shown). The V_T in ROI whole brain was also significantly lower than in the matched control group [0.75 ± 0.25 (controls) *vs.* 0.55 ± 0.08 (MDD), $p=0.030$]. The results for selected ROIs are shown in Fig. 1.

Finally, we compared the subgroup of patients with a treatment-resistant depressive episode ($n=7$) to a matched control group. This comparison gave comparable results in both approaches as the analysis of the whole group ($n=13$) did, yielding a significant decrease in V_T both at ROI level and at voxel level in the group with a treatment-resistant depressive episode (data not shown).

Discussion

This study shows a significantly lowered [^{11}C]verapamil uptake (V_T) in prefrontal cortex and temporal lobes in patients with a major depressive episode. The results of different methods of analysis were all in accordance with each other.

In functional imaging studies the prefrontal cortex and temporal lobes have often been associated with MDD (Brody *et al*. 2001; Drevets, 2000). To our knowledge this is the first study showing involvement of P-gp in medicated patients with MDD.

Seven of the 13 patients studied were considered to be treatment resistant, most patients were admitted to a psychiatric hospital, indicating either a severe depressive episode or a chronic course of the illness. Although clinical parameters indicating treatment resistance or chronicity were not significant cofactors in ANCOVA, a significant decrease in V_T (in temporal and frontal areas) was found in a subset of patients with a treatment-resistant depressive disorder (compared to a matched control group).

Treatment resistance in depression may in fact be associated with increased P-gp function. Increased P-gp function may cause low uptake of antidepressants. Similar to treatment-resistant epilepsy where increased expression of P-gp is associated with resistance to anti-epileptics and poor prognosis (Loscher & Potschka, 2002; Sisodiya *et al*. 2001), treatment resistance may also have influenced our results.

Regional differences in V_T may reflect localized regions of higher P-gp function, which may be under the influence of a functional polymorphism of MDR1. The SNP C3435T has been associated with altered P-gp function, albeit in an intestinal cell line (Hoffmeyer *et al*. 2000), which does not necessarily reflect the P-gp expression in brain cells. However, the impact of genetic variations in the *MDR1* gene on the course of MDD or the response to antidepressants is considered to be moderate or absent, and results are conflicting (Eichelbaum *et al*. 2004; Laika *et al*. 2006; Mihaljevic-Peles *et al*. 2007; Qian *et al*. 2006; Woodahl & Ho, 2004). The frequencies of the determined polymorphism in our study group did not differ from the frequencies seen in the general population (Tan *et al*. 2004; Woodahl & Ho, 2004).

Our results may also be explained by a neuroinflammatory process. There is increasing evidence for the role of cytokines in the pathogenesis of depression. Inflammation results in the release of pro-inflammatory cytokines, acting as a neuromodulator and accounting for most of the symptoms in depression (Raison *et al*. 2006; Schiepers *et al*. 2005). Experimental animal models of inflammation show that inflammation can influence P-gp expression and activity in different ways (Fernandez *et al*. 2004; McRae *et al*. 2003; Monville *et al*. 2002). Although a decrease in function and expression of P-gp seems to be the case in acute inflammatory models, the study by Tan and colleagues shows that P-gp function was increased after the acute inflammatory phase (Tan *et al*. 2002). The course of a depressive episode may be similar in such a way that in the chronic phase P-gp is up-regulated. Post- mortem studies are warranted to confirm these hypotheses. It is desirable that further (neuroimaging) studies are conducted to shed light on the neuroinflammatory events in MDD.

Regional differences in V_T could be explained by an increase in atrophy in cortical brain areas in major depression. However, the most consistent findings in studies using structural MRI is reduction in hippocampal volume (Videbech & Ravnkilde, 2004) and basal ganglia, whereas atrophy in frontal regions has been found less consistently. The most robust reduction in V_T, in our study was seen in the temporal lobes. The fact that no differences in V_T were found in the hippocampus at ROI level may be due to spill-in of radioactivity of the adjacent choroid plexus, since is has been reported that there is a high accumulation of radioactivity in the choroid plexus (Langer *et al*. 2007).

A possible limitation of the present study is the fact that patients were treated with antidepressant medication. The increase in P-gp function in our study could be caused by the use of medication. Drugs or substances that are known to enhance P-gp expression were all excluded from our study (see Table 1). Many of the antidepressant and antipsychotic drugs used are substrates for P-gp, but their effect on P-gp activity is probably not clinically relevant (El Ela *et al*. 2004; Peer *et al*. 2004; Weber *et al*. 2005; Weiss *et al*. 2003*a*).

Several antidepressants and anticonvulsants are known to inhibit the P-gp pump in a concentration-dependent manner in porcine brain cells (Peer *et al*. 2004; Weber *et al*. 2005; Weiss *et al*. 2003*a*, *b*). In *in-vitro* studies of antipsychotics inhibition of P-gp was only seen in concentrations above therapeutically relevant plasma levels, thus suggesting that the inhibitory effect of antipsychotics may not play a role in clinical practice (El Ela *et al*. 2004; Weber *et al*. 2005). Recently, it was found that venlafaxine (used by three patients) can induce P-gp in human Caco-2 cells (Ehret *et al*. 2007). Nevertheless, the implication of chronic use of antidepressant and/or antipsychotic drugs is not known. It cannot be excluded that antidepressant and/or antipsychotic agents given for a sustained period lead to an up-regulation of the P-gp pump. To rule out this possibility, future studies in medication-naive patients are needed.

In addition to a possible effect on P-gp function, medication may also have influenced the metabolism of [^{11}C]verapamil. It is known that anticonvulsants influence [^{11}C]verapamil metabolism, probably through induction of cytochrome P450 (Abrahim *et al*. 2008). Two of our patients were on valproic acid. However, leaving these two patients out of the analysis made no difference to the results. Medication may also have influenced the free fraction of [^{11}C]verapamil, leading to a lower V_T effect in the patient group. The influx parameter (K_1) suggested no difference between the two groups, due to a large variance. The fact that only certain areas (i.e. temporal lobes and prefrontal cortex) showed a significant decrease in V_T cannot be explained in this way.

In the present study no analysis of metabolites was performed. Although this can be seen as another limitation of the study, we assume that the total contribution of metabolites, that contribute to the PET signal, but have no affinity for the P-gp pump, is small. Only the *N*-demethylated fraction (so-called polar fraction) has no affinity for P-gp (Lubberink *et al*. 2007; Pauli-Magnus *et al*. 2000; Sasongko *et al*. 2005). As Lubberink *et al*. (2007) have shown the 'one tissue compartment' model gives an excellent fit of the data, irrespective of metabolite input. Their data indicate that the contribution of the polar fraction to the brain signal is small. However, it cannot be excluded that chronic use of antipsychotic and antidepressant agents may have influenced the metabolism of [^{11}C]verapamil, thereby reducing brain uptake of the radiotracer in patients. However, the fact that a decrease in V_T is only seen in specific areas can probably not be attributed to the metabolites of [^{11}C]verapamil.

In summary, in our PET study using [^{11}C]verapamil as a tracer, we have found evidence for an increased function of P-gp in patients with a major depressive episode under long-term treatment conditions, which for the first time may provide an explanation for treatment resistance in patients suffering from MDD.

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None.

Statement of Interest

None.

References

Abi-Dargham A, Martinez D, Mawlawi O, Simpson N, Hwang DR, Slifstein M, Anjilvel S, Pidcock J, Guo NN, Lombardo I, *et al.* (2000). Measurement of striatal and extrastriatal dopamine D1 receptor binding potential with [^{11}C]NNC 112 in humans: validation and reproducibility. *Journal of Cerebral Blood Flow and Metabolism* **20**, 225–243.

Abrahim A, Luurtsema G, Bauer M, Karch R, Lubberink M, Pataraia E, Joukhadar C, Kletter K, Lammertsma AA, Baumgartner C, Muller M, Langer O (2008). Peripheral metabolism of (R)-[(^{11})C]verapamil in epilepsy patients. *European Journal of Nuclear Medicine and Molecular Imaging* **35**, 116–123.

Bartels AL, Kortekaas R, Bart J, Willemsen ATM, de Klerk OL, de Vries JJ, van Oostrom JCH, Leenders KL (2008*b*). Blood-brain barrier P-glycoprotein function decreases in specific brain regions with aging: A possible role in progressive neurodegeneration. *Neurobiology of Aging*. Published online: 19 March 2008. doi:10.1016/j.neurobiolaging.2008.02.002.

Bartels AL, Willemsen AT, Kortekaas R, de Jong BM, de Vries R, de Klerk O, van Oostrom JC, Portman A, Leenders KL (2008*a*). Decreased blood-brain barrier P-glycoprotein function in the progression of Parkinson's disease, PSP and MSA. *Journal of Neurotransmission* **115**, 1001–1009.

Bauer B, Hartz AM, Fricker G, Miller DS (2005). Modulation of p-glycoprotein transport function at the blood-brain barrier. *Experimental Biological Medicine* (*Maywood*) **230**, 118–127.

Brody AL, Barsom MW, Bota RG, Saxena S (2001). Prefrontal-subcortical and limbic circuit mediation of major depressive disorder. *Seminars in Clinical Neuropsychiatry* **6**, 102–112.

Cordon-Cardo C, O'Brien JP, Boccia J, Casals D, Bertino JR, Melamed MR (1990). Expression of the multidrug resistance gene product (P-glycoprotein) in human normal and tumor tissues. *Journal of Histochemistry and Cytochemistry* **38**, 1277–1287.

Drevets WC (1999). Prefrontal cortical-amygdalar metabolism in major depression. *Annals of the New York Academy of Science* **877**, 614–637.

Drevets WC (2000). Functional anatomical abnormalities in limbic and prefrontal cortical structures in major depression. *Progress in Brain Research* **126**, 413–431.

Dunner DL (2006). Definitions and clinical characteristics of treatment-resistent depression. 159th Annual Meeting of the American Psychiatric Association, Toronto, Canada, 2006, p. 8A [Abstract].

Ehret MJ, Levin GM, Narasimhan M, Rathinavelu A (2007). Venlafaxine induces P-glycoprotein in human Caco-2 cells. *Human Psychopharmacology* **22**, 49–53.

Eichelbaum M, Fromm MF, Schwab M (2004). Clinical aspects of the MDR1 (ABCB1) gene polymorphism. *Therapeutic Drug Monitoring* **26**, 180–185.

El Ela AA, Hartter S, Schmitt U, Hiemke C, Spahn-Langguth H, Langguth P (2004). Identification of P-glycoprotein substrates and inhibitors among psychoactive compounds – implications for pharmacokinetics of selected substrates. *Journal of Pharmacy and Pharmacology* **56**, 967–975.

Elsinga PH, Franssen EJ, Hendrikse NH, Fluks L, Weemaes AM, van der Graaf WT, de Vries EG, Visser GM, Vaalburg W (1996). Carbon-11-labeled daunorubicin and verapamil for probing P-glycoprotein in tumors with PET. *Journal of Nuclear Medicine* **37**, 1571–1575.

Elsinga PH, Hendrikse NH, Bart J, Vaalburg W, van Waarde A (2004). PET Studies on P-glycoprotein function in the blood-brain barrier: how it affects uptake and binding of drugs within the CNS. *Current Pharmaceutical Design* **10**, 1493–1503.

Fernandez C, Buyse M, German-Fattal M, Gimenez F (2004). Influence of the pro-inflammatory cytokines on P-glycoprotein expression and functionality. *Journal of Pharmacy & Pharmaceutical Sciences* **7**, 359–371.

Hamilton M (1960). A rating scale for depression. *Journal of Neurology, Neurosurgery and Psychiatry* **23**, 56–62.

Hampel H, Kotter HU, Moller HJ (1997). Blood-cerebrospinal fluid barrier dysfunction for high molecular weight proteins in Alzheimer disease and major depression: indication for disease subsets. *Alzheimer Disease and Associated Disorders* **11**, 78–87.

Hampel H, Muller-Spahn F, Berger C, Haberl A, Ackenheil M, Hock C (1995). Evidence of blood-cerebrospinal fluid-barrier impairment in a subgroup of patients with dementia of the Alzheimer type and major depression: a possible indicator for immunoactivation. *Dementia* **6**, 348–354.

Hendrikse NH, de Vries EG, Eriks-Fluks L, van der Graaf WT, Hospers GA, Willemsen AT, Vaalburg W, Franssen EJ (1999). A new in vivo method to study P-glycoprotein transport in tumors and the blood-brain barrier. *Cancer Research* **59**, 2411–2416.

Hendrikse NH, Schinkel AH, de Vries EG, Fluks E, van der Graaf WT, Willemsen AT, Vaalburg W, Franssen EJ (1998). Complete in vivo reversal of P-glycoprotein pump function in the blood-brain barrier visualized with positron emission tomography. *British Journal of Pharmacology* **124**, 1413–1418.

Hoffmeyer S, Burk O, von Richter O, Arnold HP, Brockmoller J, Johne A, Cascorbi I, Gerloff T, Roots I, Eichelbaum M, Brinkmann U (2000). Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. *Proceedings of the National Academy of Sciences USA* **97**, 3473–3478.

Karssen AM, Meijer OC, vander Sandt ICJ, Lucassen PJ, de Lange ECM, de Boer AG, de Kloet ER (2001). Multidrug resistance P-glycoprotein hampers the access of cortisol

but not of cortisone to mouse and human brain. *Endocrinology* **142**, 2686–2694.

Kortekaas R, Leenders KL, van Oostrom JC, Vaalburg W, Bart J, Willemsen AT, Hendrikse NH (2005). Blood-brain barrier dysfunction in parkinsonian midbrain in vivo. *Annals of Neurology* **57**, 176–179.

Laika B, Leucht S, Steimer W (2006). ABCB1 (P-glycoprotein/MDR1) gene G2677T/a sequence variation (polymorphism): lack of association with side effects and therapeutic response in depressed inpatients treated with amitriptyline. *Clinical Chemistry* **52**, 893–895.

Langer O, Bauer M, Hammers A, Karch R, Pataraia E, Koepp MJ, Abrahim A, Luurtsema G, Brunner M, Sunder-Plassmann R, *et al.* (2007). Pharmacoresistance in epilepsy: a pilot PET study with the P-glycoprotein substrate R-[(^{11})C]verapamil. *Epilepsia* **48**, 1774–1784.

Loscher W, Potschka H (2002). Role of multidrug transporters in pharmacoresistance to antiepileptic drugs. *Journal of Pharmacology and Experimental Therapeutics* **301**, 7–14.

Loscher W, Potschka H (2005). Role of drug efflux transporters in the brain for drug disposition and treatment of brain diseases. *Progress in Neurobiology* **76**, 22–76.

Lubberink M, Luurtsema G, van Berckel BN, Boellaard R, Toornvliet R, Windhorst AD, Franssen EJ, Lammertsma AA (2007). Evaluation of tracer kinetic models for quantification of P-glycoprotein function using (R)-[^{11}C]verapamil and PET. *Journal of Cerebral Blood Flow and Metabolism* **27**, 424–433.

Matheny CJ, Lamb MW, Brouwer KR, Pollack GM (2001). Pharmacokinetic and pharmacodynamic implications of P-glycoprotein modulation. *Pharmacotherapy* **21**, 778–796.

Mayberg H (2002). Depression, II: localization of pathophysiology. *American Journal of Psychiatry* **159**, 1979.

McRae MP, Brouwer KL, Kashuba AD (2003). Cytokine regulation of P-glycoprotein. *Drug Metabolism Review* **35**, 19–33.

Mihaljevic-Peles A, Bozina N, Sagud M (2007). Pharmacogenetics in modern psychiatry. *Psychiatria Danubina* **19**, 231–233.

Monville C, Fages C, Feyens AM, D'Hondt V, Guillet C, Vernallis A, Gascan H, Peschanski M (2002). Astroglial expression of the P-glycoprotein is controlled by intracellular CNTF. *BMC Cell Biology* **3**, 20.

Pauli-Magnus C, von RO, Burk O, Ziegler A, Mettang T, Eichelbaum M, Fromm MF (2000). Characterization of the major metabolites of verapamil as substrates and inhibitors of P-glycoprotein. *Journal of Pharmacology and Experimental Therapeutics* **293**, 376–382.

Peer D, Dekel Y, Melikhov D, Margalit R (2004). Fluoxetine inhibits multidrug resistance extrusion pumps and enhances responses to chemotherapy in syngeneic and in human xenograft mouse tumor models. *Cancer Research* **64**, 7562–7569.

Qian W, Homma M, Itagaki F, Tachikawa H, Kawanishi Y, Mizukami K, Asada T, Inomata S, Honda K, Ohkohchi N, Kohda Y (2006). MDR1 gene polymorphism in Japanese patients with schizophrenia and mood disorders including depression. *Biological & Pharmaceutical Bulletin* **29**, 2446–2450.

Raison CL, Capuron L, Miller AH (2006). Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends in Immunology* **27**, 24–31.

Sasongko L, Link JM, Muzi M, Mankoff DA, Yang X, Collier AC, Shoner SC, Unadkat JD (2005). Imaging P-glycoprotein transport activity at the human blood-brain barrier with positron emission tomography. *Clinical and Pharmacological Therapeutics* **77**, 503–514.

Schiepers OJ, Wichers MC, Maes M (2005). Cytokines and major depression. *Progress in Neuro-psychopharmacology & Biological Psychiatry* **29**, 201–217.

Schinkel AH, Smit JJ, van Tellingen O, Beijnen JH, Wagenaar E, van Deemter L, Mol CA, van der Valk MA, Robanus-Maandag EC, te Riele HP (1994). Disruption of the mouse mdr1a P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. *Cell* **77**, 491–502.

Schinkel AH, Wagenaar E, Mol CA, van Deemter L (1996). P-glycoprotein in the blood-brain barrier of mice influences the brain penetration and pharmacological activity of many drugs. *Journal of Clinical Investigation* **97**, 2517–2524.

Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, Hergueta T, Baker R, Dunbar GC (1998). The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *Journal of Clinical Psychiatry* **59** (Suppl.) 20, 22–33.

Sisodiya SM, Lin WR, Harding BN, Squier MV, Thom M (2002). Drug resistance in epilepsy: expression of drug resistance proteins in common causes of refractory epilepsy. *Brain* **125**, 22–31.

Sisodiya SM, Lin WR, Squier MV, Thom M (2001). Multidrug-resistance protein 1 in focal cortical dysplasia. *Lancet* **357**, 42–43.

Talairach J, Tournoux P (1998). *Co-Planar Stereotaxic Atlas of the Human Brain: 3-Dimensional Proportional System – An Approach to Cerebral Imaging*. New York: Thieme Medical Publishers.

Tan EK, Drozdzik M, Bialecka M, Honczarenko K, Klodowska-Duda G, Teo YY, Tang K, Wong LP, Chong SS, Tan C, *et al.* (2004). Analysis of MDR1 haplotypes in Parkinson's disease in a white population. *Neuroscience Letters* **372**, 240–244.

Tan KH, Purcell WM, Heales SJ, McLeod JD, Hurst RD (2002). Evaluation of the role of P-glycoprotein in inflammation induced blood-brain barrier damage. *Neuroreport* **13**, 2593–2597.

Trentani A, Kuipers SD, ter Horst GJ, den Boer JA (2003). Intracellular signalling transduction dysregulation in depression and possible future targets for antidepressant therapy. In: Kasper S, den Boer JA, Sitsen JMA (Eds.), *Handbook of Depression and Anxiety*, 2nd edn (pp. 349–386), New York: Marcel Dekker.

Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, Mazoyer B, Joliot M

(2002). Automated anatomical labelling of activations in spm using a macroscopic anatomical parcellation of the MNI MRI single subject brain. *Neuroimage* **15**, 273–289.

Videbech P, Ravnkilde B (2004). Hippocampal volume and depression: a meta-analysis of MRI studies. *American Journal of Psychiatry* **161**, 1957–1966.

Weber CC, Kressmann S, Ott M, Fricker G, Muller WE (2005). Inhibition of P-glycoprotein function by several antidepressants may not contribute to clinical efficacy. *Pharmacopsychiatry* **38**, 293–300.

Wegman TD, Maas B, Elsinga PH, Vaalburg W (2002). An improved method for the preparation of [^{11}C]verapamil. *Applied Radiation and Isotopes* **57**, 505–507.

Weiss J, Dormann SM, Martin-Facklam M, Kerpen CJ, Ketabi-Kiyanvash N, Haefeli WE (2003*a*). Inhibition of P-glycoprotein by newer antidepressants. *Journal of Pharmacology and Experimental Therapeutics* **305**, 197–204.

Weiss J, Kerpen CJ, Lindenmaier H, Dormann SM, Haefeli WE (2003*b*). Interaction of antiepileptic drugs with human P-glycoprotein in vitro. *Journal of Pharmacology and Experimental Therapeutics* **307**, 262–267.

Woodahl EL, Ho RJ (2004). The role of MDR1 genetic polymorphisms in interindividual variability in P-glycoprotein expression and function. *Current Drug Metabolism* **5**, 11–19.