P-glycoprotein overactivity in epileptogenic developmental lesions measured in vivo using (R)-[11C]verapamil PET

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Abstract

Objective: Overexpression of the drug transporter P-glycoprotein (P-gp) is thought to be involved in drug-resistance in epilepsy by extrusion of antiepileptic drugs (AEDs). We used positron emission tomography (PET) and the P-gp substrate radiotracer (R)-[11C]verapamil (VPM) together with the third-generation P-gp inhibitor tariquidar (TQD) to evaluate P-gp function in individuals with drug-resistant epileptogenic developmental lesions.

Methods: Twelve healthy controls (7 male, median age 45, range 35-55 years), and two patients with epileptogenic developmental lesions (2 male, aged 24 and 62 years) underwent VPM-PET scans before and 60 minutes after a 30-minute infusion of 2 and 3 mg/kg TQD. The influx rate constant, VPM-K1, was estimated from the first 10 minutes of dynamic data using a single-tissue compartment model with a VPM plasma input function. Statistical parametric mapping (SPM) analysis was used to compare individual patients with the healthy controls.

Results: At baseline, SPM voxel-based analysis revealed significantly lower uptake of VPM corresponding to the area of the epileptogenic developmental lesion compared to 12 healthy controls ($P < .048$). This was accentuated following P-gp inhibition with TQD. After TQD, the uptake of VPM was significantly lower in the area of the epileptogenic developmental lesion compared to controls ($P < .002$).

Significance: This study provides further evidence of P-gp overactivity in patients with drug-resistant epilepsy, irrespective of the type of lesion. Identifying P-gp overactivity as an underlying contributor to drug-resistance in individual patients will enable novel treatment strategies aimed at overcoming or reversing P-gp overactivity.

Keywords: drug-resistant epilepsy, P-glycoprotein, positron emission tomography, tariquidar
INTRODUCTION

Despite advances in antiepileptic drug (AED) therapy, about one-third of epileptic patients are resistant to seizure-suppressant therapy. One proposed mechanism for drug-resistance is encapsulated by the "transporter hypothesis," which postulates that overactivity of drug transporter(s) at the blood–brain barrier (BBB) prevents drugs from reaching therapeutic concentrations at their targets. P-glycoprotein (P-gp), the best-studied multidrug efflux transporter, has been shown to be increased at the BBB in a variety of rodent epilepsy models and human tissue taken from surgery and postmortem brains.

Previous studies have shown that P-gp is ectopically expressed in surgically resected specimens from patients with drug-resistant epilepsy with a variety of structural abnormalities, including focal cortical dysplasia (FCD), dysplastic neuroepithelial tumors, and hippocampal sclerosis, which are three common causes of drug-resistant epilepsy.

Using positron emission tomography (PET) with the P-gp substrate radiotracer (R)-[11C]verapamil (VPM), we assessed P-gp activity and its functional relevance in vivo in patients with mesial temporal lobe epilepsy (mTLE). We showed an attenuated increase of VPM uptake after P-gp inhibition with tariquidar (TQD) in patients with drug-resistant mTLE compared to healthy controls, and this difference in the TQD response was most pronounced in the sclerotic hippocampus ($P < .0001$). One of the major drawbacks of studying patients with mTLE is that the region of most interest, the hippocampus, is difficult to quantify due to spillover of radioactivity from the adjacent choroid plexus. Other epileptogenic lesions located distant from the choroid plexus, would allow the quantitative analysis of the entire epileptogenic brain region, to better elucidate the role of P-gp in drug-resistant focal epilepsies.

Here, we report individual results of VPM-PET studies before and after the third-generation P-gp inhibitor TQD in two patients with drug-resistant epilepsy due to epileptogenic developmental lesions.

METHODS

Participants

We recruited three drug-resistant patients with epileptogenic developmental lesions (two male, aged 24 and 62 years) from consultant-led outpatient clinics at the National Hospital for Neurology and Neurosurgery in London, UK. Patients were recruited based on magnetic resonance imaging (MRI) findings suggesting FCD. Drug-resistance was defined as ongoing seizures despite two tolerated and appropriately prescribed AEDs. One patient was scanned at baseline, but the repeat scan after TQD failed due to radiochemistry failure. Therefore, the data are not included in the analysis.

Patient 1 had drug-resistant left temporal lobe epilepsy. MRI showed a lesion in the left temporal lobe, expanding into the left inferior temporal gyrus with signal change in the underlying white matter reaching the temporal horn, suggesting possible FCD or a low-grade neoplastic process. Interictal electroencephalography (EEG) showed sharp waves in the left anterior and mid-temporal regions. Repeated and prolonged video-telemetry-EEG captured habitual seizures and showed left temporal onset. The patient underwent left temporal lesionectomy after the VPM-PET scan. The histology revealed a hamartoma. Postoperatively, the patient did not become seizure-free but seizures are less severe, and overall the frequency reduced (Figure 3A,B).

Patient 2 had drug-resistant focal epilepsy. MRI showed an area of cortical thickening and hyperintensity affecting the cortex bordering the postcentral sulcus on the left with a T2-weighted hyperintense band extending from this to the ependymal surface, likely to represent an area of FCD. Repeated and prolonged video-telemetry-EEG captured habitual seizures, which were concordant with the epilepsy arising from the area surrounding the presumed focal cortical dysplasia, however, with different spread patterns into the supplementary motor area in some seizures and activation of the autonomic networks in others. Interictal EEG showed focal spikes in the left central region. Magnetoencephalography (MEG) examination results co-localized with the area of FCD. Due to the proximity of the FCD to his primary sensory cortex, the patient had intracranial EEG recording to map the seizure onset and sensory and motor cortex after the VPM-PET scan. Because of the high risk of sensory loss affecting his dominant hand, he chose not to proceed with surgery. He continues to experience seizures (Figure 4A,B). The patient’s characteristics are summarized in Table 1.
Twelve healthy controls (7 male, median age 45, range 35-55 years), with no neurological or psychiatric disorders and no regular or recent intake of drugs known to be a substrate or inhibitor for P-gp, were recruited through advertising in local newspapers, university newsletters, and word-of-mouth from the general population of Manchester, UK. Participants underwent a screening interview, with a neurological and general medical examination, routine biochemistry (liver and kidney functions), and hematology testing before inclusion.

The study was approved by the Moorfields and Whittington Research Ethics Committee, the University of Manchester Research Committee, and the UK Administration of Radioactive Substances Advisory Committee. All participants were given a detailed description of the study and gave written informed consent before enrollment.

### TABLE 1  
Clinical data of drug-resistant epilepsy patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender/age (y)</th>
<th>MRI</th>
<th>Age at onset (y)</th>
<th>Duration of epilepsy (y)</th>
<th>Average seizure frequency (per mo)</th>
<th>Last seizure before PET scan (d)</th>
<th>Current AEDs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M/62</td>
<td>Hamartoma left inferior temporal gyrus</td>
<td>17</td>
<td>45</td>
<td>10</td>
<td>5</td>
<td>ZON, LEV, CLB, OXC</td>
</tr>
<tr>
<td>2</td>
<td>M/24</td>
<td>FCD left posterior central gyrus</td>
<td>5</td>
<td>19</td>
<td>90</td>
<td>1</td>
<td>CBZ, LE</td>
</tr>
</tbody>
</table>

Abbreviations: AED, antiepileptic drug; CBZ, carbamazepine; CLB, clobazam; FCD, focal cortical dysplasia; LEV, levetiracetam; M, male; OXC, oxcarbazepine; PET, positron emission tomography; Pt, patient; ZON, zonisamide.

2.2 | PET Imaging

As described previously, to assess P-gp function, 60-minute VPM-PET scans were acquired at the Wolfson Molecular Imaging Centre in Manchester, UK, using the High-Resolution Research Tomograph (HRRT, CTI/Siemens). Each participant was injected with a smooth bolus intravenous injection of VPM: 555 MBq (range 370-740 MBq) for men, and 505 MBq (335-670 MBq) for women. Arterial blood was sampled for the duration of the PET scan. Radioactivity in arterial blood was assayed continuously for the first 15 minutes and discrete blood samples were processed using in-line solid-phase extraction and high-performance liquid chromatography. The time course of unmetabolized VPM concentration in arterial plasma was used as the input function for kinetic modeling of the dynamic PET data. The input function was generated by combining the second-by-second measurement of whole blood radioactivity concentration with the time course of plasma to whole blood ratios and the time course of fractions of unmetabolized VPM in plasma measured from the discrete arterial blood samples.

In all subjects, a 60-minute baseline VPM-PET scan was performed to assess P-gp function. To test the functional relevance of between-group baseline differences and to increase the sensitivity for group differences, drug-resistant patients and healthy controls underwent a second set of VPM-PET scans on the same day (except for two healthy controls who were studied on separate days for technical reasons) starting 60 minutes after the end of a 30-minute intravenous infusion with the third-generation P-gp inhibitor TQD (drug-resistant epilepsy patients received 3 mg/kg TQD, seven controls received 3 mg/kg TQD, five controls had 2 mg/kg TQD, Figure 1). Baseline and inhibitor scans were acquired in fixed order because of the long half-life of TQD (18-36 hours). The function of P-gp can be quantified in vivo from dynamic PET data using compartment modeling. For the kinetic modeling of the VPM-PET data a single-tissue compartment model was used, and to limit the effect of radio-labelled metabolites we undertook the kinetic analysis only on the first 10 minutes of dynamic PET data. Brain entry of the radiolabeled P-gp substrate VPM is limited by the action of the efflux transporter P-gp, and the net effect can be measured by $K_1$, the transport rate constant from blood-to-brain tissue compartment. Low VPM-$K_1$ values thus correspond to high P-gp-mediated brain-to-blood transport, and a smaller increase in VPM-$K_1$.
in response to a given dose of the P-gp inhibitor is indicative of higher P-gp function. To evaluate P-gp activity, dynamic PET scans were performed before and after partial inhibition with the third-generation P-gp modulator TQD. Successful P-gp inhibition increases VPM-K1, but this increase is attenuated in areas of high P-gp activity, since a standard dose of P-gp inhibitor is given systemically, which will inhibit only a lower percentage of P-gp binding sites in areas of high P-gp activity. For all participants, T1-weighted MRIs were acquired with a 3-Tesla GE Excite II scanner in London (General Electric). The dynamic 3D VPM-PET data acquisition was reconstructed using the ordinary Poisson ordered-subset-expectation-maximization-algorithm (OP-OSEM with 5 iterations and 16 subsets) and corrected for head motion.\(^{14}\) For the voxel-based analysis, we employed weighted generalized linear least-squares to generate parametric VPM-K1 maps\(^{15}\) and smoothed the dynamic images with a 2-mm Gaussian filter.

### 2.3 PET image analysis

The imaging processing steps were done in SPM 12 (Wellcome Trust Centre for Neuroimaging, London, UK).\(^{16}\) MRI and co-registered VPM-K1 maps were spatially normalized with a symmetrical and centered gray matter DARTEL template. To normalize for differing rates of peripheral metabolism of VPM and to allow for detection of regionally specific differences between healthy controls and patients, we created VPM-PET images corrected for differences in global means; first we calculated the population mean whole-brain gray matter from the patients and controls, and then multiplied the individual VPM-K1 parametric maps with the ratio of population to individual mean whole brain VPM-K1.\(^{11}\) The normalized VPM-K1 maps were finally smoothed using an isotropic 2-mm Gaussian filter.

For each patient an individual region of interest (ROI) of the epileptogenic developmental lesion was manually drawn by one of the investigators (M.F.) on the T1-weighted MRI images using the MRIcron software.\(^{17}\) The ROI of the epileptogenic developmental lesion was transformed into a binary mask, smoothed with a 2-mm width Gaussian kernel and spatially normalized to the symmetrical and centered gray matter DARTEL template as described above. The SPM analysis was then restricted to voxels belonging to the binary masks of the ROI of the epileptogenic developmental lesion for each individual patient analysis. In addition, we tested whether changes in P-gp activity were regional or also extended to other areas of the brain. For this analysis we used the ROI of the epileptogenic developmental lesion, which was spatially normalized to

**Figure 2** MRI of the epilepsy lesions and region-of-interest (ROI) placement. Images are displayed in neurological convention (subject’s left is displayed on image left). Patient 1: ROI of the hamartoma (left inferior temporal gyrus) displayed in red and contralateral ROI in blue (A), with surrounding ROI seen in red (B). Patient 2: ROI of the presumed FCD (left posterior central gyrus) displayed in red and contralateral ROI in blue (C), with surrounding ROI in red (D).
the DARTEL template as described above and flipped to analyze the ROI of the contralateral side individually for each patient. Furthermore, we analyzed the area surrounding the ROI by smoothing the ROI with a 6-mm-width Gaussian kernel and then subtracted this smoothed ROI from the original ROI (Figure 2; Patient 1: Figure 2A,B, Patient 2: Figure 2C,D).

Parametric 

Parametric VPM-K1 maps of drug-resistant epilepsy patients were compared to healthy controls (n = 12; "one-against-all-analysis"). For the analysis at baseline, a two-sample t test was applied between individual patient data and the control group, assuming unequal variance, with an analysis of covariance (ANCOVA) by subject, without overall grand mean scaling and explicit masks of the ROIs of the epileptogenic developmental lesions, the contralateral ROIs, and the ROIs surrounding the epileptogenic developmental lesion for each individual analysis. We used the full factorial design analysis for the analysis after P-gp inhibition with TQD with unequal variance between groups, an ANCOVA by group, and an explicit mask of the ROI of the epileptogenic developmental lesion for each individual analysis. In view of our a priori hypothesis we report difference in VPM uptake at a threshold \( P < .05 \) (uncorrected), with an extended threshold of 10 voxels for the ROIs of the epileptogenic developmental lesion. Family-wise error corrections for multiple comparisons were performed at \( P < .05 \) for the analysis of the ROIs of the contralateral sides and the ROIs surrounding the epileptogenic developmental lesion.

### 2.4 Statistical analysis

Statistical analysis was performed using SPSS 25.0. We used independent nonparametric tests to compare the two groups. The level of significance was set at \( P < .05 \).

### 3 RESULTS

There was no statistically significant difference in age, weight, or injected doses of VPM between the drug-resistant epilepsy patients and the healthy controls (\( P > .05 \)).

<table>
<thead>
<tr>
<th>Patient</th>
<th>Cluster size (voxels)</th>
<th>Peak voxel coordinates (x, y, z; mmm)</th>
<th>( Z_{\text{max}} )</th>
<th>Peak level ( P ) (uncorrected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23</td>
<td>−56 6 −34</td>
<td>4.11</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>16</td>
<td>−54 −8 −36</td>
<td>4.15</td>
<td></td>
<td>&lt;.001</td>
</tr>
<tr>
<td>16</td>
<td>−26 −4 −44</td>
<td>3.04</td>
<td></td>
<td>.001</td>
</tr>
<tr>
<td>17</td>
<td>−52 −12 −42</td>
<td>2.92</td>
<td></td>
<td>.002</td>
</tr>
<tr>
<td>2</td>
<td>11</td>
<td>−46 −30 54</td>
<td>3.07</td>
<td>.001</td>
</tr>
</tbody>
</table>

Note: Clusters reaching significance at \( P < .05 \) after blocking with TQD (with a cluster-extent threshold of 10 voxels). For Patient 1: all four clusters of focal differences in VPM-K1 after P-gp inhibition with TQD was located within the area of the developmental lesion.

### 3.1 Patient 1

At baseline, the uptake of VPM was minimally reduced in the area of the hamartoma in the left inferior temporal gyrus (Patient 1: 0.020 vs 12 controls, 0.038 mL/min/cm³, standard deviation (SD) = 0.014 mL/min/cm³, range = 0.018-0.065 mL/min/cm³, cluster size: 1 voxel; \( P < .048 \), uncorrected).

After P-gp inhibition with TQD, the uptake of VPM in Patient 1 was significantly attenuated in the area of the hamartoma in the left inferior temporal gyrus (Patient 1: 4.1% increase of VPM after 3 mg/kg TQD vs 57.5% (SD = 22%, range = 25.2%-91.6%) in controls (n = 7 for 3 mg/kg TQD), \( P < .002 \)) indicating increased P-gp activity in the area of the hamartoma (Figure 3C, Table 2).

The analysis of the contralateral ROI of the hamartoma did not show any significant changes at baseline (\( P = 1.000 \)) or after P-gp inhibition with TQD (\( P = .881 \)).

The analysis of the area surrounding the hamartoma did not show any significant changes at baseline (\( P = 1.000 \)). After P-gp inhibition with TQD, the uptake of VPM in Patient 1 was significantly attenuated in the area surrounding the hamartoma in the left temporal lobe (Patient 1: 47.9% increase of VPM after 3 mg/kg TQD vs 122.3% (SD = 61.7%, range = 30.2%-233.6%) in controls (n = 7 for 3 mg/kg TQD), cluster size: 11 voxel; \( P < .016 \)), indicating increased extended P-gp activity in the area surrounding the hamartoma (Figure 3D).

### 3.2 Patient 2

At baseline there was minimal reduced uptake of VPM corresponding to the area of the FCD in the left posterior central gyrus (patient 2: 0.020 vs 12 controls: 0.041 mL/min/cm³, SD = 0.017 mL/min/cm³, range = 0.015-0.063 mL/min/cm³, cluster-size: 1 voxel; \( P < .044 \), uncorrected).

After P-gp inhibition with TQD, VPM uptake in Patient 2 was significantly attenuated in the corresponding area of FCD in the left posterior central gyrus (after 3 mg/kg TQD Patient 2: 34.3% increase of VPM vs 122.3% (SD = 61.7%, range = 30.2%-233.6%) in controls (n = 7 for 3 mg/kg TQD), cluster size: 1 voxel; \( P < .016 \)), indicating increased extended P-gp activity in the area surrounding the hamartoma.
The analysis of the contralateral ROI of the FCD did not show any significant changes at baseline \( (P = .795) \) or after P-gp inhibition with TQD \( (P = .465) \). The analysis of the area surrounding the FCD did not show any significant changes at baseline \( (P = 1.000) \) or after P-gp inhibition with TQD \( (P = .957) \).

**DISCUSSION**

This is the first in vivo human PET study investigating P-gp function in patients with different epileptogenic developmental lesions compared to healthy controls. Previous PET studies with the radiolabeled P-gp substrate VPM were performed exclusively in patients with TLE due to hippocampal...
Evidence for P-gp expression in drug-resistant epilepsy has been derived mainly from studying epileptogenic human brain tissue resected during epilepsy surgery. Upregulation of P-gp was seen in glia, which do not normally express this protein in patients with FCD, dysembryoplastic neuroepithelial tumors, and hippocampal sclerosis. In addition, P-gp is intensely expressed in both neurons and reactive astrocytes in most dysplastic tissues and endothelial cells of patients with FCD. Although these findings support the “transporter hypothesis,” increased multidrug efflux transporter expression may not be functionally relevant. Therefore, non-invasive brain imaging of multidrug efflux transporter function in patients with drug-resistant epilepsy is a strategy to evaluate whether regional overexpression of multidrug efflux transporters at the BBB could plausibly contribute to drug-resistance in focal epilepsy.

We can only report on the postoperative outcome of Patient 1, who continued to have seizures following lesionectomy. Even after the removal of the visible lesion on MRI, residual microscopic lesions can still be epileptogenic, which means that developmental lesions tend to be more extensive than is apparent on MRI. We postulate that our widespread areas of attenuated VPM uptake suggestive of P-gp overactivity reflect these extensive lesions not visible on MRI. Our observations of extended PET changes in areas surrounding and far beyond the lesion, in association with poor outcome, are in keeping with the findings by Langer et al., who used PET and VPM to test for differences in P-gp activity between epileptogenic and non-epileptogenic brain regions of patients with drug-resistant unilateral mTLE in seven patients undergoing epilepsy surgery. Optimal surgical outcome was associated with higher temporal lobe P-gp function before surgery, higher P-gp-positive staining in surgically resected hippocampal specimens, and reduction in global P-gp function postoperatively, compared with nonoptimal surgery outcome. Their result also indicated that P-gp expression is dynamic and changes with seizure frequency or AED treatment.

Our results were externally reproduced in a study using VPM-PET and the nonselective P-gp inhibitor cyclosporine A in six patients with drug-resistant epilepsy and five patients with drug-sensitive epilepsy compared to eight healthy controls. All patients with drug-resistant epilepsy had asymmetry that was significantly different from that of the healthy controls, whereas all patients with drug-sensitive epilepsy had an asymmetry similar to that in healthy controls. In addition, specific regions that had significant asymmetry were different between the lateral and medial temporal lobe epilepsy groups. We demonstrated in our study that P-gp overactivity is also present in pathologies of focal epilepsies other than mTLE and show that this may be a relevant mechanism of drug-resistant epilepsy.

### 4.1 Limitations

Our study is limited by the small number of patients studied to date, and the difficulties in performing paired scans in this patient cohort. Group analysis in patients with different epileptogenic developmental lesions is difficult to perform, as epileptic foci are located in different locations of the brain. Because of the small number of patients, further analyses investigating genetic information (ie, polymorphisms) or seizure frequency or duration of epilepsy would not have been meaningful.

Despite the use of a high-resolution scanner, PET inherently has limited spatial resolution, which is further reduced at the bottom of the sulcus due to volume averaging or partial volume effects. This might explain why the maximum abnormality in Patient 2 with a typical bottom of the sulcus dysplasia, is seen closer to the gyral crown, rather than the the maximal site of dysmorphic neurons in this particular FCD.

### 5 Conclusions

This is the first study demonstrating P-gp overactivity in vivo in two patients with drug-resistant epilepsy due to different epileptogenic developmental lesions. The VPM uptake was found to increase after P-gp inhibition and attenuated in the epileptogenic zone, but also extended further to other ipsilateral regions, suggesting widespread
abnormalities in drug-resistant patients compared to healthy controls. Increased P-gp activity is not specific for a certain epilepsy pathology, since this has been demonstrated now in mTLE patients with hippocampal sclerosis, and in patients with FCD or hamartoma. We provide further support for the “transporter hypothesis” that overactivity of P-gp could plausibly contribute to drug resistance in epilepsy. However, it is not possible to be certain whether the observed changes in P-gp activity are the cause or consequence of seizure activity.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest to disclose. We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this article is consistent with those guidelines.

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