Chapter 6

[\textsuperscript{11}C]phenytoin quantification using parametric methods

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Abstract

**Purpose:** In this study the performance of various methods for generating quantitative parametric images of dynamic $[^{11}\text{C}]$phenytoin PET studies will be evaluated. **Procedures:** Double baseline 60 min dynamic $[^{11}\text{C}]$phenytoin PET studies, including online arterial sampling, were acquired for 6 healthy subjects. Parametric images were generated using Logan plot analysis, a basis function method (BFM) and spectral analysis (SA). Parametric distribution volume ($V_T$) and influx rate ($K_1$) were compared to those obtained from non-linear regression (NLR) analysis of time activity curves. In addition, global and regional test-retest (TRT) variability was determined for parametric $K_1$ and $V_T$ values. **Results:** Biases in $V_T$ observed with all parametric methods were less than 5%. For $K_1$, SA showed negative bias of 16%. Mean TRT variabilities of $V_T$ and $K_1$ were less than 10% for all methods. Shortening the scan duration to 45 min provided similar $V_T$ and $K_1$ with comparable TRT performance compared to 60 min data. **Conclusions:** Among the various parametric methods tested, BFM provided parametric $V_T$ and $K_1$ values with the least bias compared to NLR data and showed TRT variabilities lower than 5%, also for smaller volume of interest (VOI) sizes (i.e. higher noise levels) and shorter scan duration.
6.1 Introduction

It has been proposed that P-glycoprotein (Pgp) may play a role in treatment-resistant central nervous system disorders [1]. Pgp is an ATP-binding cassette transmembrane glycoprotein located at the luminal side of the blood brain barrier, where it functions as an efflux transporter and hence prevents its substrates from entering the brain. In common central nervous system disorders, such as epilepsy, overexpression of Pgp is thought to be an important mechanism of pharmacoresistance [2, 3].

At present, several 11C labelled Pgp substrate tracers are available, which are promising imaging tools for in vivo assessment of Pgp function at the blood brain barrier. The best characterised tracers are (R)-[11C]verapamil [4–7] or 11C-N-desmethylloperamide ([11C]dLop) [8]. In addition, tracers to measure Pgp expression, such as [11C]laniquidar [8–10] and [11C]tariquidar [11], are available.

In principle, mapping of the Pgp distribution using positron emission tomography (PET) can also be performed using relatively weak Pgp substrate tracers, as these should yield higher cerebral uptake than strong substrate tracers, such as (R)-[11C]verapamil [7]. Phenytoin is a weak Pgp substrate and, in a previous study, a reversible single tissue compartment model with blood volume parameter ($1T2k + V_b$) was identified as the best candidate model for describing [11C]phenytoin kinetics in normal subjects [12]. [11C]phenytoin showed a number of potential advantages, such as low metabolism, lower efflux rate ($k_2$) than e.g. (R)-[11C]verapamil, and the ability to shorten scan duration from 60 to 45 min without notable loss of accuracy and precision of the pharmacokinetic parameters [12].

To study differences in Pgp function at the voxel level, parametric [11C]phenytoin images may be needed. To date, no parametric methods have been applied to [11C]phenytoin data yet. Therefore, the aim of the present study was to investigate the performance of various parametric methods for describing [11C]phenytoin kinetics.

6.2 Materials and Methods

6.2.1 Scanning protocol

A total of six healthy male volunteers (mean age 28 years, range 21 to 32 years) were included in this study. All subjects were screened for medical history and underwent physical (including neurological) examination and laboratory tests. Subjects were also screened for neurological and psychiatric illness and history of drug abuse. Written informed consent was obtained from each subject after giving a written and verbal description of the study. The study was approved by the Medical Ethics Review Committee of the VU University Medical Center.
All subjects underwent two baseline dynamic PET studies on the same day. Scans were performed on a Gemini TF PET/CT scanner (Philips Medical Systems). The properties of this scanner have been reported elsewhere [13]. Prior to tracer injection, a low-dose computed tomograph scan was performed. These data were used to correct the subsequent emission scan for photon attenuation. Next, a dynamic emission scan in 3D acquisition mode was started simultaneously with an intravenous injection of 345 ± 54 (mean ± standard deviation (SD)) MBq of \( {^11}\text{C} \)phenytoin with a specific activity of 72 ± 27 MBq·μmol\(^{-1}\). \( {^11}\text{C} \)phenytoin was synthesized as described previously [14]. The radiotracer was provided in a total volume of 7 mL and administered at a rate of 0.8 mL·s\(^{-1}\), followed by a flush of 42 mL saline at 2.0 mL·s\(^{-1}\) using an infusion pump (Med-Rad).

During the 60 min \( {^11}\text{C} \)phenytoin scan, arterial blood was withdrawn continuously using an automatic on-line blood sampler (Veenstra Instruments) [15] at a rate of 5 mL·min\(^{-1}\) for the first 5 min and 2.5 mL·min\(^{-1}\) thereafter. At 2.5, 5, 10, 20, 30, 40 and 60 min after tracer injection, continuous blood sampling was interrupted briefly to withdraw 7 mL manual blood samples. After each sample the arterial line was flushed with a heparinised saline solution. In addition, all subjects underwent a structural magnetic resonance imaging scan using a 1.5T Sonata scanner (Siemens Medical Solutions). More details on the study protocols can be found elsewhere [12].

### 6.2.2 Blood and Image Analysis

Manual samples were used to determine plasma to whole blood radioactivity concentration ratios. In addition, concentrations of parent \( {^11}\text{C} \)phenytoin and its radioactive metabolites in plasma were determined using the described procedure described by Mansor et al. [12]. While for the image analysis, each MR image was co-registered with the corresponding PET image using VINCI software (Max Plank Institute). Next, VOI were defined using an automated method magnetic resonance imaging template based method (PVElab) [16]. The PgP is fairly uniformly distributed over the brain and therefore we used PVElab with the Hammers template that consisted of 67 regions covering the entire brain [17]. Grey and white matter segmentation of the co-registered MR image was performed using statistical parametric mapping version 8 (SPM8), which is incorporated in the PVElab software. These segmentations were used in combination with the above mentioned VOI to extract grey matter regional values from the dynamic PET studies.

### 6.2.3 Parametric Analysis

Three different parametric methods were evaluated, the Logan plot analysis [18], a BFM implementation of the single tissue compartment model with blood volume parameter [19] and SA [20]. For Logan, start and end times used were 12 and 140 min, respectively. For
BFM method, the start and end basis function exponentials are 0.04 and 0.1 min$^{-1}$ while for SA these are 0.03 and 0.17 min$^{-1}$ respectively. Both methods were used with 30 basis functions. The distribution volume ($V_T$) was obtained with all three methods, whilst the influx rate constant ($K_1$) was obtained using BFM and SA methods. The finding from this parametric studies will be compared with a previous NLR compartmental analysis performed on the same data [12]. Due to the sensitivity of NLR to noise, comparisons were performed at the VOI level with both small ($\leq 5$mL) and large ($>5$mL) volumes of interest to assess its effect on the test-retest variability of the studied parametric methods. After kinetic analysis, test-retest (TRT) variability of all kinetic parameters was calculated. TRT variability was calculated as the difference between test and retest kinetic parameters divided by their mean times 100%. Levene’s test was used to assess the impact of different scan durations and VOI size on TRT of $K_1$ and $V_T$. In addition, the parametric $V_T$ and $K_1$ values were averaged over each VOI. Test retest performance of VOI averaged parametric values was analysed using Bland–Altman plots [21]. Apart from assessing bias and impact of different VOIs sizes on parametric quantification, the impact of shorter scan duration on parametric test-retest variability was also studied.

### 6.3 Results

#### 6.3.1 Evaluation of bias between NLR and parametric methods

Typical [$^{11}$C]phenytoin parametric $V_T$ and $K_1$ images for a healthy subject are shown in Fig. S5. The correlations of $V_T$ obtained using the three parametric methods with that obtained using NLR are shown in Fig. 6.1. Correlations are good for all 3 methods, but at the same time a small negative bias for the parametric methods of about 5% can be seen. Fig. 6.2 shows scatter plots of $K_1$ obtained using BFM and SA versus $K_1$ obtained using NLR. Correlation are excellent with an average overestimation of about 4% for BFM and an underestimation of about 16% for SA. Results from all correlation analyses are summarized in Table 6.1.

#### 6.3.2 Test–Retest repeatability

Fig. 6.3 shows box plots of the percentage differences between test and retest values of $V_T$ and $K_1$, respectively, obtained using Logan, BFM and SA methods. For each parametric method, results are provided for both small ($\leq 5$mL) and large ($>5$mL) VOIs. Repeatability of Logan and SA derived $V_T$ seemed to be more affected by the use of small VOI BFM derived $V_T$. For $K_1$ both methods (BFM and SA) showed less than 5% (1 SD) TRT variability. In general, the median percentage difference between test and retest studies was about 5%. A somewhat wider range (larger inter-quartile range) in TRT variability of both $K_1$ and
V_T (Fig. 6.3) was observed for small compared with large VOI, but this difference was not statistically significant (Levene’s test, \( p > 0.05 \)).

### 6.3.3 Impact of Scan Duration

Figs. 6.4A and 4B show \( V_T \) and \( K_1 \) TRT variability, respectively, for different scan durations. A somewhat wider range (larger inter-quartile range) of 9.3% for 30 min, versus 6.7 and 6.3% for 45 and 60 min respectively in TRT variability of \( V_T \). Logan and SA derived \( V_T \) seemed to be more affected by shortening the scan duration than BFM derived \( V_T \). Yet, even for
Discussion

Results of the present study indicate the validity of several parametric methods for quantifying \([^{11}C]\)phenytoin kinetics in the brain. Three well established and accurate parametric methods shorter scan durations, \(K_1\) obtained with both methods (BFM and SA) showed less than 5% (1 SD) TRT variability. Shortening the scan duration to 45 min leads to slight deterioration of \(V_T\) TRT variability (from 1.7% to 2.1 for Logan, from 0.6 to 0.7 for BFM and 1.29 to 1.7 for SA), but differences were not statistically significant (Levene’s test, \(p>0.05\)). Differences between 45 min and 30 min scan durations (from 2.1% to 4% for Logan, from 0.7% to 0.75% for BFM and 1.7% to 2.9% for SA) however, were statically significant (Levene’s test, \(p<0.05\)). TRT data are shown as Bland-Altman plots in Fig S6 for 60 min of scan durations, while plot for 45 and 30 min are shown in Figs. S7 and S8. Variabilities in the Bland-Altman plots for 45 min data were similar to those of 60 min data, but became larger for 30 min data. A summary of the Bland-Altman plots is given in Table 6.2. It can be seen that shortening the scan duration from 60 to 45 min did not substantially affect TRT variability of \(K_1\) and \(V_T\).

6.4 Discussion

Results of the present study indicate the validity of several parametric methods for quantifying \([^{11}C]\)phenytoin kinetics in the brain. Three well established and accurate parametric methods
Table 6.1 – Summary of correlation, slope and intercept between various parametric methods compared with NLR.

<table>
<thead>
<tr>
<th></th>
<th>Correlation (R²)</th>
<th>slope</th>
<th>intercept</th>
<th>Correlation (R²)</th>
<th>Slope with zero intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Logan Vₜ test</td>
<td>0.81</td>
<td>0.79</td>
<td>1.45×10⁻¹</td>
<td>0.78</td>
<td>0.95</td>
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<tr>
<td>Logan Vₜ retest</td>
<td>0.87</td>
<td>0.90</td>
<td>5.30×10⁻²</td>
<td>0.87</td>
<td>0.95</td>
</tr>
<tr>
<td>BFM Vₜ test</td>
<td>0.79</td>
<td>0.84</td>
<td>1.03×10⁻¹</td>
<td>0.77</td>
<td>0.96</td>
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<tr>
<td>BFM Vₜ retest</td>
<td>0.73</td>
<td>0.83</td>
<td>1.05×10⁻¹</td>
<td>0.72</td>
<td>0.95</td>
</tr>
<tr>
<td>SA Vₜ test</td>
<td>0.83</td>
<td>0.89</td>
<td>5.95×10⁻²</td>
<td>0.83</td>
<td>0.96</td>
</tr>
<tr>
<td>SA Vₜ retest</td>
<td>0.84</td>
<td>0.94</td>
<td>1.34×10⁻²</td>
<td>0.84</td>
<td>0.96</td>
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<tr>
<td>BFM K₁ test</td>
<td>0.97</td>
<td>0.84</td>
<td>7.40×10⁻³</td>
<td>0.92</td>
<td>1.03</td>
</tr>
<tr>
<td>BFM K₁ retest</td>
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<td>0.81</td>
<td>8.30×10⁻³</td>
<td>0.85</td>
<td>1.03</td>
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<tr>
<td>SA K₁ test</td>
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<td>0.75</td>
<td>3.90×10⁻³</td>
<td>0.87</td>
<td>0.85</td>
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<tr>
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<td>0.85</td>
<td>0.74</td>
<td>4.60×10⁻³</td>
<td>0.83</td>
<td>0.86</td>
</tr>
</tbody>
</table>

Figure 6.3 – Test-retest variability of (A) Vₜ and (B) K₁ for different parametric methods for small (≤5mL, blank) and large (>5mL, diagonal line pattern) volumes of interest.
Discussion

were evaluated. Other alternatives such as multi-linear analysis [22] were not available at our institute and could not be evaluated in the current study. However, it should be noted that this method could provide improved calculation time compared with BFM because of full linear approach. For VT, strong correlations and good agreement were seen between the various parametric methods and NLR. The small negative bias of about -5% was mainly caused by the data from a single subject (p5). Unfortunately, it was not possible to identify any reason for this discrepancy. Although the Logan plot does not take blood volume (Vb) into account, the bias in VT was not substantially different from that biases seen with BFM and SA. In addition, although Logan plot analysis may suffer from noise induced bias [22, 23], this was not seen in the present study. BFM showed an overestimation in K1 of about 4% with a larger underestimation in case of SA. The incorporation of Vb as a fit parameter would result in an underestimation of K1 for SA (Fig. 6.2). Therefore, the SA analysis was repeated without incorporating Vb as fit parameter. In this case the underestimation in K1 reduced to about 5%. At the same time, however, bias in VT increased to about 15%. Overall, amongst the three parametric methods, BFM provided the smallest bias in VT of 5% underestimation compared to NLR.

Figure 6.4 – Test-retest variability of VT (A) and K1 (B) for different parametric methods and scan durations. The blank boxplot represent the 60 min, diagonal line pattern and dots pattern represent 45 and 30 min respectively.

As shown in Fig. 6.3, the TRT variabilities of VT and K1 for all three parametric methods were small (SD <5%). Overall, BFM shows the best TRT variability for VT, followed by SA and Logan, respectively. With respect to K1, SA seemed to provide better TRT variability than BFM. TRT variabilities were slightly increased for small compared to large VOI for all parametric methods. In addition, the TRT percentage of VT and K1 in parametric methods are comparable to NLR method. Even though the TRT variabilities were small, there might be a limitation for quantification of [11C]phenytoin in or near high uptake regions such
as the choroid plexus due to spill over effects as was also seen for the hippocampus in (R)-[\(^{11}\text{C}\)]verapamil studies [24]. In general TRT variabilities of \(K_1\) and \(V_T\) increased when shortening the scan duration. \(V_T\) repeatability obtained with BFM seemed to be less affected when shortening scan duration. \(K_1\) repeatability was more sensitive to reducing scan duration. Overall, scan duration could be shortened from 60 to 45 min without substantially affecting \(V_T\) and \(K_1\) repeatability for any of the methods tested. The performances of various methods, however, were solely based on data obtained from healthy (male) subjects. Further studies will be needed to assess whether various parametric method are sensitive enough to provide better quantification under other and/or pathological conditions.

Table 6.2 — Summary of the results for Bland-Altman plots for each method with different scan durations for \(V_T\) (Logan, BFM and SA) and \(K_1\) (BFM and SA).

<table>
<thead>
<tr>
<th>Method</th>
<th>(V_T)</th>
<th>Mean</th>
<th>SD</th>
<th>Lower limit (-1.96SD)</th>
<th>Upper Limit (+1.96SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Logan</td>
<td>60 min</td>
<td>(-1.64 \times 10^{-2})</td>
<td>(5.17 \times 10^{-2})</td>
<td>(-1.18 \times 10^{-1})</td>
<td>(8.49 \times 10^{-2})</td>
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<tr>
<td>BFM</td>
<td>60 min</td>
<td>(-5.54 \times 10^{-3})</td>
<td>(4.08 \times 10^{-2})</td>
<td>(-8.54 \times 10^{-2})</td>
<td>(7.43 \times 10^{-2})</td>
</tr>
<tr>
<td>SA</td>
<td>60 min</td>
<td>(4.39 \times 10^{-2})</td>
<td>(-1.22 \times 10^{-2})</td>
<td>(-9.81 \times 10^{-2})</td>
<td>(7.38 \times 10^{-2})</td>
</tr>
<tr>
<td>Logan</td>
<td>45 min</td>
<td>(-1.87 \times 10^{-2})</td>
<td>(5.50 \times 10^{-2})</td>
<td>(-1.26 \times 10^{-1})</td>
<td>(8.91 \times 10^{-2})</td>
</tr>
<tr>
<td>BFM</td>
<td>45 min</td>
<td>(-6.66 \times 10^{-3})</td>
<td>(4.22 \times 10^{-2})</td>
<td>(-8.93 \times 10^{-2})</td>
<td>(7.60 \times 10^{-2})</td>
</tr>
<tr>
<td>SA</td>
<td>45 min</td>
<td>(-1.33 \times 10^{-2})</td>
<td>(4.35 \times 10^{-2})</td>
<td>(-9.86 \times 10^{-2})</td>
<td>(7.19 \times 10^{-2})</td>
</tr>
<tr>
<td>Logan</td>
<td>30 min</td>
<td>(-3.48 \times 10^{-2})</td>
<td>(1.24 \times 10^{-1})</td>
<td>(-2.78 \times 10^{-1})</td>
<td>(2.08 \times 10^{-1})</td>
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<td>30 min</td>
<td>(-5.81 \times 10^{-3})</td>
<td>(5.41 \times 10^{-2})</td>
<td>(-1.12 \times 10^{-1})</td>
<td>(1.00 \times 10^{-1})</td>
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<tr>
<td>SA</td>
<td>30 min</td>
<td>(-1.88 \times 10^{-2})</td>
<td>(4.72 \times 10^{-2})</td>
<td>(-1.11 \times 10^{-1})</td>
<td>(7.36 \times 10^{-2})</td>
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<tr>
<td>BFM</td>
<td>(K_1) 60 min</td>
<td>(-1.71 \times 10^{-4})</td>
<td>(2.80 \times 10^{-3})</td>
<td>(-5.66 \times 10^{-3})</td>
<td>(5.31 \times 10^{-3})</td>
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<tr>
<td>SA</td>
<td>(K_1) 60 min</td>
<td>(2.08 \times 10^{-3})</td>
<td>(-3.91 \times 10^{-4})</td>
<td>(-4.46 \times 10^{-3})</td>
<td>(3.68 \times 10^{-3})</td>
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<tr>
<td>BFM</td>
<td>(K_1) 45 min</td>
<td>(-5.76 \times 10^{-5})</td>
<td>(2.82 \times 10^{-3})</td>
<td>(-5.58 \times 10^{-3})</td>
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<td>(-3.14 \times 10^{-4})</td>
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<td>(-4.39 \times 10^{-3})</td>
<td>(3.76 \times 10^{-3})</td>
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<tr>
<td>BFM</td>
<td>(K_1) 30 min</td>
<td>(2.35 \times 10^{-4})</td>
<td>(2.88 \times 10^{-3})</td>
<td>(-5.41 \times 10^{-3})</td>
<td>(5.88 \times 10^{-3})</td>
</tr>
<tr>
<td>SA</td>
<td>(K_1) 30 min</td>
<td>(-1.30 \times 10^{-5})</td>
<td>(2.18 \times 10^{-3})</td>
<td>(-4.29 \times 10^{-3})</td>
<td>(4.26 \times 10^{-3})</td>
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</table>

Overall, from our studies the finding shows that the BFM seems to be a good parametric method for the evaluation of \([^{11}\text{C}]\)phenytoin studies with potential to reduce the scan duration to 45 min.
6.5 Conclusion

Amongst the different parametric methods for quantifying $[^{11}C]$phenytoin kinetics in the brain, BFM produced the best results in term of bias and repeatability. In addition, scan duration could be shortened to 45 min, but further validation under pathological conditions is warranted.

6.6 Supplementary materials

Figure S5 – Parametric $V_T$ and $K_1$ images of 11C-phenytoin for an axial plane through the middle of the brain of a healthy subject using (from left to right) Logan graphical analysis (Logan), a basis function method (BFM) and spectral analysis (SA). The top row shows $V_T$ images, the bottom row $K_1$ images.
Figure S6 – Bland-Altman plots of test-retest for $V_T$ in the left column and $K_1$ in the right column for 60 min scan durations. (A) for Logan, (B&C) for the BFM and (D&E) for SA.
Figure S7 – Bland-Altman plots of test-retest for $V_T$ in the left column and $K_1$ in the right column for 45 min scan durations. (A) for Logan, (B&C) for the BFM and (D&E) for SA.
Figure S8 – Bland-Altman plots of test-retest for $V_T$ in the left column and $K_1$ in the right column for 30 min scan durations. (A) for Logan, (B&C) for the BFM and (D&E) for SA
Bibliography


