KINETIC MODELLING OF NICOTINIC ACETYLCHOLINE RECEPTORS WITH 5-[123]I-IODO-A-85380 AND DYNAMIC SINGLE-PHOTON EMISSION COMPUTED TOMOGRAPHY 1

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Abstract: 5-iodo-3-(2(S)-azetidinylmethoxy)pyridine (5-[123]Iiodo-A-85380) is a novel radioligand for brain nicotinic acetylcholine receptors (nAChRs), having demonstrated high specific binding to nAChRs and favourable properties for in vivo imaging. The purpose of this study was to evaluate different tracer kinetic modelling strategies for the quantification of regional nAChR binding in baboon brain using dynamic single-photon emission computed tomography (SPECT). Imaging studies were performed on two anaesthetised baboons. The radioligand (397 ± 43 MBq) was infused into the animals over 2 min and dynamic SPECT images were acquired for 3 hr. The plasma input function was determined from arterial blood samples with metabolite correction. The data were analysed using 2- and 3-compartment models. Reliable fits could be obtained with a 3-compartment model which provided significantly improved fitting as compared to the 2-compartment model, despite poor identifiability in some parameters. The 2-compartment model failed to fit the data adequately even though the identifiability of parameters was high. The 3-compartment model described the data well, and with the strategies used to obtain reliable fits, is the model of choice for analysis of 5-[123]Iiodo-A-85380 kinetics. Copyright © 2002 IFAC

Keywords: Binding, biomedical systems, modelling, parameter estimation.

1. INTRODUCTION

Neuronal nicotinic acetylcholine receptors (nAChRs) are thought to play an important role in tabacco dependence (Corrigall et al., 1992) and are affected in various neurodegenerative conditions such as Alzheimer’s and Parkinson’s diseases (Perry et al., 1995). These receptors are also involved in different CNS functions, for instance, learning and memory (Levin and Rose, 1995). In vivo studies of nAChRs, however, have been limited by the lack of suitable radiotracers for imaging. Initial attempt to study nAChRs in the living brain with positron emission tomography (PET) was made possible by using [11]C-nicotine but rapid washout of the tracer from the brain and high degree of nonspecific binding impede its applications.
for quantitative in vivo studies (Nyback et al., 1994). Current research efforts have focused on development of new, highly specific and selective nAChR radioligands that are capable of localising nAChRs in vivo with high affinity. The discovery of epibatidine, a potent nAChR agonist, has stimulated development of nAChR radioligands with favourable properties for imaging with PET and single-photon emission computed tomography (SPECT). However, the high toxicity of epibatidine-related compounds may preclude their use in human studies (Molina et al., 1997).

A series of 3-pyridyl ether compounds possessing sub-nanomolar affinity for nAChRs with reduced toxicity profile has been reported recently. One of these compounds, 3-(2(S)-azetidinylmethoxy)pyridine (A-85380) possesses high affinity for α4β2 nAChRs in human. It has been labelled with iodine-123 (5-[I]iodo-A-85380) and demonstrated high specificity and selectivity for nAChRs in mouse brain in vivo, with low acute toxicity (Musachio et al., 1998). 5-[I]iodo-A-85380 has also been used in SPECT imaging of nAChRs in Rhesus monkey and Papio anubis baboon (Chefer et al., 1998; Musachio et al., 1999). We have recently described our methodology for imaging nAChR binding using 5-[I]iodo-A-85380 and SPECT and reported the first in vivo imaging of nAChRs upregulation in response to chronic (−)-nicotine treatment in baboon (Kassiou et al., 2001). The aim of this study was to investigate different tracer kinetic modelling strategies for the quantification of 5-[I]iodo-A-85380 in baboon brain using SPECT.

2. METHODS
2.1 Radioligand synthesis
Full details of 5-[I]iodo-A-85380 synthesis have been described previously (Kassiou et al., 2001).

2.2 Animal Preparation
The study procedures were approved by the Central Sydney Area Health Service Animal Welfare Committee. Two adult male Papio hamadryas baboons were studied pre- and post-chronic treatment with saline or (−)-nicotine. The (−)-nicotine treated baboon (Evan) weighed 23.6 kg and the weight of the saline control baboon (Max) was 21.3 kg. After the baseline imaging study for the baboons, subcutaneously implanted osmotic pumps (Alzet 2ML2, Alza Corporation, Palo Alto, CA, USA) were used to infuse a saline solution of (−)-nicotine tartrate (2 mg/kg/24hr) for Evan and saline for Max, continuously during a 14 day period. Prior to pump implantation, animals were anaesthetised with ketamine (5–8 mg, intramuscularly). Venous blood samples were taken from both animals at baseline, 2 days, and 15 days after pump implantation, and 7 days after the pump had been removed for Evan. Plasma samples were then analysed to determine the levels of (−)-nicotine and cotinine in plasma.

2.3 Data Acquisition
The experimental conditions and injected dose are summarised in Table 1. Animals were anaesthetised with ketamine (5–8 mg, intramuscularly), and anaesthesia was maintained throughout the study by infusion of ketamine (13 mg/kg/hr). Baboons were placed in a Trionix Triad XLT triple head gamma camera (Trionix Research Laboratory, Twinsburg, OH) and the head was immobilised in the head holder using a thermoplastic mask. A 15-min transmission scan with a line source filled with 99mTc at the focus of a long focal length (1140 mm) fan beam collimator was performed prior to tracer administration and was used for photon attenuation correction. Dynamic emission SPECT imaging was performed with ultra high resolution, low energy (UHR-LE) short focal length (480 mm) fan beam collimators fitted to all three detectors.

Dynamic emission data acquisition and arterial blood sampling were commenced simultaneously with the start of a 2 min infusion of the ligand using a syringe pump. Data were sampled every 30 s at the beginning and were gradually extended to every 5 and 10 min for a total data acquisition time of 3 hrs. Data were collected into 128×64 projection images (pixel size = 3.6 mm × 3.6 mm) with continuous rotation and binning into 60 projections for a complete rotation. The 30 s frames were integrated into 1 min frames for further processing. Fan beam data were rebinned to parallel beam data with a zoom of 1.6, resulting in a final isotropic reconstructed voxel size of 2.2 mm × 2.2 mm × 2.2 mm.

2.4 Plasma Metabolite Analysis and Input Function
Arterial blood samples were taken throughout the study. The samples were centrifuged for 5 min at 5000 rpm to separate plasma for counting in an automated NaI well counter which was cross calibrated with the SPECT system by counting aliquots of a phantom filled with a 123I solution of known activity and by acquiring and processing SPECT studies of the phantom on the gamma camera similar to the baboon studies. Aliquots of plasma samples taken at 2, 5, 10, 15, 30 and 180 min were used to determine the metabolite fraction in plasma by acetonitrile denaturation followed by TLC. The measured unmetabolised fractions were fitted with a dual exponential function and all plasma sample counts were corrected for metabolites using the fitted curve. The metabolite corrected data were decay corrected and cross calibrated to produce the input function for kinetic modelling.

2.5 Image Analysis
Emission data were corrected for scatter using transmission dependent scatter correction (Narita et al., 1997) and reconstructed using the ordered-subset expectation-maximisation (OSEM) iterative (2 iterations, 20 subsets) reconstruction algorithm (Hudson
and Larkin, 1994). Attenuation was corrected with attenuation maps derived from the transmission scan. The reconstructed images were then convolved with a three-dimensional Gaussian kernel with a full-width at half-maximum of 3.7 mm. The resultant SPECT images were cross calibrated with the NaI well counter. The plasma do not cross the blood-brain barrier and there are either low amount of metabolites produced in the brain and/or they egress rapidly from the brain, similar to the non-specific uptake. The thalamus VOI in the reference study was not prominent. The thalamus VOI in the reference study was applied instead.

### 2.6 Compartmental Model Fitting

Tissue time-activity curves (TACs) generated for each VOI were investigated with both 2-compartment and 3-compartment models using nonlinear least squares fitting and the metabolite corrected input function, under the assumptions that labelled metabolites in the plasma do not cross the blood-brain barrier and that there are either low amount of metabolites produced in the brain and/or they egress rapidly from the brain, similar to the non-specific uptake. Therefore the labelled metabolite in tissue is negligible. Figure 1 shows the structures of the 3-compartment and 2-compartment models. Rate constants ($K_1$, $k_3$, $k_2$, $k_3$, and $k_4$) were estimated by model fitting and cerebral blood volume was assumed to be 5% in all regions. The volume of distribution ($V_d$), the binding potentials, $BP_1$ (Mintun et al., 1984), and $BP_2$ (Laruelle et al., 1994) for the 3-compartment model were calculated according to the following equations:

$$V_d = \frac{K_1}{k_2} \left(1 + \frac{k_3}{k_4}\right)$$  

(1)

$$BP_1 = \frac{k_3}{k_4}$$  

(2)

$$BP_2 = \frac{K_1 k_3}{k_2 k_4}$$  

(3)

But the binding potential could not be determined for this model.

As highlighted by Fujita et al. (2000), reliability of fitting the 3-compartment model can be problematic. The following strategies were thus employed to constrain the 3-compartment model fit: (1) The 2-compartment model was always fitted first and the initial values for the 3-compartment model were derived from the parameters obtained from the 2-compartment model fit. (2) The residual sum of square (RSSQ) from the 3-compartment model fit had to be less than $1.1 \times$ RSSQ of the 2-compartment model fit. (3) $V_d$ for 3-compartment model fit was constraint to be less than two times the $V_d$ estimated by the 2-compartment model fit. (4) The mean parameter values of the 100 bootstrap Monte Carlo realisations provided improved

<table>
<thead>
<tr>
<th>Study</th>
<th>Experimental condition</th>
<th>Dose injected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evan baseline</td>
<td>Baseline, prior to any treatment</td>
<td>400 MBq</td>
</tr>
<tr>
<td>Evan nicotine</td>
<td>End of chronic (-)-nicotine treatment, pump removed at end of scan</td>
<td>447 MBq</td>
</tr>
<tr>
<td>Evan post1</td>
<td>Day 7 post (-)-nicotine treatment and pump removal</td>
<td>392 MBq</td>
</tr>
<tr>
<td>Evan post2</td>
<td>Week 5 post (-)-nicotine treatment and pump removal</td>
<td>390 MBq</td>
</tr>
<tr>
<td>Max baseline</td>
<td>Baseline, prior to any treatment</td>
<td>434 MBq</td>
</tr>
<tr>
<td>Max saline</td>
<td>Day 15 of saline treatment, pump removed at end of scan</td>
<td>324 MBq</td>
</tr>
<tr>
<td>Max post1</td>
<td>Week 4 post saline treatment and pump removal</td>
<td>197 MBq</td>
</tr>
</tbody>
</table>

Table 1. Experimental conditions and injected dose for the treated baboon (Evan) and the control baboon (Max).
estimate of parameters. Given a curve of \( n \) data points, for each of bootstrap Monte Carlo iteration, \( n \) points are randomly selected from the curve and the model is fitted to the \( n \) randomly selected points. As the \( n \) points are randomly selected, some of the points from the original data set will be duplicated while others will not be selected. The points which are duplicated and not selected will vary randomly for each iteration (Press et al., 1992).

### 2.7 Statistical Analysis

The standard deviations (SD) of the rate constants and macroparameters \((V_d', V_p', B_{P_1}, \text{ and } B_{P_2})\) were derived from the bootstrap Monte Carlo technique with 100 iterations. The identifiability of the parameter is defined by the coefficient of variation (CV), which is defined as the ratio of SD to the value of the parameter estimate. To statistically compare the adequacy of the model fits, the \( F \)-statistic (Landaw and DiStefano, 1984), the Akaike Information Criterion (Akaike, 1974) and the Schwarz Criterion (Schwarz, 1978) were computed for each model configuration. For the \( F \)-statistic, the null hypothesis that the lower-order model (2-compartment model in this study) fits the data better is rejected if the probability is less than an acceptable level (\( P < 0.05 \) was chosen). As for the Akaike Information Criterion (AIC) and the Schwarz Criterion (SC), the model configuration with the lowest score is preferred. Nonparametric run test was also used to test whether the residuals between the measurements and the fitted data points were randomly distributed above zero. Differences with \( P < 0.05 \) were considered statistically significant.

### 3. RESULTS AND DISCUSSION

Figure 2 shows the tissue TACs of the cerebellum, frontal cortex and thalamus for one of the baseline studies (Evan\textsubscript{baseline}). The corresponding 2- and 3-compartment model fits are also shown. The increased uptake and retention of 5-\textsuperscript{[12]}Ijodo-A-85380 in the thalamus as compared to the other regions are clearly evident. The regional distribution of the radioligand was also consistent with the known densities of nAChRs in baboon brain (thalamus > frontal cortex > cerebellum). It was apparent that 2-compartment model failed to fit the data adequately, as shown in this figure.

Tables 2 and 3 summarise the compartmental model fitting results for both 2- and 3-compartment models. The CV of the parameters estimated with the 2-compartment model in all cases was <10\% except the thalamus curve of Max\textsubscript{baseline} where the CV of \( k_2 \) was 13\%. The reliability in estimating \( K_1 \) using the 3-compartment model was reasonable, with CV <10\% for all fits. The CV of the rate constant parameters estimated with the 3-compartment model, was generally higher (i.e. lower reliability) as compared to the corresponding 2-compartment model fitting. Some high correlations (\( r \approx 0.95 \)) between parameters were observed with the 3-compartment model fits but \( r > 0.9 \) was also seen between parameters in 2-compartment model fits. The high correlations between and low reliability of the rate constant parameters suggested that more than 3 compartments would not be able to fit the data. This is in agreement with the recent findings of Fujita et al. (2000) despite the differences in radioligand administration protocol. Therefore, no attempt was made to fit more than 3 compartments.

The bootstrap Monte Carlo estimate of the macroparameters, \( V_d, V_p, B_{P_1} \) and \( B_{P_2} \) and their SDs are tabulated in Tables 2 and 3. The correspondence between \( V_d \) and \( V_d' \) was very good (\( V_d' = 0.98V_d - 0.01; r = 0.99 \)). A reduction in \( V_d \) and \( V_d' \) was observed in all regions during (-)-nicotine treatment (Evan\textsubscript{nicotine}) as compared to the mean of the baseline studies (Evan\textsubscript{baseline}, Evan\textsubscript{post1}, Max\textsubscript{baseline}, Max\textsubscript{saline}, Max\textsubscript{post1}): 31\% in the cerebellum, 33\% in the frontal cortex, and 57\% in the thalamus for \( V_d \). The variability of \( B_{P_1} \) was found to be as high as 46\% and this was because of the low reliability in estimating \( k_2 \) to \( k_4 \) which are highly correlated. The reliability of \( B_{P_2} \) is generally higher (i.e. lower reliability) as compared to the corresponding 2-compartment model fitting. Some high correlations (\( r \approx 0.95 \)) between parameters were observed with the 3-compartment model fits but \( r > 0.9 \) was also seen between parameters in 2-compartment model fits. The high correlations between and low reliability of the rate constant parameters suggested that more than 3 compartments would not be able to fit the data. This is in agreement with the recent findings of Fujita et al. (2000) despite the differences in radioligand administration protocol. Therefore, no attempt was made to fit more than 3 compartments.
better than that of $BP_1$, with $CV < 20\%$ except for the cerebellum curves of Evan$\_nicotine$ and Max$\_post1^*$. The randomness of the data point distribution above the fitted curve was tested by the nonparametric run test. With the exception of the thalamus curves during nicotine treatment (Evan$\_nicotine$) and post-(-)-nicotine treatment (Evan$\_post2$), and the cerebellum curve of Max$\_post1^*$, the hypothesis of randomness was rejected for all other 2-compartment model fits. As for 3-compartment model fitting, the hypothesis was rejected in Max at baseline level (Max$\_baseline$) and the frontal cortex curve of Max$\_post1^*$. Figure 3 shows the fitted curves obtained for the frontal cortex of Max$\_baseline^*$, where the hypothesis of randomness was rejected for 3-compartment model fit. Inspection of the plot showed that the results were not caused by the inadequacy of the 3-compartment model but the problem with the data. There were technical problems during the synthesis of the radioligand for the study of post saline treatment in Max (Max$\_post1^*$), as the administered activity was only about half that of the other studies (Table 1), which may possibly account for the atypical results seen with the frontal cortex.
curve, although it is not clear whether these technical difficulties could have affected the tracer kinetics and the observed differences. To test the hypothesis of 3-compartment model fit provides a significantly better fit to the data over 2-compartment model fit, the $F$-statistic was applied and it suggested that the hypothesis could not be rejected in all cases. This finding was also supported by the AIC and SC, as presented in Tables 2 and 3.

4. CONCLUSIONS

Our results demonstrate that, with the modelling strategies used, reliable fits could be obtained with a 3-compartment model which provided a significantly improved fitting as compared to the 2-compartment model, despite poor identifiability in some parameters. Adequate reliability for the $K_1$ and macroparameter $V_d$ was obtained with the 3-compartment model. The 2-compartment model failed to fit the data completely and hence introduced biases, in particular, the $K_1$ estimate compared to the 3-compartment model. The higher reliability of the 2-compartment model may have advantage for the generation of parametric images.

5. REFERENCES


