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Nuclear Medicine and Biology 29 (2002) 115–123

NUCLEAR MEDICINE — AND — BIOLOGY

Estimate the time varying brain receptor occupancy in PET imaging experiments using non-linear fixed and mixed effect modeling approach

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Received 4 June 2001; received in revised form 21 August 2001; accepted 21 August 2001

Abstract

Positron-Emission Tomography (PET) is an imaging technology currently used in drug development as a non-invasive measure of drug distribution and interaction with biochemical target system. The level of receptor occupancy achieved by a compound can be estimated by comparing time-activity measurements in an experiment done using tracer alone with the activity measured when the tracer is given following administration of unlabelled compound. The effective use of this surrogate marker as an enabling tool for drug development requires the definition of a model linking the brain receptor occupancy with the fluctuation of plasma concentrations. However, the predictive performance of such a model is strongly related to the precision on the estimate of receptor occupancy evaluated in PET scans collected at different times following drug treatment. Several methods have been proposed for the analysis and the quantification of the ligand-receptor interactions investigated from PET data. The aim of the present study is to evaluate alternative parameter estimation strategies based on the use of non-linear mixed effect models allowing to account for intra and inter-subject variability on the time-activity and for covariates potentially explaining this variability. A comparison of the different modeling approaches is presented using real data. The results of this comparison indicates that the mixed effect approach with a primary model partitioning the variance in term of Inter-Individual Variability (IIV) and Inter-Occasion Variability (IOV) and a second stage model relating the changes on binding potential to the dose of unlabelled drug is definitely the preferred approach.

Keywords: PET imaging; Non linear model; Fixed effect; Mixed effect

1. Introduction

Positron-Emission Tomography (PET) is an imaging technology currently used in drug development as a non-invasive measure of drug distribution and interaction with biochemical target system [10,11,26]. This method is more and more frequently applied to define neurochemical correlates of illness and to explore the interaction properties of a drug with cerebral receptor and enzyme systems [2,13]. Furthermore, PET studies can supply accurate information for a rational definition of a dosage regimen suitable to achieve expected therapeutic outcomes, assuming that the brain receptor occupancy is a surrogate marker of a pharmacological drug activity [12,21,24]. Several methods have been proposed for the analysis and the quantification of the ligand-receptor interactions investigated *in vivo* from PET data [7,8,16,17,20,23,27,28]. All the *in vivo* approaches are based on mathematical models, which describe the transport of the ligand from the blood to a free ligand brain compartment and the interaction with the ligand-receptor sub-system. One of the major issues remaining unsolved is the estimate of the value and the precision of receptor time-varying occupancy accounting for the variability induced by the complex manipulations necessary to generate the time-activity data and by the intra- (or inter occasion) and inter-subject variability in individual response. Examples of abnormal (negative) fractional receptor occupancy values based on the independent modeling of time-activity data for each subject and for each PET scan time, have been reported [1]. In addition, in a recent paper has been showed that a correct inference about subject responses to activation tasks in a fMRI study can be derived through the use of a statistical model which accounts for both within- and between–subject variance applying random-effect modeling approach in the data interpretation [19].

The aim of the present study is to evaluate alternative parameter estimation strategies based on the use of non-linear mixed effect models accounting for intra and inter-

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0969-8051/02/$ – see front matter
PII: S0969-8051(01)00275-X

subject variability on the time-activity and for the identification of possible source of this variability using individual covariate measurements. The effective use of PET measurement as an enabling tool for drug development requires the definition of a model linking the brain receptor occupancy with the fluctuation of plasma concentrations. However, the predictive performance of such a model is strongly related to the precision on the estimate of the time varying receptor occupancy values.

2. Materials and methods

2.1. PET study

The aim of this study was the *in vivo* evaluation of the binding kinetics of a high affinity NK_1 receptor antagonist, [11C]GR205171, in the monkey brain. The experiments were initially conducted in 5 anesthetized rhesus monkeys. Furthermore, two additional monkeys were included in the same study on a separate occasion. Following a baseline experiment, each monkey received one or two unlabelled ligand followed by a tracer injection. The unlabelled drug was injected at the doses of: 0.05 mg/kg and 0.5 mg/kg in the monkey 1, 2, and 3, 0.1 mg/kg in the monkey 4, 1 mg/kg in the monkey 5, 0.001 mg/kg in the monkey 6 and 0.01 mg/kg in the monkey 7. Cerebellum was considered the reference region (RR) without specific receptors and Striatum the region of interest (ROI) according to the information collected on previous autoradiography studies. Each scan lasted approximately 55 minutes for monkey 1, 2, 3, 4, 5 and approximately 90 minutes for monkey 6 and 7. The time activity curves were expressed in SUV (Standardized Uptake Value), which equals the radioactivity concentration divided by dose of injected radioactivity normalized to body weight (normalized dose radioactivity). The PET studies were performed in the Uppsala University PET Center and the details on equipment, experimental conditions together with preliminary results have been reported in a recent publication [5].

2.2. Time-activity model selection

The PET modeling was organized into two consecutive steps. The first one concerned the choice of the most appropriate structural model while the second one consisted on the evaluation of the most appropriate parameters estimation procedure.

The data here presented were previously analyzed using an irreversible graphical methods (Patlak) [5]. However, we decided to re-analyze the date and to compare alternative modeling options using a kinetic approach because it was shown that the simplifying assumptions underling the graphical method can lead to substantial bias [23]. Since the arterial input function was not available three models based on the reference region were used to account for a reversible and irreversible binding hypotheses. This approach estimates receptor-bound activity by subtracting the concentration of activity in a reference region, known to be devoid of the receptors of interest (non-specific binding + free tracer), from the concentration of total uptake in the region of interest (specific + non-specific binding + free activity). The level of receptor occupancy achieved by a compound can be estimated by comparing time-activity measurements from a pre-dose PET scan using the tracer alone, with the activity measured when the tracer is given following administration of the cold (unlabelled) compound. The pre-dose scan gives an estimate of the total number of receptors available to be occupied: the binding potential (BP). In subsequent scans, the PET tracer has less specific binding because the compound is occupying a defined proportion of the specific receptors. The two-tissue compartment reference tissue model (RTM) [16] and the simplified reference tissue model (SRTM) [17] were initially used. In the last approach, a modified version of the RTM, MRTM, based on irreversible binding assumption was applied. The models were compared on the basis of weighted residuals, parameter precision, Akaike criteria using a weighted non-linear least squares procedure as implemented in the SAMII software package [4]. The minimization algorithm reached a successful convergence in 100% of data sets using SRTM, 46% using MRTM and 77% using RTM. According the Akaike criteria SRTM was the preferred model in 66% of data sets, the RTM in 15% and MRTM in 19%. The results have been presented in [6] and show that the SRTM is the most appropriate model among those evaluated to describe the [11C]GR205171 binding kinetic in monkey. This one tissue-compartment and three-parameter model assumes that only the parent tracer, crossing the blood-brain barrier, diffuses from the plasma compartment to the a region devoid of specific binding sites (C_r), and to the specific compartments associated to a region of interest (C_t). Furthermore, the level of non-specific binding is assumed identical in both tissues. Moreover, the SRTM model provided well identified estimate of the model parameters with increased convergence rate in combination with increased stability when compared with alternative models. Time-activity data were analyzed using the Simplified Reference Tissue Model, considering the Cerebellum as the reference region and the Striatum as region of interest. Three alternative data analysis approaches were investigated based on the use of non-linear fixed and random-effect models.

2.3. Model A

A non-linear fixed-effects model (Equation 1) was used to independently analyze the time-activity data collected at each PET scan time as if they come from separate animals.

$$\frac{dy}{dt} = \left(k_2 - \frac{R_1 \cdot k_2}{(1 + BP)}\right) C_r(t) - \frac{k_2}{(1 + BP)}\, y$$
$$C_t(t) = y + R_1 \cdot C_r(t) \qquad (1)$$

Were C_t and C_r are the tracer concentration in the ROI and in the RR respectively, BP is the binding potential, R_I is the ratio of the delivery in the ROI compared to that in the RR (ratio of influx), and k_2 is the efflux rate constant from the ROI. The fractional receptor occupancy value at scan time i ($RO\%_i$) was further derived from the primary model parameters using the binding potential value estimated at the baseline (BP_0) and the one estimated at the i^{th} PET scan time (BP_i) as:

$$RO\%_i = 100\,\frac{BP_0 - BP_i}{BP_0} \tag{2}$$

2.4. Model B

All the time-activity data collected in a monkey was simultaneously analyzed using a non-linear fixed-effects approach and the Model B (Equation 3). All parameters were considered as fixed-effect parameters. R_I and k_2 were assumed to have a typical value for each monkey constant across PET scan times. R_I and k_2 were estimated using all the measurements at the different times, BP_0 was estimated using only baseline data while $RO\%_i$ was estimated using the measurements at time i. The model was constrained to estimate positive $RO\%_i$ values using a model re-parameterisation: the receptor occupancy (RO%) was constrained to be equal to 0 at baseline and to assume values ranging between 0 and 100% at the different PET scan times.

$$\begin{aligned} \frac{dy}{dt} &= \left(k_2 - \frac{R_1 \cdot k_2}{(1+BP)}\right) C_r(t) - \frac{k_2}{(1+BP)}\,y \\ BP &= BP_0 - \frac{RO\%_i \cdot BP_0}{100} \\ C_t(t) &= y + R_1 \cdot C_r(t) \end{aligned} \tag{3}$$

The parameters estimated in the Model B are: BP_0, R_I, k_2 + $RO\%_i$ [i = 1, number of PET scans (including baseline) –1]

2.5. Model C

The non-linear mixed-effects approach was applied using the structural model defined by equation 4. All data for each monkey and each scan time were jointly analyzed accounting for intra (or inter occasion)-and inter- monkey variability. The modeling approach (Model C-a) was based on the assumptions that: (a) typical tracer kinetic and binding parameters exist for each monkey (fixed-effect) and (b) these parameters may vary across monkey and experimental conditions within the same monkey according to two variability sources: an Inter-Occasion Variability (IOV) and Inter-Individual Variability (IIV). IIV was estimated as a first level random-effect parameter while occasion-specific departure of the parameter from the individual typical values (IOV) was accounted by a second level random-effect model component.

Table 1
Parameter values estimated using the Model A

Monkey		R_1	k_2	BP	RO%
1	Baseline	0.840	0.0349	2.620	0
	Scan 1	1.360	0.3270	0.209	92.0
	Scan 2	1.050	0.0354	0.300	88.5
2	Baseline	0.778	0.0290	4.550	0
	Scan 1	0.778	0.0405	0.418	90.8
	Scan 2	0.848	0.1100	0.109	97.6
3	Baseline	0.863	0.0245	3.340	0
	Scan 1	0.905	0.0418	0.234	93.0
	Scan 2	1.000	0.0651	0.221	93.4
4	Baseline	*	*	*	—
	Scan 1	*	*	*	—
5	Baseline	1.070	0.0341	3.860	0
	Scan 1	*	*	*	—
6	Baseline	1.040	0.0433	0.848	0
	Scan 1	0.938	0.0151	1.020	−20.3
7	Baseline	1.090	0.0323	1.710	0
	Scan 1	1.010	0.0076	0.932	45.5

* Non-linear regression procedure failed to reach convergence.
– Parameter not estimated.

$$\begin{aligned} \frac{dy}{dt} &= \left(k_2 - \frac{R_1 \cdot k_2}{(1+BP)}\right) C_r(t) - \frac{k_2}{(1+BP)}\,y \\ C_t(t) &= y + R_1 \cdot C_r(t) \end{aligned} \tag{4}$$

2.6. Model for IIV and IOV

Denoting the *i*th subject's average parameter value P_i, and its value at the *j*th occasion P_{ij}, a general model for IOV was:

$$\begin{aligned} P_i &= f(P^*,\ \eta_i) \\ Pij &= g(Pi,\ kij) \end{aligned} \tag{5}$$

where P^* is a typical value of P in the population and η_i and k_{ij} are assumed to be independently, normally distributed parameters both with zero mean and variance ω^2 and π^2, respectively. The η represents the between individual difference (IIV) and the k the between occasion difference within an individual (IOV). The following exponential models were evaluated to describe IIV and IOV variability:

Table 2
Parameter values estimated using the Model B

Monkey	R_1	k_2	BP_0	$RO\%_1$	$RO\%_2$
1	1.1	0.0142	119	99.6	99.7
2	0.83	0.0302	2.97	82.6	87.9
3	0.946	0.0217	2.95	86.9	79.6
4	0.613	0.0211	282	100	
5	1.09	0.0246	16.8	98	
6	0.968	0.0429	0.932	63.2	
7	1.03	0.0392	1.59	83.6	

$RO\%_1$, $RO\%_2$: receptor occupancy estimated at the first and second scans time.

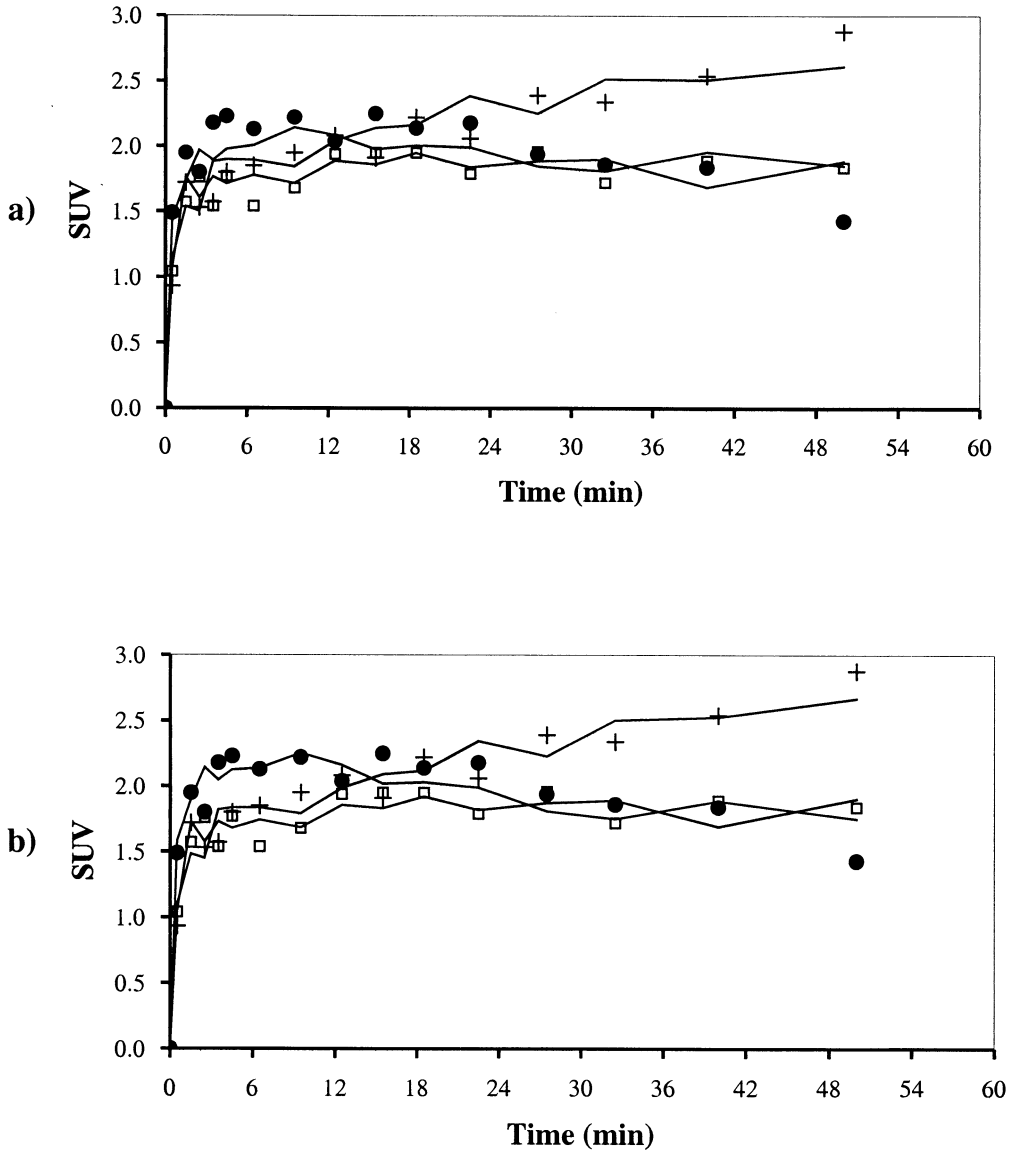

Fig. 1. Monkey 2: Individual observed time-activity (SUV) data with model predicted values (continuos line) using fixed effect model B (panel a) and random effect model C-c (panel b) at (+) baseline, ( ) scan 2, (●) scan 3.

$$P_{ij} = P^* \cdot e^{(\eta_i + k_{ij})}$$
$$\eta_i \approx N(0,\ \omega^2) \qquad (6)$$
$$k_{ij} \approx N(0,\ \pi^2)$$

Using this approach, the model parameters were partitioned in fixed-effect (R_I, k_2, BP), random-effect (ωR_I, ωk_2 and ωBP), and residual error (σ) parameters. All the parameters (fixed and random) were estimated using all the collected measurements. R_I, k_2 and BP were assumed to vary across PET scan times taking values from two distributions having typical values equal to R_I^*, k_2^* and BP* and a dispersion proportional to ωR_I, ωk_2 and ωBP to account for IIV and to πR_I, πk_2 and πBP to account for IOV variance component.

Table 3
Non-linear mixed effect modelling: comparison of the objective function values estimated using an additive and a proportional error model assumption

	Model C-a	Model C-b	Model C-c
Proportional error model	−643.112	−647.658	−687.345
Additive error model	−622.654	−630.683	−663.933

2.7. Model for residual error

The residual error on the time-activity measurements was modeled using either additive or proportional model. This error term component represents the residual departure of the model from the observations and contain contribu-

tions from unexplained variability, measurement error and model misspecification for the dependent variable.

2.8. Covariate effects

The dose of unlabelled ligand was expected to affect the BP values estimated in different occasions. Therefore, the dose of unlabelled ligand was considered as a covariate potentially explaining the variability observed on the BP fixed-effect parameter value. The procedure used to investigate the influence of the covariate was based on the analysis of the individual Bayesian parameter estimates plot vs. the covariate values [18] and on the log-likelihood ratio test. The exponential (Model C-b, Equation 7) and the sigmoid (Model C-c, Equation 8) models were investigated as potentially explanatory models.

$$BP_{ij} = BP_0 \cdot e^{-Dose_{ij} \cdot \beta} \quad (7)$$

$$BP_{ij} = BP_o - \frac{Emax \cdot Dose_{ij}}{ED_{50} + Dose_{ij}} \quad (8)$$

where BP_0 is the binding potential at baseline, BP_{ij} is the binding potential at i^{th} scan for j^{th} subject, β is a slope factor, Emax represents the maximum BP reduction and ED_{50} the dose giving 50% of the maximum BP reduction.

The model retained was included as a second stage model in the equation 4. The predictive accuracy of the individual Bayesian estimates of the time activity data was evaluated by comparing the scatter plot of the individual predictions vs. the observed data with the unitary slope reference line.

2.9. Data analysis

All analyses were performed using the first-order estimate method as implemented in NONMEM Version 5.1 [3]. Furthermore, using the population parameter the bayesian individual estimates of kinetic parameters were then estimated. Minimizing the objective function provided by NONMEM is equivalent to maximize the likelihood of data. Hypothesis testing was performed by comparing the changes in the objective function (OF) when the value of one or more parameters have been fixed in the regression model. The difference in OF values is asymptotically distributed as χ^2 with a degree of freedom equal to the difference in the number of parameters between the two regression models. Any reduction in OF greater than 3.84 and 5.99 (χ^2, $p < 0.05$ with 1 and 2 *df*) was considered to be significant and the parameter(s) concerned retained in the model according to the log-likelihood ratio test [9].

3. Results

The parameters estimated using the fixed-effect modeling approach (Model A) are shown in Table 1. The computational algorithm failed to reach convergence for monkey 4 at baseline and at scan 1 and for monkey 5 at scan 1 probably due to the variability on time-activity data. Furthermore, inconsistent negative value for receptor occupancy was estimated for monkey 6. In the Model B, all the time-activity observations collected in the same monkey at different scan times were simultaneously analyzed using a re-parameterised model where the RO% value was fixed to 0 at baseline and to a value ranging between 0 and 100% at the different scan times. The parameters estimated using this modeling approach are shown in Table 2. The scatter plot of the observed and model predicted time activity data of a typical individual (monkey 2) vs. time is displayed in Figure 1a. Two sets of analyses were conducted using the non-linear mixed effect to evaluate the influence of additive and proportional error model. The analysis database included 7 monkey with 17 time-activity curves and a total of 267 measurements. The results, shown in Table 3, indicate that the proportional error model significantly improved the OF values for all the modeling approaches used. The fixed and random parameter values estimated with the Model C-a, C-b, and C-c using the proportional error model are shown in Table 4. The results of this analysis indicate that the fixed influx/efflux parameter R_I and k_2 estimated from the 4 models have similar values at the exception of k_2 in the model C-c which shows an higher value. The comparison of random effects estimates indicates that IOV variability seems to represent the most important component of the total variability and that the inclusion of the Emax model, as a second stage regression model, significantly ($P < 0.01$) explains the observed variability on BP as a function of the unlabelled drug dose administered at the different scan times. The time activity plot of the observed and mixed effect model predicted values of a typical individual (monkey 2) is displayed in Figure 1b while the individual ob-

Table 4
Non-linear mixed effect modelling fixed and random effect parameter values.

Parameters	Model C-a	Model C-b	Model C-c
R_1	0.982	0.981	1.0
k_2	0.0171	0.0196	0.0268
BP	1.19	β = 2.19	BPo = 3.31
		BPo = 1.23	Emax = 3.05
			ED50 = 0.0000323
ωR	15	15	16
$\omega k2$	<1	<1	<1
ωBP	<1	<1	<1
πR_1	11	12	11
πk_2	35	33	<1
πBP	182	145	56
σ	8	8	7
OF	−643.112	−647.658	−687.345
ΔOF	0	4.56	44.233
Probability		df = 1	df = 2
		$P < 0.05$	$P < 0.01$

The ICV, IIV, IOV and residual error variability are expressedas CV%.

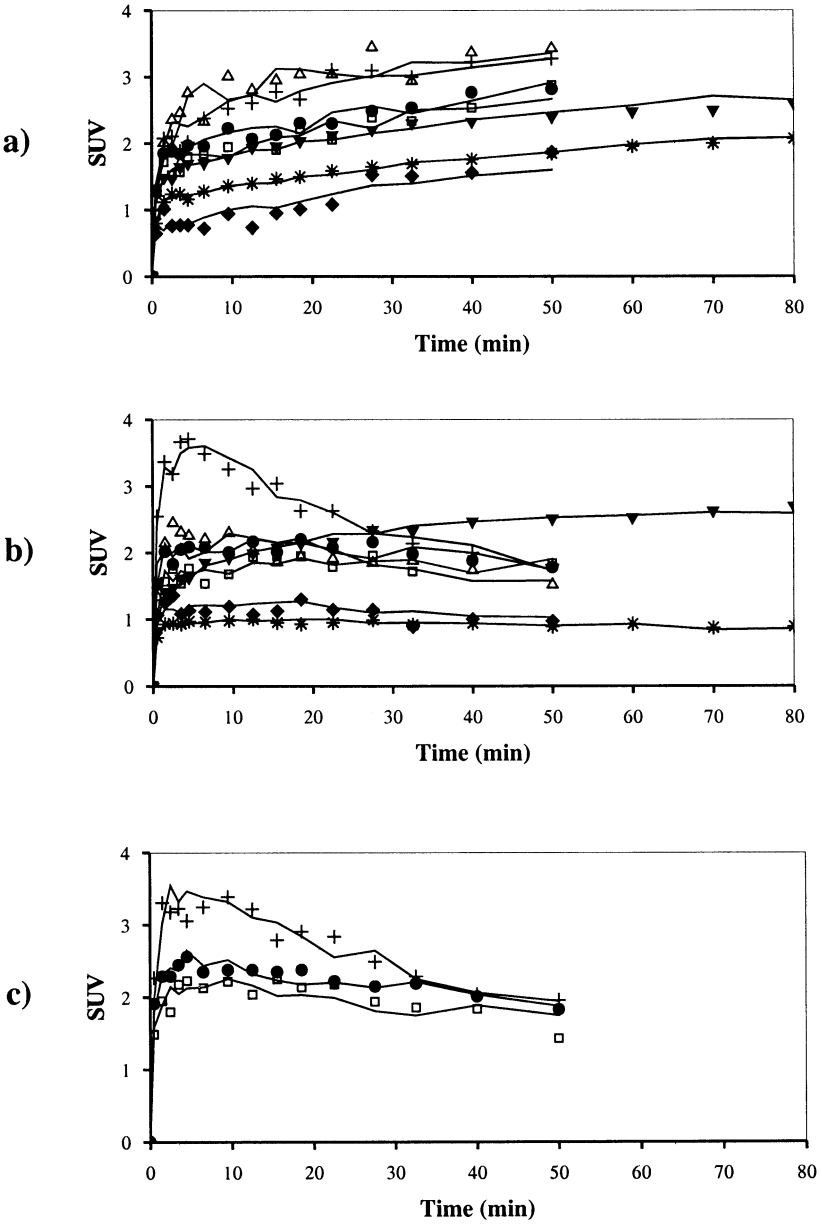

Fig. 2. Individual observed time-activity (SUV) data with the posterior individual predicted values (continuos line) at baseline (panel a), at the second scan time (panel b) and at the third scan time (panel c) for each monkey enrolled in the study: (+) monkey 1, ( ) monkey 2, (●) monkey 3, (◆) monkey 4, (△) monkey 5, (▼) monkey 6, (*) monkey 7.

served time activity data with the posterior model predicted values for the 7 monkeys at baseline and at the first and second scan time are display in Figure 2. The overall evaluation of the fitting obtained with the C-c model is illustrated by the excellent agreement between individual prediction vs. observed RO% values with the unitary slope reference line (Figure 3).

4. Discussion

PET offers unique possibilities to investigate physiology, metabolism, pharmacokinetic, pharmacodynamic, and modes of action of drugs from animal and human studies. Several methods have been proposed for the analysis and the quantification of *in vivo* ligand-receptor interactions from PET data even if no universally "best" method has been recognized [25]. In any case, the modeling approach based on the arterial plasma input function appears as the method of choice [26]. However, in absence of arterial input function, mainly dues to the impossibility of properly identify and measure metabolite concentrations, the reference tissue methods remain, at the moment, a preferred modeling strategy despite the limitation and the known problems associated to this approach. In the present paper, STRM has

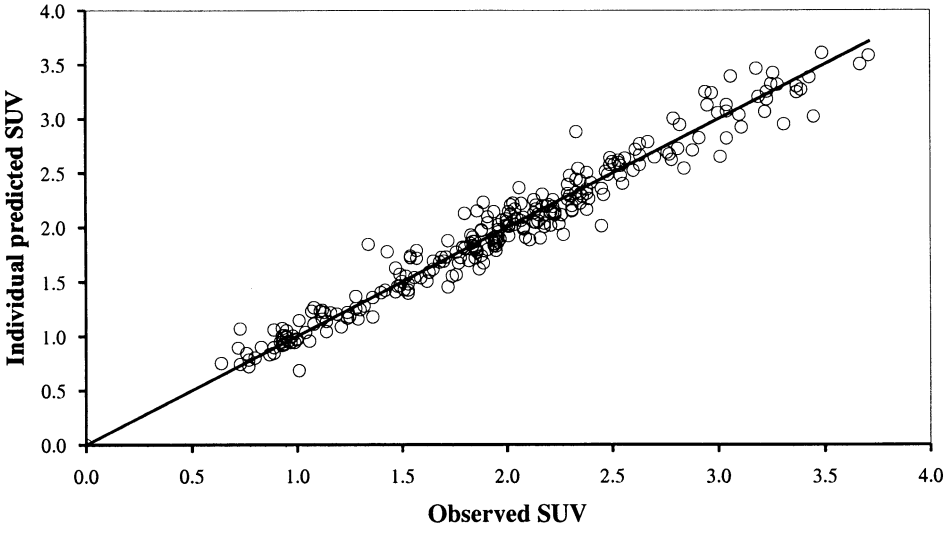

Fig. 3. Individual predicted versus observed time-activity data (SUV) with the reference unitary slope line (continuos line).

been selected according to statistical and goodness of fit criteria. At variance from the graphical method, which provides biased parameter estimates, the SRTM usually supplies well identified but, some time, underestimated parameter values.

A reliable estimate of the time-varying fraction of receptor occupancy integrated with the drug pharmacokinetic properties will enable researcher to build predictive models necessary to optimize the drug development process. Monte Carlo simulations have demonstrated that ignoring the presence of the inter-occasion variability may lead to biased and more variable parameter estimates [14,15] in pharmacokinetic/pharmacodynamic studies. For this reason, similar problems are expected in the analysis of PET experiments due to the repeated measure structure of the time-activity data and the complex mathematical models used to describe the response. The presence of intra-and inter-subject variability can be detected by inspecting the changes over time of the time-activity data measured in a RR following the same tracer injection. By definition, the RR is expected to be drug receptor free, therefore the variability observed on the time-activity kinetics in this region is assumed to reflect only inter- and intra-subject variability. This can be quantified by using the distribution property of the area under the time-activity curve estimated using the linear trapezoidal rule from 0 to 50 minutes (Mean = 82.7, Min = 42.1, Max = 110.4, S.D. = 19.2, CV% = 23.4). Some of this variation can be linked to experimental conditions associated to the PET technology (such as equipment calibration and tuning, procedures to collect and process data, sensitivity and detection limits, etc.) or to physiological processes associated to individual behavior. On these conditions, the use of non-linear mixed effect modeling approaches seems appropriate to better estimate the receptor occupancy parameter accounting for the different sources of variability. The evaluation of the different modeling approaches revealed that one of the major limitations of Model A is related to the underlying assumption considering each time-activity curve as a measurement coming from a separate individual. This assumption aggregates the within subject and the measurement error variability into an overall measurement noise, which therefore results artificially inflated. The final consequence of this assumption was the estimate of misleading parameters such as a negative receptor occupancy value and, in some cases, the impossibility to reach convergence in the minimization algorithm. This finding is in agreement with previously reported observations [1,22]. To overcome these limitations the Model B approach was proposed. In this approach the whole set of observations collected at different scan times on each monkey were simultaneously fitted together and the model was constrained to estimate positive RO% values. Furthermore, R_I and k_2 were estimated on all the individual data, assuming that these values remain constant on the same monkey, while the observations at baseline and at the different scan times were used to estimate BP_0 and the RO% at the different scan times. Using this approach we did not observe any computational problem and any inconsistency on the estimated parameter values. However, two major limitations persist: (a) the R_I and k_2 values are not constant over time for an individual but they may change on time, (b) this approach does not account for intra-individual variability which was, again, lumped into the measurement noise. Finally, three mixed effect models were investigated: the first one (Model C-a) only accounted for IIV and IOV while the Model C-b and C-c included two alternative second stage models to explain variability on BP as a function of the dose of unlabelled drug administered. The comparison of the different models indicates that the mixed effect approach with a primary model partitioning the variance in term of IIV and IOV and a second stage model relating the changes of binding potential to the dose of unlabelled drug

Table 5
Brain receptor occupancy (%) estimated using fixed and mixed effect modelling approach

Monkey	Scan	Model A	Model B	Model C
1	1	92.0	99.6	92.5
	2	88.5	99.7	91.3
2	1	90.8	82.6	86.4
	2	97.6	87.9	93.3
3	1	93.0	86.9	85.6
	2	93.4	79.6	84.8
4	1	*	100	97.
5	1	*	98	95.1
6	1	−20.3	63.2	50.1
7	1	45.5	83.6	85

* The non-linear regression procedure failed to reach convergence.

with an Emax model is definitely the preferred approach. However, the limited number of subjects (7 monkeys) and the limited number of occasions for subject (3 occasions in 3 monkeys and 2 occasions in 4 monkeys) suggests that the estimate of each variance term component must be cautiously interpreted even if the overall database used in the analysis (267 observations) was sufficiently large to allow a proper parameter estimation. In any case, the contribution of the IOV to the overall variance remains larger than the one of the IIV indicating the presence of an important intra-subject variability in the time-activity data collected during a PET experiment in the same subject. In addition, the relative error affecting the receptor occupancy seems inversely proportional to its value: the lowest is the value, the highest is the discrepancy between the RO% values estimated with the different methods as reported in Table 5. This observation indicates that the influence of the estimation procedure may become a critical factor for the appropriate evaluation of this parameter in particular at low RO% values (i.e. $< 50\%$). These findings may be of particular interest in the analysis of the experiments designed for the evaluation of receptor occupancy kinetic profile over time where several PET scans are collected in the same individual and where the extent of intra-subject variability may introduce artifact and/or bias in the evaluation of the results.

In conclusion, the non-linear mixed effect modeling seems to represent a valid alternative analysis approach mainly because it accounts for the repeated-measurement structure of the data and supply an estimate of the different variability components on the parameter values. In addition, this approach allows to integrate a second stage regression model to investigate the sources of variability in term of concomitant measurements (covariates). In our example only dose was included in this second stage model, however this approach can be easily extended to account for other factors such as demographical, pathophysiological, genetic factors which can potentially be used to investigate sources of variability in brain receptor occupancy.

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