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Non-linear mixed effects modelling of positron emission tomography data for simultaneous estimation of radioligand kinetics and occupancy in healthy volunteers

Matts Kågedal [a,*], Zsolt Cselényi [a], Svante Nyberg [a], Siv Jönsson [a,1], Patrick Raboisson [a], Per Stenkrona [b], Andrew C. Hooker [c], Mats O. Karlsson [c]

[a] AstraZeneca R&D, SE-151 85 Södertälje, Sweden
[b] Karolinska Institutet, Department of Clinical Neuroscience, Psychiatry Section, Karolinska Hospital, S-171 76 Stockholm, Sweden
[c] Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden

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ABSTRACT

The aim of this work was to develop a model simultaneously estimating ^{11}C-AZD9272 radioligand kinetics and the relationship between plasma concentration of AZD9272 and receptor occupancy in the human brain. AZD9272 is a new chemical entity pharmacologically characterised as a noncompetitive antagonist at the metabotropic glutamate receptor subtype 5 (mGluR5). Positron emission tomography (PET) was used to measure the time course of (^{11}C-AZD9272) in the brain. The study included PET measurements in six healthy volunteers where the radioligand was given as a tracer dose alone as well as post oral treatment with different doses of unlabelled AZD9272. Estimation of radioligand kinetics, including saturation of receptor binding was performed by use of non-linear mixed effects modelling. Data from the regions with the highest (ventral striatum) and lowest (cerebellum) radioligand concentrations were included in the analysis. It was assumed that the extent of non-displaceable brain uptake was the same in both regions while the rate of CNS uptake and the receptor density differed.

The results of the analysis showed that AZD9272 binding at the receptor is saturable with an estimated plasma concentration corresponding to 50% occupancy of approximately 200 nM. The density of the receptor binding sites was estimated to 800 nM and 200 nM in ventral striatum and cerebellum respectively. By simultaneously analysing data from several PET measurements and different brain regions in a non-linear mixed effects framework it was possible to estimate parameters of interest that would otherwise be difficult to quantify.

Introduction

An accurate prediction of therapeutic exposure levels is important in drug-development since it can improve go/no-go decisions as well as dose selection in clinical trials. The prediction of therapeutic doses of new CNS compounds is traditionally predicted based on plasma concentrations in preclinical efficacy models and human pharmacokinetics. However, for this prediction to be valid one needs to take into account any interspecies differences in plasma protein binding, transport across the blood brain barrier (BBB) and affinity to the receptor (Boström et al., 2008). These factors can vary considerably between species and are often challenging to estimate accurately. Estimation of receptor occupancy avoids these problems by giving a quantitative measure of the drug interaction with the target receptor at the site of action. Estimation of receptor occupancy thus has the potential to significantly improve the translation between compounds targeting the same receptor or from preclinical efficacy models to man. This bridging approach via occupancy was to be applied to a putative CNS drug, AZD9272. AZD9272 is a new chemical entity pharmacologically characterised as a noncompetitive antagonist (aka negative allosteric modulator) at the metabotropic glutamate receptor subtype 5 (mGluR5). When this study was performed, there was no validated mGluR5 radioligand for PET available, thus it was explored if a ^{11}C-labeled version of AZD9272 itself could be used as a radioligand for PET-occupancy studies. Prior to this study the suitability of [^{11}C]AZD9272 as a PET-ligand was evaluated in primate (Andersson, 2010) and in human (data on file).

The present analysis was performed by using non-linear mixed effects modelling where radioligand concentrations in the CNS from a high and low-uptake region, arterial blood concentration data of [^{11}C]AZD9272 as well as unlabelled AZD9272 concentrations in plasma from all PET-measurements were included in one simultaneous analysis.

The nonlinear mixed effects modelling approach, often referred to as population modelling, has been extensively used in pharmacokinetic/pharmacodynamic analyses and is particularly useful when data is

* Corresponding author. Fax: +46 8 553 288 96.
E-mail address: matts.kagedal@astrazeneca.com (M. Kågedal).
[1] Present address: Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden.

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sparse (Aarons, 1996). Despite the apparent richness of the data collected in PET-studies, the information from each separate PET-measurement on model parameters is often sparse and increasingly the analysis of PET-data is being performed by population methods (Liefaard et al., 2005; Lim et al., 2007; Zamuner et al., 2002).

For many receptors, including mGluR5 (Treyer et al., 2007), no reference region known to be devoid of receptors exists, making it difficult to distinguish specific from non-specific concentrations. As a consequence, the occupancy is also difficult to estimate. There are three principally different sources of information in the data supporting the distinction between specific and nonspecific uptake. First, the radioligand kinetics can be informative if the specific binding is kinetically distinguishable from the kinetics of the non-specific uptake. Secondly, the non-displaceable (non-specific plus free) concentration can be estimated by assuming that it is the same in different brain regions, leveraging the difference in specific binding between regions (Lassen et al., 1995; Cunningham et al., 2010). Thirdly, there is information even in individual regions on the non-displaceable uptake based on the saturation model where the non-displaceable uptake is the asymptote which the total uptake approaches when drug exposure tends to infinity.

The aim of the present work was to develop a model that simultaneously integrated information from all PET-measurements in order to characterize the relationship between exposure and occupancy.

Materials and methods

Overall study design

This was an open-label, non-randomised, single-centre PET study in six healthy volunteers. Each volunteer was examined by PET at four separate occasions. In all four PET examinations a tracer amount of the radioligand [^{11}C]AZD9272 was given. The first occasion was a base-line assessment where the radioligand was given alone. During the three subsequent PET examinations, the tracer amount of the radioligand was combined with single oral doses of AZD9272 that was given approximately 3 h prior to start of the PET-measurement. Doses in the range 3-24 mg were selected to provide a good description of the relationship between drug concentration in plasma and receptor occupancy (the saturation hyperbola), while not exceeding the maximum tolerated dose of 24 mg. The PET scan duration was 92 min at the base-line measurement and 62 min after pre-treatment. PET examinations after oral dosing with AZD9272 were separated by a washout period of 10 days or more in order to allow drug plasma concentrations to fall to below 5% of its peak concentration. [^{11}C]AZD9272 was synthesized by radiochemists at the PET centre from a precursor immediately before intravenous administration. The mass of the radioligand was to be less or equal to 0.04 μg/kg body weight and the radioactive dose was to be approximately 300 MBq at each PET examination. The obtained specific activity was on average 2100 Ci/mmol. The study was concluded by a follow-up visit 9 to 14 days after the last PET examination.

Derivation of data for PK-PD analysis

MRI and image analysis

Before PET each volunteer was examined by Magnetic Resonance Imaging (MRI). MRI scans were performed on a 1.5 Tesla unit (General Electric, Sigma). Two examinations were made in one session during 15 min. The first was T2-weighted for clinical evaluation regarding pathology. The second was T1-weighted for co-registration with PET and delineation of anatomical brain regions or regions of interests (ROIs). The T2 sequence was a 2-D fast spin echo protocol with the following settings: TR 5000 ms, TE 68, FOV 260×260 mm, 44×3.0 mm slices, slice gap 0.125 mm, matrix 256×256, 1 NEX. The T1 sequence was a 3-D SPGR protocol with the following settings: TR 21 ms, TE 6 ms, flip angle 35°, FOV 260 mm, matrix 256×192×156, 156×1.0 mm slices, 1 NEX. The T1 sequence was optimized for trade off between a minimum of scanning time and a maximum of spatial resolution and contrast between grey and white matter.

PET measurements were performed on an ECAT Exact HR 47 system (CTI/ Siemens, Knoxville, TN, USA) run in three-dimensional mode with dual-energy Windows scatter correction (Wienhard et al. 1994). A three-ring detector block architecture gives a 15 cm wide field of view (FOV). The transaxial resolution of the reconstructed images is 3.8 mm full width at half maximum (FWHM) at the centre of the FOV, 4.5 mm FWHM tangentially, and 7.4 mm radially at 20 cm from the centre. The axial resolution is 4 mm FWHM at the centre and 6.8 mm at 20 cm from the centre. Prior to each emission a transmission scan of 10 min was performed using three rotating 68Ge–68 Ga sources. After correction for attenuation, random and scattered events, images were reconstructed using a Hann filtre (2 mm FWHM). The reconstructed volume was displayed as 47 horizontal sections with a centre-to-centre distance of 3.125 mm and a pixel size of 2×2 mm.

After PET acquisition and reconstruction, PET images were transferred to the Statistical Parametric mapping software (SPM5) for spatial normalization and co-registration. For each subject, the MRI images were spatially normalized to position the anterior–posterior commissural (AC–PC) line in the horizontal plane, and the interhemispheric plane orthogonal to the AC–PC plane. The reoriented T1 images were then re-sliced to 1 mm voxels in a matrix of 220×220×170. The delineations of anatomical regions of interests (ROIs) were made manually on the spatially normalized MRI images in the three orthogonal projections using software developed for the Human Brain Atlas (Roland et al., 1994). For the ventral striatum ROIs were made on coronal projections anterior to the anterior commissure as described by Mawlawi et al., (2001). For the caudate nucleus, putamen, the hippocampus, temporal, anterior cingulate cortex the ROIs were made on sagittal projections, while for the dorsolateral prefrontal cortex and amygdala the ROIs were made on coronal slices. For the thalamus and cerebellum the ROIs were made on horizontal projections. The SPM5 software was used to have the PET images co-registered to the normalized MR images and re-sliced to a 2×2×2 mm matrix. The ROIs were superimposed on the PET images using the co-registration parameters. Radioactivity concentration in each ROI (CROI) was calculated for each sequential frame, corrected for ^{11}C decay, and plotted versus time. The ROIs were pooled for each region. The results were stored as time–activity (TACT) data for brain tissue, given as radioactive concentration (nCi/mL). The duration of each time frame was 20 s for the first 2 min (6 frames) followed by 1 min duration up to 6 min (4 frames) and then 3 min for the remainder of the experiment.

Derivation of [^{11}C]AZD9272 plasma input function

Arterial metabolite corrected plasma [^{11}C]AZD9272 concentration versus time was generated as described previously (Farde et al., 1989). It involved correction for metabolite and distribution into blood cells.

Bio-analytical methods and estimation of drug exposure during PET

Plasma samples for determination of unlabelled AZD9272 concentration in plasma were taken immediately prior to start of PET, mid PET and after completion of the PET measurement. The determination of the unlabeled plasma concentration of AZD9272 was performed by reversed-phase liquid chromatography and tandem mass spectrometry.

Modelling

The population analysis program NONMEM, a widely used nonlinear regression software package in population pharmacokinetic–pharmacodynamic data analysis and the first order conditional