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# A positron emission tomography study in healthy volunteers to estimate mGluR5 receptor occupancy of AZD2066 — Estimating occupancy in the absence of a reference region

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ABSTRACT

AZD2066 is a new chemical entity pharmacologically characterized as a selective, negative allosteric modulator of the metabotropic glutamate receptor subtype 5 (mGluR5). Antagonism of mGluR5 has been implicated in relation to various diseases such as anxiety, depression, and pain disorders.

To support translation from preclinical results and previous experiences with this target in man, a positron emission tomography study was performed to estimate the relationship between AZD2066 plasma concentrations and receptor occupancy in the human brain, using the mGluR5 radioligand [11C]-ABP688.

The study involved PET scans on 4 occasions in 6 healthy volunteers. The radioligand was given as a tracer dose alone and following oral treatment with different doses of AZD2066. The analysis was based on the total volume of distribution derived fro
m each PET-assessment. A non-linear mixed effects model was developed where ten delineated brain regions of interest from all PET scans were included in one simultaneous fit. For comparison the analysis was also performed according to a method described previously by Lassen et al. (1995).

The results of the analysis showed that the total volume of distribution decreased with increasing drug concentrations in all regions with an estimated Kipl of 1170 nM. Variability between individuals and occasions in non-displaceable volume of distribution could explain most of the variability in the total volume of distribution. The Lassen approach provided a similar estimate for Kipl, but the variability was exaggerated and difficult to interpret.



## Introduction

Estimation of receptor occupancy can significantly improve the translation between compounds targeting the same receptor or from preclinical efficacy models to man (Kagedal et al., 2012). The present study of the relationship between AZD2066 concentrations in plasma and occupancy in the human brain was performed in order to improve predictions of therapeutic exposure levels and aid dose-selection for following clinical studies.

AZD2066 is a new chemical entity pharmacologically characterized as a potent and selective, negative allosteric modulator (NAM, aka non-competitive antagonist) of the metabotropic glutamate receptor subtype 5 (mGluR5) (Raboisson et al., unpublished). The mGluR5 receptor is widely distributed in the central nervous system (Bear et al., 2004; Berg et al., 2011; Brodkin et al., 2001; Chiamulera et al., 2001; Johnson et al., 2009; Spooren et al., 2000; Tatarczynska et al., 2001; Varney and Gereau, 2002; Walker et al., 2001a,b) and has received attention as a potential therapeutic target in various diseases such as anxiety, depression, and pain disorders.

Using in vitro functional assays AZD2066 has been shown to inhibit human mGluR5 with mean IC50 values of 5.7 and 7.5 nM (calcium mobilization assay and phosphatidyl inositol hydrolysis assay, respectively). AZD2066 also competitively inhibited (Ki of 21 nM) [3H]MPEP, a prototypic mGluR5 NAM, from binding to human mGluR5 (Gasparini et al., 1999). AZD2066 displayed >100 to >1000-fold selectivity in vitro for mGluR5 vs. 147 other molecular targets, including all other mGluR subtypes. In vivo, AZD2066 penetrated the blood brain barrier easily and displaced the known mGluR5 radioligands [3H]methoxymethyl-MTEP (Anderson et al., 2002) and [11C]-ABP688 (Ametamey et al., 2006, 2007; Hintermann et al., 2007) in rat (Raboisson et al., Neuropharmacology submitted) and non-human primate brains (unpublished data, manuscript in preparation).

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AZD2066 safety and efficacy have been investigated in clinical studies in healthy volunteers as well as in patients with painful diabetic neuropathy, neuropathic pain with mechanical hypersensitivity and major depressive disorder. In patients suffering from neuropathic pain with mechanical hypersensitivity, AZD2066 demonstrated a clinically and statistically significant reduction in pain and hypersensitivity (Jonzon et al., 2012), while no efficacy conclusion could be drawn from the studies in the other two indications. In healthy volunteers, AZD2066 was found to be tolerable with mainly dose-dependent CNS adverse events (AEs) observed. The development of AZD2066 was terminated since some AEs observed with AZD2066, and also previously with other mGluR5 antagonists, suggested that AZD2066 risk-benefit profile was not favourable. Detailed reports of the phase 2a data and of AZD2066 safety profile will be reported elsewhere (Karlsten et al., 2012; Jonzon et al., 2012; Ståhle et al., 2012).

The study was carried out using the radioligand [$^{11}$C]-ABP688, a highly selective radioligand for in vivo imaging of mGluR5 receptors in human using PET. The radioligand has favourable kinetics allowing for quantification of central mGluR5 in a reasonable short, 45–60 min acquisition (Ametamey et al., 2006, 2007; Treyer et al., 2007). The binding on human mGluR5 of ABP688, a chemical derivative of MPEP in which the aromatic ring is replaced by a functionalized cyclohexenone moiety, is also known to be fully displaceable by MPEP (Hintermann et al., 2007), indicating that ABP688, MPEP and AZD2066 share a common binding site on mGluR5.

In humans, no reference region known to be devoid of mGluR5 receptors exists (Patel et al., 2007; Treyer et al., 2007), making estimation of occupancy more difficult. It is theoretically possible to derive the binding potential ($BP_{ND}$) as an index of specific binding based on the estimated rate parameters in each individual PET scan (Innis et al., 2007). In the absence of a reference region, this method however suffers from identifiability problems. Obviously, when occupancy becomes high, identifying rate-parameters relating to specific binding becomes futile.

While the individual rate-constants often are highly correlated, a robust estimate of the total volume of distribution (VT) is however often possible to derive (Cunningham et al., 2004). Lassen et al. (1995) proposed an approach allowing estimation of occupancy, based on VT, leveraging the difference in receptor density between regions. The Lassen approach was subsequently further developed (Cunningham et al., 2010).

In a situation when information is sparse based on each individual subject, nonlinear mixed effects modelling (NLME) has been shown to be useful (Aarons, 1996). By integrating all data into one simultaneous fit, a model that is un-identifiable based on individual subjects may become identifiable. This method may thus improve the ability to separate specific from nonspecific brain uptake in the analysis and hence allow estimation of occupancy. Increasingly the analysis of PET-data is being performed by population methods (Liefaard et al., 2005; Lim et al., 2007; Syvanen et al., 2011; Zamuner et al., 2012). Recently it was proposed that simultaneous modelling of radioligand kinetics of all PET scans in two regions of interest (ROIs) allowed quantification of the relationship between exposure and occupancy applying NLME methodology (Kagedal et al., 2012). This approach made use of all the data in an efficient way, since the radioligand kinetics as well as the difference in specific uptake between regions informed the model in one simultaneous fit. The drawback with this approach was that only two regions of interest were included and that it had rather long runtimes and more complex model-building process.

In the present analysis VT for ten different regions of interest was determined in a first step for each of the PET scans. The relationship between plasma drug concentration during the PET scan and occupancy was subsequently estimated using NLME modelling where VT from 10 brain regions in six subjects were included in one simultaneous fit. A similar approach has been proposed previously by Berges et al. (2008). For comparison, the analysis was also performed by the method proposed by Lassen et al. (1995).

The aim of the present work was to quantify the relationship between AZD2066 plasma concentrations and displacement of [$^{11}$C]-ABP688 from mGluR5 binding-sites in the CNS and to compare the results obtained with NLME modelling to the Lassen approach.

## Methods

### *Overall study design*

This was an open-label, non-randomised, single-centre, exploratory PET study in 6 healthy male volunteers aged between 23 and 40 years. The study comprised 2 panels, Panel 1 and Panel 2, with 3 subjects each. An interim analysis of preliminary data from Panel 1 including PET data and pharmacokinetic data was performed for dose selection for Panel 2.

Four PET scans were performed in each subject. The first PET scan was performed in the absence of AZD2066 (baseline PET). In the subsequent occasions the PET scan was preceded by administration of an oral solution of AZD2066. Venous blood samples for determination of AZD2066 concentration in plasma were collected regularly before, during and after the PET scan. In Panel 1, 3 single oral doses (3.5 mg, 6.9 mg and 13.5 mg) of AZD2066 were administered at separate occasions with a dosing interval of at least one week. In Panel 2, the subjects received 13.5 mg, 6.9 mg and 0 mg (a second baseline scan). These doses were selected to provide informative data for the estimation of the concentration–occupancy relationship. The repeated baseline measurement was performed since it was judged informative for the estimation of the concentration–occupancy relationship and in addition would provide some (albeit limited) test–retest data.

### *Study drugs*

The oral solution of AZD2066 was prepared by the Karolinska Hospital Pharmacy. [$^{11}$C]-ABP688 was manufactured extempore at the PET centre at Karolinska University Hospital, Solna. At each PET-scan, approximately 300 MBq of [$^{11}$C]-ABP688 in an aqueous solution was administered as an intravenous bolus injection. The total radioactivity amount for each volunteer including all four PET-scans was approximately 1200 MBq. The injected radioligand had high specific radioactivity and the total mass administrated was less than 0.3 μg per injection.

Subcutaneous lidocaine, 1 to 2 mL, was given as a local anaesthesia prior to the insertion of the arterial cannula.

### *PET related measurements*

Prior to PET scans, two anatomical 3D MRI examinations were made in one session. The first examination was T2-weighted and was used for clinical evaluation and exclusion of pathology. The second examination was T1-weighted and was used for delineation of anatomically defined ROIs. MR images were acquired using a 1.5 T General Electric Signa Unit (Milwaukee, WI, USA). Imaging parameters included a repetition time of 23 ms, echo time of 4 ms, matrix of 256 × 192 × 156, and voxel size of 1.02 × 1.02 × 1.0 mm.

The PET system was a Siemens Medical Solutions High Resolution Research Tomograph (HRRT) which follows radioactivity in 207 sections of the brain with three dimensional acquisition (Varrone et al., 2009). The spatial resolution in the reconstructed image is on average 2.3 mm full-width half-maximum (FWHM) in all directions.

In each PET scan the subject was placed supine and the head was fixed to the positron camera by the use of an individualized plaster helmet as described previously (Bergström et al., 1981). Radioactivity in the brain was measured for 63 min, following radioligand injection. The duration of the first 9 frames was 10 s each, followed by 2 × 15 s, 3 × 20 s, 4 × 30 s, 4 × 60 s, 4 × 180 s, and finally 7 × 360 s. A 63 minute acquisition was judged sufficient for obtaining stable results using [$^{11}$C]-ABP688 according to prior results (Ametamey et al., 2007).