Effect of the myeloperoxidase inhibitor AZD3241 on microglia: a PET study in Parkinson’s disease

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Impaired mitochondrial function, oxidative stress and formation of excessive levels of reactive oxygen species play a key role in neurodegeneration in Parkinson’s disease. Myeloperoxidase is a reactive oxygen generating enzyme and is expressed by microglia. The novel compound AZD3241 is a selective and irreversible inhibitor of myeloperoxidase. The hypothesized mechanism of action of AZD3241 involves reduction of oxidative stress leading to reduction of sustained neuroinflammation. The purpose of this phase 2a randomized placebo controlled multicentre positron emission tomography study was to examine the effect of 8 weeks treatment with AZD3241 on microglia in patients with Parkinson’s disease. Parkinson patients received either AZD3241 600 mg orally twice a day or placebo (in 3:1 ratio) for 8 weeks. The binding of $^{11}$C-PBR28 to the microglia marker 18 kDa translocator protein, was examined using positron emission tomography at baseline, 4 weeks and 8 weeks. The outcome measure was the total distribution volume, estimated with the invasive Logan graphical analysis. The primary statistical analysis examined changes in total distribution volume after treatment with AZD3241 compared to baseline. Assessments of safety and tolerability of AZD3241 included records of adverse events, vital signs, electrocardiogram, and laboratory tests. The patients had a mean age of 62 (standard deviation = 6) years; 21 were male, three female and mean Unified Parkinson’s Disease Rating Scale III score (motor examination) ranged between 6 and 29. In the AD3241 treatment group ($n=18$) the total distribution volume of $^{11}$C-PBR28 binding to translocator protein was significantly reduced compared to baseline both at 4 and 8 weeks ($P<0.05$). The distribution volume reduction across nigrostriatal regions at 8 weeks ranged from 13–16%, with an effect size equal to 0.5–0.6. There was no overall change in total distribution volume in the placebo group ($n=6$). AZD3241 was safe and well tolerated. The reduction of $^{11}$C-PBR28 binding to translocator protein in the brain of patients with Parkinson’s disease after treatment with AZD3241 supports the hypothesis that inhibition of myeloperoxidase has an effect on microglia. The results of the present study provide support for proof of mechanism of AZD3241 and warrant extended studies on the efficacy of AZD3241 in neurodegenerative disorders.

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Introduction

Parkinson’s disease is the second most common chronic neurodegenerative disorder. Most symptomatic treatments for Parkinson’s disease target the dopamine system and provide symptomatic relief in early to intermediate stages. However, during long-term dopaminergic therapy (>5 years) motor complications emerge (Ondo, 2011). Thus, there is a recognized therapeutic need for new medications that would modify the disease course by preserving functions of the dopaminergic and other neurochemical systems involved in the pathophysiology of Parkinson’s disease.

The aetiology of Parkinson’s disease remains largely unknown. Impaired mitochondrial functioning and oxidative stress are likely implicated in the pathogenesis of the progressive neuronal dysfunction, as they might enhance α-synuclein aggregation into inclusion bodies (Gupta et al., 2008; Gautier et al., 2014). The reactive oxygen generating enzyme, myeloperoxidase (MPO), has been found to be expressed by microglia cells in Parkinson’s disease brain (Galter et al., 2007). Oxidative stress and neuronal loss in Parkinson’s disease has been associated with persistent neuroinflammation. This hypothesis has been supported by studies examining different inflammatory markers. Increased expression of genes encoding proinflammatory cytokines and elevated density of glial cells expressing pro-inflammatory cytokines have been found in the substantia nigra of patients with Parkinson’s post-mortem (Hunot and Hirsch, 2003; Duke et al., 2007; McGeer and McGeer, 2008). Moreover, an increase in cytokine levels has been found in the CSF of patients with Parkinson’s disease (Müller et al., 1998; Lindqvist et al., 2013). Finally, increased binding of 18-kDa translocator protein (TSPO), a marker of activated microglia, has been reported in Parkinson’s disease patients in vivo (Ouchi et al., 2005; Gerhard et al., 2006; Bartels et al., 2010). TSPO binding measured with PET has been used to examine effect of minocycline and cyclooxygenase (COX-2) inhibitors in multiple system atrophy and Parkinson’s disease (Bartels et al., 2010; Dodel et al., 2010). The pilot studies, however, did not conclusively confirm effect of anti-inflammatory drugs on TSPO binding.

The novel compound AZD3241 is a selective and irreversible MPO inhibitor in development by AstraZeneca. Studies in animal models of Parkinson’s disease and multiple system atrophy AZD3241 have demonstrated suppression of microglia activity and neuroprotective effect on dopamine cell survival, i.e. preservation of TH-positive neurons in substantia nigra [MPTP model; (PLP)-α-synuclein transgenic mouse multiple system atrophy model; Stefanova et al., 2005; AstraZeneca data on file]. It is predicted that AZD3241, acting by inhibition of MPO activity, may break the self-perpetuating cycle of oxidative toxic inflammatory process and thereby slow down progression of neurodegeneration in Parkinson’s disease.

Initial PET imaging of TSPO with the radioligand 11C-(R)PK11195 have been hampered by unfavourable radioligand characteristics, e.g. high non-specific binding (Chauveau et al., 2008). A new radioligand 11C-PBR28 has more recently been developed and has been demonstrated to have higher signal-to-noise ratio compared to 11C-(R)PK11195 as well as the unique feature of ‘no binding’ in some subjects (Fujita et al., 2008; Kreisl et al., 2010). The latter has been explained by the rs6971 polymorphism of the TSPO gene that leads to a non-conservative amino acid substitution associated with different affinity of 11C-PBR28 to TSPO in vitro leading to the low, mixed and high affinity binding (Owen et al., 2010, 2011). Depending on homozygosity or heterozygosity of the mentioned polymorphism, the subjects have been shown to display apparently different TSPO binding in vivo (Kreisl et al., 2013; Owen et al., 2014).

The purpose of the present phase 2a randomized placebo controlled multicentre PET study was to examine the effect of 8 weeks treatment with AZD3241 on microglia as measured by 11C-PBR28 binding to TSPO in 24 patients with Parkinson’s disease. The patients were also examined for the polymorphism of the TSPO gene. The primary outcome measure was total distribution volume (VT) of 11C-PBR28 binding. The primary objective was to determine changes in VT after treatment with AZD3241 compared to baseline. As a part of secondary and exploratory objectives the study we also examined differences in changes of VT after treatment with AZD3241 compared to placebo, as well as safety and tolerability of AZD3241.

Materials and methods

This PET study was approved by the local Research Ethics and Radiation Safety Committees and the Medical Products Agencies in Sweden and Finland and was performed in
accordance with the current amendment of the Declaration of Helsinki and International Conference on Harmonization/Good Clinical Practice guidelines. Written informed consent was obtained from all patients. Patients were recruited by movement disorder specialists at five study centres (one centre in Finland and four centres in Sweden). PET measurements were performed at the Karolinska Institutet PET Centre, Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden and at the Turku PET Centre, University of Turku/Turku University Hospital, Finland. The study was carried out from April 2012 to January 2013.

Study design

This was a randomized, double-blind and placebo controlled study (EudraCT Number: 2011-004803-19; ClinicalTrials.gov Identifier: NCT01527695). Patients were randomized in a 3:1 ratio to one of two parallel groups (AZD3241 600 mg twice daily or placebo) and were treated for 8 weeks (Fig. 1). All doses of AZD3241 and placebo were administered orally in identical-appearing tablets of 25 mg and 100 mg (manufacturer AstraZeneca). The addition of a small placebo group was primarily motivated by the need of a control group for safety assessments, due to the early clinical phase of AZD3241 development and for blinding the study. Secondarily, the placebo group provided initial qualitative information on VT changes within the placebo group and relative to the drug treatment arm. However, the study was not powered to provide group comparisons as this was not the aim of the study (Bindelslev, 2008).

The study included four PET measurements, two of which were performed during the enrolment/screening period. The first PET measurement with 11C-PBR28 served for exclusion of ‘low affinity binders’, based on the inspection of images and typical time radioactivity curves (Kreisl et al., 2010; Owen et al., 2011), and was retrospectively confirmed by TSPO genotyping. The first 11C-PBR28 PET measurement also served as baseline. The second PET measurement was performed with the dopamine transporter (DAT) radioligand 18F-FE-PE2I and aimed to confirm the presence of nigrostriatal deficit. Analysis of 18F-FE-PE2I binding was performed by visual assessment of striatal DAT loss and criteria for inclusion were the presence of asymmetric decrease in striatal 18F-FE-PE2I binding and a more severe reduction in the posterior putamen compared to caudate nucleus (according to the criteria reported for DaTSCAN SPECT; Catafau and Tolosa, 2004; Darcourt et al., 2010). The third and fourth PET measurements were performed using 11C-PBR28 at ~4 and ~8 weeks after randomization and served for the evaluation of treatment effects on TSPO.

Subjects

Twenty-nine patients with Parkinson’s disease, aged 45–75 years were enrolled. The patients fulfilled the modified United Kingdom Parkinson’s Disease Society Brain Bank criteria for idiopathic Parkinson’s disease (Hughes et al., 1992). Other inclusion criteria were: modified Hoehn and Yahr stage (Hoehn and Yahr, 1967; Goetz et al., 2004) of 1–2, Unified Parkinson’s Disease Rating Scale (UPDRS) motor score (Part III) (Fahn et al., 1987) of at least 10 at randomization. Patients were de novo or on stable treatment for Parkinson’s disease using dopaminergic medication i.e. on a stable dose for at least 28 days before randomization and during the study. Exclusion criteria were: suspicion of atypical Parkinsonian syndromes; anatomical brain abnormalities detected on MRI other than typical for Parkinson’s disease; presence of significant dyskinesias, motor fluctuations, swallowing difficulties or loss of postural reflexes; cognitive impairment (Mini-Mental State Examination ≤ 24); significant other neurological or psychiatric disease, hypothyroidism/thyroid hypofunction or presence of anti-thyroid peroxidase antibody, major or unstable somatic disease, and patients defined as ‘low affinity binders’ (Owen et al., 2011, 2014) with respect to 11C-PBR28 binding to TSPO.

Clinical assessments

Clinical evaluations of patients included collection of demographic and general clinical information, a standardized neurological exam, assessment of disease severity and motor signs with the UPDRS (Goetz et al., 2007). Cognitive capacity was assessed with the Mini Mental State Examination (Folstein et al., 1975), a brief 30-point questionnaire testing arithmetic, memory and orientation. Neurological examinations and PET imaging were performed in the ON state, i.e. the patients had taken their anti-parkinsonian medication according to prescription.

Imaging data acquisition and analysis

MRI and PET data were acquired at each of the participating imaging centres, 18 patients examined at Karolinska Institutet and six patients at Turku University. Image analysis was performed at the Karolinska Institutet PET Centre.

Magnetic resonance imaging

Prior to PET measurements, two anatomical brain MRI examinations were made for each patient. Images were acquired with 1.5 T Siemens Avanto system (Stockholm) and 1.5 T Philips Interna system (Turku) using the clinical protocol of each imaging centre, respectively. The first T2-weighted image was used for clinical evaluation and exclusion of pathology, and was evaluated by a clinical neuroradiologist. The second T1-weighted image was used for co-registration with PET and delineation of anatomical brain regions of interest. The T1-weighted magnetic resonance images were segmented into grey and white matter, and CSF using the SPM5 software (Wellcome Department of Cognitive Neurology, UK) and co-registered to the PET summation images.

Positron emission tomography

Radiochemistry

18F-FE-PE2I was prepared from its corresponding tosyl precursor, TsOE-PE2I (PharmaSynth AS) as described previously (Stepanov et al., 2012). 11C-PBR28 was prepared from its corresponding desmethyl-PBR28 precursor (PharmaSynth AS),
Figure 1  Study design. Titration: 50 mg BID, 100 mg BID, 200 mg BID, 300 mg BID, 400 mg BID, 600 mg BID; Rx = randomization. Vertical solid lines indicate visit at the clinical centre; dashed lines indicate telephone call; asterisk indicates blood sampling for drug plasma concentration analysis. w = weeks.

with minor modifications to the description in literature (Briard et al., 2008). Each radioligand was formulated in a mixture of sterile phosphate-buffered saline and ethanol (<10%) before final sterilization by filtration (0.22 mm; Millex GV filter, Millipore). The injected radioactivity was in the range 157–217 MBq, mean, standard deviation (SD): 185 (13) MBq for 18F-FE-PE2I and in the range 298–446 MBq, mean (SD): 403 (32) MBq for 11C-PBR28. The specific radioactivity of the radioligand at time of injection was 147 (85) GBq/μmol (mean, SD) for 18F-FE-PE2I and 243 (157) GBq/μmol for 11C-PBR28, corresponding to an injected mass of 0.75 (0.37) μg and 0.86 (0.55) μg, respectively.

Positron emission tomography measurements

An individual plaster helmet (PET centre, Karolinska Institutet) or a thermoplastic mask (Turku PET centre) were made for each patient and used with a head fixation system to minimize movement artefacts. A cannula was inserted into the left or right cubital vein. The radioligand was dissolved in a sterile physiological phosphate buffer (pH 7.4) and injected as a bolus over 10 s. The cannula was then immediately flushed with 10 ml saline.

PET data were acquired over 93 min with the high-resolution research tomograph (HRRT; Siemens/CTI). List-mode data were binned and reconstructed using 3D Ordinary Poisson Ordered Subset Estimation Maximization with 10 iterations and 16 subsets, including modelling of the point-spread function (Varrone et al., 2009) into a 4D PET image containing 38 consecutive time frames (9 × 10 s, 2 × 15 s, 3 × 20 s, 4 × 30 s, 4 × 60 s, 4 × 180 s and 12 × 360 s) with a 3D array of 256 × 256 × 207 voxels having a size of 1.22 × 1.22 × 1.22 mm. Attenuation correction was acquired with a 6-min transmission measurement using a single 137Cs source.

The 4D PET data were motion-corrected by co-registering individual 3D PET frames after the first minute of acquisition to the accumulated PET data of the first minute. The rigid body transformations required to co-register the individual frames were plotted and the realigned PET data were used for the patients where the PET analyst judged the amount of head movement to be significant. For minor movements <1.5 mm, all the reconstructed frames where aligned. For larger movements, each frame had to be reconstructed separately. An ill positioned attenuation correction may result in over or under corrected image data, thus being unfit for quantitative analysis. Therefore, the transmission map was aligned to the each frame emission data. Each image was reconstructed using the realigned transmission data and then the image was realigned to the initial position of the transmission data. In the final analysis, the realigned 18F-FE-PE2I PET image was used in case of two patients (one with minor and one with larger movement) and the realigned 11C-PBR28 PET image was used in case of one patient (baseline measurement, with minor movement).

Arterial blood sampling and determination of plasma radioactive 11C-PBR28 metabolites

A catheter was inserted into the radial artery and arterial blood was collected continuously during the first 10 min using an automated blood sampling system (ABSS, Allogg AB). Thereafter, a series of arterial blood samples (2 ml) were drawn manually at ~2, 4, 5, 7, 10, 15, 20, 30, 45, 60, 75 and 90 min post-radioligand injection. The radioactivity (counts/s) in the manually drawn samples was immediately measured for 10 s in a well counter cross-calibrated with the PET system. After centrifugation 0.2 ml plasma was pipetted and plasma radioactivity was measured in a well counter.

Plasma radioactivity corresponding to unchanged 11C-PBR28 was determined in arterial blood (4 ml) sampled at 4, 10, 20, 30, 45, 60, 75, and 90 min (PET Centre, Karolinska Institutet) and 4, 7, 10, 20, 30, 40 and 50 min after injection (Turku PET Centre). The plasma (1.5 ml) obtained after centrifugation of blood was deproteinized with acetonitrile and analysed by high-performance liquid chromatography (radio-HPLC system, Halldin et al., 1995; Amini et al., 2013). Blood (4 ml) and plasma (1.5 ml) samples were counted in a NaI well counter. Acetonitrile (A) and ammonium formate (100 mM) (B) were used as the mobile phase at 6.0 ml/min, according to the following gradient: 0–4 min (A/B), 40:60 → 80:20 v/v; 4.1–6 min (A/B), 80:20 v/v; 8 min (A/B), 40:60 v/v. Peaks for radioactive
compounds eluting from the column were integrated and their areas were expressed as a percentage of the sum of the areas of all detected radioactive compounds (decay-corrected to the time of injection on the HPLC).

Analysis of blood and plasma radioactivity concentrations and measurements of $^{11}$C-PBR28 metabolites were performed at two PET centres independently (PET centre, Karolinska Institutet, Stockholm, Sweden and Turku PET centre, Finland) using a common protocol.

**Arterial plasma input function for $^{11}$C-PBR28**

The time-curve representing the plasma radioactivity of unchanged radioligand, i.e. metabolite corrected arterial input curve, was generated by sequential procedures (Farde et al., 1989). The blood curve from the ABSS was merged with the interpolated curve from manual blood samples. Plasma and blood radioactivity concentrations from manual blood samplings were divided to obtain a plasma/blood ratio curve. The ratio curve was inter- and extrapolated from 0s to end of ABSS data collection using linear interpolation. Plasma time–activity curves were generated by multiplying the ABSS curve with the extrapolated plasma/blood ratio curve and by merging the resulting curve with the plasma curve from manual blood samples. The radioactivity concentration in total blood and plasma time–activity curves was produced using the software Kaleidagraph® Version 4.03. Individual data of the parent fraction of radioligand were fitted using an empirical model consisting of a mixture of the Hill and Richards equations (Bindslev, 2008). Metabolite correction of plasma input curve was performed by multiplying uncorrected plasma time–activity curves with parent fraction model curves.

**Quantification of $^{11}$C-PBR28 binding to TSPO**

The delineations of anatomical brain regions of interest were made automatically using the automated anatomical labelling template, AAL (Tzourio-Mazoyer et al., 2002). The use of AAL in patient populations (e.g. Alzheimer’s disease) has been reported (Schain et al., 2014), supporting the validity of using this template in Parkinson’s disease having less pronounced brain atrophy. Brain regions of the nigrostriatal system (whole striatum, ventral striatum, substantia nigra) were delineated using in-house template for patients with Parkinson’s disease (see detailed description below). In total, 39 brain regions of interest were delineated. The list of regions of interest included parts of the nigrostriatal system (caudate nucleus, putamen, ventral striatum, pallidum, substantia nigra and total striatum) and cerebral cortex (frontal, parietal, limbic, temporal, and occipital cortices), thalamus, cerebellum, grey matter, and whole brain.

$^{11}$C-PBR28 binding to TSPO was estimated by calculating the total distribution volume $V_T$ (Innis et al., 2007). The wavelet-aided parametric imaging (WAPI) approach (Cselenyi et al., 2006) was used to produce parametric images of $V_T$. The underlying kinetic analysis in the WAPI approach was the multi-linear version of Logan’s graphical analysis using the metabolite-corrected arterial blood curve as input function and fitting the last 10 points of the Logan plot to obtain the $V_T$ (data 14–60 min after injection) (Logan et al., 1990; Ichise et al., 2002). Time effect on $V_T$ was examined (analysis was performed using 45-, 60- and 90-min time intervals). The choice of final data analysis was limited to the 60 min duration of PET measurements, in order to minimize the potential contribution of radioligand metabolism to the imaging signal, to optimize duration of PET and plasma radioactivity measurements across patients and centres and to preserve stability and reliability of $V_T$ (Supplementary Fig. 1).

**Quantification of $^{18}$F-FE-PE2I binding to DAT**

The delineations of anatomical brain regions of interest were made automatically using an in-house region of interest template covering 16 brain regions: caudate nucleus, putamen, ventral striatum, substantia nigra, including regions in right and left hemisphere separately, cerebellum and total striatum as a combination of regions of interest for caudate, putamen and ventral striatum. The regions of interest were displayed on the corresponding PET images and the average radioactivity concentration for the whole volume of anatomical structure was obtained by pooling data from a series of sections. The radioactivity concentration in each region of interest was calculated for each sequential time frame, corrected for $^{18}$F decay and was plotted versus time (time–activity curves). The reference region-based linear graphical method was applied using the cerebellum time–activity curve as input function (Logan et al., 1990). The last 11 points of the Logan plot were fitted to obtain the distribution volume ratio (30–90 min after injection) and WAPI approach was used (Cselenyi et al., 2006) to produce detailed parametric images and binding potential, $B_{ND}$, values of $^{18}$F-FE-PE2I binding.

**Genotyping for TSPO polymorphisms**

Genotyping for TSPO polymorphism was performed retrospectively and was used to confirm whether subject had polymorphism affecting TSPO affinity (Owen et al., 2011) and also to determine whether he/she was heterozygous or homozygous for it. The reason for retrospective genotyping was that DNA polymorphism was reported after the initiation of the present study. Genotyping of single nucleotide polymorphism of TSPO (DNA polymorphism rs6971, Ala substitution by Thr at amino acid position 147) was performed using the TaqMan® assays (Applied Biosystems) and detection via ABI 7900HT SDS (Applied Biosystems) according to manufacturer's recommends conditions. All data were analysed with Sequence Detection Systems® software Version 2.1 (Applied Biosystems). Analysis was performed at AstraZeneca, Macclesfield, UK.

**Determination of plasma AZD3241 concentrations**

For determination of plasma AZD3241 concentrations, venous blood samples were collected ($K_2$ EDTA) by a sparse sampling schedule, i.e. three samples were taken during each visit (a total of maximum 12 samples for each patient during the course of the study, Fig. 1). One was taken at arrival at the study centre, one in the middle of the visit and one was taken at the end of the visit. At one of the visits (preferably at Week 4), the first sample was taken pre-dose. Plasma concentrations of AZD3241 were determined using LC-MS/MS (Covance Laboratories, Inc.). Plasma samples collected in the placebo treatment arm were excluded from the pharmacokinetic
analysis set after study unblinding. The average plasma AZD3241 concentration on the day of PET examination was calculated.

Safety assessments

Safety and tolerability assessments included records of adverse events, vital signs (pulse, blood pressure), ECG, clinical chemistry and haematology assessments, and urinalysis. The Columbia Suicide Severity Rating Scale (C-SSRS) was administered during the on-site visits and via telephone. Adverse events and medical/surgical history were classified according to the terminology of the Medical Dictionary for Regulatory Activities, MedDRA, Version 15.1 (Sept, 2012) (Brown et al., 1999).

Statistics

The calculations of study sample were driven by the primary aim to examine the potential effect of AZD3241 on TSPO binding. The addition of a small placebo arm was motivated by the need of a control group for safety assessments and binding, because of the first use of AZD3241 in a patient population. Calculations were based on a published study (Gerhard et al., 2006) which described eight subjects with repeated PET measurements using $^{11}$C-(R)PK11195 performed 1.5–2 years apart. The standard deviation of the log relative change from baseline in $B_{PND}$ in striatum was 0.553. Assuming a true reduction in TSPO binding by 40%, the power was almost 95% to obtain a statistically significant reduction in the active arm, but only 43% to obtain a statistically significant treatment effect between active and placebo arms (secondary analysis). Thus, the study was not powered to detect statistically significant difference between the treatment arms. The dropout rate was anticipated to be ~20%, therefore 24 patients were randomized.

For each brain region, descriptive statistics (mean and SD) were presented for observed $V_T$ of $^{11}$C-PBR28, change from baseline and per cent change from baseline at Week 4 and Week 8 assessments.

The primary statistical analysis of PET data aimed to examine the relative change in regional $^{11}$C-PBR28 binding to TSPO ($V_T$) in relation to baseline. The primary analysis was carried out separately for each assessment visit (Week 4 and Week 8) using an ANOVA model on change from baseline in $V_T$ of $^{11}$C-PBR28 with a fixed effect for treatment. From this model, the point estimate for change from baseline along with the corresponding 95% confidence interval (CI) and the $P$-value for testing change from baseline equal to zero were provided for each treatment arm. In addition, effect size was also calculated as absolute value of mean change from baseline divided by SD.

The secondary statistical analysis aimed to compare $V_T$ changes in the AZD3241 versus the placebo treatment arm. The least squares mean estimate for the difference between AZD3241 and placebo along with corresponding 95% CI and the $P$-value for testing the difference equal to zero were calculated. The effect size was computed as absolute value of the difference in AZD3241 and placebo least squares means divided by square root of mean squared error (MSE) from the ANOVA model.

Before analysing changes in $V_T$ of $^{11}$C-PBR28 using the above model, significance of the two baseline variables, genotype and baseline $V_T$ of $^{11}$C-PBR28 was assessed using a repeated measures ANCOVA model with terms for treatment, visit, treatment by visit interaction, genotype and baseline $V_T$ for $^{11}$C-PBR28 as a continuous covariate under compound symmetry covariance structure for the five selected brain regions (striatum, caudate, putamen, substantia nigra and grey matter). These two baseline variables were found to be not statistically significant for each of the five selected brain regions and therefore, were not included in the statistical model for the analysis of $V_T$ of $^{11}$C-PBR28.

In all analyses, the statistical significance was set at $P = 0.05$. All statistical analyses were carried out using SAS (R) software Version 9.2.

Results

Twenty-nine patients with Parkinson’s disease were screened and five were excluded from the study (Fig. 2) based on the observation of negligible $^{11}$C-PBR28 binding on PET images and corresponding time activity curves. In total, 24 patients with Parkinson’s disease, 50–73 years of age, 21 male and three females, with an UPDRS III score (motor examination) from 6–29 were included. The diagnosis of Parkinson’s disease was supported by qualitative and quantitative evaluation of DAT binding using $^{18}$F-F-E-PE2I PET (Table 1 and Fig. 3A). Twenty-two patients completed treatment with either AZD3241 or placebo for 8 weeks according to the protocol, two patients discontinued treatment 5 days before scheduled last PET examination. One patient did not perform PET measurement at 4 weeks due to an anxiety episode at examination, but completed PET examination at 8 weeks.

Positron emission tomography imaging results

$^{11}$C-PBR28 binding to TSPO, baseline

The distribution of $^{11}$C-PBR28 binding in brain was rather homogenous. The mean $V_T$ values ranged from 3.54 in the thalamus to 2.60 in the substantia nigra, with the slight gradient thalamus > ventral striatum, frontal cortex > cerebellum > dorsal striatum > substantia nigra (Fig. 3B). Regions of the nigrostriatal pathway (all striatum, putamen, and substantia nigra) showed no conspicuous difference in TSPO binding compared to other brain regions (baseline data; Table 2). There were no significant correlations between $^{11}$C-PBR28 binding to TSPO and $^{18}$F-FE-PE2I binding to DAT neither in the striatum, nor in the substantia nigra.

Individual $V_T$ values were related to TSPO genotype. Thirteen patients were high affinity binders (homozygous, Ala/Ala) and 10 patients were mixed affinity binders (heterozygous, Ala/Thr). One patient did not consent for DNA blood sampling. High and mixed affinity binders...
were equally distributed between treatment groups: there were seven mixed and 10 high affinity binders in the AZD3241 treatment group (41:59%) and three mixed affinity binders/three high affinity binders in the placebo group (50:50%) (chi-square test $P < 0.04$).

**Effect of AZD3241 on $^{11}$C-PBR28 binding to TSPO**

**Group data analysis**

The primary statistical analysis examined the effect of AZD3241 and placebo on $^{11}$C-PBR28 binding to TSPO, within each treatment group separately. AZD3241 significantly reduced $V_T$ in regions of the nigrostriatal pathway compared to baseline $V_T$ values, in the range of 15.5–16.2% at 4 weeks and 13.2–15.7% at 8 weeks (the effect size was from 0.5 to 0.6) (Table 2 and Fig. 4). In the placebo group, no statistically significant effect on $V_T$ values was observed (Table 2). Changes in $V_T$ across nigrostriatal regions were in the range of 1.7–4.7% at 4 weeks, and 2.9–5.8% at 8 weeks as compared to baseline. In other brain regions outside the nigrostriatal pathway (e.g. cortical regions, thalamus, and cerebellum) changes in $^{11}$C-PBR28 binding were similar to those observed in regions of the nigrostriatal pathway.
In the secondary statistical analysis the effect of AZD3241 on TSPO binding was compared to placebo. There was no significant difference in changes of VT between the treatment groups.

Exploratory statistical analysis of PET data examined potential influence of co-factors, i.e. visit, TSPO genotype, and VT at baseline. There were no significant time effects on changes in VT in either of the treatment groups. None of the tested cofactors had effect on the main results (data not shown).

**Individual data analysis**

Inspection of individual data (Fig. 5) showed that the changes in VT varied between patients in the AZD3241 as well as the placebo groups. After 8 weeks treatment with AZD3241 changes in VT in the putamen were in the range of −61% to 10%, with two patients consistently showing VT values above baseline (overall variability in response, Figs 4 and 5). In the placebo arm, after 8 weeks, changes in VT in the putamen ranged from −26% to 40%, with three patients showing reduction and three patients increase in TSPO binding compared to baseline. The pattern of changes in TSPO binding was similar across all brain regions.

**Pharmacokinetics of AZD3241**

The sparse number of blood samples collected and the variability allowed in the timing of these collections did not allow for traditional analysis of pharmacokinetics or pharmacokinetic-pharmacodynamic relationships. The geometric mean of the maximum observed concentration following AZD3241 600 mg twice daily was 3.6 µmol/l (range 1.2–5.6 µmol/l) at 4 weeks and 4.0 µmol/l (range 1.3–7.0 µmol/l) at 8 weeks. This exposure was comparable to that observed in healthy subjects in a MAD study (AstraZeneca data online: the geometric mean Cmax value for the AZD3241 600 mg twice daily dose for 10 days was ~5.5 µmol/l).

**Safety and tolerability**

AZD3241 was found to be safe and well tolerated in patients with Parkinson’s disease. There were no serious adverse events. The most common adverse events were fatigue, nasopharyngitis, headache and insomnia (Table 3). Headache, nausea and insomnia were reported only by patients treated with AZD3241. All of the adverse events were of mild to moderate intensity and self-resolving except for one patient in the placebo arm who reported severe fatigue. None of the patients discontinued the study due to adverse events.

Examination of thyroid function showed increased mean thyroid stimulating hormone (TSH) levels during administration of AZD3241 relative to placebo, with no clear changes in mean tri-iodothyronine (T3) and thyroxine (T4) levels, and normal thyroxiperoxidase antibody levels (Supplementary Table 1). Three patients with elevated TSH values after administration of AZD3241 were observed, with the highest value 6.8 mU/l (mild increase, reference range 0.4–3.5 mU/l). In all cases TSH levels returned to baseline by the time of follow-up.

Biochemical analysis of plasma uric acid concentrations showed that mean levels decreased during administration of AZD3241 relative to placebo (Supplementary Table 1). Ten patients in the AZD3241 group had decrease in uric acid concentrations, with the lowest value 139 µmol/l (reference
Table 2 Effect of AZD3241 on total distribution volume $V_T$ of $^{11}$C-PBR28 in selected brain regions

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Time</th>
<th>$n^*$</th>
<th>Mean (SD)</th>
<th>Change from baseline (%) (mean, SD)</th>
<th>Difference, 95% CI (comparison to baseline)</th>
<th>P-valueb</th>
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<tr>
<td><strong>AZD3241</strong></td>
<td></td>
<td></td>
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<td>$-0.5$, $(-0.87, -0.09)$</td>
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<td>16</td>
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<td>$-0.5$, $(-0.93, -0.03)$</td>
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<td>$4.9$ (30.90)</td>
<td>$0.0$, $(-0.69, 0.77)$</td>
<td>0.91</td>
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</tbody>
</table>

95% CI, and P-value were computed using an ANOVA model on change from baseline with a fixed effect for treatment.

*aNumber of subjects who had a non-missing value at baseline. For Week 4 and Week 8, number of subjects with non-missing values at both baseline visit and that post-baseline visit.

*b*Estimate and CI for post-baseline minus baseline. P-value for change from baseline = 0.

*statistically significant difference.*
were examined with 11C-(R)PK11195 at baseline and after cycline11C-(R)PK11195 binding decreased (Dodel 24 weeks of treatment. In two patients treated with minocycline, showing increase in VT (Bartels et al. 2010). Altogether, preliminary examinations of potential effect of a COX-2 inhibitor or an antibiotic on microglia have not shown conclusive effect on TSPO. Thus, the present study is the first to demonstrate significant effect of drug on the microglia marker TSPO in a neurodegenerative disorder.

To test the hypothesis on the mechanism of action of AZD3241 on TSPO within the AZD3241-treated group. In addition, a small placebo group (n = 6) was included for binding and safety examination purposes, and the study was not powered for comparisons between treatment groups. There was no significant difference between the effect of AZD3241 and placebo on TSPO binding in this small study sample, even though there was no significant effect on TSPO binding within the placebo group.

At baseline there was no distinct regional difference in TSPO binding in patients with Parkinson’s disease. For each patient, VT values were similar across brain regions with numerically slightly higher levels in the thalamus, confirming the previously reported regional distribution of TSPO in vivo at physiological conditions (Fujita et al., 2008; Jučaitė et al., 2012). A several-fold increase in TSPO binding in the nigrostriatal system in Parkinson’s disease relative to controls and irrespective of treatment used has been reported earlier (Ouchi et al., 2005; Gerhard et al., 2006) using the radioligand 11C-PK11195. Whether 11C-PBR28 binding in brain regions related to the pathophysiology of Parkinson’s disease is increased compared to controls, to our knowledge so far has not been reported. Also, it cannot be excluded, that there is a global elevation of TSPO binding in the brain of Parkinson’s disease patients.

The results of the present study serve as indirect support for proof of mechanism and target engagement, supporting the view that AZD3241 enters the brain and interacts with the biochemical chains of oxidative cellular environment and neuroinflammation. AZD3241 was administered for 8 weeks only. Longer treatment is required to show if the observed effect on microglia is beneficial for the treatment of Parkinson’s disease or other neurodegenerative diseases.

Imaging of DAT using the radioligand 18F-FE-PE2I was used to confirm neurodegeneration in the dopamine system. All 24 patients with Parkinson’s disease had asymmetry in 18F-FE-PE2I binding to DAT in the putamen, or reduced 18F-FE-PE2I binding in posterior putamen compared to caudate nucleus on visual inspection and low DAT levels (BPND) in the dorsal, ventral striatum and substantia nigra compared to the BPND values reported in the literature (Sasaki et al., 2012; Fazio et al., 2015). Correlations between DAT levels and TSPO binding could not be demonstrated for the striatum or the substantia nigra. Lack of relationship between the marker of neuroinflammation and dopamine system and its implications in the understanding of Parkinson’s disease pathophysiology should be taken with caution. Only two patients in the study were de novo, while others were using antiparkinsonian medications that could influence DAT levels (Guttman et al., 2001). Furthermore, rate of changes in the dopamine system in Parkinson’s disease are slow and PET measurement would represent the current deficit in dopaminergic innervation, while TSPO binding may be more

Discussion

In the present PET study we demonstrated that the MPO inhibitor AZD3241 had an effect on the microglia marker TSPO in the brain of patients with Parkinson’s disease. AZD3241 was administrated for 8 weeks and this treatment was associated with a significant reduction in 11C-PBR28 binding to TSPO across all examined brain regions as compared to baseline. Two other medications with potential effect on neuroinflammation have previously been tested in clinical trials in Parkinson’s disease and multiple system atrophy. Five patients with Parkinson’s disease received 1 month of treatment with celecoxib and 11C-PK11195 binding to TSPO was quantified in two of them, showing increase in VT (Bartels et al., 2010). In a clinical trial of minocycline therapy in multiple system atrophy-parkinsonian type patients (MEMSA trial), a subgroup of eight patients (n = 3 minocycline, n = 5 placebo) were examined with 11C-(R)PK11195 at baseline and after 24 weeks of treatment. In two patients treated with minocycline 11C-(R)PK11195 binding decreased (Dodel et al., 2010). Altogether, preliminary examinations of potential effect of a COX-2 inhibitor or an antibiotic on microglia have not shown conclusive effect on TSPO. Thus, the present study is the first to demonstrate significant effect of drug on the microglia marker TSPO in a neurodegenerative disorder.

To test the hypothesis on the mechanism of action of AZD3241 the present study examined the effect of

range 230–480 μmol/l). Uric acid levels returned to baseline by the time of follow-up visit.

Otherwise, the assessment of haematology, clinical chemistry, urinalysis, ECG, and vital signs showed no clinically meaningful changes. There were no increases in suicidal ideation or behaviour (C-SSRS scale) between baseline and subsequent examinations.
dynamic and sensitive to different factors influencing the brain immune system.

In the present study no serious adverse events were reported. Headache was the most commonly reported adverse event. Mild elevation in thyroid stimulating hormone levels suggests a need for further monitoring of thyroid function in clinical studies. Plasma uric acid concentrations showed mild decrease in some patients. The clinical significance of the uric acid decrease is not clear. No clinically relevant effects on other clinical laboratory

Table 3 Most common adverse events during treatment with AZD3241 and placebo

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Number (%) of patients</th>
<th>AZD3241</th>
<th>Placebo</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects treated</td>
<td></td>
<td>18</td>
<td>6</td>
<td>24</td>
</tr>
<tr>
<td>Number of subjects with at least one adverse event</td>
<td></td>
<td>13 (72)</td>
<td>3 (50)</td>
<td>16 (67)</td>
</tr>
<tr>
<td>Most common adverse events</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td></td>
<td>4 (22)</td>
<td>0</td>
<td>4 (17)</td>
</tr>
<tr>
<td>Nasopharyngitis</td>
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<td>3 (17)</td>
<td>1 (17)</td>
<td>4 (17)</td>
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<tr>
<td>Insomnia</td>
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<td>3 (17)</td>
<td>0</td>
<td>3 (12)</td>
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<tr>
<td>Nausea</td>
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<td>0</td>
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<tr>
<td>Fatigue</td>
<td></td>
<td>2 (11)</td>
<td>2 (33)</td>
<td>4 (17)</td>
</tr>
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</table>

Only adverse events occurring in ≥10% of the cases in at least one treatment group are presented.
tests, vital signs or ECGs were observed in patients receiving AZD3241. Altogether, AZD3241 was safe and well tolerated.

**Comments**

There was considerable intra-individual variability over the three PET measurements. Individual data analysis comparing repeated measurements have to be interpreted with caution, considering the test–retest reproducibility of PET measurements. Traditional quantitative analysis of brain biochemistry benefits from regions with absence of the molecular target under examination. However, TSPO is present in all brain regions (Doble et al., 1987). Thus, imaging data analysis in the present study was dependent on the demanding procedure of generating arterial input curve and this may be the reason for high intra-individual variability of TSPO binding. As reported recently, reproducibility of $^{11}$C-PBR28 is $\sim$15% (Collste et al., 2014).

There was also high intersubject variability in TSPO binding at baseline. The radioligand $^{11}$C-PBR28 is a second generation radioligand with nanomolar affinity to TSPO (K$_i$ = 2.17 ± 0.17 nM; Owen et al., 2011)]. The important feature of $^{11}$C-PBR28 is the presence of different binding groups, depending on the TSPO genotype (Owen et al., 2010). The present study used intrasubject comparisons of TSPO binding, and also included DNA analysis for the TSPO polymorphism. No differences in the effect of AZD3241 in subgroups of mixed and high affinity binders were observed.

**Conclusions**

Administration of AZD3241 600 mg twice daily for 8 weeks produced a statistically significant reduction in $^{11}$C-PBR28 binding to TSPO in the brain of patients with Parkinson’s disease in vivo. Reduction in $^{11}$C-PBR28 binding to TSPO, a marker associated with brain microglia activation, supports the hypothesis that inhibition of MPO has an effect on neuroinflammation. The results of the present study may, thus, represent indirect support for Proof of Mechanism or target engagement of the myeloperoxidase inhibitor AZD3241 in the human brain.

**Acknowledgements**

We thank former project team at AstraZeneca, Södertälje, Sweden for valuable comments and discussions during study design, study set-up and AstraZeneca, PHB at Alderley Park for analysis of TSPO genotype. We thank the members of the PET Centres at the Karolinska Institutet and Turku University, for data collection and laboratory analysis.

Supplementary material

Supplementary material is available at Brain online.

**Funding**

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**Conflicts of interest**

A.J., Z.C., P.J., M.M., A.K., J.P., S.B., and L.F. were full-time employees of AstraZeneca at the time of the study conduct and manuscript preparation.

K.V., N.A., C.H., A.V., P.S., J.R., A.K., and SH. declare no conflict of interest. The study was carried out under research agreement between AstraZeneca and Department of Clinical Neuroscience, PET Centre, Karolinska Institutet, Stockholm, Sweden.

**References**


