Translocator positron-emission tomography and magnetic resonance spectroscopy imaging of brain glial cell activation in multiple sclerosis

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Abstract

Background: Multiple sclerosis (MS) is characterized by a diffuse inflammatory response mediated by microglia and astrocytes. Brain translocator protein (TSPO) positron-emission tomography (PET) and [myo-inositol] magnetic resonance spectroscopy (MRS) imaging was used together to assess this.

Objective: To explore the *in vivo* relationships between MRS and PET [^{11}C]PBR28 in MS with a range of brain inflammatory burdens.

Methods: A total of 23 patients were studied. TSPO PET imaging with [^{11}C]PBR28, single voxel MRS and conventional MRI sequences were undertaken. Disability was assessed by Expanded Disability Status Scale (EDSS) and Multiple Sclerosis Functional Composite (MSFC).

Results: [^{11}C]PBR28 uptake and [myo-inositol] were not associated. When the whole cohort was stratified by higher [^{11}C]PBR28 inflammatory burden, [myo-inositol] was positively correlated to [^{11}C]PBR28 uptake (Spearman's ρ=0.547, p=0.007). Moderate correlations were found between [^{11}C]PBR28 and both MRS creatine normalized N-acetyl aspartate (NAA) concentration and grey matter volume. MSFC was correlated with grey matter volume (ρ=0.535, p=0.009). There were no associations between other imaging or clinical measures.

Conclusions: MRS [myo-inositol] and PET [^{11}C]PBR28 measure independent inflammatory processes which may be more commonly found together with more severe inflammatory disease. Microglial activation measured by [^{11}C]PBR28 uptake was associated with loss of neuronal integrity and grey matter atrophy.

Introduction

Multiple sclerosis (MS) is a chronic inflammatory condition characterized by demyelination and neurodegeneration focally, as well as more diffusely in the central nervous system (CNS). The brain's chronic inflammatory response includes astrocyte activation and microglial activation, as well as recruitment of peripheral macrophages. It is likely that this chronic inflammation is causally responsibly for the neurodegeneration that leads to long-term disability progression . Conventional MRI detects acute white matter inflammatory lesions and consequent demyelination and gliosis. However, it lacks sensitivity and specificity for quantifying chronic inflammation and the associated neuronal injury.

Magnetic resonance spectroscopy (MRS) enables measurement of a range of brain metabolites *in vivo*. Two metabolites in relatively high concentration, myoinositol and N-acetyl aspartate (NAA), are amongst those showing pathology related changes in MS [1]. Myoinositol has been proposed as a glial marker, as it is found in high concentrations in activated astrocytes and can be elevated in MS [2]. Choline, a less specific marker of membrane turnover in glial cells, shows similar changes under some conditions [3]. NAA is synthesized in the mitochondria of neurons. Reduced concentrations are associated with neurodegeneration in MS and other diseases [2, 4].

The relationships between neurodegeneration and activation of the innate immune response could be explored by coupling MRS and PET using radioligands that bind to the 18 kD mitochondrial translocator protein (TSPO) [5]. Activated microglia, macrophages and astrocytes express high levels of TSPO and show high TSPO radioligand binding. Several studies using the first generation TSPO ligand [^{11}C]PK11195 have highlighted extensive multi-focal chronic innate inflammation and its association with disability and the likelihood of future clinical relapses [6-9]. More recent work by different groups have further described heterogeneity amongst lesions within individuals and the inflammatory load in the white

matter that appears normal with conventional MRI [10, 11]. Newer second generation TSPO ligands (such as [^{18}F]PBR111 or [^{11}C]PBR28) [5] with higher binding affinity for TSPO and less non-displaceable (i.e., non-specific or "off target") binding have been developed that may more specifically measure inflammatory changes [12].

However, specific interpretation of the increased brain TSPO signal in MS with any of these radioligands is confounded by their potential to reflect activated astrocytes, as well as microglia and macrophages. A limited correlative [^{11}C]PK11195 autoradiographic and histopathological study included in the original report suggested selectivity for activated microglia/macrophages in white matter lesions [6]. Additional immunohistopathological reports support this conclusion [13], but the more general relationships between astrocyte markers and TSPO radioligand binding are poorly defined. Combining TSPO PET and MRS myoinositol offers one approach to assessing their relationship *in vivo*.

In this study, we sought to explore the *in vivo* relationships between MRS measures of myoinositol and PET [^{11}C]PBR28 binding in patients with MS selected to have a wide range of brain inflammatory load and disability. This allowed us to explore also how different measures of neuronal integrity (MRS NAA) and neurodegeneration (normalised gray matter volume) were related to these measures of inflammatory response.

Material and methods

Study population

The study was approved by the West Bromley Research Ethics Committee and the Administration of Radioactive Substances Advisory Committee. Patients had a diagnosis of multiple sclerosis according to the revised McDonald criteria (2010) [14], with Expanded Disability Status Scale (EDSS) up to 7.0 and either relapsing-remitting course or a secondary progressive disease course. None of the subjects had been treated steroids or experienced a clinical relapse within 3 months of their scans. Women who were pregnant or breastfeeding were not eligible to participate. All subjects gave written informed consent in accordance with the Declaration of Helsinki. Neurological disability was scored using EDSS and the Multiple Sclerosis Functional Composite (MSFC) [15].

TSPO genotyping

TSPO genotype was assessed using a TaqMan based polymerase chain reaction (Applied Biosystems® QuantStudioTM 7) assay specific for the rs6971 polymorphism in the TSPO gene, as previously described [16]. Patients having genotypes associated with LAB were excluded, as they show negligible displaceable binding [16].

MRI Scanning

MRI scans were performed on a Siemens 3 Tesla Trio scanner (Siemens Healthcare, Erlangen Germany) equipped with a 32-channel phased-array head coil. Volumetric T1-weighted MP-RAGE images were acquired for all subjects using a 1 mm isotropic resolution 3D SPACE sequence, before and 5 minutes after intravenous gadolinium-chelate administration (0.2 mL/kg Gadoteric Acid, Dotarem®; repetition time = 2300 ms, echo time = 2.98 ms, inversion time =9 00 ms with 256 x 240 x 160mm field of view . Volumetric T2-weighted FLAIR (fluid attenuated inversion recovery) images were acquired using a 1mm

isotropic resolution 3D SPACE sequence with a 250 x 250 x 160 mm field of view, echo time = 395 ms, repetition time = 5 s, inversion time = 1800 ms, turbo factor of 141, 256 x 256 x 160 matrix, and parallel imaging factor of 2 in 5 m:52 s.

Single voxel MR spectroscopy was acquired in the same session as the other MRI sequences. A sagittal survey image was used to identify the anterior commissure (AC) and posterior commissure (PC). The spectroscopy voxel was positioned just superior to the lateral ventricles in the midline (de Stefano et al. 2001). The spectroscopy voxel measured 40 mm anteroposterior x 25 mm craniocaudal x 40 mm left–right [17].

Proton spectra were acquired using a 90^{o} –180^{o} –180^{o} sequence for volume selection (repetition time = 2000 s, echo time = 272 s). Magnetic field homogeneity was optimized to a linewidth of ~5 Hz over the spectroscopy voxel using the proton signal from water.Water suppression was achieved by a chemically selective saturation, the WET method [18].

MRS analyses

LCModel software (version 6.3) was used for metabolite quantification applying the internal water reference method, accounting for different water content in grey matter, white matter and cerebrospinal fluid [19]. Only metabolites with Cramér–Rao bounds < 20% were considered. Concentrations of N-acetylaspartate (NAA), myo-inositol, glycerophosphocholine and creatine plus phosphocreatine were included for analysis. Concentrations in millimole (mM) units were calculated for all metabolites and results are presented in institutional units.

PET scanning

[^{11}C]PBR28 was injected as an intravenous bolus over approximately 20 s at the start of a 90 min dynamic PET acquisition. Injected activities for [^{11}C]PBR28 ranged from 223.8- 379.6 MBq (325.6+/-34.6 MBq, n=44). Injected mass for different subjects ranged from 1.16 - 8.91 μg (2.75+/-1.64 μg).

Radioligand synthesis

Radiosynthesis and quality control was performed on site as previously described, as previously described, obtaining radiochemical purities of > 95% [12].

[^{11}C]PBR28 PET Image and Kinetic analysis

T2 FLAIR images were rigidly registered to T1 using FLIRT (FMRIB Software Library v5.0). WML were manually segmented on the registered T2 image using Jim software (Xinapse Systems v7). The WML mask was used for lesion- filling the T1 image before segmentation into white matter, grey matter, cerebral cortex and cerebrospinal fluid using the FSL tools FAST and FIRST (FMRIB Software Library v5.0) [20]. Normalised brain volumes were calculated using SIENAX [21]. A mask of NAWM was created by subtracting the WML mask dilated by 6mm around its edges in 3D and the resulting mask further eroded by 3 mm. The masks of WML, NAWM and grey matter were multiplied by the mask of the spectroscopy voxel to create the respective masks within the spectroscopy voxel.

The T1 image and dynamic PET images were used as inputs for the MIAKAT software package (*www.miakat.org*) for kinetic analysis of PET data. For this, PET images were motion corrected using a frame-by-frame realignment algorithm, in which all frames were individually realigned to a reference frame and rigid registered to MNI (Montreal Neurological Institute) space using SPM5 (Wellcome Trust Centre for Neuroimaging, http://www.fil.ion.ucl.ac.uk/spm) with a mutual information cost function.

These transformed 4D PET images were integrated over time to obtain 3D PET summation images in MNI space. The CIC Neuroanatomical Atlas was non-linearly deformed into the individual's space, via mapping of T1-weighted MR imaging data, to obtain a personalized anatomical parcellation of regions of interest, which were used to generate time-activity curves for the caudate and voxel-wise for whole brain.

The Logan graphical reference method [22] using a reference tissue time-activity curve as the input function and a linear start time at 35 min and model fitting performed with linear regression was used to estimate the DVR at the voxel level to produce parametric DVR maps, relative to the caudate nucleus $V_{T.}$ The whole spectroscopy voxel mask and the WML, NAWM and grey matter masks within the spectroscopy voxel were applied to the PET parametric DVR image to obtain the DVR for the respective regions of interest.

We chose to use a normalised quantitation to allow studies to be conducted without an arterial line. [^{11}C]PBR28 V_T has a high test-retest variability of approximately 20%, the major contribution to which appears to come in the blood to tissue transfer modelling [23]. Alternatively, reference based methods show less test-retest variability (5% or less) [23]. While TSPO is expressed throughout the brain, lower levels of specific binding within a proposed pseudo-reference region do not affect the reliability of the parameter estimates, although this may lead to underestimation of relative binding differences between regions of interest [24]. We used the caudate nucleus as a pseudo-reference region as relatively lower levels of microglial activity and TSPO expression is found in the caudate and normalized standardized uptake ratios are low in caudate compared to other brain regions [13, 25].

Statistical Analyses

Statistical analyses were performed using SPSS software (IBM, SPSS v22). For correlational analyses, the Spearman's correlation coefficient was calculated, unless otherwise stated. Descriptive statistics were

reported as mean +/- standard deviation (SD) unless otherwise stated. A p-value of less than 0.05 was considered significant for all statistical tests. The primary hypothesis (positing a correlation between PET [^{11}C]PBR28 DVR and [*myo*-inositol]) was tested first. Exploratory relationships reported were tested subsequently and are reported with uncorrected p-values.

Results

24 people with clinically definite multiple sclerosis underwent [^{11}C]PBR28 PET, single voxel MR spectroscopy and structural MRI scanning (Table 1, Fig. 1). One patient was excluded from the final analysis because noise artifacts precluded reliable estimates of metabolite concentrations. Of the 23 remaining patients (nine men, median age 48 years, range 22-66 years) included in the final analysis, seven had a diagnosis of secondary progressive disease and 16 had relapsing remitting disease. The median EDSS was 5.0 (range, 1.0-7.0).

Differences in [^{11}C]PBR28 uptake are not explained by brain [*myo*-inositol]

The concentration of *myo*-inositol in the spectroscopy voxel (expressed as either an absolute concentration [ρ=0.250, p=0.25] or as a ratio to total tissue creatine [ρ=0.111, p=0.65]) was not significantly correlated to TSPO binding measured as the mean PET [^{11}C]PBR28 DVR within the spectroscopy voxel. We explored *post hoc* whether an association could be found in those people with a higher inflammatory burden. To do this, we divided subjects into two groups based on the median [^{11}C]PBR28 DVR (1.26). There was a significant (un-corrected) correlation between the normalised [*myo*-inositol] and [^{11}C]PBR28 DVR weighted by WML fraction within the high inflammatory load (DVR > 1.26) subgroup (ρ=0.685, p=0.014)(Fig. 2a). We further explored whether there was an association between choline and *myo*-inositol concentrations. There was a moderately significant (un-corrected) correlation between *myo*-inositol and choline concentrations (ρ=0.547, p=0.007) (Fig 2b).

Relationships between measures of inflammatory burden and measures of neurodegeneration

We explored the relationships of inflammatory markers ([^{11}C]PBR28 DVR and *myo*-inositol with measures of neurodegeneration. A moderate correlation was found between the creatine normalized NAA concentration and [^{11}C]PBR28 DVR in WML (ρ=-0.443, p=0.034 (un-corrected) (Fig. 3a). We also found a correlation between the whole brain normalized GM volume, as a measure of relative neurodegeneration, and [^{11}C]PBR28 DVR weighted by WML fraction in the spectroscopy voxel (ρ=-0.535, p=0.009)(Fig. 3b). There was no evidence for correlations between *myo*-inositol and either NAA (p=0.274) or the normalised GM volume (p=0.574 and p=0.299, respectively).

Imaging measures and disability

Disability measured by MSFC was correlated with normalised GM volume (ρ=0.535, p=0.009). We found within a general linear model that a large component of disability, as measured by MSFC, was explained by normalised GM volume (F=19.5, p=2x10^{-4}). This relationship was driven predominantly by lower and upper limb motor scores (25FTW, F=18.8, p=0.007; 9HPT, F=11.6, p=0.003). We further tested for additional explanatory power from measures here by including the TSPO DVR, *myo*-inositol and NAA , but did not find that they added further to the model. We also did not find significant relationships between any of these measures and EDSS.

Discussion

Increased brain TSPO uptake with non-malignant brain pathology could be attributed to either an increased density of activated microglia or to increased astrocytes, as TSPO expression can be elevated in both cell types [5]. Histopathological studies in MS have shown that the [*myo*-inositol] detected by MRS signal corresponds to astrocyte activation [2] and that increased TSPO expression co-localises with activated microglia [13]. We have been able to test the independence of these markers *in vivo* for the first time in a group of MS patients with a range of inflammatory loads. We failed to find a meaningful correlation between MRS [*myo*-inositol] and PET [^{11}C]PBR28 uptake. Changes in the two measures in this population therefore must be related to distinct processes or to elements of a common process with different time courses. Similar findings were reported for HIV positive patients studied using the first generation PET radioligand [^{11}C]PK11195 in conjunction with MRS for [*myo*-inositol] [26]. The results support interpretation of TSPO PET primarily as a marker of activated microglia/macrophages in MS.

However, these results should not be interpreted as evidence that there is never an association between the independent processes of microglial activation reflected in the PET TSPO signal and astrocyte activation in the disease. Activated astrocytes and microglia are found within and outside lesions at all stages of MS [27]. Brex et al. highlight the l heterogeneity of changes in MRS neurodegenerative and inflammatory markers amongst lesions and between patients [28]. The two markers of brain inflammation thus can be related under some conditions. Our *post hoc* exploratory analysis suggests in patients with a higher inflammatory load there is a convergence of the two glial cell population inflammatory pathologies. Similar observations were made in a study that found the [*myo*-inositol] concentration is associated with at higher levels of brain inflammatory pathology in Alzheimer's disease [29].
The correlation of greater microglial activation within T2 lesion with either reduced NAA or grey matter volume, both of which are markers of neurodegeneration, provide further evidence for the potential

clinical significance of the increased brain PET TSPO radioligand uptake. This is consistent with histopathological findings in MS in which microglial activation is associated with grey matter neurodegeneration [30, 31], the association of increased PET [^{11}C]PK11195 uptake in NAWM with greater brain atrophy [9] and correlations reported between PET TSPO radioligand uptake and measures of disability or likelihood of a diagnosis of clinically definite MS after presentation with the clinically isolated syndrome [8, 9, 32]. Both populations of glial cells also could contribute to axonal damage [3]. However, the lack of a relationship between brain [*myo*-inositol] and measures of neurodegeneration in this study again emphasizes that the TSPO PET and MRS measure are reporting largely independent phenomena or phenomena with different timecourses. For example, astrocyte activation could be antecedent to neurodegeneration with a longer time course [33]. Future, longer term longitudinal studies combining MRS and PET TSPO imaging as described here could evaluate their independent predictive value for future neurodegeneration and increases in disability.

We did not find relationships between disability and the inflammatory marker measures, although a well precedented [17] correlation between grey matter atrophy and disability measured with MSFC was observed, despite the limited study power. This perhaps is not surprising, as MRS and PET TSPO are measures of inflammatory state, potentially predictive of future change rather than antecedent neurodegenerative processes [32, 34]. However, there is the additional confound of different delays between inflammatory, neurodegenerative and disability changes in patients with MS [27, 33].

This small study was powered to test for a relatively strong, general relationship between [*myo*-inositol] and PET [^{11}C]PBR28 uptake in MS. While the results clearly distinguish between results from the two measures, a limitation of the study design is that the range of disease investigated was limited; the possibility of a relationship under some conditions cannot be ruled out. If increased TSPO expression

and radioligand binding depends on the specific activation phenotypes of astrocytes, the conclusion that PET TSPO radioligand uptake reflects microglial activation predominantly could not be generalized safely. This deserves further histopathological study. A fundamental limitation lies in the use of the MRS [*myo*-inositol] as an index of astrogliosis. While there is a strong evidence in support of the approach from correlative neuropathology [2], anabolic and catabolic pathways for *myo*-inositol are expressed in other cell types, as well [3]. Additional corroborative observations with other, potential more specific markers of astroglial activation would support the argument [35].

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Declarations

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Figure Legends

Figure 1. Placement of spectroscopy voxel shown in (A) sagittal, (B) axial and (C) coronal slice. The parametric TSPO DVR surfaceplot from a representative section of the spectroscopy voxel a patient with MS with high inflammatory load (D) with (E) representative MR spectra from a patient. mIns=myoinositol, Cho= choline, Cr+PCr=creatinine and phosphocreatinine, NAA=N-acetylaspartate. The colorbar to the right of D represents the DVR for the surface plot of the PET parametric DVR.

Figure 2. Relationships of different imaging measures of glial cell activation. (A)The concentration of myo-inositol (Ins) normalised to total creatinine and phosphocreatinine concentration (Cr+PCr) compared to [^{11}C]PBR28 distribution volume ratio (DVR) of the T2 FLAIR white matter lesions weighted by the WML fraction within the spectroscopy voxel. (B) Correlation of choline to myo-inositol concentration in the spectroscopy voxel.

Figure 3. Relationships of neurodegeneration measures to [^{11}C]PBR28 DVR. (A) Normalised concentration of N-acetylaspartate (NAA) correlated with average distribution volume ratio (DVR) in T2 FLAIR weighted white matter lesions across the whole spectroscopy voxel (Spearman's ρ=-0.443, p=0.034) (B) Grey matter volume by lesion weighted [^{11}C}PBR28 DVR.

Table 1. Summary of study population characteristics.

Gender, male : female	**9:14**
Age, years, median (range)	**48 (22-66)**
Multiple sclerosis subtype, RRMS : SPMS	**16:7**
Disease duration, years, mean (SD)	**13.7 (6.7)**
EDSS, median (range)	**5.0 (1.0-7.0)**

Abbreviations: RRMS=relapsing remitting multiple sclerosis; SPMS=secondary progressive multiple sclerosis; EDSS=Expanded Disability Status Scale; SD=standard deviation

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