SUPPLEMENTAL MATERIAL

Inclusion criteria of healthy controls and SPMS patients

Main inclusion criteria for SPMS patients were: age between 18 – 75 years, definite MS diagnosis[1,2] for more than 5 years prior to enrolment, a progressive course of MS for a minimum of two years, and a moderate to heavy lesion load (> 9 T2 hyperintense MS-lesions) in brain MRI. Exclusion criteria were disease modifying treatment within 3 months, or corticosteroid treatment within thirty days of evaluation, EDSS score > 8, active neurological or autoimmune disease other than MS, or another comorbidity considered significant. The control subjects were age and sex matched healthy individuals with no known neurological symptoms or diseases. Initially, 10 healthy controls and 10 SPMS patients were enrolled in the study. One control subject (male, age 62 years) was excluded because of unexpected, multiple white matter lesions of vascular origin in the MRI, and one healthy female's (44 years, no prior history of anxiety) ^{11}C-PK11195 PET data was discarded because of poor image quality due to excessive head movements during the scan caused by sudden anxiety. Therefore, 8 control subjects were included in the final evaluation. In addition, one SPMS patient had a single right-sided, cortical occipital lesion consistent with an old ischemic infarction, but no other ischemic changes. Due to the volume reduction caused by the infarction, this patient was excluded from the volumetric correlational analyses. One healthy control had mild ischemic degenerative changes in the WM without consequent tissue loss or old infarctions. None of the ischemic lesions in either group were included in ROI analyses.

References

1. Polman CH, Reingold SC, Edan G, et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". *Ann Neurol.* Dec 2005;58(6):840-846.

2. Poser CM, Paty DW, Scheinberg L, et al. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. *Ann Neurol.* Mar 1983;13(3):227-231.

Methodology for obtaining conventional MR images and MR image parameters

Brain MRI with Philips Gyroscan Intera 1.5 T Nova Dual scanner (Philips, Best, the Netherlands) was performed on all subjects for neuroradiological analysis and acquisition of anatomical reference for the PET images. The MRI sequences included axial T1 and T2 weighted, coronal T2 weighted fluid attenuated inversion recovery (FLAIR), and axial Gadolinium-enhanced 3DT1 weighted series. Total lesion load of the white matter (WM) MS plaques was determined from coronal FLAIR scans using a semiautomated thresholding technique.[1] The measurement of total brain, WM and GM volumes was performed using SIENAX (structural image evaluation, using normalisation, of atrophy - v2.6, part of FSL) [2].

Methodology for pre-processing 3DT1 MR images for the parametric PET image analysis

The preprocessing of the 3DT1 MR images for the parametric PET image analysis was performed with Voxel-Based Morphometry, version 8 (VBM8)[3], a toolbox in Statistical Parametric Mapping, version 8 (SPM8),[4] running on MatLab 2011 (The MathWorks, Natick, MA). The 3DT1 MR images were first coregistered to the sum of motion corrected PET images and then segmented into WM and GM images. Additionally, the deformation fields used for spatial normalisation were calculated via high dimensional DARTEL [5] algorithm yielding improved anatomical precision.

References

1. Virta JR, Laatu S, Parkkola R, Oikonen V, Rinne JO, Ruutiainen J. Cerebral acetylcholinesterase activity is not decreased in MS patients with cognitive impairment. *Mult Scler.* 2011;17:931-938.

2. Smith SM. Fast robust automated brain extraction. *Hum Brain Mapp.* 2002;17:143-155.

3. Gaser C. Structural Brain Mapping Group: 2012 VBM8. http://dbm.neuro.uni-jena.de/vbm/. Accessed July 1st, 2012.

4. Wellcome Trust Center for Neuroimaging: 2012 Statistical Parametric Mapping, SPM8. http://www.fil.ion.ucl.ac.uk/spm. Accessed July 1st, 2012.

5. Ashburner J. A fast diffeomorphic image registration algorithm. *Neuroimage.* 2007;38:95-113.

^{11}C-PK11195 radioligand production

^{11}C-PK 11195 ((R)-[*N*-methyl-^{11}C]-1-(2-chlorophenyl)-*N*-(1-methylpropyl)-3-isoquinolinecarboxamide) was synthesized using the previously published methods[1,2] with some modifications. Briefly, ^{11}C-carbon dioxide was converted into ^{11}C-methyl iodide, which was trapped into a solution of desmethyl-(*R*)-PK11195 (1.0 mg) and 3.0 μl of 1.0 M tetrabutylammonium hydroxide in dimethyl sulfoxide (150 μl). Reaction mixture was heated at 80 °C for 3 minutes. Tracer was subsequently purified with semipreparative HPLC. 0.5 ml of propylene glycol/ethanol (7/3) was added to the collected fraction and the fraction was evaporated. Residue was formulated in 0.1 M phosphate buffer (pH 7.4) containing propylene glycol/ethanol (8:2) and sterile filtered through Pall Corp. Acrodisc 0.2 μm HT Tuffryn membrane. Radiochemical purity of produced ^{11}C-PK 11195 was more than 99 % and specific radioactivity was 42 ± 11 MBq/nmol (mean±standard deviation) at the time of injection.

References

1. Camsonne R, C C, D C, et al. Synthesis of N-(^{11}C) methyl, N-(methyl-1 propyl), (chloro-2 phenyl)-1 isoquinoleine carboxamide-3 (PK 11195): A new ligand for peripheral benzodiazepine receptors. *Journal of Labelled Compounds and Radiopharmaceuticals.* 1984;21(10):985-991.

2. Debruyne JC, Versijpt J, Van Laere KJ, et al. PET visualization of microglia in multiple sclerosis patients using ^{11}C-PK11195. *Eur J Neurol.* May 2003;10(3):257-264.

Supplemental Table 1. Comparison of data processing and modelling methods in analyses of [^{11}C]PK11195 positron emission tomography (PET) studies in multiple sclerosis patients by Politis et al.(*1*) and Rissanen et al.

	Politis et al. 2012	Rissanen et al.
PET motion correction	None / information not available	SPM8[*]
MRI-PET coregistration	SPM2[†]	SPM8
Spatial normalisation	Atlas based automated segmentation (MAPER)[‡]. Atlas image registered to subject space by using IRTK[§].	MR images in subject space are registered to MNI space by using DARTEL[‖] normalisation in VBM8[¶].
Variable of interest	BP_{nd}[#] within atlas based ROIs[¤] derived from parametric BP_{nd} images estimated voxel-by-voxel from dynamic images.	Regional DVR[**] estimated from regional time activity curves. Parametric DVR images estimated voxel-by-voxel from dynamic images.
ROI / VOI acquisition method (software used)	MAPER, ANALYZE[¤¤], further segmentation to white and gray matter (SPM2)	Manual delineation of individual ROIs (Carimas[††)], semiautomated segmentation into normal appearing gray and white matter, and pathological white matter (SPM8, VBM8 and LST[§§])
Modeling method	Parametric BP_{nd} image estimated using BF-SRTM[¤¤] with SVCA4[***] gray reference region input.	ROI-based regional DVR estimates and parametric DVR images calculated using Logan[†††] method with SVCA4 gray reference region input.
Model settings	Parameter bounds for basis function method not reported. Additional vascular component included in the BF-SRTM and the inverse of the frame durations used for weighting (*2*).	Time range 5-60 minutes selected based on visual inspection of Logan plot regression lines. Control and SPMS subjects' mean reference region k2-value (k2=0.145) calculated from thalamus using parameter estimates of SRTM with SVCA4 gray reference region input. Voxel-wise DVR values limited to range [-1 10].

* SPM8 = statistical parametric mapping (version SPM8, Wellcome Department of Imaging Neuroscience, UCL)
† SPM2 = statistical parametric mapping (version SPM2, Wellcome Department of Imaging Neuroscience, UCL)
‡ MAPER = multi-atlas propagation with enhanced registration (*3*)
§ IRTK = Image registration Toolkit (*4*)
‖ DARTEL image registration algorithm (*5*)
¶ VBM8 = Voxel-Based Morphometry toolbox (version VBM8, university of Jena)
# BP_{nd} = binding potential
¤ ROI = region of interest
** DVR = distribution volume ratio
†† Carimas software, version 2.4, Turku PET Centre
¤¤ ANALYZE medical imaging software (version 8.1, Mayo Foundation)
§§ LST = lesion segmentation tool (*6*)
¤¤ BF-SRTM = basis function method of simplified reference tissue model (*7*)
*** SVCA4 = Supervised clustering algorithm with 4 tissue classes.
††† Logan = Logan graphical method (*8*)

References

1. Politis M, Giannetti P, Su P, et al. Increased PK11195 PET binding in the cortex of patients with MS correlates with disability. *Neurology.* 2012;79:523-530.

2. Tomasi G, Edison P, Bertoldo A, et al. Novel reference region model reveals increased microglial and reduced vascular binding of 11C-(R)-PK11195 in patients with Alzheimer's disease. *J Nucl Med.* 2008;49:1249-1256.

3. Heckemann RA, Keihaninejad S, Aljabar P, Rueckert D, Hajnal JV, Hammers A. Improving intersubject image registration using tissue-class information benefits robustness and accuracy of multi-atlas based anatomical segmentation. *Neuroimage.* 2010;51:221-227.

4. Rueckert D, Sonoda LI, Hayes C, Hill DL, Leach MO, Hawkes DJ. Nonrigid registration using free-form deformations: application to breast MR images. *IEEE transactions on medical imaging.* 1999;18:712-721.

5. Ashburner J. A fast diffeomorphic image registration algorithm. *Neuroimage.* 2007;38:95-113.

6. Schmidt P, Gaser C, Arsic M, et al. An automated tool for detection of FLAIR-hyperintense white-matter lesions in Multiple Sclerosis. *Neuroimage.* 2012;59:3774-3783.

7. Gunn RN, Lammertsma AA, Hume SP, Cunningham VJ. Parametric imaging of ligand-receptor binding in PET using a simplified reference region model. *Neuroimage.* 1997;6:279-287.

8. Varga J, Szabo Z. Modified regression model for the Logan plot. *J Cereb Blood Flow Metab.* 2002;22:240-244.