Early and protective microglial activation in Alzheimer’s disease: a prospective study using $^{18}$F-DPA-714 PET imaging

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While emerging evidence suggests that neuroinflammation plays a crucial role in Alzheimer’s disease, the impact of the microglia response in Alzheimer’s disease remains a matter of debate. We aimed to study microglial activation in early Alzheimer’s disease and its impact on clinical progression using a second-generation 18-kDa translocator protein positron emission tomography radiotracer together with amyloid imaging using Pittsburgh compound B positron emission tomography. We enrolled 96 subjects, 64 patients with Alzheimer’s disease and 32 controls, from the IMABio3 study, who had both $^{11}$C-Pittsburgh compound B and $^{18}$F-DPA-714 positron emission tomography imaging. Patients with Alzheimer’s disease were classified as prodromal Alzheimer’s disease ($n = 38$) and Alzheimer’s disease dementia ($n = 26$). Translocator protein-binding was measured using a simple ratio method with cerebellar grey matter as reference tissue, taking into account regional atrophy. Images were analysed at the regional (volume of interest) and at the voxel level. Translocator protein genotyping allowed the classification of all subjects in high, mixed, and low affinity binders. Thirty high + mixed affinity binders patients with Alzheimer’s disease were dichotomized into slow decliners ($n = 10$) or fast decliners ($n = 20$) after 2 years of follow-up. All patients with Alzheimer’s disease had an amyloid positive Pittsburgh compound B positron emission tomography. Among controls, eight had positive amyloid scans ($n = 6$ high + mixed affinity binders), defined as amyloidosis controls, and were analysed separately. By both volumes of interest and voxel-wise comparison, 18-kDa translocator protein-binding was higher in high affinity binders, mixed affinity binders and high + mixed affinity binders Alzheimer’s disease groups compared to controls, especially at the prodromal stage, involving the temporo-parietal cortex. Translocator protein-binding was positively correlated with Mini-Mental State Examination scores and grey matter volume, as well as with Pittsburgh compound B binding. Amyloidosis controls displayed higher translocator protein-binding than controls, especially in the frontal cortex. We found higher translocator protein-binding in slow decliners than fast decliners, with no difference in Pittsburgh compound B binding. Microglial activation appears at the prodromal and possibly at the preclinical stage of Alzheimer’s disease, and seems to play a protective role in the clinical progression of the disease at these early stages. The extent of microglial activation appears to differ between patients, and could explain the overlap in translocator protein binding values between patients with Alzheimer’s disease and amyloidosis controls.

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Introduction

Emerging evidence suggests that neuroinflammation plays a crucial role in the pathophysiology of Alzheimer’s disease (Heneka et al., 2015). Microglia, the resident phagocytes of the brain, and astroglia are key players in the inflammatory response. Microglia are ubiquitously distributed in the brain and are activated by neuronal cell death or protein aggregation. In Alzheimer’s disease, the two principal misfolding and aggregating proteins are amyloid-β and tau. A marked accumulation and activation of microglia around amyloid-β plaques has been described in the post-mortem brains of patients with Alzheimer’s disease and in animal models (Prokop et al., 2013). In humans, the recent discovery of risk variants of gene encoding innate immune system molecules emphasizes the impact of neuroinflammation in Alzheimer’s disease pathogenesis (Griciuc et al., 2013; Guerreiro et al., 2013). However, the question as to whether the microglial activation plays a beneficial or detrimental role is as yet unresolved.

Many reports suggest that microglia are attracted to amyloid-β deposits, which they internalize and degrade, aiding clearance of amyloid-β from the brain. However, during the course of the disease, microglia may lose this beneficial effect as they acquire a ‘toxic’ phenotype due to chronic activation and continued production of pro-inflammatory mediators (Prokop et al., 2013; Michaud and Rivest, 2015). Several studies in transgenic mouse models have revealed diverging roles for neuroinflammation in the build-up of amyloid plaques and neurofibrillary tangles (Heneka et al., 2015) but the spectrum of glial cell actions and immune-related changes in Alzheimer’s disease is still a matter of controversy and investigation.

In humans, neuroinflammation has been investigated using 18-kDa translocator protein (TSPO) radiotracers by PET, considered as a marker of microglial activation. The first studies using 11C-PK11195 showed somewhat contradictory results (Cagnin et al., 2001; Versijpt et al., 2003; Edison et al., 2008; Okello et al., 2009; Wiley et al., 2009; Schuitemaker et al., 2013; Fan et al., 2015). There was a tendency toward increased TSPO binding in Alzheimer’s disease dementia, albeit with a large overlap with controls and no clear conclusion about binding in early Alzheimer’s disease. These results can perhaps be explained by the radiotracer itself, which suffered from many limitations, due to its highly lipophilic nature, low bioavailability, high non-specific binding and its limited capacity to detect small changes in TSPO expression (Banati et al., 2000; Lockhart et al., 2003; Belloli et al., 2004).
Recent studies using second generation of TSPO radio tracers suggest late but not early development of neuroinflammation, but again results are somewhat heterogeneous (Yasuno et al., 2008, 2012; Kreisl et al., 2013; Golla et al., 2015; Lyoo et al., 2015; Varrone et al., 2015). In addition these studies are limited by small sample sizes and methodological issues relating to the quantification of TSPO binding, which may lead to misinterpretation.

18F-DPA-714 belongs to this new generation of TSPO radio tracers, which have a greater affinity and better signal-to-noise ratio than the 11C-PK11195 (Chauveau et al., 2009).

For the first time, aiming to investigate the role of neuroinflammation in early Alzheimer’s disease, we analysed microglial activation using 18F-DPA-714 together with amyloid imaging (PiB-PET) in a large cohort of patients with Alzheimer’s disease, at both prodromal and dementia stages, with a 2 year clinical follow-up, taking into account the polymorphism of the TSPO gene responsible for different affinity profiles.

Materials and methods

Study design and participants

All participants were enrolled in the prospective longitudinal IMABio3 study (PHRC-0054-N 2010), which aimed to assess the neuroinflammation in Alzheimer’s disease. The study was approved by the Ethics Committee of the Salpêtrière Hospital. All subjects provided written informed consent prior to participating. This research has been registered on http://clinicaltrials.gov/under number NCT01775696.

Patients with Alzheimer’s disease were included according to the following criteria: (i) progressive episodic memory impairment, characterized by a low free recall not normalized with a sematic cueing (Sarazin et al., 2007; Dubois et al., 2011); (ii) absence of extrapyramidal signs; and (iii) CSF Alzheimer’s disease profile, when available, defined as score <0.8, calculated with the formula amyloid-P42/([240 + (1.18 x T-tau)] (de Souza et al., 2011).

Controls were recruited according to the following criteria: (i) Mini-Mental State Examination (MMSE) score ≥27/30 and normal neuropsychological assessment; (ii) Clinical Dementia Rating (CDR) score = 0; (iii) no history of neurological or psychiatric disorders; and (iv) no memory complaint or cognitive deficit. We did not include subjects with (i) severe cortical or subcortical vascular lesions; (ii) history of autoimmune and inflammatory diseases or chronic migraines; or (iii) history of psychiatric disorders or drugs abuse.

Among the 114 subjects screened, 103 fulfilled all the inclusion criteria. PET scans could not be achieved for technical problems for seven subjects. Finally 96 subjects were included in the study: 64 patients with Alzheimer’s disease (age = 68 ± 10.3 years) and 32 controls (age = 69.7 ± 9 years). CSF biomarkers measures were available for 56/64 patients with Alzheimer’s disease.

Patients with Alzheimer’s disease were classified in two groups according to their CDR score: 38 patients displayed a CDR score of 0.5, constituting the prodromal Alzheimer’s disease group and 26 patients displayed a CDR score ≥1 constituting the Alzheimer’s disease dementia group.

The autonomy of the patients at the prodromal stage was also documented by a normal score or only one item impaired at the first level in the four Instrumental Activities of Daily Living (IADL, ability to use the telephone, independence for transportation, self-administration of medication, ability to handle finances) (Sarazin et al., 2007).

All participants underwent the same procedure including a complete clinical and neuropsychological assessment, brain 3 T MRI, 11C-PiB and 18F-DPA-714 PET imaging. So far, 30 patients with Alzheimer’s disease were followed up annually for 2 years (n = 18 from the prodromal Alzheimer’s disease group and n = 12 from the Alzheimer’s disease dementia group).

Clinical, functional and cognitive assessment

The neurological and neuropsychological examination included the MMSE, the CDR scale, the Montgomery–Åsberg Depression Rating Scale (MADRS) and tests for assessing verbal and visual episodic memory, executive function, gesture praxis, visu-o-constructive function and language.

APOE ε4 and TSPO genotype

Blood samples were drawn to characterize APOE and TSPO genotypes. Based on the rs6971 polymorphism within the TSPO gene, we classified all subjects as high affinity binders (HAB), mixed affinity binders (MAB) or low affinity binders (LAB).

MRI acquisition

For each subject a 3D T1-weighted structural MRI acquisition was obtained on a 3 T MRI scanner (Siemens Trio, 32 channel system, with a 12 channel head coil for signal reception), to aid identification of volumes of interest for subsequent PET image analysis. This sequence provided a high grey/white matter contrast-to-noise ratio and enabled excellent segmentation and accurate co-registration with the PET images.

11C-PiB and 18F-DPA-714 PET imaging procedure

Data acquisition

MRI and PET scans were performed within 4 months of each other. 11C-PiB and 18F-DPA-714 PET scans were performed on the same day. Both PET scans were performed on a High Resolution Research Tomograph (HRRT; CTI/Siemens Molecular Imaging) (de Jong et al., 2007). A 6-min brain transmission scan was performed before injection of each radio ligand using a 137Cs point source to correct the emission scan for tissue attenuation.

11C-PiB-PET (mean 362 ± 53 MBq) and 18F-DPA-714 PET (mean 200 ± 15 MBq) were injected intravenously, and PET dynamic acquisitions in list mode lasted up to 90 min. All corrections (attenuation, normalization, random and scatter coincidences) were incorporated in an iterative OSEM reconstruction. The partial volume effect was corrected by...
directly incorporating resolution modelling (i.e. Point Spread Function modelling) inside the iterative algorithm (Sureau et al., 2008) so that no further post-correction was needed. Ten iterations using 16 subsets were used. Dynamic data were binned into 27 time frames (6 × 1 min, 7 × 2 min, 14 × 5 min). Reconstructed dynamic data were realigned for motion correction according to the process of frame-to-reference image registration in Pmod (version 3.5; PMOD Technologies Ltd.).

Parametric images were created using BrainVisa software (http://brainvisa.info). Standard uptake value (SUV) parametric images were obtained by: (i) averaging late images (intervals of 40–60 min for 11C-PiB images and of 60–90 min for 18F-DPA-714 images) (Lopresti et al., 2005; Lavisse et al., 2015); and (ii) adjusting for body weight and injected radio ligand dose. Standard uptake value ratio (SUVr) parametric images were constructed by dividing each voxel by the corresponding value obtained in the cerebellum grey matter. The cerebellar grey matter used as a pseudo reference region in both PET analysis (de Souza et al., 2011).

**Methodological consideration for the DPA-714 analysis**

SUVr have the advantage to improve subject tolerability by not requiring arterial catheterization, and are thought to be less variable than the absolute quantitation for TSPO imaging, potentially due to problems in identifying the correct input function (Guo, 2015; Lyoo et al., 2015).

The choice of the time window between 60–90 min to measure DPA-714 uptake is justified by a recent study showing that 18F-DPA-714 volume of distribution (VT) was remarkably stable between 60 and 90 min (Lavisse et al., 2015). In our population, the kinetic analysis demonstrated the validity of this time window (60–90 min), during which the ratios between volumes of interest and cerebellar grey matter were constant (Supplementary material).

Because TSPO is present in all structures in the brain, no true reference region devoid of TSPO can be identified. The acceptable alternative is to use a pseudo-reference region, relatively spared of Alzheimer’s disease pathology and for which the radiotracer uptake is independent of the pathological status. The choice of the cerebellar grey matter as pseudo-reference region was based on the following considerations:

1. Neuropathological data: cerebellum is devoid of tau pathology in Alzheimer’s disease even at the latest stages (Braak stage VI), and deposition of amyloid-β peptides is observed only in the advanced amyloid phases (Thal phase V). The cerebellar cortex did not demonstrate microgliosis or astrocytosis (Wood, 2003).

2. Recent published PET studies: the use of cerebellar grey matter as pseudo-reference region has already been validated using another second generation TSPO ligand (Lyoo et al., 2015). In addition, using 18F-DPA-714, the cerebellar grey matter displayed the lowest VT among all volumes of interest in controls (Lavisse et al., 2015).

3. In our population, 18F-DPA-714 uptake in cerebellar grey matter was (i) lowest among all volumes of interest in both control and Alzheimer’s disease groups; (ii) not different between control and Alzheimer’s disease subjects according to TSPO binding (Supplementary material); (iii) not different between prodromal Alzheimer’s disease and Alzheimer’s disease dementia accordingly to TSPO binding; and (iv) not correlated with MMSE, cortical volume and age.

To avoid overspill from sagittal sinus due to TSPO expression in endothelial cells, the cerebellar grey matter, delineated on individual 3D T1 MRI was eroded from 4 mm using spherical structuring elements of 4 mm radius, corresponding to more than 1.5 times the spatial resolution of the tomograph (HRRT).

**Volume of interest analysis**

The same method of anatomical segmentation was used for both 11C-PiB and 18F-DPA-714 PET images. An automated segmentation of grey matter was performed to each individual 3D T1-weighted MRI scans using the VBM8 package (http://dbm.neuro.uni-jena.de/vbml/) implemented in SPM8 (Institute of Neurology, London, UK; http://www.fil.ion.ucl.ac.uk/spm/). The segmented MRI scans were coregistered with both 11C-PiB and 18F-DPA-714 parametric images of the subject using a standard mutual information algorithm. The automated anatomical labelling (AAL) atlas was deformed to each individual MRI (using the deformation field extracted from VBM8). Each volume of interest was intersected with the T1 MRI grey matter mask to perform a pseudo-atrophy correction. Then this new labelling volume was registered to the individual PET space of 11C-PiB and 18F-DPA-714 parametric images using their respective transformation extracted from the PET-MRI coregistration. Similarly, cerebellar grey matter was identified for each subject and after eroded (4 mm), the region was used as pseudo-reference region.

Application of the AAL atlas to the PET data allowed the calculation of 11C-PiB and 18F-DPA-714 uptake in 76 anatomical regions. The volumes of interest were defined separately for the left and right hemispheres and were then pooled into greater anatomical volumes of interest, as previously described (de Souza et al., 2011). Briefly, we defined 10 volumes of interest: (i) the frontal; (ii) anterior cingulate; (iii) medium cingulate; (iv) posterior cingulate; (v) precuneus; (vi) parietal; (vii) temporal; (viii) hippocampus; (ix) and occipital cortex. A mean 11C-PiB and 18F-DPA-714 SUVr’s were obtained for each region. As a measure of global cortical burden, we calculated a 11C-PiB and 18F-DPA-714 Global Cortical Index (GCI), representing the subject’s mean SUVr of the neocortical regions cited above. Using this methodology, the cut-off of PiB-GCI was set as at 1.45.

**Voxel-wise analysis**

We used SPM8, implemented on a MATLAB platform (Mathworks.inc). Spatially normalized images were smoothed with an 8 mm full-width at half-maximum Gaussian filter. Groups were compared using unpaired two-sample t-test, with TSPO genotype as a covariate. For comparison of HAB + MAB patients with Alzheimer’s disease and controls, results were thresholded at the strict $P < 0.05$, family-wise error (FWE) corrected. For controls and amyloidosis controls comparison, a threshold of $P < 0.01$ was used. For all analyses, a minimum-activated voxel threshold of 20 voxels was applied.
Statistical analysis

Data were analysed using SPSS20 (SPSS Inc., Chicago, Illinois) and MedCalc (http://www.medcalc.org) software, and Figs 3 and 5 were produced using R, a software environment for statistical computing and graphics (R Core Team, 2014; http://www.R-project.org/). Normality of distribution was tested using the Shapiro-Wilk test. Differences between groups were assessed using the chi-square test, ANOVA, or Kruskall-Wallis tests, where appropriate. ANOVA, adjusted for TSPO genotype, was used to compare DPA binding between Alzheimer’s disease groups and controls and between amyloidosis controls and controls (with TSPO genotype as a covariate). To compare DPA uptake between fast and slow decliners, we used ANOVA with confounding factors (age, TSPO genotype and initial MMSE score) as covariates. A Bonferroni correction was used for multiple comparisons. Correlations were performed by using linear partial correlation analysis, taking into account covariates.

Results

Subject characteristics

All groups were matched for age (Table 1). The prevalence of APOE ε4 carriers was higher among patients with Alzheimer’s disease. In the whole population, the proportion of LAB was lower than MAB and HAB. The proportion of LAB, MAB and HAB was similar in the prodromal Alzheimer’s disease, Alzheimer’s disease dementia and control groups. Neuropsychological characteristics are presented in the Supplementary material.

11C-PiB-PET analysis

All patients with Alzheimer’s disease had a positive amyloid PET (PiB-GCI > 1.45). We found no difference of the mean PiB-GCI and the PiB uptake values in any volume of interest between the prodromal Alzheimer’s disease and Alzheimer’s disease dementia groups.

Among the controls, eight subjects had positive PiB-PET imaging (PiB-GCI > 1.45), constituting the amyloidosis-control group. The other 24 control subjects had no significant PiB retention (PiB-GCI < 1.45).

18F-DPA-714 binding: volume of interest analysis

To avoid interpretation bias due to the polymorphism of the TSPO gene, we compared Alzheimer’s disease and controls accordingly to their binding affinity (Table 2).

Among LAB subjects, the DPA-GCI and regional cortical binding (SUVr) did not differ significantly between patients with Alzheimer’s disease (n = 6) and controls (n = 4) (GCI of 1.23 ± 0.10 and 1.22 ± 0.25, respectively, P = 0.2, data not shown).

Among HAB subjects, the Alzheimer’s disease group showed higher DPA-GCI when compared to controls (P = 0.02), even at the prodromal stage. Significant higher DPA-714 binding was observed in the medium and posterior cingulate, precuneus, parietal and temporal cortex, especially at the prodromal stage. The DPA-714 binding was higher at the prodromal stage but without significant difference between prodromal Alzheimer’s disease and Alzheimer’s disease dementia groups.

In the MAB group, the Alzheimer’s disease group showed higher DPA-GCI than controls (P = 0.02), even at the prodromal stage. The highest DPA-714 uptakes were observed in the same volumes of interest than those observed in the HAB patients.

The same results were found when we pooled all HAB and MAB subjects, taking into account the TSPO affinity as a covariate in the analysis.

Table 1 Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 32)</th>
<th>Amyloidosis-controls defined by a positive PiB-PET (n = 8)</th>
<th>Alzheimer’s disease patients (n = 64)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls defined by a negative PiB-PET (n = 24)</td>
<td>Amloidosis-controls defined by a positive PiB-PET (n = 8)</td>
<td>Prodomal AD (n = 38)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>68.2 (8.4)</td>
<td>74.3 (9.8)</td>
<td>67.8 (9.1)</td>
</tr>
<tr>
<td>Sex (% male)</td>
<td>6 (25%)</td>
<td>4 (50%)</td>
<td>16 (42%)</td>
</tr>
<tr>
<td>Carrier of APOE ε4</td>
<td>0 (0%)</td>
<td>2 (25%)</td>
<td>24 (63%)*</td>
</tr>
<tr>
<td>MMSE score</td>
<td>29.5 (0.6)</td>
<td>29.1 (0.8)</td>
<td>24.1 (2.8)**</td>
</tr>
<tr>
<td>CDR</td>
<td>CDR = 0 (n = 24)</td>
<td>CDR = 0 (n = 8)</td>
<td>CDR = 0.5 (n = 38)**</td>
</tr>
<tr>
<td>HAB</td>
<td>11 (46%)</td>
<td>2 (25%)</td>
<td>17 (45%)</td>
</tr>
<tr>
<td>MAB</td>
<td>9 (38%)</td>
<td>4 (50%)</td>
<td>17 (45%)</td>
</tr>
<tr>
<td>LAB</td>
<td>4 (16%)</td>
<td>2 (25%)</td>
<td>4 (10%)</td>
</tr>
<tr>
<td>IATI†</td>
<td>N.A</td>
<td>N.A</td>
<td>0.46 (0.24)</td>
</tr>
<tr>
<td>11C-PiB-GCI</td>
<td>1.24 (0.08)</td>
<td>2.19 (0.65)*</td>
<td>2.77 (0.56)**</td>
</tr>
</tbody>
</table>

Data are mean (SD) or number (%). *P < 0.05 compared to controls, defined by a negative PiB-PET (GCI < 1.45).
†P < 0.05 compared to amyloidosis-controls, defined by a positive PiB-PET (GCI > 1.45).
‡P < 0.05 compared to prodromal Alzheimer’s disease.
AD = Alzheimer’s disease.
Compared to controls, HAB + MAB patients with Alzheimer’s disease showed significant symmetrical $^{18}$F-DPA-714 binding, throughout the temporal and parietal regions (significance threshold set at $P < 0.05$, FWE corrected) (Fig. 1). At the prodromal stage, the results remained significant in the same cortical areas. No difference was found between the prodromal Alzheimer’s disease and Alzheimer’s disease dementia groups. An involvement of the frontal cortex was observed in the prodromal Alzheimer’s disease but not in the patients with Alzheimer’s disease dementia.

### Correlations between $^{18}$F-DPA-714 binding and age or APOE

No correlation was found between the age and the DPA-GCI binding in the whole population ($r = 0.03$, $P = 0.4$ corrected for the CDR and TSPO genotype). Similarly, no correlation was found among controls or among...
MAB + HAB patients with Alzheimer’s disease. No difference of DPA-714 binding was observed between groups according to the presence or the absence of at least one APOE ε4 allele.

**Correlations between ¹⁸F-DPA-714 binding and markers of severity: MMSE and cortical atrophy**

Correlations are shown in Fig. 2. In the HAB + MAB group, partial linear correlations showed a significant positive correlation between the DPA-GCI and MMSE scores, even after correction with age and TSPO genotype as covariates ($r = 0.31$, $P = 0.016$).

We found a positive correlation between the DPA-GCI and the grey matter volume ($r = 0.35$, $P = 0.004$). The correlations were corrected for age, TSPO genotype and CDR status as covariates. The correlation remained significant, and even stronger, when we restricted the analysis to the prodromal Alzheimer’s disease group alone ($r = 0.47$, $P = 0.006$, with age and TSPO genotype as covariates).

**Comparison of ¹⁸F-DPA-714 binding between amyloidosis controls and PiB negative controls**

The DPA-GCI was significantly higher in the HAB + MAB amyloidosis controls ($n = 6$, defined by positive amyloid imaging) than in the HAB + MAB controls ($n = 20$, defined by a negative amyloid imaging) ($1.39 \pm 0.29$ and $1.21 \pm 0.10$, respectively, $P = 0.04$ with TSPO genotype as a covariate), with an overlap between both groups (Fig. 3). Analysis by volumes of interest showed a significant higher mean uptake value of the DPA-714 in the cingulum and the precuneus. The voxel-wise comparison confirms the data, showing an extension of the neuroinflammation in the frontal cortex ($P < 0.01$). When applying a stricter threshold, the comparison was no longer significant.

**Correlations between ¹¹C-PiB and ¹⁸F-DPA-714 binding**

In HAB + MAB patients with Alzheimer’s disease, the PiB-GCI and DPA-GCI were positively correlated ($r = 0.255$, $P = 0.031$) (Fig. 4). When we analysed the correlations in each volume of interest that we identified above as having increased neuroinflammation, we found a positive correlation between the PiB and DPA-714 fixation in all of them, except the occipital cortex, in which $P$-values tended to reach the significance threshold. The correlations were adjusted to age, TSPO genotype, CDR score and APOE ε4 status.

**¹⁸F-DPA-714 cortical binding and disease progression**

Forty-nine subjects were followed-up over 2 years, with a standardized protocol including a CDR. Sixteen controls and two amyloidosis-controls remained stable. HAB + MAB patients with Alzheimer’s disease ($n = 30$) were dichotomized into slow decliners or fast decliners based on the progression of the CDR score at the last visit. Ten patients with Alzheimer’s disease remained stable, showing unchanged CDR score ($n = 5$ prodromal Alzheimer’s disease and $n = 5$ Alzheimer’s disease dementia at baseline), whereas 20 patients with Alzheimer’s disease declined, with an increase of 0.5 or more of the CDR score ($n = 13$ prodromal Alzheimer’s disease and $n = 7$ Alzheimer’s disease dementia at baseline) (Supplementary material). All patients were treated with cholinesterase inhibitors. We compared the GCI of the DPA binding at...
baseline between the slow and fast decliners. After adjusting for confounding factors (age, TSPO genotype and initial MMSE score), we found a significant higher GCI-DPA-714 SUVr in the slow decliners than in the fast decliners group (1.51 ± 0.17 and 1.29 ± 0.1, respectively, \( P = 0.001 \)) (Fig. 5).

By contrast, no difference was observed for the PiB-GCI measured at baseline between both slow and fast decliner groups, with age as a covariate (2.82 ± 0.19 and 2.63 ± 0.13, respectively, \( P = 0.11 \)).

To ensure that the cortical atrophy did not affect the results, we further conducted the same analysis after excluding two patients with CDR = 2 at baseline. The TSPO binding was higher \( (P = 0.001) \) in the slow decliners group \( (n = 10) \) than in the fast decliners group \( (n = 18) \), without difference in the MMSE and cortical volume at baseline \( (\text{MMSE} = 22.7 \pm 3.7 \text{ and } 20.1 \pm 4.3, \text{respectively}; \text{cortical volume} = 677.4 \pm 57 \text{ and } 625.2 \pm 69) \). Because slow decliner patients were younger than fast decliners \( (74 \pm 11.4 \text{ years versus } 62.7 \pm 8.4 \text{ years, } P = 0.01) \), we verified that (i) the DPA-714 binding was not different in the early and late onset of Alzheimer’s disease in the whole Alzheimer’s disease group and in patients with Alzheimer’s disease who were followed-up over 2 years; and (ii) the absence of correlation between age and DPA-714 binding.

### Discussion

In this study, we used \(^{18} \text{F}-\text{DPA-714} \), a marker of microglial activation, and PiB, a marker of fibrillar amyloid deposition, by PET to investigate in vivo the role of neuroinflammation in Alzheimer’s disease. All patients with Alzheimer’s disease met the new criteria of Alzheimer’s disease (Dubois et al., 2010), including pathophysiological biomarkers with positive CSF biomarkers and amyloid PET imaging, limiting considerably the risk of including non-patients with Alzheimer’s disease. Moreover, the control group was defined by negative PiB-PET, excluding preclinical Alzheimer’s disease, whereas amyloidosis-controls defined by positive PiB-PET, suggestive of preclinical Alzheimer’s
disease, were analysed separately. TSPO binding was analysed according to individual TSPO genotype, which influences the radiotracer binding affinity (Owen et al., 2011).

Using both a volume of interest-based method and direct statistical parametric mapping comparison, we found an increase of TSPO binding in Alzheimer’s disease compared to controls, especially at the prodromal stage, involving preferentially the temporo-parietal cortex. Moreover, higher inflammation as measured by TSPO binding was associated with higher MMSE score and grey matter volume, which are both inversely correlated with Alzheimer’s disease severity. These correlations remained significant within the prodromal Alzheimer’s disease group after correction for age and TSPO genotype, excluding the risk of false positive results caused by the most severe patients with Alzheimer’s disease. Taken together, these data suggest early rather than late microglial activation in Alzheimer’s disease.

Studies using $^{11}$C-PK11195 report microglial activation in patients with mild cognitive impairment (MCI) and amyloid deposition (Edison et al., 2008; Okello et al., 2009), but this observation has not been confirmed with the new second generation TSPO tracers (Kreisl et al., 2013): increased in vivo binding of TSPO was observed in patients with Alzheimer’s disease dementia using $^{18}$F-FEMPA (Varrone et al., 2015); microglial activation was observed in Alzheimer’s disease, but not at all or only sparsely in MCI stage of Alzheimer’s disease using $^{11}$C-PBR28 (Kreisl et al., 2013; Lyoo et al., 2015); while the only published study using $^{18}$F-DPA-714 did not account for the effect of TSPO genotype, severely compromising interpretation of the results (Golla et al., 2015). Finally, it

**Figure 3** Scatter and box plots showing global and regional $^{18}$F-DPA-714 SUVr in anatomical regions across groups. (-) = Controls, defined by a negative PiB-PET (in yellow); (+) = amyloidosis controls, defined by a positive PiB-PET (in green) in the HAB + MAB population. *P < 0.05 adjusted for age, TSPO genotype and initial MMSE score.
should be noted that all these studies included very few patients with prodromal Alzheimer’s disease (from 4 to 11).

Our results are seemingly at odds with those of Kreisl et al. (2013), who reported neuroinflammation later in the disease progress, in association with increasing severity. In addition, we did not find any correlation between age and TSPO binding. This discrepancy may be explained by the difference in the method for measuring the density of TSPO. Kreisl et al. (2013) used a metabolite-corrected plasma input model (classical two-tissue compartment model) to quantify $^{11}$C-PBR-28 binding, whereas we used a simple ratio method (SUVr) with the cerebellar grey matter as reference tissue to measure the cortical $^{18}$F-DPA-714 binding.

As previously discussed, the pseudo-reference region method has a much lower coefficient of variation than absolute quantification (Lyoo et al., 2015) and, in addition for $^{11}$C-PBR28 specifically, variability in blood and tissue has been reported to lead to the high variability in VT (Guo, 2015), as used by Kreisl et al. (2013).

Of note, our cohort was larger than those previously reported, enabling us to analyse HAB and MAB groups separately, thus reinforcing our results. We found a positive correlation between PiB and TSPO binding. Previous publications combining imaging neuroinflammation and amyloid reported conflicting results, and should be interpreted with caution due to the limited sample sizes (Cagnin et al., 2001; Edison et al., 2008; Okello et al., 2009; Wiley et al., 2009; Kreisl et al., 2013; Fan et al., 2015). Our correlation, which remained significant after correction for age, severity stage of the disease, ApoE4 and TSPO genotypes, suggests a relationship between fibrillar amyloid-$\beta$ deposition and neuroinflammation.

Whilst the presence of reactive microglia around amyloid-$\beta$ plaques has been confirmed in both humans and animal models (Prokop et al., 2013), the mechanisms by which amyloid deposits provoke an inflammatory response are not fully understood. One way to address this question is to study the topography of neuroinflammation. Interestingly, the cortical regions in which the TSPO binding was highest were also affected by amyloid deposition, apart from the frontal cortex. Involvement of the frontal cortex was only observed in patients with prodromal Alzheimer’s disease using both volumes of interest and SPM methods.

Importantly, when we analysed the amyloidosis-controls, we found not only an increase on TSPO binding compared with controls, but also a more substantial involvement of the frontal cortex rather than the posterior region. This suggests that microglial activation should appear at the preclinical stage of Alzheimer’s disease and then follows a dynamic way during the course of the disease.

Longitudinal data showed that high microglial activation observed at baseline was associated with clinical stable progression after 2 years of follow-up, while low microglial activation was associated with rapid decline. Fast decliners were younger than slow decliners, as previously reported (van der Vlies et al., 2009; Koedam et al., 2010), however, our results were corrected for age, MMSE at baseline and TSPO genotype, and were not explained by different treatment. Conversely, no difference was observed in PiB binding. These results are in agreement with a recent paper by Ramanan et al. (2015) showing that lower microglial activation, assessed by $^{11}$C-PBR28, is associated with rapid cognitive decline in Alzheimer’s disease.

One important limitation of our results is the methodology used to quantify the DPA-714 binding in the absence of arterial blood sample. No prior study has yet established that DPA-714 can distinguish patients with Alzheimer’s
disease and controls using traditional kinetic modelling methods. Given the lack or arterial plasma sampling, we are not able to provide evidence that (i) DPA-714 VT was greater in Alzheimer’s disease than controls when measured with arterial input function; and (ii) that DPA-714 VT did not differ in cerebellum between patients with Alzheimer’s disease and controls. In consequence, we cannot exclude that the SUVr method may confound the results. In addition, the presence of microglial activation in the cerebellum cannot be formally excluded in Alzheimer’s disease, even in the absence of amyloid and tau pathology. Even if our data are in agreement with recent publications (Chakrabarty et al., 2015; Guillot-Sestier et al., 2015; Michaud and Rivest, 2015; Ramanan et al., 2015), further studies with arterial blood function will be necessary to confirm these clinical results.

Taken together, these data suggest that neuroinflammation appears at the prodromal and even possibly preclinical stage of Alzheimer’s disease, and plays a protective role in the clinical progression of the disease. The extent of microglial activation was heterogeneous among patients, as indicated by the overlap in TSPO binding values between patients with Alzheimer’s disease and amyloidosis-controls. Such heterogeneity may translate into significantly different rates of disease progression. Note that the strict criteria of inclusion, excluding any history of inflammatory and autoimmune diseases, limit the risk that individual microglial activation was due to other factors than Alzheimer’s disease.

Microglial activation is often considered neurotoxic, generating a proinflammatory response sustained over time that can cause neuronal death (Aguzzi et al., 2015).
2013). However, microglial stimulation in mice with Alzheimer’s disease-like pathology has been shown to decrease amyloid-β burden (Herber et al., 2004, 2007). Recent studies in mouse models of amyloid pathology suggested that in healthy individuals or in early phases of Alzheimer’s disease, activated microglia are efficient at migrating towards amyloid-β deposits and clearing them by phagocytosis. As the disease progresses, microglia become progressively dysfunctional, displaying altered activation, migration and functional differentiation (Chakrabarty et al., 2015; Guillot-Sestier et al., 2015; Michaud and Rivest, 2015).

Despite some methodological limitations discussed above, our results provide, for the first time in humans, corroborative evidence in line with this model. Neuroinflammation observed in prodromal Alzheimer’s disease is likely driven primarily by brain-resident immune cells, especially microglia, in a non-uniform manner among patients with Alzheimer’s disease. Such interindividual variability in early innate neuroinflammatory response may influence the clinical course of disease progression. Increased early microglia activation may be protective, and a failure to appropriately adapt to chronic amyloid deposition could be an underlying mechanism in neurodegeneration. Such a protective role of early microglial response in Alzheimer’s disease pathogenesis fosters the clinical interest of microglia-targeted therapeutic approaches (Shechter and Schwartz, 2013). Finally, 18F-DPA-714 PET imaging may prove a valuable innovative tool for accurately assessing neuroinflammation in early and preclinical Alzheimer’s disease.

Acknowledgements

The authors would like to thank chemical/radiopharmaceutical and nursing staff of Service Hospitalier Frédéric Joliot for the synthesis of 11C-PIB and 18F-DPA-714 and patient management, the team of CENIR (Centre de Neuroimagerie de Recherche) in Salpêtrière Hospital for patient management during the MRI acquisition, the staff of the CATT (Centre d’Acquisition et de Traitement automatisé de l’Image) for technical support, the study participants. The authors gratefully acknowledge Pr. Charles Duyckaerts for useful comments and valuable suggestions, the Fondation pour la Recherche sur la Maladie d’Alzheimer, and the CEAN2BM/Neurospin-Paris Descartes University collaboration.

Funding


Conflict of interest

G.D. received grants from F. Hoffmann-La Roche during the conduct of the study. L.H. has obtained a grant from the Fondation pour la Recherche sur la Maladie d’Alzheimer. R.A.C. is an employee of F. Hoffmann-La Roche Ltd who supported a part of the study. B.D. has collaborated with Eli Lilly and Affiris, and has received grants for his institution from Roche and Pfizer. During the last 2 years, M.S. has collaborated with Allianz and with the pharmaceutical company Novartis (lecture/speaking).

Supplementary material

Supplementary material is available at Brain online.

References


Appendix I

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