Neuroinflammation and β Amyloid Deposition in Alzheimer’s Disease: In vivo Quantification with Molecular Imaging

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Key Words
Neuroinflammation · Translocator protein · Microglia · Alzheimer’s disease · Amyloid plaques · Molecular imaging · [18 F]DPA-714

Abstract
Background/Aims: Neuroinflammation plays a crucial role in the pathogenesis of Alzheimer’s disease (AD). Its relationship with underlying β amyloid deposition remains unclear. In vivo visualization of microglial activation has become possible with the development of molecular imaging ligands when used with positron emission tomography (PET). The translocator protein (TSPO) is upregulated during neuroinflammation. Consequently, targeting TSPO with radiolabeled ligands for PET is an attractive biomarker for neuroinflammation.

Methods: A review of the research literature on PET imaging which studied in vivo neuroinflammation in AD subjects and its relationship with amyloid load was performed, including papers published between 2001 and 2012.

Results: Six studies were included using either [11 C]PK-11195 or another non-TSPO radioligand that binds to the monoaminooxidase B. All the studies evaluated amyloid load with [11 C]PIB. Microglial activation and astrocitosis are potentially early phenomena in AD. However, the individual levels of amyloid deposition and microglial activation were not correlated.

Conclusion: Noninvasive in vivo molecular imaging to visualize neuroinflammation in AD may contribute to our understanding of the kinetics of neuroinflammation and its relationship to the hallmarks of the disease. Both are important for the development of future therapeutic modalities and for quantifying the efficacy of future disease-modifying treatments.

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Alzheimer’s disease (AD) is the most common cause of dementia in elderly subjects. AD is characterized by brain lesions like extracellular deposits of β-amyloid proteins in senile plaques and intracellular neurofibrillary tangles of hyperphosphorylated tau protein, both of which are associated with the loss of neurons [1]. In AD, pathological changes precede the clinical diagnosis of dementia due to AD [2]. Consequently, a new lexicon was proposed [3, 4] defining the research criteria for the diagnosis of AD based upon the presence of a specific pattern of episodic memory disturbance (within test conditions of encoding specificity), associated with biomarker positivity, that can include cerebrospinal fluid (CSF) tau, p-tau and β-amyloid, positron emission tomography (PET) amyloid positivity, medial temporal lobe atrophy on magnetic resonance imaging (MRI) and/or hypometabolism on fluorodeoxyglucose PET. In 2011, the National Institute on Aging-Alzheimer’s Association workgroup published recommendations and distinguished the preclinical stages of AD (presymptomatic phase without any clinical signs) [5], the diagnosis of mild cognitive impairment (MCI) due to AD, also considered as a prodromal phase (MCI-AD) [6], and the final phase, dementia due to AD (AD dementia) [7]. The development of disease biomarkers for AD [8] in order to identify the pathophysiological processes underlying cognitive impairment has been incorporated into revised diagnosis guidelines [7]. Moreover, postmortem human AD [9, 10] and animal model AD studies [11–13] have reported that inflammatory processes are also implicated in the neuropathology of AD and upregulated levels of pro-inflammatory cytokines [14].

Considering the recent development of molecular imaging, the main aim of this review was to investigate the current evidence on neuroinflammation through in vivo molecular imaging and its relationship with amyloid load in AD subjects in the hope that this may lead to future developments in both diagnosis and therapeutics. Noninvasive in vivo molecular imaging of neuroinflammation may represent a biomarker for early diagnosis, as well as a useful tool for assessing the efficacy of disease-modifying therapeutic strategies.

Neuroinflammation in AD: In vivo Quantification with Molecular Imaging

Gliial and Peripheral Blood Cell Reaction in AD

AD is characterized histopathologically by neuropathological hallmarks (senile plaques, intracellular neurofibrillary tangles and diffuse loss of neurons). However, prominent activation of the inflammatory processes is also observed in human postmortem studies of AD patients [14–19]. These inflammatory components include microglia activation, which may be considered to be the resident tissue macrophages in the central nervous system (CNS), as well as astrocytosis, both of which surround the plaques. In response to an activating stimulus, activated microglia secrete a variety of inflammatory mediators including cytokines [tumor necrosis factor (TNF) and interleukin (IL)-1β, IL-α and IL-6] and chemokines that promote the inflammatory state. Various human and animal studies have demonstrated that neuroinflammation plays a role in amyloidogenesis processing [19–23], although the role of microglia activation during the course of the disease remains poorly understood. Microglial activation may be beneficial in AD by promoting amyloid clearance which may facilitate β amyloid elimination [24–28]. However, microglial activation may also produce brain damage via the release of inflammatory mediators, including IL-1β, IL-6 and TNFα [27]. Microglial cells may amplify several steps of the amyloid cascade [24, 29]. Some studies have shown that this inflammatory response was also present at the periphery with increased levels of circulating cytokines and chemokines in plasma [30–32] and in peripheral blood mononuclear cells (PBMCs) [32–34] of patients with AD compared to age-matched controls. Furthermore, in patients with AD, both activated T cells and monocytes are present peripherally [35] and infiltrate around amyloid deposits in the brain [36, 37]. Access into the CNS across the blood-
brain barrier in AD patients depends on the inflammatory environment, but the role played by chemokines remains to be elucidated. Some chemokines (e.g. CCL2, CCL5 and CX3CL1) have been quantified in the plasma or CSF of AD patients, and their role in the pathophysiology of AD has been revealed [31, 38–41]. An additional argument for the role of systemic inflammation in the pathophysiology of AD is provided by epidemiological studies which suggest that nonsteroidal anti-inflammatory drugs (NSAIDs) may decrease the risk of AD [42–45]. However, randomized controlled trials involving the systemic administration of NSAIDs have reported mixed or inconclusive results, suggesting that we need to identify different treatment effects according to the stage of the disease [46, 47].

Translocator Protein Brain Imaging of Neuroinflammation

Sustained inflammatory responses involve microglia and astrocytes. Microglial cells become activated at every stage in response to a pathological event [48]. The inflammatory reaction involves a dramatic increase in the expression of a mitochondrial transmembrane protein, the translocator protein (TSPO; 18 kDa), formerly known as the peripheral benzodiazepine receptor (PBR), a receptor located in the outer membrane of mitochondria [49, 50], whose upregulation is considered to be a hallmark of microglial activation. TSPO is an attractive target for imaging cerebral inflammation because it is only modestly expressed in normal brain parenchyma, but is dramatically upregulated during neuroinflammation [27, 51, 52].

The development of novel PET radioligands with a high affinity for TSPO offers us the possibility of exploring neuroinflammation in vivo. PET imaging of neuroinflammation is a noninvasive imaging technique which uses radioisotope-labeled ligands (such as carbon-11 or fluorine-18) that have a high affinity for binding specifically to the TSPO [10, 53]. The lipophilic nature of the radioligand facilitates its entry across the blood-brain barrier into the CNS and its binding to brain TSPO. Several different PET radioligands are available or are being developed [23]. PK-11195 [1-(2-chlorophenyl)-N-(1-methylpropyl)-3-isoquinoline carboxamide], the reference TSPO radioligand, labeled with carbon-11, was used in animal models for focal ischemia [51, 54, 55], in human brain ischemia [56, 57] and in neurodegenerative diseases [51, 58, 59].

Imaging studies of neuroinflammation in AD subjects mainly using [11C]PK-11195 reported an increase in [11C]PK-11195 binding in various cortical regions (entorhinal, frontal, temporoparietal and cingulate cortices) compared to controls [58, 60–63]. Several other putative TSPO-specific ligands with improved pharmacological properties have been developed in preclinical and human studies, including [18F]PBR06 [64], [18F]PBR28 [65], [18F]DPA-713 [15] and [11C]vinpocetine [49]. Another TSPO ligand, [11C]DAA 1106, was evaluated in postmortem tissues from patients with neurodegenerative diseases, multiple sclerosis or cerebral infarcts and showed favorable pharmacological properties [49, 50]. This tracer was evaluated in AD patients compared to controls [66] and, more recently, in MCI subjects compared to AD patients and controls [67]. [11C]DAA 1106 retention was higher in the dorsal and medial prefrontal cortex, the lateral temporal cortex, the parietal and occipital cortex, the anterior cingulate cortex and the striatum in AD and MCI patients compared to controls. There was no significant difference in [11C]DAA 1106 binding between MCI and AD patients. In contrast, Varrone et al. [68] failed to demonstrate any significant binding of [18F]DAA 1106 in AD subjects. While [11C]PK-11195 and other radioligands are now available for studying in vivo neuroinflammation, some limitations have become apparent. Labeling with carbon-11 for both [11C]PK-11195 and [11C]DAA 1106 limits its widespread clinical use (short half-life of carbon-11: 20.4 min) [48, 69]. The quantification of TSPO by PET currently uses the cerebellum as a reference tissue [56]. However, this quantification may be confounded by interindividual variability in binding affinity of tracers, suggesting a possible polymor-
phism in the TSPO binding site [70]. This variability in TSPO binding may be related to an underlying genetic mechanism [70–72]. Consequently, another radiotracer for TSPO, DPA-714, labeled with fluorine 18, has been developed. It has a longer half-life than [11C]PK-11195 (110 compared to 20 min) and is preferentially employed in multicenter clinical studies. [18F]DPA-714 has a good affinity and specificity for TSPO and no affinity at all for CBR (central benzodiazepine receptor) [73]. [18F]DPA-714 uptake is reversible and reaches a peak within 20 min after bolus injection and slowly decreases thereafter as has recently been shown in the first human study using this radiotracer [74]. Preliminary data in amyotrophic lateral sclerosis are now available [75].

The in vivo quantification of activated microglia with PET imaging may be a useful tool for attaining a better understanding of the physiopathology of AD, especially the chronological relationship between the hallmarks of the disease (especially amyloid load) and microglial activation. In fact, the role of neuroinflammation and its relationship to Aβ remain controversial. Since the hallmarks of AD appear before the clinical symptoms develop, we can suggest that microglial activation plays an early role in the physiopathology of AD. Consequently, we conducted a systematic review of the literature by including PET imaging studies in which the participants had either AD or MCI (prodromal), and where neuroinflammation was studied analyzing TSPO radioligands and their relationship to amyloid load.

Methods

Search Strategy

In accordance with the preferred reporting items for systematic reviews and meta-analysis (PRISMA) statement [76], we conducted a search on the online PubMed literature database. The search strategy included studies from 2001 to 2012, using combinations of the following terms: Alzheimer’s disease; neuroinflammation; microglial activation, astrocytes, PET imaging; TSPO; carbon 11-labeled PK11195; carbon 11-labeled Pittsburgh compound B; amyloid load. We limited our search results to adult subjects and original research studies in the English language.

Study Selection

We excluded paper reviews, single reports and molecular imaging which involved other neurological diseases than AD or nonhuman imaging studies (fig. 1). Consequently, this article consists of a review of published research studies on molecular imaging of neuroinflammation and its relationship with PET amyloid imaging in AD and MCI subjects. We focused on the characteristics of the sample (AD and MCI criteria), the study design, identifying which radiotracers had been used, the primary findings of the studies regarding the relationship between neuroinflammation and amyloid load and correlations with measures of cognition. A summary of the key studies can be found in table 1.

Results

PET Imaging of Neuroinflammation and Its Relationship to Amyloid Load in AD

PET imaging studies which assessed the regional relationship between microglial activation and Aβ deposition in AD were all cross-sectional studies and used [11C]PK-11195 for quantifying neuroinflammation and 11C-Pittsburgh compound B ([11C]PIB) [77, 78] for measuring the amyloid load (table 1). The first radiotracer to reflect the burden of amyloid plaques in AD patients in vivo was [11C]PIB [77, 78], but its use is limited because of the short half-life (20 min) of carbon-11. Newer radiotracers have been developed, using fluorine-18, which are more accessible to clinical research thanks to their longer half-life (110 min) [78], e.g. florbetapir [79–85], florbetaben [86, 87], flutemtamol [88] and FDDNP [89]. All have already shown that they are capable of distinguishing AD patients from healthy controls.
Yokokura et al. [90] reported a significant increase in \([^{11}C]PK-11195\) BP in the anterior and posterior cingulate areas in AD subjects compared to controls, who demonstrated a more robust increase in \([^{11}C]PIB\) accumulation. \([^{18}F]FDG\) uptake was also reduced in these regions as has been classically reported in AD in the early stage of the disease. However, there was also an increase in PIB binding in broader regions, such as the parietal, temporal and frontal areas, indicating a lack of coupling between amyloid deposits and microglial activation. Wiley et al. [91] confirmed that there was no difference in brain \([^{11}C]PK-11195\) retention between AD, MCI and control subjects, or in the presence or absence of Aβ deposits, as detected by \([^{11}C]PIB\) retention. Okello et al. [92] compared the in vivo pattern of amyloid deposition and microglial activation in 14 subjects with amnestic MCI to 2 other groups: 22 AD subjects who had \([^{11}C]PIB\) PET and 15 who had \([^{11}C]PK-11195\) PET. Fourteen healthy controls underwent \([^{11}C]PIB\) PET and 10 underwent \([^{11}C]PK-11195\) PET. The results confirmed the existence of an increase in \([^{11}C]PIB\) uptake in 50% of the MCI subjects (PIB positive). However, only 38% of the subjects had an increase in \([^{11}C]PK-11195\) uptake which was not correlated with \([^{11}C]PIB\), confirming the absence of correlation between the regional level of \([^{11}C]PK-11195\)
Table 1. Molecular brain imaging studies of neuroinflammation and relationship with amyloid load in human AD and MCI subjects

<table>
<thead>
<tr>
<th>Study</th>
<th>Populations</th>
<th>PET imaging of neuroinflammation</th>
<th>PET imaging of amyloid load</th>
<th>Outcome measures and statistical analysis</th>
<th>Main results</th>
<th>Correlations with cognition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edison et al. [60]</td>
<td>AD: n = 13; DSM IV and McKhann et al.’s [132] criteria; mean age 65.6 ± 4.6 years; duration of diagnosis 14.5 ± 6.5 months; subjects with significant white matter disease were excluded</td>
<td>[11C]PK-11195: significant increase in PK-11195 binding related with β amyloid load in frontal, temporal, parietal, occipital and cingulated cortex (p &lt; 0.05); PK-11195 signal was not correlated with amyloid load</td>
<td>[11C]PIB: increase in PK-11195 binding in posterior cingulate and frontal regions (r = 0.66)</td>
<td>AD: significant inverse correlation between MMSE scores and PK-11195 binding in posterior cingulate, parietal and frontal regions (p &lt; 0.05)</td>
<td>Not evaluated</td>
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<tr>
<td>Okello et al. [92]</td>
<td>AD: n = 22; McKhann et al.’s [132] criteria and DSM IV criteria; mean age 64.9 ± 6.4 years; mean duration of symptoms 5.5 ± 3.8 years; MMS 21.5 ± 3.6 MCI: n = 14; mean age 66.6 ± 9.6 years; Petersen et al.’s [133] criteria for amnestic MCI; MMS 27.7 ± 1.5; subjects with significant white matter disease were excluded</td>
<td>[11C]PK-11195: no significant differences in BP between subject groups and no significant difference between PIB-negative and PIB-positive groups; no significant correlation between indices of [11C]PK-11195 and [11C]PIB retention</td>
<td>Controls: no significant differences in [11C]PIB binding between [11C]PIB-positive and [11C]PIB-negative groups; no correlation between individual regional PK-11195 binding and PIB retention</td>
<td>AD and MCI: no correlation between MMSE, duration of the disease and [11C]PIB retention or [11C]PK-11195 binding; in the PIB-negative MCI subjects, lower MMSE score correlated with higher PK-11195 binding in anterior cingulate and temporal cortex (p &lt; 0.05)</td>
<td>Not evaluated</td>
<td></td>
</tr>
<tr>
<td>Wiley et al. [91]</td>
<td>AD: n = 6; McKhann et al.’s [132] criteria and DSM IV criteria; mean age 64.9 ± 6.4 years; MMS 13–28; no restriction for anticholinesterase drug; no details about the duration of the disease MCI: n = 6; no details about diagnosis criteria; mean age 71.8 ± 5.5 years; MMS 27–30 Controls: n = 5; mean age 72 ± 5.9 years</td>
<td>Analysis of variance with post-test Bonferroni correction to compare [11C]PIB (SUV) and [11C]PK-11195 (BP) fixation between groups; nonparametric Mann-Whitney test to compare data between [11C]PK-11195-positive and -negative groups; Pearson’s correlation coefficient to study the relationship between [11C]PK-11195 and [11C]PIB retention</td>
<td>[11C]PK-11195 BP: no significant differences in BP between subject groups and no significant difference between PIB-negative and PIB-positive groups; no significant correlation between indices of [11C]PK-11195 and [11C]PIB retention</td>
<td>[11C]PK-11195 BP: not evaluated</td>
<td></td>
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<tr>
<td>Yokokura et al. [90]</td>
<td>AD: n = 11; McKhann et al.’s [132] criteria and DSM IV criteria; mean age 70.6 ± 6.4 years; exclusion: subjects with significant white matter disease, donepezil and NSAIDs disease duration 2.4 ± 1.7 years; MMS 21.9 ± 3.5 Controls: n = 10</td>
<td>[11C]PIB: increase in [11C]PIB uptake in the anterior and posterior cingulate area; significant negative correlation between [11C]PK-11195 and [11C]PIB uptake in the posterior cingulate cortex (r = –0.84), the area with the most severe reduction in the [18F]FDG</td>
<td>[11C]PK-11195 BP in the anterior and posterior cingulate area; significant negative correlation between [11C]PK-11195 and [11C]PIB uptake in the posterior cingulate cortex (r = –0.84), the area with the most severe reduction in the [18F]FDG</td>
<td>Inverse correlation between MMSE score and [11C]PK-11195 BP values, but not [11C]PIB in left anterior cingulate cortex (r = 0.9), left precuneus (r = 0.8) and left hippocampus (r = 0.79)</td>
<td>Inverse correlation between MMSE score and [11C]PK-11195 BP values, but not [11C]PIB in left anterior cingulate cortex (r = 0.9), left precuneus (r = 0.8) and left hippocampus (r = 0.79)</td>
<td>Not evaluated</td>
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<tr>
<td>Santillo et al. [93]</td>
<td>AD: n = 9; McKhann et al.’s [132] criteria and DSM IV criteria; MMS 10–16; median age 82 years (range 60–71)</td>
<td>[11C]DED: increased PIB uptake in AD and MCI; no correlation with amyloid load</td>
<td>AD: significantly higher [11C]DED retention in frontal (35% increase), parietal (35.2% increase) and medial temporal lobes (22.3% increase) (1 subject was PIB negative, 1 subject fulfilled the diagnosis of Lewy body disease 3 months later); significant correlation (r = 0.492) between [11C]DED and PIB retention values</td>
<td>Inverse correlation between MMSE score and [11C]PK-11195 BP values, but not [11C]PIB in left anterior cingulate cortex (r = 0.9), left precuneus (r = 0.8) and left hippocampus (r = 0.79)</td>
<td>Not evaluated</td>
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and [11C]PIB binding. In contrast to these previous reports, Edison et al. [60] found an increase in [11C]PK-11195 binding in 13 AD subjects. These subjects had an increase in amyloid load, measured by the [11C]PIB in these areas. A more recent study investigated the relationship between fibrillar Aβ and astrocytes in AD and MCI subjects [69] using the PET tracer [11C]-deuterium-L-deprenyl ([11C]DED). This tracer has a high affinity and specificity for monoamine oxidase B (MAO-B) located in astrocytes. [11C]PIB was used to measure Aβ deposition. The authors reported increased [11C]DED binding in the bilateral frontal and parietal cortex in MCI subjects compared to normal controls. The increase in [11C]DED binding was more pronounced in the [11C]PIB-positive MCI subjects. Santillo et al. [93] confirmed the increased [11C]DED binding in the frontal, parietal and temporal lobes in AD compared to controls and a significant correlation between binding potential (BP) and PIB retention value. These results suggest that there was an increased number of astrocytes reacting in AD as early as in the predementia state.

Neuroinflammation and Cognitive Decline in AD

Patients with AD usually have a progressive clinical course, although the rate of progression varies widely among subjects [94]. Approximately 10–30% of AD cases undergo rapid cognitive decline [94] which is associated with a greater loss of autonomy and a higher mortality rate than in those who have a slower progression of the disease [95–98]. Why some AD subjects decline more rapidly than others is not clearly understood. Several factors have been suggested to contribute to disease progression [94, 99]: demographic factors (level of education, age), clinical features (extrapyramidal signs, age of onset of the disease, nutritional status) [100–103], presence of psychotic symptoms [104], cholinesterase inhibitor treatment [105, 106], genetic factors [apolipoprotein E (APOE) ε4 allele] [97, 107, 108], cerebrovascular disease and vascular risk factors [109–125]. Even though several demographic, clinical or genetic factors have been proposed for explaining the rate of decline in AD, they do not fully explain the variability observed in the different rates of clinical progression. Recently, it has been shown that peripheral markers of systemic inflammation are associated with an increase in cognitive decline [126]. Microglial activation could be related to disease activity and progression. Therefore, molecular imaging of microglia activation may prove to be an important biomarker of cognitive decline in AD.
Only a few studies have assessed the relationship between microglial activation measured with $[^{11}C]$PK-11195 uptake and cognition in AD or MCI subjects [60, 90, 92] (Table 1). In AD patients, $[^{11}C]$PK-11195 signals were inversely correlated with cognitive function as measured with Mini-Mental State Examination (MMSE) scores, but there was no direct relationship between $[^{11}C]$PK-11195 binding and amyloid load measured with $[^{11}C]$PIB [60]. In the AD group, Yokokura et al. [90] found a significant negative correlation between MMSE scores and $[^{11}C]$PK-11195 BP in the left anterior cingulate cortex, left precuneus, left hippocampus and left middle frontal cortex, but not between MMSE scores and the uptake of $[^{11}C]$PIB in the limbic, precuneus and prefrontal areas [90].

Considering the new diagnostic criteria of AD, it seems important to study the profile of neuroinflammation in MCI (prodromal AD). MCI subjects are more likely to have higher levels of systemic inflammation (cross-sectional relationship) [127], although we do not know the impact of systemic inflammation on the progression from MCI to AD. In MCI subjects, only a few molecular imaging studies have reported an increase in binding compared to controls [67, 69, 92] (Table 1). Yasuno et al. [67] evaluated PBR binding in MCI subjects using PET $[^{11}C]$DAA 1106, another PBR ligand. $[^{11}C]$DAA 1106 binding was increased in 6 of 7 MCI subjects compared to controls. There was no significant difference in BP between MCI and AD. Five of the 7 MCI subjects who were followed for 5 years eventually developed AD and 1 was diagnosed with Lewy body disease [67]. Schuitemaker et al. [128] studied $[^{11}C]$PK-11195 binding in 10 MCI subjects and compared them with 19 AD patients and 21 healthy controls. They reported increased binding in the occipital lobe in AD compared to controls, but no difference in PK-11195 binding between clinically stable MCI subjects and those who progressed to dementia. There was no correlation between BP and cognitive function. These results suggest that increased TSPO binding in MCI subjects may indicate that microglial activation precedes the onset of dementia. Moreover, the results of molecular imaging studies combining molecular imaging of the amyloid load and microglial activation, especially in prodromal AD, suggest that a regional increase in TSPO binding in prodromal AD reflects early changes associated with Aβ deposition which occurs before structural changes and cognitive impairment become apparent. We should expect increased TSPO binding in prodromal AD which progresses to dementia, and perhaps earlier in preclinical AD. Subjective memory impairment, also called subjective cognitive impairment, is generally considered to be a pre-MCI condition during the clinical course of AD [129, 130], if the patient’s complaint of cognitive impairment can be considered a predictor of cognitive decline [131]. Future studies designed to quantify neuroinflammation in patients who have subjective memory complaints are needed to detect preclinical microglial activation, which could be the first indication of the neurodegenerative process.

Discussion

Microglial activation can be visualized using molecular PET imaging with specific radioligands. The data obtained following the use of molecular imaging for studying neuroinflammation in vivo in AD subjects support the postulate that microglial activation and astrocytosis are potentially early phenomena. However, individual levels of amyloid deposition and microglial activation are not clearly correlated.

Limitations

All the studies reported (summarized in Table 1) are very promising but had limitations. Firstly, the size of the study group was small in all the studies which selected AD patients using McKhann et al.’s [132] criteria or Petersen et al.’s [133] criteria for MCI without details...
about the MCI subtype. Furthermore, in many of the molecular imaging studies which assessed the relationship between neuroinflammation and amyloid load, the patient’s concomitant drug therapy (cholinesterase inhibitors, NSAIDs) was not systematically reported or considered as an exclusion criterion. However, symptomatic drug therapy may influence the binding affinity. In fact, preclinical studies reported that cholinesterase inhibitors may have an influence on neuroinflammation [134] and consequently on TSPO molecular imaging. Moreover, additional genetic or age-related factors may contribute to Aβ formation and microglial activation in AD. Vascular disease may be one of them. Only 3 of the studies excluded subjects with significant cerebrovascular disease in spite of the fact that there is probably a relationship between microglial activation and vascular microangiopathy in AD. In fact, combined neuropathological and postmortem MRI studies have shown that AD subjects with white matter hyperintensities had much greater microglial activation than controls [135, 136]. These findings indicate that there is a potential link between microglial activation and vascular microangiopathy during the course of AD. Consequently, neuroinflammation could be related to vascular dysfunction, especially in elderly AD subjects.

Another limitation may be the sensitivity of the radioligands used for PET imaging which may differ according to each of their characteristics [137]. The majority of the research on neuroinflammation PET radiotracers targets PBR ligands, and [11C]PK-11195 was the TSPO-binding radioligand most frequently studied. However, new fluorine-labeling radioligands, like [18F]DPA-714, are promising because they can be used in clinical practice. Newer non-TSPO ligands that target other aspects of microglia are emerging. For example, [11C]DED binds to the monoaminooxidase B expressed in reactive astrocytes. Many efforts are currently being made to develop new PET tracers for specific receptors such as P2X, which is involved in the activation of microglial cells by the ATP provided from neuronal death, cannabinoid receptor subtype 2 (CB2) [138], which is overexpressed by activated microglial cells, and specific enzymes such as Cox-2 and MMP-9 that are upregulated. In addition, different binding affinity patterns for TSPO have been identified in humans according to the genetic status of the subjects, which could have an influence on the interpretation of the findings obtained from imaging [70, 139].

Brain imaging of microglial activation could help elucidate the biological variables involved and especially the relationship of microglial activation with the hallmarks of the disease and the temporal sequence of events leading to cognitive decline. Until now, PET imaging of neuroinflammation has concentrated on its relationship with the hallmark of the disease, amyloid deposition, which has revealed that the cognitive status of AD subjects is inversely correlated with microglial activation but not with amyloid load [60, 90]. Even if activated microglia is known to be a component of Aβ plaques in the brains of patients with AD, the association of microglia with Aβ plaques is probably a function of the plaque type. Consequently, soluble Aβ oligomers or fibrillar Aβ (plaques) should be considered to have an affinity for microglial activation tracers. In fact, preclinical studies reported that soluble Aβ oligomer could be an even more deleterious substance than fibrillar Aβ [140] that can activate microglia and stimulate secretion of cytokines. Actually, the development of Aβ-PET imaging agents has allowed us to detect fibrillar Aβ deposition in vivo [77]. But we still have to resolve the question of the relationship between microglial activation and tau pathology. Brain molecular imaging of tau pathology is limited. [18F]FDDNP [2-(1-[89]ethylidene)malononitrile] is the only molecular PET probe that provides a measure of both amyloid and tau [89, 141–143]. Additional tau PET ligands are currently under development in animal and human brain studies, for example [18F]T807 [144] or THK523i [145], and may provide a new perspective on the relationship between neuroinflammation and AD pathology which is not restricted to Aβ.
The role of inflammatory processes may be different considering AD 'subtypes', according to different levels of Aβ and tau, in addition to the presence or absence of the APOE ε4 allele (fig. 2). With respect to the molecular imaging of neuroinflammation in AD, only 2 papers assessed the relationship between neuroinflammation and other biomarkers of the disease, especially \([^{18}F]FDG\) [69, 90]. Carter et al. [69] reported no significant relationship between the different PET tracers \([^{11}C]DED, \([^{11}C]PIB\) and \([^{18}F]FDG\). CSF values (Aβ42, tau and p-tau) were reported in some of the AD and MCI subjects included in the studies, but the authors did not study the correlation between these CSF values and \([^{11}C]PIB\) BP. Yokokura et al. [90] confirmed no significant correlations between \([^{11}C]PK-11195\) and \([^{18}F]FDG\) standardized uptake values in any region involved. The APOE gene product may be an important determinant of microglial activity in AD, as was reported in a postmortem study of AD brains with the APOE ε4 gene [146]. Since \([^{18}F]FDG\) imaging reported brain changes before the onset of clinical symptoms in carriers of the APOE ε4 allele [147–150], the combination of the cerebral metabolic rate with the pattern of neuroinflammation measured with PET molecular imaging and the genetic risk factors could be useful for detecting preclinical AD subjects and for assessing experimental therapies for prevention.

Finally, future developments in the molecular imaging of microglial activation may contribute to improving our understanding of the relationship between neuroinflammation, affective symptoms and the pathophysiology of AD. The depression syndromes in geriatric patients may be mediated by inflammatory processes [151]. Indeed, epidemiological studies have demonstrated an association between elevated peripheral cytokines and depressive symptoms in elderly subjects [151–154]. Some recent preclinical studies have reported the results of antidepressant treatment on amyloid load using mouse models of AD [155, 156].

**Fig. 2.** Inflammation in AD. Amyloid accumulation forms aggregates that activate microglia. This phenomenon induces the production of reactive oxygen species (ROS), nitric oxide (NO) and the expression of cytokines.
These findings also support the idea that there is a close relationship between AD and depression which involves neuroinflammation. Moreover, recent papers have reported that systemic inflammation (with increased serum TNFα and IL-6) was associated with an increase in cognitive decline [126] and with an exacerbation of neuropsychiatric symptoms [157]. Finally, brain imaging of microglial activation could add to our understanding of the physiopathology of atypical AD syndromes [7], like Benson’s syndrome or logopenic aphasia.

Aging

The profile of microglial activation in the aging brain remains undetermined and could explain the variable results found in MCI subjects. Aging is associated with glial activation and increased production of inflammatory mediators [158]. Murine models supported the view that aging is accompanied by impairment in microglial functionality [159]. Microglial senescence and degeneration could compromise the neuroprotective function of microglial cells [160]. However, little is known about the role of microglia in healthy elderly subjects. Some studies have reported that increased levels of inflammatory markers were associated with impairment in cognitive functions, especially levels of IL-6 and -8 [161, 162]. Only a few molecular imaging studies have addressed microglial activation in healthy elderly controls. Cagnin et al. [58] studied adults aged 32–80 years and reported that \(^{11} \text{C}\)PK-11195 binding did not significantly change with age except in the thalamus. Kumar et al. [163] studied the age-related changes in the distribution and expression of TSPO with \(^{11} \text{C}\)PK-11195 in 15 healthy adults (mean age 29 ± 8.5 years) and 10 children (mean age 8.8 ± 5.2 years). The overall pattern of \(^{11} \text{C}\)PK-11195 distribution in the brain was the same in the adults and in the children, although the intensity of uptake was increased in specific regions such as the midbrain and thalamus, contrasting with a lower uptake in the frontal, parietal and temporal cortex [163]. More recently, Schuitemaker et al. [128] reported an increase in the specific binding of \(^{11} \text{C}\)PK-11195 with aging in healthy subjects (19–79 years old) in several cortical and subcortical areas (frontal cortex, anterior and posterior cingulate cortex, medial inferior temporal lobe, insula, hippocampus, entorhinal cortex, thalamus, parietal and occipital cortex and cerebellum) [128]. Gulyas et al. [164] used PET with \(^{11} \text{C}\)vinpocetine, another TSPO radioligand, to study microglial activation during normal brain aging (healthy volunteers aged between 25 and 78 years) and reported increased uptake and binding with age in the entire brain and in all the brain regions studied. The significance of the age-related increase in \(^{11} \text{C}\)PK-11195-specific binding remains undetermined. First of all, we wonder how important it is to consider the degree of neuronal brain damage. In fact, the previously reported studies were cross-sectional and did not quantify the amyloid lesions or the cognitive functions. Even if the neuropsychological performances of the subjects included were normal, we cannot totally eliminate the existence of preclinical AD in some of them. The second consideration concerns the quantitative interpretation of the signal binding pattern of subjects with respect to the TSPO radiotracer. In fact, different patients have different affinity patterns for TSPO ligands. Caution should be used in interpreting this fact because differences in PET signals according to the subjects studied are not necessarily related to differences in target density and may reflect individual variability in binding affinity related to an underlying genetic mechanism [70–72]. Binding affinity and selectivity for TSPO may vary according to the radioligands.

Conclusions

In AD, activated microglia may prove to be an important therapeutic target, since microglial activation may be involved in neurodegenerative processes as early as in the prodromal stage. PET imaging with TSPO radioligands provides an interesting tool that
should improve our understanding of the progression and the severity of neuroinflammation and perhaps provide us with an indicator of the prognosis of the disease. Longitudinal studies are needed to evaluate the role of neuroinflammation in prodromal AD in order to determine whether microglial activation, when detected early in AD, is beneficial rather than detrimental.

The association of microglial activation with disease progression could help us to determine the prognosis of AD subjects and, subsequently, to target specific subgroups of AD patients with a higher risk of rapid cognitive decline. It could also potentially improve the assessment of the probability of disease progression and specific treatment strategies could be adapted, as more invasive and potentially harmful disease-modifying treatments for AD become available [165].

Consequently, molecular imaging of brain TSPO, as well as amyloid imaging, may help identify new therapeutic targets and monitor the results of future clinical trials aimed at preventive and therapeutic treatments. Molecular imaging of microglial activation could help us document the central inflammatory status of study subjects and assist us in designing future research studies, particularly with respect to determining which subjects to enroll into clinical trials, and could help us evaluate the benefit of specific therapies in selected groups, for example, by monitoring the effects of Aβ immunization [166]. Results from clinical trials suggest that neuroinflammation plays an additional role in the different stages of the disease.

Microglial activation in vivo is a promising avenue of research in AD. It can be detected before the onset of dementia and could be predictive of the disease processes at an early stage. PET imaging of activated microglia could help elucidate the role of activated microglia in the pathogenesis of AD and supply us with prognostic information in order to stratify MCI subgroups at increased risk for developing AD and, in addition, help establish treatment strategies and monitor therapeutic efficacy.

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