CHAPTER EIGHT

Imaging Translocator Protein as a Biomarker of Neuroinflammation in Dementia

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Contents

Abstract

Neuroinflammation has long been considered a potential contributor to neurodegenerative disorders that result in dementia. Accumulation of abnormal protein aggregates in Alzheimer's disease, frontotemporal dementia, and dementia with Lewy bodies is associated with the activation of microglia and astrocytes into proinflammatory states, and chronic low-level activation of glial cells likely contributes to the pathological changes observed in these and other neurodegenerative diseases. The 18 kDa translocator protein (TSPO) is a key biomarker for measuring inflammation in the brain via positron emission tomography (PET). Increased TSPO density has been observed in brain tissue from patients with neurodegenerative diseases and colocalizes to activated microglia and reactive astrocytes. Several radioligands have been developed to measure TSPO density in vivo with PET, and these have been used in clinical studies of different dementia syndromes. However, TSPO radioligands have limitations, including low specific-to-nonspecific signal and differential affinity to a polymorphism on the *TSPO* gene, which must be taken into consideration in designing and interpreting human

Advances in Pharmacology, Volume 82
ISSN 1054-3589
https://doi.org/10.1016/bs.apha.2017.08.004

PET studies. Nonetheless, most PET studies have shown that increased TSPO binding is associated with various dementias, suggesting that TSPO has potential as a biomarker to further explore the role of neuroinflammation in dementia pathogenesis and may prove useful in monitoring disease progression.

ABBREVIATIONS

AD	Alzheimer's disease
CBD	corticobasal degeneration
DLB	dementia with Lewy bodies
FDG	fluorodeoxyglucose
FTD	frontotemporal dementia
MCI	mild cognitive impairment
PDD	Parkinson's disease dementia
PET	positron emission tomography
PSP	progressive supranuclear palsy
SNP	single nucleotide polymorphism
TSPO	translocator protein 18 kDa

1. INTRODUCTION

A number of studies have implicated neuroinflammation, loosely defined here as the activation of microglia and astrocytes into proinflammatory states, as a pathological contributor to various neurodegenerative causes of dementia. Clinically, dementia is defined as a decline in cognitive function severe enough to interfere with daily function. While some forms of dementia—for instance, that caused by cerebrovascular disease—are an exception, most neurodegenerative dementias are associated with a specific proteinopathy: the abnormal accumulation of a misfolded protein. One example is the neurofibrillary tangles associated with Alzheimer's disease (AD), which are composed of the microtubule-associated protein tau in a hyperphosphorylated state, aggregated into paired helical filaments. In vitro studies and animal models suggest that proteinopathies stimulate neuroimmune responses (Gao et al., 2011; Maezawa, Zimm, Wulff, & Jin, 2011; Serrano-Pozo et al., 2011). As a result, several clinical and nonclinical studies have been performed in an attempt to elucidate the relationship between neuroinflammation and neurodegenerative dementias.

Astrocytes and microglia are the primary building blocks of the immune system in brain, but even peripheral immune cells—including monocytes

and leukocytes—can enter brain and play important roles (Kanegawa et al., 2016). Glial cells have both pro- and antiinflammatory functions and are key to a number of processes under basal as well as disease conditions; these include cellular repair, free radical reduction, phagocytosis, and steroid release. Proinflammatory functions—for instance, cytokine or reactive oxygen species release—can damage healthy neurons, leading to synaptic dysfunction, synapse loss, and neuronal death. In neuroimmune cells, any imbalance between proinflammatory and reparatory functions can thus result in CNS injury. Although the damaging effects of such potential imbalances are recognized in classic neuroimmunological diseases (e.g., multiple sclerosis), growing evidence nevertheless suggests that chronic low-level activation of glial cells may also contribute to the pathological changes observed in many neurodegenerative diseases. Given the importance of this area of study, one goal of considerable interest is quantifying the current inflammatory state in a living human brain, which would allow investigators to measure disease severity, study pathophysiological mechanisms, and identify novel targets for treatment.

It should be noted here that while frontotemporal dementia (FTD), dementia with Lewy bodies (DLB), and Parkinson's disease dementia (PDD) are all proteinopathies, AD is by far the most studied because it is the most common. Indeed, only a handful of studies have used translocator protein 18 kDa (TSPO) PET imaging (discussed later) to study non-AD dementias. Furthermore, because it is a dual proteinopathy in which both amyloid plaques and tau tangles play a key role, the relationship between AD and inflammation may be more complex, due to different temporal order and topographic location of amyloid and tau pathologies. Thus, this chapter will largely focus on the extant state of research into neuroinflammation in AD.

2. TRANSLOCATOR PROTEIN 18 kDa

A key biomarker for measuring inflammation in the brain via positron emission tomography (PET) has been TSPO (Chauveau, Boutin, Van Camp, Dolle, & Tavitian, 2008). TSPO is a transmembrane protein found mainly in the outer mitochondrial membrane. Previously known as the peripheral benzodiazepine receptor (PBR) because it binds diazepam, TSPO was first discovered as a high-affinity receptor for Ro-4864 in lung, liver, and kidney (Braestrup & Squires, 1977). Studies have shown that TSPO binds to cholesterol and porphyrins and likely plays a role in transporting

substrates across membranes (Papadopoulos et al., 2015; Papadopoulos & Miller, 2012). However, recent findings in viable mice genetically depleted of TSPO have called into question its role in some of these functions (Banati et al., 2014; Morohaku et al., 2014; Selvaraj, Stocco, & Tu, 2015; Tu et al., 2014; Tu, Zhao, Stocco, & Selvaraj, 2015). In the periphery, low TSPO levels have been found in immune-competent cells, leukocytes, and macrophages, but TSPO is also found in microglia and astrocytes (Casellas, Galiegue, & Basile, 2002). Glial cells are known to become activated in response to cellular injury, and this morphological and functional change results in increased TSPO expression (Kuhlmann & Guilarte, 2000).

With regard to the scope of this chapter, increased TSPO density has been observed in a number of neurological disorders (see Jacobs, Tavitian, & INMind Consortium, 2012 for a review), including classic neuroimmunological disorders such as multiple sclerosis and HIV encephalopathy (Cosenza-Nashat et al., 2009). Notably, increased TSPO density has also been observed in brain tissue from patients with neurodegenerative diseases and is expected to be increased in proteinopathic dementias (that is, dementias associated with abnormal protein aggregates such as amyloid plaques).

3. RADIOLIGANDS FOR TSPO

Since the racemic version of ^{11}C-PK-11195 was used to image TSPO more than 30 years ago (Le Fur et al., 1983), new radioligands have been developed that have a much greater amount of specific binding in human brain. Nevertheless, a key limitation shared by all ligands, including PK-11195, is that a common single nucleotide polymorphism (SNP) affects the affinity of their binding to TSPO (Kreisl et al., 2010; Owen et al., 2011). This characteristic was first noted with ^{11}C-PBR28, where a 40-fold difference of affinity exists between high- and low-affinity binders (Kreisl et al., 2010). The responsible SNP (rs6971) is codominantly expressed, thereby generating three genotypes: high, mixed, and low-affinity binders (Owen et al., 2011, 2010). This SNP is variably present in different ethnic groups; for instance, it is almost nonexistent among the Japanese but present in roughly 5%–10% of subjects of European and African American descent. This SNP has such a dramatic effect on the binding of ^{11}C-PBR28 that homozygous low-affinity binders provide no quantifiable brain uptake and are typically excluded prior to any scan, based on a genotype test. Excluding low-affinity binders is important to avoid unnecessary exposure

to radiation and can be logistically difficult for subjects who live far from the imaging center.

Although still sensitive to genotype, several of the second-generation TSPO radioligands, including ^{11}C-PBR28 and ^{11}C-DPA713, appeared to have greater specific binding than ^{11}C-(*R*)-PK-11195. For example, receptor/transporter blocking studies in monkeys showed that ^{11}C-PBR28 has about 10-fold higher specific binding than ^{11}C-(*R*)-PK-11195, but comparable blocking was only recently reported in humans (Ikawa et al., 2017; Kobayashi et al., 2017; Owen et al., 2014). The blocking drug used in humans was XBD173, which was reported to reduce anxiety symptoms in rodents and to blunt panic attacks in humans (Rupprecht et al., 2009). XBD173 was developed as an anxiolytic specifically because it (and perhaps all TSPO ligands) stimulates production of steroids and neurosteroids, the latter of which may have antianxiety properties. Because XBD173 had been used in humans and found to be relatively well tolerated, research PET Centers at Imanova (London) and at the NIMH (Bethesda) were able to use it to measure the amount of specific binding of four ^{11}C-labeled radioligands for TSPO (Table 1). Baseline and blocking studies were performed in the same healthy subjects. The outcome measure was "binding potential" (BP_{ND}), the ratio at equilibrium of specific to nondisplaceable uptake, which may be roughly viewed as an overall measure of "signal to noise" in PET studies. Indeed, ^{11}C-(*R*)-PK-11195 had a woefully inadequate BP_{ND} value (0.8), followed by ^{11}C-PBR28 (1.2), ^{11}C-ER176 (4.2), and ^{11}C-DPA-713 (7.3). Based on these overall values of "signal to noise," ^{11}C-DPA-713 appeared

Table 1 Comparison of Four ^{11}C-Radiolabeled Ligands for TSPO in Healthy Subjects, Before and After Blockade by XBD173

Ligand	Genotype Sensitivity	BP_{ND} in High-Affinity Binders[a]	Radiometabolite Contamination in Low-Affinity Binders[b]
^{11}C-(*R*)-PK11195	+	0.8	+++
^{11}C-PBR28	+++	1.2	Not reliably measured
^{11}C-DPA-713	++	7.3	+++
^{11}C-ER176	+	4.2	None

[a] BP_{ND} is a unitless measure of the ratio at equilibrium of the concentration of specific to nondisplaceable uptake, which may be roughly interpreted as the overall "signal-to-noise" ratio in PET imaging.
[b] The presence of radiometabolites in brain was indirectly measured as a time-dependent increase of apparent receptor density (i.e., distribution volume (V_T)) during the course of scans in low-affinity binders.

to the best of these four radioligands for measuring TSPO. However, studying these radioligands in homozygous low-affinity binders showed that ^{11}C-ER176 had an unexpected advantage (Table 1); specifically, among the four ^{11}C-radioligands, only ^{11}C-ER176 did not generate radiometabolites that accumulate in brain and confound quantitation of the target. Such accumulation might differ between individuals or between groups and is a common reason for rejecting a radioligand for inadequate quantitation. Although these studies need to be replicated, they suggest that ^{11}C-ER176 may currently be the best radioligand for imaging TSPO. It should be noted here that ^{11}C-ER176 is sensitive to genotype, and the imaging results must thus still be corrected for genotype post hoc, but low-affinity binders need not be excluded a priori.

^{18}F-GE180, a recently developed TSPO radioligand, was reported to have increased binding in amyloid-producing transgenic mice (Brendel et al., 2016; Liu et al., 2015). However, quantitative studies in healthy human subjects have shown that it has such low brain uptake that it is unlikely to be useful in clinical populations. More specifically, ^{18}F-GE180 has very low rate of brain entry, with K_1 estimated to be 0.01 mL cm^{-3} min^{-1} and corresponding first pass extraction of approximately 1% (Feeney et al., 2016). Such a low rate of brain entry may be caused by the radioligand's being a substrate for efflux transporters, such as P-glycoprotein, at the blood–brain barrier. In our opinion, the cause of this low brain uptake should be examined before ^{18}F-GE180 is used in patient populations.

In addition to deficiencies specific to each radioligand, the methods used to quantify TSPO density can also introduce potential confounds in clinical PET studies. The "gold standard" method of quantification for reversible PET radioligands is kinetic modeling using the metabolite-corrected arterial input function. However, this method requires arterial catheterization, which may be a barrier to subject recruitment in populations suffering from dementia and that may introduce errors associated with the relatively high technical demands of precise measurement of parent radioligand concentration in plasma. To avoid these disadvantages, semiquantitative methods have been used in clinical TSPO PET studies. For ^{11}C-(*R*)-PK-11195, the most common method of analysis is a cluster-based approach. With this approach, voxels on the PET image are identified that behave as though there is no specific binding (i.e., reference tissue), and these voxels are used to correct for between-subject differences in radioligand delivery to voxels that have high amounts of specific binding (i.e., target tissue). However, TSPO is expressed throughout the brain, and the identified reference tissue will

therefore also contain radioligand that is bound to TSPO to some extent. Thus, this method likely introduces some degree of underestimation bias. Another analytic method that has been used with ^{11}C-(*R*)-PK-11195 and some second-generation radioligands is a tissue-ratio method, where radioligand binding in a target region expected to show disease-related pathology is divided by radioligand binding in a region expected to have little pathology. A previous study from our laboratory validated this approach against the "gold standard" of kinetic modeling, using the cerebellum as a "pseudo-reference" region to compare ^{11}C-PBR28 binding in patients with AD vs healthy controls (Lyoo et al., 2015). However, some studies have used this or similar tissue-ratio approaches with a different radioligand without first validating it against kinetic modeling (Bloomfield et al., 2016; Zurcher et al., 2015).

4. NEUROINFLAMMATION IN ALZHEIMER'S DISEASE

Neuroimmune response is a key pathological contributor to AD (Baik, Kang, Son, & Mook-Jung, 2016; Jin et al., 2015; Lee, McGeer, & McGeer, 2015; McGeer & McGeer, 2013; Raha et al., 2017). β-Amyloid activates microglia at physiologic concentrations (Maezawa et al., 2011), resulting in release of cytokines and activation of complement. The proinflammatory state initiated by activated microglia can cause neuronal loss (Neniskyte, Neher, & Brown, 2011). Inflammation can stimulate hyperphosphorylation of tau, resulting in the aggregation of neurofibrillary tangles (Ghosh et al., 2013). Extracellular tau can also activate microglia, potentially leading to a positive feedback loop of inflammation and tau pathology that propagates without involvement of β-amyloid (Serrano-Pozo et al., 2011). Brain tissue from AD patients revealed activated microglia and reactive astrocytes, which overexpress TSPO when proximal to β-amyloid plaques (Cosenza-Nashat et al., 2009).

Most PET studies conducted to date in AD have shown positive results (Schain & Kreisl, 2017). For instance, studies found that ^{11}C-(*R*)-PK-11195 binding was increased in AD patients and correlated with clinical severity (Cagnin et al., 2001; Edison et al., 2008; Yokokura et al., 2011). Edison and colleagues found that ^{11}C-(*R*)-PK-11195 binding did not correlate with amyloid load (measured with ^{11}C-Pittsburgh compound B (PIB)), despite both ^{11}C-(*R*)-PK-11195 and PIB being elevated in AD patients (Edison et al., 2008). One study using ^{11}C-PBR28 found that binding was greater in amyloid-positive AD patients than in controls or than in amyloid-positive

mild cognitive impairment (MCI) patients, especially in temporo-parietal regions; no difference was noted in the cerebellum, which is typically spared of pathology in AD (Kreisl et al., 2013) (Fig. 1). That study further found that ^{11}C-PBR28 binding correlated with volume loss as well as with several cognitive indices, but not with amyloid load. Another study found that ^{11}C-DAA1106 binding was greater in AD patients than controls in several brain regions, including striatum and cerebellum but that binding did not correlate with symptom severity (Yasuno et al., 2008). Autoradiography studies demonstrated greater binding of ^{3}H-DAA1106 to TSPO in both a transgenic AD mouse model (Maeda et al., 2011) and in human tissue from AD brains (Gulyas et al., 2009). In addition, binding with ^{18}F-FEPPA was shown to be greater in AD patients than in controls in hippocampus and white matter regions such as internal capsule and cingulum bundle, as well as in prefrontal, temporal, parietal, and occipital cortices (Suridjan et al., 2015). Greater ^{18}F-FEPPA binding also correlated with worse performance on some cognitive measures (Suridjan et al., 2015). Finally, in studies using ^{11}C-(*R*)-PK-11195 and ^{11}C-PBR28, TSPO binding was found to increase with progression of AD (Fan, Okello, Brooks, & Edison, 2015; Kreisl et al., 2016). Building on this work, Kreisl and colleagues similarly found that patients who showed clinical progression at follow-up had a greater increase in ^{11}C-PBR28 binding than patients who remained clinically stable (Kreisl et al., 2016).

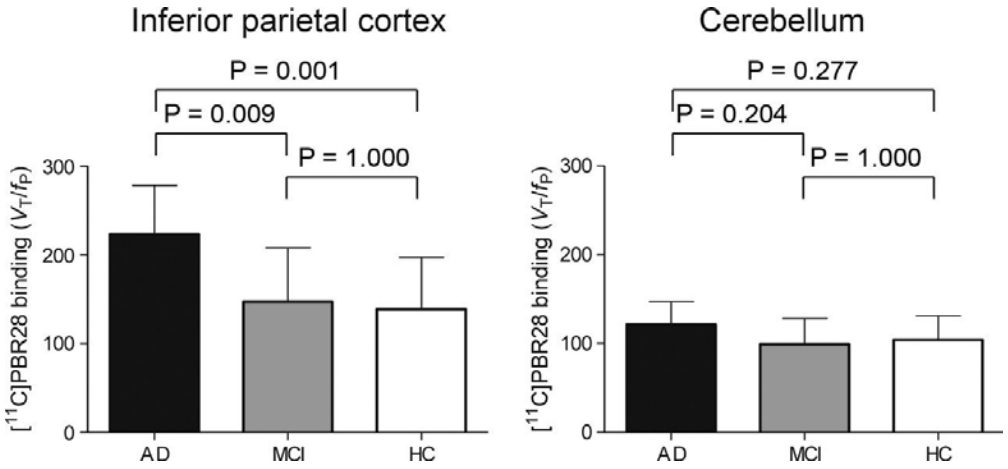

Fig. 1 ^{11}C-PBR28 binding is greater in patients with Alzheimer's disease (AD, $n = 19$) than in patients with mild cognitive impairment (MCI, $n = 10$) or age-matched healthy controls (HC, $n = 13$). Bar graphs show values for ^{11}C-PBR28 binding (V_T/f_P) in inferior parietal lobule (*left*) and cerebellum (*right*), corrected for partial volume effect. ^{11}C-PBR28 binding values were adjusted for *TSPO* genotype, age, and education. Error bars denote SD.

Nevertheless, some negative studies found no difference in TSPO binding between AD patients and controls (Groom, Junck, Foster, Frey, & Kuhl, 1995; Schuitemaker et al., 2013; Varrone et al., 2013; Wiley et al., 2009). Methodological differences may explain some of these mixed results. For instance, one of the negative studies had a control group that included seven patients with unilateral gliomas, and the unaffected hemisphere was used as comparison data (Groom et al., 1995). Also, none of the negative studies using ^{11}C-(*R*)-PK-11195 used absolute quantification of ^{11}C-(*R*)-PK-11195 binding; rather, they used a cluster-based method. In one negative study using ^{18}F-FEDAA1106, *TSPO* genotype was not determined (Varrone et al., 2013). Therefore, it is likely that variance in the PET data caused by the rs6971 SNP contributed to the negative results.

Whether changes in TSPO occur in the preclinical and prodromal stages of AD is less clear. The earliest studies comparing TSPO binding between prodromal AD patients (defined as having MCI) and controls found no difference between the groups (Kreisl et al., 2013; Lyoo et al., 2015; Schuitemaker et al., 2013; Wiley et al., 2009). In contrast, Yasuno and colleagues showed that three MCI patients with ^{11}C-DAA1106 binding >1.5 SD above the mean binding of controls had converted to dementia at follow-up; however, of the seven MCI patients imaged at baseline, two were lost to follow-up and one had ^{11}C-DAA1106 binding similar to that seen in controls but still converted to dementia at follow-up (Yasuno et al., 2012). In another study measuring ^{11}C-(*R*)-PK-11195 binding, Okello and colleagues found that only 5 of 13 MCI patients had elevated ^{11}C-(*R*)-PK-11195 binding and that this was not related to amyloid binding (Okello et al., 2009). Hamelin and colleagues found that amyloid-positive MCI patients had greater ^{18}F-DPA-714 binding than controls (Hamelin et al., 2016). However, in contrast to the findings of Kreisl et al. (2013) and Lyoo et al. (2015)—where AD patients had greater TSPO binding than MCI patients—and those of Yasuno and colleagues, which found that MCI and AD patients both had increased binding compared to controls, Hamelin and colleagues found that binding was greater in MCI than in AD patients (Hamelin et al., 2016); that study further found that ^{18}F-DPA-714 binding was inversely correlated with cognitive measures, with more affected patients having lower TSPO binding. The study by Hamelin and colleagues was the first to present results demonstrating that amyloid-positive control subjects (preclinical AD) had greater TSPO binding than amyloid-negative controls. One interpretation of these data is that TSPO is elevated in the preclinical and prodromal phase of AD and then decreases as the disease

progresses. The authors concluded that there was an early and protective microglial activation in AD. This conclusion was predicated on the assumption that decreases in TSPO in the more severely affected patients reflected a loss of protective function of microglia—such as amyloid phagocytosis—and that the loss of this protective microglial function allows AD to progress. While the study by Hamelin and colleagues included an unusually large sample size for a clinical PET study ($n = 96$), it was a cross-sectional study, and it has not yet been determined if individual subjects demonstrate increased ^{18}F-DPA-714 binding over time (Hamelin et al., 2016). The study also used a simplified tissue-ratio method to quantify ^{18}F-DPA-714 binding that has not yet been validated against the "gold standard" for reversible radioligands without a true reference region (i.e., kinetic modeling using the metabolite-corrected arterial input function).

A recent study by Fan and colleagues reported decreased ^{11}C-(*R*)-PK-11195 binding in MCI patients after longitudinal follow-up (Fan, Brooks, Okello, & Edison, 2017). The authors concluded that there may be a biphasic pattern of microglial activation, with a peak occurring during MCI that later declines, followed by a second peak during the dementia stage of the disease. In such a scenario, the evidence suggests that the amount of microglial activation in the second peak would continue to rise as the dementia process worsens (Calsolaro & Edison, 2016). These results may resolve the apparent contradictions noted earlier, where TSPO binding was greater in MCI than AD dementia, as well as the evidence from earlier TSPO PET studies that found that TSPO binding increased with disease severity in patients with dementia. However, the paper by Fan and colleagues included only eight MCI patients, half of whom showed no evidence of amyloid plaque deposition on baseline PET imaging. Also, follow-up imaging was not performed on controls in either this or the earlier longitudinal study of ^{11}C-(*R*)-PK-11195 in AD (Fan et al., 2015), and the effects of natural aging or test–retest variability on longitudinal ^{11}C-(*R*)-PK-11195 binding may have influenced these results. Larger, serial-imaging studies with harmonized TSPO-imaging methodology are needed to elucidate the relationship between TSPO and progression along the AD spectrum.

5. NEUROINFLAMMATION IN NON-ALZHEIMER'S DEMENTIAS

5.1 PDD and DLB

Neuroinflammation is a proposed pathogenic contributor to diffuse Lewy body disease (DLBD), which includes the aforementioned PDD and

DLB. DLBD is defined by aggregated α-synuclein inclusions (i.e., Lewy bodies), which activate microglia in vitro and stimulate release of proinflammatory mediators (Zhang et al., 2005). Activated microglia are found proximal to Lewy bodies at autopsy (Mackenzie, 2000; Power & Blumbergs, 2009; Togo et al., 2001). The amount of activated microglia is proportional to both the number of Lewy bodies found at autopsy (Mackenzie, 2000) and, in transgenic mice, to the amount of α-synuclein and measured loss of dopaminergic neurons (Gao et al., 2011). Prior TSPO PET studies in patients with Parkinson's disease without dementia have mostly shown positive results (Bartels et al., 2010; Gerhard et al., 2006; Iannaccone et al., 2013; Ouchi et al., 2005), although there have been a small number of negative studies (Bartels et al., 2010; Ghadery et al., 2017). Two studies also found increased TSPO in PDD or DLB (Edison et al., 2013; Iannaccone et al., 2013). One of these studies found that ^{11}C-(*R*)-PK-11195 binding was greater in basal ganglia and substantia nigra for both PDD and DLB patients than in controls. In addition, the DLB patients had increased binding in the cortex and cerebellum (Iannaccone et al., 2013). Interestingly, all PDD and DLB patients were accrued within 1 year of onset of their clinical symptoms, which suggests that increased TSPO density is detectable in the early stages of disease. The second study performed ^{11}C-(*R*)-PK-11195 PET imaging in patients with Parkinson's disease with and without dementia and found that binding was greater than controls in frontal, temporal, and occipital cortices for both PDD patients and those with Parkinson's disease without dementia; furthermore, ^{11}C-(*R*)-PK-11195 binding was found to be inversely correlated with Mini Mental State Exam score among PDD patients (Edison et al., 2013). In addition, regional increases in ^{11}C-(*R*)-PK-11195 binding overlapped with regional glucose hypometabolism in patients for whom ^{18}F-FDG PET was also performed (Edison et al., 2013).

5.2 Frontotemporal Lobar Degeneration and Related Tauopathies

Frontotemporal lobar degeneration (FTLD) refers to a collection of diseases—including behavioral variant FTD, progressive nonfluent aphasia, and semantic dementia (Ferrari et al., 2014; The Lund and Manchester Groups, 1994)—that are pathologically distinct from AD and the α-synucleinopathies and that can cause synaptic dysfunction and neuronal loss in the frontal and temporal lobes. Both familial and sporadic forms of FTLD often—though not always—result from the aggregation of abnormal tau filaments (Caroppo et al., 2016; Ferrer et al., 2014; He et al., 2016;

Laws et al., 2008; Lee et al., 2013; Lindquist, Schwartz, Batbayli, Waldemar, & Nielsen, 2009). Other disorders considered under the larger umbrella of FTLD include the "Parkinson's plus" disorders such as primary progressive palsy and corticobasal ganglionic degeneration; these are characterized by shared nigrostriatal cell loss and are pathologically defined by tau aggregation (Ioannidis, Konstantinopoulou, Maiovis, & Karacostas, 2012).

One study found that ^{11}C-(*R*)-PK11195 binding was increased in patients with a clinical diagnosis of FTLD ($n=5$), but quantification was performed without either blood sampling or use of a valid reference region (Cagnin, Rossor, Sampson, Mackinnon, & Banati, 2004). Using full quantification with arterial blood sampling, we observed increased ^{11}C-PBR28 binding in patients with behavioral variant FTD (Fig. 2; unpublished data). A study of four patients with corticobasal degeneration (CBD) found increased ^{11}C-(*R*)-PK11195 binding in several cortical regions as well as striatum and brainstem (Gerhard et al., 2004). Another small study found that four patients with progressive supranuclear palsy (PSP) displayed increased ^{11}C-(*R*)-PK11195 binding in striatum, cerebellum, brainstem, thalamus, and the frontal lobe (Gerhard et al., 2006). Both of the latter two studies used the primary visual cortex and occipital white matter as a reference for nonspecific binding. While the authors suggested that these

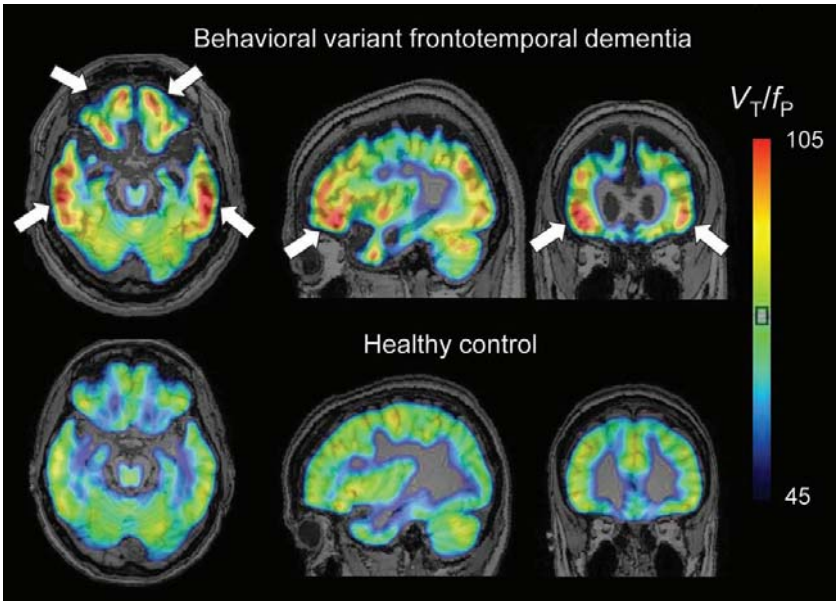

Fig. 2 Representative ^{11}C-PBR28 parametric images (V_T/f_P) for a patient with behavioral variant FTD and a healthy control. Both subjects were high-affinity binders (noncarriers of the rs6971 *TSPO* polymorphism). *Arrows* show increased binding in frontal and temporal cortices.

regions are unaffected in CBD and PSP—though not free from TSPO—the outcome measures may nevertheless be methodologically biased. However, another study found that the distribution of tau pathology commonly seen in CBD overlapped with the location of increased binding (Dickson et al., 2002).

6. ALTERNATIVE TARGETS FOR NEUROINFLAMMATION IN DEMENTIA

Given the limitations associated with TSPO PET imaging, alternative targets for measuring neuroinflammation in vivo have been explored. Monoamine oxidase (MAO)-A and MAO-B are isoenzymes that metabolize neurotransmitters and xenobiotic amines via oxidative deamination (Saura et al., 1994), and hydrolysis of substrates by MAO-B results in reactive oxygen species as a by-product (Corcia et al., 2012). Because MAO-B expression increases with age, the corresponding accumulation of reactive oxygen species may contribute to age-related neurodegeneration (Saura, Richards, & Mahy, 1994a, 1994b). In addition, reactive astrocytes overexpress MAO-B, and the density of this isoenzyme has therefore been used as a marker for neuroinflammatory response (Aquilonius, Jossan, Ekblom, Askmark, & Gillberg, 1992; Jossan, Gillberg, Gottfries, Karlsson, & Oreland, 1991; Nakamura et al., 1990; Oreland, 1980). In studies, the MAO-B radioligand ^{11}C-L-deprenyl showed greater binding in AD and MCI patients than in older controls (Carter et al., 2012; Choo, Carter, Scholl, & Nordberg, 2014; Hirvonen et al., 2009; Santillo et al., 2011), suggesting reactive astrocytosis early in AD pathogenesis.

Additional proposed targets for neuroinflammation include arachidonic acid metabolism (Esposito et al., 2008), cyclooxygenase (Shukuri et al., 2016), and the P2X purinoceptor 7, which is expressed by microglia and thought to play a regulatory role in the inflammatory pathway (Han et al., 2017). However, at present, radioligand development for these potential targets is in the early stages.

7. THE CLINICAL SIGNIFICANCE OF INCREASED TSPO IN DEMENTIA

Activated microglia have several functions, including aberrant synaptic pruning and release of reactive oxygen species, driving a more proinflammatory state that can lead to neurodegeneration. Conversely,

activated microglia may be involved in phagocytosis and release of trophic factors that promote an overall antiinflammatory state. Broad categories have been used to define two distinct microglial phenotypes: M1 and M2. In neurodegenerative dementias, the potential role of microglia becomes particularly confusing, as evidence exists for both protective and destructive actions. One current theory is that activated microglia play a more protective role early in the AD process that later fails, resulting in increased plaque and tangle deposition, and that later stages of disease are associated with activated microglia that have predominantly cytotoxic functions (Hickman, Allison, & El Khoury, 2008). Under this hypothesis, "switching" microglia from an M1 to an M2 state may be therapeutically useful in treating AD. However, the M1/M2 dichotomy may be overly simplistic, and the concept of polarization of microglial function remains controversial (Ransohoff, 2016).

While the proposed biphasic pattern of microglial activation in AD—an early protective phase followed by a later neurodegenerative phase—conforms to the concept of M1/M2 microglial phenotypes, limitations to this model exist. First, imaging and CSF studies have shown that neurodegenerative changes are frequently detected in the MCI stage of AD (Knopman et al., 2016). Elevations in CSF tau, fluorodeoxyglucose (FDG) hypometabolism, and hippocampal atrophy are all detectable prior to the onset of dementia, and FDG hypometabolism and hippocampal atrophy may accelerate as cerebral amyloid is increasing, prior to obvious clinical symptoms (Insel et al., 2017). These results argue against MCI being a "protected" stage of AD.

Second, amyloid-negative MCI should not be universally equated with early-stage AD, as considerable evidence suggests that in many of these subjects cognitive symptoms may be due to non-AD pathology. For instance, autopsy, CSF, and PET studies have shown significant cerebral amyloidosis in subjects without cognitive symptoms (Jansen et al., 2015; Serrano-Pozo et al., 2013; Toledo et al., 2015), and longitudinal studies suggest that, in most patients, amyloid plaque deposition occurs prior to functional changes in cognition (Bateman et al., 2012; Buchhave et al., 2012; Forster et al., 2012; Landau et al., 2012; Lo et al., 2011). In addition, MCI patients without evidence of amyloidopathy are less likely to have pathologically defined AD at autopsy (Vos et al., 2013), and they show less cognitive decline than amyloid-positive MCI patients (Landau, Horng, Fero, Jagust, & Alzheimer's Disease Neuroimaging Initiative, 2016). The emergence of symptoms that warrant a clinical diagnosis of MCI due to AD without evidence of amyloid

plaque pathology is not consistent with the prevailing model of the temporal order of biomarker abnormalities in AD (Jack et al., 2011).

Finally, this biphasic model of microglial activation does not take into account differences in the threshold of detection of different biomarkers (Jack et al., 2013). As noted earlier, all TSPO radioligands are associated with sensitivity limitations that confound accurate quantification. Therefore, it is possible that the amount of TSPO binding seen in prodromal and MCI patients is very close to the threshold of detection achievable with currently available radioligands. The apparent peak and decline of TSPO binding in patients with MCI may represent variation in the noise at a stage where TSPO density is relatively low and therefore difficult to accurately detect.

Most of the agreement in TSPO studies concerns patients in the dementia phase of AD, when the disease phenotype is most severe and TSPO binding is likely at its greatest. Determining the temporal relationship between TSPO binding and AD more precisely will require larger and better-powered studies. Moreover, the detection threshold for TSPO radioligands should be compared to that for other, better-characterized AD biomarkers to avoid erroneous associations between microglial activation, amyloid deposition, tau aggregation, and neurodegeneration. In addition, because human PET studies are associational in nature, the results cannot be used to conclude a cause and effect relationship between microglial function and AD progression. Because the role of microglia in the prevention or progression of AD presently remains unknown, we cannot yet determine whether increased TSPO binding reflects positive, negative, or neutral biological effects. Windows of opportunity may indeed exist for modulating microglial responses with novel therapeutics in order to attenuate the course of AD. However, in order to use TSPO PET to define those windows, we need to better understand the role that TSPO specifically, and microglia generally, plays in the AD process.

8. CONCLUSION

Considerable evidence supports the notion that neuroinflammation is closely related to the neurodegenerative changes seen in dementing disorders and that inflammatory responses can be detected with TSPO PET imaging in patients suffering from these disorders. Therefore, TSPO PET has potential as a biomarker to further study various dementias and may prove useful in monitoring disease progression. However, more information

on the role of microglial activation and the causal relationships, if any, between microglial function and dementia pathogenesis is needed if TSPO PET is to be used to monitor response to immunomodulatory treatments.

CONFLICT OF INTEREST STATEMENT

Funding for this work was supported in part by the Intramural Research Program at the National Institute of Mental Health, National Institutes of Health (IRP-NIMH-NIH). Dr. Kreisl has received research funding from AstraZeneca, Merck, and the NIA (K23AG052633). Dr. Innis and Ms. Henter have no conflicts of interest to disclose, financial or otherwise.

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FURTHER READING

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