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Imaging neuroinflammation in Alzheimer's disease and other dementias: Recent advances and future directions

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Abstract Alzheimer's disease (AD), dementia with Lewy bodies, frontotemporal dementia (FTD), and Huntington's disease (HD) are the main neurodegenerative causes of dementia. Causes and mechanisms of these diseases remain elusive. Neuroinflammation is increasingly emerging as an important pathological factor in their development. Positron emission tomography (PET) using [^{11}C]PK11195 represents a method of visualizing the microglial component of neuroinflammation via the translocator protein (TSPO) and we discuss the valuable insights this has yielded in neurodegenerative diseases. We discuss the limitations of this method and the development of second generation TSPO PET ligands which hope to overcome these limitations. We also discuss other methods of visualizing neuroinflammation and review the state of current dementia treatments targeted at neuroinflammation. It is our view that a multimodal investigation into neuroinflammation in AD, Parkinson's disease dementia, FTD and HD will yield valuable pathological insights which will usefully inform development of therapeutic targets and biomarkers.

Keywords: Alzheimer's disease; Dementia; Neurodegenerative disease; neuroinflammation; Microglial activation; PET; TSPO; MRI

1. Introduction

Neurodegenerative conditions account for significant morbidity and mortality and comprise a variety of pathologies associated with aberrant protein aggregation, including Alzheimer's disease (AD)—beta amyloid and tau; frontotemporal dementias (FTD)—tau, TDP-43, fused in sarcoma protein (FUS); Lewy body spectrum disorders which include Dementia with Lewy Bodies (DLB) and Parkinson's disease with (PDD) and without (PD) later dementia—alpha synuclein; and Huntington's disease (HD)—huntingtin. Chronic neuroinflammation and, in particular, microglial activation is associated with all these conditions [1–4]. There is an intense debate on whether neuroinflammation is a primary or secondary event in neurodegenerative diseases [5].

Microglia are of myeloid origin and comprise around 15% of the non-neuronal cells in the brain. Normally they are quiescent and their processes monitor the status of the brain milieu. Invading pathogens, trauma, infection, degenerative disease and stroke can all trigger activation of microglia with the production and release of reactive oxygen and nitrogen species, cytokines, and chemokines. This intrinsic "inflammation" in the central nervous system can have both beneficial and deleterious effects [6,7]. Microglia have different phenotypes which interchange dynamically and the cells act to strip and remodel synapses [8], remove cellular debris by phagocytosis [9] and provide central nervous system innate immunity by releasing cytokines [10]. A significant amount of current evidence points to a key role for microglia in neurodegeneration. In AD, activated microglia surround amyloid plaques and resting microglia are activated by amyloid oligomers, fibrils, and amyloid precursor protein (APP) [11,12]. Knockout mice lacking the APP gene show decreased microglial activation [13]. In PD, microglia are activated by alpha synuclein fibrils [14]. This initial activation is likely an attempt to clear the protein, however due to factors specific to the misfolded protein, the microglia are unable to accomplish this and become

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chronically activated [15]. Plaque associated microglia display dilated intracellular channels of smooth endoplasmic reticulum containing amyloid fibers [16,17], suggesting a role of microglia in clearing beta amyloid. Microglia may normally be responsible for amyloid phagocytosis in health [18]. In AD it has been argued that the microglia clustered around amyloid deposits have become dysfunctional and incapable of removing amyloid [19]. One can therefore see how a deleterious loop may be established, whereby accumulation of misfolded protein leads to microglial activation and secretion of neurotoxic factors which perpetuates neurodegeneration and further microglial activation [20]. Though the precise causal relationship remains to be fully delineated, the paradigm whereby protein misfolding is the primary trigger to microglial activation has been strengthened by a post-mortem study demonstrating reduced microglial activation in AD patients treated with an active vaccine to amyloid-β42 and subsequent decreased amyloid-plaque burden [21].

Another important issue to consider is heterogeneity of microglial subpopulations. Peripheral macrophages have been shown to have two distinct activated states. In the first state, described as classical activation (M1), macrophages respond to a micro-organism challenge with a robust proinflammatory cytokine response and enhanced microbial killing, which may also damage the host. The second state, alternative action (M2), is a more nuanced response to T-helper-2 cytokines and is associated with wound healing and tissue repair [22]. Microglia broadly follow this schema and the balance of neurotoxic M1 microglia and neuroprotective M2 microglia is increasingly thought to be central to AD pathogenesis, with evidence of M1 microglia localizing around amyloid plaques [23]. More recent evidence implicates the TREM2 gene, a gene involved in balancing M1 and M2 activation, with risk of AD [24]. Clearly further exploration of microglial behavior in vivo, assessing the behavior and heterogeneity of microglia, the temporal relationship between microglial activation and neurodegeneration and how exactly microglial activation correlates to clinical phenotype are critical issues to explore.

2. Imaging microglia using [^{11}C]PK11195

Given the potentially important role of activated microglia in neurodegeneration, imaging their function in vivo provides a tool which allows us to quantify and localize disease activity and potentially to evaluate novel therapeutic interventions. Positron emission tomography (PET) is the most widely used in vivo method for detecting microglial activation. When microglia are activated, there is an upregulation of mitochondrial translocator protein (TSPO) expression. TSPO is found throughout the body but at only low levels in healthy central nervous system (CNS). It is thought to have a role in cholesterol and amino acid transport, in CNS steroid production and mitochondrial membrane potential regulation, but the exact function of TSPO in the CNS is yet to be elucidated [25].

Experimental data in animal models of brain disorders show TSPO expression is associated primarily with activated microglia, but can also be seen in reactive astrocytes depending on the nature of the neuronal insult [26]. In rat models of focal ischemia, TSPO expression has been found to peak in microglia and then be followed by a rise in astrocyte activation, suggesting a temporal relationship between TSPO expression in microglia and reactive astrocytes [27]. Ex vivo human post-mortem study of patients suffering from stroke, multiple sclerosis (MS), AD, FTD and progressive supranuclear palsy (PSP) showed that there was a degree of observed TSPO-radioligand binding, with both first and second generation radioligands, to reactive astrocytes. There was no correlation, however, on quantification of this association whereas significant correlation was noted between TSPO-radioligand binding and activated microglia [28]. A recent contradictory study in rats, where specific astrocyte activation was achieved by lentiviral gene transfer in the absence of neurodegeneration or a generalized noxious stimulus, demonstrated specific and significant binding of first and second generation TSPO-radioligands to reactive astrocytes [29]. Other papers also support a role for reactive astrocytes in the TSPO-signal [30,31]. There is ongoing debate about the extent to which TSPO can distinguish between activated microglia and reactive astrocytes but it is clear there is a degree of overlap and therefore caution should be applied when interpreting TSPO-binding in humans and animals. This signal could be the result of reactive astrocytes and activated microglia, which play markedly different roles in neuroinflammation.

The PET marker most commonly used to target TSPO is [^{11}C]*(R)*PK11195 [1-(2-chlorophenyl)-N-methyl-N-(1-methylpropyl)-3-isoquinoline carboxamide], which was first reported to bind to the hearts of dogs and humans in 1986 [32]. [^{11}C]PK11195 has a half-life of 20.4 minutes due to its carbon-11 label and its extensive use in imaging human neurological diseases is explored below.

2.1. Dementia

AD represents 60% of dementia cases and its incidence is predicted to rise. Immunological studies have shown that activated microglia colocalize with amyloid plaques and hyperphosphorylated tau, both in post-mortem human AD studies [1,33] and in animals [34]. [^{11}C]PK11195 PET detects in vivo microglial activation in the brain of mouse models of AD [33] and in patients with AD [35,36]. In humans with AD, [^{11}C]PK11195 PET reveals microglial activation throughout the association cortex (Fig. 1) in a similar distribution to that of amyloid plaque deposition [36]. Increased cortical [^{11}C]PK11195 binding can be detected in around 60% of mild to moderate AD patients and around 40% of subjects with amnestic mild cognitive impairment (aMCI) [37]. Levels of cortical [^{11}C]PK11195

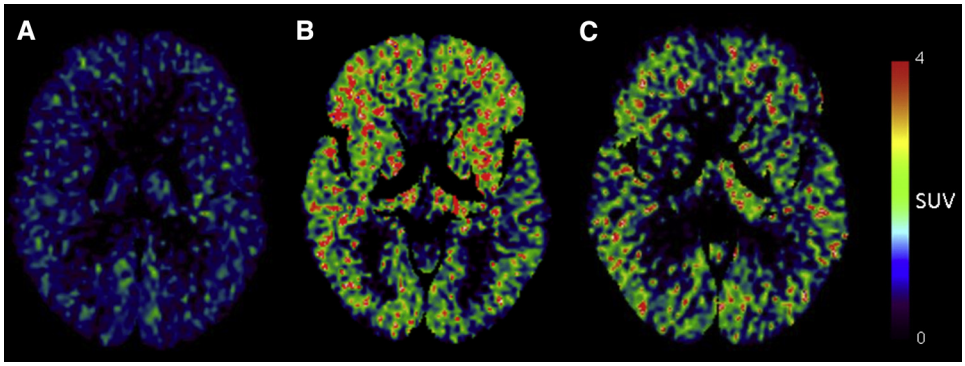

Fig. 1. [^{11}C]PK11195 BP in healthy control, AD and PDD subjects. Fig. 1 compares microglial activation in [^{11}C]PK11195 in healthy controls (A) to patients with AD (B) and patients with PDD (C), where significant activation is seen.

signal show an inverse correlation with Mini-Mental State Examination ratings [36], suggesting involvement of microglial activation in neuronal dysfunction and cognitive impairment. However, not all studies have detected increased [^{11}C]PK11195 binding in aMCI and mild to moderate AD [38], which may reflect different sensitivities of the cameras and analytical approaches used.

It has been suggested that microglia normally play a role in clearing amyloid oligomers and that failure of amyloid clearance by microglia leads to the accumulation of misfolded proteins [39]. Furthermore, in vivo studies in transgenic mouse models of AD have shown microglial aggregation around amyloid plaques in increasing numbers and the release of cytokines causing neuronal damage [40]. It has, therefore, been hypothesized that dysfunctional microglia could be a cause of amyloid plaque accumulation. Initially, microglia attempt to clear amyloid but as their phagocytic function becomes impaired by aging or chronic activation [41], their failure leads to progression [42]. However, proof of this in vivo remains lacking.

PET studies in FTD using [^{11}C]PK11195 have shown increased uptake in frontotemporal regions and basal ganglia [43], in keeping with neuropathological findings [3]. This is noteworthy as the pathology of FTD does not involve amyloid plaques and suggests that microglial activation is not specific to any pathology and may reflect part of a common neurodegenerative pathway, potentially open to amelioration.

DLB post mortem studies have shown associations between alpha-synuclein immune-positive neurons and activated microglia [2]. A recent [^{11}C]PK11195 PET study found significant binding in the substantia nigra and putamen with additional microglial activation in association cortices compared with nondemented subjects with PD [44], consistent with the pathological studies demonstrating early involvement of association cortex in DLB [45].

2.2. Parkinson's disease

PD is the second most common neurodegenerative disorder in the elderly and is characterized by degeneration of dopaminergic neurons in the substantia nigra pars compacta with resultant striatal dopaminergic deficiency. Activated microglia have been reported in the nigra and putamen in post-mortem human PD patients [1] and in animal models [46]. It has therefore been proposed that neurodegeneration may be directly caused by microglial activation and release of proinflammatory cytokines. It has also been shown that alpha-synuclein can induce microglial activation and cytokine release [47]. It is therefore necessary to study further in vivo the relationship between microglial activation and neurodegeneration in PD.

PET studies using [^{11}C]PK11195 have demonstrated increased microglial activation in midbrain and putamen (Fig. 1) [44,48,49], and more diffusely in the basal ganglia, pons and cortical regions [50]. In one of these studies [48], raised nigral [^{11}C]PK11195 signal correlated with loss of putamen dopamine transporter binding measured with [^{11}C]CFT PET and locomotor disability, supporting a direct role for microglia in causing dopaminergic projection loss. This finding, however, has not been reproduced by others. Braak staging of PD postulates that neuropathological changes occur initially in the medulla and pons, then the substantia nigra and midbrain, and then ascend to basal ganglia, cingulate and associated cortex [51]. It is therefore possible that the distribution of microglial activation in individual PD cases reflects the Braak, rather than the clinical, stage of the disease.

[^{11}C]PK11195 PET studies correlating patterns of microglial activation with known human neuropathology have been performed using in the 'Parkinson's plus' syndromes multiple system atrophy (MSA) [52], PSP [53] and corticobasal degeneration (CBD) [54]. In MSA, a randomized, controlled trial attempting to downregulate microglial activation using minocycline has been undertaken, but failed to demonstrate clinical improvement in motor scores after 48 weeks of therapy, although a decrease of microglial activation was seen in individuals on active therapy [55]. Other agents that suppress microglial activation are currently being tested.

2.3. Huntington's disease

HD is an autosomally inherited, fully penetrant neurodegenerative disease characterized by a CAG trinucleotide

repeat expansion in the huntingtin gene on the short arm of chromosome four [56]. The result is a lengthened polyglutamine chain on huntingtin protein, leading to its abnormal neuronal aggregation. The pathology targets striatum [57], however, recent advances in imaging have shown widespread changes involving cortical areas [56]. Post-mortem human studies demonstrate high levels of microglial activation adjacent to areas of neurodegeneration in HD [4]. [^{11}C]PK11195 PET has demonstrated striatal, thalamic, and association cortex microglial activation. A majority of adult pre-symptomatic HD patients also show increased microglial activation targeting the hypothalamus and striatum [58,59]. A multimodal imaging study utilizing [^{11}C]PK11195 PET found a progressive increase in microglial activation when comparing normal brains to HD mutation carriers and then to symptomatic HD patients [60]. This study also demonstrated an association between levels of microglial activation and severity of motor dysfunction, loss of independent function and, most strikingly, the predicted probability of 5-year clinical HD onset. This, therefore, suggests microglial activation is closely linked to disease progression from pre-clinical to clinical HD. Due to the detectable monogenic mutation and full penetrance of this condition, HD represents a good model for testing interventions targeting microglial activation for efficacy as neuroprotective agents.

3. Limitations of [^{11}C]PK11195 PET

Initial pharmacokinetic evaluation of PK11195 in animals demonstrated that it "washes out" quickly from the brain [61]. While in vitro studies have shown that PK11195 binds to TSPO with 3 nM affinity and that increased [^{11}C]PK11195 binding is associated with labeled activated microglia [28], in vivo, clinical studies show a low specific signal-to-background ratio due to its lipophilicity. While the most robust method to quantify radioligand binding is to use a metabolite-corrected arterial plasma input curve with compartmental modeling, this is difficult with [^{11}C]PK11195 as it binds to plastic tubing [62]. To avoid the need for an arterial input function, a simplified reference tissue model (SRTM) was developed [63]. The SRTM relies on using an appropriate tissue reference for nonspecific binding, but in neurodegenerative disease no brain region represents such a reference. Given this, cluster analysis was used to define grey matter voxels in patients' brains, that had the kinetic uptake and washout properties of normal cortex, to generate a reference input function. This method was improved by segmenting dynamic images into groups according to radiotracer activity and then defining the input cluster, which is also known as supervised cluster analysis. This improved the agreement between plasma input and reference tissue models [64]. Recently, image derived corrections to blood volume fraction have been demonstrated to further improve accuracy [65].

4. Second generation TSPO radioligands

[^{11}C]PK11195 has been used for two decades but, because of the low signal to background ratios and short 20 min half-life, several second generation TSPO markers have been developed. These include [18F]N-2-(2-fluoroethoxy)benzyl)-N-(4-phenoxypyridin-3-yl)acetamide or ([18F]-FEPPA), N-(5-Fluoro-2-phenoxyphenyl)-N-(2-(18n)F-fluoroethyl-5-methoxybenzyl)acetamide or ((18)F-FEDAA1106) [18F]FEPPA, [18F]FEDAA1106, [^{11}C]vinpocetine, [11C]DAC, [11C]DAA1106, N-Benzyl-N-methyl-2-(7-[(11)C]methyl-8-oxo-2-phenyl-7,8-dihydro-9H-purin-9-yl) acetamide or ([(11)C]DAC), [(11)C]-N-(2,5-dimethoxybenzyl)-N-(4-fluoro-2-phenoxyphenyl)-acetamide or ([11C]DAA1106), [^{11}C]N1-methyl-2-phenylindol-3-ylglyoxylamide ([^{11}C]31), [11C]CLINME, [11C]DPA-713, [18F]DPA-714, [18F](2-[6-chloro-2-(4-iodophenyl)-imidazo[1,2-a] pyridin-3-yl]-N-ethyl-N-methyl-acetamide) or ([11C]CLINME, (11)C-labeled N,N-diethyl-2-[2-(4-methoxyphenyl)-5,7-dimethylpyrazolo[1,5-alpha]pyrimidin-3-yl]acetamide or ([11C] DPA-713), (18)F-labeled N,N-diethyl-2-(2-(4-(2-fluoroethoxy)phenyl)-5,7-dimethylpyrazolo[1,5-alpha]pyrimidin-3-yl)acetamide or ([18F] DPA-714), (18)F-N-fluoroacetyl-N-(2,5-dimethoxybenzyl)-2-phenoxyaniline or ([18F]PBR06], (11)C-N-acetyl-N-(2-[(11)C]methoxybenzyl)-2-phenoxy-5-pyridinamine or ([11C]PBR28) PBR06 and [11C]PBR28 (see Supplementary Table 1). These radiotracers are in various stages of development and are being evaluated in humans, they are discussed below.

[^{11}C]PBR28 is a radioligand with an 80-fold higher affinity for TSPO than PK11195, lower signal-to-noise ratio and more favorable pharmacokinetics [66]. However, it was found that patients exhibited different binding affinities for TSPO, falling into high affinity, low affinity, or mixed affinity binding groups [67]. This variable binding was mapped to the expression of a specific polymorphism in the TSPO gene, which permits prediction of binding potential [68]. This variability in binding affects modeling of radioligand binding as a proxy for microglial activation, to the extent where some studies have chosen only to use high affinity binders, representing around 50% of Caucasians, to make their analysis more robust. This approach will result in selection bias of patients. In vitro studies with [^{11}C]PK11195 suggest that this agent is little influenced by TSPO polymorphisms [69]. Nevertheless [^{11}C]PBR28 does show significant binding in AD patients who are medium affinity binders, compared with medium affinity binding controls (Fig. 2) (Edison, unpublished data). A recent study of a novel TSPO ligand N1-methyl-2-phenylindol-3-ylglyoxylamide, [^{11}C]31, showed high binding when tested in brain tissue which demonstrated high, low and intermediate binding to PBR28 [70]. The development of [^{18}F]PBR28 will allow the tracer to be used widely, even if there is no cyclotron available on site [71].

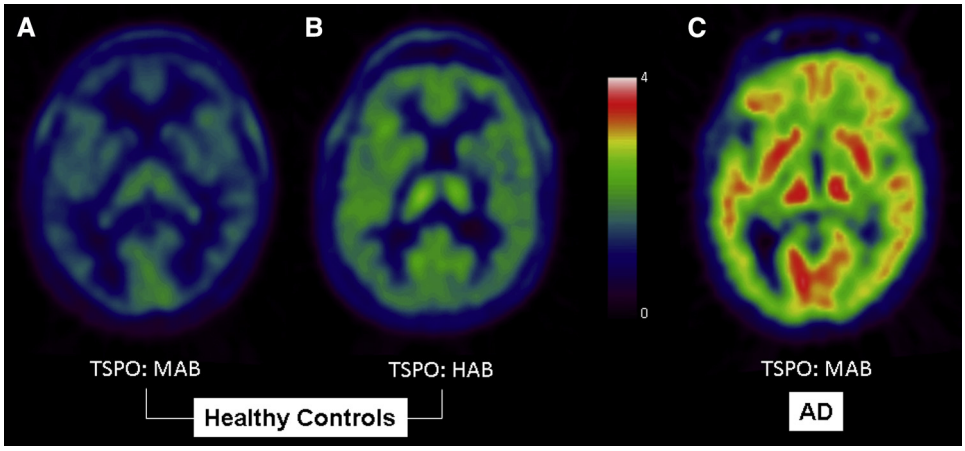

Fig. 2. [^{11}C]PBR28 BP in healthy controls compared with AD subjects. Fig. 2 compares microglial activation as quantified by the second generation TSPO ligand [^{11}C]PBR28 in (A) healthy control medium affinity binders (MABs), (B) healthy control high affinity binders (HABs) and (C) AD MAB patients.

Despite current limitations with [^{11}C]PBR28, a recent study has been able to demonstrate that increased uptake correlated with poor performance on neuropsychological scores, a diagnosis of AD and decreased grey matter volume. This is similar to findings with [^{11}C]PK11195 but in this study they failed to demonstrate increased uptake of [^{11}C] PBR28 with aMCI, suggesting inflammation occurs as part of the disease progression from aMCI into AD, a valuable addition to the argument over whether neuroinflammation is the cause or result of neurodegeneration' [72].

Another second-generation ligand is [^{11}C]DAA1106, which has higher binding affinity to TSPO than [^{11}C] PK11195, but is influenced by TSPO polymorphisms and binds to cells other than activated microglia [73]. Yasuno et al. have carried out studies in AD and aMCI, showing increased binding in aMCI compared with controls, as seen with [^{11}C]PK11195 [74] and diffuse binding in AD, with some uptake in the cerebellum [75]. Yasuno et al. also found that MCI subjects with higher [^{11}C]DAA1106 binding went on to develop AD when followed up after 5 years, suggesting microglial activation precedes clinical dementia [74]. This is intriguing though further work, including looking at how microglial activation correlates with amyloid deposition and clinical dementia, is required to definitively prove causality. A study evaluating [^{11}C] DAA1106 in schizophrenia showed no difference between controls and patients [76], contradicting previous studies. In these studies, TSPO genotyping was not performed.

[^{18}F]DPA-714 shows excellent biodistribution, low levels of breakdown to metabolites in vivo [77] and a longer (110 min) half-life allowing more flexibility in usage. It is less influenced by TSPO polymorphisms than PBR28 and has roughly the same affinity for TSPO as [^{11}C]PK11195. It has been used in newly diagnosed Amyotrophic lateral sclerosis (ALS) and showed increased uptake in primary and supplementary motor areas, in keeping with previous neuropathology studies, but also in the temporal lobe [78] however, this needs further evaluation.

Vinpocetine was initially used as vasodilatory neuroprotective agent following ischemic stroke, which was eventually shown to have no benefit. It was developed as a [^{11}C] PET ligand because of favorable pharmacokinetics [79] and its affinity toward TSPO [80]. It was also noted that, in healthy brains, there was significant nonspecific activity [79]. A study carried out in four MS patients showed that, when compared with [^{11}C]PK11195, [^{11}C]vinpocetine had a similar biodistribution with significantly higher binding potential [81]. However, a small study in six AD patients and 12 healthy volunteers, showed no statistically significant difference between these groups [82]. Another study comparing [^{11}C]vinpocetine with [^{11}C]PK11195 in stroke did not reveal any significant difference between the uptake of these two ligands in the peri-infarct zone, an area of established microglial activity [83]. Due to these negative studies, [^{11}C]vinpocetine was not considered a good marker.

While attempts have been made to improve the pharmacokinetics and specific binding, the inter-subject variability is being evaluated for [^{11}C]PBR28, [^{11}C]DAA1106 and [^{18}F]DPA-714. The prospect of [^{11}C]31, which gives better specific binding than [^{11}C]PK11195 and is not affected variable binding, might make it a better tracer, however, further clinical studies are necessary to establish this.

5. Other methods of detecting neuroinflammation in vivo

Targeting activated microglia is a challenging task as they only make up 15% of non-neuronal cells and the activated population forms only a small proportion of this. Although imaging microglial activation has yielded valuable insights, the problems with low signal-to-noise ratio has led to a search for new ways of imaging neuroinflammation.

5.1. Novel PET targets for neuroinflammation

The endocannabinoid system has two main receptors, type 1 (CB_1R) and type 2 (CB_2R). CB_1R is relevant to

neuropsychiatric disease, whereas CB_2R is involved in the immune system and is expressed at low levels in healthy CNS [84]. There is increasing consensus that CB_2R is an expression can be up-regulated on certain phenotypes of activated microglia, making it a potential PET target [85]. There is histological evidence for increased CB_2R in the spinal cord of mouse models of ALS [86] and in the lesioned zone in a rat model of ischemic stroke [87]. In post-mortem studies in AD, specific CB_2R upregulation was noted around amyloid plaques [88]. A number of tracers have been developed including [^{11}C]NE40 [89], [^{18}F] FEGW405833 [90] and [^{11}C]A-836339 [91]. [^{18}F] FEGW405833 studies showed high specific binding but slow washout, and thus this is not an appropriate PET ligand [90]. [^{11}C]NE40 and [^{11}C]1-839339 are yet to be used in humans, but all show promising radioligand characteristics in preclinical studies and in mouse models of neuroinflammation [89] and AD [91]. Further work is required, but in vivo studies in humans would be highly intriguing and provide an interesting comparator to TSPO PET studies.

Cyclo-oxygenase (COX) is the main enzyme responsible for the first step in metabolizing arachidonic acid into inflammatory mediators such as prostaglandins and thromboxanes [92]. There are two isoforms: COX-1 and COX-2, and varied patterns of overexpression of both COX isoforms have been observed in a range of neurodegenerative diseases in association with activated microglia, including AD, PD, ALS and TBI and in ischemic stroke, for a review see Choi et al [93]. The expression of COX-1 and COX-2 is dynamic and unpredictable. This, coupled with the difficulties in creating ligands specific to either COX-1 or COX-2 [94] and the high constitutional expression of COX-1, makes these targets less appealing. Despite this, the COX-1 specific radioligand [^{11}C]ketoprofen-methyl-ester has been synthesized and increased uptake shown to correspond to microglial activation on immunohistochemistry. LPS injured rat striatum showed a rapid increase in [^{11}C]ketoprofen-methyl-ester binding after 1 day, compared with a slower rise in [^{11}C] PK11195 binding which peaked after 3 days [94].

Arachidonic acid (AA) is the intermediate step between membrane phospholipid and inflammatory mediators. Correspondingly, increased levels of AA metabolites have been found in the CSF of AD patients [95]. In view of this, a method has been devised of visualizing increased AA metabolism as a proxy for neuroinflammation. 1-[^{11}C]-AA showed encouraging properties in healthy elderly volunteers [96] and a study in eight AD patients and nine age-matched controls found globally increased cerebral metabolism of AA, particularly in areas associated with plaque deposition, and some sparing of deep grey matter structures [97]. A weakness of this approach is the targeting of nonspecific pathological process and there is little evidence of close association between microglial mediated damage and AA metabolism. Further studies are awaited.

Another potential microglial target is the P2X7 receptor which is highly expressed on activated microglia associated with pathology in MS and ALS in the spinal cords in human post-mortem studies, and recently a novel tracer has been developed [98] exists. Matrix-metalloproteinase upregulation is associated with inflammation and has been targeted in PET studies to visualize macrophage association with atherosclerosis. Development of novel probes is on-going, which may eventually lead to an application to neuroinflammation [99].

While most efforts have focused on imaging activated microglia, several groups are focusing on imaging astrocytes. [^{11}C]L-deprenyl targets the monoamine-oxidase enzyme (MAO-B) [100], which is expressed specifically in astrocytes and is increasingly thought to be expressed more during neuroinflammation [101]. A study was carried out using this ligand on post-mortem brain sections of seven AD patients and compared with eight controls. It found that there was significantly increased binding of [^{11}C]L-deprenyl in the temporal lobes and white matter, compared with controls, and this correlated with immunohistological studies of adjacent slices showing reactive astrocytes [100]. Interestingly, high affinity TSPO compounds did not block [^{11}C] L-deprenyl binding, proving that this is separate to TSPO binding. Further clinical studies are necessary to evaluate this further.

β-Glucuronidase is a lysosomal protein involved in breakdown of the extracellular matrix and is released by granulocytes in areas of inflammation [102]. Studies in Huntington's disease and AD [103] have all suggested elevated levels of β-glucuronidase in key pathological sites. It has further been suggested that [^{18}F]FDG PET studies in AD, which demonstrate hypometabolism associated with post-mortem rises in β-glucuronidase, reflect active gliosis and neuronal cell death [104]. This means that β-glucuronidase could be a potentially useful imaging target for neuronal damage and gliosis. A radiotracer has been developed called [^{18}F]FEAnGA, which is cleaved specifically by β-glucuronidase to create a metabolite which is more slowly cleared than the more hydrophilic [^{18}F]FEAnGA [105]. This tracer has been tested in a rat model of herpes encephalitis and compared with [^{11}C]PK11195. It was found that despite its low brain uptake, [^{18}F]FEAnGA volume of distribution was increased in the cortex, brainstem and cerebellum of HSV-1 infected rats compared with controls [105]. This relatively recent development gives an opportunity to study the downstream effects of neuroinflammation.

5.2. *Magnetic resonance imaging techniques targeting neuroinflammation*

Magnetic resonance imaging (MRI) has higher spatial resolution than PET and is devoid of any radiation. Manganese-enhanced MRI (MEMRI) relies on the paramagnetic properties of Mn^{2+}, which shorten T1 relaxation time of bound water protons, giving contrast enhancement. It has been shown in mouse models of hypoxic-ischemic brain injury that there is manganese-enhancement at day 42

post-injury in the cortex, hippocampus and amygdala, which correlated with activated microglia and reactive astrocytes on histological examination [106]. Normally, cerebral manganese enhancement is undetectable after 3 weeks, so it is has been postulated that this prolonged enhancement is due to specific association of manganese to the cells of chronic inflammation around the lesion [106]. MnDPDP is approved for humans and has been used in healthy volunteers [107], but applications to neuroscience are limited by minimal BBB penetration at the currently accepted safe dose.

Gadolinium^{3+} diethylenetriamine penta-acetic acid (DTPA) is the predominant MRI contrast agent and works along a similar line to manganese^{2+}. This has recently been combined successfully with a second generation TSPO targeting ligand, DPA-713 and showed favorable characteristics in very early testing [108]. More preclinical and clinical studies are necessary to evaluate this technique further.

Magnetic resonance spectroscopy (MRS) is a technique whereby cerebral metabolic processes can be measured dynamically in vivo and taking metabolism as a proxy of activity. A potential microglial metabolic substrate, [1-C^{13}]acetate, has been developed and was recently used in a small trial comparing metabolic rates between age-matched controls, AD and MCI patients. They found that glial metabolic rates were raised in AD and MCI patients and that higher metabolism correlates with poorer cognitive performance on neuropsychometry [109]. Further well-designed, larger studies are required to fully elucidate whether metabolism of [1-C^{13}]acetate can be taken as a proxy for neuroinflammation and more work is required to assess the degree of region-specific localization of metabolism which is possible but this does represent another intriguing direction.

6. Results of clinical trials targeting neuroinflammation in dementia

This review has focused on how neuroinflammation is involved in dementia and the best way to image this to learn more about the process. Because it has become clearer that neuroinflammation plays a crucial role in neurodegeneration, studies have focused on downregulating neuroinflammation as a therapeutic strategy. Attempts have been made to target the inflammatory cascade generally in AD with steroidal and nonsteroidal anti-inflammatory medications, with no beneficial effect being noted in a Cochrane review of over 14 randomized controlled trials and over 2000 participants [110]. More potent untargeted immunosuppression in the form of intravenous immunoglobulin (IVIG) has been studied in AD with initial early promise, with improvement of mini-mental state scores demonstrated in pilot studies [111,112]. However, in phase II and III, double blinded placebo controlled trials in AD, no significant benefit was seen between patients receiving regular IVIG and placebo [113,114]. A further phase III trial combining IVIG and plasmapheresis in AD is underway [115] as well a phase II trial administering IVIG to aMCI patients [116].

In terms of more targeted immunomodulation we have already discussed minocycline, to target microglial activation specifically, and its lack effect in MSA [55]. Etanercept, an anti-TNF alpha monoclonal antibody, has been injected perispinally, in an open label 15 patient study with apparent improvement in cognitive function [117]. These findings are far from conclusive but have prompted a larger open-label crossover study which is currently recruiting participants [118].

What can be concluded is that so far efforts to target neuroinflammation in dementia have been unsuccessful. This is likely a combination of the lack of specificity in the target, inappropriate therapeutic intervention and most importantly the wrong timing. Considering the lack of effect of NSAIDs and steroids as disease modulating drugs in established inflammatory conditions like rheumatoid arthritis, it is unlikely that it is going to be effective in subtle inflammatory conditions like dementia. It is also likely that giving IVIG and anti-amyloid treatment to people with established dementia may be too late as damage accumulates over years before dementia manifests clinically and the results of trials targeting amyloid positive cognitively normal individuals are going to be crucial in AD treatment. This also further underlines the importance of imaging neuroinflammation as it could provide vital information about the timing and type of intervention in neurodegenerative diseases.

7. Conclusions and future directions

In this review we have considered the spectrum of neurodegenerative diseases imaged using in vivo PET imaging using TSPO markers, the novel pathological insights these have yielded, the limitations of this approach, efforts to target neuroinflammation as a therapeutic intervention and potential future directions. We have also reviewed other methods of imaging neuroinflammation in vivo using other molecular targets with PET or MRI. It is our view that the combination of other modalities with PET scanning, particularly using second generation radioligands, will provide fascinating insights into neuroinflammation and its relation to neurodegeneration. While there is more and more evidence to suggest that neuroinflammation plays a vital part in neurodegenerative diseases, the mechanism by which neuroinflammation plays a significant role in these diseases is still being debated. While there is evidence to demonstrate that neuroinflammation could be harmful, there is substantial evidence to suggest that neuroinflammation could be beneficial in clearing the debris in the central nervous system, however, this may inadvertently cause neuronal death. We believe by using novel imaging techniques to examine neuroinflammation, we should be able to disentangle the influence of neuroinflammation in different stages of the disease, along with addressing the relative timing of neuroinflammation in neurodegenerative disease in relation

to functional decline. With the recent advances in amyloid imaging, we have been able to demonstrate that amyloid deposition takes place decades before neuronal damage and cognitive dysfunction in AD, however, the relative timing of neuroinflammation is still being debated. Equally, the role of M1/M2 phenotypes of microglia and the influence of overexpression of one of these phenotypes in relation to the other on disease progression is still poorly understood. The novel methods of imaging neuroinflammation will clearly shed light on these disease processes and allow us to develop improved intervention strategies.

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jalz.2014.08.105

RESEARCH IN CONTEXT

1. Systematic review: To collect the relevant literature for our review we used initial broad search terms to collect all relevant papers, these were 'microglia; imaging' on the Pubmed database. This yielded over 1000 references which were then manually sorted into the subject headings for review. For the radiotracer specific sections of the review, the name of the radiotracer was used as a search-term and studies in humans and key animal studies were included.
2. Interpretation: Our review represents a comprehensive overview of the field with commentary by experts and represents the most thorough exploration of novel positron emission chromatography radioligands for TSPO and imaging neuroinflammation to date.
3. Future directions: We believe using the novel imaging techniques of neuroinflammation, we should be able to disentangle the influence of neuroinflammation in different stages of the disease, along with addressing the relative timing of neuroinflammation in neurodegenerative disease and its influence on the functional decline. This will also allow us to understand the influence of M1/M2 phenotype on disease progression.

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