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PET tracers for the peripheral benzodiazepine receptor and uses thereof

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The peripheral benzodiazepine receptor (PBR) is expressed on the outer mitochondrial membrane of activated microglia and is implicated in the pathophysiology of a variety of central nervous system and peripheral diseases. The abundant receptor concentration makes PBR a potential biomarker and an attractive target for quantification *in vivo* using positron emission tomography. PBR can be an important target for monitoring disease progression, for evaluating the effect of therapy, and for investigating new treatment modalities. PBR is also emerging as a potential target in the treatment of neuroinflammatory and neuropsychiatric disorders. Here, we review the positron emission tomography radioligands employed for imaging PBR in living brain and their applications.

Introduction

The peripheral benzodiazepine receptor (PBR) is a hetero-oligomeric complex located in the outer mitochondrial membrane [1]. The PBR consists of at least three different subunits, including an 18 kDa protein, a 32 kDa voltage-dependent anion channel, and a 30 kDa adenine nucleotide carrier [2]. Evidence supports three main functions of the PBR: (i) cholesterol binding and transport for biosynthesis of steroids and bile salts, (ii) protein import for membrane biogenesis, and (iii) porphyrin binding and transport for heme biosynthesis [2,3]. The PBR receptor binding site is predominantly the 18 kDa protein, which has been named the 'translocator protein', reflecting its role in binding and transport of molecules across the mitochondrial membrane [2,3]. In the central nervous system (CNS), PBR ligands have been found to stimulate synthesis of neurosteroids involved in diverse functions, from regulation of apoptosis to reduction of anxiety via modulation of the $GABA_A$ receptor [2].

The PBR is found in many regions of the body, including the human iris, ciliary-body, heart, liver, adrenal and testis, blood cells (including lymphocytes and erythrocytes), and brain [2]. In the CNS, the PBR is expressed primarily on microglia when they become activated in response to a wide variety of insults [1]. Upon activation, microglia undergo a change in morphology, migrate toward the site of neuronal damage, proliferate, synthesize numerous pro-inflammatory molecules and might release neurotoxic metabolites, resulting in progression of disease and, ultimately, loss of neurons through prolonged microglia-mediated damage [4]. Because the PBR is expressed mostly on activated microglia, it is present only in very low levels in normal brain parenchyma, except in certain areas, such as those constitutively without blood–brain barrier (BBB) (e.g. the choroid plexus and the ependymal cells lining the ventricles). That the PBR is expressed at low levels in normal brain parenchyma and is upregulated locally in response to damage makes it a potentially ideal and sensitive marker for the detection of small changes in the region of injury.

PET imaging of PBR

Positron emission tomography (PET) is an imaging technique in which tracer compounds labeled with positron-emitting radionuclides are injected into the subject of the study to track biochemical

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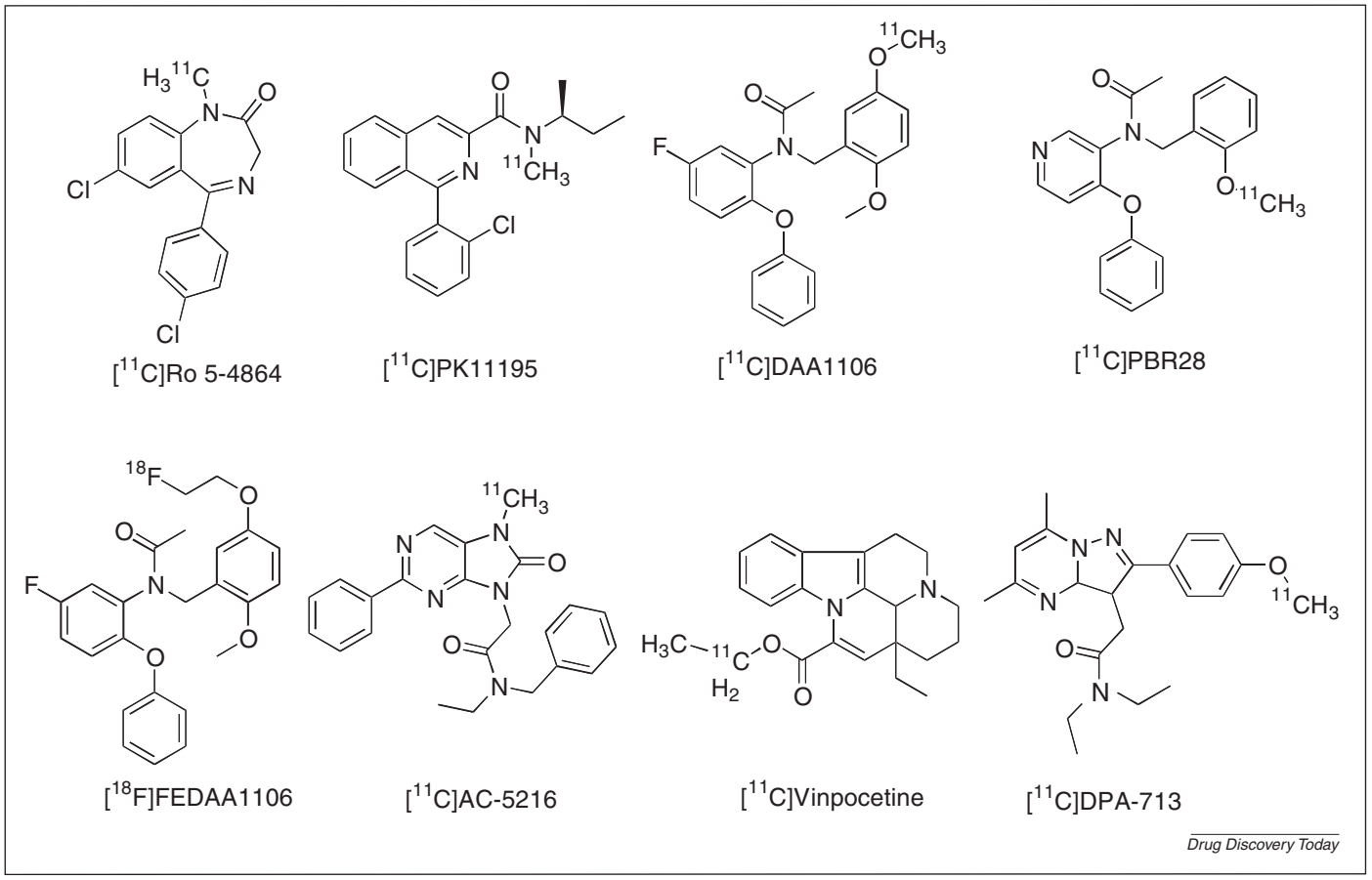

FIG. 1

PET tracers studied in human for PBR.

and physiological processes *in vivo*. Radiolabeling of PBR ligands has enabled the imaging of PBR expression using PET. Because it is based on a ligand–receptor interaction, PET imaging of the PBR benefits from well-validated concepts and tools from the neuroreceptor imaging field [5,6]. Several specific ligands for the PBR have been successfully radiolabeled and used for *in vivo* studies with human subjects [7–10] (Fig. 1). The development of radiolabeled ligands has enabled PET imaging of the PBR to be used in the study of neuroinflammatory and neurodegenerative conditions. PET imaging of the PBR strategy is already being used for quantitative assessment of disease progression and treatment response. PET findings involving the PBR in various human CNS diseases are summarized in Table 1. In this review, we focus on studies of radioligands for PET imaging of the PBR in human subjects, with a summary of findings published through March 2010.

[11C]Ro 5-4864

Ro 5-4864 is a 4′-chlorodiazepam (7-chloro-5-(4-chlorophenyl)-1-methyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one) compound. It is the only benzodiazepine with binding affinity 6 nM that has been radiolabeled with C-11 isotope in this context to date [11]. PET studies of [11C]Ro 5-4864 in human subjects with gliomas and meningiomas did not demonstrate increased uptake of this tracer in areas with tumor, known to have a high density of the PBR, as compared with uptake in normal brain tissue [12,13]. Moreover, it has been found that Ro 5-4864's binding affinity is both temperature dependent and species dependent, providing markedly different results between rats and humans [14,15]. This ultimately limits its usefulness as a tool for studying the PBR.

[11C]-PK11195 (*R*)-[11C]-PK11195

Racemic PK11195 (1-(2-chlorophenyl)-N-methyl-N-(1-methylpropyl)-3-isoquinoline carboxamide) is the first non-benzodiazepine high-affinity and selective PBR ligand (K_I = 9.3 nM) [5]. *In vivo* comparison of *R* and *S* enantiomers of [11C]PK11195 in rats with cortical focal lesion show a twofold higher affinity of (*R*)-[11C]PK11195, making it advantageous over racemic or *S*-enantiomer of PK11195 for imaging studies [16]. Although both Ro 5-4864 and PK11195 bind the PBR in a saturable and reversible manner with nanomolar affinity, they differ substantially in their kinetics and pharmacological profile in that PBR isoquinoline binding sites are more abundant in the normal human brain than PBR benzodiazepine sites by approximately threefold [17]. PK11195 has several kinetic properties that permit its use as an *in vivo* ligand: the extraction of PK11195 from blood to brain is rapid and high (>90%) and is unimpeded by the BBB (i.e. tracer delivery is similar in areas with and without a BBB) [18]. Although some have suggested that PK11195 is a substrate of the efflux transporter P-glycoprotein, other studies have not found this to be the case [19].

Quantification of [11C]-labeled PK11195 has been approached largely by either normalization of the uptake to a reference region such as the cerebellum or application of the simplified reference tissue model with a 'reference' region devoid of PBR derived from cluster analysis [20,21]. In addition to reference tissue modeling,

TABLE 1

***In vivo* PET imaging studies of the peripheral benzodiazepine receptor (PBR) in human diseases**

Disease	PET ligand	Effect	Refs
AD	[^{11}C]PK11195	No increases in binding were identified relative to control subjects	Groom *et al.*, 1995
	(*R*)-[^{11}C]PK11195	Increased binding in entorhinal, temporo-parietal and cingulate cortex	Cagnin *et al.*, 2001
	[^{11}C]DAA1106	Increased binding in various cortex regions, striatum and cerebellum	Yasuno *et al.*, 2003
	[(*R*)-[^{11}C]PK11195	Increased binding in various cortex regions	Edison *et al.*, 2008
AD and MCI	(*R*)-[^{11}C]PK11195	No increased binding	Wiley *et al.*, 2009
	(*R*)-[^{11}C]PK11195	Increased binding in the frontal cortex of some subjects	Okello, *et al.*, 2009
ALS	(*R*)-[^{11}C]PK11195	Increased binding in motor cortex, prefrontal cortex, pons and thalamus	Turner *et al.*, 2004
CV	[(*R*)-[^{11}C]PK11195	Increased binding in occipital and temporo-parietal cortex	Goerres *et al.*, 2001
CBD	(*R*)-[^{11}C]PK11195	Increased binding in caudate, putamen, substantia nigra, pons, pre-postcentral gyrus and frontal cortex	Gerhard *et al.*, 2004
	(*R*)-[^{11}C]PK11195	Increased binding in basal ganglia, temporal and parietal cortex	Henkel *et al.*, 2004
FASSc	(*R*)-[^{11}C]PK11195	Reduction of binding in lung macrophages	Branley *et al.*, 2008
FTD	(*R*)-[^{11}C]PK11195	Increased binding in frontal temporal cortex	Cagnin *et al.*, 2004
Glioblastoma	[^{11}C]PK11195	Increased binding in area of tumor	Pappata *et al.*, 1991
Glioblastoma	[^{11}C]Ro 5-4864	No increase in binding	Junck *et al.*, 1989
HE	(*R*)-[^{11}C]PK11195	Increased binding in the pallidum, right putamen and right dorsolateral prefrontal region	Cagnin *et al.*, 2006
	[^{11}C]PK11195	No increase in binding	Iverson *et al.*, 2006
Herpes encephalitis	(*R*)-[^{11}C]PK11195	Increased binding in primary and secondary projected neuron	Cagnin *et al.*, 2001
HIV encephalitis	(*R*)-[^{11}C]PK11195	Increased binding in thalamus, putamen, frontal, temporal and occipital cortex	Hammoud *et al.*, 2005
	(*R*)-[^{11}C]PK11195	Increased binding in striatum and cortical regions including prefrontal cortex and anterior cingulate	Pavese *et al.*, 2006
	(*R*)-[^{11}C]PK11195	Increased binding in striatum and cortical regions	Tai *et al.*, 2007
Ischemic stroke	[^{11}C]PK11195	Increased binding in cerebral cortex	Ramsay *et al.*, 1992
	[^{11}C]PK11195	Increased binding in the ipsilateral thalamus	Pappata *et al.*, 2000
	[^{11}C]PK11195	Increased binding in cerebral cortex	Gerhard *et al.*, 2000
	(*R*)-[^{11}C]PK11195	Increased binding in primary lesion and remote pathological changes after Wallerian degeneration	Gerhard *et al.*, 2005
	(*R*)-[^{11}C]PK11195	Increased binding in peri-infact zone	Price *et al.*, 2006
MS	(*R*)-[^{11}C]PK11195	Increased binding in MS plaques, cerebral central gray and in areas corresponding with clinical deficits	Banati *et al.*, 2000
	[^{11}C]PK11195	Increased binding in Gadolinium-enhancing lesions and in normal-appearing white matter	Debruyne *et al.*, 2003
	[^{11}C]PK11195	Increased binding in normal-appearing white matter	Versijpt *et al.*, 2005
	[^{11}C]PK11195, [^{11}C]vinpocetine	Increased binding in MS plaques	Vas *et al.*, 2008
MSA	(*R*)-[^{11}C]PK11195	Increased binding in prefrontal cortex, putamen, pallidum, pons and substantia nigra	Gerhard *et al.*, 2003
PD	(*R*)-[^{11}C]PK11195	Increased binding in midbrain	Ouchi *et al.*, 2005
	(*R*)-[^{11}C]PK11195	Increased binding in pons, basal ganglia, frontal and temporal cortex	Gerhard *et al.*, 2006
	[^{11}C]PK11195	Increased binding in contralateral putamen BP and midbrain BP, but not significantly different from normal controls	Bartels *et al.*, 2010
PSH	[(*R*)-[^{11}C]PK11195	Increased binding in motor cortex and supplementary motor region contralateral to the affected limbs	Turner *et al.*, 2005
PSP	(*R*)-[^{11}C]PK11195	Increased binding in basal ganglia, midbrain, frontal cortex and cerebellum	Gerhard *et al.*, 2006
RA	(*R*)-[^{11}C]PK11195	Higher binding in knee joints	Van der Laken *et al.*, 2008
RE	(*R*)-[^{11}C]PK11195	Increased binding in affected hemisphere	Banati *et al.*, 1999
SZ	(*R*)-[^{11}C]PK11195	Increased binding in total gray matter	van Berckel *et al.*, 2008
SZ	(*R*)-[^{11}C]PK11195	Increased binding in whole-brain gray matter, particularly in the hippocampus	Doorduin *et al.*, 2009

Abbreviations: AD, Alzheimer's Disease; ALS, amyotrophic lateral sclerosis; CBD, corticobasal degeneration; CV, cerebral vasculitis; FASSc, fibrosing alveolitis associated with systemic sclerosis; FTD, frontal temporal dementia; HE, hepatic encephalopathy; HD, Huntington's disease; MCI, mild cognitive impairment; MS, multiple sclerosis; MSA, multiple system atrophy; PD, Parkinson's disease; PSH, progressive spastic hemiparesis; PSP, progressive supranuclear palsy; RA, rheumatoid arthritis; RE, Rasmussen's encephalitis; SZ, schizophrenia.

full kinetic characterization of (*R*)-[^{11}C]PK11195 with measurement of arterial input function has been reported with the application of a model with two tissue compartments and four rate constants [22]. Logan graphical analysis with arterial input function or with reference tissue input adds an accurate method for generating binding parameters [20,23]. In general, the use of a tissue input function has the advantage over an arterial input function in that the latter requires extensive arterial blood sampling in patients with brain

injury. Although these various evolutions in technique have led to some improvements in the quantification of [^{11}C]PK11195, it is nevertheless difficult to assess their accuracy given that PET studies conducted in humans are not validated by postmortem receptor autoradiography [24,25].

Studies of whole-body distribution and metabolism of (*R*)-[^{11}C]PK11195 in humans have found a large individual variation in the amount of plasma radiometabolites [26]. The whole-body distribution of (*R*)-[^{11}C]PK11195 showed the highest radioactivity levels in urinary bladder, adrenal gland, liver, salivary glands, heart, kidneys, and vertebral column. (*R*)-[^{11}C] PK11195 seems to be eliminated through both the renal and the hepatobiliary systems.

[^{11}C]PK11195 has been used in a wide range of human CNS diseases, from Rasmussen's encephalitis and multiple sclerosis (MS) to neurodegenerative diseases (Alzheimer's, Parkinson's, amyotrophic lateral sclerosis, and Huntington's), infectious diseases (HIV and herpes encephalitis), and neuropsychiatric disorders such as schizophrenia. To date, the vast majority of PET imaging studies on PBR in human disease have been performed with [^{11}C]PK11195 as the tracer, and – with the exception of the earliest studies – most have used the (*R*)-enantiomer. What follows is a summary of PET studies using racemic [^{11}C]PK11195 or (*R*)-[^{11}C]PK11195 in human subjects.

The first PET study to use [^{11}C]PK11195 in humans was reported by Charbonneau *et al.* in 1986 [27]. The authors studied [^{11}C]PK11195 binding in the heart of dogs and humans and found specific binding in both species. Junck *et al.* [12] later reported a comparative PET study of [^{11}C]Ro 5-4864 and [^{11}C]PK11195 in glioma patients and found increased binding of [^{11}C]PK11195, whereas [^{11}C]Ro 5-4864 failed to demonstrate specific binding. Banati *et al.* [28] observed a considerable increased binding of (*R*)-[^{11}C]-PK11195 in the affected cerebral hemisphere of two Rasmussen's encephalitis patients, as compared with the unaffected contralateral hemisphere. The histologic distributions of microglial staining and distribution pattern from MRI were also correlated with areas of increased (*R*)-[^{11}C]PK11195 binding. By contrast, patients with hippocampal sclerosis showed no increase in (*R*)-[^{11}C]PK11195 binding, which the authors interpret as evidence that the *in vivo* (*R*)-[^{11}C]PK11195 signal is preferentially caused by the presence of activated microglia, as opposed to glial scar tissue.

In a study of patients with MS, Banati *et al.* [18] found increased PBR expression with (*R*)-[^{11}C]PK11195 in areas of focal pathology identified by MRI, particularly in gadolinium-enhancing lesions. Binding of (*R*)-[^{11}C]PK11195 was also increased in brain areas corresponding to ongoing or recent clinical deficit; for example, in patients with visual dysfunction, signals were observed in anatomical locations along the pathway of the neuronal network controlling visual processing or eye movement. No significant correlations were seen between the global hemispheric (*R*)-[^{11}C]PK11195 lesion load and disability (total expanded disability status scale and individual sub scores), disease duration, or the interval since the last relapse. The authors suggest that (*R*)-[^{11}C]PK11195 binding might relate better to clinical change than to cumulative measures of long-standing and recent disability as measured by the expanded disability status scale. PET studies by Debruyne *et al.* [29] in a small population of MS patients also revealed increased brain uptake of [^{11}C]PK11195 in MS patients as compared with normal controls, particularly in patients imaged during an acute MS relapse. Binding was increased in gadolinium-enhanced lesions, as well as in MRI normal-appearing white matter (NAWM). A later study by Versijpt *et al.* [30] elaborated on this finding of increased [^{11}C]PK11195 in areas of MRI-NAWM by also demonstrating a significant correlation between total NAWM [^{11}C]PK11195 uptake and disease duration, as well as a correlation between NAWM [^{11}C]PK11195 uptake and brain atrophy as measured by MRI. Vas *et al.* [31] compared [^{11}C]PK11195 and [^{11}C]vinpocetine binding in MS patients and observed greater binding of both tracers in affected brain regions than in unaffected regions; however, BP of [^{11}C]vinpocetine was higher than [^{11}C]PK11195. Taken together, these studies suggest that microglial activation is of central importance in the pathophysiology of MS and can be visualized with PET imaging using radioligands for the PBR.

Several studies have found increased binding of [^{11}C]PK11195 and (*R*)-[^{11}C]PK11195 in patients with ischemic stroke [32–35]. Binding of (*R*)-[^{11}C]PK11195 has been found to correspond with areas where T1-weighted MRI shows intensity changes [32]. In patients with chronic middle cerebral artery infarcts, [^{11}C]PK11195 has been used to visualize increased microglial activation in the ipsilateral thalamus [33]. PET imaging with [^{11}C]PK11195 thus seems to be a promising tool in the study of cerebral infarction and could also be useful in the evaluation of neuroprotective strategies, especially with respect to the consequences of microglial activation.

Molecular imaging of inflammation in Alzheimer's disease (AD) and dementia has been reviewed by Versijpt *et al.* [36]. In a study of eight patients with AD, Cagnin *et al.* [37] showed *in vivo* microglial activation in the brain of patients with mild to moderate AD in several brain regions using PET with the radioligand (*R*)-[^{11}C]PK11195. The spatial distribution of (*R*)-[^{11}C]PK11195 binding matched with the regional distribution of cerebral hypometabolism, as detected with [^{18}F]fluorodeoxyglucose, and correlated with brain atrophy assessed by longitudinal MRI scans. Cagnin *et al.* also reported high levels of (*R*)-[^{11}C]PK11195 binding in regions not traditionally thought to be involved in AD, such as the thalamus and the brainstem. The authors interpret these findings as a result of microglial activation in regions connected to areas of primary pathology, which might be amplified in the thalamus by the high density of corticothalamic connections. As one review article notes, however, it is not possible to confirm the histological presence of activated microglia in these regions for studies of human subjects [38]. These increases might reflect regional variations in the constitutive PBR expression that are independent of the disease pathology, or the increases might be a non-uniform element of non-specific (*R*)-[^{11}C]PK11195 binding. One early study in AD patients failed to show any significant increase in [^{11}C]PK11195 uptake compared with controls [39], but this study can be distinguished from later studies by several important methodological differences, the sum of which are thought to have resulted in a substantially lower sensitivity. The early study used a racemic [^{11}C]PK11195 as opposed to the higher affinity *R*-enantiomer and did not acquire PET data in 3D model. Later studies also used a different application of tracer kinetic modeling for the generation of quantitative parametric maps. Several groups have evaluated various modeling methods for quantification of (*R*)-[^{11}C]PK11195 in AD and baseline scans. Comparative study of

(*R*)-[^{11}C]PK11195 and [^{11}C]PIB, a specific tracer for imaging β-amyloid in AD and mild cognitive impairment, revealed an increase in microglial activation in cortical areas; however, the amyloid deposit load was twofold greater in cortical areas in AD patients [40–42]. More recently, Wiley *et al.* [43] observed no differences in brain (*R*)-[^{11}C]PK11195 retention when subjects were grouped by clinical diagnosis or by the presence or absence of β-amyloid pathological findings as indicated by analyses of [^{11}C]PIB retention. These findings suggest that either microglial activation is limited to later stages of severe AD or (*R*)-[^{11}C]PK11195 is too insensitive to detect the level of microglial activation associated with mild to moderate AD.

(*R*)-[^{11}C]PK11195 has been studied in many other forms of dementia, including those of infectious etiology. In a PET study of patients with AIDS, Hammoud *et al.* [44] observed greater binding of (*R*)-[^{11}C]PK11195 than in normal controls. However, patients with HIV-associated dementia did not show significant differences in binding when compared to HIV+ nondemented patients.

Increased microglial activation has been observed in the PET scanning of (*R*)-[^{11}C]PK11195 in patients with Huntington's disease [45]. The formation of microglia has been observed in both symptomatic and presymptomatic Huntington's disease gene carriers, and the degree of microglial activation in the striatum has been found to correlate with D2 receptor dysfunction as measured by [^{11}C]raclopride PET, as well as with clinical measures of disease severity [46].

In multiple system atrophy (MSA) patients, (*R*)-[^{11}C]PK11195 binding has been shown to match the known distribution of neuropathologic changes, with increased binding in the dorsolateral prefrontal cortex, putamen, pallidum, pons, and substantia nigra [47]. These findings suggest that microglial activation is an indicator of disease activity and that (*R*)-[^{11}C]PK11195 PET can thus be used to characterize the *in vivo* neuropathology of MSA. Dodel *et al.* [48] recently reported the effect of minocycline in MSA patients with (*R*)-[^{11}C]PK11195 and PET. Two out of three MSA patients treated with minocycline in this clinical trial showed decreased PBR binding with (*R*)-[^{11}C]PK11195, whereas most patients receiving placebo experienced a mean increase in binding. Further studies with larger populations are required to address the clinical relevance of (*R*)-[^{11}C]PK11195 with PET.

In patients with Parkinson's disease (PD), Gerhard *et al.* [49] reported increased (*R*)-[^{11}C]PK11195 binding in the pons, basal ganglia, frontal, and temporal cortex as compared with normal controls. These authors also performed longitudinal PET studies using (*R*)-[^{11}C]PK11195 and [^{18}F]DOPA and found that binding of (*R*)-[^{11}C]PK11195 in patients with PD remained stable over the two year period of follow-up. Microglial activation was not found to correlate with clinical index or [^{18}F]DOPA binding in this longitudinal study. These findings suggest that microglial activation might be important early in the disease process but remains stable as the disease progresses. Bartels *et al.* [50] have used [^{11}C]PK11195 in PD patients to evaluate the medication effect of celecoxib, a COX-2 inhibitor. PET analyses showed that PD patients possessed higher contralateral putamen binding potential (BP) and midbrain BP than controls, although considerable overlap was seen and differences were not statistically significant. Unexpectedly, the BP and distribution volume (DV) after celecoxib were slightly higher. These findings suggest that [^{11}C]PK11195 might not be a suitable tracer for the reliable assessment of PD.

The utility of (*R*)-[^{11}C] K11195 might be greater in patients with more severe movement disorders. For example, in patients with corticobasal degeneration, increased binding of (*R*)-[^{11}C]PK11195 has been observed in cortical regions and basal ganglia as compared with normal controls [51]. In a study of patients with progressive spastic hemiparesis, Turner *et al.* [52] reported that two of three patients showed increased binding of (*R*)-[^{11}C]PK11195 in the motor cortex and supplementary motor region contralateral to the affected limbs, as visualized on PET imaging. No focal areas of increased binding were seen in the cerebral cortex of the third patient, who had a high cervical cord lesion and was presumed to have extra-cerebral inflammatory disease. In amyotrophic lateral sclerosis, patients were found to have significantly increased binding in the motor cortex, pons, dorsolateral prefrontal cortex, and thalamus relative to healthy control subjects. Furthermore, there was a significant correlation between binding in the motor cortex and the burden of upper motor neuron signs clinically [53]. Taken together, these findings suggest that cerebral microglial activation can be detected *in vivo* during the evolution of several degenerative movement disorders.

In a PET study of patients with hepatic encephalopathy (HE), Cagnin *et al.* [54] found significant increases in glial (*R*)-[^{11}C]PK11195 binding bilaterally in the pallidum, right putamen, and right dorsolateral prefrontal region. The patient with the most severe cognitive impairment had the highest increases in regional (*R*)-[^{11}C]PK11195 binding. These findings support earlier experimental evidence from rodent models of liver failure and suggest that an altered glial cell state, as evidenced by the increase in cerebral PBR, might be causally related to impaired brain functioning in HE [55]. Iversen *et al.* [56], however, reported no significant differences in the volume of distribution (VT) of [^{11}C]PK11195 between regions studied or between the HE and control group. These latter results might be interpreted as meaning that microglial activation with expression of PBR is not an important mechanism of degeneration in patients with HE, or the results might simply reflect the inadequacy of the tracer, particularly in its racemic form.

One of the latest uses of PK11195 has been in the study of neuropsychiatric disorders such as schizophrenia. In a study of ten patients with recent-onset schizophrenia, BP of (*R*)-[^{11}C]PK11195 in total gray matter was increased relative to healthy age-matched controls, suggesting that activated microglia might play a part in the loss of gray matter associated with this disease [57]. In a study of seven patients within the schizophrenia spectrum who had recently experienced an episode of psychosis, a significantly higher BP of (*R*)-[^{11}C]PK11195 was found in the hippocampus relative to healthy age-matched volunteers. It was also found that patients with recent psychosis had 30% higher (*R*)-[^{11}C]PK11195 BP in the whole-brain gray matter, despite the fact that MR images revealed no visual abnormalities [58]. The results of these two studies suggest that neuroinflammation might have an important role in schizophrenia and that PK11195 combined with PET might provide insight into this process beyond the realm of traditional neuroimaging techniques.

[^{11}C]PK11195 has even been used to guide therapy. In a recent report involving a child with refractory seizures secondary to encephalitis, Kumar *et al.* [59] used [^{11}C]PK11195 to detect areas

of neuroinflammation, which were then removed surgically. This led to significant recovery.

[^{11}C]PK11195 has also been used in the study of inflammatory diseases outside the CNS. In a study of patients with rheumatoid arthritis, Van der Laken *et al.* [60] reported higher binding of (*R*)-[^{11}C]PK11195 in PET imaging of knee joints. PET tracer uptake in joints correlated significantly with PBR staining in the sublining of synovial tissue. In a study of patients with the chronic lung disease scleroderma fibrosing alveolitis (FASSc), Branley *et al.* [61] reported a trend of reduced uptake of (*R*)-[^{11}C]PK11195 in FASSc patients versus controls and also found that uptake correlated inversely with lung density, which was significantly elevated in FASSc. Reduced uptake of the radiotracer was thought to represent a change in the morphology of lung macrophages with disease progression and accumulation of permanent scar tissue. These results of studies outside the CNS demonstrate that inflammatory cell traffic can be reliably imaged in regions as diverse as knee joints and lung tissue and that PK11195 can be used to assess disease progression.

Limitations of PK11195

Although it is well established that (*R*)-[^{11}C]PK11195 and [^{11}C]PK11195 show increased CNS retention in a wide array of neurological disorders, there are several methodological and kinetic issues that limit the interpretation and potential of this radioligand. Lower than desirable BP has been attributed to both low affinity for its receptor and relatively low total brain uptake, which results from substantial binding of the tracer to organs in the periphery. In addition to this low level of total binding in the normal brain, [^{11}C]PK11195 has a high level of non-specific binding owing to its lipophilic nature; together, these factors lead to a poor signal-to-noise ratio. The low level of binding in normal brain also renders the modeling of this tracer particularly difficult because effects of no interest such as tissue heterogeneity and vascular signal become predominant [24]. Yet another complicating factor is that [^{11}C]PK11195 demonstrates highly variable kinetic behavior, which further complicates quantitative analysis of PBR receptor density. This variability is thought to result from its extensive binding to plasma proteins, some of which are acute-phase reactants that vary in inflammatory conditions, both systemically and locally at the site of acute injury [24]. Refinements in the modeling methods for analysis of PK11195 imaging data have led to incremental improvements in the quantification of PBR [62], but the lack of sensitivity and specificity of [^{11}C]PK11195 and (*R*)-[^{11}C]PK11195 have so far precluded the development of a standard method of analysis easily applicable to all subjects. Until now, therefore, the interpretations of results have been limited to an emphasis on the general agreement between brain areas of increased PK11195 uptake and the known distribution of a given pathology [5,10].

In addition to these drawbacks, the short half-life of ^{11}C (20.4 min) is a limitation to the dissemination of [^{11}C]-labeled PK11195 for clinical purposes. The longer half-life of ^{18}F (109.8 min) enables remote cyclotron creation of the tracer and is preferred to facilitate both distribution and multiple use of the radiotracer production batches.

Another important consideration is in regards to the extent of microglial activation that needs to be present in the CNS before a signal can be detected using PK11195. For example, in patients with mild cognitive impairment (a condition that often progresses to Alzheimer's disease), some studies have observed increased (*R*)-[^{11}C]PK11195 binding in a portion of patients [42], whereas others have not found any significant differences between patients and normal controls [43]. Similarly, (*R*)-[^{11}C]PK11195 PET imaging was not able to distinguish between HIV-infected patients with dementia and HIV-infected patients without dementia [44]. These studies suggest that low levels of microglial activation might not be detected using PK11195 and underscore the importance of developing ligands that bind to microglia with greater sensitivity and specificity.

There are also certain discrepancies in this body of work that remain to be addressed. For example, (*R*)-[^{11}C]PK11195 has shown increased binding in regions traditionally not associated with neuroinflammation such as the thalamus in several diseases such as AD, PD, and Huntington's disease [37]. One potential explanation for these findings is that the PBR is being induced in cells other than microglia, such as astrocytes, which has been suggested in animal models of neuronal injury [63,64], as well as in humans [65]. These findings might also reflect regional variations in the constitutive PBR or some degree of non-uniform non-specific binding in the CNS because it is not possible to histopathologically ascertain microglial activation in these regions in human PET studies.

These concerns highlight the importance of developing newer ligands that have greater specificity and sensitivity to activated microglia for PET imaging. In light of the limitations of PK11195, many groups worldwide are actively engaged in a search for new ligands with improved capacities to quantify PBR expression. During the past few years, more than 50 new PBR radioligands have been reported in the literature, labeled with the short-lived positron emitters carbon-11 and fluorine-18 (half-life: 109.8 min) or with the single-photon longer-lived emitter iodine-123 (half-life: 13.2 h) [7–10]. Most of these potential tracers are still in the early stages of investigation.

[^{11}C]Vinpocetine

Vinpocetine (eburanamenine-14-carboxyic acid ethyl ester), a vinca alkaloid, is an agent that is currently used in the treatment of acute and chronic stroke patients [66] because it is thought to interfere with various stages of the ischemic cascade. The uptake of [^{11}C]vinpocetine in human brain has been shown to distribute rapidly and heterogeneously among brain regions [67]. When compared with the cerebellum, the highest regional uptake has been found in the thalamus, upper brain stem, striatum, and cortex [68]. In a study with oral administration, [^{11}C]vinpocetine accumulated in stomach, liver, brain, and kidney with distribution of brain identical to that of intravenous administration [69]. Vinpocetine binds the PBR with low affinity *in vitro* (IC_{50} = 0.2 μM), but its role as a PBR ligand is supported by the finding that pretreatment with vinpocetine substantially decreases later uptake of [^{11}C]PK11195. The uptake of [^{11}C]vinpocetine is increased in brain after PK11195 pretreatment, presumably owing to blockade of PBRs in the periphery [67]. One drawback of vinpocetine is that it binds to other receptors with an affinity similar to its affinity for the PBR (including adrenergic $\alpha_2\beta$ receptors, IC_{50} = 0.9 μM), a finding that raises questions about its *in vivo*

specificity for the PBR [68]. [^{11}C]vinpocetine has been used with success in a small PET imaging study of MS patients, in which it showed greater global brain uptake than [^{11}C]PK11195 and increased BP in plaque regions for all four MS patients, whereas [^{11}C]PK11195 showed increased binding in plaque regions of only one of the four patients [70]. However, the affinity of vinpocetine for other receptors and the presence of [^{11}C]ethanol as radiometabolite make quantification of PBR difficult and clinical results less reliable [10].

[^{11}C]DAA1106 and [^{18}F]FEDAA1106

DAA1106, N-(2,5-dimethoxybenzyl)-N-(5-fluoro-2-phenoxyphenyl)acetamide, is a 2-phenoxy-5-fluoroanilide derivative with high affinity and selectivity for the PBR [71]. DAA1106 has a five-fold to six-fold higher affinity for PBR than PK11195. *In vivo* imaging in monkey brains has demonstrated fourfold higher levels of [^{11}C]DAA1106 binding compared with [^{11}C]PK11195, as well as higher levels of specific binding in experimentally lesioned areas [72]. The [^{11}C]DAA1106 binding was markedly inhibited by unlabeled DAA1106 and PK11195 in the monkey brain, suggesting that most of the [^{11}C]DAA1106 binding represents specific binding. Because of its higher affinity, DAA1106 might serve as a better ligand for labeling of the PBR, as well as for addressing some of the issues related to non-specific binding seen in studies with [^{11}C]PK11195. *Ex vivo* autoradiography and PET imaging *in vivo* showed greater retention of [^{11}C]DAA1106 compared with [^{11}C]-PK11195 in animal models of neuroinflammation induced with either lipopolysaccharide or 6-hydroxydopamine [73]. DAA1106 binds with higher affinity to microglia in rat models of neuroinflammation when compared with PK11195. In a study comparing the pharmacological binding properties of [^{3}H]PK11195 and [^{3}H]DAA1106, Venneti *et al.* [74] looked at binding patterns in postmortem tissues from patients with cerebral infarcts, amyotrophic lateral sclerosis, AD, frontotemporal dementia, and MS ($n = 10$ each). In all diseases, [^{3}H]DAA1106 showed a higher binding affinity, as reflected by lower dissociation constant values than those of [^{3}H]PK11195. Moreover, specific binding of both ligands correlated with the presence of activated microglia identified by immunohistochemistry *in situ*. These studies suggest that DAA1106 might possess binding characteristics superior to those of PK11195, which might be beneficial for *in vivo* PET imaging. Use of [^{11}C]DAA1106 in a small study of patients with AD yielded promising results [75]. Mean BP was increased significantly in the brain of AD patients compared with control subjects in areas of known AD pathology, including the dorsal and medial prefrontal cortex, lateral temporal cortex, parietal cortex, occipital cortex, anterior cingulate cortex, striatum, and cerebellum. [^{11}C]DAA1106 binding was also observed in more widespread regions in the AD patients than in earlier studies using [^{11}C]PK11195. The authors suggest that this might be due to the higher affinity and lipophilicity of DAA1106 as compared with PK11195 in the quantification of PBR *in vivo*. Interpretations of these results should be made cautiously, however, given that DAA1106 and PK11195 were not directly compared in the same subjects.

More recently, Fujimura *et al.* [76] studied various analytic methods for quantification of [^{18}F]FEDAA1106 (fluoroethyl derivative of DAA1106) in healthy humans. The DV was estimated by nonlinear least-squares (NLS), Logan plot and multilinear analysis (MA), and these methods were found to be significantly correlated. There was also significant correlation between BP with NLS and DV with NLS, Logan plot or MA; however, the interindividual differences in the DV of the free and non-specific binding compartment (K1/k2) were large. In a simulation study, variation of the DV estimated by Logan plot was small, but it was underestimated as the noise increased. By MA, the bias of DV was smaller, but the variation of DV was larger than by Logan plot. Within a 3% noise level, there was almost no difference between Logan plot and MA in both bias and variation. DVs estimated by both Logan plot and MA were underestimated by 10–20%. Although the variation of DV was larger by NLS than by Logan plot, it was small enough in the noise level of volume of interest analysis, and the bias of DV was 0–2%. These results suggest that NLS is a suitable method for the estimation of [^{18}F]FEDAA1106 binding to PBRs.

[^{11}C]PBR28

PBR28, N-(2-methoxybenzyl)-N-(4-phenoxypyridin-3-yl)acetamide, is a PBR ligand with a lower lipophilicity than PK11195 and DAA1106 [77]. [^{11}C]PBR28 has shown high brain uptake in monkey brain in areas consistent with known PBR distribution in monkey [78]. Similar results were observed in a rat model of cerebral ischemia and stroke [79]. Biodistribution of [^{11}C]PBR28 in healthy humans has been found to match known patterns of PBR distribution, with highest uptake in the PBR-rich organs: lungs, kidneys, and spleen [80]. Kinetic analysis of [^{11}C]PBR28 in healthy human subjects revealed that DVs were only approximately 5% of what had been observed in monkeys [80]. The time–activity curves in two of the twelve subjects seemed as if they had no PBR binding (i.e. rapid peak of uptake and fast washout from brain). The cause(s) of these unusual findings are unknown, but both subjects were also found to lack binding to PBRs in peripheral organs such as lung and kidney. Similarly, one in seven subjects had less binding of [^{11}C]PBR28 (60–90%) in kidneys, spleen, and lungs. The activity in the baseline monkey scans was greater than that in humans for organs with high PBR densities. For this reason, the human effective dose was overestimated by 60% with monkey biodistribution data.

[^{11}C]AC-5216

AC-5216 ([^{11}C]-AC-N-benzyl-N-ethyl-2-(7-methyl-8-oxo-2-pheyl-7,8-dihydro-9H-purin-9-yl)acetamide) is a dihydropurin with high affinity for the PBR [81]. [^{11}C]AC5216 has been validated successfully in several TPSO models, including kainic acid rat models [81,82]. PET studies in monkey brain demonstrated high uptake of [^{11}C]AC-5216 in the occipital cortex, a rich PBR-dense area in the primate brain [83]. PET studies in human showed that the highest BP, compared with nondisplaceable uptake (BPND), was in the thalamus (4.6 ± 1.0) and binding was lowest in the striatum (3.5 ± 0.7) [84]. The total VT obtained by an NLS method of graphical analysis showed regional distribution similar to BPND. There was no correlation between BPND and VT, however, because of the interindividual variation of K1/k2. BPND obtained with data from a scan time of 60 min was in good agreement with that from a scan time of 90 min ($r = 0.87$). Regional distribution of [^{11}C]AC-5216 was in good agreement with previous PET studies of PBRs in the human brain. BPND is more

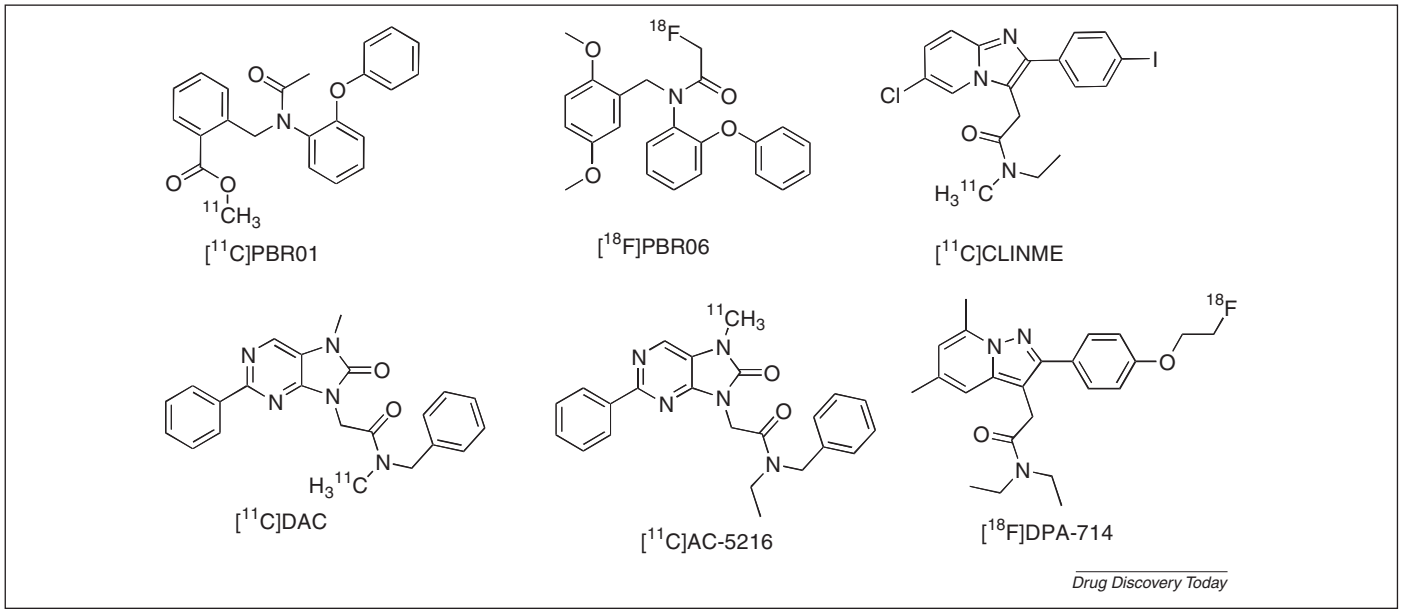

FIG. 2

Examples of promising radiotracers studied in animals for PBR.

appropriate for estimating [^{11}C]AC-5216 binding than VT is because of the interindividual variation of K1/k2, and with this method, [^{11}C]AC-5216 is a promising PET ligand for quantifying PBR in the human brain.

[^{11}C]DPA-713

DPA-713 (N-diethyl-2-[2-(4-methoxyphenyl)-5,7-dimethyl-pyrazolo[1,5-a]pyrimidin-3-yl]-acetamide) is a high-affinity PBR ligand in the pyrazolopyrimidine group [85] (Fig. 2). [^{11}C]DP-713 was successfully validated in a variety of rat inflammation models and in monkeys [86,87]. In a PET study comparing [^{11}C]DPA-713 to [^{11}C]PK11195, Endres *et al.* [88] found that in the healthy brain, the average plasma-to-tissue clearance and the total VT of [^{11}C]DPA-713 were an order of magnitude larger than those measured for [^{11}C]PK11195. Studies in patient populations are needed to determine whether [^{11}C]DPA-713 is sensitive enough to evaluate localized elevations in PBR expression.

Other promising candidates for the labeling of PBR include [^{11}C]PBR01, [^{18}F]PBR06, [^{11}C]CLINME, [^{11}C]DAC, [^{11}C]AC-5216, and [^{18}F]DPA-714 (Fig. 2). [^{11}C]PBR01 and [^{18}F]PBR06 have shown a high degree of displaceable specific binding in the brain in PET imaging studies of rhesus monkeys [87,89–92]. Of these, [^{18}F]PBR06 might have better kinetics for quantitative analysis, with relatively low non-specific uptake [90]. [^{11}C]CLINME has compared favorably with (*R*)-[^{11}C]PK11195 in PET imaging of rodents with induced local neuroinflammation, where uptake of [^{11}C]CLINME was identical to that of (*R*)-[^{11}C]PK11195 in the brain lesion but significantly lower in the intact contralateral hemisphere [91]. [^{18}F]DPA-714 has also been studied in PET imaging of rodents with induced local neuroinflammation, where it performed better than [^{11}C]DPA-713 and (*R*)-[^{11}C]PK11195, with the highest uptake ratio and BP [91,92].

Concluding remarks

Extensive research during the past decade has led to the development of several new PET radioligands for the visualization of the PBR, which have surpassed ^{11}C-labeled PK11195 in efficacy. Among these, [^{11}C]DAA1106 and [^{11}C]PBR28 have demonstrated significantly increased binding in human brain. [^{18}F]DPA-714 is also a promising PET tracer and has recently demonstrated high affinity for the PBR with better uptake and BP than [^{11}C]-(*R*)-PK11195. Comparative studies of these new radioligands relative to PK11195 are needed to further determine which will lead to the best results in human clinical research. Although (*R*)-[^{11}C]PK11195, [^{11}C]DAA1106 and [^{11}F]PBR28 are currently the most extensively studied PET ligands for the quantification of PBR in human, the development of a new PET tracer that is easier to use, more reliable, and more quantifiable is still required for clinical studies to become more widespread and productive. Most research efforts in this field are currently at the level of animal studies and are aimed at the following key issues: improving ligand metabolic stability, decreasing non-specific binding, developing reliable tracer-kinetic modeling, and finding more reliable methods for PBR quantification.

Currently available PBR ligands combined with PET have enabled the study of neuroinflammatory conditions in ways beyond the scope of conventional imaging techniques. With additional improvements, PET imaging of the PBR could offer a non-invasive modality for early diagnosis of CNS diseases, as well as a strategy for quantitative assessment of disease progression and treatment response. Although most work to date has focused on adult neurological conditions, detection of PBR expression also has potential applications in pediatric disorders, such as in the detection of perinatal brain injury in newborns exposed to intrauterine insults and as a prognostic indicator for the development of white matter injury and cerebral palsy [93]. There is also evidence to suggest that PET imaging of the PBR could be useful in guiding therapy, such as in outlining seizure foci for surgical removal. Finally, early work in animal models suggests that PBR ligands may have several potentially useful effects, from decreasing inflammation [94,95] to promoting neuronal survival

and regeneration in various models of injury [96–98]. PBR ligands have even shown promise as anxiolytic agents, acting via the production of neurosteroids that target the $GABA_A$ receptor [99,100]. These initial findings suggest that PBR ligands have potential as therapeutic agents for the treatment of neurological and psychiatric disorders and that PET imaging with PBR ligands could help in the further development of drugs for human use.

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