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Nuclear imaging of neuroinflammation: a comprehensive review of [¹¹C]PK11195 challengers

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Abstract

Neurodegenerative, inflammatory and neoplastic brain disorders involve neuroinflammatory reactions, and a biomarker of neuroinflammation would be useful for diagnostic, drug development and therapy control of these frequent diseases. *In vivo* imaging can document the expression of the Peripheral Benzodiazepine Receptor (PBR) / Translocator protein 18kDa (TSPO) that is linked to microglial activation and considered a hallmark of neuroinflammation. The prototype Positron Emission Tomography (PET) tracer for PBR, [¹¹C]PK11195, has shown limitations that until now have slowed the clinical applications of PBR imaging. In recent years, dozens of new PET and SPECT radioligands for the PBR have been radiolabelled and several have been evaluated in imaging protocols. Here we review the new PBR ligands proposed as challengers of [¹¹C]PK11195, critically analyze preclinical imaging studies and discuss their potential as neuroinflammation imaging agents.

Key words

Neuroinflammation; PET; [¹¹C]PK11195; Peripheral Benzodiazepine Receptor (PBR); Translocator protein 18kDa (TSPO)

Running title: [¹¹C]PK11195 challengers

Introduction

Inflammation, a physiological response to different types of tissue insults, is a cascade of coordinated chemical and cellular reactions. The inflammatory reaction isolates the damaged tissue and promotes immune responses; eventually, resolution of inflammation is associated with tissue regeneration [1]. Typically, the inflammatory cascade involves a local production of cytokines that underlies cell recruitment and cell differentiation by calling into action specific gene expression. The cellular responses culminate with the formation of a scar, which physically isolates the lesion from healthy tissue.

The central nervous system (CNS) is characterised by a limited regenerative capacity and immune specificities, sometimes gathered under the expression of “immune privilege”, such as the presence of a blood-brain barrier, the lack of normal lymphatic drainage, and a reduced immune surveillance [2]. Acute neuroinflammation following stroke or trauma may contribute to the initial extension of the lesion by increasing neuronal loss in the penumbra, but may also promote subsequent functional recovery by enabling neuronal plasticity [3]. On the other hand, chronic neuroinflammatory processes are suspected to sustain neuronal loss in a number of pathological conditions such as auto-immune diseases (e.g. multiple sclerosis [4]) and neurodegenerative diseases (e.g. Alzheimer disease [5]).

The cellular response during neuroinflammation involves the activation of cells of the monocyte lineage, whether they be resident (microglia) or circulating (monocytes/macrophages). A major feature of acute or chronic neuroinflammation is the activation of microglial cells [6], which drastically change their morphology to become indistinct from peripheral macrophages that infiltrate the brain parenchyma. During the early 80's it was discovered that increased binding of Ro5-4864 (a benzodiazepine) and PK11195 (an isoquinoline) was a hallmark of microglial activation. This led to the experimental validation of the binding of these ligands as an indirect index of neuronal damage [7, 8]. Their binding site in the outer mitochondrial membrane has most often been referred to as the peripheral benzodiazepine receptor (PBR), due to its high level of expression in peripheral organs (e.g. adrenals, kidney), although a new nomenclature i.e. translocator protein (TSPO) (18-kDa) was recently suggested [9]. This binding site is now recognised and used as a marker of neuroinflammation, rather than one of neurodegeneration, although these two processes are often linked. The level of this binding is minimal in normal conditions and locally enhanced following local activation of microglia or macrophage infiltration in damaged areas. Radiolabelling of PBR ligands (i.e. [¹¹C]Ro5-4864 [10] and [¹¹C]PK11195

[11]) allowed the imaging of PBR expression as a surrogate marker of microglial activation, and paved the road to the current concept of positron emission tomography (PET) imaging of neuroinflammation using PBR radioligands [12, 13].

Alternative nuclear-imaging approaches have been proposed to visualize neuroinflammatory processes: (i) imaging inducible forms of nitric oxide synthases [14-17] or cyclo-oxygenases [18-22] by radiolabelling of inhibitors of these enzymes, has not been successful so far. (ii) The use of cobalt-55-PET or cobalt-57-SPECT for calcium tracking following stroke is a non-specific approach to detect neuroinflammatory changes [23-26] and is unlikely to become useful for neurodegenerative disorders. (iii) Leukocyte labelling with [^{99m}Tc]HMPAO for SPECT imaging [27-30] has been widely used for peripheral inflammation but is unsuitable to detect early cellular changes in the brain. MRI-based techniques also challenge PET for the detection of neuroinflammation-related events. Gadolinium contrast enhancement reveals blood-brain barrier leakage [31], macrophages can be labelled by ultrasmall superparamagnetic iron oxide (USPIO) nanoparticles [32, 33], and functionalized contrast agents can document endothelial activation [34, 35]. However, these techniques reflect vascular integrity rather than microglial activation, and the specificity and accuracy of labelling/targeting strategies in molecular MRI remain to be demonstrated.

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 which greatly facilitate quantification. Through appropriate compartmental modeling, PET imaging could offer *quantitative* follow-up of neuroinflammation, an advantage for biomarker-type measurements during drug development and early clinical developments.

[¹¹C]PK11195 was the first tracer to be consistently used in PET imaging studies of neuroinflammation, although numerous limitations have been pointed out: [¹¹C]PK11195 displays a high level of non-specific binding [36, 37] and a poor signal-to-noise ratio which complicates its quantification; in addition, carbon-11 labelling of the molecule (half-life: 20.38 minutes) limits its dissemination and wide clinical use. With the aim to improve quantification of relevant parameters such as kinetic constants (k_n) and binding potential (BP), different groups have proposed several modeling methods for the analysis of [¹¹C]PK11195 imaging data. Using the simplified reference tissue model (SRTM), the group of Lammertsma obtained reasonably accurate BP values, even when arterial blood sampling was not available [38-42]. Voxel-based parametric maps [41-43] using voxels of reference may help to refine the analysis of data from patients with diffuse brain inflammation such as Alzheimer disease patients. Although this type of analysis may give more precise and reliable quantification of

[¹¹C]PK11195 binding, the resulting quantified parameters might be subject to bias (over- or underestimation depending on the model used) and should be interpreted with caution [40]. Further refinements in the processing of parametric maps were proposed recently [43, 44], but the lack of sensitivity of [¹¹C]PK11195 has so far precluded the development of a standard method of analysis easily applicable to all subjects. In particular, methods for quantification run up against the difficulty to define a true reference region, that is, a part of the brain showing no [¹¹C]PK11195 binding. Therefore, until now the interpretations of results have been limited to an emphasis on the general agreement between brain areas of increased [¹¹C]PK11195 uptake and the known distribution of a given pathology [45-50]. Venneti *et al.* [51] have reviewed PET brain imaging studies using [¹¹C]PK11195 and provided a detailed discussion on the kinetic modeling aspects and resulting quantification of PET data. For a complete overview of *in vivo* imaging of neuroinflammation using [¹¹C]PK11195, the reader is referred to their article or that of Cagnin and colleagues [52].

Alternatively, because far more than a descriptive role is awaited from PET imaging of neuroinflammation, many groups worldwide are actively engaged in a search for PBR ligands with improved capacities to quantify PBR expression over [¹¹C]PK11195. During the last years, over 40 new PBR radioligands labelled with the short-lived positron emitters carbon-11 and fluorine-18 (half-life: 109.8 minutes), or with the single-photon longer-lived emitter iodine-123 (half-life: 13.2 hours) were reported in the literature, and more are likely to come. The objective of the present review is to summarize the relevant information for all [¹¹C]PK11195 challengers reported as of June 2008. In the first part, the radiochemistry, kinetic profiles, biodistribution studies, and metabolic analysis of the candidate PBR radioligands are presented according to their classification into 7 different chemical entities. When available, results of *in vivo* imaging studies on rodent models are discussed. Part two presents the preclinical *in vivo* imaging studies of a subset of radioligands that were further studied in primates and humans, generally because they showed promising features during *in vivo* rodent studies. Evaluation of these radioligands has most often been conducted in healthy primates and humans. Finally, we discuss the methodology used for the evaluation of new PBR radioligands with special emphasis on the animal models.

By compiling all references dealing with peripheral benzodiazepine receptor ligands other than [¹¹C]PK11195 into a single comprehensive review, we intend to help the Journal readership quickly survey the literature and hope to contribute advancing this field of considerable medical interest.

PART 1: Screening of PK11195 challengers

PBR radioligands can be subdivided into 7 chemical classes or entities [53] : (1) benzodiazepines, (2) quinoline carboxamides, (3) indoleacetamides, (4) vinca alkaloids, (5) oxodihydropurines, (6) phenoxyarylacetamides, and (7) imidazopyridines and bioisosteric structures (imidazopyridazines and pyrazolopyrimidines).

Table 1 recapitulates the radiolabelled PBR radioligands that, to our knowledge, have been published at the end of June 2008 with related references for radiolabelling procedures, biodistribution, autoradiographic and *in vivo* imaging of normal or neuroinflammatory model rodent studies.

Class 1: benzodiazepines

Historically, the benzodiazepine [¹¹C]Ro5-4864 [10] was synthesized the same year as [¹¹C]PK11195. It was the first molecule able to discriminate peripheral (PBR) from central (CBR) benzodiazepine receptors. Early PET studies of glioma imaging at the end of the 80's gave disappointing results [54, 55] and the binding of this molecule was later shown to differ between species [56]. A iodo-analogue of Ro5-4864 was also radiolabelled to yield a potential SPECT tracer, and *ex vivo* autoradiography using the radioisotope iodine-125 showed accumulation in the tumor of C6 glioma-bearing rats [57].

Class 2: quinoline carboxamides (3-isoquinolinecarboxamides and quinoline-2-carboxamides)

PK11195, a 3-isoquinolinecarboxamide, belongs to this chemical family. The (*R*)-enantiomer was shown to have the highest affinity [36] and has been used in the majority of PET studies so far. Several other closely related structures were radiolabelled, using carbon-11 ([¹¹C]PK11211 and [¹¹C]PK14105 [58]) or fluorine-18 ([¹⁸F]PK14105 [59]). The first fluorine-18 PBR radiotracer, [¹⁸F]PK14105, was assessed in an excitotoxic lesion model and showed faster clearance than [¹¹C]PK11195 [60]. Recently, a non-chiral analogue of PK11195 was radiolabelled with fluorine-18 [61]. [¹²³I]iodo-PK11195 was also developed as a SPECT agent [62], and evaluated in a small series of Alzheimer patients, with results similar to those obtained with (*R*)-[¹¹C]PK11195 [63].

Three analogues of the same quinolinecarboxamide moiety, the quinoline-2-carboxamides VC195, VC193M and VC198M, were radiolabelled with carbon-11. *Ex vivo* distribution demonstrated an interesting kinetic profile with fast uptake and retention in PBR-

rich organs such as adrenal gland, heart or kidney [64]. They were further assessed in the quinolinic acid (QA) rodent model of Huntington disease [65]. *Ex vivo* measurements from 2, 5 and 3 rats at respectively 15, 30 and 60 minutes post-injection showed that the ratio of uptake in the injured striatum over uptake in the control striatum was higher for [¹¹C]PK11195 than for these compounds. In spite of its higher initial uptake in the brain, [¹¹C]VC195 performed as well as [¹¹C]PK11195. Metabolic analysis of these tracers was not performed. The distribution of [¹¹C]VC701, a chloro-derivative of VC195, was also evaluated in healthy rodents, and it was found that PK11195 co-injection affected mostly the peripheral organs [66]. One study reported PET images of the mouse with [¹¹C]VC701 [66], but the brain uptake was not quantified. Fluorine-18-labelled molecules from this class are now awaited.

Class 3: indoleacetamides

This chemical class yielded several highly potent and specific ligands [67, 68]. *In vivo* pharmacological data are available for FGIN-1-27 [69] and SSR180575 [70]. One molecule related to this group was radiolabelled with iodine-123 for SPECT imaging [71], and its biodistribution was evaluated in normal rats [72].

Class 4: vinca alkaloids

Vinpocetine, a drug presenting neuroprotective properties, was radiolabelled with carbon-11 in an attempt to decipher its mechanism of action [73, 74]. The hypothesis that this molecule binds to PBR is supported by a 30% reduction of [¹¹C]PK11195 uptake in primate brain after pre-treatment with 3mg/kg of vinpocetine [75]. However, the uptake of [¹¹C]vinpocetine is increased in brain after PK11195 pre-treatment. Moreover, this molecule has a comparable *in vitro* affinity for PBR (IC₅₀=0.2μM) and for various other receptors (e.g.: adrenergic α₂β receptors, IC₅₀=0.9μM) [74], an observation that questions its *in vivo* specificity for the PBR. Together with a possible contribution of radiolabelled ethanol as a metabolite [76], this is likely to complicate proper quantification of PBR expression using [¹¹C]vinpocetine. Nevertheless, several interesting studies have already been conducted in humans, including MS patients [74, 77, 78] (see part 2).

Class 5: oxodihydropurines

AC-5216, a molecule with *in vivo* PBR-mediated anti-anxiety and antidepressant-like effects [79], has been radiolabelled with carbon-11 and tested in a cancer model [80]. Zhang *et al.* [81] recently published its *ex vivo* distribution in mice, and demonstrated specific

binding displaceable by non radiolabelled PK11195 in the olfactory bulbs and in the cerebellum. One metabolite found in brain tissue represented 10% of total radioactivity after one hour. The authors also performed PET imaging in the primate brain (see part 2). The same group reported an *in vitro* and *ex vivo* autoradiographic study in rats with a striatal lesion induced by kainic acid [82]: as expected, binding was increased in injured areas, and no metabolites were detected in brain homogenates. However, *ex vivo* measurements showed an increased uptake in the intact parts of the brain 30 minutes after co-injection of the radiotracer and non-radiolabelled PK11195 (10mg/kg) or AC-5216 (1mg/kg). This results from displacement of the radiotracer from specific binding sites in peripheral organs and suggests a high level of non-specific binding in the brain.

Class 6: phenoxyarylacetamides

An important group of new PBR radiotracers are the derivatives of DAA1106, a high-affinity PBR ligand with a chemical structure based on the opening of the diazepine ring of Ro5-4864.

Radiolabelling of DAA1106 with carbon-11, together with its *ex vivo* biodistribution in mice, was reported in 2003 [83]. Blocking studies showed that PK11195 (1mg/kg) could inhibit 50 to 85% of tracer uptake in various brain regions at 30 minutes. Only the intact radiotracer was detected in brain homogenates after one hour. A following report showed *in vitro* and *ex vivo* autoradiographies of rats bearing hippocampal lesions induced by 10 nmoles of kainic acid (KA), together with brain distribution studies in healthy rhesus monkeys [84]. Autoradiographies performed 7 days after KA infusion showed that [¹¹C]DAA1106 binding in the area of the lesion was twice the binding of that in the non-lesioned cortex. The reason for not choosing the contralateral hippocampus as the reference region was not given by the authors. It can be speculated that perhaps diffuse contralateral microglial activation precludes the use of that region as a true control region devoid of PBR expression. Comparison with the performance of [¹¹C]PK11195 in that particular model has not been assessed. However, brain distributions of [¹¹C]DAA1106 and [¹¹C]PK11195 were compared in the same rhesus monkey (see part 2). Three recent reports also suggest a stronger *in vivo* accumulation for [¹¹C]DAA1106 than for [¹¹C]PK11195 in the rabbit kidney [85] and the rat brain [86, 87]. A direct comparison of [¹¹C]DAA1106 and [¹¹C]PK11195 has been published using three different neuroinflammatory models: intrastriatal lesions with lipopolysaccharide (LPS) and with 6-Hydroxydopamine [87], and a traumatic brain injury (TBI) model [86]. Discrepancies were found between the results of the blocking studies, which showed that PK11195, in

contrast to DAA1106, did not fully displace [^{11}C]DAA1106. DAA1106 efficiently binds to PBR *in vitro*, and although flumazenil did not displace [^{11}C]DAA1106, its *in vivo* specificity for PBR in rodents remains an open question because of its partial displacement by PK11195 and of the diffuse uptake observed around brain lesions. However, the partial displacement of [^{11}C]DAA1106 by PK11195 may also reflect heterogeneity in the PBR binding site for both ligands. [^{11}C]DAA1097 and two others carbon-11 analogues were studied using *ex vivo* autoradiography in rats and PET imaging in rhesus monkey [88] (see part 2).

Two additional candidates, [^{11}C]PBR01 and [^{11}C]PBR28, were presented during the 16th International Symposium on Radiopharmaceutical Chemistry (2005) [89], and PET imaging in primate with both tracers was recently published [90-92] (see part 2). [^{11}C]PBR28 was further assessed in a neuroinflammation imaging protocol using the permanent middle cerebral artery occlusion (MCAO) rodent model of ischaemia [93]. Brain damage and PET regions of interest (ROI) were determined by histology, and autoradiography with [^3H]PK11195 correlated with PET distribution volume obtained through modeling with arterial blood sampling. One radioactive metabolite was found in plasma and was later reported to account for 10-15% of PET signal in the brain [91, 94]. Despite the detection of metabolites, displacement by 10mg/kg PK11195 resulted in a slow decline of the uptake in the core infarct and in the peri-infarct regions until their levels reached that of the contralateral hemisphere. In this case, the bolus-infusion protocol ensured a steady state and relative independence from blood flow variations. The maximal increase reported for an individual rat in the penumbra was threefold the uptake in the contralateral area, a value similar to that of [^{11}C]PK11195 binding measured in the infarct core after a transitory MCAO as reported by Rojas *et al.* [95]. Overall, a direct comparison study between [^{11}C]PBR28 and [^{11}C]PK11195 would be interesting in order to eliminate animal model differences and appreciate the potential of this tracer. Due to partial metabolism, a deuterated analogue, *d*₃- [^{11}C]PBR28, and a fluorine-18 alternative, termed [^{18}F]FEPPA, were also developed [94].

Recently, iodine-131 labelling of two DAA1106 analogues yielded future candidate radiotracers for SPECT using iodine-123 and PET using iodine-124 [96]. Data from *ex vivo* biodistribution in mice and autoradiography in rats suggest specific binding as shown by pre-treatment studies, although the true level of non specific binding cannot be adequately appreciated in healthy animals.

Several additional fluorine-18-labelled molecules of this chemical class have also been developed for PET imaging. One, termed [^{18}F]PBR06, was produced by the same group who reported labelling and PET imaging of [^{11}C]PBR28 and [^{11}C]PBR01, and was also studied in

primates [90] (see part 2). The free fraction in plasma was found to be the highest for [¹¹C]PBR28 with a value of 5.6% [91, 92].

In addition to that of [¹⁸F]DAA1106 [97], radiosynthesis of [¹⁸F]FMDAA1106 and [¹⁸F]FEDAA1106 [98] have been reported. These two compounds readily entered the brain and showed specific uptake according to *ex vivo* autoradiography in rats and *ex vivo* biodistribution in mice [98]. However, their metabolism differed significantly, with the fluoromethyl group of [¹⁸F]FMDAA1106 undergoing intense defluorination, whereas the fluoro-ethyl group of [¹⁸F]FEDAA1106 was relatively preserved. Similar observations were made in a primate PET imaging study, which showed a high uptake of radioactivity in the skull following [¹⁸F]FMDAA1106 injection, whereas [¹⁸F]FEDAA1106 had a rather homogenous and long-lasting brain tissue uptake [99]. Deuteration of the fluoromethyl group (*d*₂-[¹⁸F]FMDAA1106) led to a slower metabolic rate, in rodents, but not in primates [100], and therefore it is likely that *in vivo* defluorination will preclude further development of [¹⁸F]FMDAA1106. In contrast, no metabolites were detected in the mouse brain homogenates with [¹⁸F]FEDAA1106, and PET imaging in rhesus monkey showed a high bioavailability and a good selectivity (see part 2). Preliminary imaging data have been reported for [¹⁸F]FEDAA1106 in the rat brain [101]. This radioligand was recently used to detect the neuroinflammatory response to anti-amyloid treatment in transgenic mice [102].

Additionally, several radioligands of this class, namely [¹¹C]DAA1106, [¹⁸F]FEDAA1106 and [¹¹C]PBR28 have been studied quantitatively in pioneer human studies [103-106] discussed in part 2.

Class 7: Imidazopyridines and bioisosteric structures (imidazopyridazines and pyrazolopyrimidines)

Alpidem, which binds potently to CBR and PBR, is the primary member of the imidazo[1,2-a]pyridine class, from which are derived bioisosteric structures such as imidazo[1,2-b]pyridazines and pyrazolo[1,5-a]pyrimidines.

New imidazo[1,2-a]pyridines selective for PBR were labelled with iodine-123 in order to generate potential SPECT tracers. Interestingly, [¹²³I]IZOL, a iodo-analogue of zolpidem, which pharmacological effect is supposed to be mediated by CBR binding, turned out to distribute in rats like a PBR radioligand [107]. Blocking experiments using PK11195, Ro5-4864, or cold IZOL, revealed a significant reduction of binding in the olfactory bulb, but increased uptake in others parts of the brain. This observation is reminiscent of similar findings with [¹¹C]PK11195, and could suggest a high non specific binding of the molecule.

A series of 6-chloro substituted imidazo[1,2-a]pyridines were radiolabelled through the same iodophenyl group [108]. One of these compounds, [¹²³I]CLINDE, was used for *ex vivo* autoradiography of spinal cord sections of rats with experimental autoimmune encephalomyelitis (EAE) [109]. [¹²³I]CLINDE uptake in the spinal cord increased with the severity of the disease, expressed as a clinical score. Spatial distribution of the radioactivity was in agreement with the level of infiltrating macrophages detected using immunohistochemistry, and the increase in uptake could be inhibited by PK11195 pre-treatment. In a different study using a cancer model, metabolic analysis suggested that [¹²³I]CLINDE was stable during the time of SPECT examination [110, 111]. Two derivatives were radiolabelled with fluorine-18 and their *ex vivo* biodistributions were studied without testing for the presence of metabolites [112]. A carbon-11 analogue, [¹¹C]CLINME, was developed [113] and compared to [¹¹C]PK11195 in an experimental model of neuroinflammation, using a small-animal dedicated PET camera [114]. CLINME has also been labelled with iodine-123 for SPECT imaging [115].

Radiolabelling procedures with carbon-11 of four other derivatives closely related to the PBR ligand CB-34, which presents interesting *in vivo* pharmacological effects [116], have recently been reported, together with preliminary biodistribution data [117].

Several imidazo[1,2-b]pyridazines were radiolabelled with iodine-123 [118] and with fluorine-18 [119]. *Ex vivo* biodistribution was reported for the latter [120].

Different groups have recently drawn attention to new pyrazolo[1,5-a]pyrimidine molecules, labelled with carbon-11 or fluorine-18. [¹¹C]DPA-713 was originally reported to slowly accumulate in the baboon brain [121, 122] (see part 2), and a similar time-activity curve was observed in pigs [123]. [¹¹C]DPA-715 is a derivative in which the two methyl groups of DPA-713 are replaced by trifluoromethyl groups [124]. [¹⁸F]DPA-714, a fluorine-18 analogue of DPA-713, was recently evaluated *in vivo* in baboons (see part 2) and *ex vivo* in QA-injured rats, in which an 8-fold increase in binding was obtained in the lesion with respect to the undamaged contralateral side [125].

[¹¹C]CLINME [114], [¹¹C]DPA-713 [126] and [¹¹C]DPA-715 [124] were evaluated comparatively to [¹¹C]PK11195, seven days after a unilateral intrastriatal injection of α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) in rats leading to a local microglial activation and macrophage invasion. Brain imaging of the three radiotracers performed at identical time points after injection showed that the uptakes of [¹¹C]CLINME and [¹¹C]DPA-713 were at the same levels to that of [¹¹C]PK11195 in the inflamed lesioned area, while the uptakes in the intact area were lower in comparison to that of [¹¹C]PK11195.

As a result, [^{11}C]CLINME [114] and [^{11}C]DPA-713 [126] achieved a significantly higher inflamed-to-intact area ratio, considered as a reliable estimate of the target-to-background ratio, than [^{11}C]PK11195. In addition, the uptakes of [^{11}C]CLINME and [^{11}C]DPA-713 were completely abolished in the lesioned areas following injection of non radiolabelled PK11195 or the unlabelled corresponding ligands (1mg/kg), and no metabolites were detected in brain homogenates up to half an hour after tracer injection. Taken together, these results favour [^{11}C]CLINME and [^{11}C]DPA-713 as promising candidates for future studies of neuroinflammation. Conversely, despite its higher lipophilicity [^{11}C]DPA-715 showed a very low level of brain uptake resulting in a very weak contrast in the lesion, results which preclude further development of this ligand [124].

Doorduyn *et al.* compared [^{11}C]DPA-713, [^{18}F]DPA-714 and [^{11}C]PK11195 in a rat model of herpes encephalitis, and found no significant difference in sensitivity between the 2 carbon-11-labelled radiotracers [127], while a better contrast was obtained using [^{18}F]DPA-714 [128].

Table 1. Summary of PBR ligands radiolabelling and animal distribution studies.

Chemical class		Radioligand code (if existing)	Structure / Radiosynthesis	<i>Ex vivo</i> biodistribution / Autoradiography	<i>In vivo</i> imaging	Neuroinflammation model
1	benzodiazepines	[¹¹ C]Ro5-4864	[10]	[8] (review)		
		[¹²³ I]iodo-Ro5-4864	[57]	[57]		C6 glioma [57]
2	3-isoquinoline-carboxamides	[¹¹ C]PK11195	[11, 58]	[51, 52] (reviews)		
		[¹²³ I]iodo-PK11195	[61]			
		[¹¹ C]PK11211 [¹¹ C]PK14105	[58]			
		[¹⁸ F]PK14105	[59]	rat [60]		kainic acid (rat, intrastriatal) [60]
		one [¹⁸ F] non-chiral derivative of PK11195	[61]			
	quinoline-2-carboxamides	[¹¹ C]VC193M [¹¹ C]VC195 [¹¹ C]VC198M	[64]	rat [65]		quinolinic acid (rat, intrastriatal) [65]
		[¹¹ C]VC701	[66]	rat [66]	mouse [66]	
3	indoleacetamides	[¹²³ I]PBR200	[71]	rat [72]		
4	vinca alkaloids	[¹¹ C]vinpocetine	[73]		primate [73, 75, 76]	
5	oxodihydropurines	[¹¹ C]AC-5216	[80]	rodent [80, 81, 82]	primate [81]	kainic acid (rat, intrastriatal) [82]

Table 1. (Continued)

Chemical class		Radioligand code (if existing)	Structure / Radiosynthesis	<i>Ex vivo</i> biodistribution / Autoradiography	<i>In vivo</i> imaging	Neuroinflammation model
6	phenoxyaryl-acetamides	[¹¹ C]DAA1106	[83]	mouse [83], rat [84]	primate [84], rabbits [85], rat [86, 87]	kainic acid (rat, hippocampus) [84], TBI (rat) [86], LPS, 6-OHDA (rat, intrastriatal) [87]
		[¹⁸ F]DAA1106	[97]			
		[¹¹ C]DAA1197 & two [¹¹ C] derivatives	[88]	rat [88]	primate [88]	
		[¹¹ C]PBR01	[89, 90, 91]		primate [89, 90, 91]	
		[¹⁸ F]PBR06	[90]		primate [90]	
		[¹¹ C]PBR28	[89, 91]		primate [89, 91, 92] rat [93]	ischemia (rat, MCAO) [93]
		[¹⁸ F]FEPPA	[94]	rat [94]		
		two [¹³¹ I] derivatives of DAA1106	[96]	rodent [96]		
		[¹⁸ F]FMDAA1106 [¹⁸ F]FEDAA1106	[98]	rodent [98]	primate [99]	alzheimer (mouse, transgenic) [102]

Table 1. (Continued)

Chemical class		Radioligand code (if existing)	Structure / Radiosynthesis	<i>Ex vivo</i> biodistribution / Autoradiography	<i>In vivo</i> imaging	Neuroinflammation model
7	imidazo-[1,2a]-pyridines	[¹²³ I]IZOL	[107]	rat [107]		
		[¹²³ I]CLINDE and two [¹²³ I] derivatives	[108]	rat [108] ([¹²³ I]CLINDE)		EAE (rat) [108] ([¹²³ I]CLINDE)
		two [¹⁸ F] derivatives of CLINDE	[112]	rat [112]		
		[¹¹ C]CLINME	[113]		rat [114]	AMPA (rat, intrastriatal) [114]
		[¹²³ I]CLINME	[115]	rat [115]		
		four [¹¹ C] derivatives of CB34	[117]	mouse [117]		
	imidazo-[1,2b]-pyridazines	two [¹²³ I] derivatives	[118]			
		one [¹⁸ F] derivative	[119]	rat [120]		
	pyrazolo-[1,5-a]-pyrimidines	[¹¹ C]DPA-713	[121, 122]		primate [121], pig [123], rat [126, 127]	AMPA (rat, intrastriatal) [126], herpes encephalitis (rat) [127]
		[¹⁸ F]DPA-714	[125]	rat [125]	primate [125], rat [128]	quinolinic acid (rat, intrastriatal) [125], herpes encephalitis (rat) [128]
		[¹¹ C]DPA-715	[124]		primate, rat [124]	AMPA (rat, intrastriatal) [124]

PART 2: Preclinical Studies of PK11195 challengers

Non-human primate studies

To our knowledge, 16 PBR radioligands have been engaged in imaging protocols in non-human primates, all in healthy animals. Table 2 summarizes the results obtained with [^{11}C]PK11195 and with the most promising compounds among each series. Apart from the animal species, the main differences between studies concern the ROIs used to generate time-activity curves, which include whole brain, putamen, cortical grey matter and occipital cortex. Occipital cortex has been reported to yield the highest [^3H]PK11195 binding sites in humans [129].

In the brains of animals unchallenged by neuroinflammation, two different biodistribution profiles emerge independently of the chemical class of the radioligands,: [^{11}C]PK11195, [^{11}C]DAA1106, [^{11}C]DAA1097 [^{18}F]FEDAA1106 and [^{11}C]AC-5216 peak early (1 to 15 minutes) after injection, whereas [^{18}F]PBR06, [^{11}C]PBR28, [^{11}C]DPA-713, [^{18}F]DPA-714 slowly accumulate over 20 to 40 minutes. [^{11}C]PK11195 uptake was shown to slowly decrease after the distribution phase, and neither pre-treatment nor displacement could modify this low basal uptake [37]. This has been regarded as a major indication that [^{11}C]PK11195 yields a high level of non-specific binding in the primate brain.

In contrast to [^{11}C]PK11195, [^{11}C]DAA1106, [^{11}C]DAA1097 and [^{18}F]FEDAA1106, as well as [^{11}C]AC-5216, displayed a high and quasi constant cerebral uptake. Pre-treatment and displacement studies pointed to the slowly reversible binding of [^{11}C]DAA1106, 50 and 75% of the initial binding being abolished by 5mg/kg of PK11195 and 1mg/kg of DAA1106, respectively. Those differences may result from the higher affinity of DAA1106 for PBR when compared to PK11195, but could also suggest different binding sites on the PBR, a different level of non specific binding or reflect differences in blood extraction due to different levels of lipophilicity (logP of DAA1106 is 3.7 vs 2.7 for PK11195). [^{11}C]DAA1097 displayed a high level of non-specific binding, since a pre-treatment by either PK11195 or DAA1106 induced only a moderate decrease in its uptake [88]. In contrast, the binding of [^{11}C]AC-5216 could be blocked to the same extent as that of [^{11}C]DAA1106, despite its slightly slower entry into brain. For these two radioligands, non displaceable binding represented 60% of total brain uptake after pre-treatment by 5mg/kg PK11195 [81].

In contrast, pre-treatment with either PK11195 or DAA1106 reduced the uptake of [^{18}F]FEDAA1106 to respectively 30 and 20% of the baseline value. This basal uptake in brain was 1.5 times higher than for [^{11}C]DAA1106 and 6 times higher than for [^{11}C]PK11195 in the

rhesus monkey [99]. However, in contrast to [^{11}C]DAA1106, reversibility of the binding in a displacement protocol has not been tested for [^{18}F]FEDAA1106. Overall, even if [^{18}F]FEDAA1106 has not yet directly been compared with [^{11}C]PK11195 in a situation of pathophysiological microglial activation, this fluorinated tracer is an interesting candidate because of its high bioavailability in brain tissue and of its reduced non specific binding, at least when compared with [^{11}C]DAA1106.

[^{11}C]PBR28 showed high brain penetration in rhesus monkeys and a decrease of about two thirds of its cortical uptake after displacement by 12mg/kg of PK11195 or 1mg/kg of DAA1106 [89]. Recent reports showed that a co-injection of the radiotracer with 3mg/kg DAA1106 resulted in a decrease of 76% of the initial radioactivity [92], and that displacement with PK11195 at 5mg/kg resulted in a 70% decrease [91]. [^{18}F]PBR06 accumulation in the brain was similar to that of [^{11}C]PBR28, and pre-treatment with DAA1106 resulted in a reduction of 80% of the [^{18}F]PBR06 uptake [90].

[^{11}C]DPA-713 [121] and [^{18}F]DPA-714 [125] exhibited both a low uptake in the brain and comparable specificity, with approximately two thirds of the total binding being abolished by PK11195 pre-treatment.

Overall, the results summarized in Table 2 tend to indicate that most of the new PBR radioligands show a lower level of non specific binding in the primate brain than [^{11}C]PK11195, with the possible exception of [^{11}C]DAA1097. However, a direct comparison between radioligands is complicated by differences in the species studied, in the choice and delineation of the ROI, and in the read out units. Moreover, in most cases the absence of compartmental modeling, as well as the various doses and agents used for blocking studies preclude any conclusive comparison of the ratio of specific to non-specific (displaceable) signal. Finally, the results obtained with the radiotracers in healthy animals in the absence of any inflammatory challenge cannot predict their ultimate performance in patients undergoing inflammatory reactions.

Table 2. Competition studies in primates

radioligand	species	ROI	control experiment		pretreatment or displacement experiments					Reference
			time to reach max uptake	max uptake ¹	agent	dose (mg/kg)	injection time (T0=PET)	min uptake ²	specific binding (%) ³	
[¹¹ C]PK11195	baboon	cortical grey matter	3-5 min	0.008% ID/ml	PK11195	1	co-injection	0.004% ID/ml	n.d.	[37]
	rhesus monkey	occipital cortex	1 min	0.015% ID/ml	PK11195	1	+ 8 min	0.004% ID/ml	n.d.	
[¹¹ C]DAA1106	rhesus monkey	occipital cortex	3 min	0.020% ID/ml	PK11195	5 & 10	- 5 min	0.010 % ID/ml	60%*	[84]
					DAA1106	0.5	- 5 min	0.010 % ID/ml	60%*	
					DAA1106	1	- 5 min	0.005 % ID/ml	80%*	
					PK11195	5	+ 30 min	0.010 % ID/ml	50%*	
					DAA1106	1	+ 30 min	0.005 % ID/ml	75%*	
[¹¹ C]DAA1097	rhesus monkey	occipital cortex	2 min	0.015% ID/ml	PK11195	5	- 2 min	0.014 % ID/ml	25%*	[88]
					DAA1106	1	- 2 min	0.012 % ID/ml	30%*	
[¹⁸ F]FEDAA1106	rhesus monkey	occipital cortex	5 min	0.030% ID/ml	PK11195	5	- 2 min	0.010 % ID/ml	70%	[99]
					DAA1106	1	- 2 min	0.005 % ID/ml	80%	
[¹¹ C]AC-5216	rhesus monkey	occipital cortex	15 min	0.015% ID/ml	PK11195	1	- 5 min	0.008 % ID/ml	50%*	[81]
					PK11195	5	- 5 min	0.006 % ID/ml	60%*	
					AC-5216	0.1	- 5 min	0.008 % ID/ml	50%*	
					AC-5216	1	- 5 min	0.006 % ID/ml	60%*	
[¹¹ C]PBR28	rhesus monkey	whole brain	40 min	300% SUV	DAA1106	3	co-injection	73% SUV	76%	[91, 92]
					PK11195	5	+ 45 min	90% SUV	70%	
[¹⁸ F]PBR06	rhesus monkey	putamen	43 min	300% SUV	DAA1106	3	- 24 min	50% SUV	80%	[90]
[¹¹ C]DPA-713	baboon	whole brain	25 min	0.006% ID/ml	PK11195	5	- 5 min	0.002% ID/ml	65%*	[121]
[¹⁸ F]DPA-714	baboon	whole brain	20 min	0.008% ID/ml	PK11195	1.5	- 5 min	0.002% ID/ml	65%*	[125]
					DPA-714	1	+ 20 min	0.003% ID/ml	60%*	

¹ Uptake at plateau (at peak value for [¹¹C]PK11195)

² Uptake at the end of the scanning time

³ Specific binding (%) = (1 - (uptake in ROI in pre-treatment or displacement experiment) / (uptake in ROI in control experiment))*100

* Recalculated from published data (+/- 5%)

Human studies

Several new PBR radioligands were tested in humans during the past two years. In line with the non-human primate studies, the cerebral uptake of [^{11}C]DAA1106 [103] and [^{18}F]FEDAA1106 [104] reached much higher levels than (*R*)-[^{11}C]PK11195, and a very slow wash-out was observed for both radiotracers. In these studies, methods were proposed to accurately quantify PBR expression using the two-tissue compartment model with an arterial input function, but no other compartment models have been explored. Concerning [^{11}C]DAA1106, the nonlinear least-squares method was shown to be more reliable than graphical and multilinear analysis for the determination of the BP [103]. Mean BP ranged from 4.4 (striatum) to 5.5 (thalamus) for the nine healthy subjects scanned. The same conclusion was drawn for BP determination of [^{18}F]FEDAA1106, with mean BP for 7 healthy subjects ranging from 4.7 (cerebellum, striatum) to 5.3 (occipital cortex, thalamus) [104]. Relatively large inter-individual differences in the distribution volume of the free and nonspecific binding compartment (K_1/k_2) were noted for both radioligands. A recent study reported an increased binding of [^{11}C]DAA1106 in Alzheimer's disease [106]. Differences between control subjects and patients were found significant in various regions of the brain, although there was an overlap in the distribution of BP values between the two groups. Similar results in Alzheimer's patients have been reported in previous studies using (*R*)-[^{11}C]PK11195 [45] and [^{123}I]iodo-PK11195 [63].

In the case of [^{11}C]PBR28, time-activity data were analyzed with both the one- and two-tissue compartment models including a plasma input function, and important differences were noted between the pharmacokinetics of [^{11}C]PBR28 in humans and non-human primates [105]. The brain biodistribution was rather uniform, peaked earlier in humans than in primates and washed out gradually. In addition, receptor binding in the human brain was much lower (20-fold) than in non human primate brain. Data modeling suggested that an increased proportion of brain tracer uptake was due to non-specific binding in humans, compared with non human primates. A continuous increase of the total distribution volume was consistent with the entry of a radiolabelled metabolite into the brain, and this has also been observed in animals. More surprisingly, in 2 subjects out of 12, [^{11}C]PBR28 distributed as if there had been no specific PBR binding in the brain nor in the periphery, a condition that is mimicked in animals by receptor blockade. Those results highlight critical inter-species differences in the binding of PBR ligands, and suggest possible intra-species differences in the binding sites of the multimeric mitochondrial PBR complex, i.e. the existence of sub-types of PBR. A

clinical trial in HIV-seropositive patients with or without minor cognitive motor disorder (MCMD) is under way [130]. It is noteworthy that two similar trials performed using (*R*)-[¹¹C]PK11195 reached different conclusions in the ability of this radioligand to distinguish between normal controls, and neurocognitively non-impaired or impaired HIV-infected patients [131, 132], highlighting both the low sensitivity of (*R*)-[¹¹C]PK11195 and the importance of the analytical method.

Vinpocetine is in pharmacological use as a neuroprotective agent, and despite the lack of conclusive data concerning its specificity for PBR in animals, [¹¹C]vinpocetine has been tested in humans in two studies designed to decipher its biodistribution after intravenous [74], and oral [77] administration. The highest brain regional activity was consistently found in the thalamus. Vas *et al.* recently published a preliminary study comparing [¹¹C]vinpocetine and [¹¹C]PK11195 in four MS patients [78]. BP values in spherical peri-plaque regions as well as in the thalamus and in the frontal cortex were determined using the Logan linear graphical analysis with the cerebellum as a reference region. BP values were higher for [¹¹C]vinpocetine than for [¹¹C]PK11195 in all regions of interest, but a different pattern of accumulation was noticed in the peri-plaque area: [¹¹C]PK11195 tended to accumulate in and around the plaque whereas [¹¹C]vinpocetine showed higher uptake in more elongated regions outside the plaque. These observations suggest different binding sites for the two radiotracers and call for further studies in experimental models of MS.

Discussion

During the last decade, 45 new ligands of the PBR were radiolabelled with carbon-11, fluorine-18 or iodine-123; biodistribution was assessed for 36 of these, and nearly 20 were evaluated in imaging studies. This unprecedented effort is sustained by the need for a biomarker of the spatio-temporal extent of neuroinflammation, in order both to explore the extent and time course of neuroinflammation in various diseases and to document the effects of drugs on the neuroinflammatory reaction. Even though it was introduced two decades ago and has been the subject of many preclinical and clinical reports, [¹¹C]PK11195 suffers from unresolved methodological issues, such as pharmacological effects independent of PBR binding, low sensitivity, and a limited capacity to quantify subtle PBR expression *in vivo*. Therefore, the ultimate aim of research for PK11195 alternatives is to obtain a radioligand that will perform better than [¹¹C]PK11195 for the quantification of PBR expression *in vivo*. Provided that the requirements for a valid neuroreceptor radioligand (i.e. high affinity, significant brain passage, absence of radiolabelled brain metabolites) are met, a key issue is to demonstrate the absence of non specific binding. This largely determines the reliability and sensitivity of kinetic modeling methods which are required to assess the level of PBR expression quantitatively.

However, low but significant levels of PBR are expressed in the normal human brain, in the absence of neuroinflammation [129]. This may create confusion between the non specific binding of a given radioligand and its binding to diffusely expressed PBR, and complicates the validation of new PBR radioligands in humans. In that respect, laboratory rodents are ideal as their brain, with the exception of the olfactory bulbs and choroid plexus, are known to express undetectable PBR levels in the absence of neuroinflammatory challenge [133]. Therefore, non specific binding can be estimated directly from the level of binding in non inflammatory parenchymal areas in these species. Conversely, because PBR expression is absent or at best very limited in the rodent intact CNS, biodistribution in healthy animals does not provide sufficient evidence to promote or reject a candidate radiotracer, and the use of a relevant animal model is indispensable for rigorous evaluation in the early screening phase. The diversity of neuroinflammatory animal models complicates a meaningful comparison amongst the different candidates. Here we discuss the choice of the animal model, an issue of particular importance for neuroinflammation imaging.

Excitotoxic lesions provide robust, well characterized glial reactions in the rat brain [134]. The damages induced by e.g. QA, KA or AMPA can be documented by GFAP and

CD11b immunohistochemistry: following neurodegeneration which occurs within a few days, activated microglia and blood-borne macrophages fill the lesion site, and reactive astrocytes form the so-called “glial scar” around the injection site. The result is a well localized area rich in PBR-overexpressing cells, while the brain’s other regions remain devoid of the PBR target. Therefore, excitotoxic lesions appear favourable for PBR radioligand assessment as they allow a simple target-to-background ratio measurement, which can be challenged by competition for binding of unlabelled PBR ligands. Inter-study comparison can be complicated by variations in both the nature and the dose of excitotoxin and in the time course of the inflammatory reaction, so it may be useful to use [¹¹C]PK11195, despite its limitations, as a comparison point.. However, one major caveat of the excitotoxic lesion models lies in the breakdown of the blood-brain barrier induced by intracerebral administration of the toxin that may influence tissue uptake of the radiotracer (i.e. leaking into the damaged area). This breakdown is however reversible within few days [135], and, depending on the toxin used, restoration of the blood-brain barrier can be completed at a time when there is still intense microglial activation around the lesion. Naturally, restoration should be controlled properly for rigorous determination of PBR radiotracer binding *in vivo*.

EAE, an auto-immune model that can be induced in mice and rats by immunization with myelin basic protein (MBP), has been proposed as an alternative to excitotoxic models. The immunization results in an evolving neuroinflammatory reaction mediated by T lymphocytes that culminates in the formation of sparse lesions in brain parenchyma and spinal cord. In several EAE models, demyelinating lesions are observed along with inflammatory infiltrates in the spinal cord and in the brain. The advantage of EAE models is that they mimic a true human neuroinflammatory disorder (i.e. multiple sclerosis). However, differences in EAE protocols and models result in important variability in the type of neuroinflammatory lesions in terms of their diffusion and intensity. In addition, most EAE models affect the brain globally, therefore precluding the use of a reference region free of inflammation to test specificity of binding. It would be interesting to test PBR imaging in the recently described focal EAE models [136].

In contrast, a localized inflammatory region may be reliably and reproducibly obtained in experimental ischaemia protocols. However, the complex time course of glial activation after ischemia, involving both the necrotic infarct core and the apoptotic-proned penumbra, complicates the accurate delineation of ROIs and may bias inter-study comparisons. Excitotoxic and ischaemic models are also feasible in primates but represent considerable experimental burden and ethical issues.

Transgenic mouse models of brain disorders have the advantage to be more clinically relevant than excitotoxic models and to show a pathological time-course that can be followed up using *in vivo* imaging studies. However, due to the small size of the mouse brain in respect to the spatial resolution of PET or SPECT, analyses are often limited to whole brain examination. Hence, *in vivo* specificity is not easily demonstrated in mice due to the lack of a realistic reference region. Fortunately, access to rat transgenic models of neurodegenerative disease is rapidly extending, through the generation of transgenic strains [137] or the use of lentiviral vectors [138].

Overall, it appears reasonable to assume that robust models of neuroinflammation, such as those created by excitotoxic injection, that can include a comparison with [¹¹C]PK11195, provide a simple screen for the initial assessment of a new PBR radioligand. However, the inconsistent use of neuroinflammation models in the present literature review is striking: for 21 molecules out of 36, their biodistribution, whether obtained *ex vivo* or *in vivo* through imaging, has only been reported in normal, non inflammatory tissue. As a result, it appears difficult to draw conclusions on which of these radiotracers has the best prospective to replace [¹¹C]PK11195. Models that are more relevant to human pathologies (EAE, ischaemia, transgenic animal models of neurodegenerative diseases...) may offer the opportunity of longitudinal studies in order to evaluate whether the observed gain in contrast with a new radioligand translates into a better sensitivity.

Radioligands of neuroinflammation that show a significantly improved target-to-background ratio over [¹¹C]PK11195 in a rodent neuroinflammatory model might be considered as promising alternatives. Nevertheless, due to species differences, translation of rodent studies might end up in disappointing results as it has been the case for [¹¹C]Ro5-4864 and [¹¹C]PBR28. Therefore, tracers should subsequently be evaluated in primates that, in addition, allow blood sampling during PET imaging, and hence enable to define the arterial input function which is necessary for modeling the data. These additional criteria allow to refine the selection of the best tracer for neuroinflammation and, accordingly, those tracers that appear to advance more quickly to clinical applications have generally followed this development scheme. Many of these tracers belong to classes 6 and 7, i.e. the phenoxyarylacetamides and the imidazopyridines and pyrazolopyrimidines. [¹¹C]CLINME and [¹¹C]DPA-713 have been tested in excitotoxic lesion models where they demonstrated a higher target-to-noise ratio than the (*R*)-[¹¹C]PK11195. Studies with [¹¹C]DAA1106 and [¹¹C]PBR28 indicated favourable pharmacokinetic properties, and their binding profile is now being deciphered in humans. An exception on what may be considered a “standardized”

screening procedure is vinpocetine, originally a drug with neuroprotective properties that has been advanced into clinical studies of MS patients even though it showed affinity for multiple binding sites other than the PBR. The appearance of promising fluorine-18 tracers, [¹⁸F]PBR06, [¹⁸F]FEDAA1106 and [¹⁸F]DPA-714, is noteworthy, and represents a decisive step for wider dissemination of PBR imaging in clinical studies. Finally, screening of other classes of molecules that remain poorly explored (e.g. indoleacetamides) could yield future interesting compounds.

Despite the multiplicity of specific radioligands characterized, there is a clear lack of knowledge concerning the functions and basal expression of the PBR. Apart from the existence of sub-types of the receptor, that remains hypothetical, cellular expression in normal brain tissue, especially in astrocytes, has been advanced as an explanation for differential basal binding pattern between radioligands [101, 106, 129, 139]. The interpretation of the PET data in pathological conditions will require those topics to be examined in greater details. Finally, recent basic research has provided a wealth of potential new biomarkers of CNS immunological reactions. These include new binding sites on microglial cells and infiltrating macrophages, like the non-angiotensin II [¹²⁵I]CGP42112 binding site [140], the Iba-1 molecule [141], the CD200 receptor [142, 143], toll-like receptors [144], matrix metalloproteinases [145], chemokine receptors [146], as well as targets expressed on endothelial cells, like selectins [35, 147] and cell adhesion molecules [148-150], or on astrocytes, such as specific gap junction constituents (e.g. Cx43 [151, 152]) and type-2 deiodinase [153]. The utility of these targets for neuroinflammation imaging will ultimately depend on the identification of radioligands with appropriate biodistribution and sensitivity.

Conclusion

The remarkably large number of candidates PBR ligands that have been radiolabelled recently witnesses a strongly growing interest for this target. Though the basic physiological role of PBR remains incompletely understood, its expression in the brain is currently the most reliable marker for the imaging of neuroinflammation, which is linked to neuronal damage. Its quantification would allow the staging of the disease severity or/and the evaluation of therapeutics. Nuclear imaging is unique in this respect, and PET or SPECT imaging of PBR expression appears indispensable in follow-up studies of neuroinflammatory diseases as well as in the evaluation and monitoring of putative therapies. A referent biomarker for neuroinflammation drug studies is highly needed, and it would be timely that a PET tracer is recognized as such, in line with the increasing use of PET for drug approval studies [154]. Finally, considering the large amount of work necessary and the number of radiolabelled candidates, exchanges of animal models or chemical precursors between PET centers would certainly save time in selecting the best molecules for clinical applications.

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