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REVIEW ARTICLE

Positron emission tomography of type 2 cannabinoid receptors for detecting inflammation in the central nervous system

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Cannabinoid receptor CB_2 (CB_2R) is upregulated on activated microglia and astrocytes in the brain under inflammatory conditions and plays important roles in many neurological diseases, such as Alzheimer's disease, amyotrophic lateral sclerosis, and ischemic stroke. The advent of positron emission tomography (PET) using CB_2R radiotracers has enabled the visualization of CB_2R distribution in vivo in animal models of central nervous system inflammation, however translation to humans has been less successful. Several novel CB_2R radiotracers have been developed and evaluated to quantify microglial activation. In this review, we summarize the recent preclinical and clinical imaging results of CB_2R PET tracers and discuss the prospects of CB_2R imaging using PET.

Keywords: cannabinoid receptor CB2; microglia; inflammation; neurological diseases; positron emission tomography

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CANNABINOID RECEPTOR CB_2R

Cannabinoid receptors, including CB_1R and CB_2R subtypes, are G-protein-coupled receptors that are involved in endocannabinoid signaling and various physiological processes, such as pain, mood, and memory [1]. CB_1R is expressed abundantly in the central nervous system (CNS), whereas CB_2R exists mainly in the peripheral organs (e.g., spleen) and is undetectable in the brain under physiological conditions. In the periphery, CB_2R is expressed on leukocytes and macrophages and regulates inflammation and immune functions, such as phagocytosis inhibition, B and T cell differentiation, and the balance of pro-inflammatory/anti-inflammatory cytokines. Under CNS inflammation, CB_2R expression is upregulated on microglia, astrocytes, and oligodendrocytes and on post-synaptic neurons [2] in the hippocampus, brainstem, and cerebellum of both mouse/rat models [1, 3–5] and humans [6]. CB_2R has mainly been associated with anti-inflammatory/immunosuppressive roles and reductions in nuclear factor NF-κB and inflammatory mediators. Its low baseline expression and disease-induced increase make CB_2R a promising diagnostic and therapeutic target for CNS inflammation.

Microglia and astrocytes are major players in the brain innate immune system. They are morphologically dynamic and undergo priming based on the local context [7]. Ramified microglia are activated in response to brain injury and immune stimuli and develop a "classical activation, pro-inflammation" status or an "alternative activation, anti-inflammation" status [8]. The extent to which inflammation is beneficial or detrimental in disease processes has not been fully elucidated. Acute inflammation consists of an immediate and early response to a stimulus, such as stroke or trauma, and a subsequent defensive response that facilitates repair. Chronic CNS inflammation resulting from persistent stimuli is an important component in the pathophysiology of many neurological diseases, such as Alzheimer's disease (AD), Parkinson's disease [9], amyotrophic lateral sclerosis (ALS) [10], multiple sclerosis (MS) [11], and Huntington disease [12].

CNS INFLAMMATION TRACERS

Positron emission tomography (PET) enables the in vivo detection of molecular changes in animal models and in humans, thus facilitating the understanding of disease mechanisms. The development of CNS inflammation tracers has been challenging, partly due to the diverse and changing states of astrocytes and microglia. Tracers for translocator protein (TSPO) have been the most applied probes in studying the microglial activation status in patients with neurological disorders [13, 14]. Several TSPO tracers have been evaluated in animal models and patients, including the first-generation [^{11}C]PK11195, second-generation molecules such as [^{11}C]DAA1106, [^{11}C]PBR28, and [^{18}F]DPA-714 [15], and third-generation rs6971-insensitive radiotracers such as [^{11}C]ER176 [16] and [^{18}F]GE180 [17, 18]. Although it has had varying degrees of utility in different neurological diseases, TSPO imaging has the limitations of specificity of binding, data quantification (reference region), and mixed binders of the second-generation tracers due to the polymorphism in the TSPO gene [14, 19]. New classes of tracers detecting, e.g., CB_2R, monoamine oxidase B [20] cyclooxygenases (COX 1, 2), and arachidonic acid [17, 21], are under development as potential tracers for CNS inflammation.

PET TRACERS FOR IMAGING CB_2R

Several CB_2R tracers have been developed, including the oxoquinoline derivatives [^{11}C]KD2 [22], [^{11}C]KP23 [23], [^{11}C]RS-016 [24, 25], [^{11}C]RSR-056 [26], [^{11}C]RS-028, and [^{18}F]RS-126 [22, 25–31], and [^{11}C]NE40 [32]; and the thiazole derivatives [^{11}C]A-836339 [33, 34], [^{18}F]JHU94620 [35, 36], and [^{18}F]2f [37]. They

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Table 1. Summary of CB_2R tracer properties

Class	Tracer	Affinity hCB_2R	Selectivity CB_1R	LogP	Human	Animal model	Ref
Thiazole	[^{11}C]A-836339	0.64 nM	>400	2.97		AD mouse model; stroke, LPS, AMPA injected rat model	[33, 34]
	[^{18}F]2f	0.1 nM	>300	3.4		Healthy Wistar rat LPS-injected CD1 mouse	[41]
	[^{18}F]JHU94620	0.38 nM	1000				[35, 36]
	[^{18}F]15	0.29 nM	>1000				[37]
Oxoquinoline	[^{11}C]NE40	9.6 nM	100	3.5	In vivo AD; healthy control	SD/Wistar rat stroke model; rhesus monkey	[32, 42–48]
	[^{11}C]RSR-056	2.5 nM	>1000	1.94	Post-mortem AD brain, ALS spinal cord	LPS-injected mouse	[26]
	[^{11}C]RS-016	0.7 nM	>1000	2.78			[25, 30]
	[^{11}C]KD2						[22]
	[^{11}C]KP23						
	[^{18}F]RS-126			1.99	ALS spinal cord		[23, 28]
	[^{11}C]RS-028						[31]
	[^{11}C]6f	5.4 nM	1851				[49]
	[^{11}C]6b	9.4 nM	1067				
Indole	[^{18}F]FE-GW405833	27 nM	6000	2.5			[50]
	[^{11}C]Methoxy-Sch225336	4.5 nM	78	2.15			[51]
Oxadiazole	[^{11}C]MA2	87 nM	19			NMRI mouse	[38]
	[^{18}F]MA3	0.8 nM	127				
Thiophene	[^{11}C]AAT-015	3.3 nM	303	4.11		Rat	[29]
	[^{11}C]AAT-778	4.3 nM	256	6			
	[^{18}F]FC0324	0.1 nM	296			Rat	[39]
Amid	[^{18}F]1,2	2.3 nM	>500			CD1 mouse	[52]
	[^{11}C]10	11.5 nM	130				[53]

have shown high-affinity binding to CB_2R and sufficient selectivity over CB_1R. The oxadiazole derivatives [^{11}C]MA2 and [^{18}F]MA3 [38] and thiophene-based tracers [^{11}C]AAT-778, [^{11}C]AAT-015 [29], and [^{18}F]FC0324 [39] show high affinity to CB_2R but suboptimal selectivity over CB_1R. Table 1 summarizes the relevant properties of the CB_2R radiotracers. Figure 1 shows the chemical structures of different classes of CB_2R tracers. For the structure-activity relationships of CB_2R tracers, see the recent review published by Spinelli et al. [40]. Some of the most important prerequisites for a useful CNS PET tracer for imaging CB_2R include: (1) high-nanomolar-affinity binding to CB_2R to obtain a high target signal in vivo (BP_{nd} or %ID/g) given the low concentration of CB_2R in the brain; (2) suitable LogP (range 1–3) for blood-brain barrier passage; (3) specific binding to CB_2R with 1000-fold higher selectivity over CB_1R; (4) no radiometabolites in the brain; and (5) low interaction with plasma proteins. This review focuses on the evaluations of CB_2R tracers in animal models and patients with neurological diseases involving CNS inflammation.

CB_2 IMAGING IN ANIMAL MODELS

Spleen tissue, which is rich in CB_2R, obtained from animals has been used to evaluate the specificity of CB_2R tracers. Several CB_2R tracers bind with high specificity in spleen tissue slices. Figure 2a, c show autoradiograms showing the specific binding using the PET tracers [^{11}C]RSR-056 and [^{18}F]RS-126. The accumulation of [^{11}C]RSR-056 and [^{18}F]RS-126 was blocked with the CB_2R antagonist GW405833 in spleen slices obtained from a CD1 mouse and a Wistar rat. Different animal models have been used for the in vivo evaluation of CB_2R tracers using PET imaging: (1) mouse and rat models with local human CB_2R overexpression, as well as rhesus monkey [42] and (2) lipopolysaccharide (LPS)-injected mice and rats. The uptake of CB_2R tracer in the brain is rather low, with a % ID/g of 0.1–0.4 at 1 h [33, 42] and a binding potential of 1.4–3 in animal models. Figure 3 shows the coronal view of [^{11}C]RSR-056-PET overlaid on structural magnetic resonance imaging, demonstrating higher radioactivity retention in the cortex and hippocampus of an LPS-injected mouse (b) compared to a vehicle-injected mouse (a) and an LPS-injected + GW405833-treated mouse (c). The PET images were generated from averaged dynamic data at 20–60 min after the intravenous injection of [^{11}C]RSR-056. The upregulation of CB_2R detected by using [^{11}C]RSR-056 appeared to be global instead of local, which might have been related to the specificity of the radiotracer. The results from a PET study using [^{11}C]A-836339 showed no specific uptake in the rat brain intrastriatally injected with LPS and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid [34], although immunostaining for CB_2R showed positive signals. Another PET study using [^{18}F]RS-126 [28] or [^{18}F]JHU94620 [35, 36] showed specific uptake in the CB_2R-rich spleen, but not sufficient signals in the brain, of LPS-treated mice. LPS-injected models have been used to represent a pro-inflammatory rather than anti-inflammatory state. Thus, they might not be the most suitable for evaluating of CB_2R.

CB_2R imaging in an animal model of stroke

Ischemic stroke involves a cascade of hemodynamic, vascular, structural, and inflammatory responses in a time-dependent manner [54–56]. Inflammation is implicated in the initial neuronal loss and consists mainly of pro-inflammatory responses and the extension of the lesion in the penumbra; the subsequent

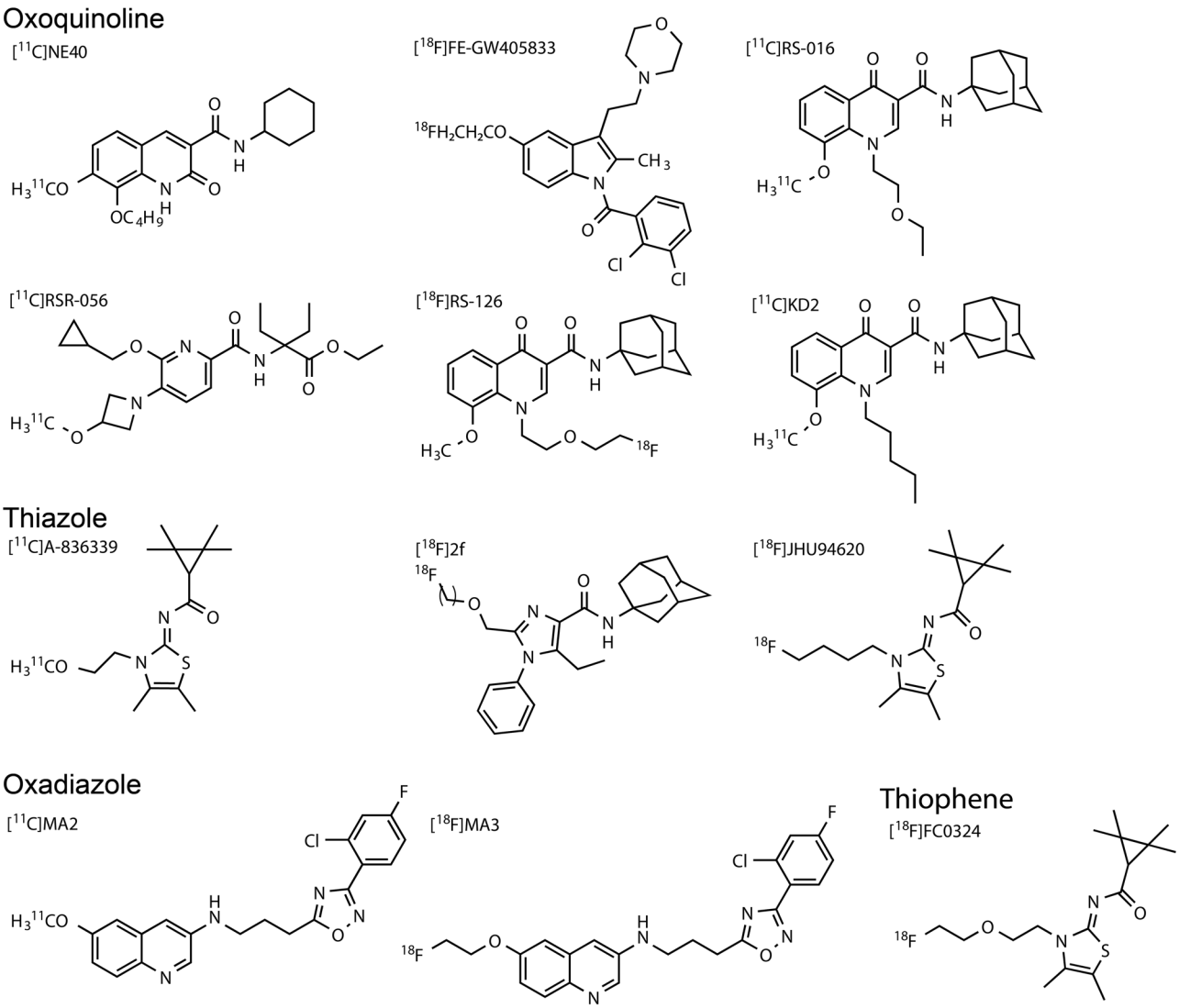

Fig. 1 Chemical structures of CB_2R tracers of oxoquinoline, thiazole, oxadiazole, and thiophene-based derivatives

functional recovery involves anti-inflammatory responses. Upregulated CB_2R mRNA was reported in a mouse focal ischemia model, peaking at 5 days and returning to normal at 10 days after occlusion [54]. Treatment with CB_2R agonists has neuroprotective effects and attenuates macrophage/microglial activation in ischemic mouse models [54, 56–59].

The models of CB_2R imaging in ischemic stroke animal have been inconclusive. An increased [^{11}C]NE40 binding with no change in the binding of TSPO tracer [^{11}C]PK11195 [43] was reported in an SD rat model of photothrombotic stroke at 24 h after surgery. Another study using [^{11}C]NE40 imaging did not show an upregulated signal in a Wistar rat model of photothrombotic stroke [48], despite the minor increase in the level of CB_2R seen by immunostaining. Low [^{11}C]A-836339 uptake was detected in a rat model over 1–28 days after occlusion, despite positive immunostaining for CB_2R and CD11 (markers for microglial activation) [34]. The utility of animal models of cerebral ischemia has been demonstrated in previous studies using TSPO tracers. Possible reasons for the different observations include the time point of assessment and differences between the stroke models. Different methods for inducing acute stroke (transient or permanent ischemia) result in variations in size, the severity of the ischemic regions, and the expression of inflammatory markers [56]. Since the pathophysiology and inflammatory levels and types evolve during the course of ischemic stroke, it is critical to reduce variations and make assessments at the optimal times. As CB_2R are mostly associated with anti-inflammation, performing imaging, flow cytometry, and mRNA measurements at a subacute window 4–5 days after ischemia might be suitable [54].

CB_2R imaging in animal models of Alzheimer's disease

AD is the most common neurodegenerative disease. The abnormal accumulation of amyloid-beta (Aβ) and tau aggregates leads to a cascade of pathophysiological changes, including inflammation, microvascular alternations, synaptic dysfunction, and neuronal loss. Microglia play a phagocytic role in the clearance of pathological protein deposits [60] and act as pro-inflammatory agents [61, 62]. Treatment with CB_2R agonists in AD mouse models reverses Aβ-induced memory impairment [63]. MicroPET using [^{11}C]A-836339 showed specific uptake in the brain areas with Aβ depositions in an APPswe/PS1dE9 mouse model of AD [33]. PET studies in some other AD mouse models using [^{11}C] deprenyl in APPswe mice [20] and TSPO tracer [^{18}F]GE180 in APP23 mice suggested increased astrocytosis and microgliosis at early disease stages. However, as age-related inflammation is also a confounding factor for the relatively mild inflammation in AD mouse models, these models might not be ideal for evaluating CB_2R tracers.

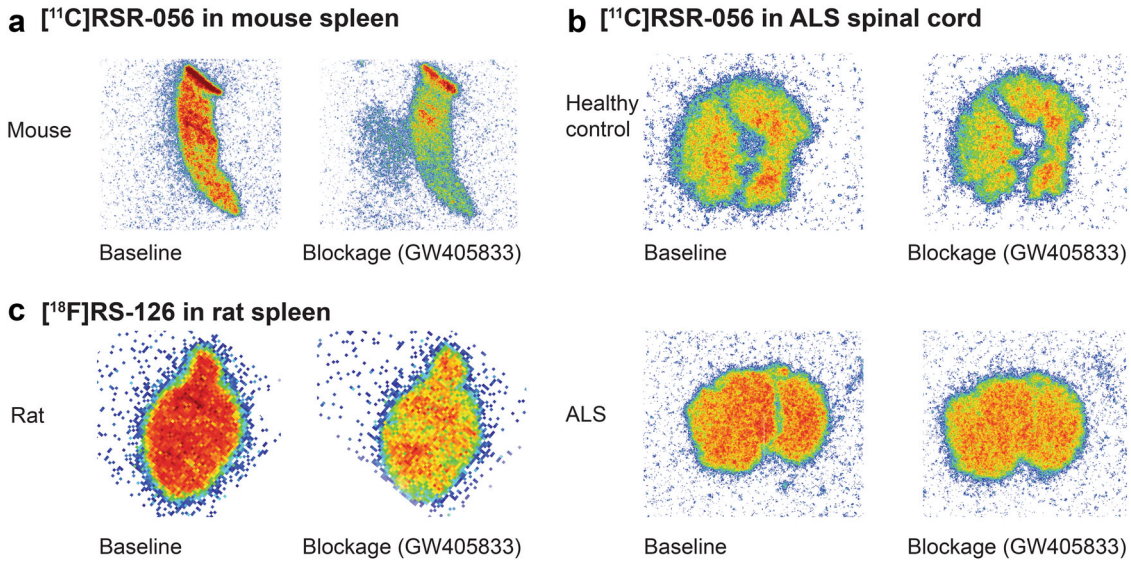

Fig. 2 Representative in vitro autoradiograms of CB_2R tracers. **a** [^{11}C]RSR-056 on spleen slices from a wild-type CD1 mouse. **b** [^{11}C]RSR-056 on cervical spinal cord from a healthy control and a patient with amyotrophic lateral sclerosis (ALS). **c** [^{18}F]RS-126 on spleen slices from a Wistar rat. Left: baseline binding; right: blockade with CB_2R antagonist GW405833

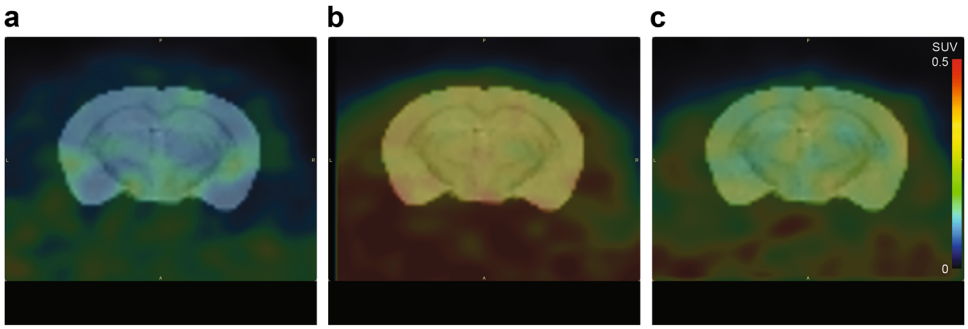

Fig. 3 Representative in vivo microPET images of coronal CD1 mouse brain sections (Bregma −1.5 mm ± 0.3 mm) by averaging dynamic data at 20–60 min after the intravenous injection of [^{11}C]RSR-056. **a** Vehicle-treated mouse. **b** LPS-treated mouse. **c** LPS-treated mouse with antagonist GW405833. Scale 0–0.5. *SUV* standard uptake value

CB_2R imaging in animal models of multiple sclerosis

MS is an autoimmune inflammatory demyelinating disease [11]. In an experimental autoimmune encephalomyelitis (EAE) model of MS [64, 65], activated microglial cells had upregulated CB_2R at the protein (10-fold) and at mRNA levels (100-fold) compared to ramified microglia. Recent clinical trials on the CB_2R inhibitor delta (9)-tetrahydrocannabinol in patients with MS showed an ameliorating effect on the symptoms, including perceived spasticity and pain [66, 67]. Thus, PET tracers for CB_2R receptors have the potential to detect the pathophysiology of MS and monitor the effects of treatment targeting CB_2R in MS. However, in the EAE animal model, a clear disease status is considered difficult to define. Therefore, the EAE animal model might not be an ideal model for the evaluation of CB_2R tracers.

POTENTIAL CLINICAL USE OF CB_2R PET IMAGING OF CNS INFLAMMATION

Few clinical evaluations of CB_2R PET imaging have been reported [45, 47]. The hurdles in CB_2R tracer development and translation may include: (1) species differences between humans and rodents in their immune systems, microglia subtypes, and levels of CB_2R expression [68], as well as distinct age-related changes, with a more pro-inflammatory type in humans [69] according to transcriptomic analysis; (2) low expression level of CB_2R in the brain, even under inflammatory conditions [70]; (3) lack of specific CB_2R antibody for post-mortem validation of in vivo imaging results [43, 55, 71]; (4) lack of full CB_2R knock-out mice [72]; and 5) developing a reference region for modeling [73].

CB_2R imaging in patients with amyotrophic lateral sclerosis

ALS is a progressive and fatal motor neuron disease [74]. A variety of genetic factors drive the degeneration of motor neurons and increase the susceptibility to ALS or influence the disease progression rate. A greater density of CB_2R-positive microglia and macrophages was observed in the post-mortem spinal cord tissues from patients with ALS compared to healthy controls. Autoradiography using [^{11}C]RSR-056 showed higher and specific binding on the post-mortem spinal cord tissues from patients with ALS compared to that from healthy controls (Fig. 2b). Similar increases were reported in other studies on ASL spinal cord tissues using [^{11}C]RS-028 [31] and [^{11}C]KD2 [22].

CB_2R imaging in patients with Alzheimer's disease

Emerging evidence indicates the involvement of CB_2R and increased levels of complements, cytokines, and chemokines in the brain of patients with AD. Reactive astrocytes, activated microglia, and upregulated CB_2R were observed in the vicinity of Aβ plaques in post-mortem AD mouse model brains and patients with AD [62, 75–88]. PET using [^{11}C]NE40 showed reduced signal along with Aβ

deposition visualized by [^{11}C]PIB in AD patients [47]. Further studies are needed to validate the utility of this tracer. A possible reason for these findings is that CB_2R upregulation is below the detection threshold, which is evident in the spinal cord from patients with ALS (Fig. 2b). In addition, as CNS inflammation is considered an early event, evaluating patients with mild cognitive impairment at the early stage of the disease process will be informative.

OUTLOOK

We propose several points for consideration during the development of CB_2R tracers: (1) Subnanomolar-affinity CB_2R tracers should be developed, considering the low expression level of the receptor. (2) We should elucidate whether CB_2R upregulation is disease-stage dependent and relates to a certain type of microglia or astrocyte. (3) Structural optimization to improve the physicochemical and pharmacological properties of this new class of CB_2R tracer will be necessary. (4) Flow cytometry should be used for surface phenotype characterization, e.g., CD45 and CD11 positivity and differentiating pro- and anti-inflammatory microglial subtypes and astrocytes. (5) Imaging results using CB_2R and TSPO tracers should be compared head to head. Given that CB_2R more closely represents anti-inflammatory features, additional knowledge of the inflammatory status in the CNS will be provided by PET imaging using both CB_2R and TSPO tracers [43]. (6) The mRNA expression of CB_2R should be measured. (7) Rational translation evaluation processes will be vital. Mouse and rat models of cerebral ischemia at 4–5 days after reperfusion might be a suitable model to test and validate CB_2R tracers.

CB_2R is also expressed on activated immune cells outside the CNS which represent potential targets for CB_2R PET imaging. Recent CB_2R imaging using [^{11}C]RS-016 shows the detection of vascular inflammation in the post-mortem carotid plaque from humans and mouse models of atherosclerosis [30].

CONCLUSION

CB_2R PET ligands will serve as useful tools for tracking the in vivo alterations in CNS inflammation during the progression of these disorders and for understanding the disease mechanisms. CB_2R PET ligands will also help illuminate new treatment strategies for neurological disorders in which CNS inflammation is implicated.

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ADDITIONAL INFORMATION

Competing interests: The authors declare no conflicts of interest.

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