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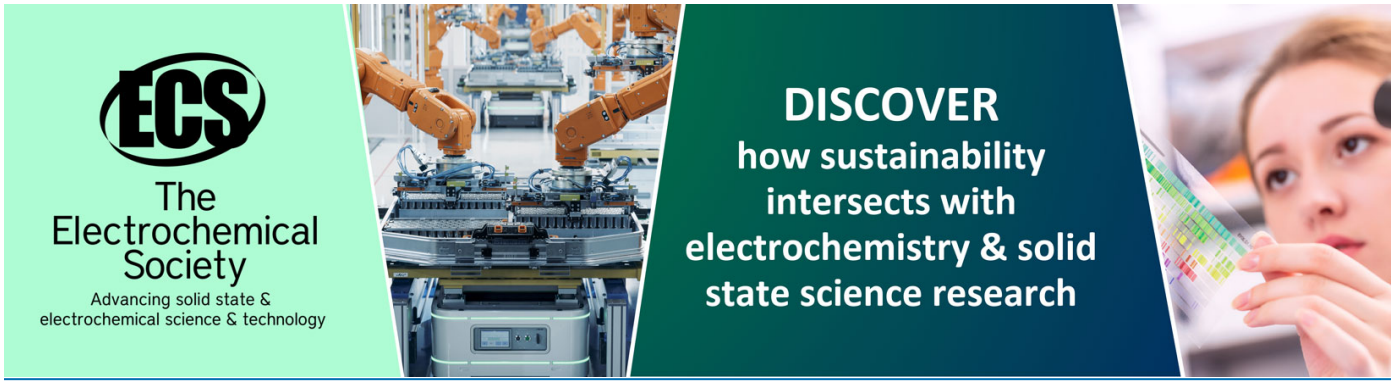

Novel targets for positron emission tomography (PET) radiopharmaceutical tracers for visualization of neuroinflammation

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Abstract. Non-invasive molecular imaging techniques can enhance diagnosis of neurological diseases to achieve their successful treatment. Positron emission tomography (PET) imaging can identify activated microglia and provide detailed functional information based on molecular biology. This imaging modality is based on detection of isotope labeled tracers, which emit positrons. The review summarizes the developments of various radiolabeled ligands for PET imaging of neuroinflammation.

Inflammation is involved in a variety of neurological diseases including stroke, Alzheimer disease, multiple sclerosis, Parkinson's disease, neurodegenerative dementias, epilepsy, psychiatric disorders such as schizophrenia, and oncologic diseases [1-6]. The inflammatory during most chronic neurodegenerative disease is dominated by the microglia [7]. Microglia are brain macrophages that emerge from early erythro-myeloid precursors and migrate to the brain mesenchyme before the blood brain barrier is formed [8]. Microglia constitute up to 10% of the total cell population of the brain. As resident macrophages of the central nervous system (CNS), the cells of microglia phagocytose cellular debris, present foreign antigens, and are the sensors of pathological events including inflammation [9]. Microglial cells can upregulate synthesis and release of various mediators, including translocator protein (TSPO), chemokines (e.g. CCL2), cyclooxygenase 1 (COX1), and cannabinoid receptor 2 (CB2) in the presence of inflammation. Blood-borne leukocytes including monocytes/macrophages, neutrophils, T-lymphocytes and B-lymphocytes extravasate into the brain through the interaction of cell surface integrins with specific endothelial adhesion molecules (e.g., ICAM-1, VCAM-1, P-/E-selectins). Stimulated cells then secrete effector molecules (e.g., matrix metalloproteinases [MMPs] and myeloperoxidase [MPO]), which trigger axonal damage and/or demyelination. Cell-to-cell

interaction between the antigen-presenting cells (APCs, e.g., B-lymphocytes, microglia, dendritic cells) is mediated via CD40 among other molecules [10].

To date, the most promising imaging techniques to visualize neuroinflammation include the methods of positron emission tomography (PET) and single photon emission computed tomography (SPECT). Identification of neuroinflammation markers using PET is a promising area of research aimed to improve the accuracy of diagnosis of these diseases, specify the prognosis and assess the effects of the therapeutic interventions [4]. PET is an imaging technology that assays the distribution of ligands labelled with positron emitters (e.g., 18F, 11C, 15O) in vivo, by measuring the emitting annihilation photons with a ring of detectors. It is a functional imaging technology that best suits the three principles of tracer measurements because PET instrumentation (1) allows absolute quantification, (2) PET labels do not modify tracer properties, and (3) PET ligands are administered at trace concentrations. These properties stem from the unique ability of the technology to utilize high energy radiation emitted from the nucleus as opposed to lower energy modalities, such as computed tomography, where X-rays are emitted by the orbiting electrons. PET labels radiate positrons, the anti-matter counterpart of the electron that annihilate with slowly moving electrons emitting two γ-rays at an 180o angle that are detected by the ring of detectors. Once a study is completed, the coincident detections of the γ-rays are reconstructed into a set of time frames that measure the distribution of the radioligand during the acquisition [11].

Molecular imaging techniques that non-invasively visualize specific targets of the inflammation cascade using sensitive and specific probes may be powerful tools to evaluate neuroinflammation in the pre-clinical and clinical settings [12]. To date, a range of novel radioligand tracers for microglia inflammatory activation is under development. Imaging with 18F-fluorodesoxyglucose (FDG) PET is used to determine sites of abnormal glucose metabolism and can be used, for example, to characterize and to localize many types of tumors [13]. However, lack of specificity of 18F-FDG PET limits its application for visualization of sites of infection and inflammation.

Several radioligands have been developed to image the activation of microglia in experimental models and in various CNS diseases using PET techniques [14]. For example, translocator protein (TSPO) is consistently elevated in activated microglia of the CNS in response to a variety of adverse factors as well as in the presence of neurodegenerative and psychiatric conditions. 11C-PK11195 PET, aimed at imaging the expression of the TSPO on activated microglia in the brain, has been used in preclinical and clinical research to investigate neuroinflammation in vivo in patients with brain diseases. Increased 11C-PK11195 uptake has been observed around the ischemic lesions for several days after the onset, but also in the regions distant from the lesion [14]. However, 11C-PK11195 suffers from two major limitations: low brain permeability and high nonspecific signal due to plasma binding resulting in a low signal-to-noise ratio [4].

Significance of brain immunity as a primary or comorbid factor of illness has sparked great interest in the TSPO as a biomarker. In recent years, novel tracers such as [11C]-DAA1106, [11C]-vinpocetine, [11C]-DPA-713, [11C]-PBR28, [18F]-FEDAA1106, [18F]-PBR06, [18F]-PBR111, [18F]-DPA-714, and [18F]-FEPPA have been developed as the second-generation TSPO radioligands aimed at improving the quality of TSPO imaging through the higher affinity of novel radioligands [2, 15]. However, such major developments have not yet resulted in the expected improvement of image quality [5]. There are some challenges: for example, the second-generation TSPO ligands show high level of vascular binding, which makes it difficult to separate the normal gray matter from other tissues [16].

Apart from TSPO, other targets, involved in the neuroinflammatory process in neurodegenerative disease, have been identified and currently are evaluated using PET ligands. One promising target is the cannabinoid receptor type 2 (CB2R), which is involved in peripheral immune system function, and is also upregulated in CNS disorders showing microglial activation [17]. This has led to the development of specific ligands, such as [11C]-NE40, which show binding to the human CB2R overexpressed in rodent striatum in preclinical PET studies [18]. First-in-man study of [11C]-NE40

showed a swift brain uptake [19], however, at this time, no clinical data on CB2R PET studies in neuronal disease have been published yet [6].

PET tracers like [64Cu]DOTA-etanercept and [64Cu]pegylated dimeric c(RGDyK) have been developed to target tumor necrosis factor (TNF) in both acute and chronic inflammation in mice. TNF may be a target for multiple sclerosis imaging in the future [10]. Another marker participating in the neuronal damage is inducible nitric oxide (NO) synthase (iNOS). The number of tracers for iNOS is minimal and the current [18F]NOS (6-(1/2)(2-[18F]fluoropropyl)-4-methylpyridin-2-amine) requires further modification and improvement [10]. The expression of another proinflammatory cytokine mediator, cyclooxygenase-2(COX-2), is extensively increased in multiple sclerosis lesions and it has been tightly linked to increase in iNOS expression [20]. PET tracers for COX-2 have been developed, but the in vivo imaging properties have not been very effective [21]. The most promising COX-2 tracer so far is [11C]Rofecoxib (4-(4-methylsulfonylphenyl)-3-phenyl-5H-furan-2-one) demonstrating in vitro usability, but lacking necessary affinity for in vivo studies [22].

Formyl peptide receptors (FPRs) are the G protein-coupled receptors (GPCR) that play an important role in leukocyte activation and chemotaxis [23]. These receptors were originally identified for their ability to bind and be stimulated by N-formyl peptides, which are produced by bacteria but can also be released from damaged mitochondria during tissue injury. The FPR are abundantly expressed on the surface of macrophages and neutrophils [23]. Moreover, FPR2 could represent an interesting target for monitoring in vivo the onset and progression of neuroinflammation in Alzheimer disease [24]. In recent years, 64Cu isotopes have been linked to FPR ligands for preclinical and clinical imaging of inflammation sites by PET. The FPR-specific peptide, cinnamoyl-F-(D)L-F-(D)L-FK (cFLFLF), was sequentially conjugated with a bifunctional polyethylene glycol moiety (PEG) and a 2,2',2",2"'-(1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrayl)tetraacetic acid (DOTA) through a lysine (K) spacer and finally labeled with 64Cu2+ to form cFLFLFK-PEG-64Cu [25]. In vitro assays for binding of the synthesized ligand to the neutrophil FPR yielded a dissociation constant of 17.7 nM. PET imaging with cFLFLFK-PEG-64Cu revealed that lung standardized uptake values correlated with lung neutrophil activity in Klebsiella-infected mice [25]. This construct represents more effective PET agent for macrophage detection compared with 18F-FDG for detection of macrophages in the pancreatic islets and aorta [26]. However, the in vivo use of peptides for neurovisualization is hampered by their low ability to cross blood–brain barrier (BBB) unless they interact with specific transport systems. Thus, non-peptidic small molecules may have some advantages because they can be suitably designed to modulate properties, such as potency, selectivity, lipophilicity, and cell permeability, which are pivotal for a potential radiotracer [27]. Recently, a series of non-peptidic potent agonists for FPR1/2 with 3-(1H-indol-3-yl)-2-[3-(4-nitrophenyl)ureido]propanamide structure was reported [28]. On the basis this scaffold compound (S)-1 was selected as a candidate for visualization of FPRs in the activated microglial cells in vivo [27]. However, (S)-[11C]-1 showed very poor BBB penetration and, thus, was unable to accumulate in the brain [27].

Therefore, at the present time, searching for new neuroinflammation markers for PET detection remains a relevant problem and requires further research efforts.

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References

[1] Heiss W-D 2014 PET imaging in ischemic cerebrovascular disease: current status and future directions *Neurosci Bull* **30** 713–32

[2] Zhang J 2015 Mapping neuroinflammation in frontotemporal dementia with molecular PET imaging *J Neuroinflammation* **12** 108

[3] Galovic M and Koepp M 2016 Advances of molecular imaging in epilepsy *Curr Neurol Neurosci* Rep. **16** 58

[4] Vivash L and O'Brien T 2016 Imaging Microglial Activation with TSPO PET: Lighting Up Neurologic Diseases. *J Nuc Med* **57** 165–8
[5] Turkheimer F E, Rizzo G, Bloomfield P S, Howes O, Zanotti-Fregonara P, Bertoldo A andVeronese M 2015 The methodology of TSPO imaging with positron emission tomography *Biochem Soc Trans* **43** 586–92
[6] Gerhard A 2016 TSPO imaging in parkinsonian disorders Clin. Transl. Imaging **4** 183–90
[7] Cunningham C 2013 Microglia and neurodegeneration: the role of systemic inflammation. *Glia* **61** 71-90
[8] Waisman A, Ginhoux F, Greter M and Bruttger J 2015 Homeostasis of Microglia in the Adult Brain: Review of Novel Microglia Depletion Systems. Trends Immunol. **36** 625-36
[9] Hanisch U K and Kettenmann H 2007 Microglia: active sensor and versatile effector cells in the normal and pathologic brain *Nat Neurosci* **10** 1387–94
[10] Poutiainen P, Jaronen M, Quintana F J and Brownell A-L 2016 Precision Medicine in Multiple Sclerosis: Future of PET Imaging of Inflammation and Reactive Astrocytes Front. *Mol Neurosci* **9** 85
[11] Beadsmoore C, Newman D, MacIver D and Pawaroo D 2015 Positron Emission Tomography Computed Tomography: A Guide for the General Radiologist Can. Assoc. *Radiol J* **66** 332–347
[12] Pulli B and Chen J W 2014 Imaging Neuroinflammation - from Bench to Bedside *J Clin Cell Immunol* **5** 226
[13] Wu C, Li F, Niu G and Chen X 2013 PET imaging of inflammation biomarkers *Theranostics* **3** 448-66.
[14] Thiel A and Heiss W D 2011 Imaging of microglia activation in stroke Stroke 42 507–12
[15] Buck A and Decristoforo C 2016 Highlights lecture EANM medicine's superheroes *Eur J Nucl Med Mol Imaging* **43** 1910–27
[16] Rizzo G, Veronese M, Tonietto M, Zanotti-Fregonara P, Turkheimer F and Bertoldo A 2014 Kinetic modeling without accounting for the vascular component impairs the quantification of [(11)C]PBR28 brain PET data *J Cereb Blood Flow Metab* **34** 1060–69
[17] Cabral G , Raborn E , Griffin L, Dennis J and Marciano-Cabral F 2008 CB2 receptors in the brain: role in immune function *Br J Pharmacol* **153** 240–51
[18] Evens N, Vandeputte C, Coolen C, Janssen P, Sciot R, Baekelandt V, Verbruggen A M, Debyser Z, Van Laere K and Bormans G M 2012 Preclinical evaluation of [11C]NE40, 2 cannabinoid receptor PET tracer *Nucl Med Biol* **39** 389–99
[19] Ahmad R, Koole M, Evens N, Serdons K, Verbruggen A, Bormans G and Van Laere K 2013 Whole-body biodistribution and radiation dosimetry of the cannabinoid type 2 receptor ligand NE40 in healthy subjects *Mol Imaging Biol* **15** 384–90
[20] Rose J W, Hill K E, Watt H E and Carlson N G 2004 Inflammatory cell expression of cyclooxygenase-2 in the multiple sclerosis lesion *J Neuroimmunol* **149** 40–9
[21] de Vries E F J , Doorduin, J, Dierckx R A and van Waarde A 2008 Evaluation of [(11)C]rofecoxib as PET tracer for cyclooxygenase 2 overexpression in rat models of inflammation *Nucl Med Biol* **35** 35–42
[22] Ji B, Kumata K , Onoe H , Kaneko H , Zhang M-R, Seki C, Ono M, Shukuri M, Tokunaga M, Minamihisamatsu T, Suhara T and Higuchi M 2013 Assessment of radioligands for PET imaging of cyclooxygenase-2 in an ischemic neuronal injury model *Brain Res* **1533** 152–62
[23] Ye RD, Boulay F, Wang JM, Dahlgren C, Gerard C, Parmentier M, Serhan CN and Murphy PM 2009 International Union of Basic and Clinical Pharmacology. LXXIII. Nomenclature for the formyl peptide receptor (FPR) family. *Pharmacol Rev* **61** 119-61
[24] Slowik A, Merres J, Elfgen A, Jansen S, Mohr F, Wruck C, Pufe T and Brandenburg LO 2012 Involvement of formyl peptide receptors in receptor for advanced glycation end products (RAGE) and amyloid beta 1-42-induced signal transduction in glial cells. *Mol Neurodegener* **7** 55

[25] Locke L W, Chordia M D, Zhang Y, Kundu B, Kennedy D, Landseadel J, Xiao L, Fairchild KD, Berr SS, Linden J and Pan D 2009 A novel neutrophil-specific PET imaging agent: cFLFLFK-PEG-64Cu 2009 J. Nucl. Med. **50** 790–97

[26] Zhang Y, Kundu B, Zhong M, Huang T, Li J, Chordia M, Chen M, Pan D, He J and Shi W 2015 PET imaging detection of macrophages with a formyl peptide receptor antagonist. Nucl Med Biol. **42** 381-6

[27] Lacivita E, Stama M, Maeda J, Fujinaga M, Hatori A, Zhang MR, Colabufo N, Perrone R, Higuchi M, Suhara T and Leopoldo M 2016 Radiosynthesis and in vivo Evaluation of Carbon-11 (2S)-3-(1H-Indol-3-yl)-2-{[(4-methoxyphenyl)carbamoyl]amino}-N-{[1-(5-methoxypyridin-2-yl)cyclohexyl]methyl}propanamide: An Attempt to Visualize Brain Formyl Peptide Receptors in Mouse Models of Neuroinflammation. Chem Biodivers. **13** 875-83

[28] Schepetkin I A, Kirpotina L N, Khlebnikov A I, Leopoldo M, Lucente E, Lacivita E, De Giorgio P and Quinn MT 2013 3-(1H-indol-3-yl)-2-[3-(4-nitrophenyl)ureido]propanamide enantiomers with human formyl-peptide receptor agonist activity: molecular modeling of chiral recognition by FPR2. Biochem. Pharmacol. **85** 404-16