General Review

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Abstract

Recently, accumulating evidences have revealed that neuroinflammation seems to be the cornerstone of many neurological diseases including stroke, multiple sclerosis, Alzheimer's disease or Parkinson's disease. Neuroinflammation causes neuronal damages by activation of plenty of cells and molecular mediators in diseases involving inflammatory process. We focus on non-invasive molecular imaging of radioligands that target inflammatory cells and molecules involved in neuroinflammation. Indeed, PET is one of the most promising imaging techniques to visualize and quantify neuroinflammation *in vivo*. We summarize here the potential neuroinflammation imaging targets and corresponding PET radioligands.

Keywords : Radiopharmaceutical – Positron Emission Tomography – Neuroinflammation – Molecular Imaging - Microglia

Résumé + mots clefs

Des données scientifiques récentes et de plus en plus nombreuses ont mis en évidence le rôle central joué par le processus de neuroinflammation dans la physiopathologie de nombreuses maladies neurologiques, telles que l'accident vasculaire cérébral, la sclérose en plaques, la maladie d'Alzheimer ou encore la maladie de Parkinson. Dans ces maladies impliquant le processus inflammatoire, la neuroinflammation cause en effet des dommages neuronaux par activation de nombreuses cellules et médiateurs moléculaires.

L'imagerie de Tomographie par Emission de Positons (TEP) apparaît comme une approche prometteuse pour visualiser et quantifier *in vivo* la neuroinflammation de façon non-invasive, grâce en particulier au développement de radioligands ciblant spécifiquement diverses molécules impliquées dans cette réaction inflammatoire cérébrale. Dans cette revue sont présentés les cibles moléculaires potentielles pour l'imagerie TEP de la neuroinflammation ainsi que les médicaments radiopharmaceutiques correspondants.

Mots-clefs : Médicament Radiopharmaceutique – Tomographie par Emission de Positons – Neuroinflammation – Imagerie moléculaire- Microglie.

1 Introduction

1.1 Neuroinflammation pathophysiology

Inflammation is a complex physiological response to different types of tissue injuries. The inflammatory reaction is an effective protective mechanism via the activation of a cascade of coordinated chemical and cellular reactions. Typically, this inflammatory cascade involves a local production of cytokines, which lead to a cell recruitment and differentiation.

The central nervous system (CNS) is characterized by a limited regenerative capacity and immune specificities such as the presence of a blood-brain barrier (BBB). Neuroinflammation is mostly mediated by microglia, which are mesoderm-derived immunocompetent cells of the CNS constituting the first line of the defence immune system of the brain when the BBB is maintained. Microglial cells, that constitute 10% of the entire cell population of the brain (1) are thus the main actors of the innate (nonspecific) immune response of the CNS. The purpose of neuroinflammation is to eliminate the initial cause of cell injury and to initiate tissue repair. However, neuroinflammation may also contribute to the disease process itself. Indeed microglia change from a resting to an activated state in response to CNS insults that stimulate them (2). Therefore, different degrees of microglia activation are seen in neurodegenerative disorders as Alzheimer's disease (AD), Multiple Sclerosis (MS) or Parkinson's disease (PD). In addition to their phagocyte role, activated microglia in chronic neuroinflammation causes long-term cerebral damage by inducing autoimmune reaction.

1.2 Neuroinflammation as a polyvalent biomarker

All neurological disorders whether degenerative (AD) or acute (stroke) induce destruction or neuronal death. Nevertheless affected neuronal population disease progression and pathophysiological process are strongly different. Despite these neuronal alterations

variabilities, they have in common the neuroinflammation process localized in affected cerebral areas.

This has led to increasing interest in visualizing neuroinflammation in a noninvasive manner that would lead to better understanding of neuroinflammation and its role in brain diseases. The *in vivo* detection of activated microglia may be used as a surrogate marker of neuronal damage and CNS disease activity. Molecular imaging can visualize, characterize, and measure the biological processes at the molecular and cellular levels in humans (3). Among the many molecular imaging techniques, Positron Emission Tomography (PET) features high sensitivity and specificity. Therefore, PET has become one the most frequently used molecular imaging method in clinical research setting.

As several neurodegenerative disorders are accompanied by increase in activated microglia, PET imaging of microglia with specific ligand may be able to assist the early detection of neuroinflammation. The large number of candidate Translocator Protein (TSPO) ligands that have been radiolabelled recently witnesses a strongly growing interest for this target. Consequently, new imaging targets and tracers for more specific neuroinflammation detection and *in vivo* characterisation are under intensive investigation.

In **Figure 1**, we summarized radiopharmaceuticals developed for PET imaging of neuroinflammation, targeting different biomarkers.

2 Potential targets for neuroinflammation molecular imaging

2.1 Current state of molecular targets for PET imaging

2.1.1 Cerebral blood flow and carbohydrate metabolism variation

Neuroinflammation is associated with several physiological parameters alteration such as cerebral blood flow variation or carbohydrate metabolism modification. Molecular imaging could use these physiological changes to visualize neuroinflammation in non-invasive procedure, in order to better understand brain diseases physiopathology, to improve the diagnosis and to assess novel therapies *in vivo*. ^{18}F-FDG (2-deoxy-2-^{18}F-fluoro-D-glucose) is the most extensively used PET tracer. It has been applied successfully in tumor detection, progression, and therapy evaluation but a high rate of glucose metabolism is also found in active white blood cells so ^{18}F-FDG PET has been performed in several studies of infection and inflammation (4).

Although cerebral blood flow modification and carbohydrate metabolism have been successful exploited to detect cerebral inflammation by PET or SPECT, these parameters are not enough specific of the inflammatory process and could be induced by other physiological causes.

2.1.2 Microglia and TSPO

2.1.2.1 TSPO structure and functions

The 18-kDa translocator protein (TSPO) is a hetero-oligomeric complex located on the outer membrane of mitochondria (5)(6). It has been first described as peripheral benzodiazepine receptor (PBR), a secondary binding site for diazepam which is distinct in its structure and whole body distribution from the central benzodiazepine receptor (CBR). Whereas CBR is mostly expressed in the brain, PBR (or TSPO) has an ubiquitous distribution in most peripheral organs including the kidney, the heart and particularly in the steroid producing tissues. TSPO remains minimally expressed in the normal healthy brain, and its basal expression rises in several acute and degenerative disorders including stroke, Alzheimer's

disease, Parkinson's disease, multiple sclerosis and amytrophic lateral sclerosis (7). The TSPO is known to be involved in modulating immune response, porphyrin transport and heme synthesis, regulation of cell proliferation, steroid biosynthesis and programmed cell death (8). Active brain disease is responsible for a change from rest to active state of microglia and this state change is associated with *de novo* expression of the TSPO. It is generally accepted that an increase in TSPO density is a reliable biomarker of microglial activation and neuroinflammation. Therefore the use of high affinity TSPO ligands for PET may greatly help for *in vivo* study of both acute and chronic neuroinflammatory conditions.

2.1.2.2 Radioligands for the TSPO

In addition to many endogenous compounds, TSPO binds a range of synthetic ligands. Thus, several classes of TSPO radioligands have already been synthesized, with, for most of them, the availability of compounds radiolabelled with carbon-11 or fluorine-18. We proposed here a classification of these compounds according to their chemical structure.

Over the last years, several structure activity relationship studies have laid out that four main domains are necessary for a ligand to interact with the TSPO (9)(10)(11). These pharmacophores present three major lipophilic regions and one hydrogen-bond donor group (12).

2.1.2.3 Benzodiazepine

Historically, the benzodiazepine ^{11}C-Ro5-4864 (**Figure 2**) was the first ligand able to discriminate peripheral from CBR. Initial PET imaging with ^{11}C-Ro5-4864 in patients with brain tumours did not show great promise because of large amounts of nonspecific binding and low *in vitro* affinity in human brain tissue (13). This nonspecific binding is related to

lipophilic and electrostatic (π- π) interactions between the radioligand and the brain tissue especially in the white matter. This limitation is not specific to TSPO PET imaging but is also met for mostly of PET brain radiopharmaceuticals.

2.1.2.4 Isoquinoline carboxamide

PK 11195 (**Figure 3**), a lipid soluble 3- Isoquinoline carboxamide was the first non-benzodiazepine type compound identified to bind to TSPO with high affinity (human Kd= 2nM)(14). *In vitro* and *in vivo* studies in both rats and mice demonstrated the superior binding affinity of PK 11195 compared to Ro5-4864. PK 11195 has been used in the majority of TSPO PET studies so far (15) and, hence has been tagged the gold standard of TSPO ligands.

The binding sites of the benzodiazepine Ro5-4864 and the Isoquinoline carboxamide derivative PK11195 on TSPO have distinct but overlapping sequences that involve amino acid residues on the first cytoplasmic loop and the C-terminal (16).

Despite ^{11}C-PK11195 has been widely used in both animals and human research, it showed several drawbacks : it displayed a poor signal-to-noise ratio, a low BBB permeability and a high level of nonspecific binding (15). For these reasons the sensitivity of PK 11195 in assessing and studying the TSPO is limited (17). In addition, ^{11}C has a very short radioactive half-time of only 20 minutes which also negatively affects widespread clinical use.

2.1.2.5 Phenoxyarylacetamides

An important group of new TSPO radiotracers are the derivatives of Phenoxyarylacetamides. N-(4-chloro-2-phenoxyphenyl)-N-(2-isopropoxybenzyl)acetamide (DAA1097) and N-(2,5-dimethoxybenzyl)-N-(5-fluoro-2-phenoxyphenyl)acetamide (DAA 1106) (**Figure 4**), are

specific ligands for TSPO (18) with a chemical structure based on the opening of the diazepine ring of Ro5-4864. They display potent anxiolytic effects in laboratory animals (19). Both ligands have been shown to inhibit ^{3}H-PK 11195 binding to mitochondrial preparations of rats whole brain with IC_{50} values of 0.92 and 0.28 nM respectively (PK 11195, IC_{50}=1.12nM)(19). DAA 1106 is not species dependent as it binds with high affinity to both monkey and rat brains having Ki values of 0.0188 nM and 0.043 nM respectively (20).

The currently leading TSPO radiotracers derivatives from DAA 1106 include the ^{11}C-PBR28 (21), ^{11}C-PBR06 (22), ^{18}F-FEPPA (23). Despite the considerable improvements exhibited by these TSPO radiotracers, most developed are less than ideal because of a significant component of nonspecific binding.

2.1.2.6 Imidazopyridines

The imidazopyridines were extensively evaluated in rodents and many of these compounds displayed improved biological characteristics, such as a higher target-to-background signal, faster clearance from nonspecific tissue and improved metabolic stability relative to ^{11}C-PK 11195 in *in vivo* animal's studies. Five of these radiotracers (^{123}I-CLINDE, ^{123}I/^{11}C-CLINME, ^{18}F-PBR111, ^{18}F-PBR102) (**Figure 5**) have further been evaluated in several rodent animal models of disease confirming that these radiotracers can image and measure activated microglia in the CNS (24)(25)(26).

2.1.2.7 Pyrazolopyrimidines

The series of pyrazolo [1,5-a] pyrimidine was published in 2001 (27), with two promising candidates for molecular imaging of neuroinflammation: The ^{11}C-DPA-713 and ^{18}F-DPA-714.

The DPA-713, or N, N-diethyl-2- [2- (4-methoxyphenyl) -5,7-dimethyl-pyrazolo [1,5-a] pyrimidin-3-yl] -acetamide (**Figure 6 (1)),** has a lower lipophilicity than PK11195, and good *in vitro* affinity for TSPO (Ki = 4.7 nM in rat kidney tissue). In baboon, a pre-injection of an excess of cold PK11195 blocks about 70% of brain binding of ^{11}C-DPA-713, indicating good radioligand binding specificity to TSPO. The relatively slow kinetics of the ^{11}C-DPA-713, with a peak maximum at 20 minutes post-injection allows accurate quantification of the measured signal. The ^{11}C-DPA-713 was evaluated in a model of inflammation in rats (intrastriatal injection of a-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)), where it showed its ability to visualize activated microglia with a specific signal TSPO and higher Binding Potential than ^{11}C-PK11195, due to lower cerebral non-specific binding (28). The ^{11}C-DPA-713 was also evaluated in healthy humans, and has shown a good *in vivo* kinetics and stability properties for human PET exploration of neuroinflammation (29).

The DPA-714, or N,N-diéthyl-2-(2-[4-(2-fluoroéthoxy)-phényl]-5,7-diméthyl-pyrazolo[1,5-a]pyrimidin-3-yl)-acétamide (**Figure 6 (2)),** exhibits a correct *in vitro* affinity for TSPO, even if slightly smaller than that of the DPA-713 (Ki = 7.0 nM rat kidney tissue, as measured by displacement of [^{3}H] -PK11195), and excellent selectivity for TSPO respectively to the CBR. The ^{18}F-DPA-714 enabled to visualize the activated microglia in rats in a model of excitotoxic lesion (30) and in a model of cerebral ischemia (31)(30). In monkeys, the ^{18}F-DPA-714 passes the BBB and accumulates in the brain with good specificity for TSPO, characterized by pretreatment with PK11195.

The ^{18}F-DPA-714 has also been successfully evaluated in healthy humans, and in several brain diseases, such as Alzheimer's disease, amyotrophic lateral sclerosis, and stroke, showing

its ability to visualize and quantify neuroinflammation in a clinical pathological setting (32)(33)(34)(35)(36).

2.1.2.8 TSPO imaging limitations

TSPO PET radiopharmaceuticals have been intensively used to explore neuroinflammation in the framework of clinical studies during these ten last years (see Part 3 below for a summary), but these studies also evidence several limitations of this approach, specifically associated with three properties of TSPO as a molecular biomarker of neuroinflammation.

- The mathematical model usually applied to quantify the TSPO radioligands binding requires a dynamic PET acquisition and the definition of a region that does not contain specific ligand binding, from which to draw the "normal" kinetic behaviour that does not contain specific ligand binding. However, microglia are distributed throughout the entire brain, and in a disease such as neurodegenerative pathology no clear reference region may *a-priori* exist. Cluster analysis should then be performed on the dynamic PET scan of each subject to determine a suitable reference region.

- A genetic polymorphism in the TSPO gene (rs6971) has been identified to cause the substitution of an amino-acid in the TSPO structure (A ^{147}T). This polymorphism affects the binding affinity properties of most of PET TSPO radiopharmaceuticals for their target, with a huge heterogeneity in PET images and their associated quantitative data (37). Three classes of individuals have been described in relation with this polymorphism: high-affinity binders (HABs, ≈ 66% of the Caucasian population); mixed-affinity binders (MABs, ≈ 29%); and low-affinity binders (LABs, ≈ 5%). In *in vivo* PET studies, the binding of the radioligand depends on the class to which the

subject belongs, but also on the radiopharmaceutical used (38). Then, genetic status regarding TSPO polymorphism is now required in clinical setting, but cross-studies analysis remains difficult when various radiopharmaceuticals are used in these studies, even if mathematical models (39) as well as new radioligands with low sensitivity to this polymorphism have (40) recently been proposed.

- Recently, there is emerging hypothesis accounting for that microglia is tuned for different types of host defense and protection, with a continuum of phenotypes from a proinflammatory and cytotoxic mediator state to a protective state that can dampen inflammation and promote tissue regeneration. Therefore, based largely on these observations, the microglial cells seem to have neuroprotective and neurotoxic roles, slowing and accelerating disease progression, respectively. Moreover, the same microglia cell may change alternatively from these two extreme states during disease progression. Yet, PET TSPO radiopharmaceuticals are not able to differentiate theses pro- or anti-inflammatory phenotypic and functional states of microglia, since TSPO is expressed similarly in both activated states (41). Therefore, such investigations would require the identification of new molecular targets and development of new radiopharmaceuticals able to differentiate the damaging from the neuroprotective microglia phenotypes, to increase the understanding of disease pathogenesis, as well as to help to develop novel therapeutic approaches.

2.2 Further neuroinflammation PET imaging targets

2.2.1 Cox2

The cyclooxygenase is an enzyme that is involved in the initial stages of the synthesis of prostaglandines. The subtype 2 (COX-2) is an inducible isoform that is activated during the

inflammatory reaction. This enzyme is expressed in very low concentration in the healthy brain, but its expression increases in a very important way further to an inflammatory stimulus, in particular in the cells of the activated microglia and in the astrocytes (42). The *in vivo* imaging of the COX-2 could supply valuable information about its exact role when it is overexpressed during an inflammatory process (43). The celecoxib is a selective inhibitor of the COX-2 and had been radiolabelled with the metastable technetium-99 (44). This radioligand showed an accumulation in cells expressing strongly the COX-2, but the specific binding has never been shown *in vitro* or *in vivo*.

More recently, several other selective inhibitors of the COX-2 as the ^{11}C-etoricoxib (45) and the ^{11}C-rofecoxib were described. Nevertheless, the evaluation of these molecules for the PET imaging in animal models of neuroinflammation has been disappointing (46).

2.2.2 Metalloproteinases and proteolytic activity

Proteases such as matrix metalloproteinases (MMPs) are crucial mediators of tissue damage secreted by microglia, astrocytes and monocytes. Altogether MMPs can degrade all the components of the extracellular matrix, and must be thus regulated finely. They are generally little expressed in healthy tissues, but their expression increases during the processes of tissular physiological or pathological remodelling under the influence of factors to modulate the expression of their genes (IL-1, TNFα, prostaglandins, cellular lesion). In the CNS, MMPs are associated with excitotoxicity, neuronal damage (47), and opening of the BBB (48). Locally increased levels of activated MMPs modulate and contribute to the progression of various diseases, such as stroke. Therefore, activated MMPs are suitable biological targets for the specific and non-invasive visualization of these pathologies *in vivo*. Recently, radiolabelled C-5-disubstituted pyrimidine-2,4,6-triones have been suggested by the

University Hospital of Münster, Germany, as a class of potent MMPs targeted radiotracers that can non-invasively visualize activated MMPs by means of PET(49). This novel MMPs radiotracer is characterized by an increased hydrophilicity compared with the lead structures and excellent MMP inhibition potencies for several MMPs(MMP-2, MMP-8, MMP-9, and MMP-13) (IC50 = 0.006−107 nM)(49).

2.2.3 Cannabinoid receptor 2 (CB2)

While the cannabinoid receptor 1 is constitutively expressed in a variety of cell types, CB2 is thought to be expressed on microglia/macrophages and is upregulated with activation of these cells (50). The cannabinoid type 2 (CB2) receptor plays an important role in neuroinflammatory and neurodegenerative diseases such as multiple sclerosis, amyotrophic lateral sclerosis, and Alzheimer's disease and is therefore a very promising target for therapeutic approaches as well as for imaging. The ^{11}C-A836339 has been evaluated and reported to be a selective CB2 agonist with high binding affinity. It shows specific cerebral uptake in the brain areas with Aβ amyloid plaque deposition in a mouse model of Alzheimer's disease (APPswe/PS1dE9 mice). These data establish a proof of principle that CB2 receptors binding in the neuroinflammation and related disorders can be measured *in vivo* in neuroinflammation and related disorders (51). Turkman and al(52), reported synthesis and results of *in vitro* receptor binding of two fluorinated 2-oxoquinoline derivatives, the ^{18}F-13 and ^{18}F-14 (**Figure 7**) as CB2 receptor ligands. *In vitro* CB2 receptor binding assay was performed using U87 cells transduced with CB2- and CB1-receptor and *ex vivo* autoradiography was performed with ^{18}F-14 on spleen and on CB2- and CB1-expressing and wild-type U87 subcutaneous tumours grown in mice. *Ex vivo* autoradiography showed accumulation of ^{18}F-14 in the CB2-expressing tumour. Compound ^{18}F-14 appears to be a potential PET imaging agent for the assessment of CB2 receptor expression; however, poor

solubility restrains its use *in vivo*.

More recently, Mu and al have identified the KD2 compound, (**Figure 8**) which belongs to a new type of structure cluster, the 4-oxoquinoline derivative compared to the published CB2 ligands such as 2-oxo-quinoline derivatives and N-arylamideoxadiazole derivatives (53). *In vitro* autoradiography with slices from rat and mouse spleen, which contain high levels of CB2, as well as *in vivo* PET of the rat spleen, demonstrated high specific and selective reversible binding. KD2 is therefore considered as a promising CB2 radiotracer for PET imaging.

2.2.4 iNOS

Nitric oxide (NO), plays a key role in various physiological processes such as peripheral blood pressure regulation and also acts as a messenger in the brain. Thus, several studies have shown that overproduction of NO is associated with central pathological states (54). Overproduction of NO by inducible enzyme nitric oxide synthase (iNOS) has been implicated in the functional tissue destruction of chronic inflammation. In an effort to develop a tracer for probing inducible nitric oxide synthase (iNOS) levels in vivo utilizing PET, two positron-emitting iNOS selective inhibitors S-[^{11}C]methylisothiourea and S-(2-[^{18}F]fluoroethyl)-isothiourea have been synthesized and evaluated by Zhang and al (55). An *in vitro* model, J774 macrophage cell line, was used to assess the uptake of radiolabeled iNOS inhibitor in response to iNOS induction at the cellular level. Increased cell uptake of these two labelled compounds to iNOS, as well as blocking under controlled *in vitro* conditions, were observed. These preliminary results are encouraging in view of further use for *in vivo* detection of iNOS.

In 2009, the ^{18}F-6-(2-fluoropropyl)-4-methylpyridin-2-amine has been assessed *in vivo* in a mouse model of lipopolysaccharide (LPS)-induced iNOS activation (56). *In vivo* biodistribution studies indicate higher tracer uptake in the lungs of the LPS-treated mice when compared to control mice. Data suggest that ^{18}F-6-(2-fluoropropyl)-4-methylpyridin-2-amine shows favourable features as a PET tracer to image iNOS activation with PET.

2.2.5 β-glucuronidase

It has been shown an increased in release of β-glucuronidase by activated microglia into the extracellular space at the site of neuroinflammation (57)(58). Therefore, β-glucuronidase might be a biomarker for neuroinflammation. Antunes et al (59), have designed a PET tracer for β-glucuronidase imaging from a glucuronide-prodrug-based structure coupled to a radioactive ^{18}F-fluoroethylaminegroup. They investigated whether β-glucuronidase is released during neuroinflammation in a rat model of herpes encephalitis (brain lysosomal hydrolase activities increase after infection with viruses) using this specific PET tracer, the ^{18}F-FEAnGA (**Figure 9**). A significant enhancement of the distribution volume of the tracer in the brains of HSV-1-infected rats was observed compared with the brains of uninfected rats. However ^{18}F-FEAnGA uptake in the brain was low in both control and in infected animals.

2.2.6 $P2X_7$

The $P2X_7$ ion channel receptor is one of the numerous P2X purinergic receptors which are found in the immune, peripheral and central nervous systems and is implicated in ATP-mediated cell death (60). $P2X_7$ is involved in the release of pro-inflammatory cytokines (61) and thus plays a role in peripheral inflammatory diseases. However several researchs have

evidence the role of $P2X_7$ on neuroinflammatory conditions such as Alzheimer's disease, epilepsy and other CNS disorders(62)(63).

In activated microglia, the purinergic $P2X_7$ receptor is upregulated. A-740003, a highly affine and selective $P2X_7$ receptor antagonist, was a promising candidate for the development of a radiotracer for imaging of neuroinflammation by PET. For this purpose, ^{11}C-A-740003 (**Figure 10**) was synthesised and evaluated *in vivo* showing little uptake in rat healthy brain (64).

Then, GlaxoSmithKline has developed GSK1482160 (**Figure 10**), a potent P2X7 antagonist with good biological activity (IC50=3nM for human P2X7)(65). This compound readily crosses the blood-brain-barrier and has been evaluated as a therapeutic agent in phase 1 human study (65) making it radiolabelled entity an attractive PET diagnostic agent candidate. The recent growth of the P2X7 antagonists development has led to the discovery of a novel series of compounds (methyl substituted 1-(5,6-dihydro-[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl)methanone) (**Figure 11**) with good drug-like properties with high P2X7 receptor occupancy in rat following oral administration (66). The compound displayed on **figure 10** appears to be viable candidate as a tracer for PET imaging. Additional efforts on related compounds should be confirm this hypothesis.

2.2.7 P2Y12

The P2Y12 receptor is an ADP-responsive G protein–coupled receptor expressed on the surface of platelets and is the pharmacologic target of several antithrombotic agents (67). In the CNS, P2Y12 expression is limited to the ramified processes of microglia. In humans, the role of P2Y12 expression in microglia is unknown. Moore et al. investigated the significance

of P2Y12 expression in the context of human brain injury and repair (68). They show that P2Y12 increases following interleukin (IL)-4 and IL-13 activation, and mediates cell migration and inflammatory responses. Identifying P2Y12 as a molecule associated with the M2 tissue regenerative phenotype may help to discover novel therapeutic targets and mechanisms that promote CNS repair.

3 Clinical Challenge and perspective

Detection of neuroinflammation *in vivo* throughout the course of neurodegenerative diseases is of great clinical interest. Studies have shown that microglia activation, as an indicator of neuroinflammation, may present at early stages of several dementias diseases, like Alzheimer's disease (AD) or frontotemporal dementia (FTD), but its role in their pathogenesis is largely unknown. PET ligands for neuroinflammation may act as surrogate markers of disease progression, as well as for the measuring of target engagement in the context of clinical trials (69)(70).

AD, the most common cause of dementia in elderly subjects has been reconceptualised as a dynamic pathophysiological process, where the accumulation of amyloid-beta (Aβ) is thought to trigger a cascade of neurodegenerative events resulting in cognitive impairment and, eventually, dementia. In addition to Aβ pathology, various lines of research have implicated neuroinflammation as an important participant in AD pathophysiology(69).

In other neurodegenerative diseases, like Parkinson's disease and Parkinson's disease dementia, microglial activation suggests a chronic inflammatory process, although there is also evidence of its association with cognitive ability and neuronal function (71).

Evidence from both human *post mortem,* and *in vivo* animal model studies implicate the neuroimmune system and activated microglia in the pathology of amyotrophic lateral sclerosis (ALS). There is growing evidence of activated microglia and inflammatory processes in the cerebral cortex in ALS. This finding might improve our understanding of the pathophysiology of ALS and might be a surrogate marker of efficacy of treatment on microglial activation (72).

In multiple sclerosis (MS), recent developments in PET imaging offer the potential to assess brain complementary informations that can not be achieved by MRI modality. Thus PET in MS could be used for the investigation of underlying pathophysiology of neuroinflammation, neuronal dysfunction, and demyelination, and remyelination. Quantitative measures of molecular targets with PET could also have future uses in clinical trials of drug development (73).

Although different mechanisms are involved in the pathogenesis of stroke, increasing evidence suggests that inflammation, mainly involving the microglial and the immune system cells, account for its pathogenic progression. Cerebral ischemia rapidly evolves to necrosis and a peri-necrosis area of ischemic penumbra in which the brain tissue is still viable for a few hours. This cerebral tissue can be preserved if treatment is initiated quickly to restore the cerebral blood flow. Thus, this area of “darkness” is the prime target for potential neuroprotective drugs (33).

A plethora of evidence points toward an undeniable role of brain inflammation in epileptogenesis. However, the exact role of this process remains unfortunately not clear. Non-invasive imaging of brain inflammation can promote our fundamental knowledge, which may

lead to better insights into the role of brain inflammation in disease ontogenesis and to investigate if anti-inflammatory therapy may play a role in treating drug-resistant epilepsy (74).

Recently studies have suggested that neuroinflammation might be an early pathology of schizophrenia that later leads to neurodegeneration, yet the exact role in the etiology, as well as the source of neuroinflammation, are still not known. The hypothesis of neuroinflammation involvement in schizophrenia is quickly gaining popularity, and thus it is imperative that we have reliable and reproducible tools and measures that are both sensitive, and, most importantly, specific to neuroinflammation. Microglial activity is elevated in patients with schizophrenia and in persons with subclinical symptoms who are at ultra-high risk of psychosis and is related to at-risk symptom severity. These findings suggest that neuroinflammation is linked to the risk of psychosis and related disorders, as well as the expression of subclinical symptoms(75)(76)(77).

The *in vivo* quantification of activated microglia with PET imaging may be an interesting tool for attaining a better understanding of the physiopathology of different diseases. For example, in AD, to evaluate the chronological relationship between the hallmarks of the disease (especially amyloid load) and microglia activation. In fact, the role of neuroinflammation and its relationship to Aß remain controversial.

As exposed previously in this review, microglia are distributed throughout the entire brain, and in a disease such as neurodegenerative pathology no clear reference region may exist. Cluster analysis was therefore performed on the dynamic PET scan of each subject to optimize the selection of a suitable reference region. But these methods are difficult to apply

in clinical setting and still require improvements (32). Recently, Lyoo et al, have measured TSPO density in AD patients and control subjects, using a simple ratio method SUVR which can substitute for, and may even be more sensitive than, absolute quantitation. The SUVR method is expected to improve subject tolerability by allowing shorter scanning time and not requiring arterial catheterization. In addition, this ratio method allows smaller sample sizes for comparable statistical significance because of the relatively low variability of the ratio values (78).

In acute and focal diseases, like stroke, a ratio method using the contralateral to the brain injury tissue as reference could be used to evaluate the TSPO density. In this kind of situations, a 10 to 20 min of PET emission acquisition performed between 40 and 80 min pi was able to differentiate acute tissue injury from normal brain tissue (33).

4 Conclusion

Neuroinflammation is a physiological response to various stimuli and is increasingly recognized as a key factor in the pathogenesis of neurodegenerative conditions. Neuroinflammation is correlated with tissue damages such as neuronal loss or demyelination. Therefore, PET radiopharmaceuticals targeting key players in the inflammation process stand for an attractive and powerful imaging biomarkers. Over the last years, many neuroinflammatory targets have been identified and corresponding molecular imaging tracers developed. Among these targets, TSPO remains currently the “gold-standard” PET target for neuroinflammation PET exploration.

However, recent findings evidenced a TSPO polymorphism resulting in differences in binding affinity of PET radioligands for TSPO. Furthermore, neuroinflammation imaging challenge needs henceforth to be based on various microglial phenotypes identification, in order to

evidence its protective or damaging role. To date, the main challenge does not seem to develop a new "best" TSPO PET radiopharmaceutical, but rather to propose a new molecular target, which would own the following "ideal" criteria:

- Identification of a brain reference region devoid of the molecular target expression, in absence of any inflammatory process.
- Lack of interindividual variability and/or affinity expression, linked to a potential genetic polymorphism, affecting PET radiopharmaceutical's binding.
- Difference of its expression between the damaging and the neuroprotective continuum phenotypic expression of microglia cells.

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Figures and Legends

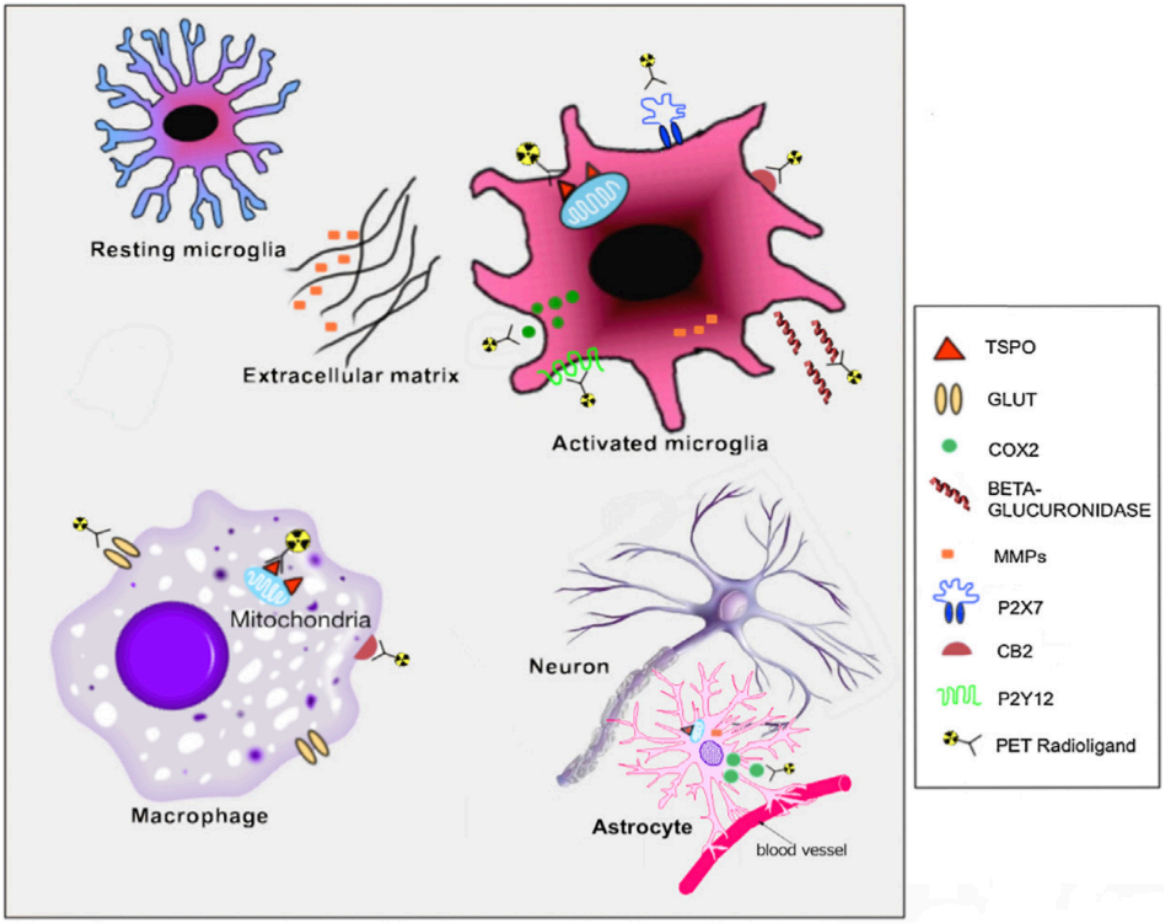


Figure 1

Neuroinflammation targets for PET imaging in brain cellular environment *(adapted from Wu, Theranostics. 2013;3(7):448-66)*

Cibles de la neuroinflammation pour l'imagerie TEP dans l'environnement cellulaire cérébral *(adapté de Wu, Theranostics. 2013;3(7):448-66)*

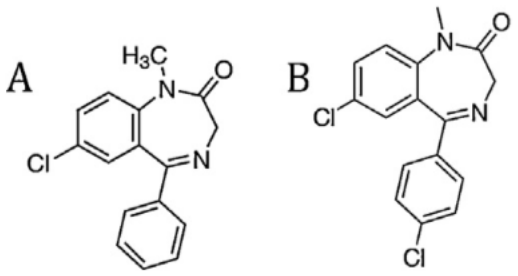


Figure 2

Chemical structure of the : (A) Diazepam ; (B) Ro5-4864

Structure chimique du : (A) Diazepam ; (B) Ro5-4864

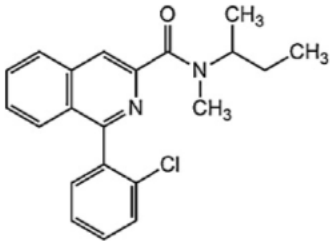


Figure 3

Chemical structure of the PK 11195

Structure chimique du PK 11195

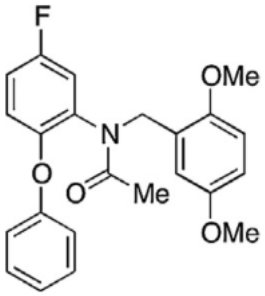


Figure 4

Chemical structure of the DAA1106

Structure chimique du DAA1106

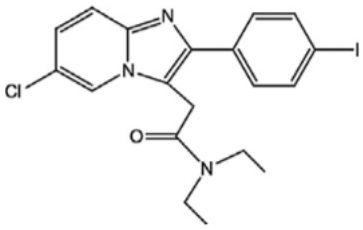
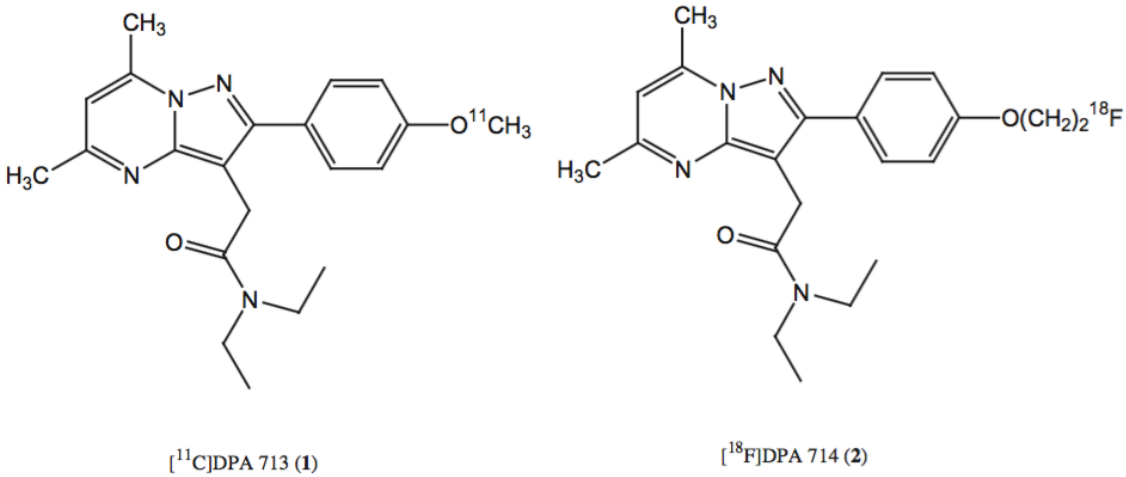


Figure 5

Chemical structure of the CLINDE

Structure chimique du CLINDE



[^{11}C]DPA 713 (**1**)

[^{18}F]DPA 714 (**2**)

Figure 6

Chemical structure of the radiolabeled compounds of the series-pyrazolo [1,5-a] pyrimidine

Structure chimique des dérivés radiomarqués de la pyrazolo [1,5-a] pyrimidine

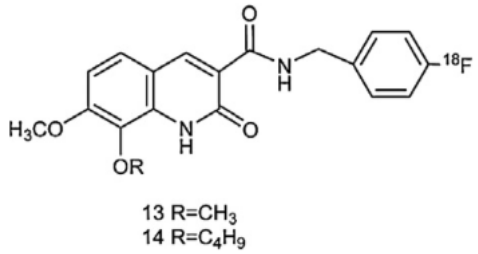


Figure 7

Chemical structure of the ^{18}F-13 and ^{18}F-14

Structure chimique des ^{18}F-13 and ^{18}F-14

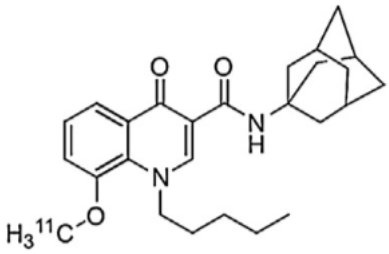


Figure 8

Chemical structure of the ^{11}C-KD2

Structure chimique du ^{11}C-KD2

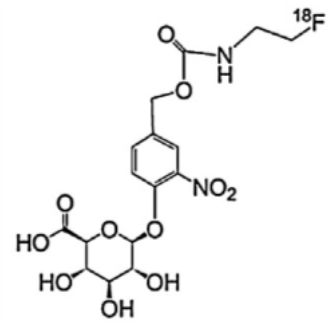


Figure 9

Chemical structure of the ^{18}F-FEAnGA

Structure chimique du ^{18}F-FEAnGA

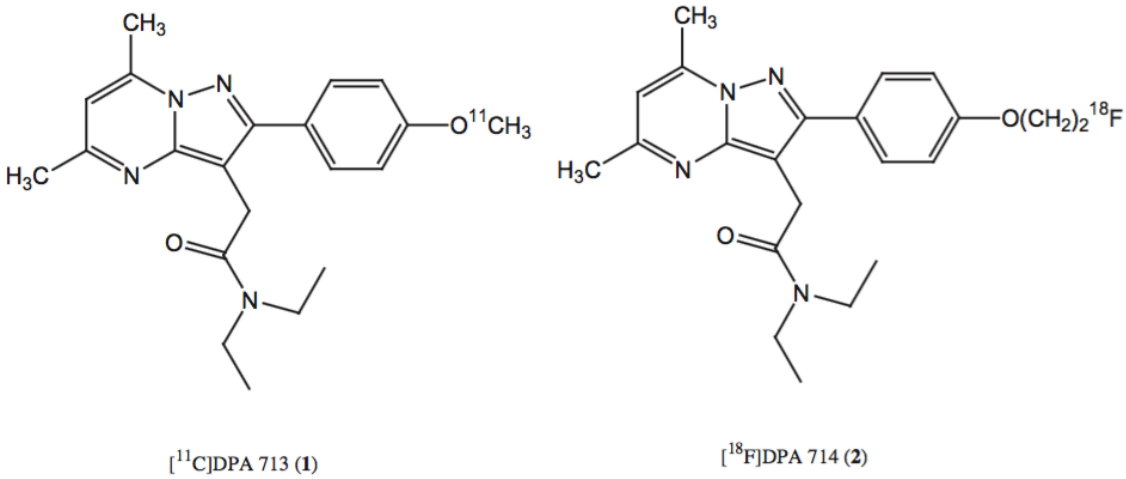


Figure 10

Chemical structure of the : (A)^{11}C-A-740003 ; (B)^{11}C-GSK1482160 ; (C)^{11}C-GSK1482160 isomer

Structure chimique du : (A)^{11}C-A-740003 ; (B)^{11}C-GSK1482160 ; (C)^{11}C-GSK1482160 isomère

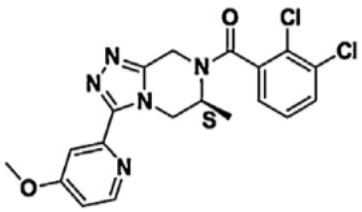


Figure 11

Chemical structure of the Methyl substituted 1-(5,6-dihydro-[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl)methanone derivative

Structure chimique du dérivé methyl substitué 1-(5,6-dihydro-[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl)methanone