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Molecular imaging of neuroinflammation in Alzheimer's disease and mild cognitive impairment

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ABSTRACT

Neuroinflammatory changes have been demonstrated to be an important feature of Alzheimer's disease (AD); however, the exact role of neuroinflammation and its progression during disease is still not well understood. One of the main drivers of the neuroinflammatory process are microglial cells. Positron Emission Tomography allows for the quantification of microglial activation by labelling the Translocator Protein 18 kDa (TSPO), which becomes overexpressed upon activation of microglial cells. Several radioligands have been designed to target TSPO and have been studied *in-vivo* in AD populations. While most studies have shown important increases in TSPO binding in AD populations compared to healthy volunteers, whether the neuroinflammatory progress occurs early on or later during disease is still unclear. In order to investigate the early changes in neuroinflammation, studies have sought to investigate microglial activation in patients with mild cognitive impairment (MCI), which is defined as a transitional stage between normal aging and dementia. In this prodromal population, conflicting results have been reported with some studies reporting increased binding in MCI, while others demonstrate no differences from controls. Here we review the TSPO PET studies in AD and MCI populations and discuss the important methodological considerations of imaging microglial activation.

1. Introduction

Neuroinflammation is considered an important pathological feature of Alzheimer's disease. Neuroinflammation is largely driven by glial cells such as microglia and astrocytes (Kreisl et al., 2013b). The first line of defense against invading pathogens and other harmful agents are microglial cells, the mononuclear phagocytes that are ubiquitously distributed in the brain and represent approximately 10% of the total cell population in the central nervous system (CNS) (Heneka et al., 2015). Microglia are assumed to exist in two states: resting and activated. In the resting state, microglia continuously extend and retract as they sample their environment for pathogens (McGeer and McGeer, 2010; Krabbe et al., 2013). During this process, microglia provide factors that support tissue maintenance (Heneka et al., 2015) and secrete various growth factors (Schwab and McGeer, 2008). Microglia are particularly sensitive to changes in brain microenvironment and can very quickly be activated in response to infection or injury (Liu and Hong, 2003). Upon activation, microglia proliferate and migrate to the site of injury and adopt a set of morphological and functional attributes (Heppner et al., 2015). The morphological changes include shortening of cellular processes and hypertrophy of the cell body (Perry et al., 2010), while the functional attributes include the upregulation of a variety of surface receptors and the release of several pro-inflammatory factors, including the tumor necrosis factor-α (TNFα) and interleukin-1β (IL-1β), free radicals such as nitric oxide (NO) and superoxide, fatty acid metabolites such as eicosanoids, and quinolinic acid (Liu and Hong, 2003). Activated microglial cells are believed to remove toxic aggregated proteins and cell debris from the CNS. Although the release of these pro-inflammatory factors is a defense mechanism of the immune system, it may ultimately have detrimental effects. Cell culture studies have demonstrated that the supernatants of highly activated microglia are toxic to neurons (Boje and Arora, 1992; Chao et al., 1992; McGuire et al., 2001; McGeer and McGeer, 2010). Thus, demonstrating that the role of microglia in the brain is complex, as their involvement may be both beneficial and detrimental. The potential dual role of microglia may be explained by the M1/M2 polarization of the cells, whereby the M1 phenotype is thought to be pro-inflammatory while the M2 phenotype is anti-inflammatory (Varrone and Nordberg, 2015). The balance between the neurotoxic M1 microglia and neuroprotective M2 microglia is thought to be central to AD pathogenesis (Varley et al., 2015).

Post-mortem evidence has demonstrated that microglia are present

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in pathologically affected areas of AD. As explained by the amyloid cascade hypothesis, the accumulation of β-amyloid (Aβ) plaques is believed to be a key factor that drives the neuroinflammatory response in AD (Schwab and McGeer, 2008). Post-mortem immunohistochemical examinations of brain slices have revealed the presence of activated microglia surrounding Aβ plaques in AD (Rogers et al., 1988; Itagaki et al., 1989; McGeer et al., 1989). Microglia have been reported to be distributed in graded concentrations in relation to their distance from Aβ deposits in transgenic mouse models of AD (Frautschy et al., 1998). Moreover, the density of activated microglia correlates with the severity of the inflammatory response (Carpenter et al., 1993). It has been proposed that microglia attempt to clear the brain of amyloid through the phagocytosis of Aβ and secretion of proteolytic enzymes that degrade Aβ (Leissring et al., 2003; Yan et al., 2006). Furthermore, activated microglia begin to produce pro-inflammatory cytokines, chemokines, complement proteins, and upon strong activation may release toxic free radicals (McGeer and McGeer, 2010; Krabbe et al., 2013). *In vitro*, Aβ peptides have been demonstrated to activate microglia and stimulate the production of nitric oxide (Maezawa et al., 2011). When microglia co-cultured with hippocampal brain slices were treated with aggregated Aβ, there was an upregulation of various pro-inflammatory molecules and neuronal death (Butovsky et al., 2005). The important findings of microglia in AD brains gave rise to an interest to quantify these cells *in-vivo*.

2. Imaging neuroinflammation with positron emission tomography

The development of different non-invasive imaging techniques has had a great impact on our ability to understand brain structure and function. Positron Emission Tomography (PET) is an analytical imaging technology used to image and measure biochemical processes of mammalian biology *in-vivo* (Phelps, 2000). PET requires the design of a ligand that binds with high specificity to a desired target, but with minimal nonspecific binding to other structures (Owen and Matthews, 2011). The molecular probe or ligand is labelled with a positron-emitting radioisotope (*e.g.*, ^{18}F, ^{11}C, ^{15}O). The radioligand is then administered intravenously at a tracer dose, such that the radioligand should only occupy a negligible amount of target sites (typically defined as $\leq 5\%$ of the total available target in the brain). Following the intravenous administration, the radioligand emits positrons (β^+) from its nucleus as it decays. The emitted positron collides with a nearby electron in the tissue, and an annihilation occurs with their masses converted into their energy equivalent through emission of two 511-keV photons 180° apart (Phelps, 2000). The photons are detected by scintillation detectors surrounding the participant, which ultimately allows for the calculation of the spatial distribution of the radioligand in the brain and indirectly the biological process being investigated.

2.1. Targeting microglia: translocator protein 18 kDa

The activation of microglia has been evidenced by the concomitant upregulation or *de novo* synthesis of a variety of cell-surface and cytoplasmic molecules (Perry et al., 2010). One particular protein that has been of great interest in quantifying neuroinflammation *in-vivo* is Translocator Protein 18 kDa (TSPO). TSPO was identified during central benzodiazepine receptor (CBR) binding studies (Braestrup and Squires, 1977). It was initially termed as a peripheral benzodiazepine receptor (PBR) as it was shown to be abundantly distributed in peripheral tissues (Chen and Guilarte, 2008). It was subsequently discovered that PBR is also present in glial and ependymal cells of the brain in addition to peripheral tissues. PBR was determined to be pharmacologically, anatomically, structurally, and physiologically distinct from the CBR, and was thus renamed to Translocator Protein 18 kDa (Chen and Guilarte, 2008).

TSPO can form a multimeric complex with the 32 kDa voltage-dependent anion channel (VDAC) also called mitochondrial porin and the 30 kDa adenine nucleotide carrier (ANC) in the outer mitochondrial membrane (Papadopoulos et al., 2006; Chen and Guilarte, 2008). The ratio of TSPO to VDAC and ANT appears to be tissue- and treatment-dependent (Veenman et al., 2007). Free TSPO, not in complex with VDAC and ANT, has also been suggested to be present in mitochondrial membranes (Veenman et al., 2007). While TSPO can be found in different parts of the cell, the primary intracellular location is the outer mitochondrial membrane (Veenman et al., 2007). The exact physiological functions of TSPO have still not been elucidated, nevertheless it is thought to participate in a variety of functions including cell growth and proliferation, steroidogenesis, bile acid synthesis, calcium flow, cholesterol transport, cell metabolism, apoptosis and neuroinflammation (Papadopoulos et al., 2006; Veenman et al., 2007; Chen and Guilarte, 2008; Gulyás et al., 2009).

In the brain, under normal physiological conditions, the levels of TSPO are low and limited to glial cells. However, during brain insults when microglia become activated, TSPO levels are dramatically upregulated (Chen and Guilarte, 2008; Gulyás et al., 2009). The increased expression of TSPO has been observed in both normal aging and in diseases involving the CNS such as AD, stroke, and multiple sclerosis (Gulyás et al., 2009). Microautoradiography and immunohistochemistry studies have confirmed that areas with increased TSPO levels coincide with the same areas in which there is an increase in microglia (Banati et al., 2000; Kuhlmann and Guilarte, 2000).

Although upregulation of TSPO has been widely confirmed, the functional significance of increased TSPO is still unknown. It has been hypothesized that increased TSPO may be associated with the proliferative, migratory and phagocytic capacity of microglia or it may be related to the secretion of inflammatory cytokines (Chen and Guilarte, 2008). The fact that TSPO levels are low in the brain parenchyma and regionally increased during brain insults makes TSPO an ideal marker for brain imaging studies.

2.2. Existing TSPO radioligands

Over the years, numerous TSPO radioligands have been developed, some of which have been studied *in-vivo* in human populations. We have chosen to review some of the radioligands studied in AD and MCI populations (Table 1). The most widely used radioligand is [^{11}C]-PK11195, which is a selective antagonist for TSPO. [^{11}C]-PK11195 was initially used as a racemate, but later studies found that the R-enantiomer has a 2-fold greater affinity for TSPO than the S-enantiomer and thus subsequent studies used [^{11}C]-(R)-PK11195 to investigate neuroinflammation *in-vivo* (Vivash and O'Brien, 2016). [^{11}C]-PK11195 has been used for almost three decades in a variety of neurological disorders such as multiple sclerosis (Politis et al., 2012), Parkinson's disease (Gerhard et al., 2006), Huntington's disease (Pavese et al., 2006), ischemic stroke (Gerhard et al., 2000), and AD (Cagnin et al., 2001). Although it has been widely used, there are some inherent limitations of this radioligand. First, it has a relatively low signal-to-noise ratio due to its high nonspecific binding and low brain permeability, and high plasma protein binding, all of which limit the accurate quantification of TSPO *in-vivo* (Chauveau et al., 2008). Second, the short half-life of carbon-11 restricts the use of the radiotracer to PET centres with an on-site cyclotron. The limitations of [^{11}C]-PK11195 have led to considerable efforts to develop improved radioligands, including [^{11}C]-PBR28 [^{11}C]-DAA1106, [^{11}C]-DPA713, [^{18}F]-PBR06, [^{18}F]-FEDAA1106, [^{18}F]-DPA714, [^{18}F]-FEMPA and [^{18}F]-FEPPA.

These radioligands generally have higher affinity and brain uptake, and an improved signal-to-noise ratio compared with [^{11}C]-PK11195 (Table 1). However their binding affinity is affected by a single nucleotide polymorphism (SNP) rs6971 in exon 4 of the TSPO gene, which causes an alanine-to-threonine substitution (Owen et al., 2012). Based on this polymorphism, individuals can be classified into one of the following three affinity patterns: high-affinity binders (HABs), mixed-