

Multi-Modal Imaging

1. Introduction

The role of glial activation in the neuropathology of Alzheimer's disease (AD) has been widely recognised both as a consequence and also as a contributing factor for the formation of amyloid and tau aggregation (Heneka *et al.*, 2015). However, astrocytes play a wide range of roles in the central nervous system (CNS) in innate immune responses, trophic support of neurons, synaptic function and ionic homeostasis (Calsolaro and Edison, 2016; Cai *et al.*, 2017). Astrocytes show highly organised coverage of the whole grey matter (Acosta *et al.*, 2017). Activated astrocytes are associated with amyloid plaques and are believed to have both direct and indirect effects on beta-amyloid (A β) deposition. Increases of inflammatory mediators and reactive oxygen species (ROS) with astrocyte activation can increase A β deposition, which, in turn, may lead to further astroglial activation (Cai *et al.*, 2017). Astrocytes are involved in tau hyperphosphorylation (Reyes *et al.*, 2008; Hallmann *et al.*, 2017; Kilian *et al.*, 2017; Logsdon *et al.*, 2017); a previous in vitro study has provided evidence that astrocytes may contribute to the production and spread of neurotoxic tau and A β 42 oligomers (Dal Pra *et al.*, 2015). In a mouse transgenic model of amyloid over-expression, activation of astrocytes can precede amyloid deposition (Heneka *et al.*, 2005a). Astrocytes may have protective roles by phagocytosing and degrading A β (Kraft *et al.*, 2013; Xiao and Hu, 2014), but, with disease progression, this may be lost (Sofroniew, 2015; Masgrau *et al.*, 2017).

Imidazoline I₂-binding sites (I₂-BS) have been located in different brain regions and are differentiated based on their binding affinity to clonidine and idazoxan (Regunathan *et al.*, 1993a). The cellular localization of the I₂-BS has been evaluated in cultured rat astrocytes and neurons. Non-adrenergic binding in astroglia is of the I₂ subclass with the putative receptor localised mainly in the mitochondria (Regunathan *et al.*, 1993a). An earlier study also demonstrated [³H]-idazoxan binding in mitochondria and also in non-nuclear fractions of cells from bovine adrenal medulla (Regunathan *et al.*, 1993b). These data have been confirmed in a study in post-mortem human brains of subjects with AD, where an up-regulation of I₂-BS has been found (Garcia-Sevilla *et al.*, 1998). However, a specific I₂-BS has not yet been cloned and there is currently no consensus regarding the nature of the I₂-BS. It may represent a heterogeneous family of proteins rather than a single one (Li, 2017).

The novel PET tracer, [¹¹C]BU99008 specifically targeting the I₂ binding site, has been developed as a potential marker of astrocyte activation (Tyacke *et al.*, 2012). [¹¹C]BU99008 showed good brain penetration in the rodent: (Tyacke *et al.*, 2012), porcine (Kealey *et al.*, 2013) and non-human primate brain (Parker *et al.*, 2014; Kawamura *et al.*, 2017) and subsequent studies showed favourable biodistribution (Tyacke *et al.*, 2018) and dosimetry profiles in humans (Venkataraman *et al.*, 2018).

In this current study, we hypothesised that uptake of [¹¹C]BU99008 would be elevated in AD/MCI subjects. The primary aim of this pilot study was to assess the regional and voxel level uptake of [¹¹C]BU99008 in AD/MCI subjects, and to evaluate the relationship between [¹¹C]BU99008 and amyloid deposition assessed using [¹⁸F]Florbetaben.

2. Results

[¹⁸F]Florbetaben ROI analysis

All healthy control subjects and patients had amyloid scans. Based on the HC's means+2SD, six out of eleven AD/MCI patients were amyloid positive. When evaluating the cohort with the single subject analysis, seven out of eleven subjects were amyloid positive. All healthy control subjects were amyloid-negative. Amyloid-positive and amyloid-negative AD/MCI subjects and healthy controls were age-matched (amyloid-positive AD/MCI 76 \pm 4.2 yrs old, Amyloid-negative AD/MCI 71.6 \pm 4.0 yrs old, healthy controls, 71.6 \pm 5.3 yrs old).

[¹¹C]BU99008 ROI analysis

The composite brain [¹¹C]BU99008 V_T of amyloid-positive AD/MCI patients was significantly higher (median 73.6, mean 73.2 ± 7.4 , range 61.4-83.6; $p=0.033$) than for the healthy controls. Multiple ROI showed increases in V_T relative to the healthy controls: frontal lobe (22%, $p=0.007$) and posterior cingulate (16%, $p=0.042$), the temporal lobe (16%, $p=0.04$) and medial temporal lobes (19%, $p=0.019$), parietal lobe (19%, $p=0.039$), occipital lobe (28%, $p=0.018$) and cerebellum (19%, $p=0.045$). The amyloid-negative population did not show any group-wise significant difference in the tracer uptake when compared with the HCs.

We extended these observations using IRF parametric maps. The AD/MCI group showed a significantly increased [¹¹C]BU99008 IRF120 in frontal (20% of increase, $p=0.02$), temporal (16% increase, $p=0.04$), parietal (18% increase, $p=0.042$), occipital lobe (19% increase, $p=0.04$), and composite brain grey matter (18% increase, $p=0.03$), as shown in Table 2B. When separating the amyloid positive subjects from the amyloid negative, significant differences relative to the IRF120 in the healthy controls, remain in the same regions, but also were found in the posterior cingulate and the thalamus.

SPM analysis

ROI-based analyses do not describe the distribution of uptake well, as they average across ROI defined by the anatomically-based atlas, rather than by the distribution of amyloid pathology. To characterise the distribution of uptake more finely, we performed a voxel level SPM analysis. As expected, this also demonstrated significant increases in [¹¹C]BU99008 uptake in frontal, temporal, parietal and occipital regions, consistent with the ROI analysis (Figure 3).

Single subject analysis

We tested whether the increase in [¹¹C]BU99008 V_T could be used to distinguish individual patients in the AD/MCI group from healthy controls using single subject SPM analysis. 7/11 of the AD/MCI patients had clusters with significantly higher uptake compared with the control group at voxel-level. 6/7 of these patients with high [¹¹C]BU99008 V_T relative to the HC were amyloid-positive. These six amyloid-positive patients demonstrated significantly higher group-wise [¹¹C]BU99008 uptake compared with the healthy controls.

Biological parametric mapping correlation between [¹¹C]BU99008 binding and amyloid deposition

The observations of increased [¹¹C]BU99008 V_T in amyloid-positive AD/MCI patients suggested a possible relationship between amyloid deposition and tissue changes giving rise to greater radioligand uptake. We tested directly for a positive correlation using voxel-based biological parametric mapping (BPM). BPM demonstrated a positive correlation between [¹¹C]BU99008 binding and amyloid in amyloid-positive subjects across multiple different cortical regions. A similar relationship was found with a BPM analysis across the whole group of 11 MCI/AD subjects.

This is the first study evaluating [¹¹C]BU99008, a novel PET tracer, in neurodegenerative disease such as AD/MCI. In this study, we have demonstrated that there was an increase in [¹¹C]BU99008 uptake in subjects with cognitive impairment at regional and voxel levels. At the voxel level, we found a positive correlation between [¹¹C]BU99008 binding and amyloid deposition in patients with cognitive impairment.

[¹¹C]BU99008 is a novel PET tracer targeting I₂-BS. Demonstration of a positive correlation between the I₂-BS density and MAO-B, which is found in glial cells, and others showing the increased extra-neuronal MAO-B activity following ageing or rat brain damage, led to the hypothesis that the

Final Project Report – EM 3

predominant I₂-BS binding site is located in glial cells (Ruiz *et al.*, 1993; Li, 2017). However, no single receptor has been characterised. Biochemical studies in rats and rabbits, aiming to characterize the I₂-BS proteins, showed four different protein bands (Li, 2017). I₂-BS are thought to be located in the mitochondria of astrocytes, and modulate GFAP expression and become up-regulated together with MAO-B with ageing, in glioblastomas and in AD (Garcia-Sevilla *et al.*, 1998).

After promising results from studies on porcine brains, preclinical PET evaluation of [¹¹C]BU99008 in rhesus monkey brain demonstrated uptake in selective regions (globus pallidus>cortex>cerebellum) consistent with *in vitro* I₂-BS localisation (Parker *et al.*, 2014). The first-in-human study recently published by our group demonstrated that [¹¹C]BU99008 has high specificity and selectivity for I₂-BS and that a two tissue compartmental model could be used to analyse the data (Tyacke *et al.*, 2018). Another recent study evaluated the biodistribution and dosimetry profiles and demonstrated a favourable radiation risk profile and concluded that [¹¹C]BU99008 is safe for consecutive studies (Venkataraman *et al.*, 2018). On the basis of this evidence, we believe that [¹¹C]BU99008 may be a potential new tracer for detection of astroglial activation with human disease *in vivo*.

In this study, we have demonstrated an increase in [¹¹C]BU99008 uptake in amyloid-positive people with MCI/AD, suggesting an association of I₂-BS with amyloid deposition, possibly relating to astroglial activation. Considerable independent data has defined a strong relationship between astrocyte activation and amyloid deposition in the brain. Animal studies and post-mortem brains have demonstrated reactive astrogliosis surrounding amyloid plaques (Nagele *et al.*, 2003; Olabarria *et al.*, 2010). Phagocytic astroglial cells that have engulfed amyloid positive material of neuronal origin appear to contribute to the generation of amyloid plaques after their lysis (Nagele *et al.*, 2003). Amyloid also contributes to astrocyte activation, the secretion of inflammatory-factor mediators (Frost and Li, 2017). Reactive astrocytes also have increased levels of BACE-1, γ -secretase and APP.

Using our single subject SPM analysis, we demonstrated that there was significant [¹¹C]BU99008 uptake in all the amyloid-positive subjects individually, and in one amyloid-negative subject. Three of these subjects had AD, while the other three had MCI. This suggests that astrocyte activation may occur early in disease progression, in conjunction with amyloid deposition (Eikelenboom *et al.*, 2010; Scholl *et al.*, 2015). Interestingly, one of our MCI subjects, who was amyloid-negative, also showed significant increase in frontal and parietal cortical regions. This suggests that expression of I₂-BS may precede or also may be driven by other, non-amyloid pathology.

While region of interest analysis averages out the uptake within a region, single subject SPM analysis could detect increased voxel-level uptake relative to the control subjects. In this study, we demonstrated that, individually, there was significant uptake in all the amyloid-positive subjects. Interestingly, when the [¹¹C]BU99008 uptake was present, it was elevated in the frontal, temporal, parietal and occipital cortices, where the amyloid load was also elevated.

Previous imaging studies have evaluated astroglial activation by targeting the MAO-B enzyme. This is widely distributed across the brain, but does show increased expression in microglia when they became activated as in neuroinflammatory conditions (Ekblom *et al.*, 1993). Both [¹¹C]-deuterium-L-deprenyl ([¹¹C]-DED) and [¹¹C]-deprenyl-D2 have been used to study reactive astrocytes. A triple tracer study in a population of MCI, mild AD and healthy controls showed increased [¹¹C]-DED binding in amyloid-positive MCI subjects, also suggested that astrocyte activation might be an early event in the AD trajectory (Carter *et al.*, 2012). This finding was further supported by a subsequent multi-tracer study conducted in a population of pre-symptomatic and symptomatic autosomal dominant AD patients (Scholl *et al.*, 2015), suggesting astrogliosis in autosomal dominant AD patients early in the disease progression (Scholl *et al.*, 2015).

Final Project Report – EM 3

Our study extends these observations using an independent approach. The I₂ tracer, [¹¹C]BU99008, binds to a different site than the deprenyl-derived tracers. We have shown that [¹¹C]BU99008 does not bind to MAO-B as its binding is not displaceable by the selective inhibitor lazabemide (Parker *et al.*, 2014), by deprenyl (unpublished observations) or the nons-selective MAO inhibitor isocabozide (Tyacke *et al.*, 2018). The exact nature and intracellular location of the I₂-BS has not yet been conclusively determined but evidence for astrocytic binding is given by a portion of its binding being co-localised with GFAP, an astrocyte marker (Dr. Magdalena Sastre, Imperial College, personal communication). Furthermore, BU99008 binding has good properties as a PET tracer, adding to confidence in the results; tissue binding is reversible and displaceable by other imidazoline I₂ ligands e.g. idazoxan in humans (Tyacke *et al.*, 2018). By contrast, deprenyl is an irreversible (suicide) inhibitor of the MAO-B enzyme, which makes accurate PET modelling of its binding more challenging.

Spectral analysis further supports our conclusions. We generated IRF-120 parametric maps to quantify [¹¹C]BU99008 PET (which correlated well with the V_T estimated from 2TCM4k kinetic modelling, see Supplementary Figure1) and, using a biological parametric mapping correlation, found a positive correlation between [¹¹C]BU99008 uptake and amyloid deposition in the MCI/AD patients.

3. Conclusion

In our study using the novel PET tracer [¹¹C]BU99008 PET, we were able to demonstrate an increase in [¹¹C]BU99008 uptake in AD/MCI patients compared to HC, which we interpret as possibly indicative of astroglial activation, and to define its distribution. We found that radioligand uptake was strongly associated with amyloid deposition in MCI/AD, suggesting a potential mechanistic link between amyloid deposition and astrocyte activation in the progression of AD. The ability to monitor this *in vivo* provides the potential for testing the mechanistic relationships directly with therapeutic approaches targeting with amyloid production or glial activation directly.

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5. Bibliography

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Final Project Report – EM 3

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Final Project Report – EM 3

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Final Project Report – EM 3

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