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Increased in vivo glial activation in patients with amyotrophic lateral sclerosis: Assessed with $^{[11}C$-PBR28

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1. Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder in which upper and lower motor neurons degenerate leading to progressive muscle weakness, respiratory failure and death often within 2–5 years (Ferraiuolo et al., 2011). Riluzole, the only FDA-approved treatment provides a modest survival benefit (Lacomblez et al., 1996), but there are no available treatments that prevent or stop disease progression and current animal models have not yet successfully predicted treatment response in people (Atassi et al., 2012; Ferraiuolo et al., 2011). A substantial body of evidence implicates the neuroimmune system and specifically activated microglia in ALS pathophysiology (Appel et al., 2011). In post mortem studies increased activated microglia are correlated with increased upper motor neuron symptoms and faster disease progression (Brettschneider et al., 2012). Despite years of research, the fundamental question of whether the immune response observed in ALS is primary or secondary, beneficial or harmful, or a combination of both, has not yet been clearly answered.

Given the disconnection between mouse models and human disease, it is critical to develop methods to examine disease biology in vivo in patients with ALS. With positron emission tomography (PET) a radiotracer is used to visualize and quantify molecular interactions with high sensitivity. Several PET radiotracers have been developed to image activated microglia and most provide contrast by binding the 18 kDa translocator protein (TSPO), formerly known as the peripheral benzodiazepine receptor (PBR), which is highly

1.1. Neuroinflammation

In vivo microglial activation assessed with $^{[11}C$-PBR28

Positive correlation with upper motor neuron symptoms

Negative correlation with healthy controls

Increased in vivo glial activation in patients with amyotrophic lateral sclerosis compared to healthy controls (p < 0.05). Region of interest analysis revealed increased $^{[11}C$-PBR28 binding in the precentral gyrus in patients (normalized standardized uptake value = 1.15) compared to controls (1.03, p < 0.05). In patients those values were positively correlated with upper motor neuron burden scores (r = 0.69, p < 0.05), and negatively correlated with the amyotrophic lateral sclerosis functional rating scale (r = −0.66, p < 0.05).

Increased in vivo glial activation in motor cortices, that correlates with phenotype, complements previous histopathological reports. Further studies will determine the role of $^{[11}C$-PBR28 as a marker of treatments that target neuroinflammation.

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expressed in activated microglia and astrocytes (Brown et al., 2007; Lavisse et al., 2012). The first application of TSPO PET imaging in patients with ALS confirmed widespread microglial activation (Turner et al., 2004). This pioneering study conducted with the radioligand $\text{[11C]-}^{\text{-(R)}}\text{PK11195}$ showed increased binding in the motor cortex, pons, dorsolateral prefrontal cortex and thalamus in a group of ALS patients. Older generation TSPO radioligands such as $\text{[11C]-}^{\text{-(R)}}\text{PK11195}$ suffered from high levels of non-specific binding and poor signal-to-background ratio (Kreisl et al., 2010). Increased TSPO expression, assessed using the radioligand $\text{[18F]-DPA-714}$, was subsequently reported in the primary motor cortex, supplementary motor area as well as temporal cortex of patients with ALS, thereby providing additional support for a role for inflammatory processes in ALS (Corcia et al., 2012).

The radioligand $\text{[11C]-PBR28}$, developed at the National Institute of Mental Health, was shown to exhibit 80 times more specific binding compared to $\text{[11C]-}^{\text{-(R)}}\text{PK11195}$ in rhesus macaques (Kreisl et al., 2010). The aim of this proof-of-concept study was to investigate $\text{[11C]-PBR28}$ binding in a group of individuals with ALS compared to a matched group including for TSPO polymorphism of healthy controls and to investigate whether the $\text{[11C]-PBR28}$ radiotracer could better sub-categorize ALS patients based on the anatomical regions with the highest disease burden.

2. Materials and methods

The study was conducted at the Athinoulia A. Martinos Center for Biomedical Imaging at Massachusetts General Hospital. The protocol was approved by the Institutional Review Board and the Radioactive Drug Research Committee. All participants provided written informed consent according to the Declaration of Helsinki.

2.1. Participants

Fourteen ALS patients were initially screened for the study. To meet inclusion criteria, participants had to fulfill the revised EL Escorial criteria (Brooks et al., 2000) for possible, probable, probable laboratory-supported or definite ALS, not have any signs of frontotemporal dementia and could not be taking any anti-inflammatory or immunosuppressant medications or benzodiazepines. None of the patients had a familial history of ALS.

$\text{[11C]-PBR28}$, along with all second-generation TSPO radiotracers to date, has differential binding affinity to TSPO depending on an Ala/147Thr polymorphism in the TSPO gene with Ala/Ala leading to high-, Ala/Thr to mixed-, and Thr/Thr to low-affinity binding (Kreisl et al., 2013; Owen et al., 2012). This binding affinity difference can be detected by standardized uptake value (SUV) measurements (Yoder et al., 2013), and needs to be controlled for in cross-sectional study designs. All participants were tested for TSPO polymorphism and low affinity binders were excluded, resulting in the exclusion of two individuals with ALS with Thr/Thr Ala174Thr polymorphism. Two additional individuals with ALS were not able to lie comfortably on the scanner table and therefore data could not be acquired.

Of the remaining 10 individuals with ALS who successfully completed scanning, seven had limb-onset ALS and three had bulbar-onset ALS. The clinical outcomes obtained from the participants with ALS included the revised ALS functional rating scale (ALSFRS-R) (Cedarbaum et al., 1999), upper motor neuron burden scale (UMNB) (Ellis et al., 1999), and vital capacity (VC). The ALSFRS-R assesses general functional status and ranges from 48 (normal level of functioning) to 0, with lower scores indicating increased disability. The UMNB measures the following deep tendon (scores 0–4) and pathological reflexes (present — 1 or absent — 0): biceps, brachioradialis, triceps, knee jerk, ankle jerk, Hoffman, Babinski, and jaw jerk. The total UMNB score ranges from 0 to 45, with 0 representing no reflex involvement, and 45 maximal abnormal UMNB. VC measures respiratory functioning and is expressed as a percentage out of 100, with 100 being normal and scores below 100 indicating decreased lung capacity. The 10 participants with ALS were compared to 10 healthy controls matched for age and TSPO binding affinity.

2.2. Radiotracer synthesis and data acquisition

$\text{[11C]-PBR28}$ was produced in-house using a procedure modified from the literature (Imaizumi et al., 2007). Briefly, the desmethyl precursor (1.0 mg in 100 µL) was loaded into a 5 mL stainless steel loop for reaction with CH$_3$I using the Wilson captive solvent method (Wilson et al., 2000). $\text{[11C]-PBR28}$ was purified by reversed-phase chromatography and reformulated by solid-phase extraction in 10% ethanol/saline and then aseptically filtered. The radioligand was injected as slow intravenous bolus, with a median administered dose of 419.49 mBq for patients with ALS and 419.08 mBq for controls. PET data were acquired over 90 min and stored in list-mode format.

Participants were scanned on a Siemens magnetic resonance (MR)/PET scanner consisting of a dedicated brain avalanche photodiode-based PET scanner operating in the bore of a 3 T whole-body MR scanner, and an 8-channel head coil was used. This combined MR/PET scanner allowed the simultaneous acquisition of MR and PET data (Catana et al., 2010). An anatomical scan, a multi-echo MP-RAGE (TR = 2530, TE 1–4 = 1.64, 3.5, 5.36, 7.22 ms, flip angle = 7°, voxel size = 1 mm isotropic) was acquired at the beginning of the scan.

2.3. Data analysis

After acquisition, PET images were reconstructed using the Ordinary Poisson Ordered Subset Expectation Maximization 3D algorithm from prompt coincidences, with corrections for normalization, dead time, isotope decay, photon attenuation and expected random and scatter coincidences. Attenuation correction maps were created using MR-based methods (Izquierdo-Garcia et al., 2014). SUV images were created for radioactivity in the field of view 60–90 min post-radioligand injection. To account for motion that may have occurred between MP-RAGE acquisition and the 60–90 min post-injection time point corresponding to the PET frame of interest, SUV$_{60–90\text{ min}}$ was generated in a two-step procedure. First, a SUV$_{60–90\text{ min}}$ image was created for each subject using an attenuation correction map computed from the native MP-RAGE. Subsequently, a new attenuation map was created based on the registration of the native MP-RAGE with the SUV$_{60–90\text{ min}}$ image obtained in this first reconstruction using FreeSurfer’s spmregister. A final SUV$_{60–90\text{ min}}$ was then reconstructed based on this new attenuation correction image well registered with the 60–90 min PET data. Individual SUV$_{60–90\text{ min}}$ images were then registered to MNI (Montreal Neurological Institute) space, spatially smoothed (6 mm full width at half maximum), and intensity-normalized to a mean of 1 (SUV$_{60–90\text{ min}}$) in order to account for differences in global signal across subjects, as previously done for $\text{[11C]-PBR28}$ (Loggia et al., 2015). SUV$_{60–90\text{ min}}$ in MNI was then fed into a voxelwise between-group analysis. FSL’s randomize was used to perform a permutation-based nonparametric two-sample unpaired $t$-test (n permutation = 10,000, 5 mm variance smoothing) with TSPO genotype added as a nuisance regressor. Threshold-free cluster enhancement (TFCE) was applied and $p$ values were family-wise error rate (FWE) corrected ($p_{\text{FWER}} < 0.05$) (Nichols and Holmes, 2002).

An a priori region of interest for the left and right precentral gyri was selected for each subject using Freesurfer’s automated parcellation. Two-tailed Mann–Whitney was used to assess between-group differences. To investigate whether $\text{[11C]-PBR28}$ binding correlated with ALS disease severity, Spearman’s $r$ was used to investigate for the presence
of correlation between SUVR\textsubscript{60–90 min} of the a priori precentral gyrus ROI and UMNB scores, ALSFRS-R, and disease duration.

3. Results

3.1. Study participants

Ten ALS patients (7 males, 3 females, 6 high- and 4 mixed-affinity binders), with a mean age of 53.2 years (SD = 10.8, range 38–68) successfully completed scanning and were compared to healthy controls matched for binding affinity and age (6 males, 4 females, 6 high- and 4 mixed-affinity). Healthy controls were on average 51.1 years old (SD = 11.0, range 33–65). See Table 1.

3.2. Individual data in patients with ALS and group means

Visual inspection of SUVR\textsubscript{60–90 min} images revealed regional increase in the precentral gyrus in patients with ALS with limb-onset disease. See Fig. 1A for individual data projected onto the MNI template and Fig. 1B for means for the ALS group, including comparisons between limb- and bulbar-onset patients, and the control group. Patients with limb-onset weakness showed increased binding in the precentral gyri and patients with bulbar-onset weakness showed increased binding in the brainstem (Fig. 1B, top two rows).

3.3. Whole brain between-group analysis

The unpaired \( t \)-test conducted on the whole brain static SUVR\textsubscript{60–90 min} image showed that the ALS group has increased \([11C]^{-}\)PBR28 binding in the bilateral motor cortices, including the primary motor cortex (M1) and supplementary motor area, as well as in the upper region of the corticospinal tract \( p_{\text{FWE}} < 0.05 \), Fig. 1C. There were no regions in which controls showed increased binding compared to individuals with ALS.

Given that to date only TSPO genotype has been shown to influence \([11C]^{-}\)PBR28 binding, we are reporting the results of a voxelwise analysis, which added only TSPO genotype (Ala/Ala vs. Ala/Thr) as a regressor of no interest. However, an additional analysis conducted using TSPO genotype, as well as age and sex as regressors of no interest, led to the same brain regions being significantly increased in the ALS vs. control group comparison as the analysis where only TSPO genotype was added as a regressor.

3.4. Region of interest analysis

Compared to controls, individuals with ALS exhibited significantly increased binding in the bilateral precentral gyri, a priori identified region of interest. ALS (median, range): 1.15, 1.05–1.30, controls: 1.03, 0.99–1.18, \( p < 0.05 \) (Fig. 2). In ALS patients, SUVR\textsubscript{60–90 min} of the right precentral gyrus was positively correlated with UMNB scores, \( r = 0.69, p < 0.05 \) (Fig. 3A) and negatively correlated with functional status measured by ALSFRS-R, \( r = -0.66, p < 0.05 \) (Fig. 3B). Disease duration did not correlate with SUVR\textsubscript{60–90 min} in the precentral gyrus.

An exploratory comparison of individuals with limb vs. bulbar onset ALS showed that individuals with limb onset (\( N = 7 \)) had a significantly higher SUVR\textsubscript{60–90 min} than individuals with ALS with bulbar onset (\( N = 3 \)) in the precentral gyrus (\( p < 0.05 \)).

4. Discussion

Our study demonstrates increased \( \text{in vivo} [11C]^{-}\)-PBR28 binding in the motor cortices and corticospinal tract in patients with ALS. This finding is consistent with the first microglial PET study in ALS patients (Turner et al., 2004), as well as histopathological studies reporting increased activated microglia near degenerating motor neurons (Brettschneider et al., 2012; Henkel et al., 2004; Kawamata et al., 1992), suggesting that \([11C]^{-}\)-PBR28 PET is a robust candidate as an \( \text{in vivo} \) biomarker of inflammation in ALS. This is the first study conducted in patients with ALS using a second-generation TSPO radioligand, while controlling for TSPO binding affinity genotype. In accordance with our findings, previous studies investigating TSPO binding reported increases in motor cortices (Turner et al., 2004). Compared to previous tracers, \([11C]^{-}\)-PBR28 SUVR images provide higher contrast in regions of activated glia, which represents a considerable advantage when evaluating neuroinflammation in individual patients.

Apart from clinical signs, there are no reliable biomarkers of upper motor neuron dysfunction in ALS. \([11C]^{-}\)-PBR28 PET could represent a marker of upper motor neuron injury that can complement electromyography as an indicator of lower motor neuron injury. Notably, the individual scores from the UMNB scale and ALSFRS-R were correlated with \([11C]^{-}\)-PBR28 binding, suggesting a clinical relevance of brain...
inflammation measured in vivo. Specifically, the UMNB score was positively correlated with microglial PET tracer uptake in the motor cortex using $[^{11}C]$-PK11195 (Turner et al., 2004). Additionally, our exploratory analysis showed that individuals with bulbar-onset did not show the increased binding in the motor cortex to the extent that it was observed in patients with limb-onset ALS. On the other hand, individuals with bulbar-onset ALS showed regional increases in PBR28 uptake in the brainstem. This suggests that $[^{11}C]$-PBR28 binding has a strong anatomical relevance to ALS clinical phenotype (Fig. 1B). The increase in PBR28 uptake in the brainstem could represent inflammation around the lower motor neurons of the cranial nerves as all three bulbar-onset subjects had evidence of lower motor neuron dysfunction on examination. This emphasizes the importance of exploring PBR28 PET imaging in the spinal cord to better characterize this observation. On the other hand,
the reason for not seeing increased PBR28 uptake values in the motor cortex in the bulbar-onset patients is unclear. Considering the very small sample size of this subgroup (N = 3) we consider these data preliminary and caution against over-interpretation of these findings.

The main limitation of our study is the relatively small sample size. However, both the two previously published PET studies in ALS using TSP0 radiotracers (Corcia et al., 2012; Turner et al., 2004) as well as \([^{11}C]PBR28\) PET studies in other diseases (Fujita et al., 2013; Oh et al., 2011) have shown that disease-related changes can be observed with comparable sample size. Based on our current findings, larger studies are needed to validate the value of \([^{11}C]-PBR28\) in ALS and our results should be considered preliminary pending further testing. Forthcoming experiments will help understand how the distribution and degree of \([^{11}C]-PBR28\) binding change as the disease progresses. Given the preliminary differences observed between individuals with ALS with bulbar onset vs. limb onset ALS, larger studies enrolling more patients with bulbar onset will also be required. In addition, studies enrolling a higher number of participants will help in addressing the topic of laterality. In our study we observed a larger group difference in the left hemisphere and correlation with disease severity only in the right hemisphere. Differences in laterality could be related to handedness of patients, site of onset, or side with most prominent symptoms at time of scan. However, given our sample size, interpretations of laterality are difficult and as previously suggested those questions will need to be addressed in larger studies or meta-analyses (Bede and Hardiman, 2014). Another potential limitation of the current study is the sole use of SUV as representation of \([^{11}C]-PBR28\) binding without performing kinetic modeling that involves arterial blood sampling. While the potential to use SUV as a binding metric is a promising characteristic for image interpretation, future studies with kinetic modeling derived from arterial blood sampling may need to be conducted to determine binding potential.

The added value of this study lies in the fact that due to the high sensitivity of \([^{11}C]-PBR28\), increased binding can be observed not only at the group level, but also at the level of individual patients. However, as it currently stands, \([^{11}C]-PBR28\) PET is not a diagnostic tool for ALS, but rather could serve as a pharmacodynamic biomarker to monitor the efficacy of treatments targeting neuroinflammation. This could be invaluable for evaluation of potential therapeutics in early phase clinical trials. Finally, the current routine ALS trial entry criteria do not include any disease biomarkers and are based solely on phenotype, disability and disease duration. Mechanism-based imaging based on enrollment of individuals with high levels of \([^{11}C]-PBR28\) binding could help with prognostic stratification or cohort enrichment in ALS clinical trials, as it would allow the selection of ALS patients that have more inflammation at baseline and thus may be more likely to respond to certain treatments. \([^{11}C]-PBR28\) PET imaging may provide important contributions to the fundamental question of immune system involvement in ALS by allowing a mechanistic investigation of the role of activated microglia. The study of individuals who are pre-symptomatic, but at high risk of developing the disease, such as superoxide dismutase-1 (SOD1) or C9orf72 gene carriers, may also provide important insights in this context, as suggested by a previous PET imaging study investigating pre-symptomatic ALS patients (Turner et al., 2005).

In conclusion, \([^{11}C]-PBR28\) PET shows increased vivo glial activation in individuals with ALS, supporting a role for glial cells in this disease. Future studies will need to determine its potential as a pharmacodynamic biomarker to monitor the efficacy of treatments targeting neuroinflammation in ALS.

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