Seizing the Neuroinflammatory Target: The Quest Continues

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Neuroinflammation in Neocortical Epilepsy Measured by PET Imaging of Translocator Protein

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Objectives: Neuroinflammation, implicated in epilepsy, can be imaged in humans with positron emission tomography (PET) ligands for translocator protein 18 kDa (TSPO). Previous studies in patients with temporal lobe epilepsy and mesial temporal sclerosis found increased [11C]PBR28 uptake ipsilateral to seizure foci. Neocortical foci present more difficult localization problems and more variable underlying pathology. Methods: We studied 11 patients with neocortical seizure foci using [11C]PBR28 or [11C] N,N-diethyl-2-(4-methoxyphenyl)-5,7-dimethylpyrazolo[1,5-a]pyrimidine-3-acetamide 713 and 31 healthy volunteers. Seizure foci were identified with structural magnetic resonance imaging (MRI) and ictal video-electroencephalography monitoring. Six patients had surgical resections: 5 had focal cortical dysplasia type 2A or B and 1 microdysgenesis. Brain regions were delineated using FreeSurfer and T1-weighted MRI. We measured brain radioligand uptake (standardized uptake values) in the ipsilateral and contralateral regions, to compare calculated asymmetry indices (AIs; 200% *[ipsilateral − contralateral]/[ipsilateral + contralateral]) between patients with epilepsy and controls, as well as absolute [11C]PBR28 binding as the ratio of distribution volume to free fraction (VT/fP) in 9 patients (5 high-affinity and 4 medium-affinity binders) and 11 age-matched volunteers (5 high affinity and 6 medium affinity) who had metabolite-corrected arterial input functions measured. Results: Nine of 11 patients had AIs exceeding control mean 95% confidence intervals in at least one region consistent with the seizure focus. Three of the 9 had normal MRI. There was a nonsignificant trend for patients to have higher binding than volunteers both ipsilateral and contralateral to the focus in the group that had absolute binding measured. Significance: Our study demonstrates the presence of focal and distributed inflammation in neocortical epilepsy. There may be a role for TSPO PET for the evaluation of patients with suspected neocortical seizure foci, particularly when other imaging modalities are unrevealing. However, a complex method, inherent variability, and increased binding in regions outside seizure foci will limit applicability.

Commentary

The relationship between epilepsy and neuroinflammation is no longer being questioned. In fact, studies have shown that, in response to tissue insult, the neuroinflammatory cascade starts with microglial activation, which then may continue even when the original insult has been removed. Neuroinflammation may underlie neurological diseases that have evaded scientists for years, with current investigations focusing on its role in multiple sclerosis, Alzheimer disease, and, of greatest significance to us, epilepsy. In the case of epilepsy, perpetual activation of the neuroinflammatory cascade likely results in dysfunction of the blood–brain barrier, giving rise to chronic neuronal hyperexcitability.[1,2] This lowers seizure threshold and promotes aberrant epileptic activity, eventually resulting in the process of epileptogenesis and the development of clinical seizures.[1,2] Prior to the dawn of advanced neuroimaging, studying neuroinflammation in humans depended on probing postmortem (or surgical) tissue samples. However, the development of modern neuroimaging techniques has allowed us targeted imaging of microglial activation via positron emission tomography (PET). Positron emission tomography imaging that utilizes specific tracers allows visualizing biochemical properties of neuroinflammation in vivo and may hold promise for localizing ictal onset zone(s) in patients with lesional and nonlesional epilepsy.

In the present study, the authors measured neuroinflammation in patients with neocortical epilepsy using 2-carbon PET radiotracers ([^{11}C]PBR28 and [^{11}C]DPA-713). These radiotracers bind to 18-kDa translocator protein (TSPO), originally designated a peripheral benzodiazepine receptor, which is overexpressed on immune cells such as microglia during active neuroinflammation.[3] Dickstein et al found increased [^{11}C]PBR28 and [^{11}C]DPA-713 binding to TSPO in regions ipsilateral and contralateral to the neocortical seizure foci. Consistent with their hypotheses and the results of the previous

studies, TSPO PET augmented the search for the seizure-onset zone, and in one case, the results of the study prompted a revision of the structural magnetic resonance imaging findings. Although the increased TSPO binding contralateral to the ictal onset zone may be nonspecific, it may also indicate that neuroinflammation in epilepsy is not a focal but rather a diffuse and widespread phenomenon, especially in patients who are lacking magnetic resonance detectable lesions.

Why is this study important? Patients with neocortical epilepsy respond poorly to antiseizure drugs, having pharmacoresistance which is associated with unfavorable personal and societal outcomes.[4] Surgical resection of the ictal onset zone is often the most efficacious treatment for achieving seizure freedom.[4] However, the surgical approach requires clearly localizing the ictal onset zone, which frequently is a challenge when seizures originate in the neocortex.[4] This study is a step in demonstrating that TSPO-based PET imaging may hold promise for delineating the ictal onset zone in patients with neocortical epilepsy when other diagnostic approaches are unrevealing. Finding focal abnormalities that are not detected by standard imaging may increase the chance of achieving seizure freedom after resection.[5]

Still, it is important to consider these results in the context of this study's limitations. The study's small sample size (N = 11) decreases statistical power, thus limiting the investigators' ability to reliably detect a difference between groups. Participants were highly variable in disease duration, age of onset, presence/absence of cortical lesions, and the location of the ictal onset zone. All of these factors limit study interpretation, especially given subtle biochemical differences that arise in chronically epileptic tissue over time. An important point is that patients were screened and genetically stratified based on the rs6971 TSPO-binding polymorphism, resulting in an inclusion of only high-affinity and mixed-affinity binders.[6] The epilepsy group included 5 high-affinity and 4 mixed-affinity binders; the healthy participants included 5 high-affinity and 6 mixed-affinity binders. In comparing mixed-affinity to high-affinity TSPO binders, high-affinity binders demonstrate a 40% increase affinity for binding TSPO.[6] This may confound the results of any study that includes both groups.[6] Even with this genetic stratification and exclusion of low-affinity binders, the lack of literature on low-affinity binders leaves a gap in our understanding that is worth further exploration. Thus, this approach limits the applicability of this technique to patients who are high- or mixed-affinity binders, leaving the low-affinity binders unable to benefit from TSPO PET.[6]

Importantly, Dickstein et al did not find significant between-group differences in absolute [^{11}C]PBR28 binding. Spatial and temporal modeling binding of [^{11}C]PBR28 to TSPO is critical for answering key questions.[7] For example, approximately how many TSPO receptors are present in the region of interest? What is the rate of influx of [^{11}C]PBR28 into the region in question? Pharmacokinetic modeling estimates the ratio of total tissue distribution volume to free, unbound [^{11}C]PBR28 over time, yielding an estimate of [^{11}C]PBR28-bound TSPO in the tissues of interest. Given the practical impossibility of finding reference brain tissue completely devoid of TSPO expression, serial blood sampling is the go-to for modeling these aspects of [^{11}C]PBR28-TSPO interactions. However, as demonstrated by this study, absolute quantification is challenging and may be unreliable. Further, the problem of absolute quantification is not unique to this study and directly stems from limitations of TSPO-based imaging.

Translocator protein PET is based on indirectly tracking activated microglia, but their functions are more complex than previously thought. Recent evidence points to the existence of 2 microglia phenotypes with rivaling functions: the neurotoxic (M1) phenotype and the neuroprotective (M2) phenotype.[8] Thus, TSPO is best regarded a general biomarker of a unified neuroinflammatory response and not a biomarker specific to the neurotoxic microglia. Furthermore, TSPO's exact function is unclear, as is the question of whether it is overexpressed in M1 or M2 microglial phenotypes. Newly developed radioligands can discriminate between immune cells and may even be specific for microglial phenotypes. For example, P2x7r is expressed on the same cell types as TSPO but may be specific for the M1 phenotype.[9] FR-β and P2Y12R receptors are microglia-specific and may preferentially bind the M2 phenotype.[9] Translocator protein is further limited by its expression on multiple cell types (eg, microglia, astrocytes, etc) at different stages of neuroinflammation.[10] Recent studies suggest a temporal variable being of importance, indicating that TSPO expression is increased on microglia at primary stages of the inflammatory process followed by increased activation and TSPO expression of astrocytes shortly thereafter—the presence of sequential events may indicate different phases of epileptogenesis or epilepsy progression as it has been recently documented in, for example, schizophrenia.[10]

Despite its limitations, TSPO has enhanced our understanding of the biochemical underpinnings of epileptogenesis. The work by Dickstein et al adds to our understanding of how TSPO PET may pinpoint neuroinflammation and localize regions of seizure onset, highlighting the exciting potential for future radiotracer-based and other neuroimaging approaches that focus on neuroinflammation. Future studies should utilize more targeted radioligands that reveal specific molecular processes and are indicative of a specific microglial phenotype. Further, PET (or nonradioactive methods) experiments that target finer aspects of neuroinflammation may guide the development of neuroimaging-based biomarkers for localizing, monitoring, and possibly preventing epilepsy.

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