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Neuroinflammation and brain atrophy in former NFL players: An *in vivo* multimodal imaging pilot study

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ABSTRACT

There are growing concerns about potential delayed, neuropsychiatric consequences (e.g, cognitive decline, mood or anxiety disorders) of sports-related traumatic brain injury (TBI). Autopsy studies of brains from a limited number of former athletes have described characteristic, pathologic changes of chronic traumatic encephalopathy (CTE) leading to questions about the relationship between these pathologic and the neuropsychiatric disturbances seen in former athletes. Research in this area will depend on *in vivo* methods that characterize molecular changes in the brain, linking CTE and other sports-related pathologies with delayed emergence of neuropsychiatric symptoms. In this pilot project we studied former National Football League (NFL) players using new neuroimaging techniques and clinical measures of cognitive functioning. We hypothesized that former NFL players would show molecular and structural changes in medial temporal and parietal lobe structures as well as specific cognitive deficits, namely those of verbal learning and memory. We observed a significant increase in binding of [^{11}C]DPA-713 to the translocator protein (TSPO), a marker of brain injury and repair, in several brain regions, such as the supramarginal gyrus and right amygdala, in 9 former NFL players compared to 9 age-matched, healthy controls. We also observed significant atrophy of the right hippocampus. Finally, we report that these same former players had varied performance on a test of verbal learning and memory, suggesting that these molecular and pathologic changes may play a role in cognitive decline. These results suggest that localized brain injury and repair, indicated by increased [^{11}C]DPA-713 binding to TSPO, may be linked to history of NFL play. [^{11}C]DPA-713 PET is a promising new tool that can be used in future study design to examine further the relationship between TSPO expression in brain injury and repair, selective regional brain atrophy, and the potential link to deficits in verbal learning and memory after NFL play.

Introduction

There have been several recent reports of memory deficits, mood disorders, and motor symptoms among former athletes exposed to repetitive, sports-related, traumatic brain injury (TBI) (Guskiewicz et al., 2005, 2007; Hart et al., 2013; McKee et al., 2013; Pearce et al., 2014; Seichepine et al., 2013; Singh et al., 2014; Weir et al., 2009). Coupled with increased public awareness, these studies have fueled scientific investigation into pathologic changes contributing to TBI-related neuropsychiatric symptoms including cognitive decline or affective changes (e.g., anxiety, mood swings, depression) in the absence of neurologic

Abbreviations: CTE, chronic traumatic encephalopathy; CVLT-II, California Verbal Learning Test, Second Edition; mTBI, mild traumatic brain injury; NFL, National Football League; RPQ, Rivermead Post-Concussive Symptom Questionnaire; SMG, supramarginal gyrus.

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signs (e.g., slurred speech, parkinsonism). Concurrently, autopsy studies of a limited number of athletes (including football players) who have suffered from TBI have led some researchers to diagnose chronic traumatic encephalopathy (CTE), a putative tauopathy characterized by global brain atrophy with a thinned corpus callosum, enlarged ventricles, and cavum septum pellucidum (Jordan, 2013). The prevalence of CTE among former athletes who have suffered from TBI is unknown, as is the prevalence of other injury-associated brain pathologies, for example those associated with Alzheimer's disease or frontotemporal degeneration. Nevertheless, there are concerns that even mild, repetitive TBI leads to the development of one or more progressive pathologies causing the delayed emergence of these neuropsychiatric disturbances (Collins et al., 1999; Matser et al., 1999; Singh et al., 2014). To best understand these relationships the development of *in vivo* tools, preferably utilizing brain imaging, is essential.

Regarding CTE, it has been hypothesized that repetitive mild TBI (mTBI) in athletes leads to axonal damage and inflammation, followed by deposition and aggregation of hyperphosphorylated tau protein (p-tau) and the formation of neurofibrillary tangles (NFTs) in susceptible areas (McKee et al., 2014; Shively et al., 2012). In stages I and II, foci of tau pathology are limited to the depths of cortical sulci and brainstem areas such as locus coeruleus. By stage III, NFTs have a more widespread distribution (Stein et al., 2014). It is unclear whether the primary pathology of CTE is deposition of p-tau or whether dysregulated inflammation drives protein deposition (Smith et al., 2013). Regional NFT distribution may promote chronic inflammation and neurotoxicity, resulting in underlying changes in local neuron morphology, more diffuse synaptic changes, and possibly changed cholinergic neurotransmission (Hellstrom-Lindahl et al., 2000; Rubio et al., 2006). NFT accumulation may also promote aggregation and diminished clearance of other pathologic proteins including amyloid β, TDP-43, and alpha-synuclein, thereby fueling further neurodegeneration, inflammatory response, and associated cognitive decline (Hazrati et al., 2013; McKee et al., 2013; Shively et al., 2012). Although neuroinflammation is not necessarily deleterious and could represent compensatory repair of these other degenerative processes, the ability to image inflammatory brain changes *in vivo* in former athletes will contribute to putting together the complex puzzle involved.

Former American football players have higher rates of delayed neurological, cognitive or affective impairments, including dementia with aging (Guskiewicz et al., 2005; Lehman et al., 2012; Weir et al., 2009). Those impairments have been attributed to the pathologic effects of repeated TBI, characterized by repetitive biomechanical shearing and inflammation of neuronal axons from rotational, linear, and/or impact decelerations of the head incurred over years of play. A recent study of retired National Football League (NFL) players found cognitive deficits and depression to be more common in this cohort compared to matched healthy controls, and reported specific deficits in naming, word finding, and visual or verbal episodic memory (Hart et al., 2013). Worse performance on verbal learning and memory testing has also been reported in Division I college varsity football and ice hockey athletes when compared to same-level athletes playing noncontact sports (McAllister et al., 2012, 2014). A functional magnetic resonance imaging (fMRI) study of former professional football players has suggested that subtle deficits in learning and memory may be due to functional inefficiencies of brain networks in medial temporal and inferior parietal lobes (Ford et al., 2013).

At present, CTE is diagnosed on the basis of findings seen at autopsy, although recent preliminary brain positron emission tomography (PET) imaging using [^{18}F]FDDNP, a radiopharmaceutical that binds to Alzheimer pathology including p-tau NFTs and amyloid, revealed increased binding in subcortical regions and in the amygdala of five former NFL players *in vivo* (Small et al., 2013). Novel technology enabling non-invasive, quantitative study of post-traumatic brain changes after TBI will improve the longitudinal study of pathologic responses to trauma, and will add value to prognostic evaluation and therapeutic monitoring. In addition to tau imaging, such technology may also include imaging of TBI-related neuroinflammation by targeting the translocator protein (TSPO).

TSPO is a five-transmembrane protein that spans the outer mitochondrial membrane (Papadopoulos et al., 2006). Although TSPO expression is low in healthy human brain tissue, brain TSPO levels are increased after TBI in animal models, likely due to increased expression by activated microglia in states of neuroinflammation and reactive gliosis. Accordingly, increased expression of TSPO has been used as a marker of brain injury or post-traumatic immune cell response (Papadopoulos and Lecanu, 2009) and has been detected in areas with other co-localized markers of microglial activation using [^{11}C]DPA-713 in animal models of neuroinflammation (Boutin et al., 2007). Using PET, two previous studies have demonstrated elevated uptake of [^{11}C]*R*-PK11195, a first-generation radioligand that targets TSPO, in the brains of patients who suffered moderate to severe TBI from several months to 17 years after injury (Folkersma et al., 2011; Ramlackhansingh et al., 2011). However, studies using [^{11}C]*R*-PK11195 are limited by poor signal-to-noise ratio related to high non-specific binding and poor brain delivery (Chauveau et al., 2008). Analysis of PET imaging data using second generation radiotracers for TSPO requires correction for rs6971 genotype because the Ala147Thr polymorphism is associated with reduced affinity for the TSPO target (Owen et al., 2012). Recently developed techniques to apply *TSPO* genotype correction to PET imaging with second generation radiopharmaceuticals not only control for the effect of this effect, but also improve the sensitivity of detecting increased TSPO density in neurodegenerative disease, as demonstrated in study of Alzheimer's disease (Kreisl et al., 2013a, 2013b) and of HIV dementia (Coughlin et al., 2014).

Building on this background, we posited a mechanistic relationship between localized, chronic neuroinflammation, altered regional morphology, and specific cognitive disruption in verbal learning and memory in American football players. Through multimodal, cross-sectional design of this pilot study, we sought to test for these changes in parallel, within a small group of former NFL players. Specifically, we hypothesized increased [^{11}C]DPA-713 binding, consistent with increased TSPO expression, in medial temporal structures and in the supramarginal gyrus (SMG) of the inferior parietal lobe, as these regions are susceptible to NFT deposition and atrophy in well-established CTE. In addition, we hypothesized volume loss and cortical atrophy in these medial temporal and inferior parietal regions. Finally, we hypothesized impaired performance on testing of verbal learning and memory in these same former players. To test these hypotheses, we used [^{11}C]DPA-713 PET to measure binding of this radiopharmaceutical to TSPO in 9 former NFL players and 9 healthy non-football players. Regional binding of [^{11}C]DPA-713 was compared after correction for the effect of rs6971 genotype on binding. Diminished volume and/or cortical thickness in the same regions of interest (ROIs) were evaluated with anatomical MRI data. Finally, we tested the former NFL players for deficits in neurocognitive function, focusing on verbal learning and memory.

Materials and methods

Human subjects

The Johns Hopkins Institutional Review Board approved this study. All subjects provided informed consent prior to participation. Former NFL players were recruited through advertisement at local chapter meetings of the NFL Players Association and through word of mouth among retired NFL players. All participants denied alcohol and illicit substance abuse (confirmed by negative urine toxicology screen). Age comparable, male healthy controls were recruited through local advertising and also were studied (including PET and MRI imaging) after careful clinical interview to ensure health. The healthy control participants were over the age of 55, were medically stable, and denied surgery in the past year. Controls denied current use of prescribed and