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14. ABSTRACT
Post traumatic stress disorder (PTSD) is a complex clinical disorder resulting from exposure to intense, life-threatening events resulting in persistent re-experiencing of the trauma, avoidance of stimuli associated with the trauma, dissociation, and heightened arousal which severely impact social and occupational functioning. Recent work has underscored morphological and functional brain alterations in PTSD patients using brain imaging with MRI, SPECT and PET imaging. Despite this encouraging preliminary work, there exists only a limited understanding of the pathophysiological changes which may subserve symptoms of PTSD. Preclinical studies now suggest that inflammatory changes may be implicated in neuronal loss in models of PTSD. Microglia represent a key inflammatory cell mediator within the CNS. Upon activation, these cells densely express an 18 kDa translocator protein (TSPO) receptors on their cell surface. Hence, it is possible to develop a radiotracer which targets TSPO as a marker for neuroinflammation. We have performed preliminary work with the TSPO imaging agent 123-I CLINDE with a goal of this proposal is to establish and validate an imaging biomarker for neuroinflammation in PTSD subjects that could both enhance our understanding of the pathophysiology and identify new therapeutic strategies for PTSD.

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Introduction

Post traumatic stress disorder (PTSD) is a complex clinical disorder resulting from exposure to intense, life-threatening events resulting in persistent re-experiencing of the trauma, avoidance of stimuli associated with the trauma, dissociation, and heightened arousal which severely impact social and occupational functioning. Recent work has underscored morphological and functional brain alterations in PTSD patients using brain imaging with MRI, SPECT and PET imaging. Despite this encouraging preliminary work, there exists only a limited understanding of the pathophysiological changes which may subserve symptoms of PTSD. Preclinical studies now suggest that inflammatory changes may be implicated in neuronal loss in models of PTSD.

Microglia represent a key inflammatory cell mediator within the CNS. Upon activation, these cells densely express an 18 kDa translocator protein (TSPO) receptors on their cell surface. This receptor is less commonly referred to as the peripheral-type benzodiazepine receptor (PBR). Hence, it is possible to develop a radiotracer which targets TSPO as a marker for neuroinflammation. A few suboptimal TSPO imaging tracers have been developed for SPECT and PET and have demonstrated inflammatory changes in human neurodegenerative disease, although to our knowledge not PTSD or traumatic brain injury. Our group has performed preliminary work with the TSPO imaging agent 123-I CLINDE with a goal of this proposal is to establish and validate 123-I CLINDE as
an imaging biomarker for neuroinflammation in PTSD subjects and controls. The proposal further serves as a model for the discovery, development and validation of radiotracers for PTSD. We have developed an efficient, focused, rapid radiotracer development program and CLINDE is the first of several tracers that could both enhance our understanding of the pathophysiology and identify new therapeutic strategies for PTSD.

Body

The overall goal of this proposal is to characterize an imaging biomarker of neuroinflammation in post traumatic stress disorder subjects and controls. Inflammatory processes are receiving intense research focus as potentially implicated in traumatic brain disease (TBI), post traumatic stress disorder (PTSD), neurodegenerative disease, and other CNS disorders. PTSD is a poorly understood entity which is now known to involve alterations in important CNS circuits implicated in arousal, memory formation, and anxiety, but for which limited human studies have been obtained.

We have a biomarker of neuroinflammation, 123-I CLINDE, an agent which binds to the 18kDa TSPO (aka peripheral benzodiazepine) receptor. These receptors are expressed on microglial cells in the context of an inflammatory response to brain and hence imaging with 123-I CLINDE potentially provides a non-invasive means for assessing regional neuroinflammation in PTSD, TBI, and other CNS diseases.

Our group has developed a highly efficient organization for discovery and development of novel imaging biomarkers for interrogation of CNS targets rapidly through preclinical to human clinical validation to applications in neuropsychiatric

populations. In the current proposal we plan to build upon our success strategy to characterize 123-I CLINDE in PTSD and control populations focusing on the initial developmental questions of optimizing the SPECT acquisition protocol, determining the best quantitative approach for measuring neuroinflammation, measuring the reproducibility of the imaging measure in PTSD subjects, such that by the end of this project, we have set the stage for wider application of the application of a neuroinflammation imaging biomarker to patients with PTSD and TBI. The development of CLINDE also serves as a model for the discovery, development and validation of other radiotracers for PTSD. CLINDE is the first of several tracers (we plan to develop that are beyond the scope of this current proposal) that could both enhance our understanding of the pathophysiology and identify new therapeutic strategies for PTSD.

Year 1
a) Initiate and complete Study Aim #1 in 6 controls and 8 PTSD subjects to assess the brain uptake, distribution, and washout of the 123-I CLINDE, measure metabolites in blood, and develop a model for quantification of the imaging signal

b) Initiate Study Aim # 2 test/retest reproducibility 123-I CLINDE in 8 PTSD subjects using the results from Study Aim #1 to determine the precision of the SPECT quantitative measure

Year 2
a) Complete Study Aim #2

b) Initiate and complete Study Aim #3 biodistribution/dosimetry of 123-I CLINDE in 4 controls to perform serial whole body planar acquisitions and 24 hour urine collections to measures the radiation absorbed dose to different target organs

c) Data collation, analysis, and preparation for scientific presentation

Key Research Accomplishments

In preparation for the initiation of the present study aims with 123-I CLINDE in PTSD subjects and controls additional information regarding the performance characteristics of the radiotracer became available to our study team. Specifically, the anticipated focal brain uptake with 123-I CLINDE in subjects for whom there was expected to be some increased binding; Parkinson's, Alzheimer's, multiple sclerosis and HIV encephalopathy showed only a very small amount of focal uptake in brain with the the image largely overwhelmed by the amount of non-displaceable uptake (i.e. radiotracer not specifically bound to the target). This made us concerned that in the present study design in PTSD, it would be similarly difficult to evaluate the potential discrete, focal areas of increased radiotracer uptake consistent with increased TSPO expression. We decided to consider additional TSPO agents with better properties prior to exposing PTSD subjects and controls to imaging studies with a poor tracer. In this vein, outside the context and funding of the present proposal, we initiated human studies with three additional TSPO radioligands with the aim of quickly deciding upon a

more optimal radioligand for the PTSD work. This report summarizes the progress of this work, even though we realize it is not being supported or part of the research initiatives funded under this grant. In addition, we have contacted the grant administrator to indicate we would wish to request a no cost extension of the work to facilitate the best scientific study possible under the current award. Summarizing this, objective progress in support of the initiation of the PTSD trial includes:

1. We have established liaisons with colleagues in the Yale Department of Psychiatry (Dr. John Krystal) for assistance in the referral of PTSD patients who we could evaluate as possible research participants.

2. Over the past months we have complete an extensive analysis and review of both volume of interest and voxel-wise parametric analyses of human 123-I CLINDE data obtained in the context of other research projects in Parkinson's, Alzheimer's, multiple sclerosis, and HIV encephalopathy. One goal of these reviews from other IND studies was to decide upon the adequacy of 123-I CLINDE to meet the scientific goals of the present study in PTSD by assessing the signal:noise issues of the radiotracer. *Based on this review we decided not to move forward with 123-I CLINDE in PTSD*.

3.We have identified three candidate alternative TSPO agents, each labeled with 18 F for PET and initiated human studies in all three to obtain a head-to-head comparison for the purpose of utilizing the best TSPO imaging biomarker in our PTSD project.

The issues and work delineated in points 2 and 3 above are described in the subsequent portion of this report.

Reportable Outcomes

Review of additional clinical imaging data from studies in controls and selected neuropsychiatric disorders

Human studies with 123-I CLINDE characterizing the uptake and washout of the radioligand from brain regions were conducted in a series of ongoing studies performed outside the context of the present award. Following the bolus intravenous injection 5 mCi of 123-I CLINDE serial dynamic SPECT acquisitions were acquired in small feasibility cohorts comprised of healthy subjects, Parkinson's, Alzheimer's, multiple sclerosis, and HIV encephalopathy.

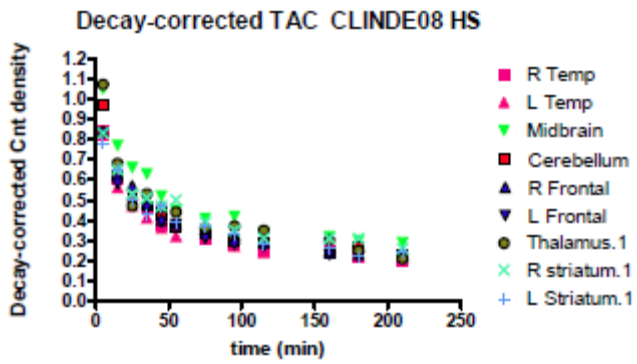


Fig. 1 Decay corrected Time-Activity Curves for several regions of interest from the healthy subject. Note the relatively rapid washout of ~70% of the signal within 30 minutes followed by a plateau of signal until nearly 4 hours post-injection. The fast washout is an important property of a successful TSPO radioligand.

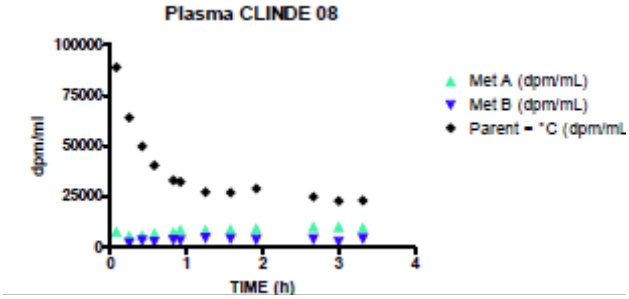


Fig. 2 Plasma concentration of 123I-CLINDE and two unidentified minor metabolites during the course of the scan depicted in Fig.1.The concentration of 123I-CLINDE,given in terms of the measured radioactivity in decays per minute (dpm), drops off rapidly during the first 60 minutes post-injection.

Some specific examples from these additional pilot studies are demonstrated in the following figures 3-5. In each case axial data from the 123-I CLINDE scan are presented at the level of the striatum summing frames from 90 min to 180 post-injection. These images were then normalized to extracranial structures for consistent visual display. Among healthy controls, there appears to be a general increase in brain signal with age.

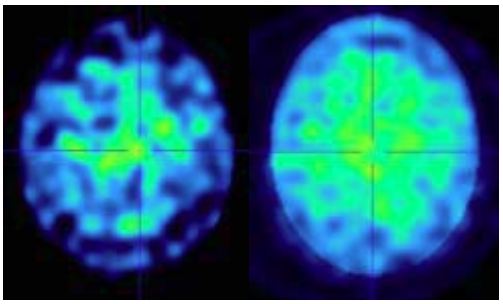


Fig. 3 Axial images at the level of the striata in two healthy subject; a 56 year old male on the left image and a 64 year old on the right. The images color tables are scaled to each other for direct visual comparison showing a general trend in the healthy subjects of higher uptake as a function of age.

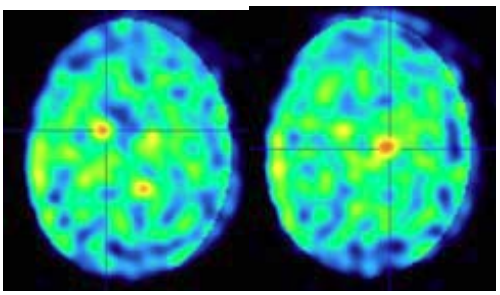


Fig. 4 123-I CLINDE images at the level of the striata in a 59 year old male with PD (left) and a 64 year olf male with PD (right). Both subjects have early, asymmetric motor disease and the CLINDE images show some focal uptake in the vicinity of the basal ganglia, but also other areas of uptake in white matter regions.

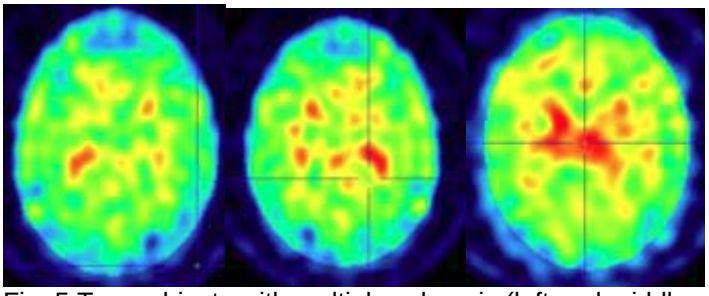


Fig. 5 Two subjects with multiple sclerosis (left and middle panel) and HIV (right). Both MS subjects show mild levels of diffusely heterogenous tracer uptake not correlated with clinical symptoms or previous or current MRI changes.

In addition to visual and volume of interest assessment of this preliminary dataset, a voxel-wise parametric analysis using SPM was performed. These analyses included:

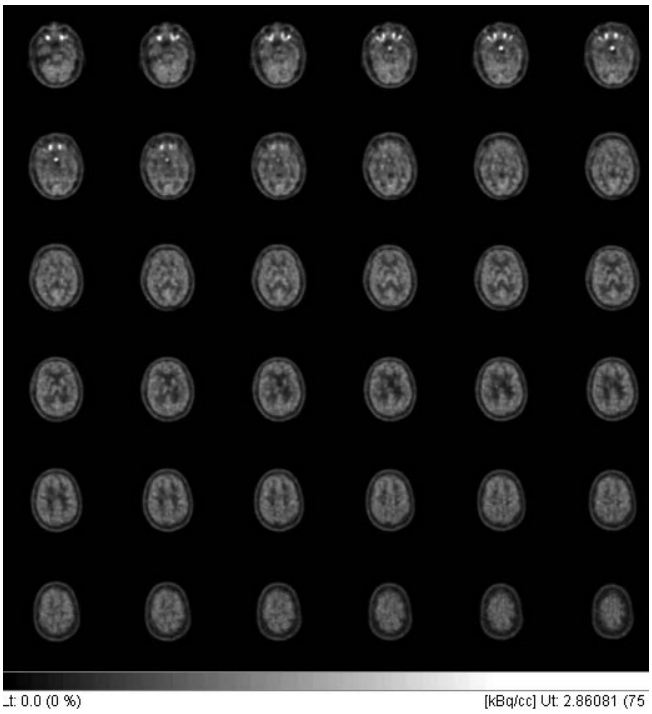
1. Comparison of age effects on CLINDE signal in healthy controls (n=12)
2. Group comparison of male versus female (n=12, 6 male, 6 female)
3. Group effects in Parkinson's disease versus older healthy control (n= 10 older controls, 7 PD)
4. Individual PD patients compared with older control template

Applying standard spatial and count normalization methods implemented in SPM 8 with modest statistical rigor for z-score cut-offs and cluster size, there were no defintive effects seen in any of these analyses. Following these analyses the decision was taken to retire CLINDE and seek a better TSPO ligand.

18F TSPO PET Radiotracers

We have been fortunate to acquire three TSPO PET tracers (18F-FEPPA, 18F-PBR06, and 18F-PBR111) and initiated characterization in both non-human primates and humans. Under the auspices of exploratory INDs for each of these tracers we have completed the following human studies:

Tracer	Total Human PET Pilot Studies
18F- FEPPA	3
18F- PBR06	5
18F- PBR111	4



FEPPA Subj3

50 min avg
image from
30-80 min pi

Fig. 6 Axial PET images from a healthy subject receiving a bolus injection of 18F-FEPPA, a potential radioligand for TSPO. Images are summed frames from 30-80 min post injection of 5 mCi of the tracer and show excellent brain penetrance and visualization of cortical structures.

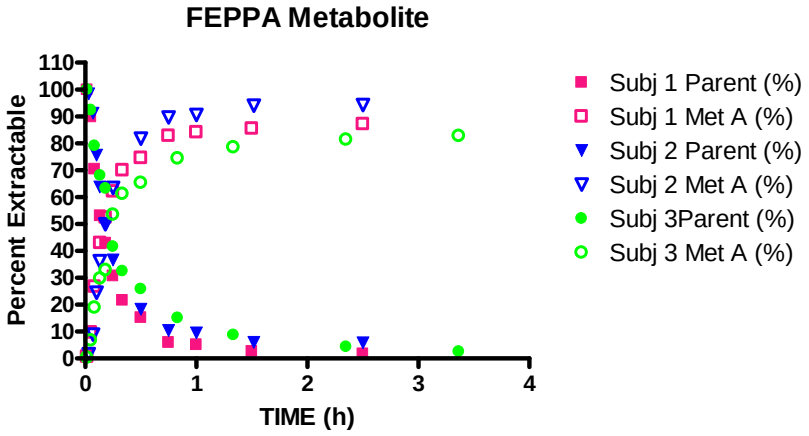


Fig. 7 Blood meatabolite analyses from subject #1, #2, and #3 showing similar pattern of metabolism of 18F FEPPA with appearance of a single metabolite which is thought not to penetrate the blood brain barrier.

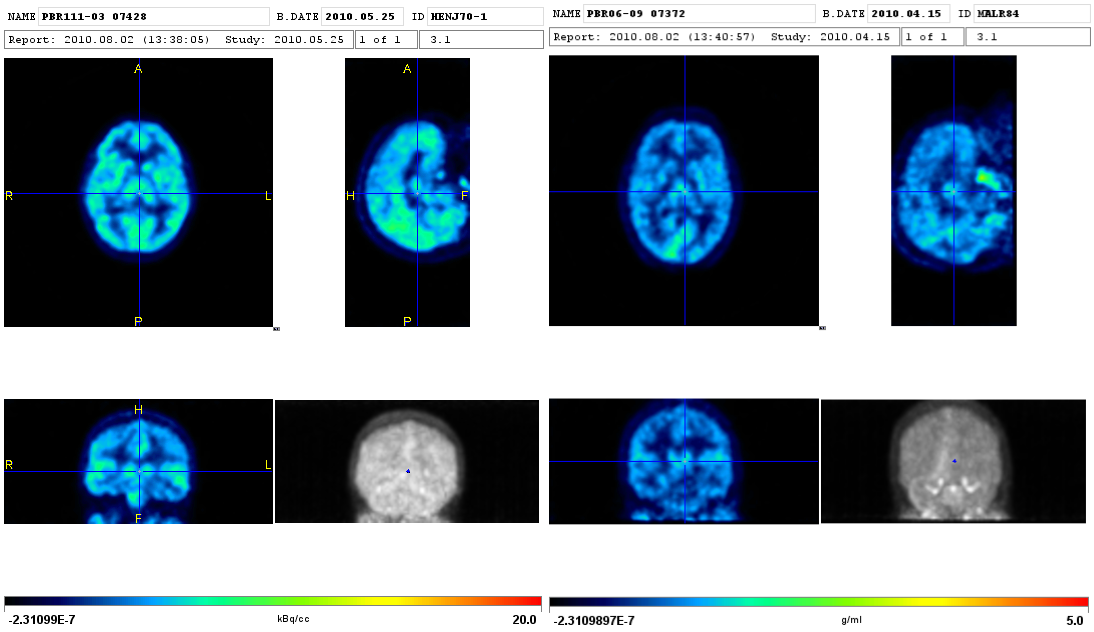


Fig. 8 Healthy subjects representative PET images after receiving a bolus injection of 18F PBR111 (left panel) and 18F PBR06 (right panel). Both tracers show good brain penetrance and visualization of cortical structures. A preliminary data set will have kinetic modeling for determining which tracer provides a better measure of TSPO is underway with the goal to use for PTSD subjects.

Conclusion

This report describes our other 123-I CLINDE research studies acquired in parallel projects evaluating neurodegenerative disease and suggests that specific uptake at TSPO sites is small relative to background brain uptake (non-displaceable uptake). In light of these data we decided to request a no cost extension on the current project to facilitate utilization of a better imaging biomarker of neuroinflammation in the present study rather than proceed with 123-I CLINDE. This assessment is moving along with the human evaluation of three separate 18-F-labelled radiotracers for PET supported through IND internal research funding. We expect to be able to select the optimal TSPO tracer within the current calendar year.

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Appendix

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