AWARD NUMBER: W81XWH-19-1-0774

TITLE: The Role of Astrocyte-Derived Tau Oligomers in Neurodegeneration Triggered by TBI.

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CONTRACTING ORGANIZATION: Indiana University, Indianapolis, IN

REPORT DATE: October 2021

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2021	Annual	01Sep2020-31Aug2021

4. TITLE AND SUBTITLE
The Role of Astrocyte-Derived Tau Oligomers in Neurodegeneration Triggered by TBI

5a. CONTRACT NUMBER
W81XWH-19-1-0774

5b. GRANT NUMBER
AZ180006

5c. PROGRAM ELEMENT NUMBER

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) AND ADDRESS(ES)
TRUSTEES OF INDIANA UNIVERSITY
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8. PERFORMING ORGANIZATION REPORT NUMBER
6030079020000

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
In AD, studies described a spatial and temporal pattern in the appearance of tau tangles in patient brains that follow neuronal networks and correlates with cognitive decline. While still a contentious topic, there is strong evidence that supports the idea that propagation of pathological tau species occurs between neurons. Microglia and astrocytes have recently been shown to be active participants in the pathological spreading of tau. Nevertheless, the functional significance of propagated glial tau remains to be established. Studies suggest that tau pathology propagation is one of the mechanisms underlying the long-term neurodegenerative effects after TBI. The mechanism by how tau propagation in TBI subsequently triggers AD/ADRD pathogenesis is still unknown.
Studies have shown how tau appears to be required for Aβ to cause synaptic and cognitive deficits in AD. It was reported that complete ablation of tau prevents deficits in spatial learning and memory after repeated mild frontal impact in WT mice. These results support the idea that reduction of tau could ameliorate the detrimental effects responsible for the association between TBI and AD. Yet no studies have addressed the contribution of exclusively reducing astrocytic tau on reverting AD/ADRD pathogenesis linked to TBI.
Hypothesis: Based in these observations in conjunction with our preliminary data, we *hypothesize that vascular damage triggered by TBI induces astrocytic-tau aggregation that subsequently promotes tau spreading throughout the brain eliciting synaptotoxicity and neurodegeneration as observed in AD and AD-related dementias*. Unfortunately, there is no clear understanding of the molecular and cellular mechanisms that underlie the contribution of astrocytic-tau aggregates to neurodegeneration and dementias associated to TBI.

15. SUBJECT TERMS
Mice

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	7	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. Introduction

Aim 1. To characterize the effect of TBI in the disruption of astrocytes-vascular coupling and reactive astrogliosis in the context of tau pathophysiology.

Sub-aim 1A. Determine the role of astrocytic-tau pathology in the neurodegeneration and synaptotoxicity associated with TBI.
In this sub-aim, WT C57BL/6 mice will be exposed to CCI and sacrificed 3 or 135 DPI to study acute or chronic injury respectively. We will perform a series of pathological and biochemical analyses to determine tau modifications associated with astrogliosis and vascular damage. We will also determine which type of reactive astrocytes (A1 or A2) is related to tau pathology. Astrocytes will be isolated to determine astrocytic-tau post-translational modifications and aggregation due to TBI. Finally, we will determine if astrocyte-derived tau oligomers induce neuronal synaptotoxicity and hippocampal LTP impairment.

Sub-aim 1B. Evaluate the role of astrocyte-derived tau oligomers in pathological spreading of tau after TBI.
In the current sub-aim, we will isolate astrocytic-tau oligomers from mice exposed to CCI and determine their ability to spread tau pathology *in vitro* and *in vivo*. First, isolated astrocytic-tau oligomers from TBI and control groups will be analyzed using a novel Forster Resonance Energy Transfer (FRET)-based biosensor cell line that specifically reports tau seeding activity. Then, isolated astrocytic-tau oligomers will be injected intracerebrally into Htau mice that over-express human tau to establish if astrocytic-tau from TBI mice accelerate the onset of behavioral impairment and neurodegeneration and propagate tau pathology *in vivo*.

Aim2. Investigate if the ablation of astrocytic-tau attenuates neurodegeneration and tau propagation throughout the brain after TBI.
It was recently reported that complete ablation of murine endogenous tau prevents deficits in spatial learning and memory after repeated mild frontal impact in WT mice[77]. Nevertheless, no studies have addressed the contribution of exclusively reducing astrocytic tau levels on TBI neurodegenerative associated phenotypes. Therefore, in this aim, we will investigate whether astrocytic tau works in concert or independently over tau spreading, pathology, glial activation, and neurodegeneration in mice subjected to TBI. Specifically, we will cross *Mapt*$^{flox/flox}$ with GFAP-Cre mice to determine if the specific ablation of astrocytic endogenous tau may revert pathological and behavioral phenotypes associated with TBI. GFAP-Cre; *Mapt*$^{flox/flox}$ and *Mapt*$^{flox/flox}$ mice will be exposed to CCI and sacrificed 3 or 135 DPI to study acute or chronic injury respectively. Mice will be subjected to a series of pathological, biochemical, electrophysiological, and behavioral analyses.

2. Keywords

Tau, oligomers, astrocytes, propagation, TBI, AD, tauopathies, seeding, aggregation.

3. Accomplishments

3.a Goal of the project

- Define the role of astrocytic tau aggregates in the synaptotoxicity due to TBI.
- Define the role of astrocyte-derived tau oligomers on tau propagation due to TBI.
- Determine if ablation of functional endogenous astrocytic tau suppresses behavioral deficit and synaptotoxicity in a mouse model for TBI**.**

3.b Accomplishment under these goals

- As part of major task 1, subtask 3, we have already isolated and characterized astrocyte-derived tau oligomers from mice expose to CCI. Astrocytes were isolated from Sham and CCI mice. Western blot analysis demonstrated how CCI induce an increase in the levels of astrocytic tau monomer (~49 KDa), the increase of a 64 KDa tau species and the induction of tau aggregation in the form of tau trimers (198 KDa).

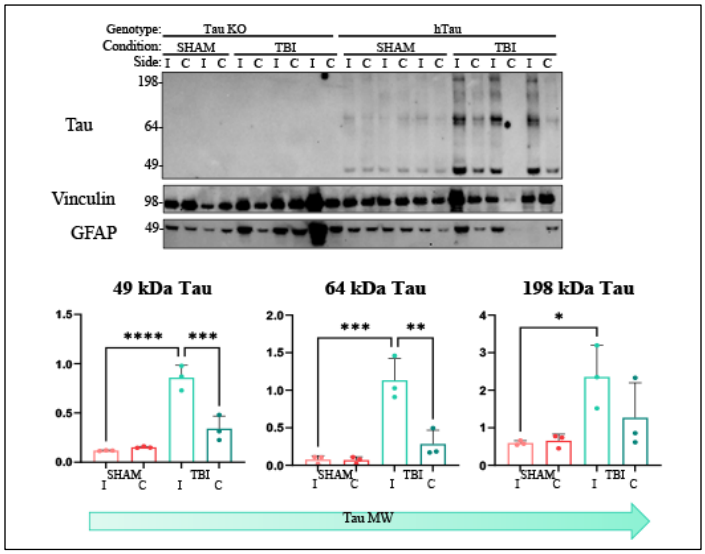

Figure 1: Control Cortical Impact induced astrocytic tau aggregation. Western blot analysis revealed the increase in the levels of different astrocytic tau species do to brain trauma in comparison to Sham controls. Specificity of tau signal was confirmed by analyzing astrocytic preparation of Tau KO mice. I = Ipsilateral and C = Contralateral

- As part of major task 2, subtask 1, we are currently characterizing the seeding activity of astrocytic-tau aggregated produced by CCI (isolated in major task 1, subtask 3). Using the FRET-based biosensor cell line that specifically reports tau seeding activity (ATCC, Cat# CRL-3275) were used to evaluated if CCI astrocyte-derived tau oligomers (isolated in Major Task 1_subtask 3) acts like a seed inducing tau aggregation *in vitro*. Our data suggested that Astrcytic-tau aggregated due to TBI do not have seeding activity in vitro measure using the Bio-sensor cell (Figure 2). We will repeat the analysis to established with certainty this results.

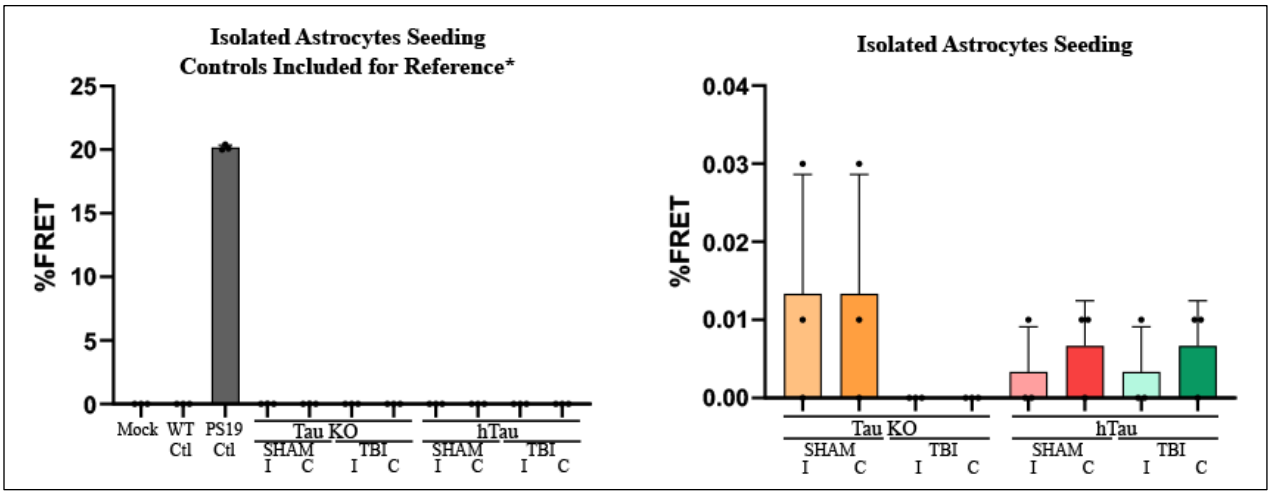

Figure 2: Control Cortical Impact do not enhance seeding activity of astrocytic tau. Using FRET-based biosensor cells, tau seeding activity was only observed in the positive control (brain lysate of PS19 mice that over-express human tau with the P301S mutation), but no seeding activity was observed in astrocytic tau of hTau mice expose to CCI nor control Sham (left panel). Right panel solely do not include positive control and only show tau seeding activity from astrocytes purified from Tau KO and hTau mice expose to CCI or control Sham. FRET values of astrocytes from Tau KO mice demonstrated that FRET values from hTau mice corresponded to background noise. I = Ipsilateral and C= Contralateral.

- As part of major task 2 subtask 2, we started stereotactically injecting astrocyte-derived tau oligomers into brains of mice expressing human tau. These mice are currently being age for further analysis.

Animal Use Regulatory Protocols

TOTAL PROTOCOL(S):

PROTOCOL (1 of 1 total):

Protocol [ACURO Assigned Number]: AZ180006.e002
Title: The Role of Astrocyte-Derived Tau Oligomers in Neurodegeneration Triggered by TBI
Target required for statistical significance: NA

Target approved for statistical significance: NA

SUBMITTED TO AND APPROVED BY:

IACUC APPROVAL DATES: 07/08/2019 – 07/08/2022

ACURO APPROVAL DAY: 01/30/2020

STATUS: APPROVED

3.c Opportunities for training.

Nothing to report.

3.d Results dissemination

Nothing to report

3.e Plan for next report period.

For our next quarterly report, we are planning to initiate the milestones described in Major Task 1 **subtask 4** and continue working in the milestone described in Major Task 2 subtask **subtask 2**.

Major Task 1 Subtaks 4:
To assess whether astrocytic-tau oligomers isolated from CCI mice exert synaptotoxicity and if this toxicity is tau dependent. We will utilize neuronal primary culture from WT (Jackson lab, Stock N: 000664) and $Tau^{-/-}$ (Jackson lab, Stock N: 007251). To prepare neuronal culture, we will require 8 WT and 8 $Tau^{-/-}$ mice. Per culture condition, n=6.

Major Task 2 Subtask 2:
Stereotactically inject astrocyte-derived tau oligomers into brains of Htau mice (Jackson lab, Stock N: 005491).

4. Impact

Traumatic brain injury (TBI) affects approximately 3.8 million people annually and costs the US more than $48 million. Furthermore, TBI has become an increasingly common feature of modern military conflicts. It is estimated that in the Iraq and Afghanistan conflicts following the terrorist attacks of September 11, 2001, the rate of TBI in military populations has dramatically increased to upwards of 10-20% of those serving, with over 250,000 soldiers exposed to some form of TBI. The long-term consequences of TBI are multifaceted and include increased risk for Alzheimer's disease (AD). The burden of AD is enormous. It is estimated that over 30 million Americans will live with AD by 2050 due to the lack of effective treatments. To date, mechanisms linking TBI to AD remain unclear. Therefore, gaining a better understanding of the mechanism(s) responsible for the association between TBI and AD/ADRD is essential not only for the development of strategies for the intervention of dementias associated to TBI, but could also open new avenues for the treatment of all AD/ADRD cases.

This proposal is based on scientific evidence that cerebrovascular dysfunction, reactive astrogliosis, and pathological accumulation of tau protein has been observed in TBI, AD, and other ADRD known as tauopathies. Therefore, in the current proposal we attempt to link these three pathological hallmarks for TBI and AD/ADRD in order to identify the contributing mechanism associated with TBI and subsequent AD/ADRD. We are confident that a major outcome of the proposal will be to dissect in detail the mechanism by which TBI leads to vascular damage followed by astrocytic-tau aggregation that subsequently is released from astrocytes inducing pathological tau propagation, synaptotoxicity, and finally neurodegeneration. As short term gains, the experiments aligned here will allow us to validate the *vascular-astrocyte-tau* axis as a key player for the mechanism linking TBI to AD/ADRD. It will also prove decreasing astrocytic-tau levels as a feasible strategy to revert synaptotoxicity and neurodegeneration associated to TBI. In the long term, the mechanisms dissected in this proposal may be expanded in the study of other neurodegenerative diseases not necessarily associated to TBI, since cerebrovascular dysfunction, reactive astrogliosis, and pathological accumulation of tau are common hallmarks of many other neurodegenerative dementias. It is also likely, due to scientific dissemination via publications or presentation, that other groups will continue dissecting with higher detail the exact mechanism of tau aggregation and release from astrocytes, increasing the number of molecular targets to revert synaptotoxicity and neurodegeneration associated to TBI.

Cumulatively, this study is devoted to understand the association between TBI and AD/ADRD, and will focus on how the connection of tau pathology with the gliovascular unit, rather than a sole neuronal connection, is a key event in the development of dementias associated to brain trauma. Ultimately, the understanding of this mechanism will yield therapeutic targets for the treatment of dementias associated to TBI that will be extremely beneficial to the military and military veteran community affected by AD/ADRD, their caregivers, and their families.

5. Changes/problems

Due to COVID-19, during the third quarterly report (04/01/2019 – 06/31/2020) our lab was totally closed. Therefore, as mentioned on the previous reports, we expect that overall our proposal will be delay solely by one quarter in relation to the proposed SOW.

6. Products

No products have been produced at the moment

7. Participants & Other Collaborating Organizations

The entire project is being perform at Indiana University

8. Special Reporting Requirements

No special requirements are reported

9. Appendices