Novel Insights into CTE Treatment and Diagnosis

Alexander Blumfelt, Eliska Mrackova, Krista Meuli
Department of Biology
Lake Forest College
Lake Forest, Illinois 60045

Summary

Chronic Traumatic Encephalopathy (CTE) has met controversy head-on since its discovery in 2002, inciting concern in both sports and research. The acute neurotrauma often affiliated with contact sports, such as repetitive traumatic brain injuries (TBIs) or blast-induced TBIs, has been directly linked to the risk of developing CTE, a neurodegenerative disease. Increased hyperphosphorylated in cis-p-tau proteins and the formation of neurofibrillary tau tangles are pathological hallmarks associated with CTE. Moreover, CTE symptoms mirror a spectrum of other neurodegenerative diseases (Alzheimer's, Parkinson's, and Amyotrophic Lateral Sclerosis), often leading to misdiagnosis. Even when CTE is suspected, there are currently no known treatments or methods of antemortem diagnosis. We hypothesize that CTE is a neurodegenerative spectrum disease that induces pathology reminiscent of PD, AD, or ALS based on genetic risk factors. To investigate CTE as a spectrum disorder, we will divide the study into three main aims: 1) to identify possible mouse models that have been widely studied in AD, PD, and ALS, 2) to identify possible forms of treatments using well-studied therapies for PD, AD, and ALS, and 3) to compare humanized disease models using iPSCs and evaluate effective treatments. Our findings will reveal potential genetic risks of CTE, establish the efficacy of known AD, PD, and ALS treatments for CTE and develop novel, humanized models and treatment techniques for CTE.

Background

The concept that repetitive concussions may have an effect on neurodegeneration was first reported in 1928 by Harrison Martland, who named this condition dementia pugilistica (1). Nevertheless, CTE was officially first described in 2002 when Dr. Bennett Omalu decided to take a closer look at the brain of Mike Webster, an NFL player. (2). During the same year, Dr. Kevin Guskiewics supported Dr. Omalu's findings by reporting that repeated concussions can lead to slower recovery of neurological functioning. Both of these reported findings were controversial and the NFL refused to accept them, suggesting in 2005 that "players who are concussed and return to the same game have fewer initial signs and symptoms than those removed from play" (1). It was not until Dr. Omalu published his second paper titled Chronic traumatic encephalopathy in a National Football League player in which he described CTE pathology in Terry Long that the NFL recognized the problems associated with repetitive head injuries (3). It was only recently that the NFL, NHL, and FIFA also started accepting the new findings. A NFL spokesman even acknowledged concussions' long term effects in 2009 (1,4). Due to these highly publicized findings in recent years, there has been an increased awareness of CTE (1,4,5).

As previously mentioned, the neuropathological aspects of CTE were first reported as individual cases of athletes who developed either parkinsonian symptoms, dementia, or depression (1,4,7). After examining the brain tissue, researchers found some common characteristics of CTE brains such as enlarged ventricles, thinning of the corpus callosum and cavum septum pellucidum, depigmentation of the substantia nigra (SN), and microscopic neurofibrillary tangles (NFT) in the cortex and midbrain (3,6,7). To further determine the extent of neuronal loss, Roberts et al. used cresyl violet staining to examine the cerebral cortex and substantia nigra of ex-boxers' brains and showed that 97% of CTE cases have Aβ deposits (8). In 2013, a set of criteria for diagnosing CTE was developed by Anne McKee et. al.showing the neocortex with p-tau immunoreactive NFTs, pretangles and dot-like or thread like neurites as well as NFTs in the cerebral cortex located in superficial layers II and III and irregular distribution of p-tau immunoreactive NFTs in cerebral sulcus (1,9). Based on the CTE diagnosis, McKee developed a system of criteria that divided the progression of CTE into 4 stages based on distinct localization of p-tau hotspots in different areas of the cortex (1,10). In the first stage, hot spots of tangled tau protein can be found in the cortex. In the second stage, the number of hot spots increases and they begin to appear in cortical sulci from which tau begins to migrate to different brain regions. In the third stage, tau begins to appear in the hippocampus and amygdala. In the fourth and final CTE stage, widespread p-tau pathology affects most brain regions as well as the spinal cord (1,10). Stieler et al. and Van der Jeugd researched the possibility of reversing the pathology of stage I and stage II of CTE, thus highlighting the need for CTE diagnosis antemortem and proposing a new area for research (11).

As most of the CTE patients exhibit symptoms similar to Alzheimer's disease (AD), Parkinson's disease (PD) and Amyotrophic lateral sclerosis (ASL), the antemortem diagnosis of CTE is still impossible, which complicates the possibility of administering adequate treatments. We hypothesize that CTE is a neurodegenerative spectrum disease whose symptoms most often resemble PD, AD, and ALS. To investigate our hypothesis we aim to 1) design possible CTE mouse models, 2) test known neurodegenerative treatments on a CTE mouse model, and 3) develop iPSCs to rescue the pathology and symptoms. We predict that these treatments will abolish the occurrence of neurodegenerative symptoms as well as behavioral deficits when symptoms mirror the treatment's target disease.

Relevance

Broader Relevance

Neurotrauma is one of the most common head injuries sustained in military combat, contact sports, and in civilians (1). In 2016, it was reported that in the United States alone, more than 1,700,000 traumatic brain injuries (TBI) were recorded (1,2,9). Although not everyone who suffers from a concussion or a blast head injury develops CTE in the future, in 2015, CBS sports reported that 95.6% of deceased NFL players tested positive for CTE postmortem (4). Patients in CTE stage III and stage IV develop parkinsonian symptoms, become suicidal or can suffer from severe headaches and memory loss (3,6,10). Both the symptoms and pathogenesis of this disease are difficult to relieve and there is no effective antemortem diagnosis or potential treatment specific for CTE patients. Therefore, researching TBI and CTE in model organisms can reveal a potential form of early diagnosis that can lead to the reversal of symptoms and development of treatments.

Intellectual Relevance:

While TBI and CTE have been relatively well studied in recent years, there are still many burning questions surrounding the mechanisms involved in the onset of CTE from TBI, the potential method of early diagnosis of CTE, and potential treatments (9). Although there are many animal models of TBI, there are currently very few effective CTE mouse models that are being used. This study will introduce new mouse models for CTE. Additionally, this study will propose different treatments for CTE, ranging from administering L-Dopa and Deep Brain Stimulation, to the potential use of induced pluripotent stem cells (iPSCs) which have never been used before in CTE research. Ultimately, we hope that this study will facilitate advancements in CTE research and will inspire future researchers to study potential treatments and means of early diagnosis in more depth.

Specific Aims

We aim to create CTE animal models using well-studied AD, PD and ALS models by varying the properties of RHI (intensity, frequency, and age of first head injury) and to test known treatments, such as L-Dopa, Deep Brain Stimulation (DBS), Tacrine, or iPSC, that could help us reverse the pathology of stage I and II or decrease the number of p-tau tangles in the brain and spinal cord. We hypothesize that CTE represents a spectrum of neurodegenerative diseases including AD, PD, and ALS. Therefore, we predict that early administration of RHI with increased speed or depth of impact, will lead to more rapid onset of AD, PD, and ALS and will also be more rapid than in control mice. We also predict that CTE models will effectively respond to AD, PD, and ALS therapies and that humanized AD, PD, and ALS symptoms and pathology can be induced by CTE iPSCs.

1. Use well studied animal models of AD, PD, and ALS to determine the effects of RHI: Mice models will be exposed to repetitive closed head injuries (RHI) with varying impact depth, recovery period length, and age of first head injury occurrence. Mice will be tested on various behavioral tasks: A53T mice will be tested on rotarod and pole test, APP mice

will be tested in the Morris water maze and using fear conditioning, and SOD1 (ALS) mice will be tested using footprint analysis and rotarod. Furthermore, immunohistochemistry will be performed postmortem. PD mice will be tested for the presence of Lewy bodies, AD mice will be tested for the presence of tau tangles, and ASL mice will be tested on neuronal motor viability.

2. Use previously established molecular therapies of PD, AD, and ALS to slow or even reverse the CTE-like neurodegeneration: If immunohistochemistry-stained mice expressing genetic predispositions to PD, AD, and ALS develop appropriate pathologies after RHI, the use of molecular therapies targeting the specific pathologies would be effective in combatting CTE-like neurodegeneration. Treatments for PD (L-Dopa and Deep Brain Stimulation), AD (Rivastigmine and an A- accumulation inhibitor), and ALS (IGF-1 and VEGF) will be used to target disease pathology. A53T, APP, and SOD1 mice will be divided into control groups and treatment groups. Afterwards, the mice will be subjected to the same behavioral tests listed above. Immunohistochemistry will again be performed postmortem, this time looking for the alleviation of PD (decrease in Lewy Bodies), AD (decrease in tau tangles), and ALS (increase in motor neuron viability).

3. Compare cellular phenotypes of CTE patient iPSCs with AD, PD, ALS, and healthy human iPSCs and evaluate efficacy of iPSC mouse models: For the first time, CTE patient iPSC-derived neurons will be developed to model CTE in vitro. It will be compared to controls to establish whether CTE embodies the pathology of AD, PD and ALS. CTE derived neurons will be transplanted into healthy mice models to determine if CTE-derived neurons sufficiently induce CTE and subsequently assess treatment efficacy. Transplantation of healthy human-derived neurons into the CTE models developed in aim 1 will demonstrate a novel rescue technique.

Research Methods and Design

1. Using well studied mouse models of AD, PD, and ALS to determine the effects of RHI

Rationale: Since first being described in 2002 by Dr. Omalu, we still do not have adequate CTE mouse models (3). Based on our hypothesis that CTE is a spectrum of neurodegenerative diseases, well studied alpha synuclein A53T, APP, and SOD1 transgenic mice models will be exposed to repetitive head injuries (11,12,13,14,15). Based on previous research, it was determined that the age of the first exposure to RHI, the intensity of RHI and the recovery period after a head injury can predict the development of CTE (7, 16). The effects of RHI will be determined by behavioral tests as well as post mortem immunohistology analysis. Varied intensity of RHI, frequency of RHI after recovery, and age of first head injury will be applied to AD, PD, and ALS mice. Furthermore, we plan to include three control groups (1. ALS, AD, or PD model without RHI, 2. Healthy mice without RHI, 3. Healthy mice with RHI). Using these three different controls, we will observe the effects of RHI on the progression of neurodegeneration. In order to induce closed head injury to the left hemisphere of the mice prefrontal cortices, we will use the weight drop model from K. Darvish and Marmarou lab (15, 21).

A: PD Mouse Model:

In this study, we will assess motor deficits and the presence of Lewy bodies in the substantia nigra in mPrp-A53T-α-synuclein transgenic male mice because this area has been reported to show age-dependent motor impairments and intracytoplasmic inclusions (11,17,18). Since motor decline in A53T mice has been well established, the effects of RHI will be assessed using a Rotarod test and pole test (17,18). After 2 months of behavioral testing, mice will be sacrificed to analyze the presence of Lewy bodies and neurites in the substantia nigra (18). Only the mouse prion promoter (mPrP) A53T alpha synuclein transgenic mice exhibit the full range of alpha synuclein pathology (alpha synuclein aggregation, fibrils and truncation as well as progressive age-dependent neurodegeneration) (11,19, 20). Therefore, we will use Tyrosine hydroxylase (TH), an enzyme that converts L-tyrosine to L-3,4-dihydroxyphelylalanine (L-Dopa), to mark the progressive loss of dopaminergic neurons in SN(17). In order to obtain another assessment of the dopaminergic neuronal loss, GIRK2 (a G-protein regulated potassium channel expressed in dopaminergic neurons of SN) will also be used (20).

B: AD Mouse Model

CTE involves tau protein aggregation and tangle formation abnormalities, similar to AD pathology (9,10,22, 24). The presence of tau tangles is crucial in the diagnosis of CTE and thus, an AD mouse model can accurately model the tau pathology of CTE. In our study, we will use APP transgenic mice that have shown memory dysfunction and the the formation of tau tangles (22,24,25). All APP mice will be assessed using the Morris Water Maze (MWM) and be fear-conditioned. After two months, mice will be sacrificed and their hippocampi will be analyzed using the immunohistochemistry protocol from Cherry et. al. (16). We will use Anti-CD68 antibody (KP1), which marks microglia, to assess the progression of AD. As a second measure, we will use AT8 which specifically recognizes phosphorylated tau (26).

C: ALS Mouse Model

In this study, we will use SOD1 male mice with glycine and alanine at transition 93. The SOD1 mice phenotype ranges from decreased grip strength, impaired coordination, motor neuron degeneration, severe muscle weakness beyond 3 months old, and hind limb tremors at the age of 14 weeks (21). Therefore, we will use the footprint analysis as well as the rotarod test to determine the effects of RHI on the motor skills decline (25). After 2 months, we will sacrifice the mice and analyze their spinal motor neurons using TDP-43 DNA-binding protein and a Gal3 marker to stain the spinal cord sections from the ALS mice. (27).

Prediction: We predict that with RHI, PD mice will show a decline in motor abilities on the rotarod as well as the pole test and TH and GIRK2 immunostaining will reveal an elevation of Lewy bodies in the SN. AD mice with RHI will exhibit spatial memory dysfunction on MWM and fear conditioning. Furthermore, anti-CD68 and AT8 will show an increased activation of microglia as well as p-tau tangles concentration. ALS mice will perform poorly on the footprint analysis and the rotarod test and will display neuronal and glial inclusions (7, 23). Control mice with no RHI should exhibit no pathology and behavioral impairments. AD, ALS, and PD control mice with no injury will exhibit less deficits than AD, ALS, and PD mice with RHI. The results will be analyzed with SPSS using ANOVA. It is possible that individual mice can respond differently to a head injury regardless of their genotype, which can have an effect on our hypothesis.

2. Use previously established molecular therapies of PD, AD, and ALS to slow or even reverse the CTE-like neurodegeneration

Rationale: Past studies have examined the potential of various molecular treatments and therapies in treating symptoms of Alzheimer's Disease (AD), Parkinson's Disease (PD), and Amyotrophic Lateral Sclerosis (ALS). Many of these studies have been conducted in animal models of these diseases. Likewise, we will be using transgenic mice. On a molecular level, research has identified and targeted the amyloid-β cascade for intervention in AD (27). Research in PD has shown L-Dopa to be a powerful treatment in the early stages of the disease to rescue motor control (32). Rab1 has also been identified in the treatment of PD via the rescue of neuron loss (28). Finally, in ALS research, there have been promising results in inducing vascular endothelial growth factor (VEGF) to lengthen the lifespan of mouse models of ALS (30). Modifying glial activity has also been shown to extend ALS mice survival through the administration of insulin-like growth factor-1 (IGF-1) (31). Therefore, these drugs and treatments were chosen for the next step in our study.

Comparing treatment effects among A53T, SOD1, and APP mice

PD, AD, and ALS mice with RHI will be assigned to a treatment group. These mice, similarly to the previous aim, will be compared to mice assigned to one of three control groups (ALS, AD, or PD mice without RHI, healthy mice without RHI, and healthy mice with RHI). Within each treatment group, A53T, SOD1, and APP mice will be further assigned to one of two or three treatments selected for each disease. PD mice will receive either intracranial doses of L-Dopa, viral vectors with the purpose of overexpressing Rab1 (28), or sessions of Deep Brain Stimulation. AD mice will receive intracranial doses of Rivastigmine, an acetylcholinesterase inhibitor (27) or be induced with a viral vector in order to knock down BACE1 for reduction in Aβ accumulation (27). Finally, ALS mice will receive either viral vectors to increase VEGF expression (30) or IGF-1 injections in the central nervous system (31).

Following introduction of treatments, A53T, SOD1, and APP mice will be subjected to the same behavioral tests as described before;

the rotarod and pole tests for PD, the MWM and a cued, classical fear conditioning paradigm for AD, and footprint analysis and the rotarod test for ALS mice. Time spent on the rotarod, as well as time spent hanging on the pole test, will be gathered for PD mice. Freezing behavior and performance on the MWM will be gathered for AD mice. Finally, stride length, sway distance, and time spent on the rotarod will be gathered for ALS mice. Mice will be tested twice: one week following treatment introduction and two months post-treatment.

Inferential statistics performed via SPSS will be used to compare the mean scores of groups in each disease measure. One-way analysis of variances (ANOVA) will be performed for each measure to determine whether any significant differences between the treatment and control groups exist.

Following treatment administration, mice will be sacrificed and their brains will be examined post-mortem by immunohistochemistry for pathological hallmarks of PD, AD, and ALS, using the staining protocols mentioned above. Results of these stains will be compared using t-tests to compare no treatment and treatment brains in PD, AD, and ALS.
Prediction: Administration of treatments previously shown to be effective for PD, AD, and ALS, if used correctly and similarly to past studies, should result in improvements in the performance of A53T, SOD1, and APP mice with RHI. ANOVAs should reveal significant differences between treatment and control groups, with treatment groups showing increased time spent on the rotarod and pole hang time (PD mice), improved MWM performance and freezing behavior (AD mice), and increased stride length, decreased sway distance, and increased time spent on the rotarod (ALS mice). On a molecular level, we expect t-tests to show a significantly decreased Lewy body presence in PD mice, reduced tau tangles in AD mice, and increased motor neuron viability in ALS mice.

3. Compare cellular phenotypes of CTE patient iPSCs with AD, PD, ALS, and healthy human iPSCs and evaluate efficacy of iPSC mouse models:

Rationale: Investigating CTE in human induced pluripotent stem cells (iPSCs) will provide a more accurate and encompassing model of this complex neurodegenerative disease (NDD) (39, 40). Furthermore, comparing these novel CTE iPSC derived models to AD (41,42), PD (43,44), ALS (45), and healthy human iPSCs, all developed from previously established protocols, will demonstrate the applicability of this new iPSC model and further elucidate the link between CTE and other NDDs. The previous 2 aims attempt to model TBI that leads to CTE as an activator of known risk genes for other NDDs. This suggests that CTE can function as an epigenetic disorder, influencing risk genes to induce particular NDDs. iPSCs have been a useful tool for studying epigenetic disorders because they have “epigenetic memory” (39). Previous research has found iPSCs particularly useful in studying complex NDDs similar to CTE but never before CTE. Neurons differentiated from iPSCs have been used to characterize molecular phenotypes of neurodegenerative diseases in vitro (47). However, studies have also shown that neuronal precursors derived from iPSCs can be transplanted into rodent brains successfully (41,42,43,44,48,49). These in vivo iPSC models have demonstrated more complexity than observed in in vitro models, including specific cortical neuronal patterns of maturation, synaptic activity, and connectivity (40, 42, 47, 50). We hope to develop a robust CTE iPSC derived mouse model that can be used to investigate the effectiveness of therapeutic treatments.

Developing human-derived induced pluripotent stem cells (iPSCs)

First, somatic fibroblasts will be collected from CTE, AD, PD and ALS patients as well as healthy humans with no known genetic risk for a NDD. Pluripotent stem cells will be induced from the fibroblasts using episomal vectors encoded with pluripotency factors as described by Okita et. al. (51). From the iPSCs, neuronal differentiation is induced by defined factors. Finally, the neural stem cells are differentiated into three different types of induced neurons: cholinergic hippocampal neurons, dopaminergic substantia nigra neurons, and motor neurons. CTE and healthy stem cells will be differentiated into each of the three types of neurons. The AD, PD, and ALS stem cells will be differentiated to one of the three types of neurons based on the expected pathology. Therefore, AD stem cells will be differentiated into cholinergic hippocampal neurons (41,42), PD stem cells will be differentiated into dopaminergic substantia nigra neurons (43, 44) and ALS stem cells will be differentiated into motor neurons (45,46). CTE patient stem cells with other or combinations of symptoms will be differentiated into varied neurons most related to those symptoms. Healthy human stem cells will be differentiated into each of the neurons developed.

Cellular Analysis

Each type of CTE neuron developed will be phenotypically and genetically compared to the respective NDD neuron and it’s healthy counterpart neuron. Some of the measures to compare phenotypes will include percent cell survival, number of dendrites and axons, and dendrite lengths. The genetic comparison will attempt to identify any similarities in mutations between CTE and respective NDD neurons that are not found in the healthy control neurons.

In vivo

CTE-induced progenitor neurons will be injected into healthy mouse models. The same behavioral tests used in aims 1 and 2 will be used to evaluate expressed symptoms. Then, the drug treatments used in aim 2 will be administered to the mice to determine if symptoms reverse. Finally, pathology will be analyzed postmortem, as described in the previous aims. Additionally, healthy induced progenitor neurons will be injected into developed mice models for CTE and rescue of pathology and symptoms will be evaluated as previously described.
Prediction: Neurons derived from CTE patient-induced neurons will express phenotypic characteristics consistent with the respective NDD that presents similar symptoms and pathology. For example, CTE cholinergic neurons will express similar cell death and dendritic growth as AD cholinergic neurons. When transplanted into healthy mice, CTE progenitor neurons will induce CTE pathology. Healthy progenitor neuron iPSCs, when transplanted into mice models developed in aim 1, will reverse behavioral symptoms and present less pathology.