Chronic Traumatic Encephalopathy: Investigating Blood-Brain Barrier Disruptions and Neuroimaging Techniques

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Abstract

Chronic traumatic encephalopathy (CTE) is a neurodegenerative tauopathy resulting from repeated cranial trauma that is often associated with contact sports, military duty, and vehicle collisions. Individuals afflicted with CTE experience a multitude of symptoms, such as depression, impaired judgement and decision-making, suicidality, disorientation, and dementia. On a molecular level, CTE is caused by the aggregation of insoluble tau protein isoforms, creating neurofibrillary tangles. Neurofibrillary tangles are known to result in the death of peripheral neurons and glial cells, although the direct mechanism is unclear. This article will discuss how disruption of the blood-brain barrier via repetitive traumatic brain injury influences the development of CTE. Furthermore, this article will also review the current imaging modalities used to support a probable diagnosis of CTE in a living patient. Studies indicate that exposure to repeated mild traumatic brain injury correlates with an increase in phosphorylated tau deposition of the perivascular space that parallels local disruption of the blood-brain barrier. Immunoreactivity patterns of examined CTE-positive neural tissue indicate blood vessels with high p-tau deposition lack many of the cellular components constituting the blood-brain barrier. Moreover, white matter degeneration is associated with delayed microvascular damage incurred through traumatic brain injury. Blood brain barrier integrity is dependent upon several transmembrane proteins belonging to the claudin and occludin superfamilies, and studies indicate that absence of these components correlates to blood-brain barrier degradation. Microvascular pathology observed in CTE-positive neural tissue contains decreased levels of claudin-5, occludin-1/zona occludens, suggesting that dysfunctional tight junctions contribute to the neuropathology of CTE. As with many proteopathies, CTE cannot be positively diagnosed antemortem. There are many imaging techniques that healthcare providers and researchers utilize to analyze the brain topography of a patient. Positron emission tomography scans permit clinicians to visualize the physiological state of a bodily compartment, such as the central nervous system.

Introduction

Chronic traumatic encephalopathy (CTE) is a tau-protein-linked neurodegenerative condition resulting from recurrent brain trauma as illustrated in contact sports and blast injuries (1). The hyperphosphorylated tau protein (p-tau) slowly accumulates throughout the brain, leading to the death of brain cells (13). The pathophysiology of this condition is not fully understood, but there has been a correlation made between consistent head trauma and the development of CTE (1). Limited autopsy findings of individuals with CTE determined the presence of a thinned corpus callosum and atrophied frontal lobes, as well as additional impacts to the medial temporal lobe, mammillary bodies, hippocampus, and substantia nigra (6). These brain regions account for the symptoms associated with CTE including altered mood and behavior, impaired cognition and executive function, and suicidal ideation (14). Additional findings through gross examination of the brain showed the presence of enlarged lateral and third ventricles, which has become a common indicator of CTE (6).

An issue that has arisen with CTE studies is the lack of non-randomized studies regarding such autopsy cases where selection bias is present (6). In accordance with a study conducted by the Boston University Center for the Study of Traumatic Encephalopathy, of the 321 known American football player deaths that occurred between February 2008 and June 2010, only 12 of the brains had undergone postmortem neuropathological examination (6). The brains that were examined provided evidence of CTE and has suggested an estimated lifetime prevalence of 3.7% (6). This correlation is a public health concern that has been brought to the attention over the last decade.

Microscopic observation demonstrates the abundance of neurofibrillary tangles along with glial tangles and neuropil thread with microtubule-associated protein tau being the main protein component of these tangles (11). This protein is known to play a role in the stabilization of microtubules (14). Tau protein pathology in CTE has been found in superficial cortical laminae (I and III) (6). It is non-uniform and is restricted to the foci of the frontal, insular, and temporal cortices (6). These neurofibrillary tangles had increased density at the level of the cortical sulci and were typically perivascular or positioned around the blood vessels (11). With this distinction, there is a possibility that blood-brain barrier disruptions that occurred during traumatic brain injury (TBI) may play a significant role in neurofibrillary tangle formation (4).

The blood-brain barrier (BBB) refers to the properties of the microvasculature of the CNS that serve to protect the brain from exposure to circulating pathogens (5). This is performed through the tight regulation of the BBB in order to maintain the CNS homeostasis for proper neuronal functioning (5). Studies have shown the partial or complete loss of the BBB functioning associated with several neurological disorders may be a major contributor to the pathology of such conditions (5). BBB breakdown permits the entry of pathogens and other damaging substances, resulting in the development of neurodegenerative diseases. For example, neuroimaging studies performed for conditions like Alzheimer's disease (AD) have shown a BBB dysfunction (16).

In relation to CTE, a study found BBB breakdown present in regions containing increased perivascular p-tau protein. The study utilized immunoreactivity patterns of claudin-5 and occluden-1, BBB associated tight junction components (5). It was determined that both tight junction components were either discontinued or absent in perivascular p-tau deposited regions (5). This resulted in the presumption that BBB dysfunction could serve as a correlate or neural dysfunction in individuals suspected of having CTE (5). More research needs to be conducted in order to further comprehend the pathway and pathophysiology of CTE.

A definitive diagnosis of CTE can only be administered through postmortem neuropathological analysis of tau protein patterns in the brain (17). There is no test that can determine if a living individual has this condition, as this is a fairly new area of research in the medical field. A definitive diagnosis of CTE can only be administered through postmortem neuropathological analysis (17). Currently there is no method of utilizing an CT, MRI, or any brain imaging technique to diagnose CTE aside from autopsy. Physicians will administer neurological exams as well as mental status testing to patients displaying symptoms of CTE. Research on developing diagnostic techniques that can be performed on live patients is currently underway.

Treatment options are limited and often arrive in the form of behavioral therapy, pain management regimes, palliative care, and memory exercises. Behavioral therapy may include the prescription of medications, such as serotonin reuptake inhibitors (SSRI), to combat depression and anxiety. Pain management includes the aforementioned medications as some individuals experiencing CTE symptoms have reported a slight reduction in mood swings, improved memory and decreased pain (17).

Methods

Databases used for research included Pubmed, sourced through the Geisinger Commonwealth School of Medicine library collection, and Google Scholar. Several keywords and phrases aided the research process: *chronic traumatic encephalopathy, traumatic brain injury, proteopathy, blood brain barrier, tight junctions, microvascular damage, neurotoxicity,* and *endothelial cells*. Articles were carefully chosen to avoid research older than a decade, with the oldest utilized paper having been published in 2009.

Discussion

Neuroimaging via positron emission tomography

Currently, there is no antemortem examination that can positively diagnose a patient suspected of CTE. Since many individuals with a neurodegenerative disease present with sudden mood changes and behavioral disorders later in their lifetime, discriminating CTE from other pathologies is challenging (18). Having a reliable diagnostic tool to detect CTE antemortem would enable medical intervention to occur at an earlier age, which is crucial for an individual with a history of repeated TBIs. Concussions may be classified as TBIs but current literature describes a TBI as an irreparable alteration to brain function caused by an external force that results in detrimental long-lasting effects (3, 10). Changes in behavior, attitude, and mood after a history of recurrent TBIs could suggest CTE has developed (10). Traditionally, neuroimaging has not been used to confirm a conclusive CTE diagnosis (18). Often, imaging results appear similar between AD and CTE-associated AD, which is why any distinct clinical characteristics must be identified prior to imaging to avoid a misdiagnosis.

PET scans are used to study different organs and tissues by injecting and tracking a radioactive drug, or tracer that is known to detect certain diseases or physiologic states. An example of one such tracer is [F-18] FDDNP, which was utilized by Barrio et al. as a tracer due to its sensitivity for tau (2). The investigative team performed PET scans on both living former football players and two control groups, which consisted of individuals who had no history of TBI and AD patients, respectively. In comparing images from different brain regions, Barrio et al. observed that the limbic, cortical, and subcortical areas contained the highest amounts of tracer; even higher concentrations were detected in the amygdala (2). These findings indicate that the aforementioned regions possessed markedly increased concentrations of tau. The investigation also compared the patterns identified via PET scans using FDDNP to actual brain tissue sampled from CTE-positive patients. The regions of the brain identified via the PET scan as having excess amounts of tau protein were similar to regions that had immunohistochemically confirmed tau deposition in CTE-positive brain tissue samples (2). However, it is interesting to note that advanced stages of CTE can be confused for advanced-stage AD, even with the use of PET imaging. As the degradation of key brain structures advances, neuroimaging results between AD and CTE begin to mimic each other, often resulting in a misdiagnosis. Finally, when comparing neural tissue from an individual with probable CTE to samples derived from an AD case, there was a statistically significant difference observed, suggesting that the CTE-positive patient suffered from advanced stages of CTE instead of AD (2).

Stern et al. investigated levels of tau in the brains of living former National Football League players with suspected CTE by using flortaucipir as a tracer in PET scans and ^{18}F-florbetapir to detect any amyloid-beta. To test how much of the marker was being taken up by tissues in the brain, researchers measured cerebellar florbetapir standard uptake value ratio (SUVR) and found that in the group comprised of former NFL players, there was a significantly higher SUVR compared to the controls (asymptomatic individuals who had no history of TBI) (18). Tracer concentrations were highest in the bilateral superior frontal area of the brain, the bilateral medial temporal portion of the brain, and left parietal area; each is known to be affected by CTE when diagnosed after death (18). Utilization of immunohistochemistry postmortem also highlighted significant amounts of tau deposition in the amygdala and midbrain in older and younger players which suggests that damage to these structures coincide with CTE rather than AD due to the mechanics of TBIs. To rule out if any of the behavioral changes that the NFL players were experiencing were related to a tauopathy other than CTE, measurements of the amount of amyloid-beta were taken via PET scan. Subsequent findings were not significant, indicating that the symptoms the players felt were not due to early onset AD, but rather were more in

line with early presentations of CTE (18). This again highlights the importance of having proper diagnostic tools so that misdiagnosis does not occur and proper intervention can take place.

The use of [F-18] FDDNP as a radioactive ligand for PET scans can be considered controversial due to FDDNP having the ability to not only bind to tau aggregates but also to amyloid-beta plaques, which are a clinical indicator used to diagnose AD (2, 13). However, that is where postmortem analysis of confirmed CTE cases is a useful tool in identifying patterns that differ markedly from AD. In a study conducted by Omalu et al., a 59-year-old football player was recruited in order to support the use of PET imaging in identifying CTE during his lifetime with the goals of confirming the condition and further supporting the utilization of such neuroimaging techniques after death (13). During his life, the player underwent numerous PET scans with [F-18] FDDNP as a tracer and upon death, postmortem tissue analysis was used to confirm a diagnosis of CTE (13). Antemortem scans utilizing [F-18] FDDNP highlighted statistically significant tau deposition with insignificant amounts of amyloid beta accumulation in the frontal and temporal lobes (13). Areas of the brain with predominant tau deposition were identified as being in the midbrain and amygdala and were confirmed postmortem via immunohistochemistry. The amygdala and midbrain are structures of the brain that tend to get damaged first during a TBI due to the rapid acceleration and sudden deceleration of the head (13, 18). These structures exhibited dense areas of tau deposition that accumulated early in life after sustaining a TBI and persisted well into older age (13, 18). This pattern of tau deposition within the amygdala and midbrain and lack of beta-amyloid highlights some of the key findings that indicate a diagnosis of CTE rather than AD (Figure 1). Once brain structures begin to develop tau neurofibrillary tangles, they can continue to spread and clinical presentation of late stage CTE can be confused for late stage AD, therefore, being able to utilize imaging techniques like [F-18] FDDNP and identifying patterns that separate CTE from AD is important.

Even with a history of TBIs and reported new onset of abnormal behavioral and cognitive abilities, there is no diagnostic test currently screening patients suspected of having CTE. However, repeated claims have been made and supported that utilizing PET scans along with a radioactively labeled tracer for a known protein of interest involved in CTE tauopathy, such as tau, can be applied antemortem to identify the onset of CTE. Thus, early intervention before advanced CTE and neurodegeneration occur may help a patient and their family adjust to a new diagnosis and remain proactive for the remainder of the prognosis.

Blood-brain barrier

Components and general structure

The BBB is responsible for maintaining separate environments between systemic circulation and the microenvironment of the central nervous system. By ensuring limited contact of the two compartments, the BBB prevents neurotoxic agents, such as antibodies, macromolecules, and certain ions, from crossing into the delicate cerebral environment. No neuron is more than 20 µm from a capillary, so BBB integrity is essential to proper neurological performance. Preserving homeostasis in the central nervous system requires a controlled microenvironment (5).

The core components of the BBB are pericytes, astrocytes, and the endothelial cells lining the cerebellar capillaries; each of these elements aids in forming a tight seal around the blood vessel(s). Endothelial cells of the central nervous system differ in structure and function from those found in the peripheral nervous system. With low rates of transcytosis and low expression of leukocyte adhesion molecules (LAMs), endothelial cells of the brain exhibit reduced trafficking and inflammation. Most importantly, these endothelial cells possess continuous intercellular tight junctions that prohibit paracellular transport of various molecules from the blood to the perivascular space (12). The perivascular space refers to the local region surrounding the microvasculature in the brain (5). Cerebral endothelial tight junctions depend on two transmembrane proteins, occludin and claudin, for their contributions to BBB stability and barrier function, respectively. Claudin-5 expression of brain endothelium predominates, with claudin-3 comparatively expressed. Studies involving mice genetically altered to lack claudin-5 demonstrated BBB breakdown, as did similar investigations utilizing human endothelial cell cultures. Occludin breakdown/absence has been correlated with an increased permeability of the BBB, but not breakdown of the structure, in analyses involving human endothelial cell cultures (9). As discussed in later sections,

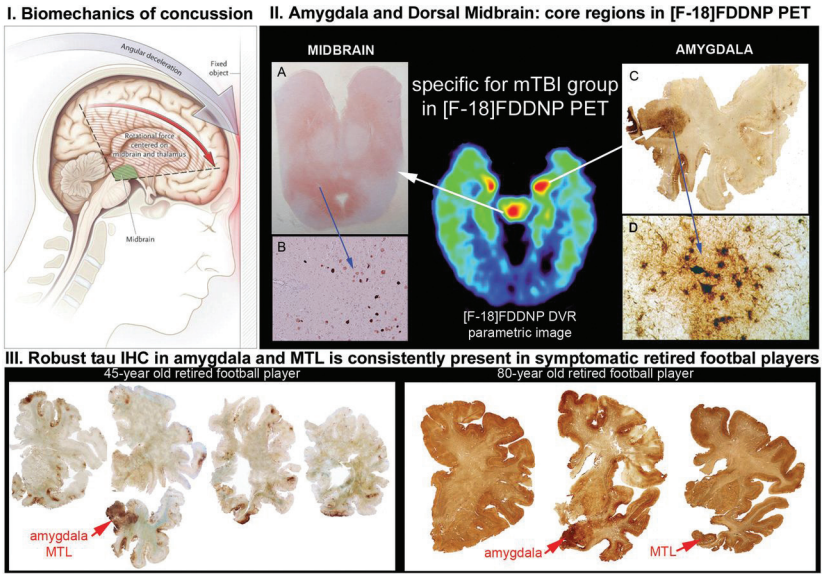

Figure 1. Usage of [F-18]FDDNP in PET to evaluate tau deposition in the midbrain and amygdala (2).

immunohistochemical examinations indicate that claudin-5 and occludin are implicated with BBB breakdown involving p-tau (5).

Blood-brain barrier disruption and p-tau

Disruption to the BBB has long been theorized to be a leading factor in the development of many neurodegenerative diseases, including CTE. Despite many years of clinical research, the role of BBB disruption in neurodegenerative pathogenesis remains inconclusive. Specifically, researchers and clinicians seek to determine if BBB degeneration occurs at the inception of neuronal decay, or if it is a subsequent event (12). Although the timeline of BBB breakdown has yet to be understood, investigations concerning the changes to BBB structure and function have determined that claudin-5 and occludin-1, subunits forming the zona occludens, or tight junctions, are heavily implicated in the compromised BBB (5). As mentioned earlier, continuous tight junctions in the endothelial cells of brain capillaries prevent paracellular movement of neurotoxic agents (9, 12). Several factors influence the tight junctions in endothelial cells, including local concentrations of p-tau.

One case report describes an investigation that sought to ascertain whether perivascular p-tau deposition promotes the degradation of the BBB. A 56-year-old man presented to the emergency room with sudden confusion and disorientation. Further investigation revealed two facts; the patient had a 5-year history of declining cognitive faculties and he was a former rugby player. Physicians diagnosed the patient with progressive supranuclear palsy (PSP), and he passed away one year after his hospitalization. PSP is a neurodegenerative disease involving a slow decrease in brain volume, resulting in a gradual loss of cognitive and physical abilities. Given the clinical similarities between PSP and CTE, it is not surprising that the patient was misdiagnosed. The study aimed to examine claudin-5 expression in a brain clinically confirmed to be positive for CTE; antibodies targeting p-tau affirmed the presence of the insoluble protein. Investigators wanted to compare the deceased patient's brain to those of individuals who had been diagnosed with PSP, but tested negative for p-tau and CTE. PSP-positive and control brains were donated from the Dublin Brain Bank. In a double immunofluorescence model, sectioned slices of the patient in question, the PSP brains, and the controls were exposed to both rabbit anti-claudin-5 antibody and mouse anti-phospho-tau. Immunoreactivity patterns of the control brains clearly revealed high levels of claudin-5 and occludin-1, but minimally detectable p-tau, indicating that the BBB was intact and there was no significant level of p-tau. Immunoreactivity of the CTE-positive brain sections exhibited a stark contrast; areas of high perivascular p-tau deposition presented absent claudin-5 and occludin-1 immunopositivity, indicating a disrupted BBB. In order to determine the additive capacity of PSP to a CTE case, researchers compared immunoreactivity patterns between the CTE/PSP brain and a PSP only brain. Claudin-5 levels of the PSP did not appear to correlate with presence of p-tau, suggesting that BBB degradation might be unique to CTE and PSP.

Regions with high p-tau deposition observed no claudin-5, or other tight junction elements. Immunoreactivity patterns showed that p-tau deposition was highest in the perivascular space, depths of sulci, and in the subpial space. Retrospective inquiry revealed that the patient had played rugby professionally for approximately 40 years, and had sustained innumerable head concussions during his career (5).

Acute versus chronic vascular changes observed with TBI

CTE pathology remains nebulous not only at the molecular level, but also in a temporal sense; how long after a TBI does CTE pathogenesis take before noticeable structural changes occur? Furthermore, how long do these changes last after the initial head trauma? Many studies compare BBB and microvascular damage through observations made during the acute and chronic time periods post-impact injury. It would appear that BBB disruptions may linger in an elevated proportion of late survivors when compared to a lower proportion of survivors with BBB damage who are in the acute stage of recovery (8). These findings are interesting, but complicate the timeline of CTE development and recognizability.

For approximately a century, neuroscientists and physicians alike have utilized the controlled cortical impact (CCI) model to elucidate the influence of a TBI on the microvasculature of the brain. Animal models permit researchers to mimic and observe the pathology that ensues after a simulated brain injury and gain invaluable insight in the process. Typically, CCI procedures combine a craniotomy with an impact injury that imitates the type of TBI that one might sustain during high contact sports and vehicle collisions, for example. A craniotomy is a small opening made between the bregma and lambda sutures of the parietal bones in rats (or other small rodents), exposing the brain. Next, a stereotaxic impactor apparatus inflicts an impact injury on the exposed portion of the brain. One such study adopted this model to assess the microvascular irregularities that followed during the acute and chronic time periods post-impact injury (7).

Male rats sustained either a bilateral CCI (bCCI), or a unilateral CCI (uCCI), of two different magnitudes, following a 5 mm craniotomy. A Benchmark Stereotaxic Impactor apparatus with a 4-mm impactor tip inflicted an impact injury on the exposed brain with a velocity of 3.5 m/second, dwell time of 200 ms, and a compression distance measuring 200 mm. Acute stages of post-impact injury were defined as 24 hours and 1 week. Control rats received a craniotomy, but no impact injury. Chronic stages of post-impact injury were defined at 1 month, 2 months, and 3 months. After reaching the designated time period of examination, the respective rats were euthanized and their corpora callosa analyzed for alteration. Investigators observed a number of different changes associated with the impact injury, including endothelial damage, BBB degradation, macrophage mediated-inflammation, astrogliosis, and evolving white matter degradation. Each of these conditions has a damage marker that indicates their degree of disturbance or immunoreactivity. It was apparent that significant white matter degeneration ensued as a consequence of TBI; uCCI histological images revealed a gradual increase in the density and diameter of white matter lesions found in the sectioned corpora callosa of rats measured from 24 hours to 3 months post-impact injury. These findings confirm the demyelination of neurons local to the region of impact. While white matter

damage predominated the findings, there were other types of brain damage present that indicated BBB disruption (7).

Microbleeding, or microvascular hemorrhaging of the corpus callosum, was not detected until 1 week post-impact injury, but the number of individual microbleeds continued to increase up to 3 months post-impact injury. Ranging from 2 to 20 µm in diameter, microbleeds were observed in both bCCI and uCCI rats, while control rats did not present with a significant increase in microbleeds. It is interesting to note that the uCCI rats with the ipsilateral impact injury demonstrated both ipsilateral and contralateral microbleeds, indicating that microbleeding can occur contralaterally to the site of impact. It can be assumed that microbleeding occurs due to damaged BBB (7). As discussed previously, endothelial cells are perhaps the most essential element to the BBB because they contain tight junctions that prevent paracellular transport (5). Researchers assessed endothelial damage by examining the immunoreactivity patterns of Intracellular adhesion molecule-1 (ICAM-1), a transmembrane protein present in endothelial cells. Results demonstrated significantly elevated levels of ICAM-1 immunoreactivity at 1 and 3 months, which is biphasic. This marked increase in free ICAM-1 concentrations correlates, temporally, with the gradual increase in microbleeds, which also peaked at 3 months. In order to evaluate BBB damage as a whole, researchers exposed primary antibodies in the corpora callosa to rat Immunoglobulin G (IgG). Immunohistochemistry revealed conspicuous, scattered IgG immunoreactivity surrounding the sites of impact injury. Maximal IgG staining occurred at 24 hours, but numerous punctate stainings were detected at 1 month. Furthermore, these punctate stainings were temporally colocalized with microbleeds, suggesting that BBB breakdown and microbleeds occurred in the same spot. Finally, gliosis of the astrocytes remained elevated at all measured time points, meaning that the astrocytes were continuing to proliferate and undergo hypertrophy. Glial fibrillary acidic protein staining of the corpus callosum measured immunoreactivity of the astrocytes. These results might suggest that as the damage to the microvasculature persisted, astrocytes continued to support the neuronal cells by attempting to repair damage, form scar tissue, and attenuate any CNS damage sustained (7).

Conclusion

As the molecular mechanisms behind CTE remain cryptic, new evidence supports previous research stating that BBB disruption is central to the pathology of this disease. Simulated TBI results in significant delayed microvascular damage surrounding the regions of impact, implicating the BBB as a foci of damage. From a clinical standpoint, the lack of an antemortem diagnostic examination complicates matters of routine for both healthcare professionals and concerned patients. There is no subsequent cure, and even the etiologic factors of CTE remain shrouded in mystery. When thinking of CTE and individuals who may be exposed to recurrent TBIs, football players often come to mind. However, they are not the only individuals prone to developing CTE. Some populations at greater risk to sustain a head injury can include individuals who engage in various high-contact sports like boxing and rugby, victims of domestic and child abuse, and military personnel. Therefore, having a way to detect the onset of CTE is vital for individuals under these circumstances so interventions can occur earlier in pathogenesis, possibly mitigating the impact that behavioral changes would have on daily life. Neuroimaging modalities refined for CTE detection have proven themselves to be a promising avenue for living patients possibly faced with a CTE diagnosis. PET scans utilized with probe sensitivity for tau may allow for further investigation of suspected CTE in living individuals who have prior history of TBI.

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