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Treating Post-Traumatic Seizures to Limit Tau Accumulation in Larval Zebrafish

Seizures Are a Druggable Mechanistic Link Between TBI and Subsequent Tauopathy

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Traumatic brain injury (TBI) is a prominent risk factor for dementias including tauopathies such as chronic traumatic encephalopathy. The mechanisms that promote prion-like spreading of Tau aggregates after TBI are not fully understood, in part due to lack of tractable animal models. Here, we test the putative role of seizures in promoting the spread of tauopathy. We introduce "tauopathy reporter" zebrafish expressing a genetically encoded fluorescent Tau biosensor that reliably reports accumulation of human Tau species when seeded via intraventricular brain injections. Subjecting zebrafish larvae to a novel TBI paradigm produced various TBI features including cell death, post-traumatic seizures, and Tau inclusions. Bath application of dynamin inhibitors or anticonvulsant drugs rescued TBI-induced tauopathy and cell death. These data suggest a role for seizure activity in the prion-like seeding and spreading of tauopathy following TBI. Further work is warranted regarding anticonvulsants that dampen post-traumatic seizures as a route to moderating subsequent tauopathy.

Commentary

Traumatic brain injury (TBI) is a devastating brain condition associated with severe disability and a high incidence of mortality. It is also a leading risk factor for neurodegeneration and dementia, such as chronic traumatic encephalopathy, a condition that has been in the spotlight as it afflicts football players and other athletes. The primary neuropathology in TBI patients is the presence of hyperphosphorylated Tau, axonal degeneration, and neuronal loss (eg, the study by Hay et al[1] and Ojo et al[2]). As observed in neurodegenerative diseases, in particular Alzheimer disease, there is a progressive accumulation and spread of Tau neuropathology, referred to as tauopathy. How TBI leads to this progressive Tau neuropathology is unclear, although seizures have been speculated to play a role in this mechanism. Traumatic brain injury is commonly followed by seizures, referred to as post-traumatic epilepsy. It is expected that more than 50% of severe TBI patients will develop seizures.[3] In addition, studies in vitro or in vivo such as in a mouse model of familial frontotemporal dementia reported that neuronal activity contributes to the release of Tau into the medium and the spread of Tau pathology.[4-6] The present study by Alyenbaawi et al directly examined whether blocking seizures with drugs could prevent the spread of Tau neuropathology.

To address this issue, they engineered transgenic zebrafish that express a tauopathy biosensor and developed a novel model of TBI in larval zebrafish. More specifically, they utilized a fluorescent Tau reporter protein that was composed of the sequence of the human Tau core-repeat domain fused to green fluorescent protein (GFP), called Tau4-GFP, and expressed the reporter in a transparent zebrafish line, the Casper background. The authors first extensively validated their sensor both in vitro and in vivo. In particular, they injected either pathogenic Tau brain homogenate from transgenic mice expressing mutant Tau or synthetic human Tau protein into the hindbrain of 2 days post-fertilization (pdf) zebrafish. In both cases, the injected larvae developed GFP+ puncta reflective of Tau aggregation in the brain, near the ventricle wall and in the spinal cord. Repeated measurements of Tau revealed progressive and significantly higher abundance of GFP+ inclusions in injected larvae over time compared to controls, showing prion-like induction of tauopathy via protein-only seeding in vivo. Unexpectedly, control larvae developed a small amount of GFP+ inclusions as well, but significantly less. Using a proteosome inhibitor to prevent Tau elimination enhanced the accumulation of inclusions.

Next, the authors developed a novel, simple and inexpensive model of TBI in larval zebrafish that resembled blast injury in humans. Larvae were placed into a syringe with a closed valve stopper. Applying a hit on the plunger produced a pressure wave through the fish body. After pressure calibration, although technical variability persisted, they found that this pressure wave led to hemorrhage, abnormalities in blood flow (eg, reduction or complete elimination), and cell apoptosis (measured with active Caspase-3). These data suggest that their

TBI model in larval zebrafish reflected typical characteristics of human TBI. Next, the authors assessed whether TBI could induce seizures. Post-traumatic seizures were visible in about 40% of the zebrafish larvae subjected to TBI. In some cases, the activity resembles stage III seizures that are the most intense seizures in zebrafish characterized by bouts of intense convulsions and arrhythmic shaking.[7] Most individuals displayed hypermotility consistent with less intense stage I or II seizures that were quantified with behavioral tracking software. The authors also reported that there was a sharp increase in neuronal activity during experimental TBI by monitoring intracellular calcium levels using a genetically encoded calcium imaging reporter.

Armed with this new model, the authors assessed whether TBI led to tauopathy and whether reducing or increasing seizure severity would alter the progression of Tau pathology. They found that TBI led to Tau4R-GFP biosensor GFP+ puncta in the central nervous system and spinal cord that increased in time following the injury. The author varied TBI parameters to show correlation with TBI severity or repetitive injuries and tauopathy, despite some expected variability. As control, the authors showed that there was no GFP+ aggregates in models expressing GFP alone or other biosensor proteins like the SOD1-GFP (another model of prion-like protein aggregation). The author next examined whether there was a correlation between tauopathy and seizures. Larvae that displayed TBI-induced seizure-like behavior exhibited significant Tau aggregates compared to larvae without seizures. To address causality between seizures and tauopathy, the authors used several drugs to either decrease neuronal activity with the anticonvulsant retigabine (a voltage-gated KCNQ potassium channel [Kv7] opener) or increase seizures with the convulsant kainate. Retigabine decreased the abundance of GFP+ Tau puncta and many larvae did not develop Tau4R-GFP aggregates. By contrast, kainate increased the abundance of GFP+ puncta in a dose-dependent manner in TBI-subjected larvae, but it did not increase them in the absence of TBI. Importantly, coapplication of retigabine and kainate did not affect the abundance of TBI-induced Tau-GFP+ aggregates suggesting that the effect of these drugs was directly due to their modulation of seizures activity. Finally, the authors found circumstantially that 4-aminopyridine (4-AP), chosen to increase seizures, reduced the appearance of GFP+ Tau puncta. Using different experimental conditions, they found that prolonged application of 4-AP was acting independent of seizures but rather through reduced endocytosis. Indeed, inhibiting endocytosis with either pyrimidyn-7 or Dyngo 4a (dynamin inhibitors) reduced TBI-induced GFP+ Tau aggregates in zebrafish larvae.

Collectively, the present study suggests a causal link between seizures and tauopathy following TBI in zebrafish larvae, and that reducing seizures with drugs can prevent the spread and even the original accumulation of pathogenic Tau. It is important to emphasize, as acknowledged by the authors, that this study was performed in larvae and not adult zebrafish. As such it most likely represents trauma experienced by the human fetus that can occur during car collisions or domestic abuse. Whether the seizure and tauopathy findings in this model apply to adult larvae remains to be validated. A recent study reported a new model of TBI (induced by ultrasound) and post-traumatic seizures in adult zebrafish[8] that would benefit from using the present Tau4R-GFP zebrafish. This recent study in adult zebrafish reinforces the idea that zebrafish provide a valid model for studying TBI-induced seizures. Zebrafish also provide an easy, high-throughput model to test drugs and assess mechanisms of Tau progression, such as endocytosis. It is nevertheless critical to validate key findings in mammalian animal models such as mice or rats prior to testing in humans. Experimentally, it would have been important to provide a more detailed timecourse analysis of the seizure development following TBI. Similarly, using a calcium indicator allowing assessing of calcium imaging over time following TBI would have been beneficial. It would also be interesting to further examine neuronal damage or loss following treatment of seizures. Perhaps more importantly, it would be important to modulate seizures using more defined approaches, such as channelrhodopsin or halorhodopsin, in addition to drugs to fully confirm the findings. Despite these limitations, the present study provides robust and clean data suggesting that treating post-traumatic seizures with anti-seizure drugs can reduce the spread of Tau aggregates. Whether a similar mechanism is true in neurodegenerative diseases such as Alzheimer disease also associated with a high incidence of seizures remains to be examined.

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