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Overcoming Barriers to Improve Treatments in Epilepsy

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Microvascular Stabilization via Blood-Brain Barrier Regulation Prevents Seizure Activity

Greene C, Hanley N, Reschke CR, Reddy A, Mäe MA, Connolly R, Behan C, O'Keeffe E, Bolger I, Hudson N, Delaney C, Farrell MA, O'Brien DF, Cryan J, Brett FM, Beausang A, Betsholtz C, Henshall DC, Doherty CP, Campbell M. *Nature Comm*. 2022;13(1):2003. doi:10.1038/s41467-022-29657-y

Blood-brain barrier (BBB) dysfunction is associated with worse epilepsy outcomes however the underlying molecular mechanisms of BBB dysfunction remain to be elucidated. Tight junction proteins are important regulators of BBB integrity and in particular, the tight junction protein claudin-5 is the most enriched in brain endothelial cells and regulates size-selectivity at the BBB. Additionally, disruption of claudin-5 expression has been implicated in numerous disorders including schizophrenia, depression and traumatic brain injury, yet its role in epilepsy has not been fully deciphered. Here we report that claudin-5 protein levels are significantly diminished in surgically resected brain tissue from patients with treatment-resistant epilepsy. Concomitantly, dynamic contrast-enhanced MRI in these patients showed widespread BBB disruption. We show that targeted disruption of claudin-5 in the hippocampus or genetic heterozygosity of claudin-5 in mice exacerbates kainic acid-induced seizures and BBB disruption. Additionally, inducible knockdown of claudin-5 in mice leads to spontaneous recurrent seizures, severe neuroinflammation, and mortality. Finally, we identify that RepSox, a regulator of claudin-5 expression, can prevent seizure activity in experimental epilepsy. Altogether, we propose that BBB stabilizing drugs could represent a new generation of agents to prevent seizure activity in epilepsy patients.

Commentary

The blood-brain barrier (BBB) is formed by endothelial cells that line the brain vasculature and proteins that create tight junctions between the endothelial cells. The BBB regulates permeability and movement of substances (e.g., ions, proteins) to and from the brain to maintain brain homeostasis. Previous studies revealed disruption of the BBB in resected tissue from patients with epilepsy and animal models of epilepsy. Furthermore, rodent models provide strong evidence that experimental induction of BBB breakdown via trauma or status epilepticus can promote epileptogenesis.[1,2] After status epilepticus in rodents, pronounced BBB leakage occurs prior to the emergence of spontaneous seizures, suggesting that disruption of the BBB does not directly induce seizures but rather contributes to the development of epilepsy.[2]

There are several potential mechanisms by which disruption of the BBB can lead to the development epilepsy. BBB disruption can allow serum albumin to enter the brain, which can be taken up by neurons, astrocytes, and microglia.[2] Uptake of albumin by astrocytes can lead to downregulation of inward rectifying potassium channels within astrocytes, activation of NMDA receptors, and subsequently, hyperexcitability.[2] In neurons, uptake of albumin can trigger neuronal death. In addition, the presence of albumin in the brain can induce transcriptional changes in genes such as transforming growth factor β (TGF-β), which in turn, can increase inflammation and lead to hyperexcitability.[2]

Proteins that form tight junctions in the brain include occludins, tricellulins, junctional adhesion molecules, and claudins. Among the claudin family of proteins, recent evidence suggests that claudin-5 is the primary claudin protein involved in

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maintaining the structure and function of the BBB.[3] Notably, mice lacking claudin-5 (homozygous knockout mice) die within 10 hours of birth although they exhibit normal brain vasculature.[4] In heterozygous claudin-5 knockout mice, Nitta et al., observed loosening of the BBB which allowed smaller particles that normally do not cross the BBB to enter the brain.[4] These observations suggest that breakdown of the tight junctions of the BBB disrupts brain homeostasis and that claudin-5 in brain endothelial cells is necessary for maintenance of the tight junctions.

Disruption of claudin-5 has been implicated in several neurological disorders, including schizophrenia and traumatic brain injury, but its role in epilepsy had not previously been elucidated. In the current study, Greene and colleagues investigated the contribution of claudin-5 to epilepsy using resected tissue from epilepsy patients and *in vitro* and *in vivo* models.[5] First, the authors observed reduced claudin-5 protein expression and increased inflammation in hippocampal tissue from patients with temporal lobe epilepsy when compared to age-matched controls, suggesting a possible association between seizures and reduced claudin-5 expression. Next, to directly investigate whether seizures can alter claudin-5 expression, the authors injected the proconvulsant kainic acid (KA) into the hippocampus of C57BL/6J wild-type (WT) mice, and similar to the human observations, reduced claudin-5 expression was observed 4 weeks later. The authors also found that hippocampal reduction of claudin-5 resulted in increased susceptibility to KA-induced seizures, deficits in recognition memory, reduced locomotion, and increased inflammation. Hippocampal knockdown of claudin-5 was achieved using a doxycycline inducible adeno-associated viral vector (AAV2/9) expressing a claudin-5 shRNA, which allowed for spatial and temporal regulation of claudin-5 knockdown via addition of doxycycline to the drinking water. Similarly, when claudin-5 was deleted from endothelial cells (using a conditional knockout mouse), the mutants also exhibited spontaneous seizures, ictal EEG activity, and increased GFAP and Iba1 expression.

The current study clearly and meticulously demonstrated that reduced claudin-5 contributes to epileptogenesis and increases seizure susceptibility, and as such, increasing claudin-5 may provide a novel therapeutic target for the treatment of epilepsy. Thus, using genetic strategies to overexpress claudin-5 might represent an attractive therapeutic approach. In future studies, it would be important to establish whether claudin-5 expression should be increased broadly throughout the brain or whether increased expression in specific brain regions might be more efficacious. Given that Greene et al., demonstrated increased seizure susceptibility and altered behavior following hippocampal reduction of claudin-5 expression, it is possible that increasing claudin-5 expression only in the hippocampus may provide sufficient protection. It is also critical to consider the potential for unwanted effects associated with targeting claudin-5, such as modulating other claudin proteins or other proteins of the BBB. For example, in retinal cells, lentiviral overexpression of claudin-5 also resulted in increased expression of claudin-1 and reduced expression of claudin-2.[6] Interestingly, Sladojevic et al., observed elevated claudin-1 expression in humans and mice with chronic stroke.[7] However, Berndt and colleagues reported downregulation of claudin-1 and an upregulation of claudin-5 in a rat stroke model.[8] Together, these observations suggest that there may be compensatory mechanisms between the claudin proteins.

Recently, it was demonstrated that inhibiting ALK5 of the TGF-β pathway, which is an important pathway in epileptogenesis, increases claudin-5 expression.[9,10] In the current study, treatment with RepSox, a potent ALK5 inhibitor, was able to increase *Cldn5* mRNA expression in hCMEC/d3 cells and improve BBB properties in an *in vitro* assay.[5] In the mouse, treatment with RepSox (2 injections of 10 mg/kg, intraperitoneal) before KA administration reduced seizure severity, BBB breakdown, and inflammation.[5] While these results are promising, it would be important to establish whether treatment with RepSox after KA administration or even following the development of spontaneous seizures would also be beneficial as these outcomes would be more clinically relevant. Furthermore, it would also be important to determine whether long-term treatment with RepSox is necessary after the development of epilepsy.

While breakdown of the BBB can facilitate epileptogenesis, it is important to note that enhancing properties of the BBB could reduce the entry of therapeutic compounds, and thus could contribute to the development of pharmacoresistance.[11] Despite these limitations, targeting the BBB or claudin-5 might provide a potential therapeutic strategy for the treatment of acquired epilepsy. In addition to models of acquired epilepsy, it would be valuable to establish whether targeting the BBB in genetic models of epilepsy would also be efficacious. Furthermore, Greene and colleagues observed schizophrenia-like behavior in heterozygous claudin-5 knockout mice, and claudin-5 may also contribute to BBB disruption and disease pathogenesis in neurodegenerative diseases like stroke and Alzheimer's disease. Therefore, targeting claudin-5 might represent a broadly therapeutic target for neurological and neurodegenerative diseases.

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