Epilepsy Currents
2023, Vol. 23(5) 321-323

DOI: 10.1177/15357597231182785
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Don't Give Me Lip: A Role for Lipid-Accumulated Reactive Astrocytes in Temporal Lobe Epilepsy

Lipid-Accumulated Reactive Astrocytes Promote Disease Progression in Epilepsy

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Reactive astrocytes play an important role in neurological diseases, but their molecular and functional phenotypes in epilepsy are unclear. Here, we show that in patients with temporal lobe epilepsy (TLE) and mouse models of epilepsy, excessive lipid accumulation in astrocytes leads to the formation of lipid-accumulated reactive astrocytes (LARAs), a new reactive astrocyte subtype characterized by elevated APOE expression. Genetic knockout of APOE inhibited LARA formation and seizure activities in epileptic mice. Single-nucleus RNA sequencing in TLE patients confirmed the existence of a LARA subpopulation with a distinct molecular signature. Functional studies in epilepsy mouse models and human brain slices showed that LARAs promote neuronal hyperactivity and disease progression. Targeting LARAs by intervention with lipid transport and metabolism could thus provide new therapeutic options for drug-resistant TLE.

Commentary

Epileptogenesis is the process by which transcriptional, molecular, biochemical, and structural changes in the brain lead to the development of spontaneous recurrent seizures.[1-3] Despite the availability of 30+ anti-seizure medications, no existing therapy exerts disease-modifying effects to prevent epileptogenesis after an inciting injury or epileptogenic event.[1] Furthering our understanding of the process of epileptogenesis could help guide design of targeted therapies. Mounting evidence suggests a key role for non-neuronal elements as contributors to epileptogenesis,[4] complementing neuron-centric changes in ion channel composition, neuronal signaling pathways, neurodegeneration, and circuit connectivity.[5] In particular, reactive astrocytosis might provide a primary target for modulation of epileptogenesis. However, whether such changes are cause or consequence of epileptogenesis and/or ongoing epilepsy remains to be disentangled.[4]

A recent study by Chen et al[6] reports a role for a newly identified subtype of reactive astrocyte in the pathogenesis of chronic temporal lobe epilepsy (TLE) in human patients and in a mouse model. The authors identified increased lipid signal in patients with TLE using magnetic resonance spectroscopy and increased lipid droplet formation in astrocytes in post-surgical tissue samples obtained after temporal lobectomy, which they refer to as lipid-accumulated reactive astrocytes (LARAs). The authors then demonstrated lipid accumulation in astrocytes from mice with chronic acquired TLE after kainic acid-induced status epilepticus. Increased lipids were observed in neurons as well as in astrocytes but not in oligodendrocytes or microglia. Using single-nucleus RNA sequencing (snRNA-Seq) of tissue obtained from surgical specimens from human patients, the authors noted differential expression of multiple transcripts related to lipid transport and metabolism, which identified this new subtype of lipid-laden reactive astrocyte hypothesized to be neurotoxic. The transcriptomically defined cluster exhibited a marked increase in expression of apolipoprotein E (APOE), a lipid transport protein implicated in Alzheimer Disease pathology.[7] Genetic deletion of *Apoe* in mice inhibited KA-induced LARA formation, and conditional deletion of *Apoe* in astrocytes (ApoE cKO) prevented astrocytic lipid accumulation in epileptic mice. Using an *in vitro* co-culture assay, conditioned media from KA-stimulated neurons was sufficient to induce LARAs, but not in the absence of Apoe. Collectively, the authors suggest that APOE mediates the transfer of lipids from neurons to LARAs during epileptogenesis, providing a potential mechanism for astrocytosis.

Apoe cKO mice demonstrated reduced seizure frequency, severity, and duration, following KA-induced TLE relative to control, and there were reduced seizure-like events in multi-electrode recordings from *ex vivo* brain slices prepared from Apoe cKO mice relative to wild-type (WT) controls. Further, *in vivo* application of cytidine-diphosphate choline (CDP-choline)—an accelerator of lipid metabolism—decreased KA-induced seizures in WT mice; however, this

effect was occluded in *Apoe* cKO mice. Collectively, these studies suggest that preventing lipid transport to astrocytes could potentially attenuate epileptogenesis in the KA animal model of TLE.

To further investigate the mechanism by which LARA formation might contribute to epileptogenesis and/or modulate epilepsy severity, the authors treated primary astrocytes from WT and *Apoe-/-* mice with free fatty acids, and evaluated changes in transcriptomics. RNA-Seq demonstrated a differential elevation in genes involved in synaptic plasticity and neurotransmission, with a notable increase in adenosine 2A receptor transcripts ($A_{2A}R$) in WT but not *Apoe-/-* mice. Viral knockdown of $A_{2A}R$ prevented the KA acid-induced increase in excitatory postsynaptic current frequency and enhanced intrinsic excitability observed via electrophysiological recordings in acute brain slices *in vitro*, and reduced seizure frequency and duration in mice *in vivo.* In addition, pharmacological inhibition of $A_{2A}Rs$ in *ex vivo* slices derived from human TLE specimens diminished the frequency and duration of seizure-like events recorded via multielectrode array. These studies indicate that activation of $A_{2A}Rs$ might regulate increased seizure susceptibility following lipid accumulation in astrocytes.

This study provides evidence for the existence of a novel subtype of reactive astrocyte that contributes to epileptogenesis via lipid accumulation and neurotoxicity through APOE and $A_{2A}Rs$. Such conclusions are supported by an impressive and diverse array of methodologies performed in both animal models and samples from human patients. However, there are several limitations to the study. While experiments demonstrate that deletion of *Apoe* modulates epileptogenesis in mice, it is not shown that accumulation of lipids in astrocytes and formation of LARAs is *sufficient* to induce epileptogenesis. Hence, it remains unclear whether LARA formation is a *causal* factor for epileptogenesis, or simply an epiphenomenon or byproduct of the complex process of epileptogenesis and/or the effect of chronic epilepsy.

The authors measure changes at 24 hours and 3 weeks following KA-induced seizure induction; however, future studies could better delineate the temporal window over which lipid accumulation occurs, which might better inform the relationship of such changes to epileptogenesis and chronic epilepsy. Such information would also be critical to guide future therapy (e.g., to establish a time window for anti-epileptogenic treatment post-traumatic brain injury or stroke). Future studies could clarify whether prevention of acute seizures or chronic epilepsy (or both) curtails lipid accumulation, for example by suppressing chronic seizures with a conventional anti-seizure medication(s) following epileptogenesis. Further, it would be of interest to validate these findings using other models of acquired TLE (such as kindling, which does not directly involve glutamatergic excitotoxicity and hence could address this potential confound), or determine if lipid accumulation contributes to pathogenesis of genetic epilepsies (e.g., Dravet syndrome, or tuberous sclerosis[8]).

Yet another unresolved question relates to the proposed mechanism(s) by which increased lipid accumulation in astrocytes leads to epileptogenesis. The authors suggest that astrocyte lipid leads to $A_{2A}R$ upregulation, which in turn potentially elevates extracellular glutamate. However, the exact cellular mechanisms connecting these steps remain unclear, as adenosine has been shown to have both pro- and anti-seizure effects.[8] Elucidating these pathways will be essential toward a better understanding of the pathological role of activity-dependent lipid signaling. For example, if future treatments target APOE, this could lead to retention of lipids in neurons, which also could be toxic.

The pathology described in this study overlaps with changes in neuroinflammatory markers and lipid metabolism seen in Alzheimer disease (AD) and multiple sclerosis, disorders of which epilepsy is a known comorbidity.[9,10] It would be important to clarify if the TLE patients included in this study might go on to develop AD. Additionally, it would be of interest to investigate whether APOE and/or LARA accumulation is a risk factor for epilepsy in patients with AD. Given the comorbidities between epilepsy and AD, future trials for antiepileptogenesis could utilize therapies that target APOE that are already under investigation in AD.

In conclusion, the discovery of LARAs could lead to the development of new anti-epileptogenic therapies and/or anti-seizure treatments that target lipid accumulation in astrocytes.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financialsupport for the research, authorship, and/or publicationof this article: This work was supported by NIH NINDS R25 NS065745 to E.C.R.

References

1. Loscher W, Klein P. The pharmacology and clinical efficacy of antiseizure medications: from bromide salts to cenobamate and beyond. *CNS Drugs*. 2021;35(9):935-963. doi:10.1007/s40263-021-00827-8
2. French JA, Bebin M, Dichter MA, et al. Antiepileptogenesis and disease modification: clinical and regulatory issues. *Epilepsia Open*. 2021;6(3):483-492. doi:10.1002/epi4.12526

3. Goldberg EM, Coulter DA. Mechanisms of epileptogenesis: a convergence on neural circuit dysfunction. *Nat Rev Neurosci*. 2013;14(5):337-349. doi:10.1038/nrn3482
4. Patel DC, Tewari BP, Chaunsali L, Sontheimer H. Neuron-glia interactions in the pathophysiology of epilepsy. *Nat Rev Neurosci*. 2019;20(5):282-297. doi:10.1038/s41583-019-0126-4
5. Rakhade SN, Jensen FE. Epileptogenesis in the immature brain: emerging mechanisms. *Nat Rev Neurol*. 2009;5(7):380-391. doi:10.1038/nrneurol.2009.80
6. Chen ZP, Wang S, Zhao X, et al. Lipid-accumulated reactive astrocytes promote disease progression in epilepsy. *Nat Neurosci*. 2023;26(4):542-554. doi:10.1038/s41593-023-01288-6
7. Wang C, Xiong M, Gratuze M, et al. Selective removal of astrocytic APOE4 strongly protects against tau-mediated neurodegeneration and decreases synaptic phagocytosis by microglia. *Neuron*. 2021; 109(10):1657-1674.e7. doi:10.1016/j.neuron.2021.03.024
8. Weltha L, Reemmer J, Boison D. The role of adenosine in epilepsy. *Brain Res Bull*. 2019;151:46-54. doi:10.1016/j.brainresbull.2018.11.008
9. Vossel KA, Beagle AJ, Rabinovici GD, et al. Seizures and epileptiform activity in the early stages of Alzheimer disease. *JAMA Neurol*. 2013;70(9):1158-1166. doi:10.1001/jamaneurol.2013.136
10. Chou IJ, Kuo CF, Tanasescu R, et al. Epilepsy and associated mortality in patients with multiple sclerosis. *Eur J Neurol*. 2019;26(2):342-e23. doi:10.1111/ene.13821