Epilepsy Currents
2021, Vol. 21(4) 282–284

DOI: 10.1177/15357597211011988
journals.sagepub.com/home/epi
SAGE

Localizing the Seizure Onset Site Through Metabolic Imaging of GABA

In Vivo Gamma-Aminobutyric Acid Increase as a Biomarker of the Epileptogenic Zone: An Unbiased Metabolomics Approach

Hamelin S, Stupar V, Maziere L, et al. *Epilepsia*. 2021;62(1):163-175. doi:10.1111/epi.16768.

Objective: Following surgery, focal seizures relapse in 20% to 50% of cases due to the difficulty of delimiting the epileptogenic zone (EZ) by current imaging or electrophysiological techniques. Here, we evaluate an unbiased metabolomics approach based on ex vivo and in vivo nuclear magnetic resonance spectroscopy (MRS) methods to discriminate the EZ in a mouse model of mesiotemporal lobe epilepsy (MTLE). Methods: Four weeks after unilateral injection of kainic acid (KA) into the dorsal hippocampus of mice (KA-MTLE model), we analyzed hippocampal and cortical samples with high-resolution magic angle spinning (HRMAS) MRS. Using advanced multivariate statistics, we identified the metabolites that best discriminate the injected dorsal hippocampus (EZ) and developed an in vivo MEGAPRESS MRS method to focus on the detection of these metabolites in the same mouse model. Results: Multivariate analysis of HRMAS data provided evidence that γ-aminobutyric acid (GABA) is largely increased in the EZ of KA-MTLE mice and is the metabolite that best discriminates the EZ when compared with sham and, more importantly, when compared with adjacent brain regions. These results were confirmed by capillary electrophoresis analysis and were not reversed by a chronic exposition to an antiepileptic drug (carbamazepine). Then, using in vivo non-invasive GABA-edited MRS, we confirmed that a high GABA increase is specific to the injected hippocampus of KA-MTLE mice. Significance: Our strategy using ex vivo MRS-based untargeted metabolomics to select the most discriminant metabolite(s), followed by in vivo MRS-based targeted metabolomics, is an unbiased approach to accurately define the EZ in a mouse model of focal epilepsy. Results suggest that GABA is a specific biomarker of the EZ in MTLE.

Commentary

Even though many patients with epilepsy can be successfully treated with medications, up to 40% are refractory to antiseizure drugs or experience significant drug-related side effects.[1] Although surgical interventions at the site of seizure onset yield excellent short-term, seizure-free outcomes in nearly 90% of all cases, about 50% of these patients experience seizure recurrence within 5 years postsurgery.[2] The reasons for the suboptimal outcomes are not fully understood, but factors such as inaccurate delineation and incomplete removal of the seizure onset site(s), may play important roles. It is also possible that new seizure onset sites develop postsurgery, causing long-term recurrence of the disease.

We do not have sufficiently safe, effective, and specific approaches for accurate delineation of all seizure onset sites in the human brain. Although scalp electroencephalography (EEG) can be used to localize seizures from the cortical surface, seizures from deeper sites are notoriously difficult to pinpoint using this technique. Intracranial EEG recordings can overcome some of these issues but are invasive and associated with risks of anesthesia and surgery. Moreover, because only limited regions of the brain can be safely monitored by intracranial electrodes, this method is subject to sampling bias and may miss seizure sites elsewhere in the brain. Lastly, the use of EEG requires that at least one typical seizure is captured, and this can take days or weeks in some patients, making long-term EEG recordings a costly and inefficient diagnostic tool.

Several alternative seizure localization approaches have been proposed, such as structural brain imaging, positron emission tomography imaging of 2-deoxyglucose[3,4] and synaptic vesicle protein 2A,[5] and metabolic imaging using magnetic resonance spectroscopy (MRS).[6] These approaches are particularly attractive because they do not require capturing of a seizure, they are minimally invasive, and they usually cover the entire brain, or large portions of it. However, the main disadvantages have been poor sensitivity and specificity, and exposure to radiation and contrast agents for some of the imaging modalities.

To overcome these issues, Hamelin et al[7] used a 2-tiered metabolomics approach to search for improved imaging

methods of the seizure onset site. To this end, the authors created a seizure focus in one of the dorsal hippocampi of laboratory mice by injecting the glutamate receptor agonist kainic acid (KA) into the structure. A separate group of mice injected with physiological saline into the same brain region was used as controls. Seven weeks later, samples of the hippocampus from both groups of mice were analyzed with ex vivo high-resolution magnetic angle spinning MRS. Nineteen metabolites were detected and quantified, including amino acids, organic acids, choline, glycerophosphocholine, glutathione, myoinositol, phosphorylcholine, and scyllo-inositol. The authors used multivariate statistics to compare the metabolomic profiles between the groups and found that γ-aminobutyric acid (GABA) and *N*-acetyl aspartate best discriminated the KA injected (seizure onset) hippocampus from the saline injected (control) hippocampus. To assess the ability of the metabolomics approach to delineate the seizure onset site from adjacent brain regions, the authors performed multivariate analysis of several other brain regions in addition to the dorsal hippocampus. They found that GABA best discriminated the seizure onset site from the other regions.

Finally, the authors used a clinically relevant approach—in vivo GABA-edited MRS—to test whether metabolic imaging of GABA in live mice can be used to identify the seizure onset site. To mimic the variability in epileptic phenotypes among human patients, 2 doses of KA were used, and to minimize acquisition bias, the GABA/(glutamate + glutamine) and GABA/creatine ratios were used, rather than GABA alone. The authors found that the GABA/(glutamate + glutamine) ratio had the best receiver operator characteristics with a specificity of 100% and a sensitivity of 88.89% for detecting the seizure onset site when the ratio was 1.674.

This study provides proof-of-concept that the 2-tiered metabolomics approach used here, that is, ex vivo metabolomics screening followed by targeted in vivo metabolic imaging, can be used to identify biomarkers of potential clinical utility. The power of the ex vivo approach is that many more metabolites can be detected than by in vivo MRS, thereby casting a wider net with respect to biomarker identification. The authors used MRS for the ex vivo quantitation; however, other, more effective analytical platforms such as liquid or gas chromatography combined with mass spectrometry could have been employed instead. The latter methods are typically more sensitive and higher throughput than MRS and are usually more readily available in high-throughput clinical and research analytical laboratories. The distinct advantage of in vivo MRS is that it is minimally invasive and can be used in live patients, often without exposure to radiation and contrast agents.

The authors postulate from the rodent data that in vivo imaging of GABA might be used to delineate the seizure onset site in human patients with epilepsy and that clinical trials are warranted. MRS imaging of GABA is indeed feasible in humans. Mattson et al used in vivo MRS to demonstrate increased brain tissue GABA levels after administration of vigabatrin to human participants,[8] and Maria et al employed edited MRS to quantify GABA and GLX (ie, the sum of glutamate and glutamine) in the neonatal human brain.[9] However, the use of GABA as a biomarker in human epilepsy may not be as straightforward as in rodents. This is partly because brain GABA metabolism is quite different in primates versus rodents. The inhibitory neuromodulator homocarnosine is closely associated with GABAergic neurotransmission in primates,[10] and homocarnosine levels are about 50% of GABA levels in the human occipital cortex, whereas rodents have very low levels of homocarnosine compared to GABA.[10] Moreover, the GABA increase in the rodent seizure onset site may be model specific and not translatable to humans because seizure foci in humans are usually not created by exogenous excitotoxins. Also, the GABA increase may relate to structural epileptogenic lesions, but not necessarily to nonlesional epilepsies.

The finding of increased GABA in the seizure onset site may seem paradoxical because of the inhibitory properties of the neurotransmitter and the notion that GABA-receptor stimulation, by benzodiazepines and barbiturates, usually suppresses seizures. Increased GABA could represent a compensatory response to counteract seizures. However, some studies have suggested that enhanced GABAergic neurotransmission may facilitate seizures through stimulation of "excitatory" $GABA_A$ receptors[11] and by increased activity of GABAergic interneurons, promoting high-frequency oscillations and neuronal hypersynchronization.[12] Another complicating factor is that MRS captures the entire pool of tissue GABA and does not differentiate between the intracellular, synaptic, and extrasynaptic (ambient) pools of the amino acid. This is an important issue because the location of the GABA increase determines its downstream effects. Brain microdialysis and immunogold electron microscopy of GABA are some of the tools that can be used to pinpoint the location and therefore better understand the role of the GABA increase.

In summary, the study by Hamelin is important and of potentially high clinical relevance because it suggests that in vivo MRS imaging may be used to delineate the seizure onset site(s) in a safe, minimally invasive, and effective manner. However, additional studies are needed to evaluate the translatability of the findings to human epilepsy, and whether GABA-edited MRS imaging is more sensitive and specific than long-term EEG monitoring and other diagnostic approaches. Furthermore, the potential applicability to nonlesional epilepsy may have the highest clinical impact for presurgical evaluations, but this remains to be investigated.

By Tore Eid

ORCID iD

Tore Eid https://orcid.org/0000-0001-9217-6393

References

1. Brodie MJ, Dichter MA. Antiepileptic drugs. *N Engl J Med*. 1996; 334(3):168-175.

2. Spencer DD, Gerrard JL, Zaveri HP. The roles of surgery and technology in understanding focal epilepsy and its comorbidities. *Lancet Neurol*. 2018;17(4):373-382.
3. Theodore WH, Newmark ME, Sato S, et al. [18F]fluorodeoxyglucose positron emission tomography in refractory complex partial seizures. *Ann Neurol*. 1983;14(4):429-437.
4. Theodore WH, Sato S, Kufta CV, Gaillard WD, Kelley K. FDG-positron emission tomography and invasive EEG: seizure focus detection and surgical outcome. *Epilepsia*. 1997;38(1):81-86.
5. Finnema SJ, Toyonaga T, Detyniecki K, et al. Reduced synaptic vesicle protein 2A binding in temporal lobe epilepsy: a [(11) C]UCB-J positron emission tomography study. *Epilepsia*. 2020; 61(10):2183-2193.
6. Pan JW, Kuzniecky RI. Utility of magnetic resonance spectroscopic imaging for human epilepsy. *Quant Imaging Med Surg*. 2015;5(2):313-322.
7. Hamelin S, Stupar V, Maziere L, et al. In vivo gamma-aminobutyric acid increase as a biomarker of the epileptogenic zone: an unbiased metabolomics approach. *Epilepsia*. 2021; 62(1):163-175.
8. Mattson RH, Petroff O, Rothman D, Behar K. Vigabatrin: effects on human brain GABA levels by nuclear magnetic resonance spectroscopy. *Epilepsia*. 1994;35(suppl 5): S29-S32.
9. Maria YL, Price AN, Puts NAJ, et al. Simultaneous quantification of GABA, Glx and GSH in the neonatal human brain using magnetic resonance spectroscopy. *Neuroimage*. 2021; 233:117930.
10. Petroff OA. GABA and glutamate in the human brain. *Neuroscientist*. 2002;8(6):562-573.
11. Cohen I, Navarro V, Clemenceau S, Baulac M, Miles R. On the origin of interictal activity in human temporal lobe epilepsy in vitro. *Science*. 2002;298(5597):1418-1421.
12. D'Antuono M, Louvel J, Kohling R, et al. GABAA receptor-dependent synchronization leads to ictogenesis in the human dysplastic cortex. *Brain*. 2004;127(pt 7):1626-1640.