0724

In vivo GABA increase as a biomarker of the epileptogenic zone: an unbiased metabolomics approach

Florence Fauvelle[1,2], Vasile Stupar[1,2], Jia Guo[3], Wafae Labriji[1], Chen Liu[3], Alicia Plaindoux[1], Emmanuel Luc Barbier[1,2], Sophie Hamelin[1], and Antoine Depaulis[1]

[1]Grenoble Institut Neurosciences, University Grenoble Alpes, La Tronche, France, [2]IRMaGE, University Grenoble Alpes, La Tronche, France, [3]Departement of Psychiatry, Columbia University, New York, NY, United States

Synopsis

New non invasive methods are required to better delimit the epileptogenic zone (EZ) during the pre-surgical exam of epileptic patients. By combining *ex vivo* NMR spectroscopy-based (MRS) untargeted metabolomics and *in vivo* MRS-based targeted metabolomics, we found that GABA was the most discriminant metabolite of the epileptogenic zone vs adjacent brain regions in a mouse model of mesio-temporal lobe epilepsy (MTLE). GABA appears therefore as a specific *in vivo* biomarker of EZ in MTLE.

INTRODUCTION

For the 30 % of drug-resistant epileptic patients who mainly suffer from focal epilepsy, a surgical resection of the epileptogenic zone (EZ) can be proposed with a mean efficiency of 50-80[1]. A precise identification of the EZ is mandatory for an effective resection. Despite a large panel of noninvasive preoperative explorations, intracranial EEG (IEEG) is often required[2], but the low spatial sampling of each electrode and/or misguided implantation may result in the resection of an insufficient brain area. Therefore, the development of non-invasive methods to better identify the EZ and to guide electrode implantation remains a challenge. In our study, we hypothesized that MRS-based metabolomics could address this issue in mesio-temporal lobe epilepsy (MTLE), the prototype of surgical epilepsy. We used KA-MTLE (kainate) mice, that leads to hippocampal sclerosis similar to human ones[3]. Using pattern recognition methods applied to *ex vivo* MRS data, we determined the metabolic profile of the EZ relative to (i) Sham and (ii) adjacent structures, and identified the metabolite that best discriminate EZ. We then performed *in vivo* MRS of this metabolite to evaluate its reliability as a biomarker for the non-invasive detection of the EZ[4].

MATERIALS AND METHODS

Animals

KA-MTLE mice were obtained by injection into the right dorsal hippocampus of 50 nL of KA while Sham received NaCl[3]. We performed 7 experimental series. For one series, a half KA dose was used instead of regular dose. Thirty days after injection, we recorded hippocampal EEG activity to check for typical hippocampal paroxysmal discharge (Fig 1A).

In vivo MRI experiments

In vivo MRI and MRS were performed on a 9.4 Tesla scanner (BioSpec 94/20 Avance III HD, Bruker Biospin) with ParaVision (6.0.1) software three weeks after KA injection. We first acquired T2-weighted images to check for hippocampal sclerosis in KA-MTLE mice (Fig 1B). Then we used a customized MEGA-PRESS[5] method for GABA level measurements into the injected hippocampus (2x1x2 mm3 voxel, 68 ms echo time, 512 averages for On and OFF spectra each using interleaved mode, 34-min acquisition). We pre-processed and quantified the data using the JET algorithm[6], which requires no manual adjustment. Creatine+phosphocreatine (CR), GABA+ (GABA + macromolecule), and glutamate+glutamine (GLX) were quantified. To limit the effect of possible acquisition bias, we calculated the ratios GABA+/GLX.

Ex vivo HRMAS MRS

One week after *in vivo* MRS, we collected 5 samples/animal: ipsilateral and contralateral hippocampus (IH and CH), divided each in posterior (P) and anterior (A), and adjacent cortex for ^{1}H HRMAS MRS. This latter was performed on a Bruker Avance III spectrometer (IRMaGe, CEA, Grenoble, France) at 500 MHz using a CPMG pulse sequence (echo time 30 ms). Quantification was performed using the QUEST procedure of jMRUI[7].

Statistical analysis

We applied multivariate statistics using the SIMCA-P software v14 (Umetrics, Umea, Sweden), to find the metabolites most involved in EZ discrimination, based on their VIP (variable importance in the projection) values[8]. We considered metabolites with VIP ≥ 1 as the most relevant.

RESULTS

For KA-MTLE mice with typical paroxysmal discharges (Fig 1A) and associated hippocampal sclerosis (Fig 1B), the principal component analysis (PCA) built with ^{1}H HRMAS MRS data of tissue showed that EZ had a specific metabolic profile, compared to sham animals and, more importantly, compared to adjacent regions (Fig 2A). 14 metabolites were stronly dysregulated in EZ compared to Sham.

Classification of the metabolites according to their discriminating power showed that GABA was the most discriminant metabolite of EZ (Fig 2B), with a more than 100% increase compared to sham homologous brain area (Fig 2C). Alanine and acetate were also increased by around 50%.

The MEGA-PRESS analysis of EZ confirmed this large GABA increase, and the decrease of GLX (Fig 3A). The *in vivo* and *ex vivo* data were largely consistent, as indicated by the correlation curve (Fig 3B).

DISCUSSION/CONCLUSION

By using an unbiased methodological strategy, we have demonstrated that a drastic increase of GABA and the associated GABA/GLX ratio are EZ-specific in a validated model of MTLE with focal seizure[4].

While this result appears somewhat paradoxical with the "GABA-deficiency theory" of epilepsy[9], it is consistent with several studies in animal model[10] and in patients[11], suggesting that GABA could contribute to epileptogenesis and ictogenesis.

Our projects are now i) to develop chemical shift imaging of GABA for animal and ii) to measure *in vivo* GABA levels in EZ of patients with a suspicion of MTLE. For this latter, *in vivo* GABA edition could be performed before surgery, followed by *ex vivo* MRS of resected tissue.

Acknowledgements

References

1. Picot MC, Baldy-Moulinier M, Daures JP, et al. The prevalence of epilepsy and pharmacoresistant epilepsy in adults: a population-based study in a Western European country. Epilepsia. 2008 Jul;49(7):1230-8.

2. Jayakar P, Gotman J, Harvey AS, et al. Diagnostic utility of invasive EEG for epilepsy surgery: Indications, modalities, and techniques. Epilepsia. 2016 Nov;57(11):1735-47.

3. Riban V, Bouilleret V, Pham-Le BT, et al. Evolution of hippocampal epileptic activity during the development of hippocampal sclerosis in a mouse model of temporal lobe epilepsy. Neuroscience. 2002;112(1):101-11.

4. 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. 2020 Dec 1.

5. Mescher M, Merkle H, Kirsch J, et al. Simultaneous in vivo spectral editing and water suppression. NMR Biomed. 1998 Oct;11(6):266-72.

6. Guo J, Gang Z, Sun Y, et al. In vivo detection and automatic analysis of GABA in the mouse brain with MEGA-PRESS at 9.4 T. NMR Biomed. 2018 ;31(1).

7. Ratiney H, Sdika M, Coenradie Y, et al. Time-domain semi-parametric estimation based on a metabolite basis set. NMR Biomed. 2005 ;18(1):1-13.

8. Galindo-Prieto B, Eriksson K, Trygg J. Variable influence on projection (VIP) for orthogonal projections to latent structures (OPLS). J Chemometrics. 2014 ;28(8):623-32.

9. Meldrum BS. GABAergic mechanisms in the pathogenesis and treatment of epilepsy. Br J Clin Pharmacol. 1989;27 Suppl 1:3S-11S.

10. Stamboulian-Platel S, Legendre A, Chabrol T, et al. Activation of GABAA receptors controls mesiotemporal lobe epilepsy despite changes in chloride transporters expression: In vivo and in silico approach. Exp Neurol. 2016 ;284(Pt A):11-28.

11. Cohen I, Navarro V, Clemenceau S, et al. On the origin of interictal activity in human temporal lobe epilepsy in vitro. Science. 2002 15;298(5597):1418-21.

Figures
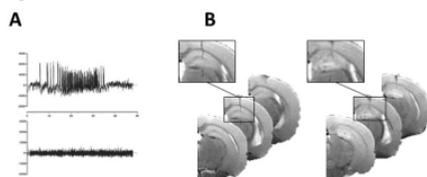
Figure 1: A/ Intrahippocampal electroencephalographic recording of KA-MTLE mouse (top) compared to Sham (bottom), showing a typical hippocampal paroxysmal discharge. B/ Three representative coronal adjacent MR images from Sham (left) and KA-MTLE (right) mice. The hippocampal sclerosis is clearly visible in EZ (right).
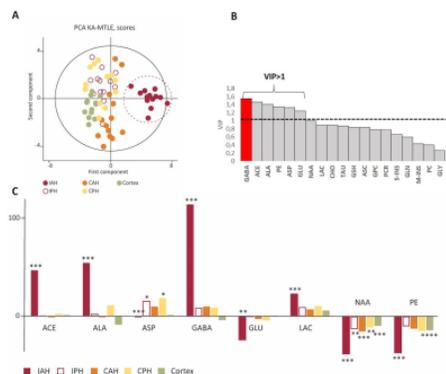
Figure 2: A/ Score plot of the principal component analysis (PCA) built with HRMAS MRS data of KA-MTLE mice and the 5 brain regions sampled (ipsilateral and contralateral hippocampus (IH and CH), posterior (P) and anterior (A), and adjacent cortex) . B/ Variable importance in the projection (VIP) ranking metabolites from most discriminative (VIP>1) to less discriminative ones in KA-MTLE mice C/ Percentage of variation of 8 metabolites (among the 19 metabolites quantified) in the 5 brain regions of KA-MTLE mice vs their sham homologous.
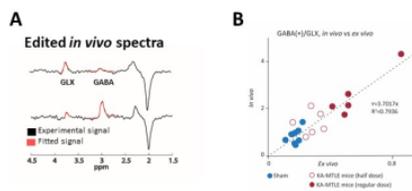
Figure 3: A/ In vivo GABA edition using MEGA-PRESS pulse sequence. Examples of GABA and GLX peaks in the subtracted spectrum, fitted using the JET algorithm, in Sham (top) and KA-MTLE (bottom) mice (regular KA dose). B/ Regression curve between in vivo GABA+/GLX and ex vivo GABA/GLX

Proc. Intl. Soc. Mag. Reson. Med. 29 (2021)
0724

https://index.mirasmart.com/ISMRM2021/PDFfiles/0724.html 2/2