JECN Journal of Epilepsy and Clinical Neurophysiology

Review article/Artigo de revisão/Artículo de revisión

PROTON MAGNETIC RESONANCE SPECTROSCOPY IN EPILEPSY – KEY FINDINGS

ESPECTROSCOPIA DE PRÓTONS POR RESSONÂNCIA MAGNÉTICA EM EPILEPSIA – PRINCIPAIS ACHADOS

ESPECTROSCOPIA DE PROTONES POR RESONANCIA MAGNÉTICA EN EPILEPSIA – PRINCIPALES HALLAZGOS

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ABSTRACT

Magnetic resonance spectroscopy (MRS) is a non-invasive technique useful both in research and neuroclinical evaluation. It relies on the same physical principles of magnetic resonance imaging providing information on chemical compounds *in vivo*. MRS uses the magnetic properties of several nuclei such as ^{13}C, ^{31}P and ^{19}F, although the ^{1}H is the most common due to its abundance and magnetic resonance signal sensitivity. Particularly in the last two decades, MRS has helped to better understand epilepsy and characterize its metabolic changes. In this review article, we aimed to point out the main contributions of MRS for epilepsy, focusing on proton magnetic resonance spectroscopy (^{1}H-MRS).

Keywords: Proton magnetic resonance spectroscopy; Partial epilepsy; Epilepsy; Generalized.

RESUMO

A espectroscopia por ressonância magnética (ERM) é uma técnica não invasiva útil tanto em pesquisa quanto em avaliação neuroclínica. Baseia-se nos mesmos princípios físicos da ressonância magnética (RM) convencional, fornecendo informações sobre compostos químicos in vivo. A ERM usa as propriedades magnéticas de vários núcleos, como ^{13}C, ^{31}P e ^{19}F, embora o ^{1}H seja o mais utilizado devido a sua abundância e à sensibilidade do sinal de ressonância magnética. Especialmente nas duas últimas décadas, a ERM tem ajudado a compreender melhor a epilepsia e a caracterizar suas alterações metabólicas. Nesse artigo de revisão, buscamos apontar as principais contribuições da ERM para a epilepsia, com foco em espectroscopia de prótons por ressonância magnética (^{1}H-ERM).

Palavras-chave: *Espectroscopia de prótons por ressonância magnética; Epilepsia parcial; Epilepsia; Generalizada.*

RESUMEN

La espectroscopia por resonancia magnética (ERM) es una técnica no invasiva utilizada en la investigación y en la evaluación neurológica clínica. Se basa en los mismos principios físicos de la resonancia magnética (RM) convencional, proporcionando información sobre compuestos químicos in vivo. Para este fin, la ERM utiliza las propiedades magnéticas de diversos núcleos tales como ^{13}C, ^{19}F y ^{31}P. Sin embargo, el ^{1}H es el más utilizado debido a su abundancia y la mayor sensibilidad de la señal de resonancia magnética. Especialmente en las últimas dos décadas, el uso de la ERM ha ayudado a comprender mejor la epilepsia y caracterizar sus cambios metabólicos. En este artículo de revisión tratamos de señalar las principales aportaciones de la ERM para la epilepsia, centrándonos en la espectroscopia de protones por resonancia magnética.

Descriptores: *Espectroscopia de protones por resonancia magnética; Epilepsia parcial; Epilepsia; Generalizada.*

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WHAT DOES MRS STAND FOR IN EPILEPSY?

Magnetic resonance spectroscopy (MRS) is a useful technique in both research and clinical neuroimage evaluation. It is based on the same physics principles of magnetic resonance imaging (MRI) providing information about chemical compounds. MRS exploits the magnetic properties of several nuclei such as ^{13}C, ^{31}P and ^{19}F although ^{1}H is the most common due to its abundance and magnetic resonance (MR) signal sensitivity. Moreover, no dedicated equipment is necessary to perform ^{1}H-MRS, what makes this technique more attractive and effective in the evaluation of several neurological conditions[1].

There are evidences of metabolic changes detectable by MRS, even subtle ones, which might not be clear in structural MRI[2,3]. This represents a great value for epilepsy in the clinical point of view. Noteworthy are MRS contributions to research in the field of epilepsy. MRS has helped to better understand and characterize metabolic alterations, identifying biomarkers of clinical parameters and dysfunction in the epileptic tissue.

In this review article, we aimed to point out the main contributions of MRS to the epilepsy field, focusing on proton magnetic resonance spectroscopy (^{1}H-MRS). This review is divided into two parts: (1) general principles of MRS and (2) main finds and contributions of ^{1}H-MRS to epilepsy. Theoretical principles showed here are relative at least to 1.5T MRI systems. Under this field strength some metabolites are not possible to be visualized or quantified by MRS. In its turn, the results referred are relative to 1.5 and 3.0 T, since are the field strengths more commonly available in clinical and research practice.

Here we refer the reader to several review papers of physical principles, technical methods of brain MRS and metabolites underpinnings, as well as metabolite quantification in higher field strengths[4-8].

PART I: A BRIEF OVERVIEW OF MRS TECHNICAL PRINCIPLES

The brain spectrum and its metabolites

MRS resulted data is called a spectrum. Metabolites are shown as peaks displayed along two axes, where the horizontal *x* axis represents the chemical shift while the vertical *y* axis brings information of relative signal amplitude. The chemical shift is read from the right to the left where metabolites have expected positions. Metabolites may be singlets, doublets, triplets or multiplets, regarding the peak structure[7]. Each peak is referred to represent the concentration (in ppm) of the metabolite measured by the area under the curve. Several approaches may be applied to quantify metabolites concentrations[9,10], either absolute or relative.

Biological underpinnings of metabolites measured by ^{1}H-MRS

Several metabolites can be identified by MRS such as N-acetylaspartate (NAA), choline-containing compounds (Cho), gamma-aminobutyric acid (GABA), glutamate (Glu), glutamine (Gln) or its sum (Glx), creatine (Cr), myo-inositol (mI), lactate (Lac), lipids, among others. However, only metabolites whose ^{1}H are in sufficient concentration can be measured by MRS in clinical scans[4]. It is important to bear in mind that metabolites measured by MRS must be in a form free to rotate and generate resonance signal. Thus, caution in interpretation of MRS results is required, since some measures may not reflect a direct or dynamic biological process.

Some metabolites are measured as combined signals of two or more molecules, being difficult to distinguish one from each other (or only resolved in higher field strengths, from 3.0T onwards). It is the case of NAA plus N-acetylapastateglutamate (NAA+NAAG), and Cr plus phosphocreatine. Others are only quantified using specific and optimized acquisition sequences, like GABA[6].

Long *versus* Short TE

MRS metabolites observed vary according to several factors, like field strength and some acquisition parameters. The general outline of a spectrum depends, at least in part, on the echo time (TE). From the choice of a certain TE advantages and disadvantages might emerge. Short TE (<40 ms) is usually better to visualize a broader group of metabolites, although it may confer wilder shaped baselines to the MR spectrum. On the other hand, a long TE (> 100 ms) usually leads to flatter baselines and thus quantification can be obtained in conditions where short TE could not be useful[11]. However, in long TE fewer metabolites might be visualized when compared to a short TE acquisition. Moreover, some are better quantified in a given TE. For instance, Glx and Ins are better visualized with short TE while Lac is better detected using long TE[1,12].

Single Voxel *versus* Magnetic Resonance Spectroscopic Imaging

MRS can be obtained through a single voxel (SV) or multiple voxels, also referred as chemical shift imaging (CSI) or magnetic resonance spectroscopic imaging (MRSI). Whereas the first allows the acquisition of metabolic information within a specific, smaller region, the second one makes possible to evaluate greater regions of interest.

In SV the shimming is more efficient, resulting in reduced magnetic field inhomogeneity (i.e. narrower line widths). Yet, the segmentation of voxel content is not possible. In small regions, like the hippocampus, structures which can interfere in the acquisition, giving raise to artifacts (i.e. bones, blood vessels, cerebrospinal fluid) might not be avoided, impairing data quantification[11].

In its turn, the main advantage of brain MRSI is to provide metabolic information from white or gray matter, i.e. MRSI is capable to outline maps of metabolic alterations[13] and even regions of maximal alteration[14]. It is possible to choose between the voxels acquired those with specific structural characteristics. However, MRSI shimming is not as efficient as in SV, leading to more variable linewidths. Besides, in small

regions the spatial resolution and voxel on the edge of the region of interest might be compromised.

The metabolites more commonly measured by ^{1}H-MRS, its biological roles and key finds associated to epilepsy are shown in the table 1. To exemplify the spectrum features, figure 1 shows a brain spectrum obtained from a TLE patient of our epilepsy service using SV ^{1}H-MRS and short TE.

PART II: ^{1}H-MRS IN THE INVESTIGATION OF EPILEPSIES

^{1}H-MRS in Focal Epilepsies

Focal epileptic seizures are those whose origin is within networks limited to one hemisphere, being less or more widely distributed. Moreover, it may originate in subcortical structures.[24] Focal epilepsies comprise those seizures clinically defined as temporal or extratemporal, whether the seizure focus is in the temporal lobe or not. Nearly 40% of these cases often become refractory to antiepileptic drugs (AED) and are referred to surgical treatment[25]. ^{1}H-MRS is widely used in focal epilepsies, mainly as part of presurgical evaluation. Temporal epilepsies are the most common form of focal epilepsy in adults[25], followed by frontal lobe epilepsy (FLE)[26], thus they are discussed in more details below. See table 2 for further details in some studies regarding focal epilepsies.

Temporal Lobe Epilepsy (TLE)

TLE comprises about 60 to 80% of all focal epilepsies in adulthood.[25] TLE is divided into mesial (MTLE) and neocortical, according to localization of the seizure focus.[35] MTLE is the most common form of TLE and hippocampal sclerosis (HS) is the most frequent underlying lesion.[36] HS

Table 1. Main metabolites measured by ^{1}H-MRS in the brain, its biological underpinnings and most prominent find associated to epilepsy.

Metabolite	Position at the chemical shift (ppm)	Biological features	Key find in Epilepsy
NAA	The highest and easier to identify peak. A singlet located at 2.01 ppm[1,6]	Synthesized in neuronal mitochondria. Marker of neuronal and axonal viability. Reflects permanent or reversible neuronal damage and more specifically mitochondrial function. Its exact role remains unknown [1,6]	A decrease in NAA reflecting neuronal or axonal loss/damage in focal epilepsies. Although there are stronger evidence of disturbed mitochondrial metabolism.[15]
Cr	A singlet positioned at 3.02 ppm in combination with PCr.[1,6]	Virtually present in all major cell types of brain tissue and in both gray and white matter. Mainly involved in brain energetics. Total creatine usually remains stable over the brain and time in normal conditions. Thus, is used to normalize other metabolites values. Further characterization is better achieved by ^{31}P-MRS. [1,6]	Its ratio is mainly used to normalize metabolic data. Cr is associated to seizure active and higher levels would be found in the post-ictal period[16]
Glu	Glu generates three multiplets arising at 2.34, 2.08 and 3.74 ppm. At 1.5T, Glu metabolite is actually a combined signal from glutamate and glutamine, plus minor glutathione and GABA contribution, often referred to as Glx.[1,6]	Major excitatory neurotransmitter. MRS measures correspond mainly to Glucytoplasmic concentrations. Found in both neurons and glia. [1,6]	An increase of Glx indicating epileptogenic process probably due to excitoxicity.[17-19]
Lac	A doublet localized at 1.32 ppm. Usually not present in detectable concentrations in the normal brain.[1,6]	Product of anaerobic metabolism of glucose. Thus, it is usually detectable under pathological conditions.[1,6]	An increase in Lac levels up to 6 hours after seizures.[2,20]
Cho	A singlet located at 3.21 ppm composed mainly by phosphorylcholine and glycerophosphorylcholine. Only those Cho molecules free from membrane (thus able to generateRM signal) account for the measure. [1,6]	Cho compounds are associated to cell membrane turnover and increased inflammatory process [1,6]	An increase of Cho would reflect cell membrane disturbance mainly associated with malformations of cortical development in extratemporal epilepsies.[21,22]
GABA	A multiplet at 3.01 ppm, a triplet at about 2.28 ppm and another multiplet 2.34 ppm. Without optimized pulse sequences (J-resolved or J-editing) the three peaks cannot be seeing at fields up to 3T due to overlapping from more intense signals.[1,6]	Most abundant inhibitory neurotransmitter.[1,6]	Elevated values in response to seizure activity.[16]
mI	Two multiplets at about 3.52 ppm and 3.61 pmm. mI peaks are usually not observable at long TE.[1,6]	Marker of glia cells. Although, there is stronger evidence of mI be equally synthesized in both neurons and glia.[1,6]	Higher levels of mI in the seizure focus, indicating gliosis processes, and lower mI levels in areas of seizure spread[23]

^{1}H-MRS: proton magnetic resonance spectroscopy; ppm: parts per million; NAA: N-acetylaspartate; Cr: creatine; ^{31}P-MRS: Phosphorus magnetic resonance spectroscopy; Glu: glutamate; GABA: gama-aminobutyric acid Glx: glutamate plus glutamine; MRS: magnetic resonance spectroscopy; Cho: choline or choline-containing compounds; Lac: lactate; TE: echo time; mI: myo-inositol.

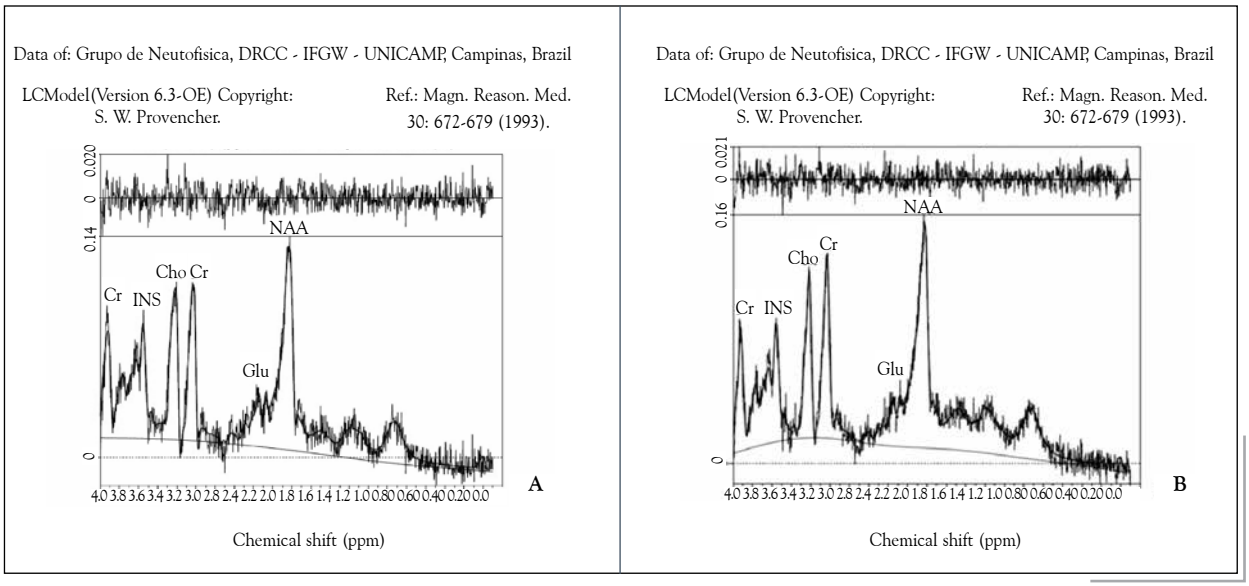

Figure 1. [1]H-MRS spectra obtained with LCModel9 from a TLE patient of our cohort. Spectra were acquired at 3.0 T system in the hippocampus, using PRESS sequence at TE = 35 ms. Panel (A) shows ipsilateral hippocampus (spectrum estimated FWHM = 0.069, SNR = 9). In (B) contralateral hippocampus (spectrum estimated FWHM = 0.053, SNR = 12). Note that the spectrum obtained ipsilaterally to the lesion displays a little less quality than the contralateral spectrum. This might be due to presence of HA, which makes acquisition more difficult. Main metabolites peaks are indicated. Cr: creatine; Ins: myo-Inositol; Cho: choline-containing compounds; Glu: glutamate; NAA: N-acetylaspartate; TLE: temporal lobe epilepsy; HA: hippocampal atrophy.

can be reliably detected by MRI exams, appearing as hippocampal atrophy (HA) and an increasing in T2 weighted signal intensity.[37]

Most of TLE patients becomes refractory to medication and are candidates to surgical treatment, undergoing extensive clinical evaluation. Correctly lateralize seizure focus is an important part of this process. Lateralization is determined by means of EEG monitoring, structural MRI and PET or SPECT results. However, studies have shown that [1]H-MRS indicates changes even in MRI negative TLE patients[17,27,35] and with normal or bilateral EEG.[30,39]

The most prominent [1]H-MRS find in MTLE-HS is a decreasing in NAA/Cr levels in the hippocampus ipsilateral to seizures focus or if bilateral reduction is found, the one ipsilateral to EEG focus usually is more evident.[3,14,17,40,41] Similarly to what is found for both ipsi-and contralateral hippocampi from MRI-positive patients, hippocampus from MRI-negative patients ipsilateral to EEG alteration also displays maximum decreasing values of NAA ratios.[42]

Actually, NAA ratios in MTLE do not seem to be confined to hippocampi but is rather a more widespread disturbance, including extrahippocampal areas, such as parietal, frontal and insula lobes. These changes occur in both MTLE and MRI negative patients although are more intense in the first ones.[26,43,44]

Relative decreasing in NAA to Cr or Cho has also been set as a predictor of AED response. SV [1]H-MRS in MTLE patients refractory to AED showed lower relative NAA levels when compared to MTLE patients with good seizure control (Mendes-Ribeiro, 1998, Campos, 2012).[29,45]

Relative NAA reduction is pointed as a result of neuronal loss.[46,47] However, NAA/Cr decreasing does not seem to explain an epileptogenic disturbance in the seizure focus alone. As mentioned above, some studies also show contralateral decreasing in NAA, even in unilateral HA cases.[17,18,28] There is also evidence of mitochondrial disturbance involved in NAA decreasing.[15] *In vivo* data appear to indicate that metabolic changes measured by 1H-MRS do not reflect only neuronal loss.[3,15,48] Normalization of NAA/Cr values after successful hippocampal resections adds information in favor of this finding.[49]

The lack of association between metabolic findings and seizure frequency in some studies[28,42] also brings the question whether is HS and neuronal loss a cause or consequence of seizures. A study aiming to prospectively evaluate the effects of acute seizures in future development of HS found decreased NAA in all seven patients evaluated and identified Lac peaks in six out of seven patients.[50] Results like this might help to better understand the association between a precipitating injury and further development of TLE. However, there were some limitations in these studies, such as the small cohort and lack of detailed MRS analyses. An acute increase of Lac levels up to 6 hours after complex partial seizure (dyscognitive) in the temporal lobe is described as a reliable indication of seizure onset.[2,20]

To sum up, [1]H-MRS findings in TLE show that key metabolic change is relative NAA reduction, with a high concordance rate with seizure focus lateralization. However, the exact underlying mechanism of NAA reduction remains controversial. Another key metabolite is Lac, which increases up to 6h after a complex partial seizure (dyscognitive). [1]H-MRS keeps its value in pre-surgical investigation and general research of TLE, where it has proven to be as useful as conventional MRI and others brain imaging techniques.

Table 2. ^{1}H-MRS studies focusing on several metabolites in focal and generalized seizure semiology.

	^{1}H-MRS technique	Seizure semiology	Cohort	Metabolites evaluated	Key finds
Doelken et al., 2008[27]	1.5T SV	Focal	17 HS MRI-positive 9 HS MRI-negative 23 Ctrls	Glx, Cho, Cr	No significant differences between groups.
Simister et al, 2002[28]	1.5T MRSI	Focal	10 Unilateral HS 10 MRI-negative 10 Ctrls	Glx, Cho, Cr, mI	Low Cr and Cho in the anterior sclerotic hippocampus; Glx increasing in contralateral anterior hippocampus of MRI-negative patients
Campos et al., 2010[29]	2.0 T SV	Focal	25TLE responsive to AED 21 TLE refractory to AED	NAA/Cr	A less intense reduction of NAA/Cr in TLE responsive to AED compared to refractory, suggesting less extent of neuronal damage and a prediction value of AED response by ^{1}H-MRS
Li et al., 2000[30]	1.5 T MRSI	Focal	21 bilateral refractory TLE 30 Ctrls	NAA/Cr	Decreasing in NAA/Cr to the side of surgery and normal NAA/Cr values in the contralateral posterior–temporal region associated with good surgical outcome
Lundbom et al., 2001[22]	1.5T MRSI	Focal	14 FLE or frontoparietal epilepsy and matched ctrls	NAA, Cho, Cr	Decreased NAA and an increasing of Cr and Cho in seizure focus. Reduced NAA/Cho+Cr in contralateral hemisphere, suggesting diffused metabolism alterations in FLE
Doelken et al., 2010[31]	3.0T MRSI	Generalized	10 GTCS 25 Ctrls	Glx, Cho	A broadly Glx increasing in both hemispheres; Cho decreasing in both grey and white matter in central regions of the brain
Simister et al., 2003[16]	1.5T SV	Generalized (different subsyndromes)	26 MRI negative	Glx, Cr, Cho, mI, GABA+homocarnosine	Elevation of Glx in frontal lobe bilaterally
Mory et al., 2003[32]	2T SV	Generalized	10 JME 10 Crtls	NAA/Cr	Low NAA/Cr values in the thalami of JME patients, suggesting thalamic dysfunction in this type of IGE
Kabay et al., 2010[33]	1.5T MRSI	Generalized	14 JAE 10 Ctrls	NAA, NAA/Cr, NAA/Cho, NAA/Cho+Cr	Lower NAA/Cr ratios in bilateral thalamus. No difference in frontal and hippocampal regions
Helms et al., 2006[19]	1.5T MRSI	Generalized	43 IGE 38 Ctrls	Glx, NAA	Increased Glx and decreased NAA in thalamus of IGE patients
Long et al., 2015[34]	3.0T MRSI	Generalized	12 BAFME from the same family 12 ctrls	NAA/Cr, NAA/Cho, Cho/Cr, and NAA/(Cr+Cho)	Lower NAA/Cho ratio in cerebellar cortex of BAFME patients

^{1}H-MRS: proton magnetic resonance spectroscopy; SV: single-voxel acquisition; MRSI: magnetic resonance spectroscopic imaging; HS: hippocampal sclerosis; MRI: magnetic resonance imaging; TLE: temporal lobe epilepsy; AED: antiepileptic drugs; FLE: frontal lobe epilepsy; GTCS: generalized tonic-clonic seizures; JME: juvenile myoclonic epilepsy; JAE: juvenile absence epilepsy; IGE: idiopathic generalized epilepsy; BAFME: benign absence familiar myoclonic epilpesy; NAA: N-acetylaspartate; Cr: creatine; Glu: glutamate; GABA: gama-aminobutyric acid Glx: glutamate plus glutamine; Lac: lactate; mI: myo-inositol; Cho: choline or choline-containing compounds.

^{1}H-MRS controversial results might be due to the heterogeneity of TLE itself, since there is a large heterogeneity of underlying pathologies. Additionally, different approaches have been applied in these studies.

Extratemporal Epilepsies

Extratemporal epilepsies (ETLE) are thus considered for having seizure foci outside temporal lobe. ETLE are less frequent, estimated in 18-30% of all focal epilepsies.[51] Frontal lobe epilepsy (FLE) is the most frequent form, followed by occipital and parietal lobes epilepsy.[52] Studies with AED refractory epilepsy show that there are tumors, trauma, vascular anomalies, and malformations of cortical development among the underlying extratemporal lesions found.[53,54] Although MRI negative casesare also a frequent finding.

Patients with ETLE are often refractory to medical treatment[25]; however, they present less favorable surgical outcome than TLE patients.[55] Identification of seizure focus usually

involve extensive presurgical evaluation in order to diminish morbidity and achieve better outcome. Presurgical evaluation is even more challenging in ETLE with MRI-negative results and often requires intracranial EEG monitoring.

NAA/Cr and NAA/Cho ratios are found to be decreased in epileptogenic regions of the frontal lobes compared to non-epileptogenic ones.[56,57] Guye and colleagues[57] studied refractory FLE using data from ^{1}H-MRSI and interictal intracranial EEG monitoring. They showed lowering of NAA/Cr and NAA/Cho+Cr ratios within regions of epileptiform abnormalities when compared to controls and regions with no electrophysiological abnormalities. The results also suggest that NAA/Cr would be more specific whereas NAA/Cho+Cr ratio would more sensitive. Thus, both values should be considered in presurgical evaluation.

Remarkably, ^{1}H-MRSI metabolic abnormalities mapping of ETLE seems to be able to detect epileptiform zones also in MRI negative cases. Similarly to what is found for TLE, metabolic findings aid detecting subtle changes in patients with ETLE, more specifically malformations of cortical development.[21,57] Krsec et al.[21] showed that ^{1}H-MRS localized the seizure focus in 5 out 7 patients with refractory FLE and added important information on the localization in other two. Histological analysis of surgical specimen revealed an FCD lesion in all patients. In Guye et al.[57] series, 4 out 7 MRI negative patients, whose seizure focus was localized by invasive examination, also presented FCD. In both studies patients showed a desirable surgical outcome.

Thus, also in extratemporal epilepsies ^{1}H-MRS in localizing seizure focus of FLE in good agreement with SPECT, seizure semiology, invasive EEG recording and histological postsurgical analysis.[21,56,57] Moreover, metabolic alterations seem to be more extensive than the seizure focus itself in extratemporal epilepsies,[21,56] as it occurs in patients with TLE.[31] Since postsurgical outcome in extratemporal epilepsies depends on successful resection of seizure focus, ^{1}H-MRS can improve presurgical evaluation as an additional tool for localization.

^{1}H-MRS in Generalized Epilepsies

In this seizure semiology are included sub-syndromes which, in general, present typical absences, tonic–clonic seizures and myoclonic jerks.[58] Juvenile myoclonic epilepsy is considered the most frequent generalized epilepsy (GE) syndrome, although numbers may vary.[59] Generalized epileptic seizures originate at some point in brain tissue and rapidly spread to bilaterally distributed networks. The so-called idiopathic generalized epilepsies (IGE, now termed genetic generalized epilepsies)[24] are usually associated with normal MRI. Although, Seneviratne and colleagues (2014)[47] listed studies which suggest an involvement of "focal" features not only in neuroimage (including MRS) but also EEG, neuropsychology and neuropathology. The findings are controversial, though data show that specific structures might play a role in GE, like frontal cortex, thalamus and even hippocampus.[33,60,61] In this context, MRS might be a useful tool in further evaluating underlying metabolic changes, where IGE is supposed to show no lesion.

There are evidence of a well characterized thalamo-cortical reduction of NAA/Cr.[33,60-62] However, other metabolites, like Glx show conflicting results for frontal lobe measures.[62] Regarding seizure control, results are also conflicting. There are studies indicating lower NAA/Cr levels, regardless seizure control[62,63] as well as more pronounced Glx elevation and NAA reduction in those patients with worse seizure control.[31]

^{1}H-MRS was successfully employed in attempt to differentiate focal from generalized non-convulsive seizures. Absence seizures do not present with the usual decrease of NAA/Cr+PCr and increasing of Lac/Cr+PCr showed in focal epilepsies. Moreover, IGE patients also do not show increasing in Lac/Cr+PCr during post-ictal or interictal periods when compared to normal controls values. The study concludes that this finding might explain the mild or lack of post-ictal confusion observed in absence seizures and the more benign course of primary generalized seizures.[2]

Even though there might not be structural alterations showed by conventional MRI, MRS is able to point substantial metabolic changes, which might help to explain GE pathophysiology. Neuronal damage might be more frequent in thalamus and frontal cortex, as indicated by changes on relative NAA. Similar to focal epilepsies, controversial results of other metabolites might be due to different methodologies applied, mainly inherent variation of the cohort studied and cortical area which have been evaluated.

Limitations in the usage of MRS

MRS hits in some technical issues. The main limitations are due to its inherent physics principles. Considering that metabolites concentrations in biological tissues are very low and that concentration measure is directly related to voxel size, the large the voxel the better the quantification.[4,5,7] Conversely, spectra must present with a minimum resolution, what is in part dependent on the acquisition time. Usually longer acquisition times are necessary compared to structural MRI.[5] Moreover; MRS is a very sensitive technique to artifacts difficult to control, like movement. Thus, is important that the subject remains still during longer periods. This may justify why MRS is not a frequent choice in epilepsy services (unless epilepsy suspected causes involve situations in which MRS is indicated, e.g. brain tumors).

Regarding brain MRS, artifacts also come from adjacent structures, like bones and blood vessels, making difficult to obtain high quality spectra depending on the regions. In some pathological alterations, this is even more problematic (e.g. the atrophic hippocampus. figure 1A). The majority of studies consider relative quantification, i.e. metabolites ratios to Cr levels. A problem that can arise from this approach is variations across subjects when comparing more than one group[7], which can be overcome by adopting absolute concentrations.[34] Moreover, as the loss of NAA may appear in some cases in parallel to an increase of Cr, the NAA/Cr ratios may actually reflects a higher relative value instead of

an absolute value in a single signal.[64] These limitations can be minimized setting well design studies, employing human resources with expertise in the field, and taking into account inherent factors like TE, acquisition sequence, water suppression, shimming, voxel positioning, post-processing techniques and others.

A long way ahead: what is left to be done with MRS?

As exposed in this review, data from MRS in epilepsy remains controversial and with many technical limitations. We need more studies with well design groups of subjects to evaluate different purposes in epileptology: pre-surgical value of MRS, biological role of its findings, MRS in animal models of epilepsy and translational studies, just to mention a few.

FINAL REMARKS

^{1}H-MRS is an MR technique that offers the benefit of reliably quantifies metabolic information *in vivo*. Quantification is possible using SV or MRSI acquisition and TE defines which metabolites are better displayed in the chemical shift. Data gathered in this review show that ^{1}H-MRS can be set as a useful tool in presurgical evaluation being able to lateralizing seizure focus in both TLE and ELTE. It is also valuable in characterizing structures more prone to be involved in GE, through its metabolic changes. ^{1}H-MRS also reliably detects subtle brain tissue alterations in patients, thus considered MRI negative. The most frequent and consistent metabolic change pointed out in literature is a decreasing in relative NAA, indicating neuronal dysfunction in both focal and GE. Relative NAA can be also used as predictive biomarker of specific features of epilepsy, like refractoriness. Association between epilepsy features and other metabolites measured by ^{1}H-MRS still needs further investigation, although an increase in Lac can be taken as a marker of epileptiform activity. Similar to any MRI technique, ^{1}H-MRS presents advantages and limitations, which can be optimized to improve its potentials.

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