MRS-lateralisation index in patients with epilepsy and focal cortical dysplasia or a MEG-focus using bilateral single voxels

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Summary

Purpose: To evaluate if single voxel proton magnetic resonance spectroscopy (SV-MRS) can help in lateralising and sometimes in localizing an epileptogenic focus. The assumption is that in MRI negative patients the underlying pathology most often is focal cortical dysplasia (FCD). Several studies have shown that in the presence of FCD there are also \textsuperscript{1}H-MRS abnormalities on the contralateral side. However, in most cases the studied group was not homogeneous and included different forms of dysplasias, including band heterotopias and polymicrogyria, and the studies used different spectroscopy protocols. In the present study, using bilateral SV-MRS we investigated the presence of a lateralisation index in two groups of patients with localisation related epilepsy: patients with focal cortical dysplasia on MRI and patients without MRI abnormalities with a focus identified by MEG. Aim of the study was to show that in both groups the expected epileptogenic side shows more pronounced metabolic alterations, making MRS a possible screening tool for clarifying lateralisation questions in patients with cryptogenic localisation related epilepsy.

Methods: In ten patients a single voxel was placed over the FCD and in nine patients over the region of interest (ROI) as indicated by MEG. In all patients a voxel was also placed in the contralateral homologus location. We used metabolite concentrations as peak ratios relative to the creatine (Cr) peak to calculate a lateralisation index.

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Results: In both groups NAA/Cr was significantly lower on the affected side whereas the results for Cho/Cr were more diverse. There were no significant differences between the two groups. The limitations of the used methods and the implications of the findings are discussed.

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Introduction

With the introduction of MRI in clinical practice the percentage of patients with cryptogenic localization related epilepsies diminished from 70% to 30%. However, despite the improvement of structural MRI techniques there are still a considerable number of patients with epilepsy left with a negative MRI study (von Oertzen et al., 2002; Phal et al., 2008). In case of intractable epilepsy of presumed extra-temporal origin this may prevent surgery or lower the chances of successful surgery in these patients. In most studies, the presence of a lesion on MRI and the possibility to resect the entire lesion are the major factors predicting successful outcome of surgery (e.g. Fauser et al., 2004; Krsek et al., 2009). Therefore, a lot of effort is put into improving imaging techniques.

With an a priori hypothesis on the probable location of a lesion more lesions can be detected by reviewing the MRI (Moore et al., 2002). MEG is a very powerful tool to determine the epileptogenic focus or the irritative zone in patients with enough spikes in their traces (e.g. Ossenblok et al., 1999; Shiraishi et al., 2001; Van ‘t Ent et al., 2003; Iida et al., 2005; Papanicolaou et al., 2005; Ossenblok et al., 2007).

There are several alternative MRI techniques that are used or investigated in epilepsy, most notably MRS. Results of MRS largely depend on determining a ROI in advance. However, even if there is a priori information, for example an identified epileptogenic lesion, the results of MRS and the conclusions based on these results are currently conflicting, especially in case of extra-temporal epilepsy. This may partly be due to the in homogeneity of the populations studied, while the methods and protocols used also differed. In extra-temporal epilepsy using MRS a decline of NAA concentration could be demonstrated in regions that were part of the epileptogenic or irritative zone (Guye et al., 2005), according to intra cerebral recordings. Six out of 12 patients in this study proved to have FCD at operation, 4 of them without abnormalities on conventional MRI, whereas 4 of the 12 patients were not operated upon. In another study 12 out of 14 patients with frontal lobe epilepsy a decline of NAA concentration was seen in the epileptogenic focus (Lundbom et al., 2001). According to structural MRI 4 had FCD (2 confirmed histological, 2 statistically significant), whereas 7 showed no abnormalities. In mesiotemporal sclerosis (MTS) MRS can show abnormalities before conventional MRI does (Hammen et al., 2003), and if bilateral hippocampal changes are found the prognosis of operation is less favorable (Lee et al., 2005). In 15 out of 20 patients with a neocortical temporal lobe epilepsy without abnormalities on MRI Shih et al. (2004) finds MRS abnormalities on the region predicted by MEG. In summary, there is increasing evidence that MRS may be more sensitive than structural MRI in detecting lesions.

Most cryptogenic epilepsies are believed to arise from FCD (Bautista, 2003). FCD has been recognised increasingly as one of the most common causes of pharmacoresistant focal epilepsy (Widders-Walsh et al., 2006). FCD is a developmental disorder in which cytoarchitectural derangements occur. Abnormal, immature cells remain present accompanied by a disruption of normal cortical and subcortical architecture (Palmini et al., 2004). As MRS is a sensitive measure of both neuronal maturation (Kok et al., 2002; Kreis et al., 2002; Tkac et al., 2003) and neuronal dysfunction (Cendes et al., 1997; Tasch et al., 1999) abnormalities are expected both in FCD and in epilepsy. As there is no support for a metabolic hemispheric asymmetry (Pouwels and Frahm, 1998) when using MRS on patients their non-affected side can be used as control side in any given investigation.

We investigated whether MRS-abnormalities in a focus as indicated by MEG in structural MRI negative epilepsy patients are comparable to MRS abnormalities in epilepsy patients with a FCD. Our goal was to determine whether SV-MRS could be supportive for localising an epileptogenic region and eventually can be used in presurgical evaluation of epilepsy patients. This study is the first to do so for foci not confined to the temporal lobe.

Methods

All patients were outpatients of Epilepsy Centre Kempenhaeghe with localisation related epilepsy. Two Groups of patients were included in the study. In the first group (the FCD group) patients were included who on structural MRI had unilateral FCD. In group 2 (the MEG group) patients were included who already had an MEG-recording for another study (Colan et al., 2009). Of these, patients in whom a plausible localisation of the epileptiform activity within the grey matter could be determined using equivalent dipole modelling and in whom structural MRI showed no abnormalities were included. Exclusion criteria were the standard exclusion criteria for MRI investigations, a cerebral lesion on MRI that clearly was not FCD, inability to cooperate and age below 18. In the FCD group 10 patients were included, in the MEG group 9. Patients age ranged from 21 to 73 years. 10 women and 9 men were included. Localisation of the ROI is described in Table 1. Permission for this study was obtained by the Local Medical Ethical Committee and all patients signed the informed consent.

In order to get the best possible signal-to-noise ratio we decided to use single voxel $^1$H-MRS (SV-MRS) and not chemical shift imaging (CSI). Even though CSI has the advantage of allowing a better spatial resolution and can be performed in a similar scan time as SV-MRS using SENSE parallel imaging, a CSI volume is more difficult to shim due to a larger shim volume. Better shimming provides smaller line widths and hence better SNR. SV-MRS spectra are therefore expected to be more robust, i.e. less variations over a large patient population. As there still is abundant discussion on the validity and on what reference to use of measuring absolute
**Table 1** Patient characteristics FCD (1–12) and MEG (20–28) groups, age in years.

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>EEG</th>
<th>MRI location FCD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>44</td>
<td>m</td>
<td>Left precentral sulcus</td>
</tr>
<tr>
<td>2</td>
<td>53</td>
<td>m</td>
<td>Left precentral sulcus</td>
</tr>
<tr>
<td>3</td>
<td>22</td>
<td>m</td>
<td>Right insula</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>m</td>
<td>Left parietal</td>
</tr>
<tr>
<td>6</td>
<td>32</td>
<td>f</td>
<td>Left frontal</td>
</tr>
<tr>
<td>7</td>
<td>39</td>
<td>m</td>
<td>Left parietal</td>
</tr>
<tr>
<td>8</td>
<td>22</td>
<td>f</td>
<td>Left parietal</td>
</tr>
<tr>
<td>10</td>
<td>44</td>
<td>f</td>
<td>Left frontal</td>
</tr>
<tr>
<td>11</td>
<td>24</td>
<td>m</td>
<td>Right insula</td>
</tr>
<tr>
<td>12</td>
<td>30</td>
<td>m</td>
<td>Right frontal</td>
</tr>
</tbody>
</table>

**MEG location**

| 20  | 21  | m    | Left peri-insular |
| 21  | 58  | f    | Left insular, right lateral temporal |
| 22  | 52  | f    | Left angular |
| 23  | 59  | f    | Left mesial fronto-parietal |
| 24  | 73  | f    | Right temporo-occipital |
| 25  | 22  | f    | Left parietal |
| 26  | 34  | m    | Right temporal |
| 27  | 35  | f    | Left insula |
| 28  | 37  | f    | Right frontal |

Sex: m: male; f: female. EEG: predominant side of found abnormalities on EEG. MRI location FCD: side and lobe. MEG: the location (side and lobe) of the irritative zone as provided by the result of the automated cluster and localization analysis procedures applied to the interictal MEG spikes.

Metabolite concentrations (Jansen et al., 2006) we decided to express metabolite concentrations as peak ratios relative to the creatine (Cr) peak, to calculate a lateralisation index.

In both groups bilateral SV-MRS was applied and a lateralisation index for the major metabolites was calculated. In all patients voxels of 1 cm × 1 cm × 2 cm were placed. In the FCD group the location of the voxel was determined by the position of the FCD, including as much of the visible FCD as possible. In the MEG group the location of the voxel was determined by the source localisation of clustered epileptiform activity in MEG (Ossenblok et al., 2007). The contralateral voxel was placed over the corresponding gyri. We avoided inclusion of cerebro-spinal fluid (CSF) as much as possible, always less than 5% of the entire volume.

MRI was performed with a 3 T Philips Intera MRI (Philips Healthcare, Best, The Netherlands). The MRI sequences consisted of 3D TFE in sagittal plane (TR 8.2 ms TE 3.7 ms, TI 1030 ms, flip angle 8, gap 0, resolution 1 mm × 1 mm), coronal FLAIR (TR 1100 ms, TE 128 ms, IR 2800 ms, 4.5 mm slice thickness, gap −0.5) and two runs of 1H-MRS (one voxel placed over the visible FCD of interest, the other over the contralateral side).

**Figure 1** Example of SV-MRS in one patient. Small MRI’s on top showing the positioning of the voxel. As this is a rectangle with fixed dimensions but positioned in slightly different ways to ensure comparable percentages of CSF shape and size seems to divers in different MRI-slices. MRS after baseline correction is depicted below including the peakfitting. A: affected side and B: healthy contralateral side.
MRS-lateralisation index using bilateral single voxels in patients with epilepsy and focal cortical dysplasia

Results

An example of the SV-MRI’s in one patient is shown in Fig. 1. In 7 out of the 10 patients in the FCD group and in 6 out of the 9 patients in the MEG group the NAA/Cr ratio was decreased on the affected side as compared to the contralateral side. This resulted in a mean lateralisation index (voxel ratio affected side/voxel ratio contralateral side) of 0.772 (p = 0.01) in the FCD group and 0.845 (p = 0.04) in the MEG group. The difference between the FCD group and the MEG group was not significant (p = 0.37). In the FCD group of 2 out of the remaining 3 patients and in the MEG group of 3 of the remaining 3 patients it was not possible to determine the peak of the NAA, Cho and Cr reliably on both sides because of the low signal-to-noise ratio of the MR spectrogram. The Cho/Cr ratios were much more diverse without statistical significant difference in both groups (p = 0.17 and 0.51 respectively) nor between both groups (p = 0.08). Results are shown in Table 2.

Discussion

Our main finding for all of the patients studied (n = 19) is a significantly decreased NAA/Cr ratio in the pathologic hemisphere as compared to the healthy side, whereas there is less consistency in the Cho/Cr ratios for both groups. We found no statistically significant differences between the FCD group and the MEG group. To our knowledge this is the only study published on MEG guided MRS findings in MRI negative patients including patients with extra-temporal foci. An earlier study by Shih et al. (2004) dealt only with temporal lobe patients. In addition we compared our SV-MRS data with SV-MRS data from patients with FCD visible at MRI.

The present pilot study has limitations. Assessment of occurrence of seizures in the 24 h prior to the MRI was based on self-report. Therefore, we cannot be absolutely sure not to have recorded post-ictal changes. However, in the context of a pilot study it was not acceptable to hospitalized the patients 24 h prior to the MRI. Furthermore, even though Simister et al. (2007) found 1H-MRS to be sensitive to metabolite changes following epileptic seizures within the immediate post-ictal period the individual NAA and NAA/Cr levels did not change significantly between studies directly post-ictal as compared to 7 h post-ictal.

We did not correct for age even though it is shown that concentrations and ratios change with age (Angelie et al., 2001). We compared Ipsilateral and contralateral side in the same patient. As far as we could investigate patients have had no other brain injuries or diseases. Concentration changes due to aging are presumed to be symmetrical. Therefore, any asymmetry can be attributed to the epilepsy or the FCD.

Concentrations of Cr are more or less equal in all brain regions including “epileptic” brain tissue and are stable, even in epilepsy (Peeling, 1992; Peeling and Sutherland, 1993; Kauppinen and Williams, 1994; Cross et al., 1996; Cendes et al., 1997). There are also indications that Cr concentration in grey matter is higher than in white matter (Pouwels and Frahm, 1998). Furthermore, cerebrospinal fluid (CSF) is considered to provide negligible signal (Lynch et al., 1993). Unfortunately, at present we are not able to perform segmentation on single voxel MRS data. While plac-
ing the voxels we took care to include as equal as possible amounts of all three components in each voxel within the same patient.

Positioning of a voxel over the MEG indicated region means that most of the time the voxel is placed very close to the convexity of the brain. Closer to the convexity, however, it is more likely that there is an effect of the scull on the SV-MRS resulting in a lower SNR. Therefore, the gain of using a single voxel instead of CSI could be lost again. In our MEG-group, despite a second recording (but not a third) we did not succeed in getting a reliable MRS-spectrum on both sides in 3 out of 9 patients. In FCD this is less of a problem as most in our group have a tail going from the dysplasia to the ventricle, allowing for a less lateralised positioning of the voxel.

We choose to use the ratios to Cr as our standard. Quantitative analysis of concentration would give a better insight. There is, however, still too much uncertainty about the viability of the different methods of quantitative analysis (Jansen et al., 2006). We therefore decided against using these methods at this stage.

It is likely that results of SV-MRS in epilepsy patients will have a normal distribution. If this is so, the use of an unpaired t-test would be appropriate in this study, resulting in more outspoken significance levels. However, as our sample size is too small to proof a Gaussian distribution we decided to use the Mann–Whitney test, also resulting in significant results.

It has been reported that in patients with temporal lobe epilepsy the temporal MRS spectrum ipsilateral to the seizure focus displays a significant increase in total Cr (and also Cho) (Connelly et al., 1994). Similar conclusions were drawn in studies on frontal lobe epilepsy (Lundbom et al., 2001). NAA concentration is considered to be a rather non-specific marker of neuroaxonal integrity and is decreased in a wide range of diseases (Bizzi et al., 2005). Combining these data the NAA/Cr ratio on the affected side is expected to decrease dramatically in epileptics. However, we found only a modest decrease in NAA/Cr lateralisation index. Our data does not support the aforementioned ipsilateral versus unilateral Cr decrease.

Although we found a decreased lateralisation index in NAA/Cr ratio in the region of the FCD, which is in agreement with the findings of Munakata et al. (2003), the decrease is less pronounced than the decreases found by Munakata et al. A reason might be the distinct references used. Munakata et al. used a remote part of the contralateral cortex. As there are regional differences we decided to use for control the homologue contralateral cortex. Even though we thoroughly screened for other regions displaying cortical dysplasia in our patients there might have been contralateral FCD below detection threshold. This statement, however, is highly speculative. Furthermore, none of the patients included in this study were recruited from the presurgical group, in order not to give them any false hope. We expect the differences found to be more outspoken in presurgical patients, because our patient group includes less severe epilepsies than in the presurgical group.

The lower NAA/Cr ratio on the location where MEG predicts an epileptogenic lesion provides extra support for the localization of the epileptogenic focus. However, it does not prove there is FCD at the investigated region. Our findings do agree with an earlier study on metabolic disturbances in epileptic patients without MRI abnormalities and/or with a focal lesion (Lundbom et al., 2001). It is an indication that there is neuronal damage in that region. Bearing in mind that metabolite changes in FCD are evidently less pronounced than in low grade gliomas (Vuori et al., 2004), combined with the fact that there were no significant differences in lateralisation index profile in the FCD and MEG group it is appealing to postulate that our findings in MRI negative patients could suggest the presence of FCD. Further study of this method in MRI-negative patients in the presurgical evaluation will be carried out and will provide us with a correlation with a histopathological substrate. However, MEG guided SV 1H-MRS does seem to be helpful in determining the presence of an epileptogenic focus.

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References


