MRI-negative refractory partial epilepsy: Role for diffusion tensor imaging in high field MRI

Qin Chen\textsuperscript{a,b}, Su Lui\textsuperscript{b}, Chun-Xiao Li\textsuperscript{c}, Li-Jun Jiang\textsuperscript{d}, Luo Ou-Yang\textsuperscript{c}, He-Han Tang\textsuperscript{b}, Hui-Fang Shang\textsuperscript{a}, Xiao-Qi Huang\textsuperscript{b,d}, Qi-Yong Gong\textsuperscript{b,e}, Dong Zhou\textsuperscript{a,*}

\textsuperscript{a}Department of Neurology, West China Hospital of Sichuan University, Chengdu, Sichuan, China
\textsuperscript{b}Huaxi MR Research Center (HMRCC), Department of Radiology, West China Hospital of Sichuan University, Chengdu, China
\textsuperscript{c}School of Science and Technology, University of Electronic Science and Technology of China, Chengdu, Sichuan, China
\textsuperscript{d}Department of Psychiatry, West China Hospital of Sichuan University, Chengdu, Sichuan, China
\textsuperscript{e}Division of Medical Imaging, Faculty of Medicine, University of Liverpool, Liverpool, L69 3GB, UK

Received 7 December 2007; received in revised form 25 February 2008; accepted 10 March 2008
Available online 28 April 2008

KEYWORDS
Diffusion tensor imaging; MRI negative; Refractory partial epilepsy; 3 T; High field MRI

Summary
Objective: Our aim is to use the high field MR scanner (3 T) to verify whether diffusion tensor imaging (DTI) could help in locating the epileptogenic zone in patients with MRI-negative refractory partial epilepsy.

Method: Fifteen patients with refractory partial epilepsy who had normal conventional MRI, and 40 healthy volunteers were recruited for the study. DTI was performed on a 3 T MR scanner, individual maps of mean diffusivity (MD) and fractional anisotropy (FA) were calculated, and Voxel-Based Analysis (VBA) was performed for individual comparison between patients and controls.

Result: Voxel-based analysis revealed significant MD increase in variant regions in 13 patients. The electroclinical seizure localization was concurred to seven patients. No patient exhibited regions of significant decreased MD. Regions of significant reduced FA were observed in five patients, with two of these concurring with electroclinical seizure localization. Two patients had regions of significant increase in FA, which were distinct from electroclinical seizure localization.

Conclusion: Our study’s results revealed that DTI is a responsive neuroradiologic technique that provides information about the epileptogenic areas in patients with MRI-negative refractory partial epilepsy. This technique may also helpful in pre-surgical evaluation.

© 2008 Elsevier B.V. All rights reserved.

* Corresponding author. Fax: +86 28 85423550.
E-mail address: zhoudong66@yahoo.de (D. Zhou).

Introduction

About a third of patients with focal epilepsy are refractory to antiepileptic drugs (Kwan and Brodie, 2000). For
these patients, the identification of the epileptogenic zone is of the most pressing interest because this can directly impact the possibility of surgical resection of the focus. Surgical treatment of patients without any obvious abnormalities on cerebral imaging is generally associated with a less favorable outcome (Cascino et al., 1992). In the last few decades, MR imaging techniques have been becoming increasingly important in localizing the seizure focus and in managing patients in a noninvasive manner (Hajek et al., 2003). However, in about 20–30% patients with refractory partial epilepsy, apparent lesions that are responsible for seizure onset cannot be found in conventional MRI (Duncan, 1997). These "MRI-negative" patients consist of an important subgroup for clinical treatment. Therefore, much effort has been made to accurately identify focal abnormalities prior to possible epilepsy surgery in these patients. Consequently, delineation of the epileptogenic zone through intracranial recording is usually required, but it is believed to be more difficult in MRI-negative cases.

Diffusion tensor imaging (DTI) is a new MRI technique that has been recently applied in locating the epileptogenic zone in patients with epilepsy (Arfanakis et al., 2002; Assaf et al., 2003; Dumas de la Roque et al., 2005; Thivard et al., 2003; Dumas de la Roque et al., 2005; Yu et al., 2006). This technique provides three-dimensional information about tissue water diffusion in each image voxel, offering a new method to quantify not only the magnitude of water diffusivity (mean diffusivity, MD) but also the extent to which diffusion has directionality (fractional anisotropy, FA) in vivo (Pierpaoli et al., 1996). Several reports referred to the role of DTI in locating epileptogenic zone in different types of epilepsy (Eriksson et al., 2001; Rugg-Gunn et al., 2001). In a large number of patients with acquired non-progressive cerebral lesions and partial seizures, DTI abnormalities were identified. The findings showed that most of these abnormalities concurred with those identified on the visual inspection of conventional MRI (Rugg-Gunn et al., 2001). Another study by Eriksson et al. (2001) found areas of reduced anisotropy in 17 out of 22 patients, and areas of increased diffusivity in 10 out of 22 patients with malformations of cortical development (MCD). They also noticed that diffusion alterations were not only within the areas of visible MCD but also in some instances beyond them. Diffusion abnormalities were also reported in hippocampal malformation (Assaf et al., 2003; Salmenpera et al., 2006) and bilateral limbic diffusion abnormalities (Concha et al., 2005) of temporal lobe epilepsy (TLE), in the subtle cortical malformation of frontal lobe epilepsy (Okumura et al., 2004), as well as in lesions of late post traumatic epilepsy (Gupta et al., 2005). These studies suggested that DTI can assist in the lateralization of the epileptogenic zone in patients with apparent lesions.

Unfortunately, only a few studies addressed the use of DTI in MRI-negative patients with epilepsy, and the results are said to be inconsistent. Rugg-Gunn et al. found diffusion abnormalities only in 27% patients (8 out of 30 patients) with "cryptogenic" partial seizure. In addition, six out of eight patients who have diffusion abnormalities and increased diffusivity corresponded to epileptiform abnormalities (Rugg-Gunn et al., 2001). The same group also reported an abnormal diffusion in the right frontal lobe of a patient with refractory epilepsy. After surgically resecting this area, the patient had a good clinical outcome, suggesting the significant role of DTI in identifying occult epileptogenic cerebral lesions (Rugg-Gunn et al., 2002). In another study, 16 patients who had partial seizure were studied. The results showed that 9 out of the 14 MRI-negative patients (64.3%) had areas of diffusion alterations, which were consistent with intracerebral EEG localization (Thivard et al., 2006). Furthermore, the inconsistency of previous studies is partly due to the heterogeneity of patient groups and limited techniques, namely, low resolution and signal-to-noise ratio on a relative low field MR system (1.5 T).

Thus, the objectives of the present study are to use the high field MR scanner (3 T) and to verify whether DTI could help in locating the epileptogenic areas in patients with MRI-negative refractory partial epilepsy.

Materials and methods

Patients and controls

The Epilepsy Center of West China Hospital recruited 15 patients (11 women and 4 men with a mean age of 31.9 ± 12.4 years and an age range of 16–53 years) who had refractory partial epilepsy (based on seizure semiology and EEG findings) and a normal conventional MRI. The clinical data and EEG findings of the patients are reported in Table 1. The suspected electroclinical localization of seizure onset was based on clinical manifestation, medical and neurological examination, ictal/interictal EEG, and video-EEG monitoring. Accordingly, seven patients were diagnosed with temporal lobe epilepsy, four patients with frontal lobe epilepsy, one patient with front-temporal lobe epilepsy, and three patients with occipital lobe epilepsy. Moreover, eight patients had secondary generalized tonic-clonus seizures, and the average duration of epilepsy was 11 years (range of 2–24 years). All patients were scanned at least 1 week after they experienced a seizure.

The control group included 40 healthy volunteers (20 men and 20 women, with a mean age of 30.5 years ± 9.7, and an age range of 16–52 years old) who do not have any history of neurological disorders and have normal conventional MRI.

This study was approved by the local ethics committee. Informed written consent to participate in the study was also obtained from the patients and healthy volunteers.

MRI study

All subjects were scanned on a 3 T MR scanner (GE, EXCITE, Milwaukee, USA) with a standard 8-channel phase array head coil at the Department of Radiology in West China Hospital. Ear plugs were provided for each subject to reduce the noise interference during the scanning process. Foam cushions were also used to increase the subject’s comfort and reduce head motion. Before the acquisition of each image, a strict quality assurance (QA) scan was applied in order to ensure a stabilized signal.

Structural MR protocol

To exclude potential abnormalities in all patients and controls, the initial MRI protocol included structural T2-weighted images and...
Table 1

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Duration (yr)</th>
<th>Seizure types and frequency (per month)</th>
<th>AEDs (mg/day)</th>
<th>Electroclinical localization</th>
<th>DTI findings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MD increased</td>
</tr>
<tr>
<td>1</td>
<td>26</td>
<td>F</td>
<td>20</td>
<td>CPS 5 GTC 3</td>
<td>CBZ/PHT</td>
<td>L. front., temp.</td>
<td>F. front.</td>
</tr>
<tr>
<td>2</td>
<td>48</td>
<td>F</td>
<td>21</td>
<td>CPS 13</td>
<td>CBZ/PHT</td>
<td>R. temp., cerebellum</td>
<td>F. temp.</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>M</td>
<td>10</td>
<td>CPS 12</td>
<td>CBZ/TPM</td>
<td>L. temp., occip.</td>
<td>F. occip.</td>
</tr>
<tr>
<td>4</td>
<td>45</td>
<td>M</td>
<td>5</td>
<td>CPS 8</td>
<td>CBZ/TPM</td>
<td>R. temp., occip.</td>
<td>F. occip.</td>
</tr>
<tr>
<td>5</td>
<td>51</td>
<td>M</td>
<td>5</td>
<td>CPS 17</td>
<td>CBZ/TPM</td>
<td>R. temp., occip.</td>
<td>F. occip.</td>
</tr>
<tr>
<td>6</td>
<td>26</td>
<td>F</td>
<td>14</td>
<td>CPS 8</td>
<td>TPA/LEV</td>
<td>R. temp., occip.</td>
<td>F. occip.</td>
</tr>
<tr>
<td>7</td>
<td>38</td>
<td>F</td>
<td>15</td>
<td>CPS 8</td>
<td>TPA/LEV</td>
<td>R. temp., occip.</td>
<td>F. occip.</td>
</tr>
<tr>
<td>8</td>
<td>45</td>
<td>M</td>
<td>10</td>
<td>CPS 14</td>
<td>TPA/LEV</td>
<td>R. temp., occip.</td>
<td>F. occip.</td>
</tr>
<tr>
<td>9</td>
<td>51</td>
<td>M</td>
<td>5</td>
<td>CPS 14</td>
<td>TPA/LEV</td>
<td>R. temp., occip.</td>
<td>F. occip.</td>
</tr>
<tr>
<td>10</td>
<td>16</td>
<td>F</td>
<td>14</td>
<td>CPS 14</td>
<td>TPA/LEV</td>
<td>R. temp., occip.</td>
<td>F. occip.</td>
</tr>
<tr>
<td>11</td>
<td>26</td>
<td>M</td>
<td>14</td>
<td>CPS 14</td>
<td>TPA/LEV</td>
<td>R. temp., occip.</td>
<td>F. occip.</td>
</tr>
<tr>
<td>12</td>
<td>38</td>
<td>F</td>
<td>15</td>
<td>CPS 8</td>
<td>TPA/LEV</td>
<td>R. temp., occip.</td>
<td>F. occip.</td>
</tr>
<tr>
<td>13</td>
<td>45</td>
<td>M</td>
<td>10</td>
<td>CPS 14</td>
<td>TPA/LEV</td>
<td>R. temp., occip.</td>
<td>F. occip.</td>
</tr>
<tr>
<td>14</td>
<td>51</td>
<td>M</td>
<td>5</td>
<td>CPS 14</td>
<td>TPA/LEV</td>
<td>R. temp., occip.</td>
<td>F. occip.</td>
</tr>
<tr>
<td>15</td>
<td>26</td>
<td>F</td>
<td>14</td>
<td>CPS 14</td>
<td>TPA/LEV</td>
<td>R. temp., occip.</td>
<td>F. occip.</td>
</tr>
</tbody>
</table>

F = female; M = male; S/CPS = simple complex partial seizures; GTC = generalized tonic-clonic seizures; ≤ = left; ≥ = right; B. = bilateral; temp. = temporal; par. = parietal; occip. = occipital; AEDs = antiepileptic drugs; CBZ = carbamazepine; LEV = levetiracetam; PHT = phenytoin; TPM = topiramate; VP A = valproate; ZNS = zonisamide.

Three dimensional SPGR T1-weighted images (TR/TE = 8.5/3.4 ms, flip angle = 12°, FOV = 240 mm, matrix = 256 × 256, slice thickness = 1 mm). Structural image data were systematically reviewed by two experienced neuro-radiologists (Dr. S Lui and Dr. QY Gong).

**DTI protocol**

DTI data were acquired using a single-shot spin-echo echo planar image (SE-EPI) sequence. The diffusion sensitizing gradients were applied simultaneously along 15 non-collinear directions (b = 1000 s/mm²) as well as an acquisition without diffusion weighing (b = 0). Moreover, 42 contiguous slices were acquired with a 3 mm slice thickness and with no gap. The other acquisition parameters were: repetition time (TR) = 10,000 ms; echo-time (TE) = 70.8 ms; number of excitations (NEX) = 2; and a 24 × 24 cm field of view (FOV). The total acquisition time was 5 min and 40 s. All scans were reviewed by an experienced neuroradiologist and a member of the research team who was blind to the group of the subjects to prevent obvious gross abnormalities.

**Voxel based analysis**

After image acquisition, the echo planar distortions induced by eddy currents were corrected using an algorithm that determines the optimum affine transformation to be applied to each diffusion-weighted images. The research team also used a mutual information similarity test and the Powell minimization scheme, which is a commercially available software application (Functool Performance, GEMS, Buc, France). Individual maps of MD and FA were calculated from the DTI data on a pixel by pixel basis according to the method proposed by Baser and Pierpaoli (1). Voxel-based analysis was performed through statistical parametric mapping (SPM2, Welcome Department of Cognitive Neurology, Institute of Neurology, University of College London, UK; available at http://www.fil.ion.ucl.ac.uk/spm/software/). First, all of the b = 0 images were normalized to standard Montreal Neurological Institute (MNI) space for estimating the normalization parameters. Using the EPI template supplied by SPM2, the original voxel size of 1.875 mm × 1.875 mm × 3 mm was interpolated to a final voxel size of 2 mm × 2 mm × 2 mm. Second, these derived parameters were applied to the MD and FA maps in order to normalize them to the MNI space. Finally, the normalized MD and FA maps were spatially smoothed with a 6 mm full width at half maximum (FWMH), isotropic Gaussian kernel to improve the signal-to-noise ratio (SNR).

Each patient’s test results were compared to those of the control group using ANCOVA, and gender and age were considered as covariates. Significant differences in diffusivity or anisotropy were defined at a threshold of P < 0.001 (FDR corrected with P < 0.05) (Rugg-Gunn et al., 2001). Additionally, each control was statistically compared with the rest of the control group.

**Results**

**DTI abnormalities**

Comparing each control subject with the remaining 39 control subjects using identical parameters and statistical thresholds as the comparison between patients and controls, the results revealed that only one subject had two clusters of increased MD located in the right temporal lobe and cerebellum. These clusters were considered to be not significant after FDR correction.

In 13 of the 15 patients, VBA detected areas of significantly diffusion abnormalities include increased MD (13 out of 15), increased FA (2 out of 15), and reduced FA (5 out
of 15). No patient exhibited areas of reduced MD. In the 13 patients with increased MD, 5 of them had reduced FA, while 1 patient had both increased MD and increased FA (detailed results in Table 1).

Correlation with electroclinical seizure localization

MD abnormalities

Patients 3, 5, and 14 exhibited widespread increased MD, which involved diffused regions distributed in the bilateral hemisphere. Assuming that they were of weak interest in terms of epileptogenic focus, these three patients were excluded in the congruent patient group.

For the 10 patients with increased MD, 7 patients had regions of increased MD concurring with the electroclinical seizure location, while 3 patients only had regions away from the electroclinical seizure location. We also observed that six out of the seven patients had regions not only limited to the electroclinical seizure location but also beyond (as shown in Fig. 1), specifically in other cerebral lobes, the thalamus, and even in the contralateral hemisphere. Only one patient had an increased MD, which was limited in the electroclinical seizure area.

FA abnormalities

In five patients with reduced FA, two of them (patient 4 and 14) had regions of reduced FA that concurred with the electroclinical seizure locations. Patient 4 had left temporal epilepsy and a reduced FA in the left temporal lobe (as shown in Fig. 2). Patient 14 was diagnosed with right temporal epilepsy, and areas of reduced FA were located in the right temporal and frontal lobe, as well as in the left thalamus. Patients 3, 5, and 13 only had areas of reduced FA away from the electroclinical seizure location.

The regions of significantly increased FA in two patients (patient 1 and 14) were distinct from electroclinical seizure location.

Discussion

In this novel imaging study, we found that with the use of a high field MR scanner, the diffusion abnormalities can be detected in a majority of MRI-negative patients with refractory partial epilepsy. In identifying the diffusion abnormality in this group of patients, MD seemed to be more sensitive than FA. Most of the diffusion abnormalities were not only found in the electroclinical seizure localization but also in other areas. To our knowledge, this is the first study that evaluated diffusion abnormalities, with particular focus on patients with MRI-negative partial refractory epilepsy, and which used the 3 T MR scanner.

Histopathological studies revealed that the surgically resected epileptogenic areas that appeared normal on MRI usually presented minor malformations such as cortical dysgenesis, mild subpial, or white matter gliosis, some of which can only be seen through a microscope (Palmini et al., 1991; Theodore et al., 1990; Zentner et al., 1995). Such occult epileptogenic regions could change the microstructural characteristics of the tissue. On the other hand, water-diffusion properties can be detected non-invasively through DTI. Hence, these findings represent a theoretical background for the application of DTI in patients with MRI-negative refractory partial epilepsy.

In the present study, the diffusion abnormalities, mostly increased MD, can be detected in most patients (13 out of 15 patients in our study). These data reinforce previ-
Figure 2  An example of a significantly reduced fractional anisotropy in an MRI-negative patient (patient 14). (A) Coronal, sagittal and axial slices of fractional anisotropy map corresponding to the site of reduced fractional anisotropy as shown in B. (B) Statistical parametric maps of reduced FA in patient 14 compared to 40 healthy controls. The major cluster is located in the right frontal lobe and temporal lobe.

ous reports (Rugg-Gunn et al., 2001; Thivard et al., 2006) and suggest that DTI is a sensitive neuroradiologic technique for detecting cerebral alterations in patients even without visible lesions on conventional MRI. The sensitivity of diffusion abnormalities is relatively higher than those in previous studies on MRI-negative patients (Rugg-Gunn et al., 2001; Thivard et al., 2006). Two possible reasons may contribute to this discrepancy. First, the number of patients in our study was carefully selected and only focused on refractory partial epilepsy. The duration of the disease as well as the high seizure frequency may potentially increase the ratio of brain tissue alteration. Second, a higher field MR scanner, more non-collinear acquisitions direction, and the thinner slice thickness used in our study may also lead to the higher sensitivity of the diffusion abnormalities.

We also found that MD presented a higher sensitivity than FA in detecting the diffusion abnormalities. This indicates that MD is a more sensitive marker than FA in this group of patients. This finding is also in accordance with the results observed by other groups (Rugg-Gunn et al., 2001; Thivard et al., 2006). The possible explanation for this observation is that the expansion of extracellular space, which caused increased MD, is associated with a relatively preserved structural organization and parallel fiber bundle arrangement of white matter tracts. In contrast to this finding, Arfanakis et al. (2002) found that anisotropy was more sensitive than diffusivity in identifying abnormalities in focal TLE through manual measurement of regions of interest (ROI). This disparity across studies reflects great variations in the methodology and in the number of sample cases.

We observed that most diffusion abnormalities were not only limited in electroclinical seizure areas, but were also concentrated in the contralateral hemisphere. This observation is said to be consistent with previous findings (Gross et al., 2006; Guye et al., 2007). The first explanation for this phenomenon is the different spatial resolution between EEG and DTI. DTI may highlight the distant components of a diffuse epileptic network that EEG might miss due to the limited number of recording electrodes (Thivard et al., 2006). Some authors speculated that these diffusion abnormalities beyond the clearly identified epileptogenic zone are likely to correlate with structural changes such as neuron loss and gliosis (Duncan, 2002a,b). Another potential explanation for the diffusion abnormalities is functional alteration (fluid shifts) as related to ongoing seizures. Reduction of diffusivity has been shown to reverse to normal in animal models (Ebisu et al., 1996; Zhong et al., 1993) and patients with status epilepticus (Diehl et al., 2001; Lansberg et al., 1999; Wiesmann et al., 1997). Similar reversible diffusion abnormalities have been reported to have been observed in the splenium of the corpus callosum after the status epilepticus and after antiepileptic drugs have been taken. Such changes may reflect the combination of cytotoxic and vasogenic edemas. However, a very recent study by Concha et al. (2007) showed that bilateral white matter diffusion abnormalities failed to return to normal after 1-year follow-up in patients with TLE and MTS. The irreversibility of white matter DTI abnormalities on seizure freedom suggested underlying structural abnormalities, which are in contrast to functional changes. In light of these reports, we considered the regions beyond the electroclinical areas in our patients as an indication of an extension of water diffusion abnormalities beyond the epileptogenic areas, and indirectly highlight the distant components of a diffuse epileptic network that may be interpreted as a cause or a consequence of repetitive epileptic discharges.

The present study has several limitations. First, we did not use stereo-electroencephalographic (SEEG) recording to identify the epileptogenic zone because of its invasive nature. As Thivard et al. proposed, we carefully
selected patients with sufficient medical information in order to accurately identify epileptogenic areas from long-time EEG monitoring. Second, a report mentioned the transient increased diffusivity in corpus callosum after taking antiepileptic drugs (Prilipko et al., 2005). However, the underlying mechanism is unclear. Moreover, all patients in our study were treated by multiple antiepileptic drugs for a long time. The chronic effect of antiepileptic drugs on the diffusion character remains unknown. Third, the lack of pathological material would lead to uncertainty on the exact cause of diffusion abnormalities in our patients. Future studies with pathological and surgical results are therefore needed to further support the underlying microstructural involvement of diffusion abnormalities. Finally, VBA was used instead of manual measurement of ROI due to the uncertainty in ROI positioning in patients with no visible lesion on MRI. However, the spatial normalization process in VBA and the process of comparing one subject with a control group may give rise to false positive clusters. As suggested in previous studies (Rugg-Gunn et al., 2001), we tried to limit the possibility of obtaining a false positive cluster by using a relatively strict restriction of $P < 0.001$ (FDR corrected with $P < 0.05$). Additionally, it is noteworthy to cite that no significant diffusion abnormality was observed in the control group. Therefore, we consider the potential risk of false positive cluster in our study to be relatively low.

**Conclusion**

In summary, the results of the current study revealed that DTI is an accurate neuroradiologic technique that provides information on the area of subtle structural abnormalities in patients with MRI-negative refractory partial epilepsy. Moreover, MD is recognized as a more accurate indicator than FA because it serves as an interictal marker of brain abnormalities for pre-surgical evaluation of MRI-negative epilepsy patients. Furthermore, instead of identifying only the epileptogenic areas, diffusion abnormalities are more likely to be multi-factorial due to the metabolic and structural changes induced by repetitive epileptic discharges. This technique can be used as part of a multimodality evaluation that includes interictal and ictal EEG recordings, neuro-psychiatric and psychological evaluations, and other imaging modalities such as PET, SPECT, or magnetoencephalography (MEG).

**Acknowledgements**

This study was supported in part by National Natural Science Foundation of China (NSFC Grant nos. 30770749, 30370513, 30625024, 30728017, and 30700256), National Basic Research Program of China (973 Program no.: 2007CB512305/1), and the National High Technology Program of China (863 Program no.: 2007AA02Z440).

**References**


