Temporal evolution of water diffusion parameters is different in grey and white matter in human ischaemic stroke

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Objectives: Our purpose was to investigate whether differences exist in the values and temporal evolution of mean diffusivity ($D_{\text{mean}}$) and fractional anisotropy (FA) of grey and white matter after human ischaemic stroke.

Methods: Thirty-two patients with lesions affecting both grey and white matter underwent serial diffusion tensor magnetic resonance imaging (DT-MRI) within 24 hours, and at 4–7 days, 10–14 days, 1 month, and 3 months after stroke. Multiple small circular regions of interest (ROI) were placed in the grey and white matter within the lesion and in the contralateral hemisphere. Values of $D_{\text{mean}}$, $D_{\text{grey}}$, $D_{\text{white}}$, FA-grey and FA-white were measured in these ROI at each time point and the ratios of ischaemic to normal contralateral values ($D_{\text{contral}}$, FA) calculated.

Results: $D_{\text{mean}}$ and FA showed different patterns of evolution after stroke. After an initial decline, the rate of increase of $D_{\text{mean}}$ was faster than $D_{\text{grey}}$ from 4–7 to 10–14 days. FA-white decreased more rapidly than FA-grey during the first week, thereafter for both tissue types the FA decreased gradually. However, FA-white was still higher than FA-grey at three months indicating that some organised axonal structure remained. This effect was more marked in some patients than in others. $D_{\text{grey}}$ was significantly higher than $D_{\text{white}}$ within 24 hours and at 10–14 days ($p<0.05$), and FA-grey was significantly more reduced than FA-white at all time points ($p<0.001$).

Conclusions: The values and temporal evolution of $D_{\text{mean}}$ and FA are different for grey and white matter after human ischaemic stroke. The observation that there is patient-to-patient variability in the degree of white matter structure remaining within the infarct at three months may have implications for predicting patient outcome.

Diffusion tensor magnetic resonance imaging (DT-MRI) provides quantitative measures of the mobility of water molecules in vivo. Parameters such as the mean diffusivity ($D_{\text{mean}}$), or the apparent diffusion coefficient (ADC) averaged over three orthogonal directions, measure the magnitude of diffusion of the water molecules, while diffusion anisotropy indices, such as fractional anisotropy (FA), indicate the deviation from pure isotropic diffusion of water mobility in vivo. These scalar parameters are thought to be useful markers of tissue cellular integrity.

Since the introduction of diffusion-weighted (DW)-MRI, many studies have investigated the temporal evolution of the parameters of diffusion in ischaemic stroke, most with DW-MRI rather than with DT-MRI. Overall these studies found that the ADC decreases immediately after stroke, then increases slowly towards normal values (pseudonormalisation). Finally, it becomes elevated, which is thought to indicate tissue necrosis. Other authors report a general reduction in diffusion anisotropy after stroke, consistent with loss of integrity of the tissue structure. These studies suggest that characterising the temporal evolution of the diffusion parameters may prove useful in differentiating acute from non-acute infarcts, as well as investigating how the brain structure is affected in ischaemic stroke.

There are, however, inconsistencies in the results of these studies. For example, some authors have reported a time of pseudonormalisation between five and 10 days, while others found that the ADC reaches normal values between 24 and 48 hours or at about one month. Generally, the diffusion anisotropy decreases after stroke, although some authors report an increase immediately after onset.

Differences in imaging methodology may partly explain the conflicting results. Another possible cause could be heterogeneity of diffusion values within the lesion in the acute stage, potentially arising from the difference in the water diffusion properties of grey and white matter. Some investigators have considered this issue and measured the parameters of diffusion in ischaemic grey and white matter. Unfortunately not all of these studies scanned individuals serially at each time point to control for between subject variability, and most did not measure the full diffusion tensor. Furthermore, some studies only imaged a small number of patients, while not all were specifically designed to analyse differences between the two types of tissue. The purpose of the present study was therefore to investigate whether differences exist in the values and temporal evolution of $D_{\text{mean}}$ and FA of lesion grey and white matter in a group of patients imaged serially from onset to 3 months.

Subjects and Methods

Subjects

Consecutive patients presenting with symptoms of moderate to severe cortical ischaemic stroke, who had no evidence of haemorrhage on the initial computed tomography (CT) or MR scan, were recruited during an effective period of 21 months between January 2001 and July 2003. A stroke team made the final decision to include or exclude patients from the study.

Abbreviations: ADC, apparent diffusion coefficient; CT, computed tomography; $D_{\text{mean}}$, mean diffusivity; DT-MRI, diffusion tensor magnetic resonance imaging; DW-MRI, diffusion-weighted MRI; EP, echo planar; FA, fractional anisotropy; ROI, regions of interest
physician examined all the patients prior to scanning, and
categorised the type of stroke according to the Oxfordshire
Community Stroke Project (OCSP) classification. The
inclusion criteria for the study were as follows: (a) the
patient could be imaged within a maximum of 24 hours after
symptom onset, (b) the patient had no contraindications to
MR, and (c) the patient could tolerate the imaging protocol.
For the present analysis, we identified those patients who
were not receiving any thrombolytic treatment and whose
visible ischaemic lesion on their first time point DW images
included both grey and white matter. Patients underwent,
where possible, repeat MRI at around 4–7 days, 10–14 days,
1 month, and 3 months after stroke. All patients were
scanned at least twice. The local ethics committee approved
the study, and in all cases informed consent was obtained
from the patient or a close family member.

**Imaging methods**

All MRI data were obtained using a GE Signa LX 1.5 T
(General Electric, Milwaukie, WI) clinical scanner, equipped
with a self shielding gradient set (22 mT/m maximum
gradient strength and 120 T/m/s slew rate) and a “birdcage”
quadrature head coil supplied by the manufacturer. The MRI
examination consisted of a standard fast spin-echo T2-
weighted sequence, a gradient-echo T1-weighted sequence,
and a previously described DT-MRI protocol based on spin-
echo echo planar (EP) imaging. The duration of the
examination was approximately 30 minutes.

To ensure that the slice locations used in the follow up
scans corresponded as closely as possible to those in the first,
the subject’s head position and tilt in the first scan were
recorded and the patient repositioned as close as possible
to this location for the follow up scans. Computational image
realignment techniques were then used to realign the images
in the follow up scans to the first, thereby minimising any
small remaining positioning errors and removing eddy
current induced artefacts.

From these MRI data, the apparent diffusion tensor of
water (D) was calculated in each voxel from the signal
intensities in the component EP images. Maps of <D> and
FA for every patient at each time point were generated on a
voxel-by-voxel basis from the sorted eigenvalues of D and
converted into Analyze (Mayo Foundation, Rochester, MN)
format.

**Region of interest analysis**

For each patient, the acute ischaemic lesion at less than
24 hours was outlined in each slice where the lesion was
visible by tracing around the region of signal hyperintensity
on the first DW-EP scan (fig 1A). The lesion outlines were
copied onto the first scan T2-weighted EP images and
multiple circular regions of interest (ROI) of 6 mm diameter
were placed in grey and white matter within the lesion using
anatomical and imaging knowledge (fig 1B). This ROI size
was chosen to enable selection of regions of grey and white
matter, while minimising the effects of mixed tissue and
cerebrospinal fluid (CSF) contamination. ROI were also
placed in comparable contralateral grey and white matter
and were used as control regions for each patient. As many
ROI as possible were placed in each slice of the T2-weighted
EP image from the within 24 hours time point and then
overlaid onto the coregistered DW-EP images, <D>, and FA
parametric maps for the same time point (fig 1C, D) and all
subsequent time points available for that patient. On average,
for each patient 14 ROI were placed in grey matter and 10 in
white matter. When tissue structures sampled by ROI chosen
at the first time point were displaced in subsequent scans by
lesion swelling or atrophy, the ROI positions were corrected
using the structural T1 and T2-weighted imaging data so that
the same anatomical piece of tissue was sampled at all time
points. Areas of coincidental periventricular white matter
disease and haemorrhagic transformation of the infarct at
later time points were avoided with the aid of the structural
MRI data. A neuroradiologist verified the positions of all ROI
and the outlines of the ischaemic lesions.

**Statistical analysis**

Mean values of <D>grey, <D>white, FAgrey and
FAwhite were calculated from all ROI in the lesion and
contralateral tissue for every patient at each time point.
However, while it is important to characterise the evolution of
<D> and FA to understand how stroke affects brain tissue
structure, these absolute parameters also reflect the intrinsic
difference between grey and white matter (<D>grey −
<D>white) and FA(FAwhite −FAgrey) for normal brain,
and inter-patient variability. Therefore, to control for
biological variation in <D> and FA and to allow group
comparisons between individuals at each time point, the ratio
of lesion to contralateral <D> and FA (<D>R and FAR) were
also calculated. Finally, a global mean for each time point
for grey and white matter <D>, FA, <D>R, and FAR was
determined from the individual patient mean data. Thus, all
lesions were weighted equally regardless of the number of
ROI in each.

Differences between <D>R and FAR values of grey and
white matter at each time point for each individual patient
were then assessed using two tailed Student’s paired t test
(null hypothesis: <D>Rgrey −<D>Rwhite = 0 and
FAgrey −FAwhite = 0), with p<0.05 considered as
statistically significant.
RESULTS

From a group of 109 patients recruited within 24 hours of stroke onset during the study period, 77 were excluded from the present analysis for the following reasons: (a) in 50 patients the lesion did not affect both grey and white matter, (b) 14 patients could not tolerate the entire first time point MRI examination, (c) in 10 patients primary haemorrhage was the cause of the stroke, (d) data from two patients were corrupted, and (e) one patient presented with evidence of other concurrent neurological disease on structural MRI. The remaining 32 patients, the majority with medium to large cortical infarcts involving both grey and white matter underwent DT-MRI examinations at least twice, with 18 patients scanned at all five time points. The demographics of the recruited patient group, stroke syndromes, and number of patients scanned at each time point are summarised in table 1.

Figure 2 shows the variation in the absolute values of lesion $<D>$ and FA after stroke for grey and white matter. After the initial reduction from normal values, both $<D>$ (grey) and $<D>$ (white) increased. The general trend in the evolution of $<D>$ (grey) and $<D>$ (white) was similar, although between time points two (4–7 days) and three (10–14 days) the rate of increase of $<D>$ (grey) was faster than $<D>$ (white). FA (white) decreased rapidly during the first week, then more gradually over the next three weeks. FA (grey) decreased less rapidly than FA (white) during the first week, then more gradually over the next three weeks. Between one and three months there was little change in either FA (grey) or FA (white). However, although FA (white) decreased more than FA (grey) it was still higher at 3 months.

Values of $<D>$ (grey), $<D>$ (white), $FA_R$ (grey), and $FA_R$ (white) are shown in table 2 for all five time points. Both $<D>$ (grey) and $<D>$ (white) were significantly reduced during the first day after stroke onset ($p < 0.0001$). $<D>$ (grey) was significantly higher than $<D>$ (white) at this time point ($p = 0.02$), with an average reduction of 26% for grey matter and 31% for white matter. Between the first and second time points $<D>$ (white) started to rise. $<D>$ (grey) and $<D>$ (white) were nearly identical at time point two. However, between time points two and three $<D>$ (grey) increased at a faster rate and pseudonormalised earlier than $<D>$ (white). $<D>$ (grey) was again significantly higher than $<D>$ (white) at time point three ($p = 0.03$). This confirms the faster recovery of $<D>$ (grey) seen between time points two and three in fig 2A. Thereafter, both $<D>$ (grey) and $<D>$ (white) increased slowly over time becoming elevated and of the same value at 3 months. Table 2 also shows that while $FA_R$ (grey) remained unchanged, $FA_R$ (white) was significantly reduced in the first day after stroke ($p < 0.0001$). Between time points one and two $FA_R$ (grey) declined less rapidly than $FA_R$ (white), with $FA_R$ (grey) being reduced by 10% and $FA_R$ (white) by 46%. At later time points both $FA_R$ (grey) and $FA_R$ (white) continued to decrease gradually. $FA_R$ (grey) was significantly greater than $FA_R$ (white) at all time points ($p < 0.0001$).

DISCUSSION

This is the largest longitudinal study to date to investigate the temporal evolution of water diffusion parameters in grey and white matter.
white matter after stroke using DT-MRI. The above results show that there are differences in the temporal evolution of $<D>_R$ and FA of grey and white matter in ischaemic lesions affecting both tissue types. Although the overall evolution of $<D>_{(grey)}$ and $<D>_{(white)}$ was similar, subtle differences were observed during the first two weeks after stroke, specifically the rate of increase of $<D>_{(grey)}$ was faster between 4–7 and 10–14 days. Differences in the evolution of grey and white matter FA were more marked, with the loss of FA_{(white)} being significantly greater than FA_{(grey)} during the first week after stroke.

Previous studies reporting changes in grey and white matter water diffusivity indices have shown contradictory results. As in the current study, Yang et al and Mukherjee et al have found that $<D>_{R}$ was significantly more reduced in white than grey matter at less than three days.$^{14, 15}$ However, Sorensen et al reported that $<D>_{R}$ was significantly more reduced in grey matter,$^{17}$ while Bastin et al and Fiebach et al did not find significant differences between grey and white matter at this stage.$^{18, 19}$ In the longitudinal studies of Yang et al and Fiebach et al, it was observed that $<D>_{R}$ of both grey and white matter remained low during approximately the first four days, increasing thereafter, and reaching normal contralateral values at approximately day 10.$^{10, 20}$ Conversely, Bastin et al only found this pattern of $<D>_{R}$ change in grey matter, which started to rise at approximately seven days, while no significant change was observed in white matter water $<D>_{R}$ during the first 14 days after the initial reduction following stroke.$^{20}$ However, all these studies, as well as the current work, observed a slightly earlier renormalisation of grey matter water diffusivity.

Our values of FA_{R} at less than 24 hours are similar to the results of Sorensen et al$^{17}$ who found that grey matter FA remained unchanged at this time point while white matter FA decreased significantly, even though only 10 of the 50 patients studied had lesions affecting both grey and white matter. Yang et al reported changes in both grey and white matter diffusion anisotropy.$^{14}$ In their longitudinal study of 26 patients, most subjects showed an increase of diffusion anisotropy for both tissue types by 12 hours, after which the diffusion anisotropy declined. At approximately 90 days, the diffusion anisotropy was further reduced in all lesions. However, for most patients in their study the diffusion anisotropy was assessed by means of an orientation-dependent parameter derived from ADC values, rather than a rotationally invariant index derived from $D$, such as FA.

Elevated $<D>$ and reduced FA indicate destruction of membrane integrity and progression towards necrosis.$^{21}$ Interestingly, fig 2A shows that grey and white matter $<D>$ was still increasing at three months, while FA did not change significantly after one month (fig 1B). Increasing water diffusion at later time points has been observed in other studies, which have attributed this to gliosis and accumulation of CSF in the lesion secondary to encephalomalacia$^1$ or to flow dephasing effects in regions that have undergone cystic degeneration.$^5$ However, no measurements of diffusion anisotropy were made in these studies to compare with ours. Another important observation is that despite the greater loss of white matter FA, it was still significantly higher than grey matter FA at three months, indicating that some organised axonal structure remains. This effect was more marked in some patients than others, which contributes to the larger standard deviations measured in FA_{(white)} than FA_{(grey)} (fig 2B). To our knowledge, this has not been reported before, as such effects are not evident when measuring whole lesion diffusion parameters, and could have implications in predicting patient outcome. For instance, DT-MRI tractography methods could be used to measure how changes in tract volume and integrity following stroke relate to clinical dysfunction and outcome.

**CONCLUSIONS**

By following individual patients over time with ischaemic lesions affecting both grey and white matter, we found evidence for the difference in the values and temporal evolution of $<D>$ and FA for grey and white matter. Specifically, the rate of increase of $<D>_{(grey)}$ was faster than $<D>_{(white)}$ from week one to week two, while FA_{(white)} decreased more rapidly than FA_{(grey)} during the first week after stroke. Thereafter, FA decreased gradually for both tissue types. However, FA_{(white)} was still higher than FA_{(grey)} at three months indicating that some organised axonal structure remained. The observation that there is patient-to-patient variability in the degree of white matter structure remaining within the infarct at three months may have implications for predicting patients’ outcome.

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**REFERENCES**