High-field quantitative transverse relaxation time, magnetization transfer and apparent water diffusion in experimental rat brain tumour

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ABSTRACT: The potential of quantitative parameter images of transverse relaxation time $T_2$, apparent diffusion coefficient (ADC) and magnetization transfer ratio (MTR) to characterize experimental brain tumours was studied. Necrosis or haemorrhage can be detected using either MTR, ADC or $T_2$ (necrosis—MTR reduced by 35%, ADC and $T_2$ increased respectively by 170% and 100% compared with normal brain tissue; haemorrhage—MTR increased by 60%, ADC and $T_2$ decreased by 40% and 20%, respectively). Normal brain tissue can only be distinguished from tumour on $T_2$ and MTR parameter images. However, for small tumours (10 μl), the best contrast is observed with MTR, ca. 30%, whereas for $T_2$ the contrast is ca. 10%. Copyright © 2000 John Wiley & Sons, Ltd.

KEYWORDS: quantitative MRI; rat-brain tumours; relaxometry; apparent diffusion coefficient; magnetization transfer

INTRODUCTION

In 1971, Damadian1 showed the potentialities of measuring proton relaxation times for the differentiation of cancerous and normal tissues. However, the demarcation of brain tumours by conventional proton relaxation-time weighting MRI is often difficult. Peritumoral oedema appears as hyperintense on heavily $T_2$-weighted images and therefore allows an indirect detection of the tumour’s contours.2–3 Nevertheless, the oedema is not always present, which limits the visibility of the tumour. Furthermore, when high field systems are used, susceptibility effects are crucial and the presence of local haemorrhaging can compromise the image quality.

Over the last decade, diffusion weighted imaging (DWI) has become established as a powerful tool providing unique information about water motion, exchange or compartmentalization in tissue. Quantitative measurements of the water diffusivity are described by an apparent diffusion coefficient (ADC), since water diffusion measurement results from the average of at least two compartments, the intracellular and interstitial spaces. The measured ADC values are generally reduced compared to the self-diffusion of free and unbounded water. DWI has been initially used to assess stroke and more recently to evaluate tumour growth and the physiopathological perturbations induced by rapid and massive cellular invasion as well as to assess tumour response to chemotherapy. Tumours are characterized by an elevated ADC compared with normal tissue.2–3 Zhao et al.4 have shown that tumour cell death in response to therapy lead to an increase in water ADC attributed to an enlargement of the interstitial space. However, in particular cases, such as highly necrotic tumours, characterized by substantially high water ADC values, the expected ADC increase due to cell death after therapy can be masked by a reduction of the necrosis contribution to the global tumour ADC.5

Magnetization transfer (MT) imaging is based on the interaction between mobile protons of ‘free’ water and protons with restricted motions in proteins and macromolecules. A selective irradiation of the ‘immobile’ proton pool causes a partial saturation of the former and a decrease in the intensity of the latter, thus creating contrast.6 Previous studies have shown that MT efficiency is different in tumours compared with healthy tissue.7 This MT efficiency can easily be quantified by calculating the MT ratio (MTR) ratio from images

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Abbreviations used: ADC, apparent diffusion coefficient; DWI, diffusion weighted imaging; MTR, magnetization transfer ratio

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performed with and without a saturation pulse. However, this ratio is not an absolute value and is highly dependent on the amplitude, offset and pattern of the saturation pulses applied to generate the contrast.\(^8\)

The purpose of this study was to attempt to analyse quantitatively, *in vivo*, on experimental C6 glioma, the biochemical changes occurring with tumour growth by calculating ADC, MTR and transverse relaxation time. We aimed to determine the most suitable parameter(s) to discriminate the different tissues that can be encountered in tumours and their surroundings.

**MATERIALS AND METHODS**

**Animal model**

Brain tumours were induced in nine female Sprague–Dawley rats (Élevage Dépré, St Doulchard, France; 200–250 g body weight) by stereotaxic inoculation of exponentially growing C6 glioma cells. The rats were anaesthetized with a mixture of Calmivet\(^8\) (Acepromazine, Vétoquinol, Lure, France) and Clorketam\(^\)\(^8\) (Kétamine, Vétoquinol, Lure, France) before being fixed in a stereotaxic holder. Through a small drilled hole (anterior 0, lateral 3 mm, depth 7 mm according to bregma), 10 µl of a suspension of 40,000 C6 glioma cells were injected over a 10 min time period into the caudate putamen of the left hemisphere. Ten days later and then every 3 days until death, six animals were scanned under halothane anaesthesia (1.5–3%, O\(_2\) 3 l/min). During MRI examination, body temperature was maintained at 36–37°C using a heating blanket.

Histological analysis was performed on rat brains at the end of the MR studies. Three additional rats were scanned 12 days post-inoculation and then sacrificed to determine the nature of the early changes observed on the MR images. Classical hematoxylin-eosin and Perls staining were performed for tumour and hemosiderin localization, respectively.
Imaging

Experiments were performed with a Bruker Avance DRX 300 equipped with a vertical superwide-bore magnet and shielded gradient insert (maximum gradient strength 144 mT/m, rising time <300 µs). The resonant circuit of the NMR probe was a 64 mm diameter birdcage. Quantitative $T_2$ MR images of the brain were obtained using a multi-spin echo sequence ($TR = 2500$ ms; $TE = 15, 30, 45, 60, 75, 90, 105$ and $120$ ms; $FOV = 3 \times 3$ cm; matrix $128 \times 128$; nine contiguous slices of 1 mm, two acquisitions) and were used for tumour localization and volume measurements.

MT and ADC images were performed on a 1 mm-thick slice, located at the tumour centre. In order to reach an acceptable signal-to-noise ratio without dramatically increasing the acquisition time, a $96 \times 96$ matrix for a $FOV = 3 \times 3$ cm was used, leading to an in-plane resolution of 312 µm. MT images were acquired using a spin echo sequence [$TR/TE = 5000/8.5$ ms, with or without saturation (10 Gaussian pulses of 5 ms, interpulse delay = 300 µs),

Figure 2. $T_2$-weighted ($TE = 75$ ms) longitudinal follow-up (10, 12, 14, 18 and 21 days after inoculation) of a C6 glioma (pointed out by arrows) and the corresponding histology section at the end point. The presence of a large necrotic core does not allow a perfect slicing and explains the burst observed on the section. It can be noted that, on day 21, the upper part of the tumour appears very bright, this spot corresponding to necrosis as confirmed by the histology section.
offset 1.5 kHz). Under these conditions, the SAR is under 1 W/kg.

Diffusion images were obtained using a Stejskal–Tanner-type
pulsed gradient stimulated echo sequence
with five diffusion weighting factor values ($b = (\gamma G \delta)^2
(\Delta - \delta/3)$), $b = 0, 11,000, 44,000, 99,000$ and $176,000$
s/cm²). The diffusion sensitizing gradient was along the
direction of the slice selection gradient. Gradient duration
$\delta = 5$ ms was used, whereas the leading edges were
separated by $\Delta = 100$ ms. The recovery time $TR$, echo time
$TE$ and mixing time $TM$ were fixed to $TR/TE/TM = 1500/
20/91.7$ ms and one acquisition was performed.

**Data analysis and statistics**

Magnetization transfer ratios (MTR) were calculated
from the signal intensity of each pixel on MTC-on image
and MTC-off image and were defined as
$100 \times [1 - (\text{MT on/MT off})]$ as described by Dousset et al.$^8$ ADC and $T_2$
were obtained by a monoexponential fitting of the
experimental points. $T_2$ was calculated after exclusion
of the first echo time point (the only one unaffected by
stimulated echoes) and after exclusion of the last one,
since the signal-to-noise ratio was usually too low ($\approx 3$).
Quantitative parameters were calculated using the Bruker
ParaVision 2.0$^1$ Software. Using a $T_2$-weighted image as
the background display, ROIs were defined on the basis
of the histological section within the tumours. In the MR
images, three to four ROIs were evaluated (normal
contralateral brain, tumour tissue, necrosis, haemorrhage).
To compare the efficiency of the three MR
parameters for the detection of brain tumour with normal
tissue, contrast ($C$) was defined as:
$C = 100 \times |(t-c)/c|$ where $t$ represents the tumour MR parameter value, and $c$
the controlateral MR parameter value.
Statistical analysis was performed using a bi-factorial analysis of variance (ANOVA) with a multiple-least-square analysis if required. The Bonferroni–Dunn procedure was used for comparing multiple mean values.

RESULTS

Ten days after glioma cell injection all rats developed a tumour. At this stage, tumours grew mainly along the needle track and their sizes were estimated at \( 8 \pm 4 \) \( \mu l \) on the multispin echo set of images and confirmed by histology. The tumours appeared homogenous without significant necrosis or haemorrhage (Fig. 1). In vivo, the C6 glioma appeared to be a rapidly growing tumour with an approximate doubling time of 2 days. The mean survival time after cell inoculation was \( 21 \pm 2 \) days for a mean tumour volume of \( 176 \pm 51 \) \( \mu l \). Figure 2 shows a \( T_2 \)-weighted longitudinal follow-up of a C6 glioma and the corresponding histology section at the end point. With growth, tumours became heterogeneous with possible necrotic or haemorrhagic cores. Figures 3 and 4 show, respectively, a highly necrotic tumour and a tumour with a haemorrhagic core observed by MRI (\( T_2 \), ADC maps or MT-weighted image) and the corresponding histology sections. ROI are defined on the basis of histology performed at the final stage. All ROI values obtained on each rat for each sub-structure were averaged for each MR parameter, respectively.

On the contralateral hemisphere, \( T_2 \) is measured at \( T_2 = 53.6 \pm 0.5 \) ms, MT ratio at MTR = 17.7 ± 0.3 and apparent diffusion coefficient at ADC = 0.72 ± 0.13 × 10\(^{-3}\) mm\(^2\)/s. These values remained unchanged throughout the whole experimental time (mean ± SEM, 

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\[ \text{Figure 4. Typical MR images (} T_2 \text{ map upper left; MT image upper right; ADC map lower left) of a tumour with a haemorrhaged core 18 days after stereotaxic inoculation of exponential growing C6 glioma cells. The hypointense signal at the higher part of the caudate putamen is due to the haemorrhage. On the lower right of the figure is presented the histological section of this haemorrhaged tumour with enlargements of the haemorrhagic spots (Perls staining—magnification } \times 100) \]
n = 25, p > 0.6). On days 10–12, tumours were homogeneous, as shown by histology. Therefore, the MR parameter values measured at this stage correspond to ‘pure’ tumour tissue and are equal to: $T_2 = 59.0 \pm 0.7$ ms, MTR = 12.2 ± 0.6 and ADC = 0.68 ± 0.25 × 10^{-3} \text{mm}^2/\text{s}$ (mean ± SEM, n = 9). The evolution of MR parameters for this tissue is shown in Fig. 5(A–C). At the end-point stage, necrosis and/or haemorrhage can be unambiguously attributed as confirmed by histology. MR parameter values in necrosis are $T_2 = 119.8 \pm 8.9$ ms, MTR = 7.9 ± 0.7, ADC = 1.84 ± 0.11 × 10^{-3} \text{mm}^2/\text{s}$ (mean ± SEM, n = 3), whereas in the haemorrhagic core, $T_2 = 45.3 \pm 2.8$ ms (values calculated from a three-point fit; at 7 T, susceptibility artefacts are crucial), MTR = 19.2 ± 0.9, ADC = 0.39 ± 0.02 × 10^{-3} \text{mm}^2/\text{s}$ (mean ± SEM, n = 3), and in ‘pure’ tumour tissue, $T_2 = 70.1 \pm 2.5$ ms, MTR = 13.0 ± 0.4, ADC = 0.71 ± 0.15 × 10^{-3} \text{mm}^2/\text{s}$ (mean ± SEM, n = 6; Fig. 6).

Figure 7 presents the contrast evolution for the different MR parameters as a function of tumour growth.

**Figure 5.** Evolution of $T_2$ (A), MTR (B) and ADC (C) as a function of time for brain tissue (●) and tumour (○). Data are presented as mean ± SEM. (All data points are significantly different between tumour and brain tissue for $T_2$ and MTR, p < 0.05—none are significantly different for ADC, p > 0.05.)

**Figure 6.** Parameter values $T_2$ (A), MTR (B) and ADC (C) of normal brain tissue, tumour, necrosis and haemorrhage measured at final time points. (*Significantly different from normal tissue defined as control, p < 0.05; #significantly different from tumour tissue defined as control, p < 0.05.)
even though, as pointed out by Eis et al., parameter on each sub-structure needs to be performed structure. Consequently, the measurement of each MR measurements of MR parameters over the entire tumour masses become heterogeneous. Due to this heterogeneity, Figs 2–4, as tumour cells invade the brain, the tumour observed in transverse relaxation parameters with tumour growth. (the histology section performed at the end point. the back-extrapolation of the sub-structure according to the basis and MR parameters for each sub-structure can be by the test of reliable demarcation. At the final stage, leads to a much more optimistic picture than is justified (C) and MTR tumour = 12.2 n ms, T2 control = 53.6 ± 0.5 ms and T2 tumour = 59.0 ± 0.7 ms, p < 0.01), and more pronounced changes in MTR (MTR control = 17.7 ± 0.3 and MTR tumour = 12.2 ± 0.6, p < 0.001). As shown in Figs 2–4, as tumour cells invade the brain, the tumour masses become heterogeneous. Due to this heterogeneity, measurements of MR parameters over the entire tumour reflect an average of the different contribution of each structure. Consequently, the measurement of each MR parameter on each sub-structure needs to be performed even though, as pointed out by Eis et al., ROI analysis leads to a much more optimistic picture than is justified by the test of reliable demarcation. At the final stage, since histology is performed, ROIs can be defined on this basis and MR parameters for each sub-structure can be unambiguously assigned. A potential flaw of this study is the back-extrapolation of the sub-structure according to the histology section performed at the end point. However, as each tumour was imaged every 2–4 days, the anatomical changes that appear gradually with time were noticed. Therefore, being conscious of the potential flaw, we decided to perform a back-extrapolation. At each stage of tumour growth, T2 of ‘pure’ tumour appears higher compared with normal brain tissue. On days 10–12, this increase is about 10% and increases with time up to about 30% [Fig. 5(A)]. For the ADC parameter, no significant changes are observed between tumour and brain tissue whatever the time [p > 0.5, Fig. 5(C)]. Previous studies reported ADC values for tumour tissue ranging between 0.6 and 1.3 mm$^2$/s depending on the tumour strain and for normal brain tissue values between 0.5 and 0.8 mm$^2$/s depending on brain structure and gradient sensitization direction. For the MT parameter, MTR of the tumour is always lower than in normal tissue, as described previously (p < 0.001). The difference between both tissues tends to be smaller as tumours grow, but is always statistically significant [Fig. 5(B)]. At the later stage of tumour growth, the detection of tumours is not a problem and the choice of MR parameter(s) to measure is not crucial. From a therapeutic point of view, the earlier the tumour can be detected the better. Figure 7 presents the tumour/brain contrast with respect to MR parameters measured. The lowest contrast is observed for ADC (5–10%) as previously reported by Ikezaki,11 but do not corroborate Eis’s results.3 For all these studies, tumour ADC is similar, whereas normal brain ADC is not. According to brain anisotropy, ADC values are dependent on the diffusion sensitizing gradient direction. It appeared that Eis used a sensitizing gradient perpendicular to the one we used and therefore measured a different striatum ADC value (Eis, 0.52 mm$^2$/s; present study, 0.72 mm$^2$/s). Transverse relaxation time is slightly better able to distinguish tumour from brain tissue, but again, as the contrast is about 10% for the early stages of a growing tumour, T2 has to be used cautiously. The moderate increase in T2 is in good agreement with all T2 measurements reported in the literature.3,11 At the early stages better contrast is obtained with MT since a value of 30% is calculated. The reduction in MT in the tumour with respect to brain tissue is due to a less efficient magnetization transfer, which can be explained by the absence of myelin in the tumour.

In conclusion, the detection of tumour tissue within normal brain can be easily performed using MRI. However, with respect to tumours and the nature of their surrounding tissues, the choice of MR sequence can be crucial. The presence of peritumoral oedema is detectable using proton relaxation time weighted MRI (longer $T_1$ and $T_2$3,14), diffusion weighted MRI (higher3,11) or magnetization transfer MRI (reduced MTR11). In this particular case, the tumour is detected indirectly, the tumour delineation being performed by the oedema. Necrosis and oedema are difficult to discriminate since their MR parameter values are similar. In the absence of

DISCUSSION

The definition of MR parameters for tumour tissue delineation and/or as a marker of tumour response to therapy have previously been assessed.2–3,4–5,10–11 In this study, we focussed on the evolution of MTR, ADC and transverse relaxation parameters with tumour growth. Measurement of MTR, ADC and $T_2$ relaxation time in the contralateral hemisphere of the rat brain showed that none of these parameters are significantly modified after C6 glioma cell inoculation, regardless of the time frame between cell injection and MRI analysis [Fig. 5 (A–C)]. Such a phenomenon has been previously reported for $T_2$ after 9L glioma inoculation12 and for MTC and ADC after RG2 glioma inoculation.11 Quantitative comparison with previously published studies cannot be performed for MTR, since magnetization transfer is highly dependent on the saturation pulse strength and the offset used.8 At the early stage of tumour growth when tumours appear homogenous (Fig. 1), no significant changes can be measured in terms of $T_2$ between tumour tissue and normal brain tissue ($p > 0.2$). Slight changes are observed in $T_2$ ($T_2$ control = 53.6 ± 0.5 ms and $T_2$ tumour = 59.0 ± 0.7 ms, $p < 0.01$), and more pronounced changes in MTR (MTR control = 17.7 ± 0.3 and MTR tumour = 12.2 ± 0.6, $p < 0.001$). As shown in Figs 2–4, as tumour cells invade the brain, the tumour masses become heterogeneous. Due to this heterogeneity, measurements of MR parameters over the entire tumour reflect an average of the different contribution of each structure. Consequently, the measurement of each MR parameter on each sub-structure needs to be performed even though, as pointed out by Eis et al.,2–3 ROI analysis leads to a much more optimistic picture than is justified by the test of reliable demarcation. At the final stage, since histology is performed, ROIs can be defined on this basis and MR parameters for each sub-structure can be unambiguously assigned. A potential flaw of this study is the back-extrapolation of the sub-structure according to the histology section performed at the end point. However, as each tumour was imaged every 2–4 days, the anatomical changes that appear gradually with time were noticed. Therefore, being conscious of the potential flaw, we decided to perform a back-extrapolation. At each stage of tumour growth, $T_2$ of ‘pure’ tumour appears higher compared with normal brain tissue. On days 10–12, this increase is about 10% and increases with time up to about 30% [Fig. 5(A)]. For the ADC parameter, no significant changes are observed between tumour and brain tissue whatever the time [p > 0.5, Fig. 5(C)]. Previous studies reported ADC values for tumour tissue ranging between 0.6 and 1.3 mm$^2$/s depending on the tumour strain and for normal brain tissue values between 0.5 and 0.8 mm$^2$/s depending on brain structure and gradient sensitization direction. For the MT parameter, MTR of the tumour is always lower than in normal tissue, as described previously (p < 0.001). The difference between both tissues tends to be smaller as tumours grow, but is always statistically significant [Fig. 5(B)]. At the later stage of tumour growth, the detection of tumours is not a problem and the choice of MR parameter(s) to measure is not crucial. From a therapeutic point of view, the earlier the tumour can be detected the better. Figure 7 presents the tumour/brain contrast with respect to MR parameters measured. The lowest contrast is observed for ADC (5–10%) as previously reported by Ikezaki,11 but do not corroborate Eis’s results.3 For all these studies, tumour ADC is similar, whereas normal brain ADC is not. According to brain anisotropy, ADC values are dependent on the diffusion sensitizing gradient direction. It appeared that Eis used a sensitizing gradient perpendicular to the one we used and therefore measured a different striatum ADC value (Eis, 0.52 mm$^2$/s; present study, 0.72 mm$^2$/s). Transverse relaxation time is slightly better able to distinguish tumour from brain tissue, but again, as the contrast is about 10% for the early stages of a growing tumour, $T_2$ has to be used cautiously. The moderate increase in $T_2$ is in good agreement with all $T_2$ measurements reported in the literature.3,11 At the early stages better contrast is obtained with MT since a value of 30% is calculated. The reduction in MT in the tumour with respect to brain tissue is due to a less efficient magnetization transfer, which can be explained by the absence of myelin in the tumour.

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peritumoral oedema, tumour delineation by conventional relaxation time MRI can barely be used as tumours appear as a slightly hyperintense area, as previously reported.2–3 In our results diffusion-weighted MRI is not better at distinguishing the tumour, since the contrast with the brain is about 5–10%, but it can be substantially increased by taking into account brain anisotropy so as to modify normal brain ADC and therefore contrast. However, magnetization transfer is more powerful and can lead to a contrast with normal brain of about 30% depending on the pre-saturation pulses used.

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