Keywords and Abbreviations

Keywords 2-HG, glioma, detection, ultra-high field

TE	echo time
PRESS	point-resolved spectroscopy
semi-LASER	semi-localisation by adiabatic selective refocusing
IDH	isocitrate dehydrogenase
2-HG	2-hydroxyglutarate
CRLB	Cramér-Rao Lower Bound
SNR	signal-to-noise ratio

ABSTRACT

Abnormally high levels of the ‘oncometabolite’ 2-hydroxyglutarate (2-HG) occur in many grade II and III gliomas and correlate with mutations in the genes of isocitrate dehydrogenase (IDH) isoforms. *In vivo* measurement of 2-HG in patients, using MR spectroscopy, has largely been carried out at 3 T, yet signal overlap continues to pose a challenge for 2-HG detection. To combat this, several groups have proposed MRS methods at ultra-high field (≥ 7 T) where theoretical increases in signal-to-noise ratio and spectral resolution could improve 2-HG detection. Long-TE semi-LASER (TE = 110 ms) is a promising method for improved 2-HG detection *in vivo* at either 3 T or 7 T owing to the use of broadband adiabatic localisation. Using previously published semi-LASER methods at 3 T and 7 T, this study directly compares the detectability of 2-HG in phantoms and *in vivo* across nine patients. Cramér-Rao Lower Bounds (CRLBs) of 2-HG fitting were found to be significantly lower at 7 T (6 ± 2)% compared to 3 T (15 ± 7)% (p = 0.0019), yet were larger at 7 T in an IDH-wildtype patient. Although no increase in SNR was detected at 7 T (77 ± 26) compared to 3 T (77 ± 30), the detection of 2-HG was greatly enhanced through an improved spectral profile and increased resolution at 7 T. 7 T had a large effect on pairwise fitting correlations between GABA and 2-HG (p = 0.004) and resulted in smaller coefficients. The increased sensitivity for 2-HG detection using long-TE acquisition at 7 T may allow for more rapid estimation of 2-HG (within a few spectral averages) along with other associated metabolic markers in glioma.

INTRODUCTION

2-Hydroxyglutarate (2-HG) has emerged as a promising marker for the diagnosis of glioma by enabling stratification based on molecular profile.[1–3] Mutations in isocitrate dehydrogenase (IDH) encoding genes (*IDH1*, *IDH2*) are indicators of glioma WHO subtypes and are found in ~80% of grade II-III gliomas and secondary glioblastomas.[4–6] The gain-of-function activity of mutant IDHs leads to the production and subsequent accumulation of 2-HG,[2] and thus robust and reliable detection of 2-HG has been the focus of recent *in vivo* methodological development at 3 T[7–11] and, increasingly, at 7 T.[12,13]

The measurement of the strongly coupled 2-HG spin system is complicated by spectral overlap from neighbouring glutamate (Glu), glutamine (Gln), γ-aminobutyric acid (GABA) and glutathione (GSH) resonances, which is exacerbated when using short-TE sequences.[7,14] For this reason, techniques such as spectral editing and 2-D correlation spectroscopy have been implemented.[9] Long-TE acquisition methods can offer detection improvements from a smaller macromolecular baseline. In particular, a long-TE PRESS[15,16] sequence (TE = 97ms) has been shown to improve 2-HG assignment over short-TE PRESS at 3 T[8,17]. Long-TE MRS with adiabatic localisation, using a semi-LASER[18,19] sequence (TE = 110 ms), has been shown to further improve detection of 2-HG at 3 T, owing to a reduced chemical shift displacement artefact resulting in full refocusing of the 2-HG resonance at 1.9 ppm.[10]

MRS at ultra-high field (≥ 7 T) benefits from a theoretical increase in

intrinsic signal-to-noise ratio[20] and spectral resolution, due to the increased chemical shift dispersion. Ultra-high field MRS has been shown to enable reliable detection of many J-coupled metabolites (e.g. lactate (Lac), myo-inositol (Ins) and γ-aminobutyric acid (GABA))[21] and can resolve nearby Glu and Gln resonances (≥ 4 T). Bisdas et al. have used a short-TE STEAM acquisition at 9.4 T to analyze 2-HG *in vivo* in glioma patients,[22] however the chemical shift displacement was large.

Ganji et al. have optimised a long-TE PRESS scheme (TE = 78 ms) at 7 T for the detection of 2-HG *in vivo*, which generated small Cramér-Rao Lower Bounds (CRLBs) of fitting for 2-HG (~5%) and reduced overlap with GABA. [13] Similarly, Emir et al. have optimised a long-TE semi-LASER[19] localisation scheme for detection of the 2.25 ppm resonance of 2-HG at 7 T.[12] Using this scheme, it was possible to distinguish between patients with IDH1 and IDH2 mutations using a spectral classifier. The shorter T2 decay times of metabolites at ultra-high field, resulting in signal loss and linewidth increases, together with a large chemical shift displacement and increased field inhomogeneity,[23] mean the advantages of using long-TE at 7 T for 2-HG remain unclear.[24,25]

There is, therefore, a need to assess the benefit that long-TE acquisition at ultra-high field may present for 2-HG detection in IDH-mutant tumours. In this study, 3 T and 7 T acquisition is compared directly using long-TE (110 ms) semi-LASER localization in phantom and *in vivo* in nine glioma patients.

METHODS

Phantom

Details of the phantom comparison are given in Supplementary Materials 1.

In vivo

Nine newly diagnosed tumour patients (6 male, 3 female, age = 40 ± 11 years) were included in the study (Table 1). Patients were scanned at 3 T and 7 T on whole body MR systems (Siemens, Erlangen) with 32 channel head coils. Written and informed consent was approved by the Oxfordshire B National Research Ethics Committee. Some of the data have previously appeared in published work at 3 T[10] and 7 T[12], as described in Table 1. Eight patients underwent surgical resection followed by immunohistochemistry and DNA sequencing to determine the IDH status of the tumour.[10,12] Six tumours had mutations in IDH1 (R132H), one in IDH2 (R172K) and one was wild-type IDH (IDH-WT). One patient is currently awaiting surgery (TBC).

MR scanning

MRS voxels of (20×20×20) mm^3 were positioned with reference to a 1 mm isotropic T1-MPRAGE acquisition (TR = 1.9/2.3 s, TI = 0.904/1.05 s, TE = 3.97/2.8 ms, at 3 T/7 T) in tumour regions of maximum homogeneity. Screenshots of the anatomical scans, showing the voxel placement in three planes, were used as a guide to place voxels during the subsequent scanning session to ensure reproducibility of placement. Previously optimised long-TE semi-LASER localisation schemes at 3 T[10] and 7 T[12]

were used for 2-HG detection (TE = 110ms, TR = 3 / 5-6 s, spectral width = 6 kHz, number of points = 2048, number of transients (NT) = 71-128). Adiabatic inversion was achieved using HS4 R25 refocusing pulses at 3 T (bandwidth = 5.5 kHz, duration = 4.5 ms, peak B_1 = 28 μT) and 7 T (bandwidth = 4.1 kHz, duration = 6 ms, peak B_1 = 19 μT). Localisation was preceded by outer volume suppression (OVS) and VAPOR water suppression.[26] First- and second-order shimming was performed with GRESHIM[27] and first-order shims were adjusted at 7 T using FASTMAP.[28] A barium titanate ($BaTiO_3$) dielectric pad was placed close to the location of the tumour to increase transmit efficiency at 7 T.[29]

Analysis

Spectra were phase and frequency corrected and fitted with LCModel[30] using simulated basis sets[10] containing 21 metabolites: 2-HG, alanine (Ala), ascorbate (Asc), aspartate (Asp), creatine (Cr), γ-aminobutyric acid (GABA), glutamine (Gln), glutamate (Glu), glycine (Gly), *myo*-inositol (Ins), lactate (Lac), *N*-acetylaspartate (NAA), *N*-acetylaspartylglutamate (NAAG), phosphocholine (PCho), phosphocreatine (PCr), phosphorylethanolamine (PE), *scyllo*-inositol (sIns), taurine (Tau), glucose (Glc), glycerylphosphorylcholine (GPC) and glutathione (GSH). Concentrations are reported relative to an internal reference of either total creatine (tCr = Cr + PCr) or total choline (tCho = PCho + GPC). No correction for T2 decay differences between metabolites was carried out in this work. The approximate 95% confidence intervals on concentration values are calculated as 2× CRLB (Cramér-Rao Lower Bound).[30] Metabolites with CRLBs ≥ 100% are considered undetected.

To compare *in vivo* spectra at 3 T and 7 T, averaged spectra of the first 64 transients were generated for each patient. Signal-to-noise ratio (SNR) was measured as the height of the total choline peak at 3.2 ppm divided by the RMS noise of the spectrum (between 10 and 14 ppm).

RESULTS

A comparison of the long-TE semi-LASER sequences at 3 T and 7 T was made in a phantom (see Supplementary Materials 1). Spectral profiles of 2-HG were identical to those of previous studies at 3 T and 7 T. As expected, the fitting of 2-HG at 7 T had lower associated Cramér-Rao Lower Bounds (CRLBs) than at 3 T across all 2-HG concentrations.

A summary of the *in vivo* results for 2-HG is given in Table 1. The only confirmed tumour with wild-type IDH (IDH-WT) in this study was observed to have the largest associated CRLB of 2-HG fitting; this was higher at 7 T (131 %) compared to 3 T (38 %). Across the IDH-mutated tumours (including unconfirmed patient P09), 2-HG was quantified with a significantly lower CRLB at 7 T (mean: 6 ± 2)% compared to 3 T (mean: 15 ± 7)% (p = 0.0019, paired two-tailed t-test).

IDH-mutated tumour spectra (including P09) were assessed using correlation and Bland-Altman analysis.[31] A strong positive correlation was found between 2-HG/tCr values measured at 3 T and 7 T, revealing a large shared variance (R^2 = 0.89, p = 0.0005) (Fig. 1A). Bland-Altman statistics revealed a bias between 3 T and 7 T (Fig. 1B), with 3 T yielding lower 2-HG/tCr ratios (-1.31 ± 1.58, mean ± CI). There was a weaker but still significant correlation between 2-HG/tCho measured at either field strength (R^2 = 0.68, p = 0.02) (Fig. 1C). The bias of these 2-HG/tCho measures

between 3 T and 7 T was found to be small (0.03 ± 0.56) (Fig. 1D).

Fig. 2A shows spectra containing averages of 2 to 64 transients from one patient (P03, IDH2-mutated). After 64 transients, 2-HG peaks from H3-H3' and H4-H4' resonances are visible in the raw spectra at 3 T and 7 T. For NT < 8, the H3-H3' resonance is distinctly less clear at 3 T. At 7 T the large inverted H4-H4' resonance remains visible in the spectrum even after only 2 spectral transients. The SNR across all spectra was similar between 3 T (77 ± 30) and 7 T (77 ± 26) ($p = 0.993$, paired two-tailed t-test), suggesting that, at long-TE, lower CRLBs of 2-HG at 7 T are not driven by higher SNR.

CRLBs of 2-HG for IDH-mutated patients (including P09) were measured as a function of the number of transients (Fig. 2B). After two transients the mean 2-HG CRLB at 7 T was found to be (19 ± 9)%; below common rejection thresholds of around 30-50%.[32] For 3 T, 8-16 transients were required to reach a CRLB < 40% and a CRLB < 20% was achieved after around NT = 64 (of 15 ± 7)%. This boost in sensitivity equates to a scan time reduction of around 3 minutes.

Voxel positioning was similar between acquisitions at 3 T and 7 T (as shown in example Fig. 3A and Supplementary Materials 3). Across all patients and fields (Fig. 3B) spectra were largely free from baseline artefacts or significant contamination over the (0.5–4.2) ppm range, apart from P05 and P06, for whom lipid contamination meant spectral analysis was restricted at both field strengths to (1.5–4.2) ppm and (0.5–4.1) ppm, respectively. The mean linewidth of the choline peak, across all spectra, was calculated to be 8.1±1.7 Hz and 13.2±3.0 Hz at 3 T and 7 T, respectively.

The mean CRLBs of metabolite fitting were compared across IDH-mutated tumour spectra (n = 8, IDH-mutated + TBC) in Fig. 4. Further to the significantly lower CRLB for 2-HG, Glu (7 T and 3 T: 16% and 36%, p = 0.10) and Lac (7 T and 3 T: 10% and 7%, p = 0.11) showed trends for reduced CRLBs at 7 T compared to 3 T. Errors of fitting *myo*-inositol (Ins) were significantly reduced (p = 0.002) at 7 T (4%) compared to 3 T (9%). GABA and GSH were detected in only n = 1 and n = 3 patients at 3 T respectively, yet this increased to n = 4 and n = 7 patients at 7 T. Larger CRLBs for glutamine (Gln) were found at 7 T, which were significantly different between field strengths (p = 0.02).

When measured relative to tCr, the largest 2-HG concentration was observed in an IDH1-mutated patient (P07, Table 1). However, upon visual inspection (Fig. 3B), the 2-HG signal appears smaller than that of P03, while the tCr resonance (at 3.03 ppm) is strongly diminished in P07. When referencing to tCho, the largest 2-HG concentration was measured in P03 at 3 T and 7 T, which matched visual inspection. P03 was the only patient found to have an IDH2 mutation.

Finally, overlap of 2-HG with GABA was smaller at 7 T, in particular at the H4-H4' resonance (at 2.25 ppm) where this peak is inverted (see simulations in Supplementary Materials 2). Moving from 3 T to 7 T also had a significant effect on LCModel pairwise correlation coefficients for 2-HG fitting with GABA (b = 0.89 ± 0.06, t = 15.7, p = 0.004, effect size + standard error), Gln (b = 0.12 ± 0.03, t = 4.3, p = 0.004), Glu (b = -0.26 ± 0.04, t = -6.2, p = 0.008) and GSH (b = 0.12 ± 0.03, t = 4.24, p = 0.008) as measured using a mixed effects linear regression model. The 2-HG:GABA correlation coefficient had a mean value of -0.62 at 3 T to 0.27 at 7 T.

DISCUSSION

The detectability of 2-HG in IDH-mutant tumours using long-TE MRS (TE=110ms), is significantly improved at 7 T compared 3 T. Improved spectral dispersion, rather than an increase in SNR, was found to result in consistently lower CRLBs of 2-HG fitting at 7 T of (6 ± 2)% compared to (15 ± 7)% at 3 T (p = 0.0019). This boost in sensitivity enabled a more rapid determination of 2-HG; only two transients (12 s acquisition duration) at 7 T were required to achieve CRLBs < 20% as compared to 3 T, where around 64 transients were needed (192 s duration). CRLBs were consistently lower at 7 T, even for very low concentrations (< 1 mM) of 2-HG in phantom. The temporal acceleration afforded by 7 T may be advantageous for development of MRS imaging (MRSI) methods for 2-HG; the semi-LASER localisation used in this study would also minimize sensitivity to B1 inhomogeneities at 7 T across an extended spatial grid.[33]

Larger signal loss from T2-relaxation at 7 T may explain the observation of identical spectral SNR at 3 T and 7 T and is the biggest limitation of the comparison presented in this work. For instance, the Cho peak, with measured T2-values in healthy brain of 170 ms at 3 T and 131 ms at 7 T,[34] experiences a signal decay of 43% and 52% at 3 T and 7 T (TE=110ms), respectively. It is also noted that T2-relaxation constants vary between metabolites by 20-30%,[35] and are affected by tissue content in different brain regions,[36] and across tumours.[37,38] T2-values for hard-to-measure, overlapping, J-coupled metabolites are rare or absent from the literature; most notably 2-HG. Thus, concentration estimates (in mM) are not reported in this work, since the accuracy of such estimates is fully dependent on the

accuracy of T2-values, which cannot feasibly be measured *in vivo* during a clinical protocol.

The tendency for 2-HG/tCr to underestimate 2-HG at 3 T compared to 7 T (or equally for 7 T to overestimate, see Fig. 1B) may be due to the shorter T2 of Cr,[35] whereby loss of tCr signal over 110 ms generates artefactual 2-HG/tCr estimates, which are larger at 7 T because of the decreased T2s. Furthermore, the tCr content in IDH-mutated glioma may be low in certain IDH-mutant tumours.[39] Thus, the use of tCr as a reference peak for 2-HG may result in inaccurate measurement of concentration (see Fig. 3B, P07). When referencing to tCho, however, there was no observed difference in the 2-HG measured at 3 T or 7 T (bias = 0.03 ± 0.56). However, the number of IDH-mutated patients in this study (n = 7) limits the conclusions of such statistics, thus studies of 2-HG detectability in larger patient cohorts may be required.

It is also worth noting that tumour spectra are sometimes observed with contamination from mobile lipids[40]. In this study, the analysis windows of two patient spectra were restricted to prevent poor spectral fitting of 2-HG and associated metabolites. Adequate shimming was achieved *in vivo* (Cho linewidth = 8.1±1.7 Hz at 3 T), such that the H3-H3’ resonance of 2-HG could be resolved from the NAA peak (2.01 ppm). Separation of these resonances would be more challenging if the NAA concentration was high. At 7 T, 2-HG has been shown to be detectable in phantom with semi-LASER (110 ms) even in the presence of a large NAA peak.[12]

Apparent line-narrowing of 2-HG multiplets at long-TE, combined with the increased spectral resolution, aided detection of 2-HG at 7 T similar to the

observations of previous studies.[13] In particular, the inverted shape of the H4-H4' resonance (2.25 ppm) at 110 ms gave improved separation amongst upright resonances. Field strength had the largest effect on the pairwise fitting correlation coefficient for GABA and 2-HG, which is similar to that noted by Ganji et al. using long-TE PRESS[13] and is suggested to be driven by a reduced overlap of 2-HG (at 1.9 ppm) and GABA (at 1.89 ppm). Further work, however, is needed to determine the extent to which 2-HG concentrations are influenced by GABA at both 3 T and 7 T; such measurements can be achieved using metabolite-specific MEGA-editing.[41]

Detection of *myo*-inositol (Ins) was improved at 7 T (p = 0.002). Ins has a triplet resonance close to the H2 resonance of 2-HG (at 4.05 ppm), and two resonances at 3.61 ppm and 3.52 ppm, overlapping with the glycine (Gly) singlet at 3.55 ppm.[42] Elevated Gly has been implicated in recurrent gliomas[43] and may reflect tumour malignancy. Without the use of optimised strategies, however, at 3 T[44] or 7 T,[45,46] separation of Gly and Ins is challenging, thus fitting of these metabolites at long-TE should be taken with caution. In particular, the reporting of Ins using long-TE 7 T is hampered by the relatively short T2 times of this metabolite.[47]

Glutathione (GSH) was detected in all confirmed IDH-mutated patients at 7 T (n = 7) and in only three patients at 3 T (Fig. 4). The sensitivity to detect GSH at 3 T using long-TE may be low partly because of overlaps with Glu and Gln.[42] Interestingly, short-TE methods at 3 T[7] and 9.4 T[22] have previously indicated that GSH concentration may be lower in IDH-mutants than wildtype. Given the issues in assigning GSH, studies using high-resolution NMR [48] or J-difference editing [49], are required to confirm the presence of GSH and 2-HG in IDH-mutated glioma.

Larger CRLBs of Gln fitting were found at 7 T (52 ± 27)% compared to 3 T (27 ± 25)% ($p = 0.02$), which may be attributed to the fact that separation of Gln from Glu signal is not possible at 3 T using single shot techniques, thus Gln is susceptible to overfitting at 3 T.[50–52] At 7 T, however, the increased separation of the H4 spins of Gln and Glu is sufficient to resolve both signals.[50] The larger fitting error at 7 T may incidentally reflect a smaller concentration of Gln and Glu in IDH-mutated tumours[53,39] and thus a higher degree of fitting uncertainty.[54] Finally, both semi-LASER sequences in this work were designed for 2-HG detection, therefore further studies are required to evaluate Glu and Gln detection at this echo time.

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TABLES

					3 T			7 T		
			Vol.		2-HG		CRLB	2-HG		CRLB
ID	Sex/Age	Tumour/Grade	cm^3	IDH	/tCr	/tCho	(%)	/tCr	/tCho	(%)
P01†	M/52	Astroglial Tumour / II	47.5	IDH1	6.9	1.7	15	8.0	1.6	6
P02†	M/53	Glioblastoma / IV	27.6	WT	2.9	0.9	38	0.3	0.1	131
P03†	F/26	Oligodendroglioma / II	34.3	IDH2	11.6	3.5	8	13.2	4.7	4
P04†	F/44	Ana. Astrocytoma / III	34.7	IDH1	2.7	0.9	24	4.1	1.2	9
P05‡	M/34	Diff. Astrocytoma * / II	30.1	IDH1	7.8	1.3	20	7.8	1.5	6
P06	F/36	Diff. Astrocytoma / II	48.1	IDH1	6.9	2.1	24	8.6	1.9	7
P07†	M/36	Diff. Astrocytoma / III	60.1	IDH1	13.4	2.9	11	18.8	1.9	4
P08	M/52	Oligodendroglioma / II	29.8	IDH1	7.6	1.8	9	8.2	1.8	5
P09†	M/24	TBC	21.8	TBC	7.2	2.8	11	6.0	2.4	7

Table 1: Summary of 2-HG measurements in nine tumour patients scanned using semi-LASER at 3 T and 7 T. Seven patients were confirmed as having an IDH mutation (IDH1 R132H, n = 6; IDH2 R172K, n = 1) and one patient (P02) was found to be IDH-wildtype (WT). The remaining patient (P09) is awaiting biopsy (TBC). † denotes 3 T data previously reported in Berrington et al. [10] ‡ Denotes 7 T data previously described in Emir et al.[12] * probable classification – too few cells for accurate histological

confirmation. 2-HG concentrations are reported relative to total creatine (tCr) and total choline (tCho) and a matched number of acquired transients (NT = 64) for both scans. Tumour volumes (Vol.) in cm^3 are also provided alongside each classification.

FIGURE LEGENDS

Figure 1: Comparison of 2-HG concentration measured at 3 T and 7 T relative to total creatine (tCr) (*top)* and choline (tCho) (*bottom)*. A and C: Correlation between 3 T and 7 T measures of 2-HG. B and D: Bland-Altman analysis of relative 2-HG concentrations showing confidence intervals on the estimated mean difference or bias (dotted) together with the zero-difference line. Statistical agreement calculated for patients who have IDH1-mutation (cross, n = 6), IDH2-mutation (triangle, n = 1) or yet to be confirmed (circle, n = 1).

Figure 2: Comparison of *in vivo* acquisition and detection using long-TE semi-LASER at 3 T and 7 T. A: Raw spectra (patient P03, IDH2) shown with increasing number of transients (NT = 2–64) and measured SNR. Vertical lines indicate 2-HG resonance positions. B: Mean CRLB of 2-HG detection over 7 patients shown with standard error (shaded, grey) and CRLB thresholds marked at 20% and 40%. Dotted vertical lines indicate positions of the 2-HG resonances, H2 (4.02 ppm), H3-H3' (1.9 ppm) and H4-H4' (2.25 ppm).

Figure 3: *In vivo* acquisition in eight tumour patients comparing 3 T and 7 T long-TE semi-LASER (NT = 64) for 2-HG detection. A: Raw spectra and LCModel fits for an example IDH1-mutated confirmed patient (P01). Corresponding CRLBs (%) are shown alongside each fit. Voxel placement is shown above overlaid onto the T1-weighted image. B: Remaining *in vivo* spectra (P02–P09) in the study, with LCModel 2-HG fits (blue) shown beneath and corresponding CRLB. NT = 64.

Figure 4: CRLBs of selected metabolites plotted for 3 T and 7 T across all IDH-mutated tumours including unconfirmed patient (P09). Standard deviation is shown in the highlighted region. * denotes significant difference between field strengths ($p < 0.05$, paired, two-sided t-test). Spectra compared between field strengths with NT = 64.