Detection of glycine as a biomarker of malignancy in childhood brain tumours using in-vivo ^{1}H MRS at short and long TE

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Introduction

In the UK brain tumours account for 25% of childhood cancer, which is the most common cause of death from disease in children. MRS provides a unique opportunity to study the metabolism of brain tumours non-invasively. Recent studies have shown that analysis of metabolite profiles derived from MRS allows accurate identification and characterisation of childhood brain tumours [1]. Most MRS studies of brain tumours have used a single echo time (TE), either short (~30ms) or long (~135ms). Short-TE MRS is advantageous due to increased signals from strongly coupled metabolites (e.g. myo-inositol), macromolecules and lipids leading to improved classification accuracy [2]. However, the interpretation of short-TE spectra is complicated by overlapping peaks. In *in-vitro* MRS, glycine (Gly), which has a singlet peak overlapping the multiplet of myo-inositol (mI) at approximately 3.6 ppm, has been reported to be high in medulloblastomas and glioblastomas and to increase with malignancy [3,4]. Accurate discrimination of these metabolites *in vivo* may improve diagnostic accuracy and provide a potential biomarker for prognosis. This is more likely to be possible at long TE, since the mI signal is attenuated relative to Gly, but at the expense of useful information where acquisition at both short and long TE is not possible. The aim of this study was to investigate Gly and mI quantitation in different types of childhood brain tumours using single-voxel MRS at short and long TE in comparison with high-resolution magic angle spinning (HR-MAS) NMR of biopsy samples.

Materials and Methods

Single-voxel ^{1}H MRS using the PRESS sequence was performed as part of a MR protocol designed for scanning children with suspected brain tumours on a 1.5 T scanner. Two cohorts were retrospectively selected from a total of over 170 children studied prior to treatment over a 5 year period. The first cohort consisted of 33 patients (7 medulloblastomas (MB), 8 pilocytic astrocytomas (PA), 4 diffuse pontine gliomas (DPG), 2 glioblastomas (GBM), 2 dysembryoplastic neuroepithelial tumours (DNT), 1 each of ependymoma, germinoma (Gm), choroid plexus papilloma (CPP), ganglioglioma (GG) and diffuse astrocytoma (DA), and 5 non-tumours) for which spectra were acquired at a TE of both 30 ms and 135 ms. A second group of 16 patients (8 MBs, 6 PAs and 2 ependymomas) were included for which HR-MAS data of a tumour sample was available with short-TE spectra acquired *in vivo*. *In-vivo* spectra were processed by LCModel™ using basis sets of metabolite signals simulated using the density matrix formalism. Fits obtained using a basis set with and without Gly were compared. Concentrations were determined relative to the internal water spectrum. Spectra were excluded if the voxel was misplaced, signal-to-noise ratio < 4, or full-width-at-half maximum > 10 Hz. Student's t-tests with unequal variances were used to compare Gly concentrations between tumour types. HR-MAS of 16 biopsy samples was performed using a pulse-acquire sequence on a Varian 600 MHz spectrometer with a gHX nanoprobe spun at 2.5 kHz (sample Temp. 6.7°C). Spectra were fit using TARQUIN [5] and relative Gly and mI concentrations were compared between tumour types and with *in-vivo* results.

Results and Discussion

Fig. 1 shows LCModel™ fits between 3.0 and 3.65 ppm of a long TE spectrum from a medulloblastoma with and without Gly in the basis set annotated to indicate metabolite contributions to the fit. A significant improvement in the quality of the fit by including Gly can be seen, with markedly reduced residuals and a flatter baseline hence improved quantitation of other metabolites, especially taurine (Tau) and mI. Fig. 2 shows that the Gly levels measured at TE 30 ms and TE 135 ms correlated significantly (correlation coefficient, r=0.8, P<0.001). The outliers tend to originate from poorer quality spectra or spectra with high levels of mI, indicating that Gly may be distinguished from mI in good quality short-TE MRS if present in high enough concentrations relative to mI. Significantly higher Gly levels were detected at both long and short TE (P<0.001) in medulloblastomas with Cramer-Rao lower bounds (CRLB) <20% indicating reliable fits. Gly was also detected in GBMs at both TE, but more reliably at long TE (CRLB <20%). Gly was not detected reliably in any of the pilocytic astrocytomas at short or long TE. Gly was detected by HR-MAS in most tumour samples, including the pilocytic astrocytomas. However, in this group Gly/(Gly+mI) was significantly higher for medulloblastomas than for pilocytic astrocytomas + ependymomas both *in vivo* (P<0.01) and *in vitro* (P<0.05).

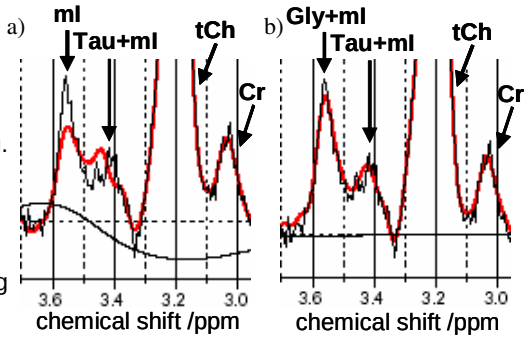

Figure 1. Example spectrum (TE 135ms) & LCModel™ fit (red) a) without and b) with Glycine in the basis set.

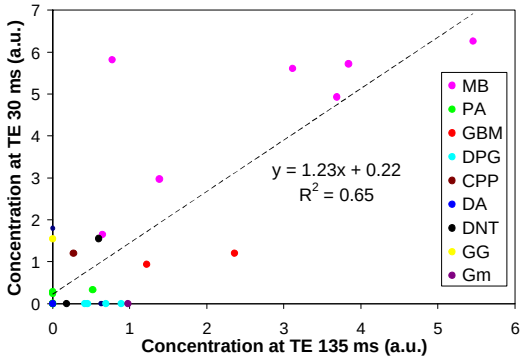

Figure 2. Correlation of Gly concentration measured at TE 135ms vs 30 ms (tumour type indicated by colour).

Conclusion

Glycine (Gly) was detected in medulloblastomas and glioblastomas in agreement with *in-vitro* studies [3,4] but not in low-grade astrocytomas, indicating that Gly is a promising biomarker in malignant childhood brain tumours that warrants investigation in a larger study. This study suggests that Gly levels can be measured by long and short TE MRS at 1.5 T and should be included in basis sets. Further investigation is required to determine the minimum Gly concentration detectable at different TEs for varying spectral quality and in the presence of varying amounts of myo-inositol.

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References

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