^{1}H NMR Spectroscopic Study of Lipid T_1- and T_2-Relaxation in BT4C Rat Gliomas *In Vivo*

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Introduction: ^{1}H NMR visible (mobile) lipids are becoming an important diagnostic and therapeutic marker for cancer. Their presence and dynamic properties have been well documented in many cell lines and tissue types, especially under pathological conditions, such as cancer. It is generally accepted that intracellular inclusions, i.e. lipid bodies are primarily responsible for these NMR resonances (1). Very little however, is currently known about the dynamics and processes involved with lipid body formation (2). In mammalian cells, their turnover appears to be regulated by cytosolic lipid body associated proteins (2). Previous NMR studies on lipid ADC values have not been able to address this issue (3,4). However, it has previously been shown, that increased protein content shortens lipid T_1 and T_2 relaxation times (5). The effects of *J*-modulation can be considered negligible in lipids, where coupling effects are weak due to the equivalence of protons in long hydrocarbon chains. In this study, we have investigated tumor lipid T_1 and T_2 relaxation by ^{1}H NMR spectroscopy to better validate lipid quantification *in vivo* and to gain more insight on the triglyceride microenvironment in lipid bodies during ganciclovir- induced apoptotic cell death.

Materials and methods: Intracranial HSV-thymidine kinase positive BT4C gliomas (n =4) were inoculated and treated with ganciclovir (GCV) for 14 days as described before (4). NMR spectroscopy data was obtained at 4.7 T on days 0, 2, 4, 6, 8, 11 and 14 of GCV treatment using a STEAM sequence (6). For T1 studies, inversion times of 530-3300 ms, with TR=5.0 s and TE=3 ms were used, and for T_2 studies TE=3-73 ms, with TR=2.5 s. In both studies, TM=30 ms and NA=16 were used. Spectra were analysed with jMRUI and the T_1 and T_2 data were best fitted using standard single exponential T_1 and T_2 models. In addition, standard 50 nm electron microscopy sections from days 0, 4, and 8 (n=3-4) were quantified for intracellular lipid bodies, the volumes of which were calculated from droplet radii corrected with a distribution function.

Results: A typical data series is shown in Fig. 1. During treatment, the T_1 and T_2 relaxation times of both 0.9 ppm (methyl) and 1.3 ppm (methylene) resonances decreased significantly (Fig. 2). The changes were more pronounced for T_2 relaxation times. The 5.4 ppm (vinyl) resonance behaved similarly to the methyl and methylene resonances (data not shown). Decreasing trends for both T_1 and T_2 could be observed for choline containing compounds at 3.2 ppm (not significant). The TEM-derived values for characteristic lipid body diameter increased from 2.7 ± 1.5 µm to 6.4 ± 4.5 µm ($p < 0.01$ student's unpaired t-test) during 8 days treatment period.

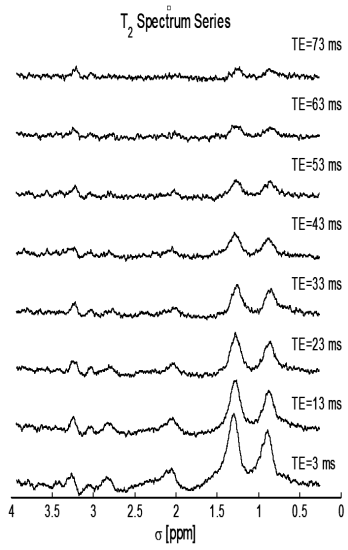
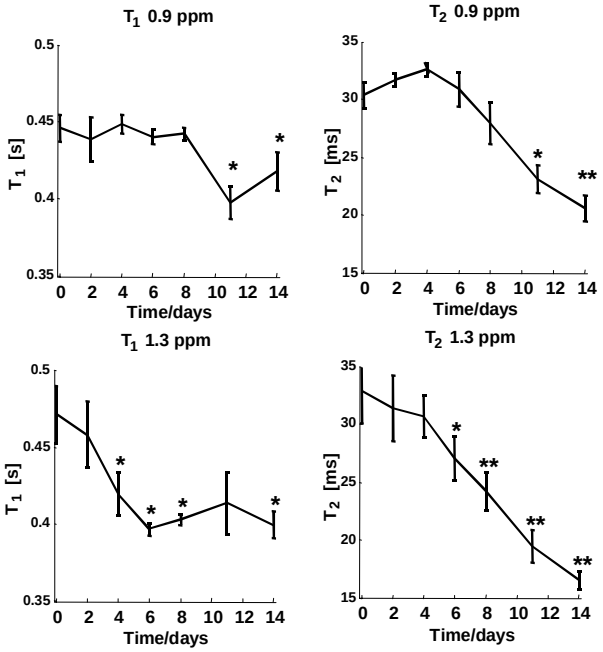


Figure 1 (left) *Typical series of T_2 weighted spectra from a BT4C glioma treated with ganciclovir (4 days). Respective echo times (TE) are shown. Number of scans = 16, TR = 2.5 seconds.*

Figure 2 (right) *T_1 and T_2 relaxation times for BT4C glioma lipid resonances at 0.9 and 1.3 ppm during ganciclovir treatment.*
** $p < 0.05$, ** $p < 0.01$, Student's paired t-test (n=4).*



Discussion and Conclusions: T_2 is considered more sensitive to changes in microcompartment size, whereas both T_1 and T_2 relaxation times inversely correlate with sample protein content (5). Interestingly, despite the increasing droplet size we observe reductions in both lipid T_1 and T_2 relaxation times during GCV treatment, which implies an increase in lipid body-associated protein concentration. The 1.3 ppm methylene resonance also appears more sensitive, with T_1 significantly decreasing after four days of treatment. This may reflect its sensitivity to changes in high frequency lipid motion, such as methylene isomerizations absent with 0.9 ppm terminal methyl groups. In conclusion, our results show dramatic changes in tumor lipid T_1 and T_2 relaxation times during apoptotic cell death, which need to be taken into account when quantifying lipid concentrations, especially at longer echo times. The results also show that relaxation studies may be used to address the *in vivo* dynamics of lipid body protein contents in many biological systems.

References:

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