Optimisation and evaluation of diffusion-weighted magnetic resonance for assessing mobile lipid resoanances in cancer.

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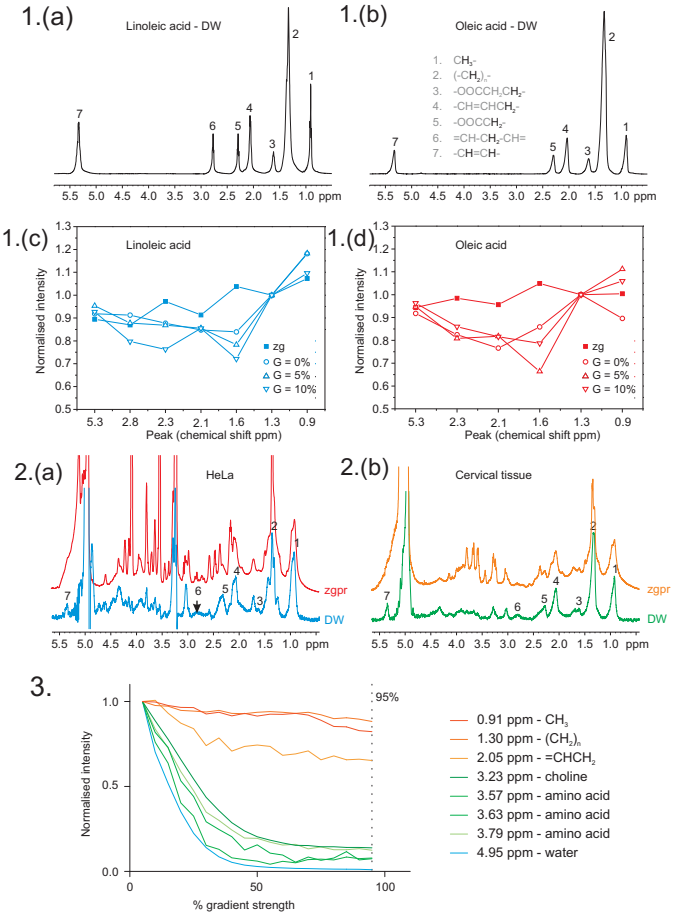
Intro: Increased levels of mobile lipid resonances (MLR) have been documented *in vivo* in a variety of tumours[1,2,3]. However, MLR changes in ^{1}H NMR spectra of cell pellets and tissues are confounded by signals from low molecular weight metabolites which complicates peak assignment and quantification. A simple and effective approach is to use diffusion-weighted sequences (DW) to attenuate the low molecular weight metabolites based on their relatively high diffusion coefficients. We have optimised a stimulated echo sequence with bipolar gradients[4] (ledbpgs, Bruker) to obtain diffusion-weighted HR-MAS spectra of model lipid solutions, cell pellets and cervical tissue samples, in order to characterise and validate the nature of the observed lipid chains in cancer cells *in vitro* and *ex vivo*.

Methods: Model substances used for validation of the diffusion-weighted sequence were oleic acid (18:1) and linoleic acid (18:2) (Sigma, UK). A HeLa cervical cell line (Cancer Research UK Cell Services, UK) was cultured under standard tissue culture conditions. Also 10 cervical punch biopsies additional to those for diagnosis were taken for *ex vivo* HR-MAS measurements. All spectra were acquired using a Bruker Avance 11.74 T spectrometer (Bruker BioSpin, Germany) equipped with a 4mm HR-MAS probe and gradient aligned along the magic angle. Samples were spun at 3 kHz and the temperature maintained at 298 K for model lipid solutions and 277 K for cell pellets and tissue samples.

Results: Diffusion-weighted ^{1}H HR-MAS spectra of linoleic and oleic acid produced seven and six peaks respectively, Figure 1 (a) and (b). All lipid peaks in the diffusion-weighted spectrum of model lipids were attenuated with respect to the spectrum obtained without gradients. Fig 1 (c) and (d) show the integral of each peak in linoleic acid and oleic acids, normalised to the number of protons and to signal intensity of 1.3 ppm peak. Figures 2(a, b) compares pulse acquire spectra with water presaturation (top) with diffusion-weighted ^{1}H HR-MAS spectra (bottom) from a pellet of HeLa cancer cells (2a) and cervical tissue biopsy (2b). Almost complete separation of major MLR, including unsaturated lipid peaks at 5.3 ppm and 2.8 ppm is achieved. Non-lipid components were highly attenuated in comparison to the pulse acquire spectrum. Removal of signals from low molecular metabolites improvemed spectral resolution in the 2.0 – 4.5 ppm region enabling visualisation of signals originating from unsaturated lipids (at 2.0 and 2.8 ppm) and protons from the glycerol backbone of triglycerides (not present in this sample). Figure 3 shows the peak area from fast diffusing metabolites, including water, and slow diffusing MLR with increasing gradient strength confirming that lipid peaks have comparable diffusion coefficients. In cell pellets, signals originating from aliphatic groups of lipids represented by peaks at 0.9 (C**H$_3$**-) and 1.3 ppm (-C**H$_2$**-) were not much attenuated even at high gradient strengths. Unsaturated lipid signal at 2.0 ppm (=CH-C**H$_2$**-) was more attenuated at low gradient strengths, suggesting a fast and slow diffusing component. The water signal at 4.9 ppm is significantly but not completely attenuated when the gradient strength reaches 95% of maximum.



Discussion: Diffusion-weighted measurements using the ledbpgs sequence revealed some attenuation of all lipid resonances in model lipids. Despite this, every resonance was visible and retained approximate proportions relative to number of protons. From our measurements of T_1 and T_2 (results not shown) small differential attenuation between lipid resonances may be attributed to differences in homonuclear scalar coupling networks. The magnitude of these couplings gives rise to a differential damping during the two spin-echo periods. For mixtures of different lipids found in cells and tissues with different extents of unsaturation it is not possible to precisely relate the peak integrals either to the number of protons or to the absolute concentration.

Application of the ledpbgs sequence to HR-MAS spectroscopy significantly reduced spectral overlap by removing signals originating from low molecular weight metabolites and non-lipid contributions to MLR peaks without significant signal loss (less than 10%). This will enable monitoring of MLR changes with a high degree of reproducibility and without the need for extract preparation. The most important limitation of the technique is that it is impossible to completely remove signals originating from low and other non-lipid metabolites. A significant water signal is still observed at high gradient strengths due to the fraction of water bound to larger macromolecules. Some signals originating from low molecular weight metabolites, for example choline-containing metabolites appeared to have fast and slow diffusing (choline moiety of phospholipids) components and their intensity was also not completely suppressed. Signals from lysine-containing, cytoplasmic polypeptides were very stable in the diffusion-weighted spectrum of cell pellets (peaks at 3.0 and 1.7 ppm) which also complicates peak assignment.

Conclusions: Diffusion-weighted HR-MAS spectroscopy aids investigation of nature and saturation of lipids in cancer cells. Due to the non-destructive nature of HR-MAS spectroscopy it will be possible to perform biochemical and cytochemical studies under different conditions of cell growth.

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