Detection of apoptotic cell death in vitro using quantitative Magnetization Transfer

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INTRODUCTION: Apoptotic cell death is an indication of tumour response and precedes the gross morphological changes in tumour size that are typically monitored by imaging[1]. Apoptosis is characterized by distinct biochemical and morphological changes at the cellular level, including condensation and breakdown of the nuclear material, reorganization of membrane lipids and membrane blebbing (for vesicle formation).

Magnetization transfer (MT) is an MRI method of saturating macromolecular protons and allowing them to exchange with free water protons. The decrease in the water signal following exchange indirectly provides information on the macromolecules and, because exchange occurs continuously throughout the saturation process, the effect on the water signal is cumulative. Through quantitative modelling, information on the exchange kinetics, concentration and frequency distribution of the macromolecular protons can be determined. This suggests potential for MT to detect the molecular breakdown and membrane reorganization that occur during apoptosis for monitoring tumour response. This study examines an *in vitro* cell sample, following induction of apoptosis, using an MT sequence with a range of saturation powers and offsets, and fits the data to a quantitative MT model.

METHODS: Acute myeloid leukemia cells (AML-5) were cultured in suspension and treated with cisplatin (10 µg/mL) to induce apoptosis. Cells were centrifuged at 2900 g to produce a sample for imaging. All data were acquired at 1.5 T (GE Signa, Milwaukee, WI) 36 or 48 hours after treatment. The field of view was 8 x 8 cm^2 and slice thickness was 2 mm. T1 data were acquired using an inversion recovery (IR) sequence (TR=2500 ms, TE=11 ms, 128^2 matrix) with eight inversion times (TI=50, 100, 200, 300, 500, 700, 900, 1500 ms). Magnetization Transfer data were acquired using a 3D spoiled gradient echo sequence (64^2 matrix, TE=4 ms) with one Hanning-windowed Gaussian[2] saturation pulse per repetition time, TR, of 200 ms. The saturation pulse width was 82 ms and peak powers of ω_1=427 and 243 Hz were used. Sixteen offset frequencies were applied (logarithmically spaced from 0.12 to 200 kHz). A 15° pulse was used for readout.

T1 data were fit as a monoexponential recovery to the equation $S = S_0\left(1-(1+a)e^{-TI/T1}+a\cdot e^{-TR/T1}\right)$ [Eq. 1], where S_0 would be the signal if all equilibrium magnetization were tipped to the transverse plane and the parameter a accounts for imperfections in the 180° pulse. MT data were fit to a two-pool model of magnetization exchange, with the fraction of free water protons (pool A) normalized to 1 and the macromolecular proton (pool B) fraction M_{0B}. The rate of magnetization exchange was modelled by the parameter R. The transverse relaxation of the free water was represented by T_{2A}. The macromolecular line shape was assumed to be super-Lorentzian (as indicated for tissues[3]), characterized by the time constant T_{2B}. T_{1obs} was fixed to the measured IR value and T_{1B} was assumed to be 1 s. For semiquantitative assessment of MT, the magnetization transfer ratio (MTR) was calculated for 427 Hz power and 2320 Hz offset frequency.

RESULTS: Sample curves for the MT signal at all offsets and ω_1=427 Hz are shown for controls, as well as 36 and 48 hours following cisplatin treatment in Fig. 1. A small but consistent increase in the magnetization transfer is observed at increasing treatment time (differences are smaller at the lower power). The fit, is shown by the solid lines. The T1 values calculated from the IR data are shown in Fig. 2a, along with the MTR (Fig. 2b) and the four parameters from the quantitative fit (Fig. 2c-f).

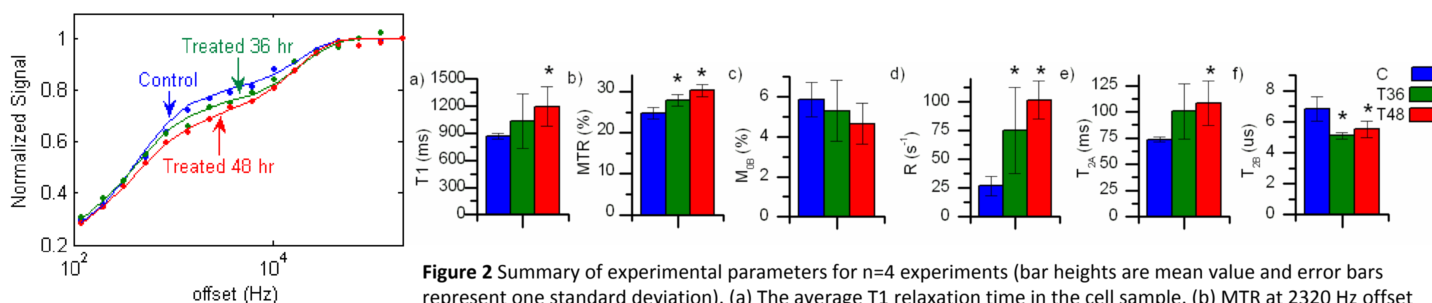

Figure 1 MT curves show increasing magnetization transfer effect with time after apoptosis induction. Points show experimental data and lines show corresponding fit to the two-pool model.

Figure 2 Summary of experimental parameters for n=4 experiments (bar heights are mean value and error bars represent one standard deviation). (a) The average T1 relaxation time in the cell sample. (b) MTR at 2320 Hz offset and 427 Hz power. Two-pool model fit parameters (c) the macromolecular proton fraction, M_{0B}, (d) the exchange rate, R, (e) the T2 relaxation time for the free water, T_{2A} and (f) the T2 relaxation time for the macromolecules, T_{2B}, which characterizes the width of the super-Lorentzian line shape. The * indicates $p < 0.05$ relative to controls.

DISCUSSION: The average T1 increases significantly only in late apoptotic stages, at 48 hours following cisplatin treatment of the AML cells. The increase in the MT effect is apparent at both 36 and 48 hours. This is emphasized by the MTR at 2320 Hz (Fig. 2b), where the effect is statistically significant at 36 hours. The fit parameters show a tendency for the exchange rate R and free water relaxation T_{2A} to increase, while the macromolecular proton fraction M_{0B} and relaxation time T_{2B} decrease. The parameters in the two-pool model, particularly R, are very sensitive to noise, making them difficult to fit, which accounts for the variation seen across experiments and the large error bars in Figures 2c-f. More accurate determination of quantitative MT parameters is needed and may be achieved by broadening the MT experimental range.

The apoptotic changes detected by MT are apparent before the changes in T1. They may also precede the changes detected by diffusion measurements that have been previously shown to be due to increased extracellular water fraction following cell shrinkage and clearance by macrophages[4]. Since water fraction may increase due to oedema and inflammation, MT offers a complementary approach for apoptosis detection, which is sensitive specifically to macromolecular interactions that are largely present inside the cell and much less sensitive to influxes of water. The parameters obtained in this study suggest that apoptosis decreases the number of macromolecular protons available for exchange, but increases the rate of proton exchange. In addition, there is a decrease in the T2 of the macromolecular pool, which suggests slower macromolecular motions and therefore changes in macromolecular structure (such as aggregation).

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