# Quantitative T1 and T2 relaxation in apoptotic cells in the presence of Gd-DTPA

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**INTRODUCTION:** Many cancer therapies attempt to limit tumour growth by inducing tumour cells to self-destruct in a systematic manner known as apoptosis. Apoptotic cells are characterized by distinct chemical and morphological changes. As demonstrated in Fig. 1, early changes include condensation of nuclear material, followed by fragmentation of the nucleus, membrane blebbing, fragmentation of the cell and a decrease in cell volume. High frequency ultrasound measurements have shown that these changes, in particular the nuclear condensation and fragmentation, can be detected as changes in the ultrasound backscatter amplitude[1]. It is, therefore, surprising that MRI parameters like T1 and T2 relaxation have shown little sensitivity to the effects of apoptosis, despite significant changes in cellular microstructure, such as cell shape and cell size (Fig.1). In this study, we have measured the relaxation properties of apoptotic cells in the presence of gadolinium (Gd) to establish whether extracellular contrast agents are useful in distinguishing between normal and apoptotic cells. We also used a two-pool model of relaxation to determine intrinsic characteristics of intra- and extracellular water and to evaluate exchange between water compartments.

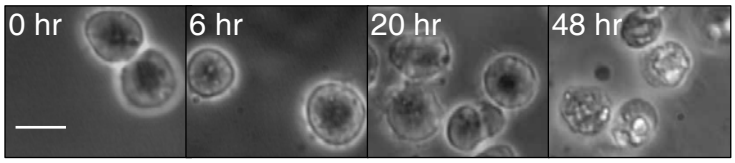


***Figure 1**. Optical microscope images with phase contrast after cisplatinum treatment show cells undergoing nuclear condensation (6 and 20 hours), nuclear fragmentation (20 hours), as well as membrane blebbing and fragmentation of the cell (48 hrs). The scale bar represents 10 μm.*

**METHODS:** Acute myeloid leukemia (AML-5) cells were grown in suspension and treated with cisplatinum (10 μg/mL) to induce apoptosis. Data collection by MRI occurred 36 hours after treatment. T1 was measured from 8 single slice inversion recovery (IR) images (TE=11ms, TR=2500ms, TI=50, 100, 200, 300, 500, 700, 900, 1500 ms, 1 NEX). T2 was measured with a CPMG sequence (TE=11 ms, TR=2500 ms, 2 NEX, 48 echoes). All data were acquired at 3 T (GE Signa, GE Medical Systems, Milwaukee, WI) with 8x8 $cm^2$ field of view, 4 mm slice thickness and 128x128 matrices. Data were fitted to a two-pool model of relaxation with exchange[2], shown in Fig. 2, to obtain the intracellular relaxation times $T1_I$ and $T2_I$, the intracellular-extracellular exchange rate $k_{IE}$ and the intracellular water fraction $M_{0I}$. Three Gd-DTPA concentrations were added to the extracellular solution and their values determined from IR images of the medium using a relaxivity of 4 mM/s and the Gd-free $T1_E$, 2910 ± 20 ms for control and 2600 ± 90 ms for treated. Extracellular T2 values were calculated with a relaxivity of 4.5 mM/s and the Gd-free $T2_E$ of 1910 ± 70 ms for control and 1520 ± 50 ms for treated. Calculated values of $T1_E$ and $T2_E$ at each concentration were then fixed, leaving $T1_I$, $T2_I$, $M_{0I}$ and $k_{IE}$ to be fit by the model. All experimental data (all Gd concentrations at all TI and TE values) were fitted simultaneously.

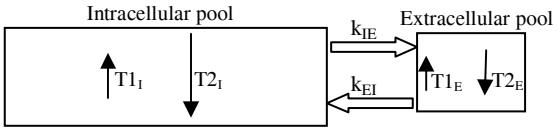


***Figure 2.** The two-pool model shows the intracellular and extracellular water fractions, $M_{0I}$ and $M_{0E}$ (where $M_{0I}+M_{0E}=1$), represented here by the sizes of the respective boxes. The intracellular pool has T1 and T2 relaxation times of $T1_I$ and $T2_I$, respectively, while those of the extracellular pool are represented by $T1_E$ and $T2_E$. Exchange of magnetization between the intracellular and extracellular pools occurs at a rate $k_{IE}$ and exchange in the reverse direction at a rate $k_{EI}$. Note $k_{EI}=k_{IE}*M_{0I}/M_{0E}$ so that the model has 6 independent parameters.*

**RESULTS:** Figure 3 shows a typical fit to the model, where T1 and T2 data were fitted simultaneously, keeping $M_{0I}$ and $k_{IE}$ the same in both cases. Table 1 gives a summary of the model parameters for control cells and cells treated with cisplatinum. With no Gd, the differences in relaxation times between control and treated samples were not significant (<T1>: 1120 ± 50 ms (control) and 1100 ± 130 ms (treated), <T2>: 74 ± 5 and 79 ± 7 ms). However, they were more pronounced with increasing Gd concentration (<T1>: 430 ± 20 and 240 ± 20 ms, <T2>: 53 ± 1 and 45 ± 3 ms for [Gd] = 4.8mM).

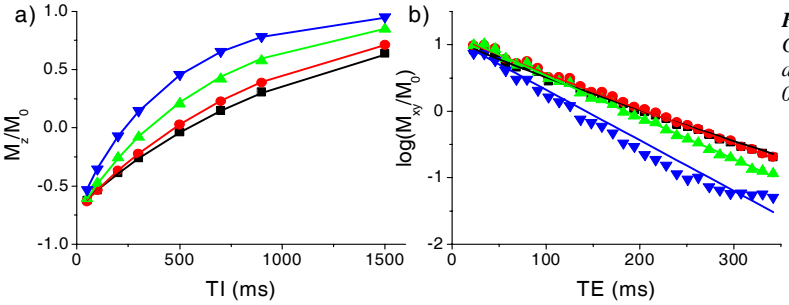


***Figure 3.** Representative fits of (a) the inversion recovery and (b) the CPMG data to the two-pool model (solid lines). Experimental data are shown for apoptotic cells at Gd concentrations of 0 mM (■), 0.35 mM (●), 1.5 mM (▲) and 4.8 mM (▼).*

**Table 1.** Results of the fit to the two-pool model for control cells and cells treated with cisplatinum.

| Parameter | $T1_I$ (ms) | $T2_I$ (ms) | $M_{0I}$ | $k_{IE}$ ($s^{-1}$) |
|---|---|---|---|---|
| Control | 1030 ± 50 | 64 ± 5 | 0.86 ± 0.01 | 1.9 ± 0.3 |
| Treated | 940 ± 10 | 59 ± 6 | 0.74 ± 0.05 | 5.5 ± 0.3 |

**DISCUSSION:** The differences in intracellular relaxation time, $T1_I$, between control and treated cells may be a result of the altered nuclear density and fragmentation occurring during apoptosis. This effect is likely due to increased magnetization transfer between intracellular water and macromolecules from the fragmented nuclei. Intracellular T2 appears to be affected by these changes as well. The decrease in $M_{0I}$ and increase in $k_{IE}$, the latter of which is proportional to the surface area to volume ratio of the cell, can be at least partially accounted for by a decrease in cell size. However, the spherical AML-5 cells should experience an increase in $k_{IE}$ of only 5% based on the decrease in intracellular volume indicated by $M_{0I}$. The main contribution to the $k_{IE}$ increase is likely an increased membrane permeability or increased surface area created by membrane blebbing. In the fast exchange approximation, the average relaxation time is $<T1>=(M_{0I}/T1_I+M_{0E}/T1_E)^{-1}$. In the absence of Gd, slight changes in intracellular T1 and $M_{0I}$ due to apoptosis are not sufficient to significantly change average T1. However, at high Gd concentrations, because extracellular relaxation times $T1_E$ and $T2_E$ are much shorter than intracellular relaxation times, changes in corresponding water fractions due to apoptosis result in significant differences in the average T1 and T2 between control and apoptotic samples. Thus Gd is able to make the cellular changes during apoptosis more visible to MRI. The results of this study also indicate that quantitative perfusion studies of tumours containing apoptotic cells should take into account the changes in the intra- and extracellular compartments.

**REFERENCES:**

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2) McConnell, H.M 1958. *J. Chem. Phys*. **28**: 430-431.