Mechanisms of apparent diffusion coefficient change at early and late stages of apoptosis

C. Bailey[1,2], A. Giles[1,3], G. J. Czarnota[1,3], and G. J. Stanisz[1,2]

[1]Medical Biophysics, University of Toronto, Toronto, ON, Canada, [2]Imaging Research, Sunnybrook Health Sciences Centre, Toronto, ON, Canada, [3]Radiation Oncology and Imaging Research, Sunnybrook Health Sciences Centre, Toronto, ON, Canada

INTRODUCTION: The apparent diffusion coefficient (ADC) has typically been applied as a measure of tumour response to therapy. The ADC usually increases in responders, a change which has been correlated with lower cell density and increased water content, including that linked to oedema and inflammatory response (1,2). Although these changes in the ADC occur earlier than visible shrinkage of the tumour, they still correspond to a relatively late stage of apoptotic cell death. Earlier changes in cell structure, including changes in shape due to membrane blebbing or increased permeability of the cell membrane should also influence the ADC. This study examines diffusion in an *in vitro* cell sample, a model that eliminates the confounding effects of inflammation and removal of dead apoptotic cells by macrophages. Additionally, the extracellular water fraction and exchange rate of these cells have been determined using a two-pool model with Gd-DTPA-BMA and corroborated by histology (3). The purpose of this study was to examine the mechanisms for changes in ADC at early (24 h post-treatment: nuclear condensation and fragmentation, see Figure 1) and late (36-48 h post-treatment: membrane blebbing, cell fragmentation and shrinkage) stages of apoptosis.

METHODS: Acute myeloid leukemia (AML-5) cells were grown in suspension and treated with 10 µg/mL of the chemotherapy drug cisplatin to induce apoptosis. Cells were centrifuged to create MRI samples. Thirty-six hours after cisplatin treatment, relaxation data were acquired at 3T (GE Signa, Milwaukee, WI) with 8 x 8 cm^2 field of view, 4 mm slice thickness and 128^2 matrices. T1 data were acquired with a multislice inversion recovery sequence (TE=11 ms, TR=2500 ms, 1 NEX, TI=50, 100, 200, 300, 500, 700, 900, 1500 ms). T2 data were acquired with a CPMG sequence (TE=11.4 ms, TR=2500 ms, 48 echoes, 2 NEX). Four concentrations of Gd-DTPA-BMA (Omniscan, GE Healthcare), ranging from 0 to 5 mM were added to the extracellular medium of the cells before centrifuging. Measurement of T1 in a slice through the extracellular medium was used to determine Gd-DTPA-BMA concentration. All relaxation data at all Gd-DTPA-BMA concentrations were then fitted to a two-pool model of relaxation with exchange, as has been described previously (3), to determine the intracellular relaxation times, the extracellular water fraction, M_{0E}, and the rate of water exchange from the intracellular to the extracellular space, k_{IE}. Diffusion data were collected at 1.5 T (GE Signa) with 12 x 12 cm^2 field of view, 3 mm slice thickness and 128^2 matrices at time points 24 and 48 hours after cisplatin treatment. A 3D axial fast spin echo sequence (TE=25.6 ms, TR=800 ms, 1 NEX) with diffusion gradients corresponding to b-values of 0, 317, 402, 500, 739, 1042, 1415, 1866 s/mm^2 was used. ADCs were determined by a weighted linear regression of the logarithm of the normalized signal plotted against b-value. In the limit of fast exchange, the measured ADC is a weighted average of the intra- and extracellular ADCs: $ADC=M_{0E}\cdot ADC_E+M_{0I}\cdot ADC_I$ [Eq. 1]. For slow diffusion in the intracellular space, the second term is negligible, so the approximation was made that $ADC\approx M_{0E}\cdot ADC_E=M_{0E}\cdot D_E\cdot(1/\lambda^2)$ [Eq. 2], where the free diffusion coefficient in the extracellular space, D_E, has been measured to be around 2 x 10^{-5} cm^2/s and λ is the tortuosity coefficient, dependent on the relative volume of the extracellular space and on the shape of the cells (4).

RESULTS: The percentage change in the MRI parameters (difference between post- and pre-treatment values over pre-treatment value) is summarized in Table 1. In the first two rows of Table 1, the average relaxation times in the absence of Gd-DTPA-BMA show no significant difference between control cells and those in the late stages of apoptosis. However, the fit of the two-pool model to the relaxation data for all Gd-DTPA-BMA concentrations showed a decrease in intracellular T1 from 1030 ± 50 ms 940 ± 70 ms when cells became apoptotic an increase in the extracellular water fraction M_{0E} from 0.14 ± 0.02 to 0.32 ± 0.06 and an increase in the exchange rate of water k_{IE} from 1.4 ± 0.6 to 6.6 ± 3.3 s^{-1}. ADC values ranged from 0.21 x 10^{-5} to 0.50 x 10^{-5} cm^2/s for controls. Because of the large biological variation, the change in ADC for apoptotic cells was compared with controls from the same experiment. The percentage change in ADC at early stages of apoptosis ranged from 10.6% decrease to 2.4% increase (no significant change from controls), but the ADC increased 35-67% at late stages of apoptosis. The average change in the ADC was used along with the value of M_{0E} to calculate the tortuosity from [Eq. 2]. This is shown in the last row of Table 1.

	Late stage change (%)
<T1>	-6 → 18
<T2>	-3 → 20
$T1_I$	-18 → -3
$T2_I$	-30 → -5
M_{0E}	11 → 28
k_{IE}	150 → 1030
ADC	35 → 67
$1/\lambda^2$	-41 → -27

***Table 1**. Summary of percentage change ((post-pre)/post) in parameters for relaxation data in the presence of Gd-DTPA-BMA (n=5) and diffusion parameters (n=4). Data are given as the range from all experiments.*

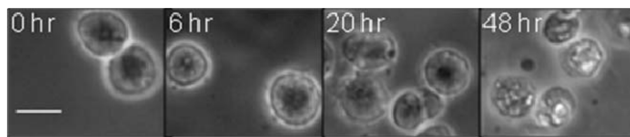

***Figure 1**. Phase contrast microscope images of the AML-5 cells in culture at the indicated time points after cisplatin treatment. Nuclear condensation and fragmentation are visible at 20 hours and membrane blebbing and some fragmentation of the cell are visible at 48 hours. The scale bar represents 10 µm.*

DISCUSSION: The results show that ADC is a better single measure of apoptotic changes, but it does not occur earlier than the two-pool model relaxation changes. The change in ADC from baseline shows that diffusion MRI can indeed indicate apoptosis, as has been shown *in vivo* previously. Unlike the changes demonstrated *in vivo*, however, this cell model is not sensitive to changes from inflammation or removal of dead cells by macrophages; it is sensitive to changes in the extracellular water content by cell shrinkage, movement of water from the intracellular space and changes in cell shape and membrane permeability

For spherical cells, the tortuosity is given by $1/\lambda^2=V_E^{0.5}$, where V_E is the extracellular volume fraction. If the spin density inside the cells is approximated as being equal to that outside then V_E is proportional to M_{0E}. This volume fraction change is not enough to account for the calculated change in $1/\lambda^2$. There must be additional changes in cell shape, such as those caused by membrane blebbing (Figure 1, 48 hour time point), to account for the increase in ADC.

There is no significant change in ADC at 24 hours, which agrees with previous findings that ADC is not a sensitive measure at early stages of apoptosis, although time points between those examined here might be investigated for ADC changes due to cellular swelling which occurs at intermediate stages of apoptosis. Although this study supports earlier findings that increase in extracellular water fraction influence the ADC, it also suggests there may be other apoptotic changes at a cellular level that are detectable with diffusion MRI.

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