Molecular magnetic resonance imaging: implications to the detection of apoptosis

P. Rapley[1], K. L. Rich[2], and M. Tassotto[1]

[1]Medical Physics, Thunder Bay Regional Health Science Centre, Thunder Bay, Ontario, Canada, [2]Physics, Lakehead University, Thunder Bay, Ontario, Canada

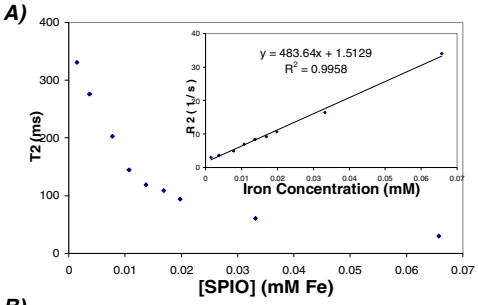
Introduction/Theory:

Molecular imaging is emerging as a potential tool for monitoring treatment response in the management of cancer patients. Recent cell biology as well as oncology research has focused on investigating apoptosis or programmed cell death as a means of measuring these induced effects and detecting response. A hallmark of early stage apoptosis is the externalization of phosphatidylserine (PS). The human protein annexin V with its high binding affinity for PS has been used ubiquitously in the biochemistry apoptosis FITC assay. As previously demonstrated in cardiomyocyte cells[1], annexin V + SPIO nanoparticles can provide sufficient MRI T_2 and T_2* contrast enhancement for *in-vivo* detection of apoptosis. The aim of the present study was to demonstrate the feasibility of imaging the therapeutic response of cancer cells by establishing a quantitative relationship between T_2 enhancement and apoptotic extent. Specifically, heat shock induced apoptosis in human leukemia HL60 cells *in-vitro* was probed by tagging with Annexin V+SPIO and subsequent analysis of the transverse relaxation (T_2) measured with a multi echo spin echo MRI acquisition. The results determined a linear relationship between spin-spin relaxivity (R_2) and the percentage apoptosis in the cell population.

Methods:

In order to detect early stage apoptosis, a number of methods were used. 100 percent apoptosis was induced in HL-60 (human leukemia) cells by placing cells in a 65ºC water bath for 15 minutes. One hour, post treatment, the level of apoptosis was determined by staining with propidium iodide and annexin V-FITC. In order to generate cell populations consisting of varying percentages of apoptotic cells, cells treated at 65ºC were mixed with untreated cells at known concentrations. The cells were analyzed by flow cytometry using a FACS Calibur flow cytometer (Becton Dickinson). The remainder of the cell population was stained with Annexin V MACS Microbeads (Mylteni Biotech) resuspended in a 50 µl volume of 1% carrageenan gel (Sigma Aldrich), placed in wells within a 384-well microplate, which was then analyzed by MRI (1.5 Tesla Seimens Avanto Clinical MRI scanner). A 16-echo spin-echo imaging sequence was used with echo times (TE) of 15-300 ms. The regions of interest (ROI) used to extract T_2 values consisted of approximately 68-70 pixels and R_2 values were obtained by taking the inverse of an average of 5 ROIs taken from 5 separate wells. Standard deviations were recorded and accurately propagated to produce error in the relaxation times. The results from the apoptosis data obtained by flow cytometry were then correlated with the T_2 relaxation rate from an MRI of the same population using SPIO molecules targeted to PS.

A)



B)

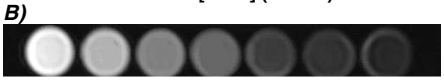


Figure 1: A) Calibration curve used to determine the influence of increasing the concentration of SPIO nanoparticles on the T_2 relaxation rate and R_2 relaxivity. B) MRI output images corresponding to the first 7 data points (SPIO concentration increases from left to right).

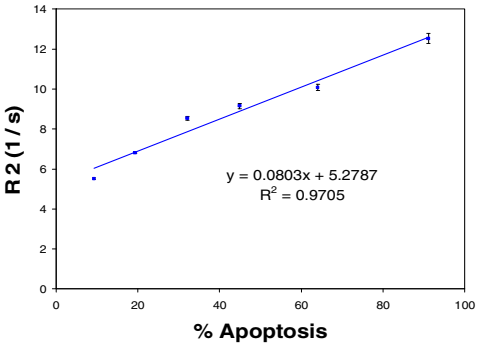


Figure 2: The correlation between level of apoptosis and spin-spin relaxivity rate (R_2). R_2 determined by MRI analysis of the same cell populations at a concentration of 120 x 10^6 cells/mL.

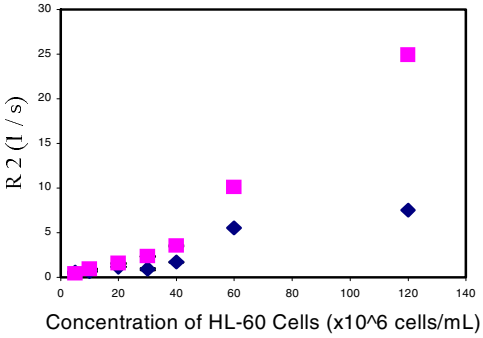


Figure 3: The effect of cell concentration on delta R_2. 100 % apoptotic cells (pink) were compared with untreated cells (navy) at various concentrations.

Results:

Figure 1 A) displays the calibration curve used to determine the influence of SPIO concentration on spin-spin relaxation time (T_2) and spin-spin relaxivity rate (R_2). This result demonstrates the fact that as the iron concentration increases its corresponding T_2 decreases exponentially and its R_2 increases linearly. Also, the increase in SPIO concentration results in a darkening of the area relative to the rest of the MRI output image (Figure 1B). Using flow cytometry in parallel with MRI analysis we correlated the spin-spin relaxivity rate (R_2) of a cell population tagged with SPIOs to the percentage of cells that were apoptotic (Figure 2). The results indicate a positive correlation between apoptotic cell death percentage and the spin-spin relaxivity rate (R_2). The slope of this relationship (which is described as delta R_2) indicates the sensitivity of this tool. A greater delta R_2 will allow a greater differentiation between certain levels of apoptosis. Figure 3 compares SPIO tagged cell concentrations for untreated and treated populations. Both untreated and treated populations are positively correlated to R_2. The interesting part of this result is that the difference between the two populations increases as the concentration of cells increases. Therefore a higher concentration of cells will create a greater difference in delta R_2 and thus increasing the sensitivity of this technique.

Discussion:

The detection of phosphatidylserine in early stage apoptosis can potentially be used to monitor the effectiveness of a given cancer therapy. In this investigation, the ease of targeting these SPIOs to specific biomarkers enabled us to detect different levels of cell death using a clinical MRI. Following cancer treatment, a patient could be injected with SPIOs and then the level of tumour cell death could be quantified using this mMRI technique.

[1] Sosnovik, D. E., E. A. Schellenberger, et al. (2005). "Magnetic resonance imaging of cardiomyocyte apoptosis with a novel magneto-optical nanoparticle." Magn Reson Med **54**(3): 718-24.