Chemotherapy-induced changes of intracellular water diffusion in cultured cells

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Introduction.

Both pre-clinical and clinical data indicates that the apparent diffusion coefficient (ADC) of tumor water increases in response to successful chemotherapy (Ctx) [1-3]. While the increase in ADC value measured at low *b*-values is believed to be correlated to a loss of cellularity, it cannot be used to determine the mode of cell death as both necrosis and apoptosis decreases the intracellular volume fraction [4]. A crucial difference between the mechanisms of cell death are the characteristics of the intracellular space due to changes in cell size (cell shrinkage during apoptosis, cell swelling during cytotoxic ischemia) as well as possible changes in the intrinsic intracellular diffusion constant. We recently presented a methodology to characterize diffusion properties of the intracellular space using a simple contrast agent (Gd-DTPA, Magnevist™) in cells cultured within a hollow-fiber bioreactor [4]. In this work we measure the intracellular water ADC in cultured cells undergoing chemotherapy to assess its usefulness for characterizing the mode of cell death.

Material and Methods.

All experiments were carried out at 9.4 Tesla on a Bruker AVANCE spectrometer (Karlsruhe, Germany) equipped with self-shielded imaging gradients (1000 mT/m max strength and 150 μs rise time). A custom made 27 mm dual tuned ($^{31}P/^{1}H$) birdcage RF probe was used for excitation and reception (Bruker Karlsruhe, Germany). Etoposide, an epipodophyllotoxin derivative which induces apoptosis by inhibiting topoisomerase II was added to the perfusate at a dose of 10 μM (IC 50: 25 μM). Rat glioma cells (C6) were obtained from ATC and routinely cultured in Dulbecco's modified Eagles medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Diffusion-weighted MRS was performed using a stimulated-echo experiment with a diffusion time Δ of 30 ms, and using 8 b values ranging from 20 to 1000 s/mm^2 (other parameters included; δ, 5ms; TE, 30 ms; TR, 1.5s). The ADC values were measured by fitting the intracellular signal decay to a mono-exponential decay.

Results and Discussion.

Fig.1 shows the fully relaxed spectrum obtained 15 min after adding the Gd-DTPA solution to the perfusate. The magnetic susceptibility shift in the resonance frequency of extracellular water induced by the Gd-DTPA uncovers the intracellular water and allows direct measurement of motional properties of the intracellular space. Fig.2 shows the ^{1}H spectra in presence of 5 mM Gd-DTPA prior to, 5 hours and 24 hours after addition of the drug. While the ^{1}H spectrum collected at 5 hours is virtually unchanged, the ^{1}H spectrum collected at 24 hours shows a decrease of the intracellular water signal consistent with the cell shrinkage (~ a 15% volume loss) characteristic of apoptosis. Concomitantly, no change could be seen in the ADC value measured 5 hours after drug exposure while a significant decrease in the ADC value is seen after 24 hours.

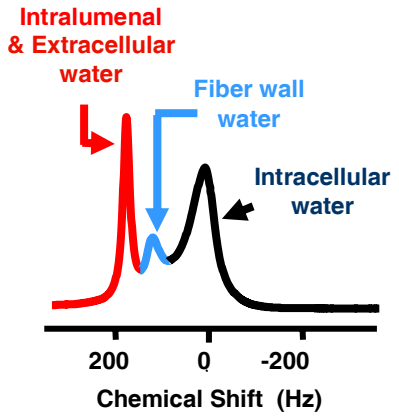

Fig.1: ^{1}H spectrum from C6 cells grown in a hollow-fiber bioreactor in presence of 5mM Gd-DTPA. Peaks assignment: — Intracellular water (+20 Hz), — Extracellular water (+185 Hz), — Intrafiber water (150 Hz).

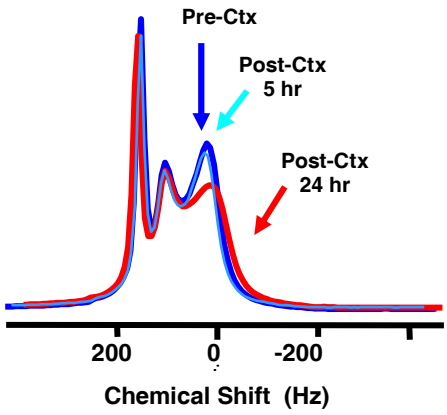

Fig.2: ^{1}H spectrum in the presence of 5 mM Gd-DTPA prior to (—) 5 hours (—) and 24 hours (—) after etoposide treatment.

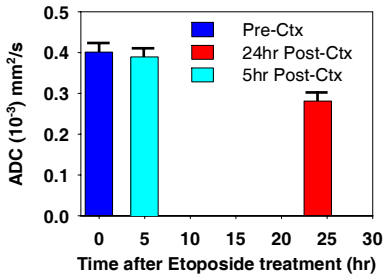

Fig.3: Intracellular ADC values prior to (■) 5 hours (■) and 24 hours (■) after etoposide treatment.

The observed decrease in intracellular ADC in C6 cells undergoing apoptosis is in contrast to the increase in ADC observed in C6 cells during ischemia [5]. The decrease in intracellular ADC could be due to an increase in restriction effect (cell shrinkage) and/or a decrease in the intrinsic intracellular apparent diffusion coefficient. This is consistent with the cell shrinkage observed in Fig.2. While the observation of an increased ADC maybe useful for clinical evaluation of Ctx response, a deeper understanding of the relationship between the diffusion properties of tumor water and the state of the cells would increase its utility in assessing treatment efficiency and in particular in characterizing the mode of cell death. Our preliminary data indicates that intracellular ADC changes in opposite directions during apoptosis and necrosis. These preliminary results support the concept of using the intracellular ADC as a marker of apoptosis in treated tumor cells.

References:

[1], Zhao *et al.*, Br J Cancer 73 : 61 (1996). [2], Chenevert *et al.*, Clin Cancer Res 3 : 1457 (1997). [3], Theilmann, *et al.*, Neoplasia 6:831 (2004). [4], Galons *et al.*, MRM 54:79 (2006). [5], Trouard et al., ISMRM Miami, (2005).