ADC increase following radiotherapy is a proxy for necrotic but not apoptotic cell death in LoVo Tumour Xenografts

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Background

ADC has been proposed as a biomarker of treatment efficacy in cancer and ADC increase may reflect cell death [1]. Several pre-clinical studies have reported increases in ADC following either radiotherapy or chemotherapy [2-4] and there is evidence that this increase in ADC may be a useful biomarker of response in clinical studies of solid tumours [5-6]. However, there is a lack of data examining the pathophysiological basis for early ADC increase and how this may relate to tumour response to therapy. Some studies have reported that apoptosis is the dominant mechanism of cell death following radiotherapy [7]. The objective of this study was to test the hypothesis that ADC changes following external beam radiotherapy and 5-FU chemotherapy represent histologically-assessed changes in necrosis and apoptosis, measured in a mouse model of colorectal cancer.

Methods

Study design: Animal studies were performed in compliance with the NCRI Guidelines for the welfare and use of animals in cancer research and UK Animals (Scientific Procedures) Act after local Ethical Committee review [8]. Tumour xenografts were initiated from LoVo colorectal tumour cells (5 x 10^6 per mouse) implanted in female nu/nu CBA mice aged 10 weeks old. When tumours reached 300-400 mm^3 in size, mice were randomised to a control group (sham; n=9 for radiotherapy or n=6 chemotherapy) or to treatment (10Gy; n=10 for radiotherapy and 5-FU 50mg/kg ip; n=6 for chemotherapy). Imaging was performed at 1 hour and 72 hours post radiotherapy and 1 hour pre and 72 hours post chemotherapy along with measurement of tumour volume by callipers. Tumours were excised 1 minute after euthanizing animals and rapidly snap frozen for histology.

Image acquisition and analysis: MRI was performed on a 7T Bruker scanner with a volume transceiver. Diffusion-weighted imaging (spin-echo sequence; TR/TE =2250/20ms; b values 150, 500 and 1000 s/mm^2; 15 contiguous slices of 0.6mm thickness; matrix 128 x 128 and FOV 2.56cm x 2.56cm) was performed after localisation with a T_2-weighted anatomical sequence. Voxel-wise values of ADC were calculated in *Matlab* (Mathworks) across the tumour using a least squares fitting algorithm and assuming Rician noise. Mean ADC was computed for both time points. ADC measurements were validated using an ice water phantom [9].

Histological analysis: 5 µm cryostat sections were prepared from the tumours. Necrosis was estimated by H&E staining sections and the degree of apoptosis using a fluorometric terminal transferase dUTP nick end labelling (TUNEL) assay that detects DNA degradation in apoptotic cells (DeadEnd™, Promega). Percentages of necrosis and apoptosis were determined by analysis of the stained sections.

Statistical analysis: Changes in tumour volume were compared by a paired Student's t test. ADC and histology between 1 hour and 72 hours post radiotherapy and 1 hour pre and 72 hours post chemotherapy were compared between the two groups with an unpaired heteroskedastic one tailed Student's t test. This test was also used to compare absolute ADC changes in the two groups. The change in ADC was plotted against percentage necrosis for both treated groups as in figure 2, with a Spearman's rho performed to test for significance. Statistical analysis was performed in *SPSS 16.0*.

Results and Discussion:

ADC values of 1.16 x10^{-3} mm^2/s (sd 0.035x10^{-3} mm^2/s) were obtained using the ice water phantom, providing measurement validation. There was no significant difference in tumour size or ADC value between the control and treated groups after 1 hour in the radiotherapy study or at baseline in the chemotherapy study. Following radiotherapy a significant difference was measured in tumour growth at 72 hours (p=0.003) and in mean ADC (p=0.03) with growth stabilisation and increase in ADC values (Figure 1). The percentage necrosis was significantly greater following radiotherapy compared with control (p=0.001) but no significant difference was seen with apoptosis (p=0.094). ADC correlated positively with necrosis but not apoptosis at 72 hours. The chemotherapy-treated group observed a small but significant decrease in ADC at 72 hours compared with control (p=0.001) and relative growth stabilisation (p=0.01). For both the radiotherapy and chemotherapy studies the ADC change on a per animal basis was significant (p=0.003 and p=0.006 respectively). Neither necrosis nor apoptosis showed significant differences between treated and control in the chemotherapy study and neither correlated with ADC at 72 hours. The percentage necrosis against ADC change (figure 2) in the radiotherapy group was significant (rho=0.65; p=0.049) but not in the chemotherapy group (rho=0.1429; p=0.80). These findings support the hypothesis that ADC increase following treatment is a proxy for induced necrotic cell death.

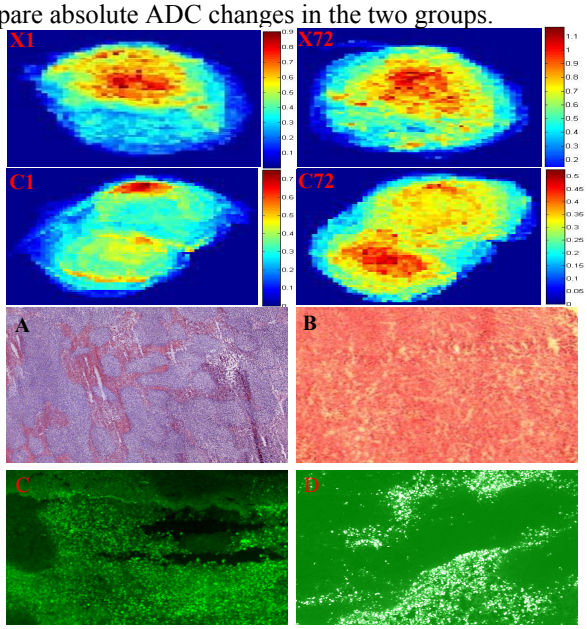

Figure 1: ADC maps in radiotherapy (X) and chemotherapy groups (C) at 1(C1/X1) and 72 (C72/X72) hours. Images A and B represent H&E stained sections for radiotherapy and chemotherapy respectively while C and D are TUNEL stained sections.

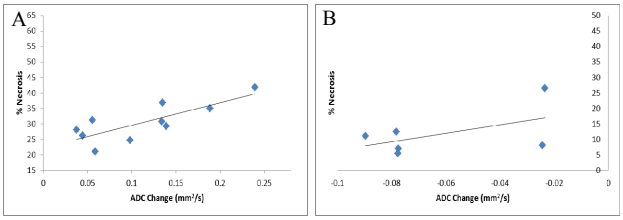

Figure 2: Correlation between ADC change (x axis) and % necrosis (y axis) for radiotherapy study in A and chemotherapy study in B.

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