In Vivo Measurement of the Hypoxia Marker EF5 using ^{19}F NMR Spectroscopy

M. N. Hoff[1], D. T. Yapp[2], A. C. Yung[3], T. S. Oliver[2], and P. Kozlowski[3,4]

[1]Physics, University of British Columbia, Vancouver, British Columbia, Canada, [2]Advanced Therapeutics, BC Cancer Research Center, Vancouver, British Columbia, Canada, [3]UBC MRI Research Centre, University of British Columbia, Vancouver, British Columbia, Canada, [4]Department of Radiology, University of British Columbia, Vancouver, British Columbia, Canada

Introduction

Due to the resistance of hypoxic cells to radiation therapy [1], the *in vivo* measurement of hypoxia in tumours could serve as a prognostic indicator for cancer treatment planning. The selective retention of bound adducts of 2-nitroimidazoles with cellular macromolecules in hypoxic regions of tumours [2] and the subsequent detection of these adducts has proven to be an accurate method of hypoxia measurement [3]. In order to determine levels of hypoxia *in vivo* in the Shionogi tumours of DDS mice, this study attempted to quantify adducts of the pentafluorinated 2-nitroimidazole EF5 using semi-quantitative fluorine (^{19}F) magnetic resonance spectroscopy (MRS). The validity of the technique was tested through comparisons to flow cytometry of excised tumours, a proven *ex vivo* method of EF5 adduct detection [3].

Materials and Methods

DD/S mice were injected subcutaneously in the back with Shionogi SC-115 androgen-dependent mouse mammary carcinoma cells. Three experimental groups of mice were prepared for MRS when tumours were 1.5 cc in size. Experimental mouse groups 2 and 3 were anaesthetized with Avertin and injected intravenously (IV) with EF5 2–3 weeks after inoculation (androgen-dependent tumours). Mice in group 1 were castrated, and 5–10 weeks following castration were anaesthetized with isofluorane and IV injected with EF5 (androgen-independent tumours). Preparations for MRS analysis occurred at two hours post-EF5-injection for mouse group 1, and at three hours post-EF5-injection for mouse groups 2 and 3. Mice were secured in a 7.05T/30cm Bruker scanner and positioned with their tumours within a 2 cm diameter solenoid coil tunable to ^{1}H and ^{19}F frequencies. A small glass sphere filled with 6 μMoles of trifluoroacetic acid (TFA) was placed below the tumour to serve as an external reference. After shimming on ^{1}H signal, a single pulse-acquisition (50μs 90° pulse, SW=25 kHz, T_R = 1.5 s, NA=1200) was used to acquire ^{19}F spectra from the tumours. The data were input into jMRUI, and the time-domain curve fitting algorithm AMARES was used to quantify peak areas in each spectrum. Following MRS, the tumours were excised, processed and examined for hypoxic cells with flow cytometry, as described previously [4].

Results

Fig. 1 shows a sample MR spectrum acquired *in vivo* five hours after EF5 injection into a mouse bearing an androgen-independent tumour. For each mouse in all three mouse groups, the EF5 CF_3/TFA peak area ratios were compared to the associated tumours' hypoxic cell percentage determined from flow cytometry. Fig. 2 shows plots of the CF_3/TFA peak ratios as a function of the hypoxic cell percentage for each mouse in group 1 (left), 2 (center), and 3 (right). Group 1 shows an inverse correlation between EF5 signal and hypoxic cell percentage that is highly significant (P-value < 0.01). Group 2 shows a similar inverse correlation, albeit with a marginal level of significance (P-value = ~0.07). No statistically significant correlation was found in group 3. The viability level (live cells/total cells) measured in cell samples prepared for flow cytometry was 34 ± 26 % for the androgen-independent tumours of group 1, and 7.2 ± 5.8 % for the androgen-dependent tumours of group 2 and 3 (5.4 ± 5.7% and 9.4 ± 5.7% respectively).

Discussion

The existence of a negative correlation between MRS and flow cytometry data in viable tumours supports the theory that MRS could be used to detect metabolites of EF5 which result from non-macromolecular binding. The prevalent theory is that due to the lack of mobility of EF5 bound to large molecules, MRS only measures sufficiently mobile adducts with low molecular weight (LMW) thiols such as glutathione (GSH) [5]. In contrast, flow cytometry measures only cellular macromolecular adducts of EF5. Macromolecular binding has been found to be inhibited by the presence of GSH [6], and the effects of 2-nitroimidazoles have been found to increase with the depletion of thiols [7]. A competition model can characterize this metabolic behaviour, elucidating the inverse correlation present in mouse group 1 (Fig. 2, left). The caveat to this theorem is that an inverse correlation will only be demonstrated if the metabolic environment is sufficiently viable to permit binding. The lack of significant correlation in mouse groups 2 and 3 is potentially due to the high levels of necrosis, which prohibit the occurrence of any form of binding. The well vascularized androgen-independent tumours of group 1 have viability levels five times greater then groups 2 and 3, and display a strong inverse correlation.

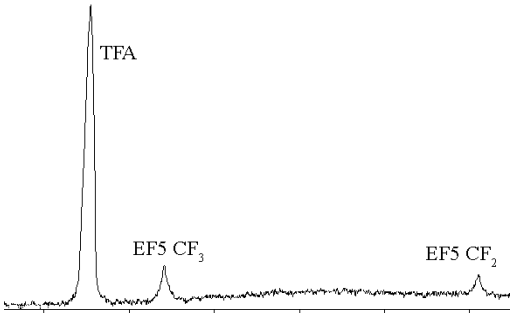

Fig. 1. *In vivo* ^{19}F MRS spectrum from a Shionogi tumour five hours after intraperitoneal injection of EF5.

Conclusion

While ^{19}F MRS of EF5 in mice with Shionogi tumours is ineffectual as a means of measuring hypoxia, there are strong indications that the technique can be used to measure adducts of EF5 with LMW thiols such as GSH.

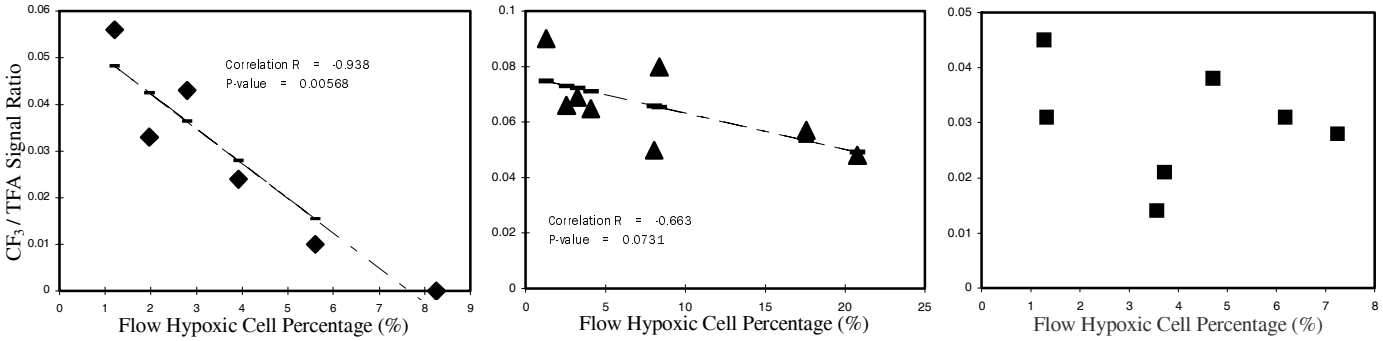

Fig. 2. ^{19}F CF_3/TFA magnetic resonance spectroscopy (MRS) signal ratio vs. flow cytometry hypoxic cell percentage for mouse group 1 (left), mouse group 2 (middle), and mouse group 3 (right). Statistical regression analysis data is given in all but group 3, where no correlation is found.

Acknowledgment

This work was supported by the Canadian Prostate Cancer Research Initiative.

References

[1] Gray LH et al. Br J Radiol. 1953;26:638; [2] Chapman JD et al. Br J Cancer. 1981;43:546; [3] Lord EM et al. Cancer Res. 1993;53:5721; [4] Skov K et al. Radiat Res. 2004;162:547; [5] Robinson SP et al. Philos Trans R Soc Lond B Biol Sci. 2004;359:987; [6] Chapman JD et al. Cancer Res. 1983;43:1523; [7] Bump EA et al. Science. 1982;217:544.