

NON-INVASIVE MONITORING OF GLUTATHIONE METABOLISM AND HETEROGENEITY IN RAT TUMOR TISSUE

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Introduction

Glutathione (GSH) is a tri-peptide of glutamate, cysteine and glycine. It is one of the primary antioxidants found in tissue and plays a major role in cancer development and therapy response (1-4). The development of a non-invasive technique to measure glutathione concentration and metabolism provides a valuable insight into tissue redox balance under normal and physiologically perturbed conditions. Studies have suggested that glutathione synthesis rate could be a biomarker for drug resistance as tumor cells recruit antioxidant defenses to protect themselves from chemotherapeutic agents (5, 6).

We have developed a novel technique to non-invasively monitor glutathione synthesis rate and concentration in *in vivo* tumor models. We are applying this to studies of therapy response and resistance in rat tumor models. Infusing tumor-bearing rats with [2-¹³C]-glycine isotopically labels the glycine pool, with resultant labeling of glutathione by the action of glutathione synthetase. Glycine and GSH distributions in the tissue can then be non-invasively imaged with ¹³C chemical shift imaging, and GSH synthesis rate determined by monitoring rate of label incorporation.

Experimental

FSA fibrosarcoma tumors were propagated by implantation of tissue fragments in the inguinal region of Fischer 344 rats. Tumors were imaged when approximately 1 cm diameter, typically 2-3 weeks after implantation (n=6). [2-¹³C]-Glycine was infused (1 mmol/kg/hr, 0.5 ml/hr) via a jugular vein catheter and Instech-Soloman infusion harness. MR data were acquired at 12 and 36 hours after the start of [2-¹³C]-glycine infusion, using the AMRIS 40cm 11.1T horizontal bore magnet (Magnex Scientific) interfaced to a Bruker console. A 1.2 cm diameter surface coil tuned to 118 MHz (¹³C frequency) and a 3 cm surface coil tuned to the 470 MHz (¹H frequency) placed orthogonally to each other, the latter used for ¹H imaging and decoupling. 2D ¹³C CSI were acquired into 8 × 8 phase encode steps, employing variable k-space averaging pulse sequences with a scan time of 70 min. The repetition time was 1.5 sec and the tip angle was approximately 45°. Excised tumors were frozen and high resolution *ex vivo* MR data were acquired from perchloric acid tumor tissue extracts.

Results and Discussion

Intravenous infusion of [2-¹³C]-glycine for 12 hours resulted in ¹³C-labelled glutathione at concentrations sufficiently high for imaging with 2D ¹³C CSI. Fig. 1 shows data from a 2D-CSI dataset acquired from *in vivo* tumor tissue, an *ex vivo* tumor spectrum from the same tissue, and images of glycine and glutathione distribution. Analysis of *ex vivo* PCA extracts demonstrated a tumor tissue glutathione concentration of 2.2 ± 0.9 μmol/g tissue (mean ± SD, n = 8), with a glutathione enrichment of 35.9 ± 1.2%. Preliminary measurements demonstrated a tumor tissue glycine concentration of 6.1 ± 1.2 μmol/g tissue (mean ± range, n=2).

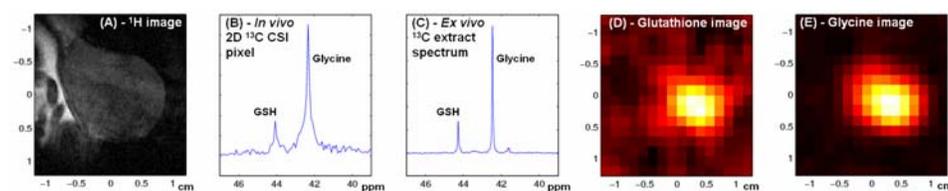


Figure 1 (A) – ¹H RARE image of FSA tumor. (B) – *In vivo* ¹H-decoupled ¹³C spectrum from one 1.5 × 1.5 mm pixel of 2D-CSI dataset. (C) – *Ex vivo* ¹H-decoupled ¹³C spectrum of a PCA tumour tissue extract. (D,E) – GSH and Glycine images generated from ¹H-decoupled ¹³C 2D-CSI dataset. All data acquired after 12h of [2-¹³C]-glycine infusion at 1 mmol/kg/h.

Discussion and Conclusions

We have developed an innovative method to monitor glutathione concentration and synthesis rate, by observing the incorporation of ¹³C-labelled glycine into cellular glutathione. 2D glutathione images with a resolution of 1.5 × 1.5 mm were obtained. Good agreement between *in vivo* and *ex vivo* extract ¹³C spectra was observed. The method has potential for increased spatial and/or temporal resolution with indirect ¹³C detection methods. Future studies will assess the impact of chemo- and radiotherapy on tumor glutathione levels, and assess whether glutathione metabolic rate and content can predict treatment response.

Acknowledgements and References

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