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. Previous studies have used ^{1}H chemical shift imaging (CSI) to measure glutathione content in human brain tumors (4), but are unable to measure the glutathione synthesis rate. Studies have suggested that glutathione synthesis rate, rather than concentration, 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-^{13}C]-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 ^{13}C chemical shift imaging, and GSH synthesis rate determined by monitoring rate of label incorporation.

Methods

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-^{13}C]-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-^{13}C]-glycine infusion, using a 40cm 11.1T horizontal bore magnet (Magnex Scientific) interfaced to a Bruker console. Rats were anesthetized with isoflurane for restraint purposes. A 1.2 cm diameter surface coil tuned to the ^{13}C frequency (118 MHz) was placed against or around the tumor, and orthogonally-oriented 3 cm surface coil tuned to the ^{1}H frequency (470 MHz) was placed below the tumor and used for ^{1}H imaging and decoupling. 2D ^{13}C 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°. Rats were euthanised on completion of the MR acquisitions and tumors excised and frozen. High resolution *ex vivo* MR data were acquired from perchloric acid tumor tissue extracts.

Results

Intravenous infusion of [2-^{13}C]-glycine for 12 hours resulted in ^{13}C-labelled glutathione at concentrations sufficiently high for imaging with 1D and 2D ^{13}C CSI. Studies of the incorporation of [2-^{13}C]-glycine into glutathione in normal brain tissue suggested that the sensitivity of this technique was <0.1 µmol/g tissue (data not shown). Figure 1 shows 1D CSI data acquired at 12 and 36 hours after the start of [2-^{13}C]-glycine infusion, glycine levels appear to reach steady state within 12 hours, but an increase in GSH can be seen between the 12 and 36 hour datasets. Figure 2 shows data from a 2D-CSI dataset acquired from *in vivo* tumor tissue, an *ex vivo* tumor spectrum from the same tissue, images of glycine and glutathione distribution and the ratio of GSH to glycine. 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).

Discussion and Conclusions

We have developed an innovative method to monitor glutathione concentration and synthesis rate, by observing the incorporation of ^{13}C-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 ^{13}C spectra was observed. The method has potential for increased spatial and/or temporal resolution with indirect ^{13}C 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.

Acknowledgments and References

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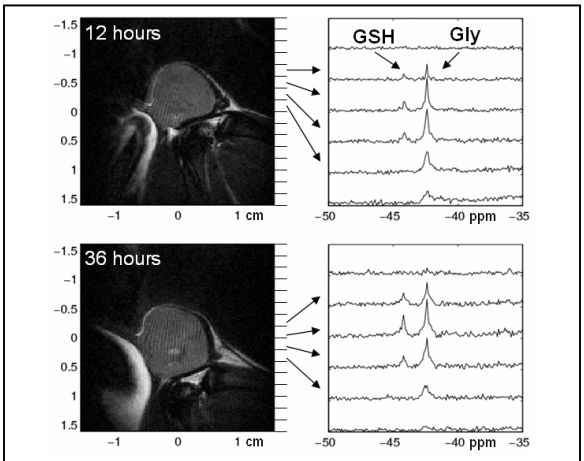

Figure 1 – ^{1}H RARE images and spectra from ^{13}C 1D-CSI datasets acquired from an FSA tumor after 12 and 36 hours of intravenous [2-^{13}C]-glycine infusion. An increase in GSH signal can be seen between the 12 and 36 hour datasets.

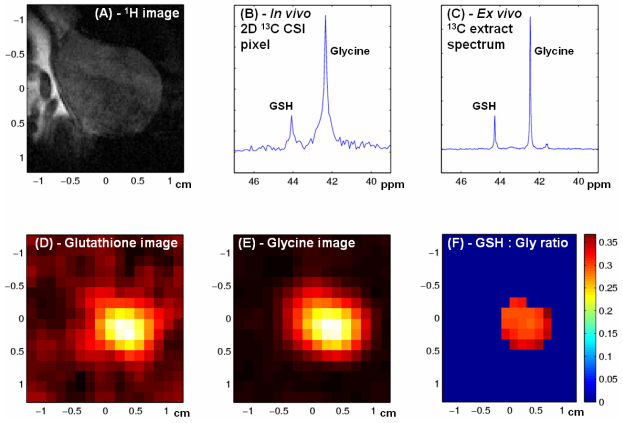

Figure 2(A) – ^{1}H RARE image of FSA tumor. (**B**) – *In vivo* ^{1}H-decoupled ^{13}C spectrum from one 1.5 × 1.5 mm pixel of 2D-CSI dataset. (**C**) – *Ex vivo* ^{1}H-decoupled ^{13}C spectrum of a PCA tumour tissue extract. (**D,E**) – GSH and Glycine images generated from ^{1}H-decoupled ^{13}C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