In vivo magnetic resonance studies of glutathione metabolism and glutathione distribution in a rat mammary tumour


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Introduction
Glutathione (GSH) is a tripeptide found in almost all tissues, where it acts as an antioxidant and forms part of the cellular defences against oxidative stress. Tumours exhibit higher reduced intracellular environments, characterized by high levels of reduced GSH compared to its oxidized disulfide counterpart (1). Higher levels of GSH and its associated enzymes appear to play a significant role in therapy-resistance (2) and reduced overall survival (3). We’ve previously used 13C MRSI to image GSH distribution in rat fibrosarcoma tumours, using 13C-glucose infusion to introduce a 13C label into GSH and thus provide a biomarker synthesis rate and concentration. The fibrosarcoma studies demonstrated high GSH levels (>2 μmol/g-tissue). We have extended our studies to the noninvasive detection of GSH in a rat mammary R3230Ac adenocarcinoma, allowing us to test our ability to detect and image GSH distribution in tumours with lower average levels of GSH, and also to probe the metabolic fate of 13C-labelled glycine via serine hydroxymethyltransferase and other pathways.

Methods
R3230Ac tumour fragments were implanted into female Fisher 344 rats and grew to 0.5 – 1cm3 tumours within 3 weeks. A catheter for 13C-glucose infusion was placed in the external jugular vein, a fitted harness and infusion lines allowed free rat movement during the infusion. [2-13C]-glucose was infused at rates of 0.5 or 1 mmole/kg/h for between 20 and 40h. MR data were acquired on an 11T spectrometer. A 15mm diameter 13C surface coil was placed around the tumour and a 3cm diameter 1H surface coil positioned orthogonally, below the tumour. Non-localised 1H-decoupled 13C spectra were acquired using a nominal 90° pulse-acquire sequence (TR = 1.5s, 400 averages, WALTZ decoupling, sw=10kHz). 2D CSI datasets were acquired into an 8x8 matrix over a 2.4 x 2.4 cm field of view, (TR = 1.5s, 1:128 averages, WALTZ decoupling, sw=10kHz). The scan duration was 72 minutes. Tumours were excised at the end of MR experiments and tissue acid extract samples prepared for high resolution MRS, HPLC and mass spectrometry.

Results
13C-glucose infusion into tumour-bearing rats had no effect on tumour GSH or cysteine levels, though an elevation in tissue glycine and serine content was observed (Table 1). Figure 1 shows in vivo non-localised 13C spectra from a control (A, no glucose) tumour, and from tumours following 30h and 24h of [2-13C]-glucose infusion at rates of 0.5 (B) and 1 (C) mmole/kg/h respectively. Resonances originate from [2-13C]-glucose at 42.4 ppm, and from incorporation of labelled glycine into the glycyl-residue of GSH at 44.2 ppm. Additional resonances are detectable at 54.8, 57.4 and 61.3 ppm, tissue extract analysis enabled assignment of these resonances to creatine, [2-13C]-serine and [3-13C]-serine respectively. 2D CSI spectra were acquired following 24h of [2-13C]-glycine infusion at doses of 0.5 and 1 mmole/kg/h. Sufficient 13C signal from labelled glycine and GSH for CSI datasets was obtained only when the higher dose was employed. Figure 2 shows a typical CSI dataset acquired after 24h of labelled glycine at a dose of 1 mmole/kg/h, the spectra matrix shows spatially localised GSH and glycine peaks, and metabolic images of 13C-labelled GSH and glycine are shown. Mass spectrometry of tissue acid extract samples provided information on the extent of GSH 13C-labeling. Our data demonstrated labelled, single 13C-labelled and doubly labelled GSH. Tandem mass spectrometry demonstrated that doubly labelled GSH had 13C at its glycine and cysteine residues, indicating that a proportion of cellular cysteine had become 13C-enriched prior to incorporation into GSH. Mass spectrometry indicated that 6% of the cysteine pool was 13C-enriched after 40h of infusion at 0.5 mmole/kg/h, formed via the transsulphuration pathway with 13C-serine as an intermediate (4).

Discussion and Conclusions
The mean GSH concentration in the R3230Ac tumours was 1.22 μmol/g-tissue, 59% of the level found in our earlier study of FSA fibrosarcoma xenografts (5). GSH levels in both R3230Ac and FSA tumours were unaltered by glycine infusion at the concentrations employed. Detection of GSH by in vivo 13C MRS requires incorporation of a significant fraction of 13C-label into GSH, and the amount of label incorporated is a function of the rate at which GSH is metabolised. Lower tissue GSH concentrations manifest as lower signal to noise ratio, but we demonstrated that raising tissue glycine and GSH 13C fractional enrichment by increasing 13C-glucose dose provided sufficient SNR for acquisition of 2D CSI datasets. As with the FSA tumours, GSH appears most prominently in the periphery of the R3230Ac tumours in CSI data, as confirmed by histology (data not shown). The ability to detect serine labelled in vivo allows us to probe another pathway in tumour biochemistry. The metabolism of [2-13C]-glycine to [2-13C]-serine occurs via the serine hydroxymethyltransferase pathway with the methylene group transferred from 5,10-methylene tetrahydrofolate (m-THF). Access to the m-THF pathway via labelling of the glycine pool may allow assessment of an alteration in m-THF reductase activity, an enzyme central to folate metabolism and a target for cancer chemotherapy. In addition, incorporation of 13C label into cysteine (via serine) provides a handle on cysteine biosynthesis and the transsulphuration pathway. Our studies have demonstrated that in vivo 13C MRS can be used to provide information on the synthesis and distribution of GSH in tumour tissue, and provide additional information on other metabolic pathways of relevance to tumour therapy.

References and Acknowledgements

Table 1. Figures 1 and 2. Table: change in tumour glutathione, cysteine, glycine and serine content after glycine infusion. Figure 1: in vivo 13C spectra before (A) and after (B) 13C-glucose infusion at 0.5 (B) and 1 (C) mmole/kg/h. Figure 2: tumour 1H image and 13C-CSI data acquired after 24h of 13C-glycine infusion at 1 mmole/kg/h, showing GSH and glycine resonances (spectra matrix) and images.