In vivo magnetic resonance studies of glycine 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. Cancerous cells exhibit highly 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 ^{13}C MRSI to image GSH distribution in rat fibrosarcoma tumours, using ^{13}C-glycine infusion to introduce a ^{13}C label into GSH and thus provide a biomarker synthesis rate and concentration. The fibrosarcoma studies demonstrated high GSH levels (>2 μmol/gram-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 ^{13}C-labelled glycine via serine hydroxymethyltransferase and other pathways.

Methods

R3230Ac tumour fragments were implanted into female Fisher 344 rats and grew to 0.5 – 1cm^3 tumours within 3 weeks. A catheter for ^{13}C-glycine infusion was placed in the exterior jugular vein, a fitted harness and infusion lines allowed free rat movement during the infusion. [2-^{13}C]-glycine 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 ^{13}C surface coil was placed around the tumour and a 3cm diameter ^{1}H surface coil positioned orthogonally, below the tumour. Non-localised ^{1}H-decoupled ^{13}C spectra were acquired using a nominal 90º 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 extracts prepared for high resolution MRS, HPLC and mass spectrometry.

Results

^{13}C-glycine 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 ^{13}C spectra from a control (A, no glycine) tumour, and from tumours following 30h and 24h of [2-^{13}C]-glycine infusion at rates of 0.5 (B) and 1 (C) mmole/kg/h respectively. Resonances originate from [2-^{13}C]-glycine at 42.4 ppm, and from incorporation of labelled glycine into the glycinyl-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-^{13}C]-serine and [3-^{13}C]-serine respectively. 2D CSI spectra were acquired following 24h of [2-^{13}C]-glycine infusion at doses of 0.5 and 1 mmole/kg/h . Sufficient ^{13}C 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 metabolite images of ^{13}C-labelled GSH and glycine are shown.
Mass spectrometry of tissue acid extract samples provided information on the extent of GSH ^{13}C-labelling. Our data demonstrated unlabelled, single ^{13}C-labelled and doubly labelled GSH. Tandem mass spectrometry demonstrated that doubly labelled GSH had ^{13}C at its glycine and cysteine residues, indicating that a proportion of cellular cysteine had become ^{13}C-enriched prior to incorporation into GSH. Mass spectrometry indicated that 6% of the cysteine pool was ^{13}C-enriched after 40h of infusion at 0.5 mmole/kg/h, formed via the transsulphuration pathway with ^{13}C-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 unperturbed by glycine infusion at the concentrations employed. Detection of GSH by *in vivo* ^{13}C MRS requires incorporation of a significant fraction of ^{13}C-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 ^{13}C fractional enrichment by increasing ^{13}C-glycine 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-^{13}C]-glycine to [2-^{13}C]-serine occurs via the serine hydroxymethyltransferase pathway with the methylene group transferred from 5,10-methylenetetrahydrofolate (m-THF). Access to the m-THF pathway via labelling of the glycine pool may allow assessment of an alteration in m-THF reductate activity, an enzyme central to folate metabolism and a target for cancer chemotherapy. In addition, incorporation of ^{13}C label into cysteine (via serine) provides a handle on cysteine biosynthesis and the transsulphuration pathway. Our studies have demonstrated that *in vivo* ^{13}C 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

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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 ^{13}C-glycine infusion at 0.5 (B) and 1 (C) mmole/kg/h. Figure 2: tumour ^{1}H image and ^{13}C-CSI data acquired after 24h of ^{13}C-glycine infusion at 1 mmole/kg/h, showing GSH and glycine resonances (spectra matrix) and images.

Infusion dose (mmol/kg/h)	Infusion time (h)	GSH	Cysteine	Glycine	Serine
		(μmol/g-tissue)			
Control (n = 4)	-	1.2 ± 0.3	0.18 ± 0.07	2.8 ± 0.6	0.9 ± 0.1
0.5 (n = 6)	31-46	1.3 ± 0.4	0.14 ± 0.04	4.5 ± 1.0	1.4 ± 0.6
1.0 (n = 3)	24	1.3 ± 0.2	0.20 ± 0.02	6.0 ± 1.0	1.5 ± 0.4