

¹³C-labelling and non-invasive detection of glutathione in human liver

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Introduction

Oxidative stress in a tissue results from an excess of reactive oxygen species, resulting in an inability of cells to maintain a reduced intracellular environment. Liver disease arises from broad range of processes, but oxidative stress is common to these processes and central to the mechanisms by which liver tissue damage occurs (1). The liver has multiple defences against oxidative stress, and glutathione (a tripeptide) is the principal intracellular antioxidant in liver tissue. Glutathione has a central role in maintaining a reduced intracellular environment, detoxifying xenobiotics and protecting against reactive oxygen species (2). Many studies have validated the importance of glutathione in liver disease, often using invasive (eg biopsy) methods to monitor glutathione content. We have developed non-invasive methods to measure *in vivo* liver glutathione concentration and synthesis rate, providing a dynamic measurement that is responsive to changing cellular oxidative stress defences (3). Our preclinical studies (3) have demonstrated that we can incorporate a ¹³C label into glutathione by administering ¹³C -labelled glycine to rats, as glycine is one of the building blocks of glutathione. By measuring the rate of appearance and magnitude of the ¹³C -label in glutathione we can report on glutathione concentration and synthesis rate, allowing quantification of tissue oxidative stress defences. Here we report the translation of our methods to human studies, successfully showing non-invasive *in vivo* detection of metabolically produced ¹³C-labelled glutathione in the liver of human subjects.

Methods

Three volunteers were recruited to the study from the staff of Newcastle University, with ethics permissions. A blood sample was collected and analysed for normal liver function prior to MR experiments. Subjects abstained from alcohol for 3 days prior to the MR study day and fasted from 11.30 pm on the evening before the study (water *ad libitum*). The study commenced at 8.30 am with acquisition of baseline liver ¹³C spectra, which was followed by commencement of ¹³C-glycine ingestion. [2-¹³C]-glycine was purchased from Cambridge Isotope Laboratories. 2.5g portions of this compound were dissolved in 50 mL water and ingested every 30 minutes for 6 hours. Liver ¹³C spectroscopy acquisitions were repeated at 2, 4, 6 and 8 hours after the start of ingestion. Volunteers ate a standardised lunch (comprising a sandwich, an apple and water) at 2h 45. No other food was ingested during the study, but water was freely available.

MR data were acquired on a Philips Achieva 3T scanner using a custom-built ¹³C/¹H surface coil (comprising a 12 cm diameter circular ¹³C surface coil and two ~20 cm oval ¹H coils operating in quadrature). Subjects lay in a supine position and the ¹³C/¹H coil was placed over the liver. Scout images were acquired to confirm that the coil was correctly located over the liver. Shimming employed a B₀ field mapping method over a volume of interest comprising liver tissue within the active volume of the ¹³C coil. Proton-decoupled ¹³C spectra were then acquired using a non-localised pulse-acquire sequence with WALTZ ¹H decoupling (TR = 1.5s, nominal tip angle = 90°, 1024 datapoints, sw=8kHz, 600 averages). Decoupler power was set to ensure that SAR limits of 4W/Kg were not exceeded. Data analysis was performed using jMRUI. Spectra were phase- and baseline-corrected and 10 Hz line broadening applied.

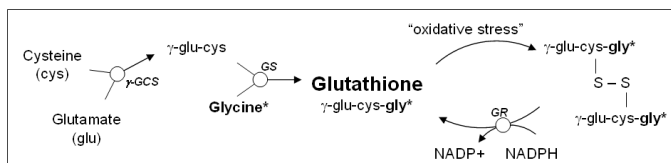


Figure 1 – Metabolic pathways showing glutathione synthesis from its component amino acids, and formation of its oxidised disulphide under conditions of oxidative stress

Results

Figure 1 shows the biochemical pathways involved for incorporation of a ¹³C label from [2-¹³C]-glycine into glutathione. Glutathione is formed in two steps from glycine, cysteine and glutamate. Figure 2 shows the custom ¹³C/¹H coil constructed for human liver spectroscopy studies. Figure 3 shows ¹³C spectra acquired before and at 2, 4, 6 and 8 hours after commencement of the 6 hour [2-¹³C]-glycine ingestion period. A clear resonance from [2-¹³C]-glycine is seen at 42.4 ppm, reaching maximum intensity at the end of the 6h glycine ingestion period. A resonance is also observed from the ¹³C-labelled glycyl residue of glutathione at 44.2 ppm. In addition, the spectra show metabolism of the ¹³C label into other compounds, such as [2-¹³C]-serine (57.4 ppm) and [3-¹³C]-serine (61.3 ppm), a resonance at 54.8 ppm corresponding to creatine and/or choline, a resonance at 45.7ppm which may be guanidinoacetate. The pattern of ¹³C labelling is similar to that observed in our previous studies of ¹³C-glycine infusion in rat tumour (3) and liver (data not shown).

Discussion and Conclusions

We observed spectral resolution that permitted discrimination of resonances from ¹³C-labelled glycine and glutathione. ¹³C-labelled glutathione was visible in spectra acquired at the first timepoint studied (2h), and the magnitude of the glutathione signal increased over the experiment duration. The data show ¹³C-labeling of the hepatic glycine pool, and the biosynthetic incorporation of this label into glutathione by the action of glutathione synthetase on γ-glutamylcysteinylglycine and [2-¹³C]-glycine. The ¹³C label was also observed in serine (formed from glycine by the action of serine hydroxymethyltransferase) and into other metabolite resonances, as has been observed in other *in situ* (4) and *ex vivo* (5) studies.

Our data show successful translation of our *in vivo* animal work into human studies, laying the groundwork for studies that gauge the strength of liver oxidative stress defences in health and in disease. The effects of therapeutic strategies to bolster hepatic glutathione content can thus be measured. Future work will focus on quantitation of hepatic glutathione concentration and synthesis rate.

References and Acknowledgements

This work was funded by the Medical Research Council (G0801239 (ID 87867)). Thanks to Philips Healthcare – Clinical Science and PulseTeq for technical assistance, to our radiographers (Louise Morris, Carol Smith and Tim Hodgeson), and to our study volunteers. References: (1) Cesaratto *et al.* Ann Hepatol, 3, 86-92 (2004). (2) Lu, Faseb J. 13: 1169-83 (1999). (3) Thelwall *et al.* Cancer Res. 65:10149-53 (2005). (4) Cowin *et al.* Biochim Biophys Acta. 1310:32-40 (1996). (5) Dringen *et al.* J Neurochem. 67:1375-82 (1996).

Figure 2 – ¹³C/¹H liver coil built for *in vivo* human ¹H decoupled ¹³C spectroscopy.

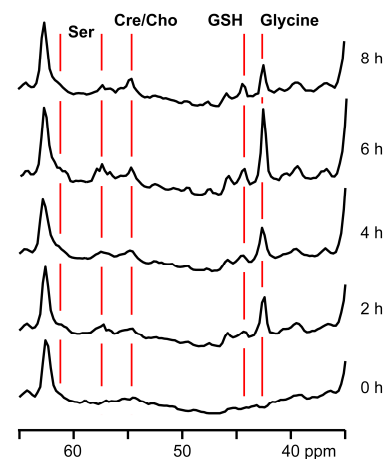


Figure 3 – Human liver ¹³C spectra showing appearance of ¹³C-labelled glycine, glutathione (GSH), serine and creatine over an 8h period, after [2-¹³C]-glycine administration