In vivo non-invasive measurement of altered hepatic glutathione concentration and synthesis rate in acute and chronic models of liver oxidative stress

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Target Audience: Research scientists and clinicians with an interest in oxidative stress, ^{13}C spectroscopy, acute or chronic liver disease, and/or dynamic in vivo spectroscopy.

Purpose: 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 (an endogenous tripeptide) is the principal intracellular antioxidant in liver tissue. Glutathione (GSH) 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 GSH concentration and synthesis rate. GSH is a tripeptide formed from glycine, cysteine and glutamate, and infusion of ^{13}C-labelled glycine allows measurement of the rate of metabolic incorporation of this MR-visible label into glutathione. In this study we tested the hypothesis that the rate of incorporation of ^{13}C-glycine into hepatic GSH is sensitive to acute and chronic oxidative stress insults, and thus this measure of GSH synthesis can serve as non-invasive biomarker of tissue oxidative stress defences. The study builds upon previous studies demonstrating that this approach can be translated directly to human studies (3), and highlights the potential of glutathione ^{13}C-labelling for non-invasive monitoring of liver disease progression and response to therapeutic strategy.

Methods: Male Sprague Dawley rats were divided into three experimental groups: control, acute oxidative stress (CCl_4-treated), and chronic oxidative stress (high fat and high carbohydrate (HFHC) diet). I/p administration of CCl_4 (1:1 mix with olive oil, 0.1mL/100g body weight) was performed 12h before MR studies. HFHC diet was provided for 8 weeks prior to MR studies and comprised TestDiet 58R2 with 60% kcal from fat, and ad libatum drinking water supplemented with 55 mM sucrose and 128 mM fructose. Data were acquired on a Varian 7T spectrometer with an in house ^{13}C/^{1}H coil for rat liver spectroscopy. Animals were anaesthetized with i/p hypnorm/midazolam, an i/v cannula placed for ^{13}C-glycine administration and an i/p cannula placed for administration of maintenance anaesthetic as required. Animals were placed in the scanner, localizer images acquired, then ^{1}H-decoupled ^{13}C spectra (TR=1.5s, SW=10kHz, tip = 90°, NA=400) were acquired every 10 minutes for 6.5 hours to monitor concentration of ^{13}C-labeled glycine and GSH. [2-^{13}C]-glycine administration (4 mmoles/kg/h for 90 min, followed by 1 mmole/kg/h for 5h) commenced after acquisition of the first ^{13}C scan. At the end of the experiment blood and liver samples taken for ex vivo analysis. Derivatised liver tissue extracts were analysed for measurement of liver GSH and glycine content by HPLC, and GSH ^{13}C fractional enrichment by mass spectrometry. MR data were analysed using the AMARES function of jMRUI and quantification performed by comparison of spectral peak areas to data acquired from phantoms containing [2-^{13}C]-glycine at known concentrations.

Results: Figure 1 shows representative in vivo ^{13}C spectra from control and CCl_4 treated rats, and from rats receiving HFHC diet, acquired after 5 hours of [2-^{13}C]-glycine infusion. Resonances at 42.4 and 44.2 ppm correspond to ^{13}C-labelled glycine and glutathione respectively. Elevated ^{13}C-GSH is observed in the CCl_4-treated rat, and decreased ^{13}C-GSH in the HFHC rat. In addition, differences in steady state concentration of hepatic [2-^{13}C]-glycine were observed between experimental groups. For the HFHC group this may be in part due to the larger size of these animals, though this may reflect differences in serine hydroxymethyltransferase pathway flux between experimental groups. Figure 2 shows the experimental timecourse of average ^{13}C-GSH content in the three experimental groups, again an elevation is seen in the acute oxidative stress group and a decrease in the HFHC group. Figure 3 shows histological sections from liver samples from the three experimental groups. Histology was normal for the control group, the CCl_4-treatment group shows hallmarks of acute oxidative stress (ballooned and swollen dead/dying hepatocytes and emergence of hepatic myofibroblasts), and the HFHC group shows steatohepatitis hallmarks (fat deposition, activation of hepatic myofibroblasts, and collagen deposition).

Discussion and Conclusions: Tissue histology demonstrated that the experiment groups successfully produced control, acute oxidative stress and steatohepatitis insults. Statistically significant differences in the concentration of hepatic ^{13}C-labelled glutathione were observed between experimental groups after infusion of [2-^{13}C]-glycine. CCl_4 has been widely used as an acute oxidative stress insult, and demonstrated to upregulate glutathione synthesis enzymes. Our data represent the first non-invasive monitoring of the effects of this insult on glutathione synthesis. Animals fed the HFHC diet for eight weeks showed a lower concentration of ^{13}C-labelled glutathione after infusion of labelled glycine, reflecting a lower steady state concentration of glutathione. This model has been shown to induce steatosis and steatohepatitis in mice, and represents a physiologically relevant model of chronic oxidative stress and liver disease. Our data demonstrate the ability to detect compromised hepatic oxidative stress defences without the need for tissue biopsy in a model of liver disease. We have successfully translated these methods to human studies in a group of healthy volunteers (3), and thus this approach has the potential as a non-invasive biomarker to report on changes in hepatic oxidative stress defences associated with disease.

References and Acknowledgements: This work was funded by the Medical Research Council (G0801239). References: 1) Cesaratto et al. Ann Hepatol, 3, 86-92 (2004). (2) Lu. Faseb J. 13: 1169-83 (1999). (3) Thelwall et al, Proc 19th ISMRM, Pg 925 (2012).

Figure 1: Hepatic ^{13}C spectra from the experimental groups

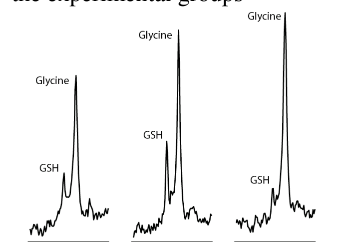

Figure 2: Timecourse of hepatic ^{13}C-Glutathione content over the experiment duration

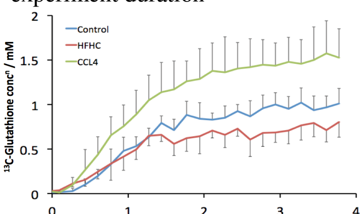

Figure 3: Liver H&E histology from the three experimental groups