Non-invasive monitoring of antioxidant prodrug metabolism in rat brain by in vivo ^{13}C MRS

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Introduction

Glutathione is an endogenous antioxidant present at millimolar concentrations in almost all tissues. It plays a central role in defending tissues against oxidative stress, where metabolism, radiation or exogenous agents result in the formation of reactive chemical species that can damage cellular DNA, proteins and membranes. Oxidative stress is a component of the progression of many disease processes, including neurodegenerative diseases. Therapeutic strategies have been proposed to limit the progression of some neurodegenerative diseases by bolstering cellular defences against oxidative stress. Some of these have targeted raising glutathione concentration and/or increasing its rate of synthesis (1).

The cysteine precursor L-2-oxothiazolidine-4-carboxylate (OTZ, procysteine) can raise the concentration of glutathione's rate-limiting component, cysteine (2,3). We have synthesized stable isotope labeled OTZ (i.e. [5-^{13}C]-2-oxothiazolidine-4-carboxylate, ^{13}C-OTZ) and tracked its uptake and metabolism *in vivo* in rat brain by ^{13}C MRS following bolus administration and 24h infusion. *Ex vivo* ^{13}C MRS, HPLC and/or mass spectrometry of brain tissue extracts augmented in vivo data. We measured the concentration of brain OTZ, observed its effect on brain glutathione *in vivo*, and observed pathways of OTZ metabolism. This provides an insight into the metabolic processes that influence OTZ efficacy for pro-antioxidant therapy.

Methods

^{13}C-OTZ was prepared from ^{13}C-cysteine (4) and administered to rats as a bolus dose by intraperitoneal injection (1100 mg/kg) or as a 24h infusion via a surgically implanted jugular vein cannula (65 mg/kg/h). Animals receiving a bolus dose of OTZ were assessed by *in vivo* ^{13}C brain MRS for a 2-8h period that commenced immediately after OTZ administration, whereas animals receiving OTZ infusions were scanned after a 20h infusion period. Data were acquired on an 11T magnet interfaced to a Bruker Avance spectrometer and console. A 15mm diameter ^{13}C surface coil was placed over the rat brain, and a 5cm diameter birdcage coil placed around the rat head. Data were acquired using a ^{13}C pulse-acquire sequence (nominal tip = 90 degrees, TR = 1.5s, sw = 10kHz, 400 averages, 1024 datapoints) with ^{1}H WALTZ decoupling. Animals were anaesthetised for restraint during MR scan sessions. Animals were sacrificed at the end of the scan sessions, the brain removed and frozen, then extracts prepared for *ex vivo* MR, HPLC and/or mass spectrometry.

Results

Figure 1 shows the timecourse of ^{13}C-OTZ appearance and clearance in rat brain over 4 hours following a bolus dose. The concentration of OTZ falls over this period. Little or no ^{13}C-glutathione is visible in the *in vivo* spectra, though ^{13}C-labeling of glutathione was observed at low concentration in *ex vivo* extract spectra. Figure 2 shows *in vivo* rat brain ^{13}C spectra before (B) and at 20h after ^{13}C-OTZ infusion (A). An *ex vivo* extract spectrum is also shown (C). Clear resonances are visible from ^{13}C-OTZ, ^{13}C-glutathione (^{13}C-GSH), [1-^{13}C]-lactate, [1-^{13}C]-taurine and [1-^{13}C]-hypotaurine. Table 1 shows concentrations of these compounds as determined by HPLC following bolus and 24h infusion of OTZ, and in control (no OTZ) rat brain.

Discussion and Conclusions

OTZ is a non-toxic cysteine precursor proposed to raise cysteine and glutathione levels in tissues, and proposed as a treatment to limit progression of neurodegenerative diseases. Our results showed that OTZ crosses the blood-brain barrier and could be observed in brain ^{13}C spectra. Our data demonstrated incorporation of ^{13}C label from OTZ into glutathione following bolus ^{13}C-OTZ administration, though the label incorporation was insufficient for *in vivo* detection. Administration of a similar quantity of ^{13}C-OTZ over a 24h period, instead of as a bolus, resulted in significant ^{13}C-labeling of glutathione that was visible *in vivo*. Appearance of the ^{13}C label in taurine, hypotaurine and lactate provided information on the metabolic fate of OTZ and the pathways that influence intracellular cysteine. We have shown that OTZ administration does not lead to considerable cysteine accumulation. It appears that cysteine produced from OTZ is quickly shunted to glutathione or to taurine/hypotaurine pathways, avoiding sustained elevated cysteine which has the potential to be toxic. HPLC data of brain tissue extracts did not demonstrate an elevated glutathione concentration, however the incorporation of ^{13}C into glutathione demonstrates metabolism of ^{13}C-OTZ into cysteine and glutathione and highlights the continual turnover of glutathione. Our previous work has shown that an elevated glutathione turnover rate can confer raised defences against oxidative stress (5), and that glutathione turnover rate can be monitored by *in vivo* MR spectroscopy (6). Here we have extended this work to probe pro-antioxidant therapeutics, paving the way for investigations of the extent to which OTZ and other antioxidants can strengthen tissue defences against oxidative stress, and to elucidate the metabolic pathways involved.

References and Acknowledgements

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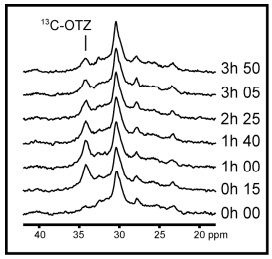

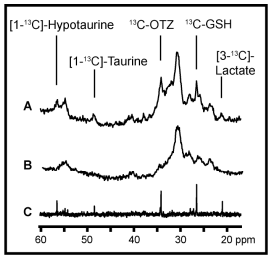

Fig 1, Fig 2, and Table 1. Effect of 13C-OTZ on glutathione, cysteine, taurine, hypotaurine and lactate

	Glutathione (μmol/g-tissue)	Cysteine (μmol/g-tissue)	Taurine (μmol/g-tissue)	Hypotaurine (μmol/g-tissue)
Control (n = 4)	1.22 ± 0.26	0.18 ± 0.05	3.76 ± 1.80	0.25 ± 0.05
OTZ 4 h (n = 3)	1.04 ± 0.17	0.39 ± 0.08*	4.35 ± 2.78	0.28 ± 0.10
OTZ 6 h (n = 3)	1.05 ± 0.05	0.25 ± 0.06	4.16 ± 1.40	0.18 ± 0.04
OTZ 20h (n = 5)	1.51 ± 0.25	0.11 ± 0.08	3.93 ± 0.55	0.40 ± 0.15**