In vivo measurement of ALDH2 activity in rat liver ethanol model using dynamic MRSI of hyperpolarized [1-^{13}C]-pyruvate

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Introduction

Aldehyde dehydrogenase-2 (ALDH2) is a detoxifying enzyme that can eliminate toxic aldehydes, whose function has been extensively researched in several disease conditions including alcoholism [1,2] and ischemia [3], as a promising target for drug development. To date, direct measurements of its enzymatic activity have been largely limited to in vitro methods. The goal of this work is to investigate using hyperpolarized [1-^{13}C]-pyruvate magnetic resonance spectroscopic imaging (MRSI) as a method for in vivo measurement of ALDH2 activity.

Ethanol is metabolized in the liver via the breakdown of ethanol to acetaldehyde and acetaldehyde to acetate, catalyzed by the enzymes alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH2) respectively. Both reactions also reduce the coenzyme nicotinamide adenine dinucleotide (NAD^+) to NADH, thus leading to accumulation of NADH in the liver. As NADH is also a coenzyme in the conversion of pyruvate to lactate with NADH levels in rat liver being rate-limiting, the altered liver metabolic state created by ethanol can be interrogated using hyperpolarized ^{13}C-pyruvate measurements [4]. The change in pyruvate-to-lactate conversion with ethanol was measured using hyperpolarized ^{13}C MRSI, with and without ALDH2 inhibition and correlated with ALDH2 enzyme activity.

Methods

All measurements were performed on a GE 3T MR scanner equipped with self-shielded gradients (40 mT/m, 150 mT/m/ms). A custom-built dual-tuned ^{1}H/^{13}C transmit/receive quadrature rat coil (dia=80 mm, length=90mm) was used for RF excitation and signal reception. Healthy male Wistar rats (424±25 g weight, n=9) were injected in a tail vein with approximately 3.2 ml of 125-mM solution of [1-^{13}C]-pyruvate. The samples were hyperpolarized via Dynamic Nuclear Polarization using HyperSense (Oxford Instruments Molecular Biotools, Oxford, UK) to get approximately 20% liquid state polarization. The time from dissolution to start of injection was ~20s.

Each animal received two pyruvate injections followed by dynamic 2D spiral MRSI of a 10-mm slice through the liver with lactate saturation every TR (5 mm resolution, TR= 5 s, start scan coincident with Pyr injection) [5]. After a baseline MRSI acquisition, a dose of 1g/kg body weight of 20% ethanol was injected targeting a blood alcohol concentration of approximately 100 mg/dL at the time of the second pyruvate injection (~45 minutes after ethanol infusion). At the end of the exam, liver tissue was harvested for ALDH2 enzyme activity assay.

The ALDH2 activity was manipulated using the FDA-approved ALDH2 inhibitor disulfiram delivered through oral gavage tube approximately 36 hours before the imaging experiments. Disulfiram reduces ALDH2 activity in rat liver to approximately 60% (with 90 mg/kg dose) and 25% (with 600 mg/kg dose) of normal at 36-48 hours after oral delivery [6]. Rats were equally divided into three groups: control group, disulfiram-treated group with 90 mg/kg dose and disulfiram-treated group with 600 mg/kg dose.

The dynamic data were analyzed using a saturable kinetics model with a Michaelis-Menten-like formulation to approximate the apparent reaction velocities of ^{13}C lactate labeling given the effective pyruvate concentration with parameters corresponding to the apparent maximal reaction velocity V_{max} and apparent lactate Michaelis constant K_M [5]. The apparent reaction velocity of the ^{13}C lactate labeling is a combination of the net chemical conversion of pyruvate to lactate and the isotopic exchange between pyruvate and lactate pools.

Results and Discussion

Figure 1 shows the saturable kinetics in liver for a control rat and a disulfiram-treated rat, both pre- and post-ethanol. A smaller increase in lactate signal was observed in the disulfiram-treated rats due to the lower ethanol-generated NADH from the partially inhibited ALDH2 activity.

Table 1 summarizes the estimated apparent V_{max} values of the pyruvate-to-lactate ^{13}C labeling process pre and post ethanol infusion. The average change of the lactate apparent V_{max} with ethanol (ΔV_{max}) was 116% ± 12% for the control group, 82% ± 10% for the disulfiram-90 group and 57% ± 9% for the disulfiram-600 group. The unit of ALDH2 enzymatic activity given in Table1 is µmole NADH formed/min/mg protein. Figure 2 shows that the ΔV_{max} of the pyruvate-to-lactate ^{13}C labeling process correlates well with the ALDH2 activity assay results. Compared to the mean ALDH2 activity of the control group, the ALDH2 activity of the disulfiram-90 group was approximately 66.2% ± 13.4% and of the disulfiram-600 group was approximately 39.2% ± 11.8%. The disulfiram-600 ALDH2 activity was somewhat higher than the 25% reported in literature, and may be due to the presence of high-K_M ALDH1 in the mitochondria and cytosol mixture of the liver tissue assayed, as disulfiram does not affect the ALDH1 activity.

This work demonstrates that the relative change of lactate production or ΔV_{max} can potentially serve as a non-invasive indicator of ALDH2 activity in this ethanol-treated rat model.

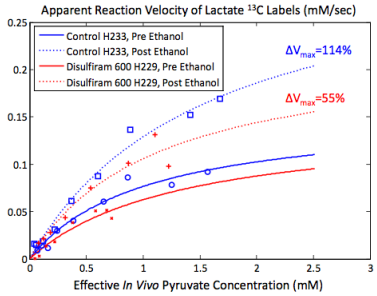

Figure1: Change in apparent V_{max} pre-to-post ethanol was lower when ALDH2 activity was inhibited via disulfiram.

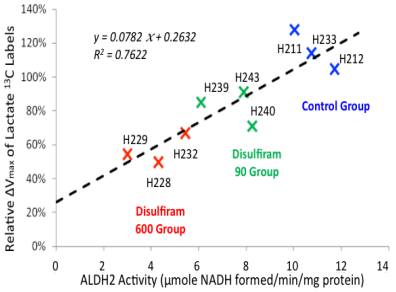

Figure2: ΔV_{max} of the pyruvate-to-lactate ^{13}C labeling process correlates well with the ALDH2 activity

	Control group			Disulfiram-90			Disulfiram-600		
Rat ID	H211	H212	H233	H239	H240	H243	H228	H229	H232
V_{max} pre-ethanol (mM/s)	0.103	0.128	0.155	0.168	0.215	0.160	0.278	0.146	0.274
V_{max} post-ethanol (mM/s)	0.234	0.261	0.332	0.310	0.369	0.305	0.417	0.22	0.459
ΔV_{max} (post-pre)/pre	128%	105%	114%	85%	71%	91%	50%	55%	67%
ALDH2 enzyme activity	10.0	11.7	10.8	6.1	8.3	7.9	4.3	3.0	5.5

References: [1] Edenberg HJ Alcohol Res Health 2007 30:p5 [2] Deng XS *et al* Curr Drug Abuse Rev 2008 1:p3 [3] Chen CH *et al* Science 2008 321:p1493 [4] Spielman D *et al* MRM 2009 62:p307 [5] Xu T *et al*, NMR Biomed 2011 24:p997 [6] Pettersson H and Tottmar O, Jour of Neurochem 1982, 39:p628
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