Assessment of acute inflammatory liver injury in a rat CCl4 model using metabolic imaging of hyperpolarized [1-13C]pyruvate

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Introduction

Hyperpolarized ^{13}C MRS provides a unique opportunity to measure dynamic metabolic processes in vivo under normal and pathologic conditions. While numerous studies have investigated hyperpolarized pyruvate (Pyr) in cancer and heart disease, few studies have reported applications in early detection of hepatic inflammatory mediated injury. Lee et al.[1] found increased hyperpolarized [1-^{13}C]Pyr to [1-^{13}C]alanine (Ala) labeling (which correlated with ex vivo hepatic alanine transaminase (ALT) activity) in a fatty-liver mouse model of type 2 diabetes. Zandt et al.[2] demonstrated detection of hepatocyte necrosis in a carbon tetrachloride (CCl_4) injured rat liver model using hyperpolarized [1,4-$^{13}C_2$]fumarate. Current standard radiologic imaging often reveals liver damage only after cirrhosis ensues. Early detection of hepatocyte injury would provide opportunity for treatments aimed at prevention of cirrhosis and the clinical sequelae of portal hypertension. The aim of this work was to investigate the use of dynamic metabolic imaging of hyperpolarized [1-^{13}C]Pyr for the detection of liver damage induced by CCl_4.

Methods

All measurements were performed on a GE 3T MR scanner (40 mT/m, 150 mT/m/ms) using a custom-built dual-tuned ^{1}H/^{13}C transmit/receive quadrature rat coil (dia=80mm, length=90mm). Male Sprague Dawley rats were divided into two groups: a treated group (n=10) that received CCl_4 through i.p. injection at a dose of 1 mL/kg body weight (dissolved in olive oil at a ratio of 1:1 v/v), and a control group (n=8) that was administered only oil. Imaging was performed 48-72 h post treatment. A 125-mM solution of hyperpolarized [1-^{13}C]Pyr was injected via tail vein (target dose 1.25 mmol/kg), with 40-mM ^{12}C-Ala added to the dissolution buffer to reduce pool size effects[3]. The samples were hyperpolarized via Dynamic Nuclear Polarization using HyperSense (Oxford Instruments, Oxford, UK).

Dynamic 3D spiral chemical shift imaging (CSI) data[4] were acquired from a volume including the liver and kidney (FOV=80x80x60 mm^3, 5 mm resolution, TR=5 s, 4.5 s acquisition, start scan coincident with Pyr injection). At the end of the exam, liver tissue was harvested for ALT enzyme activity assay and histopathology.

Results and Discussion

Figure 1 shows time-averaged ^{13}C metabolic maps superimposed onto corresponding ^{1}H MR images for a slice through the liver from a CCl_4-treated rat. Figure 2 plots the metabolite-to-substrate ratios, averaged over the first 60 s of ^{13}C CSI data, from an ROI in the liver for the control and CCl_4-treated groups. The Ala/Pyr and Lac/Pyr ratios were higher for the treated group compared to the control group (unpaired t-test P<0.02). As shown in Fig 2, the liver tissue ALT activity was also elevated in treated animals compared to control animals (P<0.02). The increased Lac/Pyr ratios for the treated group were also consistent with metabolic changes due to inflammation[5,6]. In contrast, there was no significant difference in Ala/Pyr or Lac/Pyr ratios between control and treated groups for a kidney ROI.

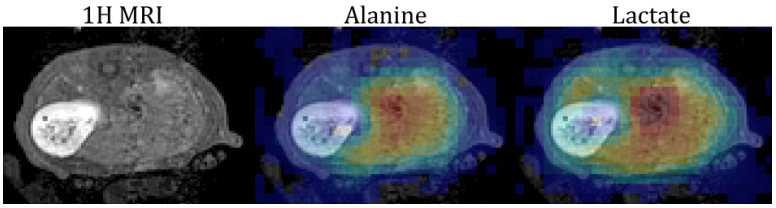


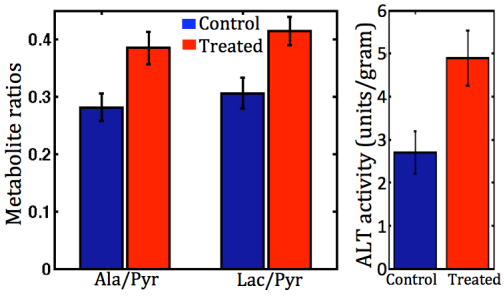
Figure1: Time-averaged metabolic 13C maps of a slice through liver for a CCl4-treated rat.

Microscopic examination of H&E stained liver sections revealed lesions consistent with CCl_4 exposure, including steatosis (lipidosis) and diffuse acute necrosis of centrilobular hepatocytes, sometimes with neutrophilic infiltration. The severity of the steatosis, inflammation and necrosis ranged from mild to moderate. Control animals displayed normal liver histology with no evidence of necrosis and minimal or no background inflammation.

While there were group differences in the Ala/Pyr ratios and the tissue ALT enzyme activity for the control and treated groups, no significant correlation was observed between the Ala/Pyr ratios and ALT activity (P=0.38). This could be due to heterogeneity in CCl_4 response across the liver tissue as only a small part of liver tissue was used for ALT assay, complicating the comparison between MR measurements and enzyme assay.

This work demonstrates that hyperpolarized ^{13}C metabolic imaging with hyperpolarized [1-^{13}C]Pyr is sensitive to inflammatory mediated liver injury. More work is needed to optimize imaging to reduce measurement variability and validate the correlation with tissue enzyme activity.

Figure2: Metabolite ratios and liver ALT activity for control and CCl4-treated rats. The Ala/Pyr ratios are higher for CCl4-treated rats corresponding to increased ALT activity. The Lac/Pyr ratios are also higher due to inflammation.



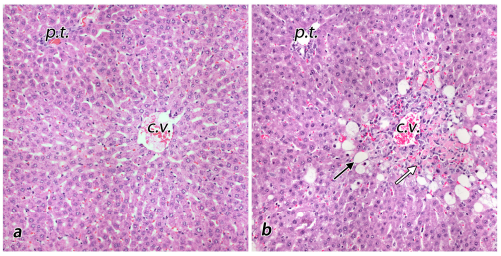


Figure3: H&E photomicrographs. (a) Liver histology from control rat with normal appearing lobule, central vein (cv), and portal triad (pt), without inflammation or steatosis. (b) Liver histology from CCl4-treated rat, with centrilobular hepatocyte steatosis (black arrow) and centrilobular necrosis with neutrophilic infiltration (white arrow).

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