Imaging cerebral ketone metabolism with hyperpolarized ^{13}C beta-hydroxybutyrate

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Highlights

Cerebral ketone metabolism is of increasing interest in Alzheimer's disease, as cognitive improvements are seen in patients treated with ketogenic diets. We hypothesized that hyperpolarized ^{13}C magnetic resonance spectroscopic imaging of hyperpolarized ^{13}C beta-hydroxybutyrate can assess treatment response *in vivo*, and performed the first proof-of-concept study of this novel approach.

Introduction

Alzheimer's disease (AD) is a devastating disease with no cure. It is thus essential, and urgent, to improve our understanding, discover treatments, and measure treatment response in AD.

Recently, a ketogenic diet (often involving a beta-hydroxybutyrate, BHB, supplement) has been proposed as a therapy for AD. However we cannot directly measure *in vivo* BHB metabolism – which would be essential to understand the treatment response. Studies exploring the relationship between the most commonly used biomarker, serum BHB, and *ex vivo* cerebral enzymatic metabolism are inconclusive, and so an *in vivo* measure of the enzyme-driven conversion of BHB to acetoacetate is needed.

We hypothesized that hyperpolarized (HP) ^{13}C magnetic resonance spectroscopy could provide this *in vivo* assessment of ketone metabolism in the brain. In this work we measured the relaxation time (T1) of HP ^{13}C BHB, confirmed enzymatic conversion could be detected, and finally carried out the first *in vivo* acquisition of cerebral metabolic data following injection of HP ^{13}C BHB.

Methods

Our HP ^{13}C BHB recipe for hyperpolarization produced 6.4M BHB in solution. Dissolution of 100uL in buffer (Tris-HCl and EDTA in dH_2O) produced a solution of 130mM BHB at pH 7 and 37°C. T1 was measured at 1.5T, 3T and 11.7T. For enzyme experiments, HP BHB was rapidly added to an NMR tube containing the enzyme beta-hydroxybutyrate dehydrogenase (BDH) and NAD, which was placed into an 11.7T MR system. Data were acquired every 4.2 seconds for 5 minutes. T1 and enzyme data were analyzed using Mestrenova.

For *in vivo* proof of concept, an anesthetized control mouse was placed into the 3T MR system (Bruker) with a dual tuned ^{1}H/^{13}C surface head coil. After hyperpolarization, 300ul HP BHB was injected via a tail vein catheter. Dynamic, non-localized data were acquired over 3 minutes. *In vivo* data were analyzed in jMRUI.

Results

The following T1 measurements were determined: 41s at 1.5T ; 37s at 3T; 28s at 11.7T. Upon injection of HP ^{13}C BHB to a pre-warmed mixture of BDH enzyme and NAD, metabolism of the probe (181ppm) to HP ^{13}C acetoacetate (175ppm) could be detected (**Figure 1A**). On injection of HP ^{13}C BHB into a mouse, we could observe the probe dynamically at 3T. When the data were subsequently summed, HP acetoacetate can be seen: this is the first observation of HP ^{13}C BHB metabolism in the mouse brain (**Figure 1B**).

Conclusion

We confirmed that enzymatic conversion of BHB to acetoacetate by BDH occurs within the lifetime of our HP probe. We have further shown that acquiring metabolic data on ketone metabolism is feasible in the mouse brain. In future studies, we will image control and ketogenic-diet-fed animals, before moving to a mouse model of AD.

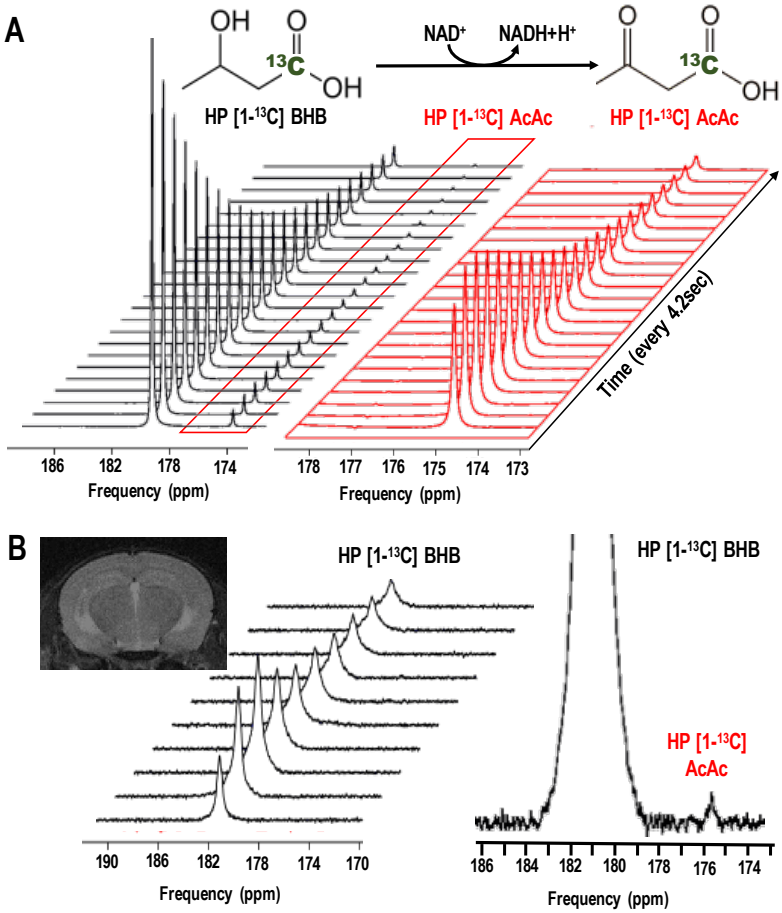

Figure 1 (A) Conversion of HP ^{13}C BHB to acetoacetate (AcAc) in the presence of BDH enzyme at 11.7 Tesla. **(B)** T_2 MRI of a mouse brain at 3 Tesla; stack plot of HP [1-^{13}C] BHB in the mouse brain; summed spectrum showing the *in vivo* simultaneous detection of HP [1-^{13}C] BHB and acetoacetate.