Hyperpolarized [1,3-$^{13}C_2$]ethyl acetoacetate is a novel diagnostic metabolic marker of liver cancer.

Sonia Colombo Serra[1], Pernille Rose Jensen[2], Luigi Miragoli[1], Magnus Karlsson[2], Claudia Cabella[1], Luisa Poggi[1], Luca Venturi[3], Fabio Tedoldi[1], and Mathilde Lerche[2]
[1]*Centro Ricerche Bracco, Bracco Imaging SpA, Colleretto Giacosa, Torino, Italy,* [2]*Albeda Research, Copenhagen, Copenhagen, Denmark,* [3]*CEIP, University of Torino, Colleretto Giacosa, Torino, Italy*

Purpose

Hepatocellular carcinoma (HCC) is the most common form of primary liver cancer[1] and, being often found in cirrhotic liver, it is hardly identifiable by imaging techniques relying on anatomical information only[2]. The emerging method based on ^{13}C magnetic resonance spectroscopy (MRS) after administration of hyperpolarized ^{13}C labeled metabolic substrate is potentially a powerful tool to report on tumor pathogenesis and metabolism. The aim of this study was to probe the potential of hyperpolarized [1,3-$^{13}C_2$]ethyl acetoacetate (EAA) as a metabolic marker of HCC providing a diagnostic tool for such a challenging disease.

Methods

Sample preparation: Finland radical free acid and Gadoteridol were dissolved in EAA (0.24 mmol) to give a concentration of 17 mM and 2.2 mM respectively. The sample was hyperpolarized at 1.2 K and 3.35 T in a custom build polarizer and then dissolved in 5 mL 40 mM TRIS buffer at pH 7 added with 100mg/L of ethylenediaminetetraacetic. *Cell growth and animal model.* Rat hepatoma cells (McA-RH7777) were grown in 90% Dulbecco´s Modified Eagles medium (DMEM) + 10% FBS at 37 °C, 5% CO_2 to confluence and harvested by trypsination. 0.2 mL of a cell suspension (5 million cells/mL of DMEM) was injected under the hepatic capsule of the liver left lobe of anesthetized Buffalo rats[3]. The tumors were grown for 21 days before the rats were imaged. *^{13}C-MRS.* Experiments were performed by a 3 T MR scanner equipped with a ^{1}H-^{13}C birdcage resonator and with a 20 mm surface coil *Chemical Shift Imaging (CSI)* maps were acquired on an 8 mm coronal slice placed in the abdominal region (matrix size = 14x14, flip angle = 5°, repetition time = 105 ms) during the first 20 s after the injection of the substrate. 64 ^{13}C s*lice selective spectra* were acquired on an axial slice placed over the tumor or the liver with a sampling rate of 3 s in order to follow the metabolic fate of the molecules and the decay of the hyperpolarized signal.

Results

^{13}C CSI maps were overlaid with anatomical ^{1}H images to yield a functional representation of EAA and [1,3-$^{13}C_2$]acetoacetate (AA) distributions (Figure 1), the latter coming from the metabolic conversion of EAA mediated by the liver isoform of carboxyl esterase (CE-1). EAA signal was detected within the tumor at high contrast-to-noise ratio (24 $\pm$ 8) relative to the healthy surrounding liver tissue. Two representative spectra from tumor and liver show similar AA signal in the two regions, but a much lower substrate signal in the liver (Figure 1D), possibly as a consequence of the higher perfusion level of the pathological tissue. In a ratio image of EAA(C1) over AA(C1) the tumor is directly identified as the bright area with approx. 4 times higher signal than in the surrounding liver tissue (Figure 2A). *Slice selective spectroscopy* data acquired on healthy liver or tumor liver slices were averaged and modeled using a set of rate equations[4] (Figure 3). The extracted rate constant for the enzymatic conversion of EAA to AA is different in the two tissue types with an approx. 2.5 times higher conversion rate in healthy liver (k_l = 0.12 s^{-1}) than in the tumor (k_t =0.05 s^{-1}).

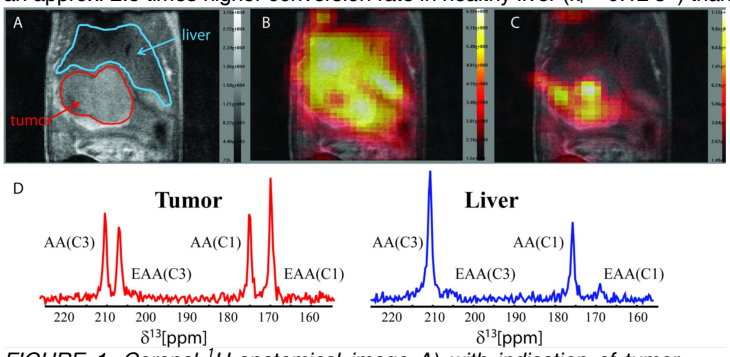

FIGURE 1. Coronal ^{1}H-anatomical image A) with indication of tumor and liver, B) overlaid with ^{13}C metabolic images of AA and C) EAA. D) Single spectra from representative voxels in liver and tumor region.

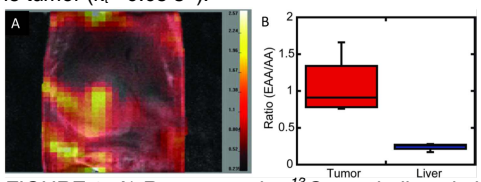

FIGURE 2. A) Representative ^{13}C-metabolic ratio image of EAA(C1) over AA(C1) ratio overlaid to the ^{1}H-anatomical image; B) an average signal ratio of all voxels in healthy liver and tumor.

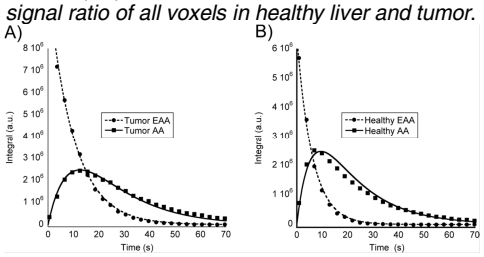

FIGURE 3. Slice selective time courses from A) tumor rat liver tissue and B) healthy rat liver tissue (n=4).

Discussion

EAA is a well-suited substrate for dissolution dynamic nuclear polarization for magnetic resonance (DNPMR): it is chemically stable, it is a liquid at room temperature and vitrifies upon flash-freezing, it is water-soluble up to a concentration of 0.2 M, it dissolves and is stable with a broad range of radicals. Thanks to its fast cellular uptake and simple metabolism, a high conversion from EAA to AA is observed. Such a conversion is mediated by the CE-1 enzyme, the most abundant CE isoform in mammalian healthy liver and liver cancer[5]. The reported expression and activity of this enzyme is about 3 times higher in human healthy liver tissue than in human cancerous liver tissue[6]. A similar difference in conversion rate between healthy and diseased rat liver tissue has been measured in the dynamic study with EAA. Such metabolic difference well explains the approx. 4 times higher substrate-to-product ratio observed in CSI ratio map.

Conclusion

A liver cancer implanted in rat is detected due to a higher substrate-to-product ratio in the tumor compared to the surrounding healthy tissue, demonstrating that the non-invasive method DNPMR can be used to monitor the real time metabolism of the liver isoform of carboxyl esterase *in vivo*.

References

1. Tinkle CL et al. Biologics: Targets and Therapy 2012;6:207-219.
2. Holland AE et al. Radiology 2005;237(3):938-944.
3. Morris, HP ed Karger 1963;3:370-411.
4. Day SE et al. Nature Med. 2007;13(11):1382-7.
5. Imai T Drug Metab. Pharmacokinet 2006;21(3):173-185.
6. Na, K. et al. Proteomics 2009;9:3989-99.