In Vivo ^{1}H-[^{13}C] Magnetic Resonance Spectroscopy Evidence of Ketone Body Metabolism in the 9L Rat Brain Tumor Model

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Introduction

The ketogenic diet (KD) (no carbohydrates) is currently receiving attention as alternative or additional treatment for the standard radiation and chemotherapy applied in the management of brain tumors. The KD induces low glucose levels while several fold increasing plasma ketone bodies. In contrast to healthy brain cells, tumor cells supposedly lack the enzymes to oxidize ketone bodies and thus have insufficient energy to grow on low glucose. The idea that brain tumor cells have limited capacity to metabolize ketone bodies is based on lower mRNA levels of β-hydroxybutyrate dehydrogenase and sucinyl-CoA:3-ketoacid CoA transferase (Fig. 1) in tumors of a mouse model compared to normal brain (1). Both these mitochondrial enzymes are necessary to metabolize ketone bodies for ATP production. However, *in vivo* evidence for the functional capacity of brain tumors to metabolize ketone bodies is lacking. We therefore applied *in vivo* ^{1}H-[^{13}C] magnetic resonance spectroscopy (MRS) combined with infusion of ^{13}C-labeled β-hydroxybutyrate (BHB) in a rat model of malignant glioma (9L) to investigate metabolic pathways of ketone bodies in tumorous and non-tumorous brain tissue.

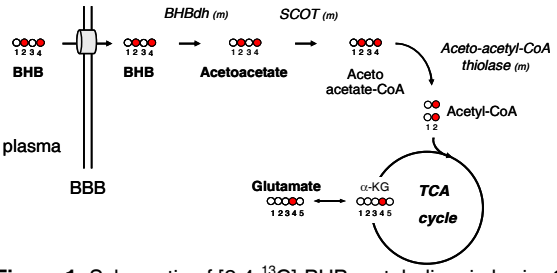

Figure 1. Schematic of [2,4-^{13}C]-BHB metabolism in brain. O = ^{12}C, ● = ^{13}C. BHBdh: BHB dehydrogenase, SCOT: Succinyl-CoA Acetoacetate-CoA transferase, Co-A: Co-enzyme A, α-KG: α-ketoglutarate, (m): mitochondrial, TCA: tricarboxylic acid cycle, BBB: blood-brain barrier.

Materials and methods

9L cells (ATCC™) were cultured in T75 flasks in DMEM containing 10% heat inactivated FBS and 1% antibiotics in an incubator at 37°C and 5% CO_2. Cells were harvested at 60-80% confluence and suspended in serum-free DMEM for inoculation. Intracerebral tumors were induced in male Fisher344/DuCrl rats by implanting cells (1×10^5 in 4 µL) in the frontal brain at 3 mm depth using a stereotactic device. All *in vivo* NMR measurements were performed using a 9.4T horizontal bore magnet interfaced to a Varian spectrometer. Rats were anesthetized with isoflurane using ~60% O_2 and ~30% N_2O. A femoral vein and artery were catheterized for infusion of [2,4-^{13}C]-BHB and blood sampling. A heating pad was used to maintain body temperature at ~37°C. A combined quadrature ^{13}C and single loop ^{1}H surface coil set-up was placed on top of the skull to acquire MR spectra from voxels (27 – 74 µL) positioned in the tumor and non-tumor brain. Fastmap (2) was used for B_0 shimming of both volumes of interest and shim settings were stored. Before [2,4-^{13}C]-BHB infusion double-inversion metabolite-nulled ^{1}H MR spectra were acquired from both voxels (inversion delay 1=1950 ms, inversion delay 2=510 ms, TR=3.5 s). A Proton-Observed-Carbon-Edited (POCE) sequence with LASER localization was used with a repetition time of 2.5 s and total echo time of 25 (17 + 8) ms (3). The ^{1}H-[^{13}C] MR spectra were acquired interleaved from tumor and non-tumor voxels by using a macro that implemented voxel-specific coordinates, shim settings and frequency offsets. MRS acquisition started together with the infusion of [2,4-^{13}C]-BHB (0.102 µL/min/g body weight, 0.75 M) and continued for 96 min. MR spectra were fitted using an LC model approach with in-house written software (4). Model ^{1}H-[^{13}C] and ^{1}H-[^{12}C+^{13}C] spectra of metabolites were simulated based on density matrices as described by Hogben *et al.* (5), while macromolecules basis sets were generated by parameterizing the voxel-specific metabolite-nulled ^{1}H MR spectra.

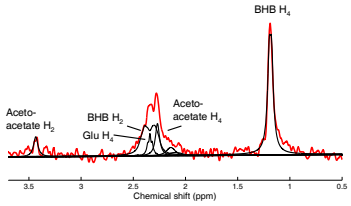

Figure 2. ^{1}H-[^{13}C] edited spectrum (red) acquired in 9L tumor (50.6 µL) 50 min following the start of [2,4-13C]-BHB infusion and individual peak fits of metabolites (black). Peak annotations: BHB: β-hydroxybutyrate, Glu: glutamate.

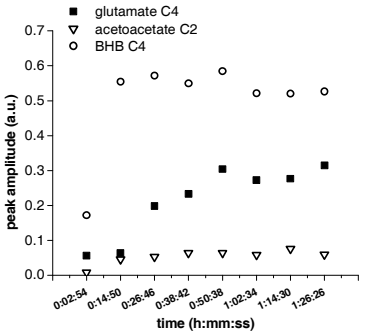

Figure 3. Time course of peak amplitudes (^{1}H-[^{13}C] edited spectra) of metabolites in 9L tumor.

Results

Upon infusion of [2,4-^{13}C]-BHB, ^{13}C-labeled BHB became quickly evident in the tumor, shortly after followed by ^{13}C-labeling of acetoacetate and glutamate (Fig. 2 and 3). Average fractional ^{13}C enrichment of BHB and acetoacetate in tumors was 26.3 and 21%, respectively. In contrast, in non-tumorous tissue, ^{13}C-labeled BHB and acetoacetate were hard to detect. Steady state fractional ^{13}C enrichment of glutamate was 9.1 and 9.9% in the two tumors studied, similar to glutamate fractional enrichment in the non-tumorous tissue: 7.9 and 7.4%.

Discussion

In healthy brain transport of BHB across the blood-brain barrier is an active process involving the monocarboxylic acid transporter 1 (MCT1). The fast appearance and abundance of ^{13}C-BHB in the tumor can be explained by a dysfunctional blood-brain barrier. The presence of ^{13}C-labeled acetoacetate and glutamate in the tumor being labeled to similar levels as in non-tumorous brain tissue is evidence of completely functional BHB metabolism (Fig.1) in the 9L rat brain tumor model.

References

(1) Zhou *et al.*, *Nutrition & Metabolism*, 4:5, 2007; (2) Gruetter R. *Magn. Reson. Med.* 29, 6, 1993; (3) de Graaf RA, *In vivo NMR Spectroscopy*, Wiley, 2007; (4) de Graaf *et al. Anal. Chem.* 83, 1, 2011; (5) Hogben HJ *et al. J Magn Reson* 208, 179, 2011.