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

Henk M. de Feyter¹, Robin A. de Graaf¹, Fahmeed Hyder¹, Kevin L. Behar³, and Douglas L. Rothman¹

¹Diagnostic Radiology, Yale University, New Haven, Connecticut, United States, ²Department of Psychiatry, Yale University, New Haven, Connecticut, United States

**Introduction**

The ketogenic diet (KD) (no carbohydrates) is currently receiving attention as an alternative or additional treatment for the standard radiation and chemotherapy 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 succinyl-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 ¹H-[¹³C] magnetic resonance spectroscopy (MRS) combined with infusion of [¹³C]-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.

**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% CO₂. Cells were harvested at 60-80% confluence and suspended in serum-free DMEM for inoculation. Intracerebral tumors were induced in male Fisher344/DuCrI rats by implanting cells (1x10⁶ 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% O₂ and ~30% N₂O. A femoral vein and artery were catheterized for infusion of [2,4-¹³C]-BHB and blood sampling. A heating pad was used to maintain body temperature at ~37°C. A combined quadrature ¹H and single loop ¹³C surface coil set-up was placed on top of the skull to acquire MR spectra from voxels (27 – 74 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 ¹H-[¹³C] and ¹H-[¹³C+¹³C] 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 ¹H MR spectra.

**Results**

Upon infusion of [2,4-¹³C]-BHB, [¹³C]-labeled BHB became quickly evident in the tumor, shortly after following by ¹³C-labeling of acetocacetate and glutamate (Fig. 2 and 3). Average fractional ¹³C enrichment of BHB and acetocacetate in tumors was 26.3 and 21%, respectively. In contrast, in non-tumorous tissue, [¹³C]-labeled BHB and acetocacetate were hard to detect. Steady state fractional ¹³C 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 [¹³C]-BHB in the tumor can be explained by a dysfunctional blood-brain barrier. The presence of [¹³C]-acetocacetate 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**