

Metabolic Changes in a Rat Glioma Model After Anti-Angiogenic Treatment Measured by MR Spectroscopic Imaging of Hyperpolarized [1-¹³C]Pyruvate

Jae Mo Park¹, Sonal Josan¹, Taichang Jang², Milton Merchant², Ralph Hurd³, Dirk Mayer⁴, Lawrence Recht², and Daniel Spielman¹

¹Radiology, Stanford University, Stanford, CA, United States, ²Neurology and Neurological Sciences, Stanford University, Stanford, CA, United States, ³GE Healthcare, CA, United States, ⁴Diagnostic Radiology and Nuclear Medicine, University of Maryland, MD, United States

Target Audience Researchers who are interested in imaging methods of cancer metabolism and treatment response.

Purpose Treatment of glioblastoma multiforme (GBM) patients with anti-vascular endothelial growth factor (VEGF) drug bevacizumab (Avastin[®]) frequently produces a dramatic, temporary imaging response that is considered primarily due to changes in permeability rather than an anti-tumor effect¹. We hypothesize that, in addition to this action, this agent also acutely and temporarily forces increased oxidative phosphorylation (OXPHOS) in glioma tissue due to nutrient depletion, increasing tumor vulnerability. To address this, flux through both the glycolytic and OXPHOS pathways must be measured *in vivo*. Metabolic imaging of hyperpolarized [1-¹³C]pyruvate (Pyr) has been successfully applied to cancer imaging as it is sensitive to the Warburg effect, i.e., the shift from OXPHOS towards glycolysis (GLY)². While the increased labeling of [1-¹³C]lactate (Lac) implies increased GLY, imaging of ¹³C-bicarbonate (Bic), reflecting flux through Pyr dehydrogenase (PDH) from Pyr to acetyl coenzyme A (acetyl-CoA), has been suggested as a surrogate marker for OXPHOS³. Using an optimized ¹³C MRS imaging sequence³, we were able to reproducibly image the changes in ¹³C-Bic in addition to ¹³C-Lac labeling in tumor-bearing rats after anti-VEGF treatment, using hyperpolarized [1-¹³C]Pyr, reflecting OXPHOS and GLY, respectively.

Methods An anti-VEGF monoclonal antibody B20.4.1.1 (Genentech) that binds rodent in addition to human VEGF was used as an anti-angiogenesis drug. Approximately 10⁶ C6-glioma cells were implanted in the right striatum of male Wistar rats 10 days prior to imaging. Each animal was anesthetized and placed in a clinical 3T GE MR scanner and high performance insert gradient³. Hyperpolarized [1-¹³C]Pyr (125 mM) was injected into four groups of rats through a tail vein catheter: untreated (n=15, 220-284g) and treated rats at 3h (n=8, 212-244g), 24h (n=8, 203-316g), and 48h (n=8, 272-300g) after a single injection of B20 (5mg/kg). For the 48h post-treatment group, B20 was injected 9 days after glioma implantation to avoid excessive growth of the tumor. Two of the rats were imaged both for baseline and 3h-post treatment. A 2D spiral chemical shift imaging sequence^{3,4} (4 spatial interleaves, spectral bandwidth=932.8Hz, 32 echoes, variable flip angle leading up to 90°, in-plane resolution=2.7x2.7mm², slice thickness=6-8mm) and a ¹³C/¹H dual-tuned quadrature RF coil (Ø=50mm) were used for data acquisitions. Metabolite maps and ratios were calculated³ and normalized relative to detected total carbon (tC) signals for assessment of the treatment response in glioma and normal brain.

Results and Discussion Fig.1 shows the effect of B20 on Lac and Bic productions after a bolus injection of hyperpolarized [1-¹³C]Pyr in a representative tumor-bearing rat brain before and 3h after B20. Lac/tC decreased and Bic/tC increased in glioma region after B20. Therefore, Lac/Bic, which reflects the balance between glycolysis and OXPHOS remarkably decreased in tumor. This trend was also found in the group study; Bic/tC increased from 0.011±0.004 (mean±se) at baseline to 0.038±0.003 at 3h-post B20, and Lac/tC decreased from 0.39±0.02 to 0.32±0.02. Accordingly, Lac/Bic decreased from 22.3±2.5 at baseline to 8.74±0.7 at 3h-post B20. However, Lac/Bic in groups imaged at 24h and 48h after the anti-VEGF treatment increased back to 11.7±2.1 and 16.1±4.5, respectively (Fig. 2).

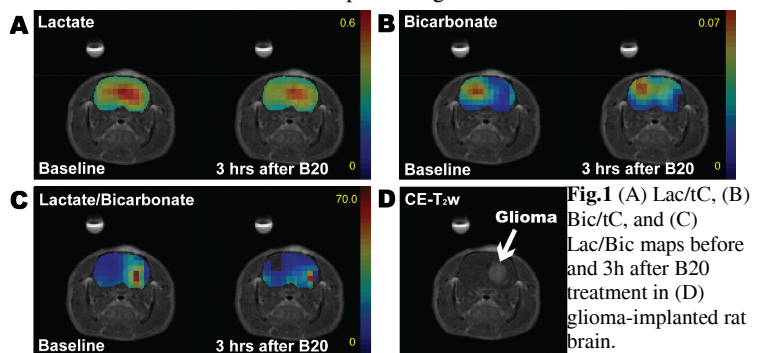


Fig.1 (A) Lac/tC, (B) Bic/tC, and (C) Lac/Bic maps before and 3h after B20 treatment in (D) glioma-implanted rat brain.

Neither Bic nor Lac levels were altered in normal-appearing brain on the contralateral side (Lac/Bic: 5.98±0.31 (baseline), 5.66±0.87 (3h), 6.50±0.66 (24h), 6.11±0.70 (48h)). These findings are consistent with the contention that sequestering VEGF is associated with a

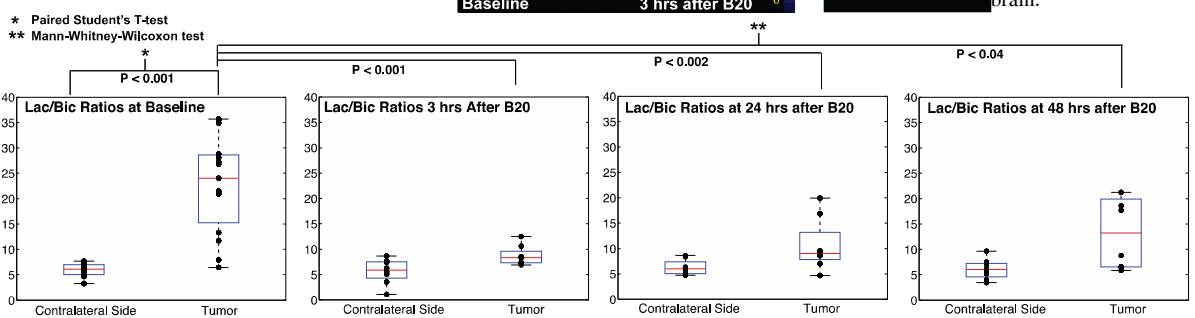


Fig.2 Distribution of Lac/Bic in glioma tumor and contralateral normal side of brain of individual animals in four groups (baseline, 3h-, 24h-, 48h-post B20 treatment). The ratio in glioma decreased after the treatment but had a trend of increasing back over time.

transient period of increased OXPHOS in glioma tissue. The immediate (3h) increase in Bic cannot be addressed by anti-angiogenesis effect (i.e., not enough time to decrease blood vessel formation), and could be due to a direct effect of VEGF on metabolism or more likely, diversion of nutrients away from the tumor tissue.

Conclusion At 3h-post B20, Bic levels were higher and Lac/Bic ratios were lower than those obtained at 24h, providing evidence for forced OXPHOS. The increased Lac/Bic at 24h and 48h relative to 3h suggests this might be a temporary phenomenon. This observation needs to be further investigated since it may give an insight as to why anti-VEGF therapy works better than agents designed primarily to treat angiogenesis, e.g., endostatin.

References 1. Von Baumgarten LD, et al. *Clin Canc Res* 2011;17(19):6192-205., 2. Kurhanewicz J, et al. *Neoplasia* 2011;13:81-97, 3. Park JM, et al. *Neuro Oncol* 2013;15(4):433-41, 4. Mayer D, et al. *MRM* 2009;62:557-564

Acknowledgements NIH: EB009070, AA005965, AA0018681, AA13521-INIA, P41 EB015891, DOD: PC100427, Lucas Foundation, Nadia's gift, and GE Healthcare