Citrate increases in gliomas in adult patients, as measured by 1H-MRS at 3T in vivo

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TARGET AUDIENCE: Neuro-oncologists/radiologists, and MR spectroscopists in brain tumors.

PURPOSE: Tumors reprogram their metabolism to meet the needs of rapid cell growth and survival in harsh environments. Changes in metabolite abundance relative to normal tissue may serve as biomarkers of malignancy, and the ability to monitor these changes noninvasively using MRS would have significant clinical utility in cancer. Citrate (Cit) is positioned at a crucial metabolic branch point, serving as an intermediate both for energy generation and for biosynthesis of lipids and related molecules [1]. Noninvasive analysis of citrate levels in tumors would therefore provide information about these pathways. Increased Cit was reported in pediatric gliomas [2], but to our best knowledge, detection of Cit in adult timorous or normal brain has not been reported to date. The goal of this study is to evaluate abnormal Cit concentrations in gliomas in adult patients using ^{1}H MRS *in vivo*.

METHODS: An MR study was carried out in 89 adult patients (ages > 25) with WHO grade 2 or 3 gliomas, determined based on histology and/or radiographic evaluation of the tumors. Written informed consent was obtained prior to the scans. Experiments were conducted in a Philips whole-body 3T scanner. A body coil was used for RF transmission and an 8-channel phased-array coil for reception. Following survey and T_2w-FLAIR imaging to identify tumor masses, single-voxel MRS data were obtained, with PRESS TE = 97 ms, from a voxel positioned within the tumor. Experimental parameters included TR = 2 s, sw = 2.5 KHz, and 2048 sampling points. The number of signal averages was 64 - 512, depending on the voxel size (4 - 8 mL). For MRS imaging, data were acquired from a 1.5 cm thick slab prescribed by the PRESS, with 1×1 cm^2 resolution. Spectra were analyzed, with LCModel, using numerically-calculated basis spectra. Metabolite concentrations were estimated with reference to water at 42 M.

RESULTS: **Figure 1** shows an *in vivo* spectrum from a patient with diffuse glioma. The spectrum shows the classic tumor pattern of increased choline and lactate, and decreased creatine and NAA. A large inverted signal was detected at 2.6 ppm. Spectral fitting was performed with two methods; one using a basis set with Cit and another using a basis set without Cit. While the LCModel fitting with Cit closely reproduced the experimental data and gave noise-level residuals at ~2.6 ppm, the analysis without Cit in the basis set did not reproduce the experimental data well in the Cit resonance region, resulting in large residuals at ~2.6 ppm. This incomplete spectral fitting affected estimation of aspartate which has resonances in the proximity of the Cit resonances, giving a ~2-fold higher estimate than that from the fitting with Cit. The NAA aspartate signals were about the same since NAA estimation was largely determined by the relatively large signal at 2.01 ppm. **Figure 2** displays data from 6 patients with gliomas. An inverted signal was clearly discernible at 2.6 ppm in all of the spectra. The data were analyzed with the two methods, similarly to Fig. 1. For the six tumor types, when Cit was not included in the basis set, residuals greater than the noise levels were observed at ~2.6 ppm in all cases, indicating that the peak at 2.6 ppm was primarily attributed to Cit. The Cit levels in these six patients were estimated to be 1.5 - 3 mM, all with Cramer-Rao lower bounds (CRLB) ≤ 10%. **Figure 3** presents ^{1}H MR spectroscopic imaging of Cit in a patient with anaplastic mixed glioma, whose single-voxel MRS spectrum is shown in Fig. 1. A map of Cit concentrations showed that Cit was concentrated at the center of the T_2w-FLAIR hyperintensity region. The Cit concentrations ranged from 0 to 3.8 mM across the brain, being 3.7 mM at the center of the tumor mass, in good agreement with the estimate by single-voxel MRS. Choline was also increased in the tumor mass, but the spatial distribution pattern was slightly different from that of Cit and, as expected, was found throughout the brain, whereas Cit showed rapid drop off in normal brain. For the 89 glioma patients enrolled in the present study, Cit was detected in 62 patients, the incidence of Cit elevation in gliomas in adult patients being 70%. The estimated concentrations ranged from 1 to 4.4 mM (mean±SD = 2.2±0.7 mM), with CRLBs between 6 and 19% (mean±SD = 11±3).

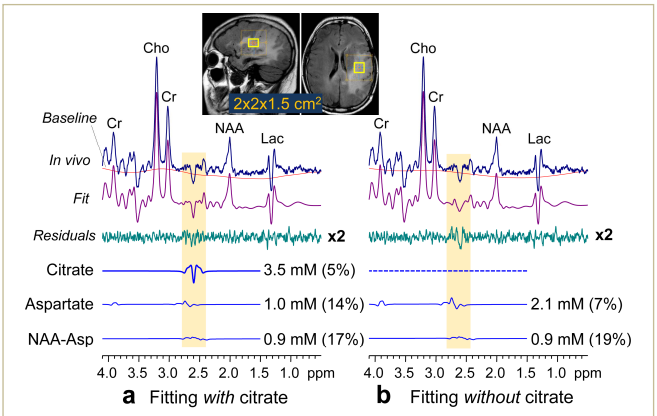

FIG 1. LCModel analysis results on a spectrum from a glioma patient, obtained using basis sets **(a)** with and **(b)** without citrate. The spectral fitting without citrate resulted in an improper fit and large residuals at ~2.6 ppm, as well as a markedly modified estimate of aspartate. Data were acquired with 256 averages (TR = 2 s). Residuals are 2-fold magnified.

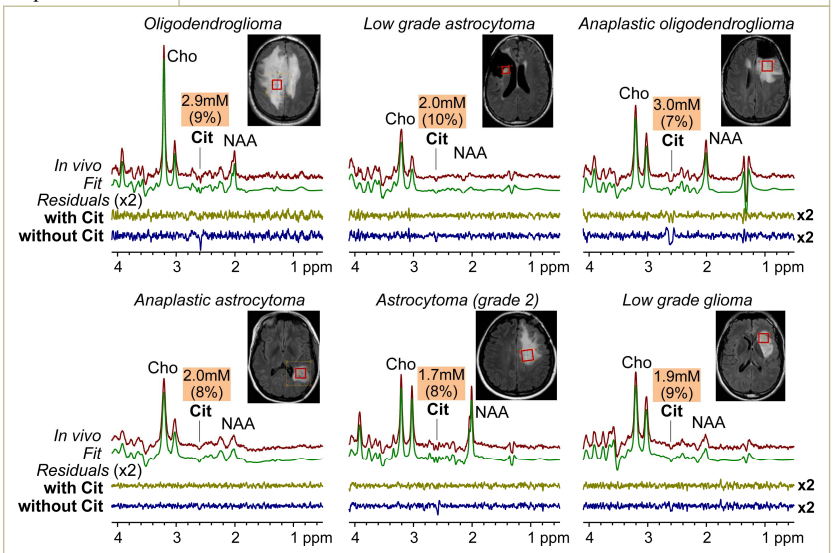

FIG 2. *In-vivo* spectra from 6 adult patients with gliomas are shown together with LCModel fits and residuals obtained from a basis set with citrate (Cit). Residuals from fitting without Cit are laid out at the bottom of individual spectra. Residuals are 2-fold magnified. The Cit concentrations and CRLBs are shown for each patient. The voxel size was 15×15×15 mm^3 (512 averages) for low-grade astrocytomas, and 20×20×20 mm^3 (128 averages) for others.

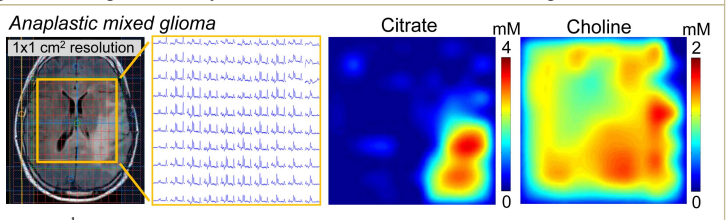

FIG 3. ^{1}H MR spectroscopic imaging of citrate in an adult patient with anaplastic mixed glioma. Left, the region of interest prescribed by PRESS is overlaid in the T2w-FLAIR image. Next, spectra of individual voxels are shown (1×1 cm^2 resolution; slab thickness 1.5 cm). The spectra are displayed between 1.9 and 4.1 ppm (right to left. Right, the estimated concentrations of citrate and choline, estimated with reference to water at 42 M, were color coded for comparison.

DISCUSSION & CONCLUSION: We have demonstrated elevation of Cit in the majority of gliomas in adult patients, using MRS *in vivo*. As Cit is unique to tumors, the specificity of Cit detection is a key advance in clinical MRS for gliomas. Interestingly, the incidence of the increased Cit in gliomas in our study (70%) is in good agreement with the reported incidence of IDH mutations (70 - 80%) [3]. Further study will be required to evaluate the potential correlation of abnormal Cit levels with IDH mutational status. Moreover, many of the patients of the present study had MR scans at multiple time points (1 - 10 scans over 1 - 3 years). Data analysis is underway to evaluate the potential clinical utility of Cit as a biomarker in the diagnosis and management of glioma patients as well as the workup of an undiagnosed mass.

REFERENCES: **1.** Icard *et al*. Biochim Biophys 2012;1825:111-116. **2.** Bluml *et al*. Neuro-Oncol 2011;13:1107-1117. **2.** Dang *et al*. Nature 2009;462:739-44.

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