Research Paper

Magnetic resonance imaging (MRI) of pharmacological ascorbate-induced iron redox state as a biomarker in subjects undergoing radio-chemotherapy

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ABSTRACT

Pharmacological ascorbate (P-AscH) combined with standard of care (SOC) radiation and temozolomide is being evaluated in a phase 2 clinical trial (NCT02344355) in the treatment of glioblastoma (GBM). Previously published data demonstrated that paramagnetic iron (Fe^{3+}) catalyzes ascorbate’s oxidation to form diamagnetic iron (Fe^{2+}). Because paramagnetic Fe^{3+} may influence relaxation times observed in MR imaging, quantitative MR imaging of P-AscH-induced changes in redox-active Fe was assessed as a biomarker for therapy response.

Gel phantoms containing either Fe^{3+} or Fe^{2+} were imaged with T2* and quantitative susceptibility mapping (QSM). Fifteen subjects receiving P-AscH plus SOC underwent T2* and QSM imaging four weeks into treatment. Subjects were scanned: pre-P-AscH infusion, post-P-AscH infusion, and post-radiation (3-4 h between scans). Changes in T2* and QSM relaxation times in tumor and normal tissue were calculated and compared to changes in Fe^{2+} and Fe^{3+} gel phantoms. A GBM mouse model was used to study the relationship between the imaging findings and the labile iron pool.

Phantoms containing Fe^{3+} demonstrated detectable changes in T2* and QSM relaxation times relative to Fe^{2+} phantoms. Compared to pre-P-AscH, GBM T2* and QSM imaging were significantly changed post-P-AscH infusion consistent with conversion of Fe^{3+} to Fe^{2+}. No significant changes in T2* or QSM were observed in normal brain tissue. There was moderate concordance between T2* and QSM changes in both progression free survival and overall survival. The GBM mouse model showed similar results with P-AscH inducing greater changes in tumor labile iron pools compared to the normal tissue.

Conclusions: T2* and QSM MR-imaging responses are consistent with P-AscH reducing Fe^{3+} to Fe^{2+}, selectively in GBM tumor volumes and represent a potential biomarker of response. This study is the first application using MR imaging in humans to measure P-AscH-induced changes in redox-active iron.
1. Introduction

Pharmacological ascorbate (P-AscH), defined as IV administered gram-doses (≥5 g–100 g) of ascorbate yielding millimolar concentrations in blood, has re-emerged as a promising adjuvant to chemotherapy and radiation therapy for the treatment of multiple cancers. Phase I clinical trials have established the safety of P-AscH in pancreatic cancer [1,2], non-small cell lung cancer [3], glioblastoma [3], and ovarian cancer [4]. Pre-clinical studies have demonstrated that ascorbate is selectively toxic to tumor cells and likely synergizes with radiation and chemotherapy agents to improve cancer cell killing [5-7].

The interaction of ascorbate with the labile iron pool (LIP) is one proposed mechanism of P-AscH’s selective cancer cell killing [3,5,8,9]. The LIP is weakly bound and easily chelated, hence called the chelatable iron pool [9-11]. The LIP is one of several pools in which iron resides and represents a small fraction of the total iron within the cell. The magnitude of the LIP in normal tissue is estimated to range from 0.2 to 5 μM [9,10,12-14]. Unlike iron bound in ferritin or transferrin, the LIP can undergo redox cycling leading to biological activity that can be harnessed for enhancing tumor response [3,20]. The LIP is proposed to catalyze the oxidation of ascorbate, leading to a high flux of hydrogen peroxide [6,15-20] while Fe$^{3+}$ is reduced to Fe$^{2+}$. Fe$^{2+}$ can then undergo cytotoxic reactions, including reactions with molecular oxygen, generating superoxide and hydrogen peroxide or directly initiate oxidation reactions via Fe$^{2+}$–O$_2$ complexes or the Fenton reaction, (Fe$^{2+}$ + H$_2$O$_2$ → Fe$^{3+}$ + OH$^-$ + HO$^-$) leading to oxidation of many biomolecules [21-23]. The ability of the LIP to redox cycle has been shown in vitro to modify the survival of P-AscH treated cancer cells [3,5]. Chelation of the LIP during ascorbate treatment with EDTA decreases cancer cell survival via an increase in iron redox cycling [3,5]. In contrast chelation with deferoxamine, increases cancer cell survival during ascorbate treatment via a decrease in the redox cycling of iron [3,5]. Iron redox cycling is thought to be greater in cancer versus normal cells, due to higher baseline cancer cell LIPs than normal cells [3,9].

Detecting changes in the LIP induced by P-AscH may serve as a biomarker to predict response. Currently, the in vivo LIP can be determined following a biopsy with tissue samples analyzed by EPR [9] or a calcinein-based assay [24]. However, biopsies are invasive and entail risk to the patient or involve terminal experiments in pre-clinical models, making repeated measurements difficult. These challenges justify the development of non-invasive methods for monitoring the redox state of the LIP as a potential biomarker for response. Two MRI methods, T$^2*$ relaxation and QSM, have shown sensitivity to the total iron concentrations in vivo [25-30]. QSM utilizes the phase of the MR signal to estimate the magnetic susceptibility of tissue, as opposed to T$^2*$ which utilizes the magnitude of the signal [31-33]. Like T$^2*$, QSM is sensitive to iron concentrations in vitro and in vivo [25,28-31,34-36]. However, the ability of these approaches to interrogate the LIP in vivo is unknown.

The highly paramagnetic properties and strong magnetic moment of Fe$^{3+}$ relative to Fe$^{2+}$ influence T$^2*$ relaxation and tissue susceptibility (QSM) [37-39]. Previous work has demonstrated that Fe$^{3+}$ exhibits greater susceptibility leading to substantially shorter relaxation times than Fe$^{2+}$ [26]. Conversion of Fe$^{3+}$ to Fe$^{2+}$ within a tumor is hypothesized to result in a measurably longer T$^2*$ relaxation time or lower tissue magnetic susceptibility in QSM, allowing for the in vivo monitoring of redox changes in the LIP.

The goal of this study was to assess the feasibility of monitoring acute changes in T$^2*$ and QSM changes (within hours) in GBM tumors treated with P-AscH. We show that T$^2*$ and QSM imaging can detect changes in the concentration of Fe$^{2+}$ in the physiologic range. These data could be used for quantitation of in vivo measurements. We then demonstrate the reliability of T$^2*$ and QSM measurements in healthy subjects and GBM patients undergoing standard of care therapy combined with P-AscH. Next, we show that T$^2*$ relaxation time increases and QSM decreases in tumors acutely after IV administration of P-AscH suggesting this approach could be used to follow changes in the LIP as a biomarker of tumor response. Finally, an animal model is used to quantify changes in the labile iron pool as a result of administration of P-AscH and the association with quantitative MRI changes.

2. Materials and methods

2.1. Phantom study

To demonstrate that T$^2*$ relaxation and QSM have differential responses to equimolar concentrations of Fe$^{3+}$ and Fe$^{2+}$, phantoms containing physiologically relevant iron concentrations (1–100 μM) were generated. Fe$^{2+}$ stock was made containing 1 mM ferrous sulfate, and 2 mM ferrozine. Ferrozine is an Fe$^{2+}$ chelator that maintains Fe$^{2+}$ in the low spin state. Ascorbate reacts with Fe$^{2+}$ present in the sample to form Fe$^{3+}$ that can then be chelated by ferrozine. Because ascorbate and ferrozine are in excess compared to Fe$^{2+}$, Fe$^{3+}$ is expected to be rapidly reduced and chelated by ferrozine. Fe$^{3+}$ stock was made using 1 mM ferric nitrate and left unchelated. A stock 1% weight to volume low temperature agarose gel was prepared. Agar was divided into two stocks; 1 mM ascorbate was added to one stock (for Fe$^{2+}$) samples. After overnight incubation, appropriate amounts of iron stocks and chelator stocks were combined in 15 mL plastic tubes to achieve the desired concentrations of Fe$^{3+}$ and Fe$^{2+}$: 0, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 μM. Falcon tubes were placed in a water bath doped with 1 mL 1. Magevisrat and imaging used a 3T TIM TRIO MR system (Siemens, Erlangen, Germany) using a multi-echo gradient-echo pulse sequence (TE = 7, 14, 21, 28, 35, 42, 49, and 56 ms; TR = 80 ms; matrix size = 192 × 256; FOV = 200 × 200 mm; slice thickness = 4 mm; flip angle = 7°).

T$^2*$ map generation: Quantitative T$^2*$ maps were generated by fitting a mono-exponential decay equation to the multi-echo gradient-echo magnitude images by least squares regression.

Quantitative susceptibility map generation: Quantitative susceptibility maps were generated using the total generalized variation QSM (TGQ-QSM) [30]. The code is graciously provided on the Langkammer group’s website (http://www.neuroimaging.at/pages/qsm.php as accessed 2019-10-14).

2.2. Mouse study

All animal experiments complied with the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

2.3. Orthotopic mouse injections

U87 GBM cells were cultured in DMEM-F12 media (15% FBS, 1% penicillin-strep, 1% Na-pyruvate, 1.5% HEPES, 0.1% insulin, and 0.02% fibroblast growth factor) and grown to 70–80% confluence at 21% O$_2$. Before injection, cells were trypsinized and the pellet resuspended in 5% methylcellulose. Three female nude athymic (NU/J) mice (Jackson Labs) were anesthetized using a ketamine (87.5 mg mL$^{-1}$) xylazine (12.5 mg mL$^{-1}$) cocktail per the University of Iowa IACUC (Protocol #71111207). Following anesthesia, a 1 cm incision was made to expose the skull and a burr hole was made approximately 3 mm lateral (right) and 2 mm posterior to the bregma. A syringe containing cells was inserted 3 mm deep to the burr hole and 4 × 10$^6$ cells in 4 μL were injected over 1 min. Following completion of the surgery, mice (n = 3) received a 5-day treatment of meloxicam (2 mg kg$^{-1}$) for pain management. Following confirmation of tumor growth as a hyperintense region on a T2-weighted anatomical image, mice were treated twice daily with ascorbate (4 g kg$^{-1}$, delivered intraperitoneally) for 7 consecutive days.
2.4. Mouse MR imaging

The mice were imaged on day 0 before the first ascorbate injection and again on day 7. Images were collected on a 7T GE small animal scanner (MR901). T2 weighted anatomical images were collected using a spoiled gradient echo sequence. T2* weighted images were collected using a gradient echo sequence (TR = 68 ms, TE = 2.5, 8.5, 14.5, and 20.5 ms, Flip angle = 16°, FOV = 25 × 20 mm, Slice thickness/gap = 0.4/0.1 mm, matrix = 256 × 204, NEX = 2). T2* maps were generated using a combination of the echo times collected and fitting each voxel to a mono-exponential curve as described for the phantom study. Images were imported into 3D Slicer software where regions of interest corresponding to the tumor and normal tissue were delineated based on the T2-weighted image. The tumor tissue was defined as the entire hyperintense region on the T2-weighted image. A similar volume of normal brain tissue was identified in the contralateral hemisphere.

2.5. EPR spectroscopy

Following euthanasia of the mice, brains were removed from the skull and separated bilaterally into tumor and contralateral normal tissue. Tissues were immediately flash frozen with liquid nitrogen and stored at −80 °C until sample preparation. Samples were prepared and analyzed for LIP concentrations using electron paramagnetic resonance (EPR) using a Bruker EMX by monitoring the high-spin Fe3+ ferrioxamine (DFO-Fe3+) complex at g = 4.3 at 100 K (Bruker ER4111VT variable temperature accessory) as previously described in Refs. [42].

2.6. Statistical analysis

The mean T2* relaxation time of voxels within the delineated region of interest were calculated. Mean T2* relaxation times were compared using a paired, two-tailed t-test. For EPR analysis, the mean signal intensity (A.U.) from triplicate measures were used to determine the labile iron concentration of samples based on a standard curve ranging from 0 to 10 μM ferrioxamine. Mean tumor and normal tissue labile iron measures were compared using a paired, two-tailed t-test.

2.7. Human studies

The work described has been carried out following The Code of Ethics of the World Medical Association. Informed consent was obtained before subject participation.

2.8. Healthy subject test-retest

Healthy subjects were invited to participate in a study exploring the repeatability of T2* and QSM imaging as an active comparison group. IRB approval was obtained from the University of Iowa Biomedical Institutional Review Board (IRB200810706; Magnotta PI). This human study did not meet ClinicalTrials.gov registration criteria. Five patients were consented and were imaged at baseline (i.e., prior to initiating radiation therapy) and at radiation fraction 20 at two time points during each visit: (scan 1) prior to the daily radiation treatment and (scan 2) three to 4 h post radiation treatment.

2.9. Imaging of subjects with glioblastoma

Subjects participating in the phase 2 clinical trial of P-AscH for GBM (NCT02344355) could opt-in for T2* MR sequences as an exploratory aim of the study. Fifteen subjects consented to undergo optional imaging and were imaged on a clinical 3T MRI (Siemens TIM TRIO, Erlangen Germany) scanner post-surgery at three time points on boost simulation day (RT fraction 19, 20, or 21, approximately four weeks into trial therapy that included an evening dose of temozolomide followed in the morning by P-AscH followed by daily radiation). T2* and QSM maps were acquired at the following times on that day: (scan 1) prior to daily therapy; (scan 2) 30–90 min post P-AscH infusion but prior to radiation treatment; and (scan 3) 4 h post P-AscH infusion and post radiation (Supplemental Fig. 1). Additional MR images collected as part of this study included T2 weighted imaging prior to P-AscH infusion and a fluid attenuated inversion recovery (FLAIR) during the second scan. T1 and contrast was acquired only after the final QSM and T2* images were acquired. Analyzed subjects were followed over time to determine progression free survival (PFS) and overall survival (OS). PFS was determined from the start of treatment to progression or last follow-up with imaging. OS was determined from the start of treatment to death or last medical follow-up.

2.10. Comparison group

GBM subjects not receiving P-AscH infusions were invited to participate in a study exploring T2* and QSM imaging as an active comparison group. IRB approval was obtained from The University of Iowa Biomedical Institutional Review Board (IRB201708773). This study did not meet ClinicalTrials.gov registration criteria. Five patients were consented and were imaged at baseline (i.e., prior to initiating radiation therapy) and at radiation fraction 20 at two time points during each visit: (scan 1) prior to the daily radiation treatment and (scan 2) three to 4 h post radiation treatment.

2.11. Image registration and contouring

The contrast-enhanced T1 weighted images served as the fixed image and the T2* maps, QSM maps, and FLAIR images were co-registered to this image using BRAINSFIT for registration [41]. For the T2* and QSM maps, registration was driven by aligning the second echo from the multi-echo GRE to the contrast enhanced T1 weighted image. The calculated transform was then applied to the T2* and QSM maps. Contours were drawn referencing the FLAIR and contrast enhanced T1 weighted images by an American Board of Radiology certified radiation oncologist. The contours were drawn blinded to the T2* and QSM maps and were eroded (contour taken in) by 2 mm. Voxels with T2* values greater than 250 ms were excluded from the volume.

2.12. Statistical analysis

Quantitative measures of T2* relaxation times and magnetic susceptibility were analyzed by comparing the slopes via analysis of covariance (ANCOVA). Comparisons between mean relaxation times and QSM in normal and tumor tissue at baseline were performed with the Wilcoxon Signed Rank test as were successive time points in each tissue type. (i.e. pre-to post P-AscH).

PFS and OS endpoints were compared to normalized T2* relaxation time changes using Harrell’s concordance index [43,44]. Tumor T2* relaxation times were normalized by dividing by the contralateral extracted. Intra-day variation was assessed by linear regression of the mean values from the two scans taken on the same day against each other. The resulting correlation coefficient (R) of the linear regression was calculated for each region. The coefficient of variation is also reported for each region.
associated white matter as an internal control. The concordance index (C-index) is interpreted as the proportion of patient pairs whose ordering of observed survival time is concordant with relaxation time changes. A C-index of 0.5 represents no concordance between the biomarker and survival while a C-index of 1.0 represents perfect concordance.

3. Results

3.1. Phantom study

Phantom studies demonstrated the differential response of T2* for Fe\(^{3+}\) compared to Fe\(^{2+}\). Comparing the slopes of the mean relaxation times for Fe\(^{3+}\) and Fe\(^{2+}\), Fig. 1A, revealed that on an equimolar basis, Fe\(^{3+}\) induced a greater change in T2* (slope = -186 \(\mu\)M \(^{-1}\) Fe\(^{3+}\)) than Fe\(^{2+}\) (slope = -33 \(\mu\)M \(^{-1}\) Fe\(^{2+}\)). ANCOVA analysis shows these slopes are significantly different (F(1,1) = 14.3, \(p < 0.001\)). Physiologic iron concentrations are highlighted on the graph in Fig. 1A.

QSM data from these phantoms show increasing magnetic susceptibility with increasing concentrations of Fe\(^{3+}\) (slope = +0.308 ppb per \(\mu\)M Fe\(^{3+}\), Fig. 1B. However, Fe\(^{2+}\) had little effect on magnetic susceptibility (slope = -0.041 ppb per \(\mu\)M Fe\(^{2+}\)). The difference in these slopes is statistically significant (F(1,1) = 7.3, \(p < 0.001\)). These data suggest that differences in Fe\(^{3+}\) and Fe\(^{2+}\) may be detected by MR imaging at physiologically relevant concentrations. Taken along with the data from T2* measurements, this phantom experiment suggested that changes in iron redox state may be visible in vivo.

3.2. Test-retest study

T2* imaging has a high degree of correlation between the first and second scans, Fig. 2A. Plotted is the second scan of a two-scan session against the first scan, R = 0.99. The coefficient of variation is approximately 0.8%. Using 40 ms as an approximation for the mean value for global T2* in the brain yields a white matter standard deviation of approximately 0.3 ms. Any change greater than 40 ms is likely due to a true biochemical change.

QSM also showed a high degree of reproducibility, Fig. 2B. The R for cerebral white matter is 0.95. The coefficient of variation is 20%, likely due to many values being so close to zero. Using the measured mean value of ~3 ppb, a standard deviation of 0.6 ppb is calculated. As shown below, experimental changes are greater than 0.6 ppb. These results suggest a high level of reproducibility in the scan methods.

3.3. Mouse study

We assessed the underlying mechanism of P-AscH-induced signal changes using an orthotopic GBM model. U87 tumors treated with P-AscH revealed a significant change (day 7 versus day 0) in T2* relaxation times between tumor and normal brain tissue (Supplemental Figure 4A). The change in T2* relaxation times was 20.3% in the tumor and ~3.5% in the contralateral normal brain. In the same study, EPR spectroscopy measurements of tissues obtained from the same mice after the 7-day course of treatment with P-AscH demonstrated a significant difference in the LIP (Supplemental Figure 4B) in the U87 GBM tumors (1.8 ± 0.05 \(\mu\)M) as compared to the normal brain tissue (1.4 ± 0.08 \(\mu\)M). These EPR results confirmed the observed T2* changes at 7 days were accompanied by changes in LIP using an independent methodology.

3.4. Clinical study evaluating acute T2* and QSM changes after P-AscH administration

The hypothesis for this study is that T2* and QSM can detect acute changes in tumor and normal tissue as a result of administration of P-AscH. Representative images from one subject are shown in Fig. 3. Fig. 3A shows the T1 contrast-enhanced anatomical image with the contrast enhancing region of tumor contoured. Fig. 3B shows a difference map of the T2* maps generated by subtracting the aligned post-P-AscH map from the pre-P-AscH T2* map. Difference maps show how the quantitative measures have changed between two successive scans. Red indicates that T2* relaxation time increased as a result of P-AscH administration (consistent with Fe\(^{3+}\) being reduced to Fe\(^{2+}\)), blue regions show a decrease in T2* relaxation time. This subject had an
average $T_2^*$ increase in the contrast enhancing region of tumor of 4.3 ms, which is reflected in the extent of red colored regions within the contour. Fig. 3C shows the difference map (post-P-AscH minus pre-P-AscH) of QSM images for the same subject at the same time points. Blue indicates that tissue susceptibility has decreased (consistent with Fe^{3+} being reduced to Fe^{2+}) and red indicates an increase in tissue susceptibility. This subject had a tissue magnetic susceptibility decrease in the contrast enhancing region of tumor by 0.95 ppb which is reflected in the extent of blue colored regions within the contour. Detectable changes in $T_2^*$ or QSM were not observed in regions of hyperintense FLAIR surrounding the tumor.

Time-courses were computed for $T_2^*$ and QSM to analyze how the contrast enhancing regions of tumor and normal tissue changed as a result of P-AscH administration and radiation therapy. The time-course showed the mean $T_2^*$ values for the contrasting enhancing region of tumor and contralateral normal brain tissue. $T_2^*$ increased 3.0 ms after administration of P-AscH ($p = 0.007$) and remained elevated compared to baseline (2.3 ms, $p = 0.02$) after radiation therapy, Fig. 4A. Contra-lateral white matter $T_2^*$ values in P-AscH treated subjects did not significantly change over the time-course, increasing only 0.4 ms post-P-AscH ($p = 0.65$), Fig. 4B. The $T_2^*$ increase in the contrast enhancing region of tumor is consistent with an in vivo reduction of Fe^{3+} to Fe^{2+} as predicted by in vitro and ex vivo studies and our pre-clinical data [3,20]. The mean values are compared in Fig. 4C demonstrating the different responses of tumor and normal tissue to P-AscH administration.

The time-course for QSM showed that tissue susceptibility in the contrast enhancing regions of tumor decreased after administration of P-AscH (1.3 ppb, $p = 0.001$) and that the decrease is persistent after radiation therapy (1.2 ppb, $p = 0.009$), Fig. 5A. Contra-lateral white matter magnetic susceptibility did not change significantly at either the post P-AscH scan ($-0.28$ ppb, $p = 0.65$) or the post-radiation scan ($-0.19$, $p = 0.39$), Fig. 5B. The QSM decrease in only the contrast enhancing regions of tumor is again consistent with an in vivo reduction of Fe^{3+} to Fe^{2+} and suggests that this reduction can be visualized with the two imaging modalities. The mean values are compared in Fig. 5C, again showing differential responses of tumor and normal tissue to P-AscH treatment.

$T_2^*$ relaxation times in GBM subjects that did not receive P-AscH treatment are demonstrated in Supplemental Figure 2. Before and after radiation therapy, $T_2^*$ relaxation times do not significantly change in either the contrast enhancing region ($p = 0.22$, Supplemental Figure 2A) or contralateral normal brain tissue ($p = 0.68$, Supplemental Figure 2B). A change was observed in QSM measurements within the contrast enhancing regions of tumor between the two scans ($-0.41$ ppb, $p = 0.04$) (Supplemental Figure 3A) while no changes were detected in normal tissue ($+0.4$ ppb, $p = 0.34$) (B). The QSM change, however, is smaller than the measured intra-day scan reliability and the difference is within the measurement error (standard deviation of 0.6 ppb) for the technique established in the test-retest portion of this study. Additional studies are needed to determine if this change in QSM is real.

To evaluate the utility of $T_2^*$ and QSM as imaging biomarkers for response to treatment with P-AscH, we compared the normalized change in $T_2^*$ relaxation times to the PFS and OS of GBM subjects included in this study. Currently, the median PFS is 9.4 months and OS is 23 months. Using Harrell’s C-index, we found moderate concordance between $T_2^*$ and QSM changes in the total tumor and T1-enhancing regions with both PFS (C-index = 0.527 and 0.600) and OS (C-index = 0.605 and 0.535) (Table 1).

4. Discussion

We hypothesized that $T_2^*$ and QSM can visualize changes in the LIP caused by treatment with P-AscH. $T_2^*$ relaxation times increased, and magnetic susceptibility decreased in contrast enhancing regions of GBM. These changes are consistent with previous in vitro results showing the reduction of Fe^{3+} to Fe^{2+} by P-AscH [3,5]. No significant change was seen in either imaging parameter in the contra-lateral normal brain. These changes in tumors were consistent across the majority of patients and were persistent for the approximate 8-h time course of the study.

These in vivo results correlate well with phantom data that show $T_2^*$ and QSM have differential sensitivity to Fe^{3+} and Fe^{2+}, with Fe^{3+} providing a significantly greater decrease in relaxation time as well as an increase in magnetic susceptibility. Gel phantoms were used to examine physiological concentrations of iron. Our $T_2^*$ measurements compare favorably to other reported work in phantoms showing shorter relaxation times for Fe^{3+} than Fe^{2+}. Our QSM results differ from Dietrich et al. [45] who report very little separation between Fe^{3+} and Fe^{2+}. We did however confirm their findings that Fe^{2+} resulted in a lower magnetic susceptibility than Fe^{3+} [45].

Based on our phantom studies, we can estimate the magnitude of the
Fig. 3. Representative images of T2* and QSM change as a result of acute P-AscH administration. A. T1 contrast enhanced anatomical showing the extent of the contrast enhancing region of tumor (contoured in red). B. Difference map (post P-AscH minus pre P-AscH) showing the change in T2* as a result of acute P-AscH administration. Red indicates an increase in T2*, consistent with Fe$^{3+}$ being reduced to Fe$^{2+}$. C. Difference map (post P-AscH minus pre P-AscH) showing the change in magnetic susceptibility as a result of acute P-AscH administration. Blue indicates a decrease in magnetic susceptibility, consistent with Fe$^{3+}$ being reduced to Fe$^{2+}$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Fig. 4. Time-course of mean T2* values in the contrast enhancing region of tumors (A) and normal tissue (B) of subjects receiving P-AscH show a persistent change in tumors post P-AscH. T2* is increased by 3.0 ms ($p = 0.007$) compared to baseline post-P-AscH infusion (approximately 4 h post baseline scan and 30 min post infusion) and remains elevated (2.3 ms, $p = 0.02$) post-radiation (approximately 8 h post baseline scan and 3.5 h post infusion). Normal tissue shows no significant changes post-P-AscH infusion (−0.4 ms, $p = 0.65$) and post radiation (−1.4 ms, $p = 0.33$). (C) Shows the differences between mean changes observed in contrast enhancing region of tumors and normal tissue. Contrast enhancing region of tumors were contoured on a contrast-enhanced T1 image by a board-certified radiation oncologist. Data are normalized per subject to the baseline scan.
changes in labile iron. Our T2* phantom studies found the slopes to be 186 μs/μM for Fe³⁺ and 33 μs/μM for Fe²⁺. From these slopes, we can estimate the concentration of iron being reduced following P-AscH administration using the following equation:

\[ [\text{iron reduction}] (\mu M) = \Delta T2^* (\text{slope ferric} - \text{slope ferrous}) \]

The mean change seen in subjects for T2* was measured to be 3.0 ms, suggesting that approximately 19 μM of ferric iron is being reduced by P-AscH.

Similarly, the same linear analysis can be applied using QSM data. QSM phantom studies indicate slopes of +0.308 ppb per μM Fe³⁺ and −0.041 ppb μM⁻¹ Fe²⁺, with a measured change of −1.3 ppb. This equates to approximately 3.7 μM of iron being reduced. The redox-active iron pool in tumors is thought to be on order of 0.5–5 μM [8–10] with transient changes of 20 μM or more possible. In an orthotopic GBM animal model, we observed a similar pattern of selective increases in T2* relaxation times. When the tumor and adjacent normal tissue was removed, this revealed significantly more labile iron in the tumor relative to the normal tissue. This supports the hypothesis that T2* mapping is capable of detecting the selective labilization of iron by P-AscH.

The proposal that QSM and T2* may capture in vivo changes in the net redox state of iron is a novel and potentially clinically useful result. This study demonstrates how both T2* and QSM methods may be used to monitor redox metabolic changes caused by manipulating LIPs with a pharmaceutical agent (P-AscH). The C-index [43, 44] shows moderate concordance between the change in tumor relaxation times following P-AscH administration in both PFS and OS. Statistical significance is not achieved likely due to the small sample size of the current study. This clinical finding suggests that T2* and QSM mapping has the capacity to provide invaluable data to studies that link iron metabolic perturbations to patient survival [12]. The results are consistent with the hypothesis that T2* and QSM may be used to assess in vivo iron redox metabolism.

This work also describes a differential response in the MR imaging of tumor and normal tissue to P-AscH. This study is the first to present a method for monitoring the in vivo changes in redox-active iron metabolism caused by the direct manipulation of the endogenous redox state of iron in cancer subjects. Future work should involve pre-clinical imaging of iron metabolism in orthotopic models and further development of the techniques to analyze the variation of LIP iron within the tumor using direct analytical methodologies.

Table 1

|                          | C-Index | 95% CI         | p-value |
|--------------------------|---------|----------------|---------|
| PFS (median = 9.4 mo)    |         |                |         |
| Total Tumor: Normal      | 0.527   | (0.237, 0.817) | 0.85    |
| T1 - Enhancing Region: Normal | 0.6     | (0.338, 0.862) | 0.45    |
| OS (median = 23 mo)      |         |                |         |
| Total Tumor: Normal      | 0.605   | (0.305, 0.904) | 0.49    |
| T1 - Enhancing Region: Normal | 0.535  | (0.262, 0.807) | 0.80    |

Fig. 5. Time-course of mean QSM values in the contrast enhancing region of tumors (A) and normal tissue (B) of subjects receiving P-AscH show a persistent change in tumors post P-AscH. QSM post-P-AscH infusion is decreased by 1.3 ppb (p = 0.001) compared to baseline (approximately 4 h post baseline scan and 30 min post infusion) and remains elevated (1.2 ppb, p = 0.009) post-radiotherapy (approximately 8 h post baseline scan and 3.5 h post infusion). Normal tissue showed no significant change post-P-AscH infusion (−0.28 ppb, p = 0.65) or post radiation therapy (−0.19, p = 0.39). (C) Shows the differences between mean changes observed in the contrast enhancing region of tumors and normal tissue. Contrast enhancing region of tumors were contoured on a contrast-enhanced T1 image by a board-certified radiation oncologist. Data are normalized per subject to the baseline scan.

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Declaration of competing interest

None.

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Appendix A. Supplementary data

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