Imaging the Progression of Iron-Loaded Glioma Tumours with FIESTA at 3T

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Introduction

Despite advances in cancer treatment, the prognosis for patients with gliomas, the most common primary tumours of the central nervous system, has not improved over the last 30 years. Single invading cells contribute to the extremely poor prognosis, as they make complete surgical resection of the tumour impossible and are resistant to conventional therapies. The inherent static nature of histological methods limits their ability to assess the dynamic process of tumour growth and invasion. It is our hypothesis that cellular imaging with MRI, using superparamagnetic iron oxide (SPIO), can provide more detailed information about tumour growth and extent and may allow the monitoring of invasion at the single cell level.

The intracellular incorporation of SPIO has enabled the detection of single cells both *in vitro* and *in vivo* with our optimized cellular imaging protocol at the clinical field strengths of 1.5 and 3 Tesla [1]. Our protocol relies on the use of a custom-built, high-performance gradient insert coil (peak gradient strength 500mT/m; peak slew rate 3000 T/m/s), custom-built solenoid RF coils, and the balanced-SSFP pulse sequence FIESTA (GE), chosen for its high SNR efficiency and sensitivity to iron. Often, however, as in the current application, it is desired to not only detect small numbers of cells, but to also track them over time. According to *in vitro* studies of cells labelled with Feridex, and we have also observed this effect with cells labelled with Bangs beads in our lab, cell division and metabolism of SPIO (in the case of Feridex) lead to the loss of sufficient iron for cell detection within 5-8 generations [2]. This translates to 5-10 days for the cells used in the current work, which have a doubling time of less than 30 hours in culture. We show here that, despite what has been observed *in vitro*, contrast due to SPIO, both in the form of biologically inert Bangs beads, as well as biodegradable Feridex, persists for at least 16 days in an *in vivo* model of glioma.

Methods

Glioma tumours were induced in C57/Bl6 mice by stereotactic injection of 10^5 GL261 cells in ~1μl into the caudate-putamen. The injected cell population consisted of either 100% unlabelled cells, 10% cells labelled with 0.9μm Bangs beads, or 25% cells labelled with Feridex (since the iron loading per cell was less).

For labelling with Bangs beads, cells were incubated overnight with 20μl of bead suspension per ml complete medium (~90μg Fe/ml). For labelling with Feridex, cells were incubated overnight with Feridex-PLL complexes at a concentration of 112μg/ml Fe and 2μg/ml PLL. In either case, cells were washed thoroughly with PBS, then resuspended with unlabelled cells to achieve the above populations for injection.

Mouse brains were imaged at 3T using our custom-built gradient insert coil, a custom-built mouse brain RF coil, and the 3DFIESTA fully-balanced SSFP pulse sequence (TR/TE=7/3.4ms, 20° flip angle, +/-21kHz bandwidth, 2 NEX, scan time ~12 mins, 100μmx100μmx200μm resolution). MRI was performed every 2-3 days starting 1 day after tumour induction until sacrifice (day 16-18).

Results

Tumours developed in all animals, regardless of iron loading, and followed similar time courses. At early time points, images of brains injected with Feridex-labelled cells were indistinguishable from those injected with Bangs bead-labelled cells (Figure 1, C & E). Over time, as the tumours grew, the iron was redistributed heterogeneously throughout the tumour volume (Figure 1, D & F). Areas of signal loss persisted in both Bangs bead-labelled tumours and Feridex-labelled tumours until sacrifice (up to 18 days) (Figure 1, D & F). Occasionally, small, discrete areas of signal loss, perhaps due to small groups of invading cells, were seen distant from the main tumour (Figure 2A).

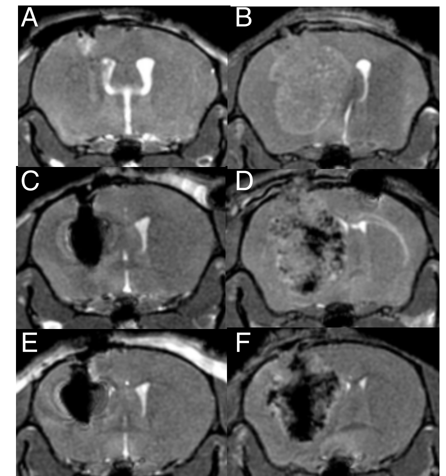

Figure 1. MR images of GL261 tumour centroid on days 1 (A, C, E) and 16 (B, D, F) after tumour induction.
A and B: unlabelled tumour
C and D: Feridex-labelled tumour
E and F: Bangs bead-labelled tumour

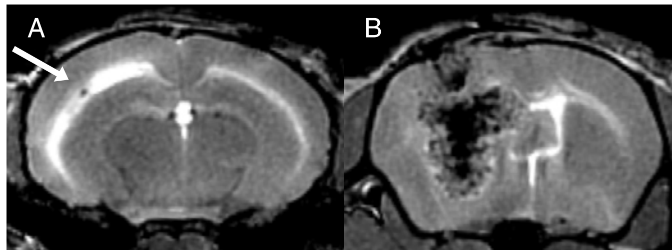

Figure 2. MR images, 3mm apart, of a mouse brain containing a Feridex-labelled tumour 14 days after tumour induction. The arrow points to a discrete signal void distant from the bulk tumour, which may be due to invading cell(s).

Discussion

In vitro results suggest that SPIO would be diluted beyond detection within 5-10 days for the cells used in this work [2]. Our data show that in a complex *in vivo* tumour system, the contrast can persist for much longer. This persistent contrast may indicate heterogeneity in cell division, proliferation and death within the tumour and may provide more detailed information about tumour progression and extent of the invasive edge. The presence of discrete signal voids near the tumour edge and distant from the bulk tumour may indicate invading cells, which have retained enough SPIO to be detected, perhaps due to a reduction in proliferation rate for migrating cells [3]. The fact that these observations are consistent for both Bangs beads and Feridex has significant implications for contrast agent targeting and clinical applications, since Feridex is a clinically approved, biologically compatible agent that can be easily conjugated to other molecules for specific targeting.

References

[1] Heyn et al. **MRM** 2006 [2] Arbab et al. **Radiology** 2003 [3] Corcoran et al. **Neurosurgery** 2003

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