Susceptibility Weighted Imaging of Iron-labeled C6-Glioma in Rats at 7T

Monika Huhndorf[1], Olga Will[2], Rolf Mentlein[3], Olav Jansen[1], and Susann Boretius[2]

[1]*Institute of Neuroradiology, University Medical Center Schleswig-Holstein, Kiel, Germany,* [2]*Biomedical Imaging, Diagnostic Radiology, Christian-Albrechts-University, Kiel, Germany,* [3]*Institute of Anatomy, Christian-Albrechts-University, Kiel, Germany*

Introduction: Annual incidence of glioblastoma is about 3 per 100.000 [1]. Standard-diagnostic is made via contrast enhanced MRI, utilizing the area of accumulated contrast agent to identify the boundaries of the tumor. However, as shown by histology this method often underestimates the area of tumor-cell-infiltration [2]. Recently a study on C6-glioma-cells in rats supposed to visualize tumor cell infiltration by *in vitro* cell labeling using iron-oxide particles in combination with *in vivo* T2*-weighted imaging [3]. Using a similar experimental setup we wanted to analyze whether susceptibility weighted imaging (SWI), as a more sensitive method of iron detection, may be able to visualize even small clusters of infiltrating tumor cells. In parallel possible tumor induced changes of T1 and T2 relaxation times, magnetization transfer and perfusion during tumor development were analyzed and compared with histology.

Methods: C6-glioma-cells were incubated with 3 mg/ml latex-coated iron-oxide-nanoparticles (310 nm; Merck, Estapor®) for 24 h. Afterwards 5 µl cell-suspensions with different cell counts (200-100.000 cel per µl) were stereotactically injected into the basal ganglia of 6 Wistar rats (male, 6 to 10 weeks old). Rats injected with either non-labeled glioma cells (n=3) or pure iron oxide nanoparticles (n = 2) served as controls. At days 5, 9, 14, 21, and 25 or 27 in case of the lower number of tumor cells, MRI was performed at 7 T (ClinScan, Bruker Biospin). MRI measurements of anesthetized rats (0.4 mg/kg medetomidine/ 70mg/kg ketamine (10%), 0.5-1.0 % isofurane via endotracheal tube) comprised SWI (3D GE, TR/TE 35/18 ms), T2-weighted images (2D FSE, TR/TE= 3150/41 ms), DCE T2*- weighted images (2D EPI, TR/TE= 400/7,3), pre and post-contrast (0.1 mmol/kg Gd-DTPA (Magnograf)) T1-weighted images (TR/TE= 10/0.9 ms, flip angle = 15°). In addition, 3 differently weighted 3D FLASH based data sets (TR/TE = 30/1 ms, flip angle=25 for T1-weighted data sets and 5° for two proton weighted data sets one without and one with additional MT-weighting by Gaussian-shaped off-resonance irradiation. The latter two yielded maps of the MT ratio. The MT saturation (δMT) correcting for signal amplitude and T1 ($T1_{app}$) was calculated as detailed in [4]. After the last MRI, rats were sacrificed and prepared for histology.

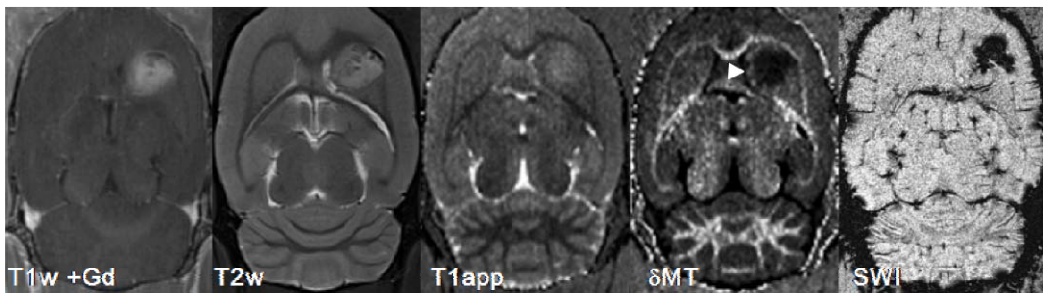

Fig. 1: Multiparameter-MRI of native C6-glioma 21 days after tumor cell injection

Results: Examples of the MRI appearance of unlabeled C6-tumors are shown in **Fig. 1**. Firstly detected at day 5 after injection the blood-brain-barrier was open for Gd-DTPA during the entire subsequent observation-period (Fig. 1, **T1w +Gd**). On **T2**-weighted images the unlabeled C6-tumor appeared well circumscribed, revealing an even slightly larger tumor circumference compared with the post Gd-DTPA image. The T1-relaxation time (**T1app**) was prolonged and the center of tumor always showed a decrease of the magnetization transfer saturation (**δMT**), which was in some cases surrounded by a rim of higher δMT values (**Fig. 1**, white arrow). In addition, the **rCBF** was increased at the tumor margin and decreased at the necrotic center.

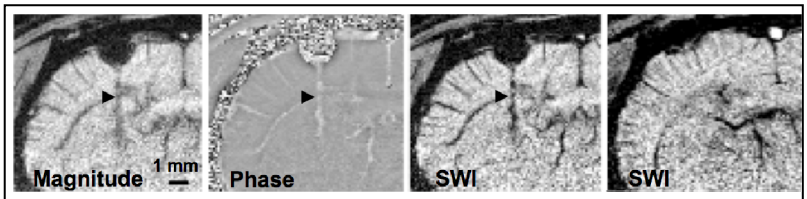

Fig.2: Magnitude, phase and susceptibility weighted image (SWI) of iron-labeled (left, black arrow) and non-labeled C6-cells (right)

High resolution **SWI** turned out to be very sensitive for iron detection. Labeled cells with a corresponding iron concentration of 0.02 µg were clearly visible on SWI, whereas the injection of native cells alone did not affect the SWI contrast (**Fig. 2**). However, no significant iron accumulation could be found in the tumor margin at times later than 14 days after injection. In fact, at earlier times after injection, the area of SWI signal decrease was always larger than the tumor circumference seen on T2w-images, but did not increase significantly with tumor growth (**Fig. 3**). Moreover, SWI signal decrease was also observed in small cortical vessels and in cases of hemorrhage independent of cell labeling (**Fig .1 and 2**).

Discussion and conclusion: SWI was very sensitive to detect iron-oxide-nanoparticles, however, this signal alteration was similar to that observed in case of hemorrhage. Here, multiparamter MRI may further improve the characterization of tumor microstructure and the distinction from hemorrhage. Although even low iron concentrations were detectable by SWI, no significant iron accumulation was found at the margin of advanced tumors, suggesting persistence of the iron particles at the site of the primary cells in the tumor center. A further increase of iron labeling seems to be no alternative in this context. It would only result in overestimation of the initial tumor volume due to massive signal attenuation. Although widely used as an animal model for human glioblastoma, with its sharp, well circumscribed margin on almost all MR contrast mechanism used here, the C6-glioma-cell model appeared less suitable for the analysis of tumor cell infiltration.

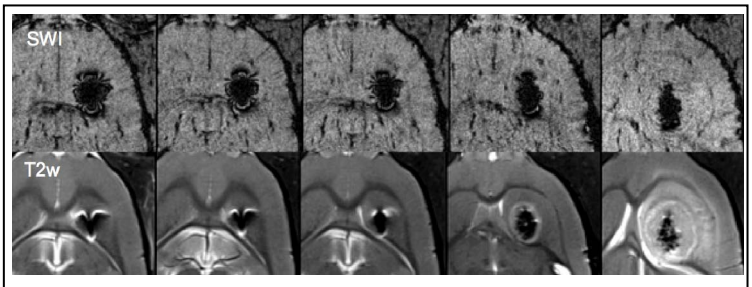

Fig. 3: Tumor growth of iron labeled C6-Glioma, from left to right: day 0, 5, 9, 14 and 21 after injection

References: [1] Dolecek et al, Neuro Oncol. Suppl 5:v1-v49 (2012), [2] Scherer, Brain 63:1-35 (1940) [3] Zhang et al., Mol Imaging Biol. 13:695-701(2011), [4] Helms et al, MRM 60:1396 (2008)