Ferumoxytol as an intravenous contrast agent for relative cerebral blood volume (rCBV) measurements by MRI in rats at 9.4 Tesla

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Introduction: Monitoring changes in blood volume with the use of intravascular contrast agents is of great interest for rodent studies of cerebrovascular diseases (such as vascular aspects of dementia), functional MRI [1], as well as cerebrovascular reactivity. Established superparamagnetic iron oxide contrast agents (Resovist, Endorem/Feridex) are no longer commercially available and not clinically approved alternatives (e.g. Feraspin, MoldayION) are of prohibitive cost for longitudinal studies in rats, where males may reach a weight of 500-800g. Ferumoxytol is a new, affordable intravenous iron preparation for treatment of the anemia of chronic kidney disease [2]. It is a carbohydrate-coated, superparamagnetic iron oxide nanoparticle (USPIO) and because of its magnetic properties, it can also be used as a magnetic resonance contrast agent [3]. In this study we investigate the use of ferumoxytol as a T_2/T_2* based MRI contrast reagent in rats at 9.4T. We investigated three aspects that are of upmost importance for a reliable measurement of rCBV, and its application in fMRI or determination of cerebrovascular reactivity: i) an unvarying particle size distribution, ii) a slow wash-out, and iii) a sufficiently high relaxivity to be used at low doses.

Materials and Methods: Experiments were conducted on male Sprague-Dawley rats (300-350g) *in-vivo* under isoflurane anesthesia (1.8-2.2% in 100% air). Coronal T_2w images (rapid single-slice: RARE, TR/TE = 2700ms / 76ms, FOV/matrix = 35x35mm^2 / 128x128; T_2 mapping: MSME, TR/TE/FA = 2000ms / 10-70ms, FOV/matrix = 35x35mm / 256x256) were acquired on a 9.4T Bruker Biospec (Ettlingen, Germany) using a four-element rat head optimized surface coil (RX) combined with a volume resonator (TX). For T_2* mapping a multi-echo gradient-echo (TR/TE/FA = 620ms / 2.14-17.2ms / 40°, FOV/matrix = 35x35mm / 256x256) was applied. Mapping used 21 slices of 1mm thickness. During rapid single-slice coronal T_2-weighted image acquisition, 5-20 mg of Fe/kg ferumoxytol (Feraheme, AMAG Pharmaceuticals, Inc) was administered using a power injector at a rate of 15 ml/h via a tail vein catheter. Ferumoxytol particle size analysis was carried out by dynamic light scattering. T_2/T_2* maps were derived from multi-echo spin-echo and multi-echo gradient-echo data using MATLAB. Time courses and dose dependency were calculated based on mean values obtained from regions of interest in the cortex and subcortical areas.

Results: During and after contrast agent injection a strong signal decrease was observed in the T_2-weighted images (-30% with 10mg/kg, Fig.2 left), which largely remained during the following observation period (-24% after 120 min). An examination of the parameter maps revealed a decrease in T_2* (-67% in cortex) and modest decrease in T_2 (-17% in cortex) throughout the brain, as demonstrated in the color-coded parameter maps (Fig.1 left) and plots of T_2/T_2* vs. time (Fig.2 left). In the 120 minutes following the injection T_2 and T_2* increased only little (8% and 4% respectively). The changes in relaxation times with increasing doses of ferumoxytol are illustrated in Fig.1 (right) and Fig.2 (center). The relaxivities r_2 / r_2* of ferumoxytol for the cortex and subcortical area were 0.2 / 6.1 and 0.4 / 5.1 s^{-1} mg^{-1} kg (derived from linear regression to R_2 / R_2* curves with a fit quality of R^2 = 0.95 / 0.98 and R^2 = 1.00 / 1.00 respectively).

Particle size analysis (samples from three 17ml Feraheme vials) showed that ferumoxytol has an average colloidal particle size of 23.38±0.37nm (zeta-average), which was almost identical for different vials (STD = 1.6%) but significantly smaller than the commonly cited 30nm [3]. The particle size distribution was narrow (Fig.2 right), ranging from 10 to 70 nm with a small poly-dispersity-index of 0.11±0.02 and did not show any other (secondary) peaks besides the one depicted in Fig.2.

Discussion and Conclusions: Our *in-vivo* results demonstrate that ferumoxytol is suitable to be used as a intravascular USPIO contrast agent in neurological rat studies at 9.4 Tesla. rCBV may be calculated from pre and post contrast agent signal intensities [4] or by parametric mapping (i.e. ΔR_2) as employed in this study. Ferumoxytol's very slow wash-out and narrow, unvarying particle size distribution suggest that it may be well suited for rCBV quantification and rCBV-based fMRI. The large changes in signal at a comparatively low dose of 10 mg Fe/kg, combined with the low cost indicate that ferumoxytol may be particularly useful as a USPIO agent for longitudinal studies in large rats.

References: [1] Gozzi A et al., Psychopharm 2010; [2] Balakrishnan VS et al., Eur J Clin Invest 2009; [3] Weistein JS et al., J Cereb Blood Flow Metab 2010; [4] Wu EX et al., MRM 2003.

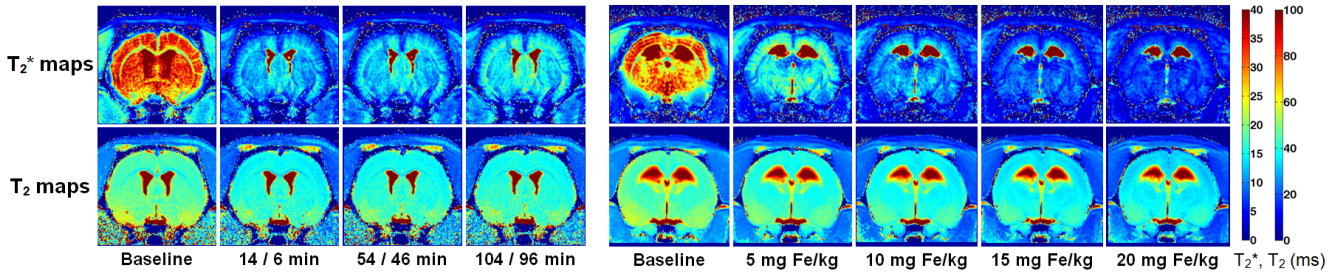

Fig.1: T_2*-maps (top row) and T_2-maps (bottom row) of the rat brain, before and after injection of ferumoxytol, for different times (left) and different doses (right). Times quoted as "*n* / *n* min" are for the T_2*-map and T_2-map respectively.

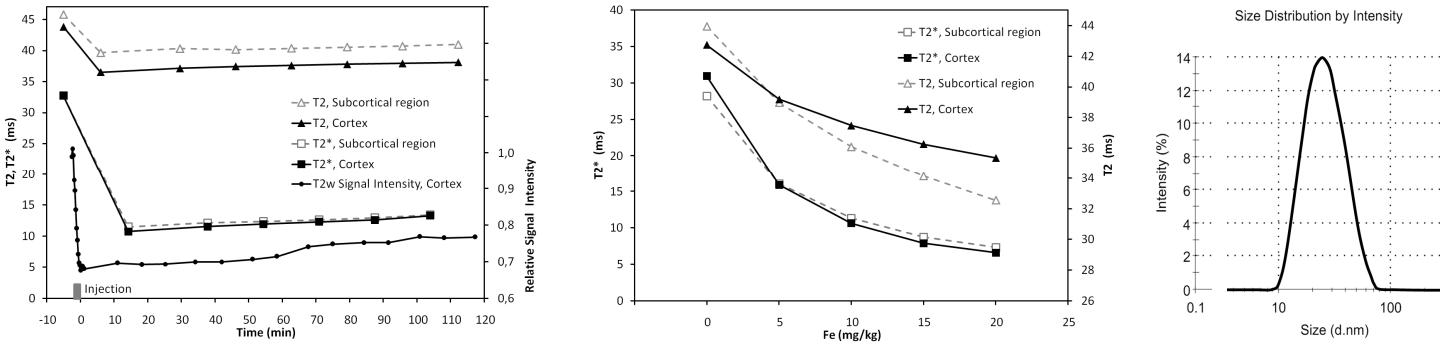

Fig.2: *Left*: Time courses of T_2, T_2*, and the T_2-weighted signal for ROIs in the cortex and subcortical regions. *Center*: Plot of T_2 and T_2* versus iron dose for ROIs in the cortex and subcortical regions. *Right*: Size distribution of ferumoxytol nanoparticles obtained from particle size analysis by dynamic light scattering.