

## Evaluation of a functional MRI Assay Using a Novel USPIO Contrast Agent (Ferumoxytol) in Normal Healthy Volunteers

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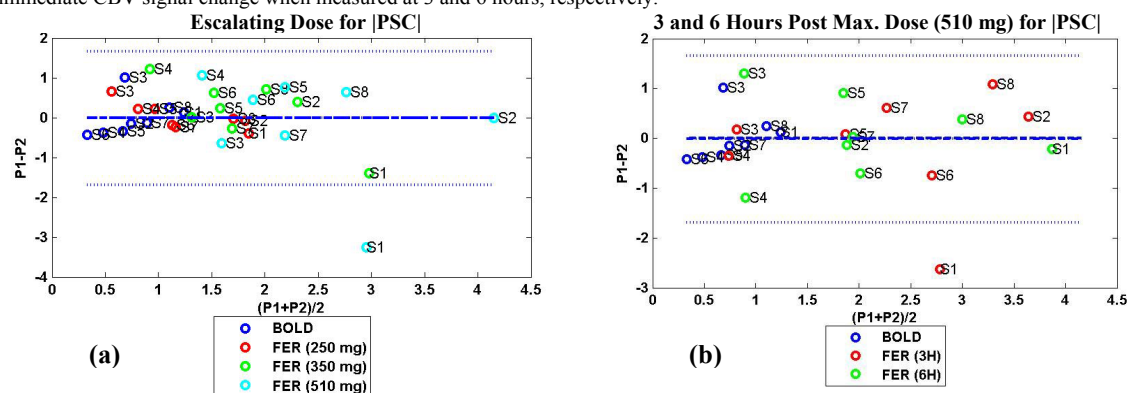
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**Introduction:** Currently there is interest in the utility of fMRI as a pharmacodynamic (PD) biomarker for centrally acting compounds. In clinical applications, fMRI has relied on endogenous Blood Oxygenation Level Dependent (BOLD) contrast. In animal experiments (1-4), Ultrasmall Superparamagnetic Iron Oxide (USPIO) nanoparticles, which function as blood pool contrast agents sensitive to cerebral blood volume (CBV), greatly enhance stimulus induced signal changes relative to BOLD. Recently, an USPIO agent ferumoxytol (Feraheme, AMAG Pharmaceuticals) has been approved for clinical use to treat iron deficiency anemia in adult patients with chronic kidney disease. We report a clinical study that explored the potential of ferumoxytol as a PD biomarker of hemodynamic changes in the brain.

**Methods:** The study was an open-label, fixed sequence design in healthy male young volunteers (n=8). Each subject was scanned on a 3 T Trio (Siemens AG) over 2 periods separated by a 3-week washout interval to examine test-retest characteristics of the fMRI assay. In each period, BOLD fMRI was followed by three doses of ferumoxytol, administered intravenously (250 mg, 100 mg, and 160 mg resulting in cumulative doses of 250 mg, 350 mg, and 510 mg) with CBV fMRI after each dose, and 3 and 6 hours after the third dose. A flashing checkerboard pattern was used as the visual stimulus and was applied for 8 cycles (15 s off / 15 s on) for each BOLD and CBV fMRI scan. Whole brain images were acquired using an EPI sequence (TR/TE=3000/30ms). Following a General Linear Model as implemented in FSL (5), average absolute percent-signal-change (PSC), (the signal increases for BOLD and decreases for CBV) was calculated from the primary visual cortex (V1) as an fMRI visual stimulus response endpoint as stipulated by the study protocol. Statistical analysis was carried out using mixed effects ANOVA with appropriate contrasts as implemented in SAS (SAS Institute Inc.) software. CBV fMRI |PSC| dose responsiveness was also examined using a trend test (NOSTASOT).

**Results:** In this study, ferumoxytol was generally safe and well tolerated. There were no clinically relevant effects on either vital signs or safety laboratory tests observed in this study.

Estimates of average |PSC| for BOLD fMRI in V1 (with 95% lower confidence limits) were 0.68 (0.43) and 0.61 (0.45) for Period 1 and Period 2, respectively. Enhancement of CBV signal compared to BOLD fMRI was observed across the whole range of ferumoxytol, (p-values range from p=0.02 to p<0.0001). Estimates of the fold increase in |PSC| were 1.53 to 3.23 across increasing doses of ferumoxytol (lower 95% confidence limits in range from 1.09-2.30). Results of the test for trend through 0-510 mg doses of ferumoxytol revealed statistically significant trend through all three doses of ferumoxytol (p<0.0001 for both periods). Importantly, the enhancement by ferumoxytol persisted at 3 and 6 hours after the last dose; the increase in |PSC| was 86-92% and 74-80% of the immediate CBV signal change when measured at 3 and 6 hours, respectively.



**Figure 1.** Bland-Altman plots of |PSC| for BOLD fMRI and CBV fMRI with escalating dose of ferumoxytol (a) and 3 and 6 hours post highest dose of ferumoxytol (510 mg cumulative) (b). Plots show (P1+P2)/2 vs. P1-P2, where P1 and P2 are |PSC| in V1 for Periods 1 and Period 2, respectively. The dotted lines correspond to the limits of agreement (95% likely range for the difference) and the dashed lines correspond to mean difference. Data corresponding to each subject labeled S1-S8.

Figure 1 depicts the Bland-Altman plots for |PSC| using BOLD and CBV fMRI. From this plot, the increase in signal enhancement for CBV fMRI appears to be dose-dependent (the higher the dose, the more to the right the response in this plot). Apart from some clear outliers (e.g., S1, probably due to the subject not attending to stimulus), the CBV fMRI exhibits test-retest repeatability comparable to that of BOLD fMRI.

**Discussion and Conclusions:** This study represents the first known fMRI clinical study with CBV fMRI. We found that ferumoxytol-based CBV fMRI provides greater visually-evoked percent signal change than BOLD fMRI within a generally safe and well tolerated ferumoxytol dose range (6). Also, persistent ferumoxytol enhancement up to at least 6 hours after the highest dose demonstrates the potential flexibility of CBV fMRI in clinical studies. For example, several studies can be performed with signal enhancement superior to BOLD fMRI up to 6 hours after a single ferumoxytol administration. When subjects were attending to the visual stimulation paradigm, good test-retest repeatability was obtained. Furthermore, the results of this study are consistent with preclinical rodent and non-human primate experiments (1-4), demonstrating vertical translatability of CBV fMRI. Overall, the increased sensitivity provided by CBV fMRI relative to BOLD should increase the utility of fMRI as a biomarker for changes in brain function in clinical studies. Future studies with brain-penetrant molecules will test the utility of CBV fMRI as a clinical pharmacodynamic biomarker.

**References:** 1) Mandeville et al, Magn Reson Med, 39(4), 615-24, 1998, 2) Leite et al. NeuroImage, 29(3),901-9, 2002, 3) Zhao et al. NeuroImage 2008, 40(1), 133-47, 4) Zhao et al, NeuroImage, 44(2), 349-62, 2009. 5) Smith et al. NeuroImage, 23, 208-19, 2004. 6) [http://www.accessdata.fda.gov/drugsatfda\\_docs/label/2009/022180lbl.pdf](http://www.accessdata.fda.gov/drugsatfda_docs/label/2009/022180lbl.pdf)