Longitudinal R_2* and R_2 Relaxation Time Measurements of (U)SPIO-loaded Murine Liver *In Vivo*

T. Kalber[1], S. Robinson[1], F. Howe[1], A. Ryan[2], J. Waterton[2], J. Griffiths[1]

[1]Basic Medical Sciences, St. George's Hospital Medical School, Tooting, London, United Kingdom, [2]AstraZeneca, Macclesfield, Cheshire, United Kingdom

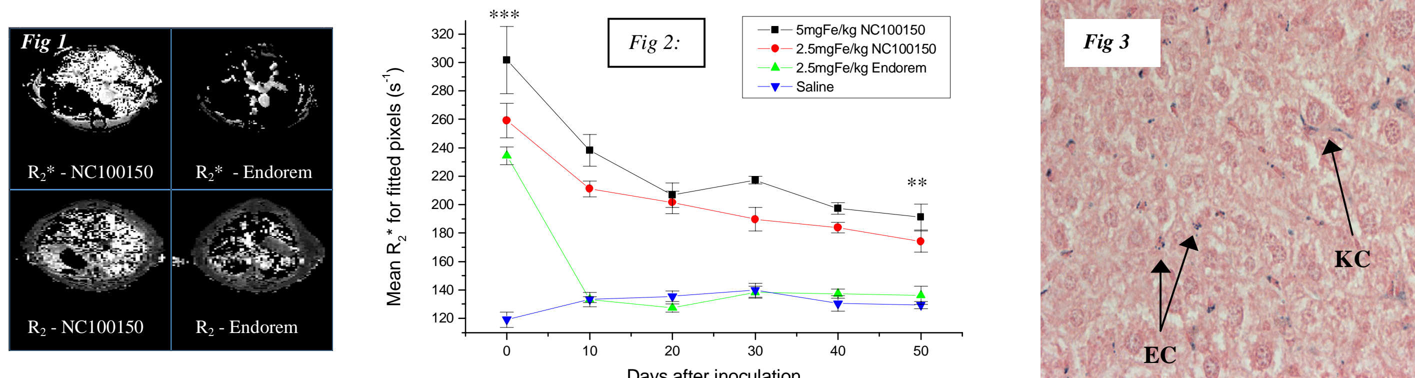
Introduction. Superparamagnetic contrast agents are made up of iron oxide particles and are based on two subclasses depending on the size of the overall hydrated particle size, superparamagnetic iron oxides (SPIO) >50 nm and ultrasmall superparamagnetic iron oxides (USPIO) <50 nm. (U)SPIO's are selectively taken up and metabolized by the macrophages of the reticuloendothelial system (RES) primarily in the liver and spleen[1-4]. The process of uptake within the RES is mainly dependent on the total size of the particle, being faster for large particles, therefore USPIO's tend to have a longer blood half life. Once internalized within the sinusoidal cells of the liver, the (U)SPIO particles are metabolized by hydrolytic enzymes into ferritin and haemosiderin, which is then passed through the normal pathway of iron metabolism, the rate of which is dependent on the sub-cellular distribution within the liver cells. However, as this distribution is largely dependent on particle coating, it is not possible to predict the rate of metabolism for one contrast agent based on the results of other iron oxides. This study uses serial measurements of R_2*, R_2, and histology to compare Endorem, an SPIO with a dextran based coating with NC100150, a USPIO with an oxidized starch coating to assess their pharmacokinetics and distribution in murine liver.

Methods. Adult 6-8 week-old female MF1 mice were used. Under halothane anaesthesia, a lateral tail vein was cannulated using a 27-gauge butterfly needle and mice administered 2.5mgFe/kg Endorem (SPIO) (AMI-25, Guerbet Europe, Advanced Magnetics Inc US) (n=5) 2.5mgFe/kg NC100150 (USPIO) (Clariscan, Amersham health) (n=5), 5mgFe/kg NC10050 (n=5) or saline (n=5). The mice were then imaged at 5 minutes (2.5mgFe/kg Endorem and NC100150 cohorts), 4 hours, 10, 20, 30, 40, and 50 days post-administration. Quantitation of liver R_2* and R_2 was performed using a Varian Unity Inova System interfaced to a 4.7T horizontal magnet, using a quadrature birdcage coil. Multi gradient-echo images with increasing R_2*-weighting (TR = 80 ms, initial TE = 4, TESPACE = 3 ms, 8 echoes) and respiratory-gated, multi spin-echo images with increasing R_2-weighting (TE = 13, 18, 23 and 28 ms) were acquired from a single transaxial 1mm slice through the liver. Apparent R_2* and R_2 maps were calculated on a pixel-by-pixel basis using ImageBrowser (Varian Inc, Palo Alto, CA, USA). A region of interest (ROI) was drawn around the whole liver. Subsequently, ROI's were drawn around the apparent vascular structures within the liver, and these regions were subtracted from the main ROI revealing pixels only from normal liver parenchyma. Any unfitted pixel values were then removed and the mean R_2* and R_2 values from the remaining fitted pixels determined. An additional 12 mice/cohort where administered (U)SPIO as above and 2 mice sacrificed at each time point. The livers were removed, fixed in formalin, cut and stained with Perl's Prussian blue iron(III) stain.

Results & Discussion. Figure 1 shows murine liver R_2* and R_2 maps acquired 5 minutes after administration of 2.5mgFe/kg NC100150 or 2.5mgFe/kg Endorem. Administration of Endorem 5 minutes before MRI resulted in liver R_2* and R_2 maps in which the majority of the pixels had relaxation rates that were too fast to be fitted. The remaining fitted pixels from the normal liver parenchyma exhibited very fast relaxation rates but were few in number. NC100150 also acutely increased the relaxation rates of the liver pixels, but not to the same extent as that induced by Endorem, supporting the hypothesis that the larger hydrated particle size SPIO agents have a shorter blood half life and faster facilitation into the RES cells.

The longitudinal changes in liver R_2* for each dose and control are shown in Figure 2. A similar response was found for liver R_2. The effects of both Endorem and NC100150 were similar 4 hours after administration, dramatically and significantly increasing R_2* (***$p<0.001$, Student's t-test) and R_2 ($p<0.001$). By day 10, the mean liver R_2* and R_2 for the Endorem dosed mice was not significantly different from saline controls. This temporal recovery of R_2* and R_2 reflects the clearance of Endorem from the liver and was confirmed by the histology. In contrast, the increase in liver R_2* and R_2 of mice administered NC100150 was sustained and was still significantly faster than saline controls at day 50 (**$p<0.01$). Figure 3 shows strong Perl's Prussian blue staining 10 days after administration of 2.5mgFe/kg NC100150. The histology revealed uptake of NC100150 not only by the Kupffer cells (KC) of the RES, but also into the endothelial cells (EC) of the liver, similar to that previously reported for rat liver[2,5]. Endothelial cells are believed to be less effective at metabolizing and degrading particulate iron compared to Kupffer cells[5], hence uptake into these cells may explain the longevity of this contrast agent. Minimal staining of Kupffer cells on day 10 can also be seen with NC100150 this also suggests that the oxidized starch particle takes longer to metabolize than a dextran based particle.

The data highlight how the differences in (U)SPIO particle coating, and particle size can affect the transport, internalization and metabolism of iron oxide contrast agents within the cells of the liver. The effects of a single injection of (U)SPIO is impressive and highlights the associated challenge of accurately measuring R_2* and R_2 from the whole liver in the presence of iron oxide particles and the need for ultra-short imaging sequences to enable complete quantification[6].



1) Brasch RC. Radiol. 1992;183:1. 2) Briley-Saebo *et al.* JMRI. 2004;20:622. 3) van Beers BE *et al*. Radiol. 1997;203:297. 4) van Beers BE *et al*. JMRI 2001;13:594. 5) Briley-Saebo *et al*. Cell Tiss. Res. 2004;316:315. 6) Chappell *et al*. JMRI 2003;18:709.

Supported by BBSRC, AstraZeneca, The Royal Society and Cancer Research UK, [CRUK] grant SP 1971/0701.