Laser Ablation ICP MS Imaging for The Detection of Contrast Agents in Tumour Tissue: Correlation to MR Images.

T. Kalber[1], J. Pugh[2], N. Kamaly[3], J. Bunch[2], J. Bell[1], A. Miller[3], and C. McLeod[2]

[1]Metabolite and Molecular Imaging Group, ISD, MRC CSC, Hammersmith Hospital, Imperial College London, London, United Kingdom, [2]Centre for Analytical Sciences, Department of Chemistry, University of Sheffield, Sheffield, United Kingdom, [3]Imperial College Genetic Therapies Centre, Department of Chemistry, Imperial College London, London, United Kingdom

Introduction:

The quantification of MRI contrast agents *in vivo* on images is a challenge in molecular imaging, especially with low molecular weight imaging probes, due to the lack of sensitivity and the low doses utilized. However, low molecular weight contrast agents conjugated to biologically functional molecules is now of great interest to facilitate drug research. Laser ablation-inductively coupled plasma mass spectrometry (LA-ICP-MS) is a powerful and versatile technique for spatially resolved measurement and mapping of elemental/isotopic composition and is capable of microanalysis of metal isotopes. In this study, we present the use of this technique correlated with MRI images for the mapping of the distribution of Gadolinium (Gd) in tumour tissue. For this purpose LA-ICP MS was used to asses the presence and distribution of Gd in solid tumours at varying time points up to 24 hours post administration of three different Gd agents. A low molecular weight tubulin binding agent (colchicine) conjugated to Gd.DOTA, which binds to tubulin within the cell with high affinity, a low molecular weight folate agent conjugated to Gd.DOTA that binds to cell surface receptors, and a passive targeting liposome preparation containing multiple Gd.DOTA's. These results will be correlated to MR images to assess the sensitivity of MR for each of these agents and how specific binding mechanism can effect this.,

Methods: Contrast agents: For low molecular weight contrast agents a previously synthesized Folic-acid coupled linker or colchicine were conjugated to DOTA.NHS.ester (Macrocyclics, USA) to produce DOTA.Folate and DOTA.Colchicinic acid respectfully. The final gadolinium containing molecules Gd.DOTA.Folate (1) and Gd.DOTA.Colchicinic acid (2), was obtained by the addition of 6H2O.GdCl3 to the DOTA conjugate. Both low molecular weight contrast agents contain one Gd ion per binding molecule. The liposome preparation contained previously synthesized paramagnetic lipid Gd.DOTA.DSA and rhodamine fluorescent tracers previously described by Kamaly *et al.* (3). They are PEGylated neutral liposomes made with a defined molar ratio of individual lipids (Gd.DOTA.DSA/DOPC/Cholesterol/DSPE PEG2000/DOPE-Rhodamine: 30/32/30/7/1 mol%) to give a predetermined total lipid concentration of 30 mg mL-1 in HEPES (20 mM, NaCl 135 mM, pH 6.5), with a size of ~100 nm and containing a total of ~ 9.87 µM of Gd.

***In vivo* experiments:** 6-8 weeks old Balb/c nude mice were inoculated subcutaneously with 5 x 10^6/0.1ml IGROV-1 cells, (a cell line that over-express folate receptors) (liposomes or Gd.DOTA.Folate), or OVCAR3 (Gd.DOTA.Colchicinic acid). Tumour bearing mice were anaesthetized with an isoflurane/O2 mix and were placed in a quadrature ^{1}H volume coil and imaged using a 4.7T Varian Direct Drive MRI scanner. A spin-echo sequence was used to obtain 20 consecutive transverse images covering the whole abdomen with the following parameters: 5 TRs ranging from 400 to 5000 ms, TE = 15 ms, FOV = 45 x 45 mm, averages: 1 matrix size: 256 x 128: 2.0 mm thickness. Mice were removed from the magnet and a tail vein cannulated for the administration of either 200 µL liposome solution, and re-imaged at 2hrs post injection, 200 µl of 1mM Gd.DOTA.Folate, DOTA.Folate, Gd.DOTA or water and re-imaged at 2, 12 and 24 hrs post injection or 200mg/kg Gd.DOTA.Colchicinic acid, DOTA.Colchicinic acid, Gd.DOTA or water and re- imaged at 2, 8 and 24 hrs post injection. Tumours and selective organs were excised and frozen either at the earliest 2 hour time point (liposomes only) or after the final scan (Gd.DOTA.Folate and Gd.DOTA.Colchicinic acid).

LA-ICP-MS: Tumour tissue sections were embedded in OCT and sections cut at 7 µm using a cryotome. The laser ablation system (LSX-200, Cetac, 266nm Nd:YAG) was configured to perform multiple line rasters for generating 2D elemental distribution maps. A beam diameter of 25µm was used for ablating tumour sections (with energy 0.3mJ) while for kidney sections a diameter of 100µm was used (1.2mJ). A scanning speed of 50µm/s and laser frequency of 10Hz was maintained for all sections. For ICP–MS (Agilent, HP4500 Series 100) the elements Gd^{157} and Zn^{66} were monitored in a time-resolved mode, isotopes were selected on the basis of high-percentage abundance and minimal isobaric and polyatomic interferences. Tumour and kidney raster lines were separated, to prevent contamination of adjacent tissue with previous raster runs, by 50µm and 200µm respectively. A typical sample area was 16-25 mm^2 (80-90 mm^2 for kidney sections). Elemental maps were produced using the Graphis software package (Kylebank Software Ltd, Ayr, UK). Adjacent sections where taken for H and E staining.

Results: Gd.DOTA.Colchicinic acid caused a reduction in T_1 8h post administration which increased over the 24 h time course (Fig 1a). As this agent is a tubulin binding agent and acts as a vascular disrupting agent resulting in central necrosis there are complex mechanisms affecting T_1. LA-ICP MS showed good correlation with the high signal intensity areas on the MR image, also LA-ICP MS showed substantial amounts of Gd throughout the centre of the tumour that was shown to be shown to now be necrotic (Fig 1b). This is most like due to the tubulin bound agent still present in the vessels that have occluded and may represent the original vascular pattern prior to therapy. The Gd.DOTA.Folate contrast agent also demonstrated a decrease in T_1 at 12 h post administration (~30%) (Fig 1c). However, the LA-ICP MS appeared to show lower levels of Gd in the tumour than that of the Gd.DOTA.Colchicinic acid molecule. The presence of Gd was mainly apparent within the peripheral areas of the tumour corresponding to well vascularised regions from H&E stained sections (Fig 1d). A similar peripheral Gd pattern in tumour tissue was also observed by LA-ICP MS for the liposome contrast agent although tumour T_1 measurements did not show a significant tumour signal enhancement at 2 h post administration compared to controls (Fig 1e). Nonetheless, substantial amounts of Gd was visible in the same tumour section when imaged for Gd using LA-ICP MS (Fig 1f). As these nanoparticles have a neutral charge due to the PEG layer, which provides prolonged circulation times *in vivo*, a gradual accumulation within tumour tissue through the EPR effect for these non-targeted liposomes has been previously shown [3]. Therefore, the 2h time points may not have been sufficient time in order to accumulation of enough Gd/particle that was sensitive enough to enhance MR. However, with the use of LA-ICP MS there is a better understanding of the nanoparticle uptake within the tumour tissue. Reduction at the earlier time points for the low molecular weight compound compared to the liposome contrast agent is probably due to receptor binding rather than accumulation as small molecules are normally cleared fairly rapidly. However, even though both low molecular weight agents contain less gadolinium per molecule compared to the liposome agent, their functionality as specific binding agents allows them to be functional contrast agents.

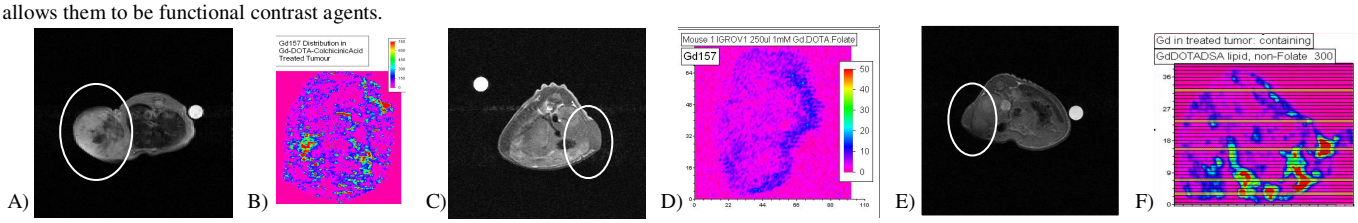

Figure1: MR images (A,C, & E) and LA-Ablation data (B, D & F) of a tumour 24 h post administration of 200mg/kg Gd.DOTA.Colchicinic acid (A & B), 12 h post administration of 200µl Gd.DOTA.Folate (C & D), or 2 h post administration of 200µl Liposomes (D & E).

Conclusions: These results demonstrate the sensitivity and potential of LA-ICP-MS for the direct mapping of Gd biodistribution and other isotopic metals within tissues of interest such as tumours. The sensitivity of this technique is ideal for furthering investigations of novel MRI contrast agents for tumour imaging. A better understanding of the amount of binding or retention needed for sensitivity in MR will help in furthering existing agent and in designing novel imaging tracers .

Refereces 1) Kalber *et al.* Proc Inter Soc Magn Reson Med. 2007;1156. 2) Kalber *et al.* Proc Inter Soc Magn Reson Med. 2008;2794. 3) Kamaly N *et al.* Bioconjugate Chemistry. 2008;19;118.