A Novel Low Molecular Weight Folate Receptor Targeted Contrast Agent For Magnetic Resonance Tumour Imaging

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Introduction: Folate receptors (FR) represent a family of membrane-anchored glycoproteins that have a high binding affinity to folic acid. We have recently synthesised a folic acid linked precursor, which has been conjugated to DOTA and complexed with gadolinium to produce a novel low molecular weight folate receptor targeted contrast agent. This molecular structure aims to improve the toxicity issues that can be associated with larger molecular weight folate-chelates. FR are present at very low levels in normal tissue but are over-expressed in a wide range of malignant cancers especially ovarian carcinomas[1]. However, as the main limitation for active targeting is the degree of magnetic label in the tissue of interest, the aim of this study has been to utilise ovarian tumour cell lines for both *in vitro* and *in vivo* MR imaging in order to assess the effectiveness of this novel folate-targeted contrast agent for the imaging of FR positive tumour cells.

Methods:

Folate contrast agent synthesis: Folate.Gd.DOTA was successfully synthesised using a combination of solid and solution phase chemistry and analysed by NMR, ESI-MS and reverse-phase HPLC. The resulting structure is shown in **Fig 1**.

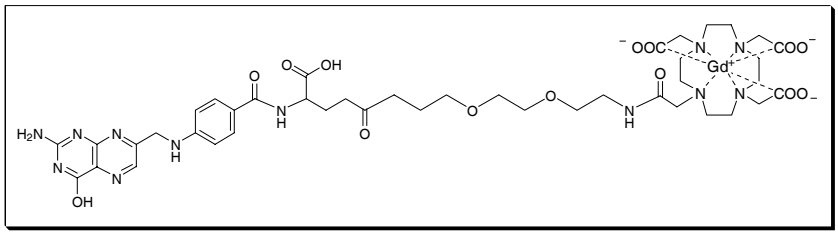

Fig 1. Folate.Gd.DOTA

In vitro experiments: *In vitro* experiments consisted of either water-soluble phantoms of Folate.Gd.DOTA compared to Folate.DOTA as the control, as well as cell pellet phantoms using IGROV-1 ovarian carcinoma cells (5 x 10^5), which were confirmed to have a high level of FR expression by both FACS analysis and immunohistochemistry. IGROV-1 cells were cultured under standard conditions, incubated with either the Folate.Gd.DOTA or Gd.DOTA control for 8 hr and pelleted for MRI. These phantoms were placed in a quadrature ^{1}H volume coil and positioned into a 4.7T Varian Inova MRI scanner. A spin-echo sequence with the following parameters was used to assess T_1 relaxation, TR = 50, 100, 200, 300, 500, 700, 1200, 3000, 2800 ms, TE = 10 ms, FOV = 45 x 45 cm^2, averages: 1 matrix size: 256 x 128: and a 2.0 mm thickness.
Preliminary in vivo experiments: For *in vivo* experiments 5 x 10^6/0.1ml IGROV-1 cells were inoculated into the flanks of 6-8 weeks old Balb/c nude mice. After ~2 weeks tumour bearing mice were anaesthetized with an isoflurane/O_2 mix and placed into a quadrature ^{1}H volume coil as described above. The mice were injected intravenously via a lateral tail vein with a 200 µl of 0.5 mM Folate.Gd.DOTA or Gd.DOTA control and imaged at 3 and 12 hrs post injection at 4.7T. Spin echo MRI images were obtained using parameters: TR = 400, 700, and 2800 ms, TE = 10 ms, FOV = 45 x 45 cm^2, averages: 1 matrix size: 256 x 128: 2.0 mm thickness, and 20 consecutive transverse slices. The tumours were then excised fixed in formalin and imaged *ex vivo* using the same parameters as in the *in vitro* experiments.

Results: *In vitro* results for water-soluble phantoms showed that the Folate.Gd.DOTA contrast agent achieved a 70% T_1 reduction compared to water and a 65% T_1 reduction compared to the control compound. The cell pellet phantoms also showed some enhancement compared to controls but the most significant T_1 enhancement was seen *in vivo*. The *in vivo* results show that within 3 hours a 45% reduction in T_1 was achieved compared to ~ 28% for Gd.DOTA control, which was sustained for at least 12 hrs after dosing (**Fig 2**.), and confirmed *ex vivo* (not shown).

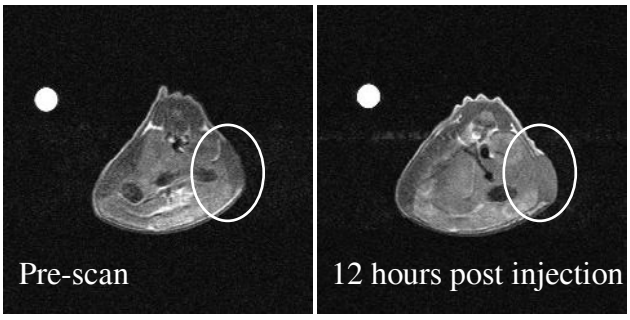

Fig 2: Pre- and post- Folate.Gd.DOTA images of IGROV-1 tumours.

Conclusion: We have shown the successful synthesis of a novel low molecular weight folate receptor targeted contrast agent and its targeting to FR positive cells *in vitro* and *in vivo* by MRI, raising the possibility of using such folate contrast agents for the detection of cancer cells *in vivo* by MRI.

1) Konda *et al.* Invest. Rad., 2000;35:50-57.