PARASHIFT contrast agents - a new approach for molecular imaging by MRI

Ian Wilson[1], Peter Harvey[2], Katie-Louise Finney[2], Alexander M Funk[2], P Kanthi Senanayake[2], Ross J Maxwell[1], David Parker[2], and Andrew M Blamire[3]
[1]*Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, United Kingdom,* [2]*Dept of Chemistry, Durham University, Durham, United Kingdom,* [3]*Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom*

Introduction

Paramagnetic contrast agents are traditionally used in MRI to enhance pathology based on differences in uptake of an injected agent. The common complexes of gadolinium act to produce a T_1 shortening of the tissue water proportional to contrast agent concentration. Other agents, such as paramagnetic iron oxide preparations (SPIO or USPIOs) act via through-space distortion of the local magnetic field leading to reduced T_2 or T_2*. In either case, the presence of the agent is detected indirectly through changes in water relaxation. Recently we have developed an approach whereby the *contrast agent molecule is detected directly* (1,2,3). Initially this approach was demonstrated using ^{19}F detection of CF_3 groups on the molecule (1,2). In the current work, we apply a similar approach for ^{1}H detection and introduce a concept we term PARASHIFT agents.

PARASHIFT concept

Proton chemical shifts normally fall within the diamagnetic 0-12ppm range and the ^{1}H spectrum *in vivo* is dominated by strong water and lipid signals at 4.7 and ~1.5ppm respectively. We sought to create a contrast agent for molecular imaging where the signal from a reporter group on the molecule was shifted away from this normal region and hence could be imaged directly, without the confound of the endogenous tissue signals. By placing the reporter group close to a lanthanide (III) paramagnetic centre, its chemical shift can, in theory, be shifted by up to ±500ppm. For a separation > 45nm the dipolar shift dominates and careful selection of the chemical structure and lanthanide ion modifies both the chemical shift of the reporter resonance and also the relaxation properties, particularly strongly reducing the T_1 allowing rapid pulsing for high sensitivity.

Methods

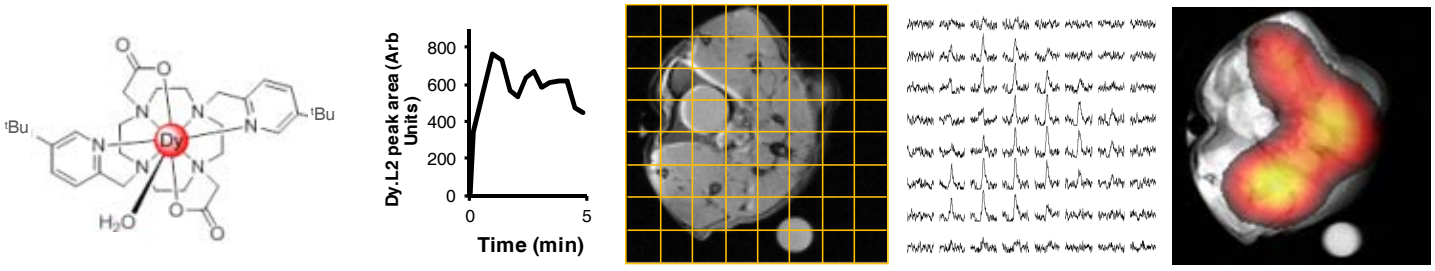
A range of paramagnetic complexes with *tert*-butyl reporter groups were synthesised. Here we report experiments using a Dysprosium complex (referred to as $[Dy.L^2]^+$, Figure 1(a). For synthesis details see ref 4). Relaxivity of the reported group was determined at a range of magnetic field strengths (4.7, 9.4, 11.7, 14.1 and 16.5T) and the frequency shift of the reporter group was also measured. MR imaging, spectroscopy and spectroscopic imaging experiments were then performed in 2mM aqueous solutions of the agent using a 7T magnet with a Varian Direct Drive console and a 39mm i.d. quadrature birdcage coil (Rapide Biomedical GmbH).

Scan sequences were optimised for TR, TE and flip angle based on the relaxivity measurements. Excitation pulse bandwidth was also optimised to minimise excitation of off-resonance water or lipid signals, which were further excluded by the receiver bandwidth of the digital filter. Final data collection *in vivo* used a MR spectroscopic imaging sequence with 2ms duration Gaussian excitation and 90° flip angle, TR/TE = 104/1.2ms, SW=5kHz 8×8 phase encoding (8mm in-plane resolution) and 16 averages. Total data acquisition time for the MRSI collection was 214s.

Finally, *in vivo* experiments were performed in tumor bearing mice. After positioning of the mice in the scanner, RF calibration, shimming and collection of conventional MRI, the scanner frequency was shifted to the t-butyl resonance and absence of any detectable signal was confirmed. Contrast agent was injected i.v. (100ul containing 0.03mmolkg^{-1} agent) and followed using an MRSI sequence.

Results

Relaxivities of the reporter group *attached* to the $[Dy.L_2]^+$ were: R_1 = 146.5s^{-1}, R_2= 203s^{-1}, such that any repetition time greater than 20ms was considered to be fully relaxed, while linewidth was around 65Hz. The resonance was found to be shifted by -6550Hz relative to water at 7T (located at -17.9ppm). The figure shows the result of one of the *in vivo* experiments in a mouse. Data were collected during the initial post-injection phase using a simple pulse-collect sequence (b) with detection of the $[Dy.L^2]^+$ complex via the signal at -17.8ppm. A rapid increase in total signal was seen over the first 60s followed by a decline as the agent begins to be cleared from tissue. Results of a spectroscopic imaging examination a shown in (c) – (e) illustrating the MRSI grid, the quality of the spectra and a color overlay of total peak integral. Contrast agent is clearly seen distributed throughout the muscle tissue, with a spectral SNR of 25.



(a) (b) (c) (d) (e)

Discussion

We have demonstrated a new class of contrast agents which present new opportunities for molecular imaging by MRI. By placing a *tert*-Butyl reporter group at a distance of 65nm from a Dysprosium ion, the dipolar shift gave rise to a frequency shifted resonance with short T_1 which could be directly detected *in vivo* without contamination from endogenous water or fat signal and with good sensitivity. Comparison of spectral peak areas against a reference standard suggested that a tissue concentration of 1micromolar was detectable in a scan duration of only ~3 mins.

Acknowledgements

This work was supported by the ERC, CRUK and EPSRC.

References

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