Eu^{3+}-based PARACEST agents with intermediate water exchange rates also act as T_2 exchange (T_{2exch}) contrast agents

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Introduction

Paramagnetic chemical exchange saturation transfer (PARACEST) agents use the combination of water exchange from a lanthanide chelating agent and RF saturation at a bound water chemical shift to create contrast in MR images **(1, 2)**. Chemical exchange of the saturated bound water protons with bulk water leads to a reduced water signal and darkening in the MR image. These agents hold great potential to further extend the functional and molecular imaging capabilities of MR **(3)**. Some published applications include measuring tumor pH, angiogenesis, and the tissue distribution of glucose and other metabolites **(4, 5)**. In our initial *in vivo* studies of PARACEST agents in mice, we observed a significant loss of MR signal in certain tissue types, most notably the kidneys (intravenous injection) and tumors grown from human cancer cell xenografts (intratumoral injection). This signal loss was present even when the CEST saturation pulse was omitted from the imaging sequence, and appeared to be caused by a local decrease in T_2 due to the presence of the CEST agent. We also observed that this effect was more pronounced when using polymerized PARACEST compounds that have a higher local concentration of exchange agent **(6)**. We hypothesized that the same proton exchange that enables the CEST effect can also cause a local decrease in T_2 for compounds with intermediate proton exchange rates. We investigated this hypothesis by acquiring *in vitro* and *in vivo* data, combined with simulations of the Bloch equations, using six different Eu^{3+} PARACEST compounds with proton exchange rates ranging from approximately zero to 10^6 sec^{-1}. Diamagnetic T_2 exchange agents have been previously used to purposely reduce or eliminate the water signal in high-resolution NMR experiments **(7)**. Also, –NH-based exchange broadening was noted recently in a report on the use of iopamidol as a CEST agent **(8)**.

Methods and Materials

In vitro data were acquired using a Maran Ultra 0.54 T spectrometer with the sample temperature controlled at 37 °C. Five different concentrations of each agent were prepared (1.25, 2.5, 5, 10, and 20 mM) with the pH and osmolarity adjusted to approximately 7.0 and 300 mOsm respectively. T_2 was measured using a CPMG sequence. *In vivo* mouse data were taken on a Varian 9.4 T small animal imaging system using a 38 mm diameter birdcage coil. Images were acquired at 37 °C using a fast spin echo sequence (TR/TE = 2500/9.12 ms, echo train = 8, average = 4). Each 128x128 pixel image took 3 minutes to acquire. Once the pre-injection baseline images were acquired, a 0.25 mmol/kg dose of agent was administered to the healthy house via tail vein injection. Images were then continuously acquired (one every 3 minutes) for one hour.

Results

Plots of *in vitro* measured R_2 versus concentration (**Fig. 1**) show that the relaxivity (r_2) is low (~0.003 $sec^{-1}mM^{-1}$) for compounds with very slow/no exchange (EuTTHA) or very fast exchange (EuDOTA), but is higher (0.020 to 0.050 $sec^{-1}mM^{-1}$) for compounds with intermediate exchange characteristics. Plotting r_2 versus the known water exchange rate (C_b) for each compound reveals that there is a peak exchange rate where r_2 is maximized (C_{bpeak} ~ $2x10^4$ sec^{-1}) (**Fig. 2**). Matlab simulated data (not shown) using a numerical solution to the Bloch equations and two pool model of chemical exchange **(9)** were in agreement with the *in vitro* r_2 data.

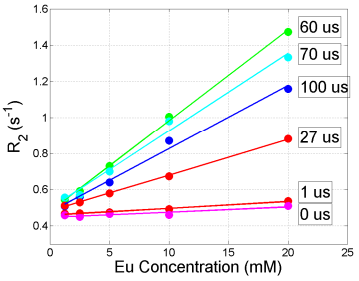

Fig.1

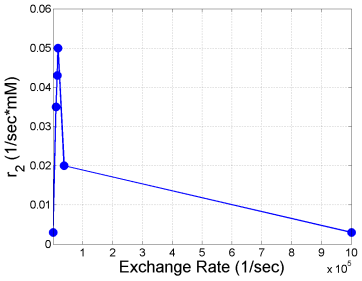

Fig.2

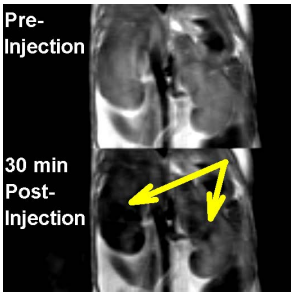

Fig.3

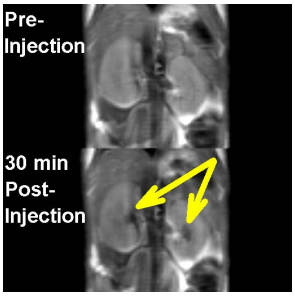

Fig. 4

Fig 1: *R_2 versus concentration for the six different Eu^{3+} compounds, each having a different bound water lifetime (τ_b=1/C_b).* ***Fig. 2:*** *A plot of r_2 versus water exchange rate (C_b) for the data from Fig. 1 reveals a peak relaxivity at approximately $2x10^4$ sec^{-1}.* ***Fig. 3:*** *Coronal images of mouse kidneys before and after the injection of EuDOTA-(gly)$_4$ (τ_b ~ 60 μs, r_2 = 0.050 $sec^{-1}mM^{-1}$) showing a severe darkening of the renal pelvis, medulla, and cortex.* ***Fig. 4:*** *Coronal images of mouse kidneys before and after the injection of EuDOTA (τ_b ~ 1 μs, r_2 = 0.004 $sec^{-1}mM^{-1}$) showing only a slight darkening of the renal pelvis.*

Conclusions

These data show that T_2-based contrast can be generated through proton exchange with Eu^{3+} PARACEST agents, where contrast is a function of exchange rate. Although this was not the intended contrast mechanism for these agents, this discovery could very well lead to a new class of contrast agents for molecular imaging using MR. Our current goal is to take advantage of this phenomenon by designing a T_2-exchange agent that is initially silent (either very fast or very slow exchange) upon *in vivo* administration, then becomes activated (to intermediate exchange) in the presence of certain molecular targets, causing the MR image to go dark in these areas. We believe this platform could be very useful for detecting cancer-related enzyme activities *in vivo*.

References

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