

A Terbium-based PARACEST MR contrast agent for *in vivo* imaging beyond the MT effect

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Introduction

Chemical exchange saturation transfer (CEST) agents create contrast in MR images by exchanging their saturated lanthanide bound protons with unsaturated bulk water protons (1, 2). CEST agents can be selectively activated by applying a 2 to 10 second long frequency-specific saturation pulse, tuned to the bound proton frequency, just before imaging. Chemical exchange of the saturated bound 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). CEST agent bound proton frequencies are typically shifted 5 to 50 ppm from bulk water (0 ppm). Unfortunately, this is the same range of the *in vivo* Magnetization Transfer (MT) effect (6). The MT effect arises from dipolar exchange of protons with endogenous tissue materials such as macromolecules and cell membranes. The MT effect typically spans from ± 100 ppm (relative to bulk water) and is proportional to saturation pulse power. As a consequence, the contrast produced by the CEST agent can be totally masked by the tissue MT effects, which greatly complicates *in vivo* imaging. In an effort to avoid the MT effect and enhance *in vivo* CEST imaging, our group has recently developed a Tb³⁺-based paramagnetic CEST (PARACEST) agent (Fig. 1) with an unusually long bound water exchange lifetime. The bound proton frequency for this agent is at -600 ppm, which is far outside the normal tissue MT window (Fig. 2). Although other Tb³⁺-based PARACEST agents have been reported (7), this agent's slower water exchange rate allows for an order of magnitude reduction in saturation pulse power, making it more suitable for *in vivo* studies. We present *in vitro* images of our Tb³⁺-based PARACEST agent to demonstrate its potential for *in vivo* imaging without the requirement of subtracting out tissue MT contributions.

Materials and Methods

The Tb³⁺-based PARACEST agent was prepared from cyclen by alkylation with four equivalents of dibutyl (2-chloroacetamido) methylphosphonate followed by treatment with lithium bromide to give the mono-butyl ester (8). Initial *in vitro* imaging was done using a 20 mM solution of the Tb³⁺ agent in a 2 mL vial. MR imaging was performed on a Varian 9.4 T small animal system using a 38 mm diameter birdcage coil. The Z-spectrum (Fig. 2) was acquired at 25 °C using a gradient echo pulse sequence (TR/TE = 3.89/1.54 ms, flip angle = 85°, average = 1). Each low resolution image (32x32 pixels, 125 ms acquire time) was preceded by a 5 second long, 20 μ T saturation pulse. The frequency of the saturation pulse was varied from 96 to -704 ppm in steps of 8 ppm yielding 101 images. The Z-spectrum was obtained by plotting the mean water signal intensity versus saturation frequency. CEST images were collected at 35 °C using a fast spin echo pulse sequence (TR/TE = 75.6/9.1 ms, echo train = 8, average = 4) with a 5 second long, 20 μ T saturation pulse for every TR. Each 128x128 pixel image took 6 minutes to acquire. The CEST image (Fig. 5) was created by subtracting the on-resonance image (saturation at -600 ppm, Fig. 4) from the off-resonance image (saturation at 600 ppm, Fig. 3).

Results

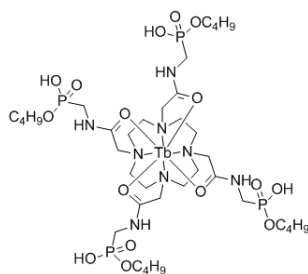


Fig. 1

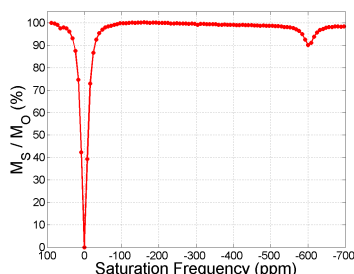


Fig. 2

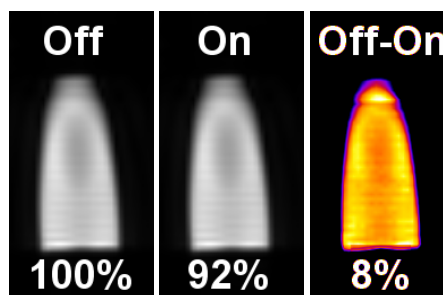


Fig. 3

Fig. 4

Fig. 5

Fig. 1: Molecular structure of the Tb³⁺-based PARACEST agent used in this study. **Fig. 2:** Z-spectrum of 20 mM of agent acquired at 25 °C shows a 10% CEST effect at -600 ppm. **Fig. 3:** Off-resonance (600 ppm) coronal image of the sample vial with an image intensity of 100%. **Fig. 4:** On-resonance (-600 ppm) image of the sample vial with an image intensity of 92%. **Fig. 5:** CEST image (Off-On) showing an 8% CEST effect.

Conclusions

Initial *in vitro* images of a Tb³⁺-based PARACEST agent with a bound proton resonance well outside of the MT window are shown. This agent has a proton exchange rate that is slow enough to allow saturation power levels acceptable for *in vivo* studies. Although a 5 second long, 20 μ T saturation pulse leads to a substantial *in vivo* MT effect, it is of little consequence for this agent, where saturation pulse power is only limited by RF heating (SAR). Subsequent polymerization of this agent should increase the CEST effect by a factor of 10, allowing for shorter or lower power saturation pulses (9). Preliminary *in vivo* experiments indicate that this new agent is nontoxic in mice at levels required for CEST imaging. Further work includes performing murine *in vivo* imaging to observe the CEST effect in the kidneys, liver, bladder, and human cancer cell xenografts.

References

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