Sub-micromolar concentrations of contrast agent have a quantifiable influence on the hyperpolarized lithium-6 relaxation time *in vivo*

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Introduction

Lithium-6 has recently been demonstrated to have longitudinal relaxation times τ on the order of minutes and to be readily hyperpolarizable by dynamic nuclear polarization (DNP, 1). In principle, these relaxation times make ^{6}Li more sensitive to contrast agents. This can be appreciated through the equation for relaxation:

$$1/\tau_{CA}=1/\tau_{pure} + r_1\cdot[CA], \quad (1)$$

where in the case of higher values for the relaxation times with (τ_{CA}) and without (τ_{pure}) contrast agent the relaxivity term ($r_1\cdot[CA]$) becomes more influential. The longitudinal relaxation time of ^{6}Li was further shown to be sensitive to the negatively charged contrast agent Gd-DOTP (2). We therefore aimed to demonstrate that these principles can be combined to quantitatively detect very low concentrations of contrast agent, both *in vitro* and *in vivo*.

Materials and Methods

Lithium-6 chloride was dissolved in a D_2O-ethanol mixture at a concentration of 15 M together with 33 mM of TEMPO. 50 µl of this mixture was then polarized at 3.35 T and 1.2 K using a custom-built polarizer (3). After dissolution into 5 ml of D_2O the concentration of lithium was ~150 mM. Within 5 s this dissolved sample was automatically transferred to a phase separator placed in the bore of a Varian Inova 9.4T 31 cm bore actively-shielded animal spectrometer. A remote controlled pump then injected 2.5 ml of the sample into the subject over 8 s. The complete sample was thus injected into the subject within 13 s of the start of the dissolution process.

To test if the sensitivity of ^{6}Li was sufficient to detect low concentrations of Gd-DOTP, a phantom consisting of a block with two 1x1 cm^2 compartments with a 0.5 mm thick bottom, separated by a 2 mm thick wall was used (n = 2 experiments). A quadrature ^{1}H coil with a single 4-loop ^{6}Li coil was placed directly under the separation wall. Both compartments were filled with 1.5 ml D_2O, while one of them was doped with Gd-DOTP such that after injection of the dissolved sample the final concentration would be 500 nM. After injection of the hyperpolarized lithium-6, the signal of the two compartments was measured with 120 separately stored repetitions of a gradient-recalled sequence (3 ms 10° BIR-4 pulse, TR = 5 s), resulting after FFT in 1D projections that consisted of 32 points (TE = 1043 µs). These projections were then zero-filled to 256 points, and the integral of the signal of each compartment was calculated. Curve fitting was done from t = 30 to 300 s in OriginPro (OriginLab Corp, USA) using an exponential decay function ($S[t]=S_0\cdot\exp[-t/\tau]$) to obtain the relaxation times τ.

In the *in vivo* studies (n = 3), male Sprague-Dawley rats (w = ~350 g) were anesthetized using 1.5% isoflurane, and a femoral vein was catheterized for injection. Blood pressure, respiration rate and temperature were maintained within normal range. The RF coil was placed on top of the head. The animal was then inserted into the 9.4 T scanner.

Signal acquisition consisted of a series of unlocalized 3 ms BIR-4 10° pulses with a 3 s interpulse delay. 45 to 50 s after the dissolution (and 31 s after the end of injection of the hyperpolarized sample), 1 ml of Gd-DOTP dissolved in saline was injected. The concentration of Gd-DOTP was adjusted such that the concentration in the total blood volume of the rat would be 1.7 µM, assuming a total blood volume of 6% of the total body weight (4).

The resulting signal was zerofilled once, after which its peak integral was measured. Two exponential decay curve fits were then made in Origin Pro: one applied to the time points before the start of the injection and one for the points after the end of the injection.

The resulting relaxation times τ of both studies were used in Eq. 1 to calculate the apparent contrast agent concentration.

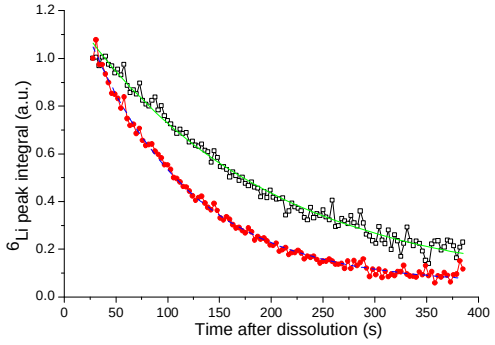

***Figure 1**. Decay curves of the two-compartment phantom. Both curves were normalized to their first point. The compartment without contrast agent (upper curve) had a relaxation time of 181 ± 8 s, while the compartment with contrast agent decayed with T= 99 ± 2 s.*

Results and Discussion

The phantom experiments displayed the expected effect of the contrast agent (Fig. 1): the signal in the contrast agent-doped compartment (red circles) decayed faster than that of the 'pure' compartment (black squares). The decay time τ_{pure} of the compartment without the contrast agent was 180 ± 12 s (and R^2=0.986), while that of the compartment containing the contrast agent was τ_{CA} = 100 ± 4 s (and R^2=0.995). From Eq. 1 with r_1= 11 $mM^{-1}s^{-1}$ (2) this resulted in [CA] = 440 nM, consistent with the target concentration of 500 nM.

A typical *in vivo* lithium-6 decay curve is displayed in Fig. 2. A significant signal lowering effect due to the contrast agent injection was observed. The curve fits before and after injection resulted in τ = 74 ± 8 and 45 ± 1 s respectively Such a change in relaxation time resulted in an apparent contrast agent concentration [CA] = 820 ± 300 nM using Eq. 1 significantly lower than the estimated concentration in blood. Since the contrast agent is limited to the blood pool, any lithium signal that had left the blood would diminish the apparent effect of the contrast agent, resulting in a lower apparent contrast agent concentration when calculated from its effect on τ.

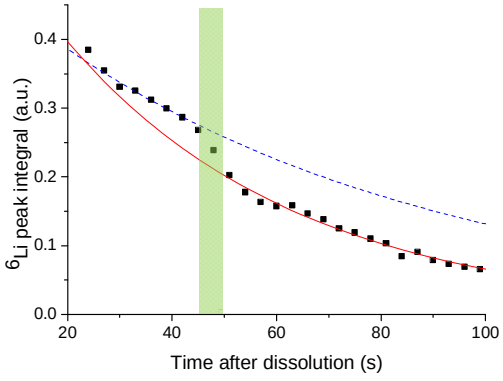

***Figure 2**. Zoom-in of a decay curve of hyperpolarized lithium-6 chloride (black squares) in a rat. The period of injection of contrast agent is indicated as a transparent bar from 45 to 50 s. Two curve fits are displayed: a dashed curve fit for the points before contrast agent injection and a solid curve for the points after injection.*

We conclude that low concentrations of Gd-DOTP can be detected *in vivo* using hyperpolarized lithium-6. When taking the magnitude of the change in relaxation times into account, it appears likely that even lower concentrations can also be detected using this technique.

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