A new R2/R1 Ratiometric Method to Measure pH with a Dendrimer-based pH-Responsive MRI Contrast Agent

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Abstract: We have recently developed a new pH-responsive dendrimer-based MRI contrast agent with excellent improvements in both overall sensitivity and responsiveness of relaxivities to pH. Therefore, the R_2/R_1 ratio of this dendritic MR agent has been used to measure pH. This pH measurement is independent of the absolute concentration of the agent so that a single MRI agent can measure pH without requiring a second MRI to account for pharmacokinetics.

Introduction: The tumor microenvironment is frequently characterized by an acidic extracellular pH_e and a neutral to alkaline intracellular pH [1]. A similar pH gradient is not observed in normal tissues. This unique pH environment in tumor tissue impacts tumor pathology and treatment. The lower pH_e has been correlated with increased gene mutation and rearrangement rates and altered gene expression in tumors, which leads to transformation of tumors from benign into metastatic [2]. Low extracellular pH combined with higher intracellular pH is also important in chemotherapy since weak acid drugs which are protonated at high pH can become trapped inside of tumor cells, conversely weak base drugs are trapped outside [3]. Therefore a method to assess pH_e throughout the tumor tissue would provide a useful tool to: 1) detect metastatic vs. benign tumors before metastasis has occurred; 2) evaluate the effect of pH-altering chemotherapies; and 3) predict efficacy before chemotherapy is applied. Since the tumor environment can be heterogeneous, ideally a method for measuring pH should have high spatial resolution.

Methods: We have proposed a novel method based on a ratiometric approach that consists of measuring the ratio between the transverse and the longitudinal paramagnetic contribution to the water proton relaxation rate, i.e. r_2/r_1. For an aqueous solution of Gd^{3+} complex containing one labile water molecule coordinated to a metal center, the inner-sphere contribution to paramagnetic water proton relaxation rates at magnetic field strength higher than 0.2T is commonly described by the following equations [4]:

(1) $$R_{1P} \cong \frac{P_M}{T_{1M}+\tau_M} \Rightarrow \frac{1}{T_{1M}} = \frac{6}{15}\frac{K^{DIP}}{r_H^6}\left(\frac{\tau_C}{1+\omega_H^2\tau_C^2}\right)$$

(2) $$R_{2P} \cong \frac{P_M}{T_{2M}+\tau_M} \Rightarrow \frac{1}{T_{2M}} = \frac{1}{15}\frac{K^{DIP}}{r_H^6}\left(4\tau_C + \frac{3\tau_C}{1+\omega_H^2\tau_C^2}\right)$$

where P_M is the molar fraction of water protons bound to Gd^{3+} ion (equal to [GdL]/55.6), τ_M is the residence lifetime, r_H their distance from metal center, ω_H their Lamor frequency (rad·s^{-1}), τ_C their molar coefficient time ($\tau_C^{-1} = \tau_M^{-1} + \tau_R^{-1} + \tau_{IS}^{-1}$ with τ_R = rotational correlation time and τ_{IS} = longitudinal electronic relaxation time). K^{DIP} is a constant value (3.887 X10^{-42} m^{6}s^{-2}). From Eqs 1-2, it is clear that the r_2/r_1 ratio is determined by τ_M, τ_c, and ω_H values, but independent of the concentration of the paramagnetic agent. A theoretical simulation, based on Eqs 1-2, indicates that at the magnetic field strengths available for MRI (>0.2 T), the r_2/r_1 ratio becomes sensitive to the rotational mobility of the Gd^{3+} complex only for τ_R values longer than 0.5 ns [4]. On the basis of available theory and in order to be effective as a ratiometric responsive probe, a Gd^{3+} complex must have a τ_R value ≥ 1 ns [4]. The NMRD (NMRD = Nuclear Magnetic Relaxation Dispersion) profile of our pH-responsive dendritic agent revealed that the τ_M value is in the range of 1.0 ns – 1.0 μs and the τ_R value is in the range of 4 - 5 ns [5]. Therefore, our dendrimer-conjugate is an ideal candidate for imaging pH of tissues by ratiometric method. To prove our hypothesis, we have prepared phantoms of [(GdDOTA-4AmP)$_{96}$-G5] (1.0 mmol per Gd^{3+}) at 6 different pH values. The liquid phantoms were scanned by a Varian 7T scanner using a set of spin-echo pulse sequences with different TRs (TR=50ms, 100ms, 300ms, 750ms, 2s, 5s with TE=8.5 and 17 msec, FOV=40mm^2, 128×128, thickness of 2 mm) as a progressive saturation study. Linear and non-linear fitting techniques were employed to calculate T_2 and T_1 of the phantoms, respectively.

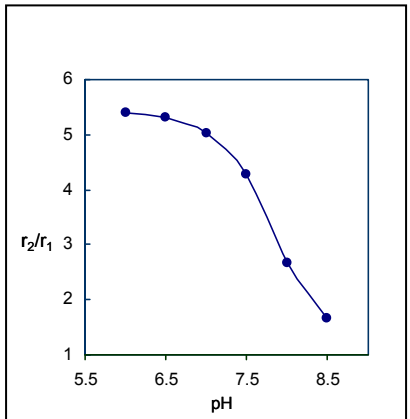

Figure 1. The pH-responsiveness of r_2/r_1 ratio of the G5Gd-PAMAM dendrimer. MR relaxivities measurements were conducted at 1.0 mM, 25°C and 7T magnetic field strength.

Result and Discussion: The calculated ratio of water proton relaxivities (r_2/r_1) of the G5-dendritic conjugate, G5-Gd$_{96}$ [5] at different pH values was plotted in Figure 1 and the ratio (r_2/r_1) showed pH-response. This ratio (r_2/r_1) increased from 1.5 to 5.5 pH units on changing the solution pH from 8.5 to 6.0. The change in r_2/r_1 ratio, 1.6 per pH unit, is a tremendous improvement (at 7T) for a relaxation-based MR contrast agent. The change in T1 relaxation time caused by a pH-responsive MRI contrast agent is also dependent on the concentration of the agent. The tissue concentration of a pH-responsive agent can be estimated by using a second pH-unresponsive agent as a surrogate. However, the ratiometric approach to measure pH does not require knowledge of the concentration of the agent. Therefore, our MRI contrast agent with T_1 and T_2 effects constitutes the only single MRI contrast agent that can accurately measure pH_e in a concentration independent manner.

Conclusion: The r_2/r_1 ratio of G5-Gd showed pH responsiveness and this pH response is independent from their absolute concentration. Now, we are interested in targeting extracellular pH-mapping of solid tumors.

References:

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