Proton Chemical Exchange Dependent Saturation Transfer (CEST):
Evaluation as a Mechanism for Non-Metal Based Exogenous MRI Contrast Agent

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Purpose To evaluate proton chemical exchange dependent saturation transfer (CEST) as a mechanism for generating exogenous MRI contrast. To define chemical groups suitable as exogenous CEST contrast agents under in vivo pH and temperature conditions. Introduction Most MR contrast agents are dependent on the T1 or T2* relaxation properties of metal chelates or metal particles which can be limited in their use at high concentrations by toxicity or 12* effects (most T1 agents). The goal of this study was to evaluate the use of saturation transfer with specific chemical groups as a non-metal based MR contrast agent. The idea is to find chemical groups with the appropriate proton exchange and chemical shift properties at physiological pH and temperature that will function as effective saturation transfer partners with water. A thousand-fold enhancement of the exchange site proton signal can result via the reduction in the water signal (Mw/Ms) based on the following equations:

\[ \frac{M_w}{M_s} = \frac{1}{1 + k_1 T_1} \]

where \( M_s \) and \( M_w \) is the water signal with and without saturation of the exchange site, \( T_1 \) is the T1 of water protons, \( k_1 \) is the pseudo first order rate constant, \( T_1 \) is the site proton lifetime, \([\text{agent}]\) is the mole fraction of the agent relative to water protons, and \( n \) is the number of exchange sites per mole of agent. \[ \text{An ideal contrast agent requires the following: 1) Slow to intermediate exchange rate. (i.e. } \frac{1}{T_1} \Delta \Delta_0 > 1 \text{ where } \Delta_0 \text{ is the chemical shift difference between the site and water. 2) Large } \Delta T_1 \text{ to support a high exchange rate and improve specificity due to } B_0 \text{ in-homogeneity can be } > 2 \text{ ppm. 3) High solubility. 4) Low toxicity. 5) Defined tissue distribution. To stay in slow exchange, } \Delta T_1 \text{ must be rather long reducing the effect of each exchange site. Given a } \Delta_0 \text{ of } 5 \text{ ppm, } \text{ and } \Delta T_1 \text{, } \Delta \Delta_0 \text{, } n \text{, and an } M_s/M_w \text{ of 0.9 requires [agent] to be } >10 \text{ mM. These concentration estimates were confirmed in our studies outlined below. Based on these considerations we searched for compounds with proton exchange sites with large } \Delta_0 \text{, high solubility and appropriate chemical exchange rates to just maintain within the slow exchange limits at physiological pH (7.4) and temperature (37°C). Methods Solutions were dissolved in HPLC grade water with inorganic phosphate buffers to maintain pH. Magnetization transfer spectra were collected at 7T using a Bruker AC-300 wide bore spectrometer at 37°C. Studies were conducted using a steady-state irradiation (15 sec) over a range of frequencies ± 8 ppm from water. The Mw data were plotted as a function of the irradiation frequency in the magnetization transfer spectra. MR images were collected on a custom designed 4T system. See figure legends for details. Results/Discussion Several classes of chemical exchange sites were evaluated. Sugar hydroxyl groups provided good chemical exchange sites at pH 7 (Mw/Ms 0.89-0.68, 250 mM sugar) but their \( \Delta \Delta_0 \) values (< 2 ppm) were too small. Sugar polymers, such as dextran, maintained these chemical exchange and shift properties but with much higher \( n \) reducing the osmotic load. Therefore, iodoside polymers will be a useful approach for reducing the osmotic load of CEST agents. Backbone amino acid amino and the arginine R group, guanidine, protons provided a better model with 2-3 ppm shifts, but were in fast exchange at pH 7.0. These studies revealed that indole ring NH groups had useful properties with \( \Delta \Delta_0 > 5 \) ppm and reasonable exchange rates at pH 7.0. We evaluated additional ring-NH groups: nucleosides, their pyrimidine and purine bases, as well as derivatives of barbituric acid and imidazole. Several of these compounds revealed promising properties with \( \Delta \Delta_0 > 5.0 \) ppm and \( M_w/M_s \) values in the range of 0.7 (62.5 mM). Barbituric acid is presented in detail in this report. The magnetization transfer spectrum of barbituric acid (62.5 mM) as a function of pH is presented in Figure 1A. The CEST effect is clearly seen in the 5 ppm region. The \( 1/T_1 \text{, } \Delta \Delta_0 \text{, } n \text{, and } M_w/M_s \) collected with a 5.3 ppm irradiation. All three solutions of barbituric acid were enhanced over the surrounding water and NH3Cl controls. Note that the contrast effect could be turned off and on depending on the saturation pulse, which is not possible with metal-based T1 or T2 agents. Barbituric acid was a model compound used in this study, but is not considered the "ideal" compound. Barbituric acid is pharmacologically inactive with an LD50=5g/kg [5]. Substitution at a side group not involved in chemical exchange is used to manufacture a wide variety of exogenous agents; this same site could be used to polymerize the compound to eliminate the osmotic stress associated with nalo hexane exchange sites. We have infused barbituric acid in rabbits as isotonic concentrations (~150 mM) with minimal short-term physiological perturbations suggesting that there is nothing inherently toxic in the short term, with this type of molecule. These studies reveal that the CEST is a feasible mechanism to generate new exogenous contrast agents that can be "turned" off and on using the saturation pulse. In addition, since these compounds do not require a metal, it may be more feasible to get the agents into cells using preexisting transport pathways. References 1. Guivel-Scharen, V., Sinnwell, T., Wolff, S., and Balaban, R., J. Magn. Res., 133:36;1998 2. Work, R. Nuclear Magnetic Resonance (N.M.R.) in Biochemistry. Applications to Enzyme Systems, (Oxford UK, 1973) 3. Goldenhal, E., Toxicol. Appl. Pharmacol., 18:185;1971.