The CEST effect of guanidine and hydroxyl protons can be used as a positive contrast in ischemia

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Target Audience Researchers interested in the chemical exchange saturation transfer (CEST) technique and MR imaging of stroke.

Purpose CEST imaging based on the endogenous amide-proton transfer (APT) effect has shown great potential in stroke studies [1]. However, the APT contrast in stroke is negative which decreases at the lesion region. When the conventional asymmetry analysis is applied to obtain APT-weighted images, its sensitivity in detecting the ischemic lesion is limited by large negative background signal from the asymmetry of magnetization transfer contrast and the nuclear Overhauser effect [2]. Moreover, the amide water proton exchange rate and the magnitude of APT effect decrease with pH exponentially [3]; therefore, its sensitivity quickly diminish at lower pH (e.g., ≤6.4), which makes it difficult to differentiate mild versus severe tissue acidosis. Alternatively, the chemical exchange (CE) effect of endogeous guanidine and hydroxyl protons may be exploited for pH-sensitive imaging. Because guanidine and hydroxyl protons exchange with water at much faster rate than amide protons, a positive contrast may be detected by judicious selection of B_1, the power of off-resonance irradiation pulse. In this preliminary study, we investigated the CE effects of guanidine and hydroxyl protons and their potential application in stroke studies.

Materials and Methods Simulation MTR_{asym} was simulated as a function of labile water exchange rate (k). Three pool exchanges between free water protons, labile protons, and bound water protons were simulated by modified Bloch Equations where the lineshape of bound water was modeled by a super-Lorentzian function [4]. We assumed a bound water proton fraction of 0.05, a labile proton fraction of 0.0005, a chemical shift between labile proton and water of 2 ppm, and the magnetization transfer rate between bound water and free water of 50 s^{-1}. The T_1 (T_2) of water, labile proton, and bound water protons were assumed to be 2 s (50 ms), 2 s (50 ms), and 2 s (13 μs), respectively.

Experiments All experiments were performed at 9.4 T. *Phantom experiment*: 15 mg/ml protamine was dissolved in phosphate buffered saline (PBS) and titrated to pH values of 6.1, 6.4, 6.7, and 7.0, and 0.15 mM $MnCl_2$ was added to each sample to shorten the T_2 values. Z-spectra of phantoms were measured at 37°C with a 0.5 μT and 4 s continuous wave pulse. *In vivo experiments*: Five Sprague-Dawley rats underwent permanent middle cerebral artery occlusion (MCAO). Z-spectra were measured after 5 hours of ischemia onset. Off-resonance irradiation was applied by a 0.8 μT and 4-s saturation pulse, and a frequency offset range from 12 ppm to -12 ppm. Apparent water diffusion coefficient (ADC) maps were also measured to identify the ischemic regions. For quantitative data analysis, Z-spectra were obtained from the regions of interest (ROI) selected at the contralateral and ipsilateral areas, based on the ADC map.

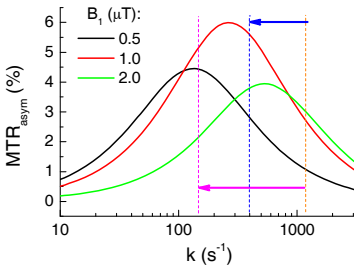

Fig. 1 Simulated MTR_{asym} as a function of exchange rate for selected B_1 values using a 3-pool exchange model.

Results and discussions At normal physiological conditions, guanidine and hydroxyl protons have a chemical shift of ~1-2 ppm relative to water, and exchange with water protons at rates of around 1000 s^{-1} [5]. Compared to amide, one distinct disadvantage of utilizing the guanidine and hydroxyl groups in CEST study is their Larmor frequencies to be closer to water resonance, resulting in high susceptibility to the direct water saturation effect. Therefore, low irradiation pulse power should be applied to reduce the direct water saturation, which inevitably reduces the saturation efficiency and consequently the CE sensitivity. Fig. 1 shows the simulated MTR_{asym} at 2 ppm as a function of k. Note with a B_1 of 0.5, 1, and 2 μT, MTR_{asym} is tuned to an exchange rate ($k_{tune} = \gamma \cdot B_1$) of 133, 266, and 532 s^{-1}, respectively [6]. Therefore, the CE contrast induced by tissue acidosis (decrease of k) can be optimized by selection of B_1. For example, the best CE sensitivity can be achieved with a B_1 of 1 μT if k decreases from 1200 s^{-1} to 400 s^{-1} (blue arrow) due to pH decrease, and a B_1 of 0.5 μT if k decreases from 1200 s^{-1} to 150 s^{-1} (pink arrow).

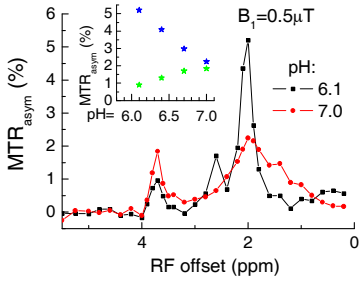

Fig. 2 MTR_{asym} of 15 mg/ml protamine in PBS show different pH dependence for the guanidine and the amide peaks. While APT sensitivity reduces at lower pH values, the guanidine CE sensitivity increases with decreasing pH (Inset).

Fig. 2 shows an example of opposite pH-induced contrast from the guanidine and amide CE effects. Protamine is a small protein with ~2/3 of its amino acid residues to be arginine [7], which has a guanidine group. A large guanidine peak can be seen at 2 ppm, in addition to the amide peak at 3.7 ppm. With an irradiation pulse power of B_1 = 0.5 μT, a decrease of pH (and consequently the exchange rate) leads to an increase of guanidine peak (blue symbols, inset plot), but a decrease of amide signal (green symbols, inset plot). The guanidine exchange rate at lower pH is closer to the tuned rate of 133 s^{-1}, while amide exchange rate at lower pH is farther from the tuned rate (see Fig. 1).

The *in vivo* Z-spectrum of contralateral ROI showed a signal dip at 3.6 ppm due to APT and a smaller dip at ~2 ppm due to guanidine-water proton echange (Fig. 3A). While the APT signal decreases at the ipsilateral ROI in the MTR_{asym} spectra due to tissue acidosis, the guanidine CE signal increases (Fig. 3B). The difference between MTR_{asym} of ipsilateral ROI and contralateral ROI (Fig. 3C) showed a dip at 3.6 ppm, but peaks at 2 ppm and at ~ 1 ppm; the latter is likely due to the hydroxl CE effect. Similar to the results of protamine in Fig. 2, *in vivo* APT signal decreases due to pH reduction, while guanidine-water proton exchange signals increase. This positive contrast is a clear benefit for CE imaging in ischemia. Note that due to the close proximity of guanidine and hydroxyl protons to the water frequency, CEST imaging based on these labile protons will be more susceptible to the B_0-inhomogeneity. In contrast to the APT signal, which mostly arises from mobile protein backbone, the guanidine and hydroxyl CE signals have concentributions from mobile protein side chains as well as metabolites such as creatine and myo-inostiol. Therefore, besides a drop in tissue pH, the change of ~1 and 2 ppm peaks in Fig. 3C may also be affected by a change in concentrations of guanidine and hydroxyl protons, and further studies would be necessary to investigate the exact source of the guanidine and hydroxyl CE effects in stroke studies.

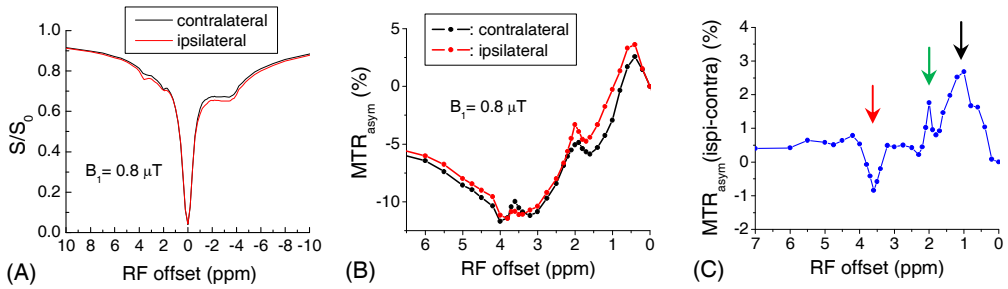

Fig. 3 Z-spectra (**A**) and MTR_{asym} spectra (**B**) measured with a 0.8 μT and 4 s saturation pulse at the contralateral ROI and ipsilateral ROI of ischemic rats. The difference between ipsilateral and contralateral SLR_{asym} (**C**) showed a decrease of the APT effect at 3.6 ppm (red arrow), an increase of the guanidine CE effect at 2 ppm (green arrow), as well as an increase at 1 ppm (black arrow) due to the hydroxyl CE effect.

References [1]. Sun PZ et al., JCBFM 27:1129 (2007). [2]. Jin T et al., MRM in press (2012). [3]. Zhou JY et al., Nat Med 9 :1085 (2003). [4]. Li, X et al., MRM 60:1197 (2008). [5]. Liepinsh E et al., MRM 35:32 (1996). [6], Jin T et al., MRM 65:1148 (2011). [7]. McMahon MT et al., MRM 60:803 (2008).