High resolution pHe Imaging of Rat Glioma using single injection of a pH-sensitive contrast reagent: Feasibility study

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Introduction. The pH-sensitive contrast reagent (CR), GdDOTA-4AmP, has been used previously to image extracellular pH (pHe) in kidneys (1) and gliomas (2) using the pharmacokinetics (PK) of a pH-insensitive CR for concentration correction. However, to transfer this methodology to a clinical environment, an alternative approach using a single injection to provide improved temporal resolution is needed. In this work, we investigate the use of T2 relaxivity to calculate the concentration of GdDOTA-4AmP. This rationale for this approach is based on the premise that T2 relaxivity is dominated by outer sphere interactions which should be pH independent. The pH-dependent T1 relaxivity is due to water exchange at the Gadolinium nucleus and hence, are dominated by inner sphere interactions (3). *In vitro* data show that SE-measured T2 relaxivities induced by Gd-4AmP follow the same pH dependence as do T1. Hence, we conclude that the T2 of Gd-4AmP is also dominated by inner sphere interactions. Consequently, we have investigated the use of an alternate lanthanide, Dy, as a doping agent to provide pH-independent T2 contrast (4). In pH and concentration titrations *in vitro*, the r2/r1 ratios determined for Dy were 10 times larger than those for Gd. A similar effect was observed *in vivo* where a DyDOTP infusion showed no effect on T1 while producing large decreases in T2 as determined by both spin echo MRI and EPSI. These data suggest that a single injection of a defined mixture of DyDOTP and Gd-DOTA-4AmP could be used to measure pHe *in vivo* with high temporal and spatial resolution.

Methods. *Glioma model.* C6 glioma cells (10^5) in DMEM were injected stereotaxically in the right caudate nucleus of rats weighing 200-250 g. Tumors were allow to grow for 2 or 3 weeks. *Animal preparation*. Rats were anesthetized with a mixture of 1-1.5% (v/v) isoflurane and O2. Body temperature was maintained at 36.5-37.5°C and monitored during all the *in vivo* experiments using a rectal fluoroptic probe. The tail vein was cannulated for CR delivery. *In vivo MRI.* All the experiments were performed on a Bruker Biospec 4.7 T system. T2 weighted (TR=4000 ms, TEeff=81, ETL=8), T1 weighted (TR=200 ms, TE=8.2 ms) spin echo images and EPSI (???) were acquired previous to any CR injection. Then, a 0.15 mmol/Kg bolus injection followed by infusion (1.5 mmol Kg-1 h-1) of DyDOTP was administered through the tail vein. Interleaved T1 T2 and EPSI images were acquired for up to 2 hours. *In vitro MRI*. T1 and T2 relaxivities of GdDOTP, DyDOTP and GdDOTA-4AmP were determined in solutions ranging from 0.1 to 25 mM in PBS at 4.7 T and 37oC. T1 and T2 measurements were done using inversion-recovery and spin-echo sequences respectively.

Results and Discussion. Both T1 and T2 relaxivities of GdDOTA-4AmP showed the same dependence on pH *in vitro* (not shown). This result was somewhat surprising, as it implies that GdDOTA-4AmP modulates T2 relaxivity mainly through inner sphere interactions. Since the pH-sensitivites are the same for both relaxation mechanisms, the Gd-induced T2 cannot be reliably used for concentration corrections. The same relationship was observed for GdDOTP, which showed a r2/r1 ratio of ~1.9 (Table 1). As expected, DyDOTP showed a larger effect on T2, with a r2/r1 ratio of ~9.4 (Table 1). To investigate this *in vivo*, animals bearing orthotopic gliomas were infused with DyDOTP and its effect on T1, T2 and linewidth were evaluated. These data showed a significant T2 decrease in the absence of any T1 effect (Fig. 2). Even more significant is the increase in the linewidth of the water peak from the EPSI data (Fig. 3), which allows for direct measurement of T2*. Since the T2/T1 ratio for Dy is approx. 10 times higher than that of Gd, we reason that a 10:1 mixture of DyDOTP : GdDOTA4AmP will allow T2-based concentration corrections and hence, pH imaging using a single injection of CRs.

Table 1

	DyDOTP		GdDOTP	
	relaxivity	r_2/r_1	relaxivity	r_2/r_1
r_1	0,16	9,38	3,17	1,9
r_2	1,5		6,02	

References.

1) Raghunand et al., *MRM* 49:249–257 (2003). **2**) Garcia-Martin et al. Magn. Reson. Med. (in preparation). **3**) Woods et al., Chem. Eur. J. 9: 4634-40 (2003). **4**) Vander Elst et al., *Acad Radiol*. Aug;9 Suppl 2:S297-9 (2002).

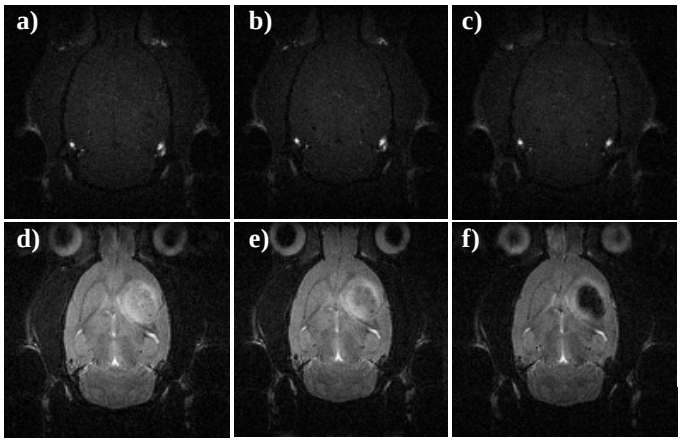

Figure 2. a) T_1 weighted images before any contrast agent, b and c) T1 weighted images after 30 and 60 minutes of DyDOTP injection, d) T_2 weighted image before any contrast agent, e and f) T2 weighted images after 30 and 60 minutes of DyDOTP infusion.

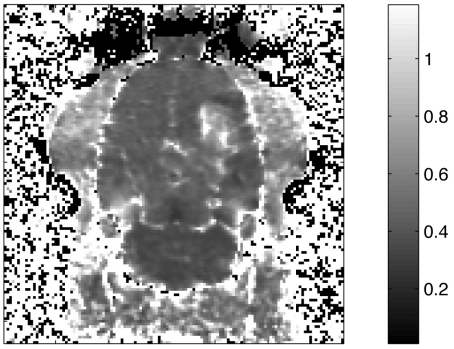

Figure 3. Linewidth map generated from a nonlinear least squares two parameter fit to EPSI data, where the spatial resolution is 256x256 . Each voxel corresponds to a ^{1}H spectrum. This map was calculated after 30 minutes of DyDOTP infusion. The level bar indicates the FWHM in ppm.