Brain tissue water comes in 2 pools: Evidence from diffusion and R2 measurements with USPIOs in non human primates

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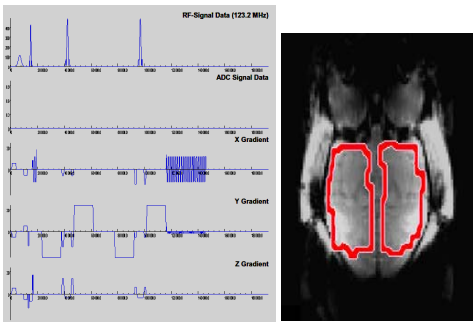

Fig.1 Left: Balanced bipolar diffusion-sensitized SE sequence (diffusion gradient pulses were set on the X, Y and Z axes), Right: Representative brain slice with ROI location

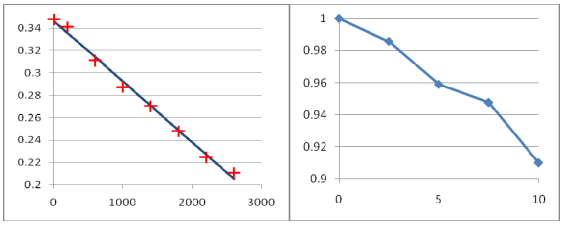

Fig.2 Model #1: Plot of $\mathbf{r_{eff}}$ (kg.mg^{-1}.s^{-1}) versus b (s/mm²) (left), plot of **D** (normalized for [**C**]=0) versus [**C**] (mg/kg iv) (right).

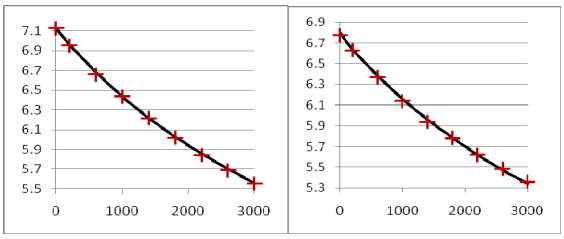

Fig.3a Model #2: Diffusion attenuation plot ln($\mathbf{S/S_0}$) of versus b (line: model, marks: data) for [**C**]=0 (left) and [**C**]=10 mg/kg (right) using the same fitted parameter set (Monkey R in Table 1).

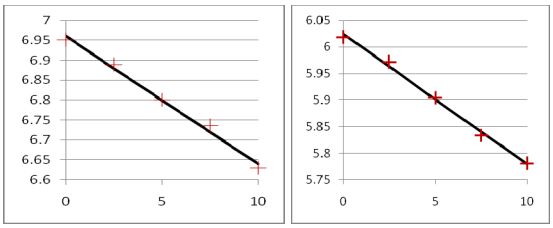

Fig.3b Model #2: Diffusion attenuation plot ln($\mathbf{S/S_0}$) of versus [**C**] (line: model, marks: data) for **b**=0 (left) and **b**=2600s/mm² (right) using the same fitted parameter set as in Fig.3a (Monkey R in Table 1, but quality of fit was the same for all animals).

	Monkey B	**Monkey R**	**Monkey K**	**Monkey C**
	TE=99ms	TE=89ms	TE=89ms	TE=89ms
V (T2w-CBV)	1.52%	1.30%	0.25%	2.95%
f_s	43.2%	43.4%	47.6%	34.7%
D_s (10^{-3}mm²/s)	0.248	0.269	0.325	0.234
D_f (10^{-3}mm²/s)	1.33	1.17	1.30	1.14
ξ	0.00	0.00	0.00	0.00
r_s (kg.mg^{-1}.s^{-1})	0.20	0.22	0.18	0.22
r_f (kg.mg^{-1}.s^{-1})	0.50	0.50	0.60	0.51

Table 1 Results of data fitting with model #2 for all animals.

Introduction - Water diffusion in biological tissues is not free (Gaussian), as the signal attenuation is not monoexponential with diffusion-weigthing (b value) [1]. Some groups have successfully characterize this attenuation with a biexponential model, which suggests the presence of 2 water pools in slow or intermediate exchange [1]. However, this model is still controversial [2] and the nature of the 2 pools (e.g., membrane-bound and bulk water) remains elusive [3]. Here we show that this 2 pools model also explains the T2 behavior of USPIO contrast agents in the brain.

Materials and Methods

Animal protocol. Four anesthetized rhesus monkeys were scanned in the sphinx position using an MR compatible stereotactic frame. Monkeys were injected inside the scanner every 15 minutes with 2.5mg/kg i.v. of USPIOs (Sinerem, Guerbet, Roissy, France) resulting in cumulative doses of 0, 2.5, 5.0, 7.5 and 10 mg/kg i.v.

MRI acquisitions. Experiments were performed on a 3T scanner (Siemens, Erlangen, Germany) using a dedicated 4 channel phased-array coil. EPI images were acquired at baseline and 2.5minutes after each injection with a gradient-echo sequence (TE/TR=18/2000ms) and a bipolar diffusion-sensitized spin-echo sequence (TE/TR= 89/1000 and 99/1000ms) with a range of b values (0, 200, 600, 1000, 1400, 1800, 2200, 2600, 3000 s/mm²). The gradient pulses were carefully balanced (Fig.1) to suppress cross-term effects between local background gradients induced by the USPIO and the gradient pulses used for diffusion [4]. Those effects are known to result in an apparent diffusion decrease when increasing the b value [5].

Data processing. ROIs were selected in brain parenchyma for each animal (Fig.1), and signal attenuation, S/So, obtained at each b value and USPIO concentration, [C], was fitted using the Generalized Reduced Gradient (GRG2) algorithm according to 2 different models:

-standard single compartment model (model #1): $\mathbf{S/S_0=exp[-TE.(R2_0+ r_{eff}[C])].exp(-bD)}$
where $\mathbf{R2_0}$ and **D** are the tissue native T2 relaxivity and diffusion coefficient, $\mathbf{r_{eff}}$ the USPIO effective relaxivity. Relaxivity ($\mathbf{r_{eff}}$) and diffusion (**D**) effects are *independent* by principle.

-2-water pool (slow and fast) model with intrinsic relaxivity and diffusion properties (model #2):
$\mathbf{S/S_0=Vexp(-TE.r_b[C]).exp(-bD^*) + (1-V)\{f_{slow}.exp(-TE.r_{slow}[C]).exp[-b(1-\xi[C])D_{slow}]}$
$\mathbf{+ (1-f_{slow}).exp(-TE.r_{fast}[C]).exp[-b(1-\xi[C])D_{fast}]\}}$
where $\mathbf{r_{slow/fast}}$ and $\mathbf{D_{slow/fast}}$ are the USPIO relaxivity and the diffusion coefficient in a slow and a fast water pools, $\mathbf{f_{slow/fast}}$ the slow/fast pool fractions (which are weighted here by their native T2 relaxivities), ξ a constant accounting for residual background gradient effects [5]. For the completeness of this model, the vascular compartment was also included (**V**=Cerebral Blood Volume weighted by its native T2 relaxivity), $\mathbf{r_b}$ = USPIO relaxivity in blood) weighted by a pseudo-diffusion coefficient, **D***, to account for IVIM effects [6]. However, the vascular component was found to be quenched for b values as low as 200s/mm².

Results - Using model #1 a single set of parameters ($\mathbf{r_{eff}}$, **D**) could not fit the data. Rather, the USPIO effective relaxivity, $\mathbf{r_{eff}}$, was found to strongly depend on b value, and **D** on USPIO concentration (Fig. 2). In the absence of cross-terms effects with local background gradients, this relaxivity/diffusion coupling cannot be explained, and this single compartment model must be clearly rejected. On the contrary, the second model accounted very well for all diffusion and T2, separate or combined, effects (Fig. 3) with a single set of parameters for each animal (Table 1). No dependence of USPIO relaxivity with b value, nor of **D** with [**C**] could be found, as expected, and fitting results were very similar across animals and experimental conditions (b value, TE, contrast concentration). Data fitting also demonstrated the absence of cross-terms effects with local gradients (ξ=0), as expected from the sequence design.

Discussion and conclusion - Overall USPIO T2 effects seem to depend on b value, even in the absence of cross-terms effects with local background gradients. This coupling effect with diffusion-weighting is well explained by the presence of 2 tissue water pools where USPIOs exhibit different relaxivities. Those pools, the nature of which deserves further investigation [3], are shared with those found to explain the biexponential behavior of the diffusion attenuation (fast & slow diffusion pools). This finding is not really surprising given that USPIO T2 effects are mainly driven by diffusion in local gradients for spin-echo sequences. In short, diffusion-weighting appears as a filter which emphasizes the contribution of the slow diffusion pool when increasing b values (decrease in effective R2). Conversely, diffusion effects are modulated by the presence of USPIOs even in the absence of background gradients (ADC decreases when USPIO concentration increases). This mechanism obviously applies to blood deoxyhemoglobin and may have implications for the interpretation of BOLD experiments [3]. Finally, the possibility to target USPIO effects to a specific water pool using diffusion-weighting might offer new contrast avenues, especially for molecular imaging.

References - [1] Niendorf Th et al. MRM (1996) 36:847-857; Clark C, Le Bihan D. MRM (2000) 44:852-859; [2] Kiselev V. MRM (2007) 57: 464-469; [3] Le Bihan. PMB (2007) 52:57-90; Kershaw J et al. NMR in Biomed (2009) 22:770-778; [4] Reese et al. MRM (2003) 49:177-182; [5] Zhong et al. JMR (1991) 95:267; Does et al. MRM (1999) 41:236-240; [6] Le Bihan et al. Radiology (1988) 168: 397-405