High sensitivity 19F MRI allows dynamic biodistribution study and oxygen tension mapping at pharmaceutical doses of a PFOB emulsion in the mouse reticuloendothelial system

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Introduction Perfluorooctylbromide (PFOB) is a promising contrast agent for *in vivo* ^{19}F MRI owing to its biocompatibility. After intravenous injection, PFOB emulsions are known to localize in the reticuloendothelial system (RES), mainly the liver and spleen. PFOB nanoparticles (PFOB NP) accumulate within macrophages [1] and thus allow quantitative MRI in these organs. However, the complex multipeak spectrum yielded by PFOB and the scalar J-coupling between the CF_2 and the CF_3 group complicate the choice of an imaging method. Moreover, when injected at pharmacological dose, the low concentration of PFOB in the RES requires a high sensivity acquisition method. We recently optimized a multi spin echo (MSE) sequence allowing cancellation of J-modulation and T_2 enhancement and yielding an excellent sensitivity [2]. Thanks to our method, we show that kinectics of accumulation of a PFOB emulsion in the RES can be quantified within the minutes following injection, allowing the evaluation of the emulsion's stealth when different quantities of polyethylene glycol (PEG) are grafted. We finally show that partial oxygen pressure (pO2) mapping can be performed in the RES after a single injection, contrary to previous studies [3].

Method Materials: NMR experiments were carried out on a 7T Bruker rodent scanner with a homemade 3.2-cm linear birdcage coil. Mice were maintained at 37°C during experiments thanks to hot air. Dynamic biodistribution: Three PFOB emulsions (20% w/w, concentration in NP ~20 nM) containing different quantities of PEG (0, 5 and 50% w/w) were synthetized. Three male Swiss mice received a 200-µL bolus of emulsion in the tail vein for each quantity of PEG and coronal ^{19}F images were acquired with our MSE sequence (60 echoes, TE = 10.5 ms, TR = 3 s, in plane resolution 1.25 mm*1.87 mm, four 5-mm-thick slices, TA=7 minutes) immediately after injection for 84 minutes. The concentration of PFOB NP in the RES was quantitatively derived in the liver and spleen using an internal reference with known concentration (slice 1). Data were fitted on an empirical single-exponential model [4] to get an accumulation time constant k (min^{-1}) and final concentration C_∞ (nM of NP). Oxygen tension mapping: Two mice were infused 3 hours before imaging with 300 µL of PFOB emulsion (PEG 5% w/w). Single-slice coronal images with the previous parameters were acquired for 7 different TR (total acquisition time = 25.5 min). Data were fitted on individual pixels to estimate T1 and the pO2 was derived by using the relationship between relaxation rate and pO2 as described by Shukla et al. [5].

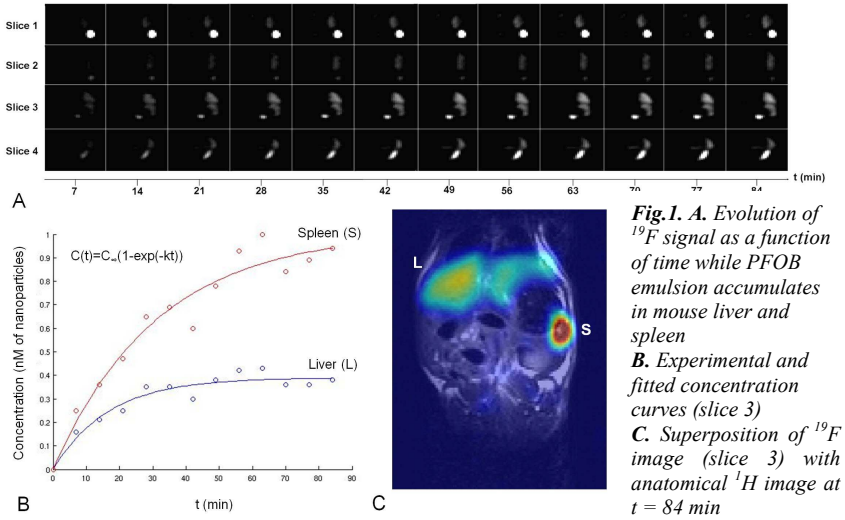

Fig.1. A. *Evolution of ^{19}F signal as a function of time while PFOB emulsion accumulates in mouse liver and spleen*
B. *Experimental and fitted concentration curves (slice 3)*
C. *Superposition of ^{19}F image (slice 3) with anatomical ^{1}H image at t = 84 min*

	k (min-1)	*C_∞ (nM)*
50% PEG		
Liver	0.059±0.031	0.366±0.186
Spleen	0.024±0.011	1.740±1.548
5% PEG		
Liver	0.126±0.066	0.419±0.358
Spleen	0.072±0.017	0.551±0.501
0% PEG		
Liver	1.350±1.822	0.144±0.105
Spleen	0.142±0.091	0.490±0.379

Table 1. *Time constant and final concentration calculated after fitting procedure*

Results and Discussion Dynamic biodistribution: ^{19}F signal appears in the liver and spleen from the first minutes and provides SNR always higher than 5 from the first image (Fig. 1) and up to 50 in the spleen after 84 minutes. No signal is found in vascular organs like the heart or lungs. We ascribe this fact to spoiling effects on flowing spins during a multi spin echo sequence. Estimated time constants and final concentrations are presented in table 1. The greater time constant and final concentration mean values found in the spleen can be explained by the higher macrophages concentration in the splenic tissues than in the hepatic tissues. The high standard deviations can be explained by interindividual variations rather than experimental noise, as confirmed by Monte Carlo simulations on individual data (not shown). Time constant is inversely correlated with the PEG content, confirming that increasing quantities of PEG improve the stealth of nanoparticles *in vivo*. Oxygen tension mapping: A pO_2 map, masked for more clarity, is shown in Fig. 2.A. Results are presented in table 2. Both experiments led to the same values in the reference (pO_2 in the atmosphere 20%) and yield values in the RES consistent with literature. As for dynamic biodistribution, no signal is found in the heart or lungs.

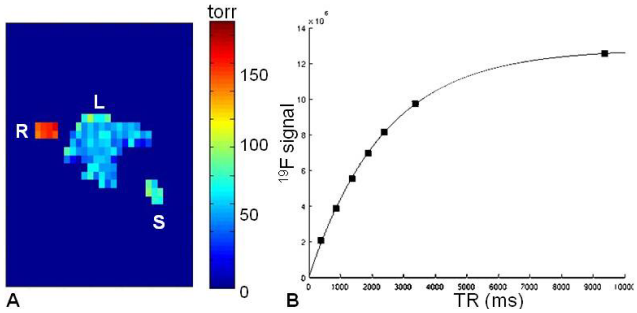

Fig. 2. (A) *pO2 map of a mouse obtained with our sequence showing the liver (L), spleen (S) and reference (R). The signal recovery curve* ***(B)*** *is shown for a single pixel, illustrating the low noise level and the accuracy of our method.*

Exp 1	T1 (ms) (mean±sd)	pO2 (torr) (mean±sd)	% p_{atm}	**Exp 2**	T1 (ms) (mean±sd)	pO2 (torr) (mean±sd)	% p_{atm}
Liver	2694±135	37±14	5	Liver	2432±185	55±16	7
Spleen	2245±220	70±19	9	Spleen	2131±120	79±11	10
Reference	1565±112	153±21	20	Reference	1560±52	154±10	20

Table 2. *Calculated T_1 values and derived pO_2 found in the organs and the reference*

Conclusion Our highly sensitive method allows non-invasive, quantitative assessment of the kinetics and the stealth of PFOB emulsions in the RES, which can be critical when investigating pharmaceutical targeting. It proved to be efficient to obtain oxygenation maps after a single injection of PFOB emulsion. These results are promising for monitoring of the RES status *in vivo* and may therefore lead to clinical applications.

[1] Ratner et al., MRM 1987; [2] Giraudeau et al., MRM 2010; [3] Barker et al., JMRI 1994; [4] Fan et al. MRM 2004; [5] Shukla et al. JMR 1995