Absolute concentration, biodistribution and pharmacokinetics of Gd-based contrast agents in lungs using UTE MRI

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Introduction:

Intratracheal administration of contrast agents based on nanostructures is a promising approach in the diagnosis of lung diseases. The characterization of contrast media pharmacokinetics (PK) and elimination pathways is fundamental to understand the potential of a given contrast agent and its toxicity, main limiting factor in the translatability of preclinical to clinical studies. We present here an *in vivo* MRI study of the PK and biodistribution of Gd-based intratracheally-administrated multimodal Ultra-Small Rigid Platforms (USRPs) and of a commercially available Gd-based contrast agent [1]. The implemented MRI PK models for lungs and kidneys were validated against optical imaging.

Material and methods:

MRI Protocol: Female Balb/c mice (12 weeks-old, 24.0 ± 0.5 g) were used in the experiment. Mice were anesthetized using an intraperitoneal injection of 50 µg/g ketamine and 5 µg/g xylazine. After the acquisition of MR baseline images, an orotracheal intubation was performed on the mice and the contrast agent solution was selectively introduced in the left lung through it. After the extubation, MR images of the mice were acquired at different times (from 5 minutes up to several hours after the administration).

The contrast agent solution volume was fixed to 20 µl and 3 different USRPs solution concentrations (10, 25 and 50 mM of Gd^{3+}) were administrated to different mice (n = 3/group) to study the PK models in the lungs; other n=3 mice received 20µl 50 mM Gd^{3+} of USRPs to study the PK models in the kidneys. Commercial gadoteric acid contrast agent (Dotarem®, Guerbet, Villepinte, F) was administrated (250 mM, 20 µl) to n = 6 mice to study the PK models in the lung and in the kidneys.

Images were acquired with a 4.7 T Biospec spectrometer (Bruker, Ettlingen, D), using a transmitter/receiver quadrature coil of 25 mm inner diameter (Bruker, Ettlingen, D). For each animal 6 axial slices of the lungs of 1 mm thickness were acquired. The acquisition was performed in *free-breathing* animals, using a 2D Ultra-Short Echo Time (UTE) sequence (804 directions/128 points, 4 averages) with a TE of 276 µs, FOV of 3 cm, TR of 84 ms and FA of 60 degrees, for a total acquisition time of about 4 minutes [1]. A standard optimized FLASH sequence was used to image the kidneys, according to the protocol described in [1].

MR image analysis: Images were reconstructed and analyzed with an in-house software implemented in IDL (RSI, Boulder, CO). Following the procedure described in Ref [1], the signal enhancement (SE) in the lungs/kidneys in each image was computed. For each animal, the SE of the lungs was evaluated on four axial slices and averaged over the mice. Assuming a typical signal shape of the form $S = \rho \sin(FA)*\exp(-TE/T_2*)[1-\exp(-TR/T_1)]/[1-\cos(FA)*(\exp(-TR/T_1)]$, with straightforward algebraic manipulations, it is possible to compute the T_1 value from the measurement of *SE*. Since the concentration of the contrast agent is related to the T_1 according to the formula $C(t) = 1/r_1 [1/T_1(t) - 1/T_1^{natural}]$, knowing the longitudinal relaxivity r_1 (82.2 $mM^{-1}s^{-1}$ for the USRPs and 4 $mM^{-1}s^{-1}$ for Dotarem®), the absolute concentration of the contrast media in the lung was computed ($T_1^{natural}$ = 1.42 s for lungs). Specific PK models were used to fit the data (oral instillation model for the lungs and multiple-compartments models for the kidneys [2]).The same procedure was repeated for the kidneys ($T_1^{natural}$ = 0.84 s). Data between different groups were compared using Mann-Whitney test with a 0.05 significance level.

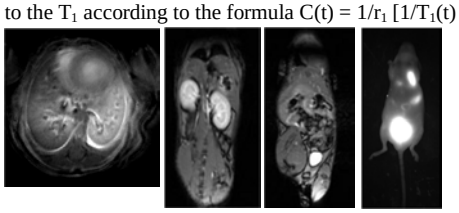

Fig 1. *MRI images showing the passage of the USRPs from the lungs (a) to the kidneys (b) and eventually to the bladder (c).The same elimination pathways were observed with optical imaging (d).*

Fluorescence imaging: 40 µl of a 50 mM Gd^{3+} solution of USRPs with Cyanine 5.5 (Cy) grafted on them was instilled in 4 mice, using the procedure described above. Whole-body mouse fluorescence imaging (ORCAII-BT-512G, Hamamatsu, Massy, F) was performed (up to day 10) on 2 mice. On the other 2 mice, 25 µl of blood were extracted from the tail vein before the instillation and at different times after (15 min, 30 min, 1 h, 3h, 5h and 24h). 10 µl of plasma were obtained and fluorescence imaging was performed on the samples.

Results:

For the same instilled volume, a 250 mM solution of gadoteric acid is required to obtain roughly the same SE achievable with a 50 mM solution of USRPs (>300%). Both MRI and fluorescence imaging showed a fast passage of the nanoparticles in the bloodstream and an accumulation in the kidneys before the final elimination through urines, as shown in Fig 1. No hepatic clearance was observed with MRI or optical imaging for the USRPs. The oral administration one-compartment model with first-order kinetics [2] is the PK model which best fitted the concentration of contrast agent measured in the lungs (MRI) and in the blood (optical imaging) after intra-tracheal administration of the USRPs or the gadoterate meglumide (Fig. 2). The USPRs showed an elimination half-life in the lungs (130±20 min) independent of the solution concentration in the explored range, confirming that the excretion mechanisms are not saturated. Such half-life was significantly longer than the one measured for Dotarem® (22±5 min, $p < 0.01$). A slightly longer half-life (184±26 min) was measured in the blood (optical imaging) for the USRPs. The USRPs diffusion half-life (MRI) in the lungs was not statistically different between the 3 different concentrations explored even though the 50 mM Gd^{3+} solution showed an average value 3 times higher than the 25 mM Gd^{3+} and 10 mM Gd^{3+} (diffusion constant of respectively 33±13 min, 11±6 min and 9±4 min). A similar average value was found for the Dotarem® instilled mice (43±5 min), supporting the hypothesis that the apparent increase in the diffusion time for the highly concentrated solutions (50 mM Gd^{3+} USRPs and 250 mM Dotarem®) is actually due to the T_2^* effect already observed in [1]. Unlike Gd-chelates, USRPs are cleared from the kidneys according to a two-compartment model with first order kinetics [2] (Fig. 3), which allows to detect a portion of nanoparticles still 24h after the instillation. The reason behind the existence of two elimination constants (61±15 min and 40±8 hours) can be easily explained considering the distribution of sizes of the nanoparticles (3.5±1 nm), with the larger USRPs presumably circulating for a longer time than the smaller ones [3]. A one-compartment model properly fitted the data relative to the Dotarem® administration with a shorter elimination half-life in the kidneys (128±25 min).

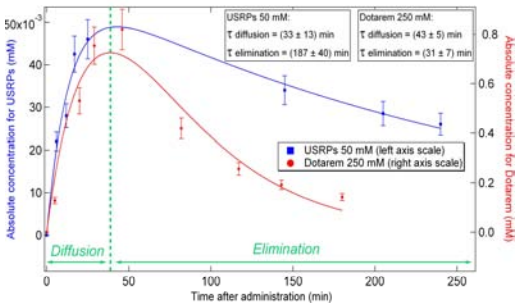

Fig 2. *Oral administration one-compartment model fit applied to lung for USRPs (blue) and Dotarem® (red).*

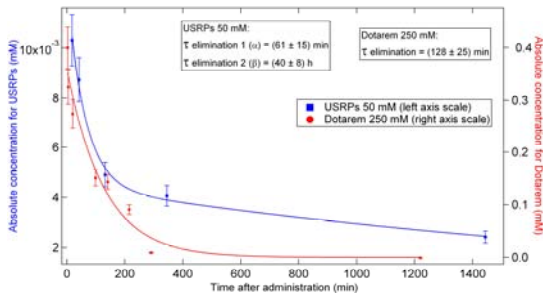

Fig 3. *Two-compartment model fit applied to kidneys for USRPs (blue) and one-compartment model applied to kidneys for Dotarem® (red).*

Discussion and conclusion:

For the two instilled Gd-based contrast agents, the MRI and the optical imaging PK studies highlighted the renal clearance and the absence of detectable hepatic clearance following instillation, ensuring negligible toxicity. The faster lung and kidney elimination of Dotarem as compared to USRPs can be related to its smaller hydrodynamic diameter (< 1 nm for Dotarem vs. 3 nm for USRPs). This translates into a wider imaging window in lungs for USRPs (6-fold longer residence time compared to gadoteric acid) and, at the same time, a good potential for accumulation in lung diseased tissues. The MRI method developed and implemented to compute the absolute concentration of the contrast agents in the lungs rely on the very short echo time of the UTE sequence and, to the exception of the highest concentrations, the negligible T_2* weighting. The MRI-based PK model has shown to give results in agreement with semi-quantitative optical imaging techniques. Exploiting the multimodality of the USRPs, a comparison of the absolute concentration measured with MRI and of the one measured with SPECT data can be envisaged to further validate the obtained results.

References: [1] *Proc. Intl. Soc. Mag. Reson. Med.* 20, p. 628 (2012) [2] G. Brenner et al., *Pharmacology*, Elsevier Health, 3rd edition (2010), 552 p. [3] *Angew. Chem. Int. Ed.*, 2011, 50, 12299-12303.

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