Optimized SPION Formulations for Molecular MRI of the Lung Using Hyperpolarized Gases

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Introduction: The recently introduced combination of hyperpolarized (HP) gas MRI with targeted superparamagnetic iron oxide nanoparticles (SPIONs) could open new avenues for MR-based molecular imaging of the lung[1]. For this approach to be clinically useful, the particles must be delivered by intravenous (iv) injection so that they reach their cellular targets quickly and efficiently. However, because the lung is the organ of interest and its fine capillary network readily traps particles larger than ~10μm, this approach critically depends on the development of stable, monodisperse SPION formulations. If SPIONs are unstable and agglomerate, they will be easily trapped in lung capillaries, thereby producing false positive results. We had previously avoided this problem by using intraperitoneal injections of SPIONs. However, this approach provides slower targeting (~24hrs) and also leads to non-specific uptake by the lymphatic system. Particularly, uptake in the right mediastinal lymph node causes detectable changes on ^{3}He MRI. Thus, the aim of the presented study is to evaluate the *in vivo* bio-distribution of several SPION formulations injected intravenously, and to evaluate their effects on ^{3}He MRI in the mouse.

Methods: We tested several SPION formulations—uncoated SPIONs, SPIONs coated with Dimercaptosuccinic acid (DMSA) and dextran-coated micrometer-sized iron oxide particles (MPIOs). For each formulation, a suspension of 1mg of particles in 400μl of physiological saline was injected through the lateral tail vein in naïve C57 male mice. HP ^{3}He MRI was performed before and 15 minutes after iv injection of the contrast agent. Images were acquired using a 3D-radial acquisition scheme with a 156μm isotropic resolution and an echo time of 1ms. At the end of the study the mice were euthanized, and lungs, liver, and spleen were excised and analyzed histologically for iron content.

Results: Uncoated SPIONs form clusters (**Fig 1c**) that accumulate in the lung parenchyma and produce localized signal defects that are clearly visible on ^{3}He MRI (**Fig 1b**). DMSA-SPIONs are more stable and do not form clusters, but show high affinity to the lung parenchyma (**Fig 1f**), where they sparsely accumulate, producing a diffuse reduction of ^{3}He signal intensity (**Fig 1d-1e**). Dextran-coated MPIOs do not accumulate in the lung parenchyma (**Fig 1i**). However, these large particles cause strong spin dephasing of the ^{3}He image during their transient time in the lung vasculature. This effect is particularly prominent near major blood vessels running perpendicular to the main B_o field (**Fig 1h**).

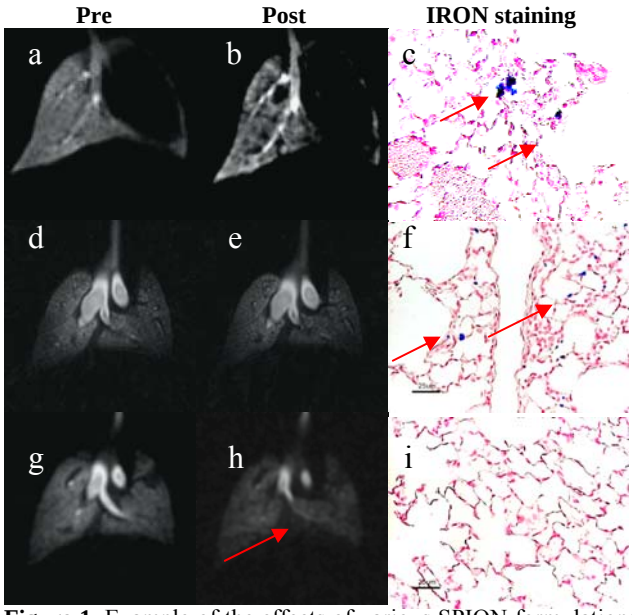

Figure 1. Example of the effects of various SPION formulations on HP ^{3}He MRI after iv injection in naive mice (**a**) HP ^{3}He MRI pre- and (**b**) post iv injection of uncoated SPIONs. Local signal loss is clearly visible in the lung parenchyma. Major airways are not affected since by the time of the imaging experiments, the large clusters were completely cleared from the blood. (**c**) Prussian blue staining of the corresponding excised lung tissue shows trapping of SPION clusters. **(d)** HP ^{3}He MRI pre- and (**e**) post iv injection of DMSA coated SPIONs. The effects of these particles is a diffuse reduction of the signal that affects mainly the lung parenchyma. Prussian blue staining of excised lung tissue (**f**) shows sparse accumulation of single SPION particles. (**g**) ^{3}He MRI pre- and (**h**) post iv injection of micrometer-sized (7μm-diameter) dextran-coated SPION. The circulating micrometer-sized particles produce major signal loss near large vessels but not in the lung parenchyma. **(f)** Indeed, prussian blue staining of excised lung tissue shows no particle accumulation in the lung parenchyma.

Conclusions: Molecular MR applications of targeted nanoparticles in the lung are particularly sensitive to SPION stability and biodistribution, which is dictated by their surface coating. Without a coating, these hydrophobic particles agglomerate, become trapped in the lung capillaries, and produce false positive results. When the particles are coated with DMSA, they become negatively charged and resist agglomeration. However, the negative surface charges interact strongly with the positively charged regions of the plasma membrane, resulting in a partial accumulation of these particles in the lung parenchyma. Dextran-coated micrometer-sized iron oxide particles are monodisperse and do not accumulate in the lungs, but do cause dephasing near the larger vessels. However, due to their larger size and their fast blood clearance, these particles cannot be used for in vivo targeting of tumor cells. For *in vivo* tumor cell targeting, smaller particles with longer circulation times are needed. To this end, SPION coated with polyethylene glycol and triethylene glycol are under investigation.

SPION effects on the HP helium images depend strongly on the particle magnetic moment and on the local iron concentration. Individual SPION particles have a small magnetic moment, and when sparsely distributed in the lung parenchyma, produce a diffuse signal reduction in the ^{3}He images that affects mainly the lung parenchyma and less the lung's airways. When SPION particles accumulate in cells, such as in cancer-targeting[1], these large clusters exhibit a larger magnetic moment, and their effect is readily observed on ^{3}He MRI as very well localized signal loss, as we have illustrated with the study using uncoated SPIONs. When large particles (MPIOs) are present in the blood stream, a major reduction of the helium signal nearby large blood vessels can be observed.

References: [1] Branca et al., *PNAS* **107** (8), 3693.

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