MRI and fluorescence imaging with upconverting nanoparticles: a new multimodal approach for lung targeting

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Introduction:
Cellular and molecular imaging are nowadays of paramount importance to understand the origin and evolution of pathological mechanisms and, even more crucial, for an early diagnosis of the disease. One of the most attractive approaches in molecular imaging is the combination of fluorescence with MRI, providing high sensitivity and resolution together with structural and functional data. To achieve this multimodality, the design of new nanomaterials appears as one of the most promising options. We present here the physicochemical characterization and the in vitro and in vivo multimodal study (MR and fluorescence imaging) of a new class of nanoparticles based on Upconverting NanoPhosphors (UCNPs) albumin-coated. These nanoparticles present the unique feature of converting low energy near infrared (NIR) light into higher visible light and/or NIR emission, which allow to overcome all the typical problems of fluorescent probes (auto-fluorescence, low penetration depth, photobleaching, high costs and toxicity) [1]. At the same time they can be combined to magnetic resonance imaging modality.

Material and methods:
Upconverting Nanophosphors synthesis and characterization: Upconverting NaGdF4 nanoparticles doped with Yb and Er were synthesized in hexagonal phase. The UCNPs were first synthesized in organic solvents, providing high quality nanocrystals, with oleic acid as coating agent [1]. After that, the particles were modified to make water soluble and suitable for biomedical applications. The innovative approach was to take advantage of the fatty acid binding sites of the albumin to directly stabilize the nanoparticles through their oleic acid ligand. The nanoassemblies were fully characterized in terms of core/hydrodynamic size, composition, fluorescence, magnetic and physiological properties (i.e. blood half-life).

Protocol: Healthy female Balbc mice (6 weeks-old, 22.0 ± 0.5 g) were used in the experiment. Isoflurane (2%) in a mixture of N2O (80:20) was used to anesthetize three mice. MRI baseline images were acquired before the intravenous administration (i.v.) of albumin-coated NaGdF4, Yb1m1Er3m3 (300 µL, 6 mM). MR images of the mice lungs were obtained at different times, from 5 minutes up to a few hours after the injection. For the fluorescence imaging, a similar protocol was followed on three different mice, with i.v. injection of 200 µL 10 mM nanoparticles. Mice were sacrificed 30 min after the administration, the lungs were extracted and 2-photon microscopy was performed on 7 µm-thick slices of the tissue.

MR imaging analysis: The images were reconstructed and analyzed with an in-house software implemented in IDL (RSI, Boulder, CO). For each image, the lungs were manually segmented (excluding the main vessels) to measure the total average signal. The noise of the images was quantified as the standard deviation of the mean signal of a region of interest selected in the image and the background noise signal was used as a reference. The signal enhancement (SE) in each image was computed as the difference between the signal to noise ratio (SNR) in the lungs after the contrast agent administration and before (baseline images), normalized to the SNR of the baseline images. For each animal 8 consecutive axial slices were acquired in order to cover the whole lungs volume. The acquisition was performed in free-breathing animals, using the 2D Ultra-short Echo Time (UTE) sequence (804 directions/128 points, 4 averages), with a TR of 112 ms, TE of 276 µs, FA of 60°, FOV of 5 cm, for a total acquisition time of about 5 minutes.

Results:
The average hydrodynamic size of the UCNPs was 83 nm (polydispersity index PDI = 0.22). Transmission electron microscopy in conjunction with X-ray and Fourier transform infrared spectroscopy confirmed the high-quality crystalline structure of the core and the composition of the coating. In vitro fluorescence excitation at 980 nm was measured and emission was found from visible to NIR according to the dopants, nature and ratio added in the nanomaterial. A great enhancement in the fluorescence signal was obtained when the core of the nanoparticles was passivated with a non doped host shell (about 5 times larger compared to non passivated UCNPs). A longitudinal relaxation of 1.05 mTm/s was measured at 1.5 T. A significantly long blood half-life of about 60 minutes was measured. For the in vivo experiments, a maximum accumulation of the nanoparticles in lung was found 33 minutes after the i.v. injection of the UCNPs, as shown in Figure 1. A maximum SE of about 35% was indeed measured (increase of the SNR from 22.8 to 30.8) in the lung parenchyma. The passive accumulation of the UCNPs after i.v. was confirmed with the ex vivo fluorescence studies. The 2-photon microscopy images (shown in Figure 2), showed the accumulation of the nanoparticles in the lungs. Other imaging modalities like CT (data not shown here) have been performed as well, confirming the multimodal potential of these nanoprobes.

Discussion and conclusion:
This study shows the feasibility of UCNPs localization in the lung parenchyma using UTE MRI. This new class of promising nanomaterials accumulates in healthy mice lungs after intravenous injection, as confirmed by the ex vivo fluorescence imaging. Such original result, in agreement with the known properties of albumin [2], is of great importance for future applications in the field of lung imaging and diagnostics. The well-known biocompatibility and biodegradability of albumin and the long residence time in the blood and lung tissues makes these nanoparticles good candidates for accumulation in diseased lung tissues. For instance, in early-stage lung cancers, the probes are expected to accumulate in large quantity in the tumor because of the enhancement permeability and retention effect. Besides, the easiness of grafting further ligands or probes to the albumin coating [3] makes the UCNPs promising for active targeting studies and/or therapeutic applications in the lungs. Finally, the natural accumulation of the nanoparticles in the lungs may also be exploited to study the preventive or curative effect of specific drugs grafted on the UCNPs in pathologies characterized by inflammation. At the light of these considerations, the combination of the high-resolution which can be reached with an optimized UTE MRI sequence and the sensitivity typical of optical imaging, strengthened by the NIR to UCNPs upconversion properties, make the UCNPs strong candidates for theranostics studies and longitudinal non-invasive preclinical experiments.

References:

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