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

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Introduction:

Cell 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:

Nanoparticles synthesis and characterization: Upconverting $NaGdF_4$ nanoparticles doped with Yb and Tm 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 them water stable 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 Balb/c mice (6 weeks-old, 22.0 ± 0.5 g) were used in the experiment. Isoflurane (2%) in a mixture of N_2/O_2 (80:20) was used to anesthetize three mice. MRI baseline images were acquired before the intravenous administration (i.v.) of albumin-coated $NaGdF_4$ Yb,Tm@$NaGdF_4$ (300 µL, 6 mM). MR images of the mice lungs were acquired 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-photons microscopy was performed on 7 µm-thick slices of the tissue.

MR acquisition: Images were acquired with a 4.7 T Biospec 47/50 spectrometer (Bruker, Ettlingen, D), using a transmitter/receiver quadrature coil of 25 mm inner diameter (Bruker, Ettlingen, D). Mice were anesthetized with 2% isoflurane in a mixture of N_2/O_2 (80:20) via facial mask and placed supine in a custom-built plastic holder. For each animal 8 consecutive axial slices (1 mm thick) were acquired, in order to cover the whole lung 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 3 cm, for a total acquisition time of about 5 minutes.

MR image 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 background and the signal to noise ratio was computed. 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, the signal enhancement of the lungs was evaluated on three axial slices and averaged over the mice.

Fluorescence imaging: Fluorescence images were collected in raster scanning mode using a 2-channels Alba spectrometer (ISS Inc, Urbana-Champaign, IL, USA) equipped with fast photomultipliers (Hamamatsu Photonics, Hamamatsu City, J). The Alba module was coupled to an inverted Ti-E microscope (Nikon Corp., Tokyo, J), equipped with a apochromatic objective, epifluorescence lamp, bright field and top stage incubator and heating chamber (Okolab Srl, Naples, I). Excitation at 995 nm was provided by a femtosecond-pulsed tunable laser (Newport Corp. Irvine, CA, USA). Emission was collected after a 610/50 nm and a 460/54 nm blocking filters (Semrock Inc., IL, USA). A series of 20-100 consecutive images of 256x256 pixels at 64 µs/pixel were averaged.

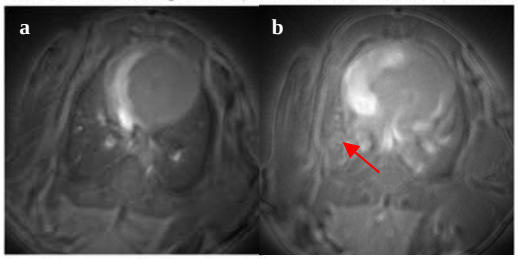

Fig 1. UTE axial slice (a) before and (b) 33 min after the i.v. administration of the UCNPs. The red arrow underlines the area of maximum SE of the lung.

Results:

The average hydrodynamic size of the UCNPs was 83 nm (polydispersity index *pdi* = 0.22). Transmission electron microscopy together 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 with 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 relaxivity of 1.05 $mM^{-1}s^{-1}$ 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-photons microscopy images (shown in Figure 2), showed the accumulation of the probes in the lung tissues. 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 sequences. 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 of albumin and the long residence time in the blood and lung tissues make 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 therapeutical 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 NIR upconversion properties, make the UCNPs strong candidates for theranostics studies and longitudinal non-invasive preclinical experiments.

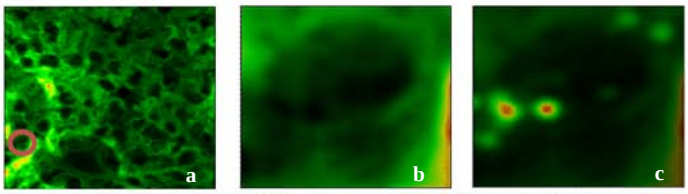

Fig 2. Fluorescence images of the H/E stained lung tissue with (a) large view of the alveolar structure (Filter 610/50 nm) and (b) zoom on the zone of interest (Filter 610/50 nm). (c) Fluorescence images of the UCNPs (Filter 460/54) merged with (b).

References:

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