Intratracheal administration of Gd-based nanoparticles: an effective approach for MRI detection and follow-up of lung tumor

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Introduction: Lung cancer is the leading cause of cancer deaths worldwide. The burden of this disease could be greatly reduced with an early diagnostics, which often results in a positive prognosis. In this context, MRI can play a major role being a noninvasive imaging technique, characterized by good soft tissue contrast, high spatial resolution, and absence of ionizing radiation. We present here an *in vivo* MRI longitudinal study of lung cancer detection in tumor-bearing immunodeficient mice through intratracheally- and intravenously- administered multimodal gadolinium-based Ultra-Small Rigid Platforms (USRPs) [1] and a commercially available Gd-based contrast agent. The localization of the tumors was validated against Bioluminescence Imaging (BLI) and histology.

Material and methods:

Study protocol: Female NMRI immunodeficient mice (6 week-old, 22.0 ± 0.5 g) were used in the experiment. At day 0, an orthotopic implantation of H358-Luc bioluminescent tumor cells (10^6 cells/mouse) was performed in mice lungs through an intratracheal (i.t.) administration. Animals (n=16) were imaged with BLI at day 30. After the acquisition of MR baseline images, the contrast agent solution was administered to the mice at different days and MR images were acquired at different times (from 5 minutes up to several hours after the administration). Between two different administrations on the same mouse, three days without any handling was foreseen in order to allow a complete elimination of the previously administered contrast agent. In detail, all the mice received an intravenous (i.v.) administration of USRPs 200µL 50 mM Gd^{3+} at day 35 and an intratracheal administration of USRPs 50 µL 50 mM Gd^{3+} at day 38. A subgroup (n=4) received an intravenous administration of USRPs 200µL 12.5 mM Gd^{3+} at Day 41, an intravenous administration of gadoteric acid contrast agent (Dotarem®, Guerbet, Villepinte, F) 200µL 50mM at Day 44 and a second intratracheal administration of USRPs 50 µL 50 mM Gd^{3+} at day 54 (follow-up study). A subgroup (n=3) received an intratracheal administration of USRPs 50µL 50 mM Gd^{3+} 3 days after the first one in order to study the reproducibility of the i.t. protocol. After the acquisition of the last images, animals were sacrificed and lungs extracted. After paraffin embedding, 4-µm-thick slices were cut every 50 µm of lungs. On average, 100 slices were obtained for each animal and stained with hematoxylin-eosin-safran (HES) in order to identify the presence of the tumor and its approximate position in the lungs. MRI Protocol: 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 *freely-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 3x3 cm, TR of 140 ms and FA of 60 degrees, for a total acquisition time of about 7 minutes. MR image analysis: Images were reconstructed with an in-house software implemented in IDL (RSI, Boulder, CO) and analyzed with a freeware software (MIPAV, NIH, MD-US). Following the procedure described in Ref [2], the signal enhancement (SE) in the lung tumor was computed for each animal and averaged over three axial slices. In addition, the contrast-to-noise ratio (CNR) was computed as the difference between the signal in tumor tissues and healthy tissues, normalized by the standard deviation of the image noise. The increase of CNR (ΔCNR) after the administration of contrast agent was computed [ΔCNR = (CNR after c.a./CNR before c.a.) -1]. The total tumor volumes were computed readapting the protocol for inflammatory volumes computation proposed in Ref. [3]. Data between different groups were compared using nonparametric Mann-Whitney test with a 0.05 significance level.

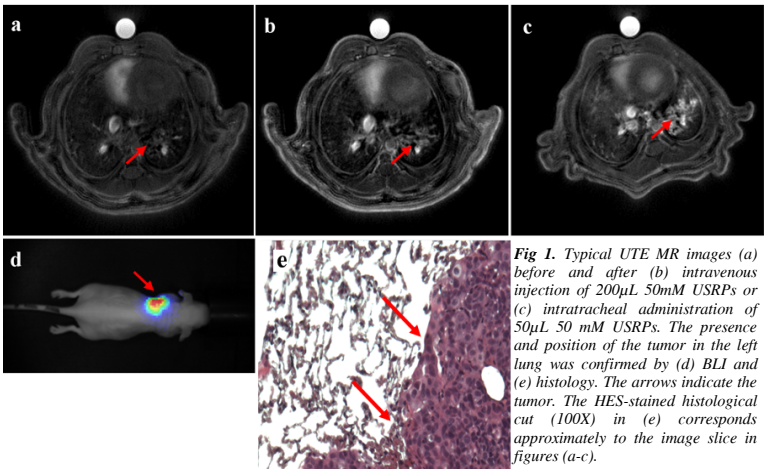

Fig 1. *Typical UTE MR images (a) before and after (b) intravenous injection of 200µL 50mM USRPs or (c) intratracheal administration of 50µL 50 mM USRPs. The presence and position of the tumor in the left lung was confirmed by (d) BLI and (e) histology. The arrows indicate the tumor. The HES-stained histological cut (100X) in (e) corresponds approximately to the image slice in figures (a-c).*

BLI imaging: Five minutes after intraperitoneal injection of luciferin (150 µg/g), the mice were anesthetized and bioluminescence images and black-and-white pictures were acquired using a back-thinned CCD camera at -80°C (ORCAII-BT-512G, Hamamatsu, Massy, F). The intensity of tumor BLI signal (approximately proportional to the number of tumor cells) was measured and correlated to the tumor volume measured with MRI [4] using a Spearman correlation coefficient.

Results: Before the administration of contrast agent, UTE MR images allowed the identification of the presence of abnormal parenchymal tissue in a number of animals (~90%). Nonetheless, the contours of the carcinogenic formations were not easily identifiable (Fig. 1a). After intratracheal or intravenous administration of USRPs, in *all* the mice a good co-localization of the position of the tumor with MRI, BLI and histology was observed, as shown in Fig. 1. The quantified tumor volumes significantly correlated with the measured bioluminescent signal both after intravenous ($R = 0.94$, $p < 0.01$) and intratracheal ($R = 0.88$, $p < 0.01$) administration of USRPs. The comparison of SE and increase of CNR in the identified tumors (Fig. 2) showed approximately two-fold higher values for the intratracheal (50µL 50mM) administration with respect to the intravenous one (200µL 50 mM), using 4 times less Gd^{3+}. Similarly, the intravenous administration of Dotarem resulted 4 times less effective than the same amount of intravenously administered USRPs. The mice that received an intratracheal administration of USRPs at 3 days distance showed no significant differences in tumor size, position, SE and ΔCNR, confirming the reproducibility of the protocol. Conversely, the comparison of the tumor size after intratracheal administration at 16 days distance showed a significant increase in the tumor volume quantified with MRI.

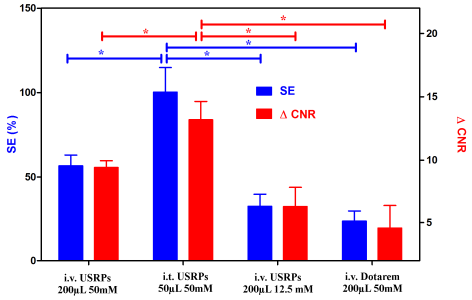

Fig2. *Bar plot comparing the SE (blue) and the ΔCNR (red) for intratracheal (i.t.) and intravenous (i.v.) administration of USRPs and Dotarem.*

Discussion and conclusion: In this work we compared two administration routes for contrast agent delivery to lung tumor tissue: intravenous injection and intratracheal administration. Both the administration modalities allowed the computation of the tumor volumes that significantly correlated with the bioluminescent signal coming from the tumor cells. The same results were obtained with fluorescence imaging (data not shown here), exploiting the multimodal potential of the USRPs. Significantly higher SEs and CNRs were observed with intratracheal administration using lower doses, reducing the toxicity issues and making the protocol especially suitable to reduce the inter-observer variability in tumor segmentation. The surprising SE and CNR achievable after intratracheal administration cannot be entirely explained in the light of the Enhanced Permeability and Retention (EPR) effect observed usually in tumor tissue following systemic administration of contrast agent. The suspected reason of the strong accumulation of the nanoparticles in the tumor may be connected to the fact that, once in the alveoli, the USRPs can directly reach the tumor since no (or very compromised) alveolar barrier is interposed between the tumor and the contrast agent (see Fig. 1e).

In conclusion, the observed high reproducibility and efficacy of the protocol, altogether, make the intratracheal administration of these Gd-based nanoparticles a good candidate for early lung cancer detection and noninvasive follow-up of the diseases. In addition, the previously demonstrated negligible acute toxicity of the USRPs and favorable pharmacokinetics [5], their theranostic properties [6] and the possibility of replacing the intratracheal administration with a simpler aerosol, altogether, make the proposed protocol potentially translatable to human studies. While the accumulation of the USRPs after intravenous injection can entirely be attributed to passive targeting, the mechanisms making the accumulation of the nanoparticles in the tumor so effective after intratracheal administration need to be further investigated. To our knowledge, this is the first time that a study clearly shows that the synergic employment of a strongly T_1-weighted MRI UTE sequence and high-relaxivity gadolinium-based nanoparticles allow the high-precision detection of lung tumor and of its contours.

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