Intratracheal administration of ultra-small Gd-based nanoparticles: a new protocol for brain tumor targeting

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Introduction:

Glioblastoma is the most aggressive and common brain tumor. The 5-year survival rate for this disease is inferior to 10% [1]. In this context, new noninvasive methods for early detection and therapy are needed in order to improve the prognosis of this pathology. We present here an *in vivo* MRI longitudinal study of brain cancer detection in tumor-bearing immunodeficient mice through intratracheally- and intravenously- administered gadolinium-based multimodal Ultra-Small Rigid Platforms (USRPs) [2].

Material and methods:

In vivo study protocol: Female NMRI immunodeficient mice (6 week-old, 22.0 ± 0.5 g) were used in the experiments. At day 0, an orthotopic implantation of U87-MG tumor cells ($5x10^5$ cells/mouse) was performed in mice brain through an intracranial administration. Animals (n=14) were repeatedly imaged with MRI between day 8 and day 15 (reproducibility and follow-up study). 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, at least 2 days without any handling was foreseen in order to allow a complete elimination of the previously administered contrast agents. In detail, a subgroup (n=4) received an intravenous (i.v.) administration of USRPs 200µL 50mM Gd^{3+}. A subgroup (n=6) received an intratracheal (i.t.) administration of USRPs 50µL 200 mM Gd^{3+}. A subgroup (n=4) received an intratracheal administration of USRPs 50µL 200 mM $Gd^{3+}$and an intravenous administration of USRPs 50µL 200 mM Gd^{3+} 2 days after.

MRI Protocol: Images were acquired with a 7 T Biospec spectrometer (Bruker, Ettlingen, D), using a transmitter/receiver quadrature coil of 25 mm inner diameter (Bruker, Ettlingen, D). For each animal 10 axial slices of the brain of 1 mm thickness were acquired. The acquisition was performed in isoflurane-anesthetized animals, using a 2D Ultra-Short Echo Time (UTE) sequence (804 directions/128 points, 2 averages) with a TE of 368 µs, FOV of 2x2 cm, TR of 140 ms and FA of 60 degrees, for a total acquisition time of about 4 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. [3], the signal enhancement (SE) in the lung tumor was computed for each animal and averaged over two axial slices. In addition, the contrast-to-noise ratio (CNR) was computed as the difference between the signal in tumor tissues and healthy tissues after the administration of the contrast agent, normalized by the standard deviation of the image noise. Data between different groups were compared using nonparametric Mann-Whitney test with a 0.05 significance level.

Results:

Before the administration of contrast agent, UTE MR images allowed the identification of the presence of brain tumor only in a small number of animals, generally only when the tumor was in its latest stages. Furthermore, the contours of the carcinogenic formations were not easily identifiable (Fig. 1a). After intratracheal or intravenous administration of USRPs, a good localization of the position of the tumor with MRI was observed, as shown in Fig. 1b and c. The comparison of SE and increase of CNR in the identified tumors (Fig. 2) showed approximately two-fold higher values for the intravenous (200µL 50mM) administration with respect to the intratracheal one (50µL 200mM), using the same amount of Gd^{3+}. The SE of the tumor was slightly longer after intratracheal administration (elimination constant = 75 ± 12 min) compared to intravenous administration (elimination constant = 55 ± 16 min). The mice that received an intratracheal administration of USRPs at a 2 days distance showed no significant differences in tumor size, position, SE and CNR, confirming the reproducibility of the protocol. Conversely, the comparison of the tumor size after intratracheal administration at 8 days distance showed a significant increase in the tumor volume as quantified with MRI.

Discussion and conclusion:

In this study we showed that the sensitivity of T_1-weighted UTE MRI for the detection of glioblastoma can be increased using T_1-shortening contrast agents. We investigated two different administration routes for contrast agent delivery to brain: a classical intravenous injection and an original intratracheal administration. Both the administration modalities allowed the visualization of tumor position and borders in all the mice, confirming the detection efficiency of UTE MRI combined with contrast media. This observation indicates that both the administration routes can be effective for tumor visualization and follow-up. The accumulation of the nanoparticles in the brain tumor after intravenous injection can be attributed to the disrupted blood-brain barrier, the enhanced permeability and retention (EPR) effect, and passive targeting mechanism. When the nanoparticles are instilled intratracheally, the USRPs, because of their small hydrodynamic diameter (4.1 nm on average), have been shown to pass from the lung parenchyma to the bloodstream, with an elimination time constant of about 3 hours [2]. Once in the bloodstream, a fraction of the nanoparticles can then passively accumulate in the brain tumor for EPR effect, in a similar way to intravenously administered nanoparticles, and be later on filtered by the kidneys and eventually excreted via urine.

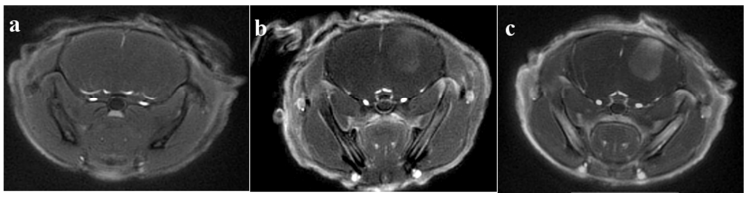

Fig 1. *UTE MR images (a) before and after the (b) intratracheal administration of 50µL 200mM USRPs or (c) intravenous injection of 200µL 50mM USRPs.*

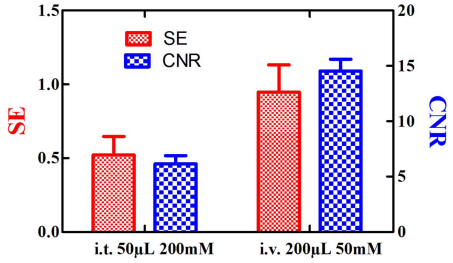

Fig 2. *Bar plot of tumor SE (left scale) and CNR (right scale) after intravenous and intratracheal administrations.*

Although lower SE and CNR were observed in the tumor after intratracheal administration of USRPs compared to intravenous injection, the intratracheal administration presents several interesting properties. First of all, it is less invasive than a standard parenteral systemic administration. This intratracheal administration modality can be considered as a first step towards a completely noninvasive administration procedure like nebulization or aerosol which can be potentially repeated *ad libitum* in preclinical or clinical studies. Secondly, the slow diffusion of the Gd-based contrast agents from the lungs to the bloodstream and then to the tumor tissue can be advantageously used in combination with interventional or therapeutic procedures (for instance radiosensitization [4] or opening of the blood-brain-barrier) or when strategies for active targeting of the tumor tissue are employed. In conclusion, the observed high reproducibility and efficacy of the protocol, altogether, make the intratracheal administration of these multimodal nanoassemblies a good candidate for early brain cancer detection and noninvasive follow-up of the diseases. In addition, the previously demonstrated negligible acute toxicity of the USRPs and favorable pharmacokinetics [5], and the possibility of lung administration with a simple aerosol, altogether, make the proposed protocol potentially translatable to human studies. To our knowledge, this is the first time that a study shows that the synergic employment of a strongly T_1-weighted MRI UTE sequence and intratracheally-administered gadolinium-based nanoparticles allow the high-precision detection of brain tumor and of its contours.

References: [1] *Lancet Oncol.*, 2009, 10, 785–793 [2] *Angew. Chem. Int. Ed.,* 2011, 50, 12299-12303. [3] *Magn. Reson. Med..* 2013, 70:1419–1426 [4] *ACS Nano*, 2011, 5(12):9566-9574 [5] *Magn. Reson. Mater. Phy.*, in press, doi: 10.1007/s10334-013-0412-5.

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