Intratracheal manganese-enhanced MRI (MEMRI) at very low dose: an effective approach for lung tumor detection

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Introduction: Manganese-enhanced MRI (MEMRI) has been extensively used in neurosciences for several applications. In addition, the small hydrodynamic diameter, high water solubility, large availability on the market and *the reduced cost* of manganese (Mn^{2+}) compounds make MEMRI a good candidate for oncological applications [1,2]. While MEMRI has been shown to have potential interest in brain, liver, neck, and breast cancers detection, no application has yet been shown for lungs. We present here for the first time an *in vivo* MRI study of lung cancer detection in tumor-bearing mice using *very low doses* of intratracheally- and intravenously- administered manganese chloride ($MnCl_2$). The localization of the tumors was validated against bioluminescence imaging (BLI) and histology. Due to the possible neurotoxicity of the manganese, the accumulation of $MnCl_2$ in the brain was evaluated using MRI.

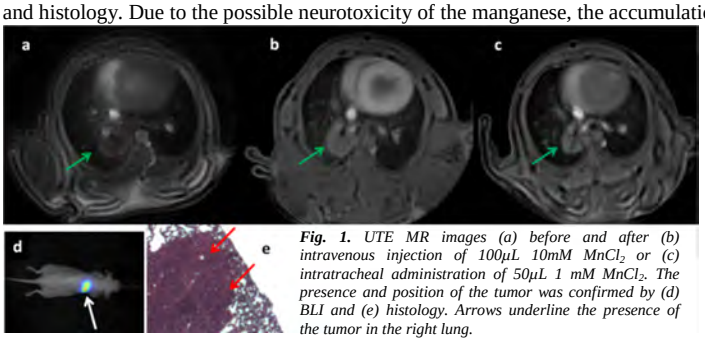

Fig. 1. *UTE MR images (a) before and after (b) intravenous injection of 100µL 10mM $MnCl_2$ or (c) intratracheal administration of 50µL 1 mM $MnCl_2$. The presence and position of the tumor was confirmed by (d) BLI and (e) histology. Arrows underline the presence of the tumor in the right lung.*

Material and methods: Study protocol: Female NMRI immunodeficient mice (6 week-old, 21.0 ± 0.4 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 (n=12) through an intratracheal administration. Animals were imaged with BLI at day 30. After the acquisition of MR baseline images, the contrast agent solution (manganese chloride solution, Sigma-Aldrich, Saint Louis, US) 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). In detail, three (n=3) mice received only an intravenous (i.v.) administration of 100µL of 10 mM $MnCl_2$ on day 45 and other three mice (n=3) received only an intratracheal (i.t.) administration of 50µL of 1 mM $MnCl_2$ on day 45. Six (n=6) mice received an i.v. administration of 100µL of 10 mM $MnCl_2$ on day 45 and an i.t. administration of 50 µL of 1 mM $MnCl_2$ on day 48. 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 (HE) 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, Germany), using a transmitter/receiver quadrature coil of 25 mm inner diameter (Bruker, Ettlingen, Germany). For each animal 10 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/256 points, 4 averages) with a TE of 337 µs, FOV of 2.5x2.5 cm^2, TR of 140 ms and FA of 60 degrees, for a total acquisition time of about 7 minutes. The same parameters were used to acquire sagittal and axial slices of the brain to assess the possible accumulation of manganese in the hippocampus. MR image analysis: Images were reconstructed with ParaVision 6.0 (Bruker, Ettlingen, Germany) 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 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.)]. The total tumor volumes were computed as described in Ref. [3]. Data between different groups were compared using nonparametric Mann-Whitney test with a 0.05 significance level. BLI: 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, France).

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 (~85%). Nonetheless, the contours of the carcinogenic formations were not easily identifiable (*Fig. 1a*). After intratracheal or intravenous administration of $MnCl_2$, 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 comparison of SE and increase of CNR in the identified tumors (*Fig. 2*) showed approximately the same values for the intratracheal (50µL of 1mM $MnCl_2$) administration with respect to the intravenous one (100µL of 10 mM $MnCl_2$), using *20 times less* Mn^{2+}. A small (25%±8%) MRI SE was observed in the hippocampus of the animals that underwent i.v. administration of $MnCl_2$ while no MRI SE was observed after i.t. administration (3%±6%), as shown in *Fig. 3*.

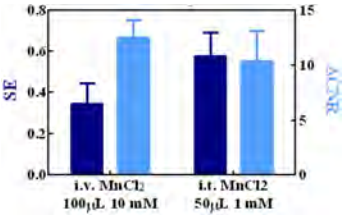

Fig. 2. *Bar plot comparing the SE (dark blue, left scale) and the ΔCNR (sky blue, right scale) for intratracheal (i.t.) and intravenous (i.v.) administrations of $MnCl_2$.*

Discussion: The main limitation in the diffusion of Mn-based T_1-shortening compounds for MRI oncological applications is related to the possible neurotoxic effect of Mn^{2+} ions. It is indeed known that the overexposure to this metal can lead to a progressive accumulation, particularly in brain regions, resulting in neurodegenerative damages. However, it is worth mentioning that there are no known reports of Mn toxicity following a single administration [4]. In this work manganese was administered to the lungs of tumor-bearing mice through two different routes: intravenous injection and intratracheal administration. Both administration modalities allowed the computation of the tumor volumes with low doses of $MnCl_2$. Similar SEs and CNRs were observed with intratracheal administration using a *20-time lower* dose compared to the intravenous injection. As a consequence, it was possible to achieve significant tumor signal enhancements using very low doses of manganese chloride (50 µL of 1 mM $MnCl_2$) when working with the i.t. route, strongly reducing the toxicity concerns. The negligible accumulation of $MnCl_2$ contrast agent in the brain following intratracheal administration was further confirmed *in vivo* using a T1-weighted UTE MRI sequence, which was previously shown to be very sensitive to detect changes due to positive contrast agents in lungs [3] and brain [5]. The possibility of using low doses of contrast agent in this study (1-20 mM [Mn^{2+}]), compared to the higher typical doses of Gd-based compounds (50-250 mM [Gd^{2+}]) in similar studies [3], is probably related to the fact that Mn ions are very similar to calcium ions and may be therefore internalized more easily into the cells, resulting in higher availability to water molecules and longer retention time in tissue. In addition, malignant tumor cells have already been shown to have a tendency to accumulate more manganese than healthy cells, owing to an overexpressed manganese-superoxide dismutase (Mn-SOD) protein [6]. Finally, the higher SE and CNR achievable after intratracheal administration (compared to i.v.) using small amounts of $MnCl_2$ are in agreement with the results presented in Ref. [3]. These results, indeed, 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 agents. The suspected reason of the strong accumulation of the contrast agents in the tumor may be connected to the direct passage of the compound from the alveoli to the tumor.

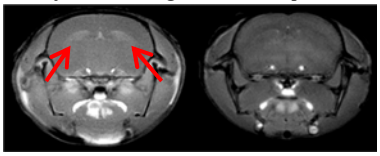

Fig. 3. *UTE T1-weighted images of the brain after (left) intravenous injection of 100µL of 10mM $MnCl_2$ or (right) intratracheal administration of 50µL of 1 mM $MnCl_2$. Arrows indicate the enhancement of the hippocampus, visible only after i.v. of $MnCl_2$ (not visible after very low dose i.t. of $MnCl_2$).*

Conclusion: In conclusion, in this work we have shown that Mn-based contrast media can be an effective, safe and *inexpensive* alternative to gadolinium-based compounds for early lung cancer detection and noninvasive follow-up of the diseases. The orotracheal administration of contrast agent used in this animal study can be easily translated in humans with simple aerosols [7]. The possibility of using *very low doses* of manganese thanks to this favorable administration route along with the possibility of replacing the intratracheal administration with a simpler aerosol, altogether, make the proposed protocol potentially *translatable to human studies*. In addition, this study has further confirmed [3] the existence of an effective mechanism of accumulation in tumor tissues after intratracheal administration that goes beyond the well-known EPR effect. To our opinion, this mechanism deserves further investigations since it may turn out to be likewise an effective method to selectively deliver therapeutic agents to pulmonary neoplastic lesions. To our knowledge, this is the first time that a study clearly allows the high-precision detection of lung tumor and of its contours thanks to the synergic employment of a strongly T_1-weighted MRI UTE sequence and manganese, an *inexpensive* contrast agent (<10$ for 1 liter 10mM).

References: [1] *Invest. Ophthalmol. Vis. Sci.* 2007; 48:963–967 [2] *Magn Reson Med* 2010; 64:902–906 [3] *Proc. Natl. Acad. Sci. USA* 2014; 111:9247-52 [4] *Contrast Media Mol. Imaging* 2012; 7:542–546 [5] *Proc. Intl. Soc. Mag. Reson. Med.* 22 (2014) p. 4031 [6] *Int. J. Cancer* 2010; 128:2138–2146 [7] *Invest. Radiol.* 2005; 40:85–88