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Magnetic particle imaging of tumor retention and leakage of magnetic nanoparticles reveals high inter-subject variability

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Abstract

Intratumoral (i.t.) injection of magnetic nanoparticles (MNPs) is a clinically relevant strategy for localized therapy, including magnetic fluid hyperthermia (MFH). However, the fate of i.t.-injected MNPs remains poorly characterized. Here, we employed magnetic particle imaging (MPI) to quantitatively and longitudinally monitor MNP retention and leakage of i.t.-Synomag®-D70 in a mouse subcutaneous 4T1 breast tumor model. Mice (n=4) were scanned serially (15 min to 8 day) using 2D MPI with fiducial calibration. At day 8, 3D MPI and CT co-registration were performed, followed by *ex vivo* imaging. A strong linear calibration (R^2=0.967) between MNP concentration and MPI signal enabled accurate iron quantification. All tumors showed strong initial MPI signal with an 1.8 to 3.0-fold signal reduction by day 8, indicating gradual tumor clearance. Liver and spleen uptake could only be observed in 2 out of 4 mice, while none of the other major organs showed uptake. The high inter-subject variability in MNP tumor retention calls for integrating MPI quantification for developing individualized MFH treatment plans.

I. Introduction

Intratumoral (i.t.) injection of therapeutic agents is an established clinical strategy for delivering high local drug concentrations while limiting systemic exposure and toxicity. This route of administration bypasses many of the biological barriers associated with systemic delivery, enabling targeted therapy directly at the tumor site.

I.t. injection of MNPs has been clinically implemented for MFH of glioblastoma multiforme [1]. However, recent studies have shown that not all i.t.-injected MNPs stay within the tumor, even immediately after injection [2]. Variabilities in tumor vascularization, extent of necrosis, extracellular matrix density, and interstitial pressure create uncertainties about the intratumoral retention and off-target leakage into the blood stream which may result in systemic uptake by the liver and spleen. These processes may significantly impact the therapeutic efficacy and safety of MFH, and have so far not been addressed *in vivo*. Magnetic resonance

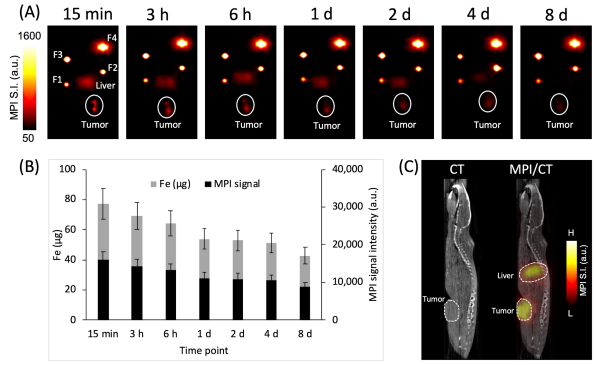

Figure 1: Longitudinal *in vivo* MPI tracking of *i.t.* injected Synomag®-D70 *NPs* in 4T1 tumor-bearing mice. (**A**) Maximum intensity projection MPI images acquired from a representative mouse that exhibited immediate liver uptake at different time points post i.t. injection. Fiducial tubes (F1–F4) were positioned for calibration during imaging. Tumor and liver regions are outlined. (**B**) Quantification of iron content and total MPI signal in tumors at each time point. Error bars represent the standard deviation of MPI signal intensity within the tumor ROI for the representative mouse shown in panel A. (**C**) Anatomical CT image and co-registered MPI/CT overlay from the same representative mouse shown in panel A, generated using fiducial-based registration in 3D Slicer, illustrating MNP retention in the tumor and leakage to the liver.

imaging (MRI), computed tomography (CT), and nuclear imaging techniques face limitations related to sensitivity, specificity, quantification accuracy, or short tracer half-lives. In contrast, MPI provides high sensitivity, zero background, and linear quantification of iron mass, making it ideally suited to track the fate of MNPs *in vivo* over extended periods [3]. We report here on the use of MPI to quantitatively and longitudinally assess the retention and leakage of MNPs injected i.t. in subcutaneous breast tumors.

II. Methods and materials

A Momentum MPI scanner (Magnetic Insight Inc., USA) was used to longitudinally monitor the retention and leakage of Synomag®-D70 (Micromod Partikeltechnologie GmbH, Germany) following i.t. injection in subcutaneous 4T1 breast tumor-bearing female Balb/c mice (n=4, 8 weeks old). A ferrozine spectrophotometric assay determined the MNP stock concentration to be 12 mg Fe/mL. A 10 µL bolus (120 µg Fe) was injected i.t. and four fiducial tubes (F1–F4) containing 30, 60, 120, and 240 µg Fe were imaged alongside the animals for signal calibration. Using "standard" MPI 2D mode, mice were scanned at 15 min, 3 hrs, 6 hrs, and 1, 2, 4 and 8 days post-injection. Tumor regions were segmented, and total MPI signal was quantified as the product of average pixel intensity and segmented area. All tumor and organ regions of interest were manually delineated on the 2D MPI images at each time point, leveraging the high signal-to-background contrast inherent to MPI. Iron content in tumors was calculated based on the fiducial calibration curve. At day 8 post-injection, animals were imaged using both 2D and 3D "standard mode" MPI, followed by micro-CT using an IVIS Spectrum/CT (Caliper Sciences) for anatomical co-registration. Animals were then euthanized, and the tumor, liver, spleen, lungs, heart, and kidneys were scanned *ex vivo* to assess systemic uptake following MNP leakage.

III. Results and discussion

A linear calibration curve was established (y= 207.46x, R^2=0.967), enabling accurate conversion of MPI signal to iron mass. All animals exhibited strong MPI signal in the tumor at 15 min post-injection (Figure 1). A gradual decline in signal was observed over 8 days in all mice, consistent with progressive clearance or redistribution of the injected MNPs. Mean iron retention in tumors decreased steadily, with a 1.8 to 3.0-fold signal reduction between 15 minutes and day 8. Immediate liver uptake was detected in one mouse but not in the other three animals as a result of a differential tumor make-up (vascularization) and/or imperfect injection hitting a vessel for that particular tumor. *Ex vivo* MPI revealed liver and spleen uptake in 2 out of 4 mice, indicating markedly different leakage profiles between animals. In the remaining mice, no detectable MPI signal was observed in these organs, and the limited sample size prevented a robust quantitative comparison of absolute organ iron content between animals. No MPI signal was observed in any of the other organs, indicating systemic clearance only by the liver and spleen.

IV. Conclusion

This study establishes a fiducial-calibrated MPI platform for quantitative, longitudinal assessment of intratumoral MNP retention and leakage. Unlike conventional imaging modalities, MPI enables noninvasive, repeated measurements of iron mass *in vivo* with high sensitivity without background signal. Our results demonstrate that MNPs are partially retained within the tumor microenvironment over 8 days but exhibit a high inter-individual variability in leakage and uptake by the liver and spleen. These findings emphasize the importance of integrating MPI for precision-guided MFH through control of spatial confinement and dosing of heating [4-6].

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Author's statement

Conflict of interest: J.W.M.B is a shareholder of SuperBranche. This arrangement has been reviewed and approved by the Johns Hopkins University in accordance with its conflict of interest policies.

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