QUANTITATIVE BIODISTRIBUTION IMAGING OF GD-LABELED NANOPARTICLES WITH PRECLINICAL MRI

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Abstract

Magnetic resonance imaging (MRI) is a powerful tool for studying nanoparticle biodistribution *in vivo.* However, conventional approaches usually rely on qualitative assessment from T1-, T2-, or T2*-weighted images. These methods provide only indirect estimates of nanoparticle concentration and are sensitive to motion artifacts. We present a new quantitative MRI methodology for evaluating biodistribution of Gadolinium-labeled nanoparticles in small animals. The method is based on T1 mapping and designed to be robust against respiratory and potentially also cardiac motion, ensuring reliable longitudinal measurements of nanoparticle concentrations.

The approach was tested in six tumor-bearing mice (Balb/c, 4T1 tumor), each imaged at five fixed time points within 48 hours after intravenous administration of Gadolinium-labeled lipid nanoparticles carrying ellipticine, or, as a reference, the standard contrast agent Gadovist. Our results demonstrate reproducible T1 quantification in the tumor, kidney, liver, and spleen, enabling direct and quantitative analysis of nanoparticle accumulation and clearance over time.

This methodology represents a robust, noninvasive strategy for assessing nanoparticle biodistribution and may facilitate the development of novel nanomedicine therapies.

Keywords: MRI, nanoparticles, elipticine, biodistribution, cancer

1. INTRODUCTION

Nanoparticles have emerged as promising vehicles for targeted drug delivery and imaging in oncology and other biomedical applications. Their ability to accumulate in tumors through passive or active targeting mechanisms makes them attractive candidates for improving therapeutic efficacy while minimizing systemic toxicity. However, successful translation of nanoparticle-based therapies depends critically on reliable methods for assessing their biodistribution *in vivo.*

Magnetic resonance imaging (MRI) is a widely used technique for noninvasive evaluation of tissue morphology, physiology, and molecular processes. Conventional MRI approaches for monitoring nanoparticles typically rely on qualitative T1-, T2-, or T2*-weighted images [1-3]. While these methods can provide indirect information about nanoparticle localization, they are limited by sensitivity to motion artifacts and lack the ability to directly quantify nanoparticle concentrations. This restricts their utility in longitudinal studies and complicates the interpretation of nanoparticle pharmacokinetics.

Quantitative MRI techniques, such as T1 mapping, offer an attractive solution by enabling absolute measurement of relaxation times that can be directly related to the concentration of paramagnetic agents [4-7]. Importantly, when designed to account for respiratory and cardiac motion [8,9], T1 mapping can provide robust and reproducible results in small animal imaging, where motion artifacts are a common challenge.

In this study, we present an MRI methodology for evaluating the biodistribution of Gadolinium-labeled nanoparticles in tumor-bearing mice. Our approach is based on T1 mapping and is optimized for robustness against physiological motion [6], ensuring reliable and reproducible quantification over time. Using this method, we examined the distribution and clearance of Gadolinium-labeled lipid nanoparticles carrying ellipticine, as well as the reference contrast agent Gadovist (Bayer Vital GmbH Germany), across multiple organs relevant to nanomedicine evaluation.

This work demonstrates the potential of quantitative MRI as a powerful tool for preclinical assessment of nanoparticles biodistribution. By providing noninvasive and longitudinal measurements of nanoparticles accumulation, this methodology may facilitate the development and optimization of novel nanomedicine-based therapies.

2. EXPERIMENTAL PART

2.1 Materials and methods

Six female Balb/c mice (Charles River, Germany) bearing subcutaneous 4T1 tumors were used in this study. Tumors were established by injection of 4T1 mammary carcinoma cells into the flank, and animals were imaged after 4 days. All procedures were performed in accordance with institutional guidelines for animal welfare and approved by the ethics committee.

All imaging was performed on a 9.4 T Bruker BioSpec 94/30 preclinical scanner (Bruker BioSpin, Ettlingen, Germany) equipped with a transmit volume coil and a 4-channel array receive-only coil. For T1 quantification, we developed an inversion recovery Look-Locker radial imaging sequence, which has been demonstrated to provide robust mapping with reduced sensitivity to respiratory and cardiac motion [9].

Prior to *in vivo* imaging, the longitudinal relaxivity (*r1*) of both the Gadolinium-labeled nanoparticles and Gadovist was determined *in vitro* using phantoms containing known concentrations of each contrast agent. These estimated contrast-agent (CA) relaxivities were used later to convert measured *in vivo* T1 values into absolute concentrations of Gadolinium in the tissue.

Each mouse was scanned prior to CA administration to obtain baseline T1 maps, and subsequently at four fixed time points after intravenous injection of either Gadolinium-labeled lipid nanoparticles carrying ellipticine or the standard Gadolinium-based contrast agent Gadovist. A total of five T1 mapping datasets per mouse were acquired over the course of the experiment. The timepoints were 0.5, 8, 24 and 48 hours after administration.

MR-image reconstruction was done using retrospective gating [9] and BART image reconstruction toolbox [10]. T1 maps were calculated for the reconstructed MR images by fitting the complex-domain T1 relaxation model [9]. The resulting T1 values were converted to Gadolinium concentrations using the relaxivity values obtained from the calibration experiments. Regions of interest (ROIs) were manually drawn in the tumor, kidney, muscle, liver, and spleen for each time point, and average concentrations were calculated.

2.2 RESULTS

T1 mapping provided consistent quantification of contrast agent distribution across all animals and time points. The results in selected tissues are shown in **Figure 1**. The reference contrast agent Gadovist did not show any noticeable accumulation in the tumor, liver, or spleen. As expected for a small-molecule Gadolinium chelate, its clearance occurred primarily through the kidneys, with elevated concentrations observed shortly after injection followed by rapid washout.

In contrast, the Gadolinium-labeled lipid nanoparticles exhibited a distinct biodistribution profile. A pronounced retention was observed in the liver, consistent with hepatic uptake of lipid-based nanoparticles by the reticuloendothelial system. Importantly, measurable accumulation of the nanoparticles was also detected in the tumors, supporting their potential for tumor-targeted drug delivery. Spleen uptake was modest but detectable, whereas renal clearance was almost identical to Gadovist.

Overall, these findings confirm that the developed quantitative MRI methodology can differentiate between the pharmacokinetics of conventional contrast agents and nanoparticle formulations. The tumor retention observed for the tested nanoparticles highlights their promise as a platform for therapeutic delivery.

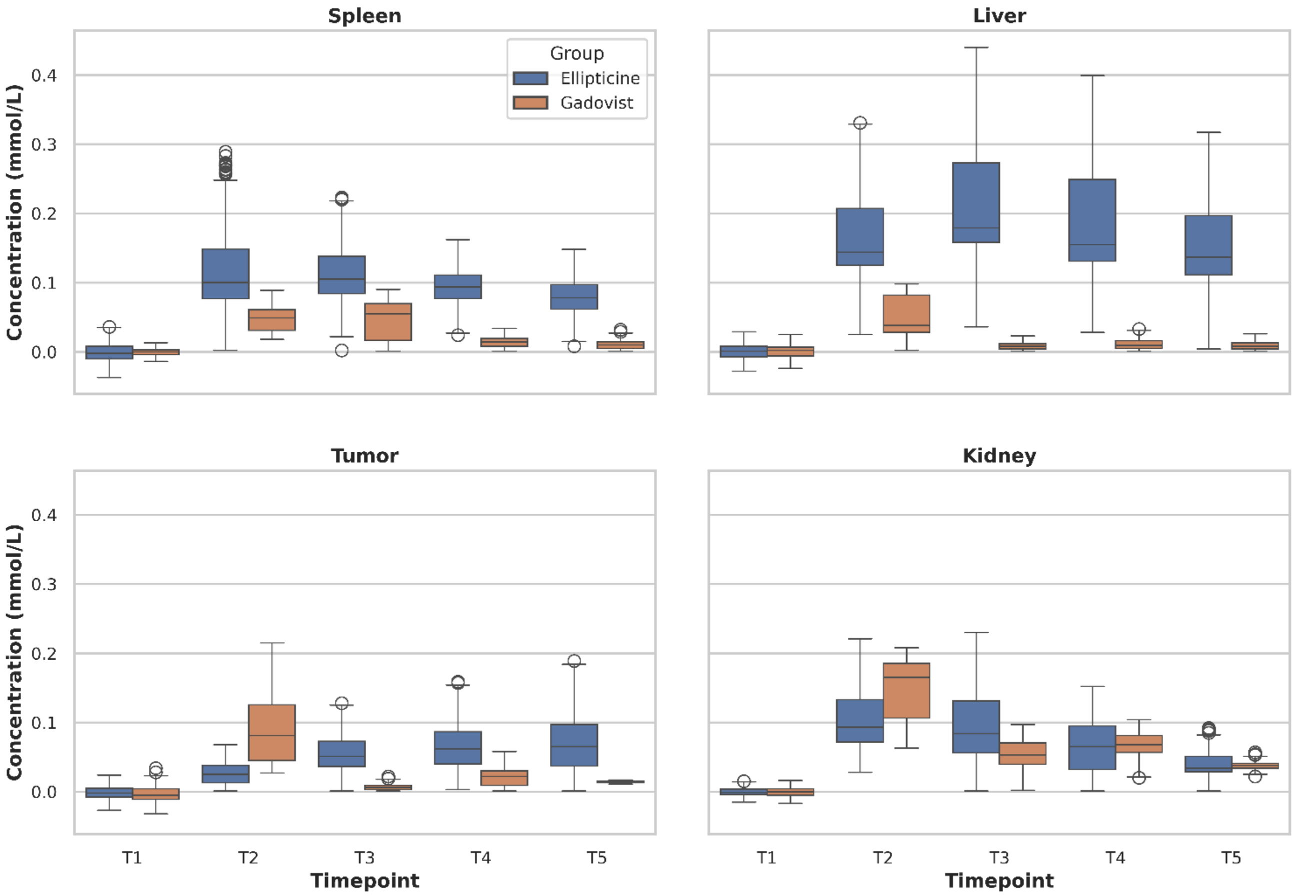

Figure 1 Experiment results in all 5 time points (0, 0.5, 8, 24 h). The boxplots show median value, 1st and 3rd quartiles and minimum and maximum excluding outliers which are visualized as circles.

3. CONCLUSION

We developed and applied a quantitative MRI methodology based on T1 mapping to assess the biodistribution of Gadolinium-labeled lipid nanoparticles *in vivo*. Unlike the conventional contrast agent Gadovist, which exhibited rapid renal clearance without significant retention in tumor or liver tissue, the tested nanoparticles demonstrated measurable accumulation in tumors and retention in the liver. These findings are consistent with the expected uptake of lipid-based nanoparticles by the reticuloendothelial system and highlight their potential for tumor-targeted drug delivery.

Our results underscore the value of quantitative MRI for noninvasive, longitudinal evaluation of nanoparticle pharmacokinetics. By enabling direct and reliable measurement of nanoparticle concentrations in different organs, this approach provides a powerful tool for preclinical development and optimization of nanomedicine formulations.

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