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Imaging and modification of the tumor vascular barrier for improvement in magnetic nanoparticle uptake and hyperthermia treatment efficacy

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Abstract

The predicted success of nanoparticle based cancer therapy is due in part to the presence of the inherent leakiness of the tumor vascular barrier, the so called enhanced permeability and retention (EPR) effect. Although the EPR effect is present in varying degrees in many tumors, it has not resulted in the consistent level of nanoparticle-tumor uptake enhancement that was initially predicted. Magnetic/iron oxide nanoparticles (mNPs) have many positive qualities, including their inert/nontoxic nature, the ability to be produced in various sizes, the ability to be activated by a deeply penetrating and nontoxic magnetic field resulting in cell-specific cytotoxic heating, and the ability to be successfully coated with a wide variety of functional coatings. However, at this time, the delivery of adequate numbers of nanoparticles to the tumor site via systemic administration remains challenging. Ionizing radiation, cisplatin chemotherapy, external static magnetic fields and vascular disrupting agents are being used to modify the tumor environment/vasculature barrier to improve mNP uptake in tumors and subsequently tumor treatment. Preliminary studies suggest use of these modalities, individually, can result in mNP uptake improvements in the 3–10 fold range. Ongoing studies show promise of even greater tumor uptake enhancement when these methods are combined. The level and location of mNP/Fe in blood and normal/tumor tissue is assessed via histopathological methods (confocal, light and electron microscopy, histochemical iron staining, fluorescent labeling, TEM) and ICP-MS. In order to accurately plan and assess mNP-based therapies in clinical patients, a noninvasive and quantitative imaging technique for the assessment of mNP uptake and biodistribution will be necessary. To address this issue, we examined the use of computed tomography (CT), magnetic resonance imaging (MRI), and Sweep Imaging With Fourier Transformation (SWIFT), an MRI technique which provides a positive iron contrast enhancement and a reduced signal to noise ratio, for effective observation and quantification of Fe/mNP concentrations in the clinical setting.

Keywords

Magnetic nanoparticle; Imaging; Tumor vascular modification; EPR effect

1) INTRODUCTION

When appropriately activated, magnetic nanoparticles (mNPs) have great potential for delivering focused and effective cell killing without incurring the complications of conventional hyperthermia-based treatment methods. To achieve such results, mNPs must have significant heating potential and be delivered to the tumor/tumor cells with a high level of specificity. The mNP used in the studies described here have ferromagnetic Fe_3O_4 cores with a biocompatible hydroxyethyl starch base coating (Micromod Partikel technologie GmbH, 18119 Rostock-Warnemuende, GERMANY). mNPs heat primarily via hysteresis when exposed to an alternating magnetic field. Although a variety of magnetic frequency and field strengths combinations can be effectively used in mNP hyperthermia, it is critical to match the mNP composition and AMF with the desired tissue/tumor result with the safety parameters (safety concerns related to eddy current heating). In the majority of the studies described here, an AMF of 165 kHz @ 400–600 Oe was used.

Our animal study endpoints, consisting of tumor regrowth delay (rodent models) and tumor control (spontaneous canine tumors) have proven effective in demonstrating the potential of mNP hyperthermia alone and in combination with other cancer treatment modalities such as radiation and chemotherapy^{1–4}. Although results have been very promising, significant therapeutic gains from mNP heating alone have so far only been observed via intratumoral mNP delivery and subsequent global tumor heating. The issue faced by systemically-delivered mNP, as with all systemic cancer therapies, is the difficulty of getting enough agent to tumor tissue without normal tissue complications. Although the enhanced permeability and retention (EPR) effect is present in varying degrees in many tumors, it is inconsistent and variable. Therefore, strategies which modify the tumor environment and improve the tumor uptake of systemically-delivered mNP are necessary in order to achieve therapeutically-relevant levels of mNP within targeted tissue. These strategies include radiation, chemotherapy, static magnetic fields as well as the use of vascular disrupting agents.

Another unmet need for the success of mNP-based therapies is the current lack of a readily available, clinically approved, noninvasive, and quantitative imaging technique. Although reliable techniques have been employed in the preclinical settings, the development of acceptable clinical imaging techniques will be necessary. Ferromagnetic, unlike superparamagnetic iron oxide nanoparticles, which are clinically approved as MRI contrast agents, incur severe image distortion due to ultra-rapid local field dephasing. This phenomenon has necessitated the use of alternative techniques for both low and high concentrations of mNP, including modifications to current MRI imaging protocols for iron oxide nanoparticles as well as the use of specific CT techniques which appear useful at high mNP concentrations. Both of these techniques will be discussed, including initial studies and limitations of each imaging modality.

2) TUMOR MODIFICATION AND VASCULAR FOR NANOPARTICLE UPTAKE IN TUMORS

2.1 Improvement of tumor permeability

While the poorly-formed vasculature of a tumor may result in enhanced permeability, other associated characteristics such as high proliferation and lack of appropriate drainage tend to inhibit nanoparticle penetration. Though this problem extends beyond nanoparticles to any small molecule imaging agent and therapy, subject to size and surface considerations, larger nanoparticles in particular suffer from poor diffusion into tumor tissues from the blood compartment. Heterogeneity both intratumorally and among tumor types exacerbates this problem, creating the need for the co-administration of a tumor modification or vascular disruption treatment to increase nanoparticle deposition intratumorally.

2.2 Vascular Normalization versus Vascular Disruption

Vascular normalization has emerged recently as a method to increase tumor penetration for small molecules. This technique relies on vascular regulatory therapeutics which slow tumor vasculature growth and “prune” irregular vasculature to obtain a semi-normal network for enhanced small molecule access. Normalizing vasculature, however, can also decrease pore size and prohibit nanoparticle penetration. Vascular disrupting agents, which temporarily increase vascular permeability in order to allow for better therapeutic penetration, may be more favorable for nanoparticle deposition however rely on tumor specificity in order to be effective.

2.3 Radiation

The use of radiation to modify tumor environment for increased permeability has been previously documented; fractionated radiation is a standard of care therapy though radiation is not widely clinically-used for this purpose. Radiation is thought to increase tumor permeability for a variety of reasons, including decreasing tumor bulk, decreasing interstitial pressure and, when fractionated, potentially also acting as a vascular normalizer. A recently published study demonstrated radiation enhancement of intravenously-delivered iron oxide nanoparticle deposition intratumorally. The study assessed the effects of radiation dose on bilateral mouse flank tumors, with one tumor serving as the radiation control as both tumors receive equal nanoparticle dose. Results showed that, for both PEG-ylated and starch-coated nanoparticles of various sizes, radiation resulted in 2–4 fold increase in nanoparticle uptake over systemic administration only.⁵

2.4 Chemotherapy

Chemotherapy, another standard of care cancer treatment, has also been shown to decrease interstitial pressure in solid tumors.⁸ This phenomenon may be the result of tumor debulking as is hypothesized with radiation, or orthogonal mechanisms. Cisplatin pre-treatment has been shown to increase iron oxide nanoparticle deposition intratumorally in a mouse model.⁶ Further studies include dose modulation, timing and chemotherapy selection in order to understand how mNP delivery can be effectively integrated with conventional chemotherapy.

2.5 Static fields

External static magnetic fields have also been successfully utilized to concentrate systemically-administered mNP in specific tissue locations. Since mNP respond to magnetic fields, a static field placed at the site of interest captures and holds circulating mNP in the local vasculature. In a study published separately, a surface-based, bilateral, noninvasive static magnetic field was utilized with a rabbit ear model to demonstrate this effect.⁹ Systemically-delivered mNP localized at the site of the magnetic field with a shape consistent with local field geometry. The study demonstrated a 10-fold increase in the local tissue accumulation of mNPs over neighboring tissue. A similar study (unpublished) conducted with C3H mice implanted with MTGB bilateral flank tumors (one tumor serving as control, the other with an applied static field) showed a multifold increase in mNP deposition in the static field tumor over the control tumor. Superparamagnetic core iron oxide (SPIO) particles of comparable size and coating were also tested in this model, displaying no increase in accumulation. This is hypothesized to be due to SPIO nanoparticles losing their induced field when the static external field is removed. The ferromagnetic mNP, by contrast, magnetically aggregate upon accumulation in the vasculature and remain this way after the static field is removed. Although further study is necessary, it is hoped enhancement of the EPR effect in combination with a static magnetic field would significantly improve mNP uptake in the tumor parenchyma.

External static magnetic fields may be a promising technique for local concentration, however various concerns must be addressed before they can be successfully implemented. Foremost is the challenge of designing a static magnetic field with enough geometric specificity and strength to manipulate mNP in deep tissue. External static fields may find the most utility in surface indications, where the external magnet can be placed directly on the tissue of interest. Another consideration is the localization and aggregation of mNP in the vascular compartment, where histology has demonstrated a lack of mNP diffusion intratumorally even after static field removal. This configuration of mNP may not be favorable for sublethal energy deposition intratumorally, however is inherently advantageous for the thermal ablation of tumor vasculature since the mNP appear to coat the vessels and are grouped in large, heat-able aggregates. Further research is necessary to explore specific applications of static field mNP concentration.

2.6 Molecular methods

Vascular disrupting agents (VDAs) have been established clinically for uses such as blood brain barrier penetration, however tumor-specific VDA therapies have faced challenges in efficacy and non-specific side effects.¹⁰ Accurate targeting of tumor vasculature and sparing of normal vasculature is contingent on exploiting a variety of differences between the two morphologies. Tumor-specific VDAs have concentrated in two classes to date: tubulin-depolymerizing and flavonoid, depending on the mechanism of action. Prominent tubulin-depolymerizing VDAs include CAP4 and AVE8062, which are both in Phase 3 clinical trials. ASA404, a novel flavonoid VDA, has shown promise in non-small cell lung cancer indications. Small molecule VDAs have been used most commonly in conjuncture with chemotherapy, although benefits to using them as an adjunct to radiation have also been explored.¹⁰ A novel vascular disrupting peptide, iRGD, has been shown to increase

penetrability of both tumor vasculature and tissue compartments through a two-step process.⁷ iRGD peptide has been shown to increase tumor accumulation of small molecule, antibody and nanoparticle therapeutic formulations.⁷ Most interestingly, given the hypothesized method of disruption, iRGD appears to function both in a conjugated and a co-administered formulation.⁷ Ongoing studies are being designed to determine if iRGD can effectively increase MNP deposition intratumorally in a co-administration setting.

3) MAGNETIC NANOPARTICLE IMAGING

Proven techniques such as confocal, light and electron microscopy, histochemical iron staining, ICP-MS, fluorescently-labeled mNPs and magnetic spectroscopy of Brownian motion (MSB), are being used to assess and quantify mNP in *in vitro* and in *ex vivo* tissues. However, a proven noninvasive mNP imaging technique which is successful in the clinical setting has not yet been developed. As illustrated in the images below, standard clinical imaging techniques (CT/MRI) are not effective methods of mNP quantification at clinically-relevant doses. In this study we examined the use of computed tomography (CT) and magnetic resonance imaging (MRI) for effectively observing and quantifying Fe/mNP concentrations in the clinical setting. Our findings suggest that both CT and MRI, specifically ultra-short T2 MRI methods such as Sweep Imaging With Fourier Transformation (SWIFT), which provides a positive iron contrast enhancement and a reduced signal to noise ratio, may be useful, however significant optimization research and technology development remains to be done.

3.1 Measurement of mNP concentration with CT

Our studies suggest that a mNP Fe concentration of approximately 3 mg Fe/gram of tissue is necessary to achieve clinically relevant thermotherapy. When used as a part of an adjuvant treatment strategy, in conjunction with radiation and/or chemotherapy, this threshold concentration for therapeutic benefit is likely to be significantly lower. Below is a graph illustrating CT (x-ray) data of mNP standards over a range of 0 to 25 mg Fe/mL. The same mNP samples were imaged on six different occasions to show repeatability and linearity of the data in the 1–25 mg Fe/mL.

3.2 CT Mouse models

The ability of CT to image mNPs *in vivo* was demonstrated using both intratumoral and intravenous injection techniques.

1. Intratumoral injection procedure: MTGB mouse mammary adenocarcinoma tumors were grown in the right flank of 8 week old female C3H mouse (Charles River Laboratories, Wilmington, MA 01887 USA). The tumor was imaged approximately 3 weeks post implantation (~150 mm³) using a clinical GE LightSpeed CT scanner. Following a pre-injection image of the tumor, the mouse was injected with mNP at 5mg Fe/g tumor (28 μ l of mNP total). Figure 3 demonstrates clear enhancement of the post-injected tumor, visible on the lower right flank of the mouse.
2. Intravenous injection procedure: A female, non-tumor bearing NU/NU mouse (Jackson Laboratories, Bar Harbor, ME 04609 USA) was injected intravenously

with 8.2 mg Fe (12 mg mNP) per 20 g mouse and imaged in the CT scanner mentioned above. Three sagittal plane images of the same mouse, shown in Figure 4, demonstrate the changes in Fe concentration in various organ compartments at 10 minutes and 24 hours post-injection. 10 minutes post-injection, high Fe concentrations are observed in the heart and liver. At 24 hours post-injection, the majority of the iron is observed in the spleen and liver. These findings are consistent with our previous distribution studies which utilized ICP-MS quantification of Fe.¹¹

3.3 MRI imaging of mNP

Both CT and MRI imaging techniques have been used *in vitro* to quantify mNP concentrations. Typically, standard MR imaging techniques are unable to visualize high mNP concentrations, while CT is unable to quantify low mNP concentrations. The primary issues associated with imaging mNP with GRE sequences were summarized by Zhou et al. as being: 1) The signal void created by the mNP is larger than the cells containing them, which in bulk tissues is manifested by large “holes” in the image, 2) Signal voids are difficult to distinguish from other sources of T2* shortening, such as the tissue/air boundaries. This results in a negative contrast, with both poor signal-to-noise ratios and specificity.¹² The Sweep Imaging With Fourier Transformation MR method (SWIFT) may be better suited for the imaging of mNP at clinically-relevant concentrations. With SWIFT, the time between excitation and signal acquisition is very short and mNP appear as positive contrast, unlike the negative contrast found with gradient-echo or spin-echo, making distinguishing mNP from other tissues or the air-tissue boundary easier^{12–14}.

An MTGB mouse mammary adenocarcinoma was grown in the flank of female C3H mouse (Charles River Laboratories, Wilmington, MA 01887 USA). Two weeks post implantation (~80 mm³), the tumor was injected with mNP 0.5 mg Fe (18.8 µl of mNP total). Thirty minutes after injection, three post-injection images were taken, two of equal resolution (10 min each) but different bandwidths and a third high-resolution reference image (30 min acquisition). The imaging parameters pre-injection were: fa=7 degrees, sw=62.5 kHz, FOV=14 cm³, 2563 pixel resolution, TR=5 ms, number of views per spiral = 4096, number of spirals= 32. SWIFT’s “TE” is actually just a dead time between the end of the RF pulse and the beginning of ADC to let the electronics settle, for this study that delay was 4.5 µs. The acquisition was 3D radial with gridding reconstruction to perform the non-uniform Fourier transform. The higher-bandwidth post-image was taken with sw=96 kHz.

The presence of mNP is clearly indicated by bright signal pile-ups as shown in the image below. The mNPs were shown to cause an image artifact rather than total signal loss. Higher bandwidth acquisition was shown to have a decrease in the artifact size with an increase in acquisition bandwidth. The technique has been demonstrated to retain signal where it would have been lost in other imaging methods. However, a dipole artifact was not expected based on earlier phantom studies, and the current size of the dipole artifact prevents high-resolution localization of the nanoparticles. It remains to be determined how to use the signal to better localize and quantify these high concentrations of mNP post-injection.¹⁵

4) DISCUSSION

In this manuscript we have shown the biologic variability and relationship that exists between mNP uptake into tumors and imaging with conventional radiologic imaging modalities (CT, MRI/SWIFT). Virtually all mNP formulations will require a threshold level to be effective in cancer therapy, however recent efforts in this field are showing that achieving this threshold is extremely challenging and that in general investigators will need to improve tumor mNP levels by many fold to be effective. Additionally, it is becoming not only desirable but necessary, from a clinical standpoint, that a noninvasive readily-available imaging modality be capable of observing and quantifying mNP in the tumor and in toxicity-sensitive normal tissues.

The studies and information presented here demonstrate the ability of two conventional cancer treatment modalities (radiation and chemotherapy) and readily available compounds and techniques (VDAs and static magnetic fields) to significantly improve mNP tumor uptake in rodent tumors. Although a significant generalization, it appears a 10 fold increase in tumor mNP levels following systemic administration will be required for therapeutic efficacy in most currently available mNP situations. This includes antibody targeting of mNP to the tumor location, however does not include additional modification of mNPs with drug loading or orthogonal methods of inducing cytotoxicity. All of this said, it is now very clear that the inherent tumor-mNP uptake potential following systemic administration is highly variable and heterogeneous for individual tumor types and organ sites. While studies to address these discrepancies therapeutically are ongoing and require significant optimization efforts, it is clear that therapeutic success with mNPs will very likely require the use multiple techniques to reach the required mNP threshold levels.

Our preliminary work on mNP imaging in tissues is demonstrating greater potential than has been previously predicted. CT and various MRI techniques all hold excellent mNP imaging potential, with CT more effective at higher mNP concentrations and MRI at lower concentrations. It is however also clear that success with any of these modalities will only be achieved through detailed and highly specific scanning parameters and experimentation. In addition, ultrasound and fluorescent imaging may also hold promise but have additional clinical limitations regarding resolution at significant tissue depth. While mNP technology has demonstrated promise as a cancer adjuvant to many conventional therapies, increasing systemically-delivered intratumoral concentrations as well as accurately imaging and quantifying tissue dose remain crucial to clinical success.

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Figure 1.

The CT scan (left) demonstrates a 3 cm³ tumor in maxilla (hard palate) of a 10 year old, 12 kg female schnauzer. The tumor extends from the ventral aspect of the nasal cavity through the dorsal hard palate. Following CT imaging, the tumor was impregnated with mNPs, and exposed to AMF for hyperthermia-based treatment. The 3 cm³ tumor received approximately 16 mg mNP (10 mg Fe) and was exposed to 165 kHz @ 400 Oe for 60 min. This MRI scan (right), taken 3 weeks after the CT, of the cross section of a canine muzzle demonstrates a significant void (arrow) in the region of the mNP impregnated maxillary tumor (see previous CT scan). Images were acquired using a 3T Philips MRI (Philips Electronics North America Corporation (Andover, MA 01810 USA)), T1 FFE/GR sequence with the following acquisition parameters: repetition time TR = 550 ms, echo time TE = 2 ms, field-of-view FOV = 200 mm, acquisition matrix = 512 x 512, 54 slices, slice thickness = 2 mm.¹⁴

Figure 2.

This CT (x-ray) data of mNP standards over a range of 0 to 25 mg Fe/mL shows imaging repeatability and linearity in the 1–25 mg Fe/mL range. Linearity of the data remained at concentrations which are in the clinical range and lower than previously thought achievable.

Figure 3.

These two sagittal CT scans of the same mouse demonstrate positive mNP enhancement of a flank tumor (lower right aspect of right image). The image on the left was taken before mNP tumor injection (5 mg Fe/g tumor tissue).

Figure 4.

Three sagittal plane images of the same mouse demonstrating the increase Fe concentration in the spleen and liver following systemic mNP delivery (0.27 mg Fe per g body weight, total of 8.2 mg Fe).

Figure 5. Left: pre-injection SWIFT MRI image (magnitude) of MTGB flank tumor bearing C3H mouse. Right: post-injection SWIFT MRI image (magnitude) of MTGB flank tumor bearing C3H mouse with 0.5mg of injected Fe. The pre-injection tumors have a homogenous appearance. Post-injection mNP are located by the creation of signal pile-ups.

Table 1

Summary of tumor modification strategies

Modification	Type	Increase in nanoparticle intratumoral deposition
Radiation	15Gy single dose, 3 days before IV mNP ⁵	3 fold, 24 hours post-injection
Chemotherapy	5 mg Cisplatin/kg body mass 6 days before IV mNP ⁶	3 fold, 24 hours post-injection
Vascular Disrupting Agents	iRGD coadministered IV with Nab-paclitaxel ⁷	10 fold, 3 hours post-injection
Static Magnetic Fields	480 T/m maximum field gradient, 1 mg mNP Fe/g body mass [*]	10 fold, 1 hour post-injection

* Data not published