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Preclinical Dosimetry of Magnetic Fluid Hyperthermia for Bladder Cancer

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Abstract

Background—Despite positive efficacy, thermotherapy is not widely used in clinical oncology. Difficulties associated with field penetration and controlling power deposition patterns in heterogeneous tissue have limited its use for heating deep in the body. Heat generation using iron-oxide super-paramagnetic nanoparticles excited with magnetic fields has been demonstrated to overcome some of these limitations. The objective of this preclinical study is to investigate the feasibility of treating bladder cancer with magnetic fluid hyperthermia (MFH) by analyzing the thermal dosimetry of nanoparticle heating in a rat bladder model.

Methods—The bladders of 25 female rats were injected with 0.4 ml of Actium Biosystems magnetite-based nanoparticles (Actium Biosystems, Boulder CO) via catheters inserted in the urethra. To assess the distribution of nanoparticles in the rat after injection we used the 7 T small animal MRI system (Bruker ClinScan, Bruker BioSpin MRI GmbH, Ettlingen, Germany). Heat treatments were performed with a small animal magnetic field applicator (Actium Biosystems, Boulder CO) with a goal of raising bladder temperature to 42°C in <10min and maintaining for 60min. Temperatures were measured throughout the rat with seven fiberoptic temperature probes (OpSens Technologies, Quebec Canada) to characterize our ability to localize heat within the bladder target.

Results—The MRI study confirms the effectiveness of the catheterization procedure to homogeneously distribute nanoparticles throughout the bladder. Thermal dosimetry data demonstrate our ability to controllably raise temperature of rat bladder 1°C/min to a steady-state of 42°C.

Conclusion—Our data demonstrate that a MFH system provides well-localized heating of rat bladder with effective control of temperature in the bladder and minimal heating of surrounding tissues.

Keywords

Bladder cancer; Hyperthermia; iron oxide; magnetite; nanoparticle heating; thermal dosimetry

1. INTRODUCTION

Bladder cancer is the fourth most common type of cancer in men and the ninth most common in women in the United States.¹ More than 50,000 men and 16,000 women are diagnosed with bladder cancer each year and more than 14,000 die annually of this disease. The most common type of bladder cancer, urothelial carcinoma, originates in cells lining the inside of the bladder wall and accounts for approximately 90% of all bladder cancers. There are two main clinical phenotypes of bladder cancer, non-muscle invasive (NMIBC) and muscle invasive (MIBC), and they differ in their biology, prognosis, and management².

For NMIBC, transurethral resection of all bladder tumors (TURBT) is the initial treatment approach and is associated with a high local recurrence rate of 40–80%. An important goal for NMIBC management is to develop treatments that reduce the risk of recurrences, which often occur repeatedly over many years. A number of therapeutic options have been explored to reduce local recurrences including intravesical chemotherapy with mitomycin-C (MMC). Despite MMC significantly reducing the NMIBC recurrence rate, many patients still experience it and methods of improving MMC efficacy such as concurrent hyperthermia have been investigated³.

Several approaches have been proposed for heating the bladder either with radiowaves, microwaves, or magnetic nanoparticle solutions combined with RF magnetic fields to enhance the effectiveness of a chemotherapeutic agent filling the bladder lumen. The largest reported clinical experience to date is with the Synergo HT intravesical microwave applicator. The technique is based on a miniature antenna inserted through a catheter into the bladder. Although clinically effective, some adverse effects such as pelvic pain and bladder wall injury have been reported^{3–6}. Other investigators have used radiofrequency capacitive electrodes^{7, 8} or radiofrequency phased array applicators⁹ to focus heat into the bladder filled with Mitomycin-C. Recent preclinical investigations have considered heating the bladder with water-cooled external microwave applicators^{10–12}, though generally this will be limited to animals or very thin patients since effective penetration depth of microwave energy is limited.

The most recent approach to be considered is Magnetic Fluid Hyperthermia (MFH) which heats the bladder by coupling energy from an external alternating magnetic field into a magnetic nanoparticle solution injected inside the bladder. As the heat source is restricted to the volume inside the bladder, the MFH approach is proposed to reduce hotspots associated with other local heating techniques. Since heating by thermal conduction falls off very rapidly, effective heating does not penetrate far into the bladder wall. Since this procedure is generally used after surgical resection of gross disease, the required depth of treatment for NMIBC is only several mm deep into the bladder wall. Therefore, by combining magnetic nanoparticles with chemotherapeutics like Mitomycin-C, the MFH approach seems well-suited to produce uniform thermochemotherapy of the bladder.

2. MATERIALS AND METHODS

2.1 Animals

Twenty-five female Fisher 344 rats, 8–15 weeks old and weighting on average 161.7 ± 16.8 g, obtained from Charles River labs were used for this study. All experiments and procedures were done with the approval of the Duke University Institutional Animal Care and Use Committee. The rats were anesthetized with ketamine/zylazine (40 mg/4 mg/kg) cocktail based on their body weight. To keep the normal body core temperature throughout the experiment the rats were lying recumbent on a wax-pad at 38°C initial temperature.

2.2 Magnetic Fluid

The magnetic fluid used in this study was iron oxide nanoparticles (with 20nm core diameter and iron oxide concentration of 100 mg/ml) coated with a surfactant and dispersed in water. After successful bladder catheterization with an 18 gauge angio catheter, 0.4 ml of magnetic fluid was injected into the bladder and the catheter was clamped. At the end of 60 min of experiment, no significant leakage of fluid was detected.

2.3 Temperature Measurements

The thermometry was carried out invasively with seven single fiberoptic temperature probes having a diameter of 0.42 mm (OpSens, Québec, Québec, Canada). Temperature probes were inserted into the vagina, rectum and into subcutaneous muscle of the upper thigh through a 18 ga catheter. Two temperature probes were inserted through an adjustable Port Seal (UPS-A, GYRUS Medical Ltd, Southborough, MA) into the bladder catheter and carefully positioned one inside the bladder and one in the urethra (8 mm) proximal to the bladder. For this monitoring configuration, body core temperature was taken as the average of the three probes located in the rectum, mouth and subcutaneous muscle sites. An additional superficial temperature measurement was performed on the skin surface above the bladder.

2.4 Experimental Heating protocol

Heat treatments were carried out using the Actium animal magnetic field applicator system, operating at a frequency of 40 kHz and with variable field strength up to 6 kA/m. The control system provides two operating modes. One mode allows manual setting of desired RF magnetic field strength and the other mode automatically adjusts field amplitude by feedback control from one temperature sensor to keep any desired steady-state temperature (e.g. 42°C) in the bladder. Hyperthermia treatments were performed without any additional cooling system.

2.5 Power deposition with magnetic nanoparticles

The specific loss power (SLP) is defined as the amount of energy converted into heat per time and per mass of magnetic material. To estimate the SLP, measurements of temperature rise as a function of time were carried out. All magnetic fluid samples, 0.4 ml filled in 1.5 ml vial, were placed in the center of the induction coil of the Actium applicator within a 3 cm thick block of styrofoam insulation. The SLP values were calculated as,

$$SLP = c \frac{m_{\text{sample}}}{m_{\text{ironoxide}}} \left(\frac{\Delta T}{\Delta t} \right) \quad (1)$$

where “c” is the specific heat capacity of sample solution (J/g/°C), m_{sample} is the total mass of the sample(g), $m_{\text{ironoxide}}$ is the mass of the iron-oxide in the sample, and $\Delta T/\Delta t$ is the initial 30 s slope of temperature rise versus time curve. As the specific heat capacity of the magnetic nanoparticle solution is unknown, we assumed “c” equal to that of water at 25°C (4.181 J/g/°C).

The SLP values were based on the temperature rise measured in 0.4 ml sample at fixed magnetic field amplitude of 5.5 kAm. The temperature was monitored with a probe placed in the center of the heated vial. In a control experiment, heating of the temperature probe in the same field and experimental configuration without iron oxide sample was found to be negligible.

2.6 MRI measurements

Images were acquired on a 7 T small animal MRI system (Bruker ClinScan, Bruker BioSpin MRI GmbH, Ettlingen, Germany) located in the “Duke Center for In-Vivo Microscopy”. During MR scanning, the mice were kept under 1–2% isoflurane anesthesia. The bladder MR contrast was enhanced with injection of Gd-DTPA (Magnevist; Bayer HealthCare Pharmaceuticals, Wayne, NJ). MR images were acquired using a multi-slice gradient echo (200 frequency encoding steps, 200 phase encoding steps, 86 slices). The echo time is 4.6 ms, and the repetition time is 1.24 s. The flip angle is 90 degrees, while the bandwidth is 50 kHz. The field-of-view is 7 cm in both in-plane directions, the slice thickness is 0.35 mm. Signal from fat is suppressed. Acquisition time is 4 minutes. No gating is used.

For detection of magnetic nanoparticle in the bladder, we performed a three-dimensional, short echo-time, radial-encoding imaging sequence acquired 134526 views from the center of Fourier Space and samples the periphery uniformly. The echo-time is 20 μ s (microsecond) and the repetition time is 8.7 ms. The flip angle is 6 degrees, the field-of-view is 7 cm in all three directions, and the bandwidth is 100 kHz. The signal from fat is suppressed. Data is regridded onto a 256^3 matrix. Acquisition time is 20 minutes. No gating is used.

3. RESULTS AND DISCUSSION

3.1 Power dissipation characteristics of magnetic fluid solution

For clinical hyperthermia applications, the ability to precisely control temperature in and around the target volume is one of the most important factors in therapeutic outcome. Complete knowledge of the heat source characteristics is essential to control heating. In Magnetic Fluid Hyperthermia, the primary considerations are the properties of the magnetic nanoparticle (MNP) and the local environment. Properties such as nanoparticle size, structure, coating, concentration, local viscosity, magnetic field amplitude and frequency play a key role in determining the power deposition into the magnetic fluids. Heating of tissue is further dependent on tissue properties and coupling of the magnetic nanoparticle into the target tissue. It is therefore appropriate to begin with characterizing power absorption directly into a vial of magnetic solution in vitro, and move into investigation of in vivo heating in the desired tissue model (rat bladder).

The heating ability of a magnetic fluid is traditionally evaluated in terms of the specific loss power (SLP) in units of W/kg. For effective treatment planning of MFH, the SLP value of the MNP solution itself is essential. However, since most of the SLP measurements are acquired in non-adiabatic conditions, we only can estimate the values. In the following sections, we characterize the effects of field strength and nanoparticle concentration on SLP of MNP solution.

3.1.1 Dependence of SLP on magnetic field strength—The widely accepted model for power dissipation in a ferrofluid in an alternating magnetic field was described by Rosensweig¹³. However, the Rosensweig description has a limited range of validation and some magnetic fluids violate these conditions. Therefore, the aim of this procedure was to verify whether the SLP dependence with the field amplitude follows the predicted square law relationship.

Figure 2 shows the estimated SLP values as a function of magnetic field amplitude for a fixed nanoparticle volume (0.4 ml) and fixed field frequency (40 kHz). As can be seen in Figure 2, the SLP shows dependence with magnetic field amplitude squared. Based on this result, the square law dependency of SLP indicates that the linear response theory may be

applied for the nanoparticle dispersion used in this study. Thus, the power dissipation may be formulated as:

$$P = \mu_0 \pi \chi'' f H^2 \quad (2)$$

where μ_0 is the permeability of free space, χ'' the AC magnetic susceptibility (imaginary part) to first order independent of H, f the frequency of the applied AC magnetic field, and H the strength of the applied AC magnetic field.

3.1.2 Variation of iron-oxide concentration—To characterize heat generation as a function of different magnetite concentrations, we diluted the original 100mg/ml nanoparticle solution to produce 0.4 ml volumes of nanoparticles in 1 ml vials with concentrations ranging from 10 to 100 mg/ml. This range in iron content allows us to estimate the minimum concentration required to reach the therapeutic temperature in a feasible time with this small 0.4 ml volume. In Figure 3, the temperature rise curves are shown for increasing concentrations of magnetite for a magnetic field strength of 5.5 kA/m.

As expected, the rate of temperature rise increases dramatically with increasing magnetite concentration. The average heating rate for a period of 10 min (or up to 50°C maximum) was calculated to estimate the appropriate range of concentration for in vivo application of this technique. From the analyses of heating rates, we estimate that the minimum concentration required for a 1°C/min heating rate in bladder is close to 50 mg/ml, which in the in vitro 0.4ml vial testing generated 1.6°C/min. The highest concentration (100mg/ml) provided a rate of 7.2 °C/min.

3.2 In vivo feasibility experiments

After the characterization of magnetite nanoparticle heating *in vitro*, we investigated the feasibility of MFH of bladder in the *in vivo* rat model. In this section, we validate our ability to fill the rat bladder with magnetic fluid via MRI image and demonstrate our ability to localize heat in rat bladder *in vivo* using a range of nanoparticle concentrations that extends well beyond the dilution range expected during typical *in vivo* treatments.

3.2.1 Nanoparticle distribution in bladder—The ability to homogeneously deliver magnetic fluid with good contact to the entire bladder wall was monitored with 7 T small animal MRI system (Bruker ClinScan, Bruker BioSpin MRI GmbH, Ettlingen, Germany). A 18 ga catheter as inserted through the urethra into the bladder and a volume of nanoparticle solution injected ranging between 0.2 ml and 0.7 ml. A visual check for leakage was carried out at each injected volume.

Since MRI cannot be used with highly concentrated nanoparticle solutions due to excessive MR signal artifacts in surrounding tissue, a low concentration 0.5 mg/ml magnetite solution was prepared for bladder imaging. We also include a control experiment with standard gadolinium (Gd-DTPA) MR contrast agent. Figure 4a gives a representative MRI image of rat bladder containing 0.25 ml saline/Gd-DTPA and showing complete filling of bladder (arrow) around the insertion catheter. Figure 4b shows the bladder filled with 0.5 ml of magnetic nanoparticle solution.

In Figure 4, the images are shown in different field of view (FOV), because the acquisition was made with distinct pulse sequences for each contrast agent. The Figure 4a image presents large dark areas due to the fat compensation acquisition. The presence of nanoparticles in the bladder is detected in the image Figure 4b. The nanoparticles greatly decrease the local voxel intensity as a consequence of shortening the transverse relaxation times of the protons. The bladder region containing nanoparticles appears gray in the image,

while the bladder filled with saline/Gd is represented by bright intensity. From detailed analyses of these images, it is possible to quantify the total volume in the bladder. The correlation between volume injected and volume measured was good, showing only minor dilution from urine during the time period of a typical heat treatment. Overall Figure 4 demonstrates that the catheterization procedure and injection of nanoparticle solution into the bladder were feasible in the rat model. Using a single injection, we demonstrated a homogeneous spatial distribution of concentrated nanoparticles in the target region (bladder).

3.2.2 Magnetic Field Control—Potential advantages claimed for MFH over other hyperthermia modalities are improved homogeneity of heating of the entire bladder wall and improved control of heat deposition. The control of temperature rise during hyperthermia treatment is a big concern for any heating approach because power deposition patterns are poorly known and difficult to control in heterogeneous *in vivo* tissues, and heating of surrounding healthy tissues is undesirable and difficult to measure. In this section, we investigate the quality and safety of the magnetic field control algorithm included in the Actium Biosystems induction heating system.

During each heat experiment, the magnetic field amplitude was automatically controlled by a feedback control algorithm, which used the temperature measured inside the bladder as control signal. The goal was to maintain the desired treatment temperature (42°C) as constant as possible. Rapid high resolution control is extremely important to deposit the desired thermal dose throughout the bladder target while avoiding damage to healthy tissue that could result from large temperature swings. Figure 5 illustrates the time-dependent magnetic field amplitude during a typical rat bladder treatment performed in constant temperature control mode.

The goal temperature of 42°C was reached in all animals tested with 100mg/ml MNP. For most treatments, the steady-state temperature was maintained with magnetic field amplitudes between 3.5 and 4.5 kA/m. It can be seen that the temperature inside the bladder was easily maintained at 42°C by the control system during the whole period of MFH treatment.

3.2.3 Heating studies

Heating efficiency of magnetite nanoparticle solution: Following catheterization and subsequent injection of MNP into the bladder, rats were placed inside the induction coil and exposed to a 40 kHz alternating magnetic field at up to 6000 A/m for about 70 min. During the initial investigation of nanoparticle heating *in vitro*, it was found that 50 mg/ml was the minimum concentration necessary to achieve the desired heating rate of 1°C/min when using a small 0.4 ml volume in an insulated vial. However, those measurements did not account for the effect of heat losses into *in vivo* bladder wall tissues. Thus additional tests were performed in the *in vivo* rat model to determine the concentration of nanoparticles required to produce a 1°C/min heating rate *in vivo*. For all experiments the rats were positioned on a preheated wax-pad at around 37°C, which implied that a gradient of 5°C would be required to reach 42°C. In an ideal scenario the 42°C needs to be reached in about 5min. Figure 6a presents representative temperature rise curves for the first 20 min of heating with 0.4 ml of magnetite nanoparticles injected into the bladder at 4 concentrations. The data of Figure 6b show the min/max range of temperatures achieved after 15 min of heating as a function of mass of injected nanoparticles, with an average of at least 3 rats for each point. The data clearly demonstrate the ability to heat rapidly and reliably to the desired 42°C setpoint in bladder when using 100 mg/ml magnetite nanoparticles.

Temperature distribution in in vivo rat bladder model: The underlying purpose of this investigation is to determine if MFH can be used to heat rat bladder locally to chemosensitizing temperatures without overheating surrounding normal tissues or raising core body temperature significantly in rats. For this study, five rats were injected with 0.4 ml of magnetic fluid and placed in the coil for 70 min. The control system was placed in temperature mode which provided automatic variation of magnetic field amplitude as necessary to maintain the bladder temperature constant at the 42°C setpoint. Figure 7 shows the average temperature curve (5 separate rats experiments) during the MFH treatments for a single fiberoptic sensor inserted in the Bladder (red line), Vagina (blue line) and multi-site Body core (black line). The standard deviation of the mean temperature is represented by the vertical error bars.

The average power-on time required to reach therapeutic temperature was less than 10 min. During the 60 min therapeutic period, the thermotherapy led to an increase in body temperature of approximately 1.0–1.5°C. The feedback control algorithm kept the temperature variation within the bladder target less than 0.2°C. After the desired 60 min at 42°C, the magnetic field was switched off and the temperature dropped sharply (data not shown). Figure 7 also shows that vaginal temperatures exhibit a wider variability than other sites. That variability of vaginal temperatures can be explained from the uncertainty in probe position for the five animals, since the vagina is not far from the bladder so is most sensitive to exact placement. Regardless, the vaginal probe shows clear separation from the bladder temperature measurements, demonstrating effective localization of heat in the bladder target.

4. CONCLUSION

This work investigates for the first time the feasibility of applying magnetic fluid hyperthermia for heating the bladder. We studied the ability to localize heat in rat bladders with a 40 kHz induction heating system following injection of magnetite based nanoparticles.

The MFH system used in this study includes an effective automatic feedback control algorithm that varies field strength during treatment to maintain constant temperature in the bladder. Bladder temperature could be reliably raised to the desired 42°C goal in 10 min with 100 mg/ml magnetite based nanoparticles. This study demonstrated successful well-localized heating of rat bladder with magnetic fluid hyperthermia with minimal heating of surrounding tissues.

Hergt et al.¹⁴ indicated that the specific heating power needed to achieve a defined temperature elevation increases dramatically with decreasing tumor size. This analysis is particularly relevant for a rat bladder heating system due to the extremely small bladder volume (0.4 ml) which requires a higher concentration of nanoparticles to produce sufficient mass of iron for coupling to the magnetic field. Once the volume of injected nanoparticles is increased to the amount typically used for treating human bladder (>40 ml), the required concentration of magnetite will decrease significantly. Future testing of nanoparticle heating and toxicity will be performed in animal models with bladder volumes closer to the human bladder to enable use of clinically practical nanoparticle concentrations.

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Figure 1.
Magnetic hyperthermia system (Actium Biosystems, Boulder CO).

Figure 2. Specific Loss Power (SLP) of 0.4ml of 100 mg/ml magnetite nanoparticle solution at a fixed frequency of 40 kHz as a function of magnetic field strength. The dashed line represents the best fit line to H^2 -law.

Figure 3. Temperature profiles for five different concentrations of 0.4ml of magnetic fluid at fixed magnetic field frequency (40 kHz) and amplitude (5.5 kA/m).

Figure 4. Pretreatment MRI image showing: (a) rat bladder filled with 0.25 ml saline/gadolinium, and (b) 0.5 ml of magnetic fluid at 0.5mg/ml. In both images, it is possible to visualize the catheter placement in the Bladder (arrows).

Figure 5.

Typical magnetic field amplitude (red line, right axis) applied during MFH (0.4 ml, 100 mg/ml) to control temperature inside the *in vivo* rat bladder (black line, left axis).

Figure 6.

Temperature achieved inside the bladder after 15 min exposure to magnetic field: a) representative heating rate profiles for 4 different rats injected with 0.4 ml of various magnetite concentrations; and b) bladder temperatures achieved after 15 min of heating with 5.5kA/m field. The bars represent the maximum and minimum values obtained in at least 3 rats per point.

Figure 7.

Average temperature obtained in 5 rats during 1 hour MFH treatment. Note excellent localization of heat in the desired bladder target and lower temperatures in surrounding normal tissues like vagina (blue) and other body sites (black). The body temperature represents the average of measurements from subcutaneous, rectum and oral probes which were similarly low compared to the bladder. Nanoparticle concentration was 100 mg/ml.