Published in final edited form as:

Proc SPIE Int Soc Opt Eng. 2007 February 13; 6440: 64400K-. doi:10.1117/12.706302.

Intratumoral Iron Oxide Nanoparticle Hyperthermia and Radiation Cancer Treatment

PJ Hoopes, RR Strawbridge, UJ Gibson, Q Zeng, ZE Pierce, M Savellano, JA Tate, JA Ogden, and I Baker

Dartmouth College Hanover, NH

R Ivkov and AR Foreman

Triton Biosystems Chelmsford, MA

Abstract

The potential synergism and benefit of combined hyperthermia and radiation for cancer treatment is well established, but has yet to be optimized clinically. Specifically, the delivery of heat via external arrays /applicators or interstitial antennas has not demonstrated the spatial precision or specificity necessary to achieve appropriate a highly positive therapeutic ratio. Recently, antibody directed and possibly even non-antibody directed iron oxide nanoparticle hyperthermia has shown significant promise as a tumor treatment modality. Our studies are designed to determine the effects (safety and efficacy) of iron oxide nanoparticle hyperthermia and external beam radiation in a murine breast cancer model.

Methods—MTG-B murine breast cancer cells (1×10^6) were implanted subcutaneous in 7 week-old female C3H/HeJ mice and grown to a treatment size of $150 \text{ mm}^3 \pm 50 \text{ mm}^3$. Tumors were then injected locally with iron oxide nanoparticles and heated via an alternating magnetic field (AMF) generator operated at approximately 160 kHz and 400 - 550 Oe. Tumor growth was monitored daily using standard 3-D caliper measurement technique and formula. specific Mouse tumors were heated using a cooled, 36 mm diameter square copper tube induction coil which provided optimal heating in a 1 cm wide region in the center of the coil. Double dextran coated 80 nm iron oxide nanoparticles (Triton Biosystems) were used in all studies. Intra-tumor, peri-tumor and rectal (core body) temperatures were continually measured throughout the treatment period.

Results—Preliminary in vivo nanoparticle-AMF hyperthermia (167 KHz and 400 or 550 Oe) studies demonstrated dose responsive cytotoxicity which enhanced the effects of external beam radiation. AMF associated eddy currents resulted in nonspecific temperature increases in exposed tissues which did not contain nanoparticles, however these effects were minor and not injurious to the mice. These studies also suggest that iron oxide nanoparticle hyperthermia is more effective than nonnanoparticle tumor heating techniques when similar thermal doses are applied. Initial electron and light microscopy studies of iron oxide nanoparticle and AMF exposed tumor cells show a rapid uptake of particles and acute cytotoxicity following AMF exposure.

BACKGROUND

For a number of well-published and generally accepted reasons, hyperthermia is an excellent adjunct to radiation in cancer treatment. However, clinical hyperthermia has not met with the type of success that experimental studies and many knowledgeable and experienced

scientists believe possible. While the general complication of tissue over-heating are reasonably well understood, the effectiveness and safety of specific thermal doses (therapeutic ratio) in various clinical settings is not documented.

Although significant gains have been made in the past 20 years, hyperthermia techniques including radiofrequency, microwave, laser and ultrasound lack the tumor treatment selectivity which can be seen with radiation and chemotherapy. Similarly, although considerable progress has been made in the development of hyperthermia treatment planning systems, the ability to significantly improve the hyperthermia therapeutic ratio has not yet materialized. Specifically, it is the inability to obtain homogeneous and precise high intra-tumoral temperatures, while limiting such temperatures to the surrounding normal tissues, that restricts clinical effectiveness and raises safety concerns.

Intratumoral iron oxide nanoparticle thermotherapy represents a recent development in the area of clinical cancer hyperthermia. In this technique, externally delivered alternating magnetic field (AMF) energy is coupled magnetically to iron or iron oxide super-paramagnetic or ferromagnetic nanoparticles to create a localized heating effect. Typically the particles being studied have a core diameter of generally less than 100 nm. Particle suspensions are injected directly into the target region (tumor) and subsequently heated via the previously mentioned externally applied AC magnetic field. In contrast to E-field dominant systems used in regional hyperthermia, boundaries of different conductive tissues do not interfere with power absorption in the field containing particles. In vitro experiments with such particles have confirmed the excellent power absorption characteristics present with a heating element present in large number and/or with a large surface area. Although nanoparticle hyperthermia cancer therapy has many variables to consider, particle composition, coating and size remain key determinants in heating efficacy. Finally, the local and/or intravenous delivery of conjugated tumor specific antibodies and iron / iron oxide particles should be able to provide a new dimension (selective particle uptake by individual cells / intracellular hyperthermia) in the nanoparticle hyperthermia cancer treatment. Although theoretically attractive, the potential usefulness and enhanced efficacy of radiation and nanoparticle hyperthermia remains largely unstudied.

MATERIALS AND METHODS

Animal and Tumor Model

Sixty female C3H/HeJ mice (Jackson Labs, Bar Harbor, ME) were used in this study. Animal care was performed in accordance with all federal and institutional guidelines. Animals received food and water ad libitum. Body weight was monitored three times per week. Tumors were induced by subcutaneous implantation of 1×10^6 MTG-B mouse mammary carcinoma cells into the shaved right flank of 7 week-old, 18-22 gram mice. Implantation was carried out under anesthesia following intraperitoneal injection of ketamine (100 mg/kg) and xylazine (5 mg/kg). Tumors were measured daily in three orthogonal directions (d_1 , d_2 , d_3), data which was used to calculate tumor volume ($\Pi \cdot d_1 \cdot d_2 \cdot d_3 / 6$). Mice were assigned to random groups once the tumor volume reached a size of $150 \text{ mm}^3 \pm 25 \text{ mm}^3$.

Tumor Irradiation

Radiation-treated mice were anesthetized with ketamine/xylazine (100/5 mg/kg) and placed in a laterally recumbent position. A single dose of 15 Gy was delivered in a 1 cm field to tumor location on the animals' right flank, using 6 MeV electrons at an SSD of 100 cm delivered via a Varian 2100C linear accelerator. The radiation dose represented a maximum depth dose of approximately 15 mm which is sufficient to extend to the depth of the implanted tumors. The chosen radiation field covered the entire tumor site and a 2 - 3 mm peri-tumor region. The peri-tumor region was assumed to be devoid of tumor cells at the time of treatment.

Nanoparticle Hyperthermia Treatment Procedure

Tumors were injected with a single dose of 80 nm iron oxide particles in the central region of the tumor. The dose was calculated according to tumor size using the following formula: 0.005 mg iron/mm³ tumor at a particle concentration of 33 mg/ml for the 80 nm particles used. For example, a 150 mm³ tumor received approximately 52 μ l of iron oxide solution. Particles were injected into a single central portion of each tumor approximately 15 minutes prior to AMF exposure (Fig 1). Each injection required approximately 5 minutes. One tumor was removed and bisected (Fig 2) for assessment of particle distribution homogeneity at 10 minutes post-injection. Although an accurate 3-D quantification was not performed, visual 2-D assessment suggested particles were present throughout the tumor but not in a homogeneous manner.

Thermometry

Three fiber optic temperature probes (FISO, Inc., Quebec, Canada) were positioned to measure temperature in the tumor core, tumor periphery and rectum (core temperature). Temperature measurements were documented at one-second intervals by each probe throughout the pre-treatment, treatment and post-treatment period.

AMF Exposure and Thermal Dose Delivery

AMF exposure (166 KHz at 400 or 550 Oe) of the mice was performed using a Huttinger TIG 10/300 generator (Huttinger, Freiburg, Germany) and a Fluxtrol Inc. water cooled coil (Fluxtrol Inc, Auburn Hills, MI) ten minutes following nanoparticle injection and 30 minutes post irradiation (when radiation was used). Under general anesthesia, with temperature monitoring probes in place, the mouse was placed inside a 50 ml conical tube and inserted into the circular AMF coil (Figure 4). Although the entire mouse receives field exposure, the tumor is positioned in a 1.5 cm central region of the coil. Comparatively, this region receives the most uniform field exposure. Temperatures were recorded in all three probes at one-second intervals throughout the experimental period (pre-heat, heat and post-heat) and graphed in real-time via software supplied by the temperature monitoring system FISO (Figure 5).

Treatment Parameters and Experimental Groups

Animals were randomly allocated to five different groups. Five mice were included in each group. Treatments were carried out under ketamine and xylazine anesthesia (as described

previously) when a tumor volume reached $150 \text{ mm}^3 \pm 50 \text{ mm}^3$. AMF treatment consisted of thermocouple implantation, a pre-treatment heating ramp-up period ($29.0 - 41.5^\circ\text{C}$) which typically ranged from 3- 9 minutes and a 10 minute treatment period (noted as initiated when the tumor reached 41.5°C). The length of treatment was dependant on the AMF strength and nanoparticle parameters. Pre-treatment core/rectal, peri-tumor and tumor temperatures averaged 32°C , 28°C and 27°C , respectively. Although the situation did not occur in these experiments, study guidelines called for immediate stoppage of AMF exposure if temperatures of 41.5° or 55°C were achieved in the rectum/core or tumor respectively.

Study arms

1. 15 Gy only (single dose, no particles)
2. Particles + AMF at 400 Oe (10 min incubation in tumor)
3. Particles + AMF at 550 Oe (10 min incubation in tumor)
4. 15 Gy + particles + AMF at 400 Oe (30 minutes between radiation and heat, 10 min particle incubation in tumor)
5. 15 Gy + particles + AMF at 550 Oe (30 minutes between radiation and heat, 10 min particle incubation in tumor)

Statistical Analysis

Due to the ongoing nature of these studies, statistical determination of variance between groups has not been performed. At the conclusion of the study such calculations will be performed using a one-way analysis of variance (ANOVA).

RESULTS

In Vitro Studies

In preparation to the in vivo studies described here a number of in vitro assays were performed to determine the relative cytotoxic association of nanoparticle heating and radiation. Briefly 1.3×10^5 MTG-B cells were harvested and mixed with the iron oxide particles at concentrations of 0, 1 and 5mg/ml in a final volume of 0.3 ml. Cells were then exposed to radiation (2 Gy, single dose) and/or a 400 Oe AMF for 15 mins. Treatment groups requiring radiation were treated on a J.L. Shepherd 137-Cs irradiator at a dose rate of 1 Gy/min prior to being treated with the AMF. After treatment, the cells were diluted and plated to determine the survival fraction based on clonogenic survival. Results were normalized against the survival fraction determined for the control group (0mg/ml, 0Gy). Results demonstrated increased cytotoxicity with increasing field strength, particle concentration and AMF exposure time. Iron oxide nanoparticle hyperthermia contributed an apparent additive cytotoxic effect to the radiation treatment.

In Vivo Studies

Although our in vivo studies are ongoing, preliminary information suggests iron oxide nanoparticle hyperthermia has the potential to be an effective and safe cancer treatment

modality, with a potentially high therapeutic ratio and radiation enhancement (Figure 6). A nominal radiation dose of 15 Gy delayed tumor regrowth approximately 2 days (as compared to no treatment). A 400 Oe AMF - nanoparticle treatment resulted in no significant regrowth delay whereas the 550 Oe group achieved a delay of approximately 9 days. Radiation treatment (15 Gy) enhanced regrowth delay in the 400 and 550 Oe groups by 2 and 15 days, respectively, suggesting a heating threshold is necessary to achieve a therapeutic advantage from the heat-radiation combination.

Although eddy currents from the AMF field can result in core body temperature increases, depending on frequency and field strength, our studies show that whole mouse AMF exposure at 160 KHz and 400 or 550 Oe, for a 20 minutes (heat-up and protocol heating), is safe.

Initial light microscopic studies of treated tumors at the time of regrowth, demonstrates two general morphologies. Some regrowth tumors contain significant necrosis with a viable tumor and fibrotic rim. Others have virtually no necrosis, consisting largely of only viable tumor and supporting stroma. Virtually all regrowth tumors contain a significant region of peri-tumoral edema and numerous iron pigment containing macrophages (Figures 7a, 7b). The peri-tumoral iron containing macrophages suggest iron from necrotic tumor cells makes its way out of the tumor to residing peri-tumoral scavenger cells.

DISCUSSION

In these preliminary studies we have shown that local hyperthermia, using AMF activated magnetic nanoparticles, alone and combined with radiation, results in significant local tumor treatment efficacy (mouse breast cancer model) at an AMF level that does not result in systemic thermal morbidity. These studies demonstrated a positive correlation between tumor thermal dose and tumor regrowth delay. Although field strength and heating time also correlate with increases in core temperature, the relationship is less direct suggesting nanoparticle hyperthermia alone may have a therapeutic ratio that is greater (more effective) than non-particle tumor heating techniques such as microwave, ultrasound and water bath, when similar thermal doses are applied. Although it is too early to judge quantitatively, our data also suggest nanoparticle hyperthermia and ionizing radiation are an effective tumor treatment combination that also has the potential for improving the therapeutic ratio of conventional radiation tumor treatment.

With respect to treatment optimization, our studies also point out the need to examine the relationship of intratumoral nanoparticle hyperthermia / radiation treatment efficacy with particle post-injection incubation times, particle composition, size and coating, treatment fractionation and scheduling, AMF frequency and field strength and delivery method.

REFERENCES

1. Wust P, Hildebrandt B, Sreenivasa G, Rau B, Gellermann J, Riess H, Felix R, Schlag PM. Hyperthermia in combined treatment of cancer. *Lancet Oncol.* 2002; 3:487–497. [PubMed: 12147435]

2. Hildebrandt B, Wust P, Ahlers O, Dieing A, Sreenivasa G, Kerner T, Felix R, Riess H. The cellular and molecular basis of hyper- thermia. *Crit Rev Oncol Hematol*. 2002; 43:33–56. [PubMed: 12098606]
3. Vernon CC, Hand JW, Field SB, Machin D, Whaley JB, van der Zee J, van Putten WL, van Rhoon GC, van Dijk JD, Gonzalez-Gonzalez D, Liu FF, Goodman P, Sherar M. Radiotherapy with or without hyperthermia in the treatment of superficial localized breast cancer: Results from five randomized controlled trials. International Collaborative Hyperthermia Group. *Int J Radiat Oncol Biol Phys*. 1996; 35:731–744. [PubMed: 8690639]
4. Valdagni R, Amichetti M. Report of long-term follow-up in a randomized trial comparing radiation therapy and radiation therapy plus hyperthermia to metastatic lymph nodes in stage IV head and neck patients. *Int J Radiat Oncol Biol Phys*. 1994; 28:163–169. [PubMed: 8270437]
5. Overgaard J, Gonzalez Gonzalez D, Hulshof MC, Arcangeli G, Dahl O, Mella O, Bentzen SM. Hyperthermia as an adjuvant to radiation therapy of recurrent or metastatic malignant melanoma. A multicentre randomized trial by the European Society for Hyperthermic Oncology. *Int J Hyperthermia*. 1996; 12:3–20.
6. Sneed PK, Stauffer PR, McDermott MW, Diederich CJ, Lamborn KR, Prados MD, Chang S, Weaver KA, Spry L, Malec MK, Lamb SA, Voss B, Davis RL, Wara WM, Larson DA, Phillips TL, Gutin PH. Survival benefit of hyperthermia in a prospective randomized trial of brachytherapy boost hyperthermia for glioblastoma multiforme. *Int J Radiat Oncol Biol Phys*. 1998; 40:287–295. [PubMed: 9457811]
7. Anscher MS, Samulski TV, Dodge R, Prosnitz LR, Dewhirst MW. Combined external beam irradiation and external regional hyperthermia for locally advanced adenocarcinoma of the prostate. *Int J Radiat Oncol Biol Phys*. 1997; 37:1059–1065. [PubMed: 9169813]
8. Algan O, Fosmire H, Hynynen K, Dalkin B, Cui H, Drach G, Stea B, Cassady JR. External beam radiotherapy and hyperthermia in the treatment of patients with locally advanced prostate carcinoma. *Cancer*. 2000; 89:399–403. [PubMed: 10918172]
9. Van Vulpen M, De Leeuw AA, Raaymakers BW, Van Moorselaar RJ, Hofman P, Lagendijk JJ, Battermann JJ. Radiotherapy and hyperthermia in the treatment of patients with locally advanced prostate cancer: Preliminary results. *BJU Int*. 2004; 93:36–41. [PubMed: 14678364]
10. Kalapurakal JA, Pierce M, Chen A, Sathiaselan V. Efficacy of irradiation and external hyperthermia in locally advanced, hormone-refractory or radiation recurrent prostate cancer: A preliminary report. *Int J Radiat Oncol Biol Phys*. 2003; 57:654–664. [PubMed: 14529769]
11. Coughlin, CT. Interstitial thermobrachytherapy. In: Nag, S., editor. *Principles and practice of brachytherapy*. Futura publishing; NY: 1997. p. 639-647.
12. Pankhurst QA, Connolly J, Jones SK, Dobson J. Applications of magnetic nanoparticles in biomedicine. *J Phys D: Appl Phys*. 2003; 36:167–181.
13. Moroz P, Jones SK, Gray BN. Magnetically mediated hyperthermia: Current status and future directions. *Int J Hyperthermia*. 2002; 18:267–284. [PubMed: 12079583]
14. Jordan A, Wust P, Fahling H, John W, Hinz A, Felix R. Inductive heating of ferrimagnetic particles and magnetic fluids: Physical evaluation of their potential for hyperthermia. *Int J Hyperthermia*. 1993; 9(1):51–68. [PubMed: 8433026]
15. Jordan, A.; Wust, P.; Scholz, R.; Tesche, B.; Fahling, H.; Mitrovics, T.; Vogl, T.; Cervos Navarro, J.; Felix, R. Cellular uptake of magnetic fluid particles and their effects in AC magnetic fields on human.
16. Johansson M, Jordan A, et al. Thermotherapy using magnetic nanoparticles combined with external radiation in an orthotopic prostate model. *The Prostate*. 2006; 66:97–104. [PubMed: 16114060]
17. DeNardo SJ, DeNardo GL, Forman AR, Ivkov R, et al. Development of tumor targeting bioprobes (¹¹¹In-Chimeric L6 monoclonal antibody nanoparticles) for alternating magnetic field cancer therapy. *Clin Cancer Res*. 2005; 11(19 Suppl):7087s–7092s. [PubMed: 16203807]
18. Ivkov R, Forman AR, DeNardo SJ, DeNardo GL, et al. Application of high amplitude alternating magnetic fields for heat induction of nanoparticles localized in cancer therapy. *Clin Cancer Res*. 2005; 11(19 Suppl):7093s–7103s. [PubMed: 16203808]

Figure 1.
demonstrates the iron oxide nanoparticle injection technique.

Figure 2. demonstrates the cut surface of tumor 10 minutes post injection. With the exception of the lower left region, there appears to be significant nanoparticle coverage of the tumor. Further studies suggest implantation method and pretreatment incubation are critical factors regarding nanoparticle tumor coverage of the tumor volume following intratumoral injection.

Figure 3.

Catheters used to house the thermometry probes were placed in the central tumor region, tumor periphery and rectum. The BD Vialon™ catheters (BD Biosciences, San Diego, CA) were used to house/guide the FISO fiber optic temperature probes. Leaving the catheters in place during treatment did not influence temperature measurement. The measurement tips of temperature probes were strategically placed so that temperatures were taken in the central and representative area of the tumor or peri-tumor tissue. Temperature probes were not moved during the treatment and placement was confirmed post treatment.

Figure 4. demonstrates a mouse positioned in cooled Fluxtrol AMF coil. The leads of three FISO thermometry probes can be seen entering the tumor, peri-tumor and rectal positions.

Figure 5.

represents a temperature change in the tumor (top curve), peri-tumor (middle curve) and rectum (bottom curve) during a typical 550 Oe exposure. As expected the highest temperature (45°C) occurred in the tumor, whereas the peri-tumor (42.5°C) and rectum /core (37°C) were lower. The significantly steeper heating curves seen in the tumor and peri-tumor region are reflective of the relative iron oxide content. The relationship of the temperatures measured at the three sites was consistent within an individual treatment group, but significantly different when treatment parameters changed.

Figure 6.

This tumor regrowth delay curve, **Figure 6**, compares the relative treatment efficacy of radiation (15 Gy) alone with iron oxide nanoparticle hyperthermia treatment alone (400 or 550 Oe) and in combined (15 Gy + nanoparticle hyperthermia) in syngeneic MTG-B tumors.

Figure 7a & b.

Figures 7a and 7b are low and high magnification photomicrographs, respectively, of a representative subcutaneous MTG-B tumor 12 days following an iron oxide nanoparticle / 550 Oe AMF treatment. Central necrosis of the tumor can be seen at the left hand margin in Fig 7a. Also visible is a rim of tumor regrowth and fibroplasia surrounding the necrosis and invading adjacent muscle tissue. A zone of edema, with many iron containing macrophages exists between the tumor region and normal muscle tissue. Figure 7b is a higher magnification photomicrograph of the tumor regrowth/fibroplasia rim and the bordering edema and iron containing macrophages (dark brown stain).