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Iron Oxide Hyperthermia And Radiation Cancer Treatment

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Abstract

It is established that heat can enhance the effect of radiation cancer treatment. Due to the ability to localize thermal energy using nanoparticle hyperthermia, as opposed to other, less targeted, hyperthermia modalities, it appears such enhancement could be accomplished without complications normally associated with systemic or regional hyperthermia. This study employs non-curative (suboptimal), doses of heat and radiation, in an effort to determine the therapeutic enhancement potential for IONP hyperthermia and radiation.

Methods—MTG-B murine breast adenocarcinoma cell are inoculated into the right flanks of female CH3/HEJ mice and grown to volumes of 150mm^3 $^+$/$_-$ 40 mm^3. A single dose of 15 Gy (6 MeV) radiation was uniformly delivered to the tumor. A pre-defined thermal dose is delivered by direct injection of iron oxide nanoparticles into the tumor. By adjusting the field strength of the 160 KHz alternating magnetic field (AMF) an intra-tumoral temperature between 41.5 and 43 degrees Celsius was maintained for 10min. The alternating magnetic field was delivered by a water-cooled 36mm diameter square copper tube induction coil operating at 160 kHz with variable magnet field strengths up to 450 Oe. The primary endpoint of the study is the number of days required for the tumor to achieve a volume 3 fold greater than the volume at the time of treatment (tumor regrowth delay).

Results—Preliminary results suggest the addition of a modest IONP hyperthermia to 15 Gy radiation achieved an approximate 50% increase in tumor regrowth delay as compared to a 15 Gy radiation treatment alone. The therapeutic effects of IONP heat and radiation combined were considered additive, however in mice that demonstrated complete response (no tumor present after 30 days), the effect was considered superadditive or synergistic. Although this data is very encouraging from a multimodality cancer therapy standpoint, additional temporal and dose related information is clearly necessary to optimize the therapy.

Keywords

Iron oxide; nanoparticle; AMF; adenocarcinoma; transmission electron microscopy; TEM; murine; MTG-B; HT-29

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Background

It is well accepted that hyperthermia can enhance the therapeutic effectiveness of radiation cancer therapy, if employed correctly [1,2]. The characteristics of hyperthermic effects make it a desirable adjuvant therapy to radiation. Utilizing very gentle heating to 40° C increases blood flow and oxygenation of heated tissues which increase the effectiveness of radiotherapy by promoting free radical formation [20, 21]. Cytotoxic effects are seen from hyperthermia at temperatures of 41.5° C and above; they are much more pronounced for cells in the S phase or in acidic conditions. At 43° C a sharp increase in cytotoxicity is seen. Of interest here is the mild hyperthermia region 41.5°C to 43°C. Mild hyperthermia is effective against cells which are normally radiotolerant, such as cells in the S phase or in acidic environments [22]. Furthermore, mild hyperthermia can radiosensitize cells by inhibiting DNA repair for finite amounts of time after application of radiation. The timing of treatments becomes an important factor in radiosensitization and ultimate efficacy of the combined treatments. Ideally one treatment is applied soon after the other. Research on murine models seems to dictate maximum effect is achieved when hyperthermia is applied three to four hours after radiation, and the optimal radiosensitization is achieved using 42°C–43°C (beyond this range greater cytotoxcicty is seen, however no further radiosensitization is achieved) [23, 24].

Despite its recognized potential, current hyperthermia modalities have not yet demonstrated the clinical cancer treatment efficacy many experts believe is possible. One major obstruction affecting the success of hyperthermia, as a stand alone or even an adjuvant therapy to radiation, is the lack of a clear understanding regarding its optimal use and delivery in the clinical setting. Additionally, despite the fact that hyperthermia is more cytotoxic to cells in low pH and hypoxic environments (like those commonly found in tumors), it appears that conventional, externally delivered, hyperthermia lacks the degree of inherent biological therapeutic advantage enjoyed by radiation therapy and possibly chemotherapy. That said, it is clear that much progress has been made by clinicians and hyperthermia teams with respect to the generation of therapeutically effective hyperthermia cancer treatment modalities [3–11]. These modalities include radiofrequency, microwave, laser and ultrasound hyperthermia techniques; all of which produce acceptable tissue heating but lack inherent tumor cell selectivity, and /or adequate spatial focusing to be highly effective as an independent agent. It has not, so far, been possible to generate high temperatures in all or most tumor cells while limiting toxic heating to surrounding normal tissues. Iron oxide nanoparticle hyperthermia is a relatively new heating modality which may be able to successfully address a number of the prior hyperthermia limitations. IONP hyperthermia shows promise as a viable heating modality which promotes therapeutic ratio by limiting heating to specifically targeted tissues [12–16, 18]. The technique involves delivering energy via an alternating magnetic field (AMF) which is absorbed by para-magnetic or super-paramagnetic iron oxide nanoparticles. The electromagnetic energy from the AMF field is converted to thermal energy (hysteretic or frictional heating) by the particles. Use of IONP -AMF therapy conveys certain advantages: first and most importantly magnetic fields readily penetrate tissue in a safe manner, if the appropriate frequency and field strength is used. Unlike electric field dominated systems power delivery

is unaffected by electrical impedance boundaries found in the body. Highly biocompatible IONP can be produced in a consistent fashion (at various sizes) and coated with a variety of materials including targeting proteins, such as antibodies or peptides [17]. Finally the Iron oxide nanoparticles can be suspended in a solution which can be accurately delivered into the tumor parenchyma or systemically. Patient geometry, the required tissue penetration and type and quality of the IONP will dictate the degree, extent and cost of the AMF delivery instrumentation.

Materials and Methods

Animal Model

Female C3H mice (Charles River Laboratory) were implanted, in the intradermal tissue of the right flank, with 1×10^6 MTG-B tumor cells as observed in Figure 1. The MTG-B cell line, first characterized by Clifton et al, was taken from a spontaneous mammary tumor of a C3H mouse. MTG-B cells were harvested and counted by hemacytometer. The cells were then centrifuged and re-suspended at a concentration of 10 million cells per milliliter. A volume of 0.1 ml of MTG-B cell suspension was injected subcutaneously into the right flanks of female C3H mice and allowed to grow into tumors. Upon reaching a size of 150 mm^3 +/− 40 mm^3, the mouse tumor was treated. Tumors were measured daily with electronic calipers in three orthogonal directions, d_1, d_2, and d_3. Tumor volume was determined using the following formula for the volume of an ellipsoid:

$$Tumor\ Volume=\frac{\pi \cdot d_1 \cdot d_2 \cdot d_3}{6}$$

Treatment Design and Methods

Study Arms

1. Gy Radiation alone (4 mice)
2. IONP + AMF @ a thermal dose of 41.5°C for 10 min. (4 mice)
3. Gy + IONP + AMF @ a thermal dose of 41.5°C for 10 min. (9 mice)

Study Arm I: Radiation Treatment

The mice were anesthetized with ketamine (100 mg/kg) and xylazine (5 mg/kg) before irradiation. Irradiation was delivered with a Varian 2100C linear accelerator. The anesthetized mouse was arranged prone on the bench of the Varian 2100C with the mouse's right leg tucked blow its abdomen. This allowed the tumor to be visible from directly above (where the x-ray source was positioned) to avoid irradiation of internal organs. The source to surface distance (SSD) used was 100cm, and the beam encompassed the entire tumor and approximately 3mm of margin. Once aligned, the linear accelerator was activated and 15Gy of 6 MeV photons were delivered to the tumor. The time elapsed between anesthetization and completion of irradiation averaged about 20 minutes. Actual irradiation time is on the order of 4 minutes.

Study Arm II: IONP Hyperthermia Treatment

Particle Injection—Iron oxide nanoparticles (supplied by Aduro Biotech, Berkeley, California) were administered to the tumor via direct injection. The IONP utilized in this experiment were dextran-coated iron oxide BNF nanoparticles with a hydrodynamic radius of approximately 120 nm manufactured by MicroMod GmBH (Rostock, Germany). Following tumor volume assessment, IONPs were injected into the tumor at an iron concentration of 5 mg/ml of tumor tissue. A single injection site was used a total injection time of 5 minutes.

Thermometry—Temperature measurements were taken immediately adjacent to the tumor, in the centre of the tumor and in the rectum throughout the experimental period. For peritumor and rectal temperatures, an 18-gauge catheter was used, positioned subcutaneously alongside the tumor and in the rectum, respectively. A 0.7 mm diameter fiber optic probe (FISO Inc, Quebec, Canada), accurate to 0.1 deg C was used for these measurements. Representative temperature measurements for the peritumor and rectal sites are demonstrated in Figure 2.

Tumor temperature measurements were taken with smaller 0.5 mm diameter fiber optic probe (Luxtron/LumaSense, Santa Clara, CA). This fiber optic probe was inserted, naked without a catheter, directly into the tumor center following a 25 gauge needle puncture of the skin covering the tumor. Data from the FISO probes was recorded electronically every second. Data from the Luxtron was recorded manually every 10 seconds.

Thermal Dose Delivery—A cylinder made from the barrel of a 50 ml conical tube was used to house the mouse while in the AMF coil (Fluxtrol Inc, Auburn Hills, MI, Figure 3). This tube provided shield so that the mouse could not come in direct contact with the coil. After placing the three temperature probes, the mouse was inserted into the housing cylinder, which in turn was placed in the AMF coil. The AMF coil was powered by a TIG 10/300 generator and cooled by running water kept at 30 degrees C by a chiller (Tek-Temp Instruments, Croydon PA). Before treatment, all mice were brought to a rectal temperature of 35–37 degrees C using a hot water compress. Upon activation of the AMF field to 450 Oe, tumor temperature was monitored; once a tumor temperature of 41.5 degrees C was reached, the ten minute treatment time countdown was initiated. During this 10 minute period, the AMF field strength was varied to ensure that the temperature remained between 41.5 and 43 degrees. Upon termination of the 10 minute treatment time, the AMF field was disengaged and intra-tumoral temperature monitored until it returned to 37 degrees C.

Study Arm III: Radiation + IONP + AMF

Mice in the third study arm were irradiated in the same method as prescribed in first study arm. Immediately upon completion of irradiation the mouse is recovered for IONP injection. The same injection technique prescribed for the mice in study arm II is repeated for mice in study arm III. Injection usually takes place with in 15 minutes of completion of the irradiation. Injection occurs no more that 30 minutes after the mouse is irradiated. After injection of the IONP, temperature probes are inserted into the mouse's rectum, tumor and peritumor region. Thermometry technique is performed precisely as prescribed for mice in

Study Arm II. After temperature probe placement thermal dose is delivered via an AMF, again the same technique used for study arm II is repeated for these mice. Application of the hyperthermia therapy is always completed within 1 hour of irradiation.

Results

Tumor Growth was monitored by tumor volume measurement every second day. Tumor regrowth was followed until the tumors reached a size of three times the volume at which they were treated (Figure 4). The 4 mice used for the 15Gy control had times to sacrifice of 16, 18, 16 and16 days. This yields an average of 16.5 days to sacrifice from treatment with a standard deviation of 1 day and a standard error of 0.5 days. The hyperthermia control showed an average time to sacrifice of 8 days with a standard deviation of 0.82 days and a standard error of 0.41 days. Two of the mice in the hyperthermia and radiation group had no measurable tumors past 40 days after treatment and were assigned tumor volume tripling times of 45 days for the purposes of these calculations. For the mice in the hyperthermia and radiation combination group displayed an average tripling time of 23.8 days with a standard deviation of 12.25 days and a standard error of 4.63 days.

Thermometry shows that application of the IONP hyperthermia in our setting, does not increase mouse core temperature to unsafe levels. The peri-tumoral and rectal temperature measurements for hyperthermia and radiation in this study were only slightly elevated above the normal mouse core temperature (Figure 3). Similarly, the tumor temperatures were also maintained at a very modest thermal dose (41.5–43.0°C), allowing for an additive or possibly synergistic effect of an also modest radiation dose.

Discussion

In this ongoing and preliminary study of iron oxide nanoparticles hyperthermia (IONP) combined with conventional external beam radiation we have demonstrated an enhanced tumor treatment response in a mouse mammary adenocarcinoma model. Although the extent of the benefit, the mechanism of cytotoxicity and optimal strategies and doses for combining IONP hyperthermia and radiation remained unclear and under study, it appears very likely combination of these two modalities is capable of providing a significantly improved therapeutic ratio and that IONP can offer a significant improvement over conventional hyperthermia delivery techniques. We believe the primary differences between IONP hyperthermia and conventional hyperthermia are based largely on the ability to direct the nanoparticles, either locally or systemically to the cancer cells and to perform intracellular, or at least, highly directed individual cell hyperthermia. Although much of the previously referenced research suggests there is a benefit to combining hyperthermia and radiation therapy, to date few if any studies have quantitatively demonstrated the effects of nanoparticle hyperthermia and radiation treatment. Previous data has suggested that the degree of enhancement is strongly dependent upon the sequence between hyperthermia and radiation, and that the greatest therapeutic ratio has generally been observed to occur when the radiation is delivered first followed 3–4 hours by hyperthermia. At this time, however, it is unclear whether nanoparticle hyperthermia combined with radiation will follow previous radiation/ hyperthermia tissue outcomes or if intracellular hyperthermia will be so different

as to completely alter the treatment effect and the manner in which the two modalities are combined.

As has been speculated and demonstrated in vitro before, these data suggest there will likely be a treatment/thermal dose regimen at which radiation and nanoparticle hyperthermia display synergy. It is also nearly certain that an IONP hyperthermia dose that is too high will overwhelm the effects of sublethal radiation and thereby negate the potential therapeutic ratio benefit. Further studies should include the narrowing the allowable range of temperature fluctuation and exploring the thermal dose space between 41.5 and 47° C for brief exposures or a reduced maximum temperature for a prolonged exposure, as well as experimenting with various radiation doses, including fractionation.

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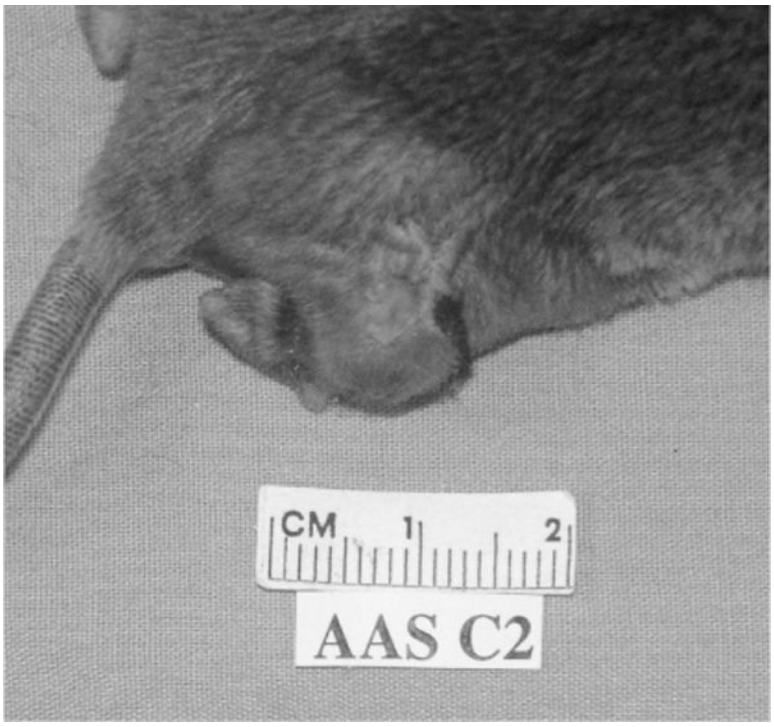

Figure 1.

MTG-B mouse mammary adenocarcinoma 12 days following intraderma implantion of 1×10^6 MTG-B cells in the right flank. Tumors were treated at a volume between 100–150 mm^3. Study endpoint was the time (days post treatment) required to for the tumor to reach a 3× increase in size (tumor regrowth delay assessment).

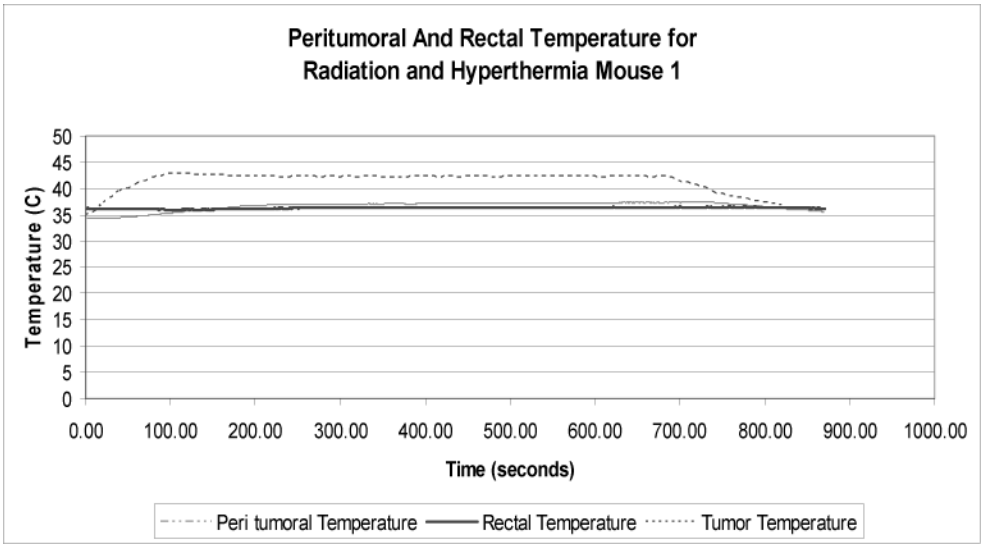

Figure 2.

This figure represents a typical peritumoral and rectal heating history. It should be noted that the peri-tumor temperature, while beginning at approximate 34° C. reached approximately 37.5° C. during the majority of the treatment. In contrast, the rectal temperature which started at approximately 36.2° C stayed relatively constant throughout the treatment period. While not depicted here, tumor temperatures were held between 41.5–43° C throughout the treatment time.

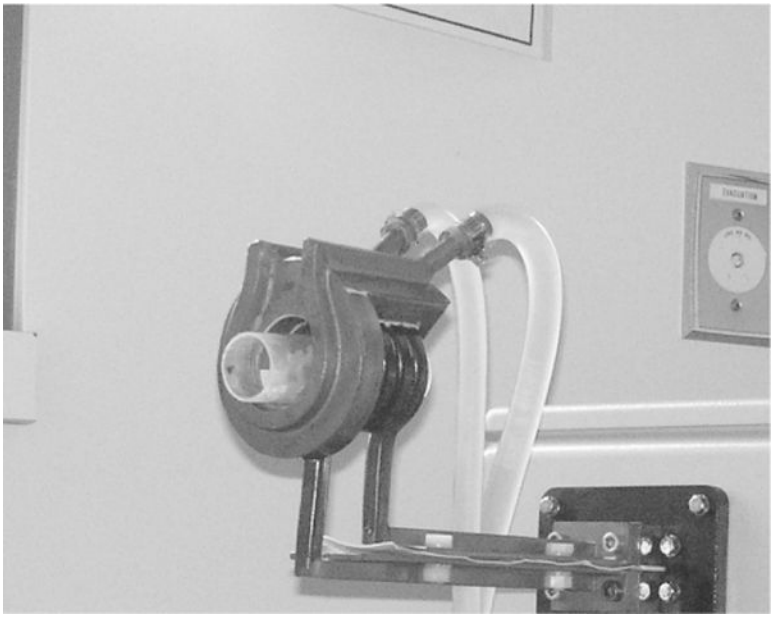

Figure 3.
Fluxtrol Water Cooled Mouse Coil used to generate the AMF shown here next to the TIG 10/300 generator.

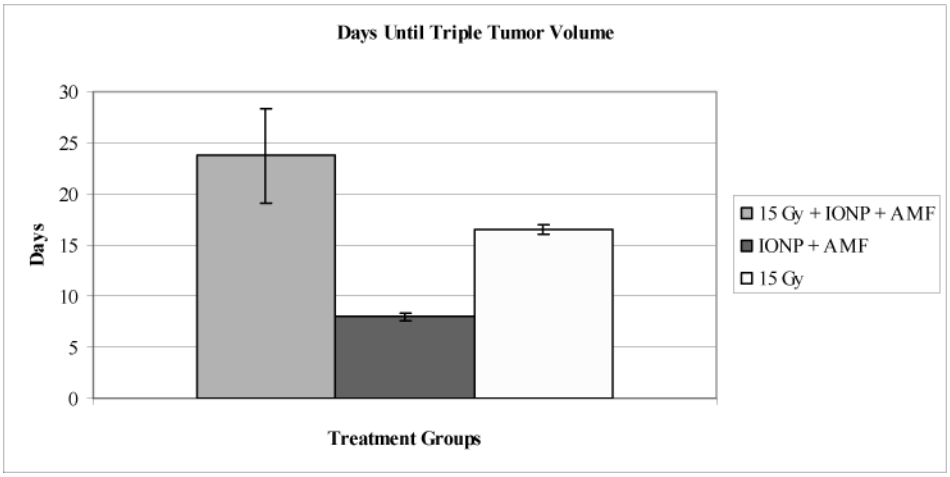

Figure 4.

This bar graph represents the average number of days for the tumor volume to triple following treatment. Error bars represent standard error calculations. The nanoparticles plus radiation group averaged approximately 23 days tumor regrowth delay, whereas the iron oxide nanoparticles hypothermia group averaged approximately 8 days (41.5–43 °C /10 min, < 5 CEM) and the 15 Gy radiation alone group average approximate 16 days