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Iron oxide nanoparticle hyperthermia and chemotherapy cancer treatment

AA Petryk[1,*], **AJ Giustini**[1,2], **P Ryan**[3], **RR Strawbridge**[2], and **PJ Hoopes**[1,2]

[1]Thayer School of Engineering, Dartmouth College, Hanover, NH 03755 USA

[2]Dartmouth Medical School, Dartmouth College, Hanover, NH 03755 USA

[3]Massachusetts College of Pharmacy and Health Sciences, Boston, MA 02115 USA

Abstract

The benefit of combining hyperthermia and chemotherapy to treat cancer is well established. However, combined therapy has not yet achieved standard of care status. The reasons are numerous and varied, however the lack of significantly greater tumor cell sensitivity to heat (as compared to normal cells) and the inability to deliver heat to the tumor in a precise manner have been major factors. Iron oxide nanoparticle (IONP) hyperthermia, alone and combined with other modalities, offers a new direction in hyperthermia cancer therapy via improved tumor targeting and an improved therapeutic ratio. Our preliminary studies have demonstrated tumor cell cytotoxicity (*in vitro* and *in vivo*) with IONP heat and cisplatinum (CDDP) doses lower than those necessary when using conventional heating techniques or cisplatinum alone. Ongoing studies suggest such treatment could be further improved through the use of targeted nanoparticles.

Methods—*In vivo:* IONPs (5mg of iron per gram of tumor) were administered into MTG-B flank tumors in female C3H-HEJ mice directly after cisplatinum chemotherapy (0.1ml/kg of body mass) was intraperitoneally injected. An 160 KHz, 350–450 Oe AMF (alternating magnetic field) was used to induce particle heating.

In vitro: Mouse mammary adenocarcinoma cells (MTG-B) cells were grown and exposed to IONP hyperthermia and cisplatinum. IONPs not associated with cells were removed by washing prior to heat induction by an AMF field. Acute cell survival, via trypan blue assay, was used to quantify the level of cytotoxicity.

Results—*In vitro* studies, using IONP + cisplatinum, have demonstrated promising cytotoxicity enhancement. Ongoing studies are being pursued to further define the mechanism of action, temporal associations and pathophysiology of combined IONP hyperthermia and chemotherapy treatment. Preliminary *in vivo* IONP /cisplatinum studies have shown a tumor growth delay/ volume reduction greater than either modality alone at comparable doses. Further enhancement of this treatment success appears to depend on a better understanding of IONP dose and tumor cell association, chemotherapy dose and administration technique, the spatial and temporal treatment relationship of the two modalities and optimal AMF - IONP coupling.

[*]Corresponding Author: Alicia.A.Petryk@Dartmouth.edu.

Keywords

Iron oxide; nanoparticle; AMF; chemotherapy; cisplatinum; murine; MTG-B; HT-29

2. BACKGROUND

The concept of combining therapeutic hyperthermia with traditional cancer treatments, such as radiation and chemotherapy, has been investigated by researchers over the past 30 years. It has been shown that the inclusion of hyperthermia with many traditional cancer treatments results in positive synergistic or additive effects.[1]

Even when applied alone, hyperthermia induces DNA fragmentation and increases intracellular calcium concentrations (inducing cellular apoptosis) as well as damaging cell membranes.[2] Different types of hyperthermia (different degrees of localization and ways of inducing elevated temperatures) have been explored in combination with both chemotherapy and radiotherapy. Variations in degree of hyperthermia localization range from no targeting (whole body hyperthermia) to regional hyperthermia (hyperthermic isolated limb perfusion and hyperthermic peritoneal perfusion) to the highly localized (individual cell) heating via membrane bound nanoparticles we show here.[1]

A critical and often limiting obstacle faced by hyperthermia cancer treatment has been the inability to localize the energy and heat to the tumor. Nanoparticles, in particular iron oxide nanoparticles, appear to have great potential as they may be localized via direct injection into the tumor and heated with an external alternating magnetic field. Further promise may also be found in the area of antibody directed iron oxide particles, which have the potential to not only treat primary tumors, but metastases as well.

Inducing localized heating with IONP and AMF for tumor treatment was first proposed by Gilchrist et al.[3] Application of this method can be divided into tumor sensitization (which uses hyperthermia to sensitize tumor cells to “traditional” treatments, i.e. chemotherapy and radiation), and tumor ablation, which depends on the cytotoxic effects of elevated temperatures alone.[4]

The effects of combined chemotherapy and hyperthermia have been investigated both *in vivo* and *in vitro*, with many methods of inducing hyperthermia, including nanoparticles. As hyperthermia modifies the tumor vasculature and tumor parenchyma, the environment surrounding the tumor cells (pH and oxygen tension) will likely be different than that found *in vitro* and may alter the potency of the chemotherapy.[5] For this reason, we chose to investigate both the effects of CDDP+IONP+AMF *in vitro* and *in vivo*.

As previously noted, the combination of chemotherapy agents with hyperthermia have been investigated with promising results. Marmor found that the effects of CDDP were potentiated *in vivo* with localized hyperthermia (RF field). It is notable that 2 to 3 separate treatments were necessary before a significant growth delay was found. The dosage used for this study was 2mg/kg i.p. and temperatures were elevated to 43°C for 30 minutes. This study also compared simultaneous administration of CDDP and hyperthermia. Results

showed that the potency of the treatment (time to tumor volume doubling) was significantly improved when CDDP and hyperthermia were given together, in comparison to treatments when hyperthermia was induced 24hrs after treatment with CDDP.[5]

Takemoto et al. compared the effects of various chemotherapeutic agents; cyclophosphamide, isofamide, melphalan, CDDP, 5-fluorouracil, mitomycin C and bleomycin and water bath based mouse limb heating to a temperature of 41.5°C in three different tumor types (mammary carcinoma, osteosarcoma and squamous cell carcinoma). This study found that growth delay time was 2–4 times greater with combined therapy as compared to single modality therapy.[6]

Previous studies conducted by our group using AMF activated IONP in mice, have shown significant tumor re-growth delay. Figure 1 shows the post-injection, pretreatment flank tumor of a C3H/HEJ mouse and the same mouse 14 days post-treatment.

3. MATERIALS AND METHODS

3.1 Iron Oxide Nanoparticles

The IONP used in these experiments are composed of Fe_3O_4 cores with a biocompatible dextran coating and have an average hydrodynamic diameter ranging between 100 and 120nm. The nanoparticles are manufactured by MicroMod GmBH, Rostock, Germany and were prepared in suspension. The lot numbers used were 84-28-102 and 04508 84-IIID.

3.2 *In vivo*: Tumor inoculation and measurement

Mice C3H/HEJ (Charles River, Wilmington, MA) were inoculated with MTG-B cells intradermally in the right rear flank. MTG-B cells were isolated, by Clifton et al.[7,8] from a spontaneous C3H/HEJ mouse mammary adenocarcinoma. MTG-B cells were cultured and counted with the use of a hemacytometer and trypan blue staining and suspended at a concentration of ten million cells per ml in 1× Alpha MEM. Using a 25 gauge needle, 100μl of the tumor cell suspension was injected. Tumors were then allowed to grow until they reached a volume of 100 mm^3 +/− 50 mm^3. Tumor volume was calculated by measuring, with digital calipers, three perpendicular diameters (d_1, d_2, d_3) and using the equation for the volume of an ellipsoid:

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

3.3 *In vivo*: Administration of nanoparticles and chemotherapy

When the tumor was found to be the correct volume, the animal was anesthetized with a Ketamine-Xylazine mixture (100mg/kg Ketamine, 5mg/kg Xylazine / kg body wt.). Cisplatinum (Bedford Laboratories, Bedford, OH) was administered intraperitoneally at a dosage of 10mg/kg. The dextran-coated iron oxide nanoparticles were then injected into the center of the tumor at a dose of 5mg of iron oxide per gram of tumor.

3.4 *In vivo*: Administration of AMF and temperature recording

The AMF field was generated by a water cooled whole body circular 10 cm diameter coil (Fluxtrol Inc, Auburn Hills, MI) powered by a Huttinger TIG 10/300 generator operating at approximately 160 KHz and 400 Oe and was maintained at a constant 30° C temperature (chiller /Tek-Temo Instruments Inc). Mouse body temperatures were recorded before, during and after treatment at three sites. Peritumoral and rectal temperatures were recorded with FISO fiber optic probes (FISO Inc, Quebec, Canada) and tumor temperature with a luxtron fiber optic probe (Luxtron/LumaSense, Santa Clara, CA). The peritumoral temperature probe was inserted next to the tumor and under the skin with the use of a 20 gauge hypodermic needle and PE catheter. The tumor temperature was measured without the use of a catheter, with the probe tip centered within the tumor. The probe was inserted in a pathway perpendicular to that of the nanoparticle-injection needle track. Peritumoral and rectal temperatures were recorded every second, while tumor temperatures were recorded every 10 seconds.

The mice were placed on heated water beds prior to treatment in order to maintain core body temperature at 36°C (±1°C). The initial AMF strength was 450 Oe. A 10 minute treatment period was started once the tumor temperature reached 41.5°C. The field was “tuned” as necessary to keep the temperature of the tumor under 43°C and above 41.5°C. Once the field was turned off, the mice were removed from the coil when the tumor temperature reached 37°C. The mice continued to be warmed until consciousness was regained.

The control mice were treated in the same fashion, excluding the injection of the iron oxide nanoparticles, and were exposed to AMF for a 15 to 30 minute duration. Tumor measurements and body mass continued to be taken post-treatment in the same manner.

The Dartmouth College Institutional Animal Care and Use Committee (IACUC) approved this study, which was conducted in accordance with all federal, institutional and AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care) guidelines.

3.5: Methods: *In vitro* administration of nanoparticles, chemotherapy and AMF

The cytotoxic effect of CCDP with or without IONP *in vitro* was also investigated. Three treatment groups (CDDP, IONP and CDDP+IONP) were used, with all three receiving an AMF or water bath exposure. MTG-B cells were cultured and counted with the use of a hemacytometer and trypan blue staining and suspended at a concentration of 10 million cells per ml in 1× Alpha MEM. 1mL samples of cell suspensions were placed into Falcon tubes.

AMF—To the CCDP alone group 3μg/ml of CDDP (3μl of stock solution) was added 5 minutes prior to a 10 minute 450 Oe AMF exposure. The IONP alone group received a total of 1 mg Fe/ml of solution of nanoparticles (a total of 54 μl of stock solution) 5 minutes prior to a 10 minute 450 Oe AMF exposure. The combined treatment group received 5μg/ml of CDDP (5ul of stock solution) and a total of 1 mg Fe/ml of solution of nanoparticles (a total of 54 μl of stock solution) 5 minutes prior to an exposure of 450 Oe AMF for 10 minutes.

Water Bath—To the CCDP alone group 3ug/ml of CDDP (3μl of stock solution) was added 5 minutes prior to a 10 minute water bath exposure (35°C ± 1°C). The IONP alone

group received a total of 1 mg Fe/ml of solution of nanoparticles (a total of 54 μl of stock solution) 5 minutes prior to a 10 minute water bath exposure (35°C ± 1°C). The combined treatment group received 5ug/ml of CDDP (5ul of stock solution) and a total of 1 mg Fe/ml solution of nanoparticles (a total of 54 μl of stock solution) 5 minutes prior to a 10 minute water bath exposure (35°C ± 1°C).

All experimental groups had an initial temperature between 33°C and 35°C, prior to exposure to the AMF field. After treatments 100 μl of each group were plated in culture flasks with MTG-B media (Eagle's Minimum Essential Medium (MEM) with 10 % fetal bovine serum, 1% L-glutamine, and 1% penicillin-streptomycin) and incubated overnight. Cell counts were performed 2 days later.

4. RESULTS

4.1 Results: *In vivo*

4.1.1 CDDP + AMF (no IONP) treatment—Unexpectedly there was significant weight loss in the group suggesting CDDP toxicity. All animals, except one, were removed from the study by day 5 post treatment. Although not statistically significant, the results suggest very modest tumor regrowth delay with CDDP alone. It is unclear why a previously published, and tolerated, CDDP dose of 10 mg/kg mouse body weight resulted in such significant morbidity.

4.1.2 Combined CDDP+IONP hyperthermia (AMF)—This treatment resulted in significant tumor shrinkage (41% by day 4 post treatment). In addition to the excellent treatment effect there was also unexpected weight loss, therefore this initial study group did not complete the prescribed endpoint.

4.1.3 *In vivo* conclusion—Overall morbidity seen in all groups was much higher than expected, limiting the usefulness of our regrowth delay data. However, there is enough information to make a preliminary assessment of increased tumor cytotoxicity CDDP + IONP hyperthermia as compared to CDDP + AMF (no heat). Additional animal studies will be required to determine the extent and type of treatment benefit.

4.2: Results: *In vitro*

The *in vitro* studies show that IONP hyperthermia (alone and combined with CDDP) results in significantly greater cytotoxicity than water bath hyperthermia alone (at the same thermal dose) or combined with AMF. It should be noted that no detectable increase in temperature was recorded for all samples which were exposed to AMF.

5. CONCLUSIONS

Besides the direct heat-induced cytotoxity, increase of temperature can dramatically enhance the antineoplastic effects of cytostatics. It can be demonstrated that there is not only a relationship between dose and response, but also between temperature and the response of a therapeutic substance. There are a number of factors causing temperature-dependent enhancement of the cytotoxicity. The biochemical reactions of some antineoplastic

substances are temperature-dependent. For example, the formation of DNA adducts by platinum containing drugs is increased by hyperthermia[9]. According to Hildebrant et al. and others, the cytotoxic effects of platinum compounds may be enhanced in a linear fashion as temperatures climb above 40.5°C.[1] This effect is, of course, not relevant at temperatures or chemotherapy doses which result in a high level of independent cytotoxicity.

In contrast to previous methods of inducing therapeutic hyperthermia, it is important to note that IONP has the potential of reaching significantly higher temperatures and in a much more localized fashion (even intracellular). Although our studies using systemic cisplatinum and IONP hyperthermia are preliminary, the data is highly suggestive that the ability to heat individual cancer cells offers a novel and unique method for improving chemotherapy sensitization and therapeutic ratio. Many important questions remain, including the effect of chemotherapy on cellular uptake of IONP, the appropriate sequencing of the two modalities and the most effective and safe dose combinations regarding optimization of chemotherapy and nanoparticle based hyperthermia.

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FINANCIAL AND COMPETING INTERESTS DISCLOSURE

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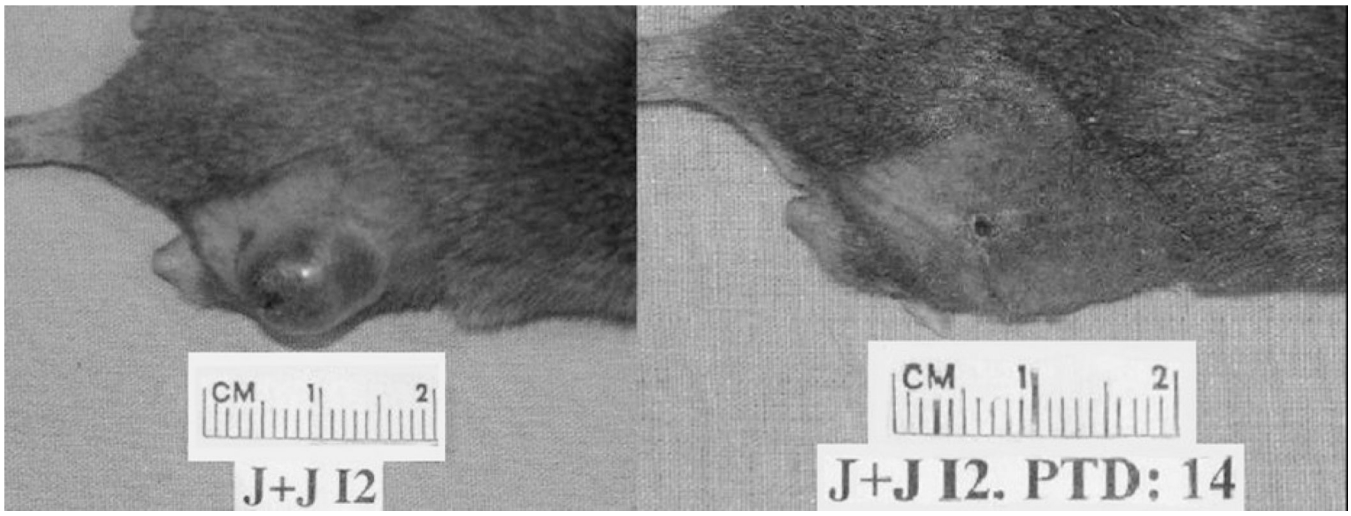

Figure 1.
Post injection pretreatment flank tumor in a C3H/HEJ female mouse (left). Same mouse 14 days post IONP and AMF treatment (right).

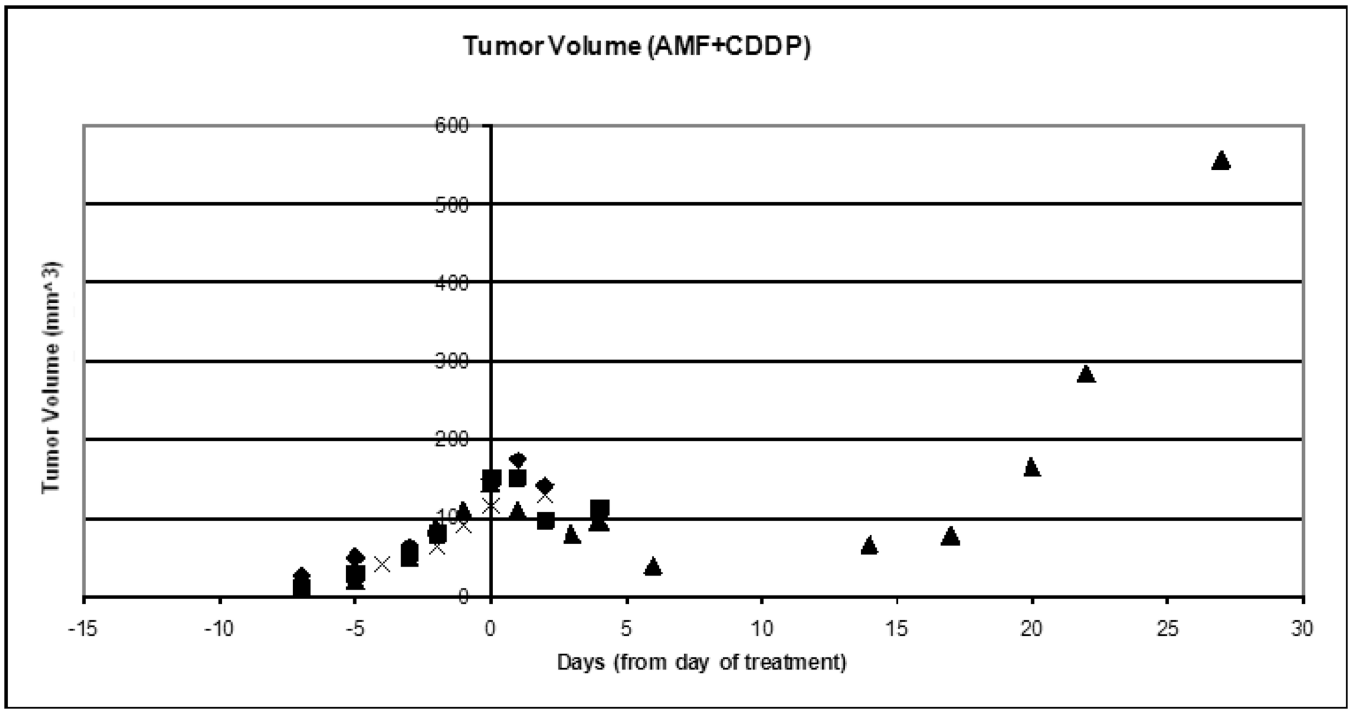

Figure 2.
Tumor volumes of mice treated with AMF and CDDP (no IONP). Each symbol is an individual mouse.

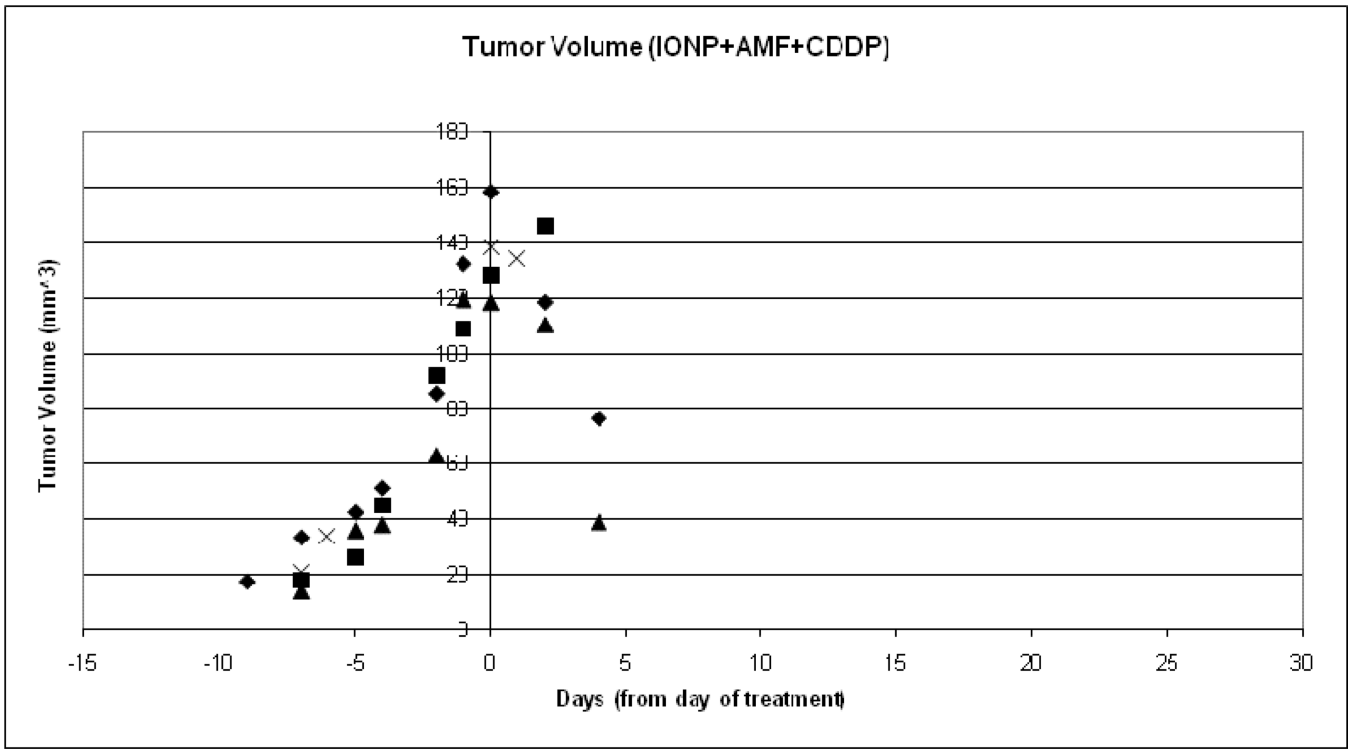

Figure 3.
Tumor volumes of mice treated with IONP, AMF and CDDP. Each symbol is an individual mouse.

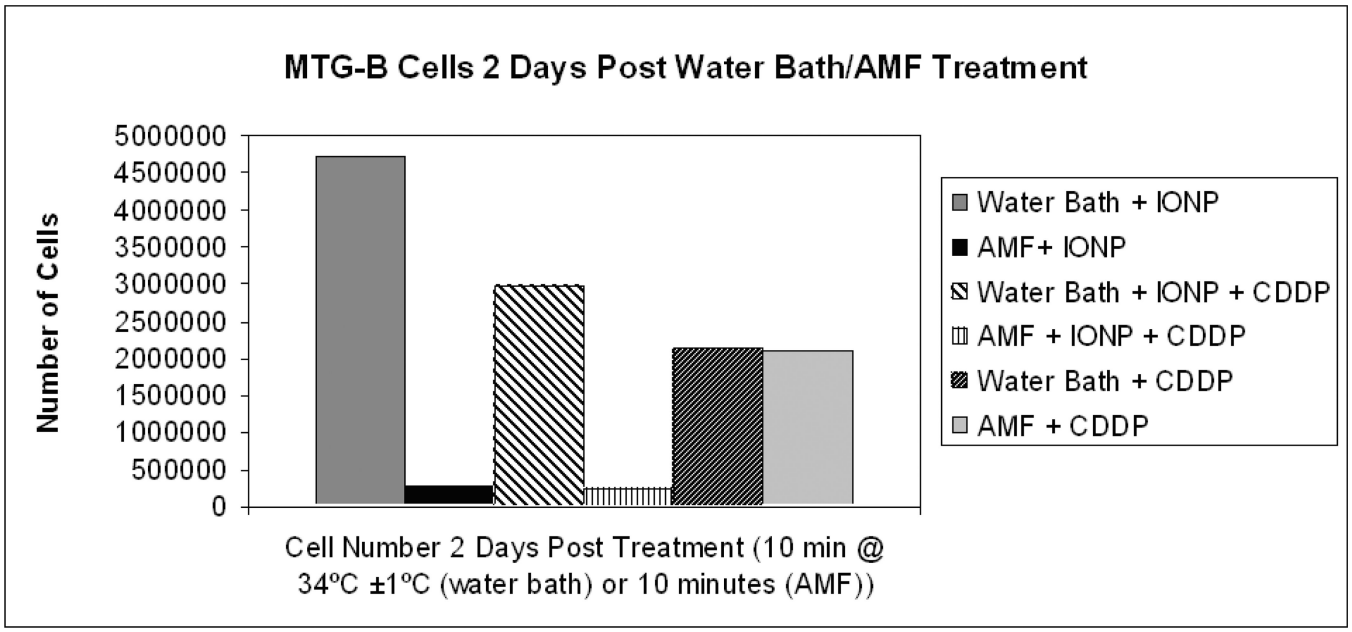

Figure 4.

The effect of IONPs, AMF, CDDP and Water Bath Heating on MTG-B cells. The greatest cytotoxicity was created by the IONP heat / CDDP and IONP heat only groups, suggesting the delivered heat dose was too much to demonstrate maximum treatment addition or synergism.