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Iron oxide nanoparticle enhancement of radiation cytotoxicity

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Abstract

Iron oxide nanoparticles (IONPs) have been investigated as a promising means for inducing tumor cell-specific hyperthermia. Although the ability to generate and use nanoparticles that are biocompatible, tumor specific, and have the ability to produce adequate cytotoxic heat is very promising, significant preclinical and clinical development will be required for clinical efficacy. At this time it appears using IONP-induced hyperthermia as an adjunct to conventional cancer therapeutics, rather than as an independent treatment, will provide the initial IONP clinical treatment. Due to their high-Z characteristics, another option is to use intracellular IONPs to enhance radiation therapy without excitation with AMF (production of heat). To test this concept IONPs were added to cell culture media at a concentration of 0.2 mg Fe/mL and incubated with murine breast adenocarcinoma (MTG-B) cells for either 48 or 72 hours. Extracellular iron was then removed and all cells were irradiated at 4 Gy. Although samples incubated with IONPs for 48 hrs did not demonstrate enhanced post-irradiation cytotoxicity as compared to the non-IONP-containing cells, cells incubated with IONPs for 72 hours, which contained 40% more Fe than 48 hr incubated cells, showed a 25% decrease in clonogenic survival compared to their non-IONP-containing counterparts. These results suggest that a critical concentration of intracellular IONPs is necessary for enhancing radiation cytotoxicity.

Keywords

Iron oxide nanoparticles; high-Z material; tumor; cancer; radiation

1. INTRODUCTION

1.1 IONP Hyperthermia

Since global hyperthermia has not proven to be consistently and inherently more damaging to cancerous cells than to healthy tissue but has shown the ability to kill tumor tissue at relatively low thermal doses, the ability to target heat to cancerous cells using iron oxide nanoparticles (IONP) excited by an alternating magnetic field (AMF) is potentially paradigm shifting^{1,2}. The efficacy of IONP-induced hyperthermia relies on the ability to deliver biocompatible nanoparticles in therapeutically relevant concentrations specifically to

tumors before activation with a safe external AMF. The challenge of delivering tumor-specific nanoparticles intravenously is being addressed worldwide, and although great progress is being made, generation of IONP that are biocompatible, tumor specific and that can reach thermally ablative concentrations in the tumor has not yet been achieved³. Therefore, it is currently prudent to use IONP-induced heating at sub-lethal doses as an adjunct to conventional cancer therapeutics.

1.2 Hyperthermia and Radiation

It has previously been shown that hyperthermia and radiation act synergistically to increase the cytotoxicity of radiation. While cancer cells are more sensitive to radiation damage than healthy cells due to their poor repair mechanisms, it is believed that heat acts to intensify this sensitization by further damaging the proteins responsible for repair. Hildebrandt et al. demonstrated that exposing cancerous tissue to 42 °C heat for increasing lengths of time decreased the surviving fraction of cells post-irradiation, and this effect was maximized when the heating occurred immediately before irradiation rather than several hours before or after⁴. It has already been demonstrated that IONPs are capable of delivering cytotoxic heat, so their use in providing radiation-sensitizing heat doses is promising. Additionally using IONPs to enhance radiation therapy would allow dual use of IONPs and increase the level of treatment synergy.

1.3 Radiation Enhancement

Radiation enhancement has previously been achieved by several groups using nanoparticles located in or around a tumor. Kong et al studied the cytotoxic effects of gold nanoparticles during X-ray irradiation, showing that intracellular gold nanoparticles increased the cytotoxicity of X-ray irradiation over extracellular nanoparticles with irradiation and irradiation alone⁵. Hainfeld et al administered gold nanoparticles to mice intravenously, achieving 8 times higher concentrations of gold in tumors than in healthy tissue for the duration of the X-ray irradiation. One-year survival of the mice was significantly increased over treatment by radiation alone and gold nanoparticles alone⁶.

Similar technology is in clinical trials in Europe, where hafnium oxide particles are being utilized to widen the therapeutic window of traditional radiation therapy. When X-ray irradiation interacts with atoms in these high-Z particles, electrons are ejected from the outermost shells, leading to DNA damage above that induced by free radicals formed from water. While these hafnium oxide particles have shown to be successful at enhancing radiation, they are not designed for hyperthermia therapy.

It is possible that the high-Z characteristics of iron oxide could also be utilized to increase the effective intracellular irradiation dose when IONPs are located within a cell at the time of irradiation⁷. This would eventually allow for the enhancement of radiation and production of heat from the same particles located within a tumor. As with IONP-induced hyperthermia, it is expected that the amount of intracellular iron will have an impact on the radiation enhancement achieved. Therefore, maximizing intracellular iron is still likely to be desirable. The questions of IONP uptake over time and potential for radiation enhancement are investigated here.

2. METHODS

2.1 Cell culture

Murine breast adenocarcinoma (MTG-B) cells were cultured in Minimum Essential Medium (MEM) with 1% Pen-Step, 1% L-Glutamine and 10% fetal bovine serum at 37°C. Cells were trypsinized, counted, and approximately 10^6 cells were plated in each well of a 6-well tissue culture dish with complete MEM. Cells were allowed to rest at least overnight before nanoparticles were added.

2.2 IONP uptake kinetics

Iron oxide nanoparticles (IONPs)(100 nm starch-coated particles, 26.5 mg Fe/mL, Micromod Partikeltechnologie GmbH, Rostock Germany) were added to complete MTG-B media (alpha-MEM) at a concentration of 0.2 mg Fe/mL. At the appropriate incubation time point, media was removed from each well and replaced with IONP-containing media. Cells were allowed to incubate with IONPs for 0, 1, 4, 6, 12, 24, 48 or 72 hours. At the end of incubation, extracellular IONPs were washed away with three PBS washes, allowing five minutes of incubation between each wash. The cells in two wells per group were counted using trypan blue dye, and four wells per group were then digested with Trace Metal Grade(TM)G-HCl, and intracellular iron was quantified by ICP-MS.

2.3 Radiation enhancement

IONPs were added to complete alpha-MEM and incubated with plated MTG-B cells for either 48 or 72 hours. Control groups without nanoparticles were incubated in fresh complete alpha-MEM for the same time. Extracellular iron was removed with three PBS washes, allowing five minutes of incubation between each wash. For each group, two wells were counted using trypan blue dye, two wells were digested with TMG-HCl, and six wells were either irradiated with 4 Gy (1 Gy/min) of 662 keV cesium-137 gamma irradiation or left on the benchtop for the duration of the treatment. Cells were allowed to rest for one hour in a 37 °C incubator post-irradiation.

Following rest, four wells per treated group were trypsinized and replated in triplicate at concentrations of 100 and 500 cells per well based on pre-irradiation counts. Cells were incubated in complete MEM for approximately two weeks or until colonies were apparent. Media was then removed and the colonies were stained with methylene blue in 10% methanol and counted.

2.4 Statistics

Each colony counted was considered to be one cell surviving post-irradiation. Percentage survival of irradiated groups was normalized to percentage survival of the corresponding non-irradiated group. The irradiated groups with and without nanoparticles were then compared with an unpaired student's t-test.

3. RESULTS

3.1 IONP Uptake Kinetics

IONP uptake kinetics in murine breast adenocarcinoma (MTG-B) cells were studied *in vitro* to determine appropriate incubation times pre-irradiation. A trend of increasing intracellular iron concentration is observed with increasing incubation time through 72 hours, and the difference between intracellular iron concentrations with 24 and 48 hour incubation is significant ($p < 0.0001$).

3.2 Radiation enhancement

Post-radiation cytotoxicity was measured by clonogenic assay. As shown in Figure 2, in samples that had incubated with IONPs for 48 hours, there was no statistically significant difference in cell viability between IONP-containing cells and control cells post-irradiation. However, with 72 hours of incubation, IONP-containing cells demonstrated a 25% decrease in clonogenic survival as compared to cells without IONPs (Figure 3). The number of surviving colonies in irradiated groups was normalized to non-irradiated surviving colonies to account for differences between trials, but no significant difference in cell survival was observed between IONP-containing and non-IONP-containing cells that were not irradiated. These results suggest that a critical concentration of intracellular IONPs may be capable of enhancing the cytotoxic effects of cesium irradiation.

4. DISCUSSION

Our preliminary results show that intracellular iron levels (IONP uptake) are not significantly different for 48 and 72 hour incubation times (Figure 1). However, there is a very strong upward trend, suggesting that additional studies will convincingly show that significantly more intracellular IONP are present after the 72 hour incubation period as compared to the 48 hour period. Samples incubated with IONPs for 48 hours showed no significant enhancement in toxicity with radiation (Figure 2), while samples incubated for 72 hours resulted in 25% enhancement with $p < 0.08$ over cells irradiated without IONPs (Figure 3). When taken together these results indicate that intracellular IONPs enhance the intracellular iron concentration with a threshold for enhancement.

Since it is unlikely that 100% of the extracellular IONPs were washed away before irradiation, it is possible that a portion of the iron associated with each cell was located extracellularly. If extracellular iron remained associated with the cells after washing, then it was measured in the iron assays and considered intracellular. While the quantity of extracellular iron was not measured explicitly in these experiments, it is likely that the quantity of extracellular iron was comparable in both groups and present in amounts much smaller than intracellular iron. At this time, it is not known whether extracellular IONPs contribute to post-irradiation toxicity.

In addition to higher levels of intracellular iron, the 72 hour incubation time may have resulted in different intracellular groupings of iron as compared to samples incubated for 48 hours, although nanoparticle configurations were not characterized here^{8,9}. Changes in configuration may have included aggregation or dispersion of IONPs within the cell,

average distance from the nucleus, or the onset of degradation of the starch coating of the particles.

While the difference in post-irradiation cell viability between nanoparticle-containing cells is not conventionally statistically significant, most of the error is due to initial cell counts conducted via hemacytometry. As such, further trials will be conducted in order to reduce the propagated error and increase significance of the result. Studies with extracellular iron will also be conducted to determine a critical distance from nanoparticle to nucleus necessary to see enhancement.

Future studies will also investigate a range of radiation energies used with intracellular nanoparticles. Our cesium gamma irradiation delivers approximately 600 keV during therapy, whereas the X-ray irradiation of Kong et al and Hainfeld et al utilized 200-250 kVp radiation. Radiation used in therapy tends to be of even higher energy. Recent studies suggest that nanoparticles such as our IONPs are best able to enhance irradiation at frequencies in the x-ray range rather than gamma irradiation's range. While cesium gamma irradiation is commonly used to treat tumors, use of lower frequency x-ray irradiation may result in a more distinct therapeutic ratio when IONPs are used as enhancers. Further work is being conducted in order to optimize the combination of intracellular iron and radiation energy that will maximize the therapeutic ratio of radiation therapy.

5. CONCLUSIONS

Iron oxide nanoparticles were investigated for their ability to enhance ^{137}Cs irradiation cytotoxicity in MTG-B cells. Cells incubated with IONPs for 72 hours demonstrated a 25% decrease in clonogenic survival post-irradiation as compared to cells irradiated without IONPs, whereas no significant difference was observed for cells incubated with IONPs for 48 hours. These results suggest that intracellular IONPs can enhance the efficacy of radiation treatment at the 137 Cesium energy of 662 keV. Theoretical calculations and recently published data suggest this effect may be further enhanced at lower radiation energies.

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Figure 1.
IONP uptake per cell at various incubation times. Values shown as mean with error bars as SE, N=4. * $p < 0.0001$, unpaired

Figure 2. Clonogenic survival of cells incubated with IONPs for 48 hours pre-irradiation. Values shown as mean with error bars as SE normalized to non-irradiated cells, N=16.

Figure 3.

Clonogenic survival of cells incubated with IONPs for 72 hours pre-irradiation. values shown as mean with error bars as SE normalized to non-irradiated cells, N= 16. * $p < 0.08$, unpaired t-test