Investigating the Role of Magnetic and Traditional Hyperthermia in Cancer Treatment

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Abstract

The results of traditional hyperthermia in combination with radiotherapy or chemotherapy were compared to results from treatments that just used chemotherapy or radiotherapy alone in order to determine if there was a statistically significant purpose of using traditional hyperthermia. Clinical trials of magnetic hyperthermia implemented in mice and hamster ovaries were analyzed to determine if the treatment has been effective in cancer treatment and if it can potentially be implemented into humans. Traditional hyperthermia trials were statistically analyzed and influenced the conclusion that it is significantly better than treatments without the traditional hyperthermia. Magnetic hyperthermia trials were analyzed and compared to each other in order to reach the conclusion that magnetic hyperthermia is, in fact, effective in cancer treatment but many limitations and side effects need to be considered before implementation in humans.

Introduction

With the rise of diseases that are harmful to humans comes the need for treatments or cure for these diseases. Cancer is one of the largest and most widespread of these diseases and causes many deaths around the world. It has been occurring since the 1700s and continues to kill millions every year (Cancer Statistics, 2018). Currently there are many treatments for cancer such as chemotherapy, radiotherapy, surgery, immunotherapy, bone marrow transplant, and cryoablation; the most relevant ones being chemotherapy and radiotherapy. Chemotherapy is a drug treatment used to kill fast-growing cells such as cancer cells. Chemotherapy is given to patients intravenously and uses a variance of different drugs in order to treat the patient.

Radiotherapy is a treatment that uses high quantities of radiation in order to kill cancer cells or reduce the size of tumors. Radiation therapy targets the cancer cell's DNA in order to make the cell stop functioning and stops the cell from multiplying and living. Although all current cancer treatments are effective to some extent, none of them provide a permanent treatment of the cancer and they also cause a certain degree of side effects that reduce the patients quality of life after the treatment (Cancer Treatments, n.d.). Recently, a treatment for cancer known as hyperthermia or traditional hyperthermia (TH) has begun to be researched and experimented with. Magnetic hyperthermia (MH) has also become a new field of research for scientists but is completely theoretical in cancer patients at the moment.

Traditional hyperthermia is a treatment for cancer that uses heat to raise the temperature of cancer cells and kill them off. There are currently three different types of TH used in clinical trials: local hyperthermia, regional hyperthermia, and whole body hyperthermia. Local hyperthermia uses large amounts of heat in order to heat up up a small part of the body such as a tumor. It uses different types of energy in order to accomplish this such as microwaves, radio waves, and ultrasound (Hyperthermia in Cancer Treatment, 2011). Depending on where the tumor is, different methods are used in order to implement local hyperthermia. Tumors just below the skin are treated with machines that are outside the patient's body. This is known as external hyperthermia. If the tumor is in or near a body cavity, then intraluminal local hyperthermia is used. In this form of local hyperthermia, probes are placed within the cavity in order to directly deliver the heat to the tumor. Radiofrequency ablation hyperthermia is used to treat tumors that are deep within the body such as brain tumors. This type of hyperthermia allows for higher temperatures to be achieved and for that reason, is the most common type of

hyperthermia treatment that is used. It uses high frequency radio waves that are omitted through a high frequency current which is produced by a thin probe placed in the tumor (Hyperthermia to treat Cancer, 2016). Regional hyperthermia is used to treat larger parts of the body that have been affected by cancer. Regional perfusion, a type of regional hyperthermia can be used in order to treat arms, legs, lungs, and livers affected with cancer. In this procedure, a portion of the patients blood is removed from the organ infected. The blood is then heated to a temperature at which the cancer cells are theorized to die and then the blood is inserted back into the patient. While the blood is being inserted back, typically chemotherapeutic drugs are also registered inside of the patient, showing a way hyperthermia and chemotherapy can be combined. Another form of regional hyperthermia is continuous hyperthermic peritoneal perfusion (CHPP), which is used to treat intestines, the stomach, and other organs located within the abdomen. During the treatment, anticancer drugs are heated and administered to the patient through a peritoneal cavity (Hyperthermia Treatment, n.d.). Lastly, the most extreme form of traditional hyperthermia is known as whole-body hyperthermia which is used to treat cancer that has spread throughout the body. Thermal chambers and hot water blankets are used in this treatment to achieve high temperatures throughout the body. Unlike MH, TH has been applied and will continue to be applied to humans, although many of the ideal benefits are still theoretical.

Magnetic hyperthermia is based off of the fact that magnetic nanoparticles (MNPs) and magnetotactic bacteria (MTB) can convert electromagnetic energy from an alternative high frequency magnetic field into heat. This heat is then applied to the cancer cells which damages the nucleus of the cells and causes the enzymes to denature (Akbarzadeh et. al, 2012). The enzymes stop functioning and eventually cause the cancer cells to stop multiplying and die off.

MH is not a direct treatment to cancer though, as it can only be used in conjunction with chemotherapy or radiotherapy (Dietzel, 1983). One of the main problems with chemotherapy and radiotherapy is that it is often hard to get full exposure of the part of the body infected with cancer and therefore a large dose of radiation is required in order to treat the cancer sufficiently. Magnetic hyperthermia provides a solution to this problem as it helps in the exposure of the organ infected or the tumor allowing for higher quality treatment, better quality of life after the treatment, less side effects, and less radiation or drug usage required.

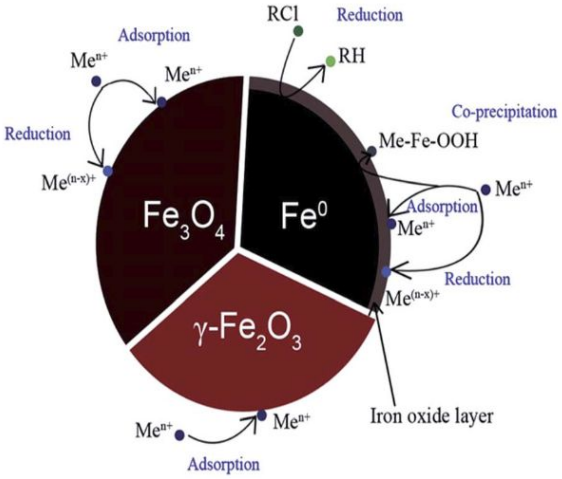

Fig. 1 Basic structure of a magnetic nanoparticle (Kharisov et. al).

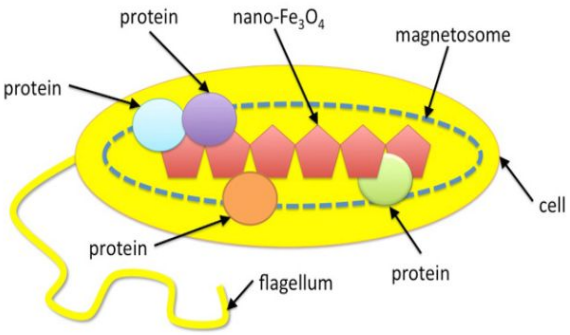

Fig. 2 Basic structure of magnetotactic bacterium (Chen et. al).

The two main substances that are the basis of magnetic hyperthermia are magnetic nanoparticles and the magnetosomes within the magnetotactic bacteria. MTB were first discovered in 1975 by Richard P. Blakemore in Massachusetts, USA. Magnetotactic bacteria are gram-negative prokaryotes that align themselves to the Earth's geomagnetic field and have many types and functions. The various types of bacteria include "coccoid-to-ovoid cells, rods, vibrios, and spirilla," each one of these having their own separate and unique functions (Lefevere & Bazylinski, 2013). Each type and size also specializes at certain tasks and for that reason,

scientists use different species of MTB based on the task. MTB is very accessible as it naturally occurs in most freshwater lakes, and ponds, especially ones that have a slow stream and a soft, muddy sediment layer (Oestreicher et al., 2015). MTB can be cultivated by anyone as the process to collect and separate them is very simple and does not require many advanced tools. As shown in Fig. 2, the basic structure of the magnetotactic bacterium is somewhat similar to the makeup of a typical bacteria. It is a rod-shaped bacteria which has a nano-iron oxide chain with proteins attached to it. This is all encapsulated by the magnetosome which is all within the cell and the flagellum attached to it. The most vital part of MTB are the magnetosomes which allow for the MTB to be used in magnetic hyperthermia. Magnetosomes are intracellular nanocrystals that "are nanometer-sized, membrane-bound crystals of the magnetic iron minerals magnetite (Fe_3O_4) or greigite (Fe_3S_4)" (Lefevre et. al, 2011). These crystals are embedded within the phospholipid bilayer membrane of the MTB. The MTB biomineralizes these magnetosomes which ultimately results in the magnetite formation which is used within the MH.

MNPs are another particle with which magnetic hyperthermia can be conducted. MNPs are nanoparticles that can be controlled by using a magnetic field. They consist of two main parts: a magnetic material and a chemical compound that makes up the nanoparticles. Magnetic nanoparticles measure approximately 1-100 nm in size, so they are larger than MTB. They do, however, have many benefits over magnetotactic bacteria. They are more compatible with antibodies and have better inductive heating properties than MTB, making them more applicable and efficient in magnetic hyperthermia. As shown in Fig. 1, the MNP has reduction, oxidation, co-precipitation, and adsorption occurring within it, making it a very versatile particle. Antibodies are proteins mainly produced by plasma cells that are used to target and fight off

pathogenic bacteria and viruses such as cancer (. Pairing the MNPs with these antibodies is beneficial as it makes the treatment even more successful than MH with the MTB. MTB have limited inductive heating properties. As seen in Fig. 3, in order to be applied effectively in hyperthermia MNPs are injected intravenously alongside chemotherapeutic drugs as well as active targeting agents. These agents are the substance that lead the MNP fluid to the site of the

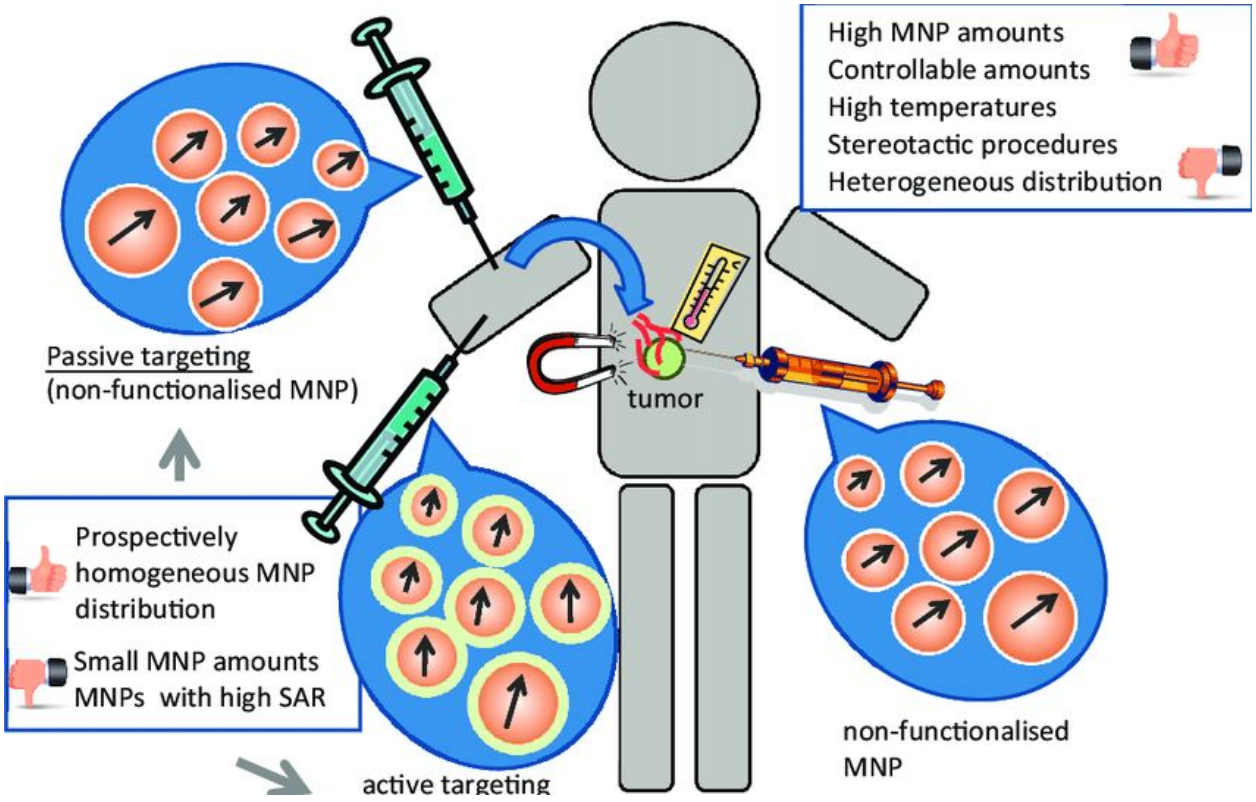

Fig. 3 Intravenous application of magnetic hyperthermia using MNPs and chemotherapeutic drugs in combination.

tumor. The MNPs within the tumor are then controlled by an alternative high frequency magnetic field which is represented by the magnet in Fig. 3. The non-functionalized MNPs are then extracted out of the patient's bloodstream to prevent any additional side effects using the active agents once again.

Purpose

Cancer contributes largely to the number of deaths by disease each year, and in order to address it a new treatment has to be developed, understood, tried, and perfected. The purpose of this study is to investigate the role of magnetic hyperthermia in cancer treatment in mice. Magnetic hyperthermia is theoretical at the moment and has been tested in humans only twice. In order to be implemented into humans, it needs to be proven to be effective and its limitations have to be considered. This study goes over the results from non-human in vivo clinical trials that have occurred. Some of these studies are compared to cancer treatments without the hyperthermia in order to show the effectiveness of the magnetic hyperthermia in cancer treatments.

Research Question

A. Is magnetic hyperthermia with MNPs in combination with radiotherapy or chemotherapy effective in mice, F344 rats, and hamsters in cancer treatments?
B. Is traditional hyperthermia in combination with radiotherapy or chemotherapy effective as compared to radiotherapy or chemotherapy used alone in cancer treatments?

Hypothesis (A)

Magnetic hyperthermia with MNPs in combination with radiotherapy or chemotherapy is effective in treating cancer in mice, hamsters, and F344 rats.

Alternative Hypothesis (B)

Traditional hyperthermia in combination with chemotherapy or radiotherapy is an effective treatment as compared to radiotherapy or chemotherapy used alone in cancer treatment.

Null Hypothesis (B)

Traditional hyperthermia in combination with chemotherapy or radiotherapy is not an effective treatment as compared to radiotherapy or chemotherapy used alone in cancer treatment.

Methods

Data Sources

The design of this study was a systematic literature review. Google Scholar, Ebscohost, PUBMED-NCBI, ResearchGate, Science Direct, PLOS, PLOS ONE, etc. were searched to gather studies investigating the role of magnetic hyperthermia in cancer treatment and its effectiveness. In order to gather relevant articles, keywords including “magnetic hyperthermia,” “magnetic nanoparticles,” “magnetotactic bacteria,” “magnetic hyperthermia in mice,” etc. were used. Further literature was found using references of articles that had already been gathered.

Filtering of Data Sources

This study focused primarily on the trials of magnetic hyperthermia which were done with MNPs as opposed to MTB. A special advisor, Dr. Ian Baker, influenced this choice of only presenting data regarding trials of magnetic hyperthermia done using only MNPs. He explained that magnetic nanoparticles are more effective in cancer treatment and are more relevant due to

the fact that they have high specific absorption rates of electromagnetic energy and that they are easier to conjugate with antibodies. This led to an exclusion of all data that regarded trials if magnetic hyperthermia done in vitro using MTBs. All papers used in this study were pertinent, full-text, peer-reviewed articles. If any source was seen to have some form of inconsistency in facts confirmed in many other papers, the paper was excluded.

Data Extraction

In order to effectively answer the research question, relevant and reliable data was extracted from various peer reviewed articles. The data extracted focused on the temperature the MH was carried out at in degrees Celsius, the percentage of cancer cells that survive after the treatment, the number of patients that are reported to have a decrease in tumor size or number of cancer cells (percent response), or the state of the cancer cells after the treatment. Only one of the figures (Fig.3), goes over the treatment being implemented in humans and compares results treatments with and without magnetic hyperthermia. The rest of the data extracted goes over treatments conducted in mice, hamsters, and Fischer 344 rats (F344 rats). The F344 rats are used since they are effective not only in determining the success of the therapy but also the side effects such as toxicity. Overall, the studies from which data was extracted and information was obtained were deeply analyzed for credibility and accuracy in order ensure reliable answers to the research questions.

Statistical Analysis

In order to determine if the treatments with traditional hyperthermia in conjunction with chemotherapy or radiotherapy were significantly better than the treatments that involved

chemotherapy or radiotherapy alone, a statistical analysis test was used. In this study a one tailed t-test was used in which unequal variances were assumed. A p-value $\leq .05$ was considered to be significant, meaning that if the p-value was greater than .05 then the null hypothesis was accepted and the alternative hypothesis was accepted, and if the p-value was less than .05 then the null hypothesis was rejected and the alternative hypothesis was accepted. Means and standard deviations were also calculated to show the spread of the data from the results of ten different studies. This analysis contributes to previous studies as no review or article has analyzed the significance of the effect of traditional hyperthermia using a t-test.

Results and Discussion

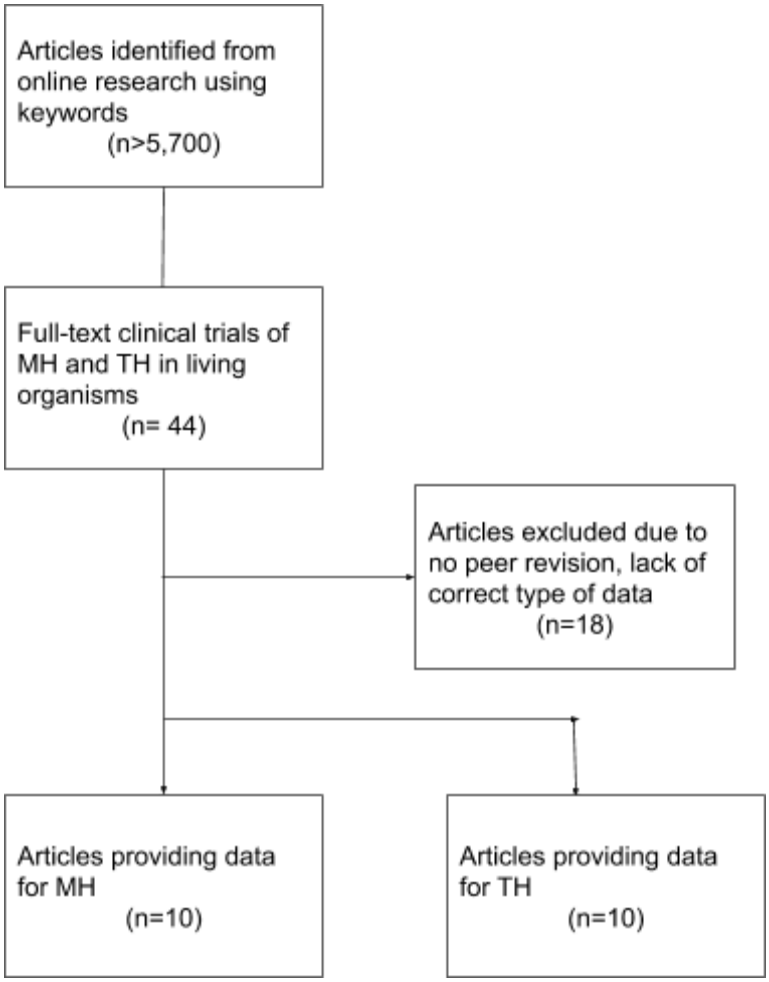

Figure 4. Diagram displaying process of article collection to find data used to answer the research question and to use in the statistical analysis.

The search results from various databases provided over 5,700 articles about magnetic and traditional hyperthermia treatments. 44 of these papers were deemed eligible to conduct this study due to the strict inclusion and exclusion criteria.

Of these 44 studies, 24 were excluded due to detected bias, lack of credibility, or incorrect type of data present. In these remaining twenty studies, ten were found to contain data supporting hypothesis (a) regarding MH and the other ten were found to contain data supporting hypotheses (b) regarding TH.

Table 1. Data comparison showing the results from different studies in which MHT was applied to F344 mice that were injected with glioblastoma cells (Sources 3, 4, 5, 6, 7, 8).

Study Extracted From	Temperature Reached (degrees Celsius)	Duration of AMF exposure (min)	Results
Yanase et. al (A)	43	60	Complete glioma cell death
Yanase et. al (B)	43-44	30	Necrotic tumor cells, some animals displayed complete tumor regression
Yanase et. al (C)	43-44	30	Some animals displayed complete tumor regression
Shinkai et. al	43-44	30	Some animals displayed complete tumor regression
Ito et. al	42	30	Reduced tumor growth
Ohno et. al	44.4	30	Significant tumor cell death
Jordan et. al	45	40	Necrotic tumor tissue

As seen in Table 1, the different studies tended to cover a similar range of temperatures, excluding Jordan et. al's study. Yanase et al.'s first study in 1997 actually ended up killing off all of the glioblastoma cells and resulted in a complete success in the removal of the cancer although the treatment did take 60 minutes as opposed to the 30 or 40 of the rest of the studies.. Then when the treatment was conducted at 43°-44° by Yanase et. al, some animals showed a complete tumor regression meaning that the cancer was completely treated. Others in the study had

necrotic tumor cells, meaning cells were self-destructive and that the cells died off to some extent. In two other studies, one conducted by Yanase et. al and the other by Shinkai et. al, also at 43°-44° , the treatment was a success again as some of the animals displayed complete tumor regression. Ito et. al conducted the treatment of glioblastoma at 42° using magnetic hyperthermia with MNPs which resulted in reduced tumor growth . Ohno et. al, conducted a study that reached the temperature of 44.4°, in which there was a reported significant number of tumor cancer cell deaths. Ultimately, all of the studies tended to result in either complete treatment of the cancer, necrotic tumor cells, or reduced tumor growth, all of these being positive and improved results.

With the relevant success of each studies research and each different temperature tested, the ideal temperature range of these studies tended to be 43°-44° as almost all studies covered this temperature range. This range also provided the most optimal results of tumor reduction and cancer cell death. This indicates that MH was a success in treating mice that were injected with glioblastoma cancer cells. The outliers within the data showed that as time of treatment increased, the results became more ideal and in some instances, led to complete treatment of the glioma.

Table 2. This table shows the effect of various temperatures on cancer cells in Chinese hamster ovaries. The following data represents the % living cancer cells after the treatment (Connor et. al, 1977; Dewey et. al, 1973; Harisidias et. al, 1975).

Temperature (degrees Celsius)	% cancer cells survived
41.5	50

42.5	.01
43.5	.02
44	.06
45.5	.06

As indicated in Table 2, the most effective temperature range of when the treatment occurred was at 42.5 - 43.5°C as in that range less than one percent of the cancer cells survived after the treatment. When the treatment was carried out at 41.5°C, 50% of the cancer cells survived, showing the treatment wasn't very effective at this temperature. When the treatment was applied at a temperature of 42.5 degrees celsius merely .01 percent of the cancer cells survived and at 43.5°C, .02 percent of the cancer cells survived. 44-45° was also an effective temperature range but the studies this data was extracted from indicated that in order to reach these results the treatment required longer periods of time when the treatment was done at these temperatures. Overall, past 42 degrees Celsius the treatment was effective in killing the cancer cells in the Chinese hamster ovaries, displaying an example of a success of MH.

As shown in Table 2, the ideal temperatures tested were 42.5°, 43.5°, and 44° as they resulted in the least percentage of cancer cells remaining within the Chinese hamster ovaries. 45.5° was also an effective treatment temperature but the study this result was extracted indicated that in order to reach the mentioned result, the treatment had to be applied longer than it had for the other temperatures. MH within the in vivo treatment was an overall success, not

only in treating the cancer cells, but also in providing a somewhat optimal temperature range for the treatment to be applied. This result also suggests that once side effects and limitations are taken into consideration and dealt with, MH has a large potential to be implemented in human cancer patients.

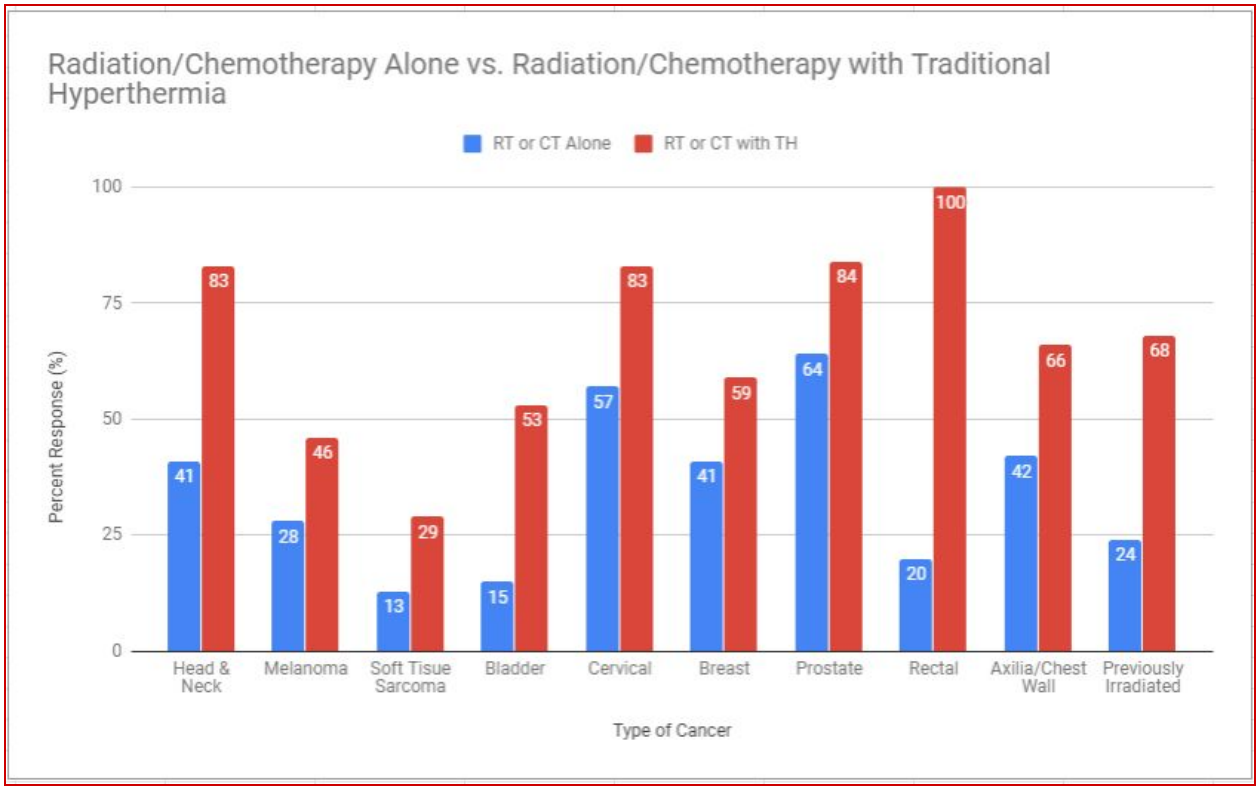

Fig. 5 This bar graph shows the treatment results in humans for traditional hyperthermia in different types of cancer. Percent response is the number of patients that were recorded to have a decreased tumor size. RT- radiotherapy, CT- chemotherapy, TH- traditional hyperthermia (Valdagni et. al, 1994; Overgaard et. al, 1995; Issels et. al 2010; Colombo et. al, 2012; Van der Zee et. al, 2000; Vernon et. al, 1996; Hurwitz et. al, 2011; Kakehi et. al, 1990; Zagar et. al, 2010).

As depicted in Figure 6, 10 different types of cancers were treated across a multitude of studies, all of them showing improved results when the TH was applied in combination with radiotherapy (RT) or chemotherapy (CT) to the patients. The percent response of Head & neck tumors, soft tissue sarcoma cells, bladder cancer cells, and previously irradiated cancer cells more than doubled when the TH was applied. When rectal cancer was treated with TH and RT or CT the percent response was 100 percent meaning every single patient treated was shown to have some decrease in amount of functioning cancer cells, an increase in necrotic tumor cells, or a decrease in overall tumor size. Melanoma, breast, axilla, cervical, and prostate cancer, when treated with TH and CT or RT all also showed a significant increase in percent response. Ultimately, the treatments with TH had a positive overall effect on the percent response and number of successful treatments.

When comparing the control group to the combination group it is evident that a higher percentage of patients were reported to have some extent of success when they were treated, as shown in Figure 6. There was some success in both groups but the combination treatment group had much better results overall, with a 36.6% better response rate on average. These trials were all conducted in real human cancer patients or voluntary cancer-injected patients, suggesting that TH also has the potential to be further implemented into humans. Further research regarding control of temperature of the tumor and surrounding body parts is required but TH is promising in becoming a standard treatment for cancer patients.

Table 3. T-test results for Fig. 5.

T-test: One-tailed, assuming unequal variances

	Combination Treatment Group	*RT or CT only Group*
Mean	67.1	34.5
Variance	21.18	17.39
P-value (both)	$7.54x10^{-4}$	
Null Hypothesis	Reject	

The average percent response of the combination treatment group was 67.1 while the RT or CT group had a mean of only 34.5. On average, the combination treatment group was 36.6% better than the control group. The standard deviation or variance of the combination treatment group was 21.18 while it was 17.39 for the RT or CT group. The TH is more efficient in treating specific types of cancers and tumors explaining this large variance in the data. The p-value of the t-test was $7.5x10^{-4}$ which is below .05. This indicates that the null hypothesis must be rejected and the alternative must be accepted. The combination group was indubitably more effective in cancer treatment than the CT or RT only group was and the statistical analysis conducted supports this alternative hypothesis.

Limitations

The lack of clinical trials conducted using magnetic hyperthermia and traditional hyperthermia was one of the major limitations of this study. Magnetic hyperthermia has yet to be applied to humans due to its various side effects and complications. Only data regarding MH in

mice, hamsters, and rats was present, and that was also quite limited. Many studies focused on the amount of time it took to heat up the tumors but not the actual reduction of the size of the tumor or the decrease in number of cancer cells. Due to this, research question(a) had to be limited to in vivo studies but still locating data covering results of the studies was a limiting factor. Also, many papers were excluded due to this restriction, leading to a further lack of data.

Traditional hyperthermia, although clinically tested, has only been experimented with in a limited number of studies. Very few clinics have the tools and the resources in order to attempt to achieve successful TH trials on voluntary patients. The treatment is not yet a standard therapy for cancer and for that reason, studies done on the application of this therapy in cancer patients, is limited. The most prevalent TH done currently is radiofrequency ablation, meaning other forms of TH are used less, researched less, and therefore cited less.

Conclusion

This systematic literature review provides evidence supporting the hypotheses provided in this study that a) Magnetic hyperthermia is effective in treating cancer in mice, hamsters, and rats and b) Traditional hyperthermia in combination with chemotherapy or radiotherapy is an effective treatment as compared to radiotherapy or chemotherapy used alone in cancer treatment. Statistical analysis resulted in a p-value of less than .05, indicating the rejection of the null hypothesis. All papers analyzed in this paper support mentioned hypotheses, suggesting that traditional hyperthermia can continue to be implemented and tested in humans and that with further research, MH has the potential to also be applied into humans. Studies analyzed regarding MH also suggested that the temperature range of 42°-44° is the ideal temperature range for the

treatment to be applied, at least in vivo. Traditional hyperthermia is most efficient when applied in combination with radiotherapy or chemotherapy as it heats up the cancer cells and amplifies the effect of the the other therapies.

Further Work

To further contribute to this study, MH can be further applied into more in vivo and in vitro experiments. Further research is required for MH to be fully viable in humans due to the toxicity of the nanoparticles and the MTB. Guidelines for further research include the increase of focus on the temperature range of 42°-44° as papers analyzed in this study were recognized to cover this temperature range. Also, this range provided the most promising results for future applications of MH, especially in vivo experiments. There is a large amount of cytotoxicity contained within the MNPs and MTB which can potentially result in large side effects. Cytotoxicity is the ability of a bacteria or particle to be toxic to a cell, meaning it is detrimental for the patient's beneficial cells that are present at the site of the tumor or organ affected with cancer to be exposed to the MNPs and MTB. The side effects of this toxicity and what can be done to limit or eliminate it is also a field that requires further research.

Another further research path could be considering the time the treatment was used and if there is a correlation between the time and results of treatment. This study focused only on the temperatures used but time was not taken into consideration in any of the tables or figures with the exception of Table 1. Duration of treatment is important as not only does it influence the actual outcome but it also could allow for more side effects to occur in the patient which would decrease the patients quality of life, hurting the treatments potential to be implemented in

humans further. This further research should be conducted regarding both TH and MH as both treatments require times to be considered.

As mentioned, there are various different types of metal material that can make up MNPs. Further research could be dedicated to determining which of the magnetic materials used within the magnetic nanoparticles is the most efficient and causes the least side effects. The various magnetic materials include iron, cobalt, nickel, and even gold. This study focused on only the iron oxide MNPs due to the fact that at the current time, it is the most researched and experimented with. MNPs containing the other magnetic materials, however, are currently being researched further and experimented with more.

As for the traditional hyperthermia, further research should focus on control of the temperatures achieved for tumors that are deep within the body. Currently, hyperthermia is not a standard treatment for cancer due to the fact that it is difficult to reach temperatures above 43°. The ideal temperature range that is theorized to achieve the best possible results is 44°-46° but this range cannot be implemented currently. Research into technology being developed in order to achieve these higher temperatures could potentially lead to higher rates of clinical applications of traditional hyperthermia as well.

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