Thermal potentiation of chemotherapy by magnetic nanoparticles

Clinical studies have demonstrated the effectiveness of hyperthermia as an adjuvant for chemotherapy and radiotherapy. However, significant clinical challenges have been encountered, such as a broader spectrum of toxicity, lack of patient tolerance, temperature control and significant invasiveness. Hyperthermia induced by magnetic nanoparticles in high-frequency oscillating magnetic fields, commonly termed magnetic fluid hyperthermia, is a promising form of heat delivery in which thermal energy is supplied at the nanoscale to the tumor. This review discusses the mechanisms of heat dissipation of iron oxide-based magnetic nanoparticles, current methods and challenges to deliver heat in the clinic, and the current work related to the use of magnetic nanoparticles for the thermal-chemopotentiation of therapeutic drugs.

KEYWORDS: magnetic fluid hyperthermia ▪ magnetic nanoparticle ▪ synergy ▪ thermal potentiation

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Magnetic nanoparticles & magnetic fluid hyperthermia

Magnetic nanoparticles typically used in biomedical applications consist of small (5–20 nm diameter) inorganic crystals of a magnetic material that is either coated with an organic layer in a core–shell configuration or embedded in an organic matrix in the form of a multicore–shell configuration. The inorganic crystals may correspond to a variety of materials known to possess either superparamagnetic, ferrimagnetic, or ferromagnetic behavior; however, the most prevalent materials are the so-called iron oxides, magnetite [1] and maghemite [1], owing to their apparent lack of toxicity and biocompatibility. In this case, the small size of the inorganic crystals results in the formation of single magnetic domain particles with superparamagnetic behavior [2]. The organic shell is typically added to confer colloidal stability [3] in aqueous and biological fluids, and as a means to add functionality, such as fluorescent tags and targeting ligands.

A wide variety of organic shells have been investigated, including polysaccharides (e.g., dextran [4–10], carboxymethyl dextran [11,12] and chitosan [5,13–16]) and biocompatible polymers (e.g., PEG [5,17–21]). In some cases, thin coatings, such as citrates [22–25] and dimercaptosuccinic acid [23,26–28], have also been used. Fluorescent tags are typically added to assist investigations of the tissue, cellular and subcellular localization of nanoparticles, using for example confocal laser scanning fluorescence microscopy. On the other hand, targeting ligands are used to enhance selective uptake of nanoparticles by tissues and cells, thereby enhancing an imaging or therapeutic outcome while minimizing side effects in nonintended tissues. Some examples of targeting ligands used with magnetic nanoparticles include simple biochemicals (e.g., folic acid) [5,17,18,29–31], antibodies [28,32,33], antibody fragments [34,35], receptor ligands [36,37] and aptamers [38–41].

The growing interest in magnetic nanoparticles for biomedical applications stems, in part, from their ability to respond to applied magnetic fields by translation (in magnetic field gradients), physical particle rotation (in alternating and rotating fields) or internal dipole rotation (in alternating and rotating magnetic fields). As a result, there is local conversion of magnetic field energy into either mechanical forces and/or torques exerted on biological structures, or thermal energy. Some of these properties, such as the possibility of externally influencing their motion and the possibility of applying mechanical forces/torques on biological structures appear to be unique to magnetic nanoparticles.

Iron oxide nanoparticles enjoy a privileged position relative to other nanoparticle platforms being developed for biomedical applications in terms of their biocompatibility. Iron oxides have been used as part of iron supplementation regimens [42] and dextran-coated iron oxide nanoparticles gained US FDA approval for commercial application as MRI contrast agents for the liver [43,44]. The above-mentioned properties of magnetic nanoparticles have enabled their application as MRI contrast agents [45,46], vectors for

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magnetically targeted drug delivery [5,47–50] and magnetically assisted gene transfection [48,51,52], actuators of cell fate through magnetomechanical actuation of cell surface receptors [27,51,53–57], agents for magnetically triggered drug release [58–61] and in cancer treatment through hyperthermia [4,6,33,34,37,62–64]. These properties make them excellent candidates for the thermal chemosensitization of anticancer drugs, the topic of this review. The following sections will provide a discussion of the mechanisms of heat dissipation of iron oxide-based nanoparticles, current methods and challenges for clinical heat applications, and current work and future perspectives regarding the use of magnetic nanoparticles for the thermal chemopotentiation of therapeutic drugs.

Mechanisms & rates of energy dissipation by magnetic nanoparticles in alternating magnetic fields

Magnetic nanoparticles are attractive for thermal sensitization of cancer cells to chemotherapeutics due to their ability to generate heat at the nanoscale. Understanding the mechanisms of such heat generation may provide the means to optimize and potentially maximize therapeutic outcomes. In the case of iron oxide, single-domain magnetic nanoparticles transform the energy of an applied alternating magnetic field (AMF) into heat by two mechanisms: internal dipole rotation and physical particle rotation (Figure 1). In both cases, there are internal or external factors that prevent the magnetic dipole from following the applied magnetic field, resulting in irreversible energy loss as dissipated heat. The dominant mechanism of energy dissipation depends on a variety of factors, but is expected to correspond to the mechanism with the shortest characteristic relaxation time.

In the first mechanism of internal dipole rotation, the magnetic dipoles are typically aligned along so-called magnetic easy axes, defined as crystal directions with minimal magnetocrystalline energy. For the dipole to change direction due to an applied AMF, the dipole must surpass an energy barrier, proportional to the so-called magnetocrystalline anisotropy constant and the particle volume. This mechanism is commonly called Néel relaxation and its characteristic relaxation time τ_N is given by:

$$\tau_N = \frac{1}{f_0} exp\left(\frac{KV_c}{k_B T}\right) \qquad \text{(Equation 1)}$$

where f_0 is a so-called attempt frequency and is commonly assumed to be 10^9 Hz, K is the magnetocrystalline anisotropy constant, V_c is the volume of the inorganic magnetic core, k_B is the Boltzmann's constant and T is the absolute temperature. The magnetocrystalline anisotropy constant in (Equation 1) depends on the nature of the magnetic material in the nanoparticle and on particle size. For example, for magnetite, a wide range of values, from close to the bulk value of approximately 11 kJ/m^3 [65,66] to over an order of magnitude higher [67,68] have been reported.

In the Brownian relaxation mechanism, particles physically rotate to align their dipoles, which are practically fixed along a crystal direction, with the magnetic field. In this case, viscous drag opposes rotation of the particle and leads to dissipation of mechanical energy in the form of heat in the fluid surrounding the nanoparticles. This mechanism is commonly called Brownian relaxation and its characteristic relaxation time τ_B is given by:

$$\tau_B = \frac{3\eta V_h}{k_B T} \qquad \text{(Equation 2)}$$

where η is the viscosity of the fluid surrounding the particles and V_h is the hydrodynamic volume of the particles.

The dominant mechanism for energy dissipation will be the one corresponding to the shorter relaxation time. Due to their distinct dependence on particle diameter, magnetocrystalline anisotropy and medium viscosity, particles below a certain critical size relaxation proceed by the Néel mechanism and above that critical size relaxation proceed by the Brownian mechanism. Figure 1 shows calculated relaxation times for the Néel and Brownian relaxation mechanisms for magnetic nanoparticles as a function of core diameter. Close to this critical diameter the particles will relax by a combination of the two mechanisms and, hence, energy dissipation will occur through a combination of the two mechanisms. Calculations of the Néel relaxation time were made for three distinct values of the magnetocrystalline anisotropy: 11 kJ/m^3, a value representative of bulk magnetite [66]; 110 kJ/m^3, a value that is an order of magnitude higher and is representative of measurements for nanoscale magnetite and for samples with magnetic interactions [68]; and 200 kJ/m^3, a value that is representative of cobalt ferrite [69]. As can be seen in Figure 1, the value of the critical diameter for transition from one dominant mechanism to another depends on the relative values of magnetocrystalline anisotropy and medium viscosity. Of these, one could control magnetocrystalline

anisotropy through selection of the magnetic material used in the nanoparticle or by using core–shell geometries. However, care must be taken to select materials with uncompromised biocompatibility if the intended application is biomedical. It is also important to realize that in a collection of particles with a wide size distribution there will be particles both above and below the threshold diameter for switching of the dominant relaxation mechanism; therefore, polydisperse collections of particles are likely to dissipate heat through a mixture of the Néel and Brownian mechanisms.

According to a theoretical calculation by Rosensweig [70], the energy dissipation rate for a given applied field amplitude and frequency can be optimized through judicious selection of particle size, modulation of magnetic relaxation time and selection of the magnetic material that the particles are composed of. This has motivated many recent studies seeking to enhance the energy dissipation rate, of which we highlight a few.

Various authors have considered changing the magnetic material used to make the nanoparticles from iron oxide to other magnetic materials, such as cobalt ferrite [71–73] or core–shell manganese oxide and cobalt ferrite structures [74]. The use of cobalt ferrite yields particles with predominantly Brownian relaxation mechanisms and with relaxation times that are close to the inverse of the typical frequencies used in magnetic fluid hyperthermia (MFH). This leads to enhanced energy dissipation. However, the intrinsic toxicity of cobalt [75] must be taken into account, along with the expectation that nanoparticles that accumulate in tissues will remain there for prolonged periods and may degrade, releasing potentially toxic cobalt ions. Furthermore, because energy dissipation by the Brownian mechanism requires physical particle rotation, under certain conditions, such as entrapment in the extracellular matrix, hindered rotation could lead to significantly lower energy dissipation rates, which is undesirable [76]. Similar arguments regarding toxicity apply to core–shell structures consisting of cobalt ferrite and manganese ferrite that have been shown to have remarkable rates of energy dissipation [77].

More recently, attention has shifted to controlled aggregation of iron oxide nanoparticles to tune particle–particle interactions, thereby increasing the effective magnetocrystalline anisotropy constant. This, in turn, shifts the optimal dissipation frequency to the typical range applied in MFH, enhancing energy dissipation. This is the subject of a recent report where energy dissipation rates as high as 2000 W/g are claimed [78]. Furthermore, one must realize that the theory by Rosensweig [70] is strictly applicable to the case of noninteracting magnetic nanoparticles and for AMFs with low amplitudes and frequencies, such that the magnetization response is linear. These assumptions are hardly applicable under actual

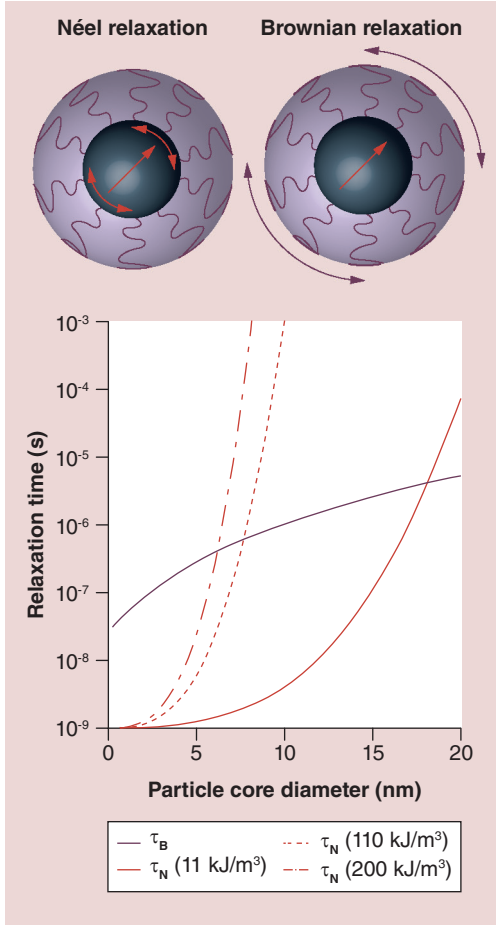

Figure 1. Magnetic nanoparticles respond to time-varying magnetic fields through either internal dipole rotation, so-called Néel relaxation, or through physical particle rotation, so-called Brownian relaxation. The characteristic relaxation times for each mechanism depend on the particle's core and hydrodynamic diameter, and the magnetocrystalline anisotropy constant of the magnetic core. Calculations shown here are for room temperature, and for the Brownian mechanism assume that the particles are dispersed in water and possess a 2-nm-thick organic shell. Representative values of the magnetocrystalline anisotropy correspond to bulk magnetite (11 kJ/m^3), nanoscale or interacting magnetite (110 kJ/m^3) and cobalt ferrite (200 kJ/m^3).
τ_B: Brownian relaxation time; τ_N: Néel relaxation time.

experimental conditions and, as such, additional work is needed to obtain a more detailed theoretical description of energy dissipation by single-domain magnetic nanoparticles in AMFs.

Clinical aspects of thermal potentiation of chemotherapeutic agents

Current methods & challenges to deliver heat in the clinic

The use of hyperthermia as a therapeutic modality dates back to the origins of medicine. This is not surprising, as our natural body response to pathogens is fever. In the case of cancer, hyperthermia treatments have been explored for more than 5000 years [79], although their translation to the clinic has only occurred during the past few decades. In the USA and Europe combined, there are more than 350 ongoing clinical trials that incorporate hyperthermia as part of the treatment [201,202]. Furthermore, hyperthermia has been explored not only as a treatment, but also as an adjuvant to enhance antineoplastic treatments such as radiotherapy and chemotherapy.

Methods for applying hyperthermia have evolved. Current techniques include ultrasound, radiofrequency, microwaves, infrared radiation, thermoseeds and hot water. These can be classified according to the extent of the area they can treat. The most common are regional or whole-body heat delivery systems, which have been reviewed in detail elsewhere [80,81]. Some of these may require invasive application to deliver homogeneous heating to the target area. However, to date, the need for heat delivery devices that could reach hyperthermia temperatures within the desired volume still remains. Therefore, the utilization of magnetic nanoparticles as a potential source to generate heat at the nanoscale has tremendous potential.

Magnetic nanoparticles as an alternative to heat delivery

The term MFH is used to describe a form of heat delivery where tissue temperature rises to a therapeutically relevant hyperthermia range (43–47°C) due to the actuation of magnetic nanoparticles under the influence of an AMF. As illustrated in Figure 2A, ideally, magnetic

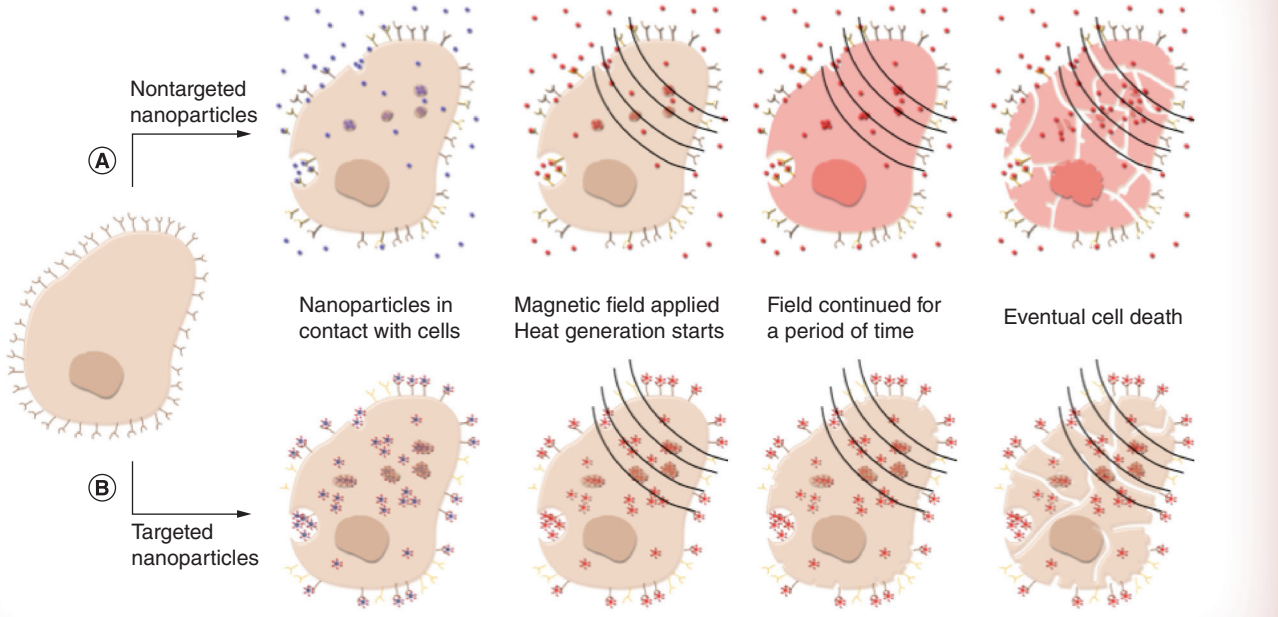

Figure 2. Progression of magnetic fluid hyperthermia. (A) Nontargeted magnetic nanoparticles reach the desired cancerous tissue; an alternating magnetic field is applied and nanoparticles dissipate heat; heat dissipation leads to an increase in the temperature of the surroundings reaching hyperthermia levels; and cell death eventually occurs (cell breakage is for illustrative purposes). **(B)** Targeted nanoparticles are internalized due the presence of a targeting ligand through vesicles, endosomes and lysosomes; an alternating magnetic field is applied only to cells with internalized nanoparticles; and cell death can occur through various mechanisms. A temperature rise may not be observed in the bulk.

nanoparticles are placed in contact with the desired tissue, either by direct injection or systemically, after which an AMF is applied, heat dissipation by the nanoparticles starts to occur until a high enough thermal dose is applied to cause cell death by various mechanisms.

MFH is attractive because of the possibility of targeted nanoscale energy delivery to deep tissues using magnetic nanoparticles [82]. It is envisioned that magnetic nanoparticles could either be directly injected into cancer tumors or delivered intravenously and targeted to tumors by a combination of the enhanced permeation and retention effect [83–90] and active targeting through surface ligands [91]. This could result in the localization of nanoparticles in the extracellular matrix surrounding cancer cells, or in cellular uptake and accumulation in intracellular structures such as vesicles, endosomes and lysosomes (FIGURE 2B).

Application of an AMF would lead to energy dissipation by the nanoparticles [70], potentially raising the tissue temperature to the therapeutically relevant hyperthermia range. Perhaps more exciting is the possibility that, due to intracellular localization of the nanoparticles, MFH could have enhanced anticancer activity over other 'external' modes of heat application. In this respect, Gordon hypothesized that internalized magnetic particles could result in a greater local temperature rise within cells or intracellular compartments [92]. However, the possibility of local nanoscale thermal effects due to magnetic nanoparticles in AMFs is perhaps the most controversial technical aspect of MFH. The controversy stems from a fundamental disagreement between expectations based on the macroscopic continuum theory of heat transfer, which indicates that there should be no advantage to delivering heat using nanoparticles, and a growing body of experimental evidence demonstrating chemical and biological outcomes that are influenced or enhanced due to energy delivery by magnetic nanoparticles. Indeed, theoretical analyses by Rabin [93] and Keblinski *et al.* [94] indicate that the immediate vicinity of an energy-dissipating nanoparticle should not suffer from a preferential increase in temperature. Furthermore, according to heat transfer models, the competition between energy deposition by the nanoparticles and heat removal by conduction in tissue and perfusion of blood through the vasculature gives rise to a lower limit for the size of a tumor that can be brought to the hyperthermia range using a given combination of nanoparticle energy dissipation rate and loading in the tissue [93,95]. This is a serious limitation, as it would preclude application of MFH in the treatment of early-stage tumors or metastatic disease. On the other hand, there is substantial experimental evidence supporting the notion that nanoscale energy delivery by magnetic nanoparticles gives rise to local effects and enhanced biological response. Examples of this are:

- Evidence of differences in temperature sensed by thermosensitive fluorophores either free in solution or bound to magnetic nanoparticles [27];
- Observations of a thermally induced transition of a thermoresponsive fluorescent polymer bound to the surface of magnetic nanoparticles immediately upon application of an AMF, despite the fact that the bulk temperature is up to 12°C below the characteristic transition temperature for the polymer [96];
- Observation of permeabilization of liposomes below their melting temperature when heat is delivered by magnetic nanoparticles localized to the liposome's lipid bilayer [59];
- Demonstrations of enhanced reductions in cell viability when subjected to MFH, compared with treatment with external heating under the same thermal dose [97];
- Observations of enhanced potentiation of anticancer drugs by hyperthermia induced by magnetic nanoparticles, compared with treatment with external heating under the same thermal dose [98];
- Demonstration that internalized, targeted magnetic nanoparticles can lead to significant reductions in cell viability without a macroscopically perceptible temperature rise [37];
- Demonstration that targeted nanoparticles accumulating in lysosomes lead to selective disruption of the lysosomal membrane upon application of an AMF without a temperature rise [99].

Taken together, there seems to be overwhelming experimental support for an advantage to deliver heat at the nanoscale using magnetic nanoparticles, even if the exact mechanisms (both physical and biological) for the observed enhanced outcomes remain unknown. Perhaps the macroscopic continuum heat transfer arguments break down at the nanoscale, as has been observed, for example, for the Stokes–Einstein

diffusivity of nanoparticles in complex fluids [100]. Or perhaps the above experimental observations require re-interpretation in another context besides differences in temperature.

There are other nanoparticle platforms being investigated for their potential to achieve hyperthermia or thermal ablation to treat cancer and perhaps the most developed is the application of so-called photothermal therapy using gold nanoparticles, nanorods and/or nanoshells. This technology is the subject of several recent reviews [101–106]. Compared with MFH, photothermal therapy has the advantage of being able to deliver thermal energy at much greater rates. However, this requires optical access to the intended area, limiting its potential to treat deep tissues due to absorption and dispersion of light by tissue. Furthermore, as the required light must be focused to achieve energy deposition, photothermal therapy requires knowledge of where the cancer tissue is located and each site must be sequentially treated, making whole-body application difficult. By contrast, because living tissues are relatively transparent to magnetic fields of the frequencies and amplitudes used in MFH, one could potentially treat the whole body using commercially available whole-body AMF applicators [107], or could treat selected regions using properly designed coils. In this sense, one could envision systemic targeted delivery of magnetic nanoparticles to all cancer tissues accessible through the vasculature, followed by simultaneous treatment without the need to individually identify the tumor masses. This could potentially be of great use in treating metastatic disease.

The other major potential advantage of using magnetic nanoparticles for the hyperthermic treatment of cancer has been mentioned multiple times already: the possibility of both passive and active targeting of systemically delivered magnetic nanoparticles to cancerous tumors and cells. The idea is to develop particles whose physicochemical properties enable their extended circulation in the bloodstream and their uptake by cancer tissues and cells through the local leaky vasculature of a growing tumor (i.e., through the so-called enhanced permeation and retention effect) [84]. The further modification of nanoparticles with targeting agents could lead to an enhancement in their uptake and potentially control their intracellular localization. Since magnetic nanoparticles have been under development for a variety of applications since the invention of ferrofluids in the 1960s, there is an extensive knowledge base with respect to their synthesis and surface modification [108–112]. Thus, it was expected that the synthesis and modification of magnetic nanoparticles to achieve selective deposition in targeted tissues such as cancer would be easily achievable. However, this has become one of the greatest obstacles to date.

As noted above, heat transfer arguments indicate that there is a minimum tumor size that can be elevated to the hyperthermia range for a given nanoparticle energy dissipation rate and particle loading. Corollary to this would be the fact that there is a minimum nanoparticle loading required to effectively achieve hyperthermia in a particular tumor using nanoparticles with a given energy dissipation rate. Thus, the potential advantages and challenges of energy delivery by magnetic nanoparticles are coupled with any potential advantages and challenges in their targeted delivery to the intended tissues.

Despite the many demonstrations of the *in vitro* efficacy of MFH in killing cancer cells, there are relatively few demonstrations *in vivo*, and most of these are only for the case of local injection of nanoparticles [6,7,113]. Although this route of delivery can certainly be of clinical use, the true potential of MFH can only be realized by targeted systemic delivery of the nanoparticles to all cancer tissues simultaneously. Although there are some demonstrations of targeted systemic delivery of magnetic nanoparticles to cancer tissues, for example in their use as MRI contrast agents, there is limited evidence of successful treatment of tumors *in vivo* following systemic delivery of targeted magnetic nanoparticles. The challenge here is achieving sufficient nanoparticle loading in the intended tissue to raise the temperature to the hyperthermia range. Perhaps this challenge could be circumvented with improved understanding of the mechanisms by which cancer cells can be destroyed using targeted magnetic nanoparticles without the need for a macroscopic perceptible temperature rise [37]. Furthermore, in addition to the challenge of enhancing magnetic nanoparticle delivery to tissues to be treated by MFH, one must also minimize nonspecific uptake in other important organs, such as the liver, if MFH is to be effectively used as a whole-body application. This is the case illustrated by recent work investigating the negative impact of nonspecific magnetic nanoparticle uptake in murine livers, followed by whole-body application of AMFs [9]. Thus, regardless of intense research, the design of magnetic nanoparticles for systemic targeted delivery to cancer tissues remains a challenge.

The potential advantages and challenges described above are also relevant to the thermal potentiation of chemotherapeutics through hyperthermia induced by magnetic nanoparticles. For this particular application, additional advantages and/or challenges can be envisioned. One potential advantage is that synergy between energy delivery by magnetic nanoparticles and chemotherapeutics could result in a positive therapeutic outcome even if the traditional hyperthermia range of 43–47°C is not achieved. This would somewhat relax the requirements of nanoparticle delivery to intended tissues and/or allow for treatment of smaller tumors, depending on the degree of synergy. However, a potential challenge would be the selective codelivery of the magnetic nanoparticles and chemotherapeutics. Here one could envision multiple strategies, such as: administration of the magnetic nanoparticles and chemotherapeutic separately (either simultaneously or in stages), leading to independent accumulation in the intended tissues (and in nonintended tissues); design of magnetic nanoparticles for the targeted transport of a passively released chemotherapeutic and for local dissipation of energy; and design of magnetic nanoparticles for targeted delivery of a chemotherapeutic whose release is triggered by local energy dissipation. This is still an area that requires further analysis and most likely the best option will differ depending on the chemotherapeutic drug used.

Sensitization of antineoplastics by heat

In order to understand the potential advantages of using MFH as a prospective adjuvant for chemotherapy, it is necessary to understand the challenges involved and the advantages of using heat to promote chemosensitivity. It is well known that the chemosensitivity of tumors is a complex phenomenon. In fact, although new antineoplastic agents have become available in recent decades, these agents alone have not necessarily increased the cure rate of a significant portion of cancers [114]. Advances in the understanding of the underlying cellular mechanisms of therapeutic action have led to the development of drug treatment combinations that pursue optimum therapeutic outcomes. Details of such methodologies have been provided elsewhere [114]. Although improved therapeutic outcomes have been found, further clinical improvement is not expected.

Drug treatment combinations pursue optimized outcomes by trying to obtain pharmacological results that are better than the addition of the outcomes of each individual drug. This idea is the basis of the concept of synergy. Very often this concept has been used interchangeably with drug enhancement. To establish synergy, a detailed and quantitative examination of the effects of each drug's concentration and treatment sequence [114,115] must be established. Several mathematical methods, including isobolograms and combination indexes have been developed for the examination of synergy in drug combinations [116,117]. To achieve such quantitative assessments, a significant number of experiments must be conducted. In the case of combination treatments of drugs with heat or radiotherapy, the concepts of thermo- and radio-chemosensitization are often used instead of the term synergy. The reason resides in the complexity of the development of a quantitative assessment of synergy given the fact that these two phenomena depend upon many variables including temperature, exposure time, exposure sequence and drug concentration [115,118].

An alternative to quantifying thermochemosensitization is defined as the thermal enhancement ratio. This parameter has been defined as the dose required to induce a certain level of cytotoxicity without heat, compared with the dose necessary to induce the same level of cytotoxicity in the presence of heat [115]. Interestingly, this ratio is not necessarily linear in nature, thus illustrating the complexity of the phenomenon [115]. Given the fact that not all chemotherapeutic drugs have been investigated specifically in combination with heat to establish synergy, the word thermochemosensitization will be employed herein to define a pharmacological enhancement caused by the presence of heat, regardless of the source.

To understand the underlying mechanisms of thermochemosensitization it is important to recognize the fundamental influence of thermal effects in an organism. The overall *in vivo* outcome is a complex relationship between thermal effects from the system to the cellular level. Comprehending the cellular and molecular changes experienced during hyperthermia is of the utmost importance for the optimization of any heat treatment. The essence of heat effects on cell death is based on the inability of the cell to control macromolecular insults, mainly protein unfolding and aggregation, which results in apoptosis or mitotic catastrophe [119]. Specifically, it has been proposed that increased membrane permeability, increased rate of DNA damage, inhibition of DNA repair mechanisms,

improved tumor perfusion *in vivo*, and the possibility that the *in vivo* hypoxic and acidic environment of the tumor are key factors that may promote selective sensitivity [120–124]. However, these factors must be further analyzed in order to understand how the method of heat application can further improve treatment.

One often overlooked yet important aspect is understanding the effect of the drug on the various stages of the cell cycle. Cells continuously undergo a series of events that eventually lead to division and replication. The most important steps are the G_1, S, G_2 and M phase. G_0 is a resting phase in which the cell is not dividing. In the G_1 phase, the cell will increase in size and checkpoint proteins will be synthesized to ensure the integrity of the DNA. If DNA integrity is acceptable, DNA replication occurs during the S phase. After replication, the cell continues to grow and another group of checkpoint proteins will be synthesized to ensure that the division phase can proceed (G_2 phase). Finally, cell division occurs (M phase). Figure 3 depicts the cell cycle, and indicates in which steps the most common families of chemotherapeutic agents cause enough damage to induce cell death. Table 1 provides a more detailed summary of various families of chemotherapeutic agents, described according to their effect on the cell cycle, mode of action and potentiation capacity with heat. Table 1 does not include all drugs that have been tested in conjunction with hyperthermia, but rather it is a summary of the chemotherapeutics most commonly investigated.

Taking into consideration that the main effects of hyperthermia in the cell cycle occur

Table 1. Common antineoplastics tested in combination with heat.

Classification	Common name	Effect on cell cycle	Mode of action	Synergy with heat	Ref.
Platinum	Cisplatin Oxaliplatin Carboplatin	G_1/S	Formation of DNA adducts	More than additive	[121]
Taxanes	Paclitaxel Docetaxel	G_2/M	Microtubule stabilization	Complex (depends on cell type)	[121]
Campothecin	Irinotecan Topotecan	S/G_2	Topoisomerase I inhibitor		[161]
Pyrimidine antagonists	5-fluorouracil Cytarabine (Ara C)	S	DNA inhibition	Independent	[81,115,118]
Dihydropyrimidine dehydrogenase inhibitor	Gimeracil	S	Inhibits DNA double-strand break repair		[162]
Nucleoside analogs	Gemcitabine	S	DNA inhibition		[132,133,163,144]
Alkylating agents	Cyclophosphamide Mephalan Ifosfamide Mitomycin C	G_1/S	Incorporates an alkyl group to DNA guanine's bases	More than additive	[81,118]
Vinca alcaloids	Vincristin Vinblastine	M	Mitotic inhibitor that prevents microtubule assembly	Independent	[121]
Proteasome inhibitors	Bortezomib	G_2/M	Binds to 26S proteasome	More than additive	[126,127,164]
ROS-generating species	Tert-butyl hydroperoxide	S		More than additive	[165–167]
Heat shock protein Inhibitors	Geldanamycin Flavonoids Tanespimycin	Effect on G_1 and G_2 checkpoint proteins	Decrease in the amount of checkpoint proteins produced	More than additive	[129,134–136]
PARP-1 inhibitors	NU1025 PJ-34	S	Repair inhibition of single-strand breaks		[168]
Antibiotics	Bleomycin Doxorubicin Actinomycin D		Breaks DNA DNA intercalation DNA transcription inhibitor	Complex	[81,118]

ROS: Reactive oxygen species.

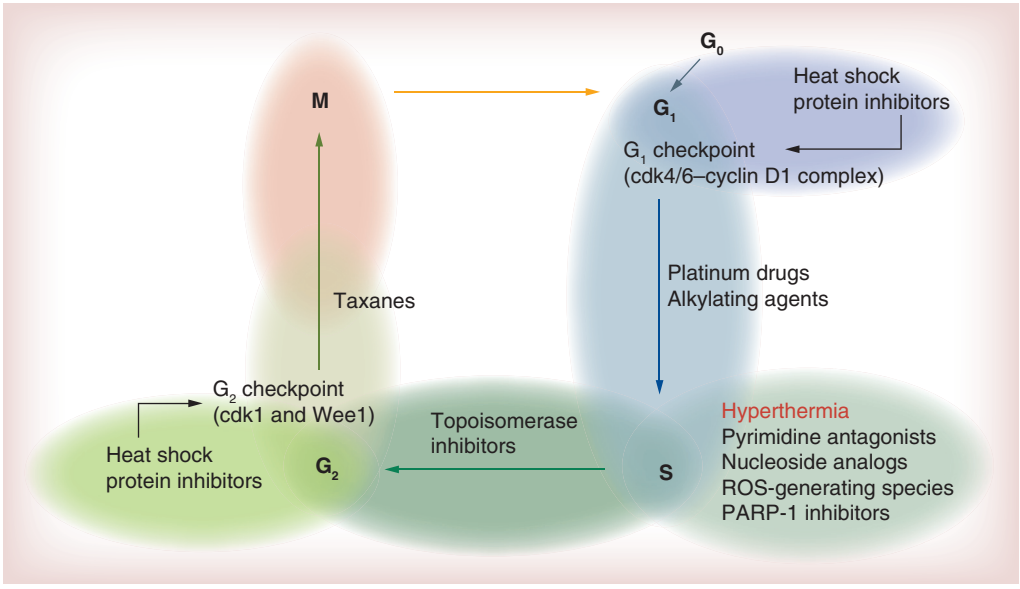

Figure 3. Cell cycle and potential proteins that require heat shock protein 90 for proper function.
ROS: Reactive oxygen species.

in the S phase (FIGURE 3), it could be argued that antineoplastics, such as pyrimidine antagonists (i.e., 5-fluoracil), vinca alkaloids (i.e., vinblastine) and taxanes should not demonstrate significant improvements [114,121,125] when treated with hyperthermia because their main mechanism of action occurs in steps of the cell cycle in the S phase or later. For these drugs, ineffective combination treatments are likely to be explained by the concept of cell cycle resistance, as proposed by Shah [114]. This concept proposes that drug resistance, in particular when more than one therapeutic agent is used, can occur due to the impact of one therapeutic agent in the cell cycle rendering the following agent ineffective [114]. When examined closely, it can be observed that the highest potential for thermochemosensitization occurs in those therapeutic agents for which the main mode of action occurs on or before the S phase.

There are some exceptions to the aforementioned rule. Take for example the case of proteasome inhibitors. Bortezomib, a FDA-approved proteasome inhibitor, has demonstrated heat potentiation *in vitro* and *in vivo* [126]. Its mode of action occurs in the G_2/M phase of the cell cycle [126,127]. Similar to other chemotherapeutic drugs, combination treatments have been explored. Unfortunately, results have demonstrated no further clinical benefit [128]. In the case of a combination treatment with hyperthermia, the fact that cells are chemosensitized by heat indicates that treatment order is essential. It is expected that proteasome inhibition prior to hyperthermia will provide the most potent combination, as significant protein unfolding will overwhelm the compromised proteasome.

Another interesting factor that could be used to explain the lack of thermochemosensitization between certain drugs and hyperthermia is the role of heat shock proteins (HSPs). These proteins, also known as chaperones, are part of the cell's heat shock response mechanism. They are involved in the response to stresses that could threaten the cell's survival [129]. The synthesis of HSPs is not only stimulated by heat, but also by any stress-causing agent such as membrane-fluidizing compounds. One could argue that if heat promotes membrane permeabilization, when used in conjunction with chemotherapeutic drugs the concentration will increase, thus promoting cell death. This has not necessarily been the case. For example, Balogh *et al.* and Dempsey *et al.* demonstrated the lack of thermochemosensitization of some drugs with fluidizing agents such as MFH [130,131]. They proposed that this could be explained by the upregulation of HSPs. Therefore, depending on cell and drug type, hyperthermia could either promote or inhibit cell death.

Adachi *et al.* proposed that the activation of HSP70 by hyperthermia inhibited the activation of NF-κB in pancreatic cancer cells, which resulted in an enhanced cytotoxicity of gemcitabine [132]. Again, treatment order was found to be important as prior or post-treatment with hyperthermia enhanced the therapeutic response, but when treatment was performed

at the same time an improved response was not observed. Similar results were found when this treatment was tested in a clinical study [133].

In order to elucidate the role of HSPs in the potentiation of heat treatments, several researchers have tested various HSP inhibitors, including quercetin (HSP70 inhibitor) [134], tanespimycin (HSP90 inhibitor) [128], and geldanamycin and its derivatives (HSP90 inhibitors) [129,135,136]. Results have indicated that the combination of HSP inhibitors and hyperthermia potentiates heat treatment. However, it is interesting to note that the previous analysis related to the cell cycle can also be applied to HSP inhibitors. Although their role in the cell cycle is complex and still not well understood, it is believed that the synthesis of several checkpoint proteins relies on the proper function of HSP70 and 90 (Figure 3) [129,137]. Their inhibition would then decrease the amount of checkpoint proteins, leading to potential problems with the successful termination of the cell cycle.

Although the mechanisms of thermochemosensitization are complex and still not well understood, the lessons learned with conventional hyperthermia methods could be applied to MFH. The capability of MFH to deliver heat at specific sites should be exploited and optimized.

Chemopotentiation using heat dissipated by magnetic nanoparticles

Nanotechnology-based systems have been employed to deliver heat to specific target areas for combination therapy or to use the nanoparticle platform to improve drug uptake. This section focuses on the utilization of magnetic nanoparticles for the thermochemosensitization of chemotherapeutic agents. Although many drugs could benefit from combined heat treatments, in the case of MFH, platinum-based drugs have been commonly employed as model drugs for the examination of controlled release and thermochemosensitization. These and other drugs tested in combination with MFH are summarized in Table 2.

Recent studies have reported the utilization of iron-based nanoparticle systems as potential drug delivery devices for cisplatin (cDDP). In some cases, the heat produced by the nanoparticle is the trigger for cDDP delivery, while, in others, particles are employed to improve uptake and allow imaging through MRI. For example, Devi-coated coprecipitated iron oxide nanoparticles with poly(lactic acid) [138]. Cisplatin loading and release was investigated without the application of a magnetic field. Similarly, Wagstaff reported a composite nanoparticle system composed of iron oxide nanoparticles coated with gold and covalently attached cDDP [139]. cDDP-containing nanoparticles were found to be more cytotoxic in resistant and native A2780 cells. Other studies have also reported the release of cisplatin from magnetic nanoparticles [140,141] and other drugs, including bleomycin [142], concanavalin A [142], cyclophosphamide [143] and gemcitabine [144].

Naik *et al.* employed iron oxide nanoparticles encapsulated in poly(glycolic acid) nanoparticles that also included cDDP [145]. Complete release of cDDP was obtained after various 2.4-GHz microwave pulses. These studies did not include any *in vivo* or cellular experiments to test for effectiveness. Jiang *et al.* examined the utilization of iron oxide nanoparticles as drug delivery carriers for cDDP and proposed that these nanoparticles could serve as MDR inhibitors [146]. *In vitro* studies with SKOV-3/DPP cells indicated that, although statistically significant, the increase in platinum uptake in the presence of the nanoparticles was only 23%. In this particular case, heat or magnetic field was not applied.

Several iron-based magnetic nanoparticles have been employed for the thermochemosensitization of antineoplastic agents. Carbon-encapsulated iron oxide magnetic nanoparticles were developed and investigated by Taylor *et al.* [147]. Cellular studies were conducted in DU-145 cells using cisplatin and MFH, and the authors concluded that the combined treatment was more effective. Babincová *et al.* examined iron oxide nanoparticles functionalized with starch domains for the ionic binding and controlled release of cDDP [148]. *In vitro* tests in BP6 cells revealed that enhanced effects were obtained when combined treatments were examined. Similarly, Kettering *et al.* provided a systematic study of particle stability and release under various environmental conditions for the system employed by Babincová, indicating that release profiles were a function of environmental properties, such as pH and protein presence [149].

A targeted system was developed by Cheng *et al.*, who designed porous, hollow, magnetic nanoparticles functionalized with herceptin for the targeted release of cDDP [150]. *In vitro*, release kinetics were pH dependent. Tests performed with SK-BR-03 cells indicated a significant reduction in cell viability when targeted nanoparticles were employed.

Table 2. Use of magnetic nanoparticles for the thermopotentiation of chemotherapeutic drugs.

Drug	Iron oxide-based nanoparticle systems	Purpose	Experimental system	Results	Ref.
Adriamycin	Nanoparticles conjugated with multidrug resistance protein inhibitors	Combination treatment	*In vivo* experiments with human chronic myeloid leukemia cell lines	Significant decrease in tumor sizes	[158]
Bleomycin	Coprecipitated chitosan-coated nanoparticles	Drug delivery	Release kinetics. Cellular experiments conducted in HeLa cells	Effective release. Increased drug activity found in nanoparticle–drug conjugated systems	[142]
Bortezomib	Coprecipitated nanoparticles coated with covalently attached carboxymethyl dextran	Combination treatment	Cellular experiments with resistant and nonresistant cell lines	Combination treatment with MFH more effective in both resistant and nonresistant cell lines when compared with hot water	[160]
Carboplatin	Iron nanopowder in chitosan nanoparticles	Combination treatment	*In vivo* studies for liver carcinoma	Higher survival rates with combination therapy	[156]
Cisplatin	Nanoparticles coated with adsorbed starch polymers	Combination treatment	Cellular combination experiments using BP6 rat sarcoma cells	Combination treatment more effective	[148]
	Magnetic nanoparticles encapsulated in poly(glycolic acid) nanoparticles	Drug delivery by polymer biodegradation and heat	Drug release using microwave pulses	Effective actuated drug release	[145]
	Nanoparticles prepared by electrochemical deposition	MDR inhibitors	*In vitro* studies with SKOV-3/DPP cells	Enhanced accumulation of platinum using nanoparticles	[146]
	Coprecipitated nanoparticles coated with adsorbed starch polymers	Drug delivery	Release kinetics	Cisplatin desorption after magnetic field application	[149]
	Porous hollow nanoparticles functionalized with herceptin	Targeted release	Release kinetics and *in vitro* cellular studies with SK-BR-03 cells	Release kinetics controlled by pH. Significant cytotoxicity found	[150]
	Carbon-encapsulated nanoparticles	Combination treatment	Release and cellular studies with DU-145 cells	Combination treatment was effective	[147]
	Coprecipitated nanoparticles coated with adsorbed carboxymethyl dextran	Combination treatment	Cellular efficacy studies using Caco-2. Compared with hot water hyperthermia	Treatment sequence is important. Significant differences between heating methods	[151]
	Nanoparticles coated with crosslinked starch	Drug delivery	Release kinetics	Release profiles influenced by crosslinking density, pH and temperature	[141]
	Coprecipitated gold-coated nanoparticles	Drug delivery/ targeted delivery	Cellular studies with resistant and native A2780 cells	Cisplatin-coated nanoparticles demonstrated higher activity	[139]

MDR: Multidrug-resistance; MFH: Magnetic fluid hyperthermia.

Table 2. Use of magnetic nanoparticles for the thermopotentiation of chemotherapeutic drugs (cont.).

Drug	Iron oxide-based nanoparticle systems	Purpose	Experimental system	Results	Ref.
Cisplatin (cont.)	Coprecipated nanoparticles coated with poly(lactic acid)	Drug delivery	Loading and release kinetics	Effective drug loading. Half of loaded drug was released	[138]
	Coprecipitated nanoparticles coated with adsorbed carboxymethyl dextran	Combination treatment/ understanding of potentiation mecahnisms	Cellular studies using Caco-2	No cytoprotective role from copper when used with MFH. Higher platinum uptake and membrane permeability with MFH when compared with hot water	[152]
Concanavalin A	Coprecipitated chitosan-coated nanoparticles	Drug delivery	Release kinetics. Cellular experiments conducted in HeLa cells	Increased drug activity found in nanoparticle–drug conjugated systems	[142]
Cyclophosphamide	Citrate-coated nanoparticles	MRI contrast/ combination treatment	*In vivo* experiments in mammary adenocarcinoma	Lifespan of mice significantly improved. MRI enhancement observed	[155]
	CO_3O_4–Fe_3O_4 hybrid nanoparticles	Drug delivery	Release kinetics. Cellular experiments conducted in mouse fibroblast	Controlled release was achieved. Particles were not cytotoxic	[143]
Geldanamycin	Micrometer-sized particles	Combination treatment	*In vitro* and *in vivo* studies using B16 melanoma	*In vivo*, 55% remission in treated group	[157]
Gemcitabine	Coprecipitated nanoparticles coated with a polyelectrolyte complex of poly(acrylic acid), chitosan and folic acid	Drug delivery	Release kinetics. Cellular experiments with PRF-5, DLD-1 and MDA-231 cell lines	Nanoparticles delivered the drug to the nuclei of cells	[144]
Melphalan	Adsorbed dextran-coated nanoparticles	MRI contrast/ combination treatment	*In vivo* experiments in P388 tumors, Ehlich carcinoma and Lewis carcinoma	Lifespan of mice significantly improved. MRI enhancement observed	[154]
Paclitaxel	Nanoparticles encapsulated in liposomes	Combination treatment	Release kinetics. Cellular experiments with HeLa cells	Combination treatment more effective	[159]
Selol	Encapsulated magnetic nanoparticles in poly(lactic)-*co*-glycolic acid nanoparticles	Drug delivery	Cellular studies using B16-F10 cells	Loaded particles produced higher cell cytotoxicity	[140]
Quercetin		Combination treatment	*In vivo* experiments with a melanoma model	Combination treatment more effective	[169]

MDR: Multidrug-resistance; MFH: Magnetic fluid hyperthermia.

More recently, Lee *et al.* [151] and Alvarez-Berrios *et al.* [152] investigated the utilization of iron oxide magnetic nanoparticles for the thermochemosensitization of cisplatin *in vitro* using the Caco-2 cell model. Lee *et al.* reported the effects of treatment sequence and heating technique [151]. The effects of treatment sequence were evident for both heat application methods, which were hot water and MFH. The highest cytotoxicity was observed when cDDP and hyperthermia were applied at the same time with an additional exposure period with the drug. Similar behavior was observed for both hot water and MFH. However, it is interesting to note that when both heat modalities were compared, MFH was more effective for all treatment sequences when compared with heat applied by a hot water bath under the same conditions. Similar observations were reported by Rodriguez-Luccioni *et al.* for the case of MFH without drugs [153]. The aforementioned results may indicate that membrane permeabilization could possess a significant role and is dependent on the type of heat treatment. To address this issue, Alvarez-Berrios *et al.* investigated the effect of membrane permeabilization on the potentiation of cDDP by MFH [152]. In this particular case, copper was employed to minimize the active transport of cDDP via the hCTR1 receptor. Results indicated that copper did not demonstrate any cytoprotective role when MFH was applied, whereas it did protect those cells that were treated with a hot water bath. Furthermore, platinum uptake experiments and fluorescence anisotropy measurements indicated that higher drug uptake and membrane permeabilization was obtained when MFH was applied. These results indicate that MFH may not only improve drug cytotoxicity by increasing drug concentration through membrane permeabilization, but other mechanisms may be at hand. Such mechanisms are complex and have not yet been elucidated.

Brusentsov *et al.* employed dextran ferrite- and citrate-coated magnetic nanoparticles and tested them for MRI contrast in combination with MFH with melphalan and cyclophosphamide *in vivo* using P388 tumors, Ehlich carcinoma, Lewis lung carcinoma and mammary adenocarcinoma [154,155]. Their results indicated that the lifespan of mice was considerably improved with significant tumor remission. They also claimed that MRI enhancement provided the means to identify tumor metastasis in all cases. In this particular case, details regarding the method of magnetic field application were not clear.

Recently, Li *et al.* encapsulated carboplatin and iron nanopowder in chitosan nanoparticles [156]. They performed *in vivo* studies for liver carcinoma, where particles were directly injected into the hepatic artery and a magnetic field was applied. Results indicated that significant survival rates were obtained for those groups treated with combination therapy in comparison to MFH alone.

Thermochemosensitization using magnetic nanoparticles has also been investigated with other drugs. These include HSP, PARP and proteasome inhibitors. Ito *et al.* investigated the combined heat treatment with geldanamycin, an HSP 90 inhibitor, with MFH *in vitro* and *in vivo* using a B16 melanoma model [157]. The particles employed were iron-based particles of 100 µm in diameter. *In vivo* results demonstrated complete remission in five out of nine mice treated with the combined treatment. More recently, Ren *et al.* investigated the thermochemosensitization of adriamycin using iron oxide nanoparticles functionalized with multidrug resistance protein inhibitors *in vivo* [158]. The results indicated that the combination treatment using multidrug resistance protein inhibitor-conjugated nanoparticles demonstrated significant decreases in tumor size when compared with those that did not. Paclitaxel-loaded magnetoliposomes were tested *in vitro* in HeLa cancer cells [159], showing that combination treatment was more effective.

Alvarez *et al.* investigated the *in vitro* thermal enhancement of bortezomib by MFH in resistant and nonresistant cells [160]. Bortezomib has been approved by the FDA for the treatment of multiple myeloma, but its use has been limited due to high clinical toxicity, and combination treatments have not provided additional benefits [126,128]. Results demonstrated decreased cell survival in those cells treated with bortezomib and MFH. Combination treatment with magnetic nanoparticles resulted in decreased cell survival when compared with a similar treatment performed under the same thermal doses using hot water hyperthermia. Interestingly, cells that demonstrated resistance to bortezomib were, in fact, more sensitive to treatment than nonresistant cells. This reveals that MFH can also sensitize otherwise drug-resistant cells.

The utilization of magnetic nanoparticles for the generation of targeted heat has tremendous potential, not only to improve current treatments, but also to serve as an alternative to improve the therapeutic window of drugs that otherwise have shown clinical toxicity or no further clinical improvements. The mechanisms

by which such improvements occur are complex and merit further investigation.

Conclusion & future perspective

The use of magnetic nanoparticles to generate heat at the nanoscale with the purpose of cancer treatment either alone or as an adjuvant for chemotherapy has an exciting future. The mechanisms of thermochemosensitization are complex and still not well understood. The lessons learned with conventional hyperthermia methods could be applied to MFH. This is certainly an area that must be addressed in order to optimize potential therapeutic outcomes. Furthermore, although the use of magnetic nanoparticles is attractive, there are still challenges that must be addressed and understood in order to successfully translate such technology to the clinic. These include: improving particle loading in the tumor tissue by selecting appropriate targets but minimizing nonspecific accumulation in relevant organs; improving particle heat generation; and optimizing *in vivo* drug and heat administration strategies. With these in mind, one can foresee that, within the next couple of years, effective targeting strategies will be further developed, including the use of aptamers and other ligands as well as in-depth understanding of local nanoscale thermal effects. With such advances, one can anticipate that within the next decade significant progress towards the development of systemically delivered externally actuated and magnetically controlled nanodelivery devices will be made. These nanoparticle platforms will not only recognize and accumulate in the desired diseased tissue, but will also improve the therapeutic index of the selected drugs. One can only dream of such platforms specifically tailored to the needs of each individual patient.

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Executive summary

Magnetic nanoparticles & magnetic fluid hyperthermia

- Magnetic nanoparticles are small inorganic crystals coated or embedded in an organic shell that possess superparamagnetic, ferromagnetic or ferromagnetic behavior.
- They respond to applied magnetic fields by translation, physical particle rotation or internal dipole rotation, converting magnetic field energy into either mechanical forces/torque or thermal energy.
- In general, magnetic nanoparticles are biocompatible.
- Magnetic fluid hyperthermia (MFH) is defined as a form of treatment where therapeutically relevant temperatures (43–47°C) are obtained through the actuation of magnetic nanoparticles.
- Magnetic nanoparticles are small enough that they could be either directly injected into cancers or intravenously delivered.
- MFH is an attractive form of therapy because energy at the nanoscale can be targeted to tissues.

Mechanisms & rates of energy dissipation by magnetic nanoparticles in alternating magnetic fields

- Iron oxide nanoparticles transform applied magnetic energy by internal dipole rotation (Néel) and physical rotation (Brownian).
- The energy dissipation rate for a given field and amplitude can be manipulated by the selection of particle size, magnetic relaxation time and the composition of nanoparticles.

Clinical aspects of thermal potentiation of chemotherapeutic drugs

- Hyperthermia modalities are various, including ultrasound, radiofrequency, microwaves, infrared radiation, thermoseeds and hot water.
- Not all therapeutic drugs demonstrate improvements in therapeutic outcome in the presence of heat.
- Thermal effects *in vivo* are a complex combination of effects from the cellular to systems level.
- Hyperthermia affects the cell in the S phase of the cell cycle.

Chemopotentiation using heat dissipated by magnetic nanoparticles

- Magnetic nanoparticles have been investigated for the local release of chemotherapeutic drugs such as cisplatin.
- *In vitro* MFH cellular studies with drugs such as cisplatin have demonstrated that combination treatments using magnetic nanoparticles are more effective than using each individually.
- *In vivo* MFH experiments in combination with drugs have indicated that patient survival and tumor remission can be achieved.
- Emerging experimental evidence supports the notion that nanoscale thermal effects occur in the vicinity of magnetic nanoparticles in alternating magnetic fields, and that these effects can lead to disruption of cellular components and cell death.
- Localized thermal effects could enhance thermal chemopotentiation of therapeutic drugs.

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