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Application of hyperthermia induced by superparamagnetic iron oxide nanoparticles in glioma treatment

André C Silva¹
Tiago R Oliveira^{1,2}
Javier B Mamani¹
Suzana MF Malheiros^{3,4}
Luciana Malavolta¹
Lorena F Pavon¹
Tatiana T Sibov¹
Edson Amaro Jr.^{1,5}
Alberto Tannús⁶
Edson LG Vidoto⁶
Mateus J Martins⁶
Ricardo S Santos⁶
Lionel F Gamarra¹

¹Instituto Israelita de Ensino e Pesquisa Albert Einstein, IIEPAE, São Paulo, Brazil; ²Instituto de Física, Universidade de São Paulo, São Paulo, Brazil; ³Departament of Neurology and Neurosurgery, Universidade Federal de São Paulo, São Paulo, Brazil; ⁴Neuro-Oncology Program of Hospital Israelita Albert Einstein, São Paulo, Brazil; ⁵Instituto de Radiologia, Faculdade de Medicina; ⁶CIERMag-Instituto de Física de São Carlos, Universidade de São Paulo, São Paulo, Brazil

Correspondence: LF Gamarra
Instituto Israelita de Ensino e Pesquisa Albert Einstein (IIEPAE) – Sociedade Beneficente Israelita Brasileira Hospital Albert Einstein (SBIBHAE), Av. Albert Einstein, 627/701-Piso Chinuch (2º. Subsolo), Morumbi – São Paulo/SP – Brazil – CEP: 05651-901
Tel +55 11 3747 1366
Fax +55 11 3813 4334
Email lgamarra@einstein.br

Abstract: Gliomas are a group of heterogeneous primary central nervous system (CNS) tumors arising from the glial cells. Malignant gliomas account for a majority of malignant primary CNS tumors and are associated with high morbidity and mortality. Glioblastoma is the most frequent and malignant glioma, and despite the recent advances in diagnosis and new treatment options, its prognosis remains dismal. New opportunities for the development of effective therapies for malignant gliomas are urgently needed. Magnetic hyperthermia (MHT), which consists of heat generation in the region of the tumor through the application of magnetic nanoparticles subjected to an alternating magnetic field (AMF), has shown positive results in both preclinical and clinical assays. The aim of this review is to assess the relevance of hyperthermia induced by magnetic nanoparticles in the treatment of gliomas and to note the possible variations of the technique and its implication on the effectiveness of the treatment. We performed an electronic search in the literature from January 1990 to October 2010, in various databases, and after application of the inclusion criteria we obtained a total of 15 articles. In vitro studies and studies using animal models showed that MHT was effective in the promotion of tumor cell death and reduction of tumor mass or increase in survival. Two clinical studies showed that MHT could be applied safely and with few side effects. Some studies suggested that mechanisms of cell death, such as apoptosis, necrosis, and antitumor immune response were triggered by MHT. Based on these data, we could conclude that MHT proved to be efficient in most of the experiments, and that the improvement of the nanocomposites as well as the AMF equipment might contribute toward establishing MHT as a promising tool in the treatment of malignant gliomas.

Keywords: brain tumor, magnetic hyperthermia, magnetic nanoparticle

Introduction

In recent decades, a major challenge for oncologists and neuroscientists has been the understanding of biological mechanisms underlying the formation of tumors in the central nervous system (CNS), as well as the development of therapies that can stabilize, reduce or even eliminate these tumors. Primary malignant CNS tumors represent 1.49% of all cancers; however, although relatively rare, they are associated with high morbidity and mortality.¹ Most of these tumors that originate from glial cells are usually referred to as gliomas.²

Gliomas are a group of heterogeneous CNS neoplasms that can be classified according to the glial cell of origin (ie, astrocytes, oligodendrocytes, ependymal cells, or choroid plexus cells).³ Gliomas are neuroepithelial tumors, which account for 33% of primary tumors and 79% of malignant CNS tumors. Astrocytomas represent 75% of all gliomas, and glioblastomas form 51.7% of cases.¹ Glioblastoma is the most

frequent and malignant astrocytoma, and despite advances in diagnosis and treatment of these tumors, their prognosis remains dismal.^{4,5} The development of new effective therapies is urgently needed. Hyperthermia induced by magnetic nanoparticles in tumor tissues is a potential therapeutic tool and has been evaluated by various ex-vivo experiments (fragments of tumor tissue removed by surgery) in animal models, with promising results, prompting Phase I studies in humans.^{6,7}

Hyperthermia is a therapeutic procedure that promotes the increase of temperature in body tissues in order to change the functionality of the cellular structures. Its activity is based on the fact that a temperature increase of between 41°C and 42°C can induce tumor cell death, as the tumor cells are less resistant to sudden increases in temperature than the normal surrounding cells.⁸ The rise in temperature changes the functioning of many enzymatic and structural proteins in the cells, in turn altering cell growth and differentiation, which can induce apoptosis.^{9,10} Changes triggered by hyperthermia in the cell membrane lead to a reduction in transmembrane transport and destabilize its potential.^{11,12} It is also known that the rise in temperature can affect the synthesis of nucleic acids and inhibition of repair enzymes, and promote changes in the conformation of DNA.¹³ The temperature increase required by hyperthermia can be achieved via different heat sources, such as electromagnetic radiation waves (hyperthermia by radiofrequency or microwave),^{14,15} ultrasound waves,^{16–18} or electrically induced hyperthermia.¹⁹ These techniques have shown good results, however, the major problem with present conventional methods is reaching a heat homogenous distribution and therapeutic temperatures in the deep region of the tumor to be treated. In this sense, treatment failure results from an insufficient temperature rise in some parts of the tumor, allowing the tumor to recur. What also must be avoided is the excessive increase in temperature which would cause peritumoral tissue damage. The magnetic hyperthermia (MHT) technique has partly solved these problems.

Studies on the application of magnetic materials to promote hyperthermia began with Gilchrist et al²⁰ in the late 1950s. After this work, many empirical studies have been carried out to evaluate the therapeutic expression in different tumor types. However, the results of these preliminary studies had their widespread use in academic circles only in the early 1990s, and today there are a small number of clinical studies in this area.^{21–24} Thus, more detailed studies on the physical limitations, degree of toxicity, and efficiency that would allow a better applicability of the method to humans are needed.

The principle of MHT comprises the administration of a magnetic fluid within the tumor for subsequent application of an alternating magnetic field (AMF), which will promote warming of the region.²⁰ At present, MHT is presented as a noninvasive promising technique, efficient in implementing hyperthermia in deep and inaccessible tissues. The advantage of MHT is related to its specificity to tumorigenic tissues, ensuring less damage to healthy tissues due to the uniform distribution of temperature and the high rate of heat transfer.

The efficiency of the MHT technique is extremely dependent on the different properties of the particles, such as size, magnetization, and the value of the Curie temperature. The Curie temperature is the maximum temperature reached by a magnetic particle. Knowledge about the Curie temperature of a magnetic particle allows an efficient temperature control to avoid overheating the tissue.²⁵ Of great importance are also the different parameters of the magnetic field used, such as intensity and frequency that will achieve the desired temperature.²⁶ The main aim in this area is to improve the parameters above in order to obtain the best treatment efficacy. At present, some preliminary studies^{23,27} that practice the clinical use of MHT to treat gliomas suggest that the superparamagnetic nanoparticles of iron oxide (SPION) have appropriate intratumoral distribution and are able to promote controlled heating of the region to hyperthermic temperatures without causing major side effects. In this sense, the objective of this review is to find in the literature, studies that discuss the relevance of hyperthermia induced by magnetic nanoparticles subjected to an AMF, in the treatment of gliomas. We have included preclinical testing (application of MHT in tumor models) and even clinical studies, which have been used with this technique, as therapy alone or even associated with other therapies. Although gliomas are exclusively CNS tumors, we have included animal model studies even where tumors have been implanted outside the CNS, as they originated from glioma cell lines (eg, T-9 rat glioma cells).

Methods

The search for publications was conducted from January 1990 to October 2010 based on the electronic data banks: ISI Web of Science and PubMed. The search keywords were as follows: [(Brain tumor), (glioma) or (glioblastoma)] and [(magnetic fluid hyperthermia), (magnetic hyperthermia) or (induction heating)]. In this review, three authors (LM, JBM, and TRO) independently checked the titles and abstracts of the articles obtained in the electronic search.

Next, three other authors (LFG, LFP, and TTS) verified the quality of the methods, inclusion criteria, and data to be collected. Following this, three more authors (ACS, SMFM, and EAJr) extracted the data and tabulated the results.

The publications had to satisfy the following inclusion criteria: 1) published in English, 2) original works, 3) hyperthermia was used for the purpose of promoting glioma tumor cell death, and 4) used nanoparticles of iron oxide under the influence of an AMF as a mediator of hyperthermia.

Results

We identified 71 publications by electronic search. Considering the inclusion criteria, we selected 15 articles: 3 *in vitro* studies,^{28–30} 10 animal model studies,^{23,29,31–37} and 2 clinical studies.^{6,27} Although there are different centers around the world that study magnetic hyperthermia as a cancer therapy, studies of application in glioblastoma tumors are concentrated mainly in two research centers, the Department of Biotechnology, School of Engineering, Nagoya University, Japan and the Department of Radiation Medicine, University of Charité Medicine, Germany.

Physical parameters for efficient heating

Physicochemical properties of magnetic nanoparticles

The studies *in vitro*, *in vivo*, and in humans, in this review, used SPION corresponding to the crystalline phase of magnetite, whose physicochemical characteristics are summarized in Table 1. In the *in vitro* studies,^{28–30} the diameter of the nucleus of the SPION ranged from 20 to 35 nm. *In vivo* applications used SPION in the range of 1–35 nm, while human studies were performed with a SPION from 12 to 15 nm. Colloidal suspensions with various concentrations of iron, ranging from 0.2 to 1×10^7 mg/mL (Table 1), were employed in these studies. In all studies of this review, the hydrodynamic diameter of SPION was not notified, but the aminosilane particles produced by MagForce Nanotechnologies AG (Berlin, Germany) had a hydrodynamic diameter of ~20 nm.³⁸

For *in vitro* applications, the coating material of SPION was a lipid layer (N-(α -trimethylammonioacetyl)-didodecyl-D-glutamate chloride [TMAG], dilauroylphosphatidylcholine [DLPC], dioleoylphosphatidyl-ethanolamine [DOPE], 1:2:2 cationic; DLPC, DOPE, 1:1 neutral)^{28,29} and PVP (polyvinylpyrrolidone) with poly(ethylene glycol)(200) methyl ether methacrylate (PEG200MMA), PEG1000MMA, triethylene glycol dimethacrylate (TEGDMA), and poly(ethylene glycol)(400) dimethacrylate (PEG400DMA).³⁰ For *in vivo* applications, the lipid layers^{29,31–34,36} carboxymethylcellulose

(CMC)³⁷ and carboxydextran DDM128P6 aminosilane²³ were used, and for human studies SPION coated with aminosilane^{6,27} was used.

Most studies used magnetite coated with cationic liposomes (MCL) and SPION coated with aminosilane. For *in vitro* and *in vivo* studies that used MCL,^{28,29,31–34,36} the affinity of MCL to the cells, compared with the liposomes of neutral charge, was enhanced due to the electrostatic interaction on the basis of the positive charge of the MCL; a higher affinity was produced by electrostatic interaction with the phospholipid cell membrane that was negatively charged. In the *in vivo* studies performed with MCL for the treatment of tumors,^{32,33} the tumor regression was associated with the high distribution of MCL, presenting a great potential for treatment due to necrosis of the tumorigenic cells, not only by heating, but also by inducing an immune response.³⁴ Le et al³⁵ have demonstrated that the MCL, besides being used for tumor hyperthermia, could also be linked to an antibody fragment, which would increase its specificity for glioma cells *in vitro* and *in vivo*. Human studies done with SPION coated with aminosilane^{6,27} showed that the SPION presented themselves as good specific markers in the therapy of tumors. The distribution of SPION was analyzed by autopsies on brains, which showed that the SPION installed were dispersed and distributed in aggregate forms in the region of the necrosis, and the distribution was restricted to the sites of instillation.²⁷

Alternating current (AC) magnetic field system to MHT therapy

From Table 1 it can be observed that the oscillation frequency of the magnetic field, collected for all studies, is mainly defined as being close to 100 kHz (ranging from 88.9 to 150 kHz); there is only one article that uses a frequency of 297 kHz.³⁰ The intensities of the magnetic field used to promote heating throughout the protocol are not commonly presented in the articles, but the maximum capacity of the magnetic field generators is between the values of 11.0 and 30.6 kA/m. With respect to magnetic field applicator configuration, the horizontal coil and ferromagnetic core coil have been used as major setup to *in vivo* experiments; however, for clinical trials, the unique magnetic field applicator available has a ferrite-core configuration. In two *in vitro* studies,^{28,30} they used the configuration of the applicator in vertical coil, and in only one study²⁹ the configuration was with a horizontal coil. In most studies, the specific loss of power or specific absorption rate are not indicated; however, studies performed by Shinkai et al,²⁸ Le et al,³⁵ and Rabias et al³⁹ reported values of 140, 96, and 286 W/g, respectively.

Table 1 Physico-chemical properties of nanoparticles based on iron oxide and features of magnetic field applicator for the treatment of gliomas in MHT

Study	Iron oxide	$[\text{Fe}_3\text{O}_4] \times 10^5$ ($\mu\text{g/mL}$)	$\langle D_{\text{core}} \rangle$ (nm)	Coating	Manufacture	Magnetic field intensity (kA/m)	Magnetic field frequency (kHz)	Coil configuration	SLP/SAR (W/g)
In vitro									
Shinkai et al ²⁸	Magnetite	$(2-8) \times 10^{-4}$ and $(2.5-10.0) \times 10^{-2}$	35	TMAG, DLPC, DOPE, 1:2:2 cationic; DLPC, DOPE, 1:1 neutral	Synthesized (nitrite oxidation method) followed by sonication method	30.6	118	Vertical coil Inner diameter: 8.5 cm Length: 21 turns	140.0
Ito et al ²⁹	Magnetite	100	35	TMAG, DLPC, DOPE, 1:2:2 cationic	Synthesized (nitrite oxidation method) followed by sonication method	30.6	118.0	Horizontal coil Inner diameter: 7 cm Length: 7 turns	N/I
Meenach et al ³⁰	Magnetite	0.0158-0.0793	20-30	PVP (PEG200MMA, PEG1000MMA, TEGDMA, PEG400DMA)	Nanostructure and amorphous materials (Los Alamos, NM) followed by free-radical polymerization	25.0	297.0	Vertical coil Inner diameter: 1.5 cm Length: 5 turns	N/I
In vivo									
Yanase et al ³¹	Magnetite	0.2×10^{-3}	35	TMAG, DLPC, DOPE, 1:2:2 cationic	Synthesized (nitrite oxidation method) followed by sonication method	30.6	118.0	Horizontal coil Inner diameter: 7 cm	N/I
Yanase et al ³²	Magnetite	0.075	1	TMAG, DLPC, DOPE, 1:2:2 cationic	Toda Kogyo Co, Ltd (Hiroshima) followed by sonication method	30.6	118.0	Horizontal coil Inner diameter: 7 cm	N/I
Yanase et al ³³	Magnetite	0.075	10	TMAG, DLPC, DOPE, 1:2:2 cationic	Toda Kogyo Co, Ltd (Hiroshima) followed by sonication method	30.6	118.0	Horizontal coil inner diameter: 7 cm	N/I
Shinkai et al ³⁴	Magnetite	0.075	10	TMAG, DLPC, DOPE, 1:2:2 cationic	Toda Kogyo Co, Ltd (Hiroshima) followed by sonication method	30.6	118.0	Horizontal coil inner diameter: 7 cm	N/I
Le et al ³⁵	Magnetite	0.05	35	Antibody-conjugated, liposomes (TMAG, DLPC, DOPE, 1:2:2 cationic)	Synthesized (nitrite oxidation method) Sonication method followed by immobilization method	30.6	118.0	Horizontal coil Inner diameter: 7 cm	96.0
Ito et al ³⁶	Magnetite	0.15	10	TMAG, DLPC, DOPE, 1:2:2 cationic	Toda Kogyo Co, Ltd (Hiroshima) followed by sonication method	30.6	118.0	Horizontal coil Inner diameter: 7 cm	N/I
Ohno et al ³⁷	Magnetite	18.3	10	CMC	Tokai Carbon Co, Ltd (Tokyo)	30.3	88.9	Horizontal coil Inner diameter: 25 cm $\times$ 25 cm	N/I
Ito et al ²⁹	Magnetite	0.075	35	TMAG, DLPC, DOPE, 1:2:2 cationic	Synthesized (nitrite oxidation method) followed by sonication method	30.6	118.0	Horizontal coil Inner diameter: 7 cm $\times$ 7 cm	N/I

Jordan et al ²³	Magnetite	2.016	3	Carboxydextran DDM128P6	Schering AG, Berlin, Germany	18.0	100.0	N/I
	Magnetite	2.24	15	Aminosilane MFL AS	MagForce Nanotechnologies AG, Berlin, Germany	18.0	100.0	N/I
Rabias et al ¹⁹	Maghemite	2.0×10^4	10–12	Dextran	Synthesized (coprecipitation method)	11.0	150.0	286.0
Human								
Maier-Hauff et al ⁶	Magnetite	1.12	15	Aminosilane MFL AS	MagForce Nanotechnologies AG, Berlin, Germany	18.0	100.0	N/I
Van Landeghem et al ²⁷	Magnetite	1.12	15	Aminosilane MFL AS	MagForce Nanotechnologies AG, Berlin, Germany	18.0	100.0	N/I
Maier-Hauff et al ⁷	Magnetite	1.12×10^5	12	Aminosilane MFL AS	MagForce Nanotechnologies AG, Berlin, Germany	15.0	100.0	N/I

Abbreviations: CMC, carboxymethylcellulose; DLPC, dilauroylphosphatidylcholine; DMA, dimethacrylate; DOPE, dioleoylphosphatidyl-ethanolamine; MHT, magnetic hyperthermia; MMA, methyl ether methacrylate; N/I, data not informed; PEG, poly (ethylene glycol); PVP, polyvinylpyrrolidone; SLP/SAR, specific loss of power or specific absorption rate; TEGDMA, triethylene glycol dimethacrylate; TMAG, N-(α -trimethylammonioacetyl)-didodecyl-D-glutamate chloride.

Effectiveness of MHT therapy

Of the 15 publications that we have selected for this review, 9 evaluated the MHT technique used in animal models of rats and mice; 2 performed experiments in cell culture, and 1 study used both animal and cell cultures. Three studies involved humans. The description of the results was subdivided as follows: in vitro experiments that included tests in cell culture; in vivo experiments in which animal models were used; ex-vivo and in vivo experiments in humans.

In vitro experiments

In 1996, Shinkai et al²⁸ verified the efficiency of MCL as a generator of intracellular hyperthermia. With the application of an AMF in glioma cells (T-9), in the presence of MCL (concentration 7.2 mg/mL), the temperature of the ‘pellet’ center reached 42.6°C in 20 minutes. Cell viability decreased when AMF was applied, reaching 0 number of cells after 40 minutes. Moreover, MCL was seen to be absorbed very well by the cells, reaching 80% of the maximum absorption in 1 hour, thereby facilitating the heating of the cells.

Posteriorly, Ito et al,²⁹ in an in vitro study, observed that hyperthermia, in addition to lysing the cells, promoted an antitumor immune response by activation of heat shock proteins (HSP70). After application of AMF, the temperature reached 42°C for 30 minutes by using MCL at a concentration of 100 µg/mL. Hyperthermia promoted necrosis in three types of T-9 cell lines developed. They observed the release of HSP70 protein mainly in the type T-9/HSP70.

In another more recent study using glioblastoma cells (M059K), Meenach et al³⁰ evaluated the ability of a hydrogel (based on polyethylene glycol) with nanoparticles, in a concentration of 7.93 mg/mL, to promote hyperthermia. There was cell death by thermal ablation (63°C) where hydrogel with the nanoparticles was applied, effective in the region of the plate, but there was no death in the surrounding region. Table 2 summarizes the main findings of the in vitro experiments.

In vivo experiments – animal model

Therapeutic protocol in animal models

Table 3 compares animal models and shows that the selected studies evaluate the effects of MHT therapy on tumor sizes between 3 and 18 mm, with the highest frequency between 10 and 18 mm, which has been included in 6 of 10 articles. One study³⁷ did not report the size of the tumor evaluated.

The cell lines used in most studies (6 of 10 studies) were T-9 cells implanted into Fischer F344 rats.^{23,29,31–34,37} The minimum number of cells inoculated ranged from 1×10^5 to

Table 2 Comparison of in vitro experiments performed to evaluate the technique of MHT in the treatment of gliomas

Study	In vitro preparation		MHT therapy								
	Tumor cell line	Culture medium	Nanoparticle	Concentration of nanoparticle	Time incubation of nanoparticles (hours)	Cell viability		Method to assess the MHT efficacy	Nanoparticle uptake by glioma cells	Temperature reached (°C)	Time of AMF exposure (minutes)
						Before MHT	After MHT				
Shinkai et al ²⁸	T-9	Dulbecco's modified Eagle's medium	Magnetite cationic liposomes	7.2 mg/mL	8	1.6×10^8 cell/mL	0 cell/mL	"Viable cell count"	55 pg/cell	42.6	60
Ito et al ²⁹	T-9	Minimum essential medium	Magnetite cationic liposomes	100 µg/mL	24	No apoptotic/necrotic cells	Apoptotic/necrotic cells	Green fluorescent dye Yo-Pro-1, propidium iodide, Vibrant apoptosis assay, flow cytometry	–	42.0	30
Meenach et al ³⁰	M059K	Dulbecco's modified Eagle's medium/Ham's F-12	PEGDMA-PEGDMA with iron oxide	7.93 mg/mL	0	No cell death	Cell death – via thermoablation	Live-dead assay/fluorescence imaging	–	63.0	5

Abbreviations: AMF, alternating magnetic field; MHT, magnetic hyperthermia; PEGDMA, poly (ethylene glycol) methyl ether methacrylate; PEGDMA, poly (ethylene glycol) dimethacrylate.

3×10^7 cells. Only in 2 studies, the cells were implanted in the brain regions,^{23,37} and in the remaining studies, the cells were applied subcutaneously. For the interval of tumor dimension studied, the amount of iron introduced into the tumor was between 3 µg and 3 mg, as per the comparison of treatment protocols presented in Table 4 and taking into consideration the nine articles that provided such information. With regard to the heating time, Table 4 shows that the authors used protocols of up to 60 minutes. However, in most studies, the duration was 30 minutes. In this sample, 7 articles favored an increase in the number of applications of AMF, with an increase in the exposure time of each application; two being the maximum number of applications observed (Table 4). The therapeutic temperature of choice was found to be between 43°C and 47°C, but most often it was found to be at temperatures of 44°C^{32,34,37} and 45°C.^{29,31,33} In most studies (8 of 10 studies) MHT was tested alone; however, 2 studies^{29,36} found that gene therapy added to MHT would promote a greater reduction in tumors.

Effectiveness of MHT in animal models

The effectiveness of MHT in models of gliomas was first reported by Yanase et al.³¹ In this study, Fisher F344 rats were used. T-9 cell cultures pre-incubated with MCL were injected subcutaneously. After 20 minutes of AMF, the temperature reached 43°C and went up to 45°C. However, rectal temperature was maintained between 35°C and 36.5°C. The application of AMF, thrice, for 60 minutes, at intervals of 12 hours, was able to completely inhibit tumor cell growth in rats, which were observed for up to 90 days.

In another publication, Yanase et al³² obtained similar results; however, the MCL was injected in Fisher rats with previously developed tumors. After 15 minutes of AMF, the maximum tumor temperature reached 44°C, with little change in rectal temperature (35°C and 37°C). The group that received three applications of magnetic irradiation for 30 minutes at intervals of 24 hours had a tumor reduction of 87.5%. Histological analysis revealed that in the control group that received no AMF, the MCL was restricted to the area of administration. However, the groups that received AMF showed a homogeneous distribution of MCL that also coincided with the necrotic regions.

In the same year, Yanase et al³³ investigated a possible anti-tumor immune response induced by hyperthermia in the same tumor model as described earlier in mice (T-9); however, tumor induction was performed on both sides of the animal. The heat induction was effective and reached 43°C in the tumor region after a 10 minute application of AMF. The group that

Table 3 Comparison of animal models used to evaluate glioma by MHT therapy

Study	Tumor cell line	Animal	Gender	Age (weeks)	Injection site	Minimum inoculation (cells)	Days to start MHT after tumor cell inoculation	Tumor size (mm)/shape
Yanase et al ³¹	T-9	Fisher rat F-344	Female	7–8	Subcutaneous (left femoral region)	1×10^6	–	3/ellipsoidal
Yanase et al ³²	T-9	Fisher rat F-344	Female	6–7	Subcutaneous (left femoral region)	1×10^7	11	13–18/ellipsoidal
Yanase et al ³³	T-9	Fisher rat F-344	Female	6–7	Subcutaneous (left and right femoral region)	1×10^7	11	13–18/ellipsoidal
Shinkai et al ³⁴	T-9	Fisher rat F-344	Female	6–7	Subcutaneous (left femoral region)	1×10^7	11	13–18/ellipsoidal
Ito et al ³⁶	U-251-SP	Athymic nude mice	Female	4	Right flank	3×10^7	–	8/–
Le et al ³⁵	U-251-SP	KSN-nu/nu nude mice	Female	4	Subcutaneous (femoral region)	–	21	10/ellipsoidal
Ohno et al ³⁷	T-9	Fisher rat F-344	Female	4	Right cerebral hemisphere	5×10^6	8	–
Ito et al ²⁹	T-9	Fisher rat F-344	Female	6	Subcutaneous space	1×10^7	–	10/–
Jordan et al ²³	RG-2	Fisher rat F-344	Male	–	Thalamus region	1×10^5	4	3–4/round
Rabias et al ³⁹	C6	Wistar rat	Male	–	Subcutaneous (anterior bregma region)	8×10^6	28	5–10/–

Abbreviation: MHT, magnetic hyperthermia.

received three sessions of 30 minutes of magnetic irradiation, with intervals of 24 hours, had a complete regression of the tumor on the left side. Although the right side did not receive an application of MCL, it also had total regression of the tumor. Histological analysis revealed the presence of immunocytes (CD3, CD4, CD8, and natural killer [NK] cells) in the group exposed to hyperthermia in the tumor tissues on both sides (left and right). After magnetic irradiation, the cured rats were injected again with T-9 cells and also with cell lines of malignant fibrous histiocytoma (MFH). The authors observed the development of MFH cells only, showing a specific immune response of the tissue to T-9 cells.

The same antitumor immune response triggered by hyperthermia, proposed by Yanase,³² was confirmed by Shinkai et al.³⁴ Soon after tumor growth (T-9 cells) in Fisher rats, after a 15-minute application of AMF, it was observed that the maximum temperature in the tumor reached 44°C. The maximum duration of application of AMF was 30 minutes, and in this time interval, animals in the group receiving magnetic irradiation had a complete regression of the tumor after repeated sessions. After regression, no

recurrence was observed for a 3-month period. Injected MCL of 80% to 90% accumulated in the tumor tissue. It was suggested that regression and no recurrence of tumor was on account of an immune response (activation of CD4, CD8, and NK cells) activated by hyperthermia.

In 2001, Ito et al³⁶ suggested that hyperthermia combined with gene therapy could be an effective therapy for the treatment of tumors. For the development of the tumors, human glioma cells (U251-SP) were implanted in the flanks of mice. The application of AMF in the tumor, in the presence of MCL, was able to produce heating to 46°C after 3 minutes. The concomitant injection in the tumor of pGadFNT, a promoter of the tumor necrosis factor (TNF) that was activated by hyperthermia and MCL, promoted an increase of gene expression, TNF- α , after the application of AMF. Application of pGadFNT and MCL and subsequent application of AMF dramatically reduced the tumor, and it was the largest decrease compared with groups in which these compounds were administered alone.

Also in 2001, Le et al³⁵ demonstrated that the effectiveness of MCL in the destruction of tumors could be increased when

Table 4 Comparison of different treatment protocols used in animal models with glioma

Study	Animals groups/ number	Main tumor length (mm)	Fe ₃ O ₄ weight administered	Time to AMF application after Fe ₃ O ₄ administration (hours)	Maximum therapeutic temperature ^a (°C)	Time to reach the therapeutic temperature (minutes)	Time of AMF application per session (minutes)	Number of AMF applications per group	Method to assess the MHT	Combined therapy
Yanase et al ³¹	3/5	3	3 µg/150 µL	N/I	≈45	20	60	1 and 3	"Tumor take"	No
Yanase et al ³²	4/10–13	13–18	3 mg/400 µL	24	≈44	15	30	1, 2, and 3	Berlin blue counter-stained with Kernechtrot or HE,	No
Yanase et al ³³	2/8, 2/1	13–18	3 mg/400 µL	24	≈45	10	30	3	"Tumor volume"	No
									"Tumor volume", HE, Immunohistochemistry (CD3, CD4, CD8, and NK)	No
Shinkai et al ³⁴	4/5	13–18	3 mg/400 µL	N/I	≈44	15	30	1, 2, and 3	HE, Immunohistochemistry (CD3, CD4, CD8, and NK)	No
Le et al ³⁵	2/5	10	0.5 mg/100 µL	24	≈43	20	30	3	Immunohistochemistry (G22–FML)	No
Ito et al ³⁶	4/5	8	3 mg/200 µL	24	≈46	3	30	1	Immunohistochemistry (TNF-α), TNF-α ELISA	TNF-α gene therapy
Ohno et al ³⁷	3/5	N/I	N/I	24	≈44.4	N/I	30	1 and 3	HE, Berlin blue, "Survival rate", Berlin blue counterstained with Kernechtrot or HE	No
Ito et al ²⁹	4/5	10	3 mg/400 µL	24	≈45	4	30	1, 2, and 3	HE, Immunohistochemistry (HSP70), TUNEL	HSP 70 gene therapy
Jordan et al ²³	10/12	3–4	1.8 mol/L 2.0 mol/L	0	39, 43–47	10	40	1	Prussian blue/immunohistochemistry (PCNA and GFAP)	No
Rabias et al ³⁹	2/–	5–10	3 mg/150 µL	0	N/I	N/I	20	1	HE	No

Note: ^aMaximum temperature reached in a period of application of AMF.

Abbreviations: AMF, alternating magnetic field; ELISA, enzyme-linked immunosorbent assay; GFAP, glial fibrillary acidic protein; HE, hematoxylin-eosin; HSP, heat shock protein; MHT, magnetic hyperthermia; N/I, data not informed; NK, natural killer; PCNA, proliferating cell nuclear antigen; TNF, tumor necrosis factor; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling; HE, hematoxylin-eosin; N/I, data not informed.

it was coupled with a fragment of a specific antibody for tumors (Fab fragment). Thus, tumors were developed in mice with human glioma cells (U251-SP). It was found that the MCL conjugated to the antibody Fab was captured by tumor cells seven times more than MCL without a specific antibody, and after application of AMF, there was a rapid increase in tumor temperature reaching 40°C in 5 minutes and 43°C after 30 minutes. Moreover, tumor growth was inhibited for up to 2 weeks.

In 2002, Ohno et al³⁷ tested the efficacy of a new SPION, CMC 'stick type', based on a magnetite, in the generation of heat. This SPION was evaluated in tumors developed in Fisher rat brains that were inoculated with T-9 cells. The nanoparticles were effectively heating the tumor tissue to 44.4°C, after AMF application, besides being widely distributed after three applications. It was found that the nanoparticles did not invade normal brain tissues surrounding the tumor. Total remission of the tumor was observed in only one animal in the group, which underwent three sessions of MHT. Moreover, this group verified higher survival when compared with other groups. Thus, the data showed a good performance by the nanoparticles in the induction of hyperthermia.

In the studies of Ito et al, hyperthermia was still identified as an inducer of antitumor immune factors.²⁹ In this study, it was hypothesized that hyperthermia activated HSPs, which in turn triggered an antitumor immune response. Tumors in Fisher rats developed from the inoculation of T-9 cells into the subcutaneous space. The application of AMF in the tumor, in the presence of MCL, was able to produce heating up to 42°C after 4 minutes, reaching 45°C. Along with the heating there was an increase in HSP70 protein expression. It was observed that the HSP70 protein was able to trigger an antitumor immune response. Moreover, it was found that on account of hyperthermia the dead cells released HSP70 protein.

Recently, Jordan et al²³ observed the efficiency of hyperthermia promoted by SPION with two different types of coatings (carboxidextran and aminosilane) in an animal model of brain tumor (RG-2). In relation to the controls, after MHT, the tumors did not differ in dimension or histopathology. There was a decrease in the level of cell proliferation in the group that used nanoparticles coated with aminosilane. In the same group there was an increase in the average survival of animals. The gain in survival was correlated with increased intratumoral temperature, observed during the treatment. Nanoparticles coated with aminosilane remained stable within the tumor when compared with those coated with carboxidextran, and were well distributed in the tumor tissue after histological analysis. It was concluded

that thermotherapy using SPION coated with aminosilane was effective in causing an extension of survival in animals submitted to the tumor model.

Finally, Rabias et al³⁹ demonstrated in a study of synthesis and characterization, the efficiency of a maghemite ferrofluid coated with dextran with high surface charge (denominated S3). The results showed that even with the injection of small quantities, there was a rapid heating and good distribution in tumor. An amount of 150 μ L of ferrofluid was injected into extracranial tumors developed from the C6 cell line in Wistar rats.

During the application of AMF of low intensity (11 kA/ma 150 kHz for 20 minutes), performed by monitoring the temperature with infrared image, they observed that the heating was restricted to the tumor area. After treatment, animals were submitted to histological analysis, and there was extensive damage or even the dissolution of the tumor tissue. This damage was restricted to the tumor area. In control groups where the ferrofluid was injected without application of a magnetic field or even in the group that there was no injection, but the animals were submitted to AMF, there was no detected tumor damage. Thus, they concluded that the ferrofluid tested, due to its high surface charge, prevented the formation of clusters, with a good dispersion in tumor tissue during heating, showing its excellent applicability in living organisms.

The main findings observed in the studies using animal models for evaluation of MHT to treat gliomas are summarized in Table 3.

In vivo and ex-vivo experiments in humans

Clinical studies were started by Maier-Hauff et al,⁶ wherein the efficacy and tolerability of hyperthermia induced by SPION-coated aminosilane combined with radiotherapy was evaluated in 14 patients for the treatment of recurrent glioblastoma multiforme. The eligibility criteria for the study were: age greater than or equal to 18 years, life expectancy greater than 3 months, tumor diameter less than 5 cm, no multilocal growth, and no metal material near the tumor focus. Each patient received an average of six sessions of thermotherapy. These sessions were preceded with instillation of approximately 0.2 mL of magnetic fluid per mL tumor volume. The average intratumoral temperature reached during the sessions was 44.6°C.

After each thermotherapy session, the patients underwent a simple fraction (2 Gy) of radiotherapy series of 30 Gy, on average. All the patients were tolerant of the instillation of SPION, not showing any complications or adverse effects.

The implementation of AMF brought few side effects, except in some patients who complained of a mild heating sensation in the head. Aspects for survival and local tumor growth were analyzed. Nine patients had their deaths related to tumor progression; however, four deaths were not related to tumor growth or thermotherapy, but were a result of prescription medication. It was concluded that MHT could be satisfactorily used in treating brain tumors because they could be safely applied in patients with glioblastoma, with minimal or no local symptoms.

In 2009, Van Landeghem et al²⁷ presented the first postmortem neuropathological study of three patients with glioblastoma undergoing MHT (Table 5). In this study, the eligibility criteria were: greater than or equal to 18 years, life expectancy greater than 3 months, tumor diameter less than 5 cm, no multilocular growth, and no metal material near the tumor focus. After recurrence of the tumor, MHT was applied as a second line of treatment and was always associated with other therapies (see Table 5). The quantity of magnetic liquid instilled into the tumor varied from 4.2 to 4.5 mL. With the application of AMF, the temperature achieved among the patients ranged between 49.5°C and 65.6°C.

After application of the MHT, survival ranged from 2.1 to 7.9 months (for details see Table 5). The anatomopathological results and other findings listed in Table 5 describe the presence of many nanoparticles in the tumor region, macrophages in the main extension, and a subsequent necrotic area inherent in the tumor. A histological analysis showed that the nanoparticles injected were dispersed or in the form of clusters, and that the necrotic regions were confined to the local instillation of nanoparticles, indicating the need for instillation in multiple locations. Immunohistochemical data revealed that the internalization of the nanoparticles was mainly driven by macrophages and minimally by tumor cells. It was suggested that the internalization by macrophages would be promoted by applying MHT and yet it promoted necrosis with subsequent infiltration and activation of phagocytes.

Later, Maier-Hauff et al⁷ presented a study of 66 patients using the MHT associated with radiotherapy. In this study, they investigated whether MHT was able to increase survival of patients with recurrent glioblastoma multiforme. The criteria for eligibility were: histological diagnosis of supratentorial glioblastoma; evidence of recurrence or progression with up to three additional foci; tumor with a maximum of 7 cm; absence of tumor infiltration in the ventricle, brain stem, corpus callosum, or the contralateral side; aged between 18 and 75 years; score ≥ 69 in the Karnofsky test; and life expectancy of up to 3 months. Seven of 66 patients did not attend the inclusion criteria, which resulted in 59 patients

Table 5 Case reports and results of the autopsies of three patients with glioblastoma who received MHT therapy

Patient	Sex	Age (years)	Glioblastoma	Primary treatment line	Secondary treatment line	Amount of magnetic liquid instilled (mL)	Intratumoral temperature (°C)	Survival after hyperthermia (months)	Brain mass (g)	Autopsy ^a	Average rate of mitosis/proliferation ^b
P1	Male	41	Left Frontoparietal	Surgery, radiotherapy, and chemotherapy	Radiotherapy and six sessions of hyperthermia	4.5	49.5	7.9	1.780	Signs of transtentorial and subfalcial herniation	$1.2 \pm 0.1/0.0625 \mu\text{m}^2/10.3 \pm 1.4\%$
P2 ^c	Male	57	Left Temporo-occipital	Untreated	–	4.2	–	–	1.610	Signs of transtentorial herniation	$1.3 \pm 0.3/0.0625 \mu\text{m}^2/15.3 \pm 2.5\%$
P3	Male	69	Left Temporal	Surgery and radiotherapy	Chemotherapy and six sessions of hyperthermia	4.6	65.6	2.1	1.640	Generalized edema with transtentorial and tonsillar herniation	$1.2 \pm 0.3/0.0625 \mu\text{m}^2/11.8 \pm 1.8\%$

Notes: ^aAll lesions showed aspects of solid tumor with necrosis and hemorrhage, and all autopsies demonstrated multifocal deposition of magnetic nanoparticles, particularly within the geographic necrosis, as the morpho-histological aspects were similar among patients expressing GFAP in one third of the tumor cells and S100 in most cells; ^bThere was no difference between surgical biopsies of patients 1 and 3 regarding the rate of mitosis and proliferation; ^cThe patient died of pneumonia 14 days after instillation of the magnetic fluid.

Source: Van Landeghem et al.²⁷

Abbreviations: GFAP, glial fibrillary acidic protein; MHT, magnetic hyperthermia.

being included in the survival statistical analysis. The average of ferrofluid injected was 4.5 mL, corresponding to an average of 0.28 mL of ferrofluid per cm³ of tumor volume. The AMF (100 kHz) applied ranged from 2 to 15 kA/m. Radiotherapy was applied before or after thermotherapy, with an average dose of 30 Gy, using fractions of 5 × 2 Gy weekly.

In general, two aspects were studied: patient survival after the diagnosis of first recurrence of the tumor (OS-2) and survival after the diagnosis of primary tumor (OS-1). In the OS-2 group, the average survival of 13.4 months was higher compared with 6.2 months in conventional treatments realized by Stupp et al.⁴⁰ The OS-1 group average was 23.2 months in contrast with the study by Stupp, where the average was 14.6 months. In this study, the increase in survival of patients could be correlated to thermotherapy in association with radiotherapy. In addition, thermotherapy showed few side effects and no serious complication, being a good treatment option when compared with conventional therapies.

Discussion

The studies selected for this review sought to evaluate the use of the technique of MHT to treat gliomas with assays in cell culture, animal models, and clinical trials. Some of these studies showed possible mechanisms triggered by hyperthermia leading to death of the tumor cells, in addition to associating hyperthermia with other therapeutic modalities.

The studies that were aimed at the use of MHT in glioma cells (T-9) began with Shinkai,²⁸ through the development of nanoparticles coated with cationic liposomes (MCL), as they were efficient in generating heat, besides having a high affinity to the cell surface, as they are positively charged. The efficiency of MCL was confirmed by Yanase³¹ with glioma T-9 cells, preincubated with MCL which were destroyed after subcutaneous implantation in Fisher rats and subsequent application of AMF. Yanase et al³² also observed that MCL had good distribution after application of AMF, and the best therapeutic strategy in Fisher rats that had developed tumors was the application of three sessions of magnetic radiation. With these studies, it was concluded that MCL could be a nanoparticle functionalized with a good performance for application in MHT.

After confirming the efficiency of MCL, these authors sought to investigate the mechanisms of destruction of glioma tumor cells that could be triggered by MHT. Yanase et al³³ proposed that MHT would be responsible for an antitumor immune response. In this study, they found that hyperthermia induced and promoted an increase of immune cells CD3, CD4, and CD8, and NK cells, in the tumor tissue of Fisher rats.

In the following year, Shinkai et al³⁴ obtained similar results to those obtained from the studies of Yanase in Fisher rats that received three sessions of hyperthermia, and they noted a significant antitumor immune response against repeated inductions of heat. Following this line of study, in 2003, Ito et al²⁹ demonstrated that the immune response observed in tumors undergoing MHT would be primarily activated by HSPs that had increased from the elevation of temperature, both in *in vitro* and *in vivo* experiments. A significant increase in HSP70 was observed in this study.

From the year 2000, there was an intention of associating hyperthermia with other therapies for the treatment of gliomas. Ito et al³⁶ found that the application of a promoter of TNF (pGadFNT) associated with the MCL in the tumor and the subsequent application of AMF would lead to overexpression of TNF- α , significantly reducing the tumor tissue. Similarly, Le et al³⁵ found an increased affinity of MCL to tumorigenic cells with the association of an antibody fragment (Fab), leading to a reduction in tumor growth. These data suggested that hyperthermia associated with other therapies could potentiate its effect.

In 2002, studies that sought new nanocomposites with different coatings, which were able to destroy glioma cells by generating heat, were initiated. In this sense, Ohno et al³⁷ evaluated the efficacy of stick-type CMC. It was noted, after three sessions of AMF, that there was a good distribution of these nanoparticles in the tumor tissue, with no detection in the tissues surrounding the tumor. The efficient heating through these nanoparticles led to remission of the tumors. By then, Jordan et al²³ had tested two new coats: carboxidextran and aminosilane. Aminosilane was more effective compared with carboxidextran, showing a decrease in cell proliferation in the region of the tumor and an increase in the survival of animals that used this nanoparticle. Yet, in the evaluation of new nanocomposites, Meenach et al³⁰ verified in *in vitro* tests, the ability of nanoparticles immersed in hydrogel (PEG) to promote hyperthermia. The application of hydrogel lysed glioblastoma cells into the culture did not affect the cells that were around the gel, showing their effectiveness in reaching only cells bathed by the gel.

Recently, Rabias et al³⁹ characterized a new nanoparticle with a high surface charge (maghemite coated with dextran) with excellent heating in both *in vitro* and *in vivo* tests. In this study, the model of glioblastoma was performed in Wistar rats (model C6). The heating was achieved satisfactorily with the instillation of small volumes of ferrofluid, and presented a good distribution in tumor. The nanoparticle was able, in some cases, to promote complete dissolution of the tumor tissue. Its efficiency was attributed to higher surface charge and the need for small volumes to get a good warming-up.

The development of new nanocomposites made the hyperthermia process effective in achieving the desired temperature in a localized manner, which brought great benefits to patients who used this treatment.

Clinical studies of MHT began with Jordan et al⁴¹ who presented a new system of application of the magnetic field to generate hyperthermia, using magnetic fluids, for future applications in the treatment of glioblastomas and prostate carcinoma. However, in 2007, Maier-Hauff et al⁶ presented a clinical study of 14 patients where MHT was applied (combined with radiotherapy) for the treatment of glioblastomas. A good tolerability to the technique was observed by the patients, without any major side effects. Four patients in this study died, although their deaths were not related to tumor progression or thermotherapy. This could be an indication that the technique of MHT would stop the development of the tumor, thus promising the use of thermotherapy in tumor control, but it is still necessary to have many more studies. In 2009, Van Landeghem et al²⁷ performed a postmortem study in patients with glioblastoma undergoing MHT. The anatomopathological examination verified the presence of many nanoparticles that were dispersed or in an aggregated form, in the tumor tissues. The necrotic regions were limited to areas of instillation of the nanoparticles, which showed that application to more areas was necessary to reach all tumor tissues. It was also found that internalization of the nanoparticles was mainly driven by macrophages and not by tumor cells.

In 2010, following the clinical studies, Maier-Hauff et al⁷ performed a study of 69 patients with recurrent glioblastoma who underwent radiotherapy in combination hyperthermia induced by magnetic nanoparticles. The results of this paper displayed differently to their initial studies, an increase in patient survival when compared with conventional therapies.⁴⁰

Another advantage was the low doses of radiation applied, suggesting that thermotherapy had a major role in increasing survival to these patients. Moreover, it was proposed that this new therapy can be applied in other tumor tissues of the body, since they have similar sensitivity to warming.

Conclusion

In this review we have discussed the application of magnetic hyperthermia for the treatment of gliomas. MHT had its efficacy demonstrated through experiments in vitro where the application of MHT to tumor cell culture promoted cell death by necrosis and/or apoptosis, or even via thermal ablation. This effectiveness was confirmed in the experimental models of rodents where an increase in survival and, in some cases,

a decrease or total regression of tumor mass was observed. In addition, in the first clinical trials, MHT was seen to be promising when combined with other conventional therapies, as a complementary therapy.

Based on these data, we can conclude that MHT has shown to be effective in many cases; however, an improvement of nanocomposites as well as the upgrading of equipment for induction of AMF, could contribute to the establishment of technique in the treatment of gliomas.

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Disclosure

The authors report no conflicts of interest in this work.

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