New Advances in Nanomedicine: Induced Hyperthermia by Magnetic Nanoparticles in the Treatment of Glioblastoma Multiforme

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Abstract
Despite technological developments in diagnosis and treatment options, glioblastoma multiforme (GBM) remains the most aggressive and difficult to treat malignant brain tumor that is associated with a high prevalence as well as a high mortality rate. More efficient therapeutic strategies that could overcome the inability to properly treat GBM tumors are needed. Hyperthermia is well-studied procedure that exposes tumor cells to a local temperature increase that results into changes in cellular structures and even cell apoptosis. However, hyperthermia in the treatment of glioblastoma has not yet been established in clinical practice due to physical limitations and toxicity. Quite recently, advances have been made in the application of magnetic nanoparticles (MNPs) that induce hyperthermia offer an attractive approach. This mini-review first addresses current challenges in the treatment of GBM and then focuses on the molecular dynamics behind hyperthermia. It also summarizes physical properties of MNPs and their role in thermotherapy. The review further highlights some exciting examples of studies that used this novel approach in the treatment of GBM.

Introduction
Glioblastoma multiforme (GBM) is the most common brain tumor in adults and has been classified by the World Health Organization as the most malignant type of all nervous system tumors [1]. Even if the tumor is detected in an early stage, the prognosis remains poor: the median survival rate for GBM patients is approximately 15 months [2, 3]. The current standard therapy for GBM involves neurosurgery, radiation therapy and chemotherapy [2]. The first choice of treatment is surgery, with the main objective improvement of symptoms and to prolong survival. Even after surgery, residual tumor cells persist because complete resection of the tumor cells is impossible due to their infiltrative growth pattern [2, 3]. They are often deeply penetrated within brain structures and extend beyond the margins of the main tumor mass that are not detected by MRI [4, 5].

Although new strategies in the treatment of GBM are constantly being developed, yet up to this point, new molecular targeted therapeutic drugs are still unable to overcome physiological obstacles in the central nervous system, and thus are unable to reach the tumor [6]. One of such physiological obstacles that causes unsuccessful drug delivery to tumor cells is the blood-brain barrier (BBB), that because of its defective vascular architecture prevents the transport of (large) molecules to the brain [7]. In addition, GBM tumors have high metabolic demands that are met via the formation of new vessels that disrupt the normal BBB and this further impairs delivery of drug agents [8].

Nevertheless, not all is lost: A nanotechnology-based drug delivery strategy could possibly beat these challenges. Recent advances in the application of magnetic nanoparticles (MNPs) that induce hyperthermia seem to be a great potential in combating glioblastoma multiforme [8, 9].

Hyperthermia
A long time ago, the Greek physician Hippocrates mentioned that high temperatures could possibly cure or diminish tumors [10]. At this time, hyperthermia has been acknowledged by several scholars due to its potential application in the treatment of cancer. Hyperthermia is a treatment that promotes the increase of temperature in tumor cells in order to change their properties [11, 12]. Previous studies
have shown that the hyperthermia temperature range (40-47°C) induces cellular apoptosis and cell cycle arrest and improves the effects of radiotherapy and chemotherapy in the treatment of cancer 13 14.

In general, the exact mechanisms by which hyperthermia induces cell damage are complex. At the cellular level, research has revealed that hyperthermia affects almost every organelle 15. Damage to cellular morphology has been observed, possibly due to a loss of microvilli and the disruption of cytoskeleton structures, such as the depolymerization of the microtubules, microfilaments, intermediate filaments and centrosome organization 15 16. Furthermore, it has also been observed that hyperthermia damages membrane transport proteins and changes the permeability of the cell membrane 15. Particularly, at specific temperatures, structural alterations of the lipid bilayer membrane structure occur that make it less rigid, resulting into impairment of ion transportation and changes in membrane potential 16. In addition, previous studies have shown that hyperthermia results into a decrease of ATP production in mitochondria 16. All in all, hyperthermia has many effects on the structure and physiology of the cell. For this reason, the question that occupies us here is to examine which molecular changes are critical with regards to the killing of tumor cells such as glioblastomas.

**Killing the tumor: the molecular mechanisms of hyperthermia**

One of the main mechanism by which hyperthermia induces cell death involves the denaturation and aggregation of nuclear proteins 15. High temperatures cause the unfolding of proteins, thereby exposing their hydrophobic groups, which can then react with each other to form aggregates that are insoluble. It is believed that denaturation and aggregation of nuclear proteins are the first steps towards nuclear thermal damage 11 14 15 because the increase of protein content in the nuclear matrix has major influences on important molecular functions 11 15.

**Inhibiting DNA Repair Systems.** Specifically, the main functions of the nuclear matrix, DNA repair, DNA replication and DNA segregation, are all inhibited by hyperthermia 11 15 17. Moreover, previous studies also suggest that heat causes DNA double-strand breaks and inhibits important repair enzymes such as DNA polymerases-β key enzymes 18. These findings are relevant for combining hyperthermia with radiotherapy, because inhibition of the cells repair system followed by radiation induces cell death 19.

**Changing the Expression of Apoptosis Genes.** As described by Wong (2011) 20, apoptosis is an “ordered and orchestrated cellular process” (p. 1) that programs induced cell death. In cancer however, tumor cells are able to evade apoptosis. The dysregulation of apoptosis in malignant cells is caused by a disruption in the balance of anti-apoptotic and pro-apoptotic proteins, such as Bcl-2 overexpression in glioblastoma cells, increased expression of inhibitor of apoptosis proteins, defects in tumor suppressor genes (e.g. defects in p53 genes), reduced expression of important signal cascades (e.g. decreased extracellular signal-regulated kinase) 16 20. Though a decreased affinity for apoptosis is present in cancer cells, hyperthermia, on the contrary, stimulates programmed cell death 16. Liang et al. (2007) 21 found a change in the expression apoptosis genes in cells after they had been exposed to hyperthermia, chemotherapy and radiation. Particularly, their study demonstrated that the expression of Bax gene (a gene that is capable of inducing cell death) increased, whereas Bcl-2 gene expression was down regulated after treatment.

**An Immune Response to Heat.** The therapeutic effects of hyperthermia treatment are not merely caused by alterations in cell morphology and function; rather, they are also caused by an immune response to thermal stress 22 23. Temperatures of about 42°C are already enough to stimulate killer cells that are powerful immunostimulants for hyperthermia 22. The mechanism of antitumor immunoimmunity by the immune system of the patient is associated with the release of heat shock proteins (HSPs). When tumor cells are exposed to thermal stress, HSPs (HSP70, HSP90 and glucose-regulated protein 96 [gp 96]) are thought to present tumor antigens 23 24. Respectively, in their proposed scenario, Ito et al. (2005) 24 argue that tumor cells have a low concentration of intracellular HSP-peptide complexes as well as a low concentration of major histocompatibility complex (MHC) class I-peptide complexes at the outer part of the cell. When cells are exposed to elevated temperatures, concentrations of both complexes rise, which enables MHC class I-restricted cytotoxic T-cells (CTLs) to identify tumor cells and kill them. Furthermore, dying tumor
cells release their HSP-peptide complexes that activate neighbouring cells to recruit antigen-presenting cells, leading to a cascade pathway by which other tumor cells are presented to CTLs as well [24].

Limitations to conventional methods of hyperthermia
To put it briefly, the effects of hyperthermia on tumor cells involve protein denaturation and aggregation, alteration in cell signaling and morphology, reduced functionality of the DNA repair mechanisms and apoptosis initiation. Conventional techniques that are used to induce hyperthermia are “ultrasound, microwaves, infrared irradiation, and tubes with hot water” (9, p. 2864). On the whole, hyperthermia seems to be an appealing approach to treat glioblastomas, because it has less side effects than chemotherapy and radiation, and it can be used side by side with these conventional forms of therapy. Although previous studies have shown beneficial effects of hyperthermia as an adjunctive therapy, yet it is still not part of the standard oncological therapy [12, 25, 26]. This is most probably due to the systematic problems that occur when using conventional methods to induce hyperthermia [9, 26, 27]. For instance, monitoring a homologous distribution, tumor-specificity, as well as the effectiveness (response rate) are still major points for improvement [25, 27, 28]. Moreover, particularly in the case of treating glioblastomas with hyperthermia it is difficult to obtain adequate high intratumoral temperatures [29]. In sum, even though hyperthermia seems to be an efficient method to add to the standard form of cancer therapy, current ways to achieve it are often inefficient, which may cause the tumor to recur.

Magnetically induced hyperthermia by nanoparticles
During the last two decades, magnetic nanoparticles (MNPs) have gained much attention by researchers due to their special properties [30]. Respectively, their physical and chemical characteristics allow a wide variety of innovative applications, such as targeted drug delivery and imaging of malignant tumor cells [31, 32]. Their reduced risk of toxicity, as well as their capacity to pass through the blood-brain barrier (BBB), offer an attractive approach in the combat against glioblastoma [32]. Regardless of the numerous studies proving that MNPs are efficient, due to the thermal conductivity of metallic nanoparticles, the heat that has been generated is depleted into the surrounding tissue [28, 36]. Previous studies have found that the MNPs employ different mechanisms in order to convert magnetic energy into heat. Fortin et al. (2008) [37] showed that the Néel relaxation contributed most to thermal production. To clarify, in the Néel relaxation mode, the magnetic moment of the nanoparticle rotates away from its axis, thereby creating internal friction that results into the release of heat [32, 38]. The main advantage of thermotherapy by MNPs is that...
a small volume of the nanoparticle containing fluid can be injected, almost continuously at the target site, thereby allowing tumor specific dose delivery and accurate heating \[^{[26][28]}\]. This ability addresses shortcomings in previous methods that induced hyperthermia, that is to say, by using MNPs; effective high temperatures are reached within the boundaries of the tumor site without harming the surrounding healthy tissue \[^{[38]}\].

**Treating glioblastomas with MNP-based hyperthermia**

MNP-based theranostics has been evaluated for feasibility and its effects have been examined in various in vivo and in vitro, animal and clinical studies. A study by Rabias et al. (2010) \[^{[39]}\] demonstrated that small quantities of nanoparticles responded with significant high temperatures and were distributed evenly in the tumor site of Wistar rats. In 2007, Maier-Hauß et al. \[^{[40]}\] presented the first clinical study where hyperthermia using magnetic nanoparticles together with radiation therapy was used in treating patients with glioblastoma multiforme. Patients reported only mild side effects and did not present with any complications. As a result, their clinical outcomes indicated a promising approach, yet they noted the need for further research. Followed by a Phase II clinical study, Maier-Hauß et al. (2010) \[^{[41]}\] found a prolonged survival rate compared with conventional methods of treatment (radiotherapy and chemotherapy with Temozolomide) for patients with recurrent GBM who underwent low-dose radiotherapy in combination with theranostics induced by MNPs. Additionally, their results also suggest that this method is suitable to use in conjunction with other therapies and this could offer a greater effectiveness as well as a promising approach to treat tumors other than glioblastomas.

Prevailing obstacles in utilizing MNPs to induce hyperthermia in the treatment of GBM involve the high concentrations of MNPs that are needed in order to reach high temperatures, the inability to undergo follow-up MRI imaging, in addition to an homologous distribution of the MNPs at the tumor site \[^{[32][42]}\]. Specifically, a heterogenous distribution of nanoparticles results into a decrease of the rate at which they oscillate when subjected to an alternating magnetic field, thereby releasing less thermal energy and thus reducing the effectiveness of the hyperthermia-based treatment \[^{[43]}\].

**Conclusion**

GBM is an aggressive brain tumor type that remains as one of the most difficult to treat. Standard forms of treatment include chemotherapy, radiation and surgery in which the tumor mass is resected. Unfortunately, infiltrative malignant cells are left behind that permit the tumor to recur despite extensive post-surgical treatment, which eventually causes the demise of the patient. New therapeutic drugs have been developed, yet without remarkable success: The majority of pharmaceutical agents administered was unable to cross the blood-brain barrier and thus never reached the tumor region. Traditional hyperthermia using external devices involves local temperature increase in order to damage tumor cells. Previous research has shown that high temperatures have a cytotoxic effect, due to alterations in cytoskeletal organization, protein aggregation at the nuclear matrix, inhibited DNA repair systems, altered expression of apoptosis genes and activation of the anti-tumor immune system. Nevertheless, conventional methods of hyperthermia lack accurate temperature control, leading to thermal underdosage at the target site and damaging healthy cells. A new advance in nanomedicine, using magnetic nanoparticles in order to induce hyperthermia, seems to be a promising approach in the combat against glioblastomas. Nanoparticle-based hyperthermia allows accurate dosage administration as well as controlling the amount of heat that is generated during the process. Regarding safety, earlier studies have shown that this type of therapy is associated with minimal side effects, although further research needs to be conducted in order to confirm these results. In addition, MNP-based hyperthermia combined with radiotherapy and chemotherapy has demonstrated a prolonged survival rate in patients with recurrent GBM. Notwithstanding, there are some challenges facing the use of MNPs in theranostics. A major problem is the heterogenous distribution of nanoparticles at their target destination. All in all, inducing hyperthermia by magnetic nanoparticles is an attractive strategy in the treatment of glioblastoma multiforme. Optimizing methods that secure adequate and homogenous delivery and a higher effective dose are the challenges for further clinical research before nanoparticle-mediated hyperthermia can be introduced in standard care.
References


