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Short Review

New Frontiers for Astrocytic Tumours

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Abstract. *Glioblastoma multiforme, the most common type of primary brain tumour, remains an unsolved clinical problem. A great deal of work has been done in an effort to understand the biology and genetics of glioblastoma multiforme, but clinically effective treatments remain elusive. It is well known that malignant gliomas develop resistance to chemo- and radiotherapy. In this review we evaluated the literature data regarding therapeutic progress for the treatment of astrocytic tumours, focusing our attention on new frontiers for glioblastoma. The research studies performed in in vitro and in vivo models show that the application of hyperthermia using magnetic nanoparticles is safe and could be a promising tool in the treatment of glioblastoma patients. Our efforts are focused towards new fields of research, for example nanomedicine and the study of the uptake and cytotoxic effects of magnetic nanoparticles. The improvement of the quality of life of patients, by increasing their survival rate is the best result to be pursued, since these tumours are considered as ineradicable.*

Glioblastoma multiforme (GBM, WHO grade IV) is the most malignant type of glioma and, in spite of the advancements in tumour therapy, has the same prognosis as it did 50 years ago: 6 months overall survival after surgery and 12 months after surgery and radiotherapy. Both genomics and proteomics studies have improved our knowledge on the pathogenesis and oncogenesis, but neither chemotherapy, radiotherapy nor gene therapy has altered the prognosis, with the exception perhaps of temozolomide (1, 2). This failure is based on the characteristics of GBM, mainly its genotypic and phenotypic heterogeneity and its clonal evolution during the transformation process (3). Additional reasons of failure are the absence of specific antigens, the complexity of the molecular pathways involved, feed-back regulation of the involved pathways and by-pass of molecular arrests due to alternative pathways. Therapeutic and diagnostic fields are changing, adapting new strategies of development to expand existing markers and to develop new ones. The emerging field of nanomedicine is an area in which technologies and abilities in nanotechnology need to be developed. Recent advances are performed in the direction of developing multifunctional nanoparticles (4-16). The magnetic nanosystems are sometimes called theranostic agents due to their simultaneous diagnostic and therapeutic use. The diagnostic function is obtained by optimizing the magnetic nanostructure (MNS) so that it acts as a contrast agent for magnetic resonance imaging (MRI) and/or as a fluorescent agent, thus allowing the mapping of the distribution of the particles in the pathological tissues. The therapeutic function is carried out through drug delivery, if the MNS is able to carry a drug, or by magnetic fluid hyperthermia (MFH).

These exciting developments are opening up opportunities for future personalised oncology in which cancer detection, diagnosis and therapy are tailored to each individual patient's profile, and also for predictive oncology, in which genetic/molecular information is used to predict development, tumour progression and clinical outcome. In this context, MFH is a new technique already utilized together with other therapeutic tools for the cure of carcinomas, mainly of glioblastoma and prostate cancer (17-19). Thus, the possibility of associating the therapeutic effect of magnetic nanoparticles (MNPs) with enhanced contrast in MRI images and with the other well-known properties of

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MNPs (such as drug delivery with subsequent *in situ* controlled drug release, and molecular targeting through their conjugation with an antibody), is extremely appealing since it would allow, for example, the particle distribution to be tracked by MRI both before heating the tissue and after thermotherapy. This would allow for immediate assessment of the efficacy of the treatment.

Our research group has been primarily devoted to studies of basic research in the field of neuro-oncology for many years. Significant data were obtained on: new markers of proliferation in glial tumours of high and low grade; proliferation and apoptosis and the response of malignant glioblastoma cells after exposure to ionizing radiation; immunobiology of malignant tumors; serum levels of cytokines and adhesion molecules (Intercellular Adhesion Molecule 1, ICAM-I) in patients; bystander effect induced by gamma radiation in different lines of human glioblastoma evaluating cell survival and the release of cytokines (Interleukin-8, Interleukin-6, Trasforming growth factor β, TGF-β) and their receptors. Proteins such as calreticulin, heat-shock protein 70, high-mobility group BOX-1, released after cell death induced by combined treatment (radiation plus chemotherapy drugs) have been studied in regard to subsequent stimulation of immune system cells to activate mechanisms of elimination of cancer cells (20-29).

New frontiers of research concern the cytotoxic effects of MNPs in cells of astrocytic tumours and subsequent *in vivo* studies in order to determine the toxicity of these particles and their risks to human health. The development of basic knowledge regarding the interaction, uptake and cytotoxicity of MNP in cell cultures of astrocytic tumours could help develop new combination therapies for the treatment of patients with malignant astrocytic tumours. Currently in the literature, there are only very few reports on effective treatment of malignant glioma by MNPs in experimental rat models or in patients with GBM. The effect of thermotherapy using MNPs was studied by Jordan *et al*. on rat malignant glioma RG-2, a well-established model of human malignant glioma. In this study Jordan *et al*. showed that thermotherapy using nanoparticle coated with aminosilane, increases the survival of glioma-bearing rats (30). In 2007 Maier-Hauff *et al*. evaluated the feasibility and tolerability of the newly developed thermotherapy using MNPs on patients with GBM recurrences. The authors concluded that magnetic hyperthermia can be applied safely in the treatment of brain tumours (31). Another study reported post-mortem neuropathological findings on patients with GBM receiving magnetic thermotherapy (32). Following instillation of magnetic nanoparticles, most of the nanoparticles tended to aggregate and preferentially located in a specific necrotic area within the tumor, close to the sites of instillation. At the borders of the aggregates, the particles were phagocytosed mainly by macrophages. Data suggest that the instillation of MNPs for MFH in patients with GBM results in the uptake of nanoparticles in glioblastoma cells to a minor extent and in macrophages to a major extent. MFH therapy further promotes the uptake of nanoparticles by macrophages, likely as a consequence of tumour inherent and therapy-induced necrosis, with subsequent infiltration and activation of phagocytes (31). In 2010, Maier-Huff *et al*. published their data regarding the efficacy and safety of intratumoral thermotherapy using iron-oxide MNPs combined with fractionated stereotactic external beam radiotherapy for patients with recurrent GBM. The authors suggested that "thermotherapy using magnetic nanoparticles in conjunction with a reduced radiation dose is safe and effective and leads to longer overall survival compared to conventional therapies in the treatment of recurrent glioblastoma" (33). Continuing these studies, in our laboratory, we obtained preliminary data regarding the uptake and cytotoxicity of MNPs *in vitro*. The study of glioblastoma cells showed that MNPs efficiently penetrate into the cells and are mainly localized within the cytoplasm (forming aggregates), on the cell membrane or around the nuclear membrane. Under our experimental conditions, we did not find cytotoxicity attributed to MNPs in cells with apoptotic features, suggesting that at the concentrations tested the glioblastoma cell line T98G could tolerate this treatment. Our studies are in progress and in order to improve our data, we should test MNPs of smaller diameter to reduce the formation of aggregates. In addition, the next step will be to evaluate the cytotoxicity of these MNPs through clonogenicity, survival and cell viability assays.

New frontiers for astrocytic tumours should be based on the acquirement of new knowledge on the interaction between nanoparticles and subcellular organelles using *in vitro* and *in vivo* experimental models of astrocytic tumours. The study of the toxic effects of MNPs that uses animal models with tumours is an experimental model comparable to the situation of patients with malignant glioma. Scientific data suggest that it is important to determine the impact of these nanoparticles on human health by studying the toxicity and the risks associated with exposure of both researchers and patients. The existing toxicological data regarding nanoparticles remain insufficient due to the small number of studies, the short exposure period, the different composition of the nanoparticles tested (diameter, length and agglomeration), and the various exposure routes in the work environment, among other factors. Regarding glioblastoma, the most aggressive and invasive cancer of the central nervous system, the survival rate remains unchanged as it is a radio- and chemo-resistant cancer. To date, understanding the basic biology of this tumour remains unsolved. It therefore makes sense to direct the efforts of scientific research to new frontiers, using theranostics and theranostic agents such as MNPs, in an attempt to reduce the tumour for subsequent neurosurgical treatment.

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