Review

Potential Role of Carbon Nanomaterials in the Treatment of Malignant Brain Gliomas

Maria Caffo [1], Antonello Curcio [1], Kumar Rajiv [2], Gerardo Caruso [1*] and Antonino Germanò [1]

[1] Department of Biomedical and Dental Sciences and Morphofunctional Imaging, Neurosurgical Clinic, University of Messina, Messina, Italy; mariella.caffo@unime.it / antonello.curcio@gmail.com / Gerardo.caruso@unime.it / germano@unime.it

[2] NIET, National Institute of Medical Science, University of Delhi, New Delhi, India; chemistry_rajiv@hotmail.com

* Correspondence: gcaruso@unime.it; Tel.: ++39-090-2212865;

Abstract: Malignant gliomas are the most common primary brain tumors in adults up to an extent of 78% of all primary malignant brain tumors. However, total surgical resection is almost unachievable due to considerable infiltrative ability of glial cells. The efficacy of current multimodal therapeutic strategies is, furthermore, limited by the lack of specific therapies against malignant cells, and, therefore, the prognosis these in patients is still very unfavorable. The limitation of conventional therapies, which may result from inefficient delivery of the therapeutic or contrast agent to brain tumors are major reasons for this unsolved clinical problem. The major problem in brain drug delivery is the presence of the blood brain barrier which limits the delivery of many chemotherapeutic agents. Nanoparticles, thanks to their chemical configuration, are able to go through the blood-brain barrier carrying drugs or genes targeted against gliomas. Carbon nanomaterials show distinct properties including electronic properties, penetrating capability on the cell membrane, high drug-loading and pH-dependent therapeutic unloading capacities, thermal properties, large surface area and easy modification with molecules, which render them as a suitable candidate to deliver drugs. In this review we will focus on the potential effectiveness of the use of carbon nanomaterials in the treatment of malignant gliomas discussing the current progress of in vitro and in vivo researches of carbon nanomaterials-based drug delivery to brain.

Keywords: Blood-Brain Barrier; Brain Drug Delivery; Carbon Nanomaterials; Cerebral Gliomas; Glioblastoma; Nanoparticles.

1. Introduction

Cerebral gliomas are the most frequent, intrinsic, primary tumors of the central nervous system (CNS). Their incidence is about 6 cases per 100,000 people per year [1]. Among them, glioblastoma (GBM) is the most frequent and most malignant histological type, the incidence of which is approximately 57% of all gliomas and 48% of all primary malignant CNS tumors [2]. It predominantly affects adults with a maximum incidence between 50years and 70years. Gliomas observed after the age of sixty account for 90% of GBM. The grading system, recently updated, proposed by WHO is the most accepted and widespread [3]. The new WHO classification combines, in addition to data relating to tumor histology and grading, also molecular data, thus obtaining a system for evaluating brain tumors, much more precise and intrinsically linked to the biomolecular characteristics of the specific cancer. GBM consists of immature astrocytes and spongioblasts and its cells have a high proliferative index. GBM has rapid growth both expansive and infiltrating the surrounding nervous parenchyma. It is variably edematous. They are characterized by the rapid evolution with the appearance, in a short time, of a focal syndrome associated with signs of intracranial hypertension.

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Surgery still remains the first step in gliomas, as it is also necessary to obtain a definitive histological examination of the lesion. Usually, the surgery must aim at the removal of the tumor as radical as possible (Figure 1). Alternatively, only a biopsy can be performed. However, rare cases of gliomas of the diencephalon, midbrain, and deep hindbrain and those with extensive extension into the corpus callosum are not amenable to surgical treatment. Adjuvant therapy in the absence of a certain diagnosis is done following the STUPP protocol [4]. STUPP protocol is based on the administration of temozolomide (TMZ) followed by radiotherapy (RT). TMZ is an oral alkylating agent that is a prodrug that activates itself, without enzymatic catalysis in the physiological pH of cells, into the active metabolite monomethyl triazenoimidazole carboxamide (MTIC). The toxic effects of MTIC are associated with alkylation of DNA, especially at the O6 and N7 positions of the nitrogenous base guanine. The chemosensitizing protocol contemplate the administration of 75mg per m2 per day, every day until the end of the radiotherapy. Subsequently, one month later, six cycles of chemotherapy began. Each cycle is defined as 5 days of TMZ every 28 days at a maximum dose of 150mg per m2 per day for the first cycle and 200mg per m2 per day for the next 5 cycles. RT consists of fractionated, targeted, doses of 2 Gy once daily for five days a week for six weeks. The STUPP protocol increased median survival to 14.6 months versus 12.1 with radiotherapy alone. The five-year survival rate increased to 9.8% versus 1.9% without STUPP protocol [5]. Another potentially useful drug in recurrent GBM is bevacizumab, a monoclonal antibody against VEGF. Irinotecan and bevacizumab demonstrated notable antitumor activity in patients with GBM, already surgically treated, in first or second relapse [6]. Procarbazine, lomustine and vincristine (PVC) are indicated as second line in patients with poor response to the STUPP protocol. However, the efficacy of current anti-cancer strategies in gliomas is limited by the blood-brain barrier (BBB) that hinders the delivery of many chemotherapeutic agents and macromolecules. Tumoral invasion is a multifactorial process, characterized by interactions between extracellular matrix protein and adjacent cells, as well as accompanying biochemical processes supportive of active cells movement [7]. Recent advances in gliomas molecular pathology and biology have evidence various genes involved in cell growth, apoptosis, and angiogenesis. The modulation of gene expression at more levels, such as DNA, mRNA, proteins and transduction signal pathways, may be the most effective modality to down-regulate or silence some specific genes functions.

Nanotechnology, which is widely used in many industrial trades, can be a valuable aid in the development of new glioma treatments. Because of their size, nanoparticles (NPs) can cross the BBB and, by acting as carriers, can deliver even more therapeutic compounds capable of interacting with multiple targets. It is possible to use nanotechnology to deliver the drug to the targeted tissue across the BBB, release the drug at a controlled rate, and avoid multidrug resistance. NPs can be designed to transport therapeutic drugs and imaging agents that are loaded onto or within the nanocarriers via chemical conjugation or encapsulation.

Carbon nanomaterials (CNs), which have been studied for some time, possess peculiar characteristics such as long stability, the ability to form stable bonds with various functional groups such as to make them suitable for numerous applications in both the industrial and biomedical fields [8]. The high biocompatibility makes them particularly functional in medical and pharmacological technologies. CNs also possess antibacterial activity [9], and can be structured into pharmacological compounds with potential use both in new anticancer therapeutic protocols and in brain drug delivery systems [10]

In this study, our goal was to report the potential and most innovative applications of CNs in the treatment of brain tumors.

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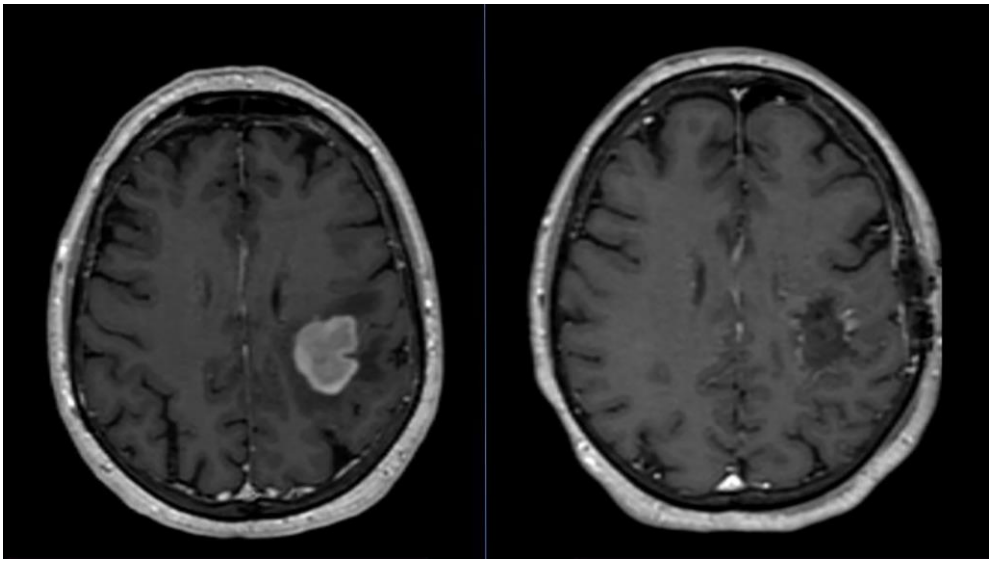

Figure 1. (**a**) Pre-operative MRI of a left frontal GBM; (**b**) Post-operative MRI. It is important to note that even if the resection is a supratotal resection, GBM has already infiltrated microscopically the nearest parenchyma.

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2. Blood-Brain Barrier

The BBB represents one of the well-defined barriers separating blood from the neuronal parenchyma. The properties of this barrier are determined by the intercellular tight junction (TJ) which reduce the paracellular permeability and the passage of large molecules. The anatomical location of the BBB is into the cerebral capillary endothelium. The nomenclature "neurovascular unit" is now used to describe the combined activity, cohesion of microvessels together with the neurons and glia that surround them. Understanding the BBB is of fundamental importance and its implications are necessary for understanding pharmacodynamic of the therapies for neurological disease, since most large molecules that could promote a benefit in the treatment of brain diseases, from cancers to neurodegenerative diseases, do not cross the BBB or cross it in part or pass-through small quantities before being degraded. New strategies are constantly being developed to overcome the BBB, especially through the bioengineered fusion of proteins that can be used as co-transporters or specific transporters to allow access to the brain parenchyma. However, the BBB is fundamental in supplying nutrients to the CNS, in allowing an outflow of waste molecules from the brain, in restricting the passage of ions and fluids through the blood and the brain thus protecting the brain from significant fluctuations that may occur within the blood proper of ionic compounds of catabolites and metabolites.

The endothelium of the cerebral capillaries is characterized by the presence of intercellular TJ as well as abundant cytoplasm, abundant mitochondria and a low rate of endocytosis and pinocytosis. The structures of the interendothelial junction that allow the formation of the BBB are the TJs, other are a group of proteins with transmembrane domains, four, and with two extracellular loops respectively defined as occludins and claudins. Another important structure are the adherens junctions which collaborate with the TJs and contain the vascular endothelial cadherin and the platelet endothelial cell adhesion molecule. Catenins represent the key point of interconnection between the intercellular structures and the cellular cytoskeleton. Other junctional elements include proteins of the immunoglobulin superfamily and are respectively junctional adhesion molecules and endothelial cell-selective adhesion molecule. The endothelial cytoplasm of the cerebral capillaries contains a large number of regulatory and signal proteins whose function is to modulate the interaction of membrane proteins with the active proteins of the cytoskeleton, such as zona occludens, calcium-dependent protein kinases. Although the anatomical site of the BBB is defined as the cerebral capillary endothelium, this distinctive endothelium also exhibits dynamic interactions with numerous other cell types. In fact, are surrounded from pericytes and astrocyte stalks, which are often considered as the cells that connect the brain barrier to the cerebral environment. Therefore, a bidirectional interaction between the capillary endothelium of the CNS and its neighboring cells actually represents today the true definition of BBB (Figure 2). Here there are important proteins that manage the maintenance of the structure both in a dynamic and physical sense, for example TGF-beta, the glial cell derived neurotrophic factor and angiopoietin1. Therefore, since all these interconnections are present between all these cells that manage the passage, how the passage of the molecules through the blood-brain barrier really takes place depends on the size and biological properties of the molecules involved: the hydrophilic molecules can pass through the interendothelial spaces; lipophilic substances and gaseous particles, just like oxygen and carbon dioxide, instead directly cross the cellular endothelium. Specific transport proteins exist for different types of molecules, for example the glucose transporter GLUT1, the LAT1 transporter for amino acids, P-glycoprotein, and a whole other series of carriers. Some barrier transporters are also polarized, showing different properties inside and outside the barrier thus allowing certain ionic passages. In fact, there is a genetic selectivity expressed in the cerebral capillaries: it allows the production of specific proteins. There is also a receptor-mediated transit mechanism to transport even larger proteins, such as plasma proteins including albumin, that would not otherwise pass.

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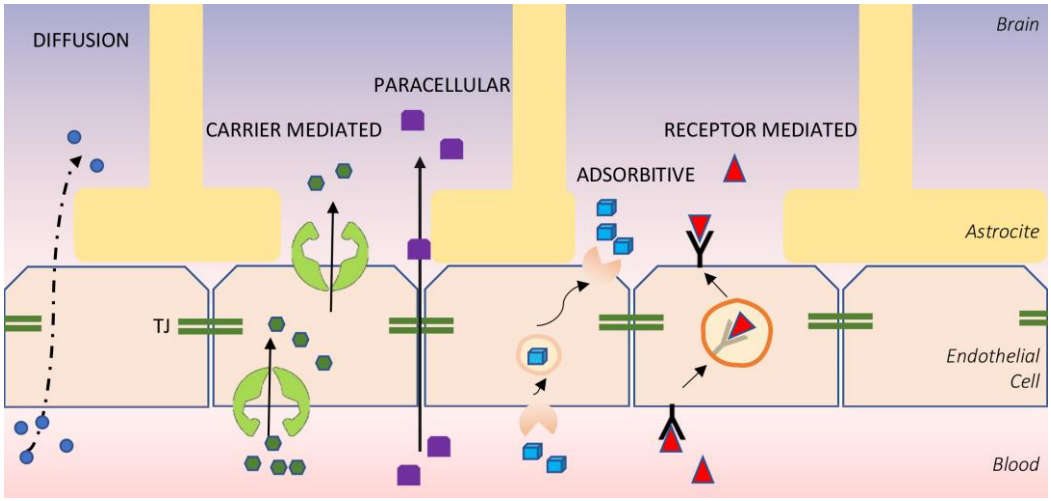

Figure 2. Scheme of BBB with its different transport mechanism.

3. Nanotechnology

Nanotechnology is regarded as a developing field with potential applications in cancer research and treatment. The manipulation of matter at the molecular and atomic levels (i.e. on a dimensional scale smaller than the micrometer, i.e. between 1 and 100 nanometers) is the domain of nanotechnologies. There are no more distinctions between chemistry, physics, engineering, mathematics, and biology in this nanoworld. In comparison to conventional treatments, NPs systems in cancer therapies provide better therapeutic and diagnostic agent penetration and lower risk [11]. Many mechanisms for brain-targeted delivery can be engineered into NPs, including receptor-mediated transcytosis, carrier-mediated transcytosis, and adsorptive mediated transcytosis. These systems can also reduce toxicity to peripheral organs and improve biodegradability. The goal of nanotechnology is to create and characterize ultra-small particles. NPs are structures with a diameter of 10-200 nm that have nearly limitless design and application possibilities in biologic systems and are used for diagnostic and therapeutic purposes. NPs can penetrate cell membranes and collaborate with biomolecules due to their extremely small dimensions, and their physical properties make them excellent imaging agents and semiconductors. The goal of using NPs for cancer treatment is to deliver the right drugs to the right patient at the right time in the right concentration [12]. This ideal concept is difficult to achieve due to the disparities in drug adhesion, distribution, metabolism, and excretion [13]. Many factors make nanomedicine superior to conventional medicine for cancer treatment: because of the increased permeability of malformed tumor vascular walls with leaky cell-to-cell junctions and dysfunctional lymphatics in tumorous tissues, their dimensions allow them to be passively accumulated in cancer cells; the expression on their surface of various targeting ligands allows the link with specific targets on tumor cells or in tumor microenvironment (TME), enhancing their accumulation [14]. Because NPs are not physically recognized as substrates, they can be used to bypass tumor escape mechanisms as drug efflux pumps [15]. In vivo and ex vivo studies show that NPs are more useful for detecting and killing cancer cells due to the delivery and release of bioactive molecules under desired temperature, pH, or enzymatic catalysis conditions [16,17]. Furthermore, encapsulation protects bioactive molecules from degradation, increasing their solubility in biological fluids. The transport of NP through blood circulation to tumor regions via blood vessels; the crossing of vasculature walls to reach surrounding tumor tissues; the introduction in the interstitial space to target cells; and cellular uptake via endocytosis and intracellular delivery are all part of the in vivo NP delivery process [18]. Phagocytosis, clathrin-mediated endocytosis, caveolin-mediated endocytosis, clathrin/caveolae-

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independent endocytosis, and micropinocytosis are the five major mechanisms involved in the endocytosis of NPs by target cells.

4. Brain Drug Delivery

The process of releasing a compound at a specific rate and location is known as drug delivery. Novel drugs necessitate effective delivery technologies that reduce side effects and improve patient compliance. Conventional anticancer agents are cytotoxic due to their low molecular weights and high pharmacokinetic volumes of distribution. High concentrations yield effective doses, but when administered alone, these drugs lack specificity and cause significant damage to non-cancerous tissues. Furthermore, the majority of chemotherapeutic agents are poorly soluble and are mixed with toxic solvents [19]. NPs-based drug delivery systems improve the penetration of therapeutic and diagnostic agents into the desired site, allowing for efficacy with lower doses and systemic drug concentration with minimal risks. NPs-based drug delivery has the potential to improve drug bioavailability, improve drug molecule timing, and enable precision drug targeting without compromising the structural and functional integrity of the BBB [20]. Size-dependent passive targeting or active targeting can be used to deliver NPs to specific sites. Passive targeting entails chemically modifying the NPs to increase permeability or stability. Insertion of ethylene oxide polymers, also known as poly-(ethylene glycol) (PEG), is the most common surface modification. PEG can increase the half-life of nanocarrier drug delivery systems by decreasing macrophage uptake due to steric repulsion effects and inhibiting plasma-protein adsorption [21]. PEGylation has been used successfully in a wide range of drug delivery systems, including lipid, polymeric, and inorganic NPs. Active targeting is typically accomplished through the incorporation of a receptor-specific ligand that promotes the targeting of drug-containing NPs towards specific cells. The use of peripherally conjugated targeting moieties for enhanced delivery of NPs systems is referred to as active targeting. This method was used to achieve high selectivity to specific tissues and to improve NP uptake into cancer cells and angiogenic microcapillaries. These compounds include an anticancer agent, a targeting moiety-penetration enhancer, such as receptors, receptor ligands, enzymes, antibodies, and surface modifications in active targeting methods. Another important feature of nanopharmaceuticals is the "triggered response," which means that they can only begin to act in response to a specific activating signal (such as the influence of a magnetic field), allowing the NPs to release the drug locally once they have reached their target within the patient's organism.

The goal of absorption-mediated transcytosis is to deliver drugs via electrostatic interactions via NPs systems functionalized with cell-penetrating peptides or cationic proteins. The adsorptive process, however, occurs in blood vessels and other organs because it is a non-specific process. This makes it difficult to achieve therapeutic concentrations in the brain while also limiting drug distribution in non-target organs. CPPs and cationic proteins (e.g., albumin) are being studied to improve brain drug delivery via adsorptive-mediated transcytosis. CPPs have effectively delivered a wide range of cargo molecules/materials into cells, including small molecules, proteins, peptides, DNA fragments, liposomes, and NPs. TAT, a transcription factor involved in the replication cycle of the human immunodeficiency virus (HIV), has been shown to enter cells [22]. Transporters for nutrients for the brain are commonly overexpressed on the BBB and can be used for brain targeted delivery [23]. Because the glutathione transporter is highly expressed on the BBB, researchers conjugated it onto liposomes to deliver drugs to the brain. Systemic administration of glycosyl cholesterol derivative liposomes containing coumarin-6 resulted in a 3.3-fold higher Cmax with less cytotoxicity to brain capillary endothelial cells than conventional liposomes [24].

Because of its high specificity, receptor-mediated transcytosis (RMT) across the BBB has received more attention. Large molecules required for normal brain function are delivered to the brain via specific receptors expressed on BBB endothelial cells. After association/ e all been shown to transcytose via receptors [25]. Tf-R is a transmembrane

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glycoprotein that is overexpressed in GBM cells. Drugs can be targeted to the Tf-R using the endogenous ligand transferrin or antibodies directed against the Tf-R. Doxorubicin (DOX) loaded into Tf-R-NPs demonstrated anti-tumor activity, with a 70% longer median survival time than DOX solution-treated brain tumor-bearing rats [26]. Endogenous ligands could bind to receptors, reducing the binding efficiency of ligand-modified NPs. Antibodies against these receptors were developed to avoid this issue. Because the binding site of antibodies to receptors differed from that of ligands with receptors, ligand competition was avoided.

Ulbrich et al. created human serum albumin (HSA) NPs conjugated to transferrin or TR-mAbs (OX26) for loperamide delivery and demonstrated efficacy in transporting the drug to the brain in mice using OX26-conjugated HSA NPs. Because it binds to an extracellular domain of TR, OX26 mAb avoids competition with endogenous transferrin in the circulation system [27]. Aktas et al. recently designed OX26 mAb-bearing chitosan-PEG NPs and demonstrated that OX26 mAb is a critical functional moiety that allows NPs to cross the BBB [28].LRP-1 and LRP-2 are ligand scavenger and signaling receptors with multiple functions. They can interact with a wide range of molecules and mediators, including ApoE, plasminogen activator inhibitor 1 (PAI-1), lactoferrin, heparin cofactor II, heat shock protein 96 (HSP-96), and engineered angiopeps [29].When associated with polysorbate 80-coated NPs, several drugs that do not cross the BBB, such as tubocurarine, loperamide, dalargin, 8-chloro-4-hydroxy-1-oxol, quinoline-5-oxide choline salt (MRZ 2/576), and DOX, show higher concentrations in the brain. When polysorbate 80, a nonionic surfactant, was conjugated on to NPs, it could adsorb ApoE in serum, and polysorbate 80-coated NPs have also been evaluated as a brain targeting delivery system by many groups [30,31]. Angiopeps are highly effective BBB targeting ligands, with angiopep 2 demonstrating increased transcytosis and parenchymal accumulation [32].

5. Carbon Nanomaterials

The family of carbon nanomaterials consists of different types of carbon-based structures. The family of carbon NPs includes many groups: fullerenes, carbon dots (CD), carbon nanotubes (CNT), which in turn can be divided into single-walled (SWNT) and multi-walled (MWNT), graphene, nanodiamonds (ND) (Figure 3). These different structures show different physical and electrochemical characteristics. Many studies in recent years on NPs are trying to identify which of these carbon nanomaterials are more suitable for the transport of drugs conjugated to them or contained by them. Mendes et al already in 2013 had noticed how drugs transported by carbon could find utility in the treatment of neurodegenerative diseases or brain tumors [33]. In 2017, Liu et al began to create specific carbon-based nanostructures that target the brain. In fact, small carbon structures if rationally functionalized on their surface can cross the BBB and therefore transport drugs [34]. Recently, Porto et al., show that carbon nanomaterials have excellent thermal and electrical conductivity, strong adsorption capacity, high electrocatalytic effect, high biocompatibility, and high surface area [35].These intrinsic characteristics would allow the structuring of pharmacological compounds and the simultaneous, potential, reduction of toxic effects. However, the ability to functionalize the surface of carbon nanoparticle structures must be well studied also on the basis of any direct and indirect toxicity that these nanoparticles can acquire.

Graphene is a sheet of carbon atoms arranged in a hexagonal grid. Each individual sheet is only one atom thick, and therefore has a comparatively enormous lateral extent. For this reason, we consider the graphene as a two-dimensional material, in which there are only two dimensions of the plane, while the third is zero. Graphene has high mechanical strength properties, over 100 times more than steel because the atoms are linked together by very strong chemical bonds. Thanks to its particular chemical configuration: graphene possesses unique physical, electronic, optical, thermal and mechanical properties. This molecule has shown promising applications not only in nanoelectronics,

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composite materials, energy technology, sensors and catalysis, but also in biomedical research [36]

. It has electrically conductive and thermally conductive properties superior to those of copper. It has a very high surface area to weight ratio. It is also totally waterproof, flexible and can be made optically transparent and it is biodegradable. It can be differently modified in space into different two- and three-dimensional forms. The most common derived chemical forms are oxidized-type graphene, reduced-type graphene, nanoribbon graphene and oxidized nanoribbon graphene, as well as quantum-dot graphene. Each of these show specific qualities in the transport of drugs.

CDs, on the other hand, are very tenacious carbon spheres, held together by covalent bonds, of small dimensions, with dimensions less than 10nm, studied since 2014 for the transport of drugs. Despite their small size, they are easy to craft. They are biocompatible, have a high capacity to penetrate and bind to receptors, are not very toxic, demonstrated by the work of Shang et al., in which CDs were put in contact with stem cells [37].

CNTs are structures in which a tube made up of carbon hexagons is closed at the end by two hemifullerene caps. They have a high penetrating power and a large surface area. This means that many molecules can be conjugated to it and all these properties can make them excellent candidates for the transport of anticancer drugs. SWNTs can be imagined as deriving from the process of rolling up a graphene plane on itself, closed at the ends by hemispherical caps of the fullerenic type. They have a high length/diameter ratio and for this reason they can be considered "almost" one-dimensional structures. MWNTs are nanotubes formed by multiple concentric SWNTs, and are therefore called "multi-walled" nanotubes. The diameter of MWNTs is usually greater than that of SWNTs, and increases with the number of walls.

The NDs are of more recent discovery. The diamond proper is an allotropic form of carbon consisting of a crystalline lattice in which there are carbon atoms arranged with a tetrahedral symmetry. In this case, NDs are produced through controlled explosions inside closed chambers: the high pressure and temperature push the carbon atoms contained in the explosive substances to fuse together, thus obtaining tiny diamonds. They have a large surface area with a microscopic diameter between 2 and 8 nanometers. They are nanocrystals with a diamond-like structure which gives them particular electronic and physical properties [38]

The fullerenes are spherical and resemble cages. Also known as buckminsterfullerene, it is a compound with a spheroidal polyhedral structure with 60 carbon atoms. Also in this case their peculiar vesicle-like shape, formed by 12 pentagonal and 20 hexagonal faces with a total of 90 edges and 60 vertices, allows both surface conjugation and the possibility of internalizing molecules

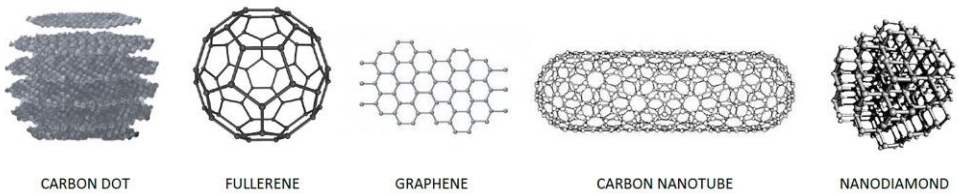

Figure 3. A schematic overview of the different types of carbon nanomaterials.

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5.1 Carbon Nanomaterials and Brain Tumors

Although there is still no selective drug for GBM treatment, the attention of researchers has focused on this issue in recent years. Many studies have been carried out to evaluate in vitro and in vivo the possibility of using these NPs, even with non-classical anticancer drugs, but which, if linked to these carbon NPs, could become promising in the therapy against GBM. Many anticancer drugs loaded into CNTs have been studied and their specificity against tumor cells or other tissues has been evaluated by conjugating them with specific target molecules. In reality, NTs by themselves can be absorbed through non-covalent hydrophobic interactions, however the functionalization of carbon nanotubes with drugs or the addition of particular proteins that allow to define a membrane target allow the controlled release of drugs in the central nervous system [37]. Indeed, it has been seen that they can overcome the blood-brain barrier via a receptor-mediated endocytosis [39]. Precursor of these studies was Zhao et al. in 2011 with the use of SWNTs conjugated to an immunostimulant oligonucleotide or cytosine-guanosine-motifs (CpG). This SWCNT-cPG was injected into mice with GL261 induced glioma observing an uptake within the tumor. However, this oligonucleotide is not currently considered an anticancer drug [40]. Differently, in the following years many research groups have tried to combine classical and non-classical anticancer drugs with NTs. Doxorubicin (DOX), which is not a first-line antiglioblastoma drug, has been successfully conjugated to transported by MWCNT. In this study, the authors crafted molecule was formed via the oxidation of MWCNT which was subsequently conjugated to Angiopeptin2 (Angiopep2) and polyethylene glycol (PEG). Once again the success of the functionalization and transport of this system to glioma target cells was tested in vitro and subsequently in vivo demonstrating once again how the created molecule MWCNT-PEG-Angiopep2 is more effective than single DOX [41]. Another similar result was obtained by another group of researchers with the use of oxaliplatin (OXA), conjugated to BBB penetrating peptide transcriptional activator (TAT), with biotin (B) and polyethyleneimine (PEI). This OXA-containing TAT-PEI-B copolymer was used in in vitro studies on murine glioma cells (C6) and human GBM cells (U87 and U251) to evaluate its absorption. In the subsequent in vivo study, the compound TAT-PEI-B-MCWTN@OXA proved to be much more cytotoxic than single OXA [42]. CNTs are considered as one of the most promising among carbon-based materials as drug carriers and the constant increase of studies represents their importance.

The attention on CD is recent, in fact they are little cited and represented in the literature as regards their conjugation with anticancer drugs useful for GBM treatment. Pioneering studies in this sense use the DOX. Transferrin-conjugated CDs bind DOX to form the molecule C-Dots–Trans–Dox which has been shown in vitro to reduce the cell viability of different pediatric brain tumor cell lines [43]. DOX was also conjugated to polymer coated carbon nanodots and IL6 fragments to give a specific target towards U87 glioma cells which was later confirmed in vivo. It has been confirmed that this molecule crosses the BBB and selectively deeply penetrates GBM cells allowing a gradual and constant release of drug. Furthermore, the presence of the IL6 fragment significantly reduces tumor cell growth, thus being able to conclude thanks to the in vivo results that this molecule increases the sensitivity towards DOX chemotherapy [44]. CDs have also been successfully conjugated with transferrin and epirubicin and TMZ for transport in GBM cells and as a result it has been noted that there is a synergistic effect of the triple-conjugated NP in reducing the viability of the tumor cell at a concentration lower than the same NP not conjugated with transferrin and compared to the two anticancer drugs used individually [45]. Even more recently CDs have been conjugated to gemcitabine with selective specificity for pediatric GBM cells. Also in this case the molecule conjugated with transferrin allowed to go beyond the BBB to reach the GBM cells. However, in this preliminary study, large amounts of drug are still needed to have an antitumor effect [46].

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Graphene was accidentally discovered in 2004 by James and Novoselov [47]. Graphene compounds, like all other compounds of carbon NPs, can modify its properties with the different combinations of molecules. The family of graphene molecules includes a wide range of nanomaterials from oxidized graphene, reduced graphene, reduced oxidized graphene, graphene nanoribbons, oxidized graphene nanoribbons, ultrathin graphite, low-layer graphene, and so on. Among all the compounds under study of nanomaterials, especially among carbon-based nanomaterials, graphene appears to be the most promising for biomedical applications thanks to its properties [48]. Already in 2012 Chen et al. had incorporated a chemotherapeutic agent, belonging to the nitrosourea family, into a molecule of oxidized graphene conjugated with polyacrylic acid (1,3-bis(2-chloroethyl)-1-nitrosourea). In vitro studies on GL261 glioma cells demonstrated the drug uptake via endocytosis and the greater efficacy of the drug conjugate compared to the virgin drug [49]. But once again DOX is the drug most studied and used as an agent conjugated to carbon NPs. DOX molecules were created with pegylated oxidized graphene both with and without transferrin and studied on mouse models demonstrating how the DOX of the pegylated graphene oxide molecule associated with transferrin (PEG-GP-transferrin-Doxorubicin) reduced the tumor volume in the rat [50]. Another molecule created with DOX is phospholipid-PEG-graphenenanoribbon-Doxorubicin, a pegylated graphene nanoribbon modified with phospholipids, studied in vitro against glioma U87 cells. This molecule once again demonstrated that the IC50 of DOX conjugated to a carbon-based NP was lower than unconjugated DOX [51]. In 2016 a study with Lucanthone, an off-the-shelf anticancer agent, allowed the creation of a molecule (Graphenenanoribbon-PEG-DSPE-Lucanthone) of oxidized graphene nanoribbon conjugated with 1,2-distearoyl-sn-glycero-3phosphoethanolamine-N -[amino(polyethylene glycol)] (PEG-DSPE) allowing a selective uptake from U251 glial cells without any effect on other neighboring cells in vitro [52]. More recently, DOX has always been associated with a graphene oxide molecule functionalized with lactoferrin and the results of the in vitro uptake study on glioma C6 cells have documented that the major uptake of DOX was that of DOX conjugated and functionalized with lactoferrin (Lactoferrin-graphene oxide-iron oxide-Doxorubicin) [53]. Between 2021 and 2022, Szczepaniak's group studied the direct effects of graphene molecules and therefore of graphene-conjugated NPs but not carrying anticancer drugs. These two studies demonstrated membrane potential changes and alteration in the viability of U87 tumor cells thus continuing to hold promise for the utility of nanoparticle compounds in the treatment of GBM [54,55]. Still new studies are needed to select specific targets on GBM cells and to select possible new transporters to functionalize the NPs. Recent research has studied, in this regard, the use of curcumin conjugated to CDs [56].

The possibility of conjugating anticancer drugs to the surface of NDs and in particular DOX has been demonstrated since 2013. Although the greater efficacy of the conjugated drug has been established, in the literature there are still few studies with the use of anticancer drugs absorbed by ND in vivo [57]. The doxorubicin-polyglycerol-nanodiamond molecule in vivo and in vitro induces the autophagy of GBM cells and also involves the expression of specific antigens which cause an increase in the immunogenicity of GBM cells. It could therefore be useful in reducing the immunosuppressive effect that occurs in patients affected by GBM [58].

For what concern fullerene, even if structurally similar to CNTs and similar in chemical and physical capacities, functionalized molecules associated with anti-tumor drugs have not yet been reported in the literature. A computer-based and computational-based predictive study on the use of fullerenes was conducted by Samantha and Das in 2017. This futuristic study demonstrates that anticancer drugs such as TMZ, procarbazine, carmustine and lomustine can be absorbed non-covalently by the surface of the fullerene [59]. The conjugation of potent anticancer drugs with fullerene nanomolecules would be a great achievement especially in relation to the effects of single fullerene on nerve cells. it

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has in fact been demonstrated in vitro and in vivo in the last 14 years that fullerene is an excellent antioxidant and in general a neuroprotective drug [60,61].

6. Discussion

The prognosis of cerebral gliomas still remains very poor today. The commonly and widely accepted therapeutic protocol is the multimodal one. A first surgical approach is followed by radio- and/or chemotherapy. Surgical techniques have evolved considerably in recent years thanks to the introduction of new technologies such as, intraoperative imaging with MRI, CT, or ultrasonography, electrophysiologic monitoring, the visualization of tumor tissue with systemically injected fluorescent dye (5-aminolevulinic acid [5-ALA]), and surgery under local anesthesia with neurolinguistic cortical language mapping. Radiotherapy and chemotherapy treatment is represented by the Stupp protocol: administration of TMZ followed by radiotherapy. Nonetheless, the prognosis, in patients affected by cerebral gliomas, remains poor, not exceeding 15 - 20 months of survival. The new data obtained in research relating to the biology of brain tumors have made it possible to identify new pathways and numerous key proteins, the triggering of which would activate the various processes of neoplastic proliferation. Various genes capable of triggering neoplastic activation processes and coding for key proteins have also been identified. Modern therapeutic approaches to brain tumors now aim to specifically target these biomolecules (VEGF, EGF, DKK...) thus attempting to slow down or stop the pathway underlying this protein. This approach, although very interesting, has some limitations. Initially, it is necessary to identify the key protein or suitable key proteins and try to target them selectively so as not to have side effects on healthy tissue. Furthermore, the presence of the BBB limits the access of these pharmacological compounds leading to an increase in the doses to be administered and the prolongation of the treatment.

The advent of nanomedicine, the use of nanotechnologies in medicine, has given new impetus to the search for new and more functional therapeutic protocols in the treatment of brain tumors. Nanoparticles have peculiar and intrinsic characteristics which make them particularly suitable for this type of therapeutic approach. Due to their size they are able to easily cross the BBB; moreover, they can be suitably engineered, thus being able to transport therapeutic agents and pharmacological compounds directly to the tumor site by interacting with membrane antigens selectively expressed by tumor cells. In this way, only the neoplastic cells would be affected reducing the therapeutic times and the quantities of drug used. The most extensively studied NPs are polymer NPs, liposomes, gold NPs, silver NPs, metal oxide, magnetic NPs, carbon nanomaterials, peptides, silica NPs, quantum dots, and dendrimers.

In this study, we have reported some interesting experimental studies using carbon nanomaterials as possible therapeutic agents or carriers in the treatment of brain gliomas. The research carried out is substantially interesting and potentially valid. They are, of course, preliminary studies on cell lines of cerebral gliomas, but with very promising results. However, these studies have some limitations such as the lack of human trials and the lack of information on the potential toxic effects in human use. Nanotoxicology studies the interactions of NPs with biological systems and the relationship between the physical and chemical properties of NPs with the induction of toxic responses. Currently, a complete evaluation of the size, shape, composition, and aggregation-dependent interactions of NPs with biological systems is lacking, so it is unclear whether the exposure of humans to engineered nanostructures could produce injurious biological responses. Some NPs such as carbon nanotubes can persist in the body for quite some time making them potentially toxic and limiting their use for prolonged and repeated treatments. It has been demonstrated that carbon nanomaterials can induce toxicity in experimental animals on the pulmonary, cardiac and reproductive systems, but they can also be responsible for

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toxic effects on the eye and on the skin. One study found that intratracheal introduction of MWCNTs in mice elicited allergic-like responses via activation of B lymphocytes and production of class E immunoglobulins [62]. Prolonged exposure of pulmonary epithelial cells to SWCNTs can cause onset of neoplastic diseases [63]. Exposure to fullerene can cause cytotoxic damage in vascular endothelial cells in humans [64]. Furthermore, in mice, an increased risk of cardiac ischemia was found after exposure to fullerene [65]. Zhu et al., demonstrated the reduction of the survival rates in zebrafish embryos, after exposure to fullerene [66]. MWCNTs can cause, on pregnant mice, impaired fetal development and brain malformations [67]. Carbon nanomaterials nanoparticles can reach the CNS through the systemic, olfactory and trigeminal pathways. Within the brain parenchyma they can induce cytotoxicity, altering the molecular pathways and triggering chronic brain inflammation, microglia activation and white matter alterations with increased risk for neurodegenerative diseases and stroke [68]. Due to the dimensional characteristics it has been observed that SWCNTs can penetrate inside the nerve cells by endocytosis and pinocytosis. The consequence of this process is the release of chemical mediators capable of inducing inflammatory processes, apoptotic processes and oxidative stress [69]. In experimental animals, the introduction of MWCNTs would induce the release of cytokines, the activation of glial cells and the triggering of inflammatory processes [70]

7. Conclusion

Our study does not, of course, arrive at definitive results. Carbon nanomaterials represent, precisely because of their peculiarities, such as the ease of passing cell membranes, the thermal properties, the large surface areas, and the easy modification with molecules, highly innovative materials and potentially suitable for being used in new therapeutic protocols against cerebral gliomas. On the other hand, there are also limitations to their use in humans, mainly linked to the onset of toxic phenomena affecting the nerve cells and the onset of inflammatory/oxidative processes. The new studies must now be directed to the search for new and more functional target molecules and, at the same time, through appropriate engineering, to the structuring of nanomaterials more suitable for human use.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S1: title; Table S1: title; Video S1: title.

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