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REVIEW ARTICLE

Nanotechnology Applications for Diffuse Intrinsic Pontine Glioma

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Abstract: Diffuse intrinsic pontine gliomas (DIPGs) are invariably fatal tumors found in the pons of elementary school aged children. These tumors are grade II-IV gliomas, with a median survival of less than 1 year from diagnosis when treated with standard of care (SOC) therapy. Nanotechnology may offer therapeutic options for the treatment of DIPGs. Multiple nanoparticle formulations are currently being investigated for the treatment of DIPGs. Nanoparticles based upon stable elements, polymer nanoparticles, and organic nanoparticles are under development for the treatment of brain tumors, including DIPGs. Targeting of nanoparticles is now possible as delivery techniques that address the difficulty in crossing the blood brain barrier (BBB) are developed. Theranostic nanoparticles, a combination of therapeutics and diagnostic nanoparticles, improve imaging of the cancerous tissue while delivering therapy to the local region. However, additional time and attention should be directed to developing a nanoparticle delivery system for treatment of the uniformly fatal pediatric disease of DIPG.

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Keywords: Brainstem glioma, DIPG, drug delivery, liposome, micelle, nanoparticle, pons, theranostics.

INTRODUCTION

Diffuse intrinsic pontine gliomas (DIPGs) are invariably fatal tumors found in the pons of elementary school aged children. These tumors are grade II-IV gliomas, with a median survival of less than 1 year from diagnosis, with prognosis unaffected by the grade of the tumor. Standard of care (SOC) therapy includes radiation without concomitant surgery; no chemotherapy has been demonstrated to be effective against a DIPG [1].

Nanotechnology could offer some therapeutic options for treatment of DIPGs, along with other brain tumors. Nanotechnology constructs (nanoparticles that are less than 100 nm, hydrodynamic radius [2]) have been used and are being developed for phase III trials for treatment of various cancers. Cancers, such as liver and lung cancers, led the field as potential recipients of nano-derived therapeutic particles [3-5]. These types of cancers were more common and more easily accessed as compared to brain tumors. To date, multiple nanoparticles have been tested to increase delivery of chemotherapies to tumors while minimizing systemic effects of the drugs. One specific advantage of treatment with nanoparticles is that these do not diffuse freely like small molecules and have increased permeability and retention in tumor tissue [6].

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In the treatment of brain tumors, nanoparticles have been developed that are able to cross the blood brain barrier (BBB) when administered through conventional delivery routes, such as intravenous injection. Once delivered, these agents can be used for both enhanced imaging [7] and for targeted delivery of therapies [2] in brain tumors. However, there are no published articles specifically focused upon the delivery of chemotherapy into DIPGs. The current literature concentrates on nanoparticles developed for the treatment of glioblastoma multiforme (GBMs), which are grade IV gliomas that can occur anywhere in the central nervous system and share some similarities with DIPGs. Nevertheless, these data are promising for the next steps in diagnosis and treatment of DIPGs. This review will discuss and highlight the data that exist for treatment of GBMs and examine potential extrapolations into treatment of DIPGs where data for DIPGs are not available.

Novel drug options for packaging into nanoparticles include CDK inhibitors, which have been demonstrated, in murine DIPG models, to prolong survival [8]. Similarly, Wee1 inhibitors in combination with radiation have been demonstrated to decrease survival of DIPG cells in culture [9]. More classic chemotherapeutic agents, such as carboplatin and temozolomide have been packaged into nanoparticles and could potentially be used against DIPGs [10, 11].

BARRIERS TO TREATMENT OF DIPGs

Surgery, whether it be debulking or resection, is not therapeutic for treatment of DIPGs due to the exquisite location of the tumors. Biopsy is safe, though not done in all

institutions at diagnosis or after progression because of concerns for morbidity of surgery while clinical presentation and imaging are fairly reliable in determining the diagnosis of a DIPG [12-14].

Unfortunately, chemotherapy does not prolong survival of DIPG patients. The theories for these limitations are various. In general, it is thought that the anatomy of the pons and the locally intact BBB limit infusion of chemotherapy to the pons in DIPGs [14]. In addition, there is also evidence that drug efflux transporters limit the efficacy of chemotherapeutic agents [15]. Finally, it is possible that the infiltrative tumor in an already restricted area increases the anisotropy of the area, diminishing the amount of systemic chemotherapy that can diffuse through the area of the tumor [16].

These limitations in such a devastating disease have lead to multiple approaches in modern medicine in an attempt to change the survival chances for affected children. One area of cancer therapy that is gaining momentum in this challenging arena, *i.e.* the pons affected by DIPG, is nanotechnology.

NANOPARTICLES FOR TREATMENT OF DIPGs

There are multiple forms of nanoparticles currently being investigated for treatment of brain tumors (Fig. **1**). Nanoparticles based upon stable elements, such as gold [17-19], silver [20], iron, and carbon [10] are made. Organic nanoparticles include liposome and micelle particles, both of which are phospholipid formulations, though of different sizes and lamellar composition. Polymer nanoparticles, such as poly-(D,L-lactic-co-glycolic acid) (PLGA) are also used [21]. In spite of the variety of nanoparticles available, delivery into the brain has remained a challenge due in large part to the non-specific and non-targeted delivery of these nanoparticles. Targeting of nanoparticles is now possible, as there are delivery techniques that address the BBB limitations mentioned above.

Solid-state Nanoparticles

Gold nanoparticles (Au NPs) are inert solid nanoparticles used for delivery of chemotherapy or detection of tumors [22, 23]. These formulations can be specifically targeted to increase uptake by glioma cells and have been demonstrated to increase overall survival in murine GBM models [24]. In one study, Au NPs were conjugated with Gd-labeled DNA and were shown to cross the BBB in glioblastoma cells and *in vivo* mouse models. They demonstrated that these agents can penetrate tumor parenchyma and silence genetic signals and subsequently decrease tumor load [25]. In another study, drug delivery vectors based on Au NPs were designed *via*

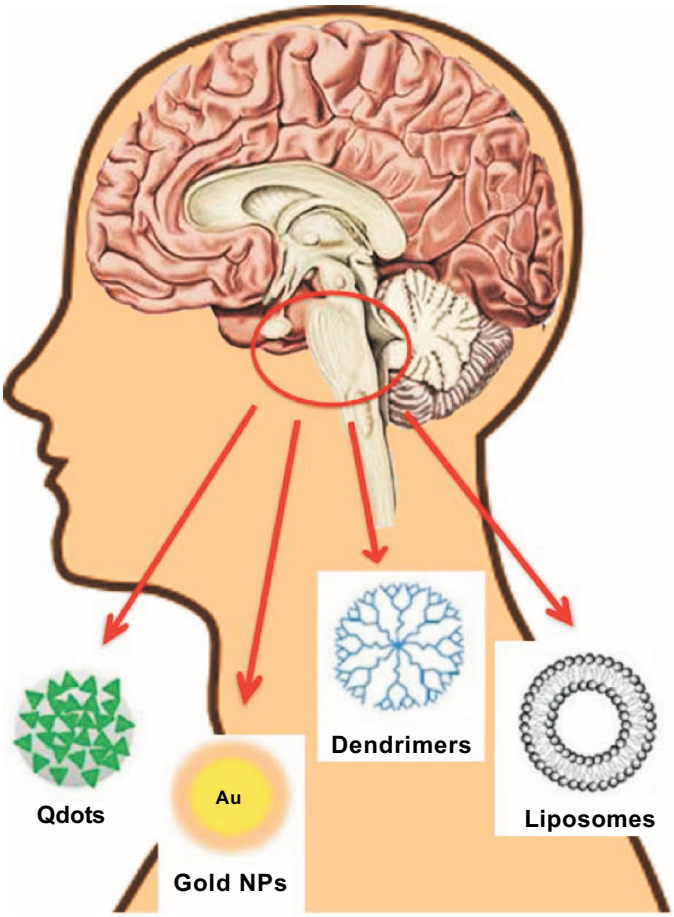

Fig. (1). Depiction of various nanoparticles: Qdots, gold nanoparticles, dendrimers, and liposomes. All of these can be utilized to transverse the blood brain barrier into the pons.

conjugation of aspartate stabilized Au NPs to temozolomide drug. These carriers delivered temozolomide, a cytotoxic drug, to treat recurring malignant glioma. These studies also revealed the low cytotoxicity of the drug delivery agent and the potential to internalize temozolomide inside glioma cells [26]. Another study reported a formulation of a Au NP-based drug carrier *via* electrostatic interaction of anionic gellan gum coating on Au NPs to cationic doxorubicin HCL (DOX, an anthracycline anti-cancer drug). Effective loading of DOX onto Au NPs increased cytotoxicity in human glioma cell lines [27]. Further, PEGylation has been utilized to prepare different formulations of RGD peptide (integrin) conjugated Au NPs to maximize the targeting efficiency to U87 glioma cell lines. The ligand density was controlled by using amine or carboxyl conjugated thiolated PEG to improve targeted nanoparticle efficacy [28]. Another study highlighted the design of DOX conjugated Au NPs using hydrazone linkage which were further made targeted *via* fabrication with Angiopep-2 decorated PEG. These multifunctional nanoparticles termed as An-PEG-DOX-AuNPs were shown to target glioma cells and displayed accumulation. Release of DOX was facilitated by low pH milieu [29].

Gold nanoparticles (Au NPs) have also been utilized for selective targeting of brain tumors and subsequent Au NP-induced radiosensitization [30]. PEGylated Au NPs as an adjuvant to the radiation therapy were delivered in *in vitro* and *in vivo* systems. Au NPs significantly increased DNA damage induced by the ionizing radiation and induced stunting of newly formed blood brain vessels. Furthermore, this combinatorial treatment increased the survival of mice with orthotopic brain tumors. In a similar study, Bobyk *et al*. [31] used commercial Au NPs to illustrate the radiosensitization efficacy of gold in killing glioma cells, which was more efficient than irradiation alone. Further advances in this approach have used chemo-radiotherapy of cisplatin-conjugated Au NPs [32]. This strategy worked *via* the synergy of irradiation with high atomic number gold and platinum molecules and cisplatin thereby producing ionizing photoelectrons and auger electrons which caused complete killing of cells *in vitro*. Another study used Au NPs in combination with cold plasma for plasma therapy of tumor cells [33]. Au NPs along with cold plasma induced cell death up to 30% more than the cells treated with plasma alone. All these strategies based upon synergistic approaches have a huge potential in killing glioma cells and reducing harm to healthy cells.

Shutao *et al*. [34] used supramolecular Au NPs prepared from 2 nm gold colloids to demonstrate enhanced photothermal effects of gold towards glioma cells. These supramolecular Au NPs were further conjugated with cRGD peptide to target the overexpressed integrins in glioma cells leading to a more targeted therapy.

Besides radiosensitization and photothermal therapy, Au NPs have also been used as a vehicle to deliver biomolecules to demonstrate therapeutic effects. Jensen *et al*. [35] covalently functionalized Au NPs with small interfering RNA (siRNA) duplexes. These Au NPs-siRNA complexes were taken up by the glial cells and transformed the cells. In the *in vivo* model, these agents crossed the BBB and increased intramural apoptosis and reduced tumor burden and progression in xenograft models without harmful side effects. In one other study, temozolomide loaded gold nanostructures [26] were found efficient in lowering chemoresistance and killed 82.7 % of cancer stem cells as compared to 42% using temozolomide alone.

Au NPs have also been used in photodynamic therapy (PDT) of glioblastoma by selectively delivering photosensitizers such as silicon phthalocyanine (Pc 4) [18]. The widespread use of PDT in brain tumor therapy has been partially hampered by non-targeted phototoxicity towards healthy tissue and lack of target specificity. Therefore, overexpressed glioma cell surface receptors, *i.e.*, epidermal growth factor receptor (EGF) and transferrin receptor (TfR) have been utilized as targeting moieties. EGF-targeted AuNPs loaded with Pc4 [18] were able to reduce cytotoxicity and increase the drug delivery efficiency. Other targets including TfR demonstrated that Tf-targeted Au NPs loaded with Pc 4 were able to cross the BBB using transcytosis and target glial cells both *in vitro* and *in vivo* to deliver Pc 4 drug for PDT therapy [36].

For targeted glioma therapy, one study designed integrin (cRGD) targeting, NIR-responsive and robust AuNR/PEG-PCL hybrid nanoparticles (cRGD-HNs) for targeted chemotherapy of human glioma xenografts in mice [24]. Ligand-directed AuNR/PEG-PCL hybrid nanoparticles demonstrated tumor-targeting as well as greater spatiotemporal and rate control over drug release *in vivo*. In another targeted therapy approach [37], Au NPs functionalized with RGD-(GC)(2) peptide were designed and demonstrated efficient cellular uptake in U87 cells.

Silver nanoparticles are primarily used due to their anti-infective properties. However, they can also be used to increase apoptosis in GBM cells, *in vitro*. These nanoparticles combine silver with chitosan and alginate complexes [20]. In addition, silver nanoparticles may be useful radiation sensitizers for glioma radiotherapy [38].

Carbon nanotubes are another form of targetable nanoparticles. These nanotubes are coated with polymers to control release of embedded chemotherapies and therefore have the potential to deliver significantly more chemotherapy than can be achieved systemically [39]. There are, however, concerns regarding toxicity to healthy tissues from the carbon nanoparticles, themselves [40]. *In vitro* glioma cytotoxicity is seen while cellular toxicity from the carbon nanotubules on initial assessment has not been noted [41].

Iron oxide nanoparticles have unique therapeutic benefits. These nanoparticles are targetable by both magnetic guidance and ligand targeting. Furthermore, they can be designed to have a staged delivery of multiple chemotherapeutic agents, such as doxorubicin and curcumin, for complex activity against glioma cells. To date, the efficacy data for these formulations are still *in vitro* [42].

Lipid-based Nanoparticles

Liposomes and micelles can be targeted with multiple types of products, including: proteins, antibodies [43], and

viral particles [44]. Liposomes are bilayer or multilayer nanoparticles, which are capable of transporting both hydrophilic and hydrophobic therapeutics. Micelles, being unilamellar, are significantly smaller than liposomes (potentially increasing their bioavailability due to increased permeability), but can carry only hydrophobic therapeutics, unless hydrophilic molecules are attached as ligands to the outer surface of the micelles. Both of these nanoparticle vehicles improve the solubility and stability of drugs being delivered to tumor cells. PEGylation (coating micelles or liposomes with polyethylene glycol) increases the stability and allows targeting of the nanoparticles [45]. Targets of DIPGs and GBMs share similarities (*e.g*. epidermal growth factor receptor, transferrin receptor, fibroblast growth factor, *etc*.), though there is heterogeneity between these tumor types and specific cellular targets for DIPGs have not been convincingly identified [9, 46, 47].

Liposomes represent one of the most promising systemic delivery strategies to improve the chemotherapy for GBM [48-50]. Liposomes can carry either hydrophobic or hydrophilic drugs, protecting them from unfavorable conditions [51, 52]. Particularly, liposomes conjugated with polyethylene glycol (PEG) can evade recognition by opsonins and the subsequent clearance by the reticuloendothelial system (RES), and hence they stay long in circulation with sustained drug release [53-57]. Size-wise, nanoscale liposomes tend to accumulate at tumor interstitium and promote drug delivery efficiency, owing to the vascular feature of tumors, referred to as enhanced penetration and retention (EPR) effect [58-60]. Further, liposomes can be constructed with special moieties for targeted delivery, achieving higher delivery efficiency and minimizing side effects [61-65].

So far, doxorubicin has been the most investigated drug for liposomal delivery to treat GBM. This drug is known to be useful against multiple tumor types [66, 67], and its free form has been demonstrated to be one of the most effective substances against glioblastoma cells *in vitro* [68]. However, free doxorubicin has no significant effect against GBM *in vivo* due to poor BBB penetration [69-72] – BBB cells express ATP-dependent efflux pump, P-glycoprotein (P-gp), restricting free doxorubicin from passing BBB [71], which works similarly in glial tumor cells against many therapeutic agents [73-75]. Besides, the therapeutic potential of doxorubicin has been limited by its chronic cardiac toxicity. Hence, the motivation for research on liposome encapsulated doxorubicin. The PEGylated liposome-encapsulated form of doxorubicin (Doxil) has been developed to treat various cancers and is commercially available. Pre-clinical and clinical studies of Doxil for GBM therapy showed that in comparison to free doxorubicin, Doxil featured intense accumulation at the tumor tissue, improved suppression of tumor growth, and reduced cardiac toxicity [63, 64, 76, 77].

Further, targeting moieties conjugated to liposomal doxorubicin are being studied to enhance the drug's therapeutic index [61-65] as well as to improve the drug's cellular uptake and internalization. For example, an immuno-liposomal doxorubicin was fabricated with the monoclonal antibody 225, targeting the epidermal growth factor receptor (EGFR) that is overexpressed in many tumors, and it showed improved therapeutic efficacy in mouse models carrying human glioblastoma tumors with respect to non-targeted liposomal doxorubicin [78, 79]. Other examples of targeting moieties which showed positive results with liposomal doxorubicin against GBM include human interlecukin-13 (IL-13) for targeting IL-13Rα2 receptor, the PGERPPR peptide for targeting Neuropilin-1 (NPR-1) [80], folate for targeting folate receptors [81], the atherosclerotic plaque specific peptide-1 (AP-1) of 9 amino acids sequence for targeting interleukin-4 receptors (IL-4R) [82], *etc*.

The enhanced drug accumulation of Doxil compared to free doxorubicin at the glioma tissues is usually ascribed to the PEGylated liposomes' capability to penetrate the BBB [63, 64, 76-82]. Counter intuitively, liposomes are not supposed to cross the normal BBB [83, 84]. BBB acts as a barrier through P-gp as well as because of its tight junction [85]. P-gp does not block liposomes, but the physiologic upper limit of pore size in the BBB of malignant glioma microvasculature is ~12 nm [86], smaller than the usual size of a liposome particle. Therefore, the penetration of the liposomes through BBB in above cases is probably due to the disrupted BBB in brain tumors [87-91]. Specifically in the GBM case, when the tumor begins to grow beyond 1-2 mm in diameter within the brain parenchyma, the BBB becomes compromised both structurally and functionally [92]. Nevertheless, liposomes can be constructed to actively penetrate BBB through receptor-mediated endocytosis. The receptors for insulin, transferrin, endothelial growth factors, amino acids, and various metabolic nutrients are expressed on the BBB [93]. Dual-targeting liposomal doxorubicin with both transferrin and folate has been proven effective in penetrating the BBB [81]. Moreover, the BBB can be disrupted by ultrasound, assisting liposomal delivery to brain tumors [82, 94, 95].

Beyond doxorubicin, many chemotherapeutic agents have been involved in preclinical studies for liposomal delivery to treat GBM, including epirubicin [79], vinorelbine [79], daunorubicin [96], docetaxel [97], irinotecan [98-100], vincristine [98], topotecan [101-104], among others. Within these various cases of liposomal delivery, observations of increased drug accumulation at tumor sites, sustained drug activity, improved drug anti-tumor efficacy, and reduced adverse side-effects with respect to free drug delivery are consistent. Cytarabine-packaged liposomes have been demonstrated to prolong the time to neurological progression in adults with neoplastic meningitis, though the response rate was no different from standard of care methotrexate [105]. Liposome encapsulated drugs are tolerable and feasible for administration to patients with brain tumors, though they have not yet been proven to improve overall survival. Likely, this is related to the formulation of the targeting moieties and the release dynamics, which remains to be optimized for maximum clinical efficacy.

Additionally, the direct intra-cerebral infusion approach, convection-enhanced delivery (CED), as an alternative to the usual intravenous administration has been attempted with liposomal irinotecan [100] and liposomal topotecan [101, 103, 104]. The advantage of CED is to bypass the BBB and minimize systemic drug exposure for fewer side effects [106,

107]. The work with liposomal irinotecan and topotecan demonstrated superior therapeutic efficacy by CED. For the benefit of clinical trials to monitor the real-time drug distribution after CED execution, the co-convection with liposomal gadodiamide, enabling MRI imaging, has been proposed [101].

Liposomes can also assist gene therapy against GBM. Traditionally, a gene therapy employs a viral vector to deliver the therapeutic DNA. In comparison, liposomes are easier to prepare, and liposomal delivery is advantageous for safety, low toxicity and absence of high neutralizing antibody levels. In general, the studies on gene therapy for GBM follow one of two approaches: (1) Suicide gene therapy using the herpes simplex thymidine kinase (HSV-tk) to sensitize tumor cells to ganciclovir (GCV) [108-110] or (2) Immune gene therapy using cytokine genes. Preclinical studies of interferon β (INF-β) and human interlukin 12 (IL-12) gene transfer using liposomes displayed positive results [111-114], and clinical studies have been warranted or already performed [115-118]. Moreover, for liposomal gene therapy, a hybrid vector of HVJ-liposome (HVJ: hemagglutinating virus of Japan or Sendai virus) has been developed [119], which can fuse with cellular plasma membrane, releasing the contents into the cytoplasm, in contrast to usual liposome vectors that are taken up into cells by endocytosis. HVJ-liposome is fabricated by fusing liposomes with UV-inactivated HVJ. The hybrid vectors deliver genes efficiently [120-124] and clinical trials are under way to treat GBM with suicide gene therapy [119] (clinicaltrials.gov # NCT02414165). Further, the gene therapy using HVJ-liposomes can be improved by targeting-modification of the hybrid vectors or rendering long-term expression of transgene *in vivo* [119].

Liposomes are also being developed to carry other genetic materials that may act as therapeutic agents for GBM therapy. For example, vascular endothelial growth factor (VEGF) siRNA can be protected from RNase degradation by liposomal packaging and delivered into gliomas *via* EPR effects and active targeting. VEGF, which is highly expressed in gliomas [125, 126], is reduced and, consequently, new blood vessel growth to gliomas is inhibited [97].

Ultrasound-sensitive nanobubbles are also being developed as nanoparticles. These are often assembled into micelles and/or liposomes for delivery of therapeutic agents, such as small interfering RNAs (siRNAs), into glioma cells [127]. These siRNAs can interact with and inhibit oncogenes to promote apoptosis and inhibit proliferation [128, 129].

Polymer-based Nanoparticles

PLGA nanoparticles have been developed that are designed to selectively penetrate the glioma parenchyma [130]. These particles are believed to be beneficial because of their biodegradable nature. They deliver chemotherapy, with current technology and development, into murine glioma models, with targeted delivery into gliosarcoma cells [131]. Similar formulations of these polymer nanoparticles demonstrate *in vitro* glioma cell death [132]. Improved survival in the animal models has not yet been demonstrated, as these nanoparticles continue to be optimized for therapy.

Therapeutic Combinations in Nanoparticles

Combination therapies of nanoparticles are also being developed. Carbon and iron nanoparticles are being made to increase biomedical applications of magnetic nanoparticles. These combinations, in addition to targeted delivery of chemotherapy, affect the C and G2/M phases of cell cycle progression leading to increased pro-apoptotic activity in murine glioma cells *in vitro* [133]. PLGA compounds are being combined with liposomes in order to increase the circulation time and enable targeting of drug and gene delivery to brain tumor tissues [134]. A combination of stem cells and nanoparticles has been demonstrated to increase distribution and retention of chemotherapy into brain tumor tissues [135, 136].

In fact, a phase I/II clinical trial of nanoparticle chemo-therapy for adults with recurrent GBM has been completed and has demonstrated prolonged overall survival compared to historical therapy on the Stupp protocol [137]. Currently, a nano-liposomal phase I trial is in its recruitment phase. This study is investigating nano-packaged irinotecan in treatment of GBM and other high-grade gliomas in adults (clinicaltrials.gov # NCT02022644).

Impact of Administration Route

Targeting and transport of nanoparticles to solid tumors have been extensively studied. Despite several advances, nanoparticle transport at high concentrations to tumors is still a challenge, especially to inaccessible locales within the brain and brainstem. Different routes of administration may result in varying patterns of biodistribution of the nano-particles and appears to be an important parameter in the delivery of therapeutic agents to DIPG. Conventional routes of administration for GBM and DIPG include intranasal, oral, intra-carotid or intravenous (i.v.) administration [138-161]. Intravenous administration *via* the carotid artery, femoral or tail vein in small animals has been the most successful in achieving significant accumulation of nanoparticles across the BBB. Oral and interperioteneal (i.p.) administration allow the nanoparticles to cross the BBB and reach the CNS after longer periods of circulation owing to the time necessary to cross other barriers such as the intestinal and peritoneal walls. During intranasal administration, nanoparticles must cross the mucosal barrier and be taken up by neurons and supporting cells before reaching the brain *via* an intra-axonal route.

In humans, intravenous administration is certainly the most common form of delivery of nanoparticles, though alternatives are being investigated. Convection enhanced delivery (CED) is a delivery system that is able to circumvent the blood brain barrier [162]. Once perfected, this could be a potential delivery route for nanoparticles, though it is an invasive approach. Another delivery route that is interesting for delivery of nanoparticles into DIPGs is intranasal, as this approach circumvents the hepatic vascular system, minimizing first pass metabolism, while enhancing noninvasive delivery into the brainstem, specifically [163]. Approaches to enteral delivery of nanoparticles are also being investigated [164], though these will perforce have to deal with the first pass metabolism of medications in the liver.

NANOPARTICLES FOR IMAGING OF DIPGs

The diagnosis of DIPG is based on conventional interpretations of radiographic findings. Using magnetic resonance imaging (MRI), the tumor appears as a large expansile brainstem mass with an epicenter within the pons. DIPG are hypo- or iso-intense on T1-weighted images, hyper-intense on T2-weighted images, and relatively homogenous on fluid attenuated inversion recovery (FLAIR) sequences [165]. Contrast enhancement is variable, but these tumors rarely enhance at diagnosis. Typical clinical presentation and MRI has thus become standard practice for diagnosing DIPG.

Surveillance imaging can be used to monitor response and progression of disease, but clinical assessment is more reliable because imaging still suffers from lack of molecular targets and contrast agents to infiltrate the DIPG. Given DIPG's invasive nature and indistinct borders, measurement of tumor size and volume is problematic and suffers significantly from inter-observer variability when using MRI [166]. In addition, MRI cannot reliably differentiate between tumor and treatment responses during the course of radiation therapy. MR perfusion and magnetic resonance spectroscopy (MRS) have shown some promise as new non-invasive imaging techniques to identify DIPG response to anti-tumor agents but thus far have not been proven clinically useful. For example, MR perfusion studies have been used to evaluate DIPG vessel density and permeability. Increased blood flow may be associated with tumor grade or malignant transformation [167]. Price *et al.* demonstrated that the relative cerebral blood volume (rCBV) determined by perfusion imaging correlated with cell proliferation in adults with high-grade gliomas [167]. In a study of children with DIPG, increased perfusion at any single time point was associated with shorter survival [168]. MRS has been used to evaluate predictive markers of response, such as the ratio of choline to N-acetyl aspartate (NAA). A higher choline:NAA ratio in children with DIPG was associated with a greater mortality when compared to a cohorts with lower ratios. Changes over time were also associated with outcome, suggesting that a dynamic increase was inversely associated with survival and vice versa [169].

Several types of nanoparticles could be developed to identify molecular targets within the DIPG or deliver chemotherapeutics that do not easily accumulate in the tumor. Nanoparticles used for imaging include magnetic nanoparticles and fluorescent nanoparticles. Theranostic nanoparticles are specific, individualized therapies that improve imaging of targeted tissue while delivering therapy to the same tissue, and are often made with iron [170, 171]. Magnetic nanoparticles are a form of theranostic nano-particles that have unique paramagnetic properties, which support their detection by MRI [172]. Alternately, fluorescent nanoparticles can increase the detection of a microscopic tumor in real time, clinically (during surgery) and with imaging [173].

Magnetic nanoparticles of particularly useful due to their helpfulness in attaining targeted imaging of brain tumors. Similar to gadolinium-based contrast agents, they rely upon diffusion across a damaged BBB to demonstrate enhancement. However, they can be targeted specifically to tumor tissue, dramatically reducing the subtleties and challenges of interpretation of MRIs when using traditional gadolinium contrast agents. Magnetic nanoparticles may also be preferable to gadolinium based nanoparticles because they have no known renal effects and they circulate for longer than gadolinium based contrast agents (24-72 hours after systemic administration) [2]. However, they are not biodegradable and may have long-term risks related to deposition of the iron or other metals [174].

Gold nanoparticles can chelate to gadolinium for improved imaging. These nanoparticles provide prolonged retention of the gadolinium, as well as targeted delivery. An exciting combination therapy includes combined drug delivery with gadolinium delivery for therapeutic drug delivery and imaging in one compound [175].

Fluorescent nanoparticles can be used to increase surgical resection of tumors, though this is not truly relevant to DIPGs, because DIPGs that are biopsied receive stereotactic biopsies, in the current therapeutic era. However, in research for DIPGs fluorescent nanoparticles are often used to determine the location of tumors with non-invasive, targeted imaging. One such interesting fluorescence imaging nanoparticle is a silver nanoplate, coated with polymers, noted to be useful in rat imaging [176].

CONCLUSION

Nanotechnology has been developed to deliver therapies in non-targeted and targeted fashion into brain tumors, especially GBM. Such development has not yet occurred in DIPGs, which are considered to be an "orphan disease." Perfection of the delivery system for nanoparticles is needed in brain tumors, in general. However, additional time and attention should be directed to developing a nanoparticle delivery system for treatment of the uniformly fatal pediatric disease of DIPG.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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