Nanocarrier-based therapies for CNS tumors

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PRACTICE POINTS

- Chemotherapies for CNS tumors are largely ineffective due to a lack of robust drug delivery.
- The blood–brain barrier hinders the passage of systemically-delivered therapeutics.
- The brain extracellular matrix limits the distribution and longevity of locally delivered therapeutics.
- Nanocarriers can be engineered to address multiple drug delivery challenges, including those posed by the CNS.
- Significant preclinical progress has been made in the fabrication and *in vivo* testing of polymer nanoparticles, liposomes, micelles and dendrimers for drug delivery to the CNS.
- Over the next 5–10 years, clinical translation of nanocarrier-based CNS drug delivery systems is anticipated.

SUMMARY: Current chemotherapeutic strategies for tumors of the CNS are largely ineffective. This is due, in part, to the lack of robust drug delivery systems. The blood–brain barrier hinders the passage of systemically delivered therapeutics, and the brain extracellular matrix limits the distribution and longevity of locally delivered agents. Drug-loaded nanocarriers represent a promising strategy to overcome these barriers. Due to their small size and versatile design, nanocarriers can be finely tuned to address specific drug delivery challenges. Here, we review the major advances in development of nanocarrier-based therapeutics for tumors of the CNS, with an emphasis on polymeric nanoparticles.

The management of CNS tumors is complex. For most tumors, a combined, multimodal approach including surgery, radiation and chemotherapy is required. Despite these intensive treatments, patients with malignant brain tumors have a universally poor survival. This is highlighted by patients with glioblastoma multiforme (GBM), the most aggressive and common primary brain tumor. In spite of massive research efforts including comprehensive genomic characterization, patients with GBM have a median survival of only 14.6 months [1]. Furthermore, although GBMs are characterized by diffuse white matter infiltration that can extend to the contralateral hemisphere, the vast majority of GBMs recur within 2 cm of the original tumor focus [2]. Unlike the central core of the GBM, which is typified by leaky vasculature and blood–brain barrier (BBB) breakdown, the 2-cm periphery generally displays an intact BBB. Thus, the therapeutic failures in this region of the tumor suggest an ongoing deficiency in drug delivery due to an inability to cross the BBB [3]. The drug delivery challenges faced in primary brain tumors are not unique. As therapies for systemic malignancies improve and patients live longer, an increasing frequency of CNS failures has been noted.

KEYWORDS

- blood–brain barrier • brain
- convection-enhanced delivery • dendrimer
- intracranial • liposome
- micelle • nanocarrier
- nanoparticle • polymer
- systemic delivery

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For example, patients with *HER2*-overexpressive breast cancer have excellent systemic responses to trastuzumab therapy. However, the CNS metastases in these same patients do not respond to intravenous trastuzumab due to the inability of the drug to cross the BBB [4]. As an increasing number of targeted therapeutics emerge, with promising systemic results, questions regarding their CNS efficacy remain unanswered.

The BBB is composed of multiple physical and metabolic barriers, which results in a tight regulation of the brain microenvironment [3,5–7]. It is estimated that the BBB prevents >98% of small-molecule drugs and approximately 100% of large-molecule drugs from accessing the CNS [8]. The primary physical barriers to BBB transport are endothelial cells and their narrow tight junctions, which severely restrict paracellular transport. There are also numerous metabolic barriers within the BBB that limit transcellular transport. Specifically, the endothelial cells allow minimal pinocytosis, the capillary endothelium contains numerous efflux transporters, and there are many intra- and extra-cellular enzymes throughout the BBB that inactivate passing compounds. Given these physical and metabolic constraints, transport across the BBB is limited to highly specific transcytotic pathways (receptor-mediated, adsorptive/charge-mediated or substrate-specific), the physically restricted paracellular aqueous pathway or the transcellular lipophilic pathway [3,5–7]. Thus, only small, lipophilic agents (<500 Da) can cross the BBB in pharmacologically relevant amounts; a physical requirement that the majority of drugs fail to meet [8,9].

Due to the formidable nature of the BBB, researchers and clinicians have attempted to bypass the BBB via direct intracranial infusion of drugs. This is a reasonable strategy that has demonstrated an acceptable safety profile; however, large-scale clinical efficacy remains unproven [10]. Local intracranial delivery of therapeutics requires infusion of a biologically efficacious agent, distribution of the agent to the target volume and retention of the agent for an appropriate therapeutic time period. The brain extracellular matrix (ECM) presents a challenge to achieving these goals. Specifically, the contents of the ECM can both degrade and clear free drug from the interstitial compartment [11]. Additionally, the pore size of the ECM is physically restrictive such that only drugs/carriers <100 nm can traverse tumorous brain and

only drugs/carriers <65 nm can traverse normal brain [12,13]. Studies have also demonstrated that movement of cationic drugs/carriers is restricted due to physical binding to ECM components and cell membranes [14].

In an attempt to overcome these barriers and improve drug delivery to CNS tumors, nanocarriers have been investigated as potential drug delivery vehicles [15–20]. Nanoparticles are solid, colloidal systems that range in size from 1 to 1000 nm and contain a therapeutic agent. Loading such agents into nanoparticle systems can protect them from degradation and clearance. As a group, nanocarriers have been used for a wide host of biological applications, including medical imaging, drug and nucleic acid delivery, interstitial thermal therapy, immunotherapy and diagnostic sensors. Nanocarriers can be fabricated from a host of organic and inorganic compounds depending on the clinical need. Each material has distinct advantages and limitations when applied to CNS drug delivery. In general, nanocarriers can improve delivery efficiency and drug kinetics while reducing off-target effects. They can be designed to deliver an assortment of therapeutics and can also be optimized for either systemic or local CNS delivery. Here, we review the published literature to summarize the current state of the art with respect to nanocarrier-based therapeutics for tumors of the CNS. We focus our review primarily on polymer nanoparticle systems; however, liposomes, micelles and dendrimers are also reviewed.

Nanocarriers

• Polymer nanoparticles

Polymers are large molecules composed of repeating subunits called monomers. Thus, polymeric nanoparticles are comprised of macromolecular materials in which the active agent is encapsulated or attached. Many natural and synthetic polymers have been used to create nanoparticles for CNS delivery, including polysaccharides, proteins, amino acids, poly(ethylenimines), poly(alkylcyanoacrylates), poly(methylidene malonates) and polyesters. Arguably, among nanocarrier-based drug delivery systems, polymer nanoparticles are safer, more stable and more tunable with respect to drug release kinetics and surface display of ligands.

• Preclinical studies: systemic delivery

Systemic delivery of polymeric nanoparticles to CNS tumors requires transport across the BBB.

This requires fabrication of a particle that can enhance existing transcytosis mechanisms and remain in the systemic circulation for a prolonged period of time. Here, we describe the major advances in development of polymeric nanoparticles for BBB bypass.

Poly(butylcyanoacrylate) (PBCA) nanoparticles were the first polymer-based nanoparticle system used to deliver drugs to the CNS. Initial studies demonstrated that intravenously administered PBCA nanoparticles, coated with polysorbate 80, could effectively deliver dalargin (a compound with opioid activity) to the CNS [21]. This work demonstrated that emulsifiers in general, and surfactants specifically, could enhance BBB transport by decreasing nanoparticle clearance by the reticuloendothelial system (RES), thereby increasing retention time [22]. Subsequent work with polyesters, including poly(lactic acid) (PLA), poly(glycolic acid) (PGA) and their copolymer poly(lactic-co-glycolic acid) (PLGA) demonstrated that pegylation improves BBB bypass and that BBB bypass occurs in a size-dependent manner [23]. Further studies with polyesters also demonstrated that surface decoration of nanoparticles with cell-penetrating peptides such as trans-activating transcription factor (TAT) increases transport across the BBB via bypass of efflux transporters [24]. Other polymers have also been employed for nanoparticle fabrication. Polysaccharides such as chitosan have been used to deliver peptides, dopamine and caspase inhibitors to the CNS following systemic administration [25]. From an engineering standpoint, at pH <6, chitosan's amino groups are protonated and the polymer becomes positively charged; this makes it particularly attractive for nucleic acid delivery. Like chitosan, poly(ethylenimines) (PEIs) are cationic polymers that are well suited for nucleic acid delivery. Intravenously administered PEI nanoparticles, surface decorated with rabies virus glycoprotein, have been shown to deliver miRNAs to the CNS [26].

The above modifications are generalizable to a host of CNS applications, including tumors. For example, a recent study has demonstrated that systemically administered siRNA–chitosan nanoparticles can be used to temporarily silence the P-gp efflux pump, which subsequently increases the efficiency of intravenous doxorubicin therapy [27]. Additional studies have demonstrated that surfactant coating of doxorubicin-loaded PBCA nanoparticles results in effective BBB bypass and pegylation of cationic albumin-conjugated

nanoparticles allows for delivery of plasmid DNA to intracranial gliomas [28,29].

• Preclinical studies: local delivery

Locoregional drug delivery to CNS tumors, via polymers, is a well-established concept [10,20,30–31]. In contrast to systemically delivered therapeutics, local delivery of therapeutics circumvents the BBB altogether. Initial work in this field led to the development of Gliadel®, a biodegradable polymer wafer impregnated with the alkylating chemotherapeutic agent bis-chloroethylnitrosourea (BCNU). Patients treated with Gliadel had only modest increases in survival because the wafer was unable to deliver therapeutic drug concentrations throughout the entirety of the infiltrative tumor zone [30,32]. In order to address this limitation in drug distribution, convection-enhanced delivery (CED) strategies have been utilized for intracranial drug delivery. CED relies on infusion of drugs into the brain under a positive pressure gradient, thereby creating bulk fluid movement in the brain interstitium, which can result in distribution of therapeutics over large volumes in the brain [33,34]. This strategy was first applied to free drug solutions and successfully increased the volume of distribution for these agents. However, because the drugs were not encapsulated or otherwise protected from the brain ECM, they were quickly degraded and cleared prior to achieving therapeutic efficacy [10]. Advances in nanocarrier design have tried to address these limitations by combining the protective, controlled-release environment of a nanocarrier system with the small size required for effective CED.

Initial local delivery studies with polymer particles began with microspheres. These particles were fabricated from a variety of materials, including PLGA, poly(methylidene malonate) (PMM), poly(epsilon-caprolactone) and chitosan. A host of therapeutics were delivered using these particles, including cyclosporine, paclitaxel, imatinib, mitoxantrone and phenytoin [35–39]. However, despite the versatility in polymer choice and therapeutic payload, these vehicles had limited clinical utility due to their relatively large size and therefore poor volume of distribution [12,13]. As discussed above, previous studies probing the ECM in tumorous brain demonstrated that nanocarriers <100 nm in diameter can be effectively distributed via CED [11,12]. Recently, a novel report was published describing the intracranial delivery of camptothecin-loaded PLGA nanoparticles via

CED. Despite the attractive biocompatibility of this PLGA system, the nanoparticles in this study were still >100 nm in diameter, thereby limiting volume of distribution [40]. A follow-up study by the same group detailed an improved fabrication procedure that, for the first time, yielded polymer nanoparticles <100 nm in size (~70 nm) with high efficiency. In addition to prolonging survival in a rodent model of malignant glioma, CED of these improved, biodegradable and biocompatible PLGA nanoparticles resulted in large, clinically relevant volumes of distribution in a large animal model [41].

Liposomes

Liposomes are artificial phospholipid vesicles, composed of a lipid bilayer, that form a core-shell structure and can easily carry both hydrophilic and hydrophobic drugs. These delivery vehicles are well characterized and have been studied for several decades [42–44]. In comparison to polymeric nanoparticles, liposomes are easier to manipulate with respect to size, but felt to be less tunable with respect to drug release kinetics. Specifically, liposomes exhibit a larger burst release within the first 48 h than polymer nanoparticles. Additionally, while many of the materials utilized for fabrication of polymeric nanoparticles are US FDA approved, the materials used to fabricate liposomes have a less robust safety profile.

• Preclinical studies: systemic delivery

There are numerous reports highlighting the ability of drug-loaded liposomes to cross the BBB and deliver a therapeutic payload. A recent study described the fabrication of a dual-targeted doxorubicin liposome that can penetrate the BBB and subsequently target glioma cells [42]. Furthermore, these liposomes increased survival time and decreased tumor volume in an *in vivo* model. Another group demonstrated that systemically administered liposomes decorated with two ligands were able to cross the BBB and deliver plasmid DNA to the rodent brain [43]. Interestingly, the liposomes utilized in both of these studies were surface decorated with transferrin, highlighting the importance of clathrin-mediated endocytosis for liposomal BBB bypass. Focused ultrasound has also been utilized in concert with liposomes with the goal of increasing intracranial drug delivery via transient BBB disruption [45]. In addition to oncologic applications, systemically administered

liposomes have been used for CNS delivery of glucocorticosteroids and rivastigmine [44,46].

• Preclinical studies: local delivery

CED of liposomal systems is well described. Due to their increased tunability with respect to size, liposomes have achieved large volumes of distribution via CED. In general, liposomes can be readily fabricated to <100 nm in size. Liposomes have been delivered via CED in numerous rodent tumor models and have carried a wide variety of therapeutic and imaging agents, including doxorubicin, topotecan, irinotecan, boron, lomeprol and gadolinium [47–50]. The clinical effectiveness of liposomal drug delivery for CNS tumors has been further validated in canine glioma models [51]. Moreover, the ability to achieve clinically relevant volumes of distribution via CED has been confirmed through primate studies [52].

Micelles

Micelles are supramolecular autoassemblies formed in an aqueous solution and composed of amphiphilic macromolecules. For the purposes of drug delivery, these macromolecules are typically block copolymers, which results in a core-shell design. This maintains the physical properties of conventional micelles but with enhanced thermodynamic stability. Polymeric micelles are typically small, stable in plasma and tunable with respect to surface modification. In general, polymeric micelles for CNS drug delivery are <100 nm in diameter, with most being <50 nm in diameter. In comparison to liposomes, micelles are generally easier to fabricate and surface modify and possess improved drug kinetics. However, as with liposomes, the long-term toxicity of micelles remains unknown.

• Preclinical studies: systemic delivery

Numerous micelles have been designed for systemic delivery to the CNS, incorporating a wide variety of block copolymers. Recent studies have demonstrated that intravenously administered micelles can cross the BBB and deliver chemotherapeutic agents, albeit in small amounts [53]. Additional research has shown that polymeric micelles can be surface modified to display a host of ligands (e.g., RGD, TGN and transferrin) that serve to enhance transport across the BBB and target glioma cells [54–57]. This strategy has been successfully utilized to deliver chemotherapeutic agents, plasmid DNA and antifungals to the CNS.

• Preclinical studies: local delivery

There are a limited number of studies describing CED of micelles. In general, CED of micelles results in similar to slightly improved volumes of distribution as compared with CED of liposomes. This is thought to be secondary to increased control over particle size. Various compounds have been loaded into micelles and administered via CED, including retinoic acid receptor agonists and doxorubicin [58,59]. These studies confirm adequate drug distribution and demonstrate therapeutic efficacy.

Dendrimers

Dendrimers are highly branched structures with a 3D architecture. They consist of an inner initiator core, followed by several repeating interior layers, and finally an exterior surface with multiple active groups. Dendrimers are characterized by ‘generation’, which is the number of branching cycles performed during its synthesis and is correlated with size. Overall, dendrimers are monodisperse and highly functional due to their extensive branching pattern. They are versatile with respect to size, and can be fabricated from a variety of materials, including poly(aminoamine) (PAMAM), poly(etherhydroxylamine) (PEHAM) and poly(propyleneimine) (PPI) [60]. Of these, the most commonly produced is the PAMAM dendrimer. Based on the generation of growth, PAMAM dendrimers can be optimized for delivery of nucleic acids and hydrophobic drugs. Cellular uptake of dendrimers is based primarily on clathrin and caveolae-mediated endocytic pathways, as well as macropinocytosis and tight junction modulation. As with liposomes and micelles, questions regarding the long-term neurotoxicity of dendrimers remain unanswered.

• Preclinical studies: systemic delivery

Dendrimers can be optimized for systemic delivery via surface modification. Numerous ligands have been attached to dendrimers, including PEG, transferrin, tamoxifen, rabies virus glycoprotein and angiopep-2, to name a few [61–64]. These ligands prolong circulation time, reduce immunogenicity, improve BBB bypass, aide in cell targeting and balance particle charge. *In vivo* studies have demonstrated that this strategy for surface functionalization can enhance uptake across the BBB by two- to three-fold. Additionally, rodents treated with drug-loaded dendrimers have prolonged survival compared with those treated with traditional chemotherapeutic agents.

• Preclinical studies: local delivery

Dendrimers have been delivered locally via CED for a variety of intracranial tumor applications. This includes siRNA delivery, MRI and CT scans, and boron neutron capture therapy [65–67]. Overall, these nanocarriers have demonstrated reasonable volumes of distribution and drug loading. Additionally, dendrimers that contain imaging moieties have confirmed the cell targeting and endosomal escape mechanisms present in this class of drug delivery systems. Further *in vivo* efficacy studies are needed to adequately assess the clinical potential of this carrier.

Delivery strategies: systemic versus local

Over the past several decades, considerable progress has been made in the development of nanocarrier-based therapeutics. Both systemic and local delivery systems have undergone significant preclinical testing. However, at present, none of these drug delivery systems are well-developed enough to be established as standard-of-care. Local delivery systems are still fraught with questions regarding volume of distribution, catheter placement and design, and safety of long-term intracranial infusion. Systemic delivery systems continue to have difficulty with efficient BBB transport; despite improvements in surface functionalization, the vast majority of intravenously administered nanocarriers never cross the BBB.

As detailed above, nanocarrier-based therapies can be administered via both systemic and local routes. Each of these techniques has unique advantages and limitations. When designing an ideal nanocarrier, a few basic requirements are common to both strategies. For the purposes of CNS delivery, nanocarriers must be: biocompatible; biodegradable; <100 nm in diameter; nonaggregating; able to efficiently encapsulate therapeutic agents; amenable to surface modification; able to adequately protect their payload; and cost effective. Systemically delivered nanocarriers must also: have prolonged circulation times; escape the RES; and possess the ability to efficiently cross the BBB. Locally delivered nanocarriers must be: neutral or negatively charged; and able to achieve high volumes of distribution via CED.

Conclusion & future perspective

Nanocarrier-based therapies are poised for significant clinical impact. Given the increasing prevalence of neurological disorders, the demand

for effective drug delivery will only continue to increase. There have been some early successes in utilizing intravenously administered nanocarriers for drug delivery to systemic malignancies. Additionally, the successes and failures of the PRECISE trial and Gliadel wafer lend credence to a CED-based nanocarrier for CNS drug delivery. Overall, a successful nanocarrier-based drug delivery regimen has the potential to reduce systemic toxicity and off-target effects, deliver a wide range of therapeutics (including small-molecule drugs and nucleic acids) and serve in a dual capacity as a diagnostic agent.

At present, there are a limited number of early clinical trials examining nanocarrier-based therapies for CNS tumors. These studies primarily evaluate CED of nanoliposomal constructs (encapsulating alkylating agents); clinical results have not yet been published. Although other chemotherapeutics have been packaged into nanocarrier systems and delivered intravenously for systemic malignancies, their efficacy in crossing the BBB and treating CNS malignancies is unknown. We now have the ability to extensively

modify nanocarriers such that size, charge, hydrophobicity, targeting and drug release can all be finely tuned. As nanocarrier-based drug delivery systems evolve, one of the major challenges to clinical translation will be the ability to scale the technology in a cost-effective manner. The future success of nanocarrier therapies rides on the demonstration of clinical efficacy. Given the extensive preclinical testing that has been performed to date, and the current lack of effective therapies for patients with malignant brain tumors, proactive translation of these systems to human clinical trial, over the next 5–10 years, is both necessary and anticipated.

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