Recent advances in nano delivery systems for blood-brain barrier (BBB) penetration and targeting of brain tumors

Shriya Reddy [a,b], Katyayani Tatiparti [a], Samaresh Sau [a], Arun K. Iyer [a,c,*]

[a] Use-inspired Biomaterials & Integrated Nano Delivery (U-BiND) Systems Laboratory, Department of Pharmaceutical Sciences, Eugene Applebaum College of Pharmacy and Health Sciences, Wayne State University, Detroit, MI 48201, USA
[b] Northville High School, Northville, MI 48168, USA
[c] Molecular Imaging Program, Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI 48201, USA

Gliomas constitute about 80% of brain tumors and have a meager two-year survival rate. The treatment options available are very few because of poor prognosis and a lack of targeted nanodelivery systems that can cross the blood–brain barrier (BBB) and the blood-tumor barrier. This short review attempts to clarify the challenges for delivery systems designed to cross the BBB, and provides a brief description of the different types of targeted nanodelivery system that have shown potential for success in delivering drugs to the brain. Further, this review describes the most recent studies that have developed nanoparticles for brain delivery in the past five years. We also provide an insight into the most recent clinical trials designed to assess the efficacy of these nanodelivery systems for glioma.

Keywords: Glioblastoma multiforme (GBM); Targeted nanoparticles; Blood–brain barrier (BBB); Clinical trials; Blood-tumor barrier (BTB)

Introduction

Gliomas are the most common forms of brain tumors, accounting for about 80% of all cases. The two-year survival rate of glioma patients has been reported to improve by only 20–25% with approved treatment strategies [1]. This poor prognosis for glioma can be attributed to the lack of targeted delivery systems that can cross the blood–brain barrier (BBB) and the blood-tumor barrier (BTB). Both these barriers, with their distinctive compositions, are known to impede the passage of drugs and their delivery systems into the affected areas of the brain. Moreover, gliomas, being solid tumors, are characterized by high metabolic rate, high cellular division, and consequently high oxygen consumption. As the tumors grow, angiogenesis is required to compensate for the increasing consumption of oxygen, and hypoxia subsequently develops due to unmet oxygen demands. These physiological changes can disrupt the BBB and make the BTB dysfunctional [2], but these barriers remain intact in most gliomas and hence continue to pose a challenge for drug delivery to the brain. The requirement to traverse both the BBB and the BTB is imperative for the success of any therapeutic strategy in gliomas. Most of the therapeutic regimens currently used for gliomas are failing pre-clinically and clinically because of their inability to cross these barriers. The search for a possible solution to overcome this challenge requires a thorough understanding of these barriers, including their structure, composition, and functions [3]. This review provides a clear understanding of these aspects of gliomas, as well as a brief discussion on the various nanoparticulate delivery systems that have been studied recently for application in the targeted delivery of drugs to brain tumors.

The blood brain barrier (BBB)

Brain

The brain constitutes about 2% of the body weight and requires 20% of the total cardiac blood supply, along with 25% of the

* *Corresponding author at: Use-inspired Biomaterials & Integrated Nano Delivery (U-BiND) Systems Laboratory, Department of Pharmaceutical Sciences, Eugene Applebaum College of Pharmacy and Health Sciences, Wayne State University, Detroit, MI 48201, USA.* Iyer, A.K. (arun.iyer@wayne.edu)

1359-6446/
https://doi.org/10.1016/j.drudis.2021.04.008

Please cite this article in press as: S. Reddy et al., Drug Discovery Today (2021), https://doi.org/10.1016/j.drudis.2021.04.008

total oxygen and glucose supply [4]. It has three distinct layers of protection: the arachnoid barrier, blood-cerebrospinal fluid (CSF) barrier, and the BBB [5]. The arachnoid barrier is avascular and has low surface area [6]. It is composed of epithelial cells and has a role in the containment of the CSF. The blood-CSF barrier forms an interface between the CSF (which is replaced every 4–5 hours, removing any medication with each replacement). The arachnoid barrier and the blood-CSF barrier are not very good targets for drug delivery because of their low surface area, poor accessibility, and high clearance rate for drugs [7].

The brain is characterized by a very complex network of neurons that communicate through neurotransmitters and neuromodulators, by the presence of synaptic potential in such networks, and by a number of capillaries in the order of 100 billion [8]. All of these components are highly protected by the complex BBB. The function of the brain, to communicate signals across to the rest of the body, requires constant exchange of ions and other molecules across the BBB.

Function of the BBB

The main function of the BBB is to maintain the composition of the ISF. In order to maintain and protect brain functions, the BBB needs to be selectively permeable to ions, nutrients and molecules, and thus the BBB is permeable to only 2–3% of small molecules and completely excludes large molecules [9]. This selective permeability is possible because of the unique composition and structure of the BBB. The BBB also contains very active efflux pumps that remove toxic, metabolic, and other waste materials out of the brain. Other functions of the BBB include the regulation of neurotransmitters at the periphery of the brain and the regulation of immune responses in the brain [10]. Together, these roles make the BBB very selectively permeable to molecules and protect the brain.

The integrity of the BBB is very crucial for a healthy brain. In gliomas, however, the BBB loses its integrity because cancer cells infiltrate the BBB and alter the neural structure, leading to down-regulation of the tight junctions, loss of microglial activity or loss of astrocytes and pericytes [11]. This disruption of BBB integrity and the mechanisms that cause are very heterogenous [12].

Composition of the BBB

BBB consists of a continuous layer of endothelial cells that have capillary properties. The endothelial cells are connected to each other by tight junctions and adherens junctions, and are enclosed by astrocyte-end-feet and basement membrane. These structures have been classified into three functional barriers: glycocalyx, endothelium, and neovascular unit (NVU).

Glycocalyx

The glycocalyx is located on the luminal side of the endothelium, where it forms the first barrier of the vasculature that is in direct contact to blood. It forms a 300-nm-thick gel-like carbohydrate-rich layer comprised of glycoproteins, proteoglycans, glycosaminoglycans [13]. These components render the BBB negatively charged [14]. The glycocalyx acts like a mesh that prevents circulating molecules from entering the BBB.

Endothelial layer

This layer is 200 nm thick and is the most important unit that separates the luminal compartments (blood) from the abluminal compartments (brain) of the BBB. It is characterized by tight junctions and adherens junctions. These two structures are responsible for the selectivity in the transport of molecules across the BBB [15]. This layer is also characterized by specific transport mechanisms, including the efflux pumps that allow exchange of nutrients and other molecules across BBB.

Tight junctions are made of transmembranous cytoplasmic proteins such as claudins, occludins, and junctional adhesion molecules (JAMs) [13]. They act as gates between the brain microvascular endothelial cells (BMECs). Their pore size of 1.4–1.8 nm makes the transport of molecules highly selective and completely eliminates the passive diffusion of large molecules [16]. Claudins are the major type of proteins forming these junctions, and they function by creating transepithelial electrical resistance (TEER) in the order of 1500–2000 Ωcm^2. Occludins and JAMs support the tight junctions and allow the movement of leukocytes across the BBB [17]. Tight junctions work in conjunction with cytoskeletal proteins, such as actin, and other cytoplasmic proteins, such as calcium-dependent serine protein kinase (CASK) and the zonula occludens proteins (ZO-1, ZO-2, ZO-3), cingulin and so on. All of these types of proteins reinforce the tight junctions.

Adherens junctions are positioned close to tight junctions in BMEC and are composed of calcium-dependent cadherins (a type of glycoproteins), such as vascular endothelial cadherin (cadherin-5 or VE-cadherin). They adhere to components of the cytoskeleton of BMEC, such as platelet-endothelial cell adhesion molecule (PECAM), the catenins (α-, β-, and γ-catenin), desmoplakin, and p120 catenin [18]. Adherens junctions support the functions of tight junctions, adding another layer of protection to the brain and maintaining microvascular integrity. Owing to their compositions and respective functions, tight and adherens junctions together create a polarity across the BBB that is important in maintaining the integrity of the BBB [19].

Neurovascular unit (NVU)

The NVU is comprised of BMEC, astrocytes, pericytes, microglia, vascular smooth muscles, and oligodendrocytes [20]. These cell function together in the NVU to regulate the permeability of the BBB. Broadly, the NVU can be described as comprising the following sections:

1. Vascular smooth muscles: the arteries, veins, arterioles that are the main supply of blood to the brain.
2. Pericytes that enclose the BMEC and the capillaries in the basal lamina BBB. These cells maintain the tight junctions, homeostasis and blood supply in the brain. They also have a role in the regulation of macrophages [8].
3. Astrocytes: stellate cells with perivascular end-feet. These cells support BMEC and have a role in maintaining the permeability of the BBB [21]. They are equipped with AQP4 water channels and other ion channels that maintain homeostasis in the brain. They further provide nutrients to the brain, and mediate cytokine-dependent inflammatory pathways [22].
4. Microglia have a role in the immune responses of the brain. They are found in the parenchyma of the brain where they perform constant surveillance of the brain [23]. Like macrophages, they have a role in the phagocytosis of cell debris

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and aberrant cells in the brain. Also like macrophages, they are activated by proinflammatory factors, including lipopolysaccharide (LPS), tumor necrosis factor-a (TNF-a), interleukin-1b (IL-1b) and reactive oxygen species (ROS) and so on, that can eventually cause disruption of the BBB if left unregulated [24].

Other protective mechanisms of the BBB

Apart from limiting the transport of molecules in and out of the brain, the BBB encompasses other mechanisms that are involved in protecting the brain:

Enzymatic activity

BBB shows some enzymatic activity that can metabolize and detoxify neuroactive toxic molecules (that have the ability to bypass the barrier) in order to protect the brain [6]. These enzymes include monoamine oxidases and cytochrome P450, peptidases, cholinesterases, and other such enzymes [25].

Immunological activity

The BBB has a highly selective and unique immunological activity that elicits immune responses by recognizing inflammatory factors such as γT cells [21,26]. The major immune cells that are active in the brain include BMECs, perivascular macrophages, microglia, T cells and mast cells. The microglia of the brain carry the major histocompatibility complex (MHC) class II, which has a role in immune responses [27]. The brain does not, however, possess antigen-presenting cells (APCs) and has very low numbers of lymphatic vessels and leukocytes. The immune responses in the brain are mediated by chemokines and the subsequently recruited T cells.

Efflux mechanisms

Efflux mechanisms facilitate the removal of waste and other toxic substances out of the brain. The main efflux transporters in the brain belong to the ATP-binding cassette (ABC) superfamily, which includes P-glycoprotein (P-gp) and the multidrug resistance protein 1 (MDR1) [20]. Other MDR proteins, such as MRP1, MRP2, MRP4, MRP5, and breast cancer related protein (BRCP) also act as efflux pumps in the brain [28], which in turn are regulated by P-gp. These transporters are active on both the luminal and abluminal sides of the BBB.

Blood-tumor barrier (BTB)

Brain tumors are associated with many structural disruptions and with compromised function in the BBB (Fig. 1). As development of the tumor progresses, the disruption leads to the loss of NVU integrity. This gives rise to the BTB. Although the BTB is more permeable than the BBB, it shows highly heterogenous permeability to molecules including drugs. Thus, it poses a great challenge for therapy [29].

Once disruption of the BBB begins, a protein called agrin (a proteoglycan) is lost from the BMECs [30]. Further, matrix metalloproteinases (MMPs) degrade components of the BBB including occludin, fibronectin, laminin, and heparan sulfate. A change in pericyte number can affect the amount of claudin and occludin present in the BBB, resulting in weakening of the tight and adherens junctions [31]. Astrocyte end-feet are also displaced [32]. Further, intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion molecule-1 (VCAM1) are upregulated in tumors, leading to enhanced immune responses [33]. All of these effects trigger immunological responses in the brain that are mediated by microglia. Microglia are upregulated in the disease state, a change that is associated with higher concentrations of pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α, and interferon-γ (IFN-γ). The NVU loses its integrity as a result of these immunological responses, affecting the permeability of the BBB [2]. The heightened proinflammatory responses trigger negative feedback responses by activating anti-inflammatory reactions mediated by regulatory T cells (T-reg) or myeloid suppressor cells (MSCs). When overactivated, these anti-inflammatory responses are also detrimental to the integrity of the BBB [34]. In high-grade gliomas, in which the tumor grows very aggressively, a condition of hypoxia is produced that, in turn, upregulates hypoxia inducible factor-1 (HIF-1). HIF-1 then stimulates VEGF production, angiogenesis, and abnormal vessel formation to compensate the growing demand for nutrition and oxygen [35]. This, in turn, results in the disruption of the BBB.

The structural and functional changes in the BBB in presence of tumors are heterogenous across the brain segments. This leads to variable permeability across the BBB. The extent of these changes depends on the tumor type, volume, stage, and location [36]. The damage is much higher in high-grade gliomas, which are the more aggressive types [37].

Properties of BBB that are advantageous for nanodelivery systems

Properties of the BBB

The selective nature of the BBB is crucial to maintaining homeostasis in the brain, but it is a major challenge for the transport of drugs into tumor tissues. The formation of tight junctions between endothelial cells severely restricts passive diffusion through the extracellular matrix. As a result, most transport must occur transcellularly, and thus lipid-soluble drugs are preferred. As the lipid solubility of drugs increases, however, they are increasingly exposed to active efflux mechanisms. Doxorubicin is a lipophilic drug that has poor BBB permeability, which has been attributed to the active efflux mechanisms present in the membranes of the BBB [38]. In addition, high lipid solubility has also been positively correlated with the accumulation of drugs at non-target sites [39]. An additional barrier to drug activity in the BBB is the degradation of drugs. The cerebral endothelial membrane is rich in mitochondria and exposes passing solutes to degrading enzymes such as neprilysin, enkephalin, and insulin-degrading enzymes [40].

Transport mechanisms of the brain

In light of increasing knowledge about the BBB and BTB, and the changes that occur in these structures in the presence of tumors, extensive research is being carried out into various mechanisms that could be applied for targeted drug delivery into the brain (Fig. 2) [5]. The various transport mechanisms and their applicability in the brain in this section.

Paracellular transport is seldom utilized because it is highly limited by endothelial cell tight junctions and intact BBB [38].

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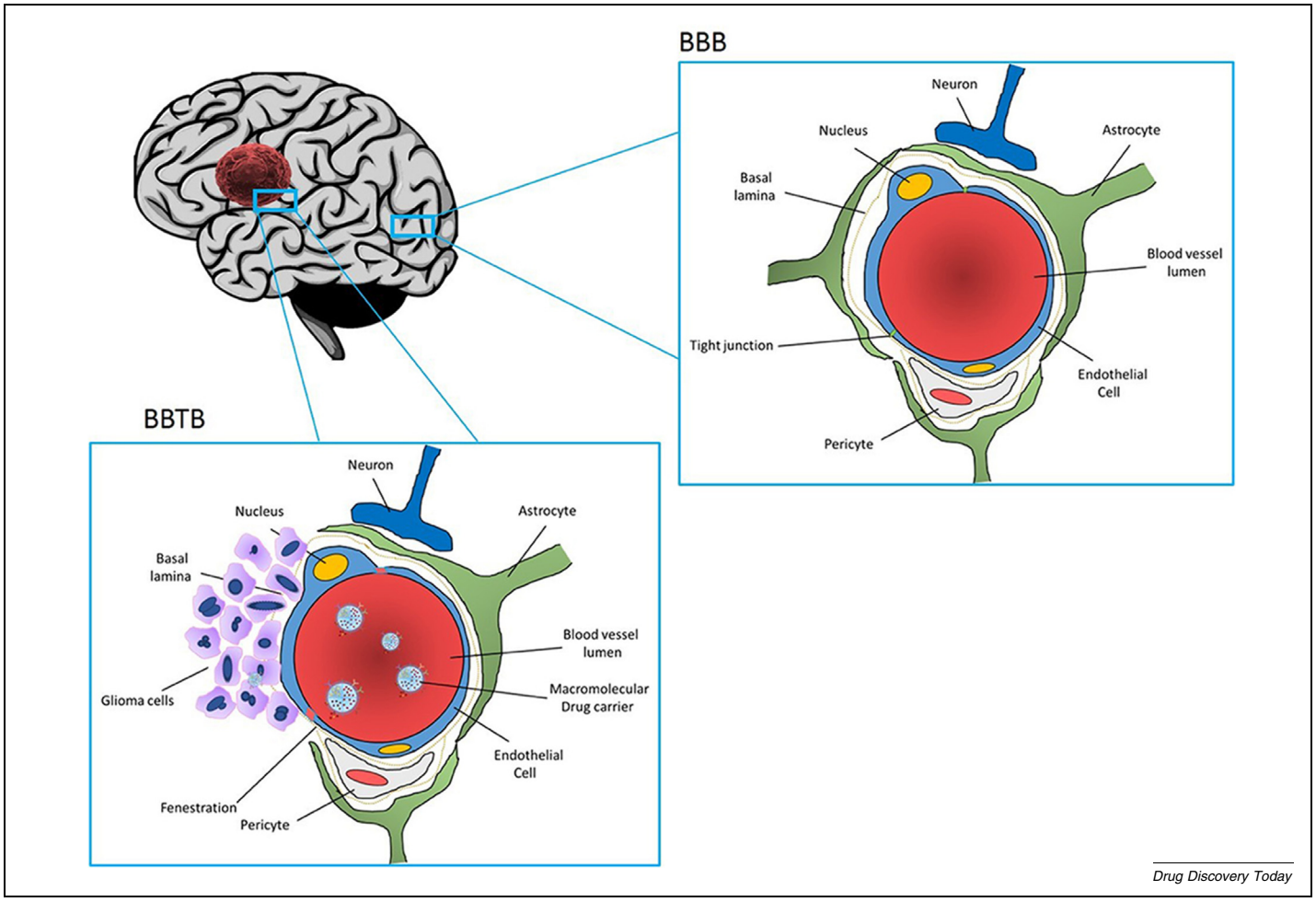

FIGURE 1

The blood–brain barrier (BBB) and the blood-tumor barrier (BTB). Major changes and disruption in the BBB that occur upon development of a tumor include, detached astrocytes, loss of tight junctions (causing increased paracellular leakage) and alteration of the efflux pump activity. The basal lamina is disrupted by factors released from immune cells and the tumor. Adapted with permission from [84].

Transport occurs to a very limited extent through the pores in BMECs and is seen mostly in disrupted BBBs [41]. Similarly, passive diffusion is very uncommon because it is only viable for lipophilic drugs that are smaller than 500 Daltons in size [42].

Carrier-mediated transport is responsible for the carriage of mostly endogenous molecules through transporters that are specific for each of these molecules. These transporters are of two types: facilitated diffusion transporters and active transporters [43]. The direction of movement is down the concentration gradient in facilitated diffusion and against the concentration gradient in active transport. Examples of such transporters in the brain include glucose transporter 1 (GLUT1), excitatory amino acid transporter 1 (EAAT1), monocarboxylate transporter 1 (MCT1), and large neutral amino acid transporter 1 (LAT1) [44]. They are responsible for the transportation of nutrients including glucose and amino acids. Carrier-mediated transport is responsible for both the uptake of substances into the brain and the release of substances back into blood circulation. Thus, drugs that are transported by this route are prone to removal by active efflux mechanisms [45]. In addition, the number of carriers in the brain is limited, so the carrier-mediated transport of a drug across the BBB is a saturable process [46].

Cell-mediated transport is observed when immune cells, such as macrophages and monocytes, traverse the BBB [47]. It has been named the 'Trojan horse' strategy because the method involves the recruitment of immune cells by natural processes such as chemotaxis and [48]. This route also facilitates the transport of large molecules of about 1.2 μm, although with some toxic effects [49]. However, the discovery, development, and production of delivery systems that take advantage of this method are difficult.

Adsorption-mediated transport occurs when macromolecules that cannot cross the BBB because their negative charge causes electrical impedance are involved. This mechanism of transport is by endocytosis of the macromolecules. The advantage of this route is that much larger-sized macromolecules can be transported through the BBB, and hence it is applicable to only very large molecules such as cell penetrating peptides or diamine- or polyamine-modified proteins [50]. Hence, the transport of such molecules by this mechanism occurs less often than their receptor-mediated transport.

Receptor-mediated transport is the most commonly used and most efficient mechanism for drug delivery. Macromolecules of 200–500 nm in size [51] need specific receptors to allow them

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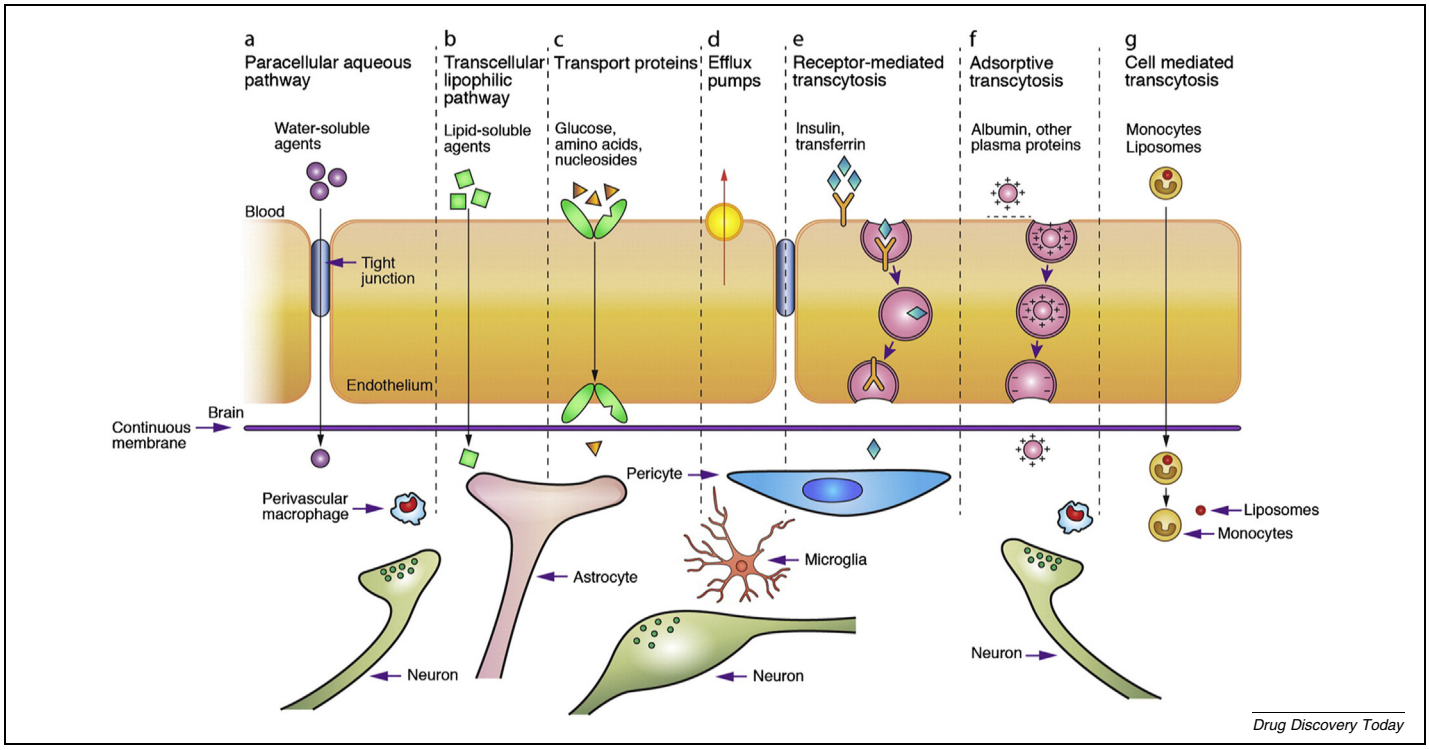

FIGURE 2

Examples of the application of mechanisms of transport across the blood–brain barrier (BBB) for effective drug delivery. Adapted with permission from [5].

to cross the BBB. The uptake of such macromolecules, which can also be referred to as ligands, usually occurs through clathrin-mediated or caveolin-mediated endocytosis. These mechanisms utilize the formation of vesicles and endosomes that are transported via the BMECs before releasing their contents into the abluminal side of the brain. Examples of ligands that are transported in this way include LRP1, LRP2, and LDLR. Proteins such as amphiphysin, endophilin, and the various adaptins, dynamins, and rab proteins found inside the BMECs act as the routers for these vesicles [52].

Nanomedicines for overcoming the BBB

There have been several advances in the treatment of gliomas over the course of the past few decades, but there is still much to be achieved and improved. To bypass the natural barriers that reduce the efficacy of standard treatments, a large variety of nanoparticles have been developed to treat tumors within the central nervous system [53]. For the convenience of discussion within the scope of this review, we divide nanoparticles into four main categories: polymeric, lipid-based, carbon-based, and inorganic nanoparticles. An extensive list of preclinical studies from the past five years, obtained using databases such as Pubmed and Embase, is provided for each of the categories, outlining the drugs and nanoparticle combinations that have been tested for glioma treatment.

Preclinical studies and their outcomes

A wide variety of the nanoparticles have been studied for their potential to deliver a drug payload in the brain to treat gliomas. Although all of these nanoparticles have been studied *in vitro*, the efficiency of some of these delivery systems has been tested *in vivo* in preclinical studies using animal models to obtain real-time efficacy data. The animal models that have been used in order to achieve this goal include syngeneic, allogeneic, orthotopic xenograft, and genetically engineered models. Syngeneic models have proven to be the most widely used because they offer a low rate of tumor rejection by the immune system [54]. The efficacy of several agents, ranging from chemotherapeutic agents (such as temozolamide, doxorubicin, and paclitaxel) to miRNA and siRNA, has been tested after delivery in a nanoparticle system. The nanoparticles offered better uptake in the brain and more sustained release of the drugs at the tumor site when compared with other delivery systems. Further, the structure of nanoparticles is flexible enough to allow surface decoration with targeting ligands so as to improve targeting to the affected tissue [55]. The targeting ligands that bind strongly to receptors that are overexpressed on the BBB include lactoferrin, folic acid, apolipoproteins, and peptides such as angiopep-2. Most of the preclinical studies have shown a prolonged survival of the animals and superior drug accumulation in the brain when nanoparticles are used as the delivery system systems [55,56]. These studies boast improved anti-tumor drug activity and impressive tumor regression [56]. Some of them even promise reduction of the toxic effects of the drug in off-site tissues. Although the preclinical studies have shown such interesting results, there has been little success in clinical trials.

Polymeric nanoparticles

Biodegradable polymeric nanoparticles are the most extensively researched nanoparticle type used to induce selective toxicity, as their drug loading capacity is higher than that of other nanoparticles. They are also highly stable, and are used in both active

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and passive targeting [57]. The several subcategories of polymeric nanoparticles include hydrogels, microspheres, chitosan particles, dendrimers, and poly(lactic-co-glycolic acid) (PLGA).

Hydrogel nanoparticles, or nanogels, are 3D structures of crosslinked hydrophilic polymer chains that hydrate water-based solutions. Their properties are similar to those of biological tissue, but they are systemically administered and have specific loading and targeting properties within their framework [58]. Microspheres are spherical nanoparticle of size 1–1000 mm that are used to encapsulate small drugs and proteins, thereby improving the bioavailability, stability, and specificity of these agents [59]. Chitosan is a polysaccharide created by the deacetylation of chitin, and chitosan nanoparticles control drug release by utilizing swelling or drug–polymer interactions [60]. Dendrimers are characterized by hyper-branched globular structure and are highly uniform, with well-defined chemical structures. The specific structure of dendrimers allows them to load therapeutic drugs by covalent conjugation or electrostatic adsorption [61]. PLGA is a very commonly used polymer composed of the ring opening polymerization of lactic and glycolic acid monomers, which are easily metabolized by the body and thus ensure that PLGA is biocompatible and biodegradable [62].

Lipid-based nanoparticles

Lipid-based nanoparticles are physiochemically stable and biocompatible, solubilize drugs well, and reduce drug-associated side effects. They are composed of one or more lipid bilayers and can encapsulate lipophilic agents that have a wide variety of sizes. Lipid nanoparticles can be divided into four big groups: liposomes (LPs), solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), and micelles. In order to maintain stability, liposomal formulations must be pegylated, by a 2–5 kDa poly (ethylene glycol) (PEG) chain [57].

Liposomes are phospholipid bilayers containing an encapsulated drug or other treatment. They are used to provide site-specific targeting, reduced toxicity, and intracellular and sustained drug release They can be used for either active or passive targeting to the site of action. When their utility in active targeting was compared to that in passive targeting, they were found to be limited in passive targeting by their low solubility, short half-life, phospholipid oxidation, and leakage of encapsulated molecules [63]. Solid-lipid nanoparticles are 50–1000 nm in diameter, and are composed of a solid lipid core matrix that can dissolve lipophilic molecules. They boast excellent physical stability, targeted drug delivery, biological biocompatibility and feasibility, and biodegradability characteristics [64]. Their disadvantages include low drug loading capacity, poor shelf life, and the tendency for burst release of the drug encapsulated in them [65]. Nanostructured lipid carriers consist of partially crystallized lipid particles of radius less than 100 nm that are dispersed in an emulsifier-containing aqueous phase [66]. Micelles are 10–100 nm in diameter and are composed of hydrophobic polymer parts such as poly (caprolactone), poly(propylene glycol) (PPG), or poly(D,L-lactide), together with a hydrophilic shell made of PEG [67].

Carbon-based nanoparticles

Carbon nanoparticles have been explored for biomedical applications and are considered to be a possible choice as a low-toxicity tumor therapy or a drug-delivery strategy [68]. Carbon has specific electrical, optical, and thermal properties that allow the nanoparticles to have very strong optical absorptions of near-infrared radiation and to generate heat [69]. They are also efficient carriers of macromolecules, leading to their potential as immunotherapies [70]. The two main subgroups of carbon nanoparticles discussed in this review are carbon nanotubes and fullerenes. Carbon nanotubes are drug carriers made of graphene sheets rolled cylindrically, which need to be functionalized in order to achieve biocompatibility with cells [71]. Buckminsterfullerene (C_{60}) is a carbon allotrope whose photophysical properties generate ROS in response to visible light. This characteristic makes it a very strong agent for photothermal therapy [72].

Inorganic nanoparticles

Inorganic nanoparticles have been studied extensively as candidates to improve radiation treatment and as contrast agents for imaging [67]. Mainly iron oxide and gold nanoparticles have been utilized as potential treatments for brain tumors, often in the form of enhancing thermotherapy [73]. Inorganic nanoparticles are relatively stable over broad ranges of temperature and pH. However, their long-term administration is a potential safety issue because of their lack of biodegradation and slow dissolution [74,75].

Status of current and completed clinical trials

Over the past two decades, multiple clinical studies have attempted to explore nanodelivery systems in the treatment of brain cancers. The most recent nanoparticle-based clinical trials are listed in Table 1. Most of these Phase I and Phase II studies have focused on drug- or siRNA-loaded nanomedicines. They aim to identify superior therapeutics and to evaluate the safety and efficacy of the nanosystem [76].

A phase II clinical trial using pegylated liposomal doxorubicin (Caelyx™, PEG-Dox) and prolonged administration of temozolomide and radiotherapy showed a 12-month progression-free survival (PFS) rate of 30.2% in newly diagnosed glioblastoma. The toxicity of the combination of PEG-Dox, prolonged administration of temozolomide, and radiotherapy was tolerable. However, neither the addition of PEG-Dox nor the prolonged administration of temozolomide created a significant improvement in patient's outcomes [77].

A phase I clinical trial investigated the safety, the tolerability and the spectrum of side effects of a gadolinium-based nanoparticle, AGuIX, combined with standard whole-brain radiotherapy in patients with multiple brain metastases [78]. The study found that gadolinium-based nanoparticles accumulated in brain metastases and could potentially be used to increase the effectiveness of radiotherapy in patients. This study has recently been extended to a phase II clinical trial including 100 patients [79].

Often, promising preclinical nanomedicines are not able to complete clinical testing for various reasons. For example, a phase I/II clinical trial studied the suppression of the glial progenitor cells that surround the ventricular system in patients with aggressive brain tumors using ITV DepoCyt® and Liposomal Encapsulated Ara-C, in combination with temozolomide. The

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TABLE 1

Recent nanoparticle-based clinical trials.

National Clinical Trial (NCT) number	Phase	Nanoparticle treatment	Status
NCT03463265	2	Nanoparticle albumin-bound rapamycin	Recruiting
NCT00944801	1, 2	Pegylated liposomal doxorubicine	Completed, 2009
NCT01044966	1, 2	Liposomal encapsulated Ara-C (DepoCyt)	Terminated
NCT00734682	1	Nanoliposomal CPT-11	Completed, 2015
NCT01906385	1, 2	Rhenium nanoliposomes	Recruiting
NCT03020017	Early 1	NU-0129 (nucleic acids arranged on the surface of a small spherical gold nanoparticle)	Active, not recruiting
NCT02340156	2	SGT-53 nanocomplex (cationic liposome encapsulating a normal human wild type p53 DNA sequence)	Terminated
NCT02766699	1	EGFR(V)-EDV-Dox (400 nm minicell containing doxorubicin with bispecific antibodies (BsAb))	Recruiting
NCT01663012	2	NKTR-102 (irinotecan molecules attached to a polyethylene glycol (PEG) polymer)	Completed, 2015
NCT03086616	1	Convection-enhanced delivery (CED) of nanoliposomal irinotecan	Recruiting
NCT02022644	1	CED of nanoliposomal irinotecan	Recruiting
NCT01386580	1, 2	2B3-101 (phase1)/trastuzumab (phase2)	Completed, 2014
NCT02861222	1	Liposomal doxorubicin	Completed, 2013
NCT02820454	1	AGuIX	Completed, 2019
NCT00019630	1	Doxorubicin HCl liposome	Completed, 2015
NCT01266096	Microdosing	124I-cRGDY-PEG-dots	Active, not recruiting
NCT00465673	2	Liposomal doxorubicin	Terminated
NCT04094077	2	AGuIX	Recruiting
NCT03818386	2	AGuIX	Recruiting
NCT03328884	2	Nanoliposomal irinotecan	Recruiting

study was terminated due to lack of adequate patient enrollment into the trial, poor KPS (Karnofsky Performance Scale, which measures the patient's functional impairment) upon disease recurrence, and the new availability of FDA approved drugs, such as Avastin® (bevacizumab), after the study's initiation (NCT01044966).

The relative lack of nanomedicines in clinical trials can be attributed to the extended testing that nanomedicines require. In addition to studies on biodistribution, safety and efficacy, testing must be carried out to study drug encapsulation, targeting efficiency, particle size and shape, and release kinetics, and to establish effective protocols [80]. Currently, validated, sensitive and standardizable assays for *in vitro*, *ex vivo*, and *in vivo* methods are needed to determine nanotoxicology during preclinical and early clinical testing [81].

Even after there is strong evidence for the safety and efficiency of a specific nanodelivery system, its potential for glioma treatment must be evidenced. As more and more nanomedicines move towards clinical trials, they face even more challenges in commercial development. The structure of most nanoparticles makes quality control and reproducibility difficult in manufacturing [82]. After development and manufacturing, intellectual property, government regulations, and overall cost-effectiveness in comparison to current therapies are also major hurdles to eventually getting nanomedicines onto the market [83].

Challenges in developing nanodelivery systems targeting the brain

Nanodelivery systems have proven to be the best strategy for overcoming the challenges to drug delivery to the brain presented by the BBB. However, there are some difficulties that need attention before nanodelivery systems can successfully translate into the clinics. The toxicity of nanomedicines is specific to the type of nanoparticles involved. Cytotoxicity or immune reactions can be induced by the size, electrical, optical or magnetic properties, surface charge, agglomeration, or chemical composition of the material [74]. Penetration of the BBB by active targeting also may create issues. Nanoparticles that are bound to physiological ligands compete with the endogenous protein that binds to their receptor, which decreases efficacy [75]. In addition, some receptors on the BBB, such insulin and transferrin (Tf), are crucial to homeostasis within the brain, and nanoparticles targeting them could reduce their activity [76]. The difficulties in scaling up the synthesis of such nanoparticles from laboratories to industrial production add to the roadblock already present in application of these nanoparticles in real time.

Conclusions

Gliomas are very lethal cancers of the brain. Surgical removal of these tumors is very risky and invasive, requiring high precision. The BBB has unique functions and properties to protect the brain, including selective transport mechanisms and efflux pumps. These mechanisms limit both passive and active transport of drug and delivery systems across the BBB. The integrity of BBB is disrupted in pathological conditions such as glioma. Nevertheless, drug delivery to the brain can be a great challenge due to the selective permeability, protective mechanisms and unique transport mechanisms of both the BBB and the BTB. Nanotechnology can offer a great solution to this problem. Targeted nanoparticles offer several advantages, including site-specific delivery, reduction of off-site drug toxicity, and improved drug

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bioavailability within tumors. But, the use of nanoparticles has disadvantages too. These include the difficulties inherent in designing nanoparticles and in scaling up the preparation of the delivery systems. Moreover, several studies that have been successful in the laboratory or preclinical stages have not seen much success in clinical trials. Evidently, further extensive research is necessary to develop a robust delivery system that is safe and efficient specifically in delivering drugs across the BBB into the brain.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

S.R. would like to acknowledge the Department of Pharmaceutical Sciences, Wayne State University (WSU) for a summer undergraduate research fellowship (SURF) in the Iyer lab. K.T. would like to acknowledge AGRADE scholarship from the Wayne State University Graduate School, EACPHS, Wayne State University. S.S. acknowledges the support of a Burroughs Wellcome Fund Collaborative Research Travel Grant (BWFCRTG). A.K.I. acknowledges a US Department of Defense CDMRP KCRP Idea Development Award #W81XWH1810471, a US National Institutes of Health, National Cancer Institute (NIH/NCI) grant R21CA179652 and Wayne State University FRAP Grant (#142174).

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Please cite this article in press as: S. Reddy et al., Drug Discovery Today (2021), https://doi.org/10.1016/j.drudis.2021.04.008

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Please cite this article in press as: S. Reddy et al., Drug Discovery Today (2021), https://doi.org/10.1016/j.drudis.2021.04.008