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REVIEW

Strategies to target drugs to gliomas and CNS metastases of solid tumors

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Abstract The treatment for central nervous system metastases of solid tumors and gliomas is limited as the blood–brain barrier (BBB) is an obstacle to systemic therapy. Here, we review the physiochemical properties of the BBB and both current and new drug strategies to penetrate brain tumors. We focus on targeting receptor- or carrier-mediated transport mechanisms over the BBB used by drug conjugates, nanoparticles, polymer-based nanocarriers, siRNA, and antibodies.

Keywords Glioma · Brain metastases · Nanoparticles · Polymer-based nanocarriers · siRNA · Antibodies

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Introduction

The options for treatment of brain tumors, such as glioma and central nervous system (CNS) metastases of solid tumors, are limited. Unfortunately, drug development in this area brought only modest improvements in the last decades [1–3].

The treatment of newly diagnosed glioblastoma (GBM), the most malignant type of glioma, consists of surgical debulking, radiotherapy, and chemoradiation with temozolomide. The median overall survival of patients with a GBM is only 12–15 months [4]. For recurrent GBM, median overall survival decreases to 7 months. Objective response rates for second-line chemotherapy in GBM do not exceed 11 % and several new therapeutic agents appeared not to be effective [5–7].

Besides primary brain tumors, 20–40 % of patients with metastasized solid tumors (mostly lung cancer, breast cancer and melanoma) develop CNS metastases [8, 9]. Current treatment for brain metastases (BM) consists of surgical resection in case of a single BM, stereotactic radiotherapy for 1–3 BM, and whole brain radiotherapy for >3 BM and/or systemic chemo- or targeted therapy. Median survival of patients with BM from solid tumors ranges from 2.8 to 25.3 months depending on Karnofsky performance status, age, primary tumor type, presence of extracranial metastases, and number of BM [10].

Patients with leptomeningeal metastases (LM) of solid tumors have a median survival of only 4–6 weeks, if left untreated. Survival can be prolonged with several months by treatment of symptomatic sites with radiotherapy, sometimes followed by systemic treatment with chemotherapy or targeted therapy [11–14].

More effective treatment for both malignant gliomas and CNS metastases (brain and LM) from solid tumors are

urgently needed. Unfortunately, promising concepts from preclinical experiments often do not translate into effective treatment modalities in the clinic [15–17]. An important reason for this is the use of preclinical brain tumor models, which poorly recapitulate the complexity of human primary brain tumors and/or CNS metastases of solid tumors, including properties of the relevant blood–brain barrier (BBB), blood–CSF barrier (BCSFB), and blood–brain tumor barrier (BTB), causing inadequate exposure of tumor cells to systemically administered drugs.

Furthermore, phase I/II clinical studies often suffer from biased patient selection, as patients with a favorable prognosis are usually being included in these studies, leading to a longer progression-free survival (PFS), which is not being confirmed in phase III studies. Moreover, drugs need to be safely administered to the patient and be effective in a specific tumor type. This implies that in case of molecular therapy, the drug needs to be specifically targeted to the brain tumor's mutation/expression status or multiple targets simultaneously without causing (neuro-) toxicity.

In conclusion, the aim of drug development for brain tumors is to achieve sufficient concentrations of an effective drug in the brain with no or limited neurotoxicity. Within this difficult trajectory of brain tumor drug development, we focus in this review on current and new strategies to transport drugs for tumors in the brain and leptomeninges across the BBB and/or the BCSFB.

Methods

The heading search terms for this review in the PubMed/Medline and ClinicalTrials.gov databases were: BBB, glioma, GBM, BM, brain tumors, blood–brain or BCSFB, LM or carcinomatosis, targeted therapies, new strategies, nanocarriers, and siRNA. Articles published in English from 2000 till 2015 were included.

Blood–brain barrier (BBB) and drug characteristics requirements

The presence of the BBB is the reason why only few systemically administered drugs can reach the brain, despite the fact that the brain is one of the best-perfused organs [18].

In brain tumors, the BBB can be partly disrupted, although the extent of disruption is considered to be different in BM, high-grade and low-grade glioma. While BM and high-grade glioma show an intensive breakdown of BBB, associated with both disruption of endothelial cell tight junctions and astrocyte–endothelial cell relationships, low-grade glioma has a relatively intact BBB [19]. Furthermore, high-grade glioma shows a heterogeneous BBB disruption, with non-contrast-enhancing parts of the tumor on MRI having a largely intact BBB and contrast-enhancing parts showing an intensive BBB breakdown [20]. Although the BBB in brain tumors is partly disturbed, this is often not sufficient for effective brain tumor treatment [20, 21]. Therefore, successful treatment strategies need to cross an intact or partly disturbed BBB.

The BBB is formed by brain endothelial cells, which are closely connected by tight junctions and limit the paracellular entry of (hydrophilic) molecules (Fig. 1). Paucity of endocytosis and the presence of specific efflux pump proteins in the endothelial cells further contribute to the barrier function of the cerebral vessels. The endothelial cells are covered by a basement membrane, pericytes, and astrocytic endfeet [22, 23].

Small lipophilic molecules can penetrate the BBB in a transcellular way via passive lipid-mediated diffusion. Alkylating agents such as temozolomide (194 Da), nitrosoureas, e.g., CCNU (lomustine, 233 Da), and procarbazine (221 Da) are small lipophilic drugs that are currently being used in glioma treatment [24] (Table 1).

Larger lipophilic drugs or hydrophilic molecules of any size cannot passively cross the BBB. Thus, an alternative way should be found to open the BBB for these types of drugs (Table 2).

Chemical, biological, and physical ways to open/cross the BBB

The BBB can be opened by chemical, biological, or physical stimuli [25–31].

A transient *chemical* disruption of the BBB can be achieved by intra-arterial (IA) administration of mannitol, an osmotic diuretic, which can lead to endothelial cell shrinkage and reversible loosening of the tight junctions for 2–3 h [32, 33]. The use of an osmotic agent is estimated to increase drug delivery in the brain with a factor of 10–100 times. Following mannitol disruption of the BBB, IA infusion of bevacizumab, cetuximab, and temozolomide has been tested in GBM patients, but no randomized trials have been performed yet [34]. Currently, hyperosmotic BBB disruption is not part of clinical practice due to an increased risk of epilepsy and stroke, unselective passage of substances to the brain, and the necessity of generalized anesthesia and repeated hospitalizations [33].

Examples of *biological* stimuli that can increase BBB permeability are bradykinin agonists. These vaso-acting agents can open the BBB by downregulating claudin-5, a tight junction protein at the BBB via calcium-induced calcium release [31, 35]. Preclinical experiments in malignant glioma bearing rats showed that carboplatin was delivered more effectively to the glioma and its

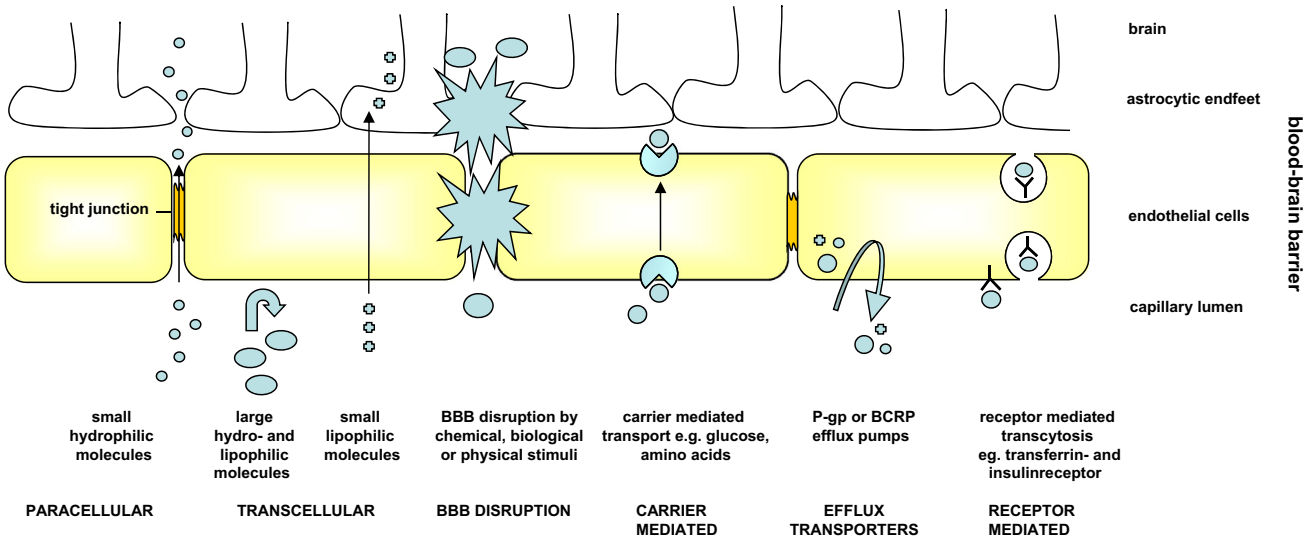

Fig. 1 Blood–brain barrier (BBB)

Table 1 BBB penetrating small liphohilic, cytotoxic drugs

Name	Indication	Route	Mechanism	Results	References
Temozolomide	Newly diagnosed GBM	Oral	Small lipophilic alkylating agent	RT with TMZ during 6 weeks + 6 adjuvant TMZ cycles: newly diagnosed GBM: 2-years OS 26 versus 10 % in radiotherapy only	Stupp et al. [4]
Nitrosourea (CCNU; lomustin) and procarbazin in PCV chemotherapy	Anaplastic oligodendroglioma with 1p19q loss	Oral	Small lipophilic alkylatic agent	Significant increase OS and PFS in combined PCV and RT versus RT EORTC 26951: median OS not reached versus 113 months RTOG 9402: median OS 14.7 versus 7.3 years	van den Bent et al. [112], Cairncross et al. [113]
Nitrosourea (CCNU; lomustin) and procarbazin in PCV chemotherapy	Recurrent GBM	Oral	Small lipophilic alkylating agent	Median PFS: 17.1 weeks 6-months PFS: 38.4 %	Schmidt [114]
Nitrosurea (CCNU; lomustin) and procarbazin in PCV chemotherapy	Low-grade glioma	Oral	Small lipophilic alkylating agent	RTOG 9802: RT followed by PCV versus RT: median PFS 10.4 versus 4.0 years; median OS 13.3 versus 7.8 years	Shaw et al. [115], Buckner et al. [116]

GBM glioblastoma, *RT* radiotherapy, *TMZ* temozolomide, *PFS* progression free survival, *OS* overall survival, *PCV* procarbazin, CCNU and vincristin [4, 112–116]

surrounding tissue after co-administration with two potent bradykinin agonists (B1R and B2R) compared to single-agent carboplatin. Moreover, co-administration with these agents was associated with increased survival [28]. In a randomized, double-blind, placebo-controlled phase II study in recurrent malignant glioma, treatment with RMP-7, a bradykinin analog in combination with carboplatin IV did not improve efficacy of carboplatin [36]. Other biological stimuli that can disrupt the BBB are chemo- and cytokines, such as interleukin-1β, tumor necrosis factor-α, and interferon-γ [27]. Bacteria and bacterial toxins (*Escherichia coli*, *Citrobacter freundii*, *Streptococcus pneumoniae*, cholera toxin, pertussin toxin), viruses and virus components (HIV-1, Measles virus), parasites, and fungal pathogens cannot only penetrate but also damage the BBB [27].

Representatives of *physical* stimuli that can open BBB are ultrasound, laser, and electric fields [37–39].

Focused ultrasound (FUS) is a noninvasive technique of low-frequency ultrasound waves that can be delivered transcranially. FUS can thermally ablate (tumor) tissue [37]. Addition of microbubbles to FUS can further enhance heating in the area of interest. At low exposure it can also be used for local and reversible BBB disruption [44, 45].

Table 2 Different transport mechanisms across the BBB

Transport mechanism across the BBB	Drug examples
Passive lipid-mediated diffusion	Small lipophilic molecules
Chemical, biological, or physical stimuli opening the BBB for drugs	Mannitol, bradykinin agonists, chemokines and cytokines, bacterial, viral components, ultrasound and electromagnetic fields
Facilitated diffusion using CMT	Levodopa, donezepil, gabapentin, baclofen
RMT—molecular Trojan horse approach	Larger (liposomal) molecules (peptides and proteins) using receptors such as LDL-receptor, glutathione receptor, insulin receptor; transferrin receptor, insulin-like growth factor receptor, or diphtheria toxin receptor
AMT mechanism	Cationic molecules
Co-administration of therapeutic agents with dual P-gp and BCRP inhibitor	Elacridar

Disruption of the BBB occurs by transient opening of the endothelial cell tight junctions by (microbubbles-) FUS-induced mechanical forces [40]. In rat glioma models, survival was increased by combining chemotherapeutic agents and FUS [41, 42]. Clinical studies on transcranial MR-guided FUS surgery in GBM patients are ongoing [43].

Laser interstitial thermotherapy (LITT) is a novel technique to ablate a tumor by laser-generated heat using an optical fiber. In a prospective trial on the use of LITT in recurrent GBM, there was a trend toward improved survival in patients treated with higher doses [39]. Thermotherapy can induce cell membrane destruction in endothelial cells, leading to BBB disruption and an area of contrast enhancement on MRI around the thermal ablation zone [44, 45]. No studies have been performed on the change of chemotherapeutic delivery during the time interval of BBB opening following LITT.

Finally, there has been a recent focus on the use of *non-thermal irreversible electroporation* (NTIRE) to cause BBB disruption. NTIRE uses a pulsed electric current to cause defects in the cell membrane leading to membrane rupture and increased BBB permeability [46]. In the brain, NTIRE-applied voltage correlates with the volume of tissue damage and BBB disruption, which can last several days post-treatment [47, 48]. NTIRE has not been used in glioma patients yet, nor has it been combined with chemotherapy.

Transcellular transport mechanisms over the BBB

Nutrients and some drugs/toxins are using active, energy-dependent, transcellular transport mechanisms over an intact BBB, in contrast to passive diffusion of molecules (Fig. 1). Most nutrients utilize facilitated diffusion using carrier-mediated transport (CMT). Glucose can cross the BBB using CMT via a glucose carrier (GLUT1). Enhanced brain penetration of drugs employing CMT requires a close structural analogy to endogenous carrier substrates [49, 50]. Brain-targeted drugs that are currently in (clinical) use and utilize CMT are levodopa for Parkinson disease, donepezil and tacrine for Alzheimer disease and gabapentin, pregabalin, valproate for epilepsy, and baclofen for multiple sclerosis [51].

The BBB transport of larger molecules, such as peptides and proteins can occur by using receptor-mediated transcytosis (RMT). Examples of RMT are transport of insulin and transferrin via insulin- or transferrin receptors [51, 52]. Once the protein is bound to its specific receptor on the BBB, the internalization of the protein into a vesicle starts. The vesicle crosses the endothelial cell and fuses with the membrane on the brain-parenchymal side, after which the protein can be released in the brain, a process called transcytosis [51]. Most likely, RMT is used by liposomes and other nanotechnology-based systems to cross the BBB. Using RMT, a toxic anti-cancer drug packed inside a liposome, being coupled to a molecule that is recognized by a receptor present on the BBB, can be shuttled across the BBB. This is called the molecular Trojan horse method [53].

Drugs currently used in drug development programs that can cross the BBB via RMT employ the following receptors: glutathione receptor, low-density lipoproteins (LDL), insulin receptor, transferrin receptor, insulin-like growth factor receptor, or diphtheria toxin receptor [18, 54–57].

Another mechanism used in drug development to target the brain is the adsorptive-mediated transport (AMT) mechanism. Molecules that originally cannot cross BBB-proteins, can be cationizated during a chemical process in which the superficial carboxyl groups on a protein are converted into extended primary amino groups. These cationized macromolecules increase the interaction with normally present anionic sites at the luminal plasma membrane of the brain endothelium. Next, the formed complexes cross the BBB via vesicle formation and enter the brain. Examples of AMT over the BBB are the uptake of cell penetrating peptides, chemically conjugated siRNA, paclitaxel, or several antibodies [58].

AMT is not as specific as RMT and CMT as cationic molecules can have a high adsorptive potential toward anionic molecules not only on the BBB but also on cell surfaces in other tissues in the body. Therefore, it can be difficult for a drug to specifically target he brain by AMT, in particular when the protein is administered intravenously [58]. Furthermore, AMT has a risk of BBB disruption and consequently neurotoxicity [58]. Another limitation of the AMT strategy is the risk of complement activation, possibly caused by the conjugation of drugs with cationized molecules, which often originate from non-human proteins, increasing the risk of immunogenicity. Therefore human proteins, recombinant humanized proteins or conjugates of cationic proteins to polyethylene glycol (PEG) are currently being used [51, 58].

Blood–CSF barrier (BCSFB)

Another important barrier of the CNS, especially for systemic treatment of LM, is the BCSFB [59, 60] (Fig. 2). The BCSFB separates the CSF from the blood in the choroid plexus and the leptomeningeal blood vessels. Transport of solutes from blood (Na^+, Cl^-, HCO_3^-) into the CSF, necessary for CNS homeostasis, occurs in the fenestrated blood capillaries of the choroid plexus of the ventricles. In blood capillaries of the leptomeninges (arachnoidea and to a lesser extent the pia mater), the BCSFB is being formed by epithelial cells that are connected by tight junctions [61–63]. The main function of the CSF is to remove brain metabolites and toxins from the brain, as the brain itself lacks lymphatic vessels [64, 65].

Similar transport mechanisms exist on the BCSFB as on the BBB (Fig. 2). However, there are a few differences: glutamate- and some Na^+-dependent transporters are only being expressed at the BCSFB and bicarbonate transporters have an increased expression on the BCSFB, as compared to the BBB. In contrast, heme-, glucose efflux- and several amino-acid transporters have a lower expression on the BCSFB as compared to the BBB [66]. (www.biopar adigms.org).

There is no barrier between the CSF and the brain interstitial brain fluid (ISF) allowing a dynamic exchange of nutrients and water between these two compartments [64]. However, the depth of diffusion of nutrients and drugs into the brain parenchyma is limited. In case of insulin-like growth factor (IGF-1) the penetration from the ventricular CSF was less than 1.25 mm into brain tissue [67].

Drug efflux transporters on the blood–brain and blood–CSF barrier

Besides the fact that the physical and chemical properties (size, lipophilicity, charge) determine the drugs' BBB permeability, entrance of drugs into the brain is also limited by drug efflux transporters [68]. ATP-binding cassette (ABC) drug efflux transporters, such as P-glycoprotein (P-gp; ABCB1) and breast cancer resistance protein (BCRP; ABCG2) are expressed at the luminal side of brain endothelial cells, thus limiting the penetration of their substrates across the BBB [69–71]. Furthermore, blood vessels in mouse and human arachnoid tissue express drug efflux transporters. For example, expression of P-gp at the luminal side of the BCSFB reduces entrance of neurotoxins into the CSF [63]. In contrast, at the BCSFB of the choroid plexus, P-gp and BCRP are located on the apical membrane of the choroid plexus epithelium, thus directing their substrates toward the CSF [72–74].

Inhibition of drug efflux transporters at the BBB can be used to increase drug accumulation in the brain, but only potent inhibitors can cause a meaningful enhancement of drug transport [75, 76]. Moreover, many drugs are substrates for both P-gp and BCRP [76, 77]. Consequently, brain penetration of these drugs can only be enhanced using a dual P-gp or BCRP inhibitor such as elacridar [76, 78].

Damaged barriers in brain tumors as a potential entrance for systemic drugs

The presence of tumor cells in the brain can cause a disruption of the BBB [27]. The damage to the BBB can be demonstrated by extravasation of gadolinium (gdDTPA, 550 Da) in the brain on gdDTPA-enhanced MRI T1 scans [79]. The extent of the disruption of the BBB in high-grade glioma is not uniform. As demonstrated by gdDTPA-enhanced T1 MRI, contrast enhancement is mainly observed in the tumor area where vascular proliferation is evident, the so-called leading/growing edge of the tumor [80, 81]. However, in the infiltrative areas of the tumor visualized on T2 or fluid-attenuated inversion recovery (FLAIR) MRI, contrast enhancement is much less prominent, indicating a more intact BBB function or leakage that could not be assessed by gdDTPA-enhanced T1-MRI. In case of low-grade glioma, contrast enhancement is absent or very discrete, angiogenesis is not intensively present and permeability of BBB is low [82]. Nduom et al. [19] showed that low-grade and non-enhancing regions of high-grade gliomas maintain the normal astrocyte–endothelial cell relationship, such as in an intact BBB.

Furthermore, in case of BM, the tumor type may determine the extent of BBB disruption. For example, patients' tumor samples of BM from triple negative and basal-type breast cancers showed different BBB leakage patterns as compared to HER2-positive breast cancer [83].

Furthermore, it is known that macroscopic BM (>2–3 mm) develop new tumor vessels that resemble the

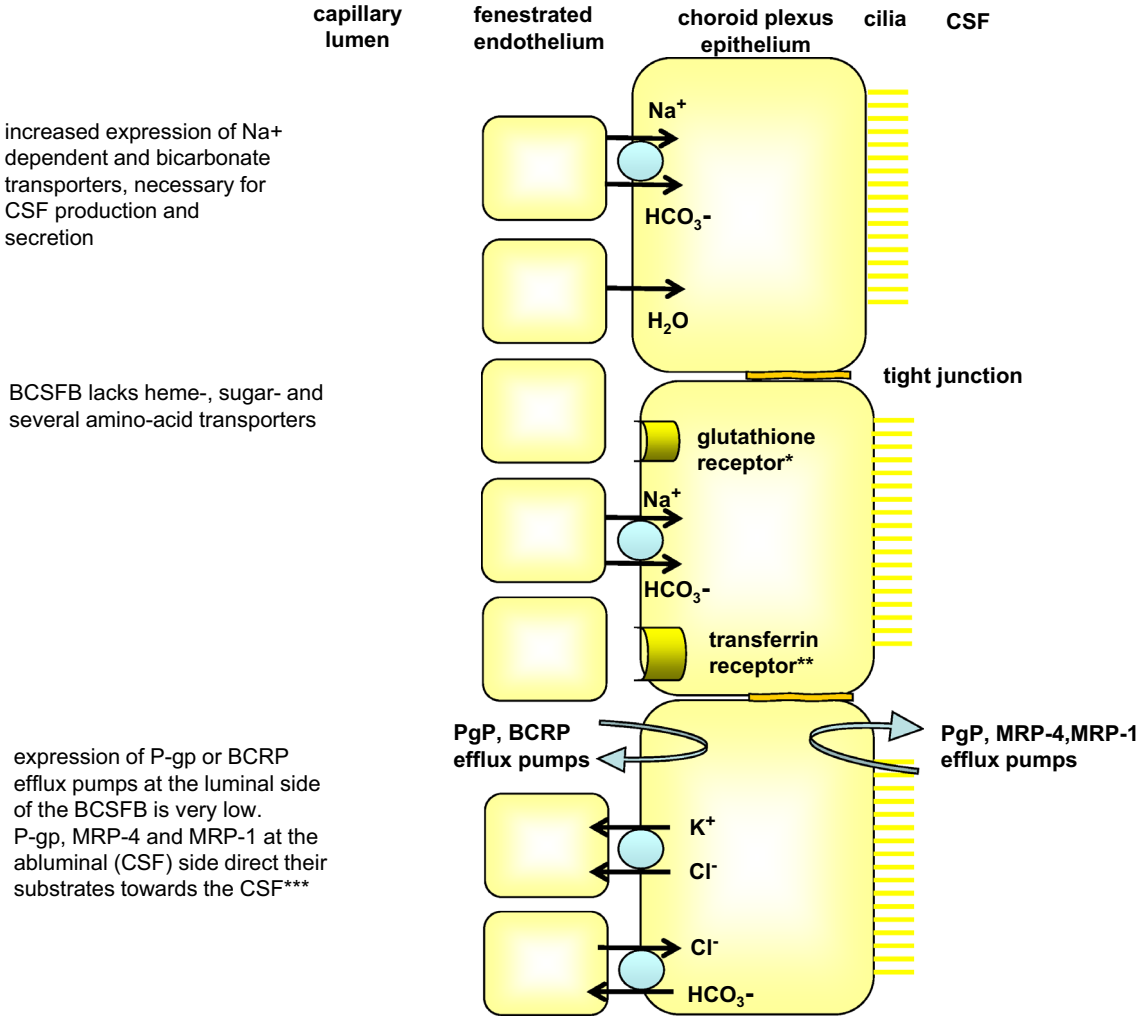

Fig. 2 BCSFB in the choroid plexus restricts and regulates para- and transcellular transport. *P-gp* P-glycoprotein, *BCRP* breast cancer resistance protein, *MRP* multidrug resistance protein. Glutathione receptor*—Otieno et al. [117], transferrin receptor**—Takeda et al. [118], efflux pump expression at abluminal side***—Roberts et al. [119]. Per author's permission, the figure was adapted from Redzic et al. [120]

vessels of the primary tumor and lack BBB characteristics. Lockman et al. [20] analyzed 2000 experimental BM in mice and demonstrated that blood–tumor barrier (BTB) permeability was increased in over 89 % of BM. However, brain uptake of 14C-paclitaxel and 14C-doxorubicin, although generally greater than in normal brain, only reached cytotoxic concentrations in about 10 % of BM [20]. This study concludes that the BTB remains a significant impediment to drug delivery and stresses the need in developing new permeable drugs for brain tumors.

Strategies to cross the BBB and target brain tumors

Several strategies have been developed to increase drug brain concentrations.

Brain-targeting receptor- or carrier-mediated transport strategies (Tables 3, 4)

Drug conjugates ANG1005 (or GRN1005) is a paclitaxel-Angiopep-2 conjugate that is shuttled over the BBB by RMT via the low-density lipoprotein receptor-related protein-1 (LRP-1). LRP-1 mediates transcytosis of physiological ligands across the BBB, such as thyroglobulin, lactoferrin, tissue-type plasminogen activator, and α-2 macroglobulin and is highly expressed on the BBB [84]. In mice studies, GRN1005 showed a broad distribution throughout the brain parenchyma while avoiding P-gp efflux transporters, in contrast with conventional paclitaxel. Furthermore, GRN1005 demonstrated anti-tumor activity in mice with GBM or BM of lung cancer [85, 86].

Table 3 Agents that can cross the BBB via carrier-mediated or receptor-mediated transport in (pre) clinical studies

Name	Current phase	Route	Mechanism	Results	References
2B3-101	Phase I/II	iv	Doxorubicin in glutathione PEGylated liposomes binding glutathione receptors on BBB	GBM in mice: fivefold higher doxorubicin brain concentration compared to Caelyx®; improved anti-tumor activity and survival in mice phase I/II studies: moderate safety profile, preliminary anti-tumor activity in patients with recurrent GBM and BM from solid tumors	Gaillard et al. [97], Milojkovic-Kerklaan et al.[98], Brandsma et al. [121]
GRN1005	Phase I/II	iv	Paclitaxel-Angiopep-2 conjugate via low-density lipoprotein receptor-related protein-1 (LRP-1) transcytosis	Intracranial responses in recurrent malignant glioma and BM from solid tumors Phase II studies ongoing	Demeule et al. [71], Regina et al. [122]
Tf-PO-DOX	In vivo	iv	Biodegradable polymersomes (PO) loaded with doxorubicin, transport via transferrin RMT	Brain tumors in rats: 70 % longer median OS compared to standard doxorubicin	Pang et al. [123]
Paclitaxel poliglumex	Phase I/II	iv	Paclitaxel conjugated with poly-L-glutamic acid, crossing BBB presumed by endocytosis due to increase in vascular leakiness by EPR effect	Phase I study in newly diagnosed HGG patients in combination with TMZ and RT: median PFS 13.5 months, OS > 22 months	Jeyapalan et al. [124]
K16ApoE	In vivo	iv	Synthetic peptide carrier of non-covalently binded cisplatin and methotrexate via transient BBB permeability between cells that express low-density lipoprotein receptor (LDL-R)	K16ApoE: 34–50-fold and 54–92-fold higher brain uptake than cisplatin and methotrexate single agent, respectively	Sarkar et al. [125]
CPP-Dox/ NGR-TSL	In vivo	iv	Thermosensitive liposome containing cell penetrating peptide as the targeting moiety-doxorubicin conjugate	Specific targeting in tumor cell lines, tumor growth inhibition in nude mice	Yang et al. [126]
GPNMB conjugated Pac-MNPs	In vivo	iv	Paclitaxel loaded magnetic nanoparticles (Pac-MNPs) manipulated by magnetic field cross BBB targeting receptor, glycoprotein non-metastatic melanoma protein B (GPNMB) overexpressed by glioblastoma cells	Prolonged blood circulation in vivo, significant accumulation of drug in rat brain tissues as compared to native paclitaxel	Dilnawaz et al. [127]
CRM197	In vivo	iv	Non-toxin mutant of diphtheria toxin (DT) receptor-specific carrier protein as carrier protein for therapeutic agents	Increased BBB permeability, pinocytotic vesicles number and redistribution of tight junction-associated proteins in brain microvessels	Wang et al. [128]

BBB blood–brain barrier, *RD* recommended dose, *HGG* high-grade glioma, *PR* partial response, *SD* stable disease, *BM* brain metastases, *GBM* glioblastoma, *EPR* enhanced permeability, and retention effect [1, 97, 98, 121–128]

In a phase I trial, GRN1005 treatment showed one complete response (CR) and two partial responses (PR) in recurrent malignant glioma patients ($n = 63$) and 5 intracranial PR in patients with BM ($n = 41$) [87, 88].

In a sub-study of nine patients with recurrent malignant glioma, GRN1005 (>200 mg/m [2] iv) was administered 3.5–6 h before intracranial resection. In resected glioma tissue, cytotoxic levels of paclitaxel (>0.3 μmol/L) were found in all nine patients. However, in a subsequent phase II study, the GRN1005 dose needed to be reduced from 650 mg/m [2] to 550 mg/m [2] because of hematological toxicity [89]. At this lower dose-level no intracranial responses in thirty enrolled patients with breast cancer and BM were seen and the study was stopped. Currently, the biotech company, Geron Corporation (California, USA) has stepped down as partner in development of GRN1005, but three clinical trials with GRN1005 treatment are ongoing: one in recurrent high-grade glioma patients with 650 mg/m [2] every 3 weeks (NCT01967810) and two in BM from breast cancer with 550 mg/m [2] every 3 weeks (NCT02048059 and NCT01480583)) all being sponsored by Angiochem Inc, Montreal, Canada.

Nanoparticles Nanoparticles are small molecules with a size of 1–100 nm and are being used as a carrier for an active pharmaceutical ingredient (API) to be eventually released at the target organ [90]. Nanoparticles can be applied as oral, topical, inhaled, or parenteral formulation

Table 4 Nanocarrier siRNA therapy

Name	Current phase	Mechanism	Results/conclusion	References
Dendrimer-conjugated magnetofluorescent nanoparticle (nanoworm)	In vivo	Internalization of the siRNA against EGFR of glioma cells	70–80 % reduction of EGFR protein levels in intracranial glioma cells	Agrawal et al. [107]
Rabies virus glycoprotein (RVG)—tagged amphiphilic cyclodextrins (CD) for siRNA delivery	In vitro	PEGylated CD-based nanoparticle tagged with a CNS-targeting peptide derived from the RVG	Potential nanocomplex for systemic delivery of siRNA targeting brain tumors	Gooding et al. [129]
Nanoparticle-based siRNA delivery vehicle for knocking down Ape-1 expression and sensitizing pediatric brain tumor cells to radiotherapy	In vitro	Nanoparticle comprising a superparamagnetic iron oxide core coated with a biocompatible, biodegradable coating of chitosan, PEG, and polyethyleneimine (PEI), able to bind and protect siRNA from degradation and deliver siRNA to the perinuclear region of target cells Carries purinic endonuclease 1 (Ape1), an enzyme in the base excision repair pathway, implicated in radiation resistance of tumor cells	Reduction of Ape-1 expression in 75 % in pediatric brain tumors (meduloblastoma and ependymoma)	Kievit et al. [130]

iv intravenous, *siRNA* short interfering RNA [107, 129, 130]

and are being divided into two groups: nanovectors, including liposomes and nanoparticulate drug carriers and polymer-based nanocarriers, including dendrimers and polymeric micelles [51]. In case of nanoparticles, RMT is facilitating drug delivery across the BBB. There are 250 nanoparticle products in various stages of clinical trials and just few of them are used for brain targeting [91]. In neuro-oncology, liposomes are the nanoparticle formulations that are in the most advanced stage of development. Liposomes are spherical phospholipid bilayer vesicles consisting of (semi)natural, biodegradable lipids with a hydrophilic inner space that contains the API. Liposomes can be protected by nonionic hydrophilic polymers, for example PEG, with the aim to avoid recognition and clearance by the mononuclear phagocyte system [90, 92]. These so-called stealthed liposomes can show long circulation times in the blood, with the drug being trapped inside the liposome and only upon release, the drug will be available at the target organ. It is being hypothesized that once liposomes reach the tumor area, they can extravasate via the leaky tumor vasculature and accumulate in the tumor by a mechanism called enhanced permeability and retention (EPR) effect [93].

Thus far, only a few liposomal drugs have been approved for CNS indications. One is liposomal amphotericin B (iv administration) for cryptococcal meningitis and another is liposomal cytarabine (Depocyte®; intrathecal administration) for LM. Liposomal amphotericin B showed significantly higher brain tissue concentrations, longer terminal half-time ($t_{1/2}$) and decreased fungal burden in the brain as compared to non-liposomal amphotericin formulations [94].

Doxil/Caelyx® (PEGylated liposomal doxorubicin) is the oldest example of a liposomal encapsulated drug that is used in oncology and is registered for metastatic breast cancer, advanced ovarian cancer, and AIDS-related Kaposi's sarcoma since 1996. Overall it has similar anti-tumor efficacy as free doxorubicin, but displays reduced cardiotoxicity [95].

Recently, 2B3-101 was developed in order to more specifically use liposomes for drug delivery to brain tumors. Like Doxil/Caelyx®, 2B3-101 is a glutathione PEGylated liposomal formulation of doxorubicin. However, in this formulation, glutathione (GSH) is attached to the PEG chains on the surface of the liposome, which targets the liposome to the active GSH transporters on the BBB (Table 3) [54]. In mice, a fivefold enhanced delivery of doxorubicin to the brain was seen after intravenous treatment with 2B3-101 as compared to Doxil/Caelyx® [96]. Furthermore, survival of mice with GBM improved after intravenous treatment with 2B3-101 as compared to Doxil/Caelyx® [97].

A phase I clinical study on 2B3-101 in 37 patients with recurrent high-grade glioma and patients with BM from solid tumors showed a moderate safety profile (i.e., hematological and mucocutaneous toxicity and infusion reactions) with preliminary anti-tumor activity.

In eight of 13 patients with recurrent malignant glioma, stable disease was seen as best response during 6–18 months. In patients with solid tumors and BM two intracranial and two extracranial partial responses were seen [98].

Other examples of liposomal brain-targeting drugs that are currently being studied (pre-) clinically are shown in Table 3.

Polymer-based nanocarriers A carrier that consists of single structure unit with multiple repetitions is called a polymer-based nanocarrier. They can be divided into dendrimers and micelles. Dendrimers are repetitively branched molecules, usually highly symmetric and spherical that can both attach drugs and other molecules. Micelles are spherical aggregates with a hydrophilic "head" region that is in contact with a surrounding solvent with a possible insoluble drug in its core [51, 99]. A fourth generation of PEGylated doxorubicin dendrimer carrier (G4-DOX-PEG-Tf-TAM) is designed for dual targeting of the BBB using transferrin (Tf) and tamoxifen (TAM). The conjugation with transferrin facilitates the transport of doxorubicin across the BBB and glioma cells, while the coupling with tamoxifen inhibits drug efflux transporters, such as P-gp, BCRP, and MDR4 [99].

In in vitro experiments, accumulation of doxorubicin was seen in C6 glioma cells only and not in in vitro murine BMVEC (brain microvascular endothelial cells) after incubation with G4-DOX-PEG-Tf-TAM. Besides coupling to liposomes, GSH has also been used to target brain delivery of doxorubicin or docetaxel-loaded polyethyleneglycol-poly lactic-*co*-glycolic acid (PEG-PLGA) nanoparticles [100, 101].

The polymeric micelle Pluronic P105, that uses dual targeting of glucose via CMT and folic acid receptors via RMT, is another brain-targeting polymer-based nanocarrier that delivers doxorubicin across the BBB. Internalization of doxorubicin into C6 glioma cells was seen in an in vitro BBB model after incubation with Pluronic P105 [102]. Mice could be treated safely with intravenous Pluronic P105 and showed significant intracranial C6 glioma cell growth suppression [102]. However, it is unclear how two different mechanisms CMT (using glucose) and RMT (using folic acid receptors) can transfer one drug i.e., doxorubicin across the BBB.

Nanocarrier siRNA delivery strategies RNA interference (RNAi) is a natural process of controlling the expression of genes. This post-transcriptional gene expression silencing can be triggered by synthetic short interfering RNA (siRNA) [103]. Because of its instability, large size, and its negative charge, siRNA needs to be delivered to a target organ by liposomes [104] (Table 4). The well-known *O*-6-methylguanine-DNA methyltransferase MGMT gene in gliomas is responsible for repair of DNA lesions that are either spontaneously present gliomas or being drug-induced (e.g., by temozolomide). In 45 % of patients with GBM, MGMT status is methylated (inactive) which is associated with a better prognosis and better response to temozolomide therapy [105]. The response to temozolomide in glioma patients with non-methylated MGMT may be improved when MGMT could be silenced or suppressed by sIRNA. A preclinical study with siRNA silencing MGMT in a locally applied cationic liposomal formulation was however disappointing, as insufficient distribution of cationic liposomes in rat and porcine brain tissue was achieved [106]. Better in vivo results in an epidermal growth factor (EGFR)-driven mice model of GBM were achieved after treatment with siRNA against the EGFR using a different nanocarrier, viz the dendrimer-conjugated magnetofluorescent nanoparticle (nanoworm). Using nanoworm internalization of the siRNA against EGFR in intracranial glioma cells in mice was shown with a 70–80 % reduction of EGFR protein levels [107].

Finally, a strategy with promising in vivo results is achieved in mice by inhibiting tumor growth in a synergistic manner by using docetaxel and LDL-1 receptor targeting liposomal formulation of siRNA that silences the vascular endothelial growth factor receptor (VEGFR) (Angiopep 2) [108]. Two other sIRNA strategies for brain targeting tested in vitro are presented in Table 4.

Strategies to deliver antibodies therapy across the BBB

Antibodies cannot cross an intact BBB or BCSFB because of their size, but can have (limited) activity on intracranial tumors when the BBB and/or BCSFB are partly disrupted.

The monoclonal antibody trastuzumab targeting human epidermal growth receptor-2 (HER2), overexpressed in HER2+ breast cancer inhibits tumor cell growth via receptor inhibition. Trastuzumab can affect BM of breast cancer because of a partly disrupted BBB in BM [109]. However, transport of antibodies over (an only partly disrupted) BBB could be improved by forming drug conjugates and using RMT for BBB transport. Recently, a new class of nanocarriers loaded with MRI tracer and combined with antibodies targeting HER-2 and EGFR receptors on the BM was developed [110]. They cross the BBB via RMT via the transferrin receptor. Another example of an antibody-drug conjugate currently tested in in vivo experiments a conjugate of Angiopep-2 (An2) and anti-HER2 monoclonal antibody, named ANG4043 [111]. ANG4043 binds to LRP1 receptor and crosses BBB also via RMT.

Conclusion

For future effective therapies for brain tumors, sustained drug brain concentrations of potentially active drugs (chemotherapy, targeted agents or siRNA) are being needed. This review gives a concise overview of the

therapeutic strategies to transfer drugs over the BBB and/or BCSFB. The majority of drugs are currently tested in preclinical studies, while several already show promising clinical (phase I/II) results. Further research on drug strategies to efficiently cross the BBB is warranted, as effective treatment strategies are needed in the treatment of brain tumors.

Compliance with ethical standards

Conflicts of interest All authors do not have any conflicts of interest to disclose.

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