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Which drug or drug delivery system can change clinical practice for brain tumor therapy?

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The prognosis and treatment outcome for primary brain tumors have remained unchanged despite advances in anticancer drug discovery and development. In clinical trials, the majority of promising experimental agents for brain tumors have had limited impact on survival or time to recurrence. These disappointing results are partially explained by the inadequacy of effective drug delivery to the CNS. The impediments posed by the various specialized physiological barriers and active efflux mechanisms lead to drug failure because of inability to reach the desired target at a sufficient concentration. This perspective reviews the leading strategies that aim to improve drug delivery to brain tumors and their likelihood to change clinical practice.

The English literature was searched for defined search items.

Strategies that use systemic delivery and those that use local delivery are critically reviewed. In addition, challenges posed for drug delivery by combined treatment with anti-angiogenic therapy are outlined.

To impact clinical practice and to achieve more than just a limited local control, new drugs and delivery systems must adhere to basic clinical expectations. These include, in addition to an antitumor effect, a verified favorable adverse effects profile, easy introduction into clinical practice, feasibility of repeated or continuous administration, and compatibility of the drug or delivery system with any tumor size and brain location.

Keywords: blood-brain barrier, brain drug delivery, brain tumor, brain tumor therapy, clinical practice, CNS, drug development, drug efflux mechanism.

Despite advances in anticancer drug discovery and development, there has been little improvement in the prognosis and outcome of malignant brain tumors. Instead, promising experimental agents for brain tumors have repeatedly demonstrated little impact on disease progression in clinical trials. These disappointing results can be partially explained by the inability to deliver therapeutic agents to the CNS across the blood-brain barrier (BBB) and failure of drugs to reach the desired target in adequate concentration.[1] This review briefly summarizes the leading strategies that intend to improve drug delivery to primary brain tumors, in view of their likelihood to change clinical practice. The evaluation and judgments expressed in this review are based on perspective obtained from clinical experience. This material does not pertain to viral- and DNA-based therapy or to immunotherapy, although some extrapolations may be relevant, particularly for the first 2 therapies discussed. Whenever pertinent, the applicability of a specific method for treatment of brain metastases is delineated, although the review does not provide a review of therapies for metastatic brain tumors.

A comprehensive literature search was performed to identify relevant studies published from 1980 through December 2012, using Medline and Google Scholar. Search terms included anti-angiogenesis, blood-brain barrier disruption, brain drug delivery, brain parenchyma, brain tumor, brain local drug delivery, convection enhanced delivery, CNS, chemotherapy delivery, CNS delivery, cerebrospinal fluid (CSF) delivery, drug bioavailability, drug carrier, drug concentration, drug development, drug efflux, drug retention, extracellular drug, focused ultrasound, infusate, intra-arterial chemotherapy, intratumoral chemotherapy, liposome, nanomedicine, nanoparticle, nanotechnology, prodrug, targeted drug delivery, and transcranial brain drug delivery.

Malignant Brain Tumors and Physiological Blood-Brain Barrier Function

Systemic chemotherapy for the treatment of primary brain tumors consists of traditional drugs, most of which are DNA-alkylating agents that intervene in the cell cycle. Recently, newer drugs that target cell surface receptors and associated pathways are being intensively

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investigated in clinical trials.[2–4] The ineffectiveness of drug therapy in the management of malignant brain tumors has frequently been attributed to physiological barriers, namely the BBB, blood-CSF barrier, and blood-tumor barrier.[1] The BBB is a significant impediment to transvascular extravasation of drugs into the extracellular compartment of brain tissue, although the barrier is often porous in malignant brain tumors, as demonstrated by the extravasation of contrast agents that produce contrast enhancement of tumors during brain imaging.[5] Because contrast agents leak into the extracellular compartment of tumor tissue, it has been argued that the BBB might not be a limiting factor for penetration of small molecular-weight drugs.[6,7] However, poor drug delivery to brain tumor is an outcome with multiple contributing factors, including low plasma concentration of drugs at the tumor site, irregular vasculature of the tumor, increased interstitial pressure, intratumoral hypoxia, and active efflux transport mechanisms at the BBB interface.[8–11] When planning drug therapy for primary malignant brain tumors, it is essential to recognize that these lesions often reside behind an intact BBB.[12] Magnetic resonance imaging (MRI) often suggests that tumor infiltrates far beyond the enhancing area; in fact, it has been demonstrated that glioblastomas contain heterogeneous cell populations with similar proliferation rates but with growth patterns that are characterized as either angiogenic or nonangiogenic.[13] Recent experience with anti-angiogenic treatment proved that glioblastomas can continue to grow and even increase cell invasion without the need for a second wave of angiogenesis.[14,15] These findings indicate that primary brain tumors proliferate behind an intact BBB function, and this barricade has to be crossed by any drug aimed to affect tumor growth.

Drug Therapy Requirements and Practical Issues

Multiple factors interact to determine a drug's ability to induce an antineoplastic effect. During the first steps of drug development and evaluation, proof must be brought from in vitro and subcutaneous xenograft experiments that the agent effectively kills tumor cells or enhances their killing by other modalities (eg, radiation therapy). In subsequent development stages, in vivo and human studies are involved and other aspects have to be considered. These include the basic requirement that the drug should reach every brain tumor cell. To achieve this aim, the drug must first reach the desired target (the brain tumor) in adequate concentration and then maintain this concentration in the tumor's extracellular space for an adequate period to be effective.[16] These prerequisites are major obstacles that directly depend on the method of drug delivery, which should be carefully contemplated. In principle, the choice is between systemic delivery and local administration. Systemic delivery should overcome the impediment posed by the BBB. It relies on the existing vascular bed to serve as the delivery vehicle that will bring the drug to the tumor, but the drug has to cross the vascular wall to the abluminal side. On the other hand, local delivery circumvents this impediment but has limited ability to reach distant infiltrating tumor cells. Almost all recent drug developments in neuro-oncology either try to improve the technique of drug delivery or fail on this account.[17–19] Apart from the delivery method, other relevant features for drug therapy include drug washout from the extracellular space and the sink effect of CSF, which become major issues when local delivery is selected. Additional factors are drug uptake, or drug efflux, by tumor cells and cellular targets and the metabolic fate of the drug in tumor cells–features that are usually investigated in preclinical models but are largely unaddressed in human studies.

A drug that demonstrates sufficient potential to finally reach the level of human studies can be evaluated for its prospects to change clinical practice on the basis of a set of 5 requirements reflecting clinical expectations from the therapy. Expectations from drug therapy cannot be separated from issues related to the strategy used for delivery to the brain. The 5 elements include (1) effectiveness; (2) favorable adverse effects profile, including systemic and neurotoxicity; (3) easy introduction (assimilation) into clinical practice; (4) repeated or continuous administration should be feasible (no agent is expected to cure a primary brain tumor by a single exposure); and (5) the agent and delivery strategy should be useful for any tumor size and CNS location if the aim is to change clinical practice and achieve more than limited local control.

In the past 3 decades, only one agent, temozolomide (TMZ), changed clinical practice for glioblastoma. If we evaluate this agent by these 5 expectations, it becomes clear that TMZ fulfills all of them. TMZ proved to significantly prolong both progression-free survival and overall survival among patients with newly diagnosed glioblastomas.[20] The treatment is well tolerated, with a favorable adverse effect profile. The regimen was easily assembled into clinical practice and became the standard therapy practiced in every clinic around the world. Repeated and continuous administration is feasible and constitutes part of the regimen, and finally, it is probably useful for any tumor size at any CNS location.

Systemic Drug Delivery to Brain Tumors and Methods to Improve Drug Transport

After systemic drug delivery is used to treat brain tumors, an optimal effect depends on the ability to maintain drug concentration at the target site for sufficient duration while avoiding systemic toxicity. These basic elements are hardly ever achieved by standard chemotherapeutic agents. For example, most small molecular weight drugs, such as nitrosoureas, are rapidly eliminated by hepatic metabolism and renal excretion, and because of their short blood half-life, only a limited fraction of the drug reaches the brain tumor.[18,19,21] In addition, the portion of the drug that fails to reach the target

can be toxic to off-target organs and induce systemic toxicity. The fraction of the drug that reaches the tumor or the plasma concentration at the target area is an elementary factor that determines the prospects to achieve adequate extracellular drug concentration at the tumor site. However, even after adequate plasma drug concentrations are achieved, drugs have to cross physiological barriers to get to the extracellular space of the tumor. Basically, there are 2 major types of drug transport mechanisms across the BBB: the passive diffusion mechanism, which is concentration gradient dependent, and endogenous carrier-mediated transport. These topics have been reviewed recently in relation to brain tumor drug therapy.[18,19,21] An additional challenge is the active efflux transporter mechanism, which limits the ability to achieve effective drug concentrations in the extracellular space and in tumor cells because of active pumping of the drug away from its target.[22–25]

Strategies to Improve Passive Drug Transport across the BBB

Many strategies have been designed to overcome poor drug transport across these barriers, and many are still under development. Strategies that rely on systemic delivery of drugs are either trying to improve passive drug transport across the barrier or exploiting the endogenous carrier transporter mechanism to carry the agents across the vascular wall. Improvement in passive drug transport may be achieved by manipulation of the drug, by increasing the plasma concentration of the drug, or by transient opening of the BBB. An alternative approach is to block the active efflux transporter mechanism and, thus, maintain the drug on the abluminal side of the BBB.

Chemical Modification of Drugs.—Drug manipulation may include chemical modification to improve the drug's capability to passively cross the barrier, its lypophylicity may be increased (by lipidization), protein binding may be reduced, or its plasma half-life may be prolonged. To increase the brain delivery of hydrophilic therapeutic agents, prodrug technologies that modify drugs to be more lipophilic are usually used.[26] The lipidization strategy must be reversible, and after in the brain, the prodrug should be converted back to the parent compound by a chemical or enzymatic process. The most successful example of a prodrug is TMZ, which converts under physiological pH to 5-(3-methyltriazen-1-yl) imidazole-4-carboxamide, the active metabolite of dacarbazine, a drug that has been in use for many years to treat systemic malignancy but is unable to cross the BBB. Many other attempts to convert anticancer drugs to prodrugs with increased BBB penetration[27] have failed because of the plasma pharmacokinetics of the drugs. A drug's pharmacokinetic profile and the area under the curve (AUC) represent important factors determining its brain availability. Indeed, prodrug technology that uses lipidization increases not only the BBB permeability coefficient but also drug uptake in other organs, thus reducing the plasma prodrug concentration. This may result in a decrease in plasma AUC, and because brain uptake of a drug is reduced in direct proportion to the decrease in plasma AUC, lower concentration in the plasma may result in little increase in brain drug concentration.[28]

A promising alternative strategy for drug engineering is the design of prodrugs that are recognized and transported by the influx transporter systems.[29–31] In principle, small-molecule drugs can be synthesized to access carrier-mediated transport systems in the BBB. Large-molecule drugs can be engineered with what is known as molecular Trojan horse delivery systems to access receptor-mediated transport systems in the BBB. Peptide and antisense pharmaceuticals may be made brain penetrating with the combined use of receptor-mediated transport-based delivery systems and the avidin-biotin technology.[32] These technologies are particularly important for delivery of recombinant protein therapeutics that target specific receptors or molecules that have been identified as drivers of tumor proliferation. These technologies are being developed and hold promise for future brain tumor–targeted therapy.

Strategies that Increase Drug Concentration in the Plasma.—Plasma concentration at the tumor site is an important factor for passive drug transport across the BBB because passive drug transport is a concentration-gradient–dependent mechanism. Multiple approaches have therefore focused on increasing the fraction of a drug that reaches the brain, including the use of high-dose chemotherapy (HD-CTx), bolus drug injection, and intra-arterial drug administration. The expected clinical impact of these strategies is discussed on the basis of the aforementioned set of 5 expectations of drug therapy.

HD-CTx increases plasma concentration of the drug, but at the cost of augmented systemic toxicity, and, therefore, requires systemic rescue maneuvers, such as the use of folinic acid in the case of methotrexate (MTX) or granulocytic colony-stimulating factors with or without autologous bone marrow stem cell support when other agents are used. The concept of systemic chemoprotection is based on drugs that effectively protect normal cells against the adverse effects of anticancer agents without exhibiting tumor protection. Amifostine and *N*-acetycysteine are examples of agents that confer protection for systemic organs exposed to antineoplastic agents,[33,34] but their applicability to brain tumor therapy has not been demonstrated. An alternative experimental approach is to enrich bone marrow stem cells with cells that are modified to incorporate drug resistance genes that convey protection against chemotherapeutic agents.[35,36] A study in rhesus monkeys showed that bone marrow protection was achieved by overexpression of a mutant O6-methylgianine-DNA methyltransferase (*MGMT*) that gives rise to an altered enzyme that is highly resistant to inactivation by alkylating agents but retains its ability to repair DNA damage.[35] This allowed for a considerable dose

escalation of TMZ, up to 450 mg/m^2, a much higher dose than the maximum tolerated without such a bone marrow protection maneuver. The safety of this approach, which combines alkylating agents and lentiviral vectors, needs to be considered, and its usefulness for brain tumor therapy is awaiting evidence.

HD-CTx is used routinely to treat both systemic malignancies and CNS tumors, but its effectiveness has been proven only in lymphoproliferative malignancies. In Burkitt's lymphoma and adult lymphoblastic lymphoma, HD-CTx significantly reduced the rate of CNS relapse.[37] Although it is widely used in other systemic lymphomas, its effect on CNS involvement has not been established;[38] however, systemic HD-CTx based on high-dose MTX is considered to be a standard therapy for primary CNS lymphoma,[39,40] despite lack of consensus on the standard regimen. Table 1 shows that HD-CTx fulfills almost all expectations from drug therapy for those indications for which effectiveness has been proven, and it is therefore not surprising that it has gained wide acceptance in clinical practice.

An alternative method to increase plasma concentration is bolus drug injection. Its effect on drug penetration into the extracellular space of a brain tumor model was demonstrated by the use of an osmotic pump device implanted in the tumor mass. This device enables dynamic in vivo evaluation of drug concentration levels in both the tumor's extracellular fluid and the plasma.[41,42] Bolus injection of MTX was shown to produce significantly higher peak drug concentrations in both locations; thus, the ratio of the AUC of the tumor's extracellular fluid/plasma (AUC_{ECF}/AUC_{plasma}) was significantly higher than the ratio obtained after continuous drug infusion. This ratio expresses drug penetration across the BBB and into the extracellular space of the tumor, with a higher ratio indicating augmented drug penetration. A single small-scale clinical study involving patients with CNS lymphoma compared results of rapid and slow infusions of MTX. Rapid MTX infusion led to a higher CSF drug level and a trend toward improved outcome.[43] Although there is a paucity of clinical evidence, this strategy has gained popularity in the clinical setting, which is easily understandable, based on the favorable profile of clinical expectations shown in Table 1.

Of note is another study that used implanted osmotic pumps in patients with malignant gliomas for evaluation of MTX concentration in the tumor extracellular space after rapid infusion. The study demonstrated the profound effect of BBB permeability on drug concentration in the extracellular space of the tumor. The calculated AUC_{ECF}/AUC_{plasma} ratio was considerably greater in the region of contrast-enhancing tumor, indicating the presence of leaky vasculature, compared with ratios in nonenhancing tissue with an intact barrier function. These findings can be expected with passive transport of a small molecular weight water-soluble drug, such as MTX.[44]

Intra-arterial drug administration has been used in various protocols in an attempt to increase plasma drug concentration and improve outcome.[45–48] Chemotherapy is injected into the artery supplying blood to the tumor and surrounding brain. The perfused tissue receives higher plasma concentration during the first passage of the drug through the circulation, and the short duration of injection (5–15 minutes) adds the effect of bolus injection. A disadvantage is the requirement for an invasive procedure to induce this transient increase in plasma concentration in the tumor area. However, a study that evaluated cisplatin concentration in resected brain metastases showed significantly higher cisplatin tumor levels after preoperative intra-arterial infusion of the drug, compared with conventional intravenous administration.[49] Table 1 shows that the strategy's profile is disadvantageous for 4 of the 5 clinical expectations; thus, it is not surprising that its clinical use is limited to experimental regimens that are used in only a small number of centers.

Strategies that Induce Transient BBB Disruption.— Strategies that aim to induce transient disruption of the BBB to enhance passive drug transport across the

Table 1. Strategies that increase the plasma drug concentration: clinical impact based on expectations from drug therapy[a]

Strategy	Effectiveness for CNS Malignancy	Toxicity/Adverse Effects Profile	Introduction into Clinical Practice	Repeated/Continuous Administration	Tumor Size and CNS Location
High-dose chemotherapy with systemic rescue maneuvers	Established in Burkitt's lymphoma, ALL and PCNSL[b]	Usually Manageable	Simple, widely practiced	Feasible but may be limited (by toxicity)	No limitations
Intravenous bolus drug injection	Suggested but not established in humans	Negligible	Simple, widely practiced	Feasible	No limitations
Intra-arterial drug injection	Suggested but not established (only phase II studies)	Invasive procedure. May be associated with SAE. Frequency varies	Limited. Requires expert team approach.	Feasible but limited by invasiveness of the procedure	No limitations

Abbreviations: ALL, adult lymphoblastic lymphoma; PCNSL, primary CNS lymphoma; SAE, severe adverse events.
[a]For references, please see text.
[b]Based on phase III clinical studies.

barrier include osmotic BBB disruption (BBBD),[48,50] biochemical disruption,[51–53] and ultrasound-mediated BBBD.[54–56]

Osmotic BBBD is the only one of these techniques used by some centers for experimental treatment of brain tumors. It entails transfemoral catheterization of the desired arterial territory and transient opening of the BBB, which is induced by rapid intra-arterial infusion of hyperosmolar mannitol. Immediately after mannitol infusion, intra-arterial and intravenous chemotherapy is administered. The procedure requires a multidisciplinary expert team approach. It is performed on 2 consecutive days under general anesthesia with assisted mechanical ventilation and is repeated monthly in accordance with protocol. Human studies have shown that normalization of BBB function after osmotic disruption is a prolonged process that takes $\geq$6 hours,[57] a period during which there is an increased risk for neurotoxic effects. Another study demonstrated a linear relationship between the degree of barrier disruption on postprocedure imaging and MTX concentrations in the ventricular CSF of humans.[58] These findings fit the model of a water-soluble drug, such as MTX, that penetrates the CNS by passive transport. In this scenario, a high degree of disruption allows more drug penetration into the CNS, but intense disruption is also associated with increased risk of complications, including seizures or even fatal brain edema;[48,59] therefore, the procedure is usually adjusted to achieve moderate and safer disruption. Available data from previous human studies[57,58] and a recent rabbit model[60,61] suggest that there are significant variations in the degree and duration of BBBD induced with intra-arterial mannitol. This inconsistency has a profound effect on passive drug delivery across the vascular wall[58,60] and on the reliability of this procedure to achieve that effect. The largest published experience with the use of BBBD in conjunction with chemotherapy has been obtained from a multicenter study enrolling patients with primary CNS lymphoma.[48] Unfortunately, this study did not clearly demonstrate results that are superior to the treatment outcome obtained with conventional administration of chemotherapy in this disease.

Biochemical BBBD relies on mediators of the inflammatory response, including leukotrienes, histamine, and vasoactive peptides, that can cause transient vascular leakage and increased permeability of blood vessels.[62–64] Bradykinin, a peripheral vasodilator, increases tight junction permeability by activating B2 receptors of the endothelial cells.[53,65] The bradykinin agonist RMP-7 transiently permeabilizes the BBB to hydrophilic compounds, with greater impact in regions of the blood-tumor barrier, compared with nontumor BBB.[51,53] Human studies showed that sequential intra-arterial injection of the agonist RMP-7 and carboplatin were needed to enhance drug penetration into the tumor; thus, this method requires an invasive procedure. In a multinational clinical trial, intra-arterial RMP-7 was evaluated in combination with carboplatin for the treatment of malignant brain tumors;[66] however, because of high levels of toxicity, it was discontinued.

Ultrasound-mediated BBBD[54–56,67,68] is a new approach to focal CNS drug delivery that has produced consistent vascular leakage without tissue damage in animal models.[69] This effect is achieved by localizing cavitation-generated mechanical stresses to blood vessel walls by intravenous injection of preformed gas bubbles just before pulsed ultrasound treatment.[56,67,70] The focal opening of the BBB is reversible and does not last beyond 24 hours. Animal studies showed that significantly higher concentrations of liposomal doxorubicin and the monoclonal antibody trastuzumab (Herceptin) were found in brain regions exposed to the focused ultrasound effect.[71,72] Clinical devices for magnetic resonance–guided focused ultrasound are available, but their use as a noninvasive treatment technique for brain disease is still under investigation. Future directions include further technology development for controlled and efficient drug transmission into the brain and demonstration of therapeutic efficacy. In addition, the cumulative effects and safety of repeated procedures must be shown before routine use in brain tumor therapy, although a recent study in rhesus monkeys showed that focal BBBD can be reliably and repeatedly produced with no evidence of histologic or functional damage.[69] The main limitation seems to be the small treatment areas, which produce only focal disruption of the barrier; thus, the positive effects that were recently reported in small animal glioma models[54,55,73] might not be duplicated in large animals or in humans. Focal and limited volume of exposure to drugs means that the technique may be applicable only for tumors of very small size and that tumor cells that have infiltrated away from the treated focus will escape the desired effect of enhanced exposure to chemotherapy.

Despite the intriguing ability to facilitate passive drug transport across the barrier, the strategies that induce transient BBBD have not gained wide clinical acceptance. This is not surprising after we evaluate these procedures according to the set of clinical expectations (Table 2). It is clear that the osmotic and biochemical BBBD procedures are either invasive or associated with severe adverse events that limit their repeated use and, thus, hamper effectiveness. In addition, the superiority of these strategies over standard, less invasive, and safer therapies has not been proved. Finally, all these procedures are limited by their inability to treat larger tumors and some CNS locations. When these findings are taken together, it becomes obvious that none of these techniques are expected to change clinical practice or become a standard therapy for invasive primary brain tumors, based on the current state of technology.

Strategies that Block Active Efflux Transporter Mechanisms.—Active efflux of anticancer drugs contributes to brain tumor drug resistance.[74–76] There are a variety of efflux transporter systems. The multidrug resistance–associated protein (MDR) family[77] includes the ATP binding cassette (ABC) transporters. Two of these transporters, P-glycoprotein (P-gp), which is the most widely researched,[74] and breast cancer resistance protein (BCRP),[75] are major components that restrict

Table 2. Strategies that induce transient disruption of the BBB in conjunction with intravenous and/or intra-arterial chemotherapy: clinical impact based on expectations from drug therapy[a]

Strategy	Effectiveness for CNS Malignancy	Toxicity/Adverse Effects Profile	Introduction into Clinical Practice	Repeated/Continuous Administration	Tumor Size and CNS Location
Osmotic BBB disruption (IA mannitol)	Not established (only phase II studies)	Invasive procedure. May be associated with SAE. Frequency varies	Limited. Requires multidisciplinary expert team approach	Feasible but limited by invasiveness of the procedure	Safety limited by tumor size, mass effect, and location
Biochemical disruption of the BBB (IA RMP-7)	Not established (only phase II studies)	Invasive procedure. Associated with SAE.	Clinical practice discontinued	Feasible but limited by invasiveness and toxicity of the procedure	Safety may be limited by tumor size, mass effect, and location
Focused ultrasound-mediated BBB disruption	No human studies (only small animals studies)	No human studies	No human studies	No human studies	Limited to small tumor size

Abbreviations: IA, intra-arterial; SAE, severe adverse events.
[a]For references, please see text.

Table 3. Strategies that use alternative methods to improve drug delivery: clinical impact based on expectations from drug therapy[a]

Strategy	Effectiveness for CNS Malignancy	Toxicity/Adverse Effects Profile	Introduction into Clinical Practice	Repeated/ Continuous Administration	Tumor Size and CNS Location
Efflux transport inhibition without/with nanocarriers	No studies	High toxicity for first generation compounds. No data for new generation compounds.	No data. Potentially simple.	No data. Potentially possible.	No data. No limitation expected.
Systemic administration of drug nanocarriers without/with targeting molecules	Not established (few phase II studies).	Varies by carrier and compound. Require further investigation.	Potentially simple.	Potentially possible.	No data. No limitation expected.
Systemic administration of CNS-targeted drug carrier exosomes	No studies.	Safe by preliminary in vivo animal studies.	Potentially simple.	Potentially possible.	No data. No limitation expected.

[a]For references, please see text.

drug penetration into the CNS. The efflux transporter MDR1 P-gp is expressed in both low- and high-grade gliomas, suggesting an intrinsic resistance of these tumors to anticancer drugs.[76,78] However, chemoresistance may be caused not only by efflux transporter expression in brain tumor cells but also by its expression in the endothelial cells of both brain tumors and normal brain capillaries.[12,25,79,80] These efflux transporters constitute an independent barrier to drug transport at the BBB interface that restricts drug penetration, regardless of the functional integrity of the endothelial tight junction mechanism. It has been demonstrated that active efflux at the BBB is a relevant obstacle for numerous drugs that are BCRP/P-gp substrates. This mechanism limits the concentration of the drugs even at the core of tumors that contain leaky blood vessels, and it provides a plausible mechanistic basis for the clinical failure of various molecularly targeted therapies.[11,81,82] Coadministration of chemotherapy or targeted therapy with inhibitors of the active efflux transporters may thus increase drug concentration in the extracellular space. Preclinical and clinical studies have been performed to explore the potential of P-gp inhibition to improve CNS penetration of drugs; however, the results were disappointing in clinical trials using first generation P-gp inhibitors (eg, verapamil and cyclosporin A) because of toxicity issues.[83] Novel P-gp inhibitors (eg, valspodar, elacridar and zosuquidar) have an improved affinity profile and may prove to have a reduced clinical toxicity.[84] Recent research has led to a new paradigm suggesting that P-gp and BCRP work as a cooperative team of gatekeepers at the BBB.[11,85] These findings suggest that inhibition of either P-gp or BCRP can be compensated by the other transporter, and therefore, only drugs that have at least a dual inhibitor effect (such as elacridar) may demonstrate clinical efficacy. Thus far, clinical evidence to support routine use of these inhibitors is lacking, although theoretically, this class of agents holds the potential to greatly impact clinical practice (Table 3). Of note, clinical trials involving systemic tumors that tested coadministration of new efflux transporter inhibitors with

chemotherapy have been disappointing because of toxicity.[82]

Strategies that Use Drug Carriers for Drug Delivery to Brain Tumors

Recent advances in nanotechnology have created exciting opportunities to improve the efficiency of drug delivery to the CNS.[18,19,86–88] In principle, a drug that is poorly distributed to the brain can be loaded onto a nanocarrier system that interacts with the microvascular endothelium at the BBB. Eventually, this may produce higher drug concentrations in brain parenchyma. These nanocarriers can be further modified for enhanced CNS selectivity and permeability with targeting moieties that will preferentially bind to putative receptors or transporters expressed at the BBB. In addition, this system can exploit the physiological barrier mechanisms for drug trafficking across the barrier structure, making use, for example, of endogenous carrier-mediated transporter processes. At this stage, no ideal nanocarrier has been identified, but several classes of nanocarrier systems have been developed in the past decade and many are still undergoing intensive investigation. A detailed description of these systems is beyond the scope of this manuscript, but several recent reviews summarize the topic.[18,86–89] In brief, nanocarrier systems can be divided into 2 major categories. nanoparticles or nanospheres, and a variety of other nanocarrier types. In principle, nanoparticles are colloidal systems with compact structure where the therapeutic agent is either entrapped in the colloid matrix or coated on the particle surface by conjugation or adsorption. Nanoparticles include polymeric or solid lipid nanoparticles, lipid or albumin nanocapsules, liposomes, and micelles. The group of other nanocarrier types includes novel nanocarriers, such as dendrimer, nanogel, nano-emulsion, and nanosuspension.

Only nanoparticles that are $\leq$12 nm in diameter can passively extravasate across the porous blood-tumor barrier microvasculature.[86,90] A subset of nanoparticles with diameter $<$10 nm was shown to maintain peak blood concentration for several hours, suggesting that they accumulate over time in the tumor extracellular space. This passive targeting is known as the enhanced permeability and retention effect. Although prolonged circulation time is an important requirement for effective drug transport to the brain, it exposes the nanocarriers to interaction with the reticular endothelial system (RES), which removes particles depending on their size, charge, and surface properties. For most nanoparticles, this is a major drawback, and polymers such as polyethylene glycol (PEG), are often attached to mask the particles from the host immune system.[91] However, pegylated carriers are not easily transported through the barriers, and their crossing via receptor-mediated transcytosis is generally inefficient; thus, strategies using targeting molecules that are conjugated to their surface are frequently used to improve CNS delivery.[89] Targeting molecules may include monoclonal antibodies to transferin receptor,[92,93] to insulin receptor,[94,95] or to EGF receptor.[96] Other targeting strategies may involve nanoparticle coating with cell-penetrating peptides[97,98] or conjugation of the carriers with endogenous molecules, such as apolipoproteins (eg, Apo A, B, or E).[99–101]

Magnetic nanoparticles have been tested as carriers in drug delivery systems.[102,103] In this system, chemotherapeutic compounds may be conjugated with magnetic nanoparticles and can be specifically targeted in vivo to localized tumors by an external magnetic field–guided delivery.[104] The external magnetic field is placed and focused over the target site (the tumor), enhancing localization of the systemically administered magnetic drug nanocarrier.[54,102] Magnetic nanoparticles are also being extensively researched as a tool for tumor imaging.[105] Multifunctional magnetic nanocarriers that act as both drug carriers and imaging tools may improve drug localization by magnetic-guided fields and facilitate timely evaluation of carrier confinement in the CNS with the use of tailored MRI protocols.

Nanocarriers may be considered as good candidates for drug delivery across the BBB if they fulfill the following requirements: the particle diameter should be $<$100 nm; they should be nontoxic, biodegradable, and biocompatible; they should be stable in blood; they should target the BBB with no activation of RES; and they should be noninflammatory. They should not induce platelet aggregation; should have prolonged circulation time; should be amenable to carrying small molecules, peptides, or nucleic acids; and should exhibit a controlled drug release profile.[87] After nanocarriers have been developed to satisfy these multiple requirements and when human efficacy and toxicity studies have revealed favorable outcomes, nanotechnology for drug delivery is expected to change clinical practice for brain tumor therapy (Table 3); however, this field is still in its infancy, and many technical issues remain before CNS nanomedicine becomes useful in a clinical setting.

Exosome Nanovesicles for Drug Delivery across Biological Barriers.—Exosomes are naturally occurring membranous nanovesicles of 40–100 nm in diameter. They arise from the endocytic cellular pathway through inward budding of the limiting late endosomal membrane, giving rise to multivesicular bodies, which then fuse with the plasma membrane to release their vesicular content (exosome).[106] Exosomes are natural carriers of protein and nucleic acids, including mRNA and microRNA,[107] and have pleiotropic biological functions, while operating as natural vectors for intercellular signaling within and between tissues. They appear to play an important role in many disease processes, most notably inflammation and cancer, where their efficient functional delivery of biological cargo seems to contribute to disease progression.[108] Recent in vivo studies showed that systemic administration of ex vivo-derived exosomes could be used to deliver exogenous cargo to a targeted tissue type.[108,109] With use of a novel targeting strategy, the systemically administered exosomes specifically targeted the brain, delivered their cargo, and induced a biological effect.[109]

Table 4. Strategies for local drug delivery: clinical impact based on expectations from drug therapy[a]

Strategy	Effectiveness for CNS Malignancy	Toxicity/Adverse Effects Profile	Introduction into Clinical Practice	Repeated/ Continuous Administration	Tumor Size and CNS Location
Implantable polymers	Failed phase III clinical trials.	Invasive procedure. Increases surgical complication rates.	Relatively simple.	Not feasible.	Limited to resectable tumors at a distance from CSF pathways.
Intracavitary drug delivery	Not established (few phase II studies).	Invasive procedure. Device-associated infections, reversible and irreversible neurotoxicity for some agents.	Limited. Requires multidisciplinary expert team approach.	Depending on the agent, may be possible for non-radioactive agents.	Limited to small tumor size or to tumors accessible for GTR
Convection-enhanced delivery	Failed phase II/III clinical trials. Some studies are still ongoing.	Invasive procedure. Some agents associated with neurotoxity or chemical meningitis.	Limited due to high complexity. Requires multidisciplinary expert team approach.	Limited and associated with increased rate of infections.	Limited by tumor size, mass effect, and CNS location.

Abbreviation: GTR, gross total resection.
[a]For references, please see text.

Capturing the full potential of exosomes in drug delivery hinges on the development of scalable approaches for exosome production and the refinement of targeting and loading methods. Establishment of a scalable source of well-characterized exosomes is important. Induced pluripotent stem cells that can be derived from the patient's skin fibroblasts hold great promise in this regard, and this approach will also eliminate immunogenicity.[110] In addition, neural stem cell–derived exosomes are likely to display intrinsic neurotropic behavior and enhanced brain specificity. Clinical translation is currently hindered by poor understanding of exosome trafficking across biological barriers and the absence of exosome-tailored nanotechnologies for purification, characterization, and loading;[106] however, if these technological hurdles can be overcome, exosomes may revolutionize drug delivery by enabling safe and effective tissue-targeted drug delivery across impermeable biological barriers (Table 3).

Local Drug Delivery to Brain Tumors

Methods that deliver drugs directly to brain parenchyma[111] aim to augment extracellular brain drug concentration by completely circumventing physiological barriers, achieving high local or interstitial drug concentration with low systemic exposure. All available techniques require invasive brain procedures.[1,17,18,111] Approaches to local drug delivery include the use of implantable controlled-release polymer systems,[112,113] various catheter devices for intracavitary drug delivery,[114,115] and convection-enhanced delivery (CED).[116–119] The major limitation of these techniques is their failure to reach distant infiltrating tumor cells, a major requirement for therapy that aims to achieve a durable effect. Because of the invasive nature of these techniques and the restricted boundaries of drug distribution, it is not surprising that these procedures largely remain experimental or that they have already failed clinical trials (Table 4).

Implanted Polymers

Drug-impregnated wafers with controlled, sustained release rates are used to provide continuous local drug delivery. These biodegradable polymers release the drug by a combination of diffusion and hydrolytic polymer degradation. The only Food and Drug Administration–approved form of local chemotherapy is carmustine wafers, which have been studied in phase III clinical trials.[112,113] Carmustine wafers are implanted into the resection cavity of the tumor; thus, a priori, the therapy is limited to the subclass of tumors that are resectable with a small volume of residual neoplasm.

To achieve good tumor control, the drug must penetrate away from the resection cavity and affect infiltrating tumor cells that invade the proximal and distal brain parenchyma. The flux of drug from the implant to the surrounding tissue is proportional to the concentration gradient. Because the concentration at the implant site is limited by the need to avoid toxicity to normal brain tissue, there is also an upper bound on the driving force for drug transport from the implant into surrounding tissue. Consequently, concentration decreases rapidly with increasing distance from the implant site. It has been found that drug diffusion delivers detectable concentrations for <0.5 mm from the implant site;[120,121] with use of a 3-dimensional computerized model, it was concluded that the drug could not diffuse $>1–2$ cm away from the implantation site.[122] This may explain why implants have not resulted in improvement in patient outcome; however, additional factors contribute to ineffective delivery. As the drug diffuses, it is degraded, taken up into the vasculature, internalized by cells, and bound to the extracellular matrix, further hindering transport. In addition, the brain

extracellular space is tortuous, and the volume available for diffusion and fluid transport is low. A further limitation has been shown in cases in which the resection cavity is connected with the CSF pathways because much of the released drug leaks into the CSF rather than diffusing into the surrounding brain. Despite clinical ineffectiveness, adverse effects have been recorded. These include mainly poor wound healing and increased risk of infection, which may sometimes add significant morbidity.

CED

CED relies on pressure-driven bulk flow of infusate as a means to deliver the desired agent to the extracellular space of the CNS. The bulk flow mechanism is created by a small pressure gradient from a pump that pushes solute through a catheter targeted in the CNS.[88,123] The pressure-driven spreading of solutes through the interstitium does not depend on their intrinsic diffusivity and continues throughout the time that CED is performed, but ends abruptly when the procedure terminates.[124] Because CED bypasses the BBB, it can be used to infuse therapeutic agents with large and small molecular weight, such as paclitaxel;[116,125] high molecular weight–targeted toxins;[117,118,126–128] and various types of nanocarriers loaded with different agents.[124] Many factors affect the final distribution of the infused agent. Factors that have been investigated include the volume of infusion and infusion rate, catheter location and cannula size, shape and back flow along the catheter track, and air bubbles.[111,129] Other determinants are infusate features (size, interstitial affinity, and octanol water coefficient) and interstitial tissue properties (binding proteins, receptor uptake or binding, and tissue isotropy). Factors that affect efflux of the agent are diffusion or loss into capillaries and the rate of metabolism. All the latter reduce concentration over time and distance.[17,124,130]

Several clinical trials, including phase III trials, have been completed using CED, and all have failed.[116,118,125–127,131–133] Table 4 shows that, if we assess the technique using the set of clinical expectations for drug delivery, it discloses an unfavorable profile for each parameter. The technique is invasive and entails placement of several catheters into the tumor bed and brain tissue. Adverse effects include surgical complications and significant neurological deterioration, which were reported in about 13% of patients.[134] Some of the agents also proved to be neurotoxic or caused aseptic meningitis when the infusate leaked to the CSF pathways.[116,125,135] The technique is highly complex and often achieves only ineffective delivery with failure to get a therapeutic dose to the target.[111,134,136] Repeated and continuous administration is limited and associated with high rate of infections.[133] The method is also limited to a certain range of tumor size and does not fit all CNS locations. Proximity to CSF pathways is an exclusion criteria, because the infusate will leak into the CSF. It has been observed that adherence to catheter placement guidelines can be hindered by lesion site, proximity to eloquent cortical areas, tissue density that interferes with trajectory, and technical limitations of stereotactic instruments.[134] When these limitations are taken together, failure to prove clinical efficacy is expected. Recent studies have aimed to improve the accuracy and safety of cannula placement and to monitor the delivery of therapeutic agents by coinfusion of radiographic and therapeutic agents or by applying an ultrasound monitoring technique.[119,137–141] New trials are planned to investigate agents that can be coinfused with radiographic tracers, novel catheters that avoid problems with backflow and potentially will provide more reliable drug distribution. These trials will indicate whether technological advances can lead to improved clinical efficacy.

Intracavitary Drug Delivery

Intracavitary drug delivery may entail simple manual drug injection into the resected tumor bed via an implanted reservoir device that can be accessed percutaneously, or injection may be performed by a motor pump that will provide prolonged and controlled intracerebral delivery of the therapeutic agent.[111]

Intracavitary delivery has been exploited recently for delivery of radioactive ligands attached to monoclonal antibodies, such as antitenascin antibodies[111]. Tenascin is a glycoproteic antigen ubiquitously present in high-grade gliomas but not in normal brain tissues. In phase II clinical trials, several types of radioactive monoclonal antibody preparations were used. The experimental agents were injected into an implanted reservoir placed in the cavity during surgery that achieved gross total resection of the tumor. Some humanized chimeric preparations were associated with hematologic toxicity and others with reversible or irreversible neurotoxicity.[111] Unfortunately, the technique has all the limitations associated with local drug delivery, as summarized in Table 4. It is thus unlikely that it will change clinical practice, although if refined, it may become applicable to a subclass of small resectable tumors after efficacy is established in phase III clinical trials.

Challenges for Drug Delivery Posed by Anti-Angiogenic Therapy

Malignant brain tumors exhibit marked and aberrant blood vessel formation, indicating that angiogenic endothelial cells are a potential target for brain tumor treatment; however, tumor vasculature often provides inefficient transport of oxygen and therapeutic agents. In addition, it is highly permeable and is thus associated with propagation of surrounding brain edema and with high interstitial pressure within the tumor mass. Expansion of tumor vasculature is stimulated by vascular endothelial growth factor (VEGF), which is a validated therapeutic target for cancer treatment.[142,143] One rationale behind the use of anti-VEGF therapy is based on the concept that normalization of tumor vasculature

with a decrease in tumor interstitial pressure will improve drug delivery and oxygen supply, which is essential for effective radiotherapy.[144,145] It remains unclear how anti-angiogenic therapy affects tumor uptake of chemotherapeutic agents and what is the expected antitumor activity of combination therapies.

Several recent publications discuss the paradoxical effect of anti-angiogenic therapy in the management of cancer and brain tumors.[144–146] The induced morphological normalization of tumor vasculature can increase the transport efficiency of drugs, but the total number of surviving blood vessels decreases, leading to increasing tumor hypoxia, which in turn, affects tumor uptake of small molecules.[145,147] In brain tumors, restoration of BBB function is associated with reduced tumor interstitial pressure, which is thought to improve delivery of chemotherapy to tumor cells,[148] but the restored barrier function impedes passive diffusion of drugs into the tumor and surrounding brain parenchyma. However, with the reduction in tumor interstitial pressure, rapid drug leakage away from the tumor bulk is decreased and a resulting increase in drug retention is observed.[145] In an experimental glioma model, it has been suggested that these complex effects on drug exposure can be exploited to improve outcome if a prodrug is used concomitantly with the anti-angiogenic therapy.[145] Increased drug retention enhanced intratumoral prodrug activation and facilitated longer retention of active compounds, escalating the antineoplastic effect of treatment.

Despite the remarkable effect of anti-VEGF therapy observed in recurrent glioblastoma,[149] clinical and histological evidence suggest that these tumors may adapt to anti-angiogenic agents with increased tumor invasiveness and vessel cooption.[150,151] Both clinical and animal studies show that the favorable effect of anti-angiogenic therapy is short-lived; thus, coupling this therapy with other antineoplastic agents is probably essential. Clearly, a better understanding of the intricate issues related to drug delivery in combination with anti-angiogenic therapy is required for future rational integration of therapeutic modalities.

Conclusions and Future Directions

Despite the tremendous efforts invested in the development of drugs and delivery systems for the treatment of brain tumors, the results are disappointing. Nonetheless, research into sophisticated, science-driven solutions is continuing. During the past decade, conceptual and practical advancements have been made in the design and implementation of various vectors that demonstrate desirable characteristics. Although optimal systems have not yet been developed, progress has been noticeable and expectations related to therapeutic efficacy have increased. Research is increasingly focused on the development of noninvasive therapies because of the repeated failure of invasive methods of drug delivery to meet clinical expectations, as delineated in this article. To achieve efficacious treatment, issues relevant to drug delivery and active efflux mechanisms need to be resolved. It seems plausible that nanomedicine approaches will improve the delivery of conventional drugs, targeted agents, and probably DNA-based therapy. If the goal is to achieve more than a temporary improvement in local control, new drugs and delivery systems must adhere to basic clinical expectations, which include, in addition to an anti-tumor effect, a favorable adverse effect profile, easy introduction into clinical practice, feasibility of repeated or continuous administration, and compatibility for any tumor size and brain location. Adherence to these essentials will enable a change in clinical practice after an antineoplastic effect is demonstrated in well-controlled clinical studies.

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