



The targeted delivery of cancer drugs across the blood–brain barrier: chemical modifications of drugs or drug-nanoparticles?

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One of the most challenging problems, if not the most challenging, in drug development is not to develop drugs to treat diseases of the central nervous system (CNS), but to manage to distribute them to the CNS across the blood–brain barrier (BBB) using transvascular routes following intravenous administration. The development of BBB targeting technologies is a very active field of research and development. One goal is to develop chemically modified derivatives of drugs or chemically modified nanoparticulate vectors of drugs, capable of crossing biological barriers, in particular the BBB. This manuscript will review the approaches that have been explored to achieve these goals, using chemical functionalization of drugs or of drug vector systems and endogenous transporters at the BBB.

Introduction

The treatment of brain cancers is limited by the inadequacy in delivering therapeutic agents in such a way that drug molecules reach the desired targets. In order to achieve efficient treatments of central nervous system (CNS) cancers, it is necessary to transport therapeutic agents across the specialized vascular system of the brain, the blood–brain barrier (BBB), which can present formidable challenges. These include the definition of the properties of the cerebral vascular system during cancer progression and the development of biotechnology to prepare biomarker-targeted delivery of multiple therapeutic agents, coupled to the possibility of avoiding various resistance mechanisms. A great deal of effort, therefore, is presently focused on improving CNS bioavailability, and tumors thereof, of therapeutic drugs that can be specifically targeted to diseased tissue, improving therapeutic opportunities, efficiency, and patient survival, while decreasing side-effects to normal cells.

The vast majority of presently used therapies for cancer capitalize on the faster rate of replication of tumor than normal cells. Most solid tumors also possess unique features and defects of their associated vasculature, such as extensive angiogenesis, defective vascular architecture, and increased vascular permeability, all of which can be used for delivering therapeutics. Devices, such as functionalized drug colloidal carriers, can take advantage of these

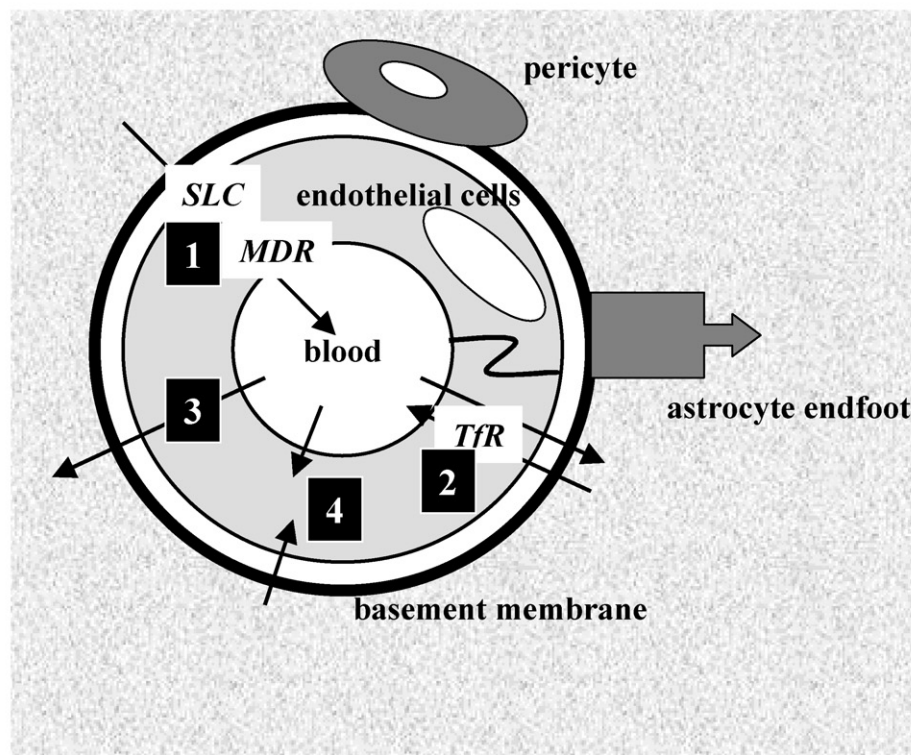
features and act as vehicles to deliver, selectively and specifically, anti-cancer drugs to tissues, either using passive mechanisms relying on increased vascular permeability in a defined location, or using active targeting of chemically modified drugs or nanoparticles. Alternatively, direct modification and/or functionalization of drugs, involving the chemical conjugation of drugs to disease-targeting biological markers, can be used to achieve direct active targeting of drugs.

In this manuscript, the attempts that have been made to use either chemical modifications of drugs or the development of drug-nanoparticulate systems to reach these objectives will be reviewed and their advantages and drawbacks discussed, to define what should be the chemical, biophysical, and biological characteristics of an optimized system.

The blood–brain barrier (BBB), brain cancers, and therapeutic options and problems

The BBB is a system of vascular cellular structures, mainly represented by tight junctions between endothelial cells, and an ensemble of enzymes, receptors, transporters, and efflux pumps of the multidrug resistance (MDR) pathways (Figure 1) (reviewed in references [1–7]) that control and limit the access of molecules to the brain, either by paracellular or transcellular pathways. Since the vascular density in the brain is very high, once molecules have penetrated the BBB, they distribute rapidly to the whole brain tissue. Whereas a few lipid-soluble molecules are able to pass freely

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- 1** Active efflux transporters Energy-independent SLC
Energy-dependent MDR
- 2** Receptor mediated transporters: Bi-directional: TfR
- 3** Carrier-mediated transporters luminal to basolateral:
Glucose, amino acids, small organic acids,
- 4** Endocytosis : ex acLDL scavenger receptor (from blood)

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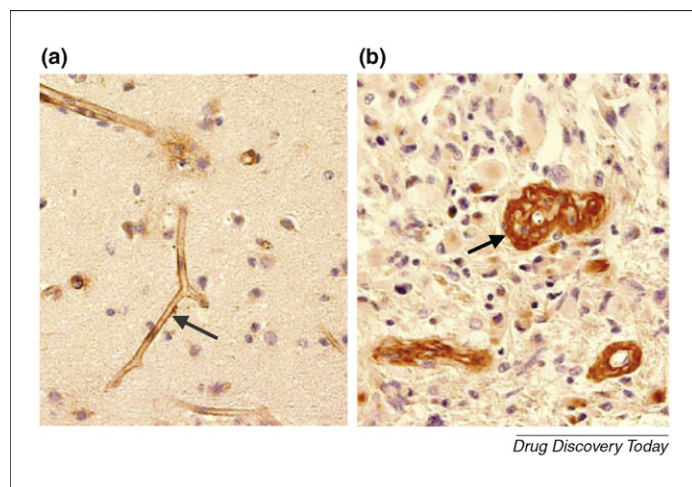
FIGURE 1

Schematic structure of cerebral capillary endothelium forming a normal BBB and active transport of drugs across the BBB.

by passive diffusion from the blood to the interstitium of the brain, ionic solutes are unable so to do. Chemical modifications of drugs that enhance lipophilicity result in an increased distribution of the drug in all organs. The design of carriers that cross the BBB at sites defined by the properties of the vasculature at that specific localization, using biology-based strategies to target specific transport systems at the BBB must, therefore, be designed. These carriers may consist of drugs or polymeric nanocarriers chemically modified with recognition and transcytosis-enhancing ligands that can allow the release of the active free drug once transendothelial transport has been performed. One particular challenge is related to finding those localized changes in the properties and biological change signatures characteristic of a diseased BBB. Functionalized

drugs must structurally resemble the normal transporter substrates, making them recognizable by the transporter, however, maintaining the biological activity of the drug is another challenge.

Cancers of the brain include gliomas (astrocytomas, oligodendrogliomas, and ependymomas), meningiomas, which are located outside the BBB, or metastases (mainly from lung, breast, melanoma, renal, and colon cancers). As low grade tumors are mainly treated by surgical resection and/or radiotherapy, drug transport across the BBB for these tumors is not relevant for this review. The most aggressive features of many high grade brain cancers, in particular glioblastoma multiforme, include widespread infiltration of surrounding tissue and high endothelial proliferation of a

**FIGURE 2**

Structure of a normal cerebral capillary forming the human BBB (a) and tumor-associated glomeruloid multi-cell-layered vessels (b) in a human glioblastoma. Vessels were labeled with lectins (dark brown coloration) and are pointed by arrows. The structure of tumor blood vessels in aggressive brain tumors, such as glioblastoma multiforme, is very different from the structure of not only a normal blood vessel of non-tumoral brain but also from a normal-looking blood vessel of the secondary invasive site of such tumors where the BBB is maintained.

glomeruloid multilayer vasculature (Figure 2). This represents an important feature when considering transporting therapeutic agents across the cerebral vasculature. Contrast-enhanced imaging indicates enhanced contrast and disrupted BBB at the primary tumor sites, but not in the infiltrative tumor areas that represent the most difficult cancerous lesions to treat. Loss of the tight junctions in the tumor vascular system does not, however, necessarily imply loss of the other biological components of the BBB, such as the detoxification and drug-resistance mechanisms [5]. Thus, therapeutic resistance of glioblastoma is due to a poor drug delivery resulting from a partial BBB preservation and high intratumoral interstitial pressure, poor blood perfusion, and the expression of various drug resistance mechanisms.

Many drugs have been or are being evaluated for the treatment of brain tumors [8]. The chemical structures and biological features of some of these drugs are given in Table 1. The BBB permits the transport across the brain vascular system of a very limited number of small hydrophobic molecules. Many anti-cancer agents, however, are large hydrophobic molecules unable to freely cross the BBB and are also substrates for the MDR efflux pumps, expressed by both the BBB vasculature and the tumor cells. Of those shown, only irinotecan (a topoisomerase I inhibitor), melphalan, and temozolomide are capable of being transported across the BBB. Nitrosoureas, in particular carmustine and vincristine [9], which have been the backbone of treatment for malignant glioma, only poorly cross the BBB. Recently, concomitant radiotherapy and temozolomide chemotherapy became standard treatment for newly diagnosed glioma, improving survival in a relevant fraction of patients [10]. The potential of drugs to reach the tumor across the BBB is not, however, the sole factor that predicts and limits efficacy. Other possibilities include drug resistance of the tumor cells, either *de novo* or acquired during treatment, poor perfusion of the neovessels of brain tumors preventing efficacious drug

concentrations at the tumor being achieved. Whereas treatment of primary tumors may be facilitated by leaky vessels at the site, the treatment at the tumor infiltration sites, where the BBB is not yet leaky, allows progression of the tumor at these sites. Malignant glioma almost always recurs and even patients who responded to first-line therapy had a poor response to second-line therapy, necessitating the development of novel therapeutic options. Surgical tissue is, however, available only rarely for evaluating the state of the BBB after the initial surgery, making evaluation of these recurrent tumors difficult.

Drugs targeting brain cancers across the blood–brain barrier: chemical functionalization of therapeutic agents or of nanoparticulate carriers?

As previously stated, the limiting factor in the treatment of brain cancers is the delivery of therapeutic agents to the brain across the BBB. A very restricted number of liposoluble small molecules (MW < 400 Da) cross the BBB by free diffusion. All the other molecules must use specific systems to be transported across the BBB (for a more detailed review see reference [5] and scheme of Figure 1). Therefore, the future for treatment of malignant brain cancers relies on the development of therapies targeting the markers and transporters of the tumor-associated cerebral endothelium, not only at the primary tumor sites but also at the invasive areas. Biological targeting involves that a specific marker (a target) is selectively expressed, or is expressed on disease-associated cells at a much higher level than on normal cells. The targeting agents may be antibodies, directed toward an antigen residing on the target tissue, or ligands for receptors or transporters, and may be covalently conjugated via an appropriate chemical bond either directly to the drug or to a vector, such as a nanoparticulate device. Most of the targets identified and evaluated until now for brain cancer have been related to molecules associated with enhanced angiogenesis or increased nutrient demand of the tumors.

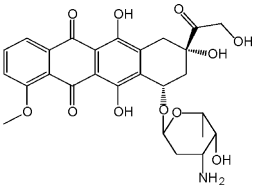
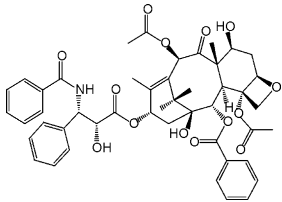
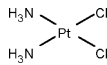
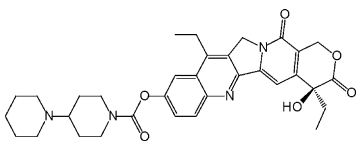
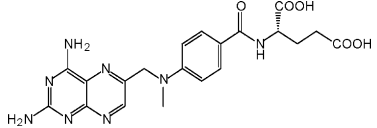
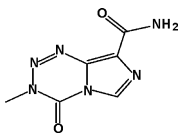
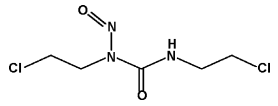
Some drugs have been conjugated to ligands or antibodies, or have been incorporated into carriers bearing ligands or antibodies for recognition by cell surface receptors expressed by target cells. Major obstacles include the physiological stability of these structures and their transport across biological barriers, in particular the blood–brain barrier, for the delivery of therapeutic drugs. In addition, for maximal efficacy, drugs must reach their targets in the appropriate location within tumor cells, that is, the cytosol, cell organelles, or the nucleus. Drug release from the carrier must also be achieved. Therefore, the combination of a drug or drug vector with a molecule recognized by a luminal blood-to-brain carrier system is mandatory, of which glucose, amino acids, monocarboxylic acid, oligonucleotides, cationic peptides, or transferrin conjugates represent potential transport systems to the brain [11–13].

Direct conjugation of drugs

Most of the approaches attempted have evaluated drug conjugation to a blood-to-brain transporter. To be successful, this approach requires the drug to mimic the endogenous ligand, since most transporters, such as the glucose transporter, are highly selective. The expression of enzymatic activities at the BBB is also important and these activities may be modified in disease. For

TABLE 1

Some of the chemotherapeutic agents in clinical use or trials for brain cancers, including metastases

Name	Structure	Characteristics	Transport across the BBB
Doxorubicin		Anthracyclins, inhibits nucleic acid synthesis very narrow therapeutic index	No
Paclitaxel		Microtubule-stabilizing,	No
Cisplatin		Inorganic Pt ²⁺ complexes DNA alkylating and intercalating, short half-life	No
Irinotecan		Inhibits DNA topoisomerase I induces single strand DNA lesions	Yes
Methotrexate		Antimetabolite of folic acid inhibits dihydrofolate reductase and DNA, RNA and protein syntheses	No
Temozolomide		Alkylating agent	Yes
Carmustine		Alkylating agent	No

example, we have shown that in brain cancers, aminopeptidase A activity is increased, whereas aminopeptidase N activity is decreased [14]. These differences are important factors for delivering intact molecules to the brain and the use of enzymatically stable (pro)drugs may be necessary, using chemical modifications such as cyclization, halogenation, methylation, pegylation or the introduction of un-natural bonds.

Approaches previously attempted fell into two categories, namely carrier-mediated and receptor-mediated transport. The former used carrier-mediated transport of small molecules that are brain nutrients. As stated above, the transporters expressed at the luminal and abluminal surfaces of the BBB are structure-specific and rarely transport drug analogs, either drugs modified to resemble their normal substrate or drugs chemically bound to their ligands. Therefore, simply coupling drugs to their ligand may

not result in their transport and only very few attempts have been successful. Rather, drugs should be modified to mimic the normal ligands, while maintaining bioactivity, which is not an obvious approach for most drugs. For example, the anti-cancer agent melphalan resembles the amino acid phenylalanine and can be transported by the LAT1 carrier [15]. Very recently, the small hydrophilic drug ketoprofen, an anti-inflammatory agent that is not a substrate for LAT1, was chemically bound via an ester linkage to the phenolic hydroxyl group of the amino acid tyrosine, a LAT1 substrate, and was recognized by the LAT1 transporter [16], opening new possibilities for small anti-cancer drugs. Melphalan has also been conjugated to L-glutamate, with some success [17]. However, for transport by amino acid transporters, the α -amino and α -carboxyl groups of the amino acid must be free, limiting chemical possibilities. The glucose transporter GLUT1 was shown

to be able to transport the anti-cancer pro-drug chlorambucil-glucose across the BBB [18].

The receptor-mediated transporters, which can transport larger molecules, have been extensively reviewed elsewhere [19–22]. The most extensively studied has been the Tf-receptor (TfR) that is expressed on highly proliferating cells, such as cancer cells and at much higher levels on endothelial cells of the BBB than on endothelial cells at other locations within the body. Diphtheria toxin-Tf conjugates [23] improved outcome in patients with chemotherapy-refractory brain tumors. Other possibilities include the LDL receptor, but this receptor is also expressed by many other tissues, whereas the LDL-related protein (LPR)-receptor, mediates only endocytosis, not transcytosis. The fact that intracellular localization depends on the characteristics of the transporter and that many antibodies and large proteins stick to the endothelium, represent significant problems; for drug delivery, however, only receptor systems capable of transcytosis (migration of the complex ligand-receptor from the luminal-to-ablumenal membranes of the endothelial cells of the BBB) present interest. Also, the active efflux transporters [4,6,7], which are responsible for drug resistance and efflux of therapeutic agents into the blood flow as soon as internalized by cells of the BBB, require that drugs should be coupled or co-injected with inhibitors of these systems to pass the BBB. A major concern with approaches using large molecules is the potential risk of immunological responses associated with long-term treatment.

Drug-nanocarriers

Drug-carrier nanoparticles [22,24,25] are defined as submicroscopic colloidal systems that may act as drug vehicles, either as nanospheres (matrix system in which the drug is dispersed) or nanocapsules (reservoirs in which the drug is confined in a hydrophobic or hydrophilic core surrounded by a single polymeric membrane). Micelles are self-assembling amphipathic colloidal aggregates of hydrophilic and hydrophobic block copolymers in which hydrophobic or hydrophilic drugs are physically trapped or covalently bound. Following systemic administration, drug delivery devices must be transported across the vascular wall into the surrounding tissues and the interstitial space. Transport of anti-cancer drugs by nanocarriers of less than 80 nm diameter may be achieved by taking advantage of the leaky vessels associated with the primary sites of human brain cancers, the enhanced permeability and retention (EPR) effect [26–29]. The extravasation of molecules is accompanied by fluid movement across the leaky vessel walls by passive diffusion or convection, dependent on the hydrostatic and osmotic pressure differences between blood and interstitial space. Small molecules mainly diffuse across, whereas large molecules are transported by convection or pressure differences. Macromolecules, extracellular matrix, and cell density are important factors in the partition of nanoparticulate therapeutic agents in tumors.

The structure of the polymer and the method of trapping drugs in the nanoparticles will define the drug release kinetics and characteristics. The detailed description and methods of preparation and characteristics for drug loading of nanocarriers fall outside of the scope of this review but have been recently reviewed by the author [25]. To achieve these goals, however, it is necessary to develop tools to entrap drugs into vectors capable of releasing the

drugs at the right place and time from their vector. In addition, the delivery systems and the therapeutic agents must resist hydrostatic, hydrophilic/hydrophobic and biophysical/biochemical barriers, resistance to treatment developed in tumors and biotransformation, degradation, and clearance mechanisms. It must be noted that the use of biodegradable polymeric matrices solves the issue of removal of the device after delivery of the drug. Surface modification of nanospheres made of polymer (synthetic or natural) aggregates or of nanoliposomes in which the drug is either dissolved, entrapped, encapsulated, or covalently attached, is possible. Drugs have been attached to their vector carriers via linkers. The design of the chemical bonds linking the drugs to their carriers is also of potential interest for the selective release of the therapeutic agents [30,31]. Therefore, the necessary synthetic routes and the design of linkers for conjugation that are appropriate and biocompatible must be developed. Drug-loaded targeting and transport-enhancing nanoparticles must match the mechanical properties and degradation rates that are needed for the application [32,33]. The most commonly used polymer, poly(ethylene glycol) (PEG), is a flexible water-soluble molecule that can be end-functionalized for chemical modification as well as for copolymerization with other polymers [34]. These polymers have features such as controllable mechanical properties and degradation rates, minimal toxicity, and immune response [35].

Conjugation of ligands targeting the BBB on the surface of colloidal carriers, either by covalent or non-covalent linkage, increases selectivity for brain cancers and the future of development for transporting anti-cancer agents across the BBB for treatment of brain cancers probably relies in the development of targeting transport-enhancing nanocarriers. Active targeting requires that reactive groups exist at the surface of nanoparticles for chemical coupling and that selective ligands for defined cell markers are presented in adequate configuration and concentration at the surface of nanoparticles. Drug release by the carrier must follow extravasation and transvascular transport and drug therapeutic dosages must be attained.

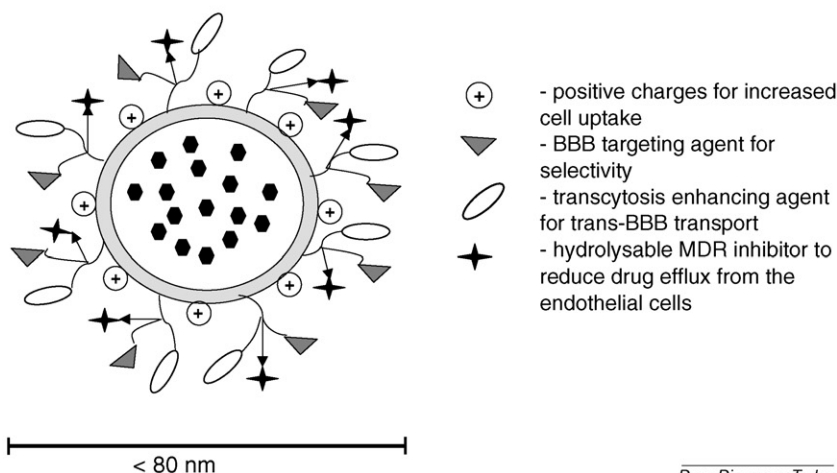
The design of nanoparticles as multifunctional platforms and some examples of their preparations and uses have been recently reviewed [22,25]. Some examples of nanoparticles able to ferry drugs across the BBB to CNS tumors have been reported and some of the necessary characteristics of such tools have been defined. Chemical derivatization or encapsulation into polymeric particles [22,25,27,36–39] has been evaluated as a possibility for enhancing drug selectivity. Nanoparticles are generally internalized into cells via fluid phase endocytosis, receptor-mediated endocytosis, or phagocytosis. Nanoparticle surface manipulations may be performed to increase cell uptake and the potential delivery of the nanoparticles in different cell compartments [40–43]. Nanocontainers have been decorated with ligands for BBB transporters. Anti-cancer agents have been loaded in Tf-coated nanoparticles, for example 5-fluorouracil [44]. The size of even a small protein, such as Tf or an antibody, however, is of the same order of magnitude as that of the container, which will prevent significant numbers of molecules to decorate the nanocontainer, thus limiting a favorable binding equilibrium. Adsorptive-mediated endocytosis, involving electrostatic interaction between a positively charged ligand and the negatively charged membrane of cells at the BBB [45], mainly sialic acid, may also be of interest. Cationized

albumin was efficiently transported across the BBB [46]. The immunogenic properties of proteins may, however, be issues in long-term treatment. Attempts using carrier-mediated systems to transport nanoparticles included the GLUT1, which showed that α -mannose, but not β -mannose derivatives incorporated on the surface of liposomes [47] induced transport across the BBB. Nanoparticles coated with a choline derivative were transported across brain-derived endothelial cells faster by the cation transporter than uncoated nanoparticles [48], possibly also dependent on the lipophilic nature of choline derivatives, whereas thiamine derivatives were not interesting for enhanced transport [49]. Another interesting system is the folic acid receptor, specifically expressed at the BBB and able to transport doxorubicin-loaded folic acid-decorated nanoparticles [50]. Some hydrophilic surfactants, in particular polysorbates, interact with the surface of the BBB [51–53]. Polysorbate-coated doxorubicin nanoparticles [52,54], but not PEG-coated nanoparticles [55], may be promising for nanoparticulate drug delivery to the brain. Adsorption of apolipoproteins onto the surfactant-coated nanoparticles may also favor receptor-mediated processes [52]. Toxicity issues, non-bio-compatibility of the surfactant, increased permeability, and tight junction disruption of the BBB by these surfactants may, however, be important issues, rejoining invasive procedures and non-specific procedures, such as EPR effects. Finally, as for direct pro-drug conjugates, the active brain-to-blood transport efflux transporters [4,6,7], which are mainly responsible of drug resistance, require additional coupling or co-injection of inhibitors of these systems to pass the BBB.

Conclusions

A few strategies exist to enhance transport of anti-cancer agents across the BBB for the treatment of high-grade brain tumors: (i) passive permeation of lipidated drugs, however, this strategy is possible only for small molecules; (ii) the development of pro-

drugs hijacking the transport mechanisms at the BBB, however, the high selectivity of these transport mechanisms limits this approach; (iii) the development of drug-loaded nanocarriers able to take advantage of any disruption of the BBB at tumor sites. The most promising tools to deliver therapeutic drugs to tumors of the brain may be nanoparticles [56,57]. Glioma vasculature exhibits physiological characteristics distinct of the intact cerebral BBB. Thus, the design of less invasive and more selective approaches for brain tumors for drug delivery must exploit these physiological differences as well as permeability differences. Colloidal systems, such as nanoparticles, show promise for brain targeting, enhanced by the ability to modify their properties, increasing drug efficacy and attenuating side effects. Technical problems associated with developing targeted nanoparticles include the increased complexity of the nanoparticles, as well as the increased risks of adverse reactions, while advantages include the increase in drug reaching its target, enhanced selectivity, and the potential for delivery of multiple agents at the same site. Creating a toolbox of molecules that can be assembled hierarchically into ordered structures, spatially and chemically controlled, is, however, essential to make nanoparticles an attractive and efficient means of encapsulating and delivering drugs to the CNS tumors. Some of the chemistry and chemical routes that can be used to achieve biomimetic assemblies comprising the polymer, a linker and a bioactive molecule, have been reviewed in reference [58]. Emphasis must be put on multi-functionality of delivery vectors: these delivery vectors must include positive charges (cations) for enhanced vascular uptake, vascular-targeting, and transcytosis-enhancing agent, and drug(s). Thus, an ideal theoretical therapeutics-delivery nanoparticle system for brain cancer (Figure 3) would be one that: (i) selectively targets diseased BBB; (ii) bears an inhibitor of the efflux pump linked by a locally hydrolysable bond, and (iii) transports drugs across the cerebral vasculature and delivers them to their target, that is, the brain cancer cells. Only nanoparticulate systems



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FIGURE 3

Structure of a model BBB drug nanoparticle delivery system. In order to be efficient and selective, nanocarriers able to ferry anti-cancer agents across the BBB to treat primary and secondary sites of aggressive brain cancers, must be very complex entities. The optimal nanocarrier will contain the anti-cancer agent in the core of a polymeric sheet, whose surface has been decorated with a BBB targeting and transport-enhancing molecule, and has enough positive charges to enhance uptake by brain tumor vasculature and inhibitor(s) for drug resistance mechanisms at the BBB and the tumor cells.

can offer this diversity, however, the biophysical, biochemical, and biological mechanisms associated with the interaction of nanoparticles with living tissue, will need to be better understood to allow widespread adoption of this technology. Challenges yet to overcome include identification of disease-associated changes of the BBB properties in brain cancers and the modification of drugs or drug-carriers with targeting and transport-enhancing agents. Antibodies have the potential to be selective; however, their size and potential immunogenic properties are a limitation to their diffusion into

tissue and use. Protein ligands, such as transferrin and its receptor, which have been widely evaluated for targeting, suffer from similar problems. There will be, in the near future, plenty of possibilities to be explored; however, in my opinion, the most promising vectors are those involving small molecules as BBB targeting/transport-enhancing agents, stable in biological media and versatile for synthesis purposes, and able to carry a large drug payload. It is unrealistic, however, to imagine that a general and unique targeting vector can be designed for all situations and purposes.

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