Review Article

Challenges in delivery of chemotherapeutic agents for the treatment of brain cancer and the scope of nanoparticulates

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ABSTRACT

Brain tumor remains one of the most dreadful, poorly prognosed cancer despite advances in understanding the molecular basis of carcinogenesis. Treatment of brain tumors requires a multidisciplinary approach that includes combination of surgery, radiation, or chemotherapy. The major hindrance to prognosis of brain tumor is the autoprotective nature of the brain and blood–brain barrier (BBB). The approaches involves deliberate opening of the tight junctions of the BBB or delivery of drugs "through" or "behind" BBB by manipulating the physicochemical properties of the drug and have major drawbacks including irreversible opening of BBB that potentially allows the entry of exogenous material such as viruses and bacteria, and allows limited spatial distribution of drug. Nanoparticles (NPs) focus on the administration of nanometric size particles loaded with chemotherapeutic agent, monoclonal antibodies, genes, proteins, peptides, and other entities. Due to its small size and modifiability warrant their selective uptake by the tumor cells. They can be formulated out of variety of substance and can carry multiple loads of drugs directing the substance toward the tumor cells. Thus in comparison to conventional therapies, NPs reduces the side effects. Number of drug delivery strategies has been developed to overcome challenges presented by the BBB. One technique that holds promise for bypassing the BBB to deliver drugs to the brain the spillover effect of drug to normal tissue is intranasal delivery. The intranasal delivery of NP loaded anticancer drug provides a practical and noninvasive method for delivering therapeutic agents to the brain due to the unique anatomical connections.

KEY WORDS: Blood–brain barrier, Cancer, Nanoparticles, Targeting

INTRODUCTION

Cancer is a common cause of death in the world; about 10 million new cases occur each year. Moreover, cancer is responsible for 12% of deaths worldwide, making it the third leading cause of death.[1]

Brain Tumor

Brain tumor remains one of the most dreadful, poorly prognosed cancer despite advances in understanding the molecular basis of carcinogenesis. The rate of incidence of brain tumors in the United States is 14 cases per 1,00,000 with the median age being 57 years.[2] Glioblastoma multiforme and anaplastic astrocytomas are the most common type of primary brain tumors in adults. The incidence rate of all primary malignant brain tumors is 78%.[3]

Depending on the tissue of origin, brain tumors can be classified as benign (primary/secondary), and malignant tumors. Secondary cancers arise due to metastasis of tumors and are seen in around 20–40% of patients.[4] Histology of primary systemic cancers forms the basis of classification of brain tumor metastases.[5] Tumor's histopathological classification and location determine the degree of the tumor, anaplasia (malignancy) and its nature. Thus primary tumors can be classified into five types: Astrocytoma, oligodendroglioma, ependymoma, gangliocytoma, and medulloblastoma. However, due to the unusual biology of central nervous system (CNS) tumor and substantial variety it has become extremely difficult to

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develop a widely accepted histological classification system.[5]

The median survival rate of brain tumor patients is approximately 1 year. The low survival rate of the patients suffering from brain tumor depicts the failure of the effectiveness of the prescribed chemotherapy. Depending on the origin of the tumor they are grouped as tumors of neuroepithelial tissue, tumors of cranial and spinal nerve, tumors of meninges, hematopoietic origin neoplasms and lymphomas, tumor of sellar region, germ cell tumors and cysts.[6] Although there are many types, tumors of the epithelial lining of neurons (glioblastomas and astrocytomas) have the highest rate of occurrence followed by the tumors of the meninges, tumors of nerves and tumors of sellar region.[7,8]

CURRENT TREATMENT APPROACHES AND ASSOCIATED PROBLEMS

Treatment of brain tumors requires a multidisciplinary approach that includes a combination of surgery, radiation or chemotherapy.[2] Tumor recurrence (96% cases), adjacent to resection margin after surgical resection is seen calling out for adjuvant therapy. Post resection, radiation therapy, and chemotherapy are used for the treatment of glioma patients.

Chemotherapeutic Agents

Temozolomide is the first generation agent used for the treatment of brain tumors and is given orally. Few other agents that can be used include irinotecan, carmustine, cisplatin, and lomustine. Many of the drugs have proved effective in the treatment of brain tumors in laboratories when used against cell lines. However, clinical failure was observed with such drugs due to insufficient barrier passage. The major fraction of such expensive molecules of the drugs is thus lost with an expression of side effects. These drawbacks are observed either due to hydrophilic nature of the molecules or due to large molecular weight [Table 1].

Currently employed chemotherapeutic agents for the treatment of gliomas with few of the relevant.

Chemotherapy Fails - A Major Reason

Though there is an advancement in understanding cellular mechanism and progress in cell study research, the prognosis of the tumor remains dismal. Improved surgical techniques, use of heavy particle radiations and discovery of new chemotherapeutic drugs are few milestone activities in the segment of prognosis.[9-11] However, there is not yet a major breakthrough therapy to eradicate this condition. This is due to deep invasion of the brain parenchyma, cells being naturally resistant to apoptosis, most cytotoxic drugs, and radiotherapy. Prognosis of the brain tumors remain very poor raising a question as to why chemotherapy fails? The major hindrance to the prognosis of brain tumor is the auto protective nature of the brain (blood brain barrier [BBB] and alignment of brain cells), genomic alterations occurring in tumor cells, efflux transporters on the barrier and properties of chemical agents used for the treatment of brain tumors.

Chemotherapeutic Agents and Associated Side Effects

The brain allows passage of some of the endogenous material, a few hydrophobic agents, and particles with a molecular weight of <500 Da. Lipophilicity of the drug is one of the important factors that should be considered while designing new entities for treatment of tumors. Improvement in the passage of drug across the BBB is possible with increased lipophilicity, but this may be associated with increased drug uptake by other tissues, causing an increased tissue burden. This nonselectivity in the delivery of drugs to the non-targeted site is detrimental; especially when cytotoxic agents are used because drug toxicity would be higher at non-target sites. Enhanced efflux along with the loss of CNS activity is another major drawback of increased lipophilicity which might lead to poor tissue retention and short biological action. Improvement in the therapy could be brought about by modulating the pharmacological properties of the drug. This approach is called as the pharmacological approach.

Lipidization of small molecules (prodrugs) has been frequently employed. Prodrugs are pharmacologically inactive compounds that result from transient chemical modifications of biologically active species. The chemical change is usually designed to improve some deficient.

The physiochemical property, such as membrane permeability or water solubility.

Thymidine phosphorylase an enzyme converting thymidine to thymine and deoxyribose is activated in some tumors especially glioma. Capecitabine is a triple prodrug involving thymidine phosphorylase for conversion to active 5-flurouracil after three steps.[12] A case study has reported the successful efficacy of capecitabine against the treatment of brain tumor.[13] Similarly; the therapeutic efficacy of paclitaxel conjugated to linolenic acid against was demonstrated by Ke *et al*.[14] Such attempts are being made, and the effects of such newly synthesized entities are being tested against brain tumors.

Table 1: Physicochemical properties of chemotherapeutics used in brain tumor

Name of the drug	Mechanism of action	Permeability coefficient	Type of brain tumor to be treated	Molecular mass (g/mol)	Half-life	Log P	BBB passage
Carmustine	Alkylating agent	$0.92*10^{-4}$ cm/s	Glioblastoma multiforme	214.049	15–30 min	1.5	Yes
Lomustine	Alkylating agent	$3.16*10^{-4}$ cm/s^{-1}	Oligodendrogliomas	233.695	94 min	3	-
Temozolamide	Alkylating agent	-	Glioblastoma multiforme, astroctomas, oligodendrogliomas	194.151	1.8 h	–0.99	Yes
Cyclophosphamide	Alkylating agents	-	Glioblastoma multiforme	261.086	3–12 h	0.8	No
Methotrexate	Inhibition of DHFR	$1.77*10^{-7}$ cm/s	CNS lymphomas	454.44	3–15 h	–0.91	-
Imatinibmesylate	Inhibition of tyrosine kinase enzyme	-	Glioblastoma multiforme, CNS lymphomas	493.603	18 h, metabolite- 40 h	1.198	-
Vincristine	Inhibition of mitosis at metaphase through its interaction with tubulin	$1.58*10^{-7}$ cm/s	Oligodendrogliomas	923.04	19–155 h	2.8	No
Procarbazine	Breaking of DNA strands	$3.01*10^{-5}$ cm/s	Glioblastoma multiforme, astroctomas, oligodendrogliomas	221.229	10 min	0.06	Yes
Cisplatin	Crosslinking of DNA	-	Glioblastoma multiforme	300.05	40–45 min	-	-
Everolimus	mTOR inhibitors, selective immunosuppressants	-	Glioblastoma multiforme, astroctomas, oligodendrogliomas	958.224	~30 h	5.01	No

BBB: Blood brain barrier, DHFR: Dihydrofolate reductase, mTOR: Mammalian target of rapamycin, CNS: Central nervous system

BBB AND BRAIN TUMOR BARRIER

Brain Physiology

There are two types of glial cells: (i) Macroglias are composed of astrocytes or oligodendrocytes. Astrocytes surround tightly the cerebral microvasculature with their end feet forming a diffusion barrier, severely restricting permeation of hydrophilic entities including drug molecules. These cells are responsible for maintaining the barrier function and maturation of cerebrovasculature. Partially they are directly connected to the endothelial cells via gap junctions. Oligodendrocytes, the second class of macroglial cells, wrap around neurons in the white matter. These are responsible for production and maintenance of the myelin sheath, an insulating multilayer stack, and (ii) pericytes intimately embrace the brain capillaries and thus might contribute to the development, maintenance, and regulation of the BBB.[15]

Nerve fibers from peripheral nerve ganglia and intrinsic brain neurons regulate cerebrovascular tone resulting in functional "neurovascular units," which have an important role in maintaining a precisely regulated microenvironment for reliable neuronal activity. Due to the tightness of the endothelial barrier, paracellular transport of substances is negligible under physiologic conditions. Consequently, only nutrients and other lipid-soluble substances enter the brain by passive transcellular diffusion. Receptor-mediated transcytosis, of insulin and transferrin, or carrier-mediated endocytosis of glucose, amino acids, purine bases, nucleosides, choline lactate and other substances takes place leading to transcellular transport of hydrophilic material or entities.[16]

BBB and Blood Tumor Barrier

The presence of BBB is the major obstacle to passage of chemotherapeutic agents. The BBB is composed of the three barriers (i) the BBB formed by the cerebrovascular endothelial cells between blood and brain interstitial fluid, (ii) the choroid plexus epithelium between blood and ventricular cerebrospinal fluid (CSF), (iii) and the arachnoid epithelium between blood and subarachnoid CSF. The three barrier layers regulate and limit the molecular exchange at the interface between the blood and the neural tissue or its fluid spaces [Figures 1 and 2].[8]

BBB protects neurons from systemically circulating potentially cytotoxic agents. Cerebral microvessels are lined by the endothelial cells covering an array of passive and active features forming a selective barrier allowing the passage of only selective material.[17,18] Brain capillaries lack fenestration and have only a

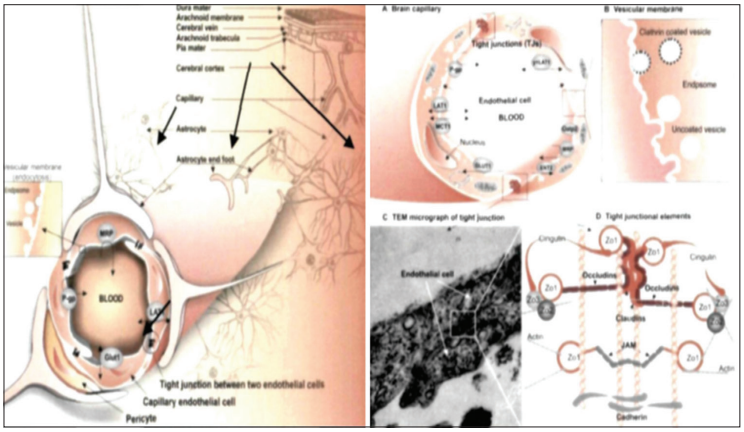

Figure 1: Blood brain barrier

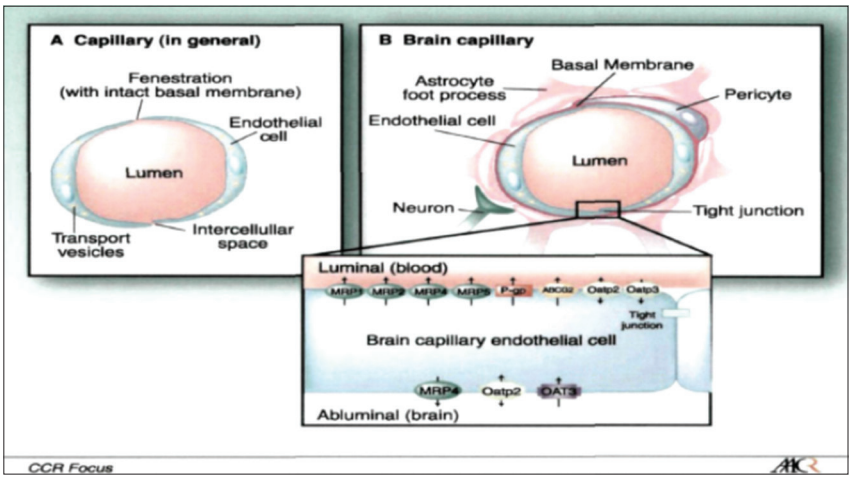

Figure 2: Efflux transporters expressed on blood brain barrier

few pinocytotic vesicles, but greater numbers and greater volumes of mitochondria than seen in a variety of peripheral tissue capillaries and other body cells. The BBB is formed by capillary endothelial cells, surrounded bya basal membrane and astrocytic perivascular end-feet. Tight junctions exhibited between endothelial cells force most molecular entities to take a transcellular route across the barrier, rather than paracellular route (seen in most endothelial cells).[19,20] Specific transporters present on the luminal and abluminal surface of the BBB endothelial membranes regulate the passage of small hydrophilic molecules, providing a selective "transport barrier." This protective mechanism permits the entry of required nutrients along with efflux of potentially harmful compounds.[21] Transport of hydrophilic molecules, if occurred is either by specific receptor-mediated transcytosis or by less specific adsorptive mediated transcytosis.[22] However, endocytosis and transcytosis takes place in the brain to a very low extent. BBB has several other roles to play not limiting to supply of essential nutrients to the brain and removal of waste products. It permits movement of only specific material and ions regulating the ionic traffic of BBB. Thus it provides protection against fluctuation in the ionic composition of the brain.[23] Large surface area and short diffusion distance between neurons and capillaries have a predominant role in regulating the brain microenvironment. Continuous turnover and fluid drainage enables clearance of large molecules and brain metabolites maintaining homeostasis of brain microenvironment.[24] Table 2 presents the list of few agents transported across the BBB along with their mechanistic pathway. From these, it is evident that BBB offers punitive action against exogenous materials including administered chemotherapeutic agents.

Brain capillaries are surrounded by or closely associated with several cell types, including the perivascular end feet of astrocytic glia, pericytes, microglia, and neuronal processes imparting them specialized barrier properties.[25]

Transport of materials required by the brain takes place by specialized transporters or enzyme

Table 2: Major transport systems for the passage of drugs across the BBB

Pathway	Mechanism	Example
Paracellular aqueous pathway	Between the endothelial cells	Water soluble agents
Transcellular lipophilic pathway	Across the endothelial cells through the cells	Lipid soluble agents
Transporter mediated endocytosis	Transporters present on the luminal and abluminal surface of the barrier for passage of certain nutrients, glucose etc.,	Glucose, amino acids, nucleosides, drugs like Cyclosporine A, azithromycin
Receptor mediated endocytosis	Identification of specific receptors on the membrane. Taken up by the receptor and transported across the membrane.	Insulin, transferrin
Adsorptive transcytosis/carrier mediated endocytosis	Carrier-mediated transport, uptake by the carrier of the drug at the luminal end of the barrier. Transport of the substrate along with the carrier. Carrier releases the substrate at the other end of the barrier.	Albumin, plasma proteins

BBB: Blood brain barrier

systems. However, these transporters possess neuroprotective function by their efflux mechanism; not allowing easy passage of exogenous material. Many chemotherapeutic agents are a substrate to these efflux transporters. For example, Astrocytes express localized transporters like P-gp and glucose transporter (GLUT) 1 and specialized enzyme systems along with the tight junctions. Hence, for better delivery of cytotoxic agents toward brain tumor; current strategies focus on either bypassing/ overcoming these efflux transporters.

Efflux Transporters

Along the BBB the efflux transporters such as P-gp, MRP's like MRP1, MRP2, MRP4, MRP5, GLUT1, Oatp2, Oatp3, ABCG2, etc., are expressed on the luminal side of the barrier offering resistance to the uptake of active components.

Pglycoprotein

It is an adenosine-5′-triphosphate (ATP) dependent efflux pathway encoded by multidrug resistance gene.

(Multidrug resistance protein [MDR] 1) and is located on the luminal side of the capillary membrane of the blood vessels. It offers resistance to the movement of chemotherapeutic agents against the concentration gradient extruding substrate back into the circulation.[16,26]

However, certain agents can be delivered for action against tumor along with efflux transporter suppressor or blockers. Verapamil, cyclosporine, erythromycin, ketoconazole, and tamoxifen are examples of agents that have demonstrated either *in vitro* or *in vivo* inhibition of P-gp function.[15,27,28] However, the relatively low P-gp inhibitory potency of these compounds is the major drawback to the use of these inhibitors in a clinical setting, requiring administration of high doses to inhibit P-gp function. At high systemic concentrations, the principal pharmacologic effect of these compounds (e.g., cardiotoxicity from calcium channel blockers, immune suppression from cyclosporine) is predominant leading to problems.[27] This limitation led to the development of novel (second-generation) compounds that exhibit less intrinsic toxicity at P-gp inhibitory concentrations than that of their "first-generation" counterparts.[29,30]

MRP Efflux Transporters

Similarly, expression of MRP family (MRP1 to MRP9) efflux transporters prevents passage of compounds through the barrier. Antharacyclines, vinca alkaloids, epidophylotoxins, camptothecin, and methotrexate, are few drugs which are substrates such transporters.[31] Breast cancer resistant protein (BCRP or ABCG2) has been detected in brain capillary endothelial cells of brain in humans. Drugs such as emethotrexate, mitoxantrone, topotecan, imatinib, erlotinib, and few others are substrates to BCRP or ABCG2 receptors.[16]

Organic Anion and Cation Transporters

Non-ATP dependent transporters; exchanges ions across concentration gradient from the blood to the brain or reverse. Thus, for successful transit into the brain parenchymatous region chemotherapeutic agent has to bypass or wins the battle over efflux transporters. Co-administration of efflux transporter inhibitors or suppressor will lead to success. However, it would be beneficial, if the delivery or carrier systems carrying the drug could by-pass or inhibit or suppress the efflux transporters

avoiding concomitant administration of suppressor and eventually their side effects. This would allow transport of the drug through the BBB without being a substrate to efflux transporters. One nanoparticulate based delivery system that was able to by-pass the P-glycoprotein mediated efflux system was poly oxy ethylene-poly oxy propylene block copolymer micelles decorated with insulin or antibodies as targeting moieties, developed by Kabanov *et al*.[32] The particles were found to accumulate in the brain in sufficient quantity. The probable reason for this action could be circumventing the multidrug resistance by overcoming and selectively inhibiting P-glycoprotein expression in tumor cells as postulated by Kabanov *et al*.[33,34] Nanoparticles (NPs) with above mechanism of action decreased the myocardial toxicity of doxorubicin in cancer treatment.[15]

Modulation of BBB in Glioblastoma Multiforme

Endothelial glial interactions are disturbed during several disorders, and thus the functions of BBB get interrupted. These alterations cause capillaries of the glial tumor/disorder condition to be leakier than those of normal tissue. Levels of inductive factors or permeability factors like vascular endothelial growth factor (VEGF) might also be upregulated in such conditions. In particular, to brain tumor downregulation of protein claudin 1/3 (maintaining the channel between two cells) has been reported.[35,36] Breakdown of the BBB in some cases of gliomas has also been reported by Doolittle.[5] The probable reason for this could be distribution and up-regulation of astrocytic AQP4 causing brain edema. Other genomic changes that occur in brain tumors are overexpression of oncogenes epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor (PDGFR) along with loss or mutation of tumor suppressor genes such as PTEN and p53, overexpression of oncogene EGFR and PDGFR is by following the RAS-MAPK and P13K/AKT pathway signaling pathway causing activation of MTOR/FRAP kinase pathway. Other important genes and proteins involved in brain tumor cell cyclecontrol, cell proliferation, programmed cell death (apoptosis)., angiogenesis, and invasiveness are bcl-2, protein kinase C-a, c-raf-1, protein kinase A Type 1, telomerase, MDM2, insulin-like growth factor 1 (IGF-1) and IGF-1 receptor, HER2 (official name ERBB2; encoded by C-ERBB2 gene), basic fibroblast growth factor (FGF), FGF receptor, transforming growth factor (TGF) alpha, TGF-P2, VEGF, integrins, and others. There are many agents being designed in the chemical laboratories either act as kinase inhibitors or inhibitors of PTEN/AKT or RAS/MAPK pathway. For example; Rapamycin and its ester inhibit the kinase activity. Understanding the molecular pathway of gliomas has opened gateways to the development of new molecular targeting agents for action against gliomas.[37]

Current Treatment Modalities Used for Gliomas

In the light of these findings; it is evident that in certain pathological conditions opening of the BBB occurs. An advantage of this knowledge is taken to deliver drugs across the BBB. The approach involves deliberate opening of the tight junctions of the BBB or delivery of drugs "through" or "behind" BBB by manipulating the physicochemical properties of the drug. BBB opening method involves the use of a hypersomolar agent (1.4 M mannitol) administered through intracarotid infusion followed by delivery of active agent**.** Chemical modification of BBB can also be brought about by administration of vasoactive compounds including bradykinin**,** interleukin, and leukotrienes. Intrathecal or intraventricular administration of drug or site administration (e.g., intratumor injection, gliadelwafer placed at the tumoral region) can also be performed for effective delivery. Convection-enhanced delivery is another method for delivery of agents to the brain**.** However, above mentioned methods have major drawbacks including the irreversible opening of BBB that potentially allows entry of exogenous material such as virus and bacteria and allows limited spatial distribution of the drug.[38]

Can Tiny Sized Particles Eradicate or Treat Gliomas?

Nanoparticulate based drug delivery systems have opened new avenues for treatment of gliomas and other types of brain tumors. Nano-carriers can be applied to medical treatment, imaging, diagnosis, drug delivery and tissue regeneration due to their promising nature. Due to small size, nano-metric material causes better permeability of therapeutic agentsinto cells compared to conventional therapy. Particles <200 nm can easily pass the blood-testis barrier due to the leaky vasculature and around 100 nm size surface modified particles can circumvent the barrier. A large number of compounds including antibodies, proteins, peptides, and chemotherapeutic agents have been delivered using NPs. NPs due to the small size and modifiability warrant their selective uptake by tumor cells. They can be formulated out of a variety of substance and can carry multiple loads of drugs directing the substance toward the tumor cells. Due to the feature of modifiability possessed by the NPs, they reduce the peripheral side - effects and increase the relative amounts of drug reaching the brain by crossing the barrier.[39] Thus, in comparison to conventional therapies, NPs reduces the side effects. This concept of nanoparticulate based drug delivery systems for treatment of brain tumors is discussed in following sections.

Biofate of Administered NPs Against Brain Tumors[15]

Post-administration; NPs get diluted with 4.7-5 L of blood. Once injected into the bloodstream; NPs move with an output speed of 0.3 m/s into the aorta coming from the heart and at a velocity of 0.6 ± 0.4 mm/s in capillary vessels**.** After that, NPs homogenously distributes over around 100,000 km of blood vessels and a total surface area of 8000 m^2 of vessel walls**.** Based on the size, extravasation of NPs occurs into the tissue, especially in the liver. NPs below 1 nm size range can extravasate freely through the tight junctions between endothelial cellsof the brain,[40] and particles with higher size range can pass due to receptor-mediated endocytosis (e.g., insulin, glucose, transferrin, etc.). In a brain of an average male subject (1.3–1.4 kg for an adult) NPs are distributed on a surface area of 100 cm^2/g brain tissue through brain microvessels, and an overall surface of 13 m^2 of the whole brain with an average intercapillary distance of 40 µm. Passage through the BBB is due to high vessel density and short intercapillary distance resulting in an almost immediate high concentration of all molecules within the brain. NPs are diluted in 120–150 ml of CSF, once they cross the 20–30 nm thick and extremely tight (trans-endothelial electrical resistance [TEER] >1500 £ >cm^2) endothelial cell layer of the BBB**.** Further, the NPs are diluted again, and distributed into 1.5 L of total brain volume (average adult)**.** After reaching the brain, NPs have to search and target a small subset of cancerous cells; an immensely challenging task. Destination reached NPs either are taken up by the glial cells or might accumulate in the semi-permeable basal membrane between the endothelial and glial cells or pass through the endothelial cells entering into CSF. Digestion of the NPs takes place due to the endocytotic-lysosomal pathway or may be replaced by other enzymes. Once the protective coating is removed, NPs release the active, trigger or inhibit cellular pathway or escape in the cytosol and interfere with transport mechanisms, DNA condensation, or mitochondrial functions or the cytoskeleton.[41,42]

Predominant Nanoparticulate Drug Delivery Systems Used for Treatment of Gliomas

Nanoparticulate drug delivery systems that are currently under investigation for treatment, diagnosis of gliomas include liposome, polymeric NPs, quantum dots, polymeric micelles, and many others. Surface engineered liposomes designed by attaching ligands, carriers, and other entities make them act as a cargo system for drug delivery within the brain.[43] These are suitable systems to deliver the drug across the barrier because of the compositional similarity of liposome and endothelial cells of the barrier. Endothelial cells of the barrier and astrocytes of the brain are composed of phosphatidylinositol, phosphatidylserine, phosphatidylcholines, and phosphatidylethanolamines. First attempts to use liposomes for active brain targeting were made by conjugating murine 0 × 26, the monoclonal antibody to PEGylated liposomes for targeting the rat transferrin receptor. Compositional similarity between solid lipid NPs (SLN)/nanostructured lipid carrier systems (NLC) and brain cells lead to its use.

Further study on the composition of endothelial cells indicates that the astrocytes are also composed of polyunsaturated fatty acids, promoting the passage of hydrophobic agents, thus SLNs and NLC (lipid composition makes delivery system hydrophobic). The flexibility offered by solid lipid nano-carrier systems and NLC in choosing the lipids make them promising delivery system for therapeutic agent delivery and brain tumor imaging. SLNs loaded with anticancer drugs such as camptothecin, doxorubicin and paclitaxel have been studied for their potential brain delivery.

Nanoemulsions can be formed by dispersing drugs in the above oils and emulsifying them with the aqueous phase. One of the proposed mechanisms for uptake of nano-emulsions is that they diffuse across the BBB and reach brain cells, without the involvement of transporters. Astrocytes and endothelial cells of the brain demonstrated expression of linolenic acid, eicosapentaenoic acid and dosapentanoic acid.[44]

Micelles due to their smaller size in the range of 10–50 nm are not taken up by reticuloendothelial system (RES) system and may circulate in the blood for longer period; they exhibit stability in plasma and flexibility of modification with targeting ligands. As compared to other delivery systems their small size makes them pass through the barrier easily. Incorporation of molecules into micelles can effectively enhance drug solubility and improve drug pharmacokinetics and biodistribution.

Dendrimeric delivery systems resemble dendrites of the neurons. The high level of control on size, branching density, surface functionality, high drug payload, targeting potential, and good colloidal, biological, and shelf stability makes dendrimers ideal carriers in the field of brain drug delivery of anticancer agents.[45] Further, decoration of dendrimers with ligand allows targeting of cancerous cells; thus passage through the BBB and active targeting to tumors.

Polymeric NPs are sub-micron sized colloidal particles (100–500 nm) with a therapeutic agent of interest encapsulated within their polymeric matrix adsorbed or conjugated onto the surface. They are promising drug delivery systems due to their capacity to release the loaded drug, relatively

high intracellular uptake, biodegradable nature, and improvement in stability of the drug.[46] The luminal and abluminal protein composition of the capillary endothelial cells of the BBB is different; indicating protein requirement of the cells. NPs of these protein structures loaded with the drug can be prepared and administered. These protein NPs might be mistaken for endogenous proteins and thus can cross the BBB. Further anchoring of these particles with endogenous nutrients such as glucose, cholines, amino acids, and monocarboxylic acids promotes passage through the barriers with increased concentrations on the other side of the barrier.

Nanoparticulate based drug delivery system using active targeting or passive targeting strategy for treatment of brain tumors are being investigated by many research groups.

HOW NPS WORK TO COMBAT GLIOMAS?

NPs take advantage of leaky vasculature and angiogenesis effect of cancer cells allowing passive diffusion of administered NPs. Cancerous cells are in the rapid stage of dividing, and during the division stage, they develop their own new blood vessels capable of supplying them nutrients. However, due to rapid multiplication, blood vessels with incomplete normal physiology are developed leading to leaky vasculature allowing permeation of larger-sized particles. This effect is termed as "enhanced permeation and retention effect" (EPR). This enhanced permeation effect (EPR) enables transport of many drugs loaded in cargo with size <100 nm at the primary tumor site.[47] Delivery of drug through the EPR effect and tumor microenvironment is termed as passive targeting. Transport of drug delivery system into surrounding tissue and interstitial space following systemic administration can be avoided by targeting the cancerous cells using substrate specific NPs. Active targeting is selective targeting that involves decorating nanosized particles with agents that will enhance selective uptake of NPs by cancer cells. These agents could be carbohydrates, proteins, acids, bases etc., depending on the receptors expressed by the cancerous cells. These decorative agents act as ligands for the specific substrate/receptor expressed on the surface of cells; thus promoting preferential uptake. NPs can also be multifunctional; where different agents are attached to the same surface. One out of these ligands promotes passage through BBB by receptor-mediated endocytosis (RMT) (e.g., insulin, transferrin) and the others promote preferential uptake by neoplasms (e.g., folic acid, alanine). NPs actively targeting the tumors interact with biomolecules on the cell surface and inside the cells that necessarily do not alter the behavior and biochemical properties of the molecules**.** Thus, gliomas may be combated by using active and passive targeting nanoparticulate based drug delivery systems.

Passive Targeting

BBB is marked with high TEER; however pathological conditions such as gliomas, multiple sclerosis, AIDS, ischemia or Alzheimer's lead to loss of TEER thus BBB integrity. This pathologic effect along with inflammation and edema opens a new and direct route to deliver the NPs through BBB. Perhaps, through an "EPR effect" passive diffusion of NPs is possible. Passive targeting of cancerous cells using NPs is represented in Figure 3.

Particles intended for passive targeting of tumors should exhibit some general characteristics. The particles should possess size below 200 nm and should be hydrophobic in nature for better permeation through the barrier. There are several research groups who have prepared such NPs and studied their activity against gliomas. Most of the particles follow general characteristics for better passage across the BBB. However, micelles have odd characteristics for this purpose since they have a particle size of 400 nm. The designed particles are in the clinical study and the probable reason for the effectiveness of such particles could be hydrophobicity of the polymer used for designing of the particles.

Performance of particles designed for passive targeting can be modulated by adsorption of solubilizer/stabilizer such as polysorbate 80, poloxamer's of various grades[48] polyethylene glycols (PEGs) and poloxamines. These polymers not only prevent RES uptake but also prevent endosomal uptake of the particles. This is significance for particles intended for long circulation and targeting to a particular organs. NPs with such coating for passive targeting of gliomas have been fabricated by various researchers and studied in detail. For an example NPs designed to coat with polysorbate 80 and such particles could enter the brain by the adsorption of apoliproteins; thus behaving like low-density lipoprotein (LDL) molecules. For the similar type of particles coated with polysorbate 80; one of the groups[49] has used polysorbate 80 as an agent for the opening of BBB allowing passage of drugs. Hence the mechanism of action of polysorbate 80 coated NPs remains debatable, but passive targeting is one of the possible mechanism by which this particles permeate.

Alternatively, transport may be realized by transcytosis of the NPs loaded with drug across the endothelial cells. NPs have better penetrability of polymeric NPs loaded with an anticancer agent

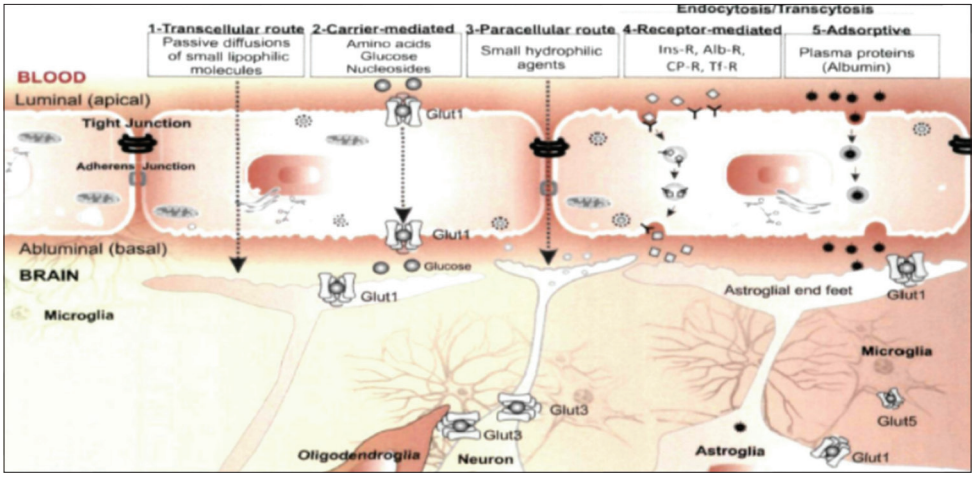

Figure 3: Various mechanisms for transport of material across the blood-brain barrier

through BBB after coating with polysorbate 80 for glioma treatment**.** Systems mimicking LDL would be beneficial for the treatment of Gliomas since LDLs are overexpressed in Gliomas**.** However, the mechanism of action of these particles should be investigated in detail to avoid any discrepancy of the opening of BBB. Since BBB opening if occurs and is irreversible in nature; the protective nature of the BBB may be hampered. Further; researchers should identify stabilizers and solubilisers avoiding damage to the intact barrier and maintaining the defensive function of the barrier.

Active Targeting

Another strategy under investigation avoids damage to the non-cancerous cells by chemotherapeutic agents. This strategy exploits and utilizes the overexpressed receptors on the tumor cells. A specific marker/target/receptor expressed on disease-associated cells at a much higher level than on normal cells. Agents that can potentially target these receptors are attached to the carrier system enabling the cargo to specifically deliver the drug to cancerous cells due to binding of Ligand to the overexpressed receptors. Such targeting agents could be antibodies used against an antigen residing on the target tissue or could be a ligand for receptors or transporters, expressed on the cancerous cells. Such agents may be directly conjugated to the drug or to the vector like a nanoparticulate device through the suitable covalent bond in corporate via a chemical reaction. Active targeting is an energy-dependent procedure allowing passage of drugs alongside the membrane against the concentration gradient. The use of nano-carriers could improve the bioavailability of drugs after their intravenous administration and penetration into the brain through different delivery systems: Transporter-mediated endocytosis (carrier-mediated transport), receptor-mediated transport, and adsorptive mediated endocytosis. As detailed by Jullieart-Jeanneret most of the targets in brain cancers are molecules associated with enhanced angiogenesis or increased nutrient demand to the tumors.[50] Monocarboxylic acid, glucose, amino acids, oligonucleotides, cationic peptides or transferrin conjugates are potential agents that can be used as ligands for transport of systems through the brain**.** This Ligand could be specific to receptors expressed by tumors or BBB or both thus allowing selective or sandwich targeting.

Transporter Mediated Endocytosis

Certain peptides and small molecules can cross the BBB; though large in size (molecular weight above 600 Daltons) due to expression of the transporters on the luminal or abluminal side of the barrier. These agents are required by the body as they are either nutrients or necessary agents for brain function. These agents when used as ligands and adhered on NPs; can allow passage through BBB, moreover, if these ligands can specifically target tumors then they can become tumor-specific and serve as dual acting ligand attached NPs. Folic acid, proteins, peptides, glucose and some fatty acids can be transported by this transport mechanism. Folic acid receptors are overexpressed on the BBB and also on brain tumors. A lipophilic prodrug, paclitaxel oleate (PO) was loaded into these NPs, and such particles are referred to as nano LDL-PO (nLDL-PO). Cell viability action of these NPs on glioma cell lines was found to be time, concentration, and cell line dependent. To confirm the mechanism of action nLDL-PO, suramin an LDL receptor inhibitor was used and the expression of LDL was blocked. Low uptake of the designed NPs by the cell after treatment indicates that the drug was delivered through the LDL receptor. Thus, active targeting of gliomas tumors through LDL receptors through Ligand attached NPs receptors could be achieved. Similarly; nanohybrids composed of camptothecins loaded micellar systems encapsulated

in NPs of magnesium-aluminum layered double hydroxides (LDHs) by an ion exchange process were fabricated by Tyner *et al*.[51] These rapidly dissolving nanohybrids when tested against glioma cells lowered the survival time compared to untreated cells, or to cells incubated with the surfactant, the pristine LDH, or water (delivery medium). Modification of the surface due to LDH provided direction to nanohybrids.

This suggested the possible use of such hybrids for a variety of drug therapies.[51] Such formulations take the advantage of transporters present on the surface of BBB for passage of nanomaterials. A few considerations to be made before administration of NPs *in vivo* include kinetics for the transport of the molecule, structural binding of the compound with the receptor, covalent attachment of the cargo system with the ligand and maintenance of activity of the drug after dissociation of the ligand and vector. This is of significance because the designed NPs could be a substrate to endosomes and hence be directly presented to lysosomes without the release of the drug in the cytosol of the cancer cells.

RMT

Large molecules required by the brain for normal functioning are transported by the receptors at the luminal side of the brain followed by movement through endothelial cells and then its exocytosis. This physiological approach involves targeting the receptor expressed on BBB by specific ligands, modified ligands, and antibodies. Transferrin receptor, insulin receptor, and diphtheria toxin receptors are a few receptors overexpressed by BBB. Antibodies, transferrin, insulin, apolipoprotein, ferritin, macroglobulins, Amyloid precursor proteins, Plasminogen Activator Inhibitor coated/conjugated NPs primarily use RMT mechanism. The advantages of using such ligands are that it makes the material more substrate specific; i.e., it becomes more site-specific and also reduces the toxicity/side effects to other cells due to their site specificity. The survival rate of the animals was enhanced indicating better efficacy of the developed formulation. Du *et al*.[52] have prepared lectins (wheat germ agglutinin [WGA]) modified liposomes and have been tested against glioma-induced model. Survival rate enhancement extended by WGA decorated particles was much higher than those particles whose surface is modulated by transferrin. The probable reason for this could be the competition experienced by the transferrin decorated NPs with the endogenous transferrin found in plasma.[53] This study indicates that competitive binding between molecules for the same receptor can be a major obstacle for effective delivery of ligand decorated particles to the target organ. Hence ligands that do not undergo competitive binding with the endogenous material for a particular receptor have been identified. He *et al*.[54] fabricated multifunctional dendrimers decorated with transferrin and WGA for dual targeting action against glioma. In such cases, one of the ligand will enhance passage through BBB and other would target the tumor cells. Authors have proved action against the C6 spheroid model, however additive effect/synergistic action *in vivo* by the ligands is yet to be demonstrated.

Monoclonal antibodies against the receptors expressed on the gliomas can be used for targeting.

The nanoparticular approach is used to deliver genetic material as well; making the therapy more effective against tumors. An attempt has been made to deliver siRNA by targeting the EGFR receptors through antibodies resulting in increased survival of intracranially implanted brain tumors in mice.[53] Antibodies against transferrin receptor have also been used to avoid competition with endogenous transferrin. 0 × 26 conjugated liposomes were the first attempted delivery systems used for targeting the brain using the antibody tagging methodology.[43] Similarity, integrins and matrix metalloproteinases (MMP's) can be targeted for treatment of Gliomas; since these are some mediators expressed in tumors for angiogenesis. Angiogenesis is one of the hallmarks and indicator of the degree of malignancy**.** Glioma progression is marked by expression of metalloproteases and integrin. Extracellular matrix hindering the easy movement of cancerous cells is degraded by MMP's, and further attachment of neoplastic cells to the new site is presented by integrins. MMP's are located on angiogenic endothelial cells and not on normal endothelial cells; thus are possibly good targets to inhibit metastasis of gliomas.

Targeting of MMP1 has been achieved by attaching mABs specific to MMP1 onto the surface of liposomes loaded with doxorubicin**.** Integrins can be targeted by using specific antibodies attached to the vectors.

VEGF receptor is another interesting site for the action of NPs since VEGFRs are upregulated in gliomas**.** Dendrimers inhibiting the growth of gliomas via VEGFR targeting have been demonstrated good activity in the cytotoxicity assay. Insulin receptor, diphtheria toxin receptor and LDL receptors are few other receptors that can be targeted with NPs for action against the tumor. Peptides or proteins (low molecular weight) binding to the above receptors can be fabricated and attached to the vector for action. Such proteins and peptides need to be fully characterized for their binding and conformational agreement with the receptors.

Further degradation of the proteins and peptides might raise issues related to toxicity and loss of target

specificity/action. Designing of receptor targeted NPs involves issues like optimal binding between the particle and receptor that needs to be addressed. Optimal binding between the particle and receptor with a low dissociation constant should be exhibited. Also after the entry into the brain; diffusion of the particles is slow and limited; hence such receptors should be exclusively expressed by the tumors or in close vicinity for better efficacy and action. Further, the accumulation of these antibodies should not exhibit any toxicity within the body because of antigen-antibody reactions.[15]

Adsorptive-Mediated Transcytosis

Adsorptive-mediated transcytosis is another modality through which NPs can be delivered for treatment of glioma. Endocytosis of the charged particles occurs by a mechanism similar to RMT. Adsorptive mediated endocytosis occurs through electrostatic interaction mainly for peptides and proteins (due to an isoelectric point). The charge(basicity of the compound) and the C-terminal are the major determinants of the AME system.

Chitosan, cationized bovine serum albumin is a few entities used for targeting NPs tobrain using the AME mechanism.

Lu *et al*.[44] have investigated the effect of cationic bovine serum albumin (CBSA) conjugated to PEG-polylactic acid as carrier system loaded with methotrexate. To assess the brain delivery property, CBSA was conjugated in different concentrations to get varying CBSA density. Brain clearance was assessed using coumarin-6 as a fluorescent probe in mice. Increased BBB permeability was observed and correlated with CBSA density and surface area of NPs. Conjugated system exhibited two folds higher concentrations in the brain compared to the unconjugated system. The probable mechanism for passage of these particles across the BBB could be adsorptive mediated transcytosis.

Specifically fabricated NPs by various research groups for active and passive targeting of tumors have all demonstrated permeation through BBB, better action against *in vitro* cell linemodels compared to free drug and enhanced survival of the brain tumor-induced animals demonstrating the promising nature of NPs as treatment modality for brain tumors especially gliomas. There are still a few future challenges for glioma treatment.

Intranasal Delivery: An Approach to Bypass BBB

Number of drug delivery strategies has been developed to overcome challenges presented by the BBB. One technique that holds promise for bypassing the BBB to deliver drugs to the brain and eliminating the surgical risk and the spillover effect of the drug to normal tissue is intranasal delivery. The intranasal delivery provides a practical, non-invasive method for delivering therapeutic agents to the brain because of the unique anatomic connections provided by the olfactory and trigeminal nerves.

Intra-nasally administered drugs reach the brain parenchyma, spinal cord, and CSF within minutes by using an extracellular route through perineural and/or perivascular channels along the olfactory and trigeminal nerves without binding to any receptor or using axonal transport. In addition to bypassing the BBB, advantages of intranasal delivery include rapid delivery to the CNS, avoidance of hepatic first-pass drug metabolism, and elimination of the need for systemic delivery, thereby reducing unwanted systemic side effects. Intranasal delivery also provides painless and convenient self-administration for patients. These are the features that encourage the use of intranasal route for delivering therapeutic agents into the CNS.[55] The difficulties that need to be overcome include an enzymatically active, low pH nasal epithelium, the possibility of mucosal irritation and large possibility of variability caused by nasal pathology. However, the obvious and most important advantage of this method is that it is non-invasive relative to other strategies.

Future Challenges

NPs have definitely opened new gateways for diagnosis, prognosis, and therapy of brain tumors as suggested by the ongoing research. However, the fact that none of these nanoparticulate drug delivery systems are in clinical trials against brain tumors raises an alarm toward the depth required in the ongoing research. NPs by-passing the BBB -by taking advantage of the leaky vasculature of the tumor can be designed and administered for treatment of gliomas. Designing of such NPs involves sound knowledge of the physicochemical properties of the drug and the excipients being used. Developed formulations need to be thoroughly characterized using well established predictive tools. Mechanism of degradation of the polymer needs to be investigated with further toxicity studies on the residual monomer units formed within the body with knowledge of mechanistic insights. There is more of in-depth research required to search for new biomarkers of brain tumors that can be achieved by understanding the molecular biology of Gliomas. Research may help in defining new receptor targets or molecular targets for brain tumors. Further, ligands those are non-toxic and non-immunogenic and specific to these receptors allowing the transport of the molecule across the barrier need to be fabricated. Further, structural binding of the ligand to these receptors, maintenance of activity and dissociation rate should be investigated. For a

drug delivery developer, controlling the size, shape, and surface characteristics of the NPs would be a challenge. The designed NPs should retain their characteristics until the time of administration calling out for a robust formulation. Another concern with the NPs is the bio-fate of the administered NPs. The absorption, distribution, metabolism and excretion pattern of the NPs needs to be completely studied and confirmed before human administration. This would involve studying the pharmacokinetics profiles and toxicity behavior of the developed NP delivery system on acute and chronic administration. These kinetic profiles need to be correlated well with the dynamic profiles of the delivery system. Correlating the available information with the target reachability and action on gliomas would give us better insights. A balanced interdisciplinary research amongst the formulators, analysts, toxicologist and chemist would enable the design of nano-based drug delivery systems that might reach the doorstep of clinical trials. However with the ongoing research; it can be postulated that NPs play a future role in the delivery of novel and conventional drugs in novel form for treatment of brain tumors.

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