

Nanotechnology in Brain Targeting

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ABSTRACT: Nanotechnology offers a vision for a 'smart' drug approach involving the design, synthesis, and characterization of materials and devices that have a functional association in nanometer scale. One area in which nanotechnology may have a significant clinical impact in neuroscience is the selective transport and delivery of drugs and other small molecules across the strictest barrier *in vivo*, the blood brain barrier (BBB). Therefore, various strategies have been proposed to improve the delivery of different antineoplastic drugs, oligonucleotides, genes, and magnetic resonance imaging contrast agents to this tissue. This review deals in brief about the status of the BBB, different pathologies of brain like neurodegenerative, cerebrovascular and inflammatory diseases. The first part of this article aims to review the strategies developed to circumvent the BBB and deliver drugs into the brain. The use of nanotechnology and advancements are discussed especially in the crucial part of the review manifesting their potentiality as non-invasive technique which overcomes the blood-brain barrier limiting characteristics in the delivery of drugs with the possibility to target specific brain tissue decreasing peripheral toxicity.

KEY WORDS: Blood Brain Barrier; Brain Cancer; Targeted Drug Delivery; Colloidal Carriers; Nanoparticles; Liposomes.

Introduction

Drug delivery to the brain is a challenge, because this tissue benefits from a very efficient protective barrier. The same mechanisms that protect the brain from foreign substances also restrict the entry of 95 % of potentially active therapeutic moieties. The blood brain barrier (BBB) is the major barrier to the passage of active molecules from the blood compartment to the brain. It is located at the level of the brain capillaries, where there is a convergence of different cell types: endothelial cells, pericytes, astrocytes and microglia (perivascular macrophages). The brain microvessel endothelial cells (BMEC) that form the BBB, display important morphological characteristics such as the presence of tight junctions between the cells, the absence of fenestrations and a diminished pinocytotic activity, that together help to restrict the passage of compounds from the blood into the extracellular environment of the brain.

Among the properties of colloidal particles that make them ideal candidates for recognizing and treating brain diseases, their ability to deliver a wide variety of payloads across the blood-brain barrier is perhaps the most important. Understanding how some nanoparticles achieve this special "permission" to enter the brain requires a closer look at how the blood-brain barrier works. The blood-brain barrier permits the exchange of essential nutrients and gases between the bloodstream and the brain, while blocking larger entities such as microbes, immune cells and most drugs from entering. This barrier system is a perfectly logical arrangement, since the brain is the most sensitive and complex organ in the human body and it

would not make sense for it to become the battleground of infection and immune response.

This biological "demilitarization zone" is enforced by an elaborate and dense network of capillary vessels that feeds the brain and removes waste products. Each capillary vessel is bound by a single layer of endothelial cells, connected by "tight junctions", thereby making it very difficult for most molecules to exit the capillaries and permeate into the brain. Outside of the central nervous system, capillaries have fenestra (the latin for "window"), which are the cracks between the cells in the vessel wall. Both small and large molecules and even cells can leave the capillary and enter into the surrounding tissue. Instead of "leaking" material, brain capillary walls closely regulate the flow of material using molecular pumps and receptors that recognize and transport nutrients such as glucose, nucleosides, and specific proteins into the brain. In other words, substances need to be pre-recognized to enter.

Tight junctions provide significant transendothelial electrical resistance (TEER) to BMEC and impede the penetration of potential therapeutic agents such as oligonucleotides, antibodies, peptides and proteins (Lo et al., 2001). Furthermore, BMEC express a variety of enzymes, both cytosolic and on the extracellular membrane which also contribute to the restrictive nature of the BBB (Bodor and Buchwald, 1999). P-glycoprotein (P-gp) is also present in the luminal plasma membrane of BMEC. This is an ATP-dependent efflux pump and a member of a family of intrinsic membrane proteins. P-gp is known to prevent the intracellular accumulation of an extensive variety of chemotherapeutic agents and hydrophobic compounds (Terasaki and Hosoya, 1999). Under normal conditions the BBB acts as a barrier to toxic agents and safeguards the integrity of the brain. Nevertheless, several disorders and

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diseases can affect the brain leading to some loss of BBB integrity.

The major neurological diseases affecting the brain may be categorized as neurodegenerative, cerebrovascular, inflammatory (infectious or autoimmune) and cancerous (see Table 1).

The challenges of CNS drug development

Effective drugs have not been developed for Most CNS disorders. Majority small molecule drugs do not cross the BBB. Of over 7000 drugs in the comprehensive medicinal chemistry (CMC) database (see Table 1), only 5% of all

drugs treat the CNS, and these CNS active drugs only treat depression, schizophrenia, and insomnia (Ghose et al., 1999). The average molecular mass of the CNS active drug is 357 Dalton. Studies shows that only 12% of drugs were active in the CNS, but only 1% of all drugs were active in the CNS for diseases other than affective disorders (Lipinski, 2000).

Limitations for drugs to enter in to CNS

Drug may be transported through the BBB by passive or active transport. Transport routes across the BBB are summarized in Fig. 1.

Table 1. The Comprehensive Medicinal Chemistry database shows that only 5 % of more than 7000 small-molecule drugs treat the CNS, and these drugs only treat four disorders: depression, schizophrenia, chronic pain, and epilepsy (Ghose et al., 1999; Lipinski, 2000). Only few effective small or large molecule drugs are there for the majority of CNS disorders, with the exception of Parkinson's disease, e.g., L-DOPA, and multiple sclerosis, e.g., cytokines.

CNS disorders treatable with small molecule drug therapy	CNS disorders largely refractory to small molecule drug therapy
Depression Schizophrenia Chronic pain Epilepsy	Neurodegenerative diseases (Alzheimer's, Huntington's, Parkinson's disease*), Inflammatory diseases (Amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), Neuro-AIDS, Brain Cancer, Stroke, Brain or Spinal cord Trauma, Autism, Lysosomal storage disorder, Fragile X syndrome, Inherited ataxias, Blindness), Cerebro vascular disease.

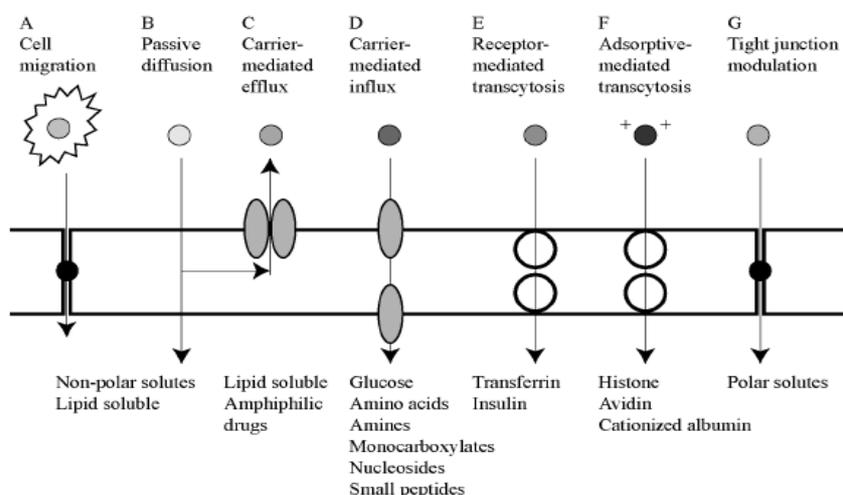


Fig 1. Transport routes across the blood brain barrier (BBB). (A) Leukocyte movement across the BBB. (B) Molecules may diffuse across the BBB by passive diffusion through the cell membranes and across the endothelial cells. (C) Active carrier-mediated efflux transporters to expel a wide range of molecules out of the BBB (D) Necessary polar metabolites and nutrients are carried into the CNS by transporters in the BBB. (E) Macromolecules, proteins and peptides are transported by receptor-mediated transcytosis (F) Some macromolecules like cationic macromolecules, induce adsorptive-mediated transcytosis. (G) To allow an increased movement of polar solutes, tight junctions may be modulated through the aqueous paracellular pathway. Adapted from (Begley and Brightman, 2003).

Passive Transport: Regarding the passive transfer through the BBB, the main factors that intervene are ionization of the drug, molecular weight, lipophilicity, and protein binding. Transport through the BBB is decreased in case of ionization of acidic compounds. Ionization of basic groups has no significant effect. Drug transport through the BBB is inversely proportional to its molecular weight. It has been demonstrated that molecular weight may be a limiting factor for values >600 daltons. Lipophilicity is probably one of the most important factors conditioning brain uptake of drugs. Transport of a drug through the BBB is generally, directly proportional to its lipophilicity. However, too high values of lipophilicity may decrease the rate of transport due to trapping of the compound inside the membrane. Values of log P falling between -0.2 and 1.3 have been described as optimal for the cerebral transport. Within these values, cerebral transfer depends on blood flow and the permeability coefficient. Moreover, a good correlation has been described between log P and the permeability coefficient as long as the molecular weight remains <800 daltons. Another important factor involved in drug transport through the BBB is protein binding. Logically, due to the size of the protein-drug complex and the characteristics of the BBB, only the free fraction of the drug should be transported through the BBB, even though this theory and protein binding as limiting factor have been questioned. Several articles have shown that the fraction of a drug that is transported through the BBB is higher than the free fraction. Different hypotheses have been suggested such as the dissociation of the protein-drug complex in the microcirculation following the binding of the protein to a specific endothelial receptor.

Active Transport: For some drugs, transfer through the BBB may be higher or lower than that expected from the chemical and physical properties. A facilitated or an active transport can be responsible for a higher rate of transfer. In case of lower rate of transfer, efflux proteins may be involved.

Strategies for drug delivery to the brain

The diffusion of drugs from the blood into the brain counts primarily upon the ability of the biologically active molecule to cross lipid membranes. Several drugs do not have adequate physicochemical characteristics such as high lipid solubility, low molecular size and positive charge which are essential to succeed in traversing BBB. This is the rationale behind development of several strategies to overcome the BBB including invasive and non-invasive techniques.

A Invasive techniques

Disruption of the BBB

The earliest technique to circumvent the BBB for therapeutical purpose and the first to be used in humans

was developed by Neuwelt *et al.* (1979). The thought behind this approach was to break down the barrier momentarily by injecting mannitol solution into arteries in the neck. The resulting high sugar concentration in brain capillaries takes up water out of the endothelial cells, shrinking them thus opening tight junctions. The effect lasts for 20–30 min, during which time drugs diffuse freely, that would not normally cross the BBB. This method permitted the delivery of chemotherapeutic agents in patients with cerebral lymphoma, malignant glioma and disseminated CNS germ cell tumors (Miller, 2002). Physiological stress, transient increase in intracranial pressure, and unwanted delivery of anticancer agents to normal brain tissues are the undesired side effects of this approach in humans. Additionally, this technique requires significant expertise for administration. Beside, vasoactive molecules such as bradykinin (Bartus *et al.*, 1996), leukotriene C4 (Hashizume and Black, 2002) and cereport (Borlongan and Emerich, 2003) have been employed to increase the permeability of brain tumor capillaries. This biochemical modulation strategy affects selective increase in blood–brain tumor barrier (BBTB) permeability to anticancer drugs without affecting the normal BBB. It is based on the discrepancy of properties between the BBB and the BBTB, because the normal brain capillaries are rich in g-glutamyltranspeptidase (g-GTP) which acts as an enzyme barrier, rapidly degrading the leukotriene – whereas receptors against bradykinin (BK type 2) are lacking which limits the penetration of this molecule in the BBB (Black and Ningaraj, 2004). A successful reduction in tumor volume and stabilization of tumor growth was noticed in gliomas, when Cereport has been co-administered with active agents such as carboplatin (Cloughesy *et al.*, 1999). Though, disrupting the BBB even for brief periods allows the brain vulnerable to infection and damage from toxins. Even substances such as albumin that circulate safely through the peripheral bloodstream can have harmful effects if they enter the brain (Miller, 2002).

Direct drug delivery

To overcome the BBB in clinical trial the drugs were administered directly by intraventricular and intracerebral routes, using a plastic reservoir implanted subcutaneously in the scalp and connected to the ventricles within the brain by an outlet catheter (Chauhan, 2002). Unfortunately, there are several problems like insufficient concentration of drug may reach the target site, secreted interstitial fluid flow works against diffusive drug penetration, the high turnover rate of the CSF (total renewal every 5–6 h in humans) continuously clears injected drug back into the blood apart from the surgical intervention required. In practice, drug injection into the CSF is a suitable strategy for sites close to the ventricles only. For drugs that need to be at elevated levels for long periods for an effective action, continuous or pulsatile infusion may be necessary (Chamberlain *et al.*, 1997).

In terms of pharmacokinetic characteristics determining brain tissue concentration intracerebral drug administration differs from systemic drug administration, where the available dose reaching the target organ is 100% (Grondin et al., 2003). Still, there are large gradients inside the tissue with very high local concentrations at the site of administration and zero concentrations at some distance for macromolecules (Misra et al., 2003).

Alternative methods to CNS drug delivery

Intracerebral implantation of controlled release systems

Brain interstitium can be directly delivered with drug by using polymeric devices which release unprecedented levels of drug directly to an intracranial target in a sustained fashion for extended periods of time. The fate of a drug delivered from the biodegradable polymer was based on: (a) rates of drug transport via diffusion and fluid convection; (b) rates of elimination from the brain via degradation, metabolism and permeation through capillary networks; and (c) rates of local binding and internalization (Guerin et al., 2004). The feasibility of polymer-mediated drug delivery by using the standard chemotherapeutic agent 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and local treatment of gliomas by this method is effective in animal models of intracranial tumors. This led to clinical trials in glioma patients, and subsequent approval of Gliadel™ by the FDA (Wang et al., 2002). A new concept of drug targeting into the CNS by stereotactic implantation of biodegradable microspheres was tried (Benoit et al., 2000). Because of their size, these microparticles can easily be implanted by stereotaxy in functional areas of the brain without damaging the surrounding tissue.

Open surgery is not required for implantation of microparticles, compared to large implants. The viability of microencapsulation of glial cell line-derived neurotrophic factor (GDNF) (Aubert-Pouessel et al., 2004; Jollivet et al., 2004) and 5-fluorouracil (5-FU) (Fournier et al., 2003a, 2003b) has enabled local delivery into neurodegenerative lesions and brain tumors, respectively. A phase I pilot study of the local and sustained delivery of 5-Fluorouracil (5-FU) was carried out in eight patients with glioma and microspheres were implanted in the tumor after a complete macroscopic surgical resection. Patient median survival was doubled in this case (Menei et al., 2004) as the diffusion distances for drugs from microspheres are much reduced. However, this invasive technique has been associated with increased risk of infection and high neurosurgical cost (Abbott and Romero, 1996).

B. Non-invasive techniques

Non-invasive techniques of delivery may be of a chemical or biological nature.

Chemical methods

The prodrug approach was involved in chemical methods, to improve some deficient physicochemical property, such as membrane permeability or solubility. For example, esterification or amidation of hydroxy-, amino-, or carboxylic acid-containing drugs, may enhance lipid solubility and enter into the brain easily. The conversion to the active form is realized generally, by an enzymatic cleavage.

A possible choice for CNS prodrugs is to link the drug to a lipid moiety, such as a fatty acid, a glyceride or a phospholipid. Such prodrug approaches were investigated for an array of acid-containing drugs, like levodopa (Misra et al., 2003). In order to target the diseased site and to release the active compound in this environment, exploiting the pH or an enzymatic process may be theoretically possible, but in the pathological conditions there are some modifications in enzymatic concentration or pH provoking the possibility of reactive metabolites.

Other problems associated with prodrugs are the poor selectivity and poor tissue retention of some of these molecules (Davis, 1997). Increasing lipophilicity also tends to increase the rate of oxidative metabolism by cytochrome P450 and other enzymes. The increased lipophilicity may improve diffusion movement across the BBB, increasing uptake into other tissues, causing an increased tissue burden (Temsamani et al., 2000; Misra et al., 2003).

Biological methods

Biological approaches involve the conjugation of a drug with antibodies which can then be directed towards an antigen residing on or within the target tissues. Antibodies to transferrin receptor (TfR) like the OX26 antibody, the 8D3 MAb or the R17-217 MAb were able to undergo receptor-mediated transcytosis across the mouse BBB via the endogenous TfR (Pardridge, 2002). It is difficult to find target tissues unfortunately, bearing specific antigens that will provide a distinct targeting effect. For example, in cancer chemotherapy tumor specific antigens are rare: tumor-associated antigens can be present not only within the target tissue but also elsewhere in the body. Other biological methods for targeting utilize ligands in the form of sugar or lectins, which can be directed to specific receptor found on cell surfaces (Davis, 1997).

An alternative route of administration

An alternative route to CNS drug delivery is the intranasal administration, which offers rapid absorption to the systemic blood preventing first-pass metabolism in the gut wall and the liver. This route has been shown to be a safe and acceptable alternative to parenteral administration of various drugs. Several studies have shown a direct route of transport from the olfactory region to the CNS in animal

models, without prior absorption to the circulating blood (Chou and Donovan, 1998; Wang *et al.*, 1998; van Laar *et al.*, 1999; Dahlin *et al.*, 2000; Chow *et al.*, 2001; Fisher and Ho, 2002; Bagger and Bechgaard, 2004). Since drugs absorbed via the olfactory route do not have to cross the BBB it may be possible to deliver substances to the CNS directly. However, the quantities of drugs reported to access the brain are very low, with concentrations in the CSF and olfactory lobes cited as nM or from 0.01% to 0.1% bioavailability (Illum, 2004). In order for a drug to travel from the olfactory region in the nasal cavity to the CSF or the brain parenchyma, it has to transverse the nasal olfactory epithelium and, depending on the pathway followed, also the arachnoid membrane surrounding the subarachnoid space. In principle, one can envisage three different pathways across the olfactory epithelium: (i) transcellularly especially across the sustentacular cells, most likely by receptor mediated endocytosis, fluid phase endocytosis or by passive diffusion, the latter pathway most likely for more lipophilic drugs; (ii) paracellularly through tight junctions between sustentacular cells or the so-called clefts between sustentacular cells and olfactory neurons; (iii) by the olfactory nerve pathway where the drug is taken up into the neuron cell by endocytotic or pinocytotic mechanisms and transported by intracellular axonal transport to the olfactory bulb (Illum, 2000).

Interesting results have been obtained by using these different strategies with some drawbacks. The linking of a lipophilic moiety or a ligand on the drug may end up in loss of therapeutic effect, the use of direct drug delivery will be difficult to develop on a large scale and the nasal route is bit experimental.

Recently studies have been carried out to explore the feasibility of using low-frequency magnetic resonance (MR) image-guided focused ultrasound as a noninvasive method for the temporary disruption of the blood-brain barrier (BBB) at targeted locations. The results demonstrated that low-frequency ultrasound bursts can induce local, reversible disruption of the BBB without undesired long-term effects (Hynynen *et al.*, 2006).

Possible systems for brain delivery

Colloidal drug carriers

The drug association without any modification to colloidal carriers is another promising approach. These vesicles could deliver drug molecules at specific site by coupling ligands to the surface of the colloids which can be administered intravenously for chronic treatment. In general, colloidal drug carriers include micelles, emulsions, liposomes and nanoparticles (nanospheres and nanocapsules). It is significant that only liposomes and nanoparticles have been largely investigated for brain drug delivery. The colloidal carriers are generally used to increase the specificity towards cells or tissues, to improve

the bioavailability of drugs and to protect them against enzyme inactivation. In addition, the colloidal systems allow access across the BBB of non-transportable drugs by masking their physico-chemical characteristics through their encapsulation in these systems.

The fate of colloidal particles after intravenous administration is determined by a combination of biological and physico-chemical events, including their interaction with plasma proteins leading to "opsonization". This process is crucial in determining the pharmacokinetics of the administered hydrophobic colloidal particles as these particles coated with plasma components are rapidly removed from the circulation. The colloidal particles which are small and hydrophilic enough can escape, at least partially, from the opsonization process and consequently remain in the circulation for a relatively prolonged period of time. Moreover, particles are coated with surfactant molecules (such as copolymers of polyoxyethylene and polyoxypropylene) or by provided with sterical stability by the direct chemical link of polyethyleneglycol (PEG) at the surface of the particles to achieve long circulation characteristics (Peracchia *et al.*, 1998, 1999a, b). In addition, active targeting can also be achieved by the attachment of a specific ligand (such as a monoclonal antibody) onto the surface of the colloidal particle, preferentially at the end of the PEG molecules since the targeted colloidal particles will be much more competent if they are also sterically stabilized.

Micelles

Polymeric micelles as drug delivery systems are formed by amphiphilic copolymers having an A–B di block structure with A, the hydrophilic (shell) and B, the hydrophobic polymers (core). In aqueous media the polymeric micelles are thermodynamically and kinetically stable. They have a size range of several tens of nanometers similar to that of viruses and lipoproteins with a considerably narrow distribution.

Several reviews have analyzed in great details the properties of the different copolymers used in the preparation of the polymeric micelles (Adams *et al.*, 2003) as well as the physical chemistry of these systems (Jones and Leroux, 1999), which may influence their properties such as size distribution and stability, drug loading capacity, the drug release kinetics, blood circulation time and biodistribution (Allen *et al.*, 1999).

Studies by Kabanov *et al.* (1992) have shown that poloxamer (Pluronic™) micelles conjugated with antibodies improved brain distribution and efficacy of a neuroleptic agent haloperidol. This result indicates that Pluronic™ micelles provide an effective transport of solubilized neuroleptic agents across the BBB. However, investigations demonstrated that only Pluronic™ unimers allowed cell penetration in bovine BMEC monolayers of

molecules such as rhodamine 123 (Batrakova et al., 2001a), digoxin (Batrakova et al., 2001b) or doxorubicin (Alakhov et al., 1999) by inhibition of the P-gp mediated drug efflux system. Other studies shown an increased analgesic effect when enkephalin, biphalin or morphine were administered as a cocktail with Pluronic P-85 at a concentration of 0.01 % (Witt et al. (2002). It is striking that the analgesia was lower with a higher concentration of Pluronic P-85 (0.1%) due to micellar trapping, which reduces the free drug concentration available for transcellular flux.

Liposomes

Liposomes are small biocompatible and biodegradable lipid vesicles composed of unilamellar or multilamellar phospholipid bilayers surrounding aqueous compartments. Their biophysical properties, such as size, surface charge, lipid composition and amount of cholesterol controls the distribution, tissue uptake and drug delivery. Liposomes have been counted for brain targeting in several pathologies through both intracerebral and intravenous administrations. Most of the studies on the transport of liposome encapsulated drugs are focused on tumor therapies to deliver doxorubicin and other antineoplastic agents with the aid of either cationic or pegylated liposomes. Overall, these treatments have led to long-term survival and inhibition of tumor growth in patients (Siegal et al., 1995; Koukourakis et al., 2000; Fiorillo et al., 2004; Saito et al., 2004).

Cationic liposomes

The capability of cationic liposomes to mediate transfection was credited to certain properties such as spontaneous electrostatic interactions between the positively charged liposomes and the negatively charged DNA, which results in an efficient condensation of the nucleic acids. A variety of mono or multivalent cationic lipids are currently available for gene transfer, such as DOTMA (*N*-[1-(2,3-dioleoyloxy)propyl]-*N,N,N*-trimethylammonium chloride) or DOTAP (1,2-dioleoyl-3-trimethylammonium-propane). These cationic lipids are often mixed with the neutral lipid dioleoyl phosphatidylethanolamine (DOPE), which is known to boost transfection efficacy due to its ability to form hexagonal phases that may contribute to the destabilization of the endosomal membrane. Cationic liposomes have been used to realize plasmid-mediated transfection of murine brain cells (Roessler and Davidson, 1994). When these liposomes were injected directly into the brain of mice, the expression of transgene could be observed at least for 21 days in the caudate putamen region. In order to deliver genetic material to scattered tumor sites, two approaches have been used. The first one is reinforcement of effectiveness of the immunotherapy. The studies on interferon (IFN)-b gene therapy using cationic liposomes have shown interesting results in the treatment of brain

tumors. The primary study developed through morphological analysis of tumor cells following liposomal IFN-b gene transfection has demonstrated that approximately 20% of the 203G (mouse glioma cell line) cells underwent morphological changes consistent with apoptosis produced by the liposomal formulation (Norimoto et al., (2003). In this way, a protocol has been developed to determine the safety and effectiveness of cationic liposomes containing the human IFN-b gene after tumor removal in five patients with recurrent malignant gliomas (Yoshida et al., 2004). The second strategy is based on the sensibilization of cancerous cells to drugs. Fusogenic liposomes have been prepared using fusogenic lipids, to attain an efficient transfer of the cationic liposomal contents into cells (Shangguan et al., 1998), by conjugation of fusogenic molecules to liposome membranes (Kono et al., 2000) or by incorporation of viral fusion proteins to bilayers. The feasibility to introduce oligodeoxynucleotides (FITC-ODN) into MBEC4 cells (mouse brain-capillary endothelial cells) by utilizing the hemagglutinin virus of Japan (HVJ)-liposomes with fusogenic activity was reported (Matsuo et al., 2000). But cationic liposomes normally require an invasive way of administration to transfer genes into the brain.

Pegylated liposomes

Pegylated liposomes have got a long blood circulation time and reduced clearance by the RES system and thus they selectively extravasate in pathological sites, like tumors or inflamed regions with a leaky endothelium. The studies on animals demonstrated an enhanced drug exposure and improved therapeutic activity (Gabizon, 1992; Siegal et al., 1995). A pegylated liposomal formulation of doxorubicin (Caelyx®) is used in clinical practice, showing effectiveness in glioblastomas and metastatic tumors (Koukourakis et al., 2000; Hau et al., 2004).

The liposomes have been found useful for drug delivery in experimental autoimmune encephalitis, a brain disorder. In inflammatory conditions, it is believed that the disruption of BBB allows the free diffusion of liposomes. Thus, prednisolone entrapped into pegylated liposomes has demonstrated an effective restoration of the BBB integrity; macrophage infiltration was diminished in the treated animals. Beside, the use of liposomes may reduce systemic side effects and could be employed for the treatment of multiple sclerosis (Schmidt et al., 2003).

Active targeting by liposomes

The most striking advancement in BBB targeting and translocation is active targeting which can be achieved by complexing the liposomes with an antibody or a ligand that will be recognized by cell surface receptor in the targeted tissue. Monoclonal antibodies (MAb) have enabled brain targeting of pegylated liposomes. The MAb are able to attach a receptor expressed on the BBB and to trigger a

receptor mediated transcytosis across the BBB. The targeting MAb acts as a molecular Trojan horse to ferry the liposomes across biological barriers in the brain via endogenous transport systems (Zhang *et al.*, 2004; Xia *et al.*, 2007). Using pegylated MAb-liposomes, specific OX26-mediated daunomycin targeting to the brain achieved successfully (Huwylar *et al.*, 1996). The digoxin encapsulated pegylated OX26-liposomes was found to enhance brain endothelial cell uptake. Pegylated MAb-liposomes have also been used to deliver genetic material to the brain (Shi *et al.*, 2001; Zhang *et al.*, 2003a, 2003b, 2004; Zhu *et al.*, 2004). These systems offer the advantage that they may be administered intravenously avoiding invasive way. These carriers were also employed for the encapsulation of an antisense gene directed to epidermal growth factor receptor (EGFR). This formulation was found to be efficient in reducing the growth of an EGFR-dependent glioma (Zhang *et al.*, 2004).

Another approach is the use of cationic liposomes decorated with transferrin ligand resulted in a significant enhancement of luciferase gene expression activity in C6 glioma cells, primary hippocampal neurons and primary cortical neurons (da Cruz *et al.*, 2004).

Therapeutic efficacy of monoclonal anticancer antibody 2C5-modified long-circulating liposomes (LCL) loaded with doxorubicin (2C5-DoxLCL) was assessed for the treatment of U-87 MG human brain tumors in an intracranial model in nude mice (Gupta and Torchilin, 2007). PEGylated immunoliposomes covalently linked to antibodies against human gliofibrillary acidic protein (GFAP) were prepared by coupling the thiolated monoclonal anti-GFAP antibodies, D4, with a maleimide derivative of the phosphatidyl ethanolamine of the liposomal membrane targeting to neural tissue astrocytes (Chekhonin *et al.*, 2005).

A neuroprotective agent Citicoline is used clinically to treat for instance Parkinson's disease, stroke, Alzheimer's disease and brain ischemia. Citicoline does not readily cross the BBB because of its strong polar nature. Hence, citicoline liposomes have been prepared by coupling Transferrin which prevents scavenging of Liposomes (Suresh *et al.*, 2006). Saposin C, a small lipid-binding protein which is derived from a single precursor protein, named prosaposin (PSAP), has several neuronal effects, including neurite outgrowth stimulation, neuron preservation, and nerve regeneration enhancement, been encapsulated into liposomes and tested for its efficacy (Chu *et al.*, 2005). In another study, pegylated liposomes loaded with horseradish peroxidase (HRP) and tagged with transferrin (Tf) to target protein drugs to the BBB (Visser *et al.*, 2005). Recently, RGD peptide was coupled with ferulic acid (FA) liposomes for binding to monocytes and neutrophils in peripheral blood for brain targeting in response to leukocyte recruitment (Qin *et al.*, 2007).

Nanoparticles

Nanoparticles are defined as a submicron drug carrier systems that are made from a broad number of materials such as poly(alkylcyanoacrylates) (PACAs), polyacetates, polysaccharides and copolymers. The methods of preparation of the nanoparticles, their characterization and medical applications have been reviewed in details earlier (Kreuter, 1992; Barratt *et al.*, 2001; Fattal and Vauthier, 2002). These successfully cross the barrier when coated with polyethylene glycol (PEG), polysorbate, or other polymer or surfactant. The exact mechanism of nanoparticle transport into the brain is not fully understood, but it is thought to depend on the particle's size, material composition, and structure. In some cases, it appears that a specialized coating of polymer or surfactant allows nanoparticles to mimic molecules that would normally be transported into the brain. For example, polysorbate-coated nanoparticles are thought to mimic low-density lipoproteins (LDL), allowing them to be transported across the capillary wall and into the brain by hitching a ride on the LDL receptor (Kreuter *et al.*, 2002). In another study nanoparticles were "decorated" with opioid peptides, short pieces of protein that act as natural painkillers. The opioid peptides bind to specific receptors on the capillary walls, which help carry the nanoparticles into the brain (Costantino *et al.*, 2005). Once inside the brain, a nanoparticle can deliver a wide variety of payloads to detect, treat cancer and other diseases. These systems are attractive because the methods of preparation are generally simple and easy to scale-up.

The major advantage of using nanoparticles for brain targeting results from their two basic properties. 1) Due to their small size, nanoparticles penetrate into even small capillaries and are taken up within cells, allowing an efficient drug accumulation at the targeted sites in the body. 2) The use of biodegradable materials for nanoparticle preparation, allows sustained drug release at the targeted site after injection over a period of days or even weeks (Vinogradov *et al.*, 2002).

Coated nanoparticles

With the aim of brain delivery, molecules such as dalargin (Kreuter *et al.*, 1995; Schroeder *et al.*, 1998), loperamide (Alyautdin *et al.*, 1997) and endomorphin-1 (Liu *et al.*, 2006) have been loaded onto nanoparticles. As they did not diffuse through the BBB after peripheral administration, dalargin loperamide or endomorphin-1 were adsorbed onto the surface of poly(butylcyanoacrylate) (PBCA) nanoparticles. Further coated with the detergent, polysorbate-80 (PS-80), a pronounced analgesic effect was obtained, reaching a maximum after 45 min of administration. The mechanism behind the translocation of nanoparticles into the brain is still not fully understood. Recent studies have suggested that the PBCA nanoparticles coated with PS-80 displayed some toxic effect towards the

BBB (Olivier et al., 1999). In addition, it was also suggested that the nanoparticles could open the tight junctions between endothelial cells in the brain microvasculature, thus creating a paracellular pathway for nanoparticle translocation. This argument was based on the observations done in an *in vitro* model of the BBB consisting of a coculture of bovine brain endothelial cells and rat astrocytes (Olivier et al., 1999). However, both *in vivo* and *in vitro* studies (Kreuter et al., 2003) did not show any disruption of the BBB by the presence of PS-80 coated nanoparticles since the permeability of the extracellular markers (sucrose and inulin) was not modified in the presence of 10 or 20 mg/ml of PBCA nanoparticles with and without polysorbate-80. This indicates, on the contrary to what was hypothesized by Olivier et al., (1999), no facilitation of the paracellular route by disruption of tight junctions due to nanoparticles was observed.

In vivo experiments in mice have clearly shown that the analgesic effect of dalargin was obtained only when the drug was pre-adsorbed onto the nanoparticles, whereas a single mixture of dalargin and PBCA nanoparticles did not show any analgesic effect. The enhancement of the drug transport through BBB by the coated nanoparticles can be explained by different mechanisms: (1) the binding of nanoparticles to the inner endothelial lining of the brain capillaries could provide a drug concentration gradient, thus improving passive diffusion (2) brain endothelial cell uptake of nanoparticles may occur through endocytosis or transcytosis. Additionally, it has been reported that apolipoproteins (APO) could be involved in the brain penetration of PBCA nanoparticles over those coated with PS-80 (Kreuter et al., 2002). A study performed using PBCA nanoparticles loaded with dalargin or loperamide and over coated with the APO-A, B, C, E or J (with or without precoating with PS-80), showed high antinociceptive effect with both PS-80-precoated and APO-B- or APO-E over coated nanoparticles. Interestingly, in APO-E-deficient mice, the antinociceptive effect was reduced comparatively to normal mice after injection of the PS-80-coated nanoparticles. Thus, it is suggested that the PS-80 could act as an anchor for APO-B and APO-E, at the surface of the nanoparticles which are then be able to interact with LDL receptor, before being taken up by the BMEC via receptor-mediated endocytosis (Borchard et al., 1994; Kreuter et al., 1995; Alyaudtin et al., 2001; Shamenkov et al., 2006; Ambruosi et al., 2006; Kreuter et al., 2007). In a recent study polymeric nanoparticle derivatised with peptide (g-7 Np) were loaded with Loperamide in order to assess their ability as drug carriers for CNS, and with Rhodamine-123, in order to qualitatively determine their biodistribution in different brain macro-areas (Tosi et al., 2007). Effect of size of nano-scaled polybutylcyanoacrylate (PBCA) and methylmethacrylate-sulfopropylmethacrylate (MMA-SPM) on the permeability of anti-human immunodeficiency virus

(HIV) agents, zidovudine (AZT) and lamivudine (3TC) (Kuo and Chen, 2006) and anti-cancer drug Methotrexate (Gao and Jiang, 2006) across the blood-brain barrier (BBB) was investigated.

Pegylated nanoparticles

Pegylated-poly(hexadecylcyanoacrylate) (PEG-PHDCA) nanoparticles have been investigated for the treatment of several CNS pathologies such as brain tumors (Brigger et al., 2002b), EAE (Calvo et al., 2002) and prion diseases (Calvo et al., 2001a). In this technology, the PEG is covalently attached to the hydrophobic block, rather than adsorbed, which considered to be the better choice to avoid the possibility of PEG desorption. These particles with PEG chains at the surface of the hexadecylcyanoacrylate hydrophobic core have shown long-circulating properties *in vivo* (Peracchia et al., 1999b). PEG-PHDCA nanoparticles have been shown to penetrate into the brain to a greater extent than all the other nanoparticles formulations tested, including the above discussed PS-80 nanoparticles (Calvo et al., 2001b). Confocal microscopy have evidenced that fluorescent-PEG-PHDCA nanoparticles were present in the epithelial cells (Brigger et al., 2002) of the brain and spinal cord surface and in the ependymal cells of the choroids plexus in EAE rats when tested for accumulation (Calvo et al., (2002). In EAE rats, PEG-PHDCA nanoparticles could reach the brain by two mechanisms: passive diffusion due to the increase of BBB permeability and transport by nanoparticles-containing macrophages which infiltrate these inflammatory tissues. This study claims that PEG-PHDCA nanoparticles had appropriate characteristics for penetration into CNS under pathological conditions, especially in neuroinflammatory diseases (Calvo et al., 2002). After intravenous administration in rats bearing intracerebral well-established gliosarcoma, PEG-PHDCA nanoparticles have accumulated preferentially in the tumoral tissue, rather than in the peritumoral brain tissue or in the healthy controlateral hemisphere.

Interestingly PEG-PHDCA nanoparticles concentrated much more in the gliosarcoma than did their nonpegylated counterparts (PHDCA nanoparticles). Based on the test of sucrose permeability, PEG-PHDCA nanoparticles did not display any toxicity towards the BBB. Using a simplified pharmacokinetic model two different mechanisms were proposed to explain the accumulation of PEG-PHDCA nanoparticles into the brain tumor, firstly due to their reduced plasma clearance and secondly, due to affinity of the PEG-PHDCA nanoparticles for the endothelial cells of the BBB, allowing their translocation (Brigger et al., 2002). The intracellular trafficking of nanoparticles by cell fractionation and confocal microscopy showed that nanoparticles are internalized by the endocytic pathway (Kim et al., 2007). Cationic bovine serum albumin (CBSA) conjugated poly(ethyleneglycol)-poly(lactide) (PEG-PLA)

nanoparticle (CBSA-NP), was designed as drug carrier to brain (Lu *et al.*, 2007). Studies have been carried out to assess the biodistribution, brain uptake and neuroprotective effect of the formulation containing vasoactive intestinal peptide, a neuroprotective peptide, which were efficiently incorporated into the poly (ethylene glycol)-poly (lactic acid) nanoparticles modified with wheat germ agglutinin (Gao *et al.*, 2007).

Solid Lipid nanoparticles (SLN)

Solid lipid NPs may be a promising sustained-release and drug-targeting system for lipophilic CNS antitumor drugs like Camptothecin (Yang *et al.*, 1999), Doxorubicin (Fundaro *et al.*, 2000). Other antineoplastic agents like 5-fluoro-2'-deoxyuridine (FUdR) (Wang *et al.*, 2002), Paclitaxel, the prodrug Cholesteryl butyrate, and anti VEGF antisense oligonucleotides have been tested in experimental animal models of cerebral gliomas by using SLN, which is elaborately reviewed in a recent article (Brioschi *et al.*, 2007).

Solid Lipid Nanoparticles (SLN) are already investigated as a pharmaceutical tool to change the pharmacokinetic and biodistribution of carried molecules and to overcome the Blood Brain Barrier (BBB). *In vivo* Magnetic Resonance Imaging (MRI) of the central nervous system (CNS) with SLN showed that super paramagnetic SLN have slower blood clearance confirming the ability of SLN to overcome the BBB and might be used as a CNS MRI contrast agent (Peira *et al.*, 2003).

Permeability of the anti-human immunodeficiency virus (HIV) agents, including stavudine (D4T), delavirdine (DLV), and saquinavir (SQV), across the *in vitro* blood-brain barrier (BBB) was studied by incorporating in SLNs, polybutylcyanoacrylate (PBCA) and methylmethacrylate-sulfopropylmethacrylate (MMA-SPM) NPs (Kuo *et al.*, 2007). Transport of the anti-HIV agents across BBB is a key factor in their applications to the therapy of the acquired immunodeficiency syndrome (AIDS).

Nanogel

A new family of carrier systems for the delivery of drugs and biomacromolecules to the brain called nanogel was developed (Vinogradov *et al.*, 1999, 2002, 2004). These so called "nanogels" systems, are made from a network of cross-linked ionic polyethylenimine (PEI) and non-ionic PEG chains (PEG-cl-PEI). Nanogels are synthesized using the emulsification solvent evaporation method. When a biologically active macromolecule is associated to the nanogel by electrostatic interactions, the PEI chains have a tendency to collapse which results in decreased volume and size of the particles. Because of the steric stabilization of the PEG chains, the collapsed nanogel forms stable

dispersions with a mean particle size of 80 nm. The surface of the nanogel could be modified with biospecific ligands for active targeting. For this purpose, various coupling strategies have been used including covalent attachment of the ligand moiety to free amino groups of the PEI fragments in the PEG-cl-PEI nanogel. Another simple way to introduce ligands in the nanogel particles consists in the partial modification of PEI fragments with biotin moieties allowing attachment of ligand using standard biotin-avidin coupling chemistry (Vinogradov *et al.*, 2002).

Nanogels have been tested as a potential carrier for oligonucleotide delivery to the brain (Vinogradov *et al.*, 2004) by using polarized monolayers of bovine BMEC. The studies performed with that model of BBB have shown an increased transport of ODN across the cell monolayers as a result of their incorporation into the nanogel. Further increase in oligonucleotide transport was observed when the nanogel carriers were modified with insulin or transferrin ligands (Kabanov and Batrakova, 2004). Permeability assays with mannitol indicated that the increased transport of ODN-nanogels did not result from single paracellular diffusion due to a disruption of the bovine BMEC monolayers. In addition, intravenous injection of ODN-nanogels in mice, no adverse toxic effects were observed and increased brain and decreased liver/spleen accumulations were noted, compared to the free ODN (Vinogradov *et al.*, 2004). Random copolymeric micelles composed of N-isopropylacrylamide (NIPAAm) and N-vinylpyrrolidone (VP) cross-linked with N,N'-methylenebisacrylamide (MBA) have been used as nanogel carriers to encapsulate N-hexylcarbamoyl-5-fluorouracil (HCFU), a prodrug of 5-FU, and have been targeted to brain tissue across blood-brain barrier (BBB) after coating with polysorbate 80. A recent review on nanogels explains the efficient intracellular penetration of nucleoside analogs that are otherwise restricted from passing across the blood-brain barrier (Vinogradov, 2007).

Recent advancements in nanotechnology

Multifunctional nanoparticles

The research team of University of Michigan has developed a tool to diagnose and treat the most virulent forms of brain cancer. That is, 20 to 200 nanometer diameter nanoparticles, they dubbed Probes Encapsulated by Biologically Localized Embedding (PEBBLEs) (Kopelman *et al.*, 2005). They designed the PEBBLEs to carry a variety of agents on their surface, each with a unique function. The major potential advantage of using these nanoparticles to treat cancer is of multifunctionality. One target molecule immobilized on the surface could guide the PEBBLE to a tumor. Another agent could be used to help visualize the target using magnetic resonance imaging (MRI), while a third agent attached to the

PEBBLE could deliver a destructive dose of drug or toxin to nearby cancer cells. All three functions can be combined in a single tiny polymer sphere to make a potent weapon against cancer (See Fig. 2). Kopelman introduced the common MRI contrast element gadolinium to the PEBBLES. When injected into the bloodstream, the nanoparticles travel their way through the bloodstream. But because they can transverse the blood-brain barrier, and because they have a targeting agent attached, the PEBBLES accumulate in the brain tumor enabling a clear MRI image within just a few hours.

The next functional step is a remarkable effort of nano-engineering. Each PEBBLE carries a photocatalyst. When stimulated by a light source through a micrometer-sized fiber optic probe inserted into the skull, the photocatalyst

converts oxygen into a so called singlet state, which effectively “bleaches” and destroys nearby cells. The PEBBLES are inert and harmless until the light is turned on. Used in combination with MRI imaging, one could now kill cancer cells at will, while tracking the effectiveness of the treatment with imaging. The targeted treatments using PEBBLES may offer a number of advantages over traditional chemotherapy. PEBBLES are highly localized to the cancer target, do very little damage to surrounding healthy tissue, and can overcome multi-drug resistance (MDR). The cancer becomes “immune” to the drug. But PEBBLES act on the outside of the cell, and the toxic payload of oxygen that they deliver acts quickly, without giving the cancer much chance to survive and develop resistance.

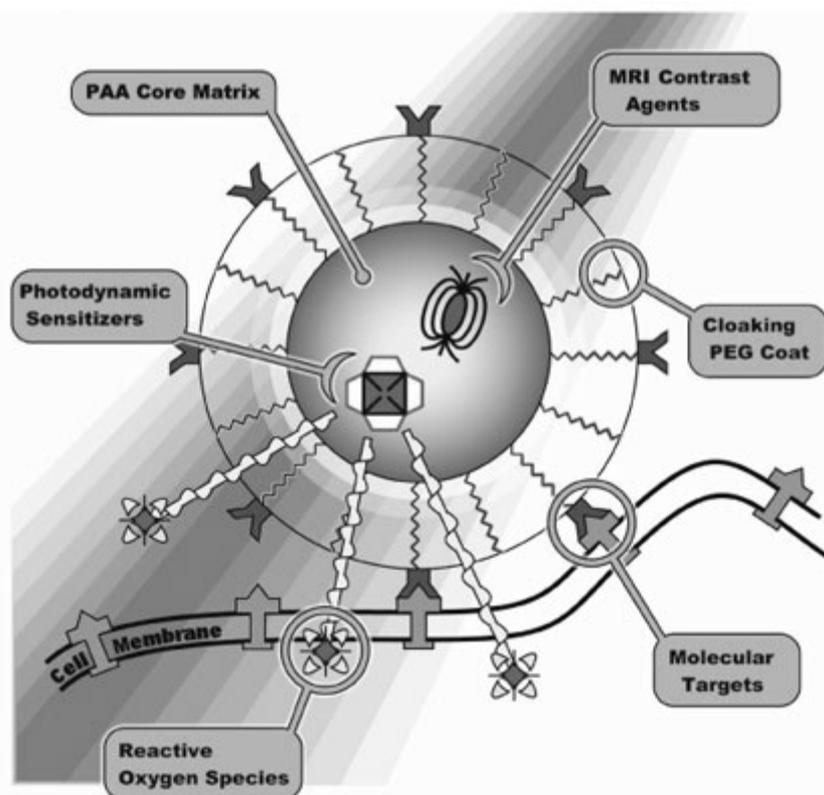


Fig. 2 The power of PEBBLES is in their multifunctionality. One tiny polymer sphere can contain a targeting agent that directs the particle to cancer cells, a protective coating (PEG) that helps it cross the blood brain barrier, photodynamic molecules that catalyze the conversion of oxygen to highly reactive oxygen singlets, magnetically dense metals for MRI contrast imaging, and a fluorescent “beacon” to visually pinpoint the nanoparticles location (Kopelman et al., 2005).

Unfortunately, the most common form of primary brain cancer, glioblastoma, is most aggressive, lethal and emerges rapidly and spread throughout the brain. Surgery is limited in its effectiveness because it is difficult to differentiate visually between cancerous and normal brain tissue, and any cancer cells left behind are likely to proliferate and form new tumors. In order to improve the odds of eliminating all the cancer during surgery and avoid

removing healthy brain tissue, researchers have devised a number of fluorophores, or “glowing” molecules that mark the tumor boundaries for removal. But the fluorescent probes are difficult to locate and use within the brain during surgery. Researchers wanted to see if surgical outcomes could be improved by a single probe that accurately marks the location of a tumor in pre-operative MRI scans, while guiding the surgeon to those same

locations in the exposed brain. Nanotechnology was the perfect solution for creating such a multifunctional probe (Veisoh *et al.*, 2005). Starting with a 10 nanometer diameter iron oxide core that serves as an MRI contrast material, coated the nanoparticles with polyethylene glycol and modified them with a fluorescent molecule called Cy5.5. The fluorescent molecule gives off light at near-infrared wavelengths, which unlike visible light can penetrate several centimeters through brain tissue. In order to selectively light up glioma tumors through imaging, a targeting agent chlorotoxin, a peptide derived from the venom of the giant Israeli scorpion, which binds specifically to a tumor surface marker found in the vast majority of gliomas had been attached. At 15 nanometers, the final particle size and composition gave it the best chance for crossing the blood brain barrier, and homing in on its target.

Researchers incorporated a drug called Photofrin along with iron oxide into nanoparticles that would target cancerous brain tumours. Photofrin is a type of photodynamic therapy (PDT), in which the drug is drawn through the blood stream to tumour cells; a special type of laser light activates the drug to attack the tumour. Iron oxide is a contrast agent used to enhance magnetic resonance imaging (MRI) (Reddy *et al.*, 2006).

Other New Technologies

A novel technology set to overcome the challenges of the blood brain barrier is being developed in an attempt to open the gates to previously unusable compounds for the treatment of central nervous system (CNS) disorders. This technology named 'LipoBridge' by biotech giant Genzyme's pharmaceuticals division, promises to offer a serious helping hand to firms hoping to come up with a new generation of CNS products, finally allowing therapeutic compounds otherwise unable to pass across the infamous blood brain barrier. About 99 per cent of all potential CNS drugs are larger than 500 Daltons, and thus automatically rejected by the blood brain barrier which simply will not allow larger molecules to pass. Using this technology, the therapeutic active agent is formulated with short chain oligoglycerolipids to facilitate transport across the blood brain barrier. The 'LipoBridge' formulation interferes with the barrier, temporarily opening up the tight

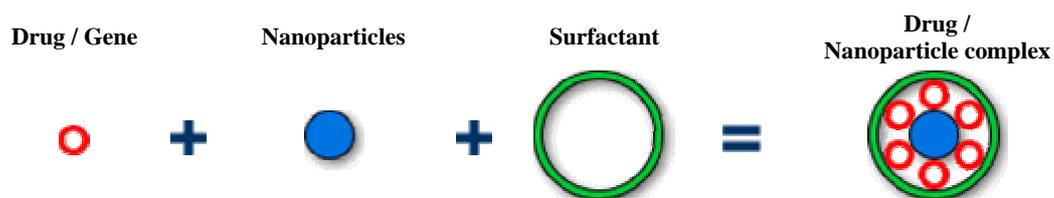
junctions and allowing only drug compounds to cross the CNS, thus reducing the risk of other unwanted molecules crossing over it.

Researchers have also developed a new way to deliver drugs into the brains of mice, which could herald a new dawn for the sufferers of brain diseases such as Alzheimer's.

This latest research uses a modified peptide, dubbed CORVUS, to enable small interfering RNA (siRNA) molecules to be delivered into the brains of mice suffering from fatal inflammation of the brain. The rabies virus glycoprotein (RVG) enables the Rabies virus to enter the brain and the researchers have modified this peptide to create the CORVUS delivery system which can carry molecules into the brain via a process known as transcytosis. Part of the RVG virus specifically binds to neuronal cells and allows the siRNA to be delivered to the brains of mice suffering from a fatal form of encephalitis, an acute inflammation of the brain commonly caused by viral infection (Kumar *et al.*, 2007). The tagging of drugs to molecules that do pass through the barrier has also been utilized and this latest method takes a similar approach. The US market for this technology is enormous as it has the potential to deliver gene therapy agents and small molecule drugs into the brain avoiding many of the disadvantages observed using other approaches.

A German company (NanoDel) has designed nanoparticles for the targeting of drugs to the brain, retina or spinal cord. A drug is attached to polybutylcyanoacrylate nanoparticles coated with a surfactant that carries the drug across the blood-brain-barrier (BBB). The technology is applicable to peptides. The nanoparticles range in diameter from 200 to 400 nm and they are suspended in an aqueous solution. Depending on the method of polymerization, drugs are either attached to the surface and/or are incorporated into polybutyl-cyanoacrylate-particles (PBCA) (see Fig. 3). The nanoparticles can be stored either in lyophilized form or in solution and can be delivered via the oral or parenteral routes. The nanoparticle/drug formulation will be supplied as lyophilized powder, which has to be dissolved in a 1% Tensid-solution (Tween® 80 solution) containing 0.9% NaCl.

Assembling of a drug loaded and coated Nanoparticle



An American based company ArmaGen provides platform technology solutions to the 'blood-brain barrier' problem, and can target small molecules, recombinant proteins and non-viral gene therapies to the brain and spinal cord. The company has programme to develop and patent AGT-2000, a non-viral gene therapy for primary and metastatic brain cancer, AGT-120 is a bi-functional genetically engineered large molecule neuroprotective drug for the treatment of acute ischemic stroke, AGT-160, AGT-140 and AGT-120 for Alzheimers disease (AD), AGT-185 for treatment of acute nerve gas exposure.

Future perspectives

Thinking outside the box is a must for developing drug systems to treat brain disorders. Nanotechnology has infiltrated into several fields like Nanoparticles, Nanofoods, Nanorobots, Nanoelectronics, Nanomedicine, Nano cosmetics, and Nanodrugs. The rush to develop and patent nanotechnology products for betterment of treating human ailments has picking up pace. Globally, the race is on between countries to develop and patent Nanotechnology. Several companies worldwide are participating in Nanotechnology research and several Billion dollars slated for Nano research in the next five years. Diseases related to CNS and other parts of the brain including brain tumor is of great concern at present and is progressively increasing day by day. An effective brain delivery carrier system is very much warranted in effective therapy of these diseases.

Conclusion

It emerges from this review that colloidal systems can easily enter brain capillaries before reaching the surface of the brain microvascular endothelial cells, when the surface of these colloids are modified in a proper way (i.e. by PEG or PS-80). These surface modified colloidal particles enhance exposure of the BBB due to prolonged blood circulation, which favors interaction and penetration into brain endothelial cells. Colloidal systems may further be modified with a variety of agents on their surface, each with a unique function leading to multifunctional therapy. Therefore, when drug is loaded, colloidal carriers may be helpful for the treatment of brain diseases, because they offer clinical benefits such as reduced drug dose, decreased side effects, increased drug viability, non invasive routes of administration and improved quality of life to patient. However, to clarify the mechanisms of drug transport to the brain, associated toxicological risks of nanotechnology and to develop cost effective newer systems to overcome major challenges are the prime need of present research.

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