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Development of Lipid Based Nanoparticulate Drug Delivery Systems and Drug Carrier Complexes for Delivery to Brain

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ABSTRACT

Many neurotherapeutics are unsuccessful in treating CNS disorders because they cannot be effectively drug delivered. Drug delivery to the brain is a challenge even though there is relatively high blood flow. There are two physiological barriers likes blood-brain barrier and blood-cerebrospinal fluid barrier which separates the brain from its blood supply controlling the transport of compounds. Many of the brain or CNS associated diseases remain untreated by effective therapies. This is not because there is a lack of candidate drugs but due to the inability of many therapeutic molecules to cross the BBB, the BCSFB or other specialized CNS barriers to reach the specific areas of brain. Hence there is a need in the modern approaches and present insights into using ligand conjugation and nanotechnology to target the BBB via different transport pathways and mechanisms. The field of novel drug delivery system has fully emerged and came into existence as an ideal approach of drug targeting and delivery to brain. The new approaches of drug delivery to brain help in successful transporting drugs across the BBB.

INTRODUCTION

Many neurotherapeutics are unsuccessful in treating CNS disorders because they cannot be effectively delivered to the brain. Drug delivery to the brain is a challenge eventhough there is relatively high blood flow. There are two physiological barriers separating the brain from its blood supply controlling the transport of compounds. One is the blood-brain barrier (BBB) and the other is the blood-cerebrospinal fluid barrier (BCSFB). Despite the rapid development in molecular structural components, progress in receptor expression at the BBB, advances in medical technology and breakthroughs in nanotechnology based approaches, many of the brain or central nervous system (CNS) associated diseases remain untreated by effective therapies. This is not because there is a lack of candidate drugs but due to the inability of many therapeutic molecules to cross the BBB, the BCSFB or other specialized CNS barriers to reach the specific areas of brain (Neuwelt *et al.*, 2008). Such a difficulty in delivering therapeutic molecules to the brain or CNS can only be overcome by a concerted effort in understanding the physiology of BBB, its permeability under different pathological or disease conditions, response to physical and chemical stimuli as well as the various transport receptors at the BBB and available delivery technologies. Many attempts to transport drugs across the BBB could be against the natural function of the BBB, effective approaches or methods should be cautiously assessed with regards to their impact on the overall protective function of BBB. There are various molecular and biological opportunities which explores at the BBB for transport of therapeutic molecules across it under various physiologic and pathological conditions. Hence there is a need in the modern approaches and present insights into using ligand conjugation and nanotechnology to target the BBB via different transport pathways and mechanisms. Internally brain is protected from foreign organisms and noxious chemicals by highly strengthened membrane system called as blood-brain barrier. BBB is a specialized system of capillary endothelial cells that protects the brain from harmful substances in the blood stream, while supplying the brain with the required nutrients for proper function. It is a semi permeable, selective barrier which was confirmed for the first time by Ehrlich who showed that cerebrospinal fluid (CSF) injection of tryptophan blue dye stained the entire brain

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parenchyma but could not enter into brain capillary microvasculature (Hawkins and Thomas, 2005). BBB is responsible for several functions like maintenance of neuronal microenvironment, tissue homeostasis, vasotonous regulation, fibrinolysis, coagulation, blood cell activation, migration during physiological and pathological processes and also helps in vascularization of normal neoplastic tissues (Risau, 1995). Physiologically BBB is made up of three layers such as inner endothelial cell layer which forms the wall of the capillary and contains tight junctions followed by presence of basement membrane upon which pericytes and astrocytic feet processes lies (Egleton and Davis, 1997). Due to the presence of such tight junctions between endothelial cells a very high electrical resistance of around 1500-2000 Ω cm^2 results as compared to 3.33 Ω cm^2 in other body tissue proving the barrier function of BBB (Crone and Olesen, 1982).

Astrocytes and pericytes helps in differentiation as well as maintenance of BBB function. Astrocytes are most abundant non-neuroncells and play many essential roles in the healthy central nervous system (CNS), including biochemical support of endothelial cells which form the blood-brain barrier, regulation of blood flow, provision of nutrients to the nervous tissue, maintenance of extracellular ion balance and a principal role in the repair and scarring process of the brain and spinal cord following traumatic injuries (Sarafian *et al.,* 2010). Pericytes are perivascular cells which are important for the maturation, remodeling and maintenance of the vascular system via the secretion of growth factors or modulation of the extracellular matrix. They are also involved in the transport across the BBB and the regulation of vascular permeability (Alltand Lawrenson, 2001). The blood-cerebrospinal fluid barrier (BCSFB) is another barrier that a systemically administered drug encounters before entering the CNS. It functions together with the BBB and the meninges, to control the internal environment of the brain.

In addition, some regions of the CNS called as circum ventricular organs (CVO) are present adjacent to the ventricles of brain where BBB capillary endothelial tight junctions are absent. These brain sites are unique in terms that they are highly vascularized as compared to other brain regions and lacks BBB because the capillary system supplying the CVOs contains fenestrated endothelial cells instead of epithelial tight junction (Cottrell and Ferguson, 2004). Examples of such areas include choroid plexus, pineal gland, neurohypophysis, medianeminence, organo sumvasculosum of lamina terminalis, subfornical organ (SFO), are a postrema of the chemo receptor trigger zone (CTZ) and nucleus tractus solitaries (NTS). Compared to the are a of tight BBB capillaries, the relative surface area of the capillaries of CVOs is very less (5000:1) which enables CVOs not to allow a significant diffusion of substances into the CNS (Johansonetal., 2005).

Physiology and biology of the blood-brain barrier

The brain is well protected and dynamically regulated to provide a sanctuary for the CNS. There are several gate ways to enter brain parenchyma, the most important two are blood circulation and cerebrospinal fluid (CSF) circulation. In the human brain, there are about 100 billion capillaries providing a combined length of brain capillary endothelium of approximately 650 km and a total surface area of approximately 20m^2 (Pardridge, 2003). Any molecules entry into the brain via parenteral administration is strictly controlled by the BBB and the BCSFB. As the surface of BCSFB faces the ventricle that is filled with CSF not the blood, which is in combination with the high turnover rate of CSF, leads to continuously flushing the injected drug (i.e. those injected into the ventricle) back to the blood ((Rip *et al.,* 2009; Pathan *et al.,* 2009). Therefore BBB is universally considered as the most important barrier in preventing molecules from reaching the brain parenchyma via extensive branches of blood capillary networks. The chief anatomical and functional site of the BBB is the brain endothelium. Physiologically, in addition to brain capillary endothelial cells, extracellular base membrane, adjoining pericytes, astrocytes, and microglia are all integral parts of the BBB supporting system. Together with surrounding neurons, these components form a complex and functional "neurovascular unit" (Hawkins and Egleton, 2008) as shown in **Fig. 1.**

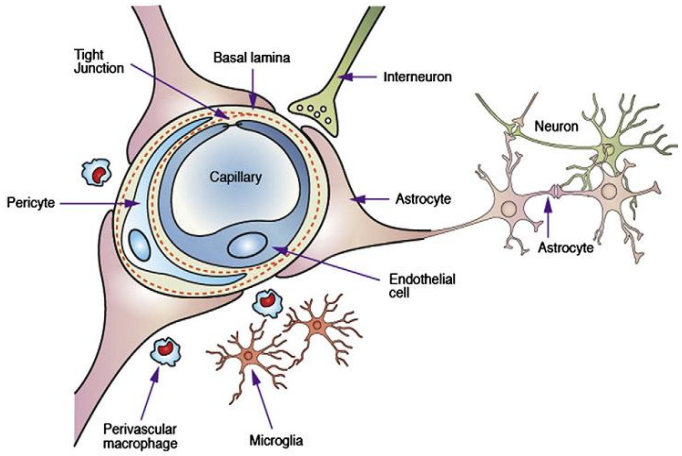


Fig. 1: Schematic representation of the blood-brain barrier (BBB) and components of a neurovascular unit (NVU) (Abbott et al., 2006).

A feature of the BBB is its low and selective permeability to molecules which can be attributed to its unique biological characteristics as mentioned following:

1) lack of fenestrations with very few pinocytotic vesicles, greater number and volume of mitochondria in endothelial cells (Stewart, 2000);
2) presence of tight junctions (TJ) between adjacent endothelial cells, formed by an intricate complex of transmembrane proteins (junctional adhesion molecule-1, occludin, and claudins) with cytoplasmic accessory proteins (zonulaoccludens 1 and 2, cingulin, AF-6 and 7H6) (Persidsky *et al.,* 2006);
3) expression of various transporters including GLUT1 glucose carrier, amino acid carrier LAT1, transferring receptors, insulin receptors, lipoprotein receptors and ATP family of efflux transporters such as p-glycoprotein

(P-gp) and multidrug resistance related proteins MRPs (Abbott *et al.*, 2006);

4) synergistic inductive functions and up regulating of BBB features by tight junctions comprising astrocytes, astrocytic perivascular end feet, pericytes, perivascular macrophages and neurons, suggested by the strong evidence from cell culture studies (Dohgu *et al.*, 2005);
5) The BBB has a strict limit for the passage of immune cells, especially lymphocytes and its immune barrieris made by the association between BBB endothelial cells and perivascular macrophages and mast cells (Williams *et al.*, 2001).

All these characteristics lead for BBB to possess multiple functions as a physical barrier (TJ), a transport barrier (P-gp), metabolic or enzymatic barrier (specialized enzyme systems and an immunological barrier).

Blood-cerebrospinal fluid barrier

The BCSFB composed of choroid plexus epithelial cells although much smaller in surface area also plays a role in the permeability of nutrients and xenobiotics. The choroid plexus is a highly vascularized branched structure with numerous villi that projecting to all four cerebral ventricles (Spector and Johanson, 1989). Although, the capillaries of the choroid plexus are fenestrated and provide little resistance to the movement of water and solutes, a barrieris formed by a monolayer of polarized epithelial cells surrounding the fenestrated capillaries that are joined together by tight junctional proteins (De Lange, 2004). These junctions form a functional barrier that restricts the movement of molecules and ions. The main function of the choroid plexus epithelial cells is to secrete and maintain the homeostatic composition of the CSF.

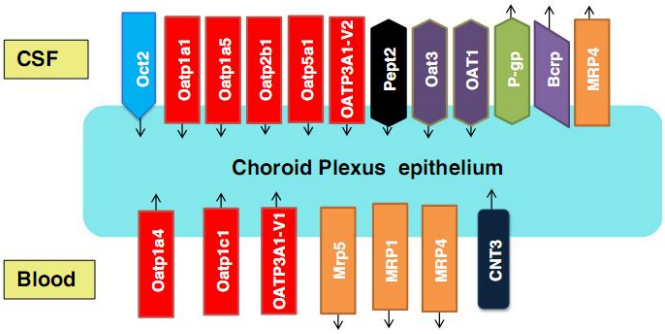


Fig. 2: Localization of selected ABC and SLC transporters in choroid plexus epithelial cells. Arrows indicates direction of the substrate transport (Kusuhara and Sugiyama, 2005).

The CSF fills the ventricles of the brain, the spinal canal and sub arachnoids space. In humans, the total volume of CSF is approximately 140 ml which is replaced four to five times daily (Garner and Brown, 1992). The CSF also provides a drainage system for the brain known as the sink effect into which products of metabolism and molecules are diluted and subsequently removed. At the level of choroid plexus epithelial cells, similar to the BBB, polarized expression of numerous receptors, ion channels and transporters has been reported as shown in **Fig. 2.** (Kusuhara and Sugiyama, 2005).

Transport systems across BBB

Unlike peripheral capillaries that allow relatively free exchange of substance across cells, the BBB rigorously limits transport into the brain. BBB not only functions as a physical barrier, but also a biochemical barrier that expresses certain enzymes like peptidases along with several cytosolic enzymes and efflux p-glycoprotein system that helps effluxing drugs from the endothelial cells back into the blood which helps in its further protecting action towards the brain microenvironment (Bernackietal.,2008). Thus the BBB is often the rate-limiting factor in determining permeation of therapeutic drugs into the brain. BBB is physiologically guided by two types of membranes such as luminal membrane and abluminal membrane. Even so, BBB has been found to be permeable in transport of nutrients like blood glucose, proteins, peptides and related peptide drugs (Egleton and Davis, 2005). Various transport mechanisms at the BBB have been explained for the transport of these substances (**Fig. 3**). These transport systems mainly operate in the luminal and abluminal membranes, i.e. from both blood-to-brain and brain-to-blood directions. But the blood-to-brain transport system is of considerable interest in drug delivery for targeting of drug molecules into brain as compared to brain-to-blood transport system. It has been well established that there are several transport routes by which solute molecules move across the BBB (Abbott and Romero, 1996). Diffusion of substances into the brain can be divided into paracellular and transcellular. Paracellular diffusion is a non-saturable and non-competitive movement of compounds (e.g., sucrose) between cells. As illustrated in **Fig. 3a**, small water-soluble molecules simply diffuse through the TJ but not to any great extent. Transcellular diffusion (transcytosis) is a non-saturable and non-competitive movement across cells of lipophilic substances by dissolving in their lipid plasma membrane (e.g. ethanol, steroid hormones, etc.) as shown in **Fig. 3b**. In the case of transport proteins or known as carrier-mediated transport (**Fig. 3c**), there is binding of a solute such as glucose or amino acids to a protein transporter on one side of the membrane that triggers a conformational change in the protein, resulting in the transport of the substance to the other side of the membrane, from high to low concentration (passive diffusion). If compounds need to be moved against a concentration gradient, ATP may provide the energy to facilitate the process (Facilitated diffusion). Carrier mediated transporter (CMT) system is expressed on both the luminal and abluminal membranes of the brain capillary endothelium and operates in both directions, i.e., from blood to brain and brain to blood directions (Frank and Pardridge, 1981). The CMT systems can be exploited for brain drug-delivery after reformulating the drug in such a way that the drug assumes a molecular structure mimicking that of the endogenous ligand. Some of examples are summarized as shown in **Table 1.**

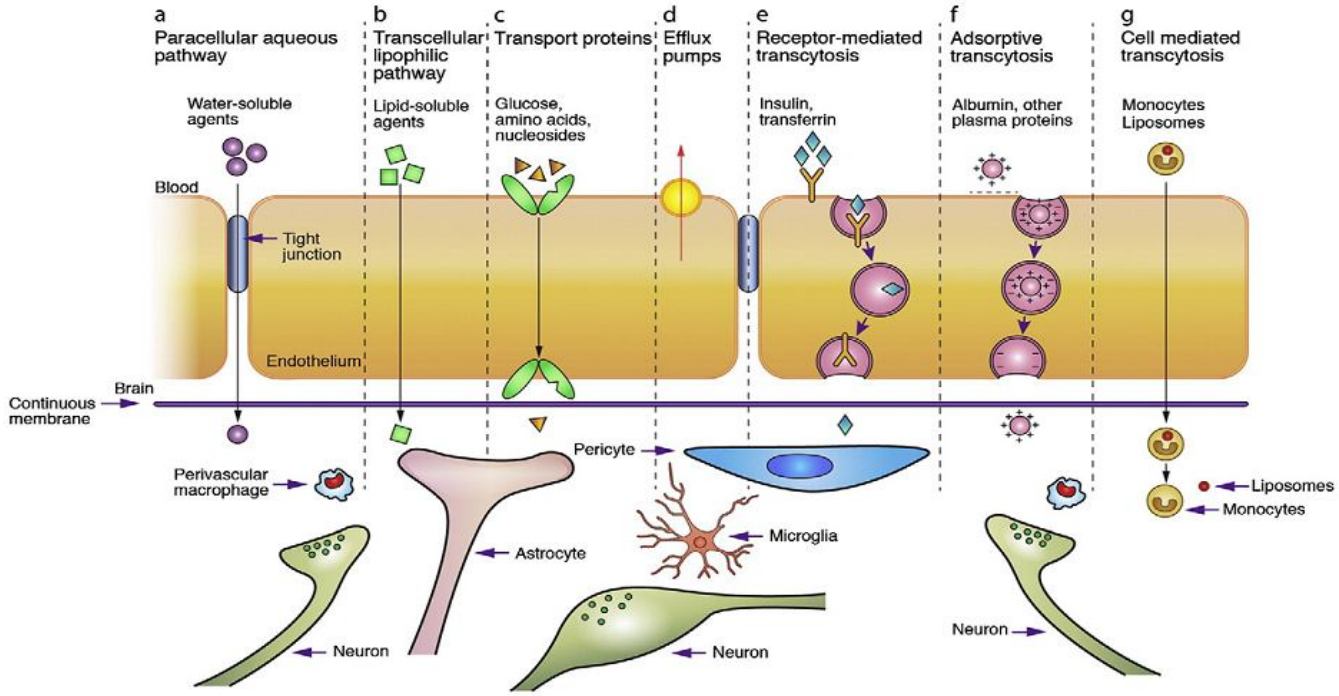


Fig. 3: Transport routes across the blood-brain barrier. Pathways "a" to "f" are commonly for solute molecules and the route "g" involves monocytes, macrophages and other immune cells which can be used for any drugs or drugs incorporated into liposomes or nanoparticles or carriers (Abbott et al., 2006).

Table 1: Carrier mediated transport system with different transporters and endogenous molecules to be transported.

Transporters	Molecules	Use
GLUT1 (Glucose transporter 1)	Glucose, hexose, 2 deoxyglucose, fluorodeoxy glucose	Positron emission tomography (PET) scanning
LAT1 (Large neutral amino acid transporter 1)	Large and small neutral amino acids, L-dopa (Levodopa), α-methyl-dopa, α-methyl-para-tyrosine or gabapentin	Parkinsonism, hypertension and in delivery of antiepileptic drugs
CAT1 (Cationic amino acid transporter 1)	Basic amino acids, like arginine or lysine	-
MCT1 (Monocarboxylic acid transporter 1)	Lactate, pyruvate, ketone bodies and monocarboxylic acid drugs like probenecid	Treatment of gout and urinary incontinence
CNT2 (Concentrative nucleoside transporter 2)	Purine nucleosides and certain pyrimidine nucleosides as uridine	Delivery of several anticancer and antiviral drugs

Table 2: Classification of active efflux transporter system (based on energy dependence) along with transporters and drug molecules to be transported.

Transporters	Classification	Drug molecules
ABCB1 (Adenosine triphposphate binding cassette transporter, subfamily B, member 1) [P-glycoprotein]	Energy and Na^+ dependent transporters (Luminal membrane)	In targeting of antitumor drugs like doxorubicin, paclitaxel to brain
ABCC (Adenosine triphposphate binding cassette transporter, subfamily C)	Energy and Na^+ dependent transporters (Luminal membrane)	Anticancer drugs
ABCG2 (Adenosine triphposphate binding cassette transporter, subfamily G, member 2)	Energy and Na^+ dependent transporters (Luminal membrane)	Drugs used for cancer treatment
OAT (Organic anion transporter)	Energy independent and Na^+ independent transporters (Abluminal membrane of brain capillary endothelial cell)	Uremictoxins, p- Amminohippuric acid (PAH), Valproic acid
OATP, OATP2 (Organic anion transporting polypeptide 2)	Energy independent and Na^+ independent transporters (Abluminal membrane of brain capillary endothelial cell)	Sulfated conjugated steroid hormones
ATA2 (Acidic aminoacid transporter 2)	Na^+ and Cl^- dependent system	Small neutral amino acids like L-alanine, L-glycine, L-proline
EAAT (Glutamic acid amino acid transporter)	Na^+ dependent high affinity system	Glutamic acid

Table 3: Receptor mediated transport system implies in the conveyance of molecules through BBB.

Transporters/ Receptors	Molecules
Insulin receptor (INSR)	Insulin
Transferrin receptor (TFR)	Transferrin
Insulin-like growth factor receptors (IGF1R & IGF2R)	Insulin like growth factor 1 & 2 (IGF-1 & IGF-2), mannose-6-phosphate
Leptin receptor (LEPR)	Leptin
Fc like growth factor receptor(FCGRT)	IgG
Scavenger receptor type B1 (SCARB1)	Modified lipoproteins, like acetylated low density lipoprotein (LDL)

Efflux pumps or transporters (**Fig. 3d**) including ATP-binding cassette (ABC) membrane associated transporter such as P-glycoprotein (P-gp), Multi drug Resistance-associated Proteins (MRPs) and Breast Cancer Resistance Protein (BCRP, ABCG2) play a significant role in restricting the permeability of several pharmacological agents including anti-cancer, anti-HIV agents and many more xenobiotics to brain microvessel endothelial cells (Bendayan *et al.,* 2002).

It requires energy in most instances and unlike CMT it is a unidirectional transport process (Lee *et al.,* 2001). Inhibition of P-gp in preclinical studies has enhanced the penetration of paclitaxel into the brain, indicating the feasibility of achieving improved drug delivery to the brain by suppression of P-gp (Kemper *et al.,* 2004). Most abundantly present component of this system is efflux P-glycoprotein, which is a product of ABCB1 gene. Several other multiple members of this gene family are available as ABCCs, ABCG2. AET is operated by broadly two groups of transporters, one is entirely energy dependent like ABC gene family found at luminal membrane (Pardridge, 2005) and others are energy independent found at abluminal membrane which are organic anion transporter1 (OAT), organic anion transporting polypeptide (OATP), glutamic acid amino acid transporter (EAAT), etc. They carry substrates to brain due to their ion transporting mechanism without utilizing energy (Conford *et al.,* 1994).Classification of active efflux transporter system along with transporters and respective drug molecules are as shown in **Table 2**.

Endocytosis can be isolated into bulk-phase (fluid phase or pinocytosis) endocytosis and mediated endocytosis (receptor and absorptive mediated). Bulk-phase endocytosis is the non-competitive, non-saturable, temperature and energy dependent non-specific uptake of extracellular fluids. It occurs to a very limited degree in the endothelial cells of the cerebral microvasculature.

Receptor mediated transcytosis (RMT) (**Fig. 3e**), provides a means for selective uptake and delivery of macromolecules to brain cells. Endothelial cells have receptors for the uptake of many different types of ligands, including growth factors, enzymes and plasma proteins. For example, insulin molecules first bind to receptors that collect in specialized areas of the plasma membrane known as coated pits. When bound to ligand these pits invaginate into the cytoplasm and then pinch free of the plasma membrane to form coated vesicles.

After acidification of the endosome, the ligand will dissociate from the receptor and cross the other side of membrane (Pardridge, 2005). Much of experimentations have been done for various receptor systems including transferring receptor (TfR), insulin receptor, lipoprotein receptors, scavenger receptors class B type I, diphtheria toxin receptor and glutathione transporter. In addition to this several other receptors are found in BBB such as receptor for leptin as LEPR, IGF-I, IGF-II, Fc fragment of IgG receptor transporter (FCGRT), etc (Rip *et al.,* 2009). Description of receptor mediated transport system responsible for the transport of molecules through BBB exemplified in **Table 3.** Adsorptive mediated transcytosis (AMT) is also known as the pinocytosis **(Fig. 3f),** which is triggered by an electrostatic interaction between appositively charged substance, usually the charged moiety of a peptide, and the negatively charged plasma membrane surface (i.e. heparin sulphate proteoglycans). Adsorptive mediated transport has a lower affinity but higher capacity than RMT. AMT based drug delivery typically involves either cationic proteins or cell penetrating peptide such as Tat-derived peptides and Syn Bvectors (Herve *et al.,* 2008). Cell mediated transcytosis is a more recently identified route of drug transport across the BBB (Jain *et al.,* 2003) is as shown in **Fig. 3g.**

It is a well-established mechanism for some pathogens such as *Cryptococcus neoformans* and HIV entry into the brain, known as "Trojan horse" model (Chretien *et al.,* 2002; Gonzalez-Scarano and Martin-Garcia, 2005). This transport route relies on immune cells such as monocytes or macrophages to cross the BBB. Unlike above mentioned transport pathways which normally permit only solute molecules whereas, cell mediated transcytosis can be used for any type of molecules or materials as well as particulate carrier systems (Park, 2008).

It has been investigated that 100% of large molecule drugs and 98% of small molecule drugs do not cross BBB. For a small molecule drug to cross the BBB in significant amounts, the molecule must have some important characteristics like such as (Chen *et al.,* 2004):

1) molecular weight is less than 500 Da;
2) compounds are unionised;
3) log P value of the drug is close to 2;
4) cumulative number of hydrogen bonds is not more than 10.

Unfortunately, only a small percentage of drugs fit these criteria. For other therapeutic molecules, their transport across the BBB will have to rely on either the integrity of the BBB or the drug or drug carrier properties and their interaction with or affinity for receptors expressed at the BBB as well as other biological or immunological processes occurring at the BBB. Several strategies have been investigated for effective clinical outcome for different CNS conditions and transport of drugs across BBB. In other words, the BBB properties and related biological processes and their roles in trafficking various types of molecules as drug delivery are fundamental to the success of its transport across the BBB.

This is the reason for the need to gain a thorough understanding of the biological and pathological properties and different probable strategies based on novel and chemistry based approaches for transport of drugs across the BBB.

Novel approaches for drug delivery to brain

The field of novel drug delivery system has fully emerged and came into existence as an ideal approach of drug targeting and delivery to brain. It mainly includes the use of small colloidal particles. The basic reason of common acceptance of these carriers is due to their controlled drug release nature as well

as their selected targeting mechanism. Targeting action may be due to the steric hindrance created by nanovectors for achieving targeting ability. These carriers are usually administered through various routes comprising oral, nasal, intra-arterial and parenteral route.

Due to their steric phenomenon, they prevent themselves from opsonisation event induced by tissue macrophages. By this way they achieve targeting ability to brain and other reticuloendothelial system (RES) organs like liver, spleen, etc. **Table 4** gives an account for drug molecules being implemented by various approaches which overcome the problems encountered in brain targeting and during drug delivery (Alam *et al.*, 2010).

Novel approaches: Implications of Nanotechnology to improve drug delivery to the brain

Principles of brain delivery using nanocarriers

The use of nanocarriers can improve brain delivery of drugs in several ways. Numerous studies have been reported about the use of different nanocarriers for delivering a broad range of therapeutic or diagnostic agentsin enhancement of *in vitro* and *in vivo* BBB permeability and drug accumulation in the brain. Many of the agents delivered are well-established substrates of ABC transporters.

These include P-gp substrates, e.g. doxorubicin, digoxin, rhodamine, vinblastine and MRP substrates, e.g. methotrexate, fluorescein (Krishna and Mayer, 2000). They usually present poor BBB permeability, but by the use of nanocarriers these compounds were able to achieve the desired therapeutic levels in the CNS with reduction in their doses as well as shorten the length of therapy evidenced by releasing the drug moiety in a sustained manner.

This approach aids for reduced risks of peripheral adverse drug effects. The circulation time can be prolonged and non-specific tissue binding reduced by coating a nanocarrier with polyethylene glycol (PEG) (Ryan *et al.*, 2008). Because only the carrier itself is engineered without the need to modify the drug molecules, dramatic alterations of the drug pharmacology can often be avoided. In addition, nanocarrier systems are known for their flexibility and versatility which can be made of different biocompatible materials. Most carriers can be engineered to obtain more desirable pharmacokinetic and bio distribution profiles for optimal treatment of the CNS.

Overview of nanocarrier-mediated drug delivery systems across the BBB

Fig. 4 represents a proposed scheme depicting about transportability of nanocarrier-mediated drug delivery systems across the BBB. Overall, nanocarriers can enhance brain delivery by three major pathways, which include:

1) increasing the local drug gradient at the BBB by passive targeting;
2) allowing drug-trafficking by non-specific or receptor-mediated endocytosis;
3) blocking drug efflux transporters at the BBB.

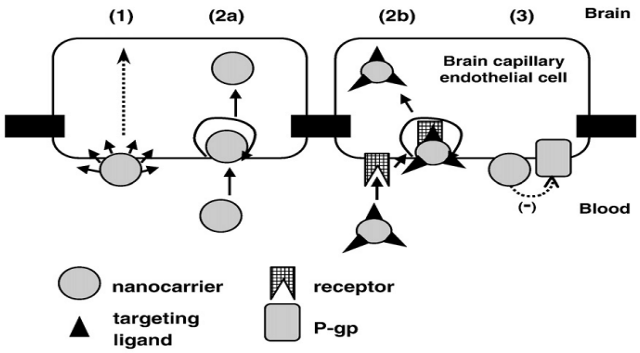


Fig. 4: Major pathways for transport of nanocarrier systems to improve drug penetration across the blood-brain barrier. (1) increasing the local drug gradient at the BBB by passive targeting, (2a& 2b) allowing drug-trafficking by endocytosis (non-specific or receptor-mediated), (3) blocking drug efflux transporters. (–): inhibitory effect (Wong et al., 2010).

Mechanism of nanocarriers and nanoparticles transport across the BBB

One of the proposed mechanisms for transport of nanoparticles across the BBB is that when nanoparticles are administered, apolipoprotein E or B adsorbed on the particle surface and then interact with the LDL receptor followed by endocytotic uptake as shown in **Fig. 5.** By this mechanism, the nanoparticles mimic the uptake of naturally occurring lipoprotein. This hypothesis was supported by dalargin-loaded poly(butyl cyanoacrylate) nanoparticles with adsorbed apo E or loperamide-loaded albumin nanoparticles in triumph of an antinoiceptive effect (Kreuter *et al.*, 2002).

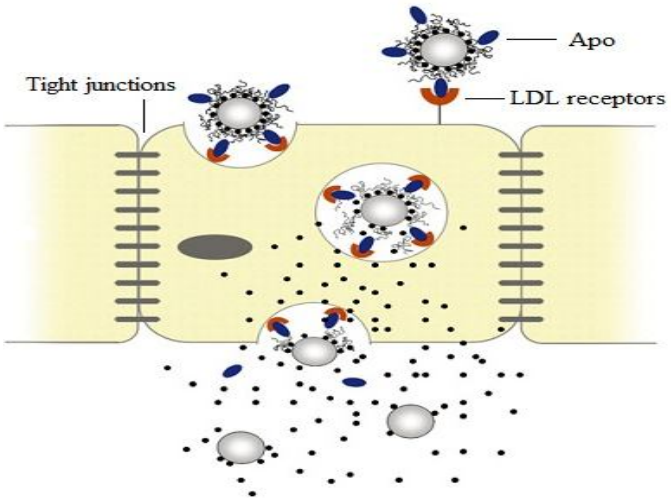


Fig. 5: Schematic representation of the proposed mechanism of polymer coated (polysorbate 80) nanoparticles/ nancarriers transport across the BBB (Blasi et al., 2007).

1. Adsorption of Apo on the surface of the coated nanoparticles (NP).
2. NP binds to the LDL receptor and interacts with it.
3. Passage of SLNs and NLCs across the BBB by endocytosis or transcytosis mechanism.
4. Release of the drug takes from NP.

Ideal attributes for transport system-mediated drug delivery systems across the blood-brain barrier

The several nanoparticulate drug delivery systems involving liposomes, nanoparticles and super molecular complexes in combination with endogenous transport systems present in the BBB have been used to transport the drug across the brain. The characteristics of these systems are (Chen and Liu, 2012):

1) the drug is either chemically modified, for instance, conjugated to a ligand or polymer as a homing device there by masking its intrinsic properties;
2) the drug is encapsulated in a surface modified drug delivery system, such as liposomes, nanoparticles or niosomes. The surface of the drug delivery system is often modified with hydrophilic polymer such as PEG to prolong the circulation time of the delivery system;
3) the drug delivery system should be non-immunogenic and capable of interacting with receptors presented at the BBB to facilitate the uptake of the drug by the BBB;
4) all systems must have controlled size, therefore their properties are uniform, consistent and their biological fate can be controlled.

Present scenario of nanocarriers for drug delivery to brain

A number of nanocarrier systems have been studied in *in vitro* or animal models specifically studied for drug delivery to brain. These nanocarriers generally fall into a few broad categories: polymer or dendrimer-based (acrylicpolymers, polyesters of lactide units and pluronicblock co-polymers), micelle-based and lipid-based (Wong *et al.*, 2010).

Polymer or dendrimer-based nanocarriers

Poly(butyl cyanoacryalate) (PBCA) is an acrylic polymer, has been extensively studied polymer for brain delivery. PBCA nanoparticles have been demonstrated good accumulation in both the brain tissues and cerebrospinal fluid without physical disruption of the BBB integrity. PBCA is biodegradable in nature and its lipophilicity facilitates efficient encapsulation of varied types of neutral and weak base compounds such as doxorubicin, dalargin, loperamide, amitriptyline and methotrexate. However, *in vivo* biodegradation of these polymers is generally so fast with generation of potentially harmful formaldehyde as by-products during the degradation (Alyaudtin *et al.*, 2001).

In case of polar or ionic compounds, due to low loading capacity of PBCA, charged acrylic co-polymers such as methyl methacrylate-sulfopropyl methacrylate (MMA-SPM) were studied as a substitute. The negative charges of these MMA-SPM nanocarrier system was responsible for higher loading capacity of polar or ionic compounds including zidovudine and lamivudine with increase in the permeability across an *in vitro* BBB model of bovine brain-microvascular endothelial cells by 8-20 and 10-18 folds, respectively (Kuoand Chen, 2006). Polyesters such as poly(D, L-lactide-co-glycolide) (PLGA) and polylactide (PLA) have shown several qualities which make them appealing for brain targeting with efficient delivery. Their metabolic degradation by-products are water and carbon dioxide which are relatively non-toxic in nature. Because of their safety profiles, PLGA and PLA are officially approved polymers for clinical use. Their molecular weights, hydrophilicity, degradation rate and release kinetics can be conveniently tailor-made by adjusting the composition. They easily form hydrolysable bonds with targeting moieties as lectin and diverse therapeutic molecules. Drug loading and brain targeting with modification of these systems using PLGA/PLA nanocarriers are quite convenient and appropriate. Multiple fold increase in brain drug concentration were observed by use of PLGA and PLA systems with dexamethasone and vasoactive intestinal peptide respectively, when both administered by intranasal route (Gao *et al.*, 2007).

Likewise polymers, dendrimers consist of repeatedly branched molecular structures of monomer units. When precisely engineered, these highly branched molecules can form spheroidal nanostructures of 1 to over 10 nm in diameter. Some of these nanostructures may contain internal void spaces or surface functional groups for encapsulation or conjugation of drug molecules, which can be used as nanocarriers for drug delivery. Dendrimeric nanocarriers have been shown to increase in BBB permeability of therapeutic agents such as DNA and methotrexate. It was also evidenced for exhibition of 21 fold increase in brain cellular uptake of lamivudine when compared with free drug solution. Despite these promising results, the drug release kinetics of dendrimers is sometimes unpredictable and their long-term safety profiles are relatively less proven as compared to polymers like PLGA (Duttaand Jain, 2007).

Micelle-based nanocarriers

A micelle is an aggregate combination formed by 50-100 amphiphilic molecules including surfactants, block-copolymers etc., when dispersed in a liquid phase. In aqueous solution, the amphiphilic molecules aggregate and orient themselves in such a way that exposure of their hydrophilic heads outside and hide their hydrophobic segments in the inner core region. The size of a micelle usually ranges from 5 to 20 nm in diameter. The small size and good drug solubilization properties make micelles potentially valuable nanocarriers for drug delivery. Pluronic micelles have shown highly effective in drug transport enhancement across BBB *in vitro* and *in vivo*.

Drug permeability enhancement of 19-fold was observed with the use of micellar drug delivery systems comprising drugs such as paclitaxel, vinblastine as well as ritonavir (Batrakova *et al.*, 2003).

Nanosuspensions

Nanosuspensions are homogenous dispersions of very fine particles of drugs which are stabilized with surfactants. These are preferably developed for highly lipophilic compounds which have partial solubility in dispersion medium and can be solubilized by excessive physical and mechanical treatment, especially designed with an aim of drug delivery. The large effective surface area of the fine drug crystals assist in increase of their

bioavailability both to the systemic circulation and the brain. Few of reports available which represented the study of atovaquone and indinavir nanosuspensions for drug delivery to the brain (Dou *et al.*, 2009).

Lipid-based nanocarriers

Lipid-based nanocarriers have been considered to be promising drug delivery system in transport of therapeutic agents to the CNS. There are a wide range of physiological lipids and phospholipids available in the development of lipid nanocarriers. These materials are biodegradable and biocompatible in nature. A number of lipid-based formulations (e.g. liposome, lipoplex) are commercially available in the market and all of them have evidenced for clinical safety. The technology transfer of these formulations on industrial scale has also been well-established (Sawant and Dodiya, 2008).

As lipophilic materials have the natural tendency to target the BBB, hence it is expected that lipid-based nanocarriers will be useful for CNS delivery of therapeutic actives. There are several classes of lipidbasednanocarriers available, including liposomes, micro- or nanoemulsion, solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC) and lipid drug conjugates (LDC).

Liposomes

Liposomes are vesicles made of one or more phospholipids bilayers. They are probably the most studied and used lipid-based nanocarriers. They are clinically well recognized nanocarriers owing to their long track record, low toxicity and ability to deliver both hydrophilic and lipophilic compounds with more efficient manner. A number of liposomal systems have been developed which were evaluated with significant improvements in brain drug levels for the treatment of various brain illnesses such as brain tumors by cisplatin, cerebral ischemia by citicholine and epilepsy by phenytoin (Anda*et al.*, 1995; Fresta *et al.*, 1994). Few of the studies also reported as use of liposomal formulations for delivery of anti retroviral drugs (ARVs), e.g. stavudine and zidovudine, which were prominently designed in case of HIV associated CNS illnesses.

Liposomal foscarnet also witnessed for increase the drug level in rat brains by 13-foldwhen compared to the free foscarnet solution. Amphotericin B is usually used to treat the opportunistic fungal infection which does not cross the BBB. Hence, the use of liposomes coupled with brain targeting peptides for delivery of amphotericin B significantly improved the drug transport across the simulated BBB model comprised of rat brain endothelial cells (Zhang *et al.*, 2003). Though liposomes exhibited the use in drug delivery of actives from varied categories, it faces some limitations as quick metabolic degradation of the phospholipids, fast systemic elimination, and instability after extended storage period of time. Few of these problems have been overcome by surface modification of these liposomes using hydrophilic polymer such as polyethylene glycol (PEG) conjugated phospholipids, known as PEGylation. Due to PEGylation approach, liposomes show prolonged blood circulation with extension in half lives of drugs as well as increase in residence time *in vivo.* A highly flexible and hydrated PEG chain attached to the liposomes surface exhibits an effective opsonins-resistant property due to its steric repulsion effect (Woodle, 1995).

Nanoemulsions and microemulsions

Nanoemulsions and microemulsions are usually oil-in-water formulations in which the oil phase is highly dispersed in the form of submicron size droplet sand stabilized by surfactants and co-surfactants. T

he average oil droplet size was in the range of 10-200 nm in diameter. Recently, highly lipophilic protease inhibitor (PI) drug such as saquinavir was the first formulated and evaluated in an oral formulation of flaxseed oil-based nanoemulsion for brain delivery in the treatment of HIV. Such approach of using nanoemulsion evidenced for increase in oral bioavailability of saquinavir to the brain and maximum saquinavir concentration in the brain of malleable mice by three and five folds respectively, when compared to its free drug solution. The study shows that; as well to the enhancement in BBB permeability, small size of the nanoemulsion and microemulsion may help in bypass the gastrointestinal tract barriers when used as oral formulations (Vyas *et al.*, 2008).

Hence there is a great potential for drugs used in CNS complications especially in brain delivery and transport by formulation and development of such lipid based drug delivery systems as nanoemulsions and microemulsions.

An overview of various nanoparticulate carrier systems especially for drug delivery to brain are mentioned in **Table 5** (Alam *et al.*, 2010).

Solid lipid nanoparticles (SLNs) and Nanostructured lipid carriers (NLCs)

Solid lipid nanoparticles (SLNs) are a relatively new class of lipid based nanocarriers, made up of a matrix of lipids. These are constituted by one or more lipids which having melting points higher than body temperatures hence carriers remain in solid state at both the room and body temperatures. These solid lipids are mostly physiological lipids such as fatty acids, mono-, di- or triglycerides, glycerin mixtures and waxes. Whereas, nano structured lipid carriers (NLCs) comprises both the solid lipids and liquid lipids (oils) creates voids for higher encapsulation of drugs. The low solubility of these nanocarrier biomaterials leads to high tolerability and low toxicity of the formulation in the body. A related class of nanocarriers is called lipid nanocapsules, which typically consist of a mixture of phospholipids and triglycerides. All of these formulations necessitate surfactants (non-ionic or ionic) for stabilization. These nanocarriers exhibit modified drug release profile including sustained and controlled release of therapeutic agents (Muller *et al.*, 1997).

Without surface modification of drugs, lipid nanocarriers are well known to cross the BBB and exhibit required therapeutic effect. SLNs and NLCs were found to bind apo lipoproteins and

target brain tissues like polymeric nanoparticles containing PBCA. Most of anti-cancer drug including paclitaxel and doxorubicin demonstrates that by use of lipid based nanoparticles including SLNs, NLCs, lipid nanocapsules or their PEGylated forms causes improvement in brain accumulation (Pandita *et al.,* 2011). This could be achieved by administering the lipid formulations by various routes such as orally, intravenously and transdermally. Camptothecin loaded SLNs containing stearic acid as a solid lipid have shown remarkable increase in drug brain concxxgv m, entration after both oral and i.v. administration in mice. Upon i.v. administration in mice, maximum concentration (C_{max}) of camptothecin was found to be increased by 180% as compared to plain drug solution with 10.4 fold of enhancement in area under the curve (AUC) (Yang *et al.,* 1999). Clozapine loaded SLNs containing tripalmit in as solid lipid having particle size of 163 nm and zeta potential of +23.2 ± 0.9mV have shown significantly increase in drug brain concentration over clozapine suspension after i.v. administration in mice (Manjunath and Venkateswarlu., 2005). Where as in case of paclitaxel, Brij 78 stabilized wax nanoparticles were represented note worthy increase in brain drug distribution as compared to paclitaxel solution containing 50:50 v/v mixture of the surfactant Cremophor ELR and dehydrated ethanol (Blasi *et al.,* 2007). Doxorubicin loaded SLNs exhibited significantly higher drug concentration in brain when animal (rat CSF tissue) groups treated with stealth SLNs. Doxorubicin coated with polysorbate 80 nanoparticles also have shown 40% cure in rats within tracranially transplanted glioblastomas (Blasi *et al.,* 2007; Alam *et al.,* 2010). Oral administration of Melatonin SLNs showed delayed onset of action with enhancement in both the AUC and half-life (Priano *et al.,* 2007). Whereas, intra peritoneal administration of baclofen SLN reveled for maintenance of sustained drug plasma concentration and prominent sedative effects in rat as compared to pure drug solution (Priano *et al.,* 2011). Hence in an account of a fore mentioned studies, lipid based nanocarrier formulations demonstrate strong potential for CNS drug delivery. Some of drugs are enlisted which are incorporated into SLNs and NLCs especially for brain targeting are mentioned in **Table 6.**

Solid lipid nanoparticles (SLNs)

SLNs are produced by replacing the oil of an o/w emulsion by a solid lipid or a blend of solid lipids, i.e. the lipid particle matrix being solid at both room and body temperatures. SLNs are composed of 0.1% (w/w) to 30% (w/w) solid lipid dispersed in an aqueous medium and stabilized with suitable concentration of surfactant, co-surfactant ranging from 0.5% (w/w) to 5% (w/w). The incorporation of pharmaceutical actives is feasible and generally carried out by using different preparation methods. The mean particle size of SLN is in the submicron rage, ranging from about 40 nm to 1000 nm (Lucks and Muller, 1996).

Nanostructured lipid carriers (NLCs)

NLCs are produced using blends of solid lipids and liquid lipids (oils). To obtain the blends for the particles matrix, solid lipids are mixed with liquid lipids, preferably in a ratio of 70:30 to a ratio of 99.9:0.1. Because of the oil presence in these mixtures, a melting point depression is observed compared to the pure solid lipid, but the blends obtained are also solid at room and body temperatures.

Due to heterogeneous nature of both the lipids it leads for creation of voids in the matrix which facilitates for maximum entrapment of drug. The overall solid content of NLCs could be increased up to 95% (Muller *et al.,* 2000).

Types of NLCs

It is well known from the study that highly ordered crystalline lipid matrices will lead to drug expulsion. Lipid nanoparticles and microparticles made from blends of solid lipids can experience this problem, especially when they are prepared from highly purified lipids, for example, tristearin. The formation of highly ordered β_i or β modifications, particularly during storage, leaves little space for drug molecules, and the expulsion of drugs leads to drug crystals in suspensions and solid dosage forms. To avoid this problem, the particles should have a controlled nanostructure that offers enough space to accommodate the drug.

Four different approaches were taken for an optimized nanostructure of NLCs. In type I, solid lipids and liquid lipids (oils) are blended. The difference in the structures of the lipids and special requirements in the crystallization process lead to a highly disordered, imperfect lipid matrix structure offering space for drug molecules and amorphous clusters of drugs (**Figure 6., I**).

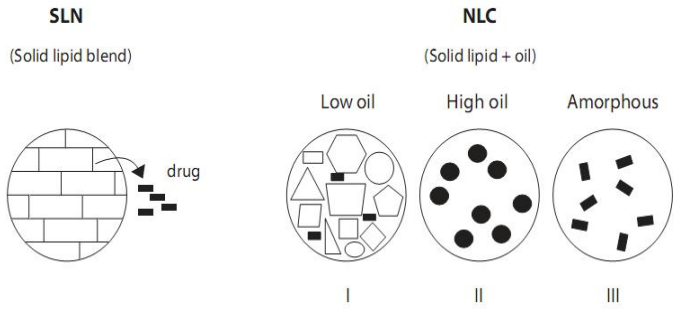


Fig. 6: Different types of NLC: I – Highly imperfect matrix; II – Multiple O/F/W type; III – Non-crystalline amorphous NLC (versus SLN with high crystallinity) (Radtke et al., 2005).

In general, drug solubility is higher in liquid lipids than in solid lipids. Based on this, particles were produced with a high content of liquid lipids (oils). During the production process, the liquid lipid particles are cooled from the molten state to room temperature leads for crystallization and form solid particles. At high oil concentrations a miscibility gap of the two lipids (solid lipid plus oil) occurs during the cooling phase, leading to phase separation, that means precipitation of tiny oily nanocompartments (**Fig. 6 II**).

In this multiple oil/fat/water, type II drug can be accommodated in the solid, but at increased solubility in the oily parts of the lipid matrix. In type III, lipids are mixed in a way that prevents them from crystallizing. The lipid matrix is solid, but in

an amorphous state (**Fig. 6 III**). The absence of crystallization avoids drug expulsion by crystallization. Lipid particles are preferentially suited to incorporate lipophillic drugs; hydrophilic drugs can only be incorporated at a low percentage (however, this is still sufficient for highly potent peptides and proteins) (Radtke *et al.*, 2005). In a further variation of the lipid matrix, water-soluble drugs were conjugated with a lipid, thus forming a water-insoluble lipidic conjugate. The lipid conjugate powder was melted and processed in the same way as the other types to yield a lipid drug conjugate (LDC) nanoparticle. Depending on the conjugate, this lipidic conjugate has a drug loading of 30-50% for water-soluble drugs. Conjugation is performed by salt formation or covalent linkage.

Modulation of drug release

Drug release from lipid particles occurs by diffusion and simultaneously by lipid particle degradation in the body. In some cases it might be desirable to have a controlled fast release going beyond diffusion and degradation.

Ideally this release should be triggered by an impulse when the particles are administered. NLCs accommodate the drug because of their highly disordered lipid structures. By applying the trigger impulse to the matrix to convert in a more ordered structure, such a desired burst drug release can be initiated shown in **Fig. 7.** NLCs of certain structures can be triggered in this way when these particles are administered as depot preparations through intravenous and intrathecal routes (Manjunath and Venkateswarlu, 2005).

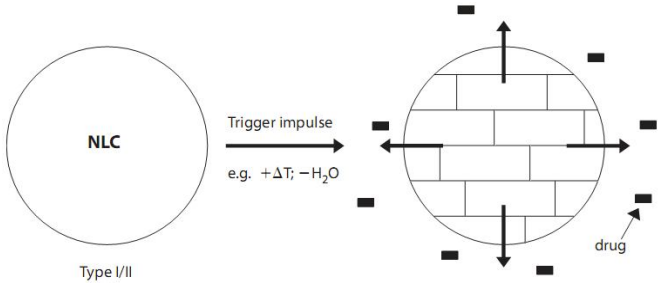


Fig. 7: Triggered drug release from NLC by initiating the conversion from a highly disordered lipid structure to more ordered stable modifications (Radtke et al., 2005).

Long term stability

During long-term storage of dispersions, particle aggregation can occur. Aggregation and shell formation were reported for SLNs.

Single particle diffuses in the dispersion medium and causes collision of particles which can lead to perikinetic flocculation (**Fig. 8a**). In the highly concentrated NLC dispersions the particles form a 'pearl-like network', thus the particles are in a fixed position and cannot undergo collision and perikinetic flocculation. After administration of the particles and dilution with fluids (for example, gastrointestinal fluids), the network is destroyed releasing single, non-aggregated particles (**Fig. 8b**). The NLC dispersions remained stable during storage and even after dilution. Hence forth, it gives evidence for long term stability with no remarkable increase in particle size (Freitas and Muller, 1999).

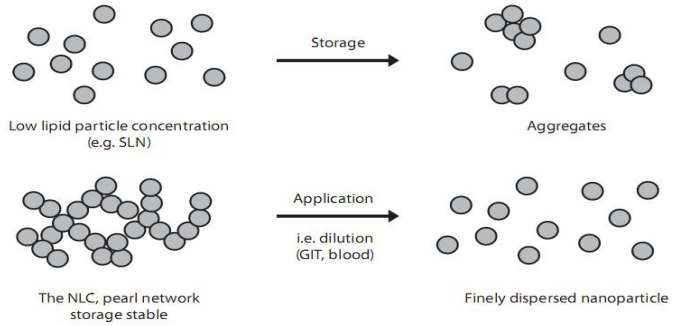


Fig. 8: a) Aggregation process in low concentrated dispersions **b)** pearl-like network in NLC dispersions with stabilizing effect (Freitas and Muller, 1999; Radtke et al., 2005).

Preparation methods of lipid nanoparticles: SLNs and NLCs

Melt-Homogenization technique

SLNs, NLCs can be produced by homogenization of the molten lipids in an aqueous phase. The preparation by this technique involves two steps. First, the lipids are heated at least 10°C above their melting point. The melted lipids are then dispersed in hot aqueous medium using a suitable dispersing agent. Dispersion is accomplished using mechanical stirring or by ultrasonication. The pre-mix formed is then passed through a thermostatized high-pressure homogenizer under optimum homogenization conditions. The second step involves the solidification of oil droplets by cooling the hot dispersions to room temperature. For drug loaded lipid nanoparticles the drug is dissolved either in melted lipid or in hot aqueous phase prior to emulsification (Liedtke *et al.*, 2000).

Microemulsification-solidification

Lipid nanoparticles can be produced by microemulsification of molten lipids, as the internal phase and subsequent dispersions of the microemulsion in aqueous medium under mechanical stirring. Such preparation does not require energy. Rapid crystallization of oil droplet on dispersion in cold aqueous medium produces lipid nanoparticles with solid matrix (Gasco, 1997).

Multiple microemulsification solidification

Multiple emulsions are complex systems, often called emulsions of emulsions, in which drops of dispersed phase contain smaller droplets that have the same composition as the external phase. Like simple emulsions, multiple emulsions are also considered of two types: oil-in-water-in-oil (o/w/o) and water-in-oil-in-water (w/o/w). Warm w/o/w multiple microemulsion can be prepared by addition of formed w/o microemulsion to a mixture of water, surfactant and co-surfactant to obtain a clear w/o/w system, vice versa conditions for o/w/o multiple microemulsion. Lipid nanoparticles can be obtained by dispersing the warm micromultiple emulsion in cold aqueous medium in a fixed ratio,

under mechanical stirring. The suspension of lipid particles is then washed with dispersion medium by ultrafiltration system (Garcý-Fuentes *et al.,* 2002).

Ultrasonication

Nanoemulsions and lipid nanoparticles can be prepared by using ultrasonication method which uses high sonication energy for breaking and minimizing the droplet size. It uses temperature, amplitude and time cycles for optimization batches of lipid nanoparticles (Puglia *et al.,* 2008).

Solvent evaporation

Such method comprises of addition of organic solution to the aqueous phase containing the stabilizing agent and stirred magnetically. The solvent was then evaporated under reduced pressure (Schubert and Muller-Goymann, 2003).

Phase inversion

The phase inversion of the emulsion occurs when the concentration of dispersed globules in the dispersion medium is high i.e. the globules are packed very closely in the suspending fluid. The concentrated o/w emulsion is thermally induced to produce w/o/w emulsion. When an aqueous solution of hydrophillic emulsifier is introduced into oil containing lipophilic surfactant, the w/o/w emulsion is obtained due to phase inversion of w/o emulsion. Phase inversion technique can be exploited to produce emulsions characterized by their fine droplet size (Matsumoto and Kanig, 1989).

Chemistry based approach

The chemical substances which help in transporting the drug substances across BBB are widely used as ideal agents for drug delivery to brain. Such kind of approaches has quite frequently being researched through aim of delivering drugs to the brain. These approaches include the use of chimeric peptides and cationic proteins for drug delivery to the brain.

Chimeric peptides

The word "chimeric" obtained from the Greek word chimera means an animal having body of lion and head of human. So this approach deals with the drug substances which are not transported through BBB are combined or covalently bonded with a transport vector to form an easily transportable or fused molecule shown in **Fig. 9** (Pardridge, 2001). Formation of such kind of peptides based on two principles; firstly the vector itself should have pharmacological activity, for example insulin. Secondly the interaction between peptide vectors with its binding receptor site must be highly specific for targeting drug to brain. Few of examples have been investigated satisfying these conditions including cationized albumin, monoclonal antibodies and histones (Bickel *et al.,* 2001). These act as ideal peptides for transferrin receptor and cross BBB.

The mechanism involves the initial binding of such vector to BBB on its exofacial epitopic site, which leads to removal of epitope of mAb from endogenous ligand binding site and freshly binds with "piggy back" across the BBB from where drug delivery occurs over the surface.

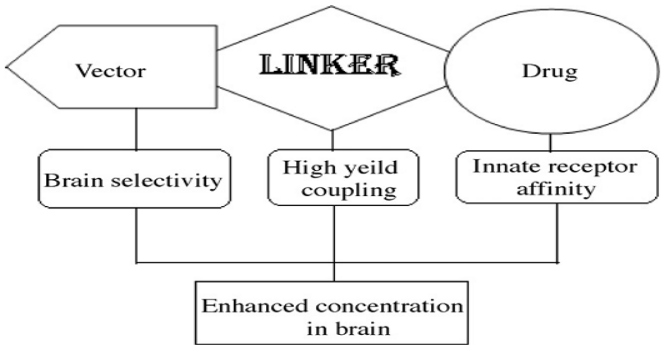


Fig. 9: Interlinked components involving vector and linker for drug delivery to brain.

Cationic proteins

In general proteins are of high molecular weight, hence cannot cross the BBB due to their very large size (Pardridge, 2002). Hence by cationization of proteins, they can easily enter the brain by electrostatic interaction with anionic functional groups present on brain surface, with transcellular adsorptive-mediated endocytosis pathways. Cationization process involves increase in the net positive charge on the polypeptide by modifying the free carboxyl groups of acidic amino acid residue on polypeptide. The free carboxyl groups of the polypeptide (e.g. NGF) may be modified with hexamethylenediamine, polylysine, diazomethane or polylysine cationization with cleavable ester bonds to enhance BBB transport as well as to produce intact growth factor (Lewis *et al.*, 1992; Pardridge, 2001).

Various cationic proteins have been studied to penetrate the BBB including avidin, histone, protamine and cationized polyclonal bovine immunoglobulin (Brasnjevic etal., 2009). Some cationic proteins or peptides have been reported to protect the brain including cationized antibodies against β amyloid depositsin patients suffering from Alzheimer's disease or viralantigens or oncogenes in tumors (Scherrmann, 2002).

Cyclodextrin complexes for drug delivery

Cyclodextrin is a tortuous shaped polysaccharide, originated from fermentation. It's occurrence found in nature with basket shapes in three different forms such as α, β and γ. It has two kinds of chemical regions. First is the core of basket which is hydrophilic and the inner cavity of basket is lipophilic in nature. Drug molecules of both hydrophilic as well as lipophilic in nature can be entrapped within it. It uses the similar "lock-in" phenomenon to entrap and release the drug. Cyclodextrin forms inclusion type of complexes and kind of chemical delivery system which releases the drug at the target site of action. Galanin peptide considered as potential applications in treating obesity have been represented remarkable concentration achievement in brain microenvironment when it was administered in the form of cyclodextrin complexes (Nonaka *et al.,* 2008). It also exhibits

number of advantages as solubility enhancement, bioavailability enhancement, biotransformation reduction, etc. (Brewster and Loftsson, 2002).

Antibody directed enzyme prodrug therapy (ADEPT)

ADEPT is an advanced method involves the use of antibody for targeting the drugs on the tumor surface of brain. The therapy involves the injection of antibody-enzyme complex into body through systemic route, which attaches to the surface of tumor expressing particular type of antigen. When prodrugs are injected, they reach the same site where antibody-enzyme complex bound on the tumor surface and are metabolized by the enzyme to get available on tumor site (Haisma *et al.,* 1998; Sharma *et al.,* 2005). Hence this technique is called as "antibody directed enzyme prodrug therapy". Most widely used antibodies are the monoclonal antibodies prepared by hybridoma technology. At very first time the prodrug of nitrogen mustard was practiced using herpes simplex virus thymidine kinase enzyme (Melton and Sherwood, 1996). Recently liposomes have been coupled to the antibody surface and prodrugs have been entrapped in liposomes instead of delivering them later, known as enzymosomes, immune liposomes or liposomal based ADEPT (Vingerhodes *et al.,* 1993). Nowadays, viruses are also used as a vector for targeting prodrugs to brain tumor named as virus directed enzyme prodrug therapy (VDEPT). The new emergent concept comprises the use of folic acid instead of monoclonal antibody (Reddy and Lows, 1998). Similarly, the use of genes is made in place of antibody for prodrug targeting to brain where a specific antigen markers are developed for selective expression of prodrug activating enzymes coded by genes. The enzyme expressed by the gene causes metabolism of prodrug with projection of active moiety in the brain (Deonarain *et al.,* 1995).

Biotin-avidin conjugated system for drug delivery

From last few years, this method has gained considerable interest in drug delivery to brain. It involves coupling between the molecules to be transported and biotin-avidin/streptavidin system where anti-transferrin receptor antibodies are present on its surface. It releases drug by binding with antigen which is expressed on the tumor surface of brain (Pardridge *et al.,* 1995a). The transport of non-transportable peptide such as vasoactive intestinal peptides (VIP) can be transported to brain with increase in blood flow. Likewise immune liposomes, biotin-avidin systems are also conjugated with liposomes by covalent conjugations for targeting drug molecules, proteins and peptides to tumor cells (Longman *et al.,* 1995).

Prodrug

Prodrugs are intended to deliver the hydrophilic drugs for brain. These are chemically modified, inert alternatives of original active pharmaceutical ingredient (API) to get a product of large bulky structure which is devoid of any biological toxicity and activity. The lesser toxicity profile of prodrugs may be due to site specificity. Prodrugs have amino acid as a promoiety differs from chimeric peptides being lacking of any kind of biological activity. Due to the enzyme specificity, whenever these moieties reach the brain, the enzymes present on the surface of BBB causes metabolism of prodrugs to give active agents, which then cross the BBB and attains effective concentration in brain. For first time, the concept of prodrug of L-dopa by conjugating dipalmitoyl glyceride moiety has been evolved as an ideal candidate for drug delivery and targeting to brain especially in treating for Parkinsonism (Garzon-Aburbeh *et al.,* 1986). Likewise, Azidothymidine prodrug was made by conjugating with dihydronicotinate system at the 5'-position of AZT showed significant increase in concentration at brain surface for treating neuropsychiatric symptoms concomitant with AIDS. Also, Zidovudine has evidenced for considerable increase in CSF concentration (Prokai *et al.,* 2000).Several other prodrugs have been intended for brain targeting include prodrugs as ganciclovir-dihydronicotinate system for cytomegalovirus infection, ribavirin-dihydronicotinate system for treating RNA virus infections, acyclovir-trigonelline hydrochloride for treating herpes simplex virus associated meningitis and varicella zoster virus infection (Brewster *et al.,* 1987).

Prodrug "Lock-in" mechanism for drug targeting

This method was first time practiced for delivering neuropeptides to brain (Bodor and Prodai, 1995). Several molecules being used by this method are estradiol, thyrotrophin, carmustine, kyotorphin, enkephalin, TRH, etc. An approach is similar to prodrug, but the difference is in the attachment of different additional functions to active moiety such as an adjuster (A), a bulkier lipophilic moiety (L), a spacer (S) and a target (T) for locking them in brain compartment. The drug is packed by lipophilic group (L) with covalently attachment to enhance lipid solubility and to disguise the nature of the molecule through an ester bond formation or sometimes through a C-terminal adjuster (A) at the carboxyl terminal and target or (T) which further then undergoes enzymatic oxidation and turns to an ionic, membrane-impermeable moiety (T+). After distribution in the body and into the CNS by crossing the BBB, the conjugate is converted to ionic compounds which are retained in brain tissue, whereas ionic conjugates produced in the rest of the body are easily eliminated. The membrane impermeable moiety locked into the brain and furthermore causes sequential metabolism and project the drug into the CNS. Amino acids are strategically used to provide a spacer (S) which ensures timely removal of the charged target by controlling the enzymatic rate of drug release (Pavan *et al.,* 2008). These systems exhibit the drug action by passive diffusion mechanism.

The lipophillic portion undergoes metabolic conversion to liberate out the free active therapeutic moiety, which entraps in brain compartment and eliminates slowly over a period of time (Boucher *et al.,* 1996). Several drugs are also being implemented by such prodrug mediated drug delivery systems include zidovudine, ganciclovir, lomustine, benzyl penicillin, etc. (Bodor and Buchwald, 1999).

Table 4: Brief account of drug molecules being implemented with various approaches for brain delivery.

Drug molecules	Problem	Approach
Dopamine	High water solubility and lower lipid solubility	Transnasal route.
Olanzapine	Lesser uptake drug due to hydrophobicity	Use of micro-emulsion formulation containing mucoadhesive polymer.
Cytosine arabinoside	Rapid turnover from cerebral environment due to leakage of CSF and lower half life	Given in a suspension formulation containing multivesicular lipid.
Etoposide	Drug shows lesser concentration in brain	Instigated in the form of reservoir type osmotic pump (Omayama, Mini Med PIMS® system) by implantation.
Lomustine	Due to lower residence time of the drug in cerebral microenvironment due to leakage by ISF	Give in the form of a matrix based depot preparation injected into brain micro blood vessels.
Dalargin	Due to high molecular weight these peptides are unable to cross junctional BBB	Peptides are given in poly butyl cyanoacrylate nanoparticles coated with polysorbate-80 to protect from opsonisation.
Doxorubicin	Lack of inefficient targeting to brain with captured by RES system and removal from blood circulation.	Given in the form of nanoparticle system which is coated by polysorbate-80.

Table 5: An overview of different nanoparticulate carrier systems for drug delivery to brain.

Therapeutic agents	Materials	Mechanisms/Inference
Polymeric nanoparticles		
Amphotericin B (AmB)	Poly(lacticacid)-b-poly(ethyleneglycol) coated with polysorbate 80 (Tween-80)	Enhanced concentration in mice brain with increased permeability across the BBB.
Loperamide	Albumin coated with apo-lipoprotein E	Higher BBB concentration with sustained release profile.
Paclitaxel	Poly(lactide) (PLA) nanoparticles decorated with D-alpha-(tocopheryl polyethyleneglycol succinate)	Increased anti-tumoral activity.
Methotrexate	Poly(butylcyanoacrylate) (PBCA) coated with polysorbate 80 (Tween-80)	Significant increase in methotrexate levels in brain. Size < 100 nm penetrated BBB.
Zidovudine	Methylmethacrylate-Sulfopropylmethacrylate ((MMA-SPM)	Increased BBB permeability by 8-20 folds.
Micelles		
Doxorubicin, Digoxin, Paclitaxel, Ritonavir, Vinblastine	Pluronic P85	Drug permeability increased from 1.6 to 19 fold across mono layered BBB model by P-gp substrates.
Biphalin, Enkephalin, Morphine	Pluronic P85	Enhancement in analgesic activity both above and below the critical micelle concentration.
Nanosuspensions		
Indinavir	Nanocrystals were loaded into bone marrow macrophages	Increased CNS concentration and drug release for 14 days and reduced HIV-1 in HIV encephalitis areas.
Atovaquone	Coatedwithapo-lipoprotein E (apoE) and stabilized by polysorbate 80, poloxamer 184, poloxamer 338	Improved uptake into mice brain and reduced *T. gonadi* infection.
Liposomes		
Phenytoin	Phospholipids and cholesterol	Increased anti-epileptic activity.
Cisplatin	Phospholipids and cholesterol	Increased drug concentration in brain tumor invaded areas and causes cell killing.
Stavudine	Phospholipids and cholesterol	Reduced HIV-p24 levels in MT 2 cells of brain.
Nanoemulsions		
Risperidone	Capmul MCM as the oil phase along with mucoadhesive polymers	Intra nasal administration showed promising cerebral and CSF concentration.
Paclitaxel	Pine-nut oil containing essential poly unsaturated fatty acid (PUFA)	Showed higher cytotoxic effect in human glioblastoma brain tumor cells.
Saquinavir	Edible oils rich in essential polyunsaturated fatty acids (PUFA), surfactant Lipoid-80 and deoxycholic acid.	Increased oral bioavailability, effective brain concentration against retroviruses.
Nanoliposome		
Tempamine	Lipid	Enhanced therapeutic activity in neurodegenerative diseases involving multiple sclerosis.

Table 6: List of drugs specifically used for brain targeting by incorporating into SLNs and NLCs (Blasi et al., 2007; Alam et al., 2010).

Therapeutic agents	Materials	Mechanisms/Inference
Etoposide	Tripalmitin as solid lipid	Achieved effective drug concentration in Dalton's lymphoma when administered by intravenous, subcutaneous and intra peritoneal routes.
Temozolomide	Stearic acid as solid lipid, poloxamer 188, lecithin and polysorbate80(Tween-80)	Attained higher cerebral concentration with reduction in cardiac and nephric cytotoxicity.
Camptothecin	Stearic acid as (solid lipid), soybean lecithin and poloxamer188	Effective brain targeting with promising sustained release profile, dose reduction and diminished systemic toxicity in mice.
5-fluoro-2'-deoxyuridine (FUdR)	Octanoic acid, Pluronic F-68 and Pluronic F-127	Improved the blood brain barrier penetration.
Paclitaxel	Brij 78, Cremophor ELR	Increased drug distribution in brain.
Clozapine	Tripalmitin, Stearylamine	Increased drug brain concentration in mice after i.v. administration.
Bromocriptine	Stearic acid	Improved penetration across rat CSF tissue with delayed onset, higher AUC and longer half-life.
Doxorubicin	Precirol ATO, Polysorbate 80 (Tween-80)	Increased drug distribution in rat brain. 40% cure rates with intra cranially transplanted glioblastomas.
Baclofen	Stearic acid	Intraperitoneal administration exhibited sustained plasma concentration and sedative effect in rats.

Table 7: CMT systems used in transport of nutrients to CNS.

Carrier	Transporter type	Representative substrate	Expression at CNS
Neutral amino acid	LAT 1	Phenylalanine	Blood- brain barrier
Hexose	GLUT 1	Glucose	Blood- brain barrier
Monocarboxylic Acid	MCT 1	Lactic acid	Blood- brain barrier
Cationic amino acid	CAT 1	Arginine	Blood- brain barrier
Nucleoside	CNT 2	Adenosine	Blood- brain barrier
Ascorbic acid	SVCT 2	Vitamin C	Choroid Plexus

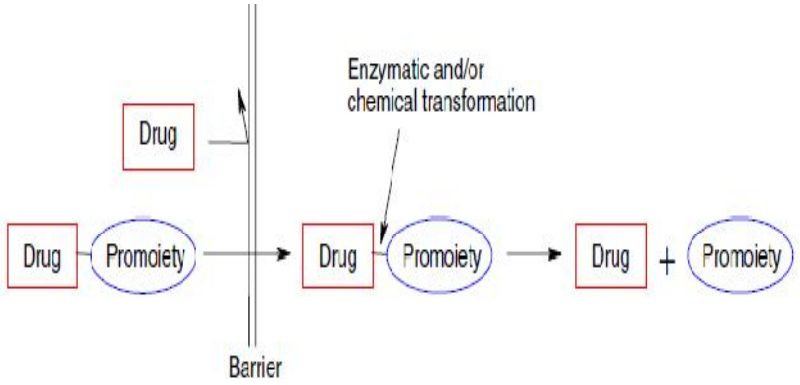


Fig. 10: Diagrammatic representation of prodrug con Covalent

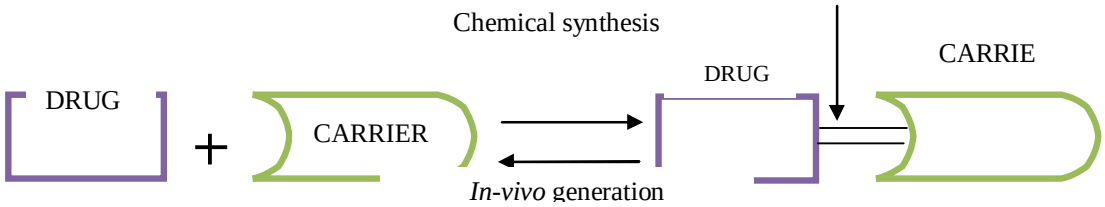


Fig. 11: Carrier linked prodrugs.

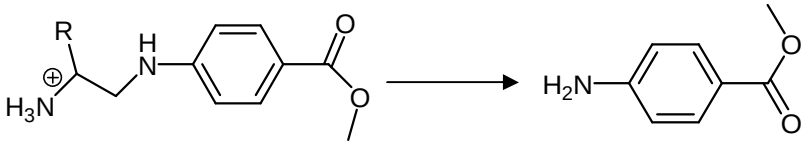


Fig. 12: Chemical structure of benzocaine with its prodrug.

Initially, the prodrug concept was introduced by Albert (1958) and his co-workers to describe 'any compound that undergoes biotransformation prior to exhibiting its pharmacological effects'. Such a broad definition includes accidental historic prodrugs (aspirin and salicylic acid), active metabolites (imipramine and desmethylimipramine) and compounds intentionally prepared to improve the pharmacokinetic profile of an active molecule. From this point of view the term, 'drug latentiation' proposed by Harper is more appropriate for prodrug concept as it indicates that there is an intention.

Drug latentiation is defined as 'the chemical modification of biologically active compound to form a new compound that, upon *in vivo* enzymatic attack, will liberate the parent compound'. Prodrug has been also defined as 'the concept of retro metabolic drug design that incorporates targeting, metabolism and the duration of action consideration into the design processes.'

Prodrug concept overcomes various short comings (Wermuth C., 2003)

1. Drugs which are not easily absorbed from GI tract because of polarity.
2. Drugs which does not cross BBB because of polarity.
3. Water insoluble, not absorbed, not capable of direct IV injection.
4. Drugs which are absorbed too quickly and where sustained release profile desired.
5. Intolerance or irritation of drugs, if absorbed as such.
6. Vulnerable drug metabolized at absorption site.
7. Chemically unstable drug in which better shelf life is needed.
8. Formulation problem, e.g. tablet formulation desired for active principle.
9. Lack of site specificity, selective transport or selective delivery desired.
10. Poor acceptance by physicians and patients due to taste or odor problems and some practical problems involving painful administration (injection).

Ideal properties of prodrugs

1. The prodrug must be readily transported to the site of action.
2. The prodrug must be selectively cleaved to the active drug utilizing special enzymatic profile of the site.
3. Once the prodrug is selectively generated at the site of action, the tissue must retain the active drug without further degradation.
4. It should not have intrinsic pharmacologic activity.
5. The metabolic fragments, apart from the active drug should be nontoxic.

Carrier linked prodrugs

The Carrier linked prodrugs result from a temporary linkage of the active molecule with the transport moiety (carrier group) that is frequently lipophilic in nature (**Fig. 11**) (Bodor and Buchwald, 1999).

Types of carrier linked prodrugs

Bipartate prodrugs

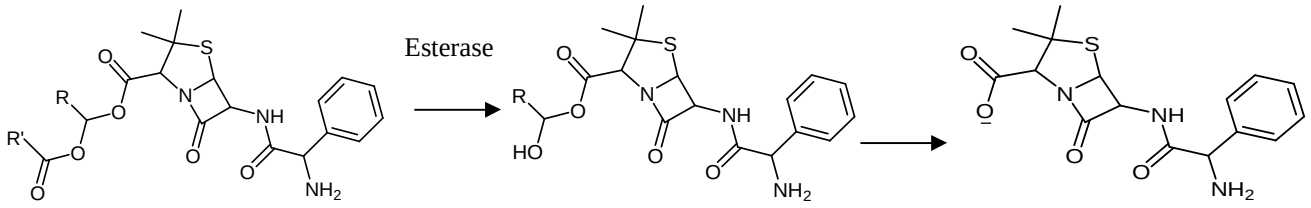
A bipartate prodrug is a prodrug comprised of one carrier attached to the drug (Pavan *et al.*, 2008). e.g. Prodrug of benzocaine

Tripartate prodrugs

When a carrier is connected to a linker that is again connected to a drug, it is called tripartite prodrug.A simple hydrolytic reaction cleaves this transport moiety at a correct moment (e.g. pivampicillin, bacampicillin). Such prodrugs are less active than the parent compound or even inactive. The transport moiety will be chosen for its non toxicity and its ability to ensure the release of the active principle with sufficient kinetics. By varying the steric and electronic properties of promoiety the rate and extent of hydrolysis can be controlled.

Ampicillin is a broad spectrum antibiotic, It suffer from poor absorption when administered orally; only about 40% drug is absorbed. In other words, to achieve the same clinical efficiency and the same blood level one must give two to three times more ampicillin by mouth than by intramuscular injection.Thus two prodrugs of ampicillin such as pivampicillin and bacampicillin were introduced as shown in **Fig. 13.**

Both results from the esterification of free carboxylic group with lipophilic, enzymatically labile ester. The absorption of these compounds is nearly quantitative (98-99%). They have same serum level and clinical efficiency as that of ampicillin. Owing to the good absorption, the drugs are given at lower dosage than ampicillin (Garzon-Aburbeh *et al.*, 1986).



Bacampicilin: R= CH_3, R= O-Et
Pivampicilin: R= H, R= tert-butyl

Amphicillin

Fig. 13: Chemical structure of ampicillinand its prodrugs as pivampicillin and bacampicillin.

Mutual prodrugs

A slight modification in carrier linked prodrugs gives mutual prodrugs, where the carrier used also has some biological activity. A mutual prodrug consists of two pharmacologically active agents coupled together so that each acts as a promoiety for the other agent and vice versa.

e.g. Mutual prodrug of ethambutol and p-amino salicylic acid (PAS). Ethambutol and p-amino salicylic acid (PAS) are potent antitubercular agents having various side effects arising due to formation of toxic metabolites. However, owing to their synergistic action, these two drugs can be prescribed together. Using this rationale, a mutual prodrug of ethambutol and p-amino salicylic acid (PAS) has been designed and its synthesis and kinetics have been reported (Pavan *et al.*, 2008).

Prodrugs and carrier mediated transport (CMT) for brain delivery

As advancement in prodrug approaches, CMT systems are used to transport nutrients, vitamins or hormones into the central nervous system. Initially, CMT transport was explored *in vivo* with physiologic techniques for determination of the Michaelis-Menten kinetic parameters (Km, expressing the substrate-transporter affinity and Vmax, expressing the transporter capacity) (Oldendorf, 1971; Pardridge, 2005; Pardridge, 2007). Nowadays, molecular cloning of transporter genes and their expression in cultured cells have extended and led to take necessary strides in the field of membrane transport mechanisms especially for the brain targeting of drugs (Pardridge, 1983; Tamai and Tsuji, 2000). CMT systems for various nutrients to be transported into the central nervous system have exemplified in **Table 7.**The transporters of neutral amino acids (LAT 1), hexose (GLUT 1), mono carboxylic acids (MCT 1), cationic amino acids (CAT 1) and nucleosides (CNT2) are widely expressed at the BBB level, whereas the ascorbic acid transporter (SVCT2) is mainly expressed in the choroids plexus (Rice, 2000). CMT systems display significant structural requirements for their substrates., in short, they are highly stereo specific in nature. Most of neuro active drugs are not transported by CMT systems; hence forth prodrugs approaches have been proposed to overcome these drawbacks. These methodologies are based on two main strategies such as: (i) the modification of drug into a "pseudonutrient" structure, able to be transported by a CMT system; (ii) conjugation of drug with a nutrient able to be transported by CMT. In both the cases, drugs are released by enzymatic cleavage from their prodrugs while being targeted into the central nervous system. Following approach of LAT1 gives detailed illustration for prodrugs as ideal prospects for drug targeting and delivery to brain.

Prodrugs and LAT 1 system

The transporters of amino acids are classified in terms of their sodium dependence (functional characteristic) and their substrate specificity. LAT subtypes are sodium-ion-independent transporters of large neutral amino acids such as leucine, phenylalanine and tyrosine which are expressed at the BBB (Tamai and Tsuji, 2000). The first cloned gene of the system L transporter named as LAT1 which were detected in the brain by Northern blot analysis method, whereas another transporters whose genes were recently cloned as LAT2. Both transporters, LAT1 and LAT2 have different substrate specificity. Presently, LAT1 is identified and evidenced as principal large neutral amino acid transporter at the BBB (Pineda *et al.*, 1999).

Currently, Parkinson's disease is usually being treated by L-DOPA which has been considered for alleviating the neurological symptoms associated with it. The example of L-DOPA in treatment of Parkinson's disease represents a suitable instance of prodrug and its transportability by LAT1 system. In this case, the dopamine does not cross the BBB, hence L-DOPA has been used by modification of the drug structure, with the aim of obtaining a prodrug as a "pseudonutrient" substrate for LAT1 while drug targeting to the brain. The α-carboxylation of dopamine allows obtaining L-DOPA which is being a large neutral amino acid and good substrate for LAT1 as shown in **Fig. 15**.

The aromatic amino acid decarboxylase induces the decarboxylation of L-DOPA, which is transported into the central nervous system and furthermore responsible for the dopamine delivery into the brain (Pardridge, 2005).

Another example is represented by L-4-chlorokynureine which can be considered a prodrug of 7-chlorokynurenic acid as shown in **Fig. 16.** This drug is deemed to be an effective as neuro protective agents while drug delivery to brain. It acts as an antagonist of NMDA receptors but due to low lipid solubility; it does not cause penetration across the BBB at therapeutic concentrations after systemic administration. The L-4-chlorokynureine, being a large neutral amino acid as a substrate of LAT1 which provides intra cerebral conversion to chlorokynurenic acid by kynurenine amino transferase (Hokari *et al.*, 1996).

Conjugation approach also plays an important role in formation of prodrugs, specifically aimed for transportation by LAT1 systems while drug targeting to brain. Example represents under such areas include conjugation of neuroactive drug nipecotic acid with neutral amino acid astyrosine as shown in **Fig. 17.**

Nipecotic acid is a potent inhibitor of neuronal GABA uptake, hence it can be effective as an anticonvulsant. Alone it does not cross the brain barriers but it has been demonstrated that intra peritoneally injected nipecotic-tyrosine ester is able to protect mice against audiogenic seizures which evidenced for the transportability of prodrug into the brain by an amino acid transport system (Bonina *et al.*, 1999). Likewise, conjugation between phosphonoformate as an antiviral agent and L-tyrosine, (**Fig. 18**) demonstrated with promising outcomes in transportation across BBB through LAT1 systems which were exemplified by *in vitro* studies. It proved that the L-tyrosine prodrug of phosphonoformate is a good substrate of LAT 1, expressed in mono layers of porcine brain micro vessel endothelial cells (Walker *et al.*, 1994).

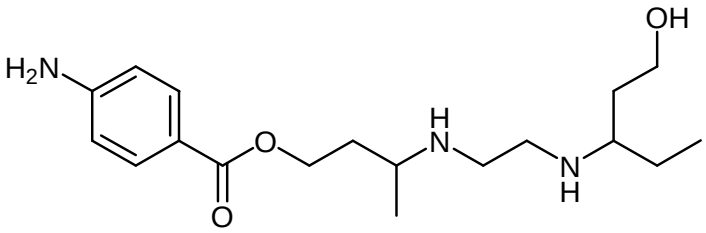
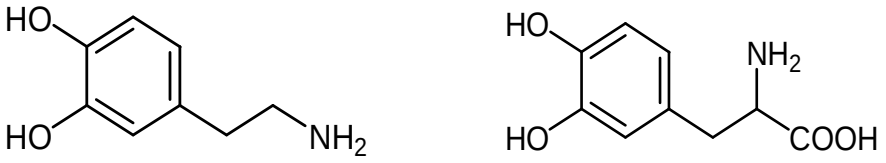
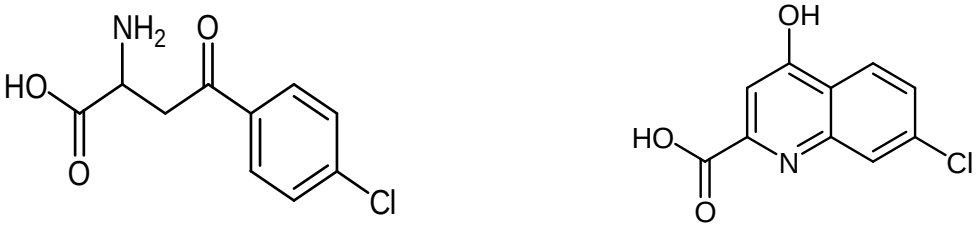


Fig. 14. Mutual prodrug of ethambutol and p-amino salicylic acid (PAS).



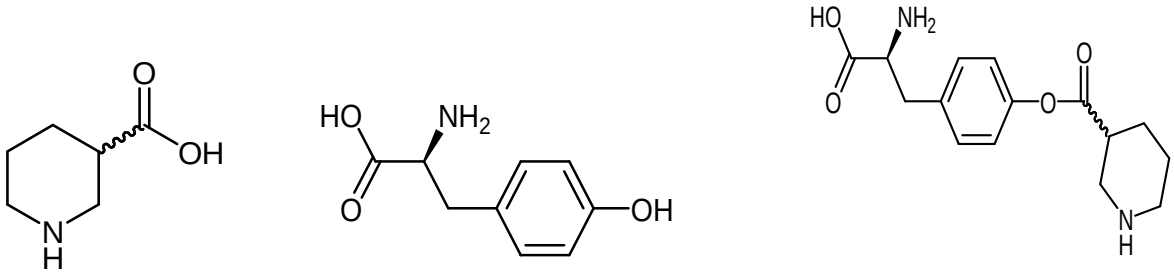
Dopamine L-Dopa

Fig. 15: Chemical structures of dopamine with its prodrug L-DOPA as "pseudonutrient" for LAT1 system.



7-Chlorokynureic acid L-4- Chlorokynureine acid

Fig. 16: Chemical structures of 7-chlorokynurenic acid with its prodrug L-4-chloro-kynureine as "pseudonutrient" for LAT1 system.



Nipecotic acid Tyrosine

Fig. 17: Chemical structures of nipecotic acid, tyrosine and their conjugate as ester.

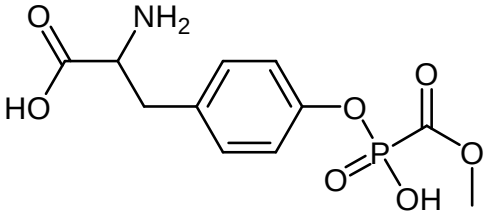


Fig. 18: Chemical structure of phosphonoformate-L-tyrosine conjugate.

CONCLUSION

In this article we provide various modern techniques to improve drug delivery in CNS disorders because there is a lack of candidate drugs not able to cross blood brain barrier. In that mainly use nanocarriers, nanoparticles and chemical approach for delivering drug to brain. The several nanoparticulate drug delivery systems like liposomes, nanoparticles, nanoemulsion, nanoliposomes and super molecular complexes in combination with endogenous transport systems present in the BBB have been used to transport the drug across the brain. The new chemical approaches are helpful in transporting the drug substances across BBB. These approaches include the use of chimeric peptides, cationic proteins, cyclodextrin complex and prodrug for drug delivery to the brain.

All above listed methods help to improve the drug concentration inside the brain which is mostly needful for the cure various CNS disorders in that mainly Alzheimer's, Epilepsy, Brain tumor, Parkinson's diseases.

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REFERENCES

Neuwelt E, Abbott NJ, Abrey L, Banks WA, Blakley B, Davis T, Engelhardt B, Grammas P, Nedergaard M, Nutt J, Pardridge W, Rosenberg GA, Smith Q, Drewes LR. Strategies to advance translational research into brain barriers. Lancet Neurol, 2008; 7: 84-96.

Hawkins BT, Thomas PD. The Blood-brain barrier/ neuro-vascular unit in health and disease. Pharmacol Rev. 2005;57:173-185.

Risau W. Differentiation of endothelium. FASEBJ. 1995; 9(10): 926-933.

Egleton RD, Davis TP. Bioavailability and transport of peptides and peptide drugs into the brain. Peptides. 1997; 18(9): 1431-1439.

Crone C, Olesen SP. Electrical resistance of brain microvascular endothelium. Brain Res. 1982; 241: 49-55.

Sarafian TA, Montes C, Imura T, Qi J, Coppola G, Geschwind DH, Sofroniew MV. Disruption of astrocyte STAT 3 signaling decreases mitochondrial function and increases oxidative stress inv itro. PLOSONE. 2010; 5(3): e9532.

Allt G, Lawrenson JG. Pericytes: Cellbiology and pathology. Cells Tissues Organs. 2001; 169: 1-11.

Cottrell GT, Ferguson AV. Sensory circumventricular organs: central roles in integrated autonomic regulation. Regul Pept. 2004; 117: 11-23.

Johanson CE, Duncan JA, Stopa EG, Baird A. Enhanced prospects for drug delivery and brain targeting by the choroid plexus-CSF route. Pharm. Res. 2005; 22(7): 1011-1037.

Pardridge WM. Blood-brain barrier drug targeting: the future of brain drug development. Mol Interv. 2003; 3: 90-105.

Rip J, Schenk GJ, deBoer AG. Differential receptor-mediated drug targeting to the diseased brain. Expert Opin Drug Deliv. 2009; 6: 227-237.

Pathan SA, Iqbal Z, Zaidi SM, Talegaonkar S, Vohra D, Jain GK, Azeem A, Jain N, Lalani JR, Khar RK, Ahmad FJ. CNS drug delivery systems: novel approaches. Recent Pat Drug Deliv Formul. 2009; 3:71-89.

Hawkins BT, Egleton RD. Pathophysiology of the blood-brain barrier: animal models and methods. Curr Top Dev Biol. 2008; 80:277-309.

Stewart PA. Endothelial vesicles in the blood-brain barrier: are they related topermeability? Cell Mol Neurobiol. 2000; 20:149-163.

Persidsky Y,Ramirez SH,Haorah J,Kanmogne GD. Blood-brain barrier: structural components and function under physiologic and pathologic conditions. J Neuroimmune Pharmacol. 2006; 1:223-236.

Abbott NJ, Ronnback L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. Nat Rev Neurosci. 2006; 7:41-53.

Dohgu S, Takata F, Yamauchi A, Nakagawa S, Egawa T, Naito M, Tsuruo T, Sawada Y, Niwa M, Kataoka Y. Brain pericytes contribute to the induction and upregulation of blood–brain barrier functions through transforming growth factor beta production. Brain Res. 2005; 1038: 208-215.

Williams K, Alvarez X, Lackner AA. Central nervous system perivascular cells are immune regulatory cells that connect the CNS with the peripheral immune system. Glia. 2001; 36:156-164.

Spector R, Johanson CE. The mammalian choroid plexus. Sci Am. 1989; 261: 68-74.

De Lange EC. Potential role of ABC transporters as a detoxification systema the blood-CSF barrier. Adv Drug Deliv Rev. 2004; 56:1793-1809.

Garner C, Brown PD. Two types of chloride channel in the apical membrane of rat choroid plexus epithelial cells. Brain Res. 1992; 591:137-145.

Kusuhara H, Sugiyama Y. Active efflux across the blood-brain barrier: role of the solute carrier family. Neuro Rx. 2005; 2:73-85.

Bernacki J. Dobrowolska A, Nierwinska K, Małecki A. Physiology and pharmacological role of the blood-brain barrier. Pharmacol Rep. 2008; 60(5):600-622.

Egleton RD, Davis TP. Development of neuropeptide drugs that cross theblood-brainbarrier. Neuro Rx 2005; 2(1):44-53.

Abbott NJ, Romero IA. Transporting therapeutics across the blood-brain barrier. Mol Med Today. 1996; 2:106-113.

Frank HJL, Pardridge WM. A direct in vitro demonstration of insulin binding to isolated brain micro vessels. Diabetes. 1981; 30:757-761.

Bendayan R, Lee G, Bendayan M. Functional expression and localization of p-glycoprotein at the blood brain barrier. Micro sc Res Tech.2002; 57:365-380.

Lee G, Dallas S, Hong M, Bendayan R. Drug transporters in the central nervous system: brain barriers and brain parenchyma considerations. Pharmacol Rev. 2001; 53:569-596.

Kemper EM, Boogerd W, Thuis I, Beijnen JH, vanTellingen O. Modulation of the blood-brain barrier in oncology: therapeutic opportunities for the treatment of brain tumours? Cancer Treat Rev. 2004; 30:415-423.

Pardridge WM. Drug and gene targeting to the brain via blood-brain barrier receptor-mediated transport systems. Int Congr Ser. 2005; 1277:49-62.

Conford EM, Hyman S, Swartz BE. The human brain GLUT-1 transporter ultra structural localization to the blood-brain barrier endothelial cells. J Cereb Blood Flow Metab. 1994; 14:106-112.

Rip J, Schenk GJ, deBoer AG. Differential receptor-mediated drug targeting to the diseased brain. Expert Opin Drug Deliv. 2009; 6: 227-237.

Herve F, Ghinea N, Scherrmann JM. CNS delivery via adsorptive transcytosis. AAPSJ. 2008; 10:455-472.

Jain S, Mishra V, Singh P, Dubey PK, Saraf DK, Vyas SP. RGD-anchored magnetic liposomes for monocytes/neutrophils mediated brain targeting. Int J Pharm. 2003; 261:43-55.

Chretien F, Lortholary O, Kansau I, Neuville S, Gray F, Dromer F. Pathogenesis of cerebral *Cryptococcus neoformans* infection after fungemia. J Infect Dis. 2002; 186: 522-530.

Gonzalez-Scarano F, Martin-Garcia J. The neuropathogenesis of AIDS. Nat Rev Immunol. 2005; 5:69-81.

Park K. Trojan monocytes for improved drug delivery to the brain. J Control Release. 2008; 132: 75.

Chen Y, Dalwadi G, Benson H. Drug delivery across the blood-brain barrier.Curr Drug Deliv. 2004; 1: 361-376.

Alam M Intakhab, Sarwar Beg, Abdus Samad, Sanjula Baboota, Kanchan Kohli, Javed Ali, Alka Ahuja, M Akbar. Strategy for effective brain drug delivery. Eur J Pharm Sci. 2010.1-19.DOI:10.1016/j.ejps.2010.05.003.

Krishna R, Mayer LD. Multidrug resistance (MDR) in cancer: mechanisms, reversal using modulators of MDR and the role of MDR modulators in influencing the pharmacokinetics of anticancer drugs. Eur J Pharm Sci. 2000; 11: 265-283.

Ryan SM, Mantovani G, Wang X, Haddleton DM, Brayden DJ. Advances in PEGylation of important biotech molecules: delivery aspects. Exp Opin Drug Deliv. 2008; 5:371-383.

Wong HL, Chattopadhyay N, Wu XU, Bendayan R. Nanotechnology applications for improved delivery of antiretroviral drugs to the brain. Advanced Drug Delivery Reviews. 2010; 62:503-517.

Kreuter J, Shamenkov D, Petrov V, Ramge P, Cychutek K, Koch-Brandt C, Alyautdin R. Apolipoprotein-mediated transport of nanoparticle bound drugs across the blood–brain barrier. J Drug Target. 2002; 10:317-325.

Blasi Paolo, Giovagnoli Stefano, Schoubben Aurélie, Ricci Maurizio, Rossi Carlo. Solid lipid nanoparticles for targeted brain drug delivery. Advanced Drug Delivery Reviews. 2007; 59:454-477.

Yan Chen, Lihong Liu. Modern methods for delivery of drugs across the blood-brain barrier.Advanced Drug Delivery Reviews.2012; 64: 640-665.

Alyaudtin RN, Reichel A, Lobenberg R, Ramge P, Kreuter J, Begley DJ. Interaction of poly (butylcyanoacrylate) nanoparticles with the blood-brain barrier *in vivo* and*in vitro*. J DrugTarget. 2001; 9:209-221.

Kuo YC, Chen HH. Effect of nanoparticulate polybutyl cyanoacrylate and methyl-methacrylate-sulfopropyl methacrylate on the permeability of zidovudine and lamivudine across the *in vitro* blood-brain barrier. Int J Pharm. 2006; 327:160-169.

Gao X, Wu B, Zhang Q, Chen J, Zhu J, Zhang W, Rong Z, Chen H, Jiang X. Brain delivery of vasoactive intestinal peptide enhanced with the nanoparticles conjugated with wheat germ agglutinin following intranasal administration. J Control Release. 2007; 121:156-167.

Dutta T, Jain NK. Targeting potential and anti-HIV activity of lamivudine loaded mannosylated poly (propyleneimine) dendrimer. Biochim Biophys Acta. 2007; 1770:681-686.

Batrakova EV, Li S, Alakhov VY, Miller DW, Kabanov AV. Optimal structure requirements for pluronic block copolymers in modifying P-glycoprotein drug efflux transporter activity in bovine brain micro vessel endothelial cells. J Pharmacol Exp Ther. 2003; 304:845-854.

Dou H, Grotepas CB, McMillan JM, Destache CJ, Chaubal M, Werling J, Kipp J, Rabinow B, Gendelman HE. Macrophage delivery of nano formulated antiretro-viral drug to the brain in a murine model of neuro AIDS. J Immunol. 2009; 183:661-669.

Sawant KK,Dodiya SS. Recentadvancesandpatentsonsolidlipid nanoparticles. RecentPatentsDrugDeliveryFormulation. 2008; 2:120-135.

Anda T, Yamashita H, Fujita HT, Yoshiharu, Shibata S. Basic experiment of BBB permeability of cisplatin encapsulated in liposome by means of *in vitro* BBB. DrugDelivSyst. 1995; 10:425-430.

Fresta M, Puglisi G, Giacomo C Di, Russo A. Liposomes as *in vivo* carriers for citicoline: effects on rat cerebral post-ischaemic reperfusion. J Pharm Pharmacol. 1994; 46:974-981.

Zhang X, Xie J, Li S, Wang X, Hou X. The study on brain targeting of the amphotericin B liposomes. J Drug Target. 2003;11:117-122.

Woodle MC. Sterically stabilized liposome therapeutics. Adv Drug Deliv Rev. 1995; 16:249-265.

Vyas TK, Shahiwala A, Amiji MM. Improved oral bioavailability and brain transport of Saquinavir upon administration in novel nanoemulsion formulations. Int J Pharm. 2008; 347:93-101.

Muller RH, Ruhl D, Runge S, Schulze-Forster K, Mehnert W. Cytotoxicity of solid lipid nanoparticles as a function of the lipid matrix and the surfactant. Pharm Res. 1997; 14:458-462.

Pandita D, Ahuja A, Lather V, Benjamin B, Dutta T, Velpandian T, Khar RK. Development of lipid-based nanoparticles for enhancing the oral bioavailability of paclitaxel. AAPS Pharm Sci Tech. 2011; 12:712-722.

Yang SC, Lu LF, Cai Y, Zhu JB, Liang BW, Yang CZ. Body distribution in mice of intravenously injected camptothecin solid lipid nanoparticles and targeting effect on brain. J Control Release. 1999; 59:299-307.

Manjunath K, Venkateswarlu V. Pharmacokinetics, tissue distribution and bioavailability of clozapine solid lipid nanoparticles after intravenous and intraduodenal administration. J Control Release. 2005; 107:215-228.

Priano L, Esposti D, Esposti R, Castagna G, DeMedici C, Fraschini F, Gasco MR, Mauro A. Solid lipid nanoparticles incorporating melatonin as new model for sustained oral and transdermal delivery systems. J Nanosci Nanotechnol. 2007; 7: 3596-3601.

Priano L, Zara GP, El-Assawy N, Cattaldo S, Muntoni E, Milano E, Serpe L, Musicanti C, Perot C, Gasco MR, Miscio G, Mauro A. Baclofen-loaded solid lipid nanoparticles: preparation, electrophysiological assessment of efficacy, pharmacokinetic and tissue distribution in rats after intraperitoneal administration. Eur J Pharm Biopharm. 2011; 79:135-141.

Lucks JS, Müller RH. Medication vehicles made of solid lipid particles (solid lipid nanospheres SLN). German Patent. 1996. EP0000605497.

Müller RH, Mäder K, Lippacher A, Jenning V. Solid-liquid (semi-solid) liquid particles and method of producing highly concentrated lipid particle dispersions. 2000. PCT/EP00/04565.

Radtke Magdalene, Souto Eliana B, Muller Rainer H. Nanostructured lipid carriers: a novel generation of solid lipid drug carriers. Pharmaceutical Technology Europe. 2005; 17(4):45-50.

Freitas C, Muller RH. Eur J Pharm Biopharm. 1999; 47:125-132.

Liedtke S, Wissing S, Muller RH, Mader K. Influence of high pressure homogenisation equipment on nanodispersions characteristics. Int J Pharm. 2000; 196(2): 183-185.

Gasco MR. Solid lipid nanospheres from warm microemuslion. Pharm Technol Eur. 1997; 9: 32-42.

Garcý-Fuentes M, Torres D, Alonso M. Design of lipid nanoparticles for the oral delivery of hydrophilic macromolecules. Colloid Surface B. 2002; 27:159-168.

Puglia C, Blasi P, Rizza L, Schoubben A, Bonina F, Rossi C, Ricci M. Lipid nanoparticles for prolonged topical delivery: An in vitro and in vivo investigation. Int J Pharm. 2008; 357(1-2): 295-304.

Schubert MA, Muller-Goymann CC. Solvent injection as a new approach for manufacturing lipid nanoparticles--evaluation of the method and process parameters. Eur J Pharm Biopharm. 2003; 55(1):125-131.

Matsumoto S, Kanig WW. Formation and applications of multiple emulsions. J Disp Sci Tech. 1989; 10(4-5): 455-482.

Pardridge WM. Brain drug targeting and gene technologies. Jpn J Pharmacol. 2001; 87: 93-103.

Bickel U, Yoshikawa T, Pardridge WM. Delivery of peptides and proteins through the blood-brain barrier. Adv Drug Deliv Rev. 2001; 46(1-3): 247-279.

Pardridge WM. Drug and gene delivery to the brain: The vascular route. Neuron. 2002; 36:555-558.

Lewis ME, Kauer JC, Smith KR, Callison KV, Baldino JF. Treating disorders by application of insulin-like growth factor. 1992. US5093317.

Brasnjevic I, Steinbusch HWM, Schmitz C, Martinez-Martinez P. Delivery of peptide and protein drugs over the blood-brain barrier. Prog Neurobiol. 2009; 87:212-251.

Scherrmann JM. Drug delivery to brain via the blood-brain barrier. Vascul Pharmacol. 2002; 38: 349-354.

Nonaka N, Farr SA, Kageyama H, Shioda S, Banks WA. Delivery of galanin-like peptide to the brain: targeting with intranasal delivery and cyclodextrins. JPharmacol Exp Ther. 2008; 325(2): 513-519.

Brewster ME, Loftsson T. The use of chemically modified cyclodextrins in the development of formulations for chemical delivery systems. Pharmazie.2002; 57(2): 94-101.

Haisma HJ, Sernee MF, Hooijberg E, Brakenhoff RH, vdMeulen-Muileman IH, Pinedo HM, Boven E. Construction and characterization of a fusion protein of single chain anti-CD20 antibody and

human beta-glucuronidase for antibody-directed enzyme prodrug therapy. Blood. 1998; 92(1): 184-190.

Sharma SK, Bagshawe KD, Begent RHJ. Advances in antibody-directed enzyme prodrug therapy. Curr Opin Investig Drugs.2005; 6(6):611-615.

Melton RG, Sherwood RF. Antibody-enzyme conjugates for cancer therapy. J Natl Cancer Inst.1996; 88(3-4):153-165.

Vingerhodes MH, Haisma HJ, Vanmuijen M, Rijh V, Cromellin DJA, Storm G.A new application for liposomes in cancer therapy: Immunoliposomes bearing enzymes (immuno-enzymosomes) for site-specific activation of prodrugs. FASEB Lett.1993; 336(3): 485-490.

Reddy JA, Lows PS.Folate mediated targeting of therapeutic and imaging agents to cancers. Crit Rev Ther Drug Carrier Syst.1998; 15(6): 587-627.

Deonarain MO, Spooner RA, Epenetos AA. Genetic delivery of enzymes for cancer therapy. Gene Ther.1995; 2:235-244.

Pardridge WM, Kan YS, Buciak JL, Yang J. Human insulin receptor monoclonal antibody under goes high affinity binding to human brain capillaries in vitro and rapidtranscytosis through the blood-brain barrier in vivo in the primate. Pharm. Res. 1995;12: 807-816.

Longman SA, Cullis PR., Choi L, de Jong G, Bally MB. A two-step targeting approach for delivery of doxorubicin-loaded liposomes to tumour cells in vivo. Cancer ChemotherPharmacol.1995; 36(2): 91-101.

Garzon-Aburbeh A, Poupaert JH, Claesen M, Dumont P. A lymphotropicprodrug of L-dopa: synthesis, pharmacological properties, and pharmacokinetic behavior of 1,3-dihexadecanoyl-2-[(S)-2-amino-3-(3,4-dihydroxyphenyl)propanoyl] propane-1,2,3-triol. J Med Chem. 1986; 29(5): 687-691.

Prokai L, Prokai-Tatrai K, Bodor N. Targeting drugs to the brain by redox chemical delivery systems. Med Res Rev. 2000; 20(5): 367-416.

Brewster ME, Venkataraghavan V, Shek E, Bodor N. Facile, one-step preparation of trigonellinate esters. Synth Commun. 1987; 17(4): 451-455.

Bodor N, Prodai L.Peptide Based Drug Design: Controlling Transport and Metabolism. In: Taylor M, Amidon G. (Eds.).,American Chemical Society, Washington, DC.1995. 317.

Pavan B, Dalpiaz A, Ciliberti N, BiondiC, Manfredini S, Vertuani S. Progress in drug delivery to the central nervous system by the prodrug approach. Molecules. 2008; 13:1035-1065.

Boucher BA, Feler CA, Dean JC, Michie DD, Tipton BK, Smith Jr KR, Kramer RE, Young B, Parks BR, Kugler AR. The safety, tolerability, and pharmacokinetics of fosphenytoin after intramuscular and intravenous administration in neurosurgery patients. Pharmacotherapy.1996; 16(4): 638-645.

Bodor N, Buchwald P. Recent advances in the brain targeting of neuropharmaceuticals by chemical delivery systems. Adv Drug Deliv Rev. 1999; 36(2-3): 229-254.

Wermuth C. The practice of medicinal chemistry. Designing Prodrugs and Bioprecursors. 561-584.

Oldendorf WH. Brain uptake of radiolabeled amino acids, amines, and hexoses after arterial injection. Am J Physiol. 1971; 221: 1629-1639.

Pardridge WM. The blood-brain barrier: bottle neck in Brain Drug Development. Neuro Rx. 2005;2: 3-14.

Pardridge WM. Drug targeting to the brain. Pharm Res. 2007; 24: 1733-1744.

Pardridge WM. Brain metabolism: a perspective from the blood brain barrier. Physiol Rev. 1983; 63: 1481-1535.

Tamai I, Tsuji A. Transporter-mediated permeation of drugs across the blood brain barrier. J Pharm Sci. 2000; 89: 1371-1388.

Rice ME. Ascorbate regulation and its neuroprotective role in the brain. Trends Neurosci. 2000; 23: 209- 216.

Pineda M, Fernandez E, Torrents D, Estevez R, Lopez C, Camps M, Lloberas J, Zorzano A, Palacin M. Identification of a membrane protein, LAT-2, Tat co-expresses with 4F2 heavy chain, an L-type amino acid transport activity with broad specificity for small and large zwitterionic amino acids. J Biol Chem. 1999; 274: 19738-19744.

Hokari M, Wu HQ, Schwarcz R, Smith QR. Facilitated brain uptake of 4-chlorokynureineand conversion to 7-chlorokynurenic acid. Neuropharmacol Neurotoxicol. 1996; 8: 15-18.

Bonina FP, Arenare L, Palagiano F, Salia A, Nava F, Trombetta D, de Caprariis P. Synthesis, stability and pharmacological evaluation of nipecotic acid prodrugs. J Pharm Sci. 1999; 88: 561-567.

Walker I, Nicholls D, Irwin WJ, Freeman S. Drug delivery via active transport at the blood-brain barrier: affinity of a prodrug of phosphonoformate for the large amino acid transporter. Int J Pharm. 1994; 104:157-167.

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