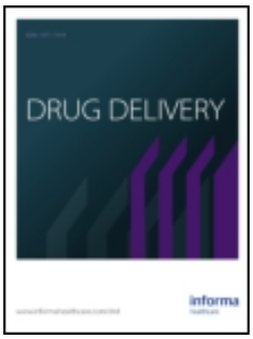

Drug Delivery

ISSN: 1071-7544 (Print) 1521-0464 (Online) Journal homepage: informahealthcare.com/journals/idrd20

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To cite this article: Narayanan Kasinathan, Hitesh V. Jagani, Angel Treasa Alex, Subrahmanyam M. Volety & J. Venkata Rao (2015) Strategies for drug delivery to the central nervous system by systemic route, Drug Delivery, 22:3, 243-257, DOI: 10.3109/10717544.2013.878858

To link to this article: https://doi.org/10.3109/10717544.2013.878858

Published online: 29 Jan 2014.

Drug Delivery

http://informahealthcare.com/drd
ISSN: 1071-7544 (print), 1521-0464 (electronic)

Drug Deliv, 2015; 22(3): 243–257
 DOI: 10.3109/10717544.2013.878858

informa
healthcare

CRITICAL REVIEW

Strategies for drug delivery to the central nervous system by systemic route

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Abstract

Context: Delivery of a drug into the central nervous system (CNS) is considered difficult. Most of the drugs discovered over the past decade are biological, which are high in molecular weight and polar in nature. The delivery of such drugs across the blood–brain barrier presents problems.
Objective: This review discusses some of the options available to reach the CNS by systemic route. The focus is mainly on the recent developments in systemic delivery of a drug to the CNS.
Materials and methods: Databases such as Scopus, Google scholar, Science Direct, SciFinder and online journals were referred for preparing this article including 89 references.
Results: There are at least nine strategies that could be adopted to achieve the required drug concentration in the CNS.
Conclusion: The recent developments in drug delivery are very promising to deliver biologicals into the CNS.

Keywords

Blood–brain barrier, central nervous system, chimeric proteins, peptidomimetics, Trojan

History

Received 3 December 2013
Revised 19 December 2013
Accepted 21 December 2013

Introduction

Central nervous system (CNS)-related diseases and injuries are difficult to treat as most of the therapeutic agents are unable to cross blood–brain barrier (BBB) and blood–spinal cord barrier (BSCB). The last decade had witnessed an increase in biological-based therapeutic agents. Most of these substances are hydrophilic in nature. Therefore, it is imperative to design a suitable delivery system for such products so that they can cross BBB and reach the target sites within CNS.

BBB helps in maintaining a homeostatic condition within CNS. However, this provides a considerable hindrance while attempting to deliver drugs by systemic route. The drugs are unable to cross the BBB and hence have to be directly administered by invasive techniques. It was first demonstrated in the early part of the twentieth century by Ehrlich that all the tissues except the brain gets stained when injected intravenously with a dye (Finlay et al., 1996). Later in 1920s, it was shown that only those substances that are capable of entering cerebrospinal fluid (CSF) could affect CNS function. After naming this selective drug permeability as ''*barriere hematoencephaique*'' it was shown in 1960 that only the substances having high lipid solubility could enter CNS (Kroll & Neuwelt, 1998). Tightly packed endothelial cells with diverse receptors, transporters and efflux pumps help BBB in maintaining homoeostatic condition within the brain (Begley & Brightman, 2003; Persidsky et al., 2006).

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BBB physiology

The BBB is tightly composed of endothelial capillaries that have less number of openings, less pinocytic activities and more number of mitochondria compared to the endothelial cell junctions observed at the other sites in the body. These cells are further surrounded by astrocytic foot process and basal membrane. These cells along with pericytes form closely knitted junctions that are permeable only to lipid soluble substances (Selmaj, 1996). Presence of astrocyte foot process further provides a high integrity to the BBB. However, solutes such as glucose, amino acids and nucleoside continuously enter CNS. These solutes are able to enter through the luminal and antiluminal portion of the BBB by carrier-mediated process (Pardridge et al., 1990b; Deeken & Loscher, 2007; Wolburg & Lippoldt, 2002). Permeability of these barriers is further influenced by the presence of astroglial cells, which regulates various signals involved in permeability of the BBB (Abbott et al., 2006).

Blood–cerebrospinal fluid barrier that starts at the choroid plexus forms an important barrier that regulates entry and exit of various substances to spinal cord. Although endothelial junctions of BCFB is not as tightly bound as that of BBB, the relative smaller surface area of this barrier compared to BBB, lower rate of diffusion and rapid rate of clearance effectively prevents entry of larger molecule and proteins and peptides (Bickel et al., 1993).

Presence of various proteins in the BBB regulates the permeability of tight junctions. Proteins in the tight junctions of the endothelial BBB differ significantly from the epithelial tight junctions. While epithelial cells have high association with P-face strand, only a low degree of association of endothelial cells with P-face cells is observed. Sealing of tight junctions is dependent on a transmembrane protein, occludin. Occludin was the first protein discovered in the endothelial junction controls. Permeability of tight junctions is regulated through phosphorylation of occludin. Claudins are another set of proteins present in BBB whose primary function is involved in restriction of permeability. Among the various types of claudins discovered, claudin 1, 3 and 5 are present in the endothelial cells. Homophilic and heterophilic interaction in BBB is regulated by junctional adhesional molecules and endothelial cell selective adhesion molecules. In addition, tight junctions contain zonula occludens proteins forming part of submembranous tight junction-associated proteins. These proteins are involved in regulation of signal transduction across the membrane (Wolburg & Lippoldt, 2002).

Presence of high concentration occludin in the brain capillaries increases their electrical resistance to an order of 1000–2000 ohm cm^2, which is very high when compared to 10 ohm cm^2 observed in the peripheral capillaries. This effectively prevents the entry of polar compounds (Butt et al., 1990; Hirase et al., 1997). High vascularization in the brain ensures that each neuron is having its own blood supply. Therefore, a substrate can gain a direct entry into a target neuron through this blood supply (Pardridge, 2003). However, some of the non-polar small molecules are not able to cross BBB due to the efficient efflux pump seen in the CNS. These molecules are efficiently recognized and removed by these pumps (Golden & Pollack, 2003).

Charge, molecular mass and lipid solubility of a molecule affects their transportation across BBB (Table 1). BBB is composed of cells such as microglial cells, pericytes, astrocytes and endothelial cells. Surface of these cells are negatively charged. BBB do not contain fenestrations and pinocytic vesicles. These properties limit the entry of molecules with size greater than 200 nm (Karnovsky, 1967; Reese & Karnovsky, 1967; Kroll & Neuwelt, 1998; Begley, 2004b). Furthermore, CSF, due to continuous circulation, produces a sink effect in the brain. This along with efflux transporters further reduces the concentration of those substances that managed to evade this protective mechanism (Davson, 1978; Begley, 1996; Pardridge, 1998). However, certain substances such as glucose, insulin and amino acids, which are required for the normal functioning of brain, can cross BBB effectively even though they are hydrophilic in nature. This is accomplished by transportation *via* specific receptors present on BBB (Kroll & Neuwelt, 1998). But still their concentration depends on their ability to overcome efflux pump, especially P-glycoprotein (Pgp) (Begley, 1996; Pardridge, 1998).

Drugs with higher lipid solubility can enter into the brain passively. But with their higher lipid solubility, their volume of distribution within the body also increases. Due to the high vascular density observed in the CNS, any molecule entering the brain is rapidly distributed throughout the brain. Therefore any drug delivery that utilizes a specific transport mechanism present at the BBB could be useful to achieve a better bioavailability at the target site. However, this requires identification of specific traits that are expressed under some pathological conditions (Juillerat-Jeanneret, 2008).

This article gives a broad outline on the various strategies involved in delivering drugs into the CNS. The strategies are classified into invasive and non-invasive techniques. The focus is on the non-invasive techniques, which are further sub-categorized into nine different approaches that are currently used for delivering drugs across BBB. Among these approaches, nanoparticulate-based delivery system and efflux pump inhibition-based delivery systems are discussed in detail. Under nanoparticulate-based drug delivery system, polymer-based drug delivery system and liposomes are discussed in detail. These delivery systems have a wider applicability compared to other drug delivery systems.

Strategies for drug delivery to the CNS by systemic route

Drug delivery to the CNS falls under two categories:
(1) Invasive techniques
(2) Non-invasive techniques

Invasive techniques

Drug delivery to the CNS by invasive techniques, such as osmotic pump and depot formulations of polifeprosan 20, cause number of complications such as damage to the neurons, inflammatory reactions, etc. Therefore, it is desirable to deliver the drug by non invasive techniques to reduce the complications. A better therapeutic concentration of the drugs can be achieved by suitable modification like prodrugs, Trojan approach, nanodrug delivery, etc. (Wohlfart et al., 2012).

Table 1. Properties of a drug molecule to enter brain.

Properties	Explanation
Nature of the compound	Unionized
Basicity	As the cell membrane is negatively charged, basic molecules are preferred over acidic substances.
Lipophilicity	Lipid solubility has a direct effect on transport across BBB. Ideally, the molecule should possess a log *p* value (octanol:water partition coefficient) near to 2 (1.5–2.7)
Size	Molecular weight: <400–500 Da
Hydrogen bonding	For each pair of hydrogen bonds or polar functional groups added, the permeability of molecule across BBB decreases by one log unit. Cumulative number of hydrogen bonds: <8–10

Disruption of BBB

This is one of the earliest methods that were tested in order to improve the drug bioavailability in the CNS. It was first proposed in 1960s that transient pores can be created in the BBB by infusion of concentrated solutions (Rapoport, 1970). Drug delivery to the brain can be achieved by disrupting BBB using hypertonic solution such as mannitol (Abbott & Revest, 1991) or by using substances such as RMP-7, which is a synthetic analogue of Bradykinin, a substance involved in the regulation of brain endothelial cellular junction (Sanovich et al., 1995). Infusion of hyperosmolar solutions such as arabinose, saline mannitol or urea into the internal carotid artery causes a temporary disruption of BBB. This occurs as a result of shrinkage of endothelial cells resulting in formation of gaps in the endothelial junction (Rapoport, 1970; Brightman et al., 1973). This technique can be utilized for the delivery of drugs into the CNS *via* transient opening created in the BBB. Gentamicin has been delivered into the CSF by causing transient disruption of BBB by administration of mannitol (Strausbaugh & Brinker, 1983).

Use of pharmacological agents for increasing the permeability of BBB

Temporary increase in the vascular permeability and vascular leakage is caused by the agents such as histamine and vasoactive peptides that are responsible for inflammatory reactions (Inamura & Black, 1994; Kroll & Neuwelt, 1998). Bradykinin, a vasodilator, increases vascular permeability by acting on B_2 receptors (Nakano et al., 1996). The success of disrupting the BBB depends on the ability to create transient pores on the BBB with the space created being large enough to permit the entry of the molecules without damaging the structure (Kroll & Neuwelt, 1998). However, most of the time disruption of BBB results in damage to the neuron.

Focused ultrasound

Recent development in technology allows focusing ultrasound of low intensity only in the area of interest. This focused ultrasound allows reversible disruption of BBB confined only to the target site. Air or perfluorocarbon entrapped in a carrier composed of lipid or albumin is injected intravenously. When ultrasound of low frequency is applied *via* transcranial route, it results in oscillation of injected air bubbles. This results in interaction of these bubbles with cerebral capillaries leading to reversible BBB disruption. Even though injection of air bubbles is not absolutely required, use of these air bubbles reduces the need for high intensity ultrasound. The disruption can last for 4–24 h (Hynynen, 2007; Burgess & Hynynen, 2013). High concentrations of drugs such as anti-Aβ antibodies (against amyloid β-plaques) (Jordão et al., 2010) and doxorubicin (for treatment of glioblastomas) (Treat et al., 2007) is achieved using FUS.

Non-invasive techniques

Table 2 gives a brief idea on various transport mechanisms available within CNS. By understanding these transport mechanisms, one could design a suitable drug delivery system without damaging the BBB. These various non-invasive approaches that could be used for delivering drugs include

(1) Changing the drug solubility
(2) Nanodrug delivery
(3) Chimeric peptide
(4) Peptidomimetics
(5) Trojan approach
(6) Immunophilins
(7) Efflux transporter inhibitors
(8) Viral vectors
(9) Prodrug approach

The focused ultrasound is still at a nascent stage. Currently, it is better to use other approaches such as carrier-mediated transport process for delivering the drugs to the CNS. A carrier can easily enter CNS when they are tagged with monoclonal antibody such as OX 26. OX 26 specifically binds to the receptor transferrin (Tf). This would help in achieving a better drug concentration in the brain if a drug is delivered using a carrier system coated with these monoclonal antibodies (Huwyler et al., 1996). Molecules are transported across the CNS either by carrier-mediated or by receptor-mediated process. Substances that provide energy, nutrition or involved in signaling are transported by carrier-mediated

Table 2. Various mechanisms involved in transportation of a molecule across blood–brain barrier.

Transport pathways	Mechanism	Features
Paracellular pathway	Presence of tight junctions prevent transport of water soluble substances	Ideal for molecules less than 20 nm in size
Transcellular diffusion	Transcytosis of lipophilic molecules of molecular weight less than 500 Da	Molecules move along concentration gradient, no energy required
Carrier-mediated transport	Takes place through facilitated diffusion or active transport	Modulates the entry of various endogenous substances such as glucose, aminoacids and purines
Adsorptive-mediated transcytosis	Based on electrostatic interaction between positively charged molecules to negatively charged cell membrane	Internalization of molecules into vesicles to reach the cell
Receptor-mediated transcytosis	(a) Endocytosis at the luminal side after receptor- ligand binding (b) Movement through the endothelial cytoplasm (c) Exocytosis of the drug or ligand attached drug at the abluminal side	The different receptors expressed on endothelial cells include insulin, insulin-like growth-factors, Tf, leptin, diphtheria toxin, dopaminergic GABAB, amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoic acid (AMPA), lipoprotein receptors, scavenger receptors class B type I and glutathione transporter
Efflux pumps	Rejects the entry of molecules	Pgp, OAT and MRP efflux pumps that expels drugs and xenobiotics.

process. Therefore, if a drug is attached to these molecules, they are also transported. However, the size of the molecule that can be attached is limited. But this limitation can be overcome by attaching the drug to a vector that is transported by a receptor-mediated process site (Pardridge, 2003; Juillerat-Jeanneret & Schmitt, 2007).

Molecules that are larger in size can be designed in such a way that they can be transported *via* specific receptors such as Tf-receptor and folic acid receptor found on the BBB that are expressed during specific pathological conditions (Juillerat-Jeanneret, 2008). Tf is transported into the brain *via* Tf receptor. The receptor mediates transcytosis when activated by its substrate. Therefore, when a drug molecule is attached to a carrier mimicking the substrate, they get transported across BBB by transcytosis (Pardridge, 2002). This technique was utilized in delivering fibroblast growth factor (Song et al., 2002). Similarly, 5-flurouracil can be delivered in to the brain by coupling the drug with Tf (Soni et al., 2005). Doxorubicin can be delivered across BBB using nanocarrier containing folic acid. Such carrier systems are able to bind with folic acid receptor expressed on the BBB and gain entry into the CNS (Brigger et al., 2004). This type of delivery is suitable even for a highly lipophilic molecule whose size is greater than 400 kDa where the passive diffusion across BBB fails.

For targeting a specific receptor-mediated transport, the ligand attached should be present at the surface of the carrier in sufficient concentration. It should be able undergo both endocytosis and transcytosis-mediated uptake (Juillerat-Jeanneret, 2008). The clearance rate of a drug can further be decreased by conjugating them with suitable water soluble polymers. In order to be transported into the CNS, lower clearance rate is required so that these conjugates are available in the circulation for longer period. But once the conjugate reaches the target site, the active drug should be released from the polymer conjugate within the expected time limit, and there should not be any toxic substance released as a result of this biotransformation (Schoenmakers et al., 2004). These conjugation steps with suitable polymers, such as polyethylene glycol (PEG), should be done carefully as some of the drugs lose their efficacy (Greenwald et al., 1995).

Changing the drug solubility

Polar drugs are poorly absorbed. A better absorption could be achieved by increasing their hydrophobicity (Jain, 2007). However, this will increase the volume of distribution of the drug within the body.

Receptor-mediated drug delivery. Endocytosis and transcytosis are two important processes that help in uptake of molecules including drugs across BBB. It is either receptor dependent or receptor independent. Receptors *viz*., transferring receptor, low density lipoprotein receptor-related protein (LRP), insulin receptor and neonatal Fc receptor present in the BBB regulates these processes. Substrates and monoclonal antibodies for these receptors are utilized in creating a Trojan-based drug delivery system. Higher expression of certain receptors during certain pathological conditions and in certain organs could also be utilized for effective concentration of a drug at that site (Xiao & Gan, 2013). Angiopep-2 is a synthetic peptide resembling Aprotinin. These peptides are readily transported across BBB as they are the ligands for LRP1 receptors. Therefore, drugs such as doxorubicin by conjugating with these peptides are easily delivered into the brain (Ren et al., 2012).

Materials can be endocytosed either through phagocytosis or pinocytosis. Phagocytosis, which is mainly concerned (but not always) with transport of solids, does not transport any therapeutic proteins. It is mainly observed in cells involved in removal of pathogens. Pinocytosis involves uptake of fluid or small particles, usually less than 100 nm, through clathrin-mediated endocytosis, macropinocytosis or caveolae-mediated endocytosis (Xiao & Gan, 2013).

Endocytosis can be either (a) clathrin-dependent or (b) clathrin-independent endocytosis (CIE)

(a) Clathrin-dependent endocytosis

Among these transport system, clathrin-mediated transport is well studied as it is the major pathway involved in uptake of nutrients (Batchelder & Yarar, 2010). In clathrin-mediated endocytosis, the particles are coated with clathrin proteins. In addition, these clathrin-coated vesicles forms complex with many other accessory proteins such as AP-1. Few other accessory proteins such as amphiphysin, dynamin and endophilin are associated during the later stage of endocytosis. These proteins are involved in pinching of the vesicles formed after the molecules are endocytosed. Among the various accessory proteins, amphiphysin I and dynamin I are found primarily in brain (Mousavi et al., 2004).

Although clathrin-meditated transport is saturable (due to the limitations in number of receptors that would be available at any given time), high concentration of clathrin vesicles in brain capillaries compared to other tissues make them ideal for drug transport (Hervé et al., 2008). In general, due to net negative charge on cell membrane, a molecule should be preferably positively charged for effective adsorptive-mediated transcytosis. Higher bioavailability of drugs such as anti-*ras* oncogene antibodies and nerve growth factors in brain has been achieved through cationization (Poduslo et al., 1998; Bickel et al., 2001). However, care should be taken since cationization in some cases could result in permanent loss in activity of molecules (further details are discussed under the section role of surface charge of nanoparticles) (Hervé et al., 2008).

(b) Clathrin-independent endocytosis

The endocytosis of a molecule can be independent of clathrin. Interest on CIE started from the understanding of the transport of bacterial toxins. CIE is primarily involved in transport of proteins attached to glycosyl phosphatidylinositol. Proteins of immune system, i.e. major histocompatibility complex classes I and II, is transported *via* CIE (Maldonado-Báez et al., 2013). CIE is further sub divided into dynamin-dependent pathway and dynamin-independent pathway. In most of the cases, CIE involves participation of guanine triphosphatases. Dynamin-dependent CI plays prominent role in caveolae-mediated endocytosis. Proteins, lipids and lipids attached to pathogen-derived proteins are internalized by this mechanism. Dynamin-independent CIE involves participation of RhoA, a small GTPase. Toxins such as ricin and cholera toxin B are known to utilize this pathway (Mayor & Pagano, 2007).

Some proteins depending upon their concentration are endocytosed either by CDE or CIE. G-protein coupled receptor is one such example where participation of both CDE and CIE is observed. For instance, epidermal growth factor when present at lower concentration, the cells uptake these proteins by CDE. But at higher concentration, these proteins are encytosed by CIE (Hansen & Nichols, 2009). Focus on CIE has grown only in the past decade (Maldonado-Báez et al., 2013). Once CIE is understood, then this pathway could be utilized for transport of drugs in the brain.

Nanodrug delivery

Higher drug concentration at the required site could be achieved by encapsulating the drugs in a suitable carrier system. Carriers such as nanospheres, nanocapsules and micelles are used in delivering the drugs to the CNS. The carrier must be biodegradable (biodegradable polymers include poly lactide-co-glycolide (PLGA) and poly lactic acid (PLA)), should be able to deliver the drug at a specific site and should have sufficient tensile strength. Carriers should have a sufficient tensile strength to remain in the circulation for a long period without degradation (Missirlis et al., 2005).

Versatility of a nanoparticulate system in terms of physical, chemical and biological modifications, which it provides, make them one of the most widely explored system of drug delivery (Tenzer et al., 2011). Smaller size of the drug-loaded nanocarrier system allows them to diffuse through the small pores present in the cell membrane. The nanoparticles due to its small size effectively mask the size-limiting properties of the BBB. One of the easiest approaches is to administer the drug by masking its physicochemical characteristics by using polymeric nanoparticles. By this method, the original drug molecule without any modification can be administered (Karnovsky, 1967; Reese & Karnovsky, 1967; Rapoport, 1970; Brightman et al., 1973; Inamura & Black, 1994; Nakano et al., 1996; Garcia-Garcia et al., 2005; Kreuter & Gelperina, 2008; Andrieux et al., 2009). These polymeric nanoparticles are transported across BBB *via* receptor-mediated endocytosis. This type of transportation is particularly prominent in the endothelial cells of brain capillaries. Drugs are attached to nanoparticles by various means like adsorption, encapsulation and covalent attachment (Kreuter et al., 1994).

The surface charge, hydrophobicity and size of the nanoparticles influence the distribution of the drug. Drugs below 200 nm are taken up by the endocytosis-mediated transport mechanism. Cellular uptake of positively charged nanoparticles is better due to the negative cell surface (Musumeci et al., 2006; Hillaireau & Couvreur, 2009).

Role of surface charge of nanoparticles. Presence of glycoprotein and glycolipid confers a net negative charge on BBB. Hence, any particles that are negatively charged will be electro statically repelled from entering BBB. These negatively charged particles can enter cell only through transport or receptor-mediated process or through endocytosis. Therefore, cationic nanoparticles could be preferred to achieve a higher and faster CNS concentration (Lockman et al., 2004; Albanese et al., 2012). However, cationic particles should be used carefully as large sized cationic nanoparticles cause transient disruption of BBB. This is primarily due to the openings of inter endothelial space created when these particles bind with them (Lockman et al., 2004). Besides, positively charged nanoparticles are eliminated from the *in vivo* system more rapidly when compared to negatively charged nanoparticles (Albanese et al., 2012). Cationization could also result in random distribution of drug molecules as all the cells in the body are negatively charged. Concerns on immunogenicity and toxicity also need to be addressed with cationized drugs (Hervé et al., 2008). Transportation of nanoparticles also depends on the size of the particles. Nanoparticles less than 100 nm are able to enter BBB irrespective of their surface charge (Lockman et al., 2004). Nanoparticles, especially those that are taken up by the endocytosis, depend on the membrane wrapping process. When these small sized particles come in contact with cell surface, a membrane is wrapped around these particles, which ultimately form the vesicles. These thermodynamically mediated process is at their optimum level when the size of the nanoparticles is around 30–50 nm (Albanese et al., 2012).

But dependency of absorption/transportation of nanoparticles across BBB on the size or shape may not be as simple as it appears. Nanoparticles upon entry into circulation are immediately coated with plasma proteins (Albanese et al., 2012). This phenomenon, which is referred as ''plasma protein corona'' formation, depends on surface properties including size and shape and type of nanomaterial (Tenzer et al., 2011). Smaller the size higher will be the energy. Hence, at higher energy, the corona formation will be thermodynamically favored. This in turn will affect the distribution of these nanoparticles (Tenzer et al., 2013). Even a size of 10 nm is known to influence ''corona'' formation. Comparatively, charge has lesser influence on ''corona'' formation (Tenzer et al., 2011). The ''corona'' proteins are believed to be the main reason behind the faster rate of clearance of cationic nanoparticles. Once the cationic nanoparticles are coated with serum proteins, they are immediately removed by mononuclear phagocyte system (MPS) (Albanese et al., 2012). However, it is difficult to predict the nature of proteins bound with these nanoparticles as more than two thousand proteins are known to exist in plasma of which at least 125 proteins have been found to be present in ''corona''. The lack of complete data on ''corona'' proteins make understanding of this phenomenon even more complicated (Tenzer et al., 2011). Until the nature of ''corona'' proteins is completely understood, it is difficult to predict the biological properties of the nanoparticles. Binding of certain proteins are known to increase the rate of elimination. This effectively reduces the circulation time (Tenzer et al., 2011). A sufficiently long circulation time is required especially for nanoparticles coated with antibodies or other vector proteins. This will allow proper interaction of these surface modified nanoparticles with their target site.

Surface modification of nanocarrier system. The carriers selected for nanoparticulate system are biodegradable with the ability to release the drug at the required site without affecting therapeutic efficacy. The surface of the carriers after/during encapsulation of the drugs is modified suitably through addition of various polymers to achieve higher

circulation time and lower elimination rate (Grazia Cascone et al., 2002; Schoenmakers et al., 2004; Musumeci et al., 2006). Surface modification with PEG is one of the most widely used methods to increase the half life of drug nanocarriers. PEGylated nanoparticles have higher circulation time compared to unmodified nanoparticles. Unmodified nanoparticles are rapidly cleared from the blood by the liver (kupffer cells) and spleen. Increase in circulation time helps in increasing the bioavailability of the drug in brain (Calvo et al., 2001). Drugs like loperamide and doxorubicin when delivered as nanoparticles are able to overcome Pgp efflux pump and cross the BBB. This results in higher drug concentration at the site of action. Coating with surfactant polysorbate 80 is found to enhance endothelial-mediated endocytosis by increasing the adsorption of apolipoprotein E on these nanoparticles (Wohlfart et al., 2012).

Nanodrug delivery system could be either (a) polymer-based system or (b) lipid-based system

(a) Biodegradable polymers

The nanomedicines are successful mainly due to biodegradable polymers. Safety, high encapsulation efficiency, ability to personalize release properties and good bioavailability makes biodegradable polymers a preferred choice in targeted drug delivery. The circulation time of nanoparticles is considerably increased using various biodegradable polymers. These polymers, by forming a cloud around particle surface, are able to prevent interaction of plasma proteins with nanoparticles (Kumari et al., 2010). These biopolymers are safe even when viewed from environmental point as these are degraded naturally without releasing any toxic substances. These biopolymers can be obtained directly from natural sources (starch), obtained from petroleum-based products (poly vinyl alcohol), synthesized chemically from monomers obtained from biological sources (PLGA and PLA or produced using microbes (polyhydroxybutyrate) (Jamshidian et al., 2010). Among various biopolymers PLGA, PLA, poly-ε-caprolactone (PCL), chitosan, gelatin and poly-alkyl-cynaoacrylates are some of the biodegradable biopolymers used in medicines for delivering drugs to the desired site. In the below section, the application of (i) PLGA, (ii) PLA and (iii) PCL is discussed in detail.

(i) PLGA

Lactic acid and glycolic acid released from hydrolysis of PLGA is utilized by the body. Therefore, PLGA is considered safe and approved for human use by the US FDA. Based on the lactic acid and glycolic acid, PLGA is classified in PLGA 50:50, 75:25, 85:15, etc. Higher the percentage of lactic acid, slower will be the degradation. Therefore, depending on the requirement of the type of PLGA has to be selected (Kumari et al., 2010; Danhier et al., 2012).

PLGA nanoparticles, which are taken up by the cells through clathrin-mediated endocytosis and pinocytosis, escape lysosome and gain entry into the cytoplasm (Danhier et al., 2012). PLGA-containing drugs are generally prepared using emulsion technique, *viz*., single emulsion and double emulsion technique. Double emulsion technique is the method of choice for hydrophilic drugs as high aqueous volume could be obtained in this technique. The surface of drug-loaded PLGA could be modified suitably to customize the drug delivery. The usefulness of PLGA in improving the drug bioavailability and stability along with the safety and ease of manipulating the pharmaceutical properties makes it suitable for delivery of wide class of drugs. However, poor drug loading and initial burst release still remains to be addressed (Danhier et al., 2012).

(ii) PLA

PLA, which was first discovered in 1932, consists of monomers of lactic acid polymerized through ring-opening reaction (Jamshidian et al., 2010). PLA is hydrolyzed into their constituent lactic acids, which are utilized by the body. The hydrolytic process is slow compared to PLGA, and therefore PLA releases the drug over a long period of time. Drugs such as flurbiprofen (Mu et al., 2013) are encapsulated in PLA for delivering into the brain. Drug-loaded PLA is made more target-specific by attaching phage display peptides (Li et al., 2013) and lactoferrin (Hu et al., 2009).

(iii) PCL

PCL is one of the earliest known synthetic biodegradable polymers. PCL is prepared from ε-caprolactone through ring opening polymerization (Natta et al., 1934; Sinha et al., 2004). It is prepared by ring opening polymerization. Ester linkages in PCL are hydrolyzed into acid and alcohol. This is a slow process and sometimes takes many months for complete hydrolysis. This property is useful in preparation of sustained release formulations and implants. PCL are useful in controlled release of drugs even for 2–3 years (Woodruff & Hutmacher, 2010). The biodegradation of PCL can be improved by adding polyglycolic acid and polylactic acid enhances (Sinha et al., 2004).

PCL is useful in the preparation of nanocapsules and nanoparticles with a size range of 10–1000 nm (Sinha et al., 2004; Woodruff & Hutmacher, 2010). It is useful for delivery of drugs, which are susceptible to acidic environment, as it does not generate any acidic by products (Sinha et al., 2004). Drugs such as phenytoin (Li et al., 2007), paclitaxel (Xin et al., 2012) and coumarin-6 (Zhang et al., 2010) are able to cross BBB when delivered in the nanoparticles encapsulated in PCL. PCL as a carrier is made more target-specific through incorporation of agents such as lactoferrin whose receptors are highly expressed in neurons (Sinha et al., 2004).

(b) Lipid-based system

Apolipoprotein- and liposomes-based delivery system are useful in enhancing the drug permeability across BBB and delivering drugs into the CNS. In the following section, a brief overview of apolipoprotein-based delivery system and a detailed overview of liposome-based delivery system is given.

(i) Apolipoprotein

Apolipoproteins aids in transportation of lipids inside the cell. Of various apolipoproteins, apolipoprotein E is of particular interest as they could be used as ‘‘Trojan horse’’ for gaining entry into the CNS. Drugs such as loperamide are delivered into the brain using this approach (Michaelis et al., 2006). It is also believed that improved bioavailability of dalargin (enkephalin) adsorbed on poly(butyl)cyanoacrylate nanoparticles is due to the coating of apolipoprotein E after their injection in to the circulation (Shamenkov et al., 2006).

(ii) Liposomes

Among the‘ various delivery system nanoparticulate deliveries, liposomes are extensively studied. An important

advantage with liposomes is its resemblance with the cell membrane. This facilitates better absorption and allows delivery of both hydrophilic and hydrophobic drugs. Almost all the drugs with various log *p* values can be encapsulated in liposomes. This is due to its biphasic nature. Depending upon the log *p* value, the drug is distributed in lipid, aqueous or partitions between these two layers (Immordino et al., 2006). Liposomes in the range of 100 nm, 200–800 nm and 500–5000 nm are referred as small unilamellar vesicles (SUV), large unilamellar vesicles and multilamellar vesicles, respectively (Torchilin, 2005). Liposomes, especially SUV's, are useful in delivering drugs to the CNS. Many drugs such as phenytoin and cisplatin have been delivered across BBB by encapsulating these drugs in liposomes (Tiwari & Amiji, 2006). Liposome-loaded drugs especially nanoliposomes are transported across BBB by endocytosis, passive diffusion or through fusion with brain endothelial cells present in the brain capillaries (Tiwari & Amiji, 2006). An important advantage with liposomal delivery is the ease with which manipulation in their properties could be achieved. Properties such as rate of release, time and extent of release and site of release could be easily altered based on the requirements. By using various surface modifiers including monoclonal antibodies and polymers, properties of a liposome are altered.

''Stealth'' liposomes have even better stability. In ''Stealth liposomes'' through incorporation of hydrophilic polymers such as PEG, chitosan, polyvinyl alcohol, the circulation time of liposomes are increased (Mufamadi et al., 2011). Addition of these polymers allows liposomes to by-pass reticuloendothelial system. These ''Stealth liposomes'' are made target specific by addition of antibodies, peptides and glycol proteins (Mufamadi et al., 2011). Development of stimuli-sensitive liposomes has made liposomes more targets specific. Using appropriate stimuli, liposomes are made to release the drug only at the desired site. The stimuli could be a pH, temperature or magnetic field (Torchilin, 2005).

pH-sensitive liposomes. pH-sensitive liposomes are stable at physiological pH and get destabilized when they encounter acidic conditions thereby releasing their contents. Depending on the type of stabilizing agents used, pH-sensitive liposomes are categorized into four types. Liposomes under category I are stabilized at neutral pH by addition of slightly acidic amphiphiles such as carboxylic acid to unsaturated phosphatidylethanolamines. ''Caged liposomes'' comes under category II. Here, lipid is chemically engineered in such a way that it will result in slow destabilization of lipids under acidic conditions. Liposomes prepared using pH-sensitive peptides is classified under category III. The category IV pH-sensitive liposomes contains polymers that are pH titratable (Drummond et al., 2000). pH-sensitive liposomes are particularly useful in pathological conditions such as cancer and inflammation and for intracellular delivery where acidic pH is encountered (Karanth & Murthy, 2007). Studies show that pH-sensitive liposomes below 150 nm are useful in treatment of brain penumbra. ATP-loaded nanoliposomes is able reduce to protect brain penumbra more effectively (Liu et al., 2010).

Thermosensitive liposomes. In thermosensitive liposomes, drug content is released when the temperature increases by more than 5 °C. These carrier systems are stable at body temperature. The drug is released from the carriers when the temperature at the target site increases to 42–45 °C. Thermosensitive liposomes are usually prepared by mixing dipalmitoyl phosphatidylcholine (DPPC), dipalmitoyl phosphatidylglycerol (DPPG), or distearoyl phosphatidylcholine (DSPC) with cholesterol. Addition of cholesterol reduces the phase transition temperature of DPPC, DPPG and DSPC. In absence of cholesterol, melting temperature of these lipids are greater than 42 °C. Therefore, it is imperative to optimize the concentration of cholesterol to be included in the formulation (Kong & Dewhirst, 1999). A drug could be made to be released at a desired site by locally increasing the temperature of the target site. The localized increase in temperature could be achieved using techniques such as focused ultrasound (Needham & Dewhirst, 2001). This property of making liposomes to release the drug without depending on the local properties of the tissues such as pH makes temperature-sensitive liposomes more attractive than pH-dependent liposomes (Kong & Dewhirst, 1999). Anticancer drugs such as *cis*-diamminedichloroplatinum (Kakinuma et al., 1996) and doxorubicin (Aoki et al., 2004) are effectively delivered to gliomas by encapsulating these drugs in thermosensitive liposomes. Further development in lipid chemistry allowing design of thermosensitive liposomes that can release the drug even at 41–42 °C would make thermosensitive liposomes more attractive.

Magnetic liposomes. A high concentration of drugs could also be achieved by using magnetic liposomes. In this technique, the drug is encapsulated in liposome-containing magnetic particles. The magnetic particles are usually nanosized magnetite (Fe_3O_4 around 15 nm) and are referred as ''ferrofluid''. The drug-loaded liposomes get accumulated at the site where the magnetic field is applied (Zhao et al., 2012). High concentration of paclitaxel and azidothymidine 5′-triphosphate in the brain have been achieved by delivering them in the form magnetic nanoliposomes (Saiyed et al., 2010; Zhao et al., 2012).

Immuno-liposomes. In immuno-liposomes, the surface is further modified by addition of modified proteins and monoclonal antibodies such as cationizied albumin and OX-26, respectively. These surface-modified immuno-liposomes undergo transcytosis, mediated either by receptors or by absorption (Schnyder & Huwyler, 2005).

Stealth liposomes. Liposomes are readily recognized and rapidly cleared by reticuloendothelial system. Therefore, polymers such as PEG, monosialoganglioside (GM1) amphiphilic polyacrylamide and poly(vinyl pyrrolidone) are added to increase their circulation time (Torchilin et al., 1994). Upon entry into circulation, liposomes are immediately covered with circulating opsonins. These opsonin-bound liposomes are immediately removed from circulation by MPS. Addition of hydrophilic polymers stearically obstructs binding of opsonins with liposomes. Polymers occupy the outer surface of liposomes and prevent other molecules from interacting with liposomes. Therefore, the circulation time of polymer-coated liposomes increases. This provides sufficient time for the drug-loaded liposomes, especially those containing antibodies, to interact with the target site thereby increasing the chances of drug uptake (Immordino et al., 2006). Among the hydrophilic polymers used in the preparation of stealth

liposomes, PEG is the most widely used. PEG is biocompatible, non-immunogenic, relatively non-toxic and soluble in polar and non-polar solvents. This together with their ability to increase drug stability, solubility and half life make them ideal candidate in design of stealth liposomes (Immordino et al., 2006).

Chimeric peptide

Over the past few years, a number of biopharmaceuticals products were developed. These are highly polar and larger in size. Because of their higher molecular weight and hydrophilicity, these molecules are effectively prevented from entering the CNS. This can be overcome by using chimeric peptides. Chimeric peptides are class of peptides and protein molecules that are coupled with a suitable vector such as OX 26 (monoclonal antibody). An important requirement is the usage of a suitable linker, which besides generating a stable conjugate could be cleaved at the target site. Avidin/biotin system is an important linking agent that is currently used for developing a stable conjugate (Bickel et al., 1993).

After administration, chimeric peptide is transported into the brain interstitial fluid by endocytosis-mediated process. Once within the fluid, the linker attaching the peptide with the vector is broken down due to the enzymatic action. The active molecule released as a result, then binds to the target site. Therefore, it is important to use a linker that could be cleaved in the fluid. If the linker is made of disulphide bond, after entering the brain, the disulphide bond will be broken down by the disulphide reductases resulting in the release of active drug from the chimeric peptide. The drug, β-endorphin is delivered in to the brain by conjugating it to a vector, cationic albumin using a disulphide linker. After reaching the brain, the disulphide bond is cleaved due to the enzymatic action of disulphide reductases resulting in the release of β-endorphin from the vector (Pardridge et al., 1990a).

Recently, two peptides *viz*., GLA (peptide sequence – GLAHSFSDFARDFVA) and GYR (peptide sequence – GYRPVHNIRGHWAPG) with ability to bind to BBB were discovered by phage display system. They were found to bind effectively to BBB. Their mechanism of binding is not yet understood. GLA is positively charged and can bind electrostatically with negatively charged cell membrane. But GYR, which is negatively charged, also shows good binding to BBB (van Rooy et al., 2010). However, when liposomes where spiked with synthetically synthesized GLA and GYR peptides, binding was less significant. It was found that the presence of naturally occurring protein coat, p3, confers conformational stability for these for effective binding with their substrate. It is suggested that these peptides could be designed along with their protein and these could be used for CNS delivery system (van Rooy et al., 2012). These studies shows that although synthetic peptides could be used in mimicking naturally occurring peptides, it should be carefully designed such that it is similar in all manners especially with respect to shape, peptide density that could be used on the surface and peptide confirmation so that it could be used effectively in drug delivery.

Vectors useful in design of chimeric peptides. Albumin and cationized immunoglobulins are regularly used as vectors in chimeric peptides.

(i) Albumin

Albumin, which is having a net negative charge at neutral pH, is chemically modified to cationized form with a pI of 8–9 (Bergmann et al., 1984). The chimeric peptide is then coupled with the single free cysteine-free group that is available of the total 35 cysteine groups present in the albumin.

(ii) Cationized immunoglobulin

Delivery of antibodies to the CNS can be greatly improved by cationization. This raises the pI and improves the CNS uptake by absorptive-mediated transcytosis (Triguero et al., 1990, 1991b). Cationized immunoglobulin, such as IgG, is being developed as a drug carrier (Triguero et al., 1989). But their tendency to accumulate in other tissues such as liver and lungs in larger quantity sometimes makes them unsuitable as a drug carrier to CNS (Triguero et al., 1991a).

Linkers for the delivery of chimeric peptides. Successful delivery of chimeric peptide depends on the stability of the linker as well. The linker should be stable in the plasma and should be cleavable at the target site. Disulphide linker is one of the widely used strategies. It is stable in the plasma; and after entering endothelial cells, it is broken down by the disulphide reductases (Letvin et al., 1986). Addition of disulphide linker is a two step process. First step in production of such linkers is the thiolation of primary amino group followed by formation of activated disulphide on the vector (Pardridge, 1991). Some of the linkages that are used for developing chimeric peptides include thioether linkage, ester linkages and Schiff's base linkages.

Some of the linkers that could be used to link the carrier with the vector are discussed below:

(i) Avidin/biotin linkers

Avidin and biotin, which bind with each other strongly, is one of the important linker used for transportation of drug, especially biotinylated molecules across BBB. These linkers are stable in the plasma and are released in the tissues (Pardridge et al., 1993).

(ii) Avidin fusion proteins

Avidin can be fused with monoclonal antibodies such as OX 26, a substrate for Tf receptor present in the BBB. Then thiolated avidin is attached to a biotinylated peptide and delivered.

Peptidomimetics (mimicking peptides)

Peptides are short amino acid sequence having specific number of monomer with a defined biological activity. In ''Peptidomimetics'', the primary structure of the peptide is retained and the backbone is altered by techniques such as chain extension, incorporation of heteroatom and use of amide bond isosteres (Patch & Barron, 2002). The pharmacokinetic properties are further improved in ''Foldamer'' where by using non-covalent bond, the primary structure is made to assume secondary structure (Gellman, 1998).

Magainin, when synthesized mimicking β-peptide, shows good antibacterial activity (Zasloff, 1987). There were also attempts to mimic somatostatin, a growth hormone, and

azapeptide, a T-cell activator (Tran et al., 1998; Hart & Beeson, 2001) using this technology. Similarly, modifications can be carried out to synthesize peptide resembling a naturally occurring biological substance with suitable modification to obtain desirable properties so that the molecules are able to cross BBB.

Trojan approach

Many of the artificially synthesized peptides show activity similar to its natural variant. But because of its ability to cross BBB and carry another substance, these are tried as vector to deliver the cargo across the BBB. Synthetic peptides such as ''Peptidomimetics'' have the ability to carry the drug coupled with them. These short peptides, which are based on natural peptides, could be synthesized and altered to improve its pharmacokinetic and therefore could serve as a good drug carrier to deliver to a target site. These synthetic peptides are able to deliver a larger cargo (Deeken & Loscher, 2007). Peptides such as magainins and dermaseptins have been tried as vectors to deliver the drug across BBB (Hariton-Gazal et al., 2002; Takeshima et al., 2003). Transcriptional activator of transcription (TAT) peptide, conjugated nanocarrier-TAT peptides and cationic peptides are some of the peptide-based delivery system currently studied for delivery drugs into the CNS using Trojan approach.

(i) TAT peptide as a carrier

Tat peptide is one of the widely explored peptide carriers. These peptides, by forming a nonspecific electrostatic bond with lipid membrane, gain entry into the CNS. This is independent of the receptor present at the surface (Dietz & Bahr, 2004). It is also shown that transduction of Tat peptide involves interaction with heparin sulfate proteoglycans, a sulfated glycosaminoglycans found at the surface of almost all the cells (Rusnati et al., 1997; Tyagi et al., 2001). Tat-BCl-X_L is transported across BBB by this mechanism (Dietz et al., 2002).

(ii) Conjugated nanocarrier-TAT peptides

Further improvement in drug delivery to CNS is achieved by combining nanocarriers with these Tat peptides. When both these delivery systems are combined, a stable drug with improved CNS penetration and reduced side effects are achieved (Torchilin, 2008; Rapoport & Lorberboum-Galski, 2009). The uptake of PEGylated ciprofloxacin improved when the surface was modified with Tat peptide (Liu et al., 2008a,b). Similarly, a higher CSF concentration of Ritonavir is achieved by conjugating its nanoformulation with Tat peptide (Liu et al., 2008a,b).

(iii) Cationic peptides as drug carrier

The antimicrobial property of cationic peptides such as magainin is attributed to their ability to induce pores on the cell membrane. This property could be utilized to deliver the drug across BBB by co-administering the drugs with these peptides. Any induction of pores on the surface of BBB by these peptides would help in diffusion of drugs through these channels. Some of these peptides are positively charged and interacts with negatively charged phospholipids resulting in translocation across the membrane. This would increase the fluidity of the cell membrane thereby increasing the drug uptake (Takeshima et al., 2003).

Immunophilins

Neuroimmunophilins are the protein receptors to which a number of immunosuppressants such as tacrolimus (FK506), cyclosporine A and sirolimus (rapamycin) bind (Snyder & Sabatini, 1995). These are otherwise called peptidyl-prolyl isomerases and cause *cis-trans* inter-conversion of amide bonds seen in peptides adjacent to proline amino acid. These isomerases are categorized into three groups namely cyclophilin A (cyclosporine A-binding protein), FK506-binding protein (FKBP) and the third parvulin, which is distinct from these two (Hamilton, 1998). These receptors are highly expressed in neurons (Snyder & Sabatini, 1995). It has been proved that these immunosuppressive agents, especially tacrolimus and its analogues, by binding with FKBP are able to produce neuroprotective and neurogenerative actions (Hamilton, 1998). These small-sized agents are able to cross the BBB easily and are useful in treating brain and spinal cord injuries (Gold, 2000). These receptors and the agents could be further explored to for CNS drug delivery.

Efflux transporter inhibitors

In many cases, it is observed that even highly lipophilic molecules are unable to accumulate within CNS. This is mainly due to the effective efflux mechanism present in BBB and BSCB. There are at least 48 members belonging to the super family of ABC transport proteins. Most of these efflux transporters present in the eukaryotes are similar to those observed in prokaryotes. This super family is sub grouped into seven sub families (ABC A–G). Among these, BBB and BSCB contain high density of efflux transporters belonging to B, C and G families. Among the efflux transporters, Pgp, belonging to ABCB, multi-drug resistance-associated proteins (MRP), belonging to ABCC, and breast cancer resistance protein (BCRP), belonging to ABCG sub family, have been studied extensively. A wide range of molecules, irrespective of their polarity, are efficiently removed by these transporters. Although they are mainly involved in removal of substances considered toxic to the CNS, the drug molecules are also removed by these transporters (Begley, 2004a).

(i) Pgp

Among the efflux transporters, Pgp is one of the efflux transporters that have been extensively studied. It was first discovered in cancer cells. But later, it was found to be distributed in number of tissues such as gastrointestinal tract and kidney apart from the brain capillaries (Chen et al., 1986).

Luminal part of the endothelial cell membrane and apical membrane of choroid plexus contain high concentration of Pgp (Begley, 2004a). Pgp can efflux wide range of drugs irrespective of their structural similarity. This makes it difficult to categorize drugs that will be a substrate for this transporter. It is believed that substrates may be similar in their pharmacophore, i.e. the possibility of a drug being substrate for this proteins could be depend on the number of hydrogen bond acceptors. It is also suggested that the possibility of a drug being strong or weak substrate for Pgp depend on their ''dwell time'', i.e. the residence time of a drug in the cell membrane. Drugs with high diffusion rate such as testosterone are weak substrates for Pgp (Seelig, 1998; Begley, 2004a). Presence of intact Pgp is important to prevent

occurrence/progression of certain diseases. However, efflux properties of these Pgp transporters also make it hard for drugs to accumulate within the CNS. Drugs such as etoposide, indinavir, cyclosporine A and doxorubicin, being a substrate for Pgp, are removed at the entry itself. This makes it difficult to achieve the required therapeutic concentration at the required site (Begley, 2004a).

A higher drug concentration could be achieved if Pgp is inhibited. Inhibitors can block the drug-binding site, interfere with ATP hydrolysis or change the permeability of cell membrane (Akhtar et al., 2011). Based on the specificity, Pgp inhibitors are classified into first, second and third generation. First-generation inhibitors are non-specific and have low affinity toward Pgp. This includes drugs such as reserpine and tamoxifen. Second-generation inhibitors have higher affinity toward Pgp but they are substrate for other ABC transportes. Dofequidar fumarate and valspodar belongs to this category. The inhibitors under first and second generation were mostly pharmacological agents with affinity toward Pgp. Inhibitors in third generation were specifically synthesized using computer-aided drug designing. Third-generation Pgp inhibitors have the highest affinity for Pgp and are more specific. They do not serve as substrates for other ABC transporters and are active even at nanomolar concentration. Biricodar, mitotane and laniquidar are some of the molecules currently studied as Pgp inhibitor (third generation) (Akhtar et al., 2011; Amin, 2013). A higher concentration of loperamide in the brain is achieved through co-administration of quinidine (Sadeque et al., 2000). There are many pharmaceutical excipients such as cremophor EL and Tween 80, which increase the drug concentration by affecting the membrane permeability. The alteration in the hydrophobic environment affects the secondary and tertiary structure of Pgp. This results in loss of Pgp function (Akhtar et al., 2011). Lipid-based excipients, such as 2-propylhexadecanoic acid, reduces the expression of *MDR1* gene expression in addition to its ability to increase membrane fluidity and block ATPase activity. Polymers, especially with thiol functional groups, can interact with cysteine group of Pgp and inhibits its function (Werle, 2008).

While inhibitors could be useful in achieving a high concentration of even less lipophilic drug, care should be taken to ensure that Pgp is not damaged irreversibly. Pgp also protects CNS by removing toxins. β-amyloid is a natural substrate of Pgp and is removed continuously from the CNS. Any mutation in Pgp leads to accumulation of β-amyloid plaques leading to onset of Alzheimer's disease (Lam et al., 2001).

(ii) BCRP

BCRP belongs to ABCG2 group. ABCG2 protein, an efflux pump initially discovered in breast cancer cell lines (Doyle et al., 1998). Later, it was found that it is distributed luminal surface of the BBB (Cooray et al., 2002). A number of molecules of sulfate and glucuronide conjugates are substrates for BCRP. BCRP is also involved in the maintenance of folate metabolism within cells. While Pgp mainly binds with weak cations and large lipophiles, BCRP can bind with large anionic compounds and large hydrophobic compounds (Nicolazzo & Katneni, 2009). BCRP is found to be elevated during some of pathological conditions such as epilepsy (Aronica et al., 2005). Drugs such as methotrexate, topotecan, daunorubicin, doxorubicin and many other drugs are substrates for BCRP (Nicolazzo & Katneni, 2009). Although some of these drugs are also substrates for Pgp, many of the Pgp inhibitors cannot inhibit BCRP. Therefore, it is important to identify and develop inhibitors specifically targeting BCRP so that sufficient concentrations of some these drugs within CNS could be achieved (Ahmed-Belkacem et al., 2005).

Fumitremorgin C (FTC) is a fungal toxin with potent BCRP inhibition. High toxicity of FTC limits its use *in vivo*. However, tetracycline analogues of FTC are much safer and are more specific. Inhibitors of tyrosine kinases such as geftinab and sorafenib and synthetic molecules such as elacridar and tariquidar can also inhibit BCRP. These inhibitors are known to act by blocking ATPase activity (Zhang et al., 2004a; Ahmed-Belkacem et al., 2005; Nicolazzo & Katneni, 2009; Agarwal et al., 2011). Drugs such as sorafenib have higher affinity toward BCRP than Pgp (Agarwal et al., 2011). Interestingly, many naturally occurring flavonoids such as 6-chrysin, prenylchrysin, tectochrysin and biochanin A are found to inhibit ABCG2. Among the flavonoids, flavones are generally found to be more potent in inhibit BCRP. These flavonoids act by interfering in the coupling between the ATP hydrolysis and transport of drug (Ahmed-Belkacem et al., 2005). Therefore, a drug could be delivered along with some of these inhibitors to achieve a better concentration in CNS.

(iii) MRP

MRP proteins are known for their ability to remove many of the anticancer drugs and antiviral agents, such as azidothymidine. MRP family consists of 13 members (Zhou et al., 2008). These are found in the apical plasma membrane of BBB (Zhang et al., 2004b). Anionic drugs and to some extent acidic ligands conjugated neutral drugs are substrates for MRP. The ligands could be glucuronate, sulfate or glutathione (Zhou et al., 2008). Among the various efflux transporters known, glutathione-S-conjugates are exclusively removed by MRP1, a member of MRP family. This transporter is able to efflux out even the heavy metal-based antineoplastic agents such cisplatin and arsenic (Borst et al., 2000).

As glutathione conjugates are good substrates for MRP, glutathione-based molecules could be designed to inhibit MRP. Ethacrynic acid conjugated with glutathione is found to be a potent inhibitor of MRP (Burg et al., 2002). Again as mentioned earlier, care should be exercised while blocking MRP. Glutathione is predominantly produced by astrocytes. These ions protect the brain against oxidative stress by removing free radicals (Minich et al., 2006). Any reduction in export of glutathione-conjugated radicals could result in accumulation of these radicals, which can produce oxidative stress within the CNS.

Viral vectors

Viruses such as Adenovirus (Di Polo et al., 1998) and Lenti virus (Kordower et al., 2000) could be used as vectors for the delivery of cDNA to the target site. But still, issues such as immunological response, inability to produce sufficient quantity of recombinant proteins and inability to express the inserted gene for long period (Eck, 1999; Dietz & Bahr, 2004)

Table 3. Advantages and limitations of various delivery systems for drug delivery to brain.

Type of delivery system	Advantages	Limitations
Focused ultrasound	Target specific	Self administration not possible. Practical application in humans is limited
Lipidization	Enhances permeability	Increases the volume of distribution
Inhibition of efflux transporters	Increases drug availability within brain	Entry of toxins
Carrier-mediated transport	Useful in delivering wide range of molecules	Requires complete understanding. Could be sometime non-specific
Peptidomimetics	Enhanced permeability	Cannot be applied for all the molecules
Nanoparticulate delivery	Target specific Useful in delivering wide range of molecules Reduces the therapeutic concentration of drugs	Distribution and rate elimination not understood completely. Role of protein corona not understood completely
Prodrug	Improved permeability	Not target specific. Cannot be applied for all the drug molecules

needs to be addressed before a viral vector could be effectively used as drug carriers.

Prodrug approach

Prodrugs are those in which the native drugs are modified by a suitable method such that they are biologically inactive and get converted to an active form at their target site (Misra et al., 2003). The conversion may be as a result of physiological change such as change in pH, chemical change or due to enzymatic action. For a site-specific delivery, a suitable enzyme could be tagged with monoclonal antibodies so that they are incorporated at the target tissue. A prodrug, which is then administered, upon reaching the target tissue, gets converted to its active form resulting in therapeutic action. β-lactamase and arylsulfatase are used for the activation of vinca alkaloids and etoposide, respectively (Kroll & Neuwelt, 1998). Lipophilicity can also be increased by administering the drugs as a prodrug, i.e. the drugs are chemically modified so that they are able to cross BBB. Upon entry, active form of the drug is released. This further prevents the exit of the drugs from the brain (Jain, 2007).

Overall view

Although a number strategies are available to improve drug bioavailability within CNS, still there is no single universal strategy that is currently available that could be used in delivering all pharmacologically active drugs across BBB. Some of the pros and cons with each delivery system are given in Table 3.

Although lipidization of drug enhances the transportation of drugs across BBB, it also increases volume of distribution. Besides high molecular weight, substance irrespective of their solubility would be retarded by BBB. Inhibition of efflux pump transporters is another way of increasing the drug concentration within CNS. But this should be done carefully as these transporters are also involved in removal of free radicals and toxins such β-amyloid peptides from the CNS. Use of carrier-mediated process requires a complete understanding of these processes under various pathophysiological conditions and at molecular level. However, these carrier systems may not be always brain specific. These would result off target delivery of the drug. While some of these problems can be overcome by using focused ultrasound, concerns over local tissue damage, non-selectivity, entry of toxins and loss in ionic balance within CNS remains to be addressed. Besides, the technology for complete implementation of this technique has not evolved. Some of the techniques, such as use of magnetic resonance imaging, may not be practically feasible when it comes to human. The type of formulation should also be decided depending upon the type of disease conditions. While diseases such as Parkinson's disease requires administration of substances that are safe and are present in normal healthy patients, conditions such as glioblastomas requires administration of substances, which are highly toxic for normal cells. These toxic drugs have to be designed in such a way that they preferentially get accumulated only in cancer cells.

Nanoparticulate delivery is one of the recent and fast emerging techniques in the area of drug delivery. Although it has established itself very well in other scientific disciplines such as imaging techniques, it is still at infancy in the area of drug delivery. The interest in nanoparticulate delivery mainly arises from the assumption that it significantly reduces the amount of the drug that needs to be administered. Higher bioavailability could be achieved with drugs in nanosize due to better absorption of a small-sized particle. But the same property of small size also increases the distribution of drugs and reduces their elimination, which can result in drug accumulation. Besides, it remains to be seen how far the pharmaceutical companies are willing to invest in this technology and take it to next level. To achieve good bioavailability within the CNS, the polymer-encapsulated drug should be below 400 Da in size. When attempts are made to achieve size as small as 200 nm, there will be huge loss in raw materials especially with regard to loss of active ingredient during formulation. This is due to poor drug loading. Sometimes, the processing loss of active ingredient can be as high as 50%. Another important phenomenon, which is not yet fully understood or given importance, is the role of ''protein corona'' on absorption, distribution, metabolism and elimination of nanoparticles, especially the pharmacokinetic behavior of the charged nanoparticles. While anionic (negatively charged) nanoparticles are electro statically limited to interact with negatively charged cell membrane and BBB, cationic (positively charged) nanoparticles are removed quickly from circulation.

Among the various delivery systems, polymer-based delivery systems appear to be promising. This could be due to the following:

(1) Variety of molecules that can be delivered
(2) Number of surface modifications that it offers.

Surface modifications are useful in making nanoparticulate delivery system more target-specific and in increasing their circulation time. However, the recent increase in protein- and peptide-based drugs has made these nanoparticulate delivery systems containing polymers complicated. Some of the harsh conditions *viz*., usage of organic solvents, homogenization and temperature during preparation of protein-loaded polymers, could result in permanent loss in activity of some of the therapeutically useful proteins. The scale-up process also remains to be addressed. Although liposomes are found to be useful in delivery of proteins, the problems with respect to stability and sterility still remains. The melting temperature (during liposome preparation), which is generally above room temperature could affect the stability of protein-based products. Delivery of drugs as prodrugs can overcome some of the above problems. But they cannot be used for all the drugs as some of the drugs either cannot be designed as prodrugs or tend to lose activity irreversibly. Therefore, the type of delivery system has to be decided based on the properties of the drug molecule, pathophysiological condition and target site.

Conclusion

With deeper understanding on the physiology of BBB, a number of newer strategies have been developed to reach CNS by systemic route, which was once considered impossible. By selecting a proper technique for a particular drug, it is possible to attain a good bioavailability in the CNS even for the molecules such as proteins and peptides that have high polarity and high molecular weight.

Acknowledgements

Authors acknowledge Manipal University authorities for providing necessary to carry out this work.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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