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Review Article

Advances in Brain Targeted Drug Delivery: Nanoparticulate Systems

Emil Joseph, Ranendra Narayan Saha*

Department of Pharmacy, Birla Institute of Technology and Science, Pilani-333031, India
***Correspondence:** rnsaha@pilani.bits-pilani.ac.in; Tel.: +91-1596 245192/515284/515269

Abstract

Drug delivery to the brain is always a challenging task for the formulation scientists because of low permeation due to presence of blood brain barrier (BBB) with tight junctions in the brain endothelial cells. Even though numerous traditional approaches such as prodrugs, disruption of blood brain barrier have shown some success to overcome these challenges, researchers are continuously working for alternatives for better delivery of drug to brain. Recent advances in nanotechnology offer an appropriate solution for the drug delivery problems associated with the brain targeted drug delivery. The present review describes various nanotechnology based formulations such as polymeric nanoparticles, solid lipid nanoparticles, liposomes, dendrimers, miscelles and nanoemulsions which have been widely used for the better delivery of the drugs across blood brain barrier. Furthermore, components of blood brain barrier, general transport mechanisms across BBB and possible mechanisms of enhanced transport of nanoformulations to the brain have been discussed in detail. Moreover several ligand based targeted systems for the active drug delivery to the brain have also been discussed.

Keywords: Nanotechnology, brain, targeted drug delivery

Introduction

Drug delivery to the central nervous system (CNS) is always a challenging task for the formulation scientists because of the presence of blood brain barrier (BBB). It has been estimated that more than 98% of CNS active drugs coming out of synthetic pipelines are not able to cross the blood brain barrier sufficiently to achieve therapeutic drug concentration.[1] Also, majority of the small molecule drugs (>98%) and almost all of the large molecule drugs, including biotechnology based products, fail to cross blood brain barrier. Overcoming the resistance of this barrier system can lead to effective treatment of several diseases of central nervous system. Numerous traditional approaches have been investigated for this purpose such as prodrugs, disruption of blood brain barrier, intra-cerebral injection, use of implants etc. Prodrugs are generally bioreversible derivatives of drug molecules that undergo a chemical or enzymatic biotransformation to convert to active drug to produce pharmacological action in the body.[2] Prodrug approach has been explored to convert the drug to a more lipophilic compound, thereby enhancing the passage across blood brain barrier. Prodrugs, based on chemical derivatives such as lipophilic esters and other hydrophobic compounds, have been studied and found to be useful in limited extent. More sophisticated prodrug approaches comprise of macromolecular delivery mechanisms (e.g., receptor-mediated prodrug transport), endogenous transporters (e.g., carrier-mediated prodrug transport), as well as gene-mediated enzyme prodrug therapy.[2,3] Prodrug approach is often limited due to the premature conversion of prodrug to the drug in plasma itself by the enzymes present in plasma.

Reversible disruption of the BBB is another traditional approach which makes the tight junctions of the endothelial cells leaky and provides access for the blood components to the brain [4]. Various techniques have been investigated for the disruption such as osmotic disruption, ultrasound disruption and disruption by bradykinin-analogue. In case of osmotic disruption, the osmotic shock causes endothelial cells to shrink, thus disrupting the tight junctions. A classic example for this is the pre-administration of a hypertonic mannitol solution with subsequent administration of drugs through intra-carotid artery, which increased the drug concentration in brain and tumour tissue as compared to the administration of drug alone.[4] MRI-guided focused ultrasound technique is another useful approach for BBB disruption. In a study performed by Feng et al, it has been demonstrated that, Evans Blue (EB) extravasation can be enhanced by the application of repeated sonication.[5] Sonications were applied at an ultrasound frequency of 1 MHz and a repetition frequency of 1Hz. There was nearly two fold increase in EB extravasation in groups with double sonication compared with the single sonication group. Extravasation was high with both the cases as compared to groups in which no sonication have been applied. The selective B2 bradykinin receptor agonist, Cereport (also called RMP-7), has found to increase permeability of drugs transiently through the blood brain

barrier. There are evidences of enhanced CNS delivery of carboplatin, loperamide, and cyclosporin-A, when administered along with RMP-7.[6] The major limitation associated with BBB disruption technique is the increased brain uptake of plasma albumin and other protein components of blood, which are toxic to brain cells.[7]

Intra-cerebral injection or use of implants is another useful traditional approach. The bolus injection of therapeutic agents and the placement of a biodegradable, drug impregnated wafer rely on the principle of diffusion to drive the drug into the infiltrated brain. Various types of paclitaxel-loaded lipidic implants and PLGA-based microparticles with controlled release kinetics for several weeks, have been prepared and characterized *in vitro* by Elkharraz *et al.*[8] These devices can directly be injected into the brain tissue (intracranial administration), overcoming the restriction that paclitaxel cannot cross the blood–brain barrier to a significant extent upon systemic administration. They have found that this type of direct administration of controlled drug delivery system is helpful to improve the local treatment of operable and inoperable brain tumors.

These traditional approaches are less successful to cross the BBB for better brain delivery and most of them are associated with numerous adverse effects. Moreover, some of these approaches are highly destructive in nature which is extremely harmful to the body in long term. Thus, it is a challenge to enhance drug permeability to brain for effective therapeutic efficacy with no or limited side effects and with better patient compliance.

Structure of BBB and Transport Mechanisms

The BBB is considered to be a dynamic and complex barrier separating blood and the central nervous system that strictly controls the exchanges between the compartments of blood and brain.[9] Blood brain barrier is a natural biological barrier that plays a crucial role in the protection of the brain by restricting the entry of untoward substances such as toxic molecules, pathogens, and numerous other external molecules and thereby maintaining the brain homeostasis. An illustration of blood brain barrier is shown in Fig.1. The endothelial cells present in brain differ significantly from cells present in other parts of the body, due to the presence of intracellular tight junctions, lesser paracellular diffusion of hydrophilic molecules, presence of relatively high number of mitochondrial cells and thereby with high metabolic activity, and a relatively higher number of active transporters.[10] The basal lamina, composed mainly of collagen, glycoproteins, and proteoglycans, is involved in the dynamic regulation of blood brain barrier with the aid of multiple basal lamina proteins, matrix metalloproteases, their inhibitors, and the tissue inhibitors of metalloproteases. The astrocytes and glial cells present in the BBB also contribute to a great extent for the barrier integrity through glial-derived neurotrophic factor, angiopoietin-1 and angiotensin II. Brain microvessels have numerous pericytes and ratio of pericytes to endothelial cells was linked with the barrier capacity. Endothelium, pericytes, perivascular astrocytes are very close contact with neuronal projections.

General Transport Mechanisms across BBB

The major transport mechanisms to the BBB are depicted in Fig. 2. These include paracellular aqueous pathway, transcellular lipophilic pathway, transport protein pathway, receptor mediated transcytosis, and adsorptive transcytosis.[11] Paracellular aqueous pathway is a rare pathway across BBB through which small water-soluble molecules diffuse into the brain. Lipophilic molecules such as alcohol, steroid hormones etc . penetrate transcellularly by dissolving in their lipid plasma membrane. In carrier-mediated transport, a protein transporter binds to glucose or amino acids which triggers a conformational change in the protein and helps in the transport of the molecule to the other side. The receptor-mediated transcytosis type of transport mechanism is for the selective uptake of macromolecules. These systems include receptors for transferrin, insulin, lipoprotein etc and those are also explored for the ligand based nanoformulations, which have been discussed in detail in later sections of this review. Another kind of transport mechanism, called adsorptive-mediated transcytosis is triggered by an electrostatic interaction between a positively charged substance such as charged moiety of a peptide, and the negatively charged plasma membrane surface such as heparin sulphate proteoglycans.

The aforementioned components of BBB and specific transport mechanisms make BBB highly dynamic and complex, thereby restricting the entry of various drug molecules. Since traditional approaches could not solve these problems, pharmaceutical scientists looked forward and found out newer approaches such as nanotechnology in order to deliver drugs across BBB successfully.

Nanotechnology in Brain Targeting

The application of nanotechnology for the drug delivery to the brain opens the doors of opportunities for the formulation scientists for the better and selective brain delivery of existing and newer potential molecules with CNS activity. It has been assumed that the delivery to brain of majority of the potential CNS drugs which have inability to cross BBB could be modified by nanotechnology in order to achieve better therapeutic action and better patient compliance. It would bring about rebirth to many drugs which have been discontinued due to their failure to gain therapeutic concentration in the brain.

Kreuter *et al*.[12-13] describe number of possibilities that could explain the mechanism of the delivery of nanoparticulate formulations across the BBB and are discussed below. As compared to the pure drugs, there is an increased retention of the nanoformulations in the brain blood capillaries combined with more adsorption to the capillary walls. These retention and adsorption create a higher concentration gradient that would enhance the transport across the endothelial cell layer and result in better delivery to the brain.

1.The nanoparticles could lead to an opening of the tight junctions between the brain endothelial cells. The drug could then permeate through the tight junctions in either free form or as nanoparticles in bound form.

2.There is a general surfactant effect of nanoformulations characterized by solubilization of the endothelial cell membrane lipids that would further lead to membrane fluidization and thereby enhanced drug permeability through blood brain barrier.

3.The nanoformulations may be endocytosed by the endothelial cells of the brain capillaries which would further result in the release of the drugs within these cells and delivery to the brain.

4.Drug loaded nanoformulations could be transcytosed through the endothelial cell layer.

5.Surfactant which is used as the coating agent could inhibit the efflux system, especially P-glycoprotein (Pgp). Endocytosis via the low density lipoprotein (LDL) receptor, mediated by the adsorption of apolipoprotein B and/or E from the blood is also a suggested mechanism for the nanoformulations coated with polysorbate such as Tween 20, 40, 60 and 80, and poloxamers such as pluronic F68.[13-14]

Nanoformulations Investigated

Numerous nanoformulations have been investigated successfully for better brain delivery which includes nanoparticulate systems (polymeric/solid lipid), liposomes, dendrimers, nanoemulsions, nanosuspensions, and ligand mediated nanosystems. Summary of these systems investigated with examples is listed in Table 1.

Polymeric nanoparticles

Nanoparticles (NPs) are, colloidal particles, less than 1000 nm, that can be used for better drug delivery and prepared either by encapsulating the drug within a vesicle and or by dispersing the drug molecules within a matrix.[15] Nanoparticulate drug delivery systems have been extensively studied in recent years for spatial and temporal delivery, especially in tumour and brain targeting. NPs have great promise for better drug delivery as found in both pharmaceutical and clinical research. As a drug carrier, NPs have significant advantages like better bioavailability, systemic stability, high drug loading, long blood circulation time and selective distribution in the organs/tissues with longer half life. These systems have been increasingly used in order to improve selective brain delivery of the therapeutic agents. Nanoparticles, prepared from a wide variety of biodegradable/biocompatible polymers such as poly(D,L-lactide-co-glycolide) (PLGA), poly(D,L-lactide) (PLA), polybutyl cyanoacrylates (PBCA), polycaprolactone (PCL) etc are extensively used for the delivery of drugs to the central nervous system. They provide numerous advantages which include protecting the drug from degradation, releasing a therapeutic load in the optimal dosage range and enabling the delivery of the therapeutic agent to the preferential site and thus decreasing the total dose.[16]

Numerous investigations have been made in order to deliver the drug across BBB by incorporating drug in polymeric nanoparticulate systems. In a study by Snehalatha *et al.,* etoposide loaded PLGA and PCL nanoparticles were prepared and biodistribution and pharmacokinetics of radiolabeled etoposide and etoposide loaded PLGA and PCL nanoparticles were carried out.[17] Etoposide loaded nanoparticles (labeled with Tc-99m) were administered by intravenous and oral routes and their respective biodistibution and pharmacokinetic parameters were determined. The results showed that overall high plasma residence of nanoparticles compared to pure etoposide signifies the advantage of PLGA and PCL nanoparticles as drug carrier for etoposide in enhancing the bioavailability and with selective distribution with higher brain permeability and possibility of reducing the etoposide associated toxicity. The extent of distribution of etoposide loaded nanoparticles in brain was found to be three times than pure drug after 24h. In another study, imatinib mesylate loaded PLGA nanoparticles were prepared and bio-distribution and pharmacokinetics were studied in rat model.[18] The results showed that nanoparticulate formulations increased the extent of drug permeation to brain with nearly 100% increase in MRT and 3 fold increase in AUC_{∞}as compared to pure drug.

There are numerous evidences for the existence of a direct nose-to-brain delivery route for delivery of the drugs to the nasal cavity and transported via the olfactory epithelium and/or via the trigeminal nerves directly to the central nervous system.[19] This route can effectively be used for administering nanoparticulate systems for better delivery of drugs to brain. Haque et al studied this approach where they prepared venlafaxine (VLF) loaded chitosan nanoparticles to enhance the uptake of VLF to brain via intranasal delivery.[20] The higher drug transport efficiency (508.59%) and direct transport percentage (80.34%) of VLF chitosan NPs as compared to other formulations suggested its better efficacy in treatment of depression.

Coating of the nanoparticles with surfactants is another useful approach as it can induce increased brain uptake. Its mechanism

has already been described in the earlier section of this review. Numerous studies have been performed in this direction and it has been found that coating with surfactant systems resulted in enhanced brain concentration of drug/dye as compared with uncoated systems.[21-22]

Solid lipid nanoparticles (SLN)

Solid lipid nanoparticles (SLN) are colloidal particles composed of biocompatible/biodegradable lipid matrix that is solid at body temperature and exhibit size in a range of 100 to 400 nm.[23] SLN offer several advantages such as controlled drug release, targeted delivery, increased drug stability, high drug payload, least biotoxicity, large scale production and ease of sterilization.[24] General ingredients used in the preparation of SLN are solid lipid(s), emulsifier(s) and water. The term "lipid" has a broader sense here and includes triglycerides (e.g. tristearin), fatty acids (e.g. stearic acid), partial glycerides (e.g. Imwitor), steroids (e.g. cholesterol) and waxes (e.g. cetyl palmitate).

SLN are widely used for the delivery of active pharmaceutical ingredients to the brain because of the advantages mentioned above and its enhanced ability to cross BBB. For instance, camptothecin-loaded solid lipid nanoparticles (SLN) were prepared using cetyl palmitate, Dynasan 114 and Witepsol E85 by Martins *et al*., using hot high pressure homogenization process.[25] A higher affinity of the SLN to the porcine brain capillary endothelial cells (BCEC) was shown in comparison to macrophages. In vivo studies in rats showed that fluorescent labelled SLN were detected highly in the brain after i.v administration. Similar studies on SLN preparation with enhanced brain uptake could be found elsewhere in the literature.[26-27]

A newer version of SLN called nanostructured lipid carriers (NLC), with increased drug loading are also becoming popular recently for brain targeting which are composed of a solid lipid and a certain amount of liquid lipid (oil), maintaining the solid state at both room and body temperature.[28-29]

Liposomes

Nanoformulations such as liposomes consists of bilayer phospholipid systems in which water-soluble drugs could reside in the aqueous phase enveloped by phospholipid bilayer and the lipophilic drugs, could directly integrate into the membrane.[30] Researchers are actively investigating on several advanced versions of liposomes such as long-circulating (PEGylated) liposomes, triggered release liposomes, liposomes containing nucleic acid polymers, ligand-targeted liposomes and liposomes containing combinations of drugs in order to achieve better drug delivery. These advances have led to numerous clinical trials of anti-cancer drugs, anti-fungal drugs, antibiotics, gene medicines, anesthetics and anti-inflammatory drugs.[30] Targeted brain delivery using liposomal systems resulted in considerable increase of drug concentration in brain/*in vitro* cell lines.[31-32]

Dendrimers

Dendrimers are a unique class of synthetic polymers which has a major role in nanotechnological advances of drug delivery.[33] The term "dendra" in "dendrimer" is derived from Greek which means tree and therefore appropriately describes its architecture. Novel dendrimer-based drug delivery systems consisting of G3 polyamidoamine (PAMAM) and surfactant conjugated dendritic nanoconjugates have been successfully applied for targeted brain delivery.[34-35] Cytotoxicity studies showed that free G3 PAMAM was relatively non-toxic while the conjugation of lauryl chains and paclitaxel molecule on the surface of G3 PAMAM dendrimer significantly increased the cytotoxicity in both human colon adenocarcinoma cell line (Caco-2) and primary cultured porcine brain endothelial cells (PBECs). Enhanced permeation of the lauryl-modified G3 PAMAM dendrimer–paclitaxel conjugates across Caco-2 cell and PBEC monolayers has also been demonstrated. Dendrimer conjugate had approximately 12-fold greater permeability across both cell monolayers than that of paclitaxel alone.

Micelles

Polymeric micelles obtained from block copolymers as colloidal carriers for drug and gene targeting have been receiving much attention in the field of drug delivery and targeting because of the high drug-loading capacity.[36] A variety of drugs with diverse characteristics, including genes and proteins, can be incorporated into the core. Researchers have demonstrated effective targeting of micelles systems to the brain by intra venous as well as intra nasal route.[37]

Nanoemulsions

Nanoemulsions have also gained considerable attention in research as well as in therapeutics due to their advantages such as ease of preparation, thermodynamic stability, optical clarity, and their ability to incorporate both hydrophobic and hydrophilic solutes etc.[38] Intranasal nanoemulsion based brain targeting drug delivery system of risperidone was studied by Mukesh Kumar *et al.*[39] They have found higher drug transport efficiency (DTE%) and direct nose to brain drug transport (direct transport percentage, DTP%) for these mucoadhesive nanoemulsions. Similar results have been obtained with saquinavir-loaded nanoemulsions which also resulted in efficient brain delivery.[40]

Ligand-Mediated Active Targeting

Advances in cell biology with respect to internalization pathways and problems associated with delivery of new macromolecular

drugs such as peptides and proteins paved the path of receptor mediated targeting for selective uptake and internalization of drugs.[41] The flexibility of nanoformulations for the attachment of ligands for specific receptors further improved the scope of ligand mediated active targeting since these systems also provide additional advantages such as controlled release of drugs and protection from external degradation before reaching the targeted site. Numerous receptors which are over expressed in brain such as transferrin receptors, insulin receptors, low density lipoprotein receptors etc have been widely explored for the ligand mediated targeted brain delivery (Table 2).

Membrane transferrin receptor-mediated endocytosis is an efficient cellular uptake pathway for drug delivery of therapeutic agents to the brain. The transferrin receptors (TfR) are present in BBB as well as many tumors cells and therefore have been widely studied for better brain delivery. Transferrin either directly or via antibodies against the transferrin receptor, such as R17217 and OX26 monoclonal antibody have been investigated to a great extent. Several researchers have explored this opportunity and conjugated transferring/antibodies against transferrin receptors with their nanoformulations in order to achieve higher concentration of drug in targeted site. Ulbrich et al prepared human

Table 1. List of nanoformulations investigated for better brain delivery

Formulation	Materials used	API/Model molecule	Advantages
Polymeric nanoparticles	PLGA and PCL	Etoposide	Selective distribution with higher brain permeability[17]
	PLGA	Imatinib mesylate	Increased the extent of drug permeation to brain[18]
	Chitosan	Venlafaxine	Better brain uptake, higher direct transport percentage[20]
	PLA-PEG-tween 80	Amphotericin B	Drug concentration in mice brain greatly enhanced, reduced the toxicity of AmB to liver, kidney etc.[21]
	PBCA-tween 80	Doxorubicin	Augumented accumulation of NP in the tumour site and in the contralateral hemisphere[22]
Solid lipid nanoparticles/ Nanostructured lipid carriers	Cetyl palmitate, dynasan, witepsol	Camptothecin	Higher affinity to the porcine brain capillary endothelial cells as compared to macrophages[25]
	Trimyristin, tripalmitin, tristearin	Clozapine	The AUC and MRT of clozapine SLNs were significantly higher in brain[26]
	Stearic acid	Idarubicin	Drug and its metabolite were detected in the brain only after IDA-SLN administration[27]
	Tripalmitin, gelucire, vitamin E	Baicalein	Brain-targeting efficiency of baicalein was greatly improved by NLCs[29]
Liposomes	DPPC, DC, Chol, DOPE, DHPE	Oregon Green	Liposomes were strongly internalized in cultured cell lines within 6h[31]
	DSPC, cholesterol, DSPE	Citicoline	Considerable increase (10-fold) in the bioavailability of the drug in the brain parenchyma[32]
Dendrimers	Polyamidoamine	Paclitaxel	12-fold greater permeability across porcine brain endothelial cells[34]
	Polypropyleneimine	Docetaxel	Higher targeting efficiency and biodistribution to the brain[35]
Micelles	Block copolymers of ethylene oxide/ propylene oxide	Olanzapine	Demonstrated higher drug targeting index (5.20), drug targeting efficiency (520.26%) and direct transport percentage (80.76%)[37]
Nanoemulsion	Glyceryl monocaprylate	Risperidone	Higher drug transport efficiency (DTE%) and increased direct nose to brain drug transport (direct transport percentage, DTP%)[39]
	Flax-seed,safflower oil	Saquinavir	Improved brain uptake[40]

serum albumin nanoparticles-conjugated with antibodies against the transferrin receptor, which could deliver loperamide across BBB whereas, pure loperamide has shown no or very poor permeation.[42] Same group have explored insulin receptors also for brain targeting of loperamide using anti-insulin receptor monoclonal antibody (29B4) and found similar results which showed many fold increase in antinociceptive effects in the tail-flick test in mice as compared to pure loperamide.[43]

Certain cell-penetrating peptides, by virtue of their structure are able to cross their way directly through the cell membrane and therefore have been investigated as ligand for improved brain delivery. Trans activating transduction protein (TAT), is an example for this, and have been shown to cross highly the BBB and accumulated in the CNS. Based on this, a novel TAT-modified liposome (TAT-LIP) of Coumarin was developed.[44] The results showed that the positive charge of the TAT-LIP played an important role in enhancing its brain delivery and it has been suggested that absorptive endocytosis might be one of the mechanisms of TAT-LIP crossing the BBB.

Another interesting receptor which is widely explored for ligand mediated brain delivery is the low-density lipoprotein receptor. A new peptide named angiopep could act as a ligand for targeting to the low-density lipoprotein receptor-related protein-1 (LRP1) and exhibit high transcytosis capacity. Based on these findings PAMAM dendrimers were modified with angiopep through bifunctional PEG and it has been found that angiopep-modified NPs showed higher efficiency in crossing blood–brain barrier than unmodified NPs in an in vitro and in vivo BBB models.[45] The biodistribution studies in mice has demonstrated more than 5 fold increase in drug concentration in brain with optimized ligand coupled formulation as compared to pure drug.

Table 2. List of ligands explored for active brain targeting

Targeting ligand	Formulation and material used	API/Model Molecules	Advantages
Transferrin/ transferrin receptor monoclonal antibodies (OX26 or R17217)	Human serum albumin nanoparticles	Loperamide	Able to transport loperamide across the BBB, induced significant anti-nociceptive effects in the tail-flick test[42]
Insulin/anti-insulin receptormonoclonal antibody (29B4)	Human serum albumin nanoparticles	Loperamide	Increased transport of loperamide across the BBB, induced significant anti-nociceptive effects in the tail-flick test[43]
Trans activating transduction (TAT)	Liposomes (soybean phospholipids,cholesterol phosphatidylethanolamine)	Coumarin	Enhanced brain delivery[44]

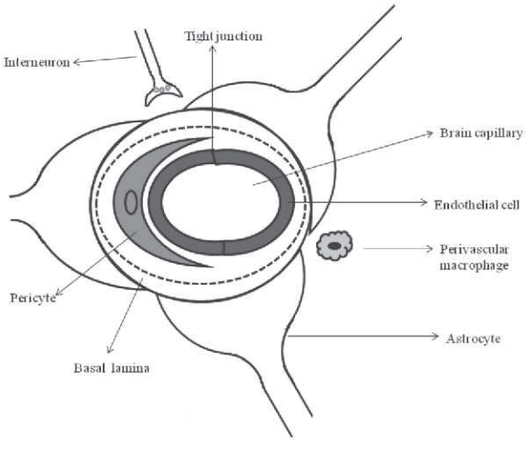

Fig. 1: Schematic representation of blood-brain barrier

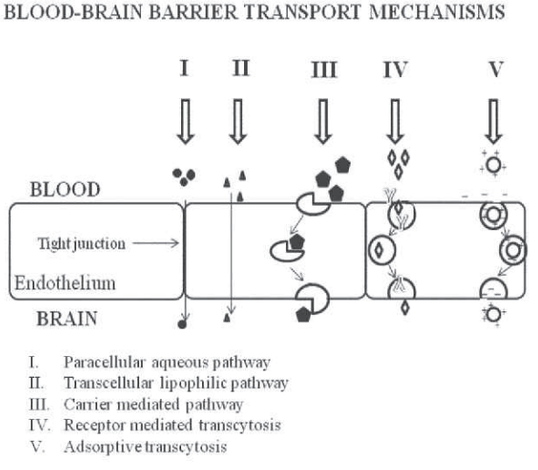

Fig. 2: General transport mechanisms across blood-brain barrier

Conclusion

The application of nanotechnology in drug delivery has opened various opportunities for the formulation scientists for the better delivery of therapeutic agents to CNS. In addition to enhanced brain transport, these systems also provide additional advantages such as extended or controlled release of drugs and protection from degradation before reaching the targeted site leading to decreased dose or lesser frequency with decreased or no side effects. Numerous drug molecules have been made to be permeated through blood brain barrier by incorporating in suitable nanoformulations which resulted in enhanced efficacy of the drug and better therapeutic action. Ligand-based active targeting is also a highly promising approach for the selective delivery of the drugs across blood brain barrier. Numerous novel targets are being identified day by day through the advances of biotechnology and sophisticated imaging techniques, which would further bring about path breaking changes in the nanotechnology based brain targeted drug delivery. It is expected, in future, more biotechnological products may be delivered better to brain by these processes.

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