

RECENT DEVELOPMENTS IN TARGETED DRUG DELIVERY SYSTEM FOR CROSSING BLOOD-BRAIN BARRIER: A REVIEW

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ABSTRACT

Targeted drug delivery systems have been used to deliver drugs in the disease sites of the body. This may reduce potential side effects of drugs and improve their therapeutic effects. Development of brain targeting drug delivery system help in the treatment of disorders related to brain. The most challenging research topic in pharmaceutical sciences is brain targeting drug delivery system. Most of the drugs do not reach brain effectively because of presence of Blood-Brain Barrier (BBB). There are various strategic approaches that have been proposed for brain drug delivery system. There is requirement of optimization balance among the physicochemical and pharmacokinetic properties of Central Nervous System (CNS) acting drugs, to make them penetrable through BBB and show excellent biological activities. This review article overviews the recent researches in the field of strategies to target brain.

Keywords: Blood Brain Barrier, Brain Targeting, Liposomes, Nanotechnology, Nanoparticles.

INTRODUCTION

Brain is one of the active and highly organised delicate organs of body. BBB in the cerebral endothelial cells and the blood- cerebrospinal fluid barrier are its parts. Barrier is an interface between the organ and blood. It consists of endothelial cells. It control/prevent transport of any agent from the stream of blood into the cell (or from the cell to the blood). BBB prevents and create obstacles in the entry of most drugs to brain such as antibiotics, anti-neoplastics, anti-parkinsonian and many other CNS acting drugs¹⁻⁵. Brain parenchymal cells (i.e., neuroglia and neurons) exist within this highly regulated environment and function in coordination. Every brain compartment has a particular set of metabolic enzymes, secretory factors, and receptor proteins that help in maintenance of the homeostatic conditions which is essential for usual functions within the compartment. The localization and expression of different hypothesised drug transporters which are present in the CNS barriers may play a key role in the influx/efflux of xenobiotics. Additionally they also have a significant impact on the general pharmacokinetic/pharmacodynamic of drugs. The cell membranes of astrocytes and microglia (both are parenchyma cells) also act as barriers to drug permeability. To improve bioavailability of CNS acting drug, targeting to diseased tissue is essential. This may also help in improving therapeutic efficacy of drug and reducing side-effects to normal cells. It is hypothesised that there may be increased vascular permeability and extensive angiogenesis of solid tumours. But these conditions may help in the delivery of therapeutic agents to their target site. Consider the example of colloidal carriers, which may act as vehicles for drug and deliver them to target site due to increased vasculature. The transport of drug may be either by passive transport mechanism on the basis of increased vascular permeability, or by active targeting process⁶. Several drugs may as well cross the BBB by active transport process, involving efflux and influx transporters^{7, 8}. In some studies it was suggested that a critical portion of BBB is made up of xenobiotic transporters which obstruct the entry of substrates from the brain to the cerebrospinal fluid. The entry of drug or any toxin into the brain endothelial cells may also be obstructing by these transporters earlier than they get entry into the brain⁹. To treat the brain disorder in an effective manner, the therapy could be provided by administration of drugs in a sustained fashion.

CNS Drug Delivery Barriers

The various barriers to the CNS drug delivery include:

Blood-Brain Barrier (BBB)

It is composed of the tight junctions between the capillary endothelial cells of vessels present in the brain^{6-7, 10-11}. These vessels have the function of perfusion in brain. The capillary walls act as a

continuous lipid bilayer and prevent the entry of microscopic, large, polar or lipid insoluble molecules into the brain except the exchange of gases (such as carbon dioxide, oxygen) and essential nutrients^{2, 6}. The important morphological characteristics of BBB include fenestrations and presence of tight junctions. BBB on molecular level is not homogenous. It may consist of a number of incompletely overlapping zones enclosed in a decidedly anisotropic lipid bilayer⁶. Most drugs, immune cells and microbes may be unable to penetrate through BBB^{2, 3}. By passive diffusion transport mechanism, most of the lipophilic drugs cross the BBB whereas polar drug molecules may be transported across the BBB by active transport mechanism and hence they are generally poor CNS agents^{2, 5, 8, 11}. Transportation of glucose into the CNS occurs through facilitative diffusion by a non-energy-dependent glucose transporter. The epithelial cells of BBB prohibit paracellular diffusion of drugs by forming tight junctions. In BBB, transcellular diffusion transport mechanism may occur through the membranes. Because cells present in the BBB possess pinocytotic vesicles. By an active transfer process, drug may be pumped back into the blood stream using an ATP efflux mechanism, through p-glycoprotein^{1, 6, 12}.

Blood-Cerebrospinal Fluid Barrier (BCB)

BCB act as barrier to drugs entering the CNS. It is formed by the plexus epithelial cells¹³. The epithelial cells have an arrangement in such a manner that it prevents the entry of molecules⁶. There may be existence of substantial inconsistencies between the composition of interstitial fluid and CSF, which suggest the presence of a barrier called as CSF-brain barrier¹⁴.

Blood-Tumour Barrier

Drug targeting to the brain tumour is more difficult⁵. There may be disruption of BBB by brain tumours, locally and nonhomogeneously¹⁵. An increase in tumour permeability results in a potentially large increase in delivery of water-soluble drugs. Permeability is a complex topic in context of brain tumour. Two major variables involve in it are tumour microvessel populations and spatial distribution of the target capillaries. There are three different types of microvessel populations are present in brain tumours. The first type consists of nonfenestrated capillaries. Tumours with this type of microvessel show no enhanced permeability to contrast agents used with CT or MRI. Second type of microvessel population consists of fenestrated capillaries. Tumours consist of these microvessels exhibit enhanced permeability to small molecules. Third type of microvessel population consists of interendothelial gaps. The gaps may be about 1 μ m large. These tumours do not exhibit selective permeability for large molecules⁵.

Factors affecting permeation rate and distribution of drug across BBB

In healthy conditions, the passage of solutes and cells from the blood to the CNS may be limited by BBB. There are numerous factors that affect permeation of drug through BBB, partition coefficient¹⁶, molecular size, ionization constant of drug^{11,17}. The transport of drug across the BBB has a relationship with drug structure and physicochemical properties. But the main important factor is lipophilicity of drug^{8,12}. Increasing lipophilicity of a drug may cause increase in its volume of distribution. Other pharmacokinetic parameters are also be affected by lipophilicity of drug. As lipophilicity of drug increases, the rate of its oxidative metabolism by enzyme cytochromes P450 may increase. Therefore, to improve bioavailability of a drug, there is a balanced effect of lipophilicity on first pass metabolism and membrane permeability¹⁶. Most of the drugs used for treatment of CNS diseases are lipoidal in nature. They may have a log octanol/water partition coefficient between 0.5 and 6.0. The molecular weight of these drugs is between 150 and 500 Da. Because lipid soluble small drug molecules possess molecular weight between the ranges 400-600 Da transported quickly through BBB by lipid mediated transport mechanism^{8, 10, 12}. Charged, highly branched, hydrophilic molecules and molecules having rotatable bonds show a poor BBB penetration¹⁸.

Causes responsible for BBB alteration

Several factors and conditions which induce alteration at BBB are listed in table-1.

Table 1: Table shows the factors that may alter BBB¹⁹

Alteration at BBB	Causing factors/conditions
Formation of pore	Drugs like anti-psychotic and anti-depressant agents
Amplified pinocytosis	Acute hypertension
Paracellular opening	Chemical mediators involved in inflammation such as bradykinin, interleukin-1, serotonin, thrombin, histamine, etc

Drug delivery by Endogeneous Transporters

A number of solute transporters are responsible to carry metabolite into the brain. These metabolites have minimal access to the CNS. Various endogenous transporters present in BBB include the following⁸:

1. Large neutral amino acid carriers: The drugs enter into the CNS by this transporter include melphalan, gabapentin and acivicin.
2. Nucleoside carrier: Abacivir is transported by this transporter.
3. Fatty acid carrier: Through this transporter docosahexanoic acid, taxol and valproic acid are delivered into the CNS.
4. Purine carrier: Example of drug transported by this carrier is oxazolamine COR 3224.
5. Cation-transporter: Diphenhydramine, lidocaine, propranolol, imipramine and mepyrmine are delivered into CNS by cation-transporter.
6. Monocarboxylic acid carrier: This carrier transports the drugs having carboxylic acid functional group. The active metabolites of lovastatin are examples of solute delivered into CNS by monocarboxylic carrier.
7. Hexose carrier: Glycosylated morphine and dehydroascorbic acid are the example of drugs transported by hexose carrier.

Strategies to target the brain

There are several strategies for delivering and targeting drugs to the CNS. The various strategies to enhance drugs uptake into the brain may include the followings:

Enhances permeability of drug through BBB by drug manipulation

There is more penetration of drug if it has low molecular weight¹², unionized at physiological pH, and better lipophilicity. For example heroin crosses the BBB about 100 times faster than morphine due to its lipophilic nature. It may be difficult to deliver hydrophilic drugs into the brain. The hydrophobic analogues of polar drugs may penetrate more readily into the BBB. The two approaches of drug manipulation may include:

(a) Lipophilic analogs

Lipophilicity favours the drug penetration through the BBB²⁰. The drug molecule should have Log P value of approximately 1.5 to 2.5 with an optimum octanol-water partition coefficient¹¹.

(b) Prodrugs

To improve the drug's pharmacokinetic properties prodrug form of them may be used¹⁰. A prodrug consists of a drug covalently linked to an inert chemical moiety¹⁹. The active drug is formed when the attached moiety in prodrug is cleaved by hydrolytic or enzymatic processes²⁰. In prodrugs the attaching chemical moieties should be such that it enhances the lipoidal nature of the drug⁸. Consider the example of various analogues of morphine. BBB is not readily crossed by morphine whereas acetylated product of morphine (heroin) readily traverses the BBB, and on subsequent cleavage of the acetyl groups by hydrolysis yields morphine. Hence in brain there is accumulation of morphine because of its hydrophilicity^{8, 18}. Prodrug formation of a drug improves the brain uptake of drugs⁵. For example, chemical modifications such as esterification of hydroxyl group, amidation of hydroxyl-, or carboxylic- groups of a drug, may increase the lipophilicity of drug and, as a result of which entry into the brain enhances¹⁹.

Intra nasal drug delivery

In current years attention has been given to utilize the nasal route for drug delivery to the brain, make use of the olfactory pathway²¹. Nose provides a direct route to the brain²². The intranasal delivery of proteins has newly emerged as a non-invasive, harmless and successful method to target proteins to the CNS, avoiding the BBB and reducing systemic exposure²³. Entrance to the CNS by the nasal route is improved by increased lipophilicity of transported molecule^{8, 18}.

Use of chemical vectors to cross BBB

The chemic peptides are used to deliver peptide to the brain^{12, 24}. A receptor-specific monoclonal antibody or cationized albumin (transportable vector) when conjugated (avidin-biotin technology facilitate the conjugation) with the therapeutic active compound forms a chemic peptide. Cationic antibodies have positive charge. These antibodies are absorbed through BBB by transcytosis. The advantage of these cationic antibodies includes minimum immunogenicity and fewer side effects to the body²⁵.

Adsorption-mediated transcytosis

In the infected brain tissue, some of the soluble factors also degrade the BBB such as malignant gliomas²⁶. In a study it was suggested that BBB may be disrupted by focused ultrasound pulses plus ultrasound contrast agent. The accurate mechanisms for the disruption are not known, but most probably correlated to the interaction between the microbubbles that make up the ultrasound contrast agent, the ultrasound field, and the microvasculature. The ultrasound frequency may affect the interaction between the ultrasound field and microbubbles⁴. In another study it was suggested that BBB can be disrupted during retroviral-associated neuromyelopathies. The virus responsible for this may be Human T Lymphotropic Virus (HTLV-1). This virus grows in body during Associated Myelopathy/Tropical Spastic Paraparesis (HAM/TSP) disease and is a neurodegenerative disease²⁷.

Brain targeting by monocytes

Monocytes have ability to cross BBB. So, these cells may be used to transport drugs into the CNS. More interestingly the infected brain

may have more population density of monocytes. Drug loaded macrophages can be utilized for transporting agents to the infected sites²⁵.

Redox system

Marcus et al prepared the chemical delivery system (CDS) for zidovudine (AZT). The system was based on redox trapping within the brain. The delivery system was tested in several animal models. On parenteral administration, AZT showed poor uptake by CNS tissues and elimination from systemic circulation was high. But when AZT was administered in the form of an aqueous formulation of the AZT-CDS, it produced a rapid uptake by CNS tissues. After that CDS was converted to corresponding quaternary salt which subsequently produce AZT. The observed concentrations of AZT in brain were 1.75 to 3.3 fold higher²⁸.

Intracerebral implants or infusion and intrathecal drug administration

In recent years, it has become clear that to circumvent the BBB, direct brain injections of drugs may be used¹⁸. The injections are injected either into brain parenchyma or intrathecally or intraventricularly into CSF. This approach may also be utilized to implant a slow-release depot containing the drug into the brain^{8, 18}. Intrathecal drug administration means administration of drug into the subarachnoid space, generally into the lumbar subarachnoid space, while intraventricular administration means injection or infusion into the lateral ventricle of brain⁵. It is a highly invasive method that has need of neurosurgery and unique equipment are the major drawbacks of this approach. Additionally, this approach requires surgical involvement if implanted directly into the brain and the solid implant may damage an area of brain¹⁸. Mainly neurosecretory cells are used for intracerebral implants purpose. A semipermeable membrane encapsulates the cells and allows continuous passive exchange of nutrients and waste products with surrounding extracellular fluid to keep cells alive. The exchange may occur through pores in the encapsulating membrane²⁵.

Intraventricular drug infusion

Drugs could easily be distributed to the surface of the brain via intraventricular drug infusion but not properly delivered to the brain parenchyma²⁵.

Intra-Arterial injection

In this method intra-arterial injection of a therapeutic agent is administered. The principle benefit of this approach is that at the time of intra-arterial injection, the tissues perfuse by that artery get a higher plasma concentration of drug during the primary passage through the circulation. The ideal property for the drug to be administered via this route is that it rapidly crosses the BBB. The problems associated with this method include incidence of local toxicity⁵.

Disrupting BBB

This technique is used widely for CNS drug delivery and involves disruption of the BBB. Exposure to X-irradiation and infusion of solvents such as dimethyl sulfoxide, ethanol²⁹ may disrupt BBB. By inducing pathological conditions such as hypertension, hypoxia³⁰, or ischemia³¹, BBB may also be disrupted²⁹. The effects of alcoholic and hypoglycaemic coma on the BBB permeability are different. The effect depends on the energy metabolism process³². Some of the important techniques for disrupting BBB are:

(i) Osmotic BBB disruption

On peripheral administration the osmotic agents (hypertonic mannitol) cause the opening of the BBB⁸. The osmotic pressure subsequently generated brings a variety of cyto-skeletal alterations that result in the transient opening of the barrier¹⁸. This may be achieved by intracranial infusion of a hypertonic solution of mannitol. The infusion is done into a cerebral artery, usually pursuing by the intra-arterial administration of a drug⁵. It results in opening of the barrier quickly; furthermore it remains open for about 30 min. At the same time if a drug is subsequently

administered through the same cannula, it can freely enter into the CNS. It is hypothesised that the hypertonic solution osmotically pulls water out from the endothelial cells, resulting shrinkage of cell. The shrinkage can cause separation of the extracellular domain (which forms and regulate the tight junctions) of the proteins⁸.

(ii) Ultrasound-induced disruption

Recently it has been studied that induction of hyperthermia may possibly increase delivery of drug to the CNS³³. The main objective to utilise ultrasound is induction of mild hyperthermia^{8, 34}. In a study it was found that ultrasound may have the ability to target a small volume within the tissue and increase BBB transport of hydrophobic drugs³⁵.

Carriers drug delivery system

There are various carriers system used in brain targeting. Some of them include the following:

(i) Liposomal technology

Multivesicular liposomes (size range 0.3-2 μ m) on peripheral administration may retain in brain due to embolization in brain microvasculature¹². To target neuropharmaceuticals to the brain, liposomes conjugating with an antibody may be utilized. Pegylated immunoliposomes are utilized to target brain using specific antibodies. These antibodies bind to endogenous receptors in brain^{8, 18}. The main disadvantage regarding the use of liposomes for BBB targeting is that in the absence of vector-mediated drug delivery there is no significant transport through the BBB³⁶. And another limitation is that when liposomes are intravenously administered, the cells lining of the reticulo-endothelial system uptake the liposomes and the structures rapidly clear from the bloodstream. These problems can be overcome by the use of chimeric peptide technology.

(ii) Nanotechnology

Nanoparticles are referred as submicroscopic colloidal drug delivery systems, either as nanospheres or nanocapsules¹⁸. Nanoparticles for pharmaceutical purposes, may also be defined as solid colloidal particles ranging in size from 1 to 1000nm (1 μ m). They are composed of polymer materials in which the active drug is dissolved, or encapsulated, or to which the drug is attached or adsorbed. After intravenous injection of polysorbate 80-coated poly (butyl cyanoacrylate) (PBCA) nanoparticles containing drug may get transported across the BBB. The extravasation of drug molecules may be fluid transport through the vessel walls³⁷. This transport depends on osmotic and hydrostatic pressure differences between interstitial space and blood. Small drug molecules may have easily diffused across, but large ones via pressure differences are transported. The release of drug from nanoparticle may be decided by the method of preparation and structure of nanoparticle. The surface modified nanoparticles may be uptaken by brain cells and hence, used for targeting BBB. Poly(ethylene glycol) (PEG) is mainly used for preparation of nanoparticles³⁸ as this polymer has less toxicity. There should be presence of active groups on surface of nanoparticles for active targeting of the brain. By receptor-mediated endocytosis or fluid phase endocytosis nanoparticles may usually enter into the cells^{10, 39}. This technology has a wide application in the delivery of drugs and other agents such as oligonucleotides⁵ and genes to CNS. The advantage of nanotechnological approach is that it carries the active form of drug to the brain in nanoparticle size. So the nanotechnology provides the active form of drug to be delivered for maximal efficacy. Nanoengineered systems for crossing the BBB have a greater potential for relatively quickly delivery of drug. Table-2 listed some of the drugs for which nanotechnology have been utilised. Nanogel is newly developed drug delivery systems, for targeted delivery to the brain. Nanogel refers to a preparation pertaining to particles of nanoscale dimension polymer composed of cross-linked nonionic PEG and ionic poly(ethyleneimine) chains¹.

(iii) Microspheres

When therapeutic agents are encapsulated in the form of microspheres, they may provide locally controlled drug release. Microspheres may be able to be implanted in the brain without damaging the functional tissue by stereotaxy, because of their

smaller size. The benefit of implantation is that there is no need of open surgery for implantation. In various studies, it has been established that there exist compatibility of poly (lactide-co-glycolide) microspheres with brain tissues. In literature the drugs

that had been encapsulated utilizing microspheres include idoxuridine, 5-fluorouracil, nerve growth factor, etc.^{56, 57} Table-3 lists some drugs which are delivered through microspherical dosage form.

Table 2: Table shows the various examples of drugs delivered for brain targeting with nanoparticles

Drug	Polymer	Inference	Reference
Paclitaxel	Poly(lactic-co- glycolic acid) (PLGA)	Nanoparticle formulations of paclitaxel prepared with natural emulsifiers have merits over the traditional emulsifiers (polyvinyl-alcohol).	40
Doxorubicin	PBCA	Nanoparticles reach intactly in the brain and showed sustained release of drug.	41
Loperamide	PBCA	Polysorbate 80-coated PBCA nanoparticles loaded with loperamide allowed the delivery of drug to the brain.	42
Hexapeptidedalargin	PBCA	Endothelial cells of blood vessel had shown the phagocytic uptake of the polysorbate 80-coated nanoparticles loaded with dalargin.	43
Tubocurarine	PBCA	Polysorbate 80-coated PBCA nanoparticles of tubocurarine showed the transport of drug into the brain.	44
MRZ 2/576	PBCA	Polysorbate 80-coated MRZ 2/576-PBCA nanoparticles prolonged the duration of the anticonvulsive activity in mice.	45
[3H]-dalargin	PBCA	It was observed that by polysorbate 80- coated nanoparticles there was no opening of the tight junctions of the brain endothelium.	46
Leu-enkephalin dalargin	PBCA	Transport of neuropeptides across the BBB with polysorbate 80.	47
Doxorubicin	PBCA	Nanoparticles may decrease the systemic toxicity of drug and offer the treatment of human glioblastoma.	48
Tubocurarine	PBCA	Nanoparticles coated with polysorbate-80 make possible the entry of drugs across BBB by following intravenous injection.	49
Loperamide	Human serum albumin (HSA)	HSA-nanoparticle conjugated with apolipoprotein E is enabling to transport.	50
Temozolomide	PBCA	Polysorbate-80-coated PBCA nanoparticles showed the higher concentration of drug in the brain.	51
Rivastigmine	Chitosan	Drug released from nanoparticles in diffusion controlled manner.	52
Doxorubicin	PBCA	Toxicity of drug bound to the nanoparticle was lower than that of free drug.	53
Loperamide	PBCA	Drug was transported into the CNS.	54
Estradiol	Chitosan	There was improved drug transportation into the brain.	55

Table 3: Table shows the various examples of drugs delivered for brain targeting with microspheres

Drug	Polymer	Inference	Reference
Paclitaxel	PLGA	Microspheres showed nearly zero- order release and may have ability of implantability. This may have great application in brain tumour chemotherapy.	58
N-Glycosylated recombinant glial cell line-derived neurotrophic factor (GDNF)	PLGA	GDNF-loaded microparticles showed <i>in vivo</i> sustained release of GDNF over up to 5 weeks.	59
Radioiodine-125-iodo-2'-deoxyuridine (125IUdR)	PLGA	Radiolabelled IUdR microspheres showed sustained release of 125IUdR over up to 35 days.	60
Recombinant humaninsulin-like growthfactor-I (rhIGF-I).	PLGA	Integrity of the protein molecules remained as such when evaluated by circular dichroism and SDS- PAGE.	61
Carmustine	Biodegradable polymer	<i>In vitro</i> studies showed that drug release in controlled mechanism.	62
5-Fluorouracil	Poly(methylidene-malonate)	Sustained release of drug in the brain.	63

12. Inhibition of efflux transport: Several ABC transporters are present in BBB. These transporters expel drugs from the CNS. The action of these transporters can be inhibited by two strategies. First include production of inhibitors for efflux transporters. Second strategy is development of drug analogues. Structure-Activity Relationship (SAR) knowledge is necessary for ABC efflux transport mechanism to achieve successful strategy⁸.

FUTURE PROSPECTIVE

It may be feasible to develop a number of systemically effective neuropharmaceuticals that will be effective following systemic administration. Novel strategies based mainly on exploitation of specific transport systems at the BBB are being planned and developed. The advancement for delivering drug or peptide across BBB requires the integration of antibody engineering, pharmacokinetics, and receptor-based drug design. The

development of a successful BBB drug delivery system seems possible. Thus, there is need of development of CNS drug delivery.

CONCLUSIONS

Brain targeting drug delivery system has essential in management of CNS disorders. It can be concluded from this review that by means of the nanotechnology, nasal routes, disruption of BBB, prodrugs, etc. the drug can be delivered across the BBB efficiently. Additional drug exposure to brain can be improved by utilizing modified colloidal particles and liposomes. Because it is assumed that they have prolong blood circulation, which helps in more interaction and penetration into brain endothelial cells. Thus these approaches can be useful in the brain targeting offers a improved clinical efficiency but still there is need of most reliable techniques or methods which high clinical significance and cost effective.

REFERENCES

- Lee G, Dallas S, Hong M, Bendayan R. Drug transporters in the central nervous system: brain barriers and brain parenchyma considerations. *Pharmacol Rev.* 2001; 53: 569-596.
- Yoon CH, Kim SJ, Shin BS, Lee KC, Yoo SD. Rapid screening of blood-brain barrier penetration of drugs using the immobilized artificial membrane phosphatidylcholine column chromatography. *J Biomol Screen.* 2006; 11: 13-20.
- Begley DJ. Delivery of therapeutic agents to the central nervous system. *Pharmacol Ther.* 2004; 104: 29-45.
- Deeken JF, Loscher W. The blood-brain barrier and cancer: transporters, treatment, and Trojan horses. *Clin Cancer Res.* 2007; 13: 1663-1674.
- Kabanov AV, Batrakova EV. New Technologies for Drug Delivery across the Blood Brain Barrier. *Curr Pharm Des.* 2004; 10: 1355-1363.
- Tamai I, Tsuji A. Transporter-mediated permeation of drugs across the blood-brain barrier. *J Pharm Sci.* 2000; 89: 1371-1388.
- Persidsky Y, Ramirez SH, Haorah J, Kanmogne GD. Blood-brain barrier: structural components and function under physiologic and pathologic conditions. *J Neuroimm Pharm.* 2006; 1: 223-236.
- Mcdannold N, Vykhotseva N, Hynynen K. Blood-brain barrier disruption induced by focused ultrasound and circulating preformed microbubbles appears to be characterized by the mechanical index. *Ultrasound Med Biol.* 2008; 34: 834-840.
- Groothuis DR. The blood-brain and blood-tumor barriers: A review of strategies for increasing drug delivery. *Neuro-Oncol.* 2000; 2: 45-59.
- Jeanneret LJ. The targeted delivery of cancer drugs across the blood-brain barrier: chemical modifications of drugs or drug-nanoparticles? *Drug Discov Today.* 2008; 13: 1099-1106.
- Pajouhesh H, Lenz GR. Medicinal chemical properties of successful central nervous system drugs. *NeuroRx.* 2005; 2: 541-553.
- Pardridge WM. CNS drug design based on principles of blood-brain barrier transport. *J Neurochem.* 1998; 70: 1781-1792.
- Bickel U. How to measure drug transport across the blood-brain barrier. *NeuroRx.* 2005; 2: 15-26.
- Pardridge WM. Drug transport in brain via the cerebrospinal fluid. *Fluids Barriers CNS.* 2011; 8: 7.
- Siegal T, Zylber-Katz E. Strategies for increasing drug delivery to the brain: focus on brain lymphoma. *Clin Pharmacokinet.* 2002; 41:171-186.
- Hansch C, Bjorkroth JP, Leo A. Hydrophobicity and central nervous system agents: on the principle of minimal hydrophobicity in drug design. *J Pharm Sci.* 1987; 76: 663-87.
- Mouritsen OG, Jorgensen K. A new look at lipid membrane structure in relation to drug research. *Pharm Res.* 1998; 15: 1507-1519.
- Chopra D, Gulati M, Saluja V, Pathak P, Bansal P. Brain permeable nanoparticles. *Recent Pat CNS Drug Discov.* 2008; 3: 216-225.
- Witt KA, Davis TP. CNS drug delivery: Opioid peptides and the blood-brain barrier. *The AAPS Journal.* 2006; 8: E76-E88.
- Rautio J, Laine K, Gynther M, Savolainen J. Prodrug Approaches for CNS Delivery. *The AAPS Journal.* 2008; 10: 92-102.
- Mathison S, Nagilla R, Kompella UB. Nasal route for direct delivery of solutes to the central nervous system: fact or fiction? *J Drug target.* 1998; 5: 415-441.
- Wolf CD. Nosy neuroprotection: intranasal administration of neuroprotective agents to the brain. *Cryonics Magazine.* 2011; 28: 18-20.
- Malerba F, Paoletti F, Capsoni S, Cattaneo A. Intranasal delivery of therapeutic proteins for neurological diseases. *Expert Opin Drug Deliv.* 2011; 8: 1277-1296.
- Bickel U, Yoshikawa T, Landaw EM, Faull K F, Pardridge WM. Pharmacologic effects in vivo in brain by vector-mediated peptide drug delivery. *Proc Natl Acad Sci.* 1993; 90: 2618-2622.
- Vyas SP, Khar RK. Targeted and controlled drug delivery novel carrier systems. 1st ed. CBS Publishers and distributors; 2007: 487-509.
- Schneider SW, Ludwig T, Tatenhorst L, Braune S, Oberleithner H, Senner V, et al. Glioblastoma cells release factors that disrupt blood-brain barrier features. *Acta Neuropathol.* 2004; 107: 272-6.
- Afonso PV, Ozden S, Prevost MC, Schmitt C, Seilhean D, Weksler B, et al. Human Blood-Brain Barrier Disruption by Retroviral-Infected Lymphocytes: Role of Myosin Light Chain Kinase in Endothelial Tight-Junction Disorganization. *J Immunol.* 2007; 179: 2576-2583.
- Brewster ME, Anderson WR, Webb AI, Pablo LM, Meinsma D, Moreno D, et al. Evaluation of a brain-targeting zidovudine chemical delivery system in dogs. *Antimicrob Agents Chemother.* 1997; 41: 122-128.
- Mayordomo F, Renau-Piqueras J, Megias L, Guerri C, Iborra FJ, Azorin I. Cytochemical and stereological analysis of rat cortical astrocytes during development in primary culture: Effect of prenatal exposure to ethanol. *Int J Dev Biol* 1992; 36: 311-21.
- Schoch HJ, Fischer S, Marti HH. Hypoxia-induced vascular endothelial growth factor expression causes vascular leakage in the brain. *Brain.* 2002; 125: 2549-2557.
- Bauer AT, Burgers HF, Rabie T, Marti HH. Matrix metalloproteinase-9 mediates hypoxia-induced vascular leakage in the brain via tight junction rearrangement. *J Cerebr Blood F Met.* 2010; 30: 837-848.
- Yorulmaz H, Seker FB, Oztas B. The effects of hyperglycemic and alcoholic coma on the blood-brain barrier permeability. *Bosn J Basic Med Sci.* 2011; 11: 108-112.
- Shivers RR, Wijsman JA. Blood-brain barrier permeability during hyperthermia. *Prog Brain Res.* 1998; 115: 413-24.
- Cho CW, Lin Y, Cobb WN. Ultrasound-induced mild hyperthermia as a novel approach to increase drug uptake in brain micro vessel endothelial cells. *Pharm Res.* 2002; 115: 413-24.
- Ka-Yun NG, Cho CW, Henthorn TK, Tanguay RL. Effect of heat preconditioning on the uptake and permeability of r123 in brain microvessel Endothelial cells during mild heat treatment. *J Pharm Sci.* 2004; 93: 896-907.
- Huwylar J, Wu D, Pardridge WM. Brain drug delivery of small molecules using immunoliposomes. *Proc Natl Acad Sci.* 1996; 93: 14164-14169.
- Garcia-Garcia E, Andrieux K, Gil S, Couvreur P. Colloidal carriers and blood-brain barrier (BBB) translocation: A way to deliver drugs to the brain? *Int J Pharm.* 2005; 298: 274-292.
- Kreuter J, Gelperina S. Use of nanoparticles for cerebral cancer. *Tumori.* 2008; 94: 271-277.

39. Olivier JC. Drug Transport to Brain with Targeted Nanoparticles. *NeuroRx*. 2005; 2: 108-119.
40. Feng SS, Mu L, Win KY, Huang G. Nanoparticles of biodegradable polymers for clinical administration of paclitaxel. *Curr Med Chem*. 2004; 11: 413-24.
41. Gulyaev AE, Gelperina SE, Skidan IN, Antropov AS, Kivman GY, Kreuter J. Significant transport of doxorubicin into the brain with polysorbate 80-coated nanoparticles. *Pharm Res*. 1999; 16: 1564-9.
42. Alyautdin RN, Petrov VE, Langer K, Berthold A, Kharkevich DA, Kreuter J. Delivery of loperamide across the blood-brain barrier with polysorbate 80-coated polybutylcyanoacrylate nanoparticles. *Pharm Res*. 1997; 14: 325-8.
43. Kreuter J, Alyautdin RN, Kharkevich DA, Ivanov AA. Passage of peptides through the blood-brain barrier with colloidal polymer particles (nanoparticles). *Brain Res*. 1995; 674:171-4.
44. Alyautdin RN, Tezikov EB, Ramge P, Kharkevich DA, Begley DJ, Kreuter J. Significant entry of tubocurarine into the brain of rats by adsorption to polysorbate 80-coated polybutylcyanoacrylate nanoparticles: an in situ brain perfusion study. *J Microencapsul*. 1998; 15:67-74.
45. Friese A, Seiller E, Quack G, Lorenz B, Kreuter J. Increase of the duration of the anticonvulsive activity of a novel NMDA receptor antagonist using poly (butylcyanoacrylate) nanoparticles as a parenteral controlled release system. *Eur J Pharm Biopharm*. 2000; 49: 103-9.
46. Alyautdin RN, Reichel A, Lobenberg R, Ramge P, Kreuter J, Begley DJ. Interaction of poly(butylcyanoacrylate) nanoparticles with the blood-brain barrier in vivo and in vitro. *J Drug Target*. 2001 Jun;9(3):209-21.
47. Schroeder U, Sommerfeld P, Ulrich S, Sabel BA. Nanoparticle technology for delivery of drugs across the blood-brain barrier. *J Pharm Sci*. 1998; 87: 1305-7.
48. Steiniger SCJ, Kreuter J, Khalansky AS, Skidan IN, Bobruskin AI, Smirnova ZS, et al. Chemotherapy of glioblastoma in rats using doxorubicin-loaded nanoparticles. *Int J Cancer*. 2004; 109: 759-767.
49. Kreuter J. Transport of Drugs Across the Blood-Brain Barrier by Nanoparticles. *Curr Med Chem: Cent Nerv Syst Agents*. 2002; 2: 241-249.
50. Michaelis K, Hoffmann MM, Dreis S, Herbert E, Alyautdin RN, Michaelis M, et al. Covalent Linkage of Apolipoprotein E to Albumin Nanoparticles Strongly Enhances Drug Transport into the Brain. *J Pharmacol Exp Ther*. 2006; 317: 1246-1253.
51. Tian XH, Lin XN, Wei F, Feng W, Huang ZC, Wang P, et al. Enhanced brain targeting of temozolomide in polysorbate-80 coated polybutylcyanoacrylate nanoparticles. *Int J Nanomedicine*. 2011; 6: 445-452.
52. Wilson B, Samanta MK, Muthu MS, Vinothapooshan G. Design and evaluation of chitosan nanoparticles as novel drug carrier for the delivery of rivastigmine to treat Alzheimer's disease. *Therapeutic Delivery*. 2011; 2: 599-609.
53. Gelperina SE, Khalansky AS, Skidan IN, Smirnova ZS, Bobruskin AI, Severin SE, et al. Toxicological studies of doxorubicin bound to polysorbate 80-coated poly(butyl cyanoacrylate) nanoparticles in healthy rats and rats with intracranial glioblastoma. *Toxicol Lett*. 2002; 126: 131-141.
54. Kreuter J, Shamenkov D, Petrov V, Ramge P, Cychutek K, Koch-Brandt C, et al. Apolipoprotein-mediated Transport of Nanoparticle-bound Drugs Across the Blood-Brain Barrier. *J Drug Target*. 2002;10: 317-325.
55. Wang X, Chi N, Tang X. Preparation of estradiol chitosan nanoparticles for improving nasal absorption and brain targeting. *Eur J Pharm Biopharm*. 2008; 70: 735-740.
56. Benoit JP, Faisant N, Venier-Julienne MC, Menei P. Development of microspheres for neurological disorders: From basics to clinical applications. *J Control Release*. 2000; 65: 285-296.
57. Menei P, Montero-Menei C, Venier MC, Benoit JP. Drug delivery into the brain using poly(lactide-co-glycolide) microspheres. *Expert Opin Drug Deliv*. 2005; 2: 363-76.
58. Ranganath SH, Kee I, Krantz WB, Chow PKH, Wang CH. Hydrogel matrix entrapping PLGA-paclitaxel microspheres: drug delivery with near zero-order release and implantability advantages for malignant brain tumour chemotherapy. *Pharm Res*. 2009; 26: 2101-2114.
59. Garbayo E, Menei CNM, Ansorena E, Lanciego JL, Aymerich MS, Prieto MJB. Effective GDNF brain delivery using microspheres-A promising strategy for Parkinson's disease. *J Controlled Release*. 2009; 135: 119-126.
60. Reza MS, Whateley TL. Iodo-2'-deoxyuridine (IUdR) and 125IUdR loaded biodegradable microspheres for controlled delivery to the brain. *J Microencapsul* 1998; 15: 789-801.
61. Carrascosa C, Aleman IT, Lopez CL, Carro E, Espejo L, Torrado S, et al. Microspheres containing insulin-like growth factor I for treatment of chronic neurodegeneration. *Biomaterials*. 2004; 25: 707-714.
62. Li KW, Dang W, Tyler BM, Troiano G, Tihan T, Brem H, Walter KA. Polylactofate microspheres for Paclitaxel delivery to central nervous system malignancies. *Clin Cancer Res*. 2003; 9:3441-7.
63. Fournier E, Passirani C, Menei CM, Colin N, Breton P, Sagodira S, et al. Therapeutic effectiveness of novel 5-fluorouracil-loaded poly(methylidene malonate 2.1.2)-based microspheres on F98 glioma-bearing rats. *Cancer*. 2003; 97: 2822-2829.