

BRAIN TARGETED DRUG DELIVERY SYSTEMVarsha Z. Gite^{1*}, Vaishali K. Ghume² and Dr. Ramanlal N. Kachave¹¹Department of Pharmaceutical Quality Assurance, Amrutvahini College of Pharmacy, Sangamner-422608.²Department of Pharmaceutics, Dr. Vitthalrao Vikhe Patil Foundation's College of Pharmacy, Viladghat, Ahmednagar-414111.***Corresponding Author: Varsha Z. Gite**

Department of Pharmaceutical Quality Assurance, Amrutvahini College of Pharmacy, Sangamner-422608.

Article Received on 24/08/2020

Article Revised on 14/09/2020

Article Accepted on 04/10/2020

ABSTRACT

Targeted drug delivery is a method of delivering medication to a patient in a manner that increases the concentration of the medication in some parts of the body relative to others. Targeted drug delivery is a method of delivering medication to a patient in a manner that increases the concentration of the medication in some parts of the body relative to others. Targeted drug delivery seeks to concentrate the medication in the tissues of interest while reducing the relative concentration of the medication in the remaining tissues. The inherent advantage of this technique has been the reduction in dose & side effect of the drug. The brain is a delicate organ, and evolution built very efficient ways to protect it. The brain is shielded against potentially toxic substances by the presence of three barrier systems: the blood brain barrier (BBB), the blood cerebrospinal fluid barrier (BCSFB) and the blood tumor barrier (BTB). General methods that can enhance drug delivery to the brain are of great interest in treatment of various CNS diseases. By localizing drugs at their desired site of action one can reduce toxicity and increase treatment efficiency. In response to the insufficiency in conventional delivery mechanisms, aggressive research efforts have recently focused on the development of new strategies to more effectively deliver drug molecules to the CNS. This review intends to detail the barriers in brain targeted drug delivery system, mechanism of transfer of drug via BBB, problems faced in brain targeted drug delivery, various approaches of brain targeted drug delivery system and its applications in treatment of various CNS diseases.

KEYWORDS: Brain targeted drug delivery, Blood cerebrospinal fluid barrier (BCSFB), Nanoparticles, Recent drug delivery.

INTRODUCTION

Drug delivery refers to approaches, formulations technologies and systems for transporting a pharmaceutical compound in the body as needed to safely achieve its desired therapeutic effect. It may involve scientific site-targeting within the body or it might involve facilitating systemic pharmacokinetics, in any case it is typically concerned with both quantity and duration of drug presence. Drug delivery technologies modify drug release profile, absorption, distribution and elimination for the benefit of improving product efficacy and safety, as well as patient convenience. Current efforts in the area of drug delivery include the development of targeted delivery in which the drug is only active in the target area of the body. The goal of a targeted drug delivery system is to prolong, localize, target and have a protected drug interaction with the diseased tissue. Targeted drug delivery system have been developed to optimize regenerative techniques. This helps maintain the required plasma and tissue drug levels in the body, thereby preventing any damage to the healthy tissue via the drug. Barriers in Brain Targeted

Drug Delivery the failure of systemically delivered drugs to effectively treat many CNS diseases can be rationalized by considering a number of barriers that inhibit drug delivery to the CNS. There are physical barriers that separate the brain extracellular fluid from the blood.

Barriers in brain targeted drug delivery

The failure of systemically delivered drugs to effectively treat many CNS diseases can be rationalized by considering a number of barriers that inhibit drug delivery to the CNS. There are physical barriers that separate the brain extracellular fluid from the blood.

1. Blood-Brain Barrier
2. Blood-Cerebrospinal Fluid Barrier
3. Blood-Tumor Barrier

1) Blood - Brain Barrier (BBB)

The blood – brain barrier (BBB) is a highly selective permeability barrier that separates the circulating blood from the brain extracellular fluid in the central nervous system. The blood brain barrier is formed by capillary

endothelial cells, which are connected by tight junctions with an extremely high electrical resistivity of at least 0.1 μm . The blood – brain barrier allows the passage of water, some gases and lipid soluble molecules by passive diffusion, as well as the selective transport of molecules such as glucose and amino acids that are crucial for neural function. On the other hand, the blood – brain barrier may prevent the entry of lipophilic potential neurotoxins by way of an active transport mechanism mediated by Pglycoprotein. Astrocytes are necessary to create the blood – brain barrier. It is estimated that more than 98% of small molecular weight drugs and practically 100% of large molecular weight drugs (mainly peptides and proteins) developed for CNS pathologies do not readily cross the BBB. Endothelial cells restrict the diffusion of microscopic objects (e.g. bacteria) and large or hydrophilic molecules into the cerebrospinal fluid (CSF), while allowing the diffusion of small hydrophobic molecules (e.g. O₂, CO₂, hormones, etc.)

Functions of BBB

The blood–brain barrier acts very effectively to protect the brain from many common bacterial infections. Thus, infections of the brain are very rare. Infections of the brain that do occur are often very serious and difficult to treat. Antibodies are too large to cross the blood - brain barrier and only certain antibiotics are able to pass. In some cases the drug has to be administered directly into the cerebrospinal fluid. However, drugs delivered directly to the CSF do not effectively penetrate into the brain tissue itself, possibly due to the tortuous nature of the interstitial space in the brain. The blood – brain barrier becomes more permeable during inflammation. This allows some antibiotics and phagocytes to move across the BBB. However, this also allows bacteria and viruses to infiltrate the BBB. The functions of BBB are shown in Fig. 1.

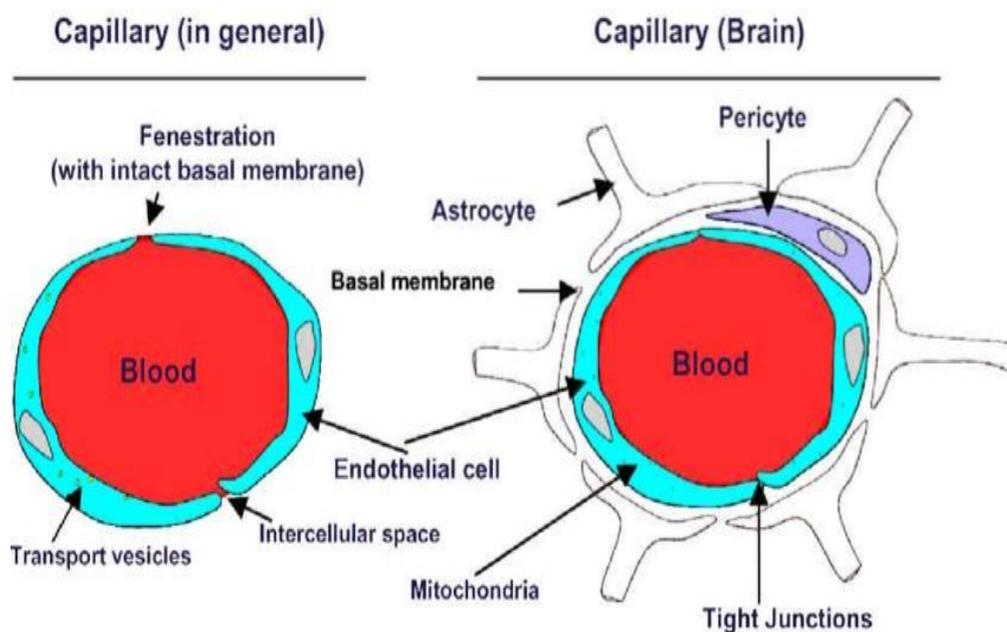


Fig. 1: Blood-Brain-Barrier.

2) Blood - Cerebrospinal Fluid Barrier (BCSFB)

The second barrier, located at the choroids plexus, is represented by the blood-cerebrospinal fluid barrier that separates the blood from the cerebrospinal fluid (CSF) which, in turn, runs in the subarachnoid space surrounding the brain. However, this barrier is not considered as a main route for the uptake of drugs since its surface area is 5000-fold smaller than that of the BBB. CSF can exchange molecules with the interstitial fluid of the brain parenchyma, the passage of blood-borne molecules into the CSF is also carefully regulated by the BCB. On the external surface of the brain the epidermal cells fold over onto themselves to form a double layered structure, which lies between the dura and pia, this is called the archnoid membrane. Within the

double layer is the subarchnoid space, which participates in CSF drainage. Passage of substances from the blood through the archnoid membrane is prevented by tight junction. The functions of blood – cerebrospinal fluid barrier are shown in Fig. 2.

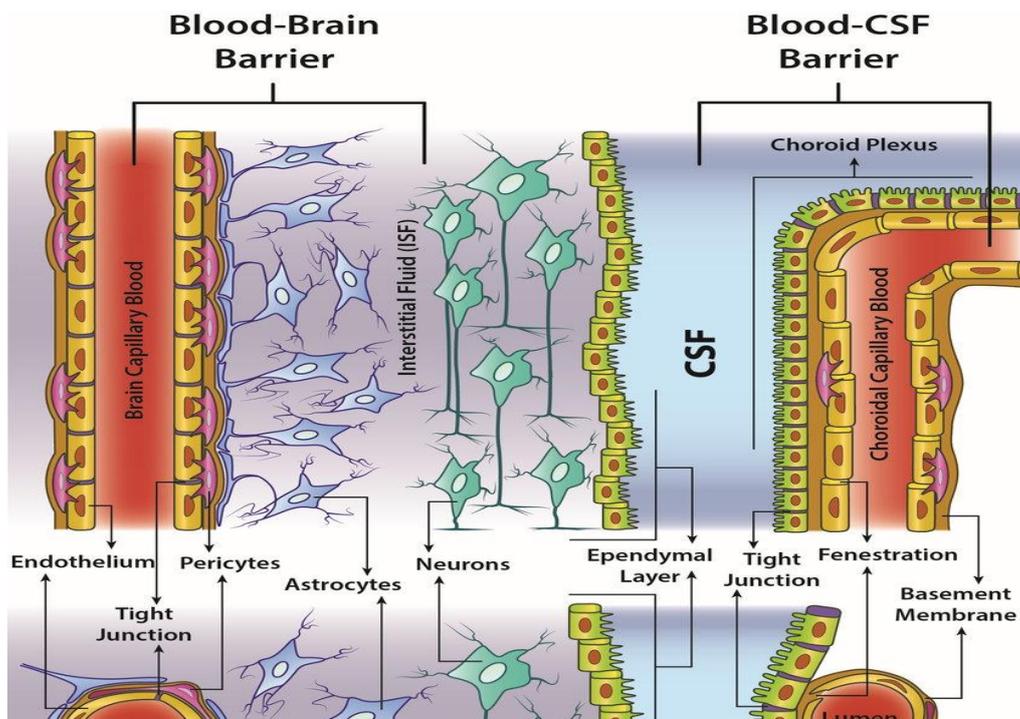


Fig. 2: Blood – Cerebrospinal Fluid Barrier.

3) Blood - Tumor Barrier

Intracranial drug delivery is even more challenging when the target is a CNS tumor. For example, even when primary and secondary systemic tumors respond to chemotherapeutic agents delivered via the cardiovascular system, intracranial metastases often continue to grow. In CNS malignancies where the BBB is significantly compromised, a variety of physiological barriers common to all solid tumors inhibit drug delivery via the cardiovascular system. Furthermore, as a tumor grows large, the vascular surface area decreases, leading to a

reduction in transvascular exchange of blood-borne molecules. At the same time, intracapillary distance increases, leading to a greater diffusional requirement for drug delivery to neoplastic cells and due to high interstitial tumor pressure and the associated peritumoral edema leads to increase in hydrostatic pressure in the normal brain parenchyma adjacent to the tumor. As a result, the brain may be less permeable to drugs than normal brain endothelium. The functions of blood – tumor barrier are shown in Fig. 3.

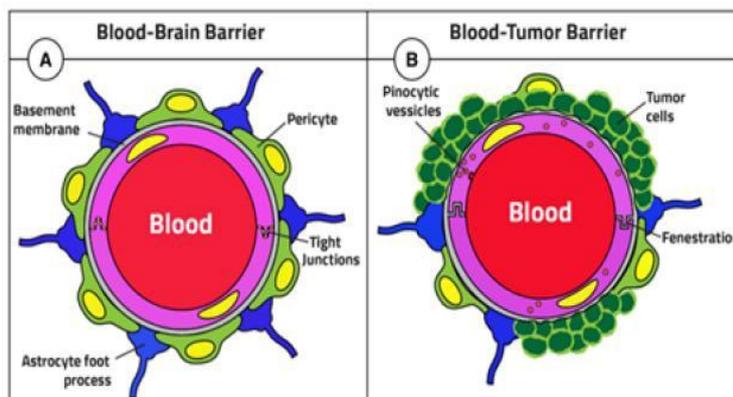


Fig. 3: Comparison between blood-brain-barrier and blood-tumor-barrier.

Mechanisms of Transfer of Drug via BBB

Substances are able to cross the vascular BBB by a variety of mechanisms like – transmembrane diffusion, saturable transport, adsorptive endocytosis and extracellular pathways.

1) Transmembrane Diffusion

Most drugs cross the BBB by transmembrane diffusion. This is a non-saturable mechanism that depend on the drug melding into the cell membrane. A low molecular weight and high degree of lipid solubility favour crossing by this mechanism. However, a drug taken up by the membranes that form the BBB must then partition into

the aqueous environment of the brain's interstitial fluid to exert an effect. As a result, a substance that is too lipid soluble can be sequestered by the capillary bed and not reach the cells behind the BBB. The percent of administered drug entering the brain is determined by both the rate of transport across the BBB and the amount of drug presented to the brain. The largest substance found to cross the BBB by the mechanism of transmembrane diffusion is cytokine - induced neutrophil chemoattractant - 1 (CINC-1) at 7,800 Dalton.

2) Saturable Transport System

Some drugs or substances used for drug like effects cross the BBB by use of saturable transport system. L - DOPA and caffeine are examples. The uptake rate across the BBB for an endogenous ligand of a transporter is roughly about 10 times higher than would be expected if it crossed by transmembrane diffusion. Additionally, many of the transporter for regulatory molecules, such as peptides and proteins, are taken up selectively by specific brain regions. The rate at which saturable system transport their ligands across the BBB is often regulated. For flow-dependent substances such as glucose, transport rate is a function of cerebral blood flow. For substances that are more slowly transported, a variety of agent have been found to alter transport. For example, leucine regulates the transport rate of peptide transport system - 1 (PTS-1).

Problems faced in brain targeted drug delivery

The first of these is that a lot of times even if a compound transverses the barrier it does not do it in a way that the drug is in a therapeutically relevant concentration. This can have lots of causes, the simplest being that the way the drug was produced only allows a small amount to pass through the barrier. Another cause of this would be the binding to other proteins in the body rendering the drug ineffective to either be therapeutically active or able to pass through the barrier with the adhered protein. Another problem that must be accounted for is the presence of enzymes in the brain tissue that could render the drug inactive. All of these are problems that must be addressed and accounted for in trying to deliver effective drug solutions to the brain tissue.

Approaches for brain targeted drug delivery

Basically, two methods have been described to actively enhance drug delivery to the brain after systemic administration: either opening/disruption of the neuroprotective BBB by osmotic imbalance, ultrasound or vasoactive compounds (e.g., bradykinin or P-glycoprotein inhibitors). While the first method has the disadvantage that those neurons may be damaged (semi)-permanently due to unwanted blood components entering the brain. As a third alternative (using a combination of aspects of both methods), positive charge has also been applied to compounds or drug carriers to effectively enhance the absorptive-mediated transport across the BBB. However, a beneficial therapeutic window of this

basically toxic transport mechanism has thus far not been established.

To overcome the multitude of barriers restricting CNS drug delivery of potential therapeutic agents, numerous drug delivery strategies have been developed. These strategies generally fall into one or more of the following categories: invasive, non-invasive or miscellaneous techniques.

A. Invasive

- Intracerebroventricular (ICV) infusion
- Convection-enhanced delivery (CED)
- Intra-cerebral injection or implants
- Disruption of the BBB.

B. Non-invasive

- Chemical techniques
 - a. Prodrug
- Colloidal Techniques
 - a. Nanoparticles
 - b. Liposomes.

C. Miscellaneous techniques

- a. Intranasal delivery

Invasive Approach

Drugs can be delivered to the brain by first drilling the hole in the head, and then implant is placed by intra-cerebral (IC) or infusion is given by intra-cerebroventricular (ICV). An advantage of this route is that a wide range of compound and formulation can be considered for ICV or IC administration. Thus both large and small molecules can be delivered, either alone or in various polymer formulations to achieve sustained release. Different invasive approaches are:

1. Intra-cerebro-ventricular infusion
2. Convection-enhanced delivery
3. Intracerebral Implants
4. Disruption of the BBB

1. Intra-cerebro-ventricular infusion (ICV)

It has been reported that the concentration of a drug in the brain is only 1–2% of the CSF concentration at just 1–2 mm from the surface. Drugs could easily be distributed to the surface of the brain via intra ventricular drug infusion but not properly delivered to the brain parenchyma. Pharmacologic effects can be seen after ICV administration, if the target receptors of the drug are located near the ependymal surface of the brain.

Limitations

The diffusion of the drug in the brain parenchyma is very low. Unless the target is close to the ventricles it is not an efficient method of drug delivery.

Example: Glycopeptide and an aminoglycoside antibiotics used in meningitis.

2. Convection-enhanced delivery (CED)

The general principle of CED involves the stereotactically guided insertion of a small-caliber catheter into the brain parenchyma. Through this catheter, infusate is actively pumped into the brain parenchyma and penetrates in the interstitial space. The infusion is continued for several days and the catheters are removed at the bedside. CED has been shown in laboratory experiments to deliver high molecular weight proteins 2 cm from the injection site in the brain parenchyma after as little as 2 hr of continuous infusion.

Limitations

Some areas of the brain are difficult to saturate fully with infusate, particularly infiltrated tissues surrounding a cavity. Proper drug delivery depends on the placement of catheters.

3. Intra-cerebral injection or use of implants

Delivery of drugs directly into the brain parenchymal space, the drugs can be administered by:

- Direct injection via intrathecal catheter.
- Control release matrices.
- Microencapsulated chemicals.

The basic mechanism is diffusion. Useful in the treatment of different CNS diseases e.g. brain tumor, Parkinson's Disease etc.

Limitations

Distribution in the brain by diffusion decreases exponentially with distance. The injection site has to be very precisely mapped to get efficacy and overcome the problem associated with diffusion of drugs in the brain parenchyma.

4. Disruption of the 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, ethanol^[16] may disrupt BBB. By inducing pathological conditions such as hypertension, hypoxia, or ischemia. BBB may also be disrupted. The effects of alcoholic and hypoglycaemic coma on the BBB permeability are different. The effect depends on the energy metabolism process. Some of the important techniques for disrupting BBB are:

• Osmotic disruption

The osmotic shock causes endothelial cells to shrink, thereby disrupting the tight junctions. Intracarotid administration of a hypertonic mannitol solution with subsequent administration of drugs can increase drug concentration in brain and tumour tissue to reach therapeutic concentration.

• MRI-guided focused ultrasound BBB disruption technique

Ultrasound has been shown to be capable of BBB disruption. The combination of microbubbles (performed microbubbles of ultrasound contrast agent, optison, with

a diameter of 2-6 μm which is injected into the blood stream before exposures to ultrasound). This technique has been shown to increase the distribution of Herceptin in brain tissue by 50% in a mice model.

Limitations of Invasive approach

- All these approaches are relatively costly, require anaesthesia and hospitalization.
- These techniques may enhance tumour dissemination after successful disruption of the BBB.
- Neurons may be damaged permanently from unwanted blood components entering the brain.

B. Non-invasive approaches

A variety of non-invasive brain drug delivery methods have been investigated, that make use of the brain blood vessel network for drug distribution. Non-invasive techniques usually depend upon drug manipulations which may include alterations as:

- Chemical techniques
 - a. Prodrug
 - Colloidal Techniques
 - a. Nanoparticles
 - b. Liposomes

• Chemical technique

a. Prodrug

Prodrug which is lipid soluble and can cross the BBB. Prodrug is metabolized within the brain and converted to the parent drug. Apart from the diacetylation of morphine to create heroin. Prodrugs are pharmacologically inactive compounds. Chemical change is usually designed to improve some physicochemical property such as solubility and membrane permeability. A prodrug consists of a drug covalently linked to an inert chemical moiety. The active drug is formed when the attached moiety in prodrug is cleaved by hydrolytic or enzymatic processes. In prodrugs the attaching chemical moieties should be such that it enhances the lipoidal nature of the drug.

Examples: levodopa, GABA, Niflumic acid, valproate.

Limitations of the prodrug

- Approach are the adverse pharmacokinetics.
- The increased molecular weight of the drug that follow from lipidation.

Colloidal Techniques

The vesicular systems are highly ordered assemblies of one or several concentric lipid bilayer formed, when certain amphiphilic building blocks are confronted with water. Drug carrier can be engineered to slowly degrade, react to stimuli and be site-specific. The ultimate aim is to control degradation of drug and loss, prevention of harmful side effects and increase the availability of the drug at the disease site. Vesicular drug delivery system has some of the advantages like:

- Prolong the existence of the drug in systemic circulation, and perhaps, reduces the toxicity if selective uptake can be achieved due to the delivery of drug directly to the site of infection.
- Improves the bioavailability especially in the case of poorly soluble drugs.
- Both hydrophilic and lipophilic drugs can be incorporated.
- Delays elimination of rapidly metabolizable drugs and thus function as sustained release systems.

A. Nanoparticles

Nanoparticles (NPs) are solid colloidal particles made up of polymeric materials ranging in size from 1-1000 nm. This definition includes both nanocapsules, with a core-shell structure (a reservoir system), and nanospheres (a matrix system). NPs are used as carrier systems in which the drug is dissolved, entrapped, encapsulated, adsorbed or chemically linked to the surface. Nanoparticle systems in CNS targeted drug therapy provide better penetration of therapeutic and diagnostic agents, and a reduced risk in comparison to conventional treatments. By using nanotechnology it is possible to deliver the drug to the targeted tissue across the BBB, release the drug at a controlled rate, and avoid degradation processes. Reduction of toxicity to peripheral organs and biodegradability can also be achieved with these systems.

Mechanism for transport

The mechanism for transport of lipoprotein to be endocytosis via the Low Density Lipoprotein (LDL) receptor of the endothelial cells after adsorption of lipoproteins from blood plasma to the nanoparticles. It is suggested that the recognition and interaction with lipoprotein receptors on brain capillary endothelial cells is responsible for the brain uptake of the drug.

Advantages of using nanoparticles for CNS targeted drug delivery

- Nanoparticles protect drugs against chemical and enzymatic degradation.
- Due to their small size nanoparticles penetrate into even small capillaries and are taken up within cells, allowing an efficient drug accumulation at the targeted sites in the body. Degradation.
- The use of biodegradable materials for nanoparticle preparation, allows sustained drug release at the targeted site after injection over a period of days or even weeks.
- They are also able to reduce side effects of some active drugs.

Limitations of using nanoparticles for CNS targeted drug delivery

- Their small size and large surface area can lead to particle-particle aggregation, making physical handling of nanoparticles difficult in liquid and dry forms.

- In addition, small particles size and large surface area readily result in limited drug loading and burst release.

Example

Radiolabelled polyethylene glycol coated hexadecylcyanoacrylate nanospheres targeted and accumulated in a rat gliosarcoma. However, this method is not yet ready for clinical trials due to the accumulation of the nanospheres in surrounding healthy tissues.

B. Liposomes

Liposomes or lipid based vesicles are microscopic (unilamellar or multilamellar) vesicles that are formed as a result of self-assembly of phospholipids in an aqueous media resulting in closed bilayered structures. Since lipid bilayered membrane encloses an aqueous core, both water and lipid soluble drugs can be successfully entrapped into the liposomes. Lipid soluble or lipophilic drugs get entrapped within the bilayered membrane whereas water soluble or hydrophilic drugs get entrapped in the central aqueous core of the vesicles. Liposomes are potential carrier for controlled drug release of tumours therapeutic agents and antibiotic, for gene and antisense therapy through nucleic acid sequence delivery, immunization through antigen delivery and for anti-Parkinson's.^[24]

Advantages

- Liposomes supply both a lipophilic environment and aqueous "milieu interne" in one system and are therefore suitable for delivery of hydrophobic, amphipathic and hydrophilic drugs and agents.
- Liposomes could encapsulate not only small molecules but also macromolecules like superoxide dismutase, haemoglobin, erythropoietin, interleukin-2 and interferon-g.
- Liposomes reduced toxicity and increased stability of entrapped drug via encapsulation (eg. Amphotericin B, Taxol).
- Liposomes help to reduce exposure of sensitive tissues to toxic drugs.
- Alter the pharmacokinetic and pharmacodynamic property of drugs (reduced elimination, increased circulation life time).

Limitations

- High production cost
- Leakage and fusion of encapsulated drug / molecules.
- Sometimes phospholipid undergoes oxidation and hydrolysis
- Short half-life
- Low solubility
- Less stability

C. Miscellaneous techniques

Intranasal drug delivery

In nasal drug delivery system drug are delivered in nasal cavity. The nasal mucosa used for delivering the drugs for CNS disorders and systemic administration of analgesics, sedatives, hormones, cardiovascular drugs, and vaccines, corticosteroid hormones.

Mechanism for transport

There are two mechanisms underlying the direct nose to brain drug delivery:

- a. Intracellular transport mediated route
- b. Extracellular transport mediated routes.

The intracellular transport mediated route is a relatively slow process, taking hours for intranasally administered substances to reach the olfactory bulb. Extracellular transport mediated routes is rapid.

In the first extracellular transport based route intranasally administered substances could first cross the gap between the olfactory neurons in the olfactory epithelium which are subsequently transported into the olfactory bulb. In the second extracellular transport based route, intranasally administered substances may be transported along trigeminal nerve to bypass BBB. After reaching the olfactory bulb the drug enters into other regions of brain by diffusion, which may also be facilitated by perivascular pump.

Advantages of intranasal drug delivery

- Rapid drug absorption via highly vascularized mucosa.
- Drugs which cannot be absorbed orally may be delivered to the systemic circulation through nasal drug delivery system.
- Convenient route when compared with parenteral route for long term therapy.
- Bioavailability of larger drug molecules can be improved by means of absorption enhancer or other approach.
- Self-administration
- Large nasal mucosal surface area for dose absorption.

Disadvantages of intranasal drug delivery

- Some drugs may cause irritation to the nasal mucosa
- Nasal congestion due to cold or allergies may interfere with absorption of drug.
- Drug delivery is expected to decrease with increasing molecular weight.

Recent Advances

- 1) Dendrimers
 - 2) Scaffolds
 - 3) Lipoplexes and Polyplexes
 - 4) Polyanhydrides
 - 5) Modified nanoparticles
- Multifunctional nanoparticles

- Magnetic nanoparticles.

6) Receptor-mediated transport (RMT)

- Monoclonal antibody (MAb) molecular Trojan horses (MTH)
- Trojan horse liposomes for CNS gene therapy
- In vivo brain imaging of gene expression

7) Transporter-independent mechanisms to circumvent the BBB

- Convection-enhanced drug delivery (CED)
- Ultrasound-mediated BBB opening.
- Bradykinin receptor-mediated BBB opening

Recent Advances in Brain Targeting

1. Dendrimers

Dendrimers are branched polymers, reminding the structure of a tree. A dendrimer is typically symmetric around the core, and when sufficiently extended it often adopts a spheroidal three-dimensional morphology in water. A central core can be recognized in their structure with at least two identical chemical functionalities; starting from these groups, repeated units of other molecules can originate, having at least one junction of branching. These repetitions of chains and branching result in a series of radially concentric layers with increased crowding. The structure is therefore tightly packed in the periphery and loosely packed in the core, leaving spaces which play a key role in the drug entrapping ability of dendrimers. Poly(amidoamine), or PAMAM, is perhaps the most well-known molecule for synthesis of dendrimers. The core of PAMAM is a diamine (commonly ethylene diamine), which is reacted with methyl acrylate and then with another ethylene diamine to make the generation PAMAM. Successive reactions create higher generations. Albertazzi *et al.* showed that functionalization of PAMAM dendrimers has a dramatic effect on their ability to diffuse in the CNS tissue *in vivo* and penetrate living neurons as shown after intraparenchymal or intraventricular injections showed that systemically administered polyamidoamine dendrimers localize in activated microglia and astrocytes in the brain of newborn rabbits with cerebral palsy, providing opportunities for clinical translation in the treatment of neuro-inflammatory disorders in humans.

2. Scaffolds

Scaffolds are implantable and can be used to treat a variety of conditions associated with brain injury and diseases, for delivering drugs to treat neurological diseases such as Parkinson's disease and Alzheimer's disease. Delivery of therapeutic agents from scaffolds potentially helps to limit the damage to neurons while helping to preserve their function.

Although scaffolds have wide range of potential applications for neural tissue engineering, the brain presents similar obstacles when designing scaffolds. Considerations include.

- Minimizing cell death and inflammation after implantation of scaffolds, by choosing biocompatible materials.
- Controlling drug release over an appropriate time period to prevent multiple surgeries or injections.
- Making the whole process minimally invasive to preserve the integrity of the BBB.
- Scaffolds should be small and minimally invasive.

• Scaffolds for brain drug delivery

Woerly S *et al.* studied efficacy of poly (hydroxyl phenyl methacrylate) [PHPMA] and PHEMA scaffolds containing glucosamine or N-acetyl glucosamine groups when implanted between the septum and the hippocampus in a fimbria-fornix lesion cavity. It was found that PHEMA scaffolds showed markedly less infiltration of connective tissue than PHPMA.

3. Lipoplexes and Polyplexes

In order to improve the delivery of the new DNA into the cell, the DNA must be protected from damage and its entry into the cell must be facilitated. Lipoplexes and polyplexes serve this purpose. Both have the ability to protect the DNA from undesirable degradation during the transfection process. Plasmid DNA can be covered with lipids in an organized structure like a micelle or a liposome. When the organized structure is complexed with DNA it is called a lipoplex. There are three types of lipids.

- Anionic (negatively charged)
- Neutral
- Cationic (positively charged)

Initially, anionic and neutral lipids were used for production of lipoplexes for synthetic vectors. Lipoplexes are compatible with body fluids, there is a possibility of adapting them to be tissue specific and there is very little toxicity associated with them. The only disadvantage is that they are complicated and time consuming to produce.

Thus, attention was turned to the cationic versions. Cationic lipids, because of the positive charge on them, have ability to naturally complex with the negatively charged DNA. Also because of the positive charge, they interact with cell membrane, endocytosis of the lipoplex occurs and the DNA is released into the cytoplasm. The cationic lipids also protect against degradation of the DNA by the cell. Cationic lipids include Dioleoyl phosphoethanolamine (DOPE), dioleoyloxy trimethylammonium chloride (DOTMA). Complexes of polymers with DNA are called polyplexes. Most polyplexes consist of cationic polymers and they are formed by ionic interactions. One large difference between the methods of action of lipoplexes and polyplexes is that polyplexes cannot release the associated DNA into the cytoplasm. For release to take place cotransfection with endosome-lytic agents which lyse the endosome must occur and the polyplex enters the cell. However this is not always the case, polymers

such as Polyethylenimine (PEI), chitosan and trimethyl chitosan have their own method of endosome disruption. PEI forms dense nanosized particulates and complexes with negatively charged DNA by electrostatic interactions. The PEI/DNA complex takes overall positive charge and interacts with negatively charged components of cell membranes and enter cells by endocytosis. The PEI/DNA complex enters the cells by non-specific adsorption mediated endocytosis. Upon endocytosis, PEI undergoes further protonation as the endosomal compartment acidifies. Protonation of PEI occurs by capturing protons, which is called as Proton Sponge mechanism. This leads to osmotic swelling and subsequent endosomal disruption. Zhanta *et al.* demonstrated that only one nuclear localization signal (NLS) peptide covalently linked to DNA could increase the transfection efficacy following an intracellular injection of the plasmid.

4. Polyanhydrides in brain tumor

Brain tumor Glioblastoma multiforme (GBM) accounts for about 80% of adult primary brain tumors and are usually found in the cerebral hemispheres. Many of the anti-cancer drugs have large molecular structures, ionic charge or are hydrophilic and thus are unable to cross the BBB, and intolerably high systemic levels are required to achieve the therapeutic doses within the CNS.

Use of polyanhydrides for direct localized delivery is one of the simplest approaches.

- Gliadel® wafers are approved by FDA in 1996. These are commercially available and one of the most successful delivery systems made of polyanhydrides. These are made up of Poly (Carboxy phenoxy) Propane:Sebacic Acid, in the ratio of 20:80. These are dimesized wafers that deliver BCNU (Carmustine), for the treatment of GBM. The wafers are directly placed in the resection cavity after tumor excision. After the debulking of the tumor, upto eight Gliadel® wafers are implanted for the treatment along the walls of tumor cavity. It was found that Gliadel® wafers release drug for about 5 days.
- Walter *et al.* have formulated polymeric disc of Poly (Carboxy phenoxy) Propane:Sebacic Acid, in the ratio of 20:80 with 20-40% of Taxol loading, and found that intact Taxol was released up to 1000 hrs, *in vitro*. They have reported that polyanhydride maintained concentration of 75- 125 ng Taxol/ mg brain tissue, within a 1-3 mm radius of the disc and concluded that Taxol shows promising results in malignant glioma when delivered via the polyanhydride system.
- Local delivery of Minocycline for treatment of intracranial glioma is another application of polyanhydrides. Minocycline was incorporated in Poly (CarboxyPhenoxy Propane:SebacicAcid) in a ratio of 50:50 by weight. It was found that combination of intracranial minocycline and systemic BCNU extended median survival by 82%.

5. Modified nanoparticles

The utilization of the nanoparticles as a vector for brain drug delivery has the following advantages.

- Excellent engineerability
- Non-toxicity.
- Controllable loading/releasing of active agents (drugs/contrast agents)
- Targeted nanoparticles can achieve the delivery of large amounts of therapeutic or imaging agents.
- The nanoparticles with enhanced surface properties (targeting and/or hydrophilic coating) may be able to deliver a high amount of drugs/contrast agents selectively to tumor sites and improve the efficacy of existing imaging and treatment of cancer in general

Types of nanoparticles

• Multifunctional nanoparticles

Multifunctional nanoparticles are 20-200 nm diameter nanoparticles. These are also called as Probes Encapsulated by Biologically Localized Embedding (PEBBLEs). Their components include:

- A targeting agent that directs the particle to cancer cells
- A protective coating (PEG) that helps it cross the BBB
- Photodynamic molecules that catalyze the conversion of oxygen to highly reactive oxygen singlets
- Magnetically dense metals for MRI contrast imaging
- Fluorescent “beacon” to visually pinpoint the nanoparticles location
- The major potential advantage of using PEBBLES to treat cancer is their multifunctionality.
- One target molecule immobilized on the surface could guide the PEBBLE to a tumor.
- Another agent could be used to help visualize the target using (MRI)
- MRI contrast element: Gadolinium
- Third agent attached to the PEBBLE could deliver a destructive dose of drug or toxin to nearby cancer cells.

All three functions can be combined in a single tiny polymer sphere to make a potent therapeutic system against cancer. When injected into the bloodstream, the nanoparticles travel through the bloodstream. As they can transverse the BBB and have a targeting agent attached, the PEBBLEs accumulate in the brain tumor enabling a clear MRI image within just a few hours. Each PEBBLE carries a photocatalyst. When stimulated by a light source through a micrometer-sized fiber optic probe inserted into the skull, the photocatalyst converts oxygen into singlet state, which effectively bleaches and destroys nearby cells. The PEBBLEs are inert and harmless until the light is turned on. Used in combination with MRI imaging, cancer cells can be killed along with tracking the effectiveness of the treatment with imaging. The targeted treatments using PEBBLEs has a number of

advantages over traditional chemotherapy. As PEBBLEs are highly localized to the cancer target, very little damage is caused to surrounding healthy tissue. PEBBLEs act on the outside of the cell and the toxic oxygen which is delivered by them acts quickly, without giving the cancer cells chance to survive and develop resistance. In this way, they overcome multiple drug resistance. In the most common form of primary brain cancer is glioblastoma, surgery has limited effectiveness because of difficulty in visually differentiating cancer cells and normal brain tissue and any cancer cells left behind are likely to proliferate and form new tumors. Thus, Fluorophores, i.e. glowing molecules were developed which mark the tumor boundaries and facilitate detection and removal of tumors.

• Magnetic nanoparticles for MRI

MRI of the CNS is usually performed with short-lived Gadolinium-based contrast agents. Iron oxide nanoparticle-based MRI contrast agents show excellent potential for the CNS imaging. The iron oxide contrast agents are known as super-paramagnetic iron oxide (SPIO) or ultra small super-paramagnetic iron oxide (USPIO) agents, depending on the size distribution of the nanoparticles. Some of the SPIO and USPIO are already clinically approved or on preclinical trial.

6. Receptor-mediated transport (RMT)

The BBB expresses RMT systems for the transport of endogenous peptides, such as insulin or transferrin. The RMT systems operate in parallel with the classical carrier-mediated transporters (CMT), which transport certain small molecule nutrients, vitamins, and hormones. Just as the CMT systems are portals of entry for small molecule drugs that have a molecular structure that mimics that of an endogenous CMT substrate, the RMT systems are portals of entry for large molecule drugs that are attached to endogenous RMT ligands.

• Monoclonal antibody (MAb) molecular Trojan horses (MTH)

Genetic engineering is used to produce either chimeric or humanized forms of the monoclonal antibody. The most potent antibody-based MTH known to date is monoclonal antibody against the human insulin receptor. Recently, this antibody has been humanized, and shown to cross the BBB in vivo in non-human primates. Certain peptidomimetic MABs act as ligands for the RMT systems. These BBB RMT-specific antibodies bind epitopes on the receptor which are spatially removed from the endogenous ligand binding site. The peptidomimetic MABs act as MTH to ferry across the BBB an attached drug, protein, antisense agent, or non-viral plasmid DNA. A number of non antibody delivery systems have been evaluated, including histone, receptor-associated protein (RAP), the tat transduction domain peptide, and other cationic peptides or polymers. Whereas the transport of ligands such as RAP is hypothesized to be receptor-mediated, the transport of cationic peptides is believed to be mediated by

absorptive-mediated endocytosis systems that are based on charge interactions. Delivery of biopharmaceuticals across the BBB has been reported recently using a related RMT system. A carrier protein known as CRM197 was used as safe and effective carrier protein in human vaccines and more recently in anti-cancer trials. CRM197 uses the membrane-bound precursor of heparin-binding epidermal growth factor (HB-EGF) as its transport receptor, which is also known as the diphtheria toxin receptor (DTR). In fact, CRM197 is a nontoxic mutant of diphtheria toxin. Membrane bound HB-EGF is constitutively expressed on various tissues and cells such as blood-brain barrier endothelial cells and several other cells. This means that major sanctuary sites (brain) and cellular reservoirs (T-lymphocytes, monocytes, macrophages) can be reached. Moreover, HB-EGF expression is upregulated strongly under (inflammatory) disease conditions, which will enhance targeted delivery considerably. CRM197 can deliver siRNA across the blood brain barrier by this mechanism. Other applications may relate to other neurotropic infections (e.g. poliovirus, West Nile virus) or other brain-related diseases (e.g. multiple sclerosis, Parkinson, Alzheimer).

- **Trojan horse liposomes for CNS gene therapy**

Gene delivery across the BBB may be ineffective owing to the rapid degradation of extracellular nucleic acids, as well as the pro-inflammatory effects of naked DNA. Encapsulation of plasmid DNA inside PEGylated liposomes eliminates the nuclease sensitivity and pro-inflammatory effects of the nucleic acid. PEGylated liposomes, per se, are not transported across the BBB. However, the attachment of a MTH to the tips of the polyethylene glycol strands allows the liposome to engage the BBB RMT system, and this triggers transport of the PEGylated immunoliposomes, also called Trojan horse liposomes, across the BBB. The administration of this new technology, to mice, rats, or monkeys is followed 24-48 hrs later by global expression of the non-viral transgene in brain. The BBB delivery of immunoliposomes carrying an expression plasmid encoding tyrosine hydroxylase allowed for complete restoration of striatal tyrosine hydroxylase enzyme activity in a model of experimental Parkinson's disease. The intravenous injection of immunoliposomes carrying an expression plasmid encoding a short hairpin RNA directed against the human epidermal growth factor led to a 90% increase in survival time of mice with intracranial human brain cancer. The PEGylated immunoliposomes gene transfer technology enables intravenous RNA interference (RNAi) of the brain.

- **In vivo brain imaging of gene expression**

Antisense radiopharmaceuticals hold promise for imaging gene expression in the brain using nuclear medicine imaging modalities, such as PET or SPECT. However, antisense radiopharmaceuticals do not cross the BBB on their own and must be modified if they are to be useful brain gene imaging agents. Peptide nucleic acids can be biotinylated and radiolabelled with ¹¹¹-

indium. In parallel, a conjugate or fusion protein, of avidin and a BBB molecular Trojan horse can be synthesized. The peptide nucleic acid is then coupled to the MTH via the avidin-biotin bridge. Such targeted antisense radiopharmaceuticals cross the BBB, and the brain cell membrane, and enable the in vivo imaging of gene expression in brain.

7. Transporter-independent mechanisms to circumvent the BBB

- **Convection-enhanced drug delivery (CED)**

CED is a method for local/regional micro infusion targeted directly to brain tissue. A continuous infusion pressure gradient over hours to days results in distribution of therapeutic agents into the interstitial space. The CED technique is used primarily for large molecular weight agents that show minimal leakage across the BBB and/or have significant systemic toxicity, including viruses, oligonucleotides, nanoparticles, liposome, and targeted immunotoxins. Parameters that affect CED volume of distribution include infusion parameters (rate, volume, duration, cannula size), infusate characteristics (molecular weight, surface properties, tissue affinity), and tissue properties (tissue density, extracellular space, vascularity, and interstitial fluid pressure). Animal studies have demonstrated that the volume of distribution achieved by CED can be imaged by magnetic resonance in real time by including contrast agents within the infusate. The major clinical use of CED will be for targeted therapy of glioblastoma. Recent studies have included interleukin 13/pseudomonas exotoxin alone or in combination with radiation/temozolomide, and radio immunotherapy with mAbs targeting tenascin or tumor necrosis factor. Despite promising early results, it appears that two industry-sponsored phase III trials of CED immunotoxins have been negative. Mechanisms for CED treatment failure include distribution inhomogeneity, high interstitial fluid pressure, and rapid efflux of agent from the injection site. To overcome these issues, increased residence time must be achieved to enhance targeted toxin receptor binding and uptake by the cancerous cells. Although primarily targeting brain tumors, the CED technique may also gain use for localized neurodegenerative disorders. For example, CED has been used to infuse 6 glucocerebrosidase into the frontal lobe and brainstem of a patient with neuronopathic Gaucher disease. Infusion of adenovirus vectors or glial-derived neurotrophic factor has been assessed in Parkinson disease.

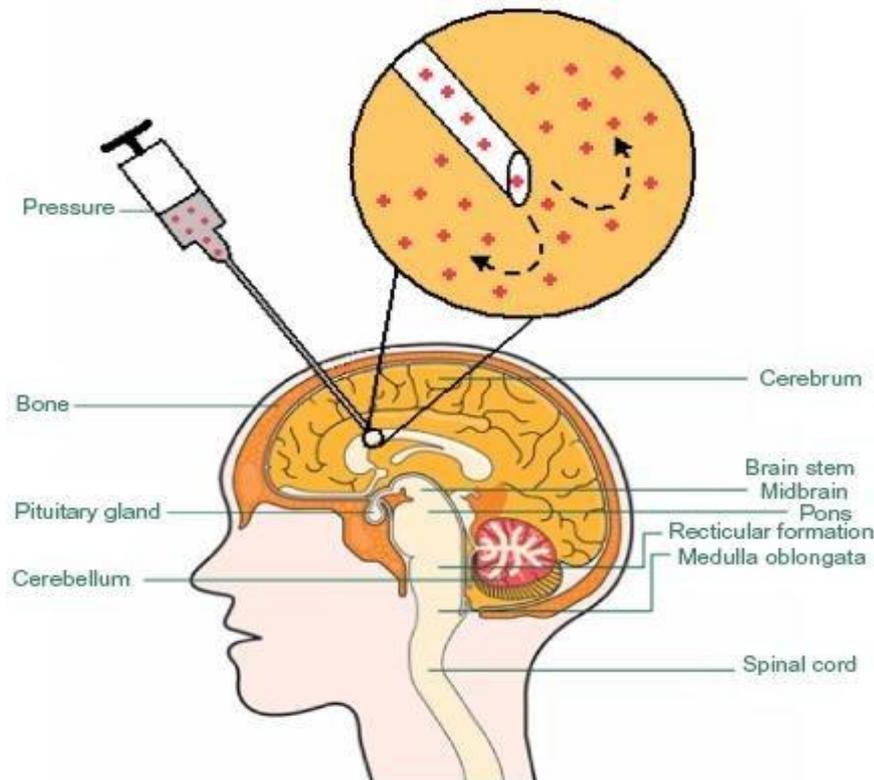


Fig. 4: Illustration convection-enhanced drug delivery.

- **Bradykinin receptor-mediated BBB opening**

Bradykinin, an endogenous peptide mediator of the inflammatory response, can induce transient increases in blood vessel permeability that can be highly specific for tumor vasculature. RMP-7 (lobadimil) is a synthetic 7 bradykinin analog that is specific for the B2 receptor and is 100-fold more potent than bradykinin in mice. Pharmacological manipulation of the BTB offers the possibility of highly specific opening and targeted drug delivery to tumor, albeit with the possibility that increases in delivery may only be modest and dependent on the tumor type or model treated. Clinical studies in the past 5 years have demonstrated the safety of concurrent RMP-7 and carboplatin, with or without radiation therapy, for both adults and children with gliomas. However, RMP-7 had no effect on the pharmacokinetics or toxicity of carboplatin, and two studies have shown no objective responses of RMP-7 and carboplatin in brain stem glioma or high-grade glioma. Higher doses of RMP-7 may be required to increase carboplatin delivery to tumor, but may also result in increased toxicity in normal brain.

- **Ultrasound (US)-mediated BBBD strategy**

US consists of pressure waves having frequencies of 20 kHz or greater. Like optical and audio waves, ultrasonic waves can be focused, reflected, and refracted through a medium. A major limitation in the utilization of US for BBBD has been the poor penetration of US through the skull, and for several decades it was believed that the skull bone had to be removed to perform US treatments

in the brain. However, experimental and theoretical studies have shown that it is feasible to achieve focal, trans-skull focused US (FUS) exposure of brain tissue by using large surface area phased arrays. Recently developed image-guided (eg, magnetic resonance imaging [MRI]-guided) FUS clinical systems have made it possible to deliver therapeutics to the targeted regions in the brain through the intact skull, and both animal studies and clinical trials have shown encouraging results. As shown in Figure, ultrasonic microbubbles combined with FUS can be used as drug carriers for targeted delivery. Preformed microbubbles with narrow size distribution have been used to achieve a repeatable cavitation environment with controlled source of cavitation nuclei. Cavitation is defined as the oscillation of bubbles in an acoustic field. Cavitation can produce strong stresses on cells to achieve various “bioeffects.” For instance, it may increase drug interaction by upregulating pathways of various types of stress response, or result in physical shearing of the cell membrane to promote direct passage of therapeutics into the cytosol. With ultrasonic microbubbles in blood vessels, the acoustic energy required by the cavitation will be greatly reduced. This technique makes the procedure more practical for application through the intact skull, since the risks of overheating the skull would be significantly reduced. Furthermore, with the use of these agents, the interaction of the US with the endothelial cells can be limited, so the chance of damage to other brain structures can be minimized.

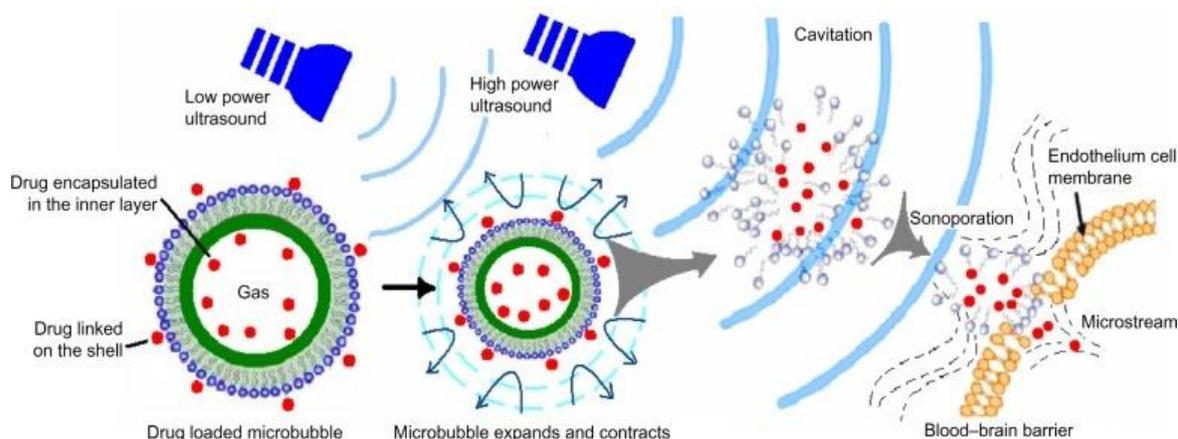


Fig. 5: Illustration of ultrasonic microbubbles for drug targeted delivery.

CONCLUSION

Delivering drugs effectively for treatment of CNS-related disorders is affected by lack of specific and efficacious approaches. Despite these obstacles, significant progress has been made in the strategies for brain targeting. But none has been proved to be satisfactory. It can be concluded from this review that by means of the above mentioned approaches the drug can be delivered across the BBB efficiently. Recent developments in drug delivery across the BBB have proven to be helpful for overcoming barriers associated with brain drug delivery. 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.

ACKNOWLEDGEMENT

The authors are thankful to Dr. Ramanlal N. Kachave, Amrutvahini College of Pharmacy, Amrutvahini for their all time guidance and encouragement.

REFERENCES

- Bummer PM, Physical chemical considerations of lipid based oral drug delivery, solid lipid nanoparticles, *Critical Review, Therapeutic Drug Carrier System*, 2004; 21(2): 1-20.
- Shrikant CS, Mahale NB, Chaudhari SR, Thorat RS, Recent advances in brain targeted drug delivery system: a review, 2015; 4(5): 542-559.
- Ganesh S, Shahiwal A, Shrenik P, Drug delivery to the central nervous system: a review, Received 16 June 2003, Revised 26 June 2003.
- Bickel U, How to measure drug transport across the bloodbrain barrier, *NeuroRx*, 2005; 2: 15-26.
- Tamai I, Tsuji A, Transporter-mediated permeation of drugs across the blood-brain barrier. *J Pharm Sci.*, 2000; 89: 1371-1388.
- Pardridge WM, Drug transport in brain via the cerebrospinal fluid. *Fluids Barriers CNS.*, 2011; 8: 7.
- Rip J, Schenk GJ, de Boer AG, Differential receptor-mediated drug targeting to the diseased brain. *Expert Opin. Drug Deliv*, 2009; 6(3): 227-237.
- Bickel U, Yoshikawa T, Pardridge WM, Delivery of peptides and proteins through the blood-brain barrier. *Adv. Drug Deliv. Rev.*, 2001; 46(1-3): 247-279.
- Lu W, Wan J, She Z, Jiang X. Brain delivery property and accelerated blood clearance of cationic albumin conjugated PEGylated nanoparticle. *J. Control. Release*, 2007; 118(1): 38-53.
- Kabanov AV, Batrakova EV, New technologies for drug delivery across the blood brain barrier, *Curr Pharm Des*, 2004; 10: 1355-1363.
- Mishra A, Ganesh S, Shahiwala A, Shah SP, Drug delivery to the central nervous system: a review, *J Pharm Pharm Sci.*, 2003; 6(2): 252-273.
- Huwlyer J, Wu D, Pardridge WM, Brain drug delivery of small molecules using immunoliposomes, *Proc Natl Acad Sci USA*, 1996; 93: 14164-14169.
- Pardridge WM, Huwlyer, J, WO022092A1, 1998.
- Tosi G, Costantino L, Ruozi B, Forni F, Randelli MA, Polymeric nanoparticles for the drug delivery to the central nervous system, *Exp, Opin, Drug Deliv*, 2008; 5(2): 155-174.
- Vyas SP, Khar RK, Targeted and controlled drug delivery novel carrier systems, 1st ed. CBS Publishers and distributors, 2007: 487-509.
- 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.
18. Yorulmaz H, Seker FB, Oztas B, The effects of hypoglycemic and alcoholic coma on the blood-brain barrier permeability, *Bosn J Basic Med Sci.*, 2011; 11: 108-112.
 19. Witt KA, Davis TP, CNS drug delivery: Opioid peptides and the blood-brain barrier. *The AAPS Journal*, 2006; 8: 76-88.
 20. Rautio J, Laine K, Gynther M, Savolainen J, Prodrug Approaches for CNS Delivery. *The AAPS Journal*, 2008; 10: 92-102.
 21. Mcdannold N, Vykhodtseva 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.
 22. Yang S, Zhu J, Liang B, Yang C, Body distribution of camptothecin solid lipid nanoparticles after oral administration. *Pharm. Res.*, 1999; 16: 751-757.
 23. Varsha A, Riddhi P, Brain targeted drug delivery system. *Int. J. Drug Dev. & Res.*, October-December, 2014; 6(4): 15-27.