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RECENT APPROACHES IN BRAIN TARGET VIA OLFACTORY ROUTE

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ABSTRACT

Many therapeutic drugs are difficult to reach the central nervous system (CNS) from the systemic blood circulation because the blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (BCSFB) form a very effective barrier which prevents most molecules from passing through. To bypass BBB, drugs can be delivered through olfactory region for nose-to-brain targeting. Peptide and protein drugs have been developed for the treatment of various neurodegenerative diseases. Drug delivery of these therapeutic proteins is facing several challenges because of the instability, high enzymatic metabolism, low gastrointestinal absorption, rapid renal elimination, and potential immunogenicity. New genetically engineered biotechnology products, such as recombinant human nerve growth factor, human VEGF, and interferons, are now possible to be delivered into the brain from the non-invasive intranasal route. For gene therapy, intranasal route is also a promising alternative method to deliver plasmid DNA to the brain. This review provides an overview of recent approaches to improve the drug delivery to the brain and the latest development of protein, peptide, and gene intranasal delivery for brain targeting.

Keywords: Brain target, olfactory, BBB, Approaches of Brain targeting.

INTRODUCTION

Many neurotherapeutics are unsuccessful in treating CNS disorders because they cannot be effectively delivered to the brain. Drug delivery to the brain is a challenge even though there is relatively high blood flow. There are two physiological barriers separating the brain from its blood supply controlling the transport of compounds. One is the blood–brain barrier (BBB) and the other is the blood–cerebrospinal fluid barrier (BCSFB). Internally brain is protected from foreign organisms and noxious chemicals by highly strengthened membrane system called as blood–brain barrier. BBB is a specialized system of capillary endothelial cells that protects the brain from harmful substances in the blood stream, while supplying the brain with the required nutrients for proper function. It is a semi permeable, selective barrier which was confirmed for the first time by Ehrlich who showed that cerebrospinal fluid (CSF) injection of tryptophan blue dye stained the entire brain parenchyma but could not enter into brain capillary microvasculature [1]. BBB is responsible for several functions like maintenance of neuronal microenvironment, tissue homeostasis, vasotonous regulation, fibrinolysis and coagulation, blood cell activation and migration during physiological and pathological processes, and also helps in vascularisation of normal neoplastic tissues [2]. Physiologically BBB is made up of three layers such as inner endothelial cell layer which forms the wall of the capillary and contains tight junctions followed by presence of basement membrane upon which pericytes and astrocytic feet processes lies [3]. Due to the presence of such tight junctions between endothelial cells a very high electrical resistance of around 1500–2000cm^2 results [4] as compared to 3.33cm^2 in other body tissue proving the barrier function of BBB [5]. Astrocytes and pericytes helps in differentiation as well as maintenance of BBB function. Astrocytes are most abundant non-neuron cells and play many essential roles in the healthy central nervous system (CNS), including biochemical support of

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endothelial cells which form the blood–brain barrier, regulation of blood flow, provision of nutrients to the nervous tissue, maintenance of extracellular ion balance, and a principal role in the repair and scarring process of the brain and spinal cord following traumatic injuries [6]. Pericytes are perivascular cells which are important for the maturation, remodeling and maintenance of the vascular system via the secretion of growth factors or modulation of the extracellular matrix. They are also involved in the transport across the BBB and the regulation of vascular permeability [7]. The blood–cerebrospinal fluid barrier (BCSFB) is another barrier (after BBB) that a systemically administered drug encounters before entering the CNS. It functions together with the BBB and the meninges, to control the internal environment of the brain. It is sited at the choroid plexus epithelium, secretes CSF, which circulates through the ventricles and around the outside of the brain and spinal cord [8]. In the last 20 years the choroidal epithelium has emerged as a complex organ with many additional functions that include neuroendocrine signaling, neuroimmune and neuroinflammatory responses, drug and toxin metabolism and transport. The improved knowledge has established the role of the choroid plexus in brain function which has the potential to open new avenues for the treatment and prevention of neurological disorders. Choroid plexus function changes with disease and aging and knowledge of the processes involved is important for understanding CNS disease states, such as Alzheimer's, Parkinson's, HIV, and disorders of CSF circulation [9]. In addition, some regions of the CNS called as circumventricular organs (CVO) are present adjacent to the ventricles of brain where BBB capillary endothelial tight junctions are absent. These brain sites are unique in terms that they are highly vascularised as compared to other brain regions and lacks BBB because the capillary system supplying the CVOs contains fenestrated endothelial cells instead of epithelial tight junction [10]. Examples of such areas include choroid plexus, pineal gland, neurohypophysis, median eminence, organosum vasculosum of lamina terminalis, subfornical organ (SFO), area postrema of the chemoreceptor trigger zone (CTZ) and nucleustractus solitarius (NTS). These sites require intimate contact to closely monitor the composition of the blood and to respond accordingly. Compared to the area of tight BBB capillaries, the relative surface area of the capillaries of CVOs is very less (5000:1) which enables CVOs not to allow a significant diffusion of substances into the CNS [11].

BBB Transport System

Unlike peripheral capillaries that allow relatively free exchange of substance across cells, the BBB rigorously limits transport into the brain. BBB not only functions as a physical barrier, but also a biochemical barrier that expresses certain enzymes like peptidases along with several cytosolic enzymes and efflux p-glycoprotein system that helps effluxing drugs from the endothelial cells back into the blood which helps in its further protecting action towards the brain microenvironment [12]. Thus the BBB is often the rate-limiting factor in determining permeation of therapeutic drugs into the brain. BBB is physiologically guided by two types of membranes such as luminal membrane and abluminal membrane. Even so, BBB has been found to be permeable in transport of nutrients like blood glucose, proteins, peptides and related peptide drugs [13]. Various transport mechanisms at the BBB have been explained for the transport of these substances (Fig. 1). These transport systems mainly operate in the luminal and abluminal membranes, i.e. from both blood-to-brain and brain-to-blood directions. But the blood-to-brain transport system is of considerable interest in drug delivery for targeting of drug molecules into brain as compared to brain-to-blood transport system. Different substances are basically transported through free diffusion mechanism either paracellularly or transcellularly [14]. Paracellular diffusion is a non-saturable and noncompetitive movement of compounds (e.g., sucrose) between cells. It occurs to a limited extent at the BBB, due to the "tight junctions". Transcellular diffusion (transcytosis) is a non-saturable and noncompetitive movement across cells of lipophilic substances (e.g. ethanol). Facilitated diffusion is a form of carrier-mediated endocytosis in which solute molecules bind to specific membrane protein carriers that trigger a conformational change in the protein; which results in carrying through of the substance to the other side of the membrane, from high to low concentration (passive diffusion). This mechanism contributes to transport of various substances including amino acids, nucleoside, small peptide, monocarboxylates, glutathione [13,15]. Endocytosis can be isolated into bulk-phase (fluid phase or pinocytosis) endocytosis and mediated endocytosis (receptor and absorptive mediated). Bulk-phase endocytosis is the noncompetitive, non-saturable, temperature and energy dependent non-specific uptake of extracellular fluids. It occurs to a very limited degree in the endothelial cells of the cerebral microvasculature [16]. Cells have different receptors for the uptake of many different types of ligands, including hormones, growth factors, enzymes, and plasma proteins. It occurs at the brain for macromolecular substances, such as transferrin, insulin, leptin, and IGF-I & IGF-II, and is a highly specific type of energy dependent transport. In addition to this several other receptors are found in BBB, e.g., receptor for leptin as LEPR, Fc fragment of IgG receptor transporter (FCGRT) [17,18]. Absorptive-mediated transport is triggered by an electrostatic interaction between a positively charged substance (charge moiety of a peptide) and the negatively charge plasma membrane surface (i.e. glycocalyx). It has a lower affinity and higher capacity than receptor-mediated endocytosis [19]. Carrier mediated transporter (CMT) system is expressed on both the luminal

and abluminal membranes of the brain capillary endothelium and operates in both directions, i.e., from blood to brain and brain to blood directions [17,20]. The CMT systems can be exploited for brain drug-delivery after reformulating the drug in such a way that the drug assumes a molecular structure mimicking that of the endogenous ligand. For example, pseudonutrients are the polar small drug molecules which are made by mimicking the structure of nutrients. Gabapentin (a γ-amino acid) successfully cross the BBB because the structure does mimic that of an γ-amino acid and is recognized by large neutral amino acid transporter [21]. They have the tendency to penetrate BBB, as these are the camouflage of true transporters and hence they use the carrier mediated transporting systems [22]. Several other drugs which have been successfully transported into the brain include melphalan for brain cancer, laevodopa (L Dopa) for Parkinson's disease and α-methyl-DOPA for treatment of high blood pressure and endogenous substances or nutrients utilizing different transporters of CMT system[23]. The uptake of nutrients from blood into the brain is facilitated by the solute carrier (SLC) transporter families. These influx carriers are involved in the transport of a broad range of substrates including glucose, amino acids, nucleosides, fatty acids, minerals and vitamins in various human tissues, including brain. There are 43 families summarized based upon amino acid homology. Amongst these, SLCO/SLC21, the organic anion transporting superfamily (OATPs), and SLC22, the organic cation/anion/zwitterions transporter family, are heavily involved in the uptake of many diverse substrates. Of the three SLC families, i.e., SLC15, SLC22, and SLCO are considered especially to have a role in xenobiotic drug uptake [24, 25]. The active efflux transport (AET) is involved in extruding drugs from the brain and is a major obstacle for many pharmacological agents, with the ABC (ATP binding cassette) transporter P-glycoprotein being the principle efflux mechanism of these agents [26]. It requires energy in most instances and unlike CMT it is a unidirectional transport process [27]. Most abundantly present component of this system is efflux P-glycoprotein, which is a product of ABCB1gene. Several other multiple members of these gene families are available as ABCCs, ABCG2. AET is operated by broadly two groups of transporters, one is entirely energy dependent like ABC gene family found at luminal membrane and others are energy independent found at abluminal membrane are organic anion transporter1 (OAT), organic anion transporting polypeptide (OATP), glutamic acid amino acid transporter (EAAT), etc. The substrates to brain due to their ion transporting mechanism without utilizing energy [28].

The Problem of Drug Transport to the Brain

The idea of a BBB that segregates the blood and brain was developed 100 years ago, following the demonstration that most organs could be stained by dye injected intravenously, with the exception of the brain and spinal cord [29]. The capillaries of the brain have evolved to constrain the movement of molecules and cells between blood and brain, providing a natural defense against circulating toxic or infectious agents. The relative impermeability of the BBB results from tight junctions between capillary endothelial cellswhich are formed by cell adhesion molecules. Brain endothelial cells also possess few alternate transport pathways (e.g., fenestra, transendothelial channels, pinocytotic vesicles), and express high levels of active efflux transport proteins, including P-glycoprotein (P-gp), Multidrug Resistance Protein- 1 (MRP-1), and Breast Cancer Resistance Protein (BCRP). The BBB also has additional enzymatic aspects which serve to protect the brain. Solutes crossing the cell membrane are subsequently exposed to degrading enzymes, ecto- and endo-enzymes, present in largenumbers inside the endothelial cells that contain large densities ofmitochondria, metabolically highly active organelles. Some small molecules with appropriate lipophilicity, molecular weight (mw) and charge will diffuse from blood into the CNS. However, the overwhelmingmajority of smallmolecules (mwN500 daltons, D), proteins and peptides do not cross the BBB. It has been reported that approximately 98% of the small molecules and nearly all large molecules (mwN1 kD, kilodaltons), such as recombinant proteins or gene-based medicines do not cross the BBB [30]. Therefore, to reach the brain, most molecules must cross the BBB through interaction with specific transporters and/or receptors expressed at the luminal (blood) side of the endothelial cells. 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. Crossing the BBB remains a key obstacle in the development of drugs for brain diseases despite decades of research. A schematic representation of different mechanisms used to cross the BBB is shown in Fig. 3.

• Small hydrophilic molecules such as amino acids, glucose, and other molecules necessary for the survival of brain cells use transporters expressed at the luminal (blood) and basolateral (brain) side of the endothelial cells.

• Larger and/or hydrophilic essential molecules such as hormones, transferrin for iron, insulin, and lipoproteins use specific receptors that are highly expressed on the luminal side of the endothelial cells. These receptors function in the endocytosis and transcytosis of compounds across the BBB.

• Small lipophilic molecules can diffuse passively across the BBB into the brain but will be exposed to efflux pumps (P-glycoprotein [P-gp], some Multidrug Resistance

Proteins [MRP], Breast cancer Resistance Protein [BCRP] and others) expressed on the luminal side of the BBB and exposed to degrading enzymes (ecto- and endo-enzymes) localized in the cytoplasm of endothelial cells before brain penetration.

Approaches in Brain Targeting

i) Invasive approach

These are physical based techniques include the use of: 1) Intracerebro-ventricular infusion, 2)Convection-enhanced delivery and 3) polymer or microchip systems. Invasive approaches deliver drug to the brain by mechanically breaching the BBB and are summarized below:

a) Intra-cerebro-ventricular (ICV) infusion

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[37]. The drug eventually distributes to the general circulation, where the drug then enters the brain parenchyma following transport across the BBB. This result is similar to a slow intravenous infusion rather than a direct administration of drugs into the brain. Pharmacologic effects can be seen after ICV administration, if the target receptors of the drug for example, opioid peptides) 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.

b) 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. In contrast to the mm distances obtained with simple diffusion, 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 h of continuous infusion. The success of CED relies on precise placement of the catheters and other infusion parameters for delivery into the correct location in the brain parenchyma.

c) Intra-cerebral injection or use of implants

Both the bolus injection of chemotherapy agents and the placement of a biodegradable, chemotherapeutic impregnated, wafer into a tumour resection cavity, rely on the principle of diffusion to drive thendrug into the infiltrated brain have demonstrated the presence of high drug concentration (0.5–3.5mMfor carmustine, 0.2–1 mM for paclitaxel) within the first 3 mm from the polymer implants in monkeys; significant concentrations (0.4 μM for carmustine, 0.6 μMfor paclitaxel) were measured up to approx. 5 cmfrom the implant as long as 30 days after implantation.

d) Disruption of the BBB

Disruption of the BBB can open access of the brain to components in the blood by making the tight junction between the endothelial cells of the brain capillaries leaky. Different techniques are used to disrupt the tight junctions:

- 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 (preformed 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.

- Application of bradykinin-analogue (RMP-7, Cereport® from Alkermes Inc.): There is evidence of the opening of the tight junctions to occur by activation of bradykinin B2 receptors through a calcium-mediated mechanism. This technique was abandoned due to lack of efficacy in Phase II and III studies when administered in combination with carboplatin [31,32].

ii) Pharmacological approach

The pharmacological approach to crossing the BBB is based on the observation that some molecules freely enter the brain, e.g. alcohol nicotine and benzodiazepine. This ability to passively cross the BBB depends on the molecular size being less than 500 D), charge (low hydrogen bonding capabilities) and lipophilicity (the more lipophilic, the better the transport). This approach consists of modifying, through medicinal chemistry, a molecule that is known to be active against a CNS target to enable it to penetrate the BBB. Modification of drugs through a reduction in the relative number of polar groups increases the transfer of a drug across the BBB. Lipid carriers have been used for transport, and there are successful examples of both these approaches. Modification of antioxidants with pyrrolopyrimidines increases their ability to access target cells within the CNS. Enhanced delivery of ganciclovir to the brain was observed by covalently attaching 1-methyl-1,4-dihydronicotinate to an hydroxymethyl group . Fatty acid such as N-docosahexaenoyl (DHA) have been incorporated in small drugs to increase their brain uptake. Incorporation of low

molecular mass drugs into pluronic micelles can increase drug solubility and drug stability, and can improve drug pharmacokinetics and biodistribution. Polymeric micelles have been utilized for delivery of CNS drugs across the BBB, and for oral delivery of drugs and tumour-specific delivery of antineoplastic agents. For example, in one early study, pluronic P85 micelles loaded with a neuroleptic drug were targeted to the brain by conjugating micelles with neurospecific antibodies, or using insulin as targeting moieties. Amphiphilic chitosan-based polymers (mwb20 kD) self-assemble in aqueous media at low micromolar concentrations to give previously unknown micellar clusters of 100–300 nm of size . Intravenous anaesthetic propofol was used as a model drug. It is known that the sleep times obtained with the carbohydrate propofol formulations are up to 10 times those obtained when using either the commercial Fresenius or Diprivan formulations. A loss of righting reflex time could not be recorded as animals were asleep by the end of the injection period; evidence that delivery of the centrally active drug across the blood–brain barrier is rapid and efficient [31].

iii) Physiological approaches

The brain requires essential substances for metabolism and survival, such as glucose, insulin, growth hormone, low density lipoprotein (LDL), etc. These substances are recognized by specific receptors or transport mechanisms, resulting in specific transport into the brain. Since almost every neuron in the brain is perfused by its own capillary as a result of the small distance separating capillaries (on average 40 μm) and the brain's very high perfusion rate. Therefore, the most effective way of delivering neuroactive drugs is via transporters or internalizing receptors on these capillaries. Drugs can be modified to take advantage of native BBB nutrient transport systems or by conjugation to ligands that recognize receptors expressed at the BBB. This will result in their being carried across the BBB after receptor-mediated transcytosis. This physiological approach is recognized by the scientific community as the one with the most likely chance of success.

a) Transporter-mediated delivery

Peptides and small molecules may use specific transporters expressed on the luminal and basolateral side of the endothelial cells forming the BBB to cross into the brain. At least 8 different nutrient transport systems have been identified, with each transporting a group of nutrients of similar structure. Only drugs that closely mimic the endogenous carrier substrateswill be taken up and transported into the brain.Drugsmay bemodified such that their transport is increased by using a carrier-mediated transporter expressed on the endothelial cells forming the BBB. Use of small molecules that directly target transporters to overcome BBB restrictions eliminate the need for the drug to be transformed for example by conjugation to antibodies and to deliver the metabolic precursor of dopamine. Use of BBB transport proteins such as the choline transporter and the amino acid transporter has been achieved successfully for a few drugs, for example, the large neutralamino acid carrier has been used to deliver dopamine's metabolic precursor, L-Dopa, to patients with Parkinson's disease, resulting in clear clinical benefit; dopamine itself is non-brain penetrant

b) Receptor-mediated transcytosis

Receptors at the blood–brain barrier: Large molecules which are necessary for the normal function of the brain are delivered to the brain by specific receptors. These receptors are highly expressed on the endothelial cells forming the BBB. These include the insulin receptor, transferrin receptor, LDL receptor and its related protein, and others. Research is still on-going to identify new receptors. The receptor-mediated transcytosis occurs in 3 steps:

1. Receptor-mediated endocytosis of the compound at the luminal (blood) side.
2. Movement through the cytoplasm of the endothelial cell.
3. Exocytosis of the drug at the abluminal (brain) side of the brain capillary endothelium.

The precise mechanism of transcytosis across polarized endothelial cells has not been determined. Additional molecules may be involved in the transcytosis across the BBB and bypassing of lysosomes in the cytoplasm which could degrade the molecules being transported. The physiologic approach comprises targeting these receptors at the BBB by specific ligands, modified ligands and antibodies. Therapeutic compounds are able to cross the BBB after association/ conjugation to these specific ligands forming molecular Trojan horses (MTH). To delivery larger amounts of therapeutics liposomes decorated with specific ligand have also been developed [31,32].

c) Transferrin receptor (TR)

The function of the TR is to provide iron to cells. Drug targeting to the TR can be achieved by using the endogenous ligand transferrin, or by using antibodies directed against the TR.

- For transferrin (Tf) the in-vivo application is limited due to high endogenous concentrations of Tf in plasma. Transferrin is an essential protein needed for iron delivery to cells and is found at mg/ml amounts in plasma.
- Using the antibody approach against TR, the receptor specific mAb binds to the receptor onthe endothelial cells, and allowsthe associated therapeutic agent to cross the BBB via receptor-mediated transcytosis. For antibodies against the TR, proof of concept studies in rats have demonstrated that amAb that binds to a distinct epitope from Tf (OX26) can be used as a brain delivery agent.

iv) Chemistry based approach

The chemical substances which help in transporting the drug substances through BBB are taken as ideal agents for delivery. From the last two decades these approaches have quite frequently being researched where various ideologies have being evolved for delivering the neurotherapeutics. Such ideas include the use of chimeric peptides and cationic proteins for drug delivery [32].

a) Chimeric peptides

The word "chimeric" obtained from the Greek word chimera means an animal having body of lion and head of human. The same stands here about the drug substances which are not transported through BBB are combined with a transport vector to form an easily transportable or fused molecule. The conjugated vector may be endogenous peptides, monoclonal antibodies (mAbs), modified proteins, peptidomimetic antibodies, etc. (Fig. 4).

These chimeric peptides are formed by covalent binding of a BBB non-permeable neuropeptide with the vector. After formation of such peptides, they are transported to brain by various transporting pathways like peptide-specific receptor. For example, insulin and transferrin are the two circulating peptides which undergo ranscytosis by their insulin and transferrin receptor present at BBB. Similarly the absorptive-mediated transcytosis system also operates for lectins. Before getting a peptide, two principles are kept in mind, firstly the vector itself should have pharmacological activity, for example insulin—a natural peptide has its own transport mechanism. Secondly the interaction between peptide vectors with its binding receptor site must be highly specific for targeting drug to brain. Several endogenous peptides have been investigated satisfying both these two conditions including cationized albumin, monoclonal antibodies and histones. These act as ideal peptides for transferrin receptor and cross BBB. The basic mechanism by which these act is the initial binding of such vector to BBB on its exofacial epitopic site, which leads to removal of epitope of mAb from endogenous ligand binding site and freshly binds with "piggy back" across the BBB on the RMI system. By this it enters into brain and act as effective vector for drug and gene delivery attached on its surface.

b) Cationic protein

It is the best suited technique for delivering proteins and peptides with a basic isoelectric point to the brain. In general proteins are of high molecular weight, hence cannot cross the BBB due to their very large size. Hence this method provides an additional advantage for delivering them by making them charged into cationic form, which can easily enter brain by electrostatic interaction with anionic functional groups present on brain surface. After cationization they easily enter by using the transcellular adsorptive-mediated endocytosis pathways. Cationization is a process which increases the net positive charge on the polypeptide by modifying the free carboxyl groups of acidic amino acid residues on a polypeptide. The free carboxyl groups of the polypeptide (e.g., IGF-I, IGF-II, NGF) may be modified with hexamethylenediamine, polylysine, diazomethane or polylysine cationization with cleavable ester bonds to enhance BBB transport, as well as to yield intact growth factor following transport. Several cationic proteins/peptides have been used to protect the brain including cationized antibodies against viral antigens or oncogenes in tumors or to image specific antigens in tumors or β-amyloid deposits in patients suffering from Alzheimer's disease. Similarly, various other cationic proteins have been reported to penetrate the BBB including avidin, histone, protamine and cationized polyclonal bovine immunoglobulin.

c) Prodrugs

Prodrugs are designed for delivering the hydrophilic drugs to brain. These are the chemically modified, inert alternatives of original API to get a product of large bulky structure which has neither biological toxicity nor activity. Prodrugs that have amino acid as a promoiety differ from chimeric peptides being devoid of any biological activity. These prodrugs give active moiety at the site of action, i.e., in brain due to the enzyme specific nature of them. Whenever these moieties reach the brain through blood, the enzymes present on the surface of BBB help in metabolism of prodrugs to give active agents, which then cross the BBB and attains concentration in brain. For the first time proposed the concept of prodrug as an ideal candidate for drug targeting to brain.

1. Prodrug "Lock-in" mechanism for drug targeting

Several molecules being used are carmustine, enkephalin, kyotorphin, estradiol, thyrotrophin, TRH, etc. The method is similar to that of prodrug formation, but the difference lies in the attachment of four different additional functions to active moiety like an adjuster (A), a bulkier lipophillic moiety (L), spacer (S) and a targetor (T) for locking them in brain. The drug is packed by covalently attached lipophilic group (L) to enhance lipid solubility and to disguise the nature of the molecule through an ester bond or sometimes through a C-terminal adjuster (A) at the carboxyl terminal and "targetor" (**T**) that undergoes enzymatic oxidation and turns to an ionic, membrane-impermeable moiety (**T+**). After distribution in the body and into the CNS by crossing the BBB, the conjugate is converted to ionic compounds which are retained in brain tissue, but ionic conjugates produced in the rest of the body are easily eliminated. The membrane impermeable conjugates "locked" into the brain undergo sequential metabolism and yield the drug in the CNS. A spacer (S) consisting of strategically used amino acids is provided to ensure timely removal of the charged targetor (by controlling the enzymatic rate of drug release).

2. Cyclodextrin complexes for drug delivery

Cyclodextrin is a tortuous shaped polysaccharide which originated from fermentation. It is found in nature as basket shapes in three different α, β, γ forms, having two kinds of chemical region. First is the core of basket which is hydrophilic and the inner cavity of basket is lipophilic nature. Drug molecules of both hydrophilic as well as lipophilic in nature can be entrapped in it. Cyclodextrin forms inclusion type of complexes which is a type of chemical delivery system and releases the drug at the site of action. It uses the similar "lock-in" phenomenon to entrap and release the drug. Other advantages include, for example, solubility enhancement, bioavailability enhancement, biotransformation reduction, etc. Though several applications of beta cyclodextrins are available in literature, recently the study reported by evolves the area of application of cyclodextrins towards brain targeting. The galanin like peptide is a major agent found to have potential applications in treating obesity when given with cyclodextrins was able to achieve good concentration in brain microenvironment.

3. Antibody directed enzyme prodrug therapy (ADEPT)

It is an advanced method used for targeting drug molecules to tumors on the surface of brain. Here the drugs are chemically modified to a prodrug form. This method uses the antibody for targeting the drugs on the tumor surface. Antibodies are proteinaceous substances synthesized in the body at the time of induction of immunological responses. Human body synthesizes antibody whenever the immune system encounters a particular type of foreign substance 'antigen' which is found to be harmful for the body. Hence antibodies are always complementary to antigens and react with them by attachment on their surface. Most widely used antibodies are the monoclonal antibodies prepared by hybridoma technology. These antibodies are bound on the surface of enzyme, which is required for activation of prodrug.

4. Biotin-avidin conjugated system for drug delivery

This method has attracted considerable interest for delivering neurotherapeutics to brain. Here the molecules to be transported are coupled with biotinavidin/ streptavidin system where anti-transferrin receptor antibodies are present on its surface. It releases drug molecules to the brain site on binding with antigen which is expressed on the tumor surface. The transport of non-transportable peptide, i.e., vasoactive intestinal peptides (VIP) can be transported easily to brain which shows increase in blood flow as a pharmacological effect. Similarly like immunoliposomes, biotin-avidin systems are also conjugated with liposomes by covalent conjugations which are bilayered lipids for targeting proteins and peptides to tumor cells. By conjugation with liposome, the targeted molecule can be easily incorporated into it or adsorbed on its surface and releases the drugs at the site of tumor cells.

v) Novel approaches for brain targeting

The field of novel drug delivery has fully emerged and has come into existence as an ideal approach of drug targeting to brain. It mainly includes the use of small colloidal particles. Targeting actionmaybe due to the steric hindrance created by nano-vectors for achieving targeting ability. These carriers are usually administered through parenteral route and due to their steric phenomenon they conceal themselves from opsonisation event induced by tissue macrophages. By this way they achieve targeting ability to brain and other reticuloendothelial system (RES) organs like liver, spleen, etc.

a) Liposomes: Liposomes are defined as non-toxic, biocompatible and biodegradable lipid body carrier made up of animal lipid like phospholipids, sphingolipids, etc. They possess advantages of carrying hydrophilic, lipophilic as well as amphoteric drug molecules either entrapped inside it or on its micellar surface. The liposomal technology is quite advanced to design with better site specific action. The surface modified liposomes can be used to directly encapsulate drug molecules to diseased tissues or organs. The brain distribution of long circulating liposomes can be modulated by conjugation of appropriate targeting vectors. Examples of brain targeting vectors include monoclonal antibody (mAb to anti-transferrin receptor, mAb to insulin receptor), cationized proteins (cationized human serum albumin), endogenous peptides or plasma proteins. The basic mechanism by which these liposomes achieve brain concentration by crossing BBB is by coupling with brain drug transport vector through absorptive-mediated transcytosis or by receptor-mediated transcytosis. The steric hindrance phenomenon for producing sterically stabilized liposomes has emerged as a new growing advance technology by which brain tumors are being easily cured. The mechanism that lies behind it is the hindrance of liposome from opsonisation and then escape from reticuloendothelial cells. These are also known as long circulatory liposomes due to their longer residence time in blood circulation and consequently increased extravasation probability through the tumor vascular endothelium. The synthetic polymeric materials have been found to be more successful and polyethylene oxide has shown considerable appreciation in tumor targeting. Other synthetic materials being tested include propylene glycol, polypropylene oxide, PEG (polyethyleneglycol) and a block polymer polyoxyethylene-polyoxypropylene which have shown better enhancement in circulation time. After surface modification of liposomes with these substances, they behave as sterically stabilized one due to enhanced hydrophilicity imparted by polymers' hydrophilic chains, lower contact angle between particles and phagocytic cells

of body and due to the lesser interaction between serum opsonins thereby preventing opsonisation.

b) Nanoparticles: nanoparticles have attracted considerable nterest in targeting drug molecules to brain. Nano delivery systems have great potential to facilitate the movement of drugs across barriers (e.g., BBB). Nanosystems employed for the development of nano drug delivery systems in the treatment of CNS disorders include polymeric nanoparticles, nanospheres, nanosuspensions, nanoemulsions, nanogels, nano-micelles and nano-liposomes, carbon nanotubes, nanofibers and nanorobots, solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC) and lipid drug conjugates (LDC). The correct mechanism of barrier opening by nanoparticles is not exactly known. But the delivered nanoparticles enter into the brain by crossing the BBB by various endocytotic mechanisms. The polymeric nanoparticles made from albumin or poly(butylcyanoacrylate) are reported to enter into the brain by their small size mediated endocytosis. Various drug molecules have been successfully delivered using nanoparticles, e.g., dalargin, kyotorphin, loperamide, tubocurarine and doxorubicin. These peptide based molecules were delivered to brain using polybutylcyanoacrylate nanoparticles coated with polysorbate-80. Other drugs which are being experimented include clioquinol, thioflavin-T, D-penicillamine, paclitaxel, tacrine, rivastigmine, dexamethasone, 5-fluorouracil, tubocurarine, loperamide. These nanoparticles travel intact and release the drug in brain microenvironment directly which is finally biodegraded due to endocytotic uptake because of very small size by BBB [32].

vi) Biotechnology based approach

The field of biotechnology has gained considerable interest by the scientists in the various other fields for applicative purposes. There are various examples where concepts of biotechnology were applied including protein engineering, polymerase chain reaction, recombinant DNA, etc. But from the last few years where targeting of drug molecules to brain is a challenge for the pharmaceutical scientists, biotechnology has given a way for effective targeting of neuropharmaceuticals to brain as well as brain tumors.

a) ***Monoclonal antibodies for targeting:*** The monoclonal antibodies (mAb) which had a promising interest in biotechnology now are being used for their application in brain targeting of drugs, proteins and peptides pharmaceuticals. They are generally prepared by hybridoma technology by combining malenoma (tumor) cells with antitumor antibodies against a particular type of antigens found on malignant cells in animals like rat. But instead of using mAb directly for brain targeting, they are modified structurally to get genetically engineered monoclonal antibodies.

b) ***Application of genomics in brain drug delivery:*** The word genome represents the total DNA contained in an organism or in a cell. Hence genomics is defined as the study related with structure and function of genome. This is the concept of modern era of biotechnology where salient features of bioinformatics are applied. It is applied in several aspects like genetic engineering, identification of complete nucleotide sequence of human nuclear genome, identification of genes responsible for producing diseases. Hence it is considered as a more efficient tool in the field of biotechnology, which helps in solving various problems of BBB drug targeting. Majority of the CNS disorders (e.g., Alzheimer's disease, cerebral AIDS, stroke, brain cancer) that have not been beneficially treated by small molecule therapy could be treated with large molecule pharmaceuticals, which do not cross the brain capillary endothelial wall. These drugs are transported through the BBB using gene technologies. It involves genetic engineering to produce monoclonal antibodies that target endogenous BBB transporters, conjugation of sequence-specific antisense radiopharmaceuticals to BBB drug targeting vectors for imaging gene expression in the brain in-vivo, intravenous injection of a non-viral therapeutic gene for expression of an exogenous gene throughout the CNS and discovery of novel transport systems (carrier-mediated transport systems, receptor-mediated transcytosis systems, etc.) expressed at the BBB by BBB genomics program, which enables the delivery of small as well as large molecules to the brain. The most important applications of genomics is in identifying the different molecular vectors, carriers, transporters which express on the membranes of blood–brain barrier and helps in transporting the drug molecules and peptides to brain microenvironment.

c) Molecular ***Trojan Horses (monocytes) for brain targeting:*** Monocytes are the agranulocytic cells present in blood. It may be used as a carrier for entrapping drug inside it to transport into the brain and hence called as "Trojan horses". The basic mechanism involves the loading of drug molecules inside it, by receptor mediated endocytosis when it interacts with suitable ligands. Human insulin receptor (HIR) is found to be the most potent mAb discovered till today and is found to be more effective at BBB. Hence, these Trojan horses are used for delivering drug molecules to brain. Epidermal growth factor, a potential peptide delivery radiopharmaceutical is of wide interest for brain cancer detection or its imaging. It cannot be delivered via i.v. route as it cannot cross the BBB; hence they are given with Trojan horses in combination with rat TfR-mAb (transferrin receptor-monoclonal antibody) for achieving effective brain concentration [32].

Fig. 1. A plan diagram of cerebral capillary that form BBB and their associations with the astrocytes and glia cells. The major paths for the transport of molecules across the BBB are shown. (1) Paracellular pathway: usually, the tight junctions limit transport of hydrophilic compounds, including polar drugs. However, due to aberrant permeability water soluble molecules manage to cross the BBB. (2) Adsorptive transcytosis: cationization increases the uptake of poorly transportable local plasma proteins such as albumin by adsorptive-mediated endocytosis and transcytosis. (3) Transport proteins: the endothelial cells contain carrier proteins for choline, amino acids, glucose, purine bases, nucleosides, etc. The transport by some transport proteins is energy-dependent (e.g., P-glycoprotein) and act as efflux transporters which extrude lipophilic molecules (e.g., Azidothymidine, AZT). (4) Transcellular pathway: it is an effective diffusive route for lipid-soluble agents (most CNS drugs enter via this route). (5) Receptor mediated transcytosis: specific receptor-mediated endocytosis and transcytosis are offered for the transport of certain proteins, such as insulin and transferring.

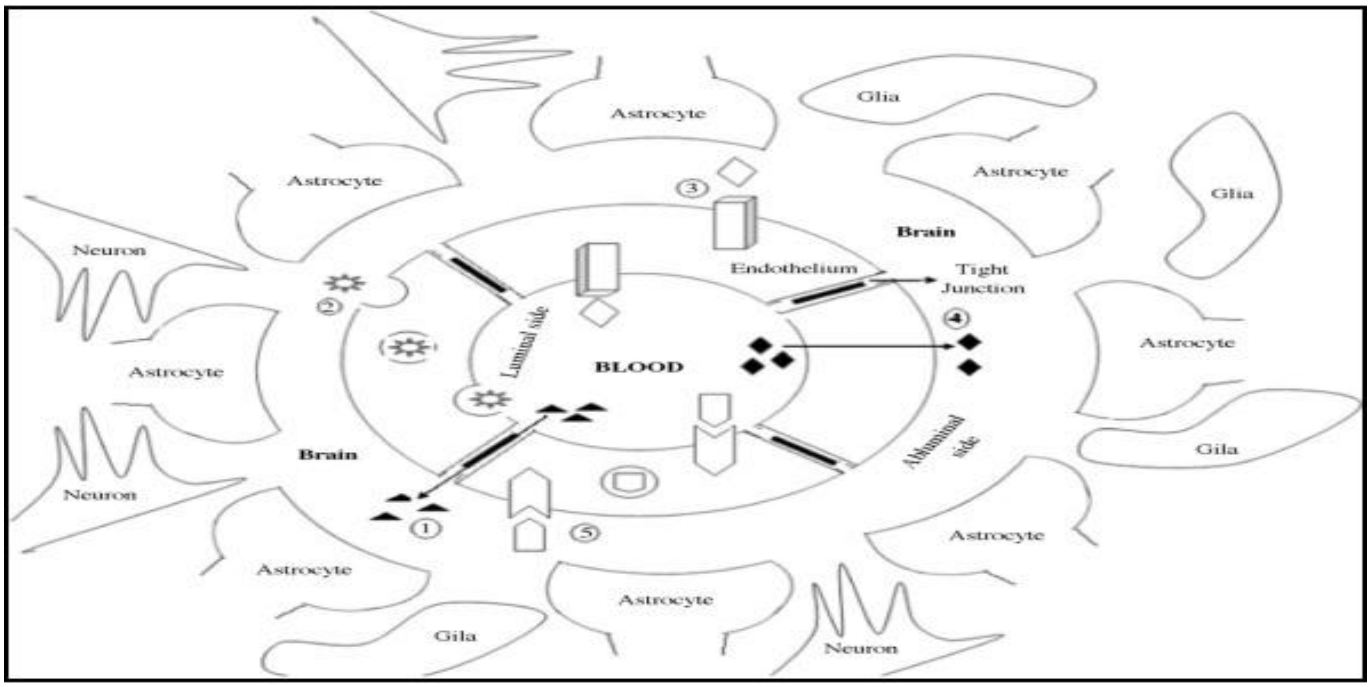


Fig. 2. Overview of different approaches of brain targeting. (ANS – autonomic nervous system; CNS – central nervous system; BBB – blood brain barrier; mAbs –monoclonal antibodies).

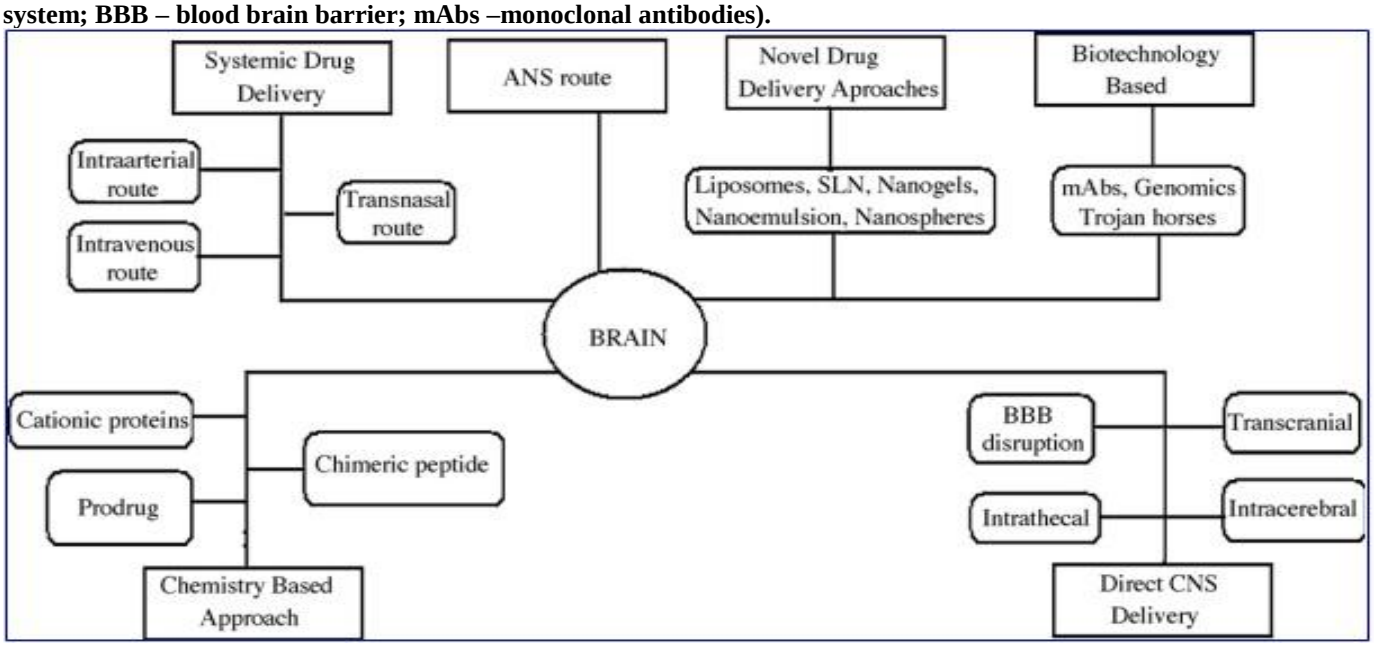


Fig. 3. Schematic representation of the transport of molecules across the BBB.

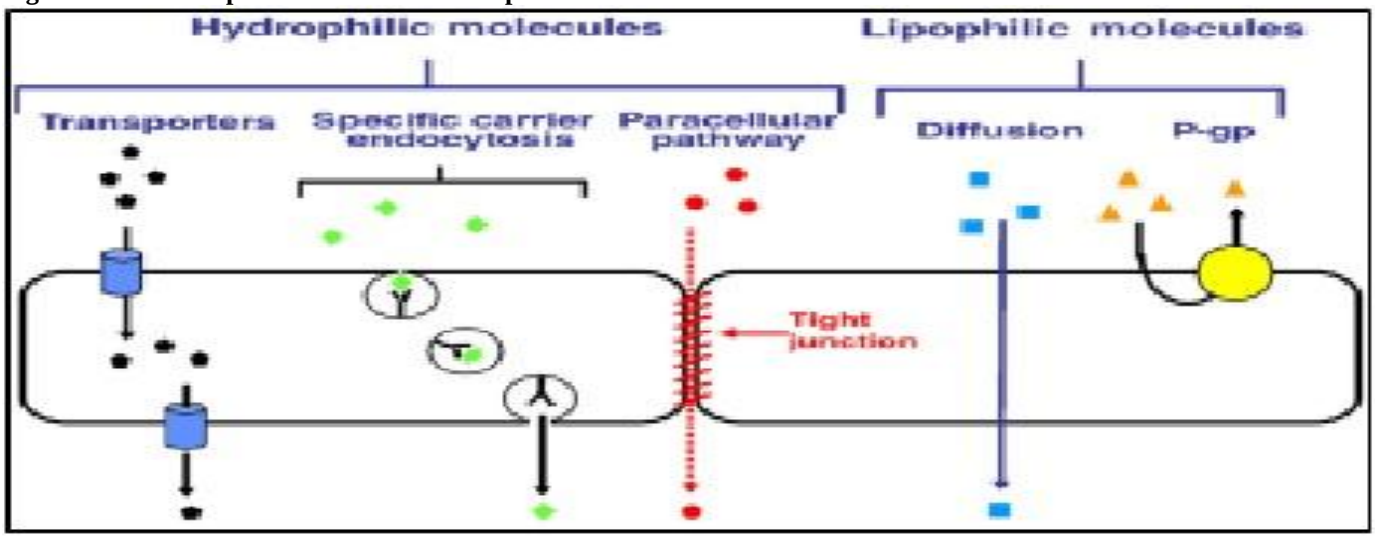
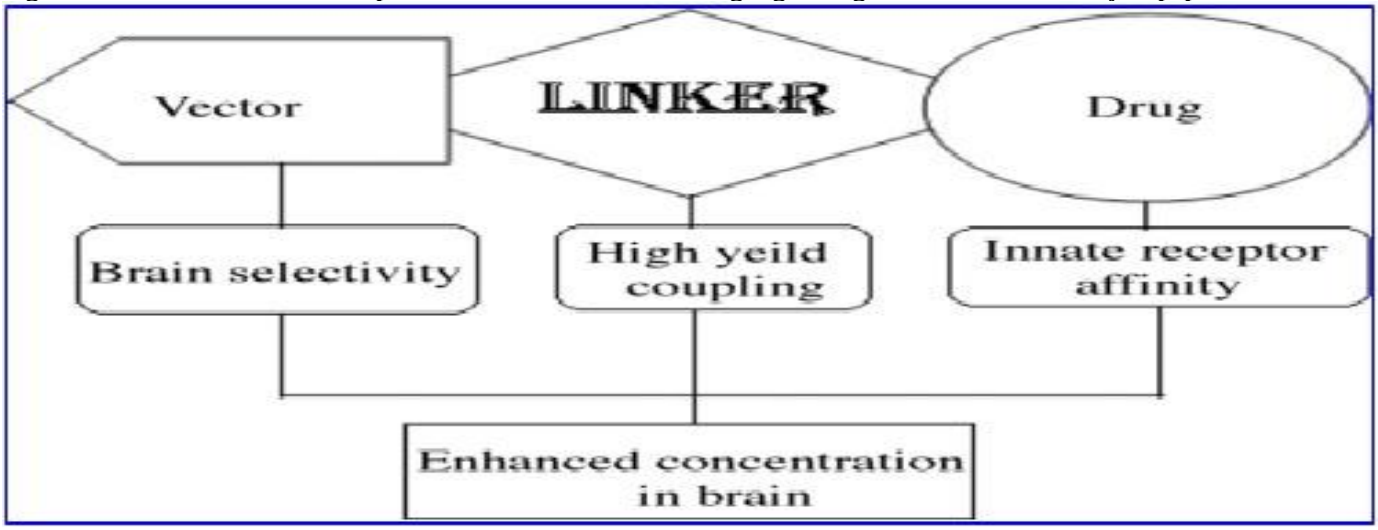


Fig. 4. The three interlinked components; vector, linker and drug together gives enhanced delivery of peptides in brain.



CONCLUSION

In this review the current techniques and new approaches in development to deliver small and large molecules such as biologics to the brain are described. The approaches like BBB disruption, use of permeation enhancers and polymer depots are procuring extensive curiosity in the field of research. Especially several different types of polymer systems used like Gliadel® wafer, Cereport TM have been found to be effective under clinical trials and are also marketed, while many other potential systems are in the childhood stages in various phases of clinical trials. Although a wide range of investigations have been made in the area of brain transporter chemistry and other mediators leading to solving the difficulty in delivery of peptides into brain effectively. But despite of this, the area of transporter chemistry is still a cumbersome and requires potential interest by researchers. Transnasal route of drug delivery give the impression to be superior strategy for achieving enhanced bioavailability in brain. It not only bypasses the BBB and hepatic first pass metabolism (when orally administered) but is also a shortest pathway for rapid drug absorption and quick onset of action. This route also, needs more attention of researchers for safe trafficking of neurotherapeutics to the brain.

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