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Review Article

Ligands as tools for Brain Targeting

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ABSTRACT

Blood brain barrier is one of the most efficient barriers in the body which regulates brain homeostasis and protects the brain from chemical substances via means of tight junctions and thus BBB is considered as the most important barrier in preventing molecules from reaching the brain parenchyma via extensive branches of blood capillary networks. Therefore for decades scientists have tried to bypass this insurmountable barrier and the use of ligands as targeting molecules have provided a sound approach for the delivery. This class of ligands is wide in nature and includes various transporters, receptors, antibodies, surfactants. This article presents various approaches used in design and delivery of nanoparticles to brain mediated by the ligands and shows how these could target the brain and provide drug treatment for diseases such as Schizophrenia, Alzheimer's, Parkinson's.

Keywords: Ligands**;** peptides; blood brain barrier; receptors; transporters

1. INTRODUCTION

Various Brain diseases such as brain tumors, brain tuberculosis, Alzheimer's and Parkinson's cause heavy burden both to the affected individual and the society. Currently, there are 1.5 billion people worldwide afflicted with CNS disease and the number is likely to increase to 1.9 billion by 2020 (Pardridge, 2001) which is a reason for great concern. BBB (Blood Brain Barrier) offers an insurmountable obstacle for the delivery of various drugs to the brain. BBB is composed of the endothelial tight junctions and thereby protect the brain from harmful and toxic substances and at the same time hampers the systemic delivery of therapeutically important drugs from the blood into the brain. It is located at the level of the brain capillaries, where there is a convergence of different cell types: endothelial cells, pericytes, astrocytes and microglia's (perivascular macrophages). The brain micro vessel endothelial cells (BMEC) that form the BBB, display important morphological characteristics such as the presence of tight junctions between the cells, the absence of fenestrations and a diminished pinocytic activity, that together help to restrict the passage of compounds from the blood into the extracellular environment of the brain. Tight junctions provide significant transendothelial electrical resistance to BMEC and impede the penetration of a large number of drugs, such as antibiotics, antineoplastic agents, neuropeptides and other active drugs for the central nervous system(CNS) (Lo, 2001),(Garcia-Garcia, 2005a). For a drug to effectively cross BBB the therapeutically active moiety must be < 400 Da, lipophilic, non-substrate to the efflux system such as P-glycoprotein. Majority of the pharmaceutically active moieties fail to lie in this criterion. Proteins and gene drugs are restricted to enter the CNS from systemic circulation due to their hydrophilicities, protein bound properties and large molecular weights. Therefore, to reach the brain, most substances must cross the BBB through interaction with specific transporters and/or receptors expressed at the luminal (blood) side of the endothelial cells thereby presenting a challenge to the scientists all over the world to make drugs enter the brain. To solve the problem of low bioavailability of drug in brain various approaches have been studied and ligand based targeting has been extensively studied. Targeted drug delivery to selected brain cell types is a crucial step in enhancing therapeutic effects while limiting side effects in non-target cells regions or cells. By coupling drug-loaded vehicles with ligands which specifically recognize receptors on the BBB, the RMT strategy combines the advantages of brain targeting, high incorporation capacity, reduction of side effects, and circumvention of the multidrug efflux system.

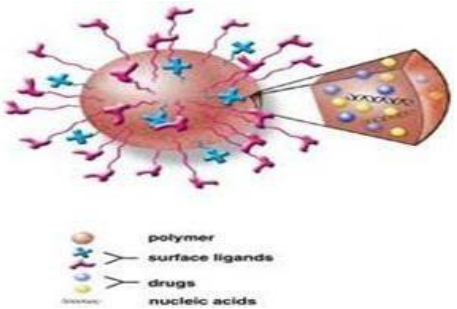

Figure 1: Ligand attached delivery system

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The enhancement of the drug transport through BBB by the coated nanoparticles can be explained by different mechanisms:

(1) The binding of nanoparticles to the inner endothelial lining of the brain capillaries could provide a drug concentration gradient, thus improving passive diffusion, and

(2) Brain endothelial cell uptake of nanoparticles may occur through endocytosis or transcytosis.

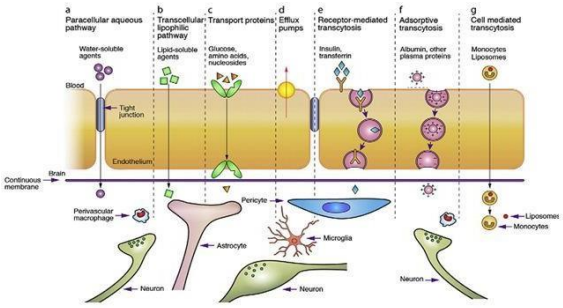

Figure 2: Various transport routes along Blood Brain Barrie (Abbott, 2006)

There are obvious reasons for the design of ligand coated long-circulating drug carriers:

1) Ligand (an antibody, protein, peptide, sugar moiety, folate or carbohydrate) attached to the carrier surface may increase the rate of elimination from the blood and uptake in the liver and spleen. However, the presence of the PEG protecting polymer may compensate for this effect;

2) Longevity of the specific ligand-bearing nanocarrier may allow for its successful accumulation in targets with diminished blood flow or with low concentration of the surface antigen.

Specific ligands on the surface allowed the Nanoparticles to cross the Blood-Brain Barrier (BBB) carrying model drugs within the brain district after their iv administration in experimental animals.

These ligands can be classified as follows:

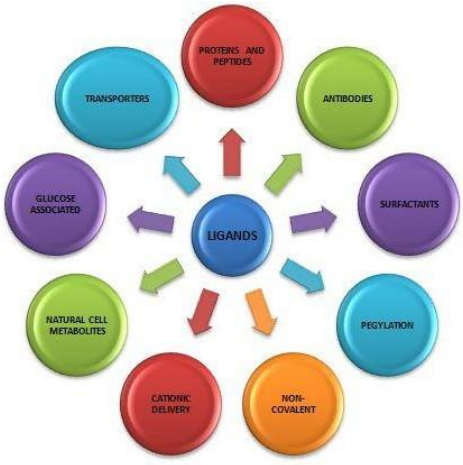

Figure 3: Classification of Ligands

2. Peptides and proteins

These have been extensively studied as ligands for specifically targeting the brain and thereby enhancing the bioavailability in brain. Peptides have many combined advantages: they are smaller than antibodies, can be synthesized by chemical methods at a large scale, and can achieve high specificity (Zhang, 2012). These have been extensively studied as ligands for specifically targeting the brain and thereby enhancing the bioavailability in brain.

2.1 Angiopep-2 peptide and EGFP-EGF1 protein

Initial studies were carried out by Demeule et al who first reported a family of peptides called angiopep, derived from the Kunitz domain of aprotinin. One of these was angiopep-2 (TFFYGGSRGKRNNFKTEEY, molecular weight 2.4 kDa), which possessed a higher BBB penetration capability (Demeule, 2008b). As a small targeting ligand, angiopep-2 has been effectively used in brain targeting. Huele et al performed various In vitro studies and showed that both bEnd.3 cells and neuroglial cells had a higher uptake of angiopep-2 and EGFP-EGF1 conjugated nanoparticles (AENP) as compared to unmodified nanoparticles. Ex vivo imaging was also carried out and it was observed that AENP had higher accumulation in the brain over unmodified nanoparticles and EGFP-EGF1 modified nanoparticles. These peptides employ a cascade type of targeting delivery system with two successive stages of targeting. Angiopep-2 penetrates the blood brain barrier and then EGFP-EGF1 binds neuroglial cells after penetrating the BBB. Tissue factor (TF) could bind with coagulation factor Ⅶ (FⅦ) and initiate the coagulation cascade that results in thrombus (Huang, 2008b, Eddleston, 1993). EGFP-EGF1 is a fusion protein derived from FⅦ which retains the specific TF binding capacity but does not cause coagulation (Huile, 2011) . Recently, Mei et al have also employed EGFP-EGF1 as a targeting moiety and conjugated it with NP and the results showed that they had an ideal targeting effect on thrombus (Mei, 2010) .It was also shown that TF were highly expressed in neuroglial cells while minimally expressed in endothelial cells. Therefore, EGFP-EGF1 is effective during second stage targeting delivering NP to neuroglial cells.

2.2 Cell penetrating peptides

CPPs are positively charged peptides with amphipathic characteristics and are capable of rapidly entering living cells without producing cytolytic effects (Herve, 2008). Due to this they have been successfully used as vectors for delivery of drugs that are P-gp substrates by effectively by-passing the P-gp in the BBB. These have potentially increased doxorubicin transport into the rat brain up to 30-fold (Rousselle, 2000) .

2.3 SynB3 (RRLSYSRRRF)

SynB3 (RRLSYSRRRF) increase brain uptake of poorly brain-penetrating drugs (Adenot, 2007). SynB peptides

are derived from a natural mammalian antimicrobial peptide with high affinity for biological membranes. It is one class of CPPs that have emerged, facilitating the intracellular delivery of polar biomolecules in vitro and in vivo (Chen, 2011). It has been shown to enhance the transport of morphine-6-glucuronide to the brain in a clinical trial (de Boer, 2007). The application of CPPs is based on the premises that biologically active cargo can be attached to CPPs and translocated into cells. The link between the CPPs and cargo is most commonly a covalent bond and seldom in non-covalent bond. A large variety of cargo molecules/materials have been effectively delivered into cells via CPPs, including small molecules, proteins, peptides, fragments of DNA, liposomes and nanoparticles (Zorko, 2005). Some can enter brain capillary endothelia cells or are even translocated into the brain tissues. Some examples are highlighted wherein Adenot and colleagues studied brain uptake of a number of free and SynB3 vectorized chemotherapeutic agents using both in situ brain perfusion and in vitro BBB/cell model (Adenot, 2007). They reported that SynB3's conjugation with various poorly brain-penetrating drugs enhanced their brain penetration by a factor of 30 for doxorubicin, 7 for benzylpenicillin, 22 for paclitaxel, 18 for dalargin and 50 for morphine-6-glucuronide with no effect on tight junction integrity. Brain uptake of the enkaphalin analogue, dalargin, a hexapeptide, was enhanced significantly when conjugated to SynB and injected intravenously in mice (Rousselle, 2003). This study signaled the potential for delivery of peptides or drugs for treatment of brain cancer, through the targeting of brain tissue after systemic delivery.

2.4 TAT

TAT is a HIV-1 trans-activating transcriptor involved in nuclear and nucleolar localization with 101 amino acids. Cao et al studied the efficacy of this peptide ligand by fusing the antiapoptotic protein Bcl-xL to TAT and injecting this into mice that were affected by stroke. The results indicated that the Bcl-xL protein is expressed in adult neurons of the CNS (Cao, 2002). Kilic et al conducted similar studies and showed that brain tissue was progressively transduced with TAT proteins within 3–4 h after intravenous delivery and this treatment reduced infarct volume and neurological deficits after long ischemic insults lasting 90 min (Kilic, 2002). TAT-shuttled nanocarriers were effectively transported across the BBB by TAT-conjugated CdS: Mn/ZnS quantum dots (Qdots) (Santra, 2005) . Histological data clearly showed that TAT-Qdots migrated beyond endothelial cells and reached the brain parenchyma. Liu et al have produced compelling evidence that TAT facilitates human brain endothelia cell uptake of nanoparticles self-assembled from TAT-PEG-*b*-cholesterol in vitro and more importantly, the nanoparticles with TAT were able to cross the BBB and translocate around the cell nucleus of neurons (Liu, 2008). Wang et al, prepared cationic nanoparticles fabricated fromcholesterol-CG_3R_6TAT via self-assembly and they showed that these crossed the BBB and produced antimicrobial activity against the pathological strains in the brain tissue with a similar efficacy as amphotericin B strong antimicrobial activity (Wang, 2010). Qin et al prepared liposomes using cholesterol-PEG_{2000}-TAT (TAT-LIP) and compared them to liposomes fabricated from cholesterol-PEG_{2000} polymer (LLIP) and conventional cholesterol formulation (LIP) in vitro and in vivo and they concluded that TAT-LIP accumulated most in the brain (Qin, 2011) all these studies confirms that nanocarriers conjugated with TAT could be a promising carrier system for transporting drug across the BBB for the treatment of brain disorders.

2.5 Phage-displayed peptides

The in vivo phage display was first introduced by Pasqualini in 1996 (Pasqualini, 1996).It provides tissue-specific peptides as targeting moieties for tumors and organs. The three-amino-acid sequence ArgeGlyeAsp (RGD) is one of the most successful targeting ligands for tumor vascular endothelial cells screened by phage display technique .Wan XM et al gained a peptide sequence (ACTTPHAWLCG) from the brain tissue that can bypass the BBB through the nasal-to-brain passage (Wan, 2009). Rooy et al selected two 15 amino acid-peptides (GLA and GYR) that can bind to the murine brain in an in situ brain perfusion model (van Rooy, 2010). A longer circulation time was obtained by using a consensus sequence of TGNYKALHPHNG (denoted as Pep TGN Phage Clone 12-2 displaying Pep TGN revealed a significant superiority on brain transport efficiency compared with native M13phage.When conjugated on nanoparticles, Pep TGN facilitated the targeted delivery of nanoparticles across the BBB, leading to significant higher bEnd.3 cells uptake and in vivo brain accumulation.

2.6 RVG

Neuronal cells specifically containing acetylcholine receptors specifically bind to a 29 amino acid sequence proteins denoted as RVG and it has been modified to RVG 9 by adding nonamer arginine residues at the carboxy terminus of RVG. When GFP siRNAs were complexed with the positively charged RVG-9R peptide and injected intravenously into GFP transgenic mice for three consecutive days, GFP expression was significantly decreased in the brain but not in the liver or spleen, confirming the specificity of brain targeting. This siRNA-peptide complex has been extensively studied by Pulford et al who incorporated this ligand into a liposome for effective brain targeting as this was specifically taken up by the brain and subsequently exosomes to target brain were developed with RVG 9 as the targeting ligand by Erviti et al (Alvarez-Erviti, 2011).

2.7 Chimeric peptides

Creation of chimeric peptides to which the active agents are conjugated is also investigated for the brain targeting. Such a system has been discussed in US Patent No. 4902505 where the chimeric peptide, such as histone is used to carry drug molecules through the BBB by transcytosis.

3. Glucose associated systems

3.1 Sialic acid and glycopeptides

Sialic acid and glycopeptides conjugated nanoparticles have been used for central nervous system targeting. Sialic acid (SA) residues and its derivatives are known to interact with specific receptors belonging to Siglec family called sialoadhesins, Sn in the brain (Crocker, 1997, Kelm, 1992). After crossing the BBB SA conjugated to Nanoparticles surface interacts with the specific SA cerebral receptors allowing Nanoparticles to be retained for a long time by the brain without being discharged. Tosi et al prepared the Nanoparticles using 25% of PLGA conjugated with glycopeptides g7 and SA and these were loaded with Loperamide (LOP) and Rhodamine(ROD) . LOP was chosen as model drug since it is unable to exert any antinociceptive effect when administered iv, being unable to cross the BBB (Tosi, 2008, Tosi, 2007, Garcia-Garcia, 2005a). ROD was used since this fluorescent dye is able to cross the BBB only in a negligible amount and it is quickly metabolized after iv administration .g7 causes the nanoparticles to cross the BBB and then SA interacts with their receptors in the brain. It was shown that with SA modification length of the pharmacological effect can last upto 24 h supporting the hypothesis on the possible interaction with the brain sialoadhesin receptors. Therefore SA-g7-Np has been shown to cross the BBB and remain within the brain parenchyma for a prolonged time (Tosi, 2010).

3.2 Glu-cholesterol as ligands

Nutrients are transferred into the brain by their respective receptors present in the BBB such as large neutral amino acid transporter (LAT1), monocarboxylic acid transporter (MCT1) glucose transporter (GLUT1) (W., 2007). It has been established by various studies that the glucose consumption of the brain is about 30% of the whole body glucose consumption, and the brain endothelium transports about ten times its weight of glucose per minute(Dick, 1984) . Therese et al have indicated in their experimental studies the use of c6 substituent of glucose analogues as more effective substrates for GLUT-1 receptors and this concept has been utilized by Yong et al to increase the concentration of Tegafur, an anti-cancer drug for brain tumors in the brain by encapsulating it into the liposomes which have been modified as The cholesterol links glucose residue via ethylene glycols at C-6 position of glucose. The tegafur concentrations in brain following administration of coupled liposomes at different time intervals was found to be about 2.5-7.0 times higher in comparison to uncoupled liposomes(Lei, 2011).

3.3 Lactoferrin

Lactoferrin (Lf) is a mammalian cationic iron-binding glycoprotein belonging to the transferrin family Proteins such as lactoferrin and transferring could be effectively used for specific brain targeting. The uptake of Lf-modified vectors and NPs by brain capillary endothelial cells (BCECs) is related to clathrin-dependent endocytosis, caveolae-mediated endocytosis, and macropinocytosis. Lf has been reported to transport across the BBB through a unidirectional receptor-mediated process (Fillebeen, 1999).Huang etal prepared polyamidoamine (PAMAM)-based non-viral gene vector to the brain and lactoferin was investigated as the brain targeting ligand and it was found that the brain gene expression of the PAMAM-PEG-Lf/DNA complex was about 2.3-fold when compared to that of the PAMAM-PEG-Tf/DNA complex(Huang, 2008a). Huang etal prepared nanoparticles for specific delivery of DNA into the cells using Lactoferin as the ligands and Nanoparticles with Lactoferin on their surface showed increased efficacy (Huang, 2009). Ji et al demonstrated that the brain uptake of Lf in rats was much greater than that of transferrin and OX26 (Ji, 2006). Hu etal showed that when Lf-conjugated PEG-PLA nanoparticles were administrated to mice intravenously, there was a 3-fold increase in brain uptake (Hu, 2009). Hu etal also assessed the biodistribution of coumarin-6-loaded Lf-conjugated PEG-PLA nanoparticles in mice and therapeutic efficacy of urocortin-loaded Lf nanoparticles on a 6-OHDA rat model of Parkinson's disease (Hu, 2011) . Their data showed 2.5 times increase in AUC by Lf nanoparticles compared to the unconjugated nanoparticles in 24 h. They concluded that the Drug-loaded Lf-nanoparticles were successfully taken up by the brain and produced therapeutic efficacy.

3.4 Transferrin (Tf)

The Tf receptor is a transmembrane glycoprotein consisting of two subunits of 90 kDa linked by a disulfide bridge. Each subunit can bind one transferrin molecule (Moos, 2000).Transferrin is considered to have a great potential in brain targeting and is extensively employed as a ligand (Li, 2002). The Tf receptor is of particular interest because its expression in capillaries throughout the body is restricted to brain capillaries (Jefferies, 1984).It must be dually noted that the transferrin receptor is expressed both at the BBB and neuronal cell membrane (Schlachetzki, 2004). The transvascular delivery of genes is possible by accessing transferrin-conjugated transport systems, which might cross the BBB and deliver genes to the doorstep of every neuron in the brain (Shi, 2000, Zhang, 2003) . Harashima etal have also demonstrated that the Tf-conjugated DNA-loaded complexes are more easily released into the cytoplasm and this type of interaction

with the receptor-ligand is considered to be a physiological process.(Harashima, 2001) Huang showed that the conjugation of Tf at the tip of the PEG tail profoundly increased the targeting capability of PAMAM-PEG-Tf to the brain as well as the greatly increased gene expression in the brain (Huang, 2007) . Soni etal Tf-liposomes was used for the delivery of antimetabolic drug 5-fluorouracil (5-FU) to the brain .In vivo experiments by the workers revealed that their accumulation within Brain was 13-fold higher than that of non-modified liposomes (Soni, 2005).

3.5 P97

Another interesting targeting molecule is melanotransferrin (P97) which has been reported to undergo active transcytosis, possibly via the LRP1 receptor (Demeule, 2002) . It was revealed that recombinant human melanotransferrin (P97) was readily taken up by mouse brain following intravenous injection and in situ brain perfusion. This P97 transcytosis across the bovine brain capillary endothelial cell monolayers was at least 14-fold higher than that of transferrin, with no apparent intra-endothelial degradation. When the effectiveness of P97 as a brain targeting ligand was tested in a mouse model, P97 and P97-adrimycin conjugates showed 6–8-fold higher than that of BSA or lactoferrin in terms of transport into the brain. More importantly their efficacy against intracranial rat C6 glioma and human ZR-75-1 mammary tumors in athymic mice was very significant with improved survival rates compared to free adriamycin (Gabathuler, 2005). This technology has now been patented worldwide and the technology (NeroTrans^{TM} transporter platform) is the proprietary property of Raptor Pharmaceutical Corp (Novato, CA). In June 2009, Raptor and Roche entered a collaboration and licensing agreement to evaluate the delivery of Roche's investigative molecules attached to Raptor's proprietary NeuroTrans™ transporter platform.

4. Transporters

The drug must be reformulated such that the drug assumes a molecular structure mimicking that of the endogenous ligand (Pardridge, 2003).

4.1 Insulin receptor

Insulin is an important hormone secreted by the B-cells of the pancreas and is involved in glucose homeostasis. The insulin receptor is a large protein having a molecular weight of 300 kDa and is a heterotetramer of two extracellular alpha and two transmembrane beta subunits (de Boer, 2007). Pardridge etal tested a genetically engineered human/mouse chimeric form of the human insulin receptor monoclonal antibody (HIRMAb) on an adult anesthetized Rhesus monkey and they concluded that humanized HIRMAb was rapidly transported into all parts of the primate brain after intravenous administration, suggesting that it can also be used for delivery of various drugs and genes across the BBB in human(Boado, 2007) .

4.2 GLUT-1 (Glucose) Transporter

Glucose is the main source of energy in the body and the human brain consumes around 30% of the total body glucose consumption (Dick, 1984). The GLUT1 isoform is mainly expressed in the luminal surface of brain capillaries as well as the choroid plexus (Tsuji, 2005). The GLUT1 transporter promotes the transport of d-glucose from the blood to the brain. Like most of the nutrient transporters, it mediates the passage through the BBB of substances exhibiting similar structures, including 2-deoxyglucose, galactose, mannose, and glucose analogs(M., 1995) . This high intake of the glucose by the brain tissues is due to the reason that d-glucose transporters are widely present and these are referred to as GLUT-1, passive glucose transporter is an extremely useful carrier for efficient and selective glucose-targeted drug delivery to the brain as this transporter is located in the Brain capillary endothelial cells composing the blood brain barrier Halmos etal (Halmos, 1996) in 1997 prepared chlorambucil-glucose derivatives and indicated that GLUT 1 mediated transport of glucose. Dufesa etal prepared Glucose-appended niosomes to deliver vasoactive intestinal peptide (VIP) to the brain and concluded that VIP reach the brain parenchyma in an intact form after encapsulation in a glucose-targeted system and allowed a significantly higher VIP brain uptake compared to control niosomes (up to 86%, 5min after treatment) (Dufes, 2004).

4.3 CRM197

It is a mutated form of Diptheria Toxin in which the enzymatic activity responsible for the toxic effect is missing due to a single point mutation at position 52 (Gly→Glu). Because of its non-toxic, yet, ability to bind to DT_R (Kaefer, 2000), makes CRM197 a useful and attractive targeting ligand. CRM 197 uses DT_R as its transport receptor and delivers its cargo molecules across the BBB by means of receptor mediated-transcytosis (Gaillard, 2005). It has been shown that CRM197 can achieve brain targeting in vitro and in vivo. It has been proved to be a safe and effective carrier protein for human vaccines and has been successfully tested as a therapeutic protein for cancer treatment in a clinical trial (Anderson, 1983, Buzzi, 2004) .Yung-Chih etal investigated the capability of CRM197-grafted polybutylcyanoacrylate (PBCA) nanoparticles (NPs) (CRM197/PBCA NPs) to carry zidovudine (AZT) across the blood–brain barrier (BBB) and found that CRM197 enhanced the permeability coefficient of AZT across the BBB and the uptake quantity of AZT-loaded CRM197/PBCA NPs by human brain-micro vascular endothelial cells (Kuo, 2012).

4.4 Type 1 large neutral amino acid transporter (LAT1)

LAT subtypes are sodium-ion-independent transporters of large neutral amino acids (as an example, phenylalanine, tyrosine or leucine) and are expressed in the BBB (Tamai, 2000).These are basically two types: LAT1 and LAT2 but only LAT 1 is widely characterized and used for brain targeting (Pardridge, 2007). Large neutral amino acids (LAT1) displays several properties that make it well suited as a brain drug delivery vector which includes a large Michaelis–Menten maximal transport capacity (V_{max}~40–60 nmol/min/g) and an appreciable binding affinity (affinity≈1/K_m,; K_m=10–200 µM) so that rapid rates of BBB exchange can be obtained (K_{in}>10^{-3} ml/s/g) with half times for brain equilibration of <15 min for high affinity substrates (Smith, 1993)

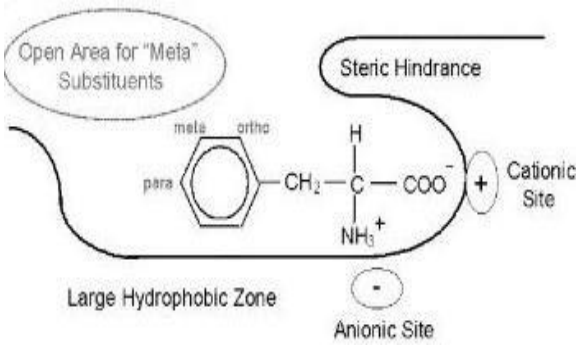

Figure 4: Model of the binding site of the BBB LAT1 in relation to the amino acid, phenylalanine (Smith, 2005).

Table 1: Various drugs utilizing lat 1 transporters

S.No	Drugs delivered via lat1	Use	Reference
1	L-Dopa	Parkinson disease	(Mena, 1975)
2	Melphalan	brain cancer	(Cornford, 1992)
3	L-methyl-DOPA	high blood pressure	(Markovitz, 1977)
4	Gabapentin	Epilepsy	(Uchino, 2002)

4.5 Choline transporters

These transporters have anionic sites as the binding areas and as such strongly interacts with cations thus actively deliver choline. neurotransmitor acetylcholine to the brain .due to their ability to bind with the positively charged species they actively deliver drugs such as carnitine and thiamine into the brain (Béduneau, 2007). fenart etal prepared dipalmitoyl-sn-glycero-3-phosphatidylcholine coated nanoparticles and observed that their passage through the endothelial cell monolayer was three- or four-fold higher than that of uncoated nanoparticles, without any modification of paracellular permeability, suggesting the effect of the BBB choline transporter (Fenart, 1999). Lockman etal prepared solid nanoparticles and coated with thiamine (thiamine-PEG-DSPE). The brain uptake transfer coefficient (Kin) of thiamine nanoparticles was significantly greater than that of untargeted nanoparticles (Lockman, 2003)

4.6 Low-density lipoprotein receptor related proteins 1 and 2 (LRP1 and LRP2 receptors)

4.6.1 Angiopeptides

Angiopeps belong to a family of peptides which are derived from Kunitz domains of aprotinin and other human proteins. They are known to have affinity for LRP receptor (Demeule, 2008b)The most studied is angiopep 2 with a sequence TFFYGGSRGKRNNFKTEEY and molecular weight of 2.4 kDa and it has shown greater transcytosis capacity and parenchymal accumulation than transferrin, lactoferrin, and avidin (Demeule, 2008a). Ke etal prepared their dendrimers and confirmed their ability in efficiently facilitating nanocarrier transport across the BBB (Ke, 2009) shao etal prepared amphotericin B-loaded polymeric micelles and proved their efficacy in drug transport across BBB (Shao, 2010). Thomas etal chemically conjugated angiopep 2 with 3 molecules of paclitaxel (ANG1005) and it was shown to be particularly effective in enhancing drug uptake into the brain, with an 86-fold increase compared to the free drug using an *in situ* rat brain perfusion model(Thomas, 2009).

4.6.2 Apolipoprotein

Apolipoproteins B and E (apo B and apo E) showed a rapid onset of the antinociceptive reaction and, moreover, even statistically higher effects …2p, 0:05† than polysorbate 80 alone. With apo E this effect remained high for the entire observation period. Apo E-overcoating achieved a prolonged anti-nociception. In these experiments apo E induced slightly higher antinociceptive effects than apo B (Kreuter, 2002). Luck (1997) observed that after incubation of nanoparticles coated with apo E alone in the plasma, the apo E was replaced to a significant extent by other plasma components. Kreuter et al (1997)(Kreuter, 1997) indicated that the nanoparticles which are coated with apo B or E appear to mimic lipoprotein particles and thus are able to interact with members of the LDL receptor family followed by their endocytotic uptake. The assumption of LDL receptor family-mediated uptake by the brain capillary endothelium is further supported by other in vitro findings: Ramge et al (2000) (Ramge, 2000). The apolipoprotein- overcoated nanoparticles thus would mimic lipoprotein particles and could interact with and then be taken up by the brain capillary endothelial cells via receptor-mediated endocytosis. In this scenario, nanoparticles would act as Trojan Horses for bound drugs. The drug then may be further transported into the brain by diffusion following release within the endothelial cells or, alternatively, by transcytosis. This, however, does not imply that apolipoproteins E or B themselves are taken up together with the nanoparticles. The apolipoproteins also

merely could facilitate the interaction of the particles with the endothelial cells. Apo A-I would be anchored on the surface of the nanoparticles allowing the interaction with the scavenger receptor class B type I located at the BBB and then the translocation of the nanoparticles (Petri, 2007a). Ku etal prepared Angiopep-modified complexes for specific brain targeting to enhance the uptake of gene drugs in the brain. It was observed and proved with the help of various experiments that the transferring efficiency of PAMAM-PEG-Angiopep/DNA was much higher than PAMAM/DNA. It was also conclude that the brain distribution of PAMAM-PEG-Angiopep/DNA in mice was higher than PAMAM/DNA. These results suggested that PAMAM-PEG-Angiopep can be exploited as a potential non-viral gene vector targeting to the brain via non-invasive administration (Ke, 2009).

5. Surfactants

Various workers demonstrated that the surfactant coated nanoparticles can successfully transport drugs across the BBB (Das, 2005, Kreuter, 1995, Schroder, 1996, Schroeder, 1998). Coating of the particles with polysorbates, especially polysorbate 80 (Tween 80) has been widely characterized and coating of nanoparticles with these materials leads to the adsorption of apolipoprotein E from blood plasma onto the nanoparticles surface which then seem to mimic low- density lipoprotein (LDL) particles and interact with the LDL receptor leading to their uptake by the endothelial cells lining the BBB (Gessner, 2001).

Two types of mechanisms may encompass the release of drug into the BBB either the drug bound to the nanoparticles may be released in these cells and then diffuse into the interior or the nanoparticles may as such be transcytosed. It has also been hypnotized that processes such as tight junction modulation or P-glycoprotein active efflux system may occur resulting in brain uptake of nanoparticles. Kreuter etal has evaluated many different surfactants (Kreuter, 1997) and only Tween 80 overcoat has been able to produce the most brain targeting effect via intravenous administration and the specific role of Tween 80 in brain targeting has also been conclusively proved.(Sun, 2004) T2P2 PBSþdrugþnanoparticlesþTween (2%)þPEG (2%) showed the maximum anti-nociceptive effect after dosing scoring a % MPE of 93.8+_6.58 thus concluding that surface engineered PBCA-NDSs with overcoats of Tween 80 and PEG 20000 represent a feasible method to deliver and target peptides to brain via the oral route. Olivier et al have suggested that the PBCA nanoparticles coated with PS80 displayed some toxic effect toward the BBB and that these nanoparticles could open the tight junctions between endothelial cells in the brain microvasculature, thus creating a paracellular pathway for nanoparticles translocation (Olivier, 1999). Kreuter has also proved that apolipoproteins (Apos) could be involved in the brain penetration of PBCA nanoparticles overcoated with PS80. Various drugs which have been used in the transport of the drugs achieved via coating of surfactants have been shown below,

Table 2: drugs targeted using polysorbate

S.No	Surfactant	Drug used	Reference
1	Polysorbate 80	Dalargin	(Alyautdin, 1995)
2	Polysorbate 80	Dalargin	(Kreuter, 1995)
2	Polysorbate 80	Kytorphin	(Schroeder, 1998)
3	Polysorbate 80	Loperamide	(Alyautdin, 1997)
4	Polysorbate 80	Tubocurarine	(Alyautdin, 1998)
5	Polysorbate 80	Doxorubicin	(Friese, 2000)
6	Polysorbate 80	NMDA receptor antagonist MRZ 2/576	(Gulyaev, 1999, Petri, 2007b)

6. Antibodies

6.1 OX26

It is an antibody directed against the Tfr which binds to a different site from that of transferring which gives it the advantage of being less affected by / or interfere with endogenous transferring. Ulbrich et al studied human serum albumin (HAS) nanoparticles with covalently coupled transferrin or transferrin receptor monoclonal antibodies (OX26 or R17-217) for brain delivery of loperamide, a molecule that is normally unable to cross the BBB on its own (Ulbrich, 2009). Results showed that significant anti-nociceptive effects were detected with loperamide-loaded HAS nanoparticles with covalently bound transferrin or the OX20 or R17-217 antibodies following iv injection

6.2 RI7217

It is an anti-rat TfR monoclonal antibody which is more selective for the brain, as it is poorly taken up by the liver and kidney (Lee, 2000). Ulbrich et al covalently coupled RI7217 to human serum albumin nanoparticles and found that it is able to transport loperamide across the BBB (Ulbrich, 2009) Rooy et al have prepared liposomes conjugated with R17217. The RI7217 liposome dose found in the parenchyma fraction was 2.6 times higher compared to the untargeted liposomes. The uptake in the brain capillaries was up to 10 times higher compared to the untargeted liposomes, and the uptake in the brain parenchyma was up to 4.3 times higher. The experiments clearly indicate the potential of RI7217 as a ligand for specific targeting to the brain (van Rooy, 2011)

7. Pegylation

Calvo etal have suggested that PEG-PHDCA nanoparticles penetrate into the brain to a greater extent than all the other nanoparticles formulations tested (Calvo,

2001). Various other workers by in vivo studies have evidenced that PEG-PHDCA nanoparticles (without drug loading) were able to penetrate into the brain of healthy animals in a significantly higher proportion than other colloids(Brigger, 2002, Calvo, 2001). Garcia hypnotized that endocytosis was the reason for cellular uptake (Garcia-Garcia, 2005b). This opens new perspectives for treatment of various neurodegenerative diseases like allergic encephalomyelitis (EAE), prion diseases, brain tumors by using these PEG-PHDCA nanospheres. It has been concluded that the PEG-PHDCA nanoparticles reached the brain by two mechanisms: passive diffusion due to the increase of the BBB permeability and transport by nanoparticles-containing macrophages, which infiltrated these inflammatory tissues. The PEGylated PHDCA nanoparticles accumulated in the brain at a 4–8 fold higher concentration than the non-PEGylated PHDCA nanoparticles after intravenous injection into rats with 9 L gliosarcoma.

8. Natural cell metabolites

Polyamines/natural cell metabolites are unevenly distributed in the brain. Various workers have postulated that despite being water soluble, cationic like molecules, polyamines are able to cross the blood brain barrier effectively(Khan, 1991, Shin, 1985). Poduslo etal has postulated that the natural cell metabolites like putrescine, spermidine and spermine seem to be successful in enhancing the ability of a drug to penetrate the blood brain barrier .This depends upon the covalent linking of a protein to polyamines (Poduslo, 1996, Wengenack, 2000) or through synthetic insertions of asparagyl/glutamyl-4-amino-butane(Poduslo, 2004).

9. Non-Covalent Delivery

A recent approach for the delivery of drug to the brain is the use of non-covalent delivery. Sarkar etal synthesized a bi-partite peptide comprising the ApoE peptide linked with sixteen lysine residues (henceforth K16ApoE), and it was evaluated for its potential to cross the BBB. K16ApoE-mediated non-covalent delivery of three different proteins (beta-galactosidase, IgG and IgM) across the BBB was investigated and demonstrated the ability of a synthetic peptide, K16ApoE, as a transporter of target proteins in the brain employing a simple 'mix-and-inject' approach. This does not need to be chemically linked to a protein 'load' to be transported across the BBB. Thus, K16ApoE is regarded as a universal protein delivery agent for the brain. Transport of proteins to the brain by K16ApoE was evaluated by employing three different strategies (enzyme activity, radiologic imaging and immunostaining), and by delivering three different proteins of diverse molecular weight intense beta-galactosidase staining in virtually all areas of the brain, and that most or all beta-amyloid plaques in the brain become labeled with antibodies delivered in the AD mice through the transporter (Sarkar, 2011).

10. Cationic delivery

Charged molecules can easily enter the brain by electrostatic interaction with anionic functional groups present on brain surface therefore it provides an additional advantage for delivering such molecules on the surface. After cationization they easily enter by using the transcellular adsorptive-mediated endocytosis pathways. Cationization increases positive charge on the polypeptide by means of modifying the free carboxyl groups of acidic amino acid residues on a polypeptide. Modification with hexamethylenediamine, polylysine, diazomethane or polylysine cationization with cleavable ester bonds of these free carboxyl groups of the polypeptide such as IGF-I, IGF-II, NGF enhances BBB transport (E., 1997). Schermann has shown by his studies that these cationic proteins/peptides including cationized antibodies are used to protect the brain against viral antigens or oncogenes in tumors or to image specific antigens in tumors or amyloid deposits in patients suffering from Alzheimer's disease(Scherrmann, 2002). Various other cationic proteins SUCH as avidin, histone, protamine and cationized polyclonal bovine immunoglobulin have been reported to penetrate the BBB(Alam, 2010).These are postulated to enter the brain via an adsorptive-mediated mechanism and Poduslo and Curran by their studies have demonstrated that polyamine modification of proteins (insulin, albumin and IgG) can dramatically increase the permeability of proteins at the BBB with 1.7–2.0 fold increase for insulin, 54–165 folds for albumin and 111–349 fold for IgG in normal adult rats. Lu et al prepared cationic bovine serum albumin (CBSA) conjugated PEG-PLA nanoparticles (CBSA-NP) and compared this to native PLA bovine serum albumin conjugated nanoparticles (BSA-NP) and CBSA unconjugated PEGylated nanoparticles (NP) in brain transcytosis across the BBB coculture and brain delivery in mice using a fluorescent probe (Lu, 2005). The studies confirmed that adsorptive-mediated mechanism is responsible for brain delivery of CBSA-NP. It was also postulated that by Increasing the surface density of CBSA conjugated per nanoparticle the transcytosis ability of nanoparticles was increased while their blood AUC decreased. With the optimized CBSA number conjugated per nanoparticle of 1:10, CBSA-NP achieved the highest % injection dose/g brain by 2.3-fold compared with NP. Therefore efficacy of this delivery system in increasing brain drug concentration has been well proved and hypnotized.

11. P-gp Inhibitors

P-gp acts as a transport protein by expelling drugs from the cytoplasm to the extracellular location (Borst, 1997). Another hypothesis for the p-gp action is the hydrophobic vacuum cleaner model which suggests that P-gp binds directly to the substrates on the plasma membrane and pumps them out of the cell by recognizing them as foreign to the membrane(Fardel, 1996). P-Gp inhibitors also referred to as the reversal agents

inhibit P-gp mediated drug transport and increase the intracellular concentration of the therapeutic agent thereby these agents can be used along as ligands to improve the efficacy of the administered molecule. Pluronics are the most suitable agents and the concept of using of Pluronic® block (or poloxamers) copolymers to inhibit the P-gp efflux pump was derived from an early study conducted in Kabanov laboratory by Miller et al these have the ability to enhance the BBB permeability of a wide range of drugs, including doxorubicin, etoposide, taxol, 3'-azido-3'-deoxythymidine, valproic acid and loperamide, in the bovine brain microvessel endothelia cell monolayer as studied by miller (Miller, 1997)thus it can be concluded by studies conducted by various et al that Pluronic copolymers could be used in the formulation of all these types of drug delivery systems, providing additional function as a biological response modifier(Batrakova, 2008)

11. CONCLUSION

Therefore having extensively studied the targeting ligands we can conclude that to successfully target the brain, the selectivity of a BBB receptor is extremely critical. Wide ranges of ligands are available each with their own advantages and disadvantages but the selection should be based on the specific use desired. This means, ideally, the receptor should be brain specific, or at least, preferentially expressed at the BBB. In addition to this Brain-targeted delivery systems must be assessed for their safety, risk and benefit for patients and the delivery system, including the targeting ligands should be designed based on knowledge of the diseased BBB.

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