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Potential of surface functionalized nanoparticles for improved therapy of refractory central nervous system disorders

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ABSTRACT

During the last decade pharmaceutical drug delivery research has been focused on enhancement of the drug efficacy by improving their selectivity to the target organ in the body. In most of drug delivery system, only a small fraction of the administered dose of the drug reaches to its therapeutic site of action, central nervous system (CNS) disorders likewise not devoid of this conception. The incidence of central nervous system disorders in humans increases with age; this, along with the fact that very few drugs can cross the blood brain barrier (BBB), exacerbates the problem of drug delivery to the central nervous system, as it act as major impediment for drug penetration in to brain. One of the virtually promising approach is to deliver therapeutic agents to the brain by using nanoparticles. Nanoparticulate drug delivery systems can pervade across the blood brain barrier and target to the central nervous system by modifying the surface and sequestering different ligands to them. Nanoparticles (NPs) in this regard can serve as a potential module for ferrying large doses of drugs across the blood brain barrier by various approaches like inhibiting P-glycoprotein efflux pump, adsorptive mediated endocytosis, etc. The current review explores the different impediments for brain drug delivery, diverse possible mechanisms by which the NPs can effectually deliver potential bioactive agents to the central nervous system and the prospects of surface modified NPs to enhance the transport of therapeutic agents across the brain, providing refined drug delivery.

Key words: Blood Brain Barrier, Nanoparticles, P-glycoprotein

INTRODUCTION

The incidence of central nervous system disorders in humans increases with age; this, along with the fact that only a few drugs can cross the blood brain barrier (BBB), exacerbates depleted drug delivery to the brain. It is predicted that by 2020, the number of people in the US alone aged >65 years will increase by 50%,[1] which will lead to concurrent increase in central nervous system disorders. This would result in an annual expenditure in the US for Alzheimer's disease alone of $ US 0.5 trillion. Because one in every three people will experience a nervous system disorder during his/her lifetime so that the neuropharmaceutical market is portended to become the most orotund sector of the pharmaceutical industry.[2] Important egress is that most central nervous system diseases, such as Parkinson's (PD), Alzheimer's (AD), multiple sclerosis and amylotropic lateral sclerosis do not respond to small molecule therapeutics.[3] Study conducted by Gosh et al.[4] revealed that from the 7000 drugs in the Comprehensive Medicinal Chemistry database,[5] only 5% are used for the treatment of brain diseases (i.e. mainly depression, schizophrenia and insomnia). In another study[6] Lipinski et al. has shown that although 12% of drugs are contrive to act specifically on the central nervous system but from that only 1% are active in the brain for intervention of only affective disorders, virtually brain diseases are largely refractory to small molecule drug therapy. (Table 1)

Table 1. List of central nervous system disorders largely refractory to small molecule drug therapy [7, 8, 9]

Central Nervous System disorders largely refractory to small molecule drug therapy
Neurodegenerative diseases : Alzheimer's, Huntington's, Parkinson's disease Inflammatory diseases: Amytrophic Lateral Sclerosis (ALS), Multiple sclerosis Neuro-AIDS, Brain cancer, Stroke, Brain or Spinal cord trauma, Inherited ataxias, Blindness, Cerebro vascular disease.

Treating central nervous system diseases is a huge challenge because of the presence of various obstacles.[10,11] The major impediment for brain drug delivery is BBB. Apart from BBB, Blood-Cerebrospinal Fluid Barrier (BCFB) and various efflux transporter proteins also obstruct the ingress of extraneous elements into the central nervous system.[12] These protective barriers throttle the entry to the brain from the periphery, of variety of compounds including therapeutically active agents utilized for the treatment of fatal central nervous system diseases, such as brain tumors, HIV encephalopathy, epilepsy, cerebrovascular disease and neurodegenerative disorders, and of other pathologies. The existing conventional drug delivery systems are inefficient in selectively targeting drugs to the central nervous system. Preventing the drug from penetrating into brain by aforementioned impediments results in the low efficacy of many potential therapeutic agents.[14, 15] With this regard, aggressive research efforts have been riveted on the development of new drugs as well as novel drug delivery strategies for more effectual delivery of variety of drug molecules to the brain.[13]

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The developmental work for new drugs for the treatment of central nervous system disorders has not kept pace with progress in molecular neurosciences because most of the newly discovered drug molecules are unable to cross the BBB. The clinical failure of brain / central nervous system diseases may be attributed largely to a lack of appropriate drug delivery systems. Localized and controlled delivery of drugs at their desired site of action is preferred because it reduces toxicity and increases treatment efficiency. During past decade, considerable endeavors were made in the field of brain-targeted drug delivery. Various more or less sophisticated approaches, such as intracerebral delivery, intracerebroventricular delivery, intranasal delivery, BBB disruption, nanoparticles, receptor mediated transport (vector-mediated transport or 'chimeric' peptides), cell-penetrating peptides, pro-drugs, and chemical delivery systems and many more approaches have been attempted. These approaches may offer many intriguing possibilities for brain delivery and targeting, but only some have been strove at the phase where they can provide safe and effective human applications.[16, 17]

From the aforementioned approaches substantial research work in the area of particulate carrier systems are currently going on to enhanced brain drug delivery. Particulate systems like nanoparticles, microspheres etc. have been used as a physical approach to modulate and improve the pharmacokinetic and pharmacodynamic properties of diverse types of drug molecules.[9] Nanoparticles can serve as a potential module for ferrying large doses of drugs across the BBB. This delivers high therapeutic payload of drug in the brain, availing to obtain optimal therapeutic response with the commencement of minimal side effects.[18, 19]

Special attention on nanoparticles based drug delivery was given in the years between 1970 and the early 1980s. Further developments resulting from these works were also followed, and riveted on especially interesting improvements such as nanoparticles for the delivery of drugs across the BBB and PEGylated nanoparticles with a prolonged blood circulation time.[17] Polymeric nanoparticles offer some specific advantages over liposomes, Niosomes and other vesicular drug delivery system. For instance, they help to ameliorate the stability of drugs / proteins and possess utile controlled release properties and hence polymeric nanoparticles are accepted as more preferable vehicle for targeted as well as controlled drug delivery.[20, 21]

CHALLENGES FOR BRAIN DRUG DELIVERY:

Unlike most other organs, the cerebral blood compartment is not in free diffusional transposition with the interstitium of the brain. The brain is tightly dissevered from the circulating blood by a unique membranous barrier, the BBB.

Drug delivery to the brain is severely haltered due to the exceptionally low permeability of the BBB. It composed of Brain Capillary Endothelial Cells (BECE) that is tightly connected and responsible for the extremely selective permeability attributes of the cerebrovasculature. (Figure 1)[22, 23, 24] It is considered as homeostatic denial mechanism of the brain against pathogens and toxins. It also screens the biochemical, physicochemical and structural features of solutes at its periphery. It also restrains solute ingress into the brain, by the transcellular route, due to an increased electrical resistance between the endothelial cells at the tight junctions (TJ).[25, 26] In brain capillaries of the endothelial cells are differ from the capillaries of other parts of the body i.e. intercellular cleft, pinocytosis, and fenestrate are virtually absent and mitochondria present

profusely; hence, diffusion occurs transcellularly. Therefore, only lipid soluble minuscule solutes that can freely diffuse through the capillary endothelial membrane passively. [27, 28, 29]

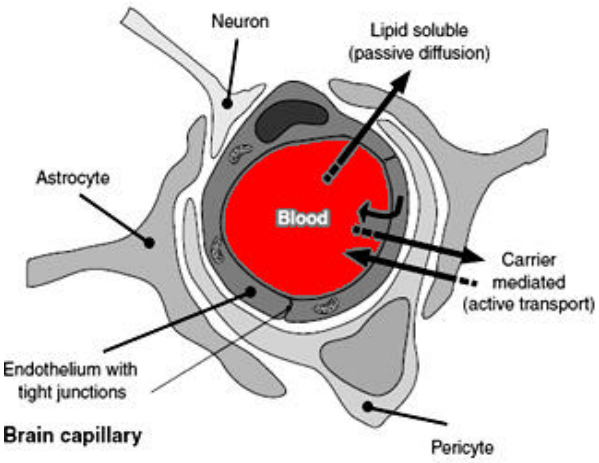

Figure 1 Schematic presentation of blood brain barrier

The BBB also have additional, enzymatic aspects: solutes crossing the cell membrane are subsequently queered to degrading enzymes present in large numbers inside the endothelial cells that contain large density of mitochondria i.e. metabolically highly active organelles.[30] Enzymes and receptors ascertained in the BBB, are adenylate cyclase, guanylate cyclase, Na+/K+ adenosine triphosphate (ATP)ase, alkaline phosphatase, catechol O-methyl transferase, monoamine oxidase, γ-aminobutyric acid (GABA) transaminase, and DOPA decarboxylase. [31,32,33,34] These enzymes have potential to recognize and degrade most peptides, including naturally occurring neuropeptides.[35, 36] The factors which are affecting brain drug delivery have been classified in following table.

Table 2. Factors affecting brain drug delivery [37]

Factors at blood brain barrier	Peripheral factors
•Concentration gradient of drug / polymer	•Systemic enzyme stability
•Molecular weight of drug	•Affinity for plasma protein
•Flexibility, conformation of drug / polymer	•Metabolism by other tissue
•Lipophilicity	•Clearance rate of drug / polymer
•Affinity for efflux protein (P-gp)	•Effect of existing pathological condition
•Cellular enzyme stability	
•Molecular charge of drug / polymer	
•Affinity for receptors and carriers	

In addition to metabolizing enzymes, the shielding effect of the BBB / Blood Cerebrospinal Fluid Barrier (BCFB) is further potentiated by the presence of certain principal efflux transporters such as P-glycoprotein, multi drug resistance protein (MRPs) and breast cancer resistance protein (BCRP). [38] MRP expressions may show species variation like in relation to brain drug incursion, P- glycoprotein and BCRP appear to be the major players with certainly multi drug resistance protein i.e. MRP2 having a lesser but significant role. The surface area of the BBB is known to be 5000-times greater than that of the BCFB and so BBB may play a prevailing role for drug and nutrient transfers into the brain.[39, 40, 41]

1. Permeability glycoprotein (P-gp) efflux transport system:

P-glycoprotein is a brain microvascular endothelial cell protein, which possesses several indispensible pharmacological functions of drug transport and expulsion. [38] It expressed in high density on the apical surface of brain endothelial cells. P-glycoprotein is involved in protecting the brain exposure to a variety of extraneous material including pharmacologically active agents and hence it is a prime hurdle for the delivery of drug used for the treatment of various central nervous system diseases such as neurodegerative disorders, primary brain tumors, cerebral HIV infection etc. P-glycoprotein has affinity for a broad range of structurally unrelated large hydrophobic compounds and it actively effluxes such compound from the brain.

P-glycoprotein is encoded by a small group of related genes called multidrug resistance genes (MDR/mdr), [42]. In rodents three P-glycoprotein gene products are identified mdr1a, mdr1b and mdr2 [43] and in humans these are identified as MDR1 and MDR2 [44]. The mdr1a/b gene products have a 90% homology with each other based on their amino acid sequence [45] and human MDR shows an 80% homology compared with the rat gene products [46]. In rodent's mdr1a, mdr1b and in humans only MDR1 gene products confer multidrug resistance and exhibit drug transport [47]. P-glycoprotein is also expressed into the luminal cell membrane of the cerebral endothelial cells and transports a very wide range of substrates with an immense structural diversity out of these cells. In general, the substrates of P-glycoprotein are lipophilic, planar molecules and are either neutral or cationic. Many drugs are transported by P-glycoprotein and its activity is responsible for limiting their penetration into the central nervous system. P-glycoprotein, mdr1a and MDR1 are principally expressed in the luminal membrane of rat / mouse and human BCEC respectively and transport substrates directly into the vascular lumen [48-56], they are also expressed in the apical membrane of the choroid plexus [55] where in, they will apparently transport substrates into cerebrospinal fluid. Thus, P-glycoprotein may remove substrates from the stroma of the choroid plexus into the relatively large volume of the cerebro spinal fluid, quashing the choroid plexus burden of drug, without adding to the overall drug content to the brain. The role of P-glycoprotein in the drug transport across BBB was proved from the mice studies where the mdr1a gene has been knocked out [57-59]. The distribution from plasma to brain of a large number of drugs, investigated in these knockout animals, and it was found that transport of drug was much greater in mice lacking the mdr1a gene than in wild-type mice. These experiments clearly demonstrate the role of P-glycoprotein in limiting the entry of many of its substrates into the central nervous system.

Besides that P-glycoprotein also contributes to efflux of undesirable substances such as amyloid-ß (Aß) proteins (protein that causes Alzheimer disease) from the brain into the blood as well as many neurotoxic drugs. Ultimately, the inhibition of P-glycoprotein system has both favorable and unfavorable effects on living bodies. [60,61]

2. BCRP (Breast cancer resistant protein) efflux system:

BCRP is principally expressed in the luminal membrane of human cerebral endothelial cells and is a crucial component of the efflux activity of the BBB. [62] Recently a porcine brain multidrug resistance protein (BMDP) has been delineated which again explicit in the luminal membranes of the cerebral endothelial cells and it is closely related to human BCRP with an 86% structural homology between the molecules.[63] Interestingly BCRP is fundamentally a half transporter when compared with P-glycoprotein, has only six trans membrane domains and one nucleotide binding site. Therefore it is assumed to form a homo-dimer in the cell membrane to function as a transporter. The porcine BCRP has a low manifestation in pericytes and does not seem to be functionally expressed in the epithelium of the choroid plexus. There is a lack of rBCRP manifestation in rat astrocytes. Degree of BCRP expression in porcine endothelial cells appears to be higher than P-glycoprotein as measured by mRNA levels but as homo-dimerisation of the molecule in the cell membrane may be mandatory for functional activity this may not correlate very directly with functional transport activity.[64, 65] BCRP is also a substrate specific like P-glycoprotein and causes expulsion of therapeutic molecules from the brain. But activity of BCRP is obstructed by P-glycoprotein reversal agent like GF120918 i.e. *N*-{4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)-ethyl]-phenyl}-9,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamide. [66, 67]

3. MRP (Multidrug resistant protein) efflux system:

The multidrug resistance-associated proteins also comprise a group of closely related gene products, [68, 69]. The degree of structural homology between the MRPs and P-glycoprotein is relatively small being in the region of 15% [70], although overall similarities between the molecules are apparent. MRP1, MRP2, MRP3 and MRP6 are large molecules possessing an additional domain at the N terminus named N-terminal transmembrane domain (TMDo) which is lacking in P-glycoprotein [68], The other MRP molecules are smaller in size and more like P-glycoprotein in overall structure but nevertheless all MRPs are full transporters and do not appear to have to associate with other molecules or dimerize in order to function as efflux transporters. Seven iso forms of MRP with known functions are presently described as MRP1 to MRP7 [71, 72, 73]. It has been demonstrated by immune histochemistry the multi drug resistant protein type 1 i.e. mrp1, is explicit in rat endothelial cells [74] but more robustly in brain parenchyma than endothelium [75] and mrp2 is explicit in the luminal membrane of the endothelial cells forming the blood brain barrier [76]. Microdialysis studies in rats have shown that mrp2 is active in the BBB and modulate the penetration of phenytoin into the brain [77]. MRP5 are explicit in the cerebral endothelial cells [69] although the precise location of the expressed protein remains to be elucidated. The role of MRP5 in nucleoside transport is crucial and thus important in the transport of antiviral drugs into the brain.

Inhibitors of the MRPs are not well delimitated than for P-glycoprotein and none seem particularly specific. Probenecid, sulfinpyrazone and benzbromarone inhibit MRP1 and 2 activity [77, 78] and probably interact with and inhibit a number of other members of the ATP-binding cassette transporters (ABC-transporter) family. It has been reported that indomethacin conquers MRP activity but not P-glycoprotein activity [79].

4. Basement membrane beneath blood brain barrier:

Besides BBB and its efflux system, US researchers have discovered a second barrier that might preclude some BBB permeant agents from reaching their target cells in the brain. This second barrier could add yet another layer of chemical bureaucracy to the design of therapeutic agents that target the brain. Neuroscientist Leslie Muldoon and neurosurgeon Edward Neuwelt, in their research in rat indicated an additional barrier that might be even more impenetrable to some agents than previously thought. Muldoon and her colleagues, while studying virus-sized particles of iron oxide in rat brain, found that their magnetic resonance images (MRI) did not reflect the distribution of iron oxide particles seen at brain autopsy. The particles had crossed the BBB, as revealed by the MRI, but were not distributed evenly among the neurons (Figure 2); instead they seemed stuck up against another barrier – the basement membrane on the basal surface of the vascular endothelial cells. To mimic viral vectors, the researchers have used two types of iron oxide particles of similar size, coated with different amounts of dextran, a polyanionic sugar polymer that allows the iron oxide particles to bind to proteins and membranes. The particles coated to a greater magnitude with dextran cross the second barrier, and at the microscopic level were well distributed within the brain. However, less coverage meant the particles got stuck at the basement membrane.

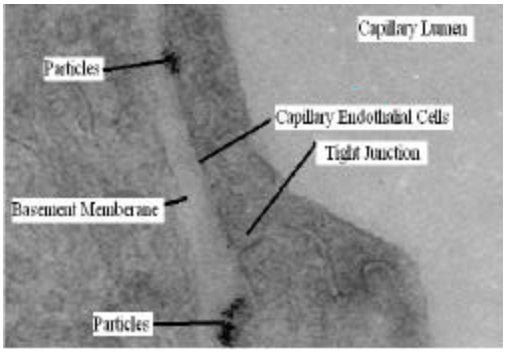

Figure 2. Transmission electron micrograph showing partially-coated iron oxide particles that have crossed the BBB but are prevented from penetrating the brain by the basement membrane

Further, Neuwelt explicates that the basement membrane is not a physical barrier but probably an electrically charged barrier similar to that in the kidney, which might explain the selective transit of particles depending on their charge.[80]

Because of aforementioned obstacles, the therapeutic value of many promising drugs, such as protease inhibitors for HIV-1 encephalitis (ritonavir, nalfinavir, and indinavir)[81], anti-inflammatory drugs (prednesolone, dexamethasone and indomethacin) for treatment of microglial inflammation during idiopathic PD and AD [82, 83], neuroleptic agents (amitriptyline and haloperidol) [84], analgaesic drugs (morphine, beta-endorphin, and asimadoline) [85], anti-fungal agents (itraconazole and ketoconazole) [86] anticancer agents (Doxorubicin, vinblastine, paclitaxel etc.) , as well as antiepileptic agents (carbamazepine, phenobarbital, phenytoin, and lamotrigine) [87, 88] is atrophied, and cerebral diseases have proven to be most refractory to therapeutic interventions.

MECHANISM OF NANOPARTICLE / DRUG TRANSPORT ACROSS BLOOD BRAIN BARRIER:

The BBB not only impedes the influx of intravascular substances from blood to brain, but also promotes transport of substances from blood to brain or from brain to blood through several transport systems such as carrier-mediated transport, active efflux transport, and receptor-mediated transport systems. These mechanisms can explicate the movement of therapeutic bioactive agents across the BBB. (Figure 3)

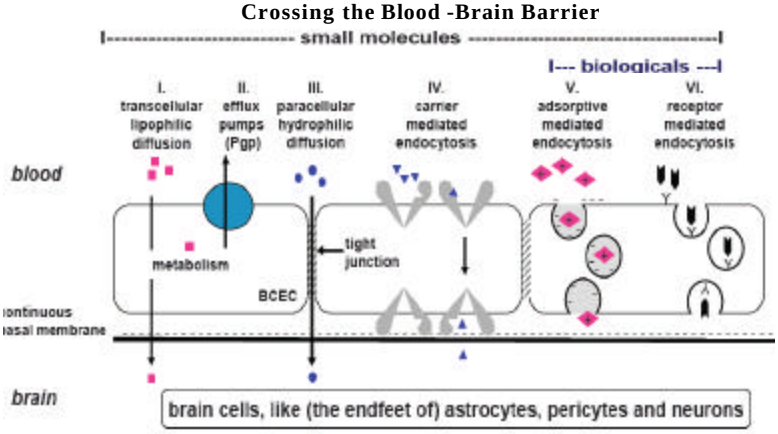

Figure 3. Mechanism of nanoparticle / drug transport across BBB

1. Receptor mediated transcytosis / Transcytosis:

Therapeutic peptides are relatively large, hydrophilic, and unstable, thus efficient incursion into the brain generally does not occur. [89] However, relatively small peptides are transported by carrier-mediated transport mechanisms, and others cross the BBB by transrytosis including receptor-mediated transrytosis and absorptive-mediated transcytosis. Nanoparticles coated with polysorbate 80/LDL, transferrin receptor binding antibody (such as OX26), lactoferrin, cell penetrating peptides and melanotransferrin have been depicted capability of delivery of a self non transportable drug into the brain via the chimeric construct that can undergo receptor-mediated transcytosis.[90, 91, 82, 93]

2. Carrier-mediated transport:

Carrier-mediated transport (CMT) involves the modification of a drug (small molecule) into a compound with a similar structure that mimics a nutrient and can thus make use of one of the several specialized carrier mediated transport systems within the BBB that exist for the transport of essential compounds, such as amino acids, hexoses, vitamins and neuropeptides, into the brain (Table 3). Transport of glutathione across the BBB is saturable [94, 95] carrier-mediated transport has a high efficiency comparable to the brain uptake of single amino acids such as phenylalanine and cysteine may be involved. The availability of endogenous carrier mediated transport pathways serves as portals for circulating potential bioactive agents to the brain. [96, 97]

Table 3. Carrier mediated transport system with examples of transported compound

Transport System	Molecule (Example)
Hexose transport system	Glucose, Mannose
Neutral amino acid transport system	Phenylalanine, Leucine
Acidic amino acid transport system	Glutamate, Aspartate
Basic amino acid transport system	Arginine, Lysine
Monocarboxylic acid transport system	Lactate and Short-chain fatty acids such as Acetate, Propionate etc. [98-101]
Choline transport system	Choline, Thiamine [102-107]
Amine transport system	Mepyramine
Nucleoside transport system	Only for Purine bases such as Adenine, Guanine
Peptide transport system	Enkephalins

Utilization of differences in the affinity and the maximal transport activity among these transport systems expressed at the BBB can be attractive strategy for controlling the delivery and retention of drugs into the brain.

3. Adsorptive-mediated endocytosis:

Cationic modification of proteins such as albumin and antibodies (IgG) can contribute to enhance cellular uptake. The cationized proteins mainly cross the BBB by adsorptive-mediated transcytosis (AMT). An electrostatic interaction exists between the cationized albumin and anionic charges on the BBB. This interaction also occurs amongst sialic acid moieties on the luminal surface of BBB and heparin sulfate groups on the abluminal surface.[108] In adsorptive mediated endocytosis initial binding of cationized substrate to the luminal plasma membrane was triggered by electrostatic interactions between the positively charged moiety of the peptide and the negatively charged plasma membrane surface region. [110-112] Structural specificity of adsorptive mediated endocytosis at the BBB was accomplished by studying uptake of different synthetic peptides having varied molecular sizes, basicities and hydrophobicities, and carboxyl-terminal using primary cultured bovine endothelial cells. These results indicated that the C-terminal structure and the basicity of the molecules, are significant determinants of uptake by the AME system at the BBB. [113]

4. Receptor-mediated endocytosis:

It is accomplished by synthesis of chimeric peptides. They are formed by covalent binding of the non permeable but pharmacologically effectual portion of the peptide to an appropriate vector which can be transported across the BBB. In such case, the chimeric peptide is first transported into the brain endothelial cytoplasm by receptor-mediated or absorptive-mediated endocytosis. The intact chimeric peptide is then transferred into the brain's interstitial space by receptor-mediated exocytosis. Subsequently, the binding between the vector and the pharmacologically active peptide is cleaved and finally, the released peptide reagins its pharmacological effect in the brain [110, 111, 114, 115]. Multiple classes of bioactive therapeutics have been delivered to the brain via chimeric peptide technology, including peptide- based pharmaceuticals, such as a vasoactive peptide analog or neurotrophins such as brain-derived neurotrophic factor, anti-sense therapeutics including peptide nucleic acids (PNAs), and small molecules incorporated within liposomes [116, 117].

5. Inhibiting P-glycoprotein Efflux Pump:

P-glycoprotein molecules act as ATP-dependent drug transport pumps, which serves to efflux the drugs out of the cells and decrease their intracellular concentration in the cytoplasm, which subsequently, diminishing their efficiency. Several strategies have been tried to shunt P-glycoprotein efflux. Reversal agents such as R-verapamil, PSC 833 (cyclosporine analog), quinidine, quinine, cyclosporine A etc. increases the influx of therapeutic agents when they are co-administered with impermeable bioactive agents. Such reversal agents subdue P-glycoprotein liaised efflux drug transport. [118] However most of the reversal agents ascertained to be pharmacologically active and elicit significant toxicity at doses required for P-glycoprotein inhibition.

6. Membrane Permeabilization Effect:

Solubilization of the endothelial cell membrane lipids with the aid of surfactants would contribute to the membrane fluidization and ameliorate permeability of the BBB. This can boost the bioactive to shunt the BBB and provide increased accretion of drugs in the brain.[119] Calvo et al., affirmed by conducting the studies that brain capillaries could take up nanoparticles coated with surfactants. They observed that the velocity of uptake of nanoparticles depends on the type of surfactant and its quantity, and the phenomenon was attributed to membrane permeabilization effect due to surfactants. [120,121]

SURFACE FUNCTIONALIZED NANOPARTICLES AS A POTENTIAL TOOL FOR TREATMENT OF REFRACTORY CENTRAL NERVOUS SYSTEM DISORDERS:

Nanoparticles are solid colloidal particles with diameters ranging from 1-1000 nm. They comprise of macromolecular materials and can be used therapeutically as adjuvant in vaccines or drug carriers, in which the active ingredient is dissolved, entrapped, encapsulated, adsorbed or chemically attached.[88]

Constructing nanoparticles with targeting ligands or coating them with suitable surface modifying agent can conduce to the development of carrier systems of radiated nature and target specific. The nanoparticles can gain access in the brain via number of aforementioned possible mechanisms depending on the nature of coating material.

Nanoparticles twinned with surface functionalized material like transferrin, lactoferrin or OX-26 could festinate the drug delivery to the brain through the different mechanism. The use of nanoparticles as carriers for drug and gene delivery has been area of an intensive research and development in this arena.[122-128] The surface of nanoparticulate carriers are often modified by a PEG sweep that increase the stability of nanoparticles in dispersion and gallop circulation time of nanoparticles in the body. [124, 129-134]

1. Polysorbate 80 coated nanoparticles:

Dalargin nanoparticles coated with polysorbate 80 (Tween) [135, 136] administered intravenously has shown pronounced analgesic effect, with a maximum concentration after 45 minutes of administration. The mechanism behind the translocation of nanoparticles into the brain is still not fully understood. In vivo experiments in mice have clearly depicted that the analgesic effect of dalargin was prevailed only when the drug was pre-adsorbed onto the nanoparticles, whereas a single mixture of dalargin and PBCA nanoparticles did not express significant analgesic effect. The enhancement of the drug transport through BBB by the polysorbate 80 coated nanoparticles can be explicated 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 (2) brain endothelial cell uptake of nanoparticles may occur through endocytosis or transcytosis. Additionally, involvement of apolipoproteins (APO) in the brain penetration of poly butylcyanoacrylate nanoparticles over those coated with polysorbate-80 was manifested [137]. A study performed using poly butylcyanoacrylate nanoparticles loaded with dalargin or loperamide and over coated with the apolipoprotein-A, B, C, E or J (with or without pre-coating with polysorbate-80), showed high antinociceptive effect with both polysorbate-80-precoated and apolipoprotein-B- or apolipoprotein-E over coated nanoparticles. Interestingly, in apolipoprotein-E-deficient mice, the antinociceptive effect was reduced comparatively to normal mice after injection of the polysorbate-80-coated nanoparticles. Thus, it is proposed that the polysorbate-80 could act as an anchor for apolipoprotein-B and E, at the surface of the nanoparticles which are then be able to interact with low density lipoprotein receptors and taken up by the brain microcapillary endothelial cells via receptor-mediated endocytosis.[138-140]

2. Pegylated nanoparticles:

Pegylated-poly (hexadecylcyanoacrylate) (PEG-PHDCA) nanoparticles have been investigated for the treatment of several central nervous system pathologies such as brain tumors [141] and prion diseases [142]. These nanoparticles shown higher incursion in to the brain than all the other nanoparticles formulations tested.[143] Confocal microscopy has evidenced that fluorescent-PEG-PHDCA nanoparticles were present in the epithelial cells [144] of the brain and spinal cord surface and in the ependymal cells of the choroids plexus in rats when tested for drug accumulation. PEG-PHDCA nanoparticles could reach to the rat brain by two mechanisms: (1) passive diffusion due to the increased permeability of BBB and (2) transport by nanoparticles-containing macrophages which infiltrate these inflammatory tissues. This study claims that PEG-PHDCA nanoparticles had appropriate characteristics for penetration into central nervous system under pathological conditions, especially in neuro inflammatory diseases.

3. Peptide functionalized nanoparticles:

On the basis that some naturally occurring peptides can effectively cross the BBB due to receptor-mediated transport (transcytosis), Costantino et al. [145] developed an elegant system, using modified poly(d,l-lactide-co-glycolide) (PLGA) copolymers obtained by conjugating poly(d,l-lactide-co-glycolide) and five short synthetic peptides, which bear some resemblance to the synthetic opioid peptide MMP-2200 (H_2N–L-Tyr-D-Thr- Gly-L-Phe-L-Leu-L-Ser–O–b-D-lactose–$CONH_2$). The tyrosine present in this peptide was substituted with phenyl alanine in order to avert a potential opioid effect and the permeability was supposed to be enhanced by the presence of glycosidic moieties (glucose, lactose, etc.). Fluorescent and confocal microscopy studies have been shown that these peptide-derivatized biodegradable NP were able to cross the BBB, although more studies are required to quantify their presence in the central nervous system and to elucidate their BBB crossing mechanisms.

4. Glutathione functionalized nanoparticles:

to-BBB's® technology platforms open novel gateways to treat devastating brain disorders like brain tumors, Alzheimer's disease and lysosomal storage diseases by combining with established and marketed drugs. In order to target potential therapeutic agent to the brain most efficient & safe way is to hijack the endogenous uptake-machinery of the blood brain barrier by associating the drug with compounds that are naturally transported into the brain. Glutathione is a natural antioxidant and found at high levels in the brain and its receptor is abundantly expressed at the blood-brain barrier. It also uniquely derogates common risks like adverse immunological reactions or interference with life-essential physiological pathways. From the experiments to-BBB® technology has proven that as higher the amount of glutathione coating on the liposomes / Nanoparticles, the greater pool of free drug was actually delivered to the brain. to-BBB® technology has developed by G-technology which utilizes an endogenous receptor-targeted mechanism in combination with liposomes coated with glutathione-conjugated PEG to mediate safe targeting and enhance the delivery of drugs to the brain. This approach is unique as it does not require drug modification and at the same time it gives rise to metabolic protection during transport and increased bioavailability at the target site. [146]

5. Pluronic / Poloxamer Coated Nanoparticles:

One emerging strategy to enhance drug delivery to the central nervous system is the co-administration of a drug with a pharmacological modulator that seizes drug efflux transport through the brain microvessel endothelial cells of BBB. One promising example of such pharmacological modulator is class of pluronic block copolymer (also known under non-proprietary name "poloxamers"). These block copolymer consists of hydrophilic ethylene oxide (EO) block and hydrophobic propylene oxide (PO) block. Due to their amphiphilic nature this copolymer displays surfactant properties including ability to interact with hydrophobic surfaces and biological membranes. In aqueous solutions at concentrations above critical micelle concentration (CMC) these copolymers self-assemble into micelles. Studies in multidrug resistant (MDR) cancer cells, polarized intestinal epithelial cells, caco-2, and polarized brain microvessel endothelial cell monolayers provided evidence that selected pluronic block copolymers can inhibit drug efflux transport systems. [147-154] The effects of pluronic block copolymers were most apparent at concentrations below the critical micellar concentration on P-glycoprotein and multi drug resistant proteins i.e. drug efflux transporters. [147, 148] Recent findings suggest that effects of pluronic on drug efflux transport proteins involve interactions of the block copolymers with the cell membranes. [150, 155] The hydrophobic propylene oxide chains of pluronic immerse into the membrane hydrophobic areas, resulting in alterations of the membrane structure, and diminution of its microviscosity ("membrane fluidization"). At relatively low concentrations (e.g. 0.01 %) pluronic inhibits the P-glycoprotein ATPase activity; possibly, due to conformational changes in the transport protein.[150] Pluronic P-85 also displayed the effects characteristic of a mixed type enzyme inhibitor - decreasing maximal reaction rate, V_{max} and increasing Michaelis constant, K_m for ATP as well as P-glycoprotein-specific substrates. In contrast, at the high concentrations (e.g. 1 %), binding of pluronic to the membrane actually results in restoration of P-glycoprotein ATPase activity. This could be due to the segregation of the block copolymer molecules in the 2D clusters in the membrane, which diminishes its interactions with the transport proteins. It was demonstrated that a fine balance between hydrophilic (ethylene oxide) and lipophilic (propylene oxide) components in the pluronic molecule should be accomplished to achieve potential diminution of the drug efflux systems.[154] Ideally most efficacious pluronic are those with intermediate lengths of hydrophobic block with a HLB value < 20. Hydrophilic block copolymers, which have an extended ethylene oxide block, do not incorporate into lipid bi layers and practically do not transport into the cells. [154] Very lipophilic block copolymers with long propylene oxide blocks anchor in the plasma membranes and remain there for an extended period of time. As a result,

although they are potent inhibitors of P-glycoprotein, they are not efficiently transported into the cell, do not cause ATP depletion and have little net effect on P-glycoprotein efflux system in brain microvessel endothelial cells. In contrast, the block copolymers displaying intermediate lipophilicity can transport across the BBB and spread throughout the cytoplasm to reach mitochondria as well as nuclei. They conquer P-glycoprotein ATPase activity and decrease ATP intracellular levels, which combined results in effective inhibition of drug efflux transport systems and enhanced drug transport to the brain.[150, 154]

6. Transferrin / Antibody directed against TfR coated Nanoparticles:

The most widely characterized receptor-mediated transcytosis system for targeting of drugs to the brain is the transferrin (TfR), a trans membrane glycoprotein consisting of two 90 kDa subunits. A disulfide bridge links these subunits and each subunit can bind one transferrin molecule. [156] The transferrin is expressed mainly on hepatocytes, erythrocytes, intestinal cells and monocytes and on endothelial cells of the BBB. [157]Transferrin is also expressed on choroid plexus epithelial cells and neurons. [155] The transferrin mediates cellular uptake of iron bound to transferrin.

Drug targeting to the brain can be achieved either by using the endogenous ligand transferrin or by using an antibody directed against the transferrin (OX-26 antirat TfR). For transferrin, the in vivo application is limited by high endogenous concentrations of transferrin in plasma and the likely overdose of iron when one tries to displace the endogenous transferrin with exogenously applied transferrin-containing systems. Recent studies have shown that liposomes tagged with transferrin are suitable for drug delivery across the brain microvessel endothelial cells in vitro, even in the presence of serum. OX-26, antirat transferrin does not bind to the transferrin-binding site and is therefore not displaced by endogenous transferrin. Some reports have proposed transcytosis of transferrin across the brain microvessel endothelial cells, whereas others have claimed endocytosis of transferrin followed by intracellular release of iron and a subsequent return of apotransferrin to the apical side of the brain microvessel endothelial cells. [158, 159, 160] The mechanism of transcytosis of OX-26 is not yet fully elucidated. Pardridge and colleagues have shown efficient drug targeting and delivery to the brain in vivo by applying OX-26. [161] In contrast, Broadwell et al.[162] have shown that both transferrin and OX-26 are able to cross the BBB, but that the transcytosis of transferrin is more efficient.

Nanoparticles have been investigated extensively for brain drug delivery, from that some of the key findings are summarized in table 4.

Table 4. Polymeric nanoparticulate carrier to Shunt blood brain barrier

Formulation	Drug	Indication	Vector to Shunt BBB	Reference
PBCA Nanoparticles	Gemcetabine	Brain Tumor	Polysorbate 80	Chun X.W.et al. [163]
PBCA Nanoparticles	Rivastigmine	AlzhiemersDisease	Polysorbate 80	Barnsbas W.et al.[164]
Nanoparticles	-	-	Polysorbate 80	Kepan G.et al.[165]
PBCA Nanoparticles	Doxorubicin	Brain Tumor	Polysorbate 80& Poloxamer188	Petri B.et al.[166]
Nanoparticles (Polymer)	-	-	PEG	Calvo et al.[167]
PEG-PLA Nanoparticles	-	-	Lactoferrin	Kaili Hu et al. [168]
PBCA Nanoparticles	-	-	Polysorbate 80	Alyautdin et al.[169]
Nanoparticle	Paclitaxel	Brain Tumor	Cetyl Alcohol / Polysorbate	Koziara et al.[170]
Nanoparticle	Penicillamine	Alzheimers Disease	-	Cui Z et al.[171]
PLGA Nanoparticles	-	-	Short Chain Synthetic Peptide	Costantino et al.[172]
Chitosan Nanoparticles	Peptide(Z-DEVD-FMK)	Cerebral Ischemia	PEG / OX 26	Aktas et al.[173]
PLGA Nanoparticles	Loperamide	Opioid Analgesic	Glycosylated Heptapeptide	Tosi et al.[174]
PBCA NPs	Loperamide, Dalargin	Opioid Analgesic	Apolipoprotein B & E	Kreuter et al.[175]
PBCA NP	Kytophin	Analgesic	Polysorbate 80	Schroeder et al.[176]

CONCLUSION:

The preceding depicted that nanoparticulate systems have great potentials as a drug delivery vehicle, as it able to convert poorly soluble, poorly absorbed and labile biologically active agents into promising deliverable drugs. To optimize this drug delivery system, greater understanding of the different mechanisms of biological interactions, and particle engineering, is still required. Further advances are needed in order to turn the concept of nanoparticle technology into a realistic practical application as the next generation of drug delivery system. From this review it emerges that nanoparticulate drug delivery systems prognosticate new opportunities in the therapy of acute and chronic brain disease. The transport of the drugs to the brain can be improved by inhibition of drug efflux transport proteins, such as Pgp, which are important gatekeepers in the BBB. Alternative strategies, involve the use of polymeric nanocarriers which can be targeted to the brain by attaching specific peptides / ligands to their surface. Colloidal nanoparticulate systems can easily enter brain capillaries before reaching the surface of the brain microvascular endothelial cells, when the surfaces of these colloids are modulated in a proper way (i.e. by PEG or PS-80). These surface modified colloidal particles enhance exposure of the BBB due to prolonged blood circulation, which favors interaction and penetration into brain endothelial cells. Colloidal systems may further be modified with a variety of agents on their surface, each with a unique function leading to multifunctional therapy.

Overall, drug delivery to the brain / central nervous system has recently been markedly enhanced through the rational design of polymer-based nanoparticulate drug delivery systems. Substantial progress will only come about, however, if continued vigorous research efforts to develop more therapeutic and less toxic drug molecules are paralleled by the aggressive pursuit of more effective mechanisms for delivering those drugs to their central nervous system targets.

REFERENCES:

1. Regier DA, Boyd JH, Burke JD., (1988) One-month prevalence of mental disorders in the United States: based on five epidemiologic catchment area sites. Arch Gen Psychiatry 45, 1988, 977-986.
2. Pardridge W., Blood-brain barrier delivery. Drug Discov Today 12, 2008, 54-61.
3. Habgood MD et al., Cell. Mol. Neurobiol. 20, 2000, 231-238.
4. Ghose AK et al., A knowledge- based approach in designing combinatorial or medicinal chemistry libraries for drug discovery: I. A qualitative and quantitative characterization of known drug databases. J Comb Chem 1, 1999, 55-68.
5. MDLR comprehensive medicinal chemistry [online]. Available from URL: http://www.mdl.com/products/knowledge/ medicinal_chem/index.jsp [Accessed 2008 Nov 26]
6. Lipinski CA., Drug-like properties and the causes of poor solubility and poor permeability. J Pharmacol Toxicol Methods 44, 2000, 235-249.
7. Dwibhashyam V, Nagappa AN., Strategies for enhanced drug delivery to the central nervous system. Indian J Pharm Sci 70, 2008, 145-53.
8. Pardridge WM., Blood-brain barrier delivery. Drug Discov. Today 12, 2009, 54–61.
9. Reichel, DB, Abbott N., Methods in Molecular Medicine: The Blood Brain Barrier: Biology and Research Protocols, Humana Press Inc. Totowa, New Jersey, 2003, p 307– 325.
10. Ricci M et al., Delivering drugs to the central nervous system: a medicinal chemistry or a pharmaceutical technology issue? Curr Med Chem 13, 2006, 1757-1775.
11. Pardridge WM., Brain drug targeting: the future of brain drug development. Cambridge: Cambridge University Press, 2001, 230-251.
12. Dwibhashyam V, Nagappa AN., Strategies for enhanced drug delivery to the central nervous system. Indian J Pharm Sci 70, 2008, 145-153.
13. Martin B et al., Encyclopedia of controlled drug delivery. New York: John Wiley and Sons Inc. (1), 1999, 184-210.
14. Mohanraj VJ, Chen Y., Nanoparticles – A Review. Trop. J. of Pharma. Res., 5 (1), 2006, 561-573.
15. Kreuter J., Nanoparticles—a historical perspective. Int. J. Pharm. 331, 2007, 1–10.
16. Liu X, Chen C., Strategies to optimize brain penetration in drug discovery. Curr. Opin. Drug Disc. 8 (4), 2007, 505–512.
17. Pardridge WM., Drug and gene targeting to the brain via blood–brain barrier receptor-mediated transport systems. Int. Cong. Ser. 49, 2005, 49–62.
18. Kreuter J., Nanoparticles. In: Kreuter J, Ed. Colloidal Drug Delivery Systems. Marcel Dekker: New York, 1994, 219-242.
19. Ringe K et al. Nanoparticle Drug Delivery to the Brain. Encyclopedia of Nanoscience and Nanotechnology 7, 2004, 91-104.
20. Bhadra D et al., Pegnology: a review of PEGylated systems. Pharmazie 57, 2002, 5-29.
21. Mu L, Feng SS., A novel controlled release formulation for the anticancer drug paclitaxel (Taxol(R)): PLGA nanoparticles containing vitamin E TPGS. J Control Release 86, 2003, 33-48.
22. Tamai A, Tsuji A., Drug delivery through the blood brain barrier. Adv. Drug Deliv. Rev. 19, 1996, 401–424.
23. Huber JD, Egleton R, Davis TP., Molecular physiology and pathophysiology of tight junctions in the blood–brain barrier, Trends Neuro sci. 24, 2001, 719.
24. Bazzoni G, Dejana E., Endothelial cell-to-cell junctions: molecular organization and role in vascular homeostasis. Physiol. Rev. 84, 2004, 869–901.
25. Crone C, Olesen S., Electrical resistance of brain microvascular endothelium. Brain Res. 241, 1982, 49–55.
26. Krause D, Mischeck U, Galla H, Dermietzel R., Correlation of zonua occludens ZO-1 antigen and transendothelial resistance in porcine and rat cultured cerebral endothelial cells. Neurosci. Lett 128, 1991, 301– 304.
27. Pardridge WM., Recent advances in blood-brain barrier transport. Annu Rev Pharmacol Toxicol 28, 1988, 25-39.
28. Tamargo RJ, Brem H., Transporting therapeutics across the blood-brain barrier. Neurosurg 2, 1992, 259-79.
29. Abbott NJ, Romero IA., Transporting therapeutics across the blood-brain barrier. Mol Med Today 3, 1996, 106-13.
30. Chen Y, Dalwadi G, Benson H., Drug delivery across the blood-brain barrier. Current Drug Delivery 1, 2004, 361-376.
31. Bodor N, Brewster ME., Problems of delivery of drugs to the brain. Pharmacol Ther 19, 1983, 337-86
32. Crone C., The blood-brain barrier: a modified tight epithelium. In: Suckling AJ, Rumsby MG, Bradbury MWB, editors. The blood-brain barrier in health and disease. Chichester: Ellis Horwood , 1986, 17-40.
33. Minn A, Ghersi-Egea JF, Perrin R, et al., Drug metabolizing enzymes in the brain and cerebral microvessels. Brain Res Rev16, 1991, 65-82.
34. Ghersi-Egea JF, Leninger-Muller B, Suleman G, et al., Localization of drug metabolizing enzyme activities to blood-brain interfaces and circumventricular organs. J Neurochem 62, 1964, 1089-96.
35. Brownlees J, Williams CH., Peptidases, peptides, and the mammalian blood-brain barrier. J Neurochem 60, 1993, 793-803
36. Witt KA, Gillespie TJ, Huber JD, et al., Peptide drug modifications to enhance bioavailability and blood-brain barrier permeability. Peptides 22, 2001, 2329-43.
37. Pardridge, WM, Triguero D, Yang J, Cancilla, PA., "Comparison of in vitro and in vivo models of drug transcytosis through the blood–brain barrier", J. Pharm. Exp. Ther. 253(2), 1990, 884–891.
38. Varma, MV, Yasvanth A, Chinmoy SD, Ramesh P., P-glycoprotein inhibitors and their screening: A perspective from bioavailability enhancement. Pharmacol Res 48, 2003, 347-359.
39. Pardridge WM, Brain metabolism: a perspective from the blood-brain barrier. Physiol. Rev., 63, 1983, 148l-1535.
40. Klecker Jr RW, Collins JM, Yarchoan R, Thomas R, Jenkins JF, Broder S, Myers CE., Plasma and cerebrospinal fluid pharmacokinetics of 3'-azido- 3'deoxythymidine: a novel pyrimidine analog with potential application for the treatment of patients with AIDS and related diseases. Clin. Pharmacol. Ther. 41., 1987, 407-412.
41. Terasaki T, Pardridge WM., Restricted transport of 3'-azido-3'-deoxythymidine and dideoxynucleosides through the blood-brain barrier. J. Infect. Dis. 158, 1988, 630-632.
42. Deely RG, Cole SP., Function, evolution and structure of multidrug resistance protein (MRP). Seminars in Cancer Biol. 8, 1997, 193- 204.
43. Hsu SIH, Cohen D, Kirchner LS, Hartstein M, Horwitz SB., Stuctural analysis of the mouse mdr1a (P-glycoprotein) promoter reveals the basis for differential transcripts heterogeneity in multi drug resistant J774.2 cells. Mol Cell Biol. 10, 1990, 3596- 3606.

44. Chin JE, Soffir R, Noonan KE, Choi KY, Roninson IB., Structure and expression of the human MDR (P-glycoprotein) gene family. Mol Cell Biol. 9, 1989,3808-3820.
45. Devault A, Gros P., Two members of the mouse mdr family confer multidrug resistance with overlapping but distinct drug specificities. Mol. Cell. Biol. 1990, 1652-1663.
46. van der Bliek AM, Kooiman PM, Schneider C, Borst P., Sequence of mdr3 cDNA encoding a human P-glycoprotein. Gene. 71, 1998, 401-411.
47. Ng WF, Sarangi F, Zastawany RL, Veintot-Drebot L, Ling V., Indentification of members of the P-glycoprotein multigene family. Mol Cell Biol 9, 1989, 1224-1232.
48. Smit JJM, Schinkel AH, Oude Elferinck RPJ, Groen AK, Wagenaar E, van Deemter L, et al. Homozygous disruption of the murine mdr2 P-glycoprotein gene leads to a complete absence of phospholipid from bile and to liver disease. Cell. 75, 1993, 451-462.
49. Cordon Cardo C, O'Brien JP, Casals D, Ritman-Grauer L, Biedler JL, Melamed MR, et al. Proc Natl Acad Sci USA 86: (1989) 695- 698.
50. Theibaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willingham MC ., Immunohistochemical localization in normal tissues of different epitopes in the multidrug transport protein P170: Evidence for localisation in brain capillaries and cross reactivity of one antibody with a muscle protein. J Histochem Cytochem 37, 1989, 159-164.
51. Tasuta T, Naito M, Oh-Hara T, Sugawara I, Tsuruo T., Functional involvement of P-glycoprotein in blood brain barrier. J Biol Chem 267, 1992, 20383-20391.
52. Stewart PA, Beliveau R, Rogers KA., Cellular localisation of Pglycoprotein recognised by MRK16 monoclonal antibody in brain and spinal cord. J Histochem Cytochem 44, 1996, 679-685.
53. Beaulieu É, Demeule M, Ghitescu L, Béliveau R., P-glycoprotein is stongly expressed in the luminal membranes of the endothelium of blood vessels in the brain. Biochem J; 326, 1997, 301-307.
54. Begley DJ, Khan EU, Rollinson C, Abbott NJ, Régina A, Roux F., The role of brain extracellular fluid production and efflux mechanisms in drug transport to the Brain In: Begley DJ, Bradbury MWB, Kreuter J. Eds, The Blood-brain barrier and drug delivery to the CNS. New York, Dekker, 2000, 93-108.
55. Demeule M, Régina A, Jodoin J, Laplante A, Dagenais C, Berthelet F, et al., Drug transport to the brain: Key roles for the efflux pump P-glycoprotein in the blood-brain barrier. Vascular Pharm 38, 2002, 339-348.
56. Virgintino D, Robertson D, Errede, Benagiano V, Girolamo F, Maiorano E, Roncali L, Bertossi M., Expression of P-glycoprotein in human cerebral cortex microvessels. J Histochem Cytochem 50, 2002, 1671-1676.
57. Schinkel AH, Smit JJM, van Telligen O, Beijnen JH, Wagenaar E, van Deemter L, et al., Disruption of the mouse mdr1a Pglycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. Cell 77, 1994, 491-502.
58. Schinkel AH, Wagenaar E, van Deemter L., Absence of the mdr1a P-glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethazone, digoxin and cyclosporine. A. J Clin Invest 96, 1995, 1698-1705.
59. Schinkel AH, Wagenaar E, Van DL., Pglycoprotein in the blood-brain barrier of mice influences the brain penetration and pharmacological activity of many drugs. J Clin Invest 97, 1996, 2517-2524.
60. Begley DJ., ABC Transporters and the Blood-Brain Barrier: Current Pharmaceutical Design. 10, 2004, 1295-1312
61. Gelperina SE, Khalansky AS, Skidan IN, Smirnova ZS, Bobruskin AI, Severin SE, et al., Toxicological studies of doxorubicin bound to polysorbate 80-coated poly (butyl cyanoacrylate) nanoparticles in healthy rats and rats with intracranial glioblastoma. Toxicol Lett. 126, 2002, 131-41.
62. Cooray H, Blackmore CG, Maskell L, Barrand MA. Localisation of breast cancer resistance protein in microvessel endothelium of human brain. Neuroreport. 13, 2002, 2059-2063.
63. Eisenblätter T, Galla HJ., A new multidrug resistance protein at the blood-brain barrier. Biochem Biophys Res Comm 293, 2002, 1273- 1278.
64. Litman T, Brangi M, Hudson E, Fetsch P, Abati A, Ross DD, et al., The multidrug-resistant phenotype associated with over expression of the new ABC half-transporter, MXR (ABCG2). J Cell Sci. 113, 2002, 2011-2021.
65. Özvegy C, Litman T, Szakács G, Nagy Z, Bates S, Váradi A, et al. (2001) Functional characterisation of the human multidrug transporter, ABCG2, expressed in insect cells. Biochem Biophys Res Comm 285: 111-117.
66. Hyafil F, Vergely C, Du Vignaud P, Grand-Perret T., In vitro and in vivo reversal of multidrug resistance by GF120918, an acrid on carboxamide derivative. Cancer Res. 53, 1993, 4595-4602.
67. Evers R, Kool M, Smith AJ, van Deemter L, de Haas M, Prost P., Inhibtory effect of the reversal agents V-104, GF120918 and Pluronic L61 on MDR1 Pgp-, MRP1- and MRP2- mediated transport. Br J Cancer. 83, 2000, 366-374.
68. Borst P, Evers R, Kool M, Wijnholds J., A family of drug transporters: The multidrug resistance-associated proteins. J Natnl Cancer Inst. 92, 2000, 1295-1302.
69. Zhang Y, Han H, Elmquist WF, Miller DW., Expression of various multidrug resistance associated protein (MRP) homologues in brain microvessel endothelial cells. Brain Res. 876, 2000, 148-153.
70. Kusuhara H, Sugiyama Y., Role of transporters in the tissueselective distribution and eleimination of drugs: Transporters in the liver, small intestine, brain and kidney. J Contr Rel. 78, 2002, 43- 54.
71. Minn A, El-Bachra RDS, Bayol-Denizot C, Lagrange P, Suleman FG., Drug Metabolism in the brain: Benefits and risks. In: Begley DJ, Bradbury MW, Kreuter J. Eds. The Blood-brain barrier and drug delivery to the CNS New York, Dekker Inc. 2000, 145-170.
72. Minn A, Leclerc JM, Heydel AL, Minn C, Denizot C, Catarelli P et al., Drug transport into the mammalian brain: The nasal pathway and its specific metabolic barrier. J Drug Targ. 10, 2002, 285-296.
73. Kusuhara H, Suzuki H, Naito M, Tsuruo T, Sugiyama Y., Characterization of efflux transport of organic anions in a mouse brain capillary endothelial cell line. J Pharm Exp Ther. 285, 1998, 1260-1265.
74. Régina A, Koman A, Piciotti M, El Hafny B, Center MS, Bergman R et al., Mrp1 multidrug resistance-associated protein and P-glycoprotein expression in rat brain microvessel endothelial cells. J Neurochem. 71, 1998, 705-715.
75. Miller DS, Nobmann SN, Gutman H, Toeroek M, Drewe J, Fricker G., Xenobiotic transport across isolated brain microvessels studied by confocal microscopy. Mol Pharm 58, 2000, 1357-1367.
76. Potschka H, Fedrowitz M, Löscher W., Multidrug resistance protein MRP2 contributes to blood-brain barrier function and restricts antiepileptic drug activity. J Pharm Exp Ther. 306, 2003, 124-131.
77. Dean M, Rzhetsky A, Allikmets R., The human ATP-binding cassette (ABC) transporter superfamily. Genome Res 11, 2001, 1156-1166.
78. Gerk PM, Vore M., Regulation of expression of the multidrug resistance-associated protein 2 (MRP2) and its role in drug disposition. J Pharm Exp Ther 302, 2002, 407-415.
79. Draper MP, Martell RL, Levy SB., Indomethacin-mediated reversal of multidrug resistance and drug efflux in human and murine cell lines over expressing MRP, but not P-glycoprotein. Br J Cancer 75, 1997, 810-815.
80. Muldoon L et al., A physiological barrier distal to the anatomic blood brain barrier in a model of transvascular delivery, Am. J. Neuroradiol. 20, 1999, 217–222
81. Kim RB, Fromm MF, Wandel C, Leake B, Wood AJ, Roden DM, Wilkinson GR., The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV-1 protease inhibitors. J Clin Invest. 101, 1998, 289–294.
82. Tsuji A., P-glycoprotein-mediated efflux transport of anticancer drugs at the blood-brain barrier. Ther Drug Monit. 20, 1998, 588–590.
83. Perloff MD, von Moltke LL, Greenblatt DJ., Ritonavir and dexamethasone induce expression of CYP3A and P-glycoprotein in rats. Xenobiotica. 34, 2004, 133–150.
84. Uhr M, Steckler T, Yassouridis A, Holsboer F., Penetration of amitriptyline, but not of fluoxetine, into brain is enhanced in mice with blood-brain barrier deficiency due to mdr1a P-glycoprotein gene disruption. Neuropsychopharmacology. 22, 2004, 380–387.
85. Moriki Y, Suzuki T, Fukami T, Hanano M, Tomono K, Watanabe J., Involvement of P-glycoprotein in blood-brain barrier transport of pentazocine in rats using brain uptake index method. Biol Pharm Bull. 27, 2004, 932–935.
86. Miyama T, Takanaga H, Matsuo H, Yamano K, Yamamoto K, Iga T, Naito M, Tsuruo T, Ishizuka H, Kawahara Y, Sawada Y., Pglycoprotein- mediated transport of itraconazole across the bloodbrain barrier. Antimicrob Agents Chemother 42, 1998, 1738–1744.
87. Potschka H, Fedrowitz M, Loscher W., P-Glycoprotein-mediated efflux of phenobarbital, lamotrigine, and felbamate at the blood-brain barrier: Evidence from microdialysis experiments in rats. Neurosci Lett. 327, 2002, 173–176.
88. Vinogradov SV, Bronich TV, Kabanov AV., Nanosized cationic hydrogels for drug delivery: preparation, properties and interactions with cells. Adv. Drug Deliv. Rev. 54, 2002, 135–147.
89. Banks WA, Kastin AJ, Barrera CM., Delivering Peptides to the Central Nervous System: Dilemmas and Strategies. Pharm. Res. 8, 1991, 1345-50.
90. Kreuter J., Influence of the surface properties on nanoparticle-mediated transport of drugs to the brain. J Nanosci Nanotechnol 4, 2004, 484-89.
91. Ji B, Maeda J, Higuchi M, Inoue K, Akita H, Harashima H, Suhara T., Pharmacokinetics and brain uptake of lactoferrin in rats. Life Sciences. 78, 2006, 851- 855.
92. Scherrmann JM, Temsamani J., The use of Pep: Trans vectors for the delivery of drugs into the central nervous system. International Congress Series 1277, 2005, 199-211.
93. Gabathuler R, Arthur G, Kennard M, Chen Q, Tsai S, Yang J, Schoorl W, Vitalis TZ, Jefferies WA., Development of a potential protein vector (NeuroTrans) to deliver drugs across the blood brain barrier. International Congress Series. 1277, 2005, 171-184.
94. Kannan R, Kuhlenkamp JE, Jeandidier E, Trinh H, Ookhtens H, Kaplowitz N ., Evidence for carrier-mediated transport of glutathione across the blood-brain barrier in the rat J. Clin. Invest. 1990, 85-93.
95. Kannan R, Kuhlenkamp JE, Jeandidier E, Trinh H, Ookhtens H., Transport of glutathione at blood-brain barrier of the rat: inhibition by glutathione analogs and age-dependence. J. Pharmacol. Exp. Ther. 263, 1992, 964-70.
96. Pardridge, WM., Transport of small molecules through the blood–brain barrier: biology and methodology. Adv Drug Deliv Rev. 15, 1995, 5–36.
97. Bergley DJ., The blood–brain barrier: principles for targeting peptides and drugs to the central nervous system. J Pharm Pharmacol. 48, 1996, 136–146.
98. Pan W, Kastin AJ, Zankel TC, et al., Efficient transfer of receptor-associated protein RAP across the blood-brain barriendothelium er. J. Cell Sci. 2004; 117 (21): 5071-80
99. Garcia-Garcia E, Andrieux K, Gil S, Couvreur P., Colloidal carriers and blood-brain barrier (BBB) translocation: a way to deliver drugs to the brain? Int. J. Pharm. 298, 2005, 274–292.
100. Terasaki T, Täkakuwa S, Moritani S, Tsuji A., Transport of monocarboxylic acids at the blood-brain barrier: studies with monolayers of primary cultured bovine brain capillary endothelial cells. J. Pharmacol. Exp. Ther. 258, 1991, 932-37.
101. Terasaki T, Täkakuwa S, Moritani S, Tsuji A., J. Pharm. Pharmacol. 43, 1991, 172-180.
102. Takanaga H, Thmai I, Inabe S, Sai Y, Higashiga H, Yamamoto H, Tsuji A (1995) cDNA Cloning and Functional Characterization of Rat Intestinal Monocarboxylate Transporter. Biochem. Biophys. Res. Commun.217, 1995, 370-78.
103. Kurihara A, Suzuki H, Sawada Y, Sugiyama Y, T Iga, and M. Hanano,J . Pharmacol Sci.76, 1987, 759.
104. Pardridge WM., Brain metabolism: a perspective from the blood-brain barrier. Physiol. Rev. 63, 1983,148l-1535.
105. Kang YS, Terasaki T, Onishi T, Tsuji A., Small Molecular Drug Transfer across the Blood-Brain Barrier via Carrier-Mediated Transport Systems. J. PharmacobioDyn. 13, 1990, 353-60.
106. M. Shimon, Y. Egozi, Y. Kloong, M. Sokolovski, and S. Cohen, I. Neurochem. 501, 1988, 719-26.
107. Conford EM, Braun LD, Oldendod WH, Carrier mediated blood-brain barrier transport of choline and certain choline analogs. J. Neurochem. 1978, 299-308.
108. Yamazaki M, Fukuoka H, Nagata O, Kato H, Terasaki T, Tsuji A., Transport Mechanism of an H_1- Antagonist at the Blood Brain Barrier : Transport Mechanism of Mepyramine Using the Carotid Injection Technique Biol. Pharm. Bull. 71, 1994, 676.
109. Patel M. et al., Getting into the Brain: Approaches to Enhance Brain Drug Delivery. CNS Drugs; 23 (1), 2009, 35-58
110. A. K. Kumagi, J. Eisenberg, and W M. Pardrige, J. Biol. Chem. 262, 1987, 15214.
111. Pardridge M, Triguero D, Buciak J., Transport of histone through the blood-brain barrier. J. Pharmacol. Exp. Ther. 251, 1989, 821-26.
112. Verrecchia T, Spenlehauer G, Bazile DV, Murry-Brelier A, Archimbaud Y, Veillard M, Non-stealth (poly(lactic acid/albumin)) and stealth (poly(lactic acid/ polyethylene glycol)) nanoparticles as injectable drug carriers. J. Control. Release 36, 1995, 49-61.
113. Smith KR, Borchardt RT., Permeability and Mechanism of Albumin, Cationized Albumin, and Glycosylated Albumin Transcellular Transport Across Monolayers of Cultured Bovine Brain Capillary Endothelial Cells. Pharm. Res. 6, 1989, 466-473.
114. Tamai I, Sai Y, Kobayashi H, Kamata M, Wakamiya T, Tsuji A., Structure-internalization relationship for adsorptive-mediated endocytosis of basic peptides at the blood–brain barrier. J Pharmacol Exp The., 280, 1997, 410–415.
115. Pardridge WM, Buciak JL, Frieden PM., Selective transport of an anti-transferrin receptor antibody through the blood-brain barrier in vivo. J. Pharmaco Exp. Ther. 259, 1991, 66 -70.
116. Pardridge WM, Kumagai AK, Eisenberg JB., Chimeric peptides as a vehicle for peptide pharmaceutical delivery through the blood-brain barrier. Biochem Biophys. Res. Commun. 146, 1987, 307-13.
117. Pardridge WM.,Vector-mediated drug delivery to the brain. Adv Drug Deliv Rev. 36, 1999, 299–321.
118. Pardridge WM., Drug and gene targeting to the brain with molecular Trojan horses. Nat Rev Drug Discov 1, 2002, 131-139.
119. Gelperina SE, Khalansky AS, Skidan IN, Smirnova ZS, Bobruskin AI, Severin SE, et al., Toxicological studies of doxorubicin bound to polysorbate 80-coated poly (butyl cyanoacrylate) nanoparticles in healthy rats and rats with intracranial glioblastoma. Toxicol Lett. 126, 2002, 131-41.
120. Misra A, Ganesh S, Shahiwala A, Shah SP., Drug delivery to the central nervous system: A review. J Pharm Pharmaceut Sci 6(2), 2003, 252-73.
121. Calvo P, Gouritin B, Chacun H, Desmaele D, D'Angelo J, Noel JP, et al., Long-circulating PEGylated polycyanoacrylate nanoparticles as new drug carrier for brain delivery. Pharm Res 18(8), 2001, 1157-66.
122. Abhinav Agarwal et al., Nanoparticles as Novel Carrier for Brain Delivery: A Review. Current Pharmaceutical Design. 15, 2009, 917-925.
123. Terasaki T, Täkakuwa S, Moritani S, Tsuji A., J. Pharm. Pharmacol. 43, 1991, 172-180.
124. Takanaga H, Thmai I, Inabe S, Sai Y, Higashiga H, Yamamoto H, Tsuji A., Biochem. Biophys. Res. Commun. 1995, 217:370
125. Kurihara A, Suzuki H, Sawada Y et al. (1987) Pharmacol Sci.76:759.
126. Alle´mann E, Gurny R, Doelker E, Drug-loaded nanoparticles-preparation methods and drug targeting issues. Eur. J. Pharm. Biopharm. 39, 1993, 173-191.
127. Moghimi S, Illum L, Davis S., Physiopathological and physicochemical considerations in targeting of colloids and drug carriers to the bone marrow. Crit Rev Ther Drug Carrier Syst 7, 1990, 187–209.
128. Gref R, Minamitake Y, Peracchia M, Trubetskoy V, Torchilin V, Langer R., Biodegradable long-circulating polymeric nanospheres. Science 263, 1994, 1600–1603.
129. Torchilin V., Polymer-coated long-circulating microparticulate pharmaceuticals. J Microencapsul

15, 1998, 1–19.
130. Vinogradov S, Batrakova E, Kabanov A., Poly (ethylene glycol)- polyethyleneimine NanoGel particles: Novel drug delivery systems for antisense oligonucleotides. Coll Surf B: Biointerfaces 16, 1999, 291–304.
131. Lemieux P, Vinogradov SV, Gebhart CL, Guerin N, Paradis G, Nguyen HK, Ochietti B, Suzdaltseva YG, Bartakova EV, Bronich TK, St-Pierre Y, Alakhov VY, Kabanov AV., Block and graft copolymers and NanoGel copolymer networks for DNA delivery into cell. J Drug Target 8, 2000, 91–105.
132. Alyaudtin RN, Reichel A, Lobenberg R, Ramge P, Kreuter J, Begley DJ., Interaction of poly(butylcyanoacrylate) nanoparticles with the blood-brain barrier in vivo and in vitro. J Drug Target 9, 2001, 209–221.
133. Roney C, Kulkarni P, Arora V, Antich P, Bonte F, Wu A, Mallikarjuana NN, Manohar S, Liang HF, Kulkarni AR, Sung HW, Sairam M, Aminabhavi TM., Targeted nanoparticles for drug delivery through the blood-brain barrier for Alzheimer's disease. J Control Release 108, 2005, 193–214.
134. Peracchia M, Vauthier C, Desmaele D, Gulik A, Dedieu J, Demoy M, D'Angelo J, Couvreur P., Pegylated nanoparticles from a novel methoxypolyethylene glycol cyanoacrylate-hexadecyl cyanoacrylate amphiphilic copolymer. Pharm Res. 15, 1998, 550–556.
135. Calvo P, Gouritin B, Villarroya H, Eclancher F, Giannavola C, Klein C, Andreux JP, Couvreur P., Quantification and localization of PEGylated polycyanoacrylate nanoparticles in brain and spinal cord during experimental allergic encephalomyelitis in the rat. Eur J Neurosci 15, 2002, 1317–1326.
136. Schroeder U, Sommerfeld P, and Sabel BA. Efficacy of oral dalargin-loaded nanoparticle delivery across the blood–brain barrier. Peptides. 19, 1998, 777–780.
137. Kreuter J, Alyautdin RN, Kharkevich DA, Ivanov AA., Passage of peptides through the blood–brain barrier with colloidal polymer particles (nanoparticles). Brain Res. 674, 1995, 171–174.
138. Kreuter J, Shamenkov D, Petrov V, Ramge P, Cychutek K, Koch- Brandt C, Alyautdin R., Apolipoprotein-mediated transport of nanoparticle-bound drugs across the blood-brain barrier. J Drug Target. 10, 2002, 317-25.
139. Ambruosi A. elperina S, Khalansky A, Tanski S, Theisen A, Kreuter J., Influence of surfactants, polymer and doxorubicin loading on the anti-tumour effect of poly(butyl cyanoacrylate) nanoparticles in a rat glioma model. J Microencapsul. 23, 2006, 582-92.
140. Kuo YC, and Chen HH., Effect of nanoparticulate polybutylcyanoacrylate and methylmethacrylatesulfopropylmethacrylate on the permeability of zidovudine and lamivudine across the in vitro blood-brain barrier. Int J Pharm. 327, 2006, 160-169.
141. Gao K, Jiang X., Influence of particle size on transport of methotrexate across blood brain barrier by polysorbate 80- coated polybutylcyanoacrylate nanoparticles. Int J Pharm. 310, 2006, 213-219.
142. Brigger I, Morizet J, Aubert G, Chacun H, Terrier-Lacombe MJ, Couvreur P, Vassal G., Poly(ethylene glycol)-coated hexadecylcyanoacrylate nanospheres display a combined effect for brain tumor targeting. J. Pharmacol. Exp. Ther. 303, 2002, 928–936.
143. Calvo P, Gouritin B, Brigger I, Lasmezas C, Deslys J. Williams A, Andreux JP, Dormont D, Couvreur P., PEGylated polycyanoacrylate nanoparticles as vector for drug delivery in prion diseases. J. Neurosci. Meth. 111, 2001, 151–155.
144. Calvo P, Gouritin B, Chacun H, Desmaele D, D'Angelo J, Noel JP, Georgin D, Fattal E, Andreux JP, Couvreur P., Long circulating PEGylated polycyanoacrylate nanoparticles as new drug carrier for brain delivery. Pharm. Res. 18, 2001, 1157– 1166.
145. Calvo P, Gouritin B, Villarroya H, Eclancher F, Giannavola C, Klein C, Andreux JP, Couvreur P., Quantification and localization of PEGylated polycyanoacrylate nanoparticles in brain and spinal cord during experimental allergic encephalomyelitis in the rat. Eur. J. Neurosci. 15, 2002, 1317–1326.
146. Costantino L, Gandolfi F, Tosi G, Rivasi F, Vandelli MA, Forni F., Peptide-derivatized biodegradable nanoparticles able to cross the blood–brain barrier. J Control. Release. 108, 2005, 84-96.
147. Technical document of to-BBB., "G-Technology"., www.toBBB.com
148. Alakhov VY, Moskaleva EY, Batrakova EV, Kabanov AV., Hypersensitization of multidrug resistant human ovarian carcinoma cells by pluronic P85 block copolymer. Bioconjug Chem. 7(2), 1996, 209-216.
149. Venne A, Li S, Mandeville R, Kabanov A, Alakhov V., Hypersensitizing effect of pluronic L61 on cytotoxic activity, transport, and subcellular distribution of doxorubicin in multiple drug- resistant cells. Cancer Res 56(16), 1996, 3626-3629.
150. Miller DW, Batrakova EV, Waltner TO, Alakhov V, Kabanov AV., Interactions of pluronic block copolymers with brain microvessel endothelial cells: evidence of two potential pathways for drug absorption. Bioconjug Chem. 8(5), 1997, 649-657.
151. Batrakova EV, Han HY, Miller DW, Kabanov AV., Effects of pluronic P85 unimers and micelles on drug permeability in polarized BBMEC and Caco-2 cells. Pharm Res. 15(10), 1998, 1525-1532.
152. Batrakova EV, Lee S, Li S, Venne A, Alakhov V, Kabanov A., Fundamental relationships between the composition of pluronic block copolymers and their hypersensitization effect in MDR cancer cells. Pharm Res 16(9), 1999, 1373-1379.
153. Batrakova EV, Li S, Vinogradov SV, Alakhov VY, Miller DW, Kabanov AV., Mechanism of pluronic effect on P-glycoprotein efflux system in blood-brain barrier: contributions of energy depletion and membrane fluidization. J Pharmacol Exp Ther. 299(2), 2001, 483-493.
154. Batrakova EV, Miller DW, Li S, Alakhov VY, Kabanov AV, Elmquist WF., Pluronic P85 enhances the delivery of digoxin to the brain: in vitro and in vivo studies. J Pharmacol Exp Ther 296(2), 2001, 551-557.
155. Batrakova EV, Li S, Alakhov VY, Elmquist WF, Miller DW, Kabanov AV., Sensitization of cells overexpressing multidrug resistant protein by Pluronic P85. Pharm Res. 88, 2003, 198-206.
156. Batrakova EV, Li S, Alakhov VY, Miller DW, Kabanov AV., Optimal structure requirements for Pluronic block copolymers in modifying P-glycoprotein drug efflux transporter activity in bovine brain microvessel endothelial cells. J Pharmacol Exp Ther 304(2), 2003, 845-854.
157. Moos T, Morgan EH., Transferrin and transferrin receptor function in brain barrier systems. Cell Mol Neurobiol. 20 (1), 2000, 77-95.
158. Ponka P, Lok CN., The transferrin receptor: role in health and disease. Int J Biochem Cell Biol. 31(10), 1999, 1111-37.
159. Cuervo AM., Autophagy: many paths to the same end. Mol Cell Biochem. 263(12), 2004, 55-72
160. Moos T, Morgan EH., The significance of the mutated divalent metal transporter DMT1 on iron transport into the Belgrade rat brain. J. Neurochem. 88 (1), 2004, 233-45
161. Pardridge WM., Drug and gene targeting to the brain with molecular Trojan horses. Nat Rev Drug Discov. 1 (2), 2002, 131-39.
162. Broadwell RD, Baker-Cairns BJ, Friden PM, et al., Transcytosis of protein through the mammalian cerebral epithelium and endothelium. III. Receptor-mediated transcytosis through the blood-brain barrier of blood-borne transferrin and anti-body against the transferrin receptor. Exp Neurol. 142(1), 1996, 47-65.
163. Chun XW et al., Antitumor effects of polysorbate-80 coated gemcitabine polybutylcyanoacrylate nanoparticles in vitro and its pharmacodynamic in vivo on C6 glioma cells of a brain tumor model. Brain Research. 1261, 2009, 91-99.
164. Barnabas W et al., Poly (n-butylcyanoacrylate) nanoparticles coated with polysorbate 80 for the targeted delivery of rivastigmine into the brain to treat Alzheimer's disease. Brain Research 1200, 2008, 159-168.
165. Kepan Gao, Xinguo Jiang., Influence of particle size on transport of methotrexate across blood brain barrier by polysorbate 80-coated polybutylcyanoacrylate nanoparticles"; Int. J. Pharm. 310, 2006, 213–219.
166. Petri B et al., Chemotherapy of brain tumour using doxorubicin bound to surfactant-coated poly(butyl cyanoacrylate) nanoparticles: Revisiting the role of surfactants. J. Control Release. 117, 2007, 51–58.
167. Clavo P et al., PEGylated polycyanoacrylate nanoparticles as vector for drug delivery in prion diseases. J. of Neurosci. Met. 111, 2001, 151–155.
168. Kaili Hu et al., Lactoferrin-conjugated PEG–PLA nanoparticles with improved brain delivery: In vitro and in vivo evaluations. J. Control. Release. 134, 2009, 55–61.
169. Alyautdin RN, Petrov VE, Langer K et al., Delivery of Loperamide across the blood brain barrier with polysorbate 80-coated poly butyl cyanoacrylate nanoparticles. J Pharm Res. 14, 1997, 325-38.
170. Koziara JM, Lockman PR, Allen DD et al., Paclitaxel Nanoparticles for the potential treatment of brain tumors. J Control Release, 99, 2004 259-69.
171. Cui Z, Lockman PR, Atwood CS, et al., Novel D-penicillamine carrying nanoparticles for metal chelation therapy in Alzheimer's and other CNS diseases. Eur J Pharm Biopharm. 59, 2005, 263-72
172. Costantino L, Gandolfi F, Tosi G, et al., Peptide-derivatized biodegradable nanoparticles able to cross the blood-brain barrier. J Control Release 108, 2005, 84-96.
173. Aktas Y, Yemisci M, Andrieux K, et al., Development and brain delivery of chitosan-PEG nanoparticles functionalized with the monoclonal antibody OX26. Bioconjugate Chem. 16, 2005, 1503-11.
174. Tosi G, Costantino L, Rivasi F, et al., Targeting the central nervous system: in vivo experiments with peptide-derivatized nanoparticles loaded with Loperamide and rhodamine-123. J Control Release. 22, 2007, 1-9.
175. Kreuter J, Shamenkov D, Petrov V, Ramge P, Cychutek K, Koch-Brandt C, Alyautdin R., Apolipoprotein-mediated Transport of Nanoparticle-bound Drugs Across the Blood-Brain Barrier. Journal of Drug Targeting 10, 2002, 317-325.
176. Schroeder U, Sommerfeld P, Ulrich S, Sabel BA., Nanoparticle technology for delivery of drugs across the blood-brain barrier. J Pharm Sci. 87(11), 1998, 1305-7.

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