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Review Article

NOVELISTIC STRATEGIES TO CROSS CNS BARRIERS: AN UPDATE.

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ABSTRACT

The two main interfaces of brain, which protects neurons from the substances present in the blood including drugs. It also helped in maintaining water homeostasis and proper milieu. These are important for neuronal functions i.e. Blood CSF interface and Blood Brain Barrier. The BBB stand a major challenge for drug delivery to brain so that the treatment of neurodegenerative disorders remains unsolved. The structure of the brain is very specific due to tight junction or zonula occludens mainly composed of interconnected endothelial cells which controls the passage of molecules. For more than 25years various efforts have performed to cross the barrier. The current challenge is to deliver the drug molecule through BBB in a safe and effective manner. The Blood cerebrospinal fluid acts as a supplement provider for brain and spinal cord. The biological properties of CSF associated with initiation of brain neurogenesis. CSF is secreted by the choroid plexus epithelium which is responsible for neuroendocrine signaling and neuroimmune response. BBB completely restricts the free movement of hydrophilic compounds it may be paracellular or may be transcellular. BBB is responsible for the transport of essential nutrients and discharge of metabolites. In this review, a focus has tried on various novel invasive techniques to overcome this unsolved issue.
Key words: *Blood-brain barrier, Neuro-degenerative disorders, CNS.*

INTRODUCTION

The blood–brain barrier: structure and regulation[1]

The blood–brain barrier (BBB) is a diffusion barrier, which impedes influx of most compounds from blood to brain. Three cellular elements of the brain microvasculature compose the BBB - endothelial cells, astrocyte end-feet, and pericytes (PCs). Tight junctions (TJs), present between the cerebral endothelial cells, form a diffusion barrier, which selectively excludes most blood-borne substances from entering the brain. Astrocytic end-feet tightly ensheath the vessel wall and appear to be critical for the induction and maintenance of the TJ barrier, but astrocytes are not believed to have a barrier function in the mammalian brain. Dysfunction of the BBB, for example, impairment of the TJ seal, complicates a number of neurologic diseases including stroke and neuro-inflammatory disorders. We review here the recent developments in our understanding of the BBB and the role of the BBB dysfunction in CNS disease. We have focused on intra-ventricular haemorrhage (IVH) in premature infants, which may involve dysfunction of the TJ seal as well as immaturity of the BBB in the germinal matrix (GM). A paucity of TJs or PCs, coupled with incomplete coverage of blood vessels by astrocyte end-feet, may account for the fragility of blood vessels in the GM of premature infants. Finally, this review describes the pathogenesis of increased BBB permeability in hypoxia– ischemia and inflammatory mechanisms involving the BBB in septic encephalopathy, HIV-induced dementia, multiple sclerosis, and Alzheimer disease.

Anatomy of blood brain barrier[2]

Cells in the brain require a very stable environment to ensure controlled and selective stimulation of neurons. As a result, only certain materials are allowed to pass from blood vessels to the brain. Substances such as O_2, glucose, H_2O, CO_2, essential amino acids, and most lipid-soluble substances enter the brain readily. Other substances, such as creatine and urea (wastes transported in the blood), most ions (Na^+,K^+,Cl^-), proteins, and certain toxins either have limited access or are totally blocked from entering the brain. Unfortunately, most antibiotic drugs are equally blocked from entering, while other substances such as caffeine, alcohol, nicotine, and heroin readily enter the brain (because of their lipid solubility). This blood-brain barrier is established by the following:

- Brain capillaries are less permeable than other capillaries because of tight junctions between the endothelial cells in the capillary walls.
- The basal lamina (secreted by the endothelial cells) that surrounds the brain capillaries decreases capillary permeability. This layer is usually absent in capillaries found elsewhere.
- Processes from astrocytes (a type of neuroglial cell) cover brain capillaries and are believed to influence capillary permeability.

The blood-brain barrier (BBB) is the regulated interface between the peripheral circulation and the central nervous system (CNS). Although originally observed by Paul Ehrlich in 1885, the nature of the BBB was debated well into the 20th century. The anatomical substrate of the BBB is the cerebral micro vascular endothelium, which, together with astrocytes, pericytes, neurons, and the extracellular matrix, constitute a “neurovascular unit” that is essential for the health and function of the CNS. Tight junctions (TJ) between endothelial cells of the BBB restrict paracellular diffusion of water-soluble substances from blood to brain. The TJ is an intricate complex of transmembrane (junctional adhesion molecule-1, occludin, and claudins) and cytoplasmic (zonula occludens-1 and -2, cingulin, AF-6, and 7H6) proteins linked to the actin cytoskeleton. The expression and sub cellular localization of TJ proteins are modulated by several intrinsic signalling pathways, including those involving calcium, phosphorylation and G-proteins. Disruption of BBB TJ by disease or drugs can lead to impaired BBB function and thus compromise the CNS.

Physiology of blood brain barrier[3]

1. The specifically regulated restrictive permeability barrier to cells and molecules is the most important feature of the blood–brain barrier (BBB). The aim of this review was to summarize permeability data obtained on in vitro BBB models by measurement of trans endothelial electrical resistance and by calculation of permeability coefficients for para cellular or trans endothelial tracers.

2. Results from primary cultures of cerebral micro vascular endothelial cells or immortalized cell lines from bovine, human, porcine, and rodent origin are presented. Effects of co-culture with astroglia, neurons, mesenchymal cells, blood cells, and conditioned media, as well as physiological influence of serum components, hormones, growth factors, lipids, and lipoproteins on the barrier function are discussed.

3. BBB permeability results gained on in vitro models of pathological conditions including hypoxia and reoxygenation, neurodegenerative diseases, or bacterial and viral infections have been reviewed. Effects of cytokines, vasoactive mediators, and other pathogenic factors on barrier integrity are also detailed.

4. Pharmacological treatments modulating intracellular cyclic nucleotide or calcium levels, and activity of protein kinases, protein tyrosine phosphatises, phospholipases, cyclooxygenas-es, or lipoxygenases able to change BBB integrity are outlined. Barrier regulation by drugs involved in the metabolism of nitric oxide and reactive oxygen species, as well as influence of miscellaneous treatments are also listed and evaluated.
5. Though recent advances resulted in development of improved in vitro BBB model systems to investigate disease modeling, drug screening, and testing vectors targeting the brain, there is a need for checking validity of permeability models and cautious interpretation of data.

Blood-brain barrier: morphology[4]

The blood-brain barrier (BBB) separates the brain and cerebrospinal fluid from the blood and regulates the exchange of substances between the blood and the brain. It is comprised chiefly of brain capillaries, choroid plexus cuboidal epithelium, and the arachnoid membrane. Barrier-type capillaries are located also in the retina, iris, inner ear, and within the endoneurium of peripheral nerves. All BBB sites are characterized by the presence of tight junctions (zonulae occludens, less than 2 ram in width) between contiguous cells, the absence of endothelial pores, and a paucity of pinocytic vesicles. Further, brain capillaries contain a several-fold increase in the numerical density of endothelial mitochondria as compared with capillaries from other regions of the body. The cells constituting the BBB, connected by tight junctions, lacking endothelial pores, and possessing relatively few pinocytic vesicles act almost like a continuous cell layer, permitting solute exchange primarily by the transcellular route only. Thus lipid soluble solutes easily penetrate the BBB while electrolytes, lipid-insoluble nonelectrolytes, and protein enter the brain from blood more slowly than they enter non-nervous tissues.

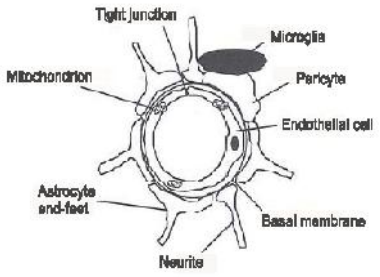

Fig 1: Morphology of the capillary epithelium in CNS.[5]

Fine Structural Localization of a Blood Brain Barrier to Exogenous Peroxidase[5]

Horseradish peroxidase was administered to mice by intravenous injection, and its distribution in cerebral cortex studied with a recently available technique for localizing peroxidase with the electron microscope. Brains were fixed by either immersion or vascular perfusion 10–60 min after administration of various doses of peroxidase. Exogenous peroxidase was localized in the lumina of blood vessels and in some micropinocytotic vesicles within endothelial cells; none was found beyond the vascular endothelium. Micropinocytotic vesicles were few in number and did not appear to transport peroxidase while tight junctions between endothelial cells were probably responsible for preventing its intercellular passage. Our findings therefore localize, at a fine structural level, a "barrier" to the passage of peroxidase at the endothelium of vessels in the cerebral cortex. The significance of these findings is discussed, particularly with reference to a recent study in which similar techniques were applied to capillaries in heart and skeletal muscle.

Polarity of the Blood-Brain Barrier[6]

The sub cellular distribution in brain capillaries of alkaline phosphatase and Na^+, K^+-ATPase was investigated by two methods. Cytochemical studies using whole brain perfusion and electron microscopic examination indicated that alkaline phosphatase activity was located in both the luminal and antiluminal cytoplasmic membranes of the brain capillary endothelial cells. By contrast, the K^+-dependent phosphatase activity associated with Na^+, K^+-ATPase was located in only the antiluminal membrane. Biochemical studies using membranes prepared by homogenization of isolated brain capillaries and density gradient centrifugation resulted in identification of two plasma membrane fractions. The light fraction contained alkaline phosphatase but very little Na^+, K^+-ATPase while the heavier fraction contained both enzyme activities. In addition, γ-glutamyl transpeptidase showed a distribution similar to alkaline phosphatase while 5′-nucleotidase activity was distributed with the Na^+, K^+-ATPase activity. We conclude that the luminal and antiluminal membranes of brain capillaries are biochemically and functionally different. This polarity should permit active solute transport across brain capillary endothelial cells which are the cells responsible for the blood-brain barrier.

Strategies to Overcome Blood-Brain Barrier[7]

Within drug discovery, it is desirable to determine whether a compound will penetrate and distribute within the central nervous system (CNS) with the requisite pharmacokinetic and pharmacodynamic performance required for a CNS target or if it will be excluded from the CNS, wherein potential toxicities would mitigate its applicability. A variety of in vivo and in vitro methods for assessing CNS penetration have therefore been developed and applied to advancing drug candidates with the desired properties. In silico methods to predict CNS penetration from chemical structures have been developed to address virtual screening and prospective design. In silico predictive methods are impacted by the quality, quantity, sources, and generation of the measured data available for model development. Key considerations for predictions of CNS penetration include the comparison of local (in chemistry space) versus global (more structurally diverse) models and where in the drug discovery process such models may be best deployed. Preference should also be given to in vitro and in vivo measurements of greater mechanistic clarity that better support the development of structure-property relationships. Although there are numerous statistical methods that have been brought to bear on the prediction of CNS penetration, a greater concern is that such models are appropriate for the quality of measured data available and are statistically validated. In addition, the assessment of prediction uncertainty and relevance of predictive models to structures of interest are critical. This will address these key considerations for the development and application of in silico methods in drug discovery.

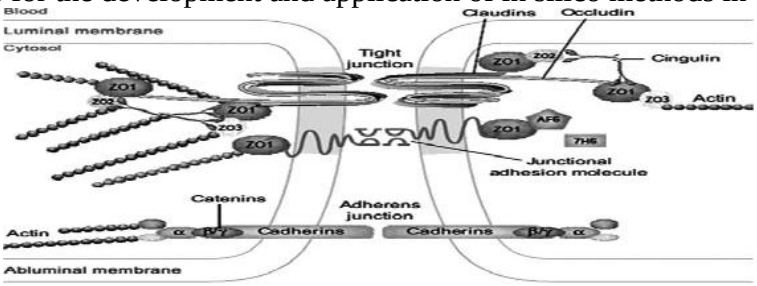

Fig 2: Structure of the tight junction complex at the BBB. [8]

1 Inconsistent blood brain barrier disruption by intra-arterial mannitol in rabbits: implications for chemotherapy[8]

The novel ability to quantify drug and tracer concentrations in vivo by optical means leads to the possibility of detecting and quantifying blood brain barrier (BBB) disruption in real-time by monitoring concentrations of chromophores such as Evan's Blue. In this study, experiments were conducted to assess the disruption of the BBB, by intra-arterial injection of mannitol, in white rabbits. Surgical preparation included: tracheotomy for mechanical ventilation, femoral and selective internal carotid artery (ICA) catheterizations, skull screws for monitoring electro cerebral activity, bilateral placement of laser Doppler probes and a small craniotomy for the placement of a fiber optic probe to determine tissue Evan's Blue dye concentrations. Evans Blue (6.5 mg/kg) was injected intravenously (IV) just before BBB disruption with intracarotid mannitol (25%, ml/40 s). Brain tissue concentrations of the dye in mannitol-treated and control animals were monitored using the method of optical pharmacokinetics (OP) during the subsequent 60 min. Hemodynamic parameters, heart rate, blood pressure, and EKG remained stable throughout the experiments in both the control and the mannitol-treated group. A brain tissue concentration of Evan's Blue and the brain: plasma. Evan's Blue partition coefficient progressively increased during the period of observation. A wide variation in brain tissue of Evan's Blue concentration was observed in the mannitol group. The experiments demonstrate the feasibility of measuring tissue concentrations of Evan's Blue without invading the brain parenchyma, and in real-time. The data suggest that there are significant variations in the degree and duration of BBB disruption induced with intra-arterial mannitol. The ability to optically monitor the BBB disruption in real-time could provide a feedback control for hypertonic disruption and/or facilitate dosage control for chemotherapeutic drugs that require such disruption.

2 Intranasal delivery bypasses the blood-brain barrier to target therapeutic agents to treat neurodegenerative disease[9].

Intranasal delivery provides a practical, non-invasive method of bypassing the blood-brain barrier (BBB) to deliver therapeutic agents to the brain and spinal cord. This technology allows drugs that do not cross the BBB to be delivered to the central nervous system within minutes. It also directly delivers drugs that do cross the BBB to the brain, eliminating the need for systemic administration and its potential side effects. This is possible because of the unique connections that the olfactory and trigeminal nerves provide between the brain and external environment. Intranasal delivery does not necessarily require any modification to therapeutic agents. A wide variety of therapeutics, including both small molecules and macromolecules, can be targeted to the olfactory system and connected memory areas affected by Alzheimer's disease. Using he intranasal delivery system, researchers have reversed neurodegeneration and res-cued memory in a transgenic mouse model of Alzheimer's disease. Intranasal insulin-like growth factor-I, deferoxamine, and erythropoietin have been shown to protect the brain against stroke in animal models. Intranasal delivery has been used to target the neuroprotective peptide NAP to the brain to treat neurodegeneration. Intranasal fibroblast growth fac-tor-2 and epidermal growth factor have been shown to stimulate neurogenesis in adult animals. Intranasal insulin improves memory, attention, and functioning in patients with Alzheimer's disease or mild cognitive impairment, and even improves memory and mood in normal adult humans. This new method of de-livery can revolutionize the treatment of Alzheimer's disease, stroke, and other brain disorders.

3 Approaches to transport therapeutic drugs across the blood–brain barrier to treat brain diseases[10]

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The central nervous system is protected by barriers which control the entry of compounds into the brain, thereby regulating brain homeostasis. The blood–brain barrier, formed by the endothelial cells of the brain capillaries, restricts access to brain cells of blood–borne compounds and facilitates nutrients essential for normal metabolism to reach brain cells. This very tight regulation of the brain homeostasis results in the inability of some small and large therapeutic compounds to cross the blood–brain barrier (BBB). Therefore, various strategies are being developed to enhance the amount and concentration of therapeutic compounds in the brain. In this review, we will address the different approaches used to increase the transport of therapeutics from blood into the brain parenchyma. We will mainly concentrate on the physiologic approach which takes advantage of specific receptors already expressed on the capillary endothelial cells forming the BBB and necessary for the survival of brain cells. Among all the approaches used for increasing brain delivery of therapeutics, the most accepted method is the use of the physiological approach which takes advantage of the transcytosis capacity of specific receptors expressed at the BBB. The low density lipoprotein receptor related protein (LRP) is the most adapted for such use with the engineered peptide compound (EPiC) platform incorporating the Angiopep peptide in new therapeutics the most advanced with promising data in the clinic.

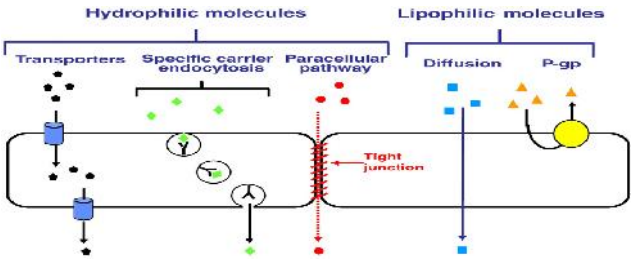

Fig 3: Transport of molecules across the BBB.[10]

4 ABC transporters in the blood-brain barrier relevance in stroke therapy[11]

There is a great demand to find effective treatment for stroke patients. Much effort has been made to target medication specifically to the affected brain area. The blood-brain-barrier strictly controls the amount of xenobiotics that can enter the brain in order to protect the fragile and sensitive homeostasis. The penetration of drug molecules through the blood brain- barrier is restricted by various mechanisms such as tight junctions and transmembrane transporter proteins. Even if a drug gets across the membrane of the brain capillary endothelia, it is liable to be pumped back to the blood stream by efflux transporters. Transporters may further influence stroke therapy via regulating the bioavailability and pharmacokinetic characteristics of drugs. Co-administration of drugs may result drug-drug interaction which is another aspect that must be considered at therapeutic interventions. This review gives insight into methods to detect transporter-drug interactions and discusses the interface of transporters and stroke.

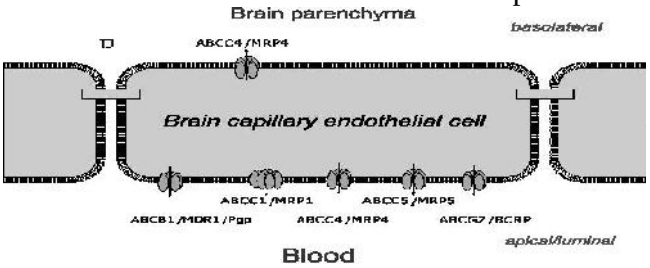

Fig 4: Efflux transporters expressed in the BBB.[11]

5 Nanotechnology breakthrough: penetration of the blood brain barrier[12]

Nanotechnology has the potential to greatly improve public health through enhanced diagnosis and treatment of increasing numbers of conditions. At present, nanotechnology for drug delivery is extremely encouraging, especially in the direction of drugs across the blood-brain barrier (BBB). The exploration of the prospects of nanoparticle drug delivery across the BBB, beginning with a description of a current application: the use of nanoparticles to deliver antiretroviral drugs to the brain for the treatment of HIV. In our discussion, we will demonstrate how this research could be applied to transport dopamine across the BBB to the substantia nigra to treat the motor symptoms presented by Parkinson's disease (PD). These approaches, which are often based on the design and engineering of plethora of nanoparticulate entities with high specificity for brain capillary endothelial cells, are currently being applied to early AD diagnosis and treatment. In addition, nanoparticles (NPs) with high affinity for the circulating amyloid-β (Aβ) forms may induce "sink effect" and improve the AD condition. There are also developments in relation to in vitro diagnostics for AD, including ultrasensitive NP-based bio-barcodes, immunosensors, as well as scanning tunnelling microscopy procedures capable of detecting Aβ_{1-40} and Aβ_{1-42}.

Table 1: Nanotechnology neuroprotective agents for treatment of AD.

Nanosystem	**Neuroprotective Function**	**Study Mode**
Nanogels	**Aβ Anti-assembly (Incorporate Aβ monomers)**	***In vitro***
Fullerene (C60): Fullerenol Carboxyfullerene,	**Aβ Anti-Assembly Anti-oxidant**	***In vitro* (cell culture) *In vivo* (Rats)**
Dendrimers:Polyamidoamine	**Aβ Anti-Assembly**	***In vitro***
Nanoceria (CeO2)	**Anti-oxidant**	***In vitro***
Gold Nanoparticles (AuNP)	**Aβ Anti-Assembly**	***In vitro***
Diamondoid Derivatives (Memantine)	**NMDA receptor Antagonism**	***In vivo* (FDAapproved)**

6 Colloidal carriers and blood–brain barrier translocation: A way to deliver drugs to the brain[13]

The major problem in drug delivery to the brain is the presence of the blood–brain barrier (BBB) which limits drug penetration even if in certain pathological situations the BBB is partly disrupted. Therefore, various strategies have been proposed to improve the delivery of drugs to this tissue. The status of the BBB in healthy patients and in pathologies like neurodegenerative, cerebrovascular and inflammatory diseases. The invasive and non-invasive strategies developed to circumvent the BBB and deliver drugs into the brain. The use of nanotechnologies (liposomes, nanoparticles) is especially discussed in the ultimate part of the review evidencing their potentiality as non-invasive technique in the brain delivery of drugs with the possibility to target specific brain tissue thanks to ligand linked to carrier surface. The idea behind this approach was to break down the barrier temporarily by injecting a sugar solution (mannitol) into arteries in the neck. The resulting high sugar concentration in brain capillaries sucks water out of the

endothelial cells, shrinking them thus opening tight junctions. In current practice, the effect lasts for 20–30 min, during which time drugs that would not normally cross the BBB diffuse freely. This method allows the delivery of chemotherapeutic agents in patients with malignant glioma, cerebral lymphoma and disseminated CNS germ cell tumor, with a subsequent decrease in morbidity and mortality compared with patients receiving systemic chemotherapy alone. However, this approach also causes several undesired side effects in humans, including physiological stress, transient increase in intracranial pressure, and unwanted delivery of anticancer agents to normal brain tissue. In addition, this technique requires considerable expertise for administration

7 Blood-brain barrier:its implications in drug transport: novel strategies in drug delivery to the brain[14]

The brain is a fragile organ as well as complicated. The brain is protected from many toxic substances and various chemicals by the presence of two barriers namely blood brain barrier (BBB) and blood cerebrospinal fluid barrier (BCSFB). BBB it protects the brain because of restrictive angio-architecture with endothelial cells, tight junctions and peculiar transport system. The routes of drug targeting to the brain now become an important tool in the pharmaceutical field because of many complicated diseases of the brain. There are some limitations for the trans-nasal drug delivery, trans cranial drug, BBB disruption, lipidization of molecules for delivery of drugs, Therefore various novel technologies are entered like nanotechnology, liposomal drug delivery and Molecular Trojan Horses. This review includes endogenous transporters which place a role in transport of drug in to the brain, drug delivery in to the brain along with limitations and discussed the novel routes of drug delivery in to the brain.

8 Mast cells migrate from blood to brain[15]

It is well established that mast cells (MCs) occur within the CNS of many species. Furthermore, their numbers can increase rapidly in adults in response to altered physiological conditions. In this study we found that early postpartum rats had significantly more mast cells in the thalamus than virgin controls. Evidence from semi thin sections from these females suggested that mast cells were transiting across the medium-sized blood vessels. We hypothesized that the increases in mast cell number were caused by their migration into the neural parenchyma. To this end, we purified rat peritoneal mast cells, labeled them with the vital dyes PKH26 or Cell Tracker Green, and injected them into host animals. One hour after injection, dye-filled cells, containing either histamine or serotonin (mediators stored in mast cells), were located close to thalamic blood vessels. Injected cells represented; 2–20% of the total mast cell population in this brain region. Scanning confocal microscopy confirmed that the biogenic amine and the vital dye occurred in the same cell. To determine whether the donor mast cells were within the blood–brain barrier, we studied the localization of dye-marked donor cells and either Factor VIII, a component of endothelial basal laminae, or glial fibrillary acidic protein, the intermediate filament found in astrocytes. Serial section reconstructions of confocal images demonstrated that the mast cells were deep to the basal lamina, in nests of glial processes. This is the first demonstration that mast cells can rapidly penetrate brain blood vessels, and this may account for the rapid increases in mast cell populations after physiological manipulations.

9 Computational prediction of blood-brain barrier permeability using decision tree induction[16]

Predicting blood-brain barrier (BBB) permeability is essential to drug development, as a molecule cannot exhibit pharmacological activity within the brain parenchyma without first transiting this barrier. Understanding the process of permeation, however, is complicated by a

combination of both limited passive diffusion and active transport. Our aim here was to establish predictive models for BBB drug permeation that include both active and passive transport. A database of 153 compounds was compiled using *in vivo* surface permeability product (logPS) values in rats as a quantitative parameter for BBB permeability. The open source Chemical Development Kit (CDK) was used to calculate physico-chemical properties and descriptors. Predictive computational models were implemented by machine learning paradigms (decision tree induction) on both descriptor sets. Models with a corrected classification rate (CCR) of 90% were established. Mechanistic insight into BBB transport was provided by an Ant Colony Optimization (ACO)-based binary classifier analysis to identify the most predictive chemical substructures. Decision trees revealed descriptors of lipophilicity (aLogP) and charge (polar surface area), which were also previously described in models of passive diffusion. However, measures of molecular geometry and connectivity were found to be related to an active drug transport component.

10 Delivery of peptide and protein drugs over the blood–brain barrier[17]

Peptide and protein (P/P) drugs have been identified as showing great promises for the treatment of various neurodegenerative diseases. A major challenge in this regard, however, is the delivery of P/P drugs over the blood–brain barrier (BBB). Intense research over the last 25 years has enabled a better understanding of the cellular and molecular transport mechanisms at the BBB, and several strategies for enhanced P/P drug delivery over the BBB have been developed and tested in preclinical and clinical– experimental research. Among them, technology-based approaches (comprising functionalized nanocarriers and liposomes) and pharmacological strategies (such as the use of carrier systems and chimeric peptide technology) appear to be the most promising ones. This review combines acomprehensive overview on the current understanding of the transport mechanisms at the BBB. Exposure of endothelium to proinflammatory cytokines (IFN-g, TNF-a and IL-1b) interrupts the BBB by disorganizing cell-cell junctions, decreases the brain solute barrier, enhances leukocyte endothelial adhesion and migration as well as increases expression of class II MHC and promotes shedding of endothelial 'microparticles' (EMP). In this review we examine interactions between cytokines/chemo kines, activated leukocytes, adhesion molecules and activated C EC in the pathogenesis of BBB failure in MS.

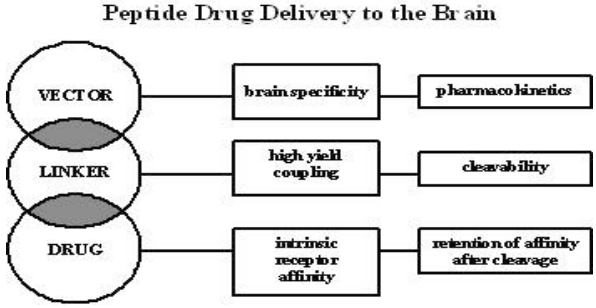

Fig 5: Three interwoven areas of vector, linker and drug development.[17]

The Status of Blood Brain Barrier in Neurological Diseases:

1 Blood-brain barrier disruption in multiple sclerosis[18]

The blood-brain barrier (BBB) is a complex organization of cerebral endothelial cells (CEC), pericytes and their basal lamina, which are surrounded and supported by astrocytes and perivascular macrophages. Collectively these cells separate and form the compartments of the

cerebral vascular space and the cerebral inter stitium under normal conditions. Without the BBB, the 'interior milieu' of the central nervous system (CNS) would be flooded by humoral neurotransmitter s and formed blood elements that upset normal CNS functions and lead to vascular/neural injury. Dysregulation of the BBB and transendothelial migration of activated leukocytes are among the earliest cerebrovascular abnormalities seen in multiple sclerosis (MS) brains and parallel the release of inflammatory cytokines/chemokines. Mechanisms for breakdown of the BBB in MS are incompletely under stood, but appear to involve direct effects of these cytokines/ chemokines on endothelial regulation of BBB components, as well as indirect cytokine/chemokine-dependent leukocyte mediated injury. Unique endothelial structural features of the BBB include highly organized endothelial tight junctions, the absence of class II major histocompatibility complex, abundant mitochondria and a highly developed transport system in CEC.

2 Alzheimer disease (AD)[19]

Alzheimer's disease is a prevalent form of adult onset dementia. It results in the progressive deterioration of cognitive ability and memory, which is related to the degeneration of basal forebrain cholinergic neurons. Amyloid_ (A_), a heterogeneous 39–43 amino acid peptide is the main constituent of the senile plaques and cerebrovascular deposits, the primary lesions in AD. The origin of the A_ deposited in cerebral vasculature and brain is uncertain. According to the "neuronal theory", A_ is produced locally in the brain. On the contrary, the "vascular theory" proposes that A_ originates from the circulation and that circulating A_ could contribute to neurotoxicity by crossing the BBB. Transport of several peptides and proteins through the BBB is possible via receptor-mediated transcytosis. In this way, it was suggested that the receptor RAGE is involved in the transcytosis of a synthetic peptide (125I-sA_1–40) homologous to human A_ and it could play an important role in the development of AD. Currently, the only specific pharmacological therapeutic option available for AD patients is the treatment with cholinesterase inhibitors, which provides moderate benefits in a subset of patients for a limited period. Additionally, recent studies have shown that nerve growth factor (NGF) may also be useful to prevent cholinergic neuron death following acute trauma.

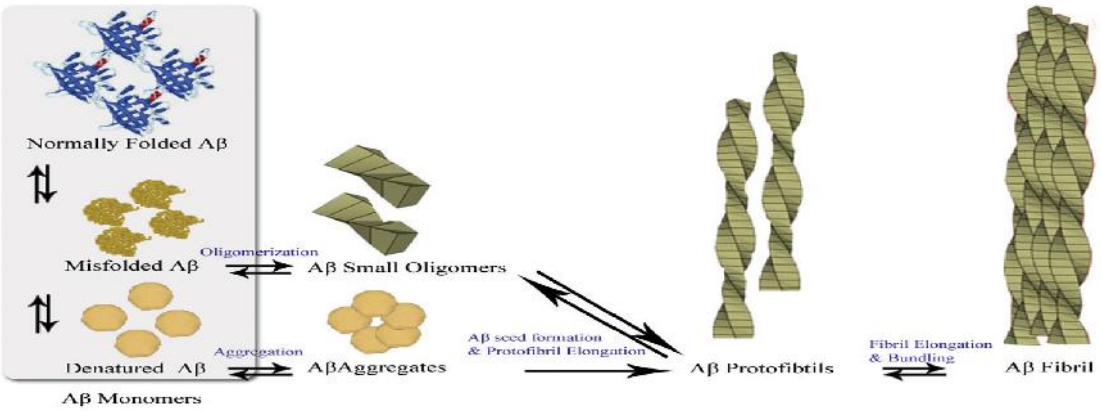

Fig 6: Sequential formation of amyloid aggregates, protofibrils and fibrils.[19]

3 Parkinson's disease (PD)[20]

Parkinson's disease is characterized by the behavioural symptoms like akinesia/bradykinesia, rigidity and tremor. At the cellular level, PD manifests in a progressive loss of midbrain dopaminergic neurons of the *substantia nigra* over several years and a concomitant development of a dopaminergic deficit in the projection area, the *striatum*. Among the factors suspected of contributing to the preferential vulnerability of dopaminergic neurons, the oxidative stress is associated with dopamine metabolism. Although current drug therapy of PD is more successful compared to treatments of AD, it does not stop the degenerative process, and pharmacotherapy unfortunately looses effectiveness with progression of the disease. Drugs aimed at the reduction of the dopaminergic deficit (L-DOPA, monoamine oxidase B inhibitors, dopamine agonists) remain the mainstay of symptomatic drug treatment. In addition to a loss of effect over time, therapy is accompanied accompanied by an increase in frequency and severity of side effects.

4 Cerebrovascular diseases[21]

The integrity of the BBB in cerebrovascular disease due to hypertension or cardiac bypass is variable, because the underlying cerebral ischemia varies with respect to the mechanism, the severity and the duration. After ischemia, drastic reductions in cerebral blood flow in the core of the lesions typically result in rapid cell death within minutes. Activation of cytokines such as tumor necrosis factor (TNF) and interleukin-1 (IL-1) and the upregulation of cell adhesion molecules can also be observed during this disease. Lymphocytes can then penetrate the BBB, releasing proteases, particularly metalloproteinases, which induce the opening of BBB. By virtue of this opening, migration and adhesion of neutrophils, monocytes and macrophag- es, to the site of injury occur.

5 Brain tumours[22]

Gliomas are the most frequent primary CNS tumours in humans. They are classified into four clinical grades, grade4 or glioblastoma multiform (GBM) being the most aggressive. The different types of glioma can be differentiated by histological characteristics reflecting cellular differentiation lineages: astrocytomas, oligodendrogliomas and mixed oligo astrocytomas. The vascular microenvironment determines the pathophysiological characteristics of gliomas, such as edema formation, tumor cell invasiveness. Indeed, endothelial cells, pericytes, and the basement membrane of tumor vessels reveal significant abnormalities when compared to cerebral vessels. An increase in vessel wall thickness is one common feature of glioma vasculature and is attributed to endothelial cell hyperplasia, leading to an increase in non-selective transendothelial transport. Tight junction opening is functionally the most important abnormality and becomes more pronounced with increasing malignancy. Thus, compared with normal human brain, astrocytomas fail to express or express a non-functional form of occludin. Moreover, fenestrations and increase in the number and size of pinocytic vacuoles has been reported. The gliomas present a particular therapeutic problem because of their poor response to chemotherapy. The resistance of tumors to therapeutic intervention may be due to cellular mechanisms, which are categorized in terms of alterations in the biochemistry of malignant cells. They comprise altered activity of specific enzymes, altered apoptosis regulation, or transport based mechanisms, like the P-gp efflux system, responsible for multi-drug-resistance (MDR). The understanding of structural and functional characteristics of vascular microenvironment in gliomas is essential for the design of successful future therapeutic strategies against this type of tumor.

Table 2: Blood brain barrier endogenous transporters[23]

Carrier-mediated transporters (CMT)	Active-efflux transporters (AET)	Receptor-mediated transporters (RMT)
Glucose transporter (GLUT1)	Adenosine triphposphate binding cassette(ABC/P-gp)	Insulin receptor (INSR)
Large neutral amino acid transporter(LAT1)	ABC transporter, subfamily C (ABCC)	Transferrin receptor (TFR)
Cationic amino acid transporter (CAT1)	ABC transporter, subfamily G (ABCG2)	Insulin-like growth factor receptor (IGF1R)
Mono carboxylic acid transporter (MCT1)	Organic anion transporter (OAT / SLC22)	Insulin-like growth factor receptor (IGF2R)
Concentrative nucleoside transporter (CNT2)	Organic anion-transporting polypeptide (OATP /SLC21)	Leptin receptor (LEPR)
Choline transporter (CHT)	Glutamic acid amino acid transporter (EAAT/ SLC1)	Fc fragment of IgG receptor transporter (FCGRT)
Nucleobase transporter (NBT)	Taurine transporter (TAUT /SLC6)	Scavenger receptor, class B (SCARB1)

Changes in blood-brain barrier permeability induced by radiotherapy[24]:

The brain requires a stable internal environment, which is established by the integrity of the blood-brain barrier (BBB). The efficacy of chemotherapeutics in the treatment of brain malignancies is often hampered by the presence of the BBB. BBB disruption can be performed either by osmotic disruption, bradykinin or irradiation. Radiotherapy with doses of 20 to 30 Gy with fraction size of 2 Gy may be used to increase the permeability of the BBB. These radiation doses by themselves will not give rise to serious side effects or long-term complications. Disruption of the BBB by radiotherapy might have implications in the treatment of primary brain tumours, cerebral metastases, and prophylactic cranial irradiation in small cell lung cancer since irradiation will cause cell kill and may enhance the effect of chemotherapy. We present a review on the effects of irradiation on the BBB and subsequently discuss the potential value for therapeutic applications.

Clinical application of BBB disruption by irradiation[25]:

BBB disruption by irradiation has the advantage that irradiation will cause cell kill and may enhance the effect of chemotherapy. One study suggests that, in order to increase the permeability of the BBB by irradiation, a total dose of 20 to 30 Gy with fractions sized up to 3 Gy are needed. To reduce long-term toxicity a maximum fraction size of 2 Gy is preferable (56). The study confirmed that opening of the BBB by irradiation with total doses administered at 2 Gy per fraction may optimise the effects of intracranial chemotherapy. These radiation doses will not give rise to serious side effects or long-term complications. These findings should be taken

into account considering radiotherapy to open the BBB. Radiation-induced BBB disruption may be considered for the treatment of primary brain tumors, e.g., gliomas, prophylactic cranial irradiation in small cell lung cancer (SCLC) and cerebral metastases. High-grade gliomas are usually treated by (postoperative) radiotherapy. The prognosis is dismal; the 2-year survival is only 5-10%. Studies on the efficacy of chemotherapy alone show hardly any benefit. Delivering the chemotherapeutic drugs to the target area is a major problem due to the BBB. Intra-arterial application of chemotherapy for patients with glioblastoma multiforme, delivered prior to radiation therapy, appears to result in a median survival three times longer than that achieved with concomitant chemotherapy/ radiation therapy. They concluded that the best treatment is intra-arterial chemotherapy with cisplatin and etoposide given prior to radiation therapy with doses in the range of 61 to 63 Gy administered with a fraction dose of 1.8 Gy. Not explored was the sequence radiotherapy followed by chemotherapy. New developed drugs such as temozolomide, a second-generation alkylating agent, may be promising. Recently an EORTC study (EORTC 26981) on temozolomide and concomitant radiotherapy has started. A combination of radiation therapy also with the intention to disrupt the BBB followed by chemotherapy may improve treatment results.

OBJECTIVE OF THE STUDY:

The main objective behind this review article is to find out the various techniques which help us in disruption of the blood brain barrier, which is the today big issue. In brief, review a number of key transporters known to express at the blood brain barrier. Our main concern is BBB penetration by neurotherapeutics because many drugs failed to cross the blood brain barrier. So the design of drugs capable of penetrating the BBB and effecting the desired biological response is a forbidden challenge. The main objectives of the present study were to investigate the effects of drug lipophilicity, as we know higher the drug is lipophilic higher it will penetrate the barrier easily. The question that arises is what physical- and medicinal-chemical characteristics do the drugs possess that will enhance the design of new therapeutic agents at the chemistry and biology intersection. We mainly focus on the new drug moieties that come into the market and act via different mechanism. We able to collect the various data on the permeation techniques with the help of which drugs can penetrate the barrier with much better influence and ability. Today there are various disorders regarding brain which are fatal due to their unknown treatment, so it is very important to find out the known treatment via exact drugs so that it can helpful. On the other hand peripherally acting drugs need to possess specific physical-chemical properties that prevent them from crossing the blood brain barrier.

REVIEW OF LITERATURE:

Blood-brain barrier (BBB) regulates the passage of solutes between the CNS and the blood. The BBB not only restricts the entry of serum proteins into the CNS, but it also controls the passage of nutrients, electrolytes, vitamins, minerals, free fatty acids, peptides, and regulatory proteins in both the brain to blood and blood to brain direction. The BBB performs these functions through a number of saturable and non-saturable mechanisms. Influx mechanisms control the homeostatic environment of the CNS, supply the brain with nutrients, and help to integrate CNS and peripheral functions. The blood-brain barrier (BBB) is a monolayer of cells that regulates the passage of solutes between the CNS and the blood. The restrictive properties of the BBB are formidable, essentially equaling that of a continuous cell membrane. However, the BBB is much more than a physical barrier. The endothelial cells that form the barrier at the capillaries, venules, and arterioles and at the epithelial cells that form the barrier at the choroid plexus control the

homeostatic environment of the CNS, determine the passage of peptides and regulatory proteins, and govern the entry of metabolic fuels, neurotransmitter precursors, and essential nutrients into the CNS. The cells forming the BBB are also enzymatically active, are a source of cytokines and nitric oxide, and can secrete toxic factors. As such, the BBB is appropriately viewed as a regulatory interface and increasingly found to be a source of disease and a target for therapeutic interventions. Mannitol is a sugar alcohol; that is, it is derived from a sugar by reduction, with a molecular weight of 182.17 g/mol and a density of 1.52 g/mL.

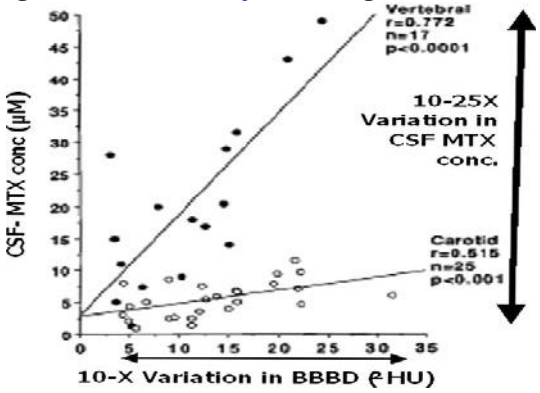

Fig 9: Variation in BBB disruption.[8]

Fairly large volumes of intracarotid mannitol (20% to 25%) are required to disrupt the blood brain barrier (BBB), that is, 200 to 300 ml/30s in humans or 10 ml/40s in rabbits. During transient cerebral hypoperfusion blood flow to the rabbit brain is decreased to 0.2 to 0.3 ml/30s.We therefore hypothesized that if the disruption of the BBB by intracarotid mannitol was primarily due to its osmotic effects, injection of 0.2 to 0.3 ml of mannitol during transient cerebral hypoperfusion will be sufficient to disrupt the BBB, thereby dramatically (by 20-folds) decrease the dose requirements compared with injections during normal blood flow. After preliminary studies, 4 doses of intracarotid mannitol were first tested: (1) 2 ml with cerebral hypoperfusion, (2) 4 ml with cerebral hypoperfusion, (3) 4 ml without cerebral hypoperfusion, and (4) 8 ml without cerebral hypoperfusion. Next, we compared the extent to which methods of drug delivery (infusion vs. bolus injection) affected BBB disruption in 12 rabbits. Finally,it assessed the duration of BBB disruption with intracarotid mannitol in another 12 rabbits. We observed that BBB disruption during injection of 4 ml of mannitol with cerebral hypoperfusion was comparable to 8 ml mannitol without cerebral hypo per-fusion. Bolus injections of 4 ml mannitol were more effective than steady-state infusions. The BBB disruption with intracarotid mannitol lasted for 60 minutes postinjection. We conclude that cerebral hypoperfusion decreases the dose of intracarotid mannitol by a modest 2-fold. Our results suggest that mechanical factors may play a significant role in the osmotic disruption of the BBB by intracarotid mannitol[26].

Certainly, in the simplest terms, it is desirable to determine whether a compound will penetrate and distribute within the CNS with the requisite pharmacokinetic and pharmacodynamic performance required for a CNS target or if it will be excluded from the CNS, wherein potential toxicities would mitigate its applicability. As a result, a variety of in vivo and in vitro methods for assessing CNS penetration have been developed and applied to advancing drug candidates with the desired properties. However, such methods are inevitably resource-intensive in terms of skilled personnel, animals, cell culturing, and bioanalytical support, and they are of course retrospective in their application, requiring the existence of synthesized compound. In silico

prediction methods address this limitation, supporting the prospective design and selection of candidate structures prior to synthesis[27].

Log blood (plasma)-brain partitioning (BB) is a measure of the *partitioning* between blood (plasma) and brain tissue, quantified by the ratio of the solute concentrations in brain and plasma. Log BB is generally obtained by methods such as the brain uptake index, which measures first-pass extraction from a single intravenous injection to yield log BB. Log BB can also be determined from the bolus carotid intravenous method with area under the curve plasma quantitation and single-point brain concentration determination. In this case, a permeability surface area coefficient is determined, and log BB can be calculated from log PS with certain assumptions regarding the endothelial surface area. It is an apparent value because the perfusate contains serum protein and other blood components to which the compound of interest may bind. Note that BBB permeability is implicitly captured in this measure and that it does not distinguish free and plasma-bound solute. It also does not address intracerebral distribution, differentiating between intracellular and extracellular concentrations. As a consequence, log BB is but a crude assessment of the likely concentrations at the targeted site of CNS activity, whether extracellular or cytosolic. Results from log BB measurements also depend upon experimental conditions, particularly dosing regimen (single bolus dose versus multiple doses versus continuous infusion) as well as sampling time from dose. Ideally, brain concentrations are measured at the plasma *t*max for the solute; however, differences in brain and systemic clearance can lead to variations in the measured log BB for a given compound depending upon sampling time. It is appropriate to consider the various contributions to experimental uncertainty when employing such data in model development, particularly when attempting to infer improved performance of a given method or set of computational descriptors relative to others. When considering CNS penetration, perhaps the most appropriate in vivo measure is the log of the permeability surface area coefficient (log PS), which represents the permeability of a given solute across the brain capillary endothelium (the anatomical representation of the BBB). This measure reflects the free (unbound) extracellular solute concentrations and is most often performed following the perfusion method established. This method eliminates serum binding and provides a direct measure of trans-BBB apparent permeability; however, it is a resource-intensive measure that requires microsurgical expertise and therefore is of relatively low throughput. Solubility of the solute of interest in the perfusate can also be a limiting factor. As with log BB, this measure does not address specific intracerebral solute tissue distribution and represents the potential combination of passive and active transport mechanisms. Considering the greater mechanistic clarity of log PS compared with log BB, the former property is likely to be more informative as a measure of CNS penetration for use in lead optimization[28].

The increased ease, speed, and throughput of in silico methods do not come without a cost, viz., the increased risk that such predictions may be somewhat or totally inaccurate for the structures of interest. Such inaccuracies would derive from two primary sources: the nature of the chemistry space represented by the compounds used in training these models and the mechanistic clarity and relevance represented in the data as they are measured. A common refrain at the present time in the field of predictive ADMET is the need for better and larger data sets from which to build the next generation of models. The area of BBB permeation is no exception to this. Even after more than a decade of intense interest in predicting BBB permeation, the number of log BB measurements available in the public domain is still probably fewer than 200. If, as has been suggested above, the focus should now shift to log PS, the need for additional data are

even more acute, since few log PS data number are available. The composition and size of the data sets also need attention. To date, the publicly available data sets comprise a motley assortment of compounds from various sources, with little guarantee of their being derived by a common experimental protocol. If the science of BBB permeation prediction is to advance as we would wish, then what is required is bespoken data generation for the purposes of model building, creating data sets that span appropriate ranges in the biological endpoint, molecular diversity, and physicochemical properties[29].

In-vivo methods, although offering a more direct measure of performance and greater acceptance in discovery decision making as a result, often cannot meet the time constraints placed on such decision making due to their low throughput. In addition, in vivo methods are not as readily amenable to the mechanistic deconvolution of the various determinants of CNS penetration, including passive and active transport. Therefore, in vitro methods that can supplant more resource intensive in vivo methods such as the use of wild-type and transformed/transfected cell lines i.e. Madin-Darby canine kidney transfected with human multidrug resistance 1 or other transformed cell lines have emerged and have found utility in combination with in vivo methods for assessing CNS penetration with greater throughput and increased mechanistic clarity and relevance. Such mechanistic clarity, along with more rapid turnaround times for the generation of data, are critical for the development and application of predictive models of CNS penetration to be applied in discovery decision making [29, 30].

The more appropriate setting for determining CNS penetration is arguably in the lead optimization stage in drug discovery. At this point in the discovery process, there is generally a sufficient investment of synthetic capacity and medicinal chemistry support for compound design and scale-up to support experimental measurements. Furthermore, the data resulting from in vitro and in vivo assessments of CNS penetration may be used for the development of more locally (in the sense of chemistry space) relevant and quantitatively predictive models. In addition, there usually exist sufficient resources for the confirmation of predicted CNS penetration and in turn the application of such data to iterative model development for improved prospective design of additional analogs.

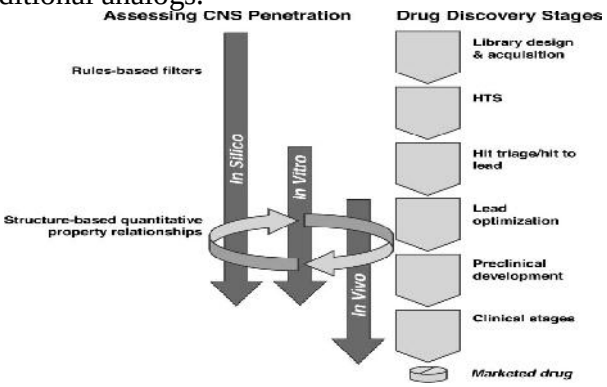

Fig 8: Application of in silico, *in-vitro*, and *in-vivo* CNS

Regardless of the stage in the drug discovery process, it is the responsibility of the user to determine the appropriateness of any predictive model that they may choose to apply, whether made available from the scientific literature or developed within their own organizations. Model appropriateness will be dictated by the quality (mechanistic clarity and relevance to the process to be modeled, statistical robustness provided by replicate measurements, and range and distribution of measured data) quantity, and whether the chemical structures in the training set

represent the chemical space of interest. There have been numerous statistical methods developed and applied to the modeling of CNS penetration, and aside from concerns regarding the appropriateness of qualitative predictions (uncertainty increases as predictions approach category boundaries), the utilization of one or more of these methods is basically a matter of user preference.[31]

CONCLUDATORY COMMENTS:

The utility of methods for predicting CNS penetration fundamentally depends upon what is to be decided and at what point in the drug discovery process. This will impact the type of data to be generated (in silico, *in-vitro*, or *in-vivo*) and will determine the requirements for the mechanistic relevance and clarity of the measured endpoint and how they can be related to chemical structure. The discovery scientist will need to determine how much uncertainty associated with data (predicted or measured) can be accommodated in the decision process (risk assessment). They should consider the advantages of employing local as opposed to global models and integration with in vitro and in vivo measurements to validate and iteratively refine models and extend their utility. These issues will continue to drive the measurement of data of appropriate statistical and mechanistic clarity for the development and improvement of in silico models. This will in turn lead to a reduction of uncertainty and will improve efficiencies by focusing on local models into lead refinement where greater resources are made available and where the opportunities exist for ongoing validation of in silico and in vitro methods against in vivo measurements. In this review article the current techniques and new approaches in development to deliver small and large molecules such as biologics to the brain are described. The different methods described in the physiological approach are summarized. The techniques used to this day involve direct injection or infusion of therapeutic compounds into the brain, the cerebro-ventricles or the CSF, but all these approaches are severely limited by poor distribution into brain parenchyma. Only the use of technologies able to transport molecules through the endothelial cells of the BBB will allow a homogenous distribution of therapeutics in the brain and thus provide a uniform and rapid exposure to brain cells. The importance of BBB for reaching CNS drugs to their targets and also undesired penetration of non CNS drugs to avoid their CNS side effects are briefly discussed. Short review of measurement methods of drug's penetration to CNS is presented along with a summary of computational aspects used for modelling purposes. The molecular and cellular properties of BBB have been reviewed and the role of its compartments in the regulating of drugs and xenobiotics penetration to the brain has been discussed. Working as a regulatory interface BBB is able to work as a physical and physiological barrier which prevents peripheral drugs to penetrate the brain and reduce their CNS side effects. This barrier activity causes some difficulties in CNS drug delivery and different measurement methods have been developed to study the rate and extent of drug delivery to the brain and the mechanism of delivery methods have studied using these methods. Beyond the experimental methods, prediction of these properties are studied in order to provide cheaper, simpler and more rapid methods for medicinal chemists who work in brain drug development field. It is well recognized that the BBB plays an important role in regulating the internal milieu of the central nervous system. Several different mechanisms are certainly involved; however, promotion of active transport across brain capillaries is potentially the most important as well as perhaps the most susceptible in disease. Comparison of these results with the well documented low permeability of

the luminal aspect of the BBB in vivo to these substances led us to propose a polar model of the brain capillary endothelial cell. Using two different methods, we showed a distinct distribution of plasma membrane markers between the luminal and ant luminal membranes. These data confirm that the brain capillary endothelial cell is polar, and therefore, potentially capable of active trans-cellular transport.

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