INTRODUCTION

The blood-brain barrier (BBB) plays a major role in controlling the microenvironment of the brain. Drug delivery to the brain is restricted by the presence of the BBB. It is actually selectively permeable to nutrients necessary for healthy brain function. Material and Methods: Various search engines like pubmed, medline and cochrane database were searched for various articles. Result and Conclusion: Prodrugs with increased lipophilicity and Chemical delivery system (CDS) are under research. A diverse collection of molecular transport systems is present on the brain endothelium to accomplish the task of nutrient transport. Receptor-mediated transcytosis (RMT) systems can be used to transport a wide range of therapeutics into the brain. Molecular identification and functional analysis of influx transport proteins and efflux transport proteins, the most active being ABC-transporters (ATP binding cassette) at BBB have progressed rapidly. This review focuses on the role of increased understanding of the pharmacology of the transport systems in the uptake of xenobiotics across the BBB into the brain for rational drug design to increase drug delivery to the brain.

KEY WORDS: Blood brain barrier, CNS drug delivery, transport across BBB

TIGHT JUNCTIONS (TJ):

Ultrastructurally TJs appear as sites of apparent fusion involving the outer leaflets of plasma membranes of adjacent endothelial cells. The TJ consist of three integral membrane proteins namely claudin, occludin, and junction adhesion molecules (JAM) and a number of cytoplasmic accessory proteins, ZO-1, ZO-2, ZO-3, cingulin and others. Cytoplasmic proteins link membrane proteins to actin which is the primary cytoplasmic protein for the maintenance of structural and functional integrity of the endothelium.

ADHERENCE JUNCTIONS (AJ):

AJs are made up of protein cadherin which join the actin cytoskeleton via intermediary proteins namely, catenins to form adhesive contact between endothelial cells. The AJs hold the cells together giving the tissues structural support. They are essential for the formation of tight junctions and disruption of AJs leads to barrier disruption.

The complete barrier function as a combination of physical barrier formed of TJs between cells decreasing flux via the intercellular cleft and paracellular cleft, transport barrier with various transport mechanism for solutes and a metabolic barrier with enzyme metabolizin molecules in transit.

TRANSPORT ACROSS BBB

Passive Diffusion , Prodrug technology and Chemical delivery system (CDS)

A wide range of lipid soluble molecules can diffuse through BBB and
enter the brain passively. This is a non saturable mechanism. Many factors which are important for CNS penetration are lipophilicity, molecular weight, hydrogen bonding, polar surface area, molecular volume and flexibility and charge of the drug molecule. Lipinski also made a set of rules after analyzing 1500 drugs from USAN or INN names for good CNS penetration. According to these rules CNS penetration is likely if: Molecular weight is < 400 ,Log P < 5 , Hydrogen bond donors < 3 and Hydrogen bond acceptors < 7. This study showed that for CNS penetration various parameters observed have lower range compared to general absorption and permeability. However a drug taken up by the membrane that form the BBB must than partition into aqueous compartments of brain’s interstitial fluid to exert an effect. As a result a substance that is too lipid soluble can be sequestered by the capillary bed and not reach the cells behind the BBB. Lipid solubility also favors uptake by the peripheral tissues, this in turn lowers concentration of drug in the blood. Thus while lipid solubility can increase transport across BBB, it can also lowers amount of drug presented to BBB. The percent of administered drug entering the brain is determined by both the rate of transport across BBB and the amount of drug presented to BBB.

By using prodrug technology various drugs can be made more clinically useful without modifying the pharmacological activity of parent drug. However the design of prodrug structure must be considered at the early stages of preclinical development keeping in mind that prodrugs although not common may alter the tissue distribution, efficacy and the toxicity of parent drug. Prodrug technology is used to improve drug delivery and CNS drug targeting. Prodrugs are made more lipophilic to utilize passive drug uptake processes into CNS by chemically modifying the drug. In chemical delivery system (CDS) there is release of active species from a lipophilic prodrug through a chemical and / or enzymatic multistep conversion. The CDS approach can be based on a dihydropyridine pyridium salt equilibrium type redox molecular carrier similar to endogenous NADH / NAD+ coenzyme system. The carrier dihydropyridine pyridium is a specific functional group attached to the drug which in addition to increasing BBB penetration by means of lipophilicity, can be converted by enzymatic oxidation to a water soluble quaternary pyridinium salt which facilitates its entry into brain’s interstitial fluid to exert an effect.

**Using Solute Carriers as transport modalities:**

BBB also separates brain from many polar nutrients like glucose and amino acids essential for brain. Therefore BBB endothelium must contain specific solute carriers (transporters) to supply CNS with these substances. The blood to brain influx transporters supply hydrophilic nutrients and other essential molecules like glucose, lactate / monocarboxylates and creatine. GLUT -1 is the glucose transporter type-1 responsible for the transport of glucose, and other hexoses including mannose, galactose, deoxyglucose or 3 o- methyl glucose. GLUT -1 is present in the micro vascular endothelial cells and GLUT-3 in the neuronal cells. Lactate which is the metabolic product of glucose is transported into and out of the neuronal cells by the monocarboxylate transporters (MCT). MCT -1 is present in the BBB and astrocytes while MCT -2 is present in the neurons. In micro vascular endothelial cells GLUT-1 is present in both the luminal and abluminal membrane of endothelial cells as well as in a substantial intracellular pool. The ratio of luminal to abluminal GLUT-1 has an impact on the delivery of glucose into the brain with increased glucose uptake linked with the increased luminal GLUT-1.

In addition L- tyrosine, L- tryptophan, L- Histidine are precursors of neurotransmitters and are transported from blood to brain via a Na+ independent neutral amino acid transporters (system L) at BBB. The system L is very important for drug delivery to the brain. L- Dopa is transported to brain through system L. L- type amino acid transporter 1 (LAT1) is a Na+ independent neutral amino acid transporter sub serving the amino acid transport system L. A remarkable feature of system L is its broad substrate selectivity. It is because of this property that system L is being regarded as a transporter of drugs. In addition to naturally occurring amino acids system L also transport drugs like L-dopa, melphalan, thyroid hormones, and gabapentin. It is proposed that manipulation of system L would have important therapeutic implications. It is seen that after a protein rich diet there is decreased efficacy of L-Dopa. This decrease in efficacy of L-Dopa was explained by saturation of system –L due to high levels of amino acids from the meal. This indirectly shows the significance of system –L in the L-Dopa transport across BBB.

**BBB transport of macromolecules: Receptor mediated transcytosis (RMT)**

Macromolecules are transported across BBB by endocytotic mechanisms through which large molecular weight solutes such as proteins and peptides can enter the CNS. These vesicular mechanisms are of two types: receptor mediated transcytosis (RMT) and adsorptive mediated transcytosis (AMT). In RMT the macromolecular ligand binds to the specific receptors on the cell surface triggering endocytotic event. A caveolus is formed containing ligand receptor complex which pinches off into a vesicle, then both receptor and ligand are internalized and move across the cytoplasm to get exocytosed on the...
other side. In AMT excess positive charge is required on the molecule, then by interacting with the cell surface binding sites induces endocytosis and subsequent process. Examples of RMT is transferrin transport system. In this Fe - transferrin binds to transferrin receptor (TfR) and transport is unidirectional from blood to brain. Other examples of transport through RMT are insulin, leptin, epidermal growth factor (EGF) and tumor necrosis factor (TNF). While cationised proteins e.g. cationised albumin binds with some nonspecific non receptor mediated area on cell surface and through AMT enters from blood to brain.

AMT method lacks specific targeting and may lead to widespread absorption. For drug delivery to CNS endogenous RMT systems can be of important use. The therapeutic carrier of interest must be conjugated to a molecule that has the capability of targeting an RMT system (RMT delivery vector). So there are two important things, one is the therapeutic carrier which carries the drug of interest and another is the vector that has the capability of targeting an RMT system (RMT delivery vector). The vector-conjugated drug cargo gains access to the brain interstitium by “piggybacking” on the natural RMT system.

Different types of nanoparticles (NPs) as carrier systems for drugs to cross BBB are studied. According to surface characteristics neutral nanoparticles and high concentration of anionic nanoparticles have no effect on CNS integrity. Anionic nanoparticles when used at lower concentration have superior BBB permeability in comparison to neutral and cationic nanoparticles formulations at the same concentration. The mechanism for transport across BBB suggested is via endocytosis. The probable mechanism is by recognition and interaction with lipoprotein receptors on the brain capillary endothelial cells. Different types of nanoparticles for targeted drug delivery to CNS are inorganic NPs, polymeric NPs, solid lipid NPs, nanocrystals, carbon nanotubes, dendrimers and quantum dots. In addition to above types there are other types under intensive research gold NPs, carbon nanotubes, dendrimers and quantum dots. In addition to above types there are other types under intensive research gold NPs, carbon nanotubes, dendrimers and quantum dots.

Liposomes are small vesicles (usually submicron sized) made up of one or more concentric bilayers of phospholipids separated by aqueous compartments. Pegylated immunoliposomes have been employed to target and nonpermanently transflect beta galactosidase and luciferase into the brain. The gene is introduced into the centre of Liposome surface of Liposome is coated with polyethylene glycol (PEG) to increase the circulation time of Liposomes by decreasing the clearance by reticuloendothelial system. In addition this 2% of PEG strands have monoclonal antibodies attached to them against the transferrin receptors. Liver and brain both have high expression of transferrin receptors. So these immunoliposomes are targeted to these organs. But if a promoter which is brain specific is encapsulated with beta galactosidase gene an example is the promoter for glial fibrillary acidic protein (GFAP), then the galactosidase enzyme expression is confined to brain only. The mechanism by which immunoliposome carries genes across BBB and transfect it into brain is not known.

The design of BBB delivery vector-drug conjugates is most importantly requires the appropriate choice of BBB delivery vector and its cognate RMT system. One of the goals has been to identify a BBB-specific RMT system with a high transport capacity. One approach to identify new BBB vectors and their conjugate RMT systems has been to use the power of combinatorial antibody library technology. Combinatorial antibody libraries are large pools of antibodies (~108-1012) having diverse specificities. These libraries can be “searched” for antibodies that perform a specific function such as binding to the plasma membranes of BBB endothelium and triggering transcytosis.

ATP - binding cassette transporters (ABC transporters) in the BBB: Inhibition of efflux mechanisms:

The most active ABC-transporters at BBB are P-glycoprotein (P-gp) encoded by the multi drug resistance genes (MDR or ABCB1), multidrug resistance proteins (MRPs or ABCB1 proteins) and the breast cancer resistance proteins (BCRP or ABCG2). The main function of ABC-transporter in the BBB is to function as active efflux pumps by consuming ATP and thereby transporting a diverse range of lipid soluble compounds out of the brain capillary endothelium and the CNS. By this means, they are removing from brain potentially neurotoxic endogenous or xenobiotic molecules and are carrying out a vital neuroprotective and detoxifying function. Drug efflux by P-gp at the BBB represents a major obstacle in the delivery of pharmacological compounds which are P-gp substrates to the brain and has been linked to the treatment failure in various brain disorders like epilepsy and brain cancers.

Two strategies have emerged from avoiding these efflux transporters, thus giving the drugs greater access to the CNS. One is by developing the specific inhibitors of efflux transporters and other is designing drugs which have poor reactivity with efflux transporters. In a microdialysis study in conscious freely moving rats it was seen that inhibition of P-gp enhances transport of imipramine across BBB and increases intracerebral concentration of imipramine.

Some of the examples of efflux transporters is verapamil, quinidine...
and cyclosporine A. These are also difficult to synthesize because this requires detailed understanding of structure activity relationship (SAR) of ABC efflux transporter mechanism, which is difficult to obtain. For designing the drugs to circumvent efflux mediated by P-gp / ABC transporters Seelg et al and Gatlak Landwojtwicz et al described a series of recognition elements required for drug P-gp interaction by analysis of over 100 known P-gp substrates. They found that the number of clusters of spatially distinct H-bond acceptors correlated with the strength of interaction. Work by Turunen et al showed that introducing anionic and amide structures into paclitaxel a P-gp substrate improved brain uptake. Overall these approaches however are not very successful.

**Cell movement across BBB:**

The healthy CNS contains resting microglial cells (innate immunity). Under physiological conditions this lymphocyte surveillance activity does not lead to the inflammation or alter BBB integrity. But when these patrolling lymphocytes encounters their specific antigens in the CNS they initiate an auto-inflammatory response leading to disruption of BBB and invasion of large number of leukocytes into CNS parenchyma. CNS inflammation may occur in the CNS due to localized glial cell activation and is hallmark of some of neurodegenerative disorders of brain. During physiological conditions mononuclear cells enter the BBB through transcellular route by diapedesis while during pathological states the tight junctions between endothelial cells may be opened as a result of cytokines and other agents and mononuclear cells may then enter the CNS via both transcellular and extracellular routes.

**CONCLUSION:**

It is seen that BBB is a dynamic system. Regulation of transport across BBB may be of various macromolecules or immune cells is important for maintenance of optimal state of functionality of brain. Any discrepancy in this transport system may lead to diseases of CNS. The evolving neuroscience has provided a number of targets for new drug development and is successful to some extent. But translation from laboratory to clinics is possible only if the greatest hurdle of BBB is overcome i.e the drug has to cross BBB to reach brain. Various transport mechanisms like increasing lipophilicity via produgs, CDS, solute carriers GLUT-1, system-L, and RMT are at various level of research. A new approach of nanotechnology especially the gold nanoparticles are also expected to bring revolution in the field of drug delivery across BBB. A better understanding of Physiology and Pharmacology of transport processes at the BBB in the CNS is very important for the treatment of CNS disorders may be CNS malignancies or neurodegenerative disorders. These various processes provide a promising area of research in the CNS disorders.

**REFERENCES**


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