REVIEW ARTICLE

Blood Brain Barrier: Smuggling Past the Barricades

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Getting onto the other side of the blood brain barrier presents the most formidable obstacle for agents intended to treat central nervous system disorders like Alzheimer's disease, Huntington's chorea, Schizophrenia, Parkinsonism and multiple sclerosis. The blood brain barrier impedes drug delivery at potential target sites. Current strategies to aid drug delivery to the central nervous system are lipidisation, chemical modification and molecular antibody technology. The future directs towards development of strategies that harness receptors, vesicles and viral genes to deliver therapeutic agents to the central nervous system.

Blood brain barrier (BBB) discovered by Paul Ehrlich in 1929, is a physical, metabolic and immunological barrier that protects the brain microenvironment from toxins[1,2]. BBB develops in the first trimester of fetal life. It occupies 2 % of the body space and utilizes 15 % of the body glucose. It is seen in the subarachanoid space, arachanoid membrane and choroid plexus[3,4]. The BBB is formed by the endothelium that lines the cerebral microvessels of the brain. Special cells called astrocytes are present in the BBB and their main function is regulation of enzyme expression in the endothelium. The other biochemical entities present in the BBB[5] include enzymes like monamine oxidase A and B, catechol-O-methyl transferase; hormones like adrenaline, dopamine, histamine, prostaglandins, bradykinins and proteins such as, p-glycoprotein also called multi drug resistant protein (MDRP) and transferrin.

The characteristic feature of the BBB is a tight junction, Zonula Occludens[6,7]. It is made up of the proteins occludin, cingulin, 7H6 (antigen protein), F-actin, spectrin, zonulin and zot. The tight junction has a very high transelectrical resistance of 2000 Ω and is characterised by high mitochondrial current and low hydraulic conductance. It serves two functions, gate function that regulates intracellular passage of substances and fence function that prevents apical and basolateral plasma membrane molecules from intermixing.

The agents which alter the integrity of the tight junction includes cytoskeletal agents phallidin (a poisonous alkaloid); endoproteases, antipapain, trypsin and elastase; oxidising agent such as hydrogen peroxide; toxins, pertusis toxin and exotoxin A; surfactants like Tween, palmitoyl carnitine, sodium dodecyl sulfate and the calcium chelator, ethylenediaminetetraacetic acid (EDTA).

Factors responsible for breakdown of BBB[7]:

Microwaves and radiowaves emitted from gadgets like mobile phones disrupt the blood brain barrier. Tumours produce abnormal capillaries with pinocytic vesicles and thus increase the permeability of BBB. Bacteria and viruses gain access to the CNS via valveless veins and endothelial cells and breach the BBB. Loss of cerebral autoregulation as seen in hypertension and prolonged hypercapnea cause stretching of endothelium and hence the breakdown of BBB. Infusion of high osmolality solutions like mannitol, arabinose and urea increase the permeability of the BBB. Increase in pressure causes breakdown of the BBB.

Evaluation of the integrity of BBB[7]:

Tests for integrity include the ability of the cerebral cap-

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i::ry to exclude most water-soluble molecules. There are two different methods for *in vitro* evaluation. The first includes introduction of a dye-protein complex into the vascular system so as to stain the brain and analysing the pattern of staining. A radiolabelled marker can replace the dye molecule. The other method involves administration of a constant infusion or bolus injection into the carotid artery and evaluation of the diffusion process by indicator diffusion technique or brain uptake method. *In vivo* methods are employed in human models and there are two different methods for it. An external detection of radionuclide activity with sodium iodide crystal contrast enhancement by magnetic resonance imaging (MRI), computerised tomography (CT) scan and intravascular rhubedium 82 study in conjunction with positron emission tomography (PET).

Transportation across the BBB:

Various metabolic substrates, therapeutic agents, neuropeptides and plasma-derived proteins are able to utilise the specialized shuttle services at the BBB[8]. These are lipid-mediated transport of small lipophilic molecules such as barbiturates, carrier-mediated transport of hydrophilic nutrients like glucose, plasma-mediated transport of acidic drugs and peptides, receptor/adsorption-mediated transcytosis of peptides such as somatostatin analogs and bulk flow transcytosis, which is minimal under normal conditions and increases in pathological conditions.

Kinetics of transport across the BBB:

Various methods are used to study the kinetics of transport across the BBB[9]. *In vitro* co-culture system comprises of a culture of bovine capillary endothelial cells or rat astrocytes. It is used to study transcellular-paracellular drug transport and influence of MDR-protein on drug transport. *In vivo* method comprises of intracerebral microdialysis in rats and mice which permits the estimation of local drug concentrations in the brain. Other techniques such as vascular brain perfusion (VBP) and capillary depletion technique (CDT) are also used in the study of various transport processes.

FACTORS AFFECTING THE PERMEABILITY OF THE BBB[9-12]

Morphological factors:

Presence of cerebral endothelial tight junction, cerebral plexus epithelial tight junction and epididymal gap junction regulate the permeability of substances across BBB. Lack of pinocytic vesicles, surface area of the microvessel and local blood flow also play an important role in regulating the permeability across BBB. Other important factors include active efflux of drugs from the brain by MDR protein. e.g. loperamide and vincristine.

Physicochemical factors:

Any molecule with optimal lipophilicity can pass through the BBB. Increase in lipophilicity causes complex formation with the proteins of the membrane and hence decreases permeability across the BBB. Low molecular weight substances (500-1000 daltons) with optimal size can cross the BBB easily. Positively charged species interact with the negatively charged tight junction and are transported across the BBB. Drugs in the unionised form at physiological pH can permeate the BBB. As the hydrogen bonding potential increases the drug is partitioned into the blood and vice versa. A protein bound drug cannot permeate the BBB.

Exogenous and endogenous substances:

Exogenous substances like α-adrenergic agonists, protamine sulfate, hypertonic solutions increase the permeability of the BBB. β-adrenergic agonists and dexamethasone decrease the permeability of the same. Endogenous substances like histamine, serotonin, bradykinin, phospholipase A_2, platelet-activating factor and macrophage inflammatory protein increase the permeability of the BBB. Angiotensin-II and 2-deoxyglucose decrease the permeability of the BBB.

MODELS FOR EVALUATION OF PERMEABILITY ACROSS BBB

BBB permeation can be evaluated by different methods, such as a model based on linear free energy relationship (LFER), another model based on 3D molecular interaction fields (Volsurf method) and an *in vitro* model.

Model based on linear free energy relationship (LFER):

Abraham and Mitchell[15-18] gave the general free energy relationship as Log $SP = c + rR_2 + S\pi^H_2 + \alpha\Sigma\alpha^H_2 + \beta\Sigma\beta^H_2 + v\ V_x$; where, SP is the property of solute in a given system, R is the excess molar refraction, π is the solute dipolarity/polarizability, α is the solute hydrogen bond acidity, β is the solute hydrogen bond basicity and V_x the Macgowan volume. These terms are called solute descriptors and they aid in prediction of solute permeability across the BBB. The solute descriptors are determined by various methods[13-20]. In fragmentation method substance is broken down into a number of well defined substructures and the constants for each of the fragments are added up to give the value of the descriptor for the substance under study. In differential distribution method the partition of the drug in three different solvent

systems octanol/water, cyclohexane/water and chloroform/water system is studied and the modified LFER equation is derived for each of the systems. From the modified LFER equation the values of the solute descriptors can be obtained.

Model based on 3D molecular interaction fields (MIFs)/Volsurf method[21-30]:

This method helps in pharmacological screening of preclinical drug molecules on the basis of physicochemical and structural features. The present method involves steps like building the energy minimized 3D structure of the compounds and generation of the 3D MIFs of the same. Conversion of 3D MIFs of the compounds into molecular descriptors (solute properties) and statstical analysis of the data obtained by the above methods using chemometric tools like principal component analysis (PCA) and partial least squares (PLS) discriminant analysis describe permeability across the BBB.

Volsurf method takes 2 min for the external prediction of 100 compounds under low resolution and 20 min for the same under high resolution. Polar water accessible surface area (PWASA)[30] and hydrogen bonding potential[28] are important examples of Volsurf descriptors, which influence the permeability of solutes across BBB significantly. An increase in PWASA of compounds increases their permeability across BBB. The optimal PWASA required for permeation across the BBB is 60-100 A^2. An increase in the hydrogen bond potential decreases the capacity of the molecules to cross the BBB and vice versa. Other Volsurf descriptors are integral moments, amphiphilic moments, critical packing and hydrophilic lipophilic balance (HLB).

In vitro model[31]:

The in vitro model employed is Madine Darby Canine Kidney (MDCK) cell permeability assay. MDCK cells cultured on a porous membrane can be used to mimic the endothelial cell barrier for permeability screening of new chemical entities (NCEs). A MDCK cell monolayer is placed between the donor and the receiver chamber. The drug is added to the donor chamber and samples are collected at the receiver end. Drug concentrations in the sample are measured at regular intervals. The rate of transport is calculated from the data obtained and is expressed as apparent permeability co-efficient P_{app}. Reference standards like mannitol, salicylic acid, haloperidol and testosterone are used in the assay. The integrity of the MDCK monolayer is determined by using two methods: Measuring the trans epithelial electrical resistance (TEER) and leakage of Lucifer Yellow dye across

the membrane. The MDCK cell permeability assay is highly reliable for in vitro screening of drugs, takes very less time (4 days) for the completion of one assay and generates consistent assay to assay results.

NEUROTHERAPEUTICS

Targeting of therapeutics to the CNS faces one major hurdle, the impermeability of the BBB coupled with complete absence of paracellular transport and the presence of active Pgp efflux system. An antineoplastic agent like vincristine is ineffective in treating malignancies of the CNS due to their active efflux into the blood by Pgp efflux system. The methods employed for optimal drug deliveries across the blood brain barrier are:

Circumvention of the BBB:

This method includes techniques that bypass the BBB. Direct instillation into tumour bed[32] is achieved by using Ommaya reservoirs™ or microcatheters for drug delivery. Tissue implants[33] involves implantation of a cell suspension or a tissue graft into the CNS. e.g implantation of nigrostriatial tissue or adrenomedullary tissue is carried out to treat Parkinsonism. Alternative route of administration[34] comprises of intrathecal or intracerebral route of administration.

Physicochemical methods to open the BBB:

The permeability of the BBB to drugs can be improved by either osmotic or chemical opening. Osmotic opening of the BBB[35] can be achieved by intracarotid injection of an inert hypertonic solution of 1.4 molar mannitol or 1.6 molar arabinose/urea. These agents cause shrinkage of the endothelial cells and hence open the tight junctions in the BBB. Chemical opening of the BBB [36,37] involves development of a drug cocktail that opens the barrier and then closes it after giving a precise time window for drug delivery. The chemical entities that serve this purpose are: bradykinin analogs, RMP7 or BK2 receptor agonists like cereport (lobradimil™). Administration of leukotriene-4, histamine, serotonin, leucine and α-adrenergic agonists aid in increasing the permeability of the BBB to drugs and agents like saralasin, angiotensin, dexamethasone and β-adrenegic agonists decrease the same.

Chemical modification of drugs:

By making the suitable changes in the drug molecule penetration into the brain can be improved. Widely used methods are increasing lipophilicity and chemical delivery systems (CDS). Drugs are modified chemically so as to increase their lipohilicity and to aid their passive diffusion into

the brain, e.g. thiopental[38] can cross the BBB and has potent hypnotic activity in comparision with other barbiturates. Three analogs of somatostatin[39,40] RC161 (Ac-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH$_2$); RC160 (D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH$_2$) RC121 (D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Tyr-NH$_2$) have enhanced BBB permeability than somatostatin. RC161 has an acetylated group at one end thus increasing lipophilic nature and permeability across the BBB. RC 160 has two aromatic moieties on either end, thus increasing the lipoid character and hence the blood brain barrier permeability.

Chemical delivery system is called retrometabolic drug design approach. The drug is coupled to two "bioremovable" moieties, a targeter (T) and a modifier function (F$_1$-F$_n$). The former aids in site specific drug delivery and is based on 1,4-dihydrotrigonelline system and the latter serve as "lipophilisers". The drug targeter complex (D-T) is converted in vivo to the hydrophilic quaternary form (D-T$^+$) which gets locked in the brain, thus providing sustained site specific release of the active drug. For example AZT (azidothymidine) used in treating AIDS-encephalopathy precipitates severe anemia, as it does not reach the CSF. AZT is converted into a chemical delivery system by treating with nicotinyl chloride and then conversion of the 5-nicotinate into 1,4-dihydro form and finally into a quaternary salt using methyl iodide (fig. 1). Pulmonary dysfunction caused by ribavirin can be avoided by using its nicotinate CDS.

Harnesing endogenous transporters:

Endogenous transporters like amino acid carriers[42,43], membrane proteins[44,45] and vesicles are used to target drug delivery across the BBB. Melphalan, an antineoplastic agent, is transported via large neutral amino acid (LNAA) system. Gabapentin, an anticonvulsant is also transported by LNAA system. System-L transports L-dopa to the CNS because of its structural resemblance to phenylalanine. The GLUT-1
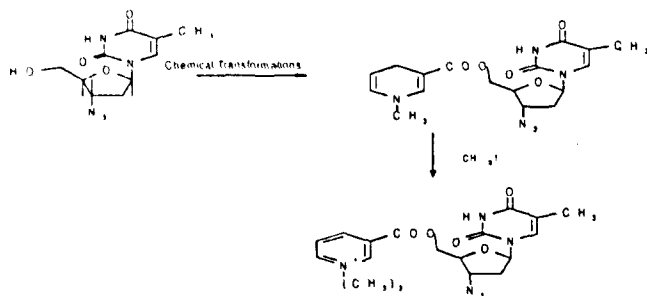

Fig. 1: Chemical delivery system (CDS) of azidothymidine (AZT).

transporter in the brain transports enkephalin, a glycopeptide causing long lasting analgesia.

The membrane proteins like organic anion transporter protein (OATP) and P-glycoprotein acts as efflux pumps causing efflux of drugs from the CSF. Agents that inhibit these proteins aid in maintaining drug concentrations in the brain. OATP system is present in the choroid plexus and it clears acidic drugs like penicillin, azidothymidine, methotrexate from the CSF. Probencid, an OATP inhibitor is therefore administered along with these drugs so as to retain them in the CSF. P-glycoprotein (Pgp)/multi-drug resistant protein (MDRP)[46,47] is a unique protein having 12 membrane spanning domains and two nucleotide binding sites (NBSs) for attachment of ATP. Drugs accumulate in the brain by passive diffusion and are cleared from the CSF by active efflux mediated by Pgp, thus precipitating multi drug resistance (MDR). P-glycoprotein inhibitors also called as chemosensitizers cause reversal of this resistance and are often used as co-drugs along with the active drug. Examples of Pgp inhibitors are verapamil, diltiazem, cyclosporin, progesterone, propranolol, cephalosporins, erythromycin, amiodarone, chlorpromazine, dipyrimidole, tamoxifen and Tween 80.

Adsorptive endocytosis[48,49] is a potential route of delivery for drugs that can be cationised. Cationised antibodies, human serum albumin and conjugates of avidin are used to target agents like biotinyl phosphodiesterase antisense oligonucleotides to the brain. In receptor-mediated endocytosis[50,51], the endogenous plasma protein transferrin is conjugated with insulin fragments and OX 26, a murine monoclonal antibody (produced against transferrin and prevents immune reaction between endogenous and administered transferrin). This protein complex stimulates survival of cholinergic neurons in the fetal brain tissue grafted in the eye. Synapse technologies Inc. devised a method of conjugation of drugs to P-97 (a family of transferrin proteins) for development of "molecular trojan horses" that deliver drug molecules across the BBB.

Gene therapy:

Gene targeting to the brain faces the obstacle that the target population is post-mitotic and is isolated behind the BBB. Several methods[52] are used to overcome this obstacle. Grafting of modified cells is an effective method in which the viral vectors such as retroviral vectors, herpes simplex virus, adeno virus and liposomes, that are directly inoculated into CSF by convection enhanced delivery (CED) or by disruption of the BBB using mannitol, glucose, urea,

bradykinin analogs, leukotriens or vasoactive peptides. Gene may activate the prodrugs, e.g. thymidine kinase gene from the HSV phosphorylates acyclovir and ganciclovir and activates it. Activation of immune response e.g. Interleukin-4 gene and gene coding for antisense insulin growth factor-I can improve immunity. Angiogenesis modulation by introduction of antisense vascular endothelial growth factor cDNA, which markedly reduces blood vessel formation and therefore reduces angiogenesis in tumours. Apoptosis and tumor suppression genes such as P53 and oncogenes such as *ras* are potential targets for gene therapy. Gene therapy for treatment of neurodegenerative disorders employs *ex vivo* modification of cells to provide local trophic factors e.g. nerve growth factor, brain-derived neurotrophic factor, fibroblast growth factor, ciliary neurotrophic factor and neurotrophin-3.

CONCLUSION

The crux of the issue lies in designing of NCEs for optimizing and rationalizing drug delivery to the CNS so as to treat a wide range of disorders from neoplasms to neurodegenerative conditions. The most scientific and validated techniques for evaluating BBB permeability of NCEs include *in vivo, in vitro* and virtual mathematical models based on a comprehensive analysis of molecular structure, pharmacokinetic profile and physicochemical properties of the NCEs. Design of chemical entities that mimic endogenous substrate at target sites offers a potential route for CNS targeting of drugs. Development of selective permeability enhancers, "molecular trojan horses" and CDS offer targeted drug delivery to the CNS.

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