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INTEGRATED PERSPECTIVES ON BRAIN-TARGETED DRUG DELIVERY: BARRIERS, RECEPTORS, PERMEABILITY DETERMINANTS, AND ASSESSMENT METHODS

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ABSTRACT

Achieving effective drug transport to the brain is one of the relevance of contemporary pharmaceutical sciences, given the rising incidence of neurological and brain disorders associated with the aging population. However, because of the presence of these barriers the blood-brain barrier (BBB), the blood-brain tumor barrier (BBTB), efflux pumps, and the blood cerebrospinal fluid barrier (BCSFB) which regulate the path of circulating drug molecules into the brain, brain delivery of a drug is a complex task for treating CNS diseases such as neurodegenerative disorders (Parkinson's, Alzheimer's disease), schizophrenia, stroke, epilepsy, brain tumor, migraine, dementia, and meningitis. For the treatment of many CNS illnesses, general techniques that can improve drug transport to the brain are very desirable. Reducing toxicity and improving treatment effectiveness can be achieved by localizing medications at the intended site of action. The following topics will be covered in detail in this review: receptor-mediated drug delivery, brain permeation parameters and their evaluation methods, obstacles in the brain targeted drug delivery system, the mechanism of drug transfer via BBB, issues encountered in the brain targeted drug delivery system, and its applications in the treatment of various CNS diseases.

KEYWORDS: Brain targeting, Barriers, Receptors, Transporters, Permeability.

INTRODUCTION

A smart drug delivery system that has been enhanced to maximize regenerative approaches is one that targets the drug delivery system. This approach is predicated on a procedure that administers a certain dosage of medication to specific sick parts of the body over an extended period of time. pharmaceuticals are absorbed through the biological membrane in the conventional drug delivery system, while pharmaceuticals are released from the dosage form in the target release system. In order to minimize the harmful and therapeutic index, targeted drug delivery is widely utilized to selectively and effectively localize a pharmacologically active moiety at a defined target while preventing it from accessing the normal non-targeting cellular linings. Drug targeting also aids in avoiding first-pass metabolism, allowing the administration of the drug to reach the receptor site at an adequate dose without upsetting the cells of extraneous tissue.[1] Retention, evasion of the target, and release are the four main criteria for effective targeting drug delivery systems. This means that the drug must be loaded into the right drug delivery vehicle, processed, and able to escape from bodily secretions that could cause it to degrade, resulting in a lengthy residence time in the circulation and reaching the targeted area.[2]

Reducing the frequency of doses, minimizing adverse effects, and minimizing fluctuations in blood levels are

the benefits of targeted medication delivery. The following factors make targeted drug delivery systems better than traditional ones: low solubility, increased drug instability, poor absorption, shorter half-life, a bigger volume of distribution, limited specificity, and a low therapeutic index of the drug molecule.[3]

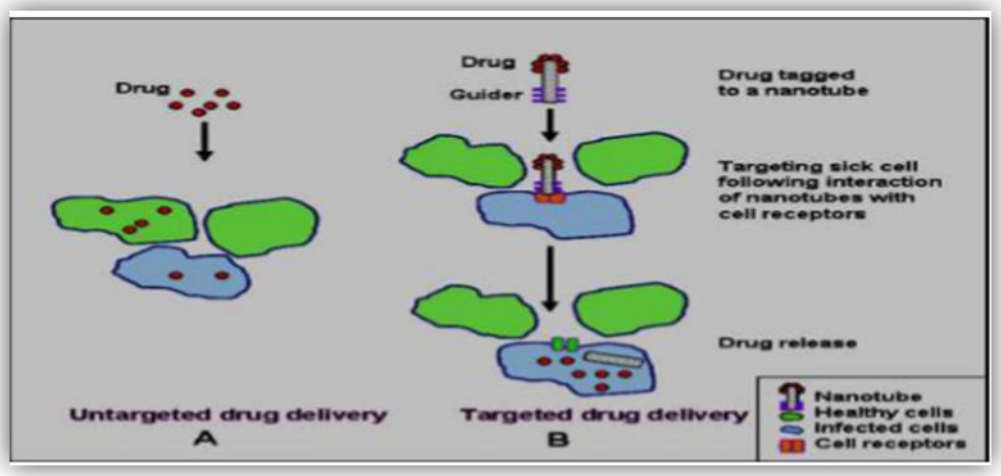


Fig. 1: Drug delivery technology.[4]

OBJECTIVE OF TARGETED DRUG DELIVERY[5]

- To ensure the safety, localization, and targeting of the medication while extending its effects on the ill tissue.
- To achieve the desired pharmacological response at a selected site without causing negative interactions at other sites, the medication must have a specialized action with a higher therapeutic index and maximal side effects.
- While the targeted release system releases the dose form of the medication, the typical drug delivery system absorbs the substance through a biological membrane.

BRAIN TARGETING: POSSIBLE OBSTACLES TO OVERCOME

The majority of medications used to treat illnesses connected to the central nervous system are lipophilic, with molecular weights below 400 Da and log P values between -0.5 and 6.0. The migration of drug molecules through the passive diffusion process is caused by the unionized fraction of pharmaceuticals that are ionized at physiologic pH and that establishes the concentration gradient across the blood-brain barrier. Given these facts, a medication should be made in a way that allows its molecule to have the best lipid solubility to cross the blood-brain barrier and maintain its advantageous concentration in the brain. Achieving this objective is not an easy task because systemic bioavailability and solubility may be decreased if the desired pharmacokinetic effects are not achieved by merely increasing the drug molecule's lipophilicity through trustworthy chemical changes. greater protein binding and greater absorption by the liver and reticuloendothelial system, which eventually results in increased metabolism, are the causes of decreased drug concentration at the target location. Apart from their lipid solubility, several therapeutic molecules that cross the blood-brain barrier are known to do so because of polar components that facilitate carrier-mediated transport across tight junctions.[6]

ROLE OF LIPOPHILICITY IN CNS ENTRY

Cytochrome P450 and other enzymes speed up oxidative metabolism, but increased lipophilicity to increase membrane permeability may make handling chemicals more difficult. Therefore, to improve bioavailability, the effects of lipophilicity on membrane permeability and first-pass metabolism must be modified. Furthermore, increased lipophilicity affects all other pharmacokinetic parameters and typically results in an increase in distribution volume.[7]

MECHANISM OF TRANSFER OF DRUG VIA BLOOD-BRAIN BARRIER (BBB)[8]

Transmembrane diffusion- The majority of drugs use transmembrane diffusion to cross the blood-brain barrier. This non-saturable mechanism requires drug binding to the cytomembrane. High lipid solubility and tiny molecular mass support this process. For a medication to be effective, it must first partition into the hydrated medium of the brain interstitial fluid after being absorbed by the blood-brain barrier membranes. Therefore, the capillary bed may capture a lipid-soluble molecule and block its passage to the cells behind the blood-brain barrier . The percentage of administered medications that enter the brain depends on both the number of pharmaceuticals present in the brain and the pace of transport through the blood-brain barrier .Cytosine-induced neutrophil chemoattractant-1 (CINC-1), with a molecular weight of 7800 Dalton, is the biggest molecule known to cross the blood-brain barrier by transmembrane diffusion.

Saturable transport system

Some drugs or substances with drug-like properties cross the blood-brain barrier via a saturable transport pathway. Examples include caffeine and levo dopa (L-DOPA). Approximately ten times as quickly as would be expected if a transporter's endogenous ligand crossed the blood-brain barrier by transmembrane diffusion. A number of regulatory molecule carriers, such as proteins and peptides, are also preferentially absorbed by specific brain regions. Often, saturated systems control how quickly their brigands pass over the blood-brain barrier. The transport rate of blood flow-dependent substances, such as glucose, is a function of blood flow. Slow-moving objects can be carried differently by a variety of agents. One peptide that regulates the rate of transfer in the peptide transport system (PTS-1) is leucine. Figure 2 depicts the movement of molecules across the brain's barriers.

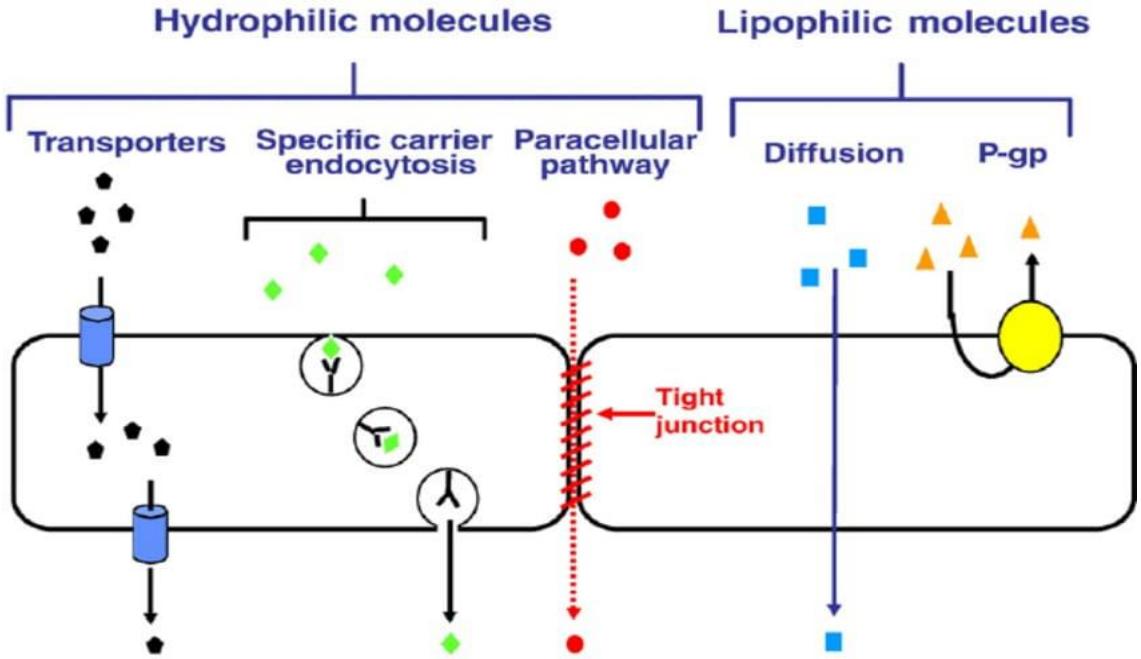


Fig 2: Schematic representation of the transport of molecules across BBB.[9]

ADVANTAGES[10]

Enhanced pharmacological efficacy: Treatment effectiveness can be raised by directing medications to certain brain cells. This is so that the medications are not wasted on other cells and are sent straight to the cells that require them.

Decreased adverse effects: Treatment side effects can be minimized by directing medications to particular brain cells. This is due to the medications' inability to interact with other cells or tissues and their lack of distribution throughout the body.

Increased patient compliance: It is feasible to increase patient compliance by directing medications to particular brain cells. This is due to the fact that when patients are aware that their medication is safe and effective, they are more likely to take it.

DISADVANTRAGES[11]

- High dose frequency is caused by the body's rapid medication excretion.
- The targeted medication delivery system's carrier may trigger an immunological reaction.
- There is not enough time for the medication delivery mechanism to remain localized in the tumor tissue.
- The spread and re-distribution of medications that have been unleashed.
- High levels of knowledge are needed for the targeted drug delivery system's manufacture, storage, and administration.
- Drug accumulation at the target location may increase toxicity.
- It will be challenging to achieve product stability.

BARRIERS IN BRAIN TARGETED DRUG DELIVERY

A number of obstacles that prevent pharmaceuticals from reaching the central nervous system can be taken into account in order to explain why systemically administered medications have failed to effectively treat many CNS illnesses. The extracellular fluid in the brain and the blood are physically separated by barriers.

1. Blood-Brain Barrier
2. Blood-Cerebrospinal Fluid Barrier
3. Blood-Tumor Barrier

1. Blood - Brain Barrier (BBB)

A highly selective permeability barrier called the blood-brain barrier (BBB) divides the extracellular fluid of the brain from the blood in circulation in the central nervous system. The capillary endothelial cells that make up the blood-brain barrier are joined by tight junctions and have

a very high electrical resistance of at least 0.1µm. Water, some gases, lipid-soluble molecules, and selective transport molecules like glucose and amino acids—all essential for CNS function—can flow through the blood-brain barrier by passive diffusion. On the other hand, an active transport mechanism mediated by P glycoprotein may block the entry of lipophilic potential neurotoxins via the blood-brain barrier. It takes astrocytes to form the blood-brain barrier. More than 98% of tiny molecular weight medications and almost all high molecular weight medications (mostly peptides and proteins) created to treat CNS disorders are thought to have difficulty crossing the blood-brain barrier. While small hydrophobic molecules (such O_2, CO_2, hormones, etc.) can diffuse into the cerebrospinal fluid (CSF), endothelial cells prevent the diffusion of microscopic items (like bacteria) and big or hydrophilic molecules.[12]

Structure[13]

The blood-brain barrier (BBB) is an intriguing structure that keeps the brain in its ideal environment and shields it from dangerous substances. The BBB's structure is made up of the following parts:

Endothelial cells: Tight connections hold the endothelial cells that comprise the blood-brain barrier firmly together. Large chemicals and particles cannot pass through these tight connections from the blood to the brain.

Pericytes: The cells that envelop the BBB's endothelial cells are called pericytes. They aid in controlling the BBB's permeability and give the endothelial cells structural support.

Astrocytes: These glial cells have a tight relationship with the BBB's endothelial cells. They support the preservation of the BBB's integrity and the control of endothelial cells' metabolism.

Microglia: The brain contains immune cells called microglia. They contribute to the brain's protection against damage and infection.

Functions of BBB

The brain is effectively shielded from numerous common bacterial infections by the blood–brain barrier. Therefore, brain infections are quite uncommon. When brain infections do happen, they are frequently extremely dangerous and challenging to cure. Only specific antibiotics can pass through the blood-brain barrier, and antibodies are too big to do so. In certain situations, the medication must be injected straight into the brain fluid. However, because of the brain's interstitial space's tortuous structure, medications that are administered directly to the CSF are unable to efficiently permeate the brain tissue itself. Inflammation makes the blood-brain barrier more permeable. This makes it possible for some phagocytes and antibiotics to pass through the BBB. But this also makes it possible for viruses and bacteria to enter the BBB. Fig. 3 depicts the BBB's functions.

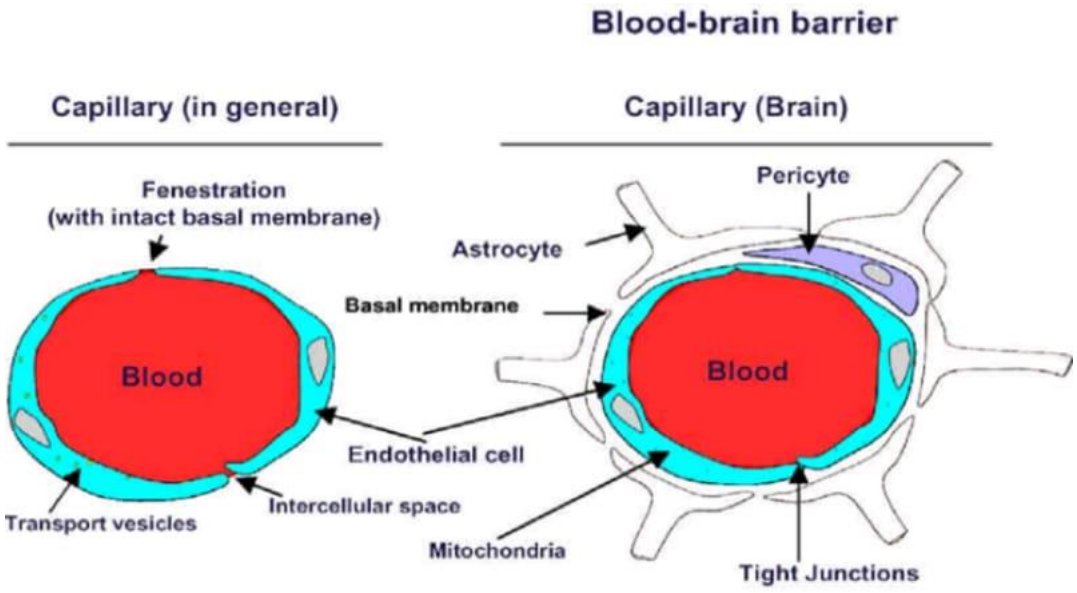


Fig. 3: Representation of Normal capillary & Blood- Brain-Barrier.

2. Blood - Cerebrospinal Fluid Barrier (BCSFB)

The blood-cerebrospinal fluid barrier, which divides the blood from the cerebrospinal fluid (CSF), which runs in the subarachnoid space around the brain, is a representation of the second barrier, which is situated in the choroids plexus. However, because its surface area is 5000 times lower than the BBB's, this barrier is not thought to be a major pathway for drug absorption. The brain parenchyma's interstitial fluid and CSF can exchange molecules, and the BCB carefully controls how blood-borne chemicals enter the CSF. The archnoid membrane is a double-layered tissue that forms on the outside of the brain between the dura and pia. It is made up of epidermal cells that fold over into themselves. The

subarachnoid space, which contributes to CSF drainage, is located within the double layer. Tight junctions stop chemicals from the blood from passing through the archnoid membrane. Fig. 4 illustrates how the blood-cerebrospinal fluid barrier works.[14]

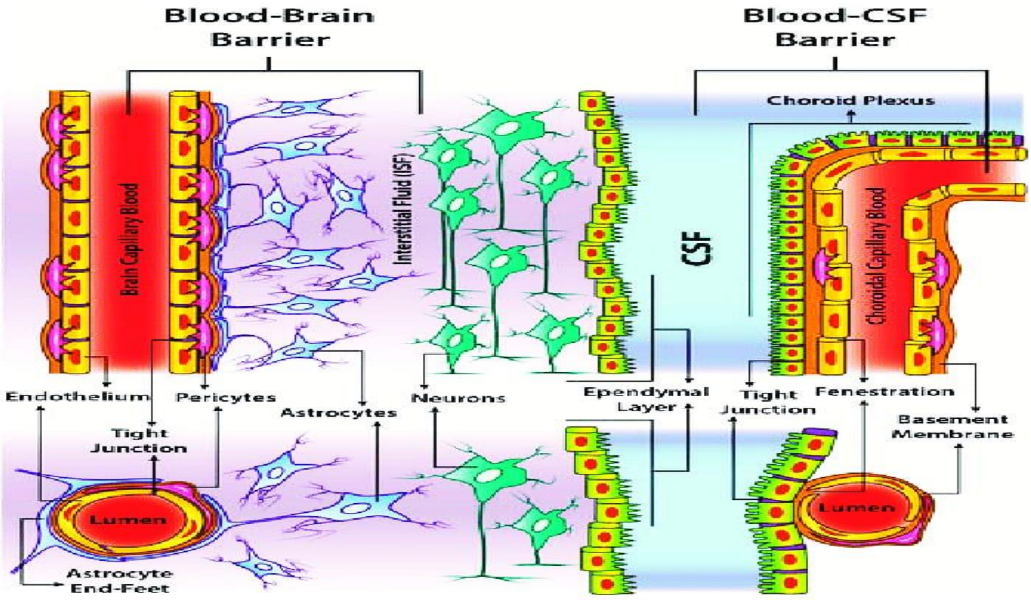


Fig. 4: Representation of BBB & Blood - Cerebrospinal Fluid Barrier.

3. Blood - Tumor Barrier (BTB)

Targeting a CNS tumor makes intracranial medicine administration much more difficult. When chemotherapeutic medicines are administered through the circulatory system to primary and secondary systemic cancers, for instance, brain metastases frequently persist in growing. All solid tumors have a number of physiological barriers that prevent drug delivery through the circulatory system in CNS cancers where the blood-brain barrier is severely damaged. Additionally, the vascular surface area shrinks with tumor size, which reduces the amount of blood-borne chemicals that may be exchanged across the arteries. Hydrostatic pressure in the normal brain parenchyma next to the tumor rises as a result of high interstitial tumor pressure and the accompanying peritumoral edema. At the same time, intracapillary distance increases, increasing the diffusion requirement for drug delivery to neoplastic cells. The brain may therefore be less drug-permeable than the typical brain endothelium. Fig. 5 illustrates how the blood-tumor barrier works.[15]

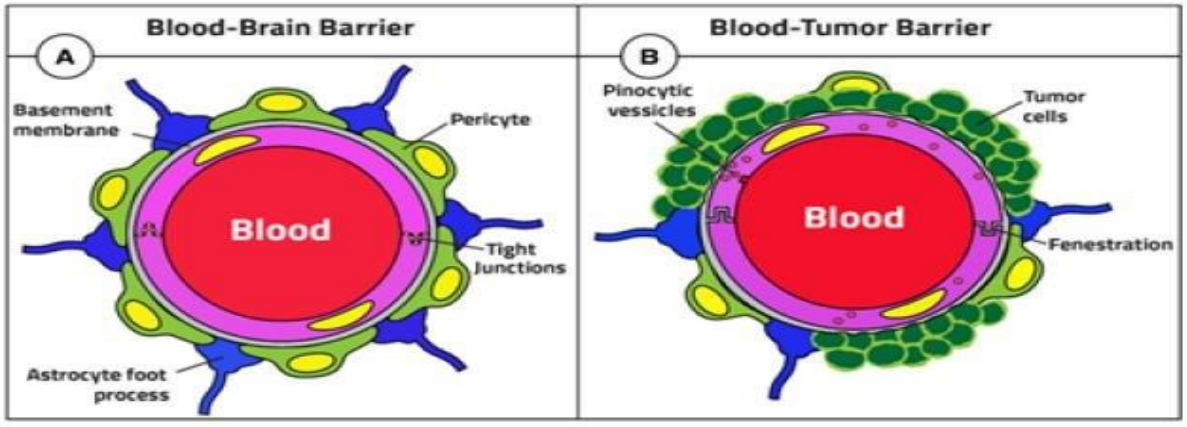
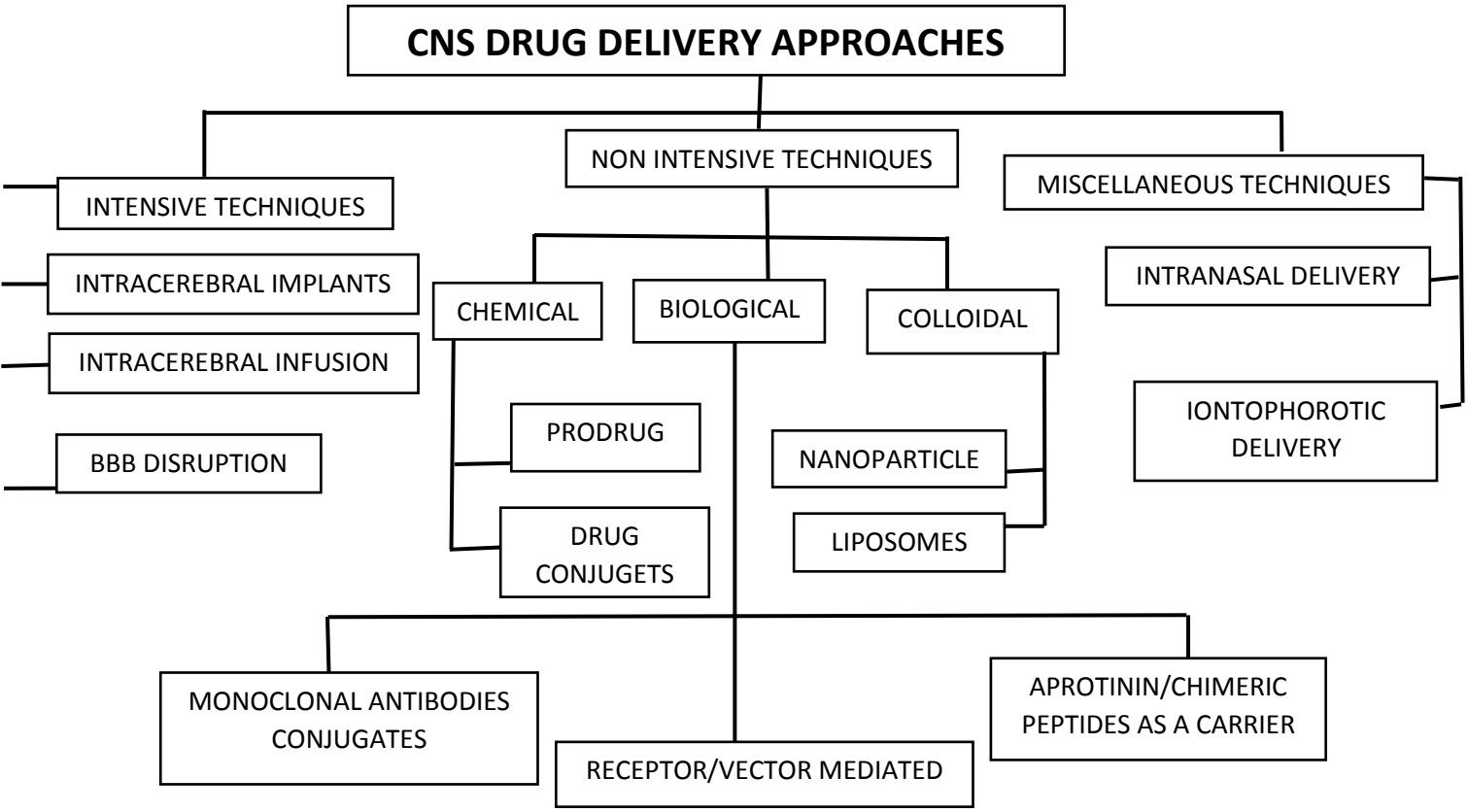


Fig. 5: Comparison between blood-brain-barrier and blood-tumor- barrier.

4. Efflux pumps

Similar to the endothelial cells lining the brain, efflux pumps serve as extra obstacles in the delivery of drugs to the brain. These pumps are composed of protein complexes known as adherens junctions, which primarily regulate the endothelium barrier's permeability.

APPROACHES TO CNS DRUG DELIVERY[16]



INVASIVE APPROACHES OR NEUROSURGICAL APPROACHES

Drilling a hole in the skull and then administering an intracerebral (IC) or intra-cerebroventricular (ICV) infusion of medication is one method of injecting drugs into the brain.These are intracranial implants that allow the medicine to circumvent the blood-brain barrier and release the drug molecule locally and continuously into the brain. Both large and small chemicals can be dispersed, either alone or in various polymer compositions, to guarantee extended release. Several invasive techniques consist of:

1. Infusion into the cerebroventricular
2. Delivery improved by convection
3. Implants into the spine
4. Interrupting the BBB

Intra-cerebroventricular (ICV) infusion

A drug's concentration in the brain is just 1-2 mm from the surface, which is roughly 1% to 2% of the concentration in the cerebrospinal fluid, according to some sources. Drugs might be easily delivered to the surface of the brain through intraventricular drug infusion, but not to the brain parenchyma. Pharmacologic effects may be seen after ICV injection if the target receptors for the drug are located near the ependymal surface of the brain.[17]

Limitations

The drug diffuses through the brain parenchyma comparatively little. As a medication delivery method, it is useless unless the target is close to the ventricles. For example, glycopeptide and an aminoglycoside antibiotic are used to treat meningitis.

Convection-enhanced delivery (CED)

The fundamental concept of CED is the stereotactically guided insertion of a small-caliber catheter into the brain parenchyma. Through this catheter, the infusate is actively forced into the brain parenchyma and enters the interstitial space. After a few days of continuing the infusion, the catheters are removed at the patient's bedside. Laboratory investigations have shown that CED may deliver high molecular weight proteins to the brain parenchyma two centimeters from the injection site after as little as two hours of continuous infusion.[18]

Limitations

Certain parts of the brain, particularly the infiltrated tissues surrounding cavities, can be difficult to fully saturate with infusate.

Catheters must be positioned correctly to distribute medications.

Intra-cerebral injection or use of implants

One of the following techniques can be used to deliver drugs straight into the parenchymal space of the brain

- Injection via intrathecal catheter.
- Release matrix control.
- Microencapsulated chemicals.

The basic mechanism is diffusion. helpful in the treatment of a number of central nervous system disorders, such as brain tumors and Parkinson's disease.

Limitations
In the brain, diffusion-based dispersion drastically decreases with distance. The injection site needs to be mapped with high precision in order to achieve efficacy and circumvent the problem of drug diffusion in the brain parenchyma.

BBB Disruption[19]
This approach, which involves rupturing the blood-brain barrier, is commonly used to provide CNS medications. Exposure to x-ray radiation and solvent infusion, such as ethanol and dimethyl sulfoxide, can disrupt the blood-brain barrier (BBB) by resulting in pathological conditions such ischemia, hypoxia, or hypertension. Disruption of the BBB is also conceivable. Alcoholic and hypoglycemic comas affect the blood-brain barrier's permeability differently. The effect is determined by the energy metabolism process.

The following are some important ways to disrupt the BBB:
• **Osmotic disturbance:** The osmotic shock causes endothelial cells to contract by rupturing tight connections. By administering a hypertonic mannitol solution intravenously and then administering the medication, it is possible to increase the concentrations of the drug in the brain and tumor tissue to therapeutic levels.

• **MRI-guided focused ultrasound BBB disruption technique**
The blood-brain barrier can be broken by ultrasound, as shown by the MRI-guided targeted ultrasound BBB disruption approach. The combination of prepared ultrasound contrast agent (optison) microbubbles, which have a diameter of 2–6 μm and are injected into the bloodstream prior to ultrasonic exposure. This technique has been shown to boost Herceptin's dispersion in brain tissue by 50% in a mouse model.

NON-INVASIVE TECHNIQUES
Prodrugs, lipophilic analogues, chemical drug delivery, carrier-mediated drug delivery, receptor-mediated drug delivery, and other variations are examples of drug manipulation techniques that are typically associated with this technique.

Chemical methods: They enhance certain physiological properties that are lacking, including membrane permeability. Chemical techniques entail altering the different functions of medications by a chemical transformation.

Prodrug: Prodrug that can pass across the blood-brain barrier and is lipid soluble. Pharmacologically inert substances known as prodrugs are created when physiologically active species undergo temporary chemical change. In the brain, it undergoes metabolism and transforms into the original medication. Prodrugs are used to enhance solubility, taste, and shelf life, as well as to boost systemic availability and gastrointestinal tolerance. Levodopa, GABA, niflumic acid, and valproate are a few examples.[20]

Drug conjugates: Compounds are encapsulated in glycosyl, maltosyl, diglucosyl, and dimaltosily derivatives of cyclodextrin in drug conjugates. Alpha linolinic acid, eicosapentaeoic acid, and their derivatives are examples of omega-3 fatty acids that are included in the therapeutic complexes.

Colloidal Techniques
Nano particles
Nanoparticles, which range in size from 1 to 1000 nm, are solid colloidal particles that have been micronized from polymeric materials. The medicine is dissolved, entrapped, encapsulated, adsorbed, or chemically bonded to the surface in these carrier systems. Compared to traditional therapies, CNS targeted drug therapy systems offer a lower risk and improved penetration of therapeutic and diagnostic agents. Drugs can be administered orally, nasally, parenterally, intraocularly, etc., using nanoparticles. Since parenteral administration became the most effective method of treating many chronic diseases, nanoparticles make it simple to modify particle size, which leads to the achievement of both active and passive drug targeting.[21]

Liposome
Liposomes are vesicular, colloidal structures made up of an equal number of aqueous compartments encircled by one or more lipid bilayers. A free medication injected in blood stream normally achieves therapeutic level for brief duration due to metabolism and elimination. A drug-encapsulated liposome maintains its therapeutic level for an extended period of time. In drug delivery systems, liposomes are used as drug carriers because they are biodegradable and basically nontoxic vesicles that can encapsulate both hydrophilic and hydrophobic molecules. Numerous liposome-based DNA delivery methods, comprising molecular components for escaping from the lysosomal compartment or targeting specific cell surface receptors, have been described.[22]

MISCELLANEOUS TECHNIQUES
Intranasal drug delivery
The nasal cavity is where drugs are delivered in this approach. Drugs for central nervous system problems and the systemic administration of analgesics, sedatives, hormones, cardiovascular medications, vaccinations, and corticosteroid hormones are delivered through the nasal mucosa.

RECEPTOR-MEDIATED DRUG DELIVERY TO THE BRAIN

Receptor-mediated endocytosis of the substance at the luminal (blood) side, passage through the endothelial cytoplasm, and exocytosis of the medication at the abluminal (brain) side of the brain capillary endothelium are the three stages that generally comprise receptor-mediated transcytosis.

1.The transferrin receptor

A transmembrane glycoprotein consisting of two 90-kDa subunits is the transferrin receptor (TfR). It is mostly expressed on intestinal cells, monocytes, hepatocytes, erythrocytes, and blood–brain barrier (BBB) endothelial cells.[23] The high plasma concentration of transferrin and the possibility of iron overload from endogenous transferrin displacement limit its use in vivo. Transferrin-tagged liposomes, however, can transport medications to BBB endothelial cells even when serum is present. Without interfering with endogenous transferrin, the monoclonal antibody OX-26 binds to TfR. While some research indicates endocytosis, iron release, and apotransferrin return to the apical side, other studies demonstrate transferrin transcytosis across BBB cells.[24]

The latter mechanism is supported by Moos and Morgan's discovery that iron crosses the BBB more effectively than transferrin. They suggested that iron is released into the brain via the TfR–transferrin complex after it travels to the basolateral side and stays attached to the receptor. It's unknown exactly how OX-26 transcytoses. While Broadwell et al. discovered that transferrin crosses the blood-brain barrier more effectively than OX-26, Pardridge and associates demonstrated successful brain targeting with OX-26.[25] Additionally, Moos and Morgan noted that OX-26 primarily builds up in BBB endothelial cells as opposed to deeper brain tissue.

2. The insulin receptor

Another important receptor-mediated transcytosis mechanism for the transfer of drugs to the brain is the insulin receptor (IR). It is a 300 kDa heterotetramer made up of two transmembrane β-subunits and two extracellular α-subunits. Tyrosine kinase activity is present in the β-chains. Through conformational changes brought about by insulin's binding to the α-chains, glucose transport and receptor autophosphorylation are made possible, ultimately resulting in endocytosis. Insulin signaling and breakdown are controlled by the endosomal system. Similar to transferrin, the risk of insulin overdose restricts the direct use of insulin as a carrier. Rather, the α-subunit of the human IR is targeted by the murine monoclonal antibody 83-14, which is employed for brain delivery.[26] The BBB permeability of this antibody is nine times greater than that of anti-TfR antibodies.

3. Lipoprotein-related protein 1 and lipoprotein related protein 2 receptor

The LDL receptor family includes the multifunctional cell-surface receptors LRP1 and LRP2 (megalin). Lipoprotein lipase, α2-macroglobulin, lactoferrin, receptor-associated protein (RAP), and plasminogen activator complexes are among the ligands they bind. Melanotransferrin (P97) is known to penetrate the blood-brain barrier through the LRP1 receptor, according to research by Beliveau's team.[27] The majority of the bound iron is transported to the liver and kidney by melanotransferrin, a transferrin homologue that is widely expressed on melanoma cells and has little uptake in the brain.[28] In animal experiments, it effectively administered doxorubicin to brain tumors following conjugation. This technique, now known as NeuroTransTM, is being developed for brain enzyme replacement therapy by BioMarin Pharmaceuticals. LRP1/2 receptors were also used by Pan et al. to show effective RAP transport across the BBB.[29] One serine protease inhibitor that has been used safely in people is aptinin (Trasylol®), which may be a viable peptide-based carrier.

4. Diphtheria toxin receptor

A human-safe carrier protein for BBB drug transport has been shown to be CRM197, a non-toxic diphtheria toxin mutant. It has long been utilized in anticancer trials and vaccinations.[30] CRM197 mediates receptor-mediated transcytosis by binding to the precursor of heparin-binding EGF-like growth factor (HB-EGF).[31] Clinical investigations indicate that following repeated CRM197 therapy, antibody levels decrease, even though pre-existing antibodies from the diphtheria vaccination may decrease efficacy. Since CRM197 has already received approval for use in humans, it is generally regarded as a safe and efficient BBB carrier. To evaluate its brain distribution and therapeutic efficacy, more in vivo research is required.

PARAMETERS FOR STUDYING BRAIN PERMEATION

1. Influx Clearance into Brain (K_{in})

Kin is the unidirectional influx constant from blood to brain (ml/min/g brain). After intravenous administration of drug, Kin is calculated using equation

$$K_{in} = Q_{br} / AUC_{(0-T)}$$

where $AUC_{(0-T)}$ is the integral of plasma concentration from t = 0 to t = T; Q_{br} is the amount of drug in the brain, without intravascular content (mass/g brain); and K_{in} is the unidirectional influx constant from blood to brain (ml/min/g brain). [^{3}H]-nicotine and brain radioactivity measurements were used to quantify nicotine transport across the rat blood-brain barrier.[32]

2. Permeability Surface Area Product (PS)

According to the same unit as K_{in} (ml/min/g brain), the permeability surface area product (PS) quantifies

unidirectional clearance from blood to brain. It is often ascertained via intravenous injection or in situ perfusion of the substance and can be evaluated for a particular brain region. PS is determined by applying the Crone–Renkin model as follows:

$$PS = -F\ ln(1 - K_{in}/F$$

where F is regional perfusion fluid flow. Chikhale et al. studied PS values of [^{14}C]-acivicin using in situ rat brain perfusion.[33]

3 Brain/plasma ratio (Kp)

The drug's distribution between the brain and plasma is expressed by Kp, which shows the degree of brain penetration rather than its rate. [^{3}H]-nicotine was used to test the blood-to-brain transit of nicotine in rats. The quantity of ^{3}H-radioactivity per gram of brain divided by that per milliliter of plasma, computed over the course of the experiment, was the apparent brain-to-plasma concentration ratio (Kp,app,brain). The initial slope of an integration plot of Kp,app,brain(t) vs AUC(t)/Cp(t) was used to determine the value of CLbrain, influx. The following equation was used to determine the uptake rate:

$$K_{p,app,brain(t)} = CL_{brain,influx} * \frac{AUC_{(t)}}{CP_{(t)}} + V_i$$

where AUC(t) is the area under the plasma concentration time curve from time 0 to t, CLbrain, inflow is the apparent blood-to-brain clearance, and Cp(t) and Vi stand for the plasma concentration at time t and the rapidly equilibrated distribution volume, respectively .In order to test brain targeting, didanosine- and NM-loaded nanoparticles were also evaluated for Kp using UPLC/MS.[34]

4. Brain uptake index

The relative absorption of a drug in relation to a reference substance is represented by the brain uptake index (BUI).[35] The radioactivity in brain tissue and the injected buffer is measured after the chemical to be examined is radiolabeled with 3H and compared with readily diffusible radiolabeled 14C-butanol as a reference. The following formula can be used to get the BUI:

$$BUI = (\frac{3_H brain}{14_C\ brain})/(\frac{3_H\ injected}{14_C\ Injected})$$

The BUI can also be represented as a percentage by multiplying it by 100. BUI is a direct function of the single pass extraction (E) and is expressed as:

$$E = 1 - e^{-PS/F}$$

where PS is the permeability surface area product (ml/min/g brain) and F is the cerebral blood or perfusion flow rate (ml/min/g brain). Using [^{3}H]-nicotine and [^{14}C]-n butanol (1 mM), a highly diffusible internal reference, Tega et al. calculated the BUI of nicotine.

5. Log BB

In order to anticipate the brain permeability, in silico approaches for evaluating blood-brain partitioning of pharmaceuticals use a range of physiochemical descriptors derived from the chemical structure of the respective substance. The distribution coefficient of the total drug between the brain and plasma on a logarithmic scale, or log BB, is determined in in silico research using the following equation:

$$LogBB = log\frac{C_{brain}}{C_{Plasma}} = \log\left(\frac{Brain}{Plasma} * ratio\right) = logK_p$$

Similar to Kp, log BB is a measure of the degree of brain partitioning. As a matter of fact, the complete brain/blood partitioning mechanism is merely an inactive drug partitioning procedure into lipid material.[36] This is due to the fact that drug binding affinities for both sides' protein and lipid contents have a significant impact on these parameters.

6. Brain unbound concentration (Cu,brain)

Understanding the pharmacokinetics and pharmacodynamics of CNS-targeted drug candidates requires knowledge of the brain unbound concentration (Cu, brain), since only the unbound drug may produce a pharmacological impact. It is measured in ng/ml. Interstitial fluid (ISF) levels in the brain are modeled by the unbound concentration in the brain. The target affinity has a direct bearing on it.[37] The unbound brain drug concentration, not the total brain drug concentration, determines a drug's in vivo effectiveness, according to the brain free drug theory. The drug concentration at the biophase is represented by the unbound brain concentration, which is what should be used to assess brain penetration.

7. Unbound brain volume of distribution (Vu,brain)

Another pharmacologically significant metric is Vu,brain, which shows whether a substance is distributed only in ISF (Vu,brain = 0.2 ml/g brain), throughout the brain water space (Vu,brain = 0.8 ml/g brain), or has a propensity to bind to brain tissue nonspecifically (Vu,brain > 0.8 ml/g brain). A liquid scintillation counter was used to measure the 3 H-radioactivity, and Tega et al. also used the brain slices approach to determine the distribution volume of [3 H]-nicotine in the brain.[38]

8. Brain free fraction (fu)

Brain unbound fraction is another name for brain free fraction. One often used technique for figuring out the free fraction of medication candidates is a brain tissue binding experiment employing equilibrium dialysis (using rat brain homogenates).

Drug binding to different tissues was estimated using the fraction of unbound drug, and a reliable and comprehensible quantitative structure-activity relationship (QSAR) for fupredictionwas developed for evaluation of CNS drug delivery. In order to determine the rate and extent of drug delivery to the brain, it is

therefore usually recommended to consider at least three factors: permeability clearance, unbound drug in the brain, and intra-brain distribution of drug.[39]

EVALUATION OF BRAIN UPTAKE/PENETRATION

Drug permeability across the BBB has been studied using a variety of methodologies. The kinetics of drug permeation across the blood-brain barrier have been studied using a variety of in vivo, in vitro, and in silico techniques. These methods are useful for determining the critical elements for steady-state brain penetration.

IN VIVO TECHNIQUES TO EVALUATE NP UPTAKE/BBB PERMEABILITY OF DRUG

The size, physicochemical characteristics, and uptake methods of NPs influence the design of brain uptake investigations. The next section discusses the various methods utilized for NPs' brain-targeting investigations.

1. Visualization methods

The most popular qualitative technique for examining in vivo BBB uptake of nanoparticles (NPs) is microscopy. NPs are loaded or coupled with fluorescent dyes for fluorescence microscopy, which is extremely sensitive. Endothelial markers like lectin are used to image brain slices following intravenous injection. Sensitive cameras can detect emissions in living animals by in vivo fluorescence imaging with near-infrared fluorophores. Plasmids expressing fluorescent proteins aid in the visualization of gene expression in brain tissue during brain-targeted gene delivery.[40] Comparisons of fluorescence intensity reveal the efficiency of NP distribution. High-resolution observation of NPs in brain regions such as capillaries and neurons is made possible by electron microscopy , which validates the processes of ligand-coated NPs . Despite its remarkable sensitivity, it only measures the entire drug—not the unbound fraction.

2. Pharmacodynamic studies

Drugs that act on the central nervous system are distinct from those that act on any other organ. By disrupting brain signaling, they produce particular behavioral effects that can be assessed using behavioral tests that have been specially created. These investigations allow for the detection of the compound's pharmacodynamic effects as well as the amount that enters the brain. By encapsulating them in NPs, drugs can be delivered across the blood-brain barrier, however they might not reach the brain in therapeutically enough amounts. The brain uptake of loperamide through polysorbate 80-coated poly-microbutylcyanoacrylate NPs and dalargin through polysorbate 80-coated PBCA Ns andP double-coated PBCA NPs was calculated via nociceptive behavioral models.[41]

3. Biodistribution studies

In pharmacokinetic and biodistribution investigations, nanoparticles (NPs) or the medications they contain are either labeled with radioisotopes like ^{3}H, ^{14}C, or ^{125}I, or their quantification is done using HPLC. Tissues such the brain, liver, and spleen are taken from animals that are slaughtered at predetermined intervals following intravenous injection. Pharmacokinetic parameters are computed using AUC values, and drug or NP concentrations in plasma and brain are assessed. For blood and brain, important metrics include mean residence time (MRT), half-life (t½), Cmax, Tmax, AUC, and so on.[42] These models aid in estimating the pace and scope of NP removal and dispersion. Even though HPLC offers precise quantification, it takes longer than visualization or microdialysis methods and calls for specific analytical knowledge.

4. Microdialysis

A semi-permeable membrane probe is inserted into the brain and perfused with physiological buffer during intracerebral microdialysis. Following oral, intravenous, or subcutaneous drug delivery, the concentration of free drugs in the brain can be measured because molecules that breach the blood-brain barrier (BBB) diffuse from the brain's interstitial fluid (ISF) into the perfusate . For pharmacokinetic studies, this method allows for the simultaneous monitoring of drug levels in the blood and brain at various time points. Correlation between pharmaceutical effects and brain extracellular fluid (ECF) concentrations is facilitated by microdialysis. Additionally, it has contributed to the explanation of gabapentin's delayed anticonvulsant activity, which suggests the inclusion of time-dependent mechanisms beyond simple BBB distribution. Gabapentin's peak impact occurs later than other antiepileptic medicines.[43]

5. Quantitative autoradiography

This method involves administering the test drug to tiny animals, radiolabelling it, and then measuring its distribution in particular brain areas. Brains are removed, sectioned (≈20 µm), and subjected to X-ray film containing radioactive standards. Blood samples are taken periodically. A BBB-impermeable marker, such as radiolabeled sucrose, is used to measure intravascular volume, which enables the computation of Kin and PS product. For determining drug localization, this approach offers great spatial resolution and sensitivity, but it is not selective; it cannot differentiate the parent drug from metabolites or bound from free fractions. It provides site-specific information without causing tissue injury or anesthetic, but it lacks time precision and does not allow for the reuse of animals.[44]

IN VITRO TECHNIQUES TO EVALUATE NP UPTAKE/BBB PERMEABILITY OF DRUG

With the advent of combinatorial chemistry and the requirement for high throughput screening, animal models are not very appropriate for both ethical and financial reasons. Furthermore, the cost and duration of in vivo biodistribution investigations are high. In order to assess BBB permeability, a variety of in vitro techniques with varying degrees of sophistication have been developed.

An in vitro model should have the following traits and be able to replicate the BBB both physiologically and physically:[45]

1) Extremely consistent and facilitate TJ expression between neighboring endothelial cells.
2) Endothelial cells have very little paracellular diffusion.
3) Have asymmetric and selective permeability to ions that are essential to the body, like CI^-, K^+, and Na^+.
4) Show that efflux systems and selective transport mechanisms (such as P-gp, MRPs, hexose, amino acids, monocarboxylic acid, and other pertinent transporters) are functionally expressed.
5) Express enzymes that break down drugs, such as monoamine oxidase (MAO) and P450s.
6) Expose endothelial cells to glia (basal membrane), laminar shear stress (apical membrane), and other permissive conditions that encourage endothelial cell differentiation and growth inhibition.
7) Adaptable and responsive to endogenous and exogenous stimuli that can impact the integrity and function of the blood-brain barrier, including permeability modulators such hyperosmolar mannitol.
8) Have the capacity to replicate the effects of a variety of physiological and pathological stimuli that impact the blood-brain barrier in vivo, such as inflammation, flow arrest, hypertension, etc.
9) Adaptable and steer clear of complicated job environments.
10) Easy to use, completely scalable, and affordable in comparison to in vivo animal drug permeability testing.

STATIC IN VITRO BBB MODELS

1. Octanol--buffer partition coefficient method

One easy, traditional way to measure drug permeability is to use the octanol–water partition coefficient. Each phase involves mixing, centrifuging, and measuring the radiolabeled medication after mutually saturating it with n-Octanol and Krebs-Ringer (KR) buffer (pH 7.4).

C(octanol)/C(buffer) is the formula used to compute the partition coefficient. This approach is simple but inaccurate because it simulates a physical barrier as opposed to a biological one. Although liposome–buffer partitioning is a more accurate representation, it only shows half of a membrane. Although it frequently fails for larger compounds, the octanol–water technique accurately predicts penetration for tiny molecules.[46]

2. Liposome-buffer partition coefficient method

The BBB is better and more accurately reflected by this method. KR buffer is used to create and dilute the multilamellar liposome suspension, as explained by Oliveira et al. After being added, the medication is separated into aliquots and placed in separate microcentrifuge tubes. The first portion is used to determine the initial medication concentration. Following incubation, the tubes are centrifuged, and a lipid pellet is obtained.[47] Drug concentration is assessed in both the lipid and buffer phases, and the associated ratio, known as the liposome-buffer partition coefficient, is computed as follows:

Liposome - buffer partition coefficient = [conc.(lipid)/ conc. ((buffer)]

3. Film balance measurements method

The Wilhelmy film balance equipment is used in this procedure to make the measurement. The lateral film pressure is measured after the lipid monolayers are dispersed at the air-water interface. KR buffer is used to spread the 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) monolayer as a subphase, and the surface pressure is kept at 10 mN/m to immobilize it. Changes in surface pressure inside the lipid monolayer and the medication solution injected into subphase are continually monitored.[48] Although this method only depicts half of the membrane, it is more similar to the BBB than the octanol--water partition coefficient method.

4. Endothelial cells monoculture method

Monolayer culture of brain microvascular endothelial cells can be obtained from bovine, pig, rodent, primate, or human sources. However, distinctive features may be lost during isolation, and permeability values frequently exceed in vivo outcomes. Because the monolayer is grown over a microporous membrane, it may selectively enter substances, exchange nutrients, and limit cell migration across compartments. Intercellular interactions, which are necessary to preserve the characteristics and functionality of the BBB, are absent from this straightforward configuration. An "edge effect," which leads to erratic cell adhesion and unchecked multilayer growth, can occur when anti-mitotic regulation is lacking. The physiological relevance is diminished in this model since both sides are exposed to serum proteins, whereas in vivo the luminal side is exposed to serum proteins and the abluminal side to astrocytic/parenchymal factors.[49]

DYNAMIC IN VITRO BBB MODELS

1. Cone plate apparatus model

The continually rotating cone creates the shear stress, and the cone angle and angular velocity define the level to endothelial cells. The endothelial cell layer is continuously exposed by the shear stress while being cultured on the plate's bottom. This model examines the relative contributions of fluid viscosity, exposure duration, and other physiological parameters, as well as the impact of laminar or turbulent flow of fluid shear stress on cells.[50]

2. 3D extracellular matrix-based BBB model

The dynamic 3D extracellular matrix model creates scaffolds that provide tight intercellular contacts and quasi-physiological biochemical gradients by using self-polymerizing extracellular matrix proteins, including as collagen, fibronectin, and laminin. Angiogenesis and cancer metastasis can be studied using this system. Cells in this three-dimensional microenvironment may be

repeatedly monitored thanks to advanced imaging techniques like tomography and confocal microscopy. However, the natural discontinuities found in vivo are absent from the uniform matrix. Molecular characterisation is less successful in bovine and porcine cells due to genetic variations than in human or rat cells. Due to the BBB's dynamic response to xenobiotics, damage, and rheological changes, these in vitro models are not very reliable. More physiologically accurate BBB models are being developed as a result of flow and microenvironmental parameters.

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