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A REVIEW ON CNS TARGETING

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ABSTRACT

Still, CNS targeting is at its infancy state; and CNS-acting candidates have the poorest success rate. In this context, this article discusses the various approaches taken for CNS targeting with their limitations. The list of approaches employed for CNS targeting starts from pro-drug, lipid mediated transport, chemical delivery system viz. dihydropyridine- pyridinium type redox delivery system to neurosurgical invasive brain delivery viz. direct intra-cerebroventicular injection. However, whether we have been successful in CNS targeting with the above mentioned strategies is a question of note. On the other hand, it discusses the presence of various specialized transport mechanisms on brain microvessel endothelial cells (BMEC) that forms the BBB. This article, further, throws light on Carrier mediated transportation (CMT) and CNS transportation of therapeutics achieved through CMT strategies.

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INTRODUCTION

Drug delivery to the brain has remained one of the most vexing problems since long ago. Diagnosis and treatment of CNS disorders represents a considerable challenge. This is mainly due to the unique and complicated environment imposed by the CNS. BBB represents a formidable obstacle not only for hydrophilic and macromolecules, but also for lipophilic and micromolecules [1]. Thus, BBB is often the rate-limiting factor in determining permeation of therapeutics into the brain. The pharmacological treatment of neurological and psychiatric disorders is often complicated by the inability of potent drugs to permeate the BBB. Pardridge says that 'Future growth in the neuropharmaceutical market is limited by the inability to target drugs through the blood–brain barrier. We all know the fast growth of neuropharmaceutical in market and yet 98% of all new drugs discovered for such disorders do not cross the BBB' [2]. Each day promising treatments of brain diseases emerge from laboratories, across the world. Many therapies that have been effective in laboratory settings have failed in clinical trials. The clinical impact of these innovative treatments will go unrealized unless effective means of drug delivery are concurrently developed. To overcome this, it is necessary to understand the molecular basis of transport functions at the BBB, and to utilize this knowledge during drug development.

This review rationalizes the various approaches employed for CNS targeting along with their limitation. This review also documents the expression of variety of transporters on both cytoplasmic and luminal plasma membrane of BBB and their bilateral role in CNS targeting. Further, it promotes the importance of Carrier mediated transportation (CMT); and CNS transportation of therapeutics achieved through CMT strategies.

Blood-brain barrier

Blood-brain barrier is a unique, selective barrier which is formed by the brain microvessel endothelial cells (BMEC) that line cerebral capillaries of the brain vessels, the basal membrane, and neurological cells. The endothelial cells of the BBB are distinguished from those in the periphery by increased mitochondrial content, lack of fenestrations, minimal pinocytotic activities [3] and presence of complex tight junctions formed by the interaction of several transmembrane proteins (such as occludin, claudins and junctional adhesion molecules-JAMs). The tight junctions seal the paracellular pathway effectively [4,5] and divide the membranes of the endothelial cells into two distinct sides, luminal (blood side) and abluminal (brain side) [6,7].

Functions of BBB

1. Neuroprotection since neuronal replacement is virtually absent in the CNS of mammals.
2. Maintenance of brain interstitial fluid (ISF) and the cerebrospinal fluid (CSF) composition.
3. Protection from fluctuations in ionic composition that can occur after a meal or exercise; and avoid disturbance of synaptic and axonal signaling.
4. Separation of centrally and peripherally acting neurotransmitters.

Various approaches employed:- Invasive techniques

Disruption of BBB

This method was first developed by Neuwelt et al [8]. The idea behind this approach is to break down the barrier temporarily by injecting a sugar solution especially mannitol into arteries in the neck. The resulting high sugar concentration in brain capillaries sucks water out of the endothelial cells, shrinking them and thus opening tight junctions. The effect of osmotic opening of BBB lasts for 20–30 min, during that time drugs that would not normally cross the BBB diffuse freely. This method shows improved advantages than cancer patients who receive systemic chemotherapy alone [9] with a subsequent decrease in morbidity and mortality in patients with malignant glioma, cerebral lymphoma and disseminated CNS germ cell tumors.

Limitation

1. Transient increase in intracranial pressure.
2. Patient can be dropped under physiological stress.
3. Also, enables the entry of unwanted substances like toxin & infectious agent into the brain. Even substance of vulnerable nature, e.g. Albumin that circulates harmlessly through the peripheral bloodstream can have deleterious effects if they enter the brain [9].
4. Need a high expertise person for administration

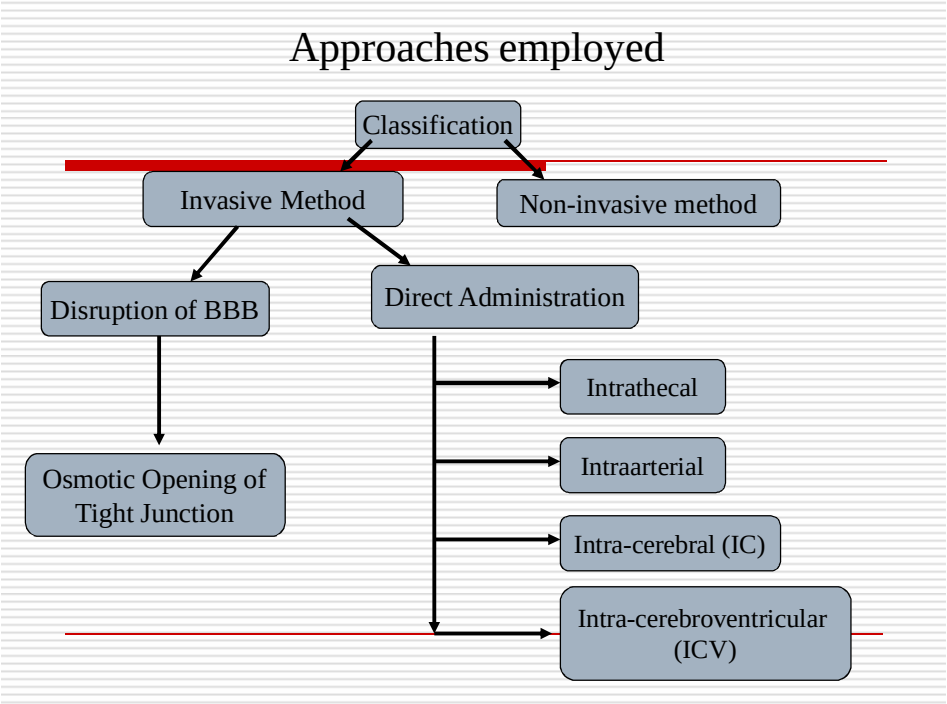

Fig.1. Classification of various approaches taken

Direct administration / Neurosurgical based invasive brain drug delivery

These are premedial trans-cranial approaches employed for the delivery of drugs to superficial, ventricular and parenchyma portion of the brain. They include intra-cerebroventricular (ICV) injection, intra-cerebral (IC) injection, convection-enhanced diffusion (CED), intraarterial and intrathecal.

Limitation

1. Surgical intervention is required.
2. For drugs relying only on diffusion for penetration, insufficient concentration of drug may reach the target site, even though, in the human brain, the diffusion distances from cerebrospinal fluid (CSF) to a drug target site may only be a few centimeters
3. Interstitial fluid secreted by microvessels of the brain flow towards the CSF spaces, which also works against drug penetration through diffusion.
4. Because of the high turnover rate of the CSF (total renewal in every 5–6 h in humans), injected drug is continuously being cleared back into the blood.
5. Direct injection into the CSF is a suitable strategy only for sites close to the ventricles.
6. Suitable only for drugs that relay on diffusion mechanism for penetration.
7. Invasive and hence are less patient friendly; more laborious and require skill.

8. Possibility of damaging the brain permanently.
9. Intra-cerebral injection or craniotomy-based brain drug delivery is not only invasive but costly (US$15,000 per patient).

Non-Invasive techniques

Lipid –mediated transport

It is a venerable approach of medicinal chemistry; and it is designed by blocking existing hydrogen bond-forming groups on the parent drug molecule. This artificial hydrophobization strategy has principally been employed for peptides and proteins molecules by the addition of fatty acid residues that facilitates the delivery of these peptides and proteins across BBB.

Limitations

1. Despite the extensive application of medicinal chemistry, there presently is not a single FDA approved drug that exemplifies the conversion of a poorly brain penetrating molecule into a high brain-penetrating molecule.
2. Again, this approach is restricted to drugs having -hydroxy, -amino, or -carboxylic acid groups for the incorporation of lipid moiety.
3. Increasing lipid solubility with a intent to increase permeability would increase permeation across all biological membranes in the body, including the BBB [10,11]; and result in increased plasma clearance due to increased volume of distribution (V_d) and reduced area under the curve (AUC) in the plasma concentration – time profile. Such occurrence has been illustrated with a lipidized form of chlorambucil.
4. Minimal transport of drug to the brain, because, the amount transported to the brain is directly comparative to the amount present in the plasma..
5. Medicinal chemistry modifications to a parent drug invariably lead to an increase in molecular weight of the drug. Any increase of the molecular weight above threshold (400 Da) can have deleterious effect on brain penetration. Since BBB permeability decreases 100-fold as the surface area of the drug is increased from 52 A° 2 (molecular weight=200 Da) to 105 A° 2 (molecular weight=450 Da).
6. Increasing the lipid solubility of a drug may enhance binding to plasma proteins, which could offset the enhanced membrane permeation caused by lipid solubility; even though, a few plasma protein-bound drugs are available for transport across the BBB in vivo, via a mechanism of enhanced dissociation at the brain capillary endothelia surface.
7. However, increasing the lipophilicity increases the rate of oxidative metabolism by cytochrome P450 as well [12-14].

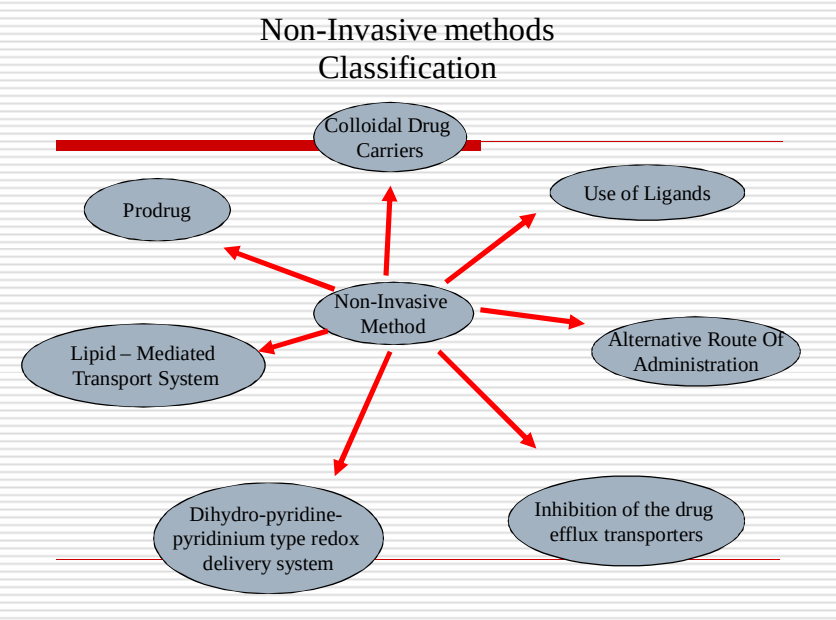

Fig.2. Classification of non-invasive methods

Prodrug

It is usually designed to improve some of the deficiency in physicochemical property, such as membrane permeability or solubility. Like hydrophobization/ lipidization strategy, this approach bode well only with drugs having -hydroxy, -amino, or -carboxylic acid groups. The parent drug molecule is transformed into prodrug generally through esterification or amidation of such groups or incorporation of lipid moiety*;* and the same is converted back to the active form in the site of action via an enzymatic cleavage. Under prodrug approach, the latter i.e. incorporation of lipid moiety (linking to a lipid moiety), such as a fatty acid, a glyceride or a phospholipid, has been explored for a large extent. Again, drug candidates containing carboxylic acid group has been utilized for large extent [11]. For examples: Levodopa into Dopamine, Primidone into Phenobarbitol, Paliperidone into Risperidone, Codeine into Morphine

Limitation

Main problems associated with prodrugs are,

1. It is valid only for drug candidates that have -hydroxy, -amino, or -carboxylic acid groups; so often the prodrug approach is not feasible with drug molecules that are lacking these groups.
2. When a prodrug is obtained through lipidization of molecules or incorporation of lipid moiety, it just encounters all the problems that a molecules undergo in previous approach i.e. lipidization of molecules. They are increasesed volume of distribution (V_d), increased plasma clearance, reduced area under the curve (AUC), enhanced plasma protein binding and enhanced susceptibility to oxidative metabolism by cytochrome P450 and other enzymes, and inevitable increase in molecular weight of the parent drug.
3. Poor selectivity and poor tissue retention [15].

Alternative route of administration/ Intranasal administration

An alternative route to CNS drug delivery as well as parenteral administration of various drugs has been investigated. Intranasal route of drug administration has various added advantages such as direct transport from the olfactory region into the CNS without coming across the BBB [16-19]. Therefore, it may be possible to deliver substances to the CNS that would otherwise have been blocked by BBB from entering the brain. Since

drugs absorbed via the olfactory route need not pass through the systemic circulation, absorption through this route has been shown to be rapid, safe, and by-passing the first-pass metabolism in the gut wall and the liver [20-22].

Limitation

However, the quantities of drugs reported to access the brain through this route are, indeed, very low, with concentrations in the CSF and olfactory lobes quoted as 0.01% to 0.1% [23,24].

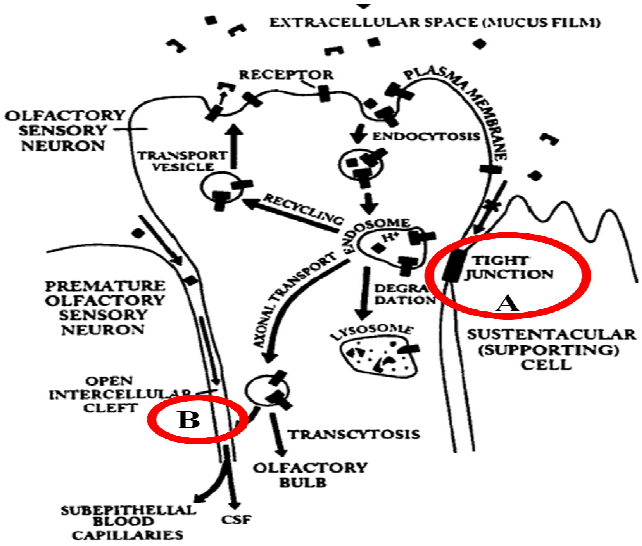

Fig.3. Depicts Intranasal - CSF route. A and B show the presence and absence of tight junction, respectively.

Colloidal drug carriers

Colloidal drug carriers include micelles, liquid crystals, vesicles, emulsions, liposomes and nanoparticles. If forthrightly spoken, extensive studies have been done only on liposomes and nanoparticles for brain drug delivery. The aim of using colloidal carriers is generally to improve the bioavailability of drugs by increasing their diffusion through biological membranes and/or to protect them against enzyme inactivation. Moreover, the colloidal systems allow access across the BBB of non-transportable drugs by masking their physico-chemical characteristics through their encapsulation in these systems.

Nanoparticles

Nanoparticles could be polymeric or lipidic (SLNs). The vital advantages about the nanoparticles are their ability to get escaped or bypassed liver and spleen filtration, particularly when they are in the range of 120–200 nm [25]. Cellular internalization is essential for a drug candidate to produce its pharmacological effect, and particles size plays an important role in cellular internalization. If the particle size is small enough to be swallowed up by the cells, cellular internalization i.e. uptake of drug particles becomes realistic. Only those molecules which are taken up by cells to their interior can produce pharmacological action. Further, due to small size, these carriers can gain access to the blood compartment easily and supplemented with prolonged circulation time in blood. Other possible advantageous of the nanoparticles are reduction in therapeutic dose, which in turn, reduces the side effects of therapeutics [26-28].

Limitation

1. One of the main problems in the targeted delivery using nanoparticles, in particular, solid lipid nanoparticles, is rapid opsonization and uptake of the carrier systems, mainly after intravenous administration, by macrophages of the reticuloendothelial system (RES), in liver and spleen.
2. Residual contamination from the production process, for example, by organic solvents [29,30].
3. Other problems include expensive production methods, a lack of large scale production method, and a suitable sterilization method [31, 32].
4. The number of products on the market is limited mainly because of the cytotoxicity of the polymers used in case of polymeric nanoparticles.
5. The main limitation is that CNS drug delivery by nanoparticles alone is not still fully elucidated.

Reason for the failure of drug delivery to the brain

However, whether we have been successful with the above mentioned strategies is a question of note. Because, the brain microvessel endothelial cells (BMEC) that form the BBB, principally offer three kind of obstacles, one is poor penetration of the drug molecule across the BBB due to the presence of tight junction, second is transendothelial electrical resistance (TEER) provided by the tight junction, and third is expression of variety of transporters on both cytoplasmic and luminal plasma membrane. These obstacles need to be addressed properly for an effective mean of CNS targeting.

Expression of transporters on BBB

The presence of various specialized transport mechanisms of solute transfer across endothelial cells and into the brain interstitium have been confirmed within the BBB. Positron emission tomography has been used to evaluate the activity of human BBB transport systems in vivo. Proteomic studies have also provided important insights into human BBB function. The brain microvessel endothelial cells (BMEC) that line cerebral capillaries of the brain vessels has been equipped with 3 different specialized transport mechanisms. Namely, *blood-to-brain influx transport system* that supplies nutrients, including glucose, amino acids and nucleotides, to the brain. Consequently, xenobiotic and drugs recognized by this influx transporter are expected to have high permeability across the BBB. *Brain-to-blood efflux transport system* that acts to eliminate metabolites and neurotoxic compounds from brain interstitial fluid, and molecules recognized by this efflux transporter are expelled from the brain parenchyma. *Drug efflux pump* that prevents entry of xenobiotics into the brain by pumping them out into the circulating blood [33].

Efflux Pump Transport

Adenosine triphosphate (ATP)-Binding Cassette (ABC) transporter is a chief member of efflux pump transporters. Prevention of intercalation and diffusion of xenobiotics into cell membranes is carried out by these transporters as protective means. They are transmembrane proteins transporters situated in BBB as well as other part of the body [34,35]. These transporters are named after a biochemical process that involves hydrolysis of ATP upon exporting substrates. This ABC family of transporters consists of A to G (ABCA-ABCG) subfamilies, and 48 subtypes [34,36]. Among the subfamily, ABCB (MDR1/ P-gp/ ABCB1), ABCC (MRP) and ABCG (BCRP/ABCG2), are the main efflux transporters.

Table.1. Shows ABC transporters and drugs back transported by them

Transporters	Drugs back transported	Reference
MDR1/ABCB1	Cyclosporin A, Verapami, Chloroquine	[37]
	Vinblastine, Actinomycin D	[38]
	Vincristine, Digoxin	[39]
	HSR-903, ciprofloxacin, norfloxacin, sparfloxacin	[40,41]
BCRP/ABCG2	Doxorubicin, Daunorubicin	[42]
	Methotrexate	[43]
	Pantoprazole	[44]
MRP4/ABCC4	Folate, Methotrexate	[45]
	Topotecan	

Carrier mediated transport (CMT)

Human body has been equipped well with many endogenous carrier-mediated transporters (CMT); and substances/ therapeutics that mimic the structures of any one of the endogenous molecule are transported through this CMT transporters. In this fashion, the parent drug will be modified chemically such that the drug candidate could mimic the structure of one of several endogenous molecules. Till date, carrier system for monosaccharides, monocarboxylic acid, large neutral amino acids, basic amino acid, acidic amino acids, amines, purine bases, nucleosides, vitamins, and hormones has been evidenced [46]. For example, BBB penetration of the catecholamine is very low, but acarboxylation of the water-soluble catecholamine results in the formation of a neutral amino acid. This amino acid may then penetrate the BBB at pharmacologically significant rates via CMT of large neutral amino acid transporter type 1 (LAT1).

Drug transported using CMT strategy

Novel NSAIDs-glucose conjugates such as, indomethacin-glucose conjugate and ketoprofen-glucose conjugate have been studied for their efficient in transportation [50]. Chlorambucil, *L*-dopa and 7-chlorokynurenic acid conjugated with glucose have been transported through GLUT-1[51-53].

Drugs that mimic the structure of phenylalanine, an endogenous amino acid, such as, *L*-Dopa, gabapentin, melphalan, and baclofen have been transported through LAT1 [54-58]. 7-Chlorokynurenic acid (7-Cl-KYNA), a potent glycine/*N*-methyl-*D*-aspartate (NMDA) receptor antagonist, has restricted BBB penetration. Conversely, *L*-4-chlorokynurenine (4-Cl-KYN), metabolite of L-tryptophan and a precursor of 7-Cl-KYNA, has readily been taken up into the brain by LAT1 [59]. Transportation of molecules which are non-substrates for LAT1 and have no resemblance with amino acids has been achieved after conjugating to amino acids. For example: ketoprofen -*L*-tyrosine conjugate and valproic acid-phenylalanine conjugates [60,61].

CONCLUSION

Till date, many approaches have been employed for CNS targeting; however, nearly all approaches have evolved with their own limitations. Invasive methods are sophisticated and economically not suitable for poor patients, besides having landmark side effects, e.g. Neurosurgical based invasive brain drug delivery. Expression of variety of transporters on both cytoplasmic and luminal plasma membrane of BBB has been

evidenced. It is obvious that molecules would not be transported into the brain unless they imitate the structure of substrate of the endogenous transporter expressed at BBB. Otherwise it should not be recognized by brain-to-blood efflux transport system. Brain targeting can effectively be achieved through carrier mediated transport (CMT); and we, human, has been bestowed with many endogenous carrier-mediated transporters. Apart from these endogenous carriers, transporter /biomarkers over expressed by certain diseased cells could be exploited for CNS targeting as well.

Table.2. Therapeutics transported through Carrier mediated transportation (CMT)

Drug	Ligand Recognized/ Ligand attached	Target used	Disease/ condition treated	References
Met^5enkephalin	D-glucopyranose	GLUT-1	Neurological pain	[47]
L-dehydroascorbic acid	D-glucopyranose	GLUT-1	----	[48,49]
Indomethacin	D-glucopyranose	GLUT-1	-----	[50]
Ketoprofen	D-glucopyranose	GLUT-1	-----	[50]
7-Chlorokynurenic acid	D-glucopyranose	GLUT-1	Convulsion	[52]
L-Dopa	D-glucopyranose	GLUT-1	Parkinson's syndrome	[53]
L-Dopa	Phenylalanine	LAT1	Parkinson's syndrome	[54,55]
Melphalan	Phenylalanine	LAT1	Brain cancer	[54,56]
Gabapentin	Phenylalanine	LAT1	Convulsion and neuropathic pain	[57]
Baclofen	L- Leucine	LAT1	Pasticity	[58]
7-Chlorokynurenic acid	L-tryptophan	LAT1	Convulsion	[59]
Ketoprofen	L-Tyrosine	LAT1	-----	[60]
Valproic acid	Phenylalanine	LAT1	Convulsion	[61]

Conflict of interest

Authors state that there is no conflict of interest.

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