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Strategic Drug Delivery Targeted to the Brain: A Review

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ABSTRACT

The central nervous system (CNS), efficiently isolated from the systemic circulation by the blood–brain barrier (BBB), represents a challenging therapeutic target. For CNS targeted agents, augmenting brain exposure by increasing blood drug concentrations often is prohibited by systemic toxicity. Therefore, a means for selectively increasing brain exposure, while minimizing systemic exposure, would be desirable. Many existing pharmaceuticals are rendered ineffective in the treatment of cerebral diseases due to our inability to effectively deliver and sustain them within the brain. General methods that can enhance drug delivery to the brain are, therefore, of great interest. Despite aggressive research, patients suffering from fatal and/or debilitating central nervous system (CNS) diseases, such as brain tumors, HIV encephalopathy, epilepsy, cerebrovascular diseases and neurodegenerative disorders, far outnumber those dying of all types of systemic cancer or heart disease. The clinical failure of much potentially effective therapeutics is often not due to a lack of drug potency but rather to shortcomings in the method by which the drug is delivered. Although several promising molecules have the potential in the in vitro settings but lack of in vivo response is probably because the molecule cannot reach the brain in a sufficient concentration. Drug delivery across the BBB is a major limitation in the treatment of central nervous system (CNS) disorders and CNS infections. As pharmacological strategies improve, there will be less need for invasive procedures for treating CNS diseases. Considerable strides have been made in intravascular delivery and neurosurgical invasive procedures to deliver therapeutic substances into the brain. This review deals with the role of several strategies and rational drug design directed at delivering drugs to the brain.

Key words: Blood brain Barrier, Drug targeting, Nose to Brain Delivery, Central Nervous System, Transport System.

INTRODUCTION

Diseases of the central nervous system (CNS) are numerous and affect a large part of the world's population. Stroke, ischemia, human immunodeficiency virus-1 (HIV-1) infection, epilepsy, and other psychiatric disorders such as anxiety, depression and schizophrenia are debilitating conditions that markedly affect the morbidity and mortality in modern society. The neurodegenerative diseases, such as Alzheimer's (AD), Parkinson's diseases (PD) and multiple sclerosis are characterized by symptoms related to movement, memory, and dementia due to the gradual loss of neurons. Brain tumors, including gliomas, astrocytomas and glioblastomas, constitute a relevant and unsolved clinical problem and the treatment of brain cancers are major challenges [1]. Unfortunately, few safe and effective methods are known for diagnosis and treatment of CNS disorders and this is mainly due to the anatomical characteristics of the CNS.

The major problem in drug delivery to brain is the presence of the BBB. Drugs that are effective against diseases in the CNS and reach the brain via the blood compartment must pass the BBB. In order to develop drugs which penetrate the BBB well to exhibit the expected CNS therapeutic effects, it is of great importance to understand the mechanisms involved in uptake into and efflux from the brain. The function of the BBB is dynamically regulated by various cells present at the level of the BBB [1]. This realization implies better understanding of the relationship of transport at the BBB to drug structure and physicochemical properties.It is estimated that more than 98% of small

molecular weight drugs and practically 100% of large molecular weight drugs (mainly peptides and proteins) developed for CNS pathologies do not readily cross the BBB [2]. To improve the brain penetration of potential therapeutic agents numerous medicinal chemistry- and pharmaceutical technology-based strategies have been explored and developed.

The aim of the present paper is to review the latest developments, evaluating both the scope and limitations of some strategies as well as the evidence supporting the importance of the therapeutic molecule features such as molecular weight and lipophilicity. The various barriers that impede the delivery of the drugs to the brain are reviewed. This is followed by a discussion of the use of both chemical modifications and nanocarriers (i.e., technological approach) for overcoming these barriers in order to affect delivery of drugs to sites in the CNS.

Barriers to CNS Drug Delivery

The failure of systemically delivered drugs to effectively treat many CNS diseases can be rationalized by considering a number of barriers that inhibit drug delivery to the CNS. There are physical barriers that separate the brain extracellular fluid from the blood.

Blood-Brain Barrier

The BBB is constituted by the brain capillary endothelial cells. This physical barrier is characterized by tight junctions between endothelial cells, by the absence of fenestrations and low occurrence of pinocytic activity [3]. These features restrict the movement of compounds from the blood into the extracellular environment of the brain (Fig. (I)).

Blood-Cerebrospinal Fluid Barrier

The second barrier, located at the choroid plexus, is represented by the blood-cerebrospinal fluid barrier (BCSFB) that separates the blood from the cerebrospinal fluid (CSF) which, in turn, runs in the subarachnoid space surrounding the brain. Unlike the capillaries that form the BBB, the capillaries in the choroid plexus allow free movement of molecules via intracellular gaps and fenestrations [4]. The epithelial cells in the choroid plexus that form the BCSFB have complex tight junctions on the CSF (apical) side of the cells. These tight junctions of the epithelial cells in the choroid plexus are slightly more permeable than those found in the endothelial cells of the BBB [16] (Fig. (I)).

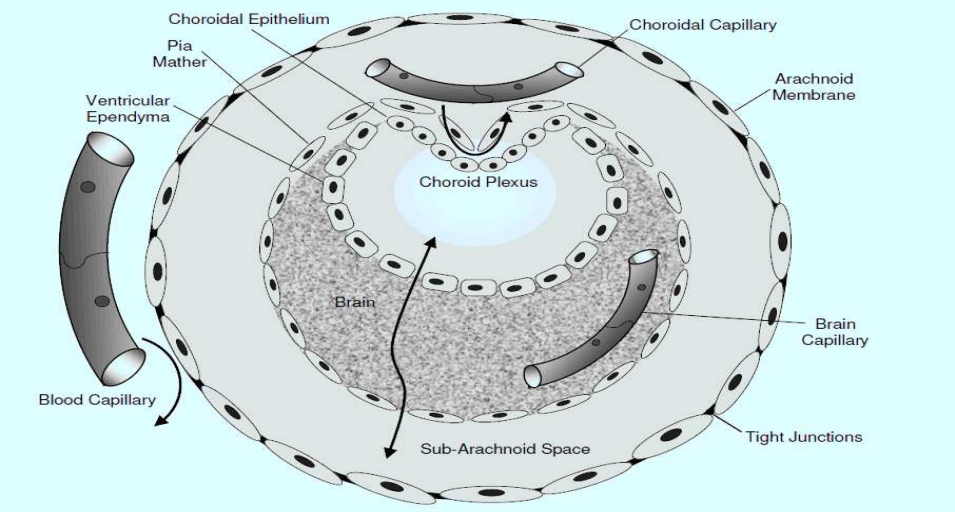

Figure (I). Schematic representation of the two main barriers in the CNS.

Blood-Tumor Barrier

Drug delivery is even more challenging when the target is a CNS tumor. The presence of the BBB in the microvasculature of CNS tumors has clinical consequences. Drug delivery to neoplastic cells in a solid tumor is compromised by a heterogeneous distribution of microvasculature throughout the tumor interstitial, which leads to spatially inconsistent drug delivery. Furthermore, as a tumor grows large, the vascular surface area decreases, leading to a reduction in trans-vascular exchange of blood-borne molecules. At the same time, intra-capillary distance increases, leading to a greater diffusional requirement for drug delivery to neoplastic cells and due to high interstitial tumor pressure and the associated peri-tumoral edema leads to increase in hydrostatic pressure in the normal brain parenchyma adjacent to the tumor. As a result, the cerebral microvasculature in these tumor adjacent regions of normal brain may be even less permeable to drugs than normal brain endothelium, leading to

exceptionally low extra-tumoral interstitial drug concentrations [5]. Brain tumors may also disrupt BBB, but these are also local and nonhomogeneous disruptions [6].

In conclusion, the delivery of drugs to the CNS via the cardiovascular system is often precluded by a variety of formidable barriers including the BBB, the BCB, and the BTB.

Furthermore, recent studies highlighted the possibility to reach the brain following the nasal route of administration. In fact, it has been shown that, by using this pathway, the transport of drug across the olfactory region in the nasal cavity occurs thus reaching directly the brain tissue or the CSF. It is based on the connection existing between the nose and the brain, that is, the olfactory bulb. In fact, the olfactory epithelium is situated between the nasal septum and the lateral wall of each side of the two nasal cavities and just below the cribriform plate of the ethmoid bone separating the nasal cavity from the cranial cavity [7].

EFFLUX MECHANISMS IN DRUG TRANSPORT TO THE BRAIN

A detailed understanding of the uptake and efflux mechanisms at the BBB would be very helpful for targeting drugs to the brain to provide the expected CNS pharmacological effect or for the reduction of BBB penetration of drugs in order to minimize side effects in the CNS. Most *in-vivo* experimental methods describing drug uptake into brain will automatically incorporate any activity of CNS efflux

into their apparent determination of brain penetration. Within the CNS are a number of efflux mechanisms that will influence drug concentrations in the brain. Some of these mechanisms are passive while others are active. Active efflux from the CNS via specific transporters may often reduce the measured penetration of drug at the BBB to levels that are lower than might be predicted from the physicochemical properties of the drug, for example, its lipid solubility. The activity of these efflux mechanisms influence the concentration in brain extracellular fluid of free drugs that are available to interact with drug receptor sites. Recently much attention has been focused on the so called multi-drug transporters; multi-drug resistance protein (MRP), P-glycoprotein (Pgp) and the multi-specific organic anion transporter (MOAT), which belong to the members of the ABC cassette (ATP-binding cassette) of transport protein [8,9]. Pgp is the product of the multidrug resistance (MDR) gene in humans and accepts a wide range of lipid-soluble substrates and will actively efflux these from cells expressing the gene product. The MOAT in the choroid plexus shows some similarity in its substrate preferences with MRP. Hence, strategies directed at increasing brain uptake of drugs that are substrates for specific efflux mechanisms need to be focused on designing reactivity with a transporter out of a drug molecule or by examining ways of inhibiting the activity of an efflux mechanism by Co-administering a competitive or non-competitive inhibitor of the efflux pump together with the desired drug. For example, for certain Pgp substrates, coadministeration of a Pgp inhibitor can increase not only oral absorption, but also BBB permeability [10, 11]. Co administration of the Pgp blocker valspodar has recently been shown to not only increase the brain levels pf paclitaxel, but also to considerably improve its therapeutic effect on tumor volume in mice [12].

Approaches for Increasing Brain Penetration

Generally, there are three approaches for increasing the penetration of drugs into the brain. The first is an invasive route that circumvents the obstacle of the BBB and/or BCSFB by direct administration of the drug into the brain. An alternative approach consists in generating a transient disruption of the BBB, allowing the therapeutic agents to enter into the brain from the blood through a more permeable BBB. The third approach concerns the chemical modification of the drug improving its penetration into the CNS.

In addition to the previous mentioned approaches, a fourth option has been increasingly investigated in the last decades and consists in the use of formulation approaches. These technological strategies are essentially non-invasive methods and are based on the use of colloidal carriers including mainly liposomes, polymeric- and solid lipidnanoparticles. However, work in this area has been primary limited to drug delivery to tumors of the brain.

To bypass the BBB, direct injection of drugs into the brain involves intracerebral and intrathecal administration. This approach is invasive and requires a craniotomy. An advantage of this pathway is that a wide range of compounds including large-and small-molecules can be administered. As mentioned above, another option to by-pass the BBB is the intranasal administration based on the connection existing between the nose and the brain that is the olfactory bulb [7]. This pathway provides a mean for the administration of various compounds into the CNS including toxic agents such as pathogens, viruses, and toxic metals as well as various therapeutic agents including small molecules and proteins. Small molecules such as cocaine and cephalexin as well as a number of protein therapeutic agents, such as insulin have been successfully delivered to the CNS using intranasal delivery [13-15] although for polar drug molecules this route is questionable with respect to the quantity of drug that can be

delivered. This administration route has been found as a promising approach for rapid-onset delivery of some medications to the CNS bypassing the BBB.

The strategy consisting in generating a transient disruption of the BBB includes the systemic administration of hyper osmotic solutions or vasoactive compounds such as bradykinin and related analogs, or various alkylglycerols. The use of osmotic agents such as mannitol or arabinose involves expansion of the blood volume caused by the addition of the hyperosmotic agent and disruption of the BBB. This barrier resumes its normal integrity and function returning the osmolarity of the blood to normal value. During this period when the tight cellular junctions between the brain capillary endothelial cells have been compromised, paracellular diffusion [Fig. (II)] of water-soluble drugs and solutes into the brain is increased [16].

The BBB can also be disrupted by pharmacological means. Several endogenous proinflammatory vasoactive agents, such as bradykinin, histamine, nitric oxide are known to induce increases in BBB permeability in a concentrationand time-dependent manner. However, although capable of producing increases in BBB permeability, these endogenous agents cannot be applied safely for CNS drug delivery. Thus, the effects of bradykinin on BBB permeability are shortlived, requiring carotid artery infusion. Better results were obtained with bradykynin analogs such as labradimil. The effect of labradimil is characterized by a significant greater plasma and tissue stability than seen with bradykinin [17].

The transient disruption of the BBB has also been observed by the systemic administration of various alkylglycerols. The extent of BBB disruption is seen to depend on the length of the alkyl group and the number of glycerols present in the structure. The exact mechanism(s) for the transient BBB disruption observed with the alkylglycerols is unknown [18]. Moreover, as with the bradykinin analogs, the disruption of the BBB by alkylglycerols is very short lived.

The third strategy for improving the brain penetration of therapeutic agents utilizes the chemical modification of the drug to improve transcellular migration. As shown in Fig. (II), the transcellular routes available include passive diffusion, specific transport systems, and endocytic processes in brain capillary endothelial cells. Overall, this strategy should lead to lower neurotoxicity compared to that associated with BBB disruption.

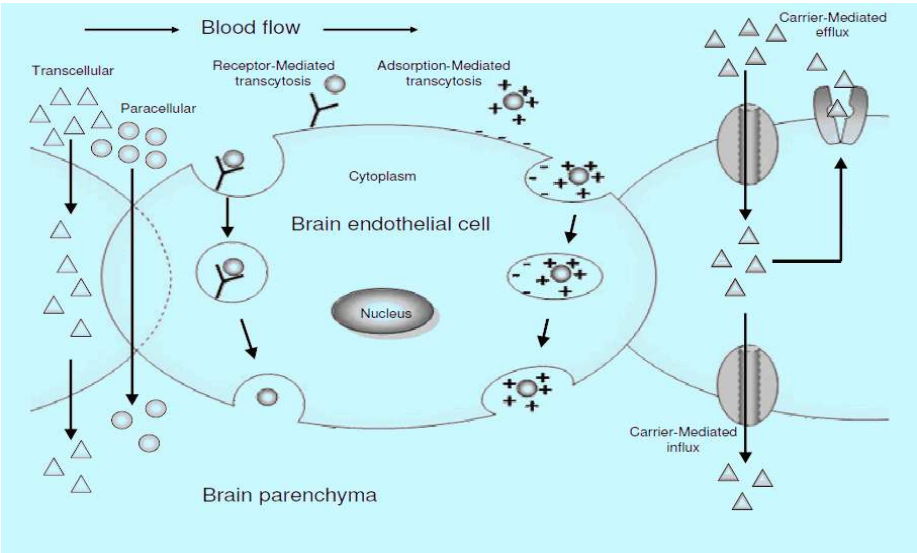

Figure (II). Blood-brain barrier transport mechanisms

Physiological Factors Affecting Drug Delivery to CNS

Many transport mechanisms for the uptake of nutrients into CNS exist in the brain (Fig. II). These transport mechanisms may be exploited for brain drug delivery. These include:

a) Passive Diffusion. The main factors affecting the passive diffusion of drugs across the BBB involve an adequate lipophilicity, neutral or uncharged nature, low hydrogen bonding potential and small molecular size (< 500 g/mol). Thus, the improvement of the passive diffusion of drugs across the BBB can often be achieved by either increasing

lipophilicity or reducing molecular size. As lipophilicity is dependent on polarity and ionization, modification of functional groups on drugs provides a method for improving passive diffusion across the BBB.

b) Carrier-mediated (Active) Transport. More than 20 carrier-mediated transporter proteins have been identified in cerebral capillaries of the BBB including transporters for glucose, amino acids, vitamins, and nucleotides. The carriers for the large amino acids (*e.g.*,amino acid transporter of type 1, LAT 1) and glucose (*e.g.*, glucose transporter of type 1 GLUT 1), especially, have a sufficiently high transport capacity [19]. Therefore, an approach for increasing the transcellular passage of drugs across BBB is to design drugs that structurally resemble or can be linked to endogenous compounds that are transported into the brain by the carriers or transporters expressed in the brain micro vessel endothelial cells [20]. Transporters that have received the greatest attention are:

i) Amino Acid Transporters. Together with the large neutral amino acid transporters, LA transporters, cationic-, anionic- and neutral-amino acid transporters have also been identified. LA transporters have been most exploited for drug delivery purposes [20]. L-Dopa is the most well-known example of a drug that is transported by LA transporters in the BBB. L-Dopa is an endogenous large amino acid and is a precursor of the neurotransmitter dopamine. LA transporters are also involved in the transport of other drugs such as L-melphalan, baclofen, and gabapentin across the BBB [21].

ii) Glucose Transporters. The most important glucose transporter present in the brain capillary endothelial cells is the type 1, glucose transporter, GLUT 1. Compared to other nutrient transport/carrier systems in the BBB, GLUT 1 has the highest transport capacity (more than 10-50 times greater than that of amino acid and carboxylic acid transporters) and therefore represents an attractive target for drug delivery to the CNS. Glycosylated analogs of various opioid compounds have shown increased CNS analgesic properties compared to the non-glycosylated compounds [22].

iii) Monocarboxylic Acid Transporter. The best-characterized organic acid transporter in the BBB is the monocarboxylic acid transporter (MCT). Examples of drugs entering the CNS through the MCT are salicylic acid and various cholesterol-lowering 3-hydroxy-3- methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors [23].

iv) Nucleoside Transporters. There are two general types of nucleoside transporter expressed in the brain capillary endothelial cells: the facilitative nucleoside transporters that carry selective nucleosides either into or out of the cell and active and the sodium-dependent transporters that can move selective nueleosides into the cell against a concentration gradient. There are several examples of drugs that are substrates for nucleoside transport systems such as the anticancer agent, gemcitabine, the antiviral agents, 3'-azidodeoxythymidine (AZT) and 2',3'-dideoxycytidine (ddC) [24, 25].

v) Peptide Transport Systems. Peptide transport systems are present in the brain capillary endothelial cells forming the BBB. The exact molecular nature of these peptide transporters remains to be determined. However, specific saturable transport systems have been identified in the BBB for glutathione [26], peptide hormones [27] and growth factors [28].

c) Vesicular Transport. Two types of vesicular transport processes are known: the fluid-phase endocytosis and the adsorptive endocytosis. However, only adsorptive endocytosis involves an initial binding or interaction with the plasma membrane of the cell. Vesicular transport due to adsorptive endocytosis is a saturable, ligand selective process. Several large macromolecules are transported from the blood into the brain through receptor-mediated endocytosis. Thus, these specific receptor-mediated transport processes represent another approach for enhancing transcellular permeability across the BBB. The most well-known processes are the transferrin- and insulin receptor-mediated vesicular transport.

i) **Transferrin** is a glycoprotein that controls the transport of iron throughout the body. The brain capillary endothelial cells have a high density of transferrin receptors on their surface. Iron enters the cell as a complex with transferrin through an endocytic process that is initiated by the binding of transferrin to its receptor. Inside the brain endothelial cell, the iron is removed from the transferrin in the endosome. However, transferrin shows a limitation as a brain delivery vector because it is recycled back to the luminal surface of the brain capillary endothelial cell. As an alternative, a murine monoclonal antibody (MAb), OX-26 has been identified, which appears to be suitable for use as a drug carrier for this transport system. First, this MAb binds to the receptor and triggers endocytosis. Second, the OX-26 MAb binds to an extracellular epitope on the transferrin receptor that is distinct from the transferring ligand-binding site; thus, the OX-26 MAb does not interfere with transferrin binding to its receptor on the brain endothelial cells. The OX-26 antibody has proven to be an effective brain delivery vector, as it has been conjugated to a variety of drugs including methotrexate and nerve growth factor [29, 30].

ii) **Insulin** is a peptide hormone involved in glucose metabolism. The presence of insulin receptors in the CNS, suggest that insulin may have important functions also within the brain. Studies demonstrating the presence of high-affinity insulin receptors on the luminal plasma membrane of brain microvessel endothelial cells indicate that the peptide penetrates the BBB through a receptor-mediated transport process. Other studies support the potential use of insulin as brain delivery vector of therapeutic agents and macromolecules to the brain. For example, insulin has been used as a BBB transport vector for proteins [31]. Many other receptors such as insulin-like growth factor receptor, and a leptin receptor may be used for the same purpose.

IN VIVO AND IN VITRO MODELS TO STUDY DRUG TRANSPORT ACROSS THE BLOOD-BRAIN AND BLOODCSF BARRIERS

The pharmacokinetics and pharmacodynamics of drugs in the CNS are understood by their unbound concentrations in the extracellular fluid of the brain. Various in-vivo and in-vitro techniques are available to study this property. The *in-vivo* techniques include the brain uptake index (BUI) [32], the brain efflux index (BEI) [33], brain perfusion [34], the unit impulse response method [35] and microdialysis [36]. The efflux transport across the BBB is a very important process for explaining the mechanism of the apparent restricted cerebral distribution of drugs after their systemic administration. In order to examine the BBB efflux transport mechanism under in-vivo conditions, the intracerebral microinjection technique has been developed and recently established as the BEI. The BEI value is defined as the relative percentage of drug effluxed from the ipsilateral (that is, they do not cross to the opposite hemisphere) cerebrum to the circulating blood across the BBB compared with the amount of drug injected into the cerebrum,
i.e.:

$$\text{BEI}\ (\%) = \frac{\text{Amount of drug effluxed at the BBB}}{\text{Amount of drug injected into the brain}}\ \text{X100}$$

The advantages of the BEI method are its ability to allow determination of the apparent in vivo drug efflux rate constant across the BBB, monitoring the concentration dependency of the test drug and the performance of inhibition studies. By contrast, the limitations of the BEI method are that only one data point can be obtained for a single intracerebral microinjection. The drug concentration in the cerebrum cannot be accurately determined. In other words, at the present time, the drug concentration in the brain is estimated by using the dilution factor, i.e. 30.3- to 46.2-fold dilution [33].

The brain interstitial fluid (ISF) concentration is a determinant for the effect of a drug in the CNS *in-vivo*. If the drug would cross the BBB in significant quantities by passive diffusion, the brain ISF concentration will equal the plasma unbound drug concentration after its administration. In this case, the plasma unbound drug concentration will be very important in predicting the CNS effect. However,

if the brain ISF concentration of a drug is significantly lower than the plasma unbound drug concentration, it will be very important to identify the mechanism involved. For the direct measurement of brain ISF drug concentration, many researchers have found brain microdialysis to be a useful technique [37, 38]. Micro-dialysis is a method of choice in the study of *in-vivo* drug transport across the BBB, based on brain's physiological and anatomical characteristics considering it to be a non-homogeneous compartment. In addition, drug disposition in the brain is determined by protein binding, blood flow, BBB transport, and the exchange between brain extracellular fluid (ECF) and brain cells. Nevertheless, intra-cerebral micro-dialysis is an invasive technique: it involves the implantation of a probe, which may cause tissue trauma, and hence may have consequences for BBB function. Therefore it is necessary to determine whether intra-cerebral micro-dialysis provides meaningful data on drug transport across the BBB and drug disposition in the brain. In-vitro models that closely mimic the in-vivo system, at least with respect to barrier properties, are in high demand. Blood-brain barrier models now available make use of cerebral capillary endothelium (porcine brain capillary endothelial cells) or choroid plexus epithelial cells (porcine choroid plexus) [39, 40]. Both cell types need serum in the growth medium to proliferate. Serum, however, inhibits the formation of tight cell-cell contacts.

Electrical resistance is an easy measure of junctional tightness [41]. A very sophisticated but highly reliable and reproducible new method is impedance spectroscopy (IS) [42], in which AC potentials are applied over a wide frequency range. At a single fixed frequency, AC potentials may be applied and analyzed if only relative changes after substrate application are expected. IS yields information about both conductivity and dielectric constant (capacitance) of the interfacial region of the cell monolayer. Essentially three types of brain capillary endothelial cell culture are currently used by researchers: primary cultures, cell lines and co-culture systems. The limitation of primary cultures has been their higher para-cellular permeability, reflected by the measurement of the electrical resistance across the monolayer. The strong correlation between the in-vivo and in vitro values demonstrated that this in-vitro system is an important tool for the investigation of the role of the BBB in the delivery of nutrients and

drugs to the CNS [43]. The main advantage of this model is the possible rapid evaluation of strategies for achieving drug targeting to the CNS or to appreciate the eventual central toxicity of systemic drug and to elucidate the molecular transport mechanism of substances across the BBB.

Strategies for drug delivery to the brain

To circumvent the multitude of barriers inhibiting CNS penetration by potential therapeutic agents, numerous drug delivery strategies have been developed. These strategies generally fall into one or more of the following three categories: manipulating drugs, disrupting the BBB and finding alternative routes for drug delivery [44-46].

Drug manipulating

Lipophilic analogues

CNS penetration is favored by low molecular weight, lack of ionization at physiological pH, and lipophilicity. Delivery of poorly lipid-soluble compounds to the brain requires some way of getting past the BBB. There are several possible strategies, such as transient osmotic opening of the BBB, exploiting natural chemical transporters, high dose chemotherapy, or even biodegradable implants. But all of these methods have major limitations: they are invasive procedures, have toxic side effects and low efficiency, and are not sufficiently safe. A possible strategy is to smuggle compounds across as their lipophilic precursors. Because drug's lipophilicity correlates so strongly with cerebro-vascular permeability, hydrophobic analogues of small hydrophilic drugs ought to more readily penetrate the BBB [47]. This strategy has been frequently employed, but the results have often been disappointing Immunoliposomes (antibody-directed liposome) have been recognized as a promising to ol for the site-specific delivery of drug s and diagnostic agents. However, the *in vivo* use of classical Immunoliposomes is hampered by the very rapid clearance of immunoliposomes from the circulation by the reticuloendothelial system. Avoidance of this obstacle is possible if gangliosides or PEG-derivatized lipids are inserted within the bilayer of conventional liposomes, as these modifications prolong considerably the liposome half-life in the circulation. Liposomes coated with the inert and biocompatible polymer PEG are widely used and are often referred to as "sterically stabilized" or "stealth liposomes". PEG coating is believed to prevent recognition of liposomes by macrophages due to reduced binding of plasma proteins. Unfortunately, it has been difficult to combine steric stabilization of liposomes with efficient immunotargeting. PEG coating of liposomes can create steric hindrances for antibody-target interaction. It has therefore been proposed to attach a cell-specific ligand to the distal end of a few lipid-conjugated PEG molecules rather than conjugate the ligand to a lipid head group on the surface of a PEG-conjugated liposome. This has been done recently with folic acid and monoclonal antibodies to target liposome to cells in tissue culture and organs *in vivo* . The application of PEG-conjugated immunoliposomes to *in vivo* brain targeting of drug s has not been attempted thus far. Conventional liposomes are not delivered to brain *in vivo* , because these agents are not transported through the brain capillary endothelial wall, which makes up the blood- brain barrier (BBB) *in vivo* .

Table I: Strategies for Linking Drugs to Transport Vectors

Class	Target AA	Agent	Linkage	Cleavability
Chemical	Lys Lys	MBS Traut's	Thio-ether (-S-)	No
	Lys Lys	SPDP Traut's	Disulfide (-SS)	Yes
Avidin-biotin	Lys	NHS-SS-biotin	Disulfide	Yes
	Lys	NHS-XX-biotin	Amide	No
	Lys	NHS-PEG-biotin	Extended Amide	No
	Asp, Glu	Hz-PEG-biotin	Extended Hydrazide	No
Genetic Engineering	Fusion Gene Element	Recombinant Protien	Recombinant Vector	No
		Recombinant Vector	Recombinant Avidin	Flexible

Abbreviation: NHS- N-Hydroxy Succinimide; PEG-Polyethelene Glycol; Hz-Hydrazine; MBS-m maleimidobenzoyl N-hrdroxy succinimide ester; SPDP-N-Succinimidyl-3-2-pyridyldithio propionate; Lys-Lysine; Asp-Aspartic Acid; Glu-Glutamic Acid; AA-Amino Acid

However, certain receptor specific monoclonal antibodies (mAbs) undergo recep to r-mediated transcytosis through the BBB, and mAb-gold conjugates are transcytosed through the BBB *in vivo*. Therefore, the present studies were designed to achieve the following goals. First, PEG-conjugated immune liposomes were synthesized using thiolated mAb and a bifunctional 2000-Da PEG (PEG 2000) that contains a lipid at one end and a maleimide at the other end [48] . Second, the pharmacokinetics and brain uptake of [3 H] daunomycin was examined following intravenous administration of the drug in free form, as a conventional liposome, as a PEG-conjugated liposome, and as a PEG-conjugated Immunoliposomes. The mAb used in these studies is the OX26 mAb to the rat transferrin receptor, which is abundant on brain micro vascular endothelium [49].

Prodrugs

Brain uptake of drugs can be improved via prodrug formation. Prodrugs are pharmacologically inactive compounds that result from transient chemical modifications of biologically active species. The chemical change is usually designed to improve some deficient physicochemical property, such as membrane permeability or water solubility. After administration, the prodrug, by virtue of its improved characteristics, is brought closer to the receptor site and is maintained there for longer periods of time. Here it gets converted to the active form, usually via a single activating step [50]. Unfortunately, simple prodrugs suffer from several important limitations. Going to extremes on the lipophilic precursor scale, a possible choice for CNS prodrugs is coupling the drug to a lipid moiety, such as fatty acid, glyceride or phospholipids. Such prodrug approaches were explored for a variety of acid-containing drugs, like levodopa, GABA, Niflumic acid, valproate or vigabatrin are coupled to diglycerides or modified diglycerides[51]. While increased lipophilicity may improve movement across the BBB, it also tends to increase uptake into other tissues, causing an increased tissue burden.

Chemical Drug Delivery

Chemical drug delivery systems (CDDS) represent novel and systematic ways of targeting active biological molecules to specific target sites or organs based on predictable enzymatic activation. They are inactive chemical derivatives of a drug obtained by one or more chemical modifications so that the newly attached moieties are monomolecular units (generally comparable in size to the original molecule) and provide a site-specific or site enhanced delivery of the drug through multi-step enzymatic and/or chemical transformations [52-55] . During the chemical manipulations, two types of bio removable moieties are introduced to convert the drug into an inactive precursor form. A targetor (T) moiety is responsible for targeting, site-specificity, and lock-in, while modifier functions (F1...Fn) serve as lipophilizers, protect certain functions, or fine-tune the necessary molecular properties to prevent premature, unwanted metabolic conversions. The CDDS is designed to undergo sequential metabolic conversions, disengaging the modifier functions and finally the targetor, after this moiety fulfils its site- or organ-targeting role.

Carrier Mediated Drug Delivery

Carrier-mediated transport (CMT) and receptor-mediated transport (RMT) pathways are available for certain circulating nutrients or peptides. The availability of these endogenous CMT or RMT pathways means that portals of entry to the brain for circulating drugs are potentially available. In the braincapillary endothelial cells, which make up the BBB, there are several transport systems for nutrients and endogenous compounds. They are (a) the hexose transport system for glucose and mannose, (b) the neutral amino acid transport system for phenylalanine, leucine and other neutral amino acids, (c) the acidic amino acid transport system for glutamate and aspartate, (d) the basic amino acid transport system for arginine and lysine, (e) the b-amino acid transport system for b-alanine and taurine, (f) the monocarboxylic acid transport system for lactate and short-chain fatty acids such as acetate and propionate, (g) the choline transport system for choline and thiamine, (h) the amine transport system for mepyramine, (i) the nucleoside transport system for purine bases such as adenine and guanine, but not pyrimidine bases, and (j) the peptide transport system for small peptides such as enkephalins, thyrotropin-releasing hormone, arginine vasopressin etc [56-57] . Utilization of differences in the affinity and the maximal transport activity among these transportsystems expressed at the BBB is an attractive strategy for controlling the delivery and retention of drugs into the brain.

Receptor/Vector Mediated Drug Delivery

Receptor-mediated drug delivery to the brain employs chimeric peptide technology, wherein a non-transportable drug is conjugated to a BBB transport vector. The latter is a modified protein or receptor-specific monoclonal antibody that undergoes receptor-mediated transcytosis through the BBB in-vivo. Conjugation of drug to transport vector is facilitated with chemical linkers, avidin–biotin technology, polyethylene glycol linkers, or liposomes. Multiple classes of therapeutics have been delivered to the brain with the chimeric peptide technology, including peptide- based pharmaceuticals, such as a vasoactive peptide analog or neurotrophins such as brain-derived neurotrophic factor, anti-sense therapeutics including peptide nucleic acids (PNAs), and small molecules incorporated within liposomes. The attachment of the drug that normally does not undergo transport through the BBB to a BBB transport vector such as the MAb, results in the formation of a chimeric peptide, provided the bifunctionality of the conjugate is retained. That is, the chimeric peptide must have not only a BBB transport function, but also a pharmaceutical function derived from the attached drug. Certain drugs may not be pharmacologically active\ following attachment to a BBB transport vector. In this case, it may be desirable to attach the drug to the transport vector via a cleavable disulfide linkage that ensures the drug is still pharmacologically active following release from the transport vector owing to cleavage of the disulfide bond. Depending on the chemistry of the disulfide linker, a molecular adduct will remain attached to the drug following disulfide cleavage, and the molecular adduct must not interfere with drug binding to the drug receptor [58-61]. A second consideration with respect to the use of a disulfide linker is that virtually all of the cell disulfide reducing activity may be

contained within the cytosol Therefore, the chimeric peptide must undergo endosomal release following receptor-mediated endocytosis into the target brain cell, in order to distribute to the reductase compartment. Figure III Illustrate the multiplicity of approaches for linking drugs to transport vectors, and the availability of these multiple approaches allows for designing transport linkers to suit the specific functional needs of the therapeutic under consideration.

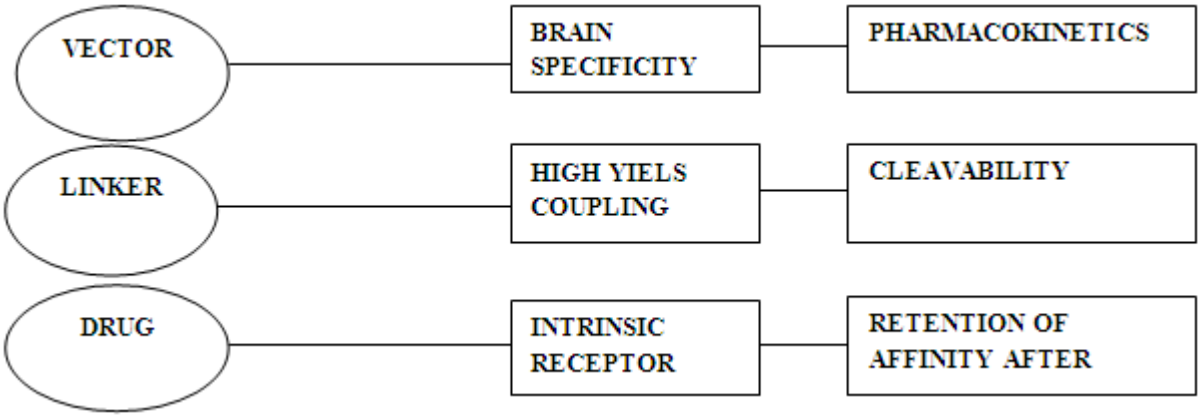

Figure III: Multiplicity of approaches for linking drugs to transport vectors Disrupting the Blood Brain Barrier

The second invasive strategy for enhanced CNS drug delivery involves the systemic administration of drugs in conjunction with transient BBB disruption (BBBD). Theoretically, with the BBB weakened, systemically administered drugs can undergo enhanced extravasations rates in the cerebral endothelium, leading to increased parenchymal drug concentrations. A variety of techniques that transiently disrupt the BBB have been investigated; however, albeit physiologically interesting, many are unacceptably toxic and therefore not clinically useful. These include the infusion of solvents such as dimethyl sulfoxide or ethanol and metals such as aluminum; X-irradiation; and the induction of pathological conditions including hypertension, hypercapnia, hypoxia or ischemia [62-63]. The mechanisms responsible for BBBD with some of these techniques are not well understood.

Osmotic blood brain barrier disruption

Osmotic opening of the BBB was developed. Intracarotid injection of an inert hypertonic solution such as mannitol or arabinose has been employed to initiate endothelial cell shrinkage and opening of BBB tight junctions for a period of a few hours, and this permits delivery of antineoplastic agents to the brain. Though this treatment is still investigational, the fact that some patients who fail systemic chemotherapy have responded to similar or lower doses of intracarotid drugs is an often-cited argument in favor of the method. One reason for the unfavorable toxic/therapeutic ratio often observed with hyper osmotic BBBD is that this methodology results in only a 25% increase in the permeability of the tumor microvasculature, in contrast to a 10-fold increase in the permeability of normal brain endothelium [64]. Osmotic disruption of the BBB has also been suggested as a delivery strategy for recombinant adenoviral vectors for gene transfer to intracerebral tumors, and for magnetic resonance imaging agents for diagnosis of brain metastases using iron oxide conjugates, but there are problems which must be overcome before the routine clinical use of this technique can be realized. Osmotic disruption seems to be most successful in treating primary non-AIDS CNS lymphoma. The risk factors include the passage of plasma proteins, the altered glucose uptake, and the expression of heat shock proteins, micro embolism or abnormal neuronal function.

Biochemical Blood-Brain Barrier Disruption

Recently, new and potentially safer biochemical techniques have been developed to disrupt the BBB. Selective opening of brain tumor capillaries (the blood–tumor barrier), by the intracarotid infusion of leukotriene C4 was achieved without concomitant alteration of the adjacent BBB. In contrast to osmotic disruption methods, biochemical opening utilizes the novel observation that normal brain capillaries appear to be unaffected when vasoactive leukotriene treatments are used to increase their permeability. However, brain tumor capillaries or injured brain capillaries appear to be sensitive to treatment with vasoactive leukotrienes, and the permeation is dependent on molecular size [65].

Alternative Routes to CNS Drug Delivery

Despite advances in rational CNS drug design and BBBD, many potentially efficacious drug molecules still cannot penetrate into the brain parenchyma at therapeutic concentrations. A third class of strategies aimed at enhancing CNS penetration of drug molecules is composed of delivery methodologies that do not rely on the cardiovascular system [65-68]. These alternative routes for controlled CNS drug delivery obviate the need for drug manipulation to

enhance BBB permeability and/or BBBD by circumventing the BBB altogether. Since, most aforementioned techniques aim to enhance the CNS penetration of drugs delivered via the circulatory system; the result is higher drug penetration throughout the entire body and frequently unwanted systemic side effects. Additionally, systemically administered agents must penetrate the BBB to enter the brain, which is a formidable task.

Intra-ventricular / Intra thecal Route

One strategy for bypassing the BBB that has been studied extensively both in laboratory and in clinical trials is the intralumbar injection or intreventricular infusion of drugs directly into the CSF. Drugs can be infused intraventricularly using an Ommaya reservoir, a plastic reservoir implanted subcutaneously in the scalp and connected to the ventricles within the brain via an outlet catheter. Drug solutions can be subcutaneously injected into the implanted reservoir and delivered to the ventricles by manual compression of the reservoir through the scalp [69-70].

When compared to vascular drug delivery, intra-CSF drug administration theoretically has several advantages. Intra-CSF administration bypasses the BCB and results in immediate high CSF drug concentrations. Since, the drug is somewhat contained within the CNS, a smaller dose can be used, potentially minimizing systemic toxicity. Furthermore, drugs in the CSF encounter minimized protein binding and decreased enzymatic activity relative to drugs in plasma, leading to longer drug half-life in the CSF. Finally, because the CSF freely exchanges molecules with the extracellular fluid of the brain parenchyma, delivering drugs into the CSF could theoretically result in therapeutic CNS drug concentrations [71].

However, this delivery method has not lived up to its theoretical potential for several reasons. These include a slow rate of drug distribution within the CSF and increase in intracranial pressure associated with fluid injection or infusion into small ventricular volumes. It results in to high clinical incidence of hemorrhage, CSF leaks, and neurotoxicity and CNS infections. The success of this approach is limited by the CSF-brain barrier, composed of barriers to diffusion into the brain parenchyma. Because the extracellular fluid space of the brain is extremely tortuous, drug diffusion through the brain parenchyma is very slow and inversely proportional to the molecular weight of the drug. For macromolecules, such as proteins, brain parenchymal concentrations following intra-CSF administration are undetectable. For these reasons, intra-CSF chemotherapy in the treatment of intraparenchymal CNS tumors has not proven to be effective. The greatest utility of this delivery methodology has been in cases where high drug concentrations in the CSF and/or the immediately adjacent parenchyma are desired, such as in the treatment of carcinomatous meningitis or for spinal anesthesia/analgesia [72].

Intrathecal and intracerebral drug administration differs fundamentally from systemic drug administration in terms of pharmacokinetic characteristics determining brain tissue concentration, where the available dose reaching the target organ is 100%. However, there are large gradients inside the tissue with very high local concentrations at the site of administration (the ventricular surface or tissue site of injection) and zero concentration at some distance for macromolecules. Since, they have low diffusion coefficients, the gradients will be even steeper than what has been measured for small molecular weight drugs. After intracerebroventricular (icv) injection, the rate of elimination from the CNS compartment is dominated by cerebrospinal fluid dynamics. Clinical examples of intrathecal small drug delivery are the icv administration of glycopeptide and aminoglycoside antibiotics in meningitis, the intraventricular treatment of meningeal metastasis, intrathecal injection of baclofen for treatment of spasticity and the infusion of opioids for severe chronic pain [73-74] . These examples have in common the fact that the drug targets in all instances are close to the ventricular surface. Superficial targets may also be accessible for some macromolecular drugs [75-76].

Olfactory Pathway

An alternative CNS drug delivery strategy that has received relatively little attention is the intranasal route. Drug delivered intranasally are transported along olfactory sensory neurons to yield significant concentrations in the CSF and olfactory bulb. In recent studies, intranasal administration of wheat germ agglutinin horseradish peroxidase resulted in a mean olfactory bulb concentration in the nanomolar range. In theory, this strategy could be effective in the delivery of therapeutic proteins such as brain-delivered neurotropic factor (BDNF) to the olfactory bulb as a treatment for Alzheimer's disease. The nasal drug delivery to the CNS is thought to involve either an intraneuronal or extraneuronal pathway. Recent evidence of direct nose-to-brain transport and direct access to CSF of three neuropeptides bypassing the bloodstream has been shown in human trials, despite the inherent difficulties in delivery [77-78]. The difficulties that have to be overcome include an enzymatically active, low pH nasal epithelium, the possibility of mucosal irritation or the possibility of large variability caused by nasal pathology, such as common cold. An obvious advantage of this method is that it is noninvasive relative to other strategies. In practice, however, further study is required to determine if therapeutic drug concentrations can be achieved following intranasal delivery.

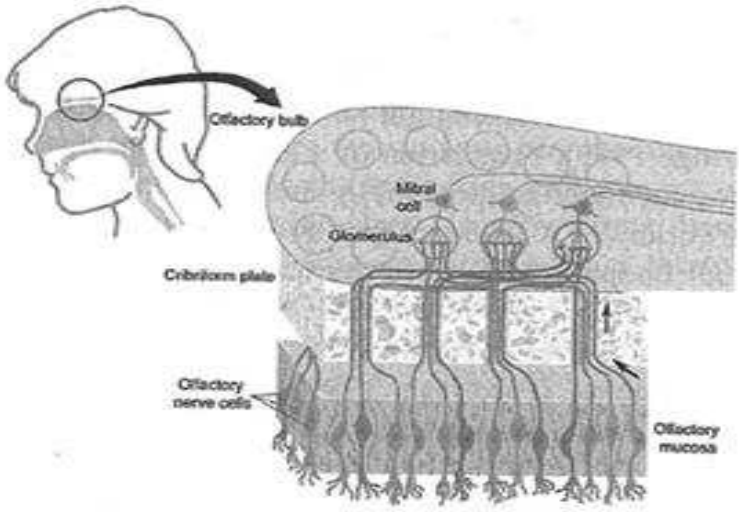

Figure IV: The olfactory bulb, olfactory mucosa, and olfactory nerve cells in humans.

Nasal transport routes:
After nasal delivery drugs first reach the respiratory epithelium, where compounds can be absorbed into the systemic circulation utilizing the same pathways as any other epithelia in the body: Tran cellular and Para cellular passive absorption, carrier-mediated transport, and absorption throughtrancytosis. Although absorption across the respiratory epithelium is the major transport pathway for nasally-administered drugs and may represent a potentially timesaving route for the administration of certain systemic drugs delivered in cryonics medication protocols (e.g., epinephrine or vasopressin), problem of BBB-mediated exclusion of brain-therapeutic agents to be of greater immediate concern. Accordingly, the remainder of this article will deal primarily with the transport of drugs to the CNS by way of the olfactory epithelium. [79]

When a nasal drug formulation is delivered deep and high enough into the nasal cavity, the olfactory mucosa may be reached and drug transport into the brain and/or CSF via the olfactory receptor neurons may occur. The olfactory pathways may be broadly classified into two possible routes: the olfactory nerve pathway (axonal transport) and the olfactory epithelial pathway. [81]

Axonal transport is considered a slow route whereby an agent enters the olfactory neuron viaendocytotic orpinocytotic mechanisms and travels to the olfactory bulb by utilizing the sameanterograde axonal transport mechanisms the cell uses to transport endogenous substances to the brain. [86]Depending on the substance administered, axonal transport rates range from 20-400 mm/day to a slower 0.1-4 mm/day. [87]The epithelial pathway is a significantly faster route for direct nose-to-brain transfer, whereby compounds passparacellularly across the olfactory epithelium into theperineural space, which is continuous with the subarachnoid space and in direct contact with the CSF. Then the molecules can diffuse into the brain tissue or will be cleared by the CSF flow into the lymphatic vessels and subsequently into the systemic circulation. [79-88]

Factors Affecting Nasal Drug Delivery to the Brain
The size of the molecule is the major determinant in whether a substance will be absorbed across the nasal respiratory epithelium and/or transported along the olfactory pathway. Fisher et al. demonstrated an almost linear relationship between the log (molecular weight) and the log (% drug absorbed) of water-soluble compounds. [84]

Other factors affecting delivery to the brain include the degree of dissociations andlipophilicity (higherlipophilicity results in better transport). Once a drug is in the brain, it can be further influenced by BBB efflux transporter systems like P-glycoprotein (P-gp). Graff and Pollack (2003), however, found that uptake into the brain was enhanced when drugs were administered in combination with the P-gp efflux inhibitor,rifampin. [81-83]

Nose-to-Brain Research
Researching nose-to-brain transfer of drugs in humans must, for obvious reasons, either employ indirect visualization of drug transfer (e.g., effects on event-related-potentials), measurement of drug concentrations in the CSF during surgery, or simple monitoring of CNS effects. Such studies have clearly indicated that drugs can be delivered to the brain in this manner, but they give no clear-cut evidence regarding the role of transfer. Because of this limitation, studies of the olfactory pathway as a conduit for transmission of drugs to the CNS have mostly made use of animals having substantially different ratios of olfactory-to-respiratory epithelium than humans.

[85]However, the mechanisms of transfer remain the same and are worthy of thorough investigation. To date, more than 50 drugs and drug-related compounds have been reported to reach the CNS after nasal administration in different species. [81]

A growing number of recent reports have demonstrated the effectiveness of intranasal administration ofneuroprotective agents in decreasing ischemic brain injury. For example, Ying et al. (2007) recently reported that intranasal administration of NAD+ profoundly decreased brain injury in a rat model of transient focal ischemia. Similarly, Wei et al. (2007] showed that intranasal administration of the PARG inhibitor Gallo tannin decreased ischemic brain injury in rats. Such agents are believed to provideneuroprotection by diminishing or abolishing activation of poly (ADP-ribose] polymerase-1 (PARP-1], which plays a significant role in ischemic brain damage. NAD+ was observed to reduce infarct formation by up to 86% even when administered at 2 hours after ischemic onset. Because PARP activation appears to be a downstream ischemic event, it may be worthwhile to also investigate the ability of IN (intranasal] administration of agents such asantiporters or NMDA receptor blockers to provideneuroprotection against the more upstream events of global ischemia such as membrane depolarization andexcitotoxicity. [84]

Interstitial Delivery

The most direct way of circumventing the BBB is to deliver drugs directly to the brain interstitium. By directing agents uniquely to an intracranial target, interstitial drug delivery can theoretically yield high CNS drug concentrations with minimal systemic exposure and toxicity. Furthermore, with this strategy, intracranial drug concentrations can be sustained, which is crucial in treatment with many chemotherapeutic agents [89].

Injections, Catheters, and Pumps

Several techniques have been developed for delivering drugs directly to the brain interstitium. One such methodology is the Ommaya reservoir or implantable pump as discussed earlier under intraventricular/intrathecal route. This technique, however, does achieve truly continuous drug delivery. More recently, several implantable pumps have been developed that possess several advantages over the Ommaya reservoir. This can be implanted subcutaneously and refilled by subcutaneous injection and are capable of delivering drugs as a constant infusion over an extended period of time [90]. Furthermore, the rate of drug delivery can be varied using external handheld computer control units. Currently each of the three different pumps available for interstitial CNS drug delivery operates by a distinct mechanism. The Infusaid pump uses the vapour pressure of compressed Freon to deliver a drug solution at a constant rate; the MiniMed PIMS system uses a solenoid pumping mechanism, and the Medtronic SynchroMed system delivers drugs via a peristaltic mechanism. The distribution of small and large drug molecules in the brain can be enhanced by maintaining a pressure gradient during interstitial drug infusion to generate bulk fluid convection through the brain interstitium or by increasing the diffusion gradient by maximizing the concentration of the infused agent as a supplement to simple diffusion. Another recent study shows that the epidural (EPI] delivery of morphine encapsulated in multivesicular liposomes (DepoFoam drug delivery system] produced a sustained clearance of morphine and a prolonged analgesia, and theresults suggest that this delivery system is without significant pathological effects at the dose of 10mg/ml morphine after repeated epidural delivery in dogs [91-94] .

Biodegradable polymer Wafers, Microspheres and Nanoparticles

Though interstitial drug delivery to the CNS has had only modest clinical impact, its therapeutic potential may soon be realized using new advances in polymer technologies to modify the aforementioned techniques. Polymeric or lipidbased devices that can deliver drug molecules at defined rates for specific periods of time are now making a tremendous impact in clinical medicine. Drug delivery directly to the brain interstitium using polyanhydride wafers can circumvent the BBB and release unprecedented levels of drug directly to an intracranial target in a sustained fashion for extended periods of time [95]. The fate of drug delivered to the brain interstitium from the biodegradable polymer wafer was predicted by a mathematical model based on (a] rates of drug transport via diffusion and fluid convection; (b] rates of elimination from the brain via degradation,metabolism and permeation through capillary networks; and (c] rates of local binding and internalization. Such models are used to predict the intracranial drug concentrations that result from BCNU-loaded pCPP:SA (1,3 bis-para-carboxyphenoxypropane: sebacic acid] wafers as well as other drug-polymer combinations, paving the way for the rational design of drugs specifically for intracranial polymeric delivery. Conjugation of a polymerically delivered chemotherapeutic agent to a water-soluble macromolecule increases drug penetration into the brain by increasing the period of drug retention in brain tissue. Hanes et al have recently developed IL-2-loaded biodegradable polymer microspheres for local cytokine delivery to improve the immunotherapeutic approach to brain tumor treatment. In theory, polymeric cytokine delivery has several advantages over delivery from transducted cells, including obviatingthe need for transfecting cytokine genes, producing longer periods of cytokine release *in-vivo* and yielding more reproducible cytokine release profiles and total cytokine dose [96-97]. Microparticles can also be easily implanted by stereotaxy

in discrete, precise and functional areas of the brain without damaging the surrounding tissue. Nanoparticles were found to be helpful for the treatment of the disseminated and very aggressivebrain tumors. Intravenously injected doxorubicin-loaded polysorbate 80-coated nanoparticles were able to lead to 40% cure in rats with intracranially transplanted glioblastomas. Another Study shows that PEGylated PHDCA (n-hexadecylcyanoacrylate] nanoparticles made by PEGyalated amphiphilic copolymer penetrate into the brain to a larger extent than all the other tested nanoparticle formulations, without inducing any modification of the BBB permeability. And the result defines two important requirements to take into account in the design of adequate brain delivery systems, long-circulating properties of the carrier and appropriate surface characteristics to permit interactions with endothelial cells. Valproic acid-loaded nanoparticles showed reduced toxic side effects of valporate therapy, not by reducing the therapeutically necessary dosage but by inhibition of formation of toxic metabolites [99-101]. In conclusion, the capacity of the biodegradable polymer delivery methodology to deliver drugs directly to the brain interstitium is vast.

Recent advances in nanotechnology

The research team of University of Michigan has developed a tool to diagnose and treat the most virulent forms of brain cancer. That is 20 to 200 nanometer diameternanoparticles; they dubbed Probes Encapsulated by Biologically Localized Embedding (pebbles]. They designed the pebbles to carry a variety of agents on their surface, each with a unique function. The major potential advantage of using these nanoparticles to treat cancer is of multifunctional. One target molecule immobilized on the surface could be used to help visualize the target using magnetic resonance imaging (MRI], while a third agent attached to the PEBBLE could deliver a destructive dose of drug or toxin to nearby cancer cells. All three functions can be combined in a single tiny polymerspere to make a potent weapon againstcancer.Kopelman introduced the common MRI contrast element gadolinium to the pebbles. When injected into thethe bloodstream, thenanoparticles travel their way through the bloodstream. And because they can transverse they have a targeting agent attached, the pebbles accumulate in the brain tumor enabling a clear MRI image within just a few hours. [102]

Researchers incorporated a drug called Photofrin along with iron oxide intonanoparticles that would target cancerous brain tumors.Photofrin is a type of photodynamic therapy (PDT], in which the drug is drawn through the blood stream to tumors cells; a special type of laser light activates the drug to attack the tumor. Iron oxide is a contrast agent used to enhance magnetic resonance imaging (MRI]

Chimeric peptide technology

Chimeric peptides are formed when a drug that is normally not transported through the BBB is conjugated to a brain drug-targeting vector. [103]The latter is an endogenous peptide, modified protein, orpeptidomimetic monoclonal antibody (mab] that undergoes RMT(Rapid metabolic transfer] through the BBB on endogenous receptor systems such as the insulin receptor or the tfr.Peptidomimeticmabs bind toexofacialepitopes on the BBB receptor that are removed from the endogenousligand binding site and piggyback across the BBB on the endogenous RMT system within the BBB, In this , a drug ismonobiotinylated in parallel with the production of a vector/ avid in or a vector/streptavidin (SA] fusion protein. [103]Thebiotinylated drug is produced in one vial and the vector/avid in fusion protein is produced in another vial, and the 2 vials are mixed before administration.

Owing to the extremely high affinity of avid in or SA binding of biotin, there is instantaneous capture of thebiotinylatedneurotherapeutic agent by the vector/avid in or vector/SA fusion protein. [104]Monoclonal antibody/avid in andmab/SA fusion genes and fusion proteins are produced with genetic engineering. Brain drug delivery in rats is possible with the OX26 mousemab to the rat tfr. Brain drug delivery in humans is possible with the genetically engineeredchimeric HIRmab. The activity of the genetically engineeredchimeric HIRmab is identical to that of the originalmurine HIRmab and thechimeric antibody is avidly taken up by the primate brain. The brain uptake of the HIRmab in the rhesus monkey is 2% to 4% of the injected dose which is a level of brain uptake that is 1 to 2 log orders greater than the brain uptake of aneuroactive small molecule such as morphine. [103-105]

Neuroprotection with peptide radiopharmaceuticals

The practice of brain imaging uses small-molecule radio chemicals that bind to monoamine or amino acid neurotransmitter systems. Whereas there are less than a dozenmonoaminergic or aminoacidergic neurotransmitter systems, there are hundreds ofpeptidergic neurotransmission systems [108].]Therefore, the use of peptide radiopharmaceuticals could greatly increase the diagnostic potential ofneuroimaging technology. [107] Potential candidates forneuroimaging include epidermal growth factor (EGF] peptide radiopharmaceuticals for the early detection of brain tumors and A_ peptide radiopharmaceuticals as a diagnostic brain scan for Alzheimer disease. Many malignantgliomas over express the EGF receptor (EGF-R] and EGF are a potential peptide radiopharmaceutical for the imaging of brain tumors. [106 -109]

Protein Neurotherapeutic agent andneuroprotection in stroke

Virtually all small-moleculeneuroprotective agents have failed in clinical stroke trials because either (*a*] these molecules have unfavorable safety profiles or (*b*] the drugs do not cross the BBB. The therapeutic window forneuroprotection is the first 3 hours after stroke, and during this time, the BBB is intact. [112]The BBB is disrupted in later stages following stroke, but at this time, chances forneuroprotection have been lost. Therefore, if effective neuroprotective agents for stroke are to be developed, these molecules must have favorable safety profiles and must be able to cross the BBB. [113]A model neurotrophin, brain-derived neurotrophic factor (BDNF], was reformulated to enable BBB transport, and the BDNF chimeric peptide isneuroprotective following delayed intravenous administration in either regional or global brain ischemia. [110-119].

Drug Delivery from Biological Tissues

Another strategy to achieve interstitial drug delivery involves releasing drugs from biological tissues. The simples approach to this technique is to implant into the brain a tissue that naturally secretes a desired therapeutic agent. This approach has been most extensively applied to the treatment of Parkinson's disease. Transplanted tissue often did not survive owing to a lack of neovascular innervation. Recently the enhanced vascularization and microvascular permeability in cell-suspension embryonic neural grafts relative to solid grafts has been demonstrated [92-93]. An alternative extension of this method is to use gene therapy to develop optimized biological tissue for interstitial drug delivery. Prior to implantation, cells can be genetically modified to synthesize and release specific therapeutic agents. The therapeutic potential of this technique in the treatment of brain tumor was demonstrated. The use of nonneuronal cells for therapeutic protein delivery to the CNS has recently been reviewed. The survival of foreign tissue grafts may be improved by advancements in techniques for culturing distinct cell types. Co-grafted cells engineered to release neurotropic factors with cells engineered to release therapeutic proteins may enhance the survival and development of foreign tissue. Ideally it would be possible to perform *in-vivo* genetic engineering to cause specific endogenous brain tissue to express a desired protein, circumventing the ischemic and immunogenic complications encountered with the implantation of foreign tissue grafts. One such technique that has been successfully used for the treatment of CNS malignancies involves *in-vivo* tumor transduction with the herpes simplex thymidine kinase (HS-tk] gene followed by treatment with anti-herpes drug ganciclovir was achieved by intra-tumoral injection of retroviral vector-producing cells containing the HS-tk gene, rendering the transfected tumor cells susceptible to treatment with ganciclovir. Other vector systems used in CNS gene transfer studies include retroviruses, adenoviruses, adeno-associated viruses, encapluation of plasmid DNA into cationic liposomes and neutral and oligodendrial stem cells [94-96].

Although this approach holds remarkable therapeutic potential in the treatment of CNS diseases, its efficacy has thus far been hindered by a number of obstacles: restricted delivery of vector systems across the BBB, inefficient transfection of host cells, and nonselective expression of the transgene and deleterious regulation of the transgene by the host [97].

CONCLUSION

The treatment of CNS diseases is particularly challenging because the delivery of drug molecules to the brain is often precluded by a variety of physiological, metabolic and biochemical obstacles that collectively comprise the BBB, BCB and BTB. The present outlook for patients suffering from many types of CNS diseases remains poor, but recent developments in drug delivery techniques provide reasonable hope that the formidable barriers shielding the CNS may ultimately be overcome. Drug delivery directly to the brain interstitium has recently been markedly enhanced through the rational design of polymer-based drug delivery systems. Substantial progress will only come about, however, if continued vigorous research efforts to develop more therapeutic and less toxic drug molecules are paralleled by the aggressive pursuit of more effective mechanisms for delivering those drugs to their CNS targets.

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