Drug delivery to the central nervous system: a review.

Ambikanandan Misra, Ganesh S., Aliasgar Shahiwala
Pharmacy Department, M.S. University of Baroda

Shrenik P. Shah
Sun Pharma Advanced Research Center, Baroda

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Abstract The brain is a delicate organ, and evolution built very efficient ways to protect it. Unfortunately, the same mechanisms that protect it against intrusive chemicals can also frustrate therapeutic interventions. 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. Treating CNS diseases is particularly challenging because a variety of formidable obstacles often impede drug delivery to the brain and spinal cord. By localizing drugs at their desired site of action one can reduce toxicity and increase treatment efficiency. In response to the insufficiency in conventional delivery mechanisms, aggressive research efforts have recently focused on the development of new strategies to more effectively deliver drug molecules to the CNS. This review intends to detail the recent advances in the field of brain-targeting, rational drug design approach and drug delivery to CNS. To illustrate the complexity of the problems that have to be overcome for successful brain targeting, a brief intercellular characterization of the blood–brain barrier (BBB) is also included.

INTRODUCTION

Despite enormous advances in brain research, brain and central nervous system disorders remain the world's leading cause of disability, and account for more hospitalizations and prolonged care than almost all other diseases combined. 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.

Despite successful examples of drug delivery to the CNS, but only some have reached the phase where they can provide safe and effective human applications. 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 will prove invaluable to researchers interested in the fundamental function of the BBB and those in the pharmaceutical industry interested in rational drug design directed at delivering drugs to the brain.

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.

Blood-Brain Barrier

It is now well established that the BBB is a unique membranous barrier that tightly segregates the brain from the

Corresponding Author: Ambikanandan Misra, Pharmacy Department, Faculty of Technology & Engineering, M.S.University of Baroda, Kalabhavan, Vadodara – 390001. Gujarat. misraan@satyam.net.in, misraan@hotmail.com

circulating blood (2, 3). The CNS consist blood capillaries which are structurally different from the blood capillaries in other tissues; these structural differences result in a permeability barrier between the blood within brain capillaries and the extracellular fluid in brain tissue. Capillaries of the vertebrate brain and spinal cord lack the small pores that allow rapid movement of solutes from circulation into other organs; these capillaries are lined with a layer of special endothelial cells that lack fenestrations and are sealed with tight junctions. Tight epithelium, similar in nature to this barrier, is also found in other organs (skin, bladder, colon, and lung) (4).This permeability barrier, comprising, the brain capillary endothelium, is known as the BBB. Ependymal cells lining the cerebral ventricles and glial cells are of three types. Astrocytes form the structural frame work for the neurons and control their biochemical environment. Astrocytes foot processes or limbs that spread out and abutting one other, encapsulate the capillaries are closely associated with the blood vessels to form the BBB. Oligodendrocytes are responsible for the formation and maintenance of the myelin sheath, which surrounds axons and is essential for the fast transmission of action potentials by salutatory conduction. Microglias are blood derived mononuclear macrophages. The tight junctions between endothelial cells results in a very high trans-endothelial electrical resistance of 1500-2000 Ωcm^2 compared to 3-33 Ωcm^2 of other tissues which reduces the aqueous based para-cellular diffusion that is observed in other organs (5, 6).

Micro-vessels make up an estimated 95% of the total surface area of the BBB, and represent the principal route by which chemicals enter the brain. Vessels in brain were found to have somewhat smaller diameter and thinner wall than vessels in other organs. Also, the mitochondrial density in brain micro-vessels was found to be higher than in other capillaries not because of more numerous or larger mitochondria, but because of the small dimensions of the brain micro-vessels and consequently, smaller cytoplasmic area. In brain capillaries, intercellular cleft, pinocytosis, and fenestrae are virtually nonexistent; exchange must pass trans-cellularly. Therefore, only lipid-soluble solutes that can freely diffuse through the capillary endothelial membrane may passively cross the BBB. In capillaries of other parts of the body, such exchange is overshadowed by other nonspecific exchanges. Despite the estimated total length of 650km and total surface area of 12 m2 of capillaries in human brain, this barrier is very efficient and makes the brain practically inaccessible for lipid- insoluble compounds such as polar molecules and small ions. As a consequence, the therapeutic value of many promising drugs is diminished, and cerebral diseases have proved to be most refractory to therapeutic interventions. Given the prevalence of brain diseases alone, this is a considerable problem. Practically all drugs currently used for disorders of the brain are lipid-soluble and can readily cross the BBB following oral administration. Although antimicrobial b-lactam antibiotics, when administered intracerebroventricularly, cause severe convulsion, fortunately these antibiotics, when administered intravenously or orally, do not cause such central nervous system (CNS) side effect because their limited transport across the blood–brain barrier (BBB). Further, in spite of being well distributed into various tissues, a lipophilic new quinolone antimicrobial agent, grepafloxacin, cannot enter the brain, resulting in the avoidance of CNS side effects such as headache and dizziness due to the displacement of g-aminobutyric acid (GABA) from the GABA receptor binding sites. On the other hand, benzodiazepines such as diazepam have been used as sedative-hypnotic agents, because these lipophilic drugs readily cross the BBB. However, the BBB transport of an immunosuppressive agent, cyclosporin A, which is more lipophilic than diazepam, is highly restricted. Similarly, almost all of the lipophilic anticancer agents such as doxorubicin, epipodophylotoxin and Vinca alkaloids (e.g., vincristine and vinblastine) hardly enter the brain, causing difficulty in the treatment of brain tumors. Although levodopa, which is useful for treatment of Parkinson's disease, is very hydrophilic, it can readily penetrate the BBB. What mechanisms underlie these diverse BBB transport characteristics of drugs which are apparently structurally and pharmacologically unrelated? In order to avoid overlap with this section, the drug transport across the BBB of small-molecular drugs by carrier-mediated transport and of peptide drugs by the adsorptive-mediated transcytosis are discussed in section 7.1.4 and 7.1.5 respectively.

Some regions of the CNS do not express the classical BBB capillary endothelial cells, but have micro-vessels similar to those of the periphery. These areas are adjacent to the ventricles of the brain and are termed the circumventricular organs (CVOs). The CVOs include the choroid plexus, the median eminence, neurohypophysis, pineal gland, organum vasculosum of the lamina terminalis, subfornical organ, subcommisaral organ and the area postrema. Though in the CVO brain regions the capillaries are more permeable to solutes, the epithelial cells of the choroid plexus and the tanycytes of other regions form tight junc-

tions to prevent transport from the abluminal extracellular fluid (ECF) to the brain ECF. The choroid plexus may be of importance when considering the transport of peptide drugs, because it is the major site of cerebrospinal-fluid (CSF) production, and both the CSF and brain ECF freely exchange (7).

The BBB also has an additional enzymatic aspect. Solutes crossing the cell membrane are subsequently exposed to degrading enzymes present in large numbers inside the endothelial cells that contain large densities of mitochondria, metabolically highly active organelles. BBB enzymes also recognize and rapidly degrade most peptides, including naturally occurring neuropeptides (8, 9).

Finally, the BBB is further reinforced by a high concentration of P-glycoprotein (Pgp), active –drug-efflux-transporter protein in the luminal membranes of the cerebral capillary endothelium. This efflux transporter actively removes a broad range of drug molecules from the endothelial cell cytoplasm before they cross into the brain parenchyma. Figure-1 gives a schematic representation of all these BBB properties using a comparison between brain and general capillaries.

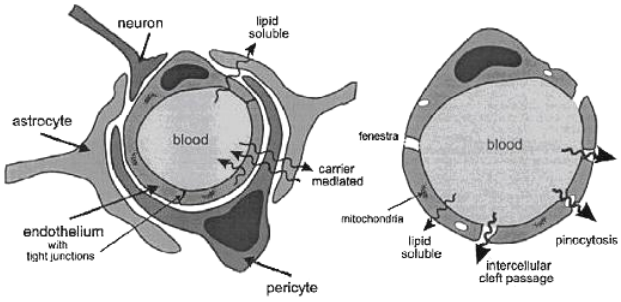

Figure 1: Schematic comparison between general (left) and brain (right) capillaries.

Blood-Cerebrospinal Fluid Barrier

The second barrier that a systemically administered drug encounters before entering the CNS is known as the blood-cerebrospinal fluid barrier (BCB). Since the CSF can exchange molecules with the interstitial fluid of the brain parenchyma, the passage of blood-borne molecules into the CSF is also carefully regulated by the BCB. Physiologically, the BCB is found in the epithelium of the choroids plexus, which are arranged in a manner that limits the passage of molecules and cells into the CSF. The choroid plexus and the arachnoid membrane act together at the barriers between the blood and CSF. On the external surface of the brain the ependymal cells fold over onto themselves to form a double layered structure, which lies between the dura and pia, this is called the arachnoid membrane. Within the double layer is the subarachnoid space, which participates in CSF drainage. Passage of substances from the blood through the arachnoid membrane is prevented by tight junctions (10). The arachnoid membrane is generally impermeable to hydrophilic substances, and its role is forming the Blood-CSF barrier is largely passive. The choroid plexus forms the CSF and actively regulates the concentration of molecules in the CSF. The choroid plexus consist of highly vascularized, "cauliflower-like" masses of pia mater tissue that dip into pockets formed by ependymal cells. The preponderance of choroid plexus is distributed throughout the fourth ventricle near the base of the brain and in the lateral ventricles inside the right and left cerebral hemispheres. The cells of the choroidal epithelium are modified and have epithelial characteristics. These ependymal cells have microvilli on the CSF side, basolateral interdigitations, and abundant mitochondria. The ependymal cells, which line the ventricles, form a continuous sheet around the choroid plexus. While the capillaries of the choroid plexus are fenestrated, non-continuous and have gaps between the capillary endothelial cells allowing the free-movement of small molecules, the adjacent choroidal epithelial cells form tight junctions preventing most macromolecules from effectively passing into the CSF from the blood (11). However, these epithelial-like cells have shown a low resistance as compared the cerebral endothelial cells, approximately 200 Ωcm^2, between blood and CSF (12).

In addition, the BCB is fortified by an active organic acid transporter system in the choroids plexus capable of driving CSF-borne organic acids into the blood. As a result a variety of therapeutic organic acids such as the antibiotic penicillin, the anti-neoplastic agent methotrexate, and the antiviral agent zidovudine are actively removed from the CSF and therefore inhibited from diffusing into the brain parenchyma. Furthermore, substantial inconsistencies often exist between the composition of the CSF and interstitial fluid of the brain parenchyma, suggesting the presence of what is sometimes called the CSF-brain barrier (13). This barrier is attributed to the insurmountable diffusion distances required for equilibration between the CSF and the brain interstitial fluid. Therefore, entry into the CSF does not guarantee a drug's penetration into the brain.

Blood-Tumor Barrier

Intracranial 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. For example, even when primary and secondary systemic tumors respond to chemotherapeutic agents delivered via the cardiovascular system, intracranial metastases often continue to grow. In CNS malignancies where the BBB is significantly compromised, a variety of physiological barriers common to all solid tumors inhibit drug delivery via the cardiovascular system. 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 (14). Brain tumors may also disrupt BBB, but these are also local and nonhomogeneous disruptions (15).

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.

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 (16, 17). The MRP in humans appears to be five isoforms, and there are different levels of expression of these various isoforms in different tissues. 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. Noticeably, brain exposure can be increased not only by enhancing influx, but by restricting efflux through the BBB as well. 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 noncompetitive 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 (18, 19). Coadministration 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 (20). On the contrary, among the brain drug delivery strategies to be discussed later, chemical drug delivery systems (CDDS) are the only ones attempting to not only increase influx, but also to decrease efflux. This strategy is done by exploiting a sequential metabolic approach that first increases influx by passive diffusion through increased lipophilicity and then decreases efflux by a 'lock-in' mechanism.

Physicochemical Factors that influence Brain Uptake

Brain penetration, brain uptake, and ability to cross the BBB need to be defined exactly to understand concepts

involved in brain uptake. Hence, the various ways in which transfer across the BBB are defined in table-1.

Table 1: Measures of "Brain Uptake".

Biological activity
Maximal brain concentration
The brain uptake index from single-pass experiments
PS-product and permeability coefficient from:
Indicator dilution during single pass
Intravenous infusion or bolus injection
Vascular perfusion of brain in situ
Blood-brain distribution

Biological activity is a general measure of brain uptake. The hypnotic activity of a number of congeneric series of CNS depressants reached a maximum when log octanol–water partition coefficient (log $P_{o/w}$) was near to 2. Various researchers confirmed this finding and the "rule of 2" became generally accepted (21). But the difficulty here is that the biological activity will depend on at least two factors:

- rate of transfer from blood to brain, or distribution between blood and brain; and
- interaction between drug and some receptors in the brain.

If these two factors cannot be distinguished, then it is impossible to use biological activity as a measure of either rate or equilibrium transfer.

The log $P_{o/w}$ probably still represents the most informative physicochemical parameter used in medicinal chemistry and countless examples where it proved as useful descriptors are available in the literature (22). On the other hand, increasing lipophilicity with the intent to improve membrane permeability might not only make chemical handling difficult, but also increase the volume of distribution in particular plasma protein binding and tends to affect all other pharmacokinetic parameters (23, 24). Furthermore, increasing lipophilicity tends to increase the rate of oxidative metabolism by cytochromes P450 and other enzymes (23, 25). Hence, to improve bioavailability, the effects of lipophilicity on membrane permeability and first pass metabolism have to be balanced.

The brain uptake index (26) is a more rigorous measure of brain uptake in which there is a relative measure of brain uptake by intra-carotid injection of a mixture of ^{14}C-labeled compound and ^{3}H-labeled water (i.e. a saline solution in ^{3}H-labeled water). The radioactivity in brain tissue is recorded 15 seconds after administration, and a brain uptake index (BUI) is defined in equation-1:

$$BUI = 100 \times \frac{(^{14}C / ^{3}H)\text{tissue}}{(^{14}C / ^{3}H)\text{saline}} \qquad \text{(equation-1)}$$

where the BUI for water is 100. Although, the BUI is useful as a rank order index of brain uptake, is not easily amenable to analysis by physicochemical methods.

A more well-defined measure of rapid brain uptake is the permeability, expressed either as a permeability-surface area product (PS) or as a permeability coefficient (PC), obtained by intravenous injection and measurement of the drug profile in arterial blood. Both the PS product and PC are quantitative measures of the rate of transport obtained by in-situ vascular perfusion technique (27) and so are amenable to analysis through standard physicochemical procedures. An advantage of the perfusion technique as a measure of brain uptake is that the time scale for determination of PS products is very short, so that back transport and biological degradation are minimized. Although there are numerous physicochemical studies on brain perfusion, it is not possible to reach any general conclusions.

Following systemic drug administration, uptake from the circulation into parenchyma by a specific organ of interest will be determined by the following factors: (a) blood flow to the organ, (b) permeability of the micro-vascular wall, and (c) the amount of drug available for uptake, which is inversely related to systemic clearance and is represented by the area under the plasma concentration-time curve (AUC). For the quantification of brain tissue accumulation (C_{brain}) at time T during the phase of unidirectional uptake, the following equation-2 holds:

$$C_{brain}(T) = PS \times AUC_{0 \to T} \qquad \text{(equation-2)}$$

where PS is the brain capillary permeability surface area product, an expression equivalent to the organ clearance and AUC is the area under the plasma concentration time curve. It should be mentioned that this equation does not take into account efflux of either intact drug or metabolism and efflux of degradation products from the brain. Measurement of efflux is covered in section 6 of this review.

Based on the relationship between the octanol / water partition coefficient (PC) divided by the square root of the molecular weight ($PC/ Mw^{1/2}$) and the BBB permeability coefficient (PS), one can classify at least three different groups: (a) substrates exhibiting a good correlation, (b)

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