Chapter

Principles of Neuropharmacodynamics: As Applied to Neuro-Oncology

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Abstract

The blood-brain barrier (BBB) is a highly selective semi-permeable membrane that separates the cerebral blood circulation from the brain and extracellular fluid in the central nervous system (CNS). The BBB is composed of endothelial cells, astrocyte end-feet and pericytes embedded in the capillary basement membrane. This system allows the passage of water, some gases and lipid-soluble molecules by passive diffusion, as well as, selective molecules such as glucose and amino acids. This review discusses pharmacodynamic concepts and methods that allow drugs to penetrate the BBB structure and enter the CNS and spinal nervous systems (SNS).

Keywords: blood-brain barrier (BBB)

1. Introduction

The blood-brain barrier (BBB) was discovered over 100 years ago by Paul Ehrlich during his studies on the brain [1]. Ehrlich's early observations that water soluble dyes stained all organs of animals except for their brains and components of the central nervous system (CNS) was the key to our present day understanding of the BBB system. Subsequently, other researchers observed that dyes injected into the blood stream did not enter the brain hence a barrier existed between the two compartments [1].

The BBB differs from normal membranes in that it possesses tight junctions between an endothelial cell/astrocyte wall with no pores to allow for transport unless materials are lipophilic, water, and/or an actively transported. The BBB is also lipophilic, free of aqueous electrolytes and highly electrical resistant. However, the BBB compartment can be traversed by lipophilic substances through passive diffusion, while other molecules that are substrates for transferases cross by direct transport [1, 2].

The BBB prevents most systemic therapies from penetrating the brain; however, when cancer cells do penetrate the BBB from a peripheral origin, they generate neo-vascularization or cancer associated "vascular mimicry" structures (new blood vessels associated with tumor-generated penetrate breaks in the BBB) which can connect intracranial metastatic cancer cell colonies with the cerebral blood circulation [3]. If undesirable or toxic materials pass through the BBB into the CNS, a protective mechanism—the P-glycoprotein (P-gP) transfer system will transport toxic materials out of the CNS [4].

Thus, developing new drugs that can exploit the cancer associated CNS "neo-angiogenic" vascularization that are not substrates for the P-gP system is of major interest and would be very useful in managing CNS and SNS malignancies.

Figure 1 shows a simplified diagram describing the BBB in relation to the brain (CNS) or spinal nervous system (SNS), the cerebral circulation and tumors growing in the CNS or SNS (**Figure 2**).

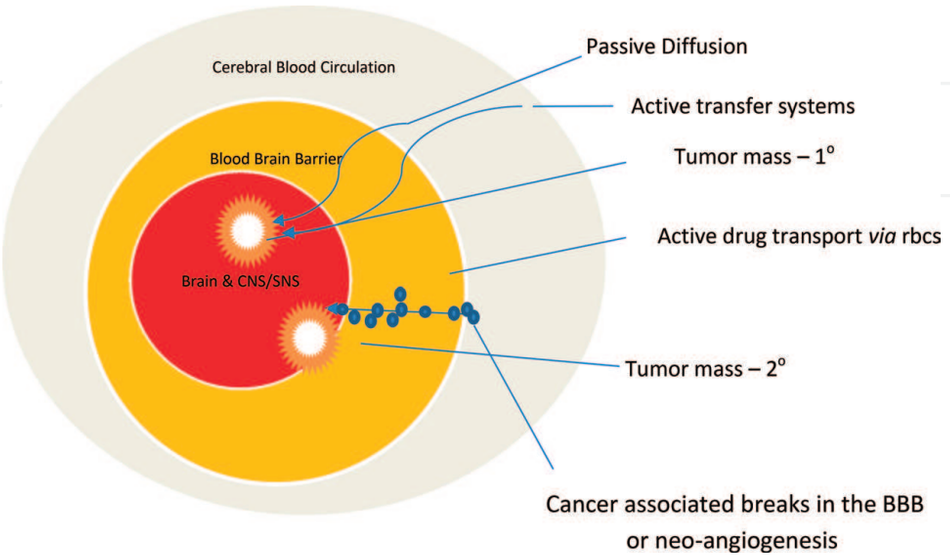


Figure 1.
Depicts three modes of drug transport to primary and secondary brain cancer tumors—direct passive permeability, active transport/transfer, and transport in association with RBCs (modified from Ref. [5]).

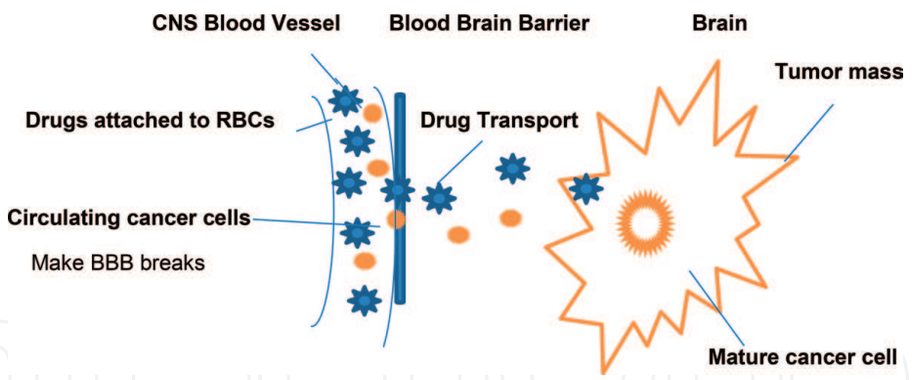


Figure 2.
Describes breaks in the BBB and neoangiogenesis that can be initiated by both metastatic and primary cancers in the CNS, allowing RBCs with drugs to cross the BBB and penetrate the CNS and tumor masses (modified from Ref. [5]).

2. Extravascular transport of cancer cells into the CNS

Not all cancer cells infiltrate the CNS or SNS by breaching the BBB. A recent report by Yao et al. describes how acute lymphocytic leukemia (ALL) cells possess a6 integrin receptors that bind to laminin, a glycoprotein molecule that covers the surfaces of the meninges, its nerve sheaths and blood vessels [6]. Tiny blood vessels pass directly from the bone marrow through the vertebrae to the meninges tissue that line the spinal cord, brain and the cerebral spinal fluid (CSF) circulation. ALL cells can attach to the outside of blood vessels and nerves in the bone marrow and migrate over the scaffolding proteins. Thus, ALL cells can slide into the CNS and

SNS via the scaffolding of the cerebral vascular circulation [6]. Other types of cancer may be able to do the same.

The next step is to design new agents to block the a6 integrin receptor [6].

3. Principles for selecting a drug for brain and spinal nervous systems—primary and secondary

Most therapy regimens for CNS and SNS cancers involve empirical protocols [7]. However, with the advent of tumor targeted and gene mutation designed immunotherapies, there are more selective therapeutic approaches to the management of cancers [8].

However, if a tumor lacks a specific tumor target antigen, genetic mutation or receptor glycoprotein, then a more individualized (personalized) approach is possible. Through in vitro sensitivity studies, cytotoxicity parameters (IC_{50}) vs. selected drugs can be identified for each individual tumor [9]. Obtaining tumor tissue for tumor molecular profiling can now be easily accomplished using liquid biopsy techniques and stem cell cultures [9, 13].

In addition, since drugs penetrate the tumors in the CNS and SNS by lipophilic and/or selective transport mechanism(s), the partition coefficient, *P* value, is also helpful in appreciating whether a drug has a chance of penetrating the lipid rich BBB membrane or if a more selective transportation system is required.

The log *P* value is an accurate and important molecular characteristic that defines lipophilicity and the ability of a drug to diffuse across the lipophilic BBB. This is can be easily measured by dissolving the drug in n-octanol and shaking with equal volumes of water. The concentration of drug is then measured in both phases and the ratio of concentration in n-octanol/water evaluated according to **Eq. 1** [10].

$$log\ P_{oct/wat} = log\left(\frac{[solute]_{octanol}}{[solute]_{water}^{un-ionized}}\right) \quad (1)$$

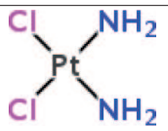
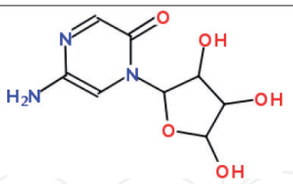
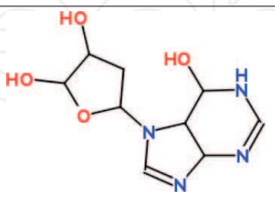
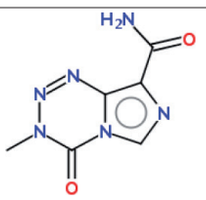
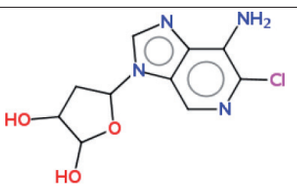
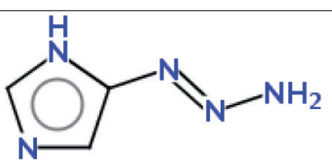
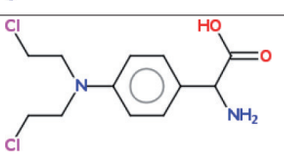
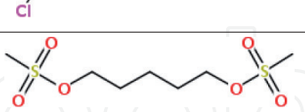
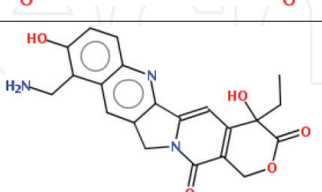
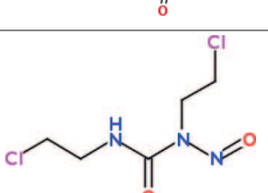
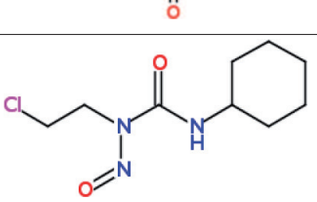
The estimation of drug penetrating through the BBB (log BB) is the concentration of drug in the brain divided by concentration in the blood [11].

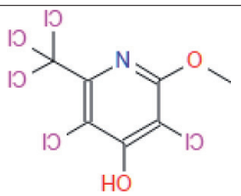
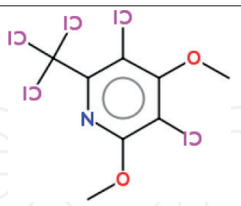
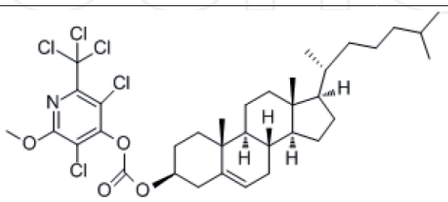
Very lipophilic compounds also tend to be highly protein bound. For a drug to diffuse from the plasma (at pH 7.4) across the BBB (log BB) into the CSF, the ideal octanol-water partition coefficient is usually 1–10 and corresponds to a log *P* of 0–1 [12]. Others recommend higher values—log $P \leq 5$ [11].

In addition, when selecting a drug, the maximum concentration of drug in the plasma initially (C_{max}) and the total drug concentration available after a single treatment—area under the curve (AUC), will be of assistance to predict if sufficient concentration of the drug is present [13].

Thus, the combination of log *P*, AUC and an $IC_{10}/_{50}$ values will be of assistance with the selection for a potentially active/useful drug for a specific individual with cancer (**Table 1**).

The above introductory information provides the general principles which govern the entry of anti-cancer cells—passively or actively, into the CNS and SNS that must be considered. Plus, after entering the brain, chemicals and drugs must not be substrates for P-glycoprotein (P-gP) transfer systems; or at least not before they can penetrate cancer cells and perform their anti-cancer effects.

Compound	Structure	Calculated log *P*	Calculated BB[11]	Actual BB[12]
Cis-platinum		−2.83	0.09	0.05–1
Cytarabine		−2.77	0.1	1
Pentostatin		−2.35	0.13	0.1–0.13
Temozolomide		−1.9	0.18	0.19
Cladribine		−0.38	0.64	0.25
Dacarbazine		−0.35	0.69	0.14
Melphalan		−0.01	0.86	0.01–0.1
Busulfan		0.08	0.9	1
Topotecan		1.41	≥1	0.42
Carmustine		1.67	≥1	0.15–0.9
Lomustine		2.96	≥1	>0.5

Compound	Structure	Calculated log *P*	Calculated BB[11]	Actual BB[12]
DM-PEN		4.32	≥1	TBD
Penclomedine (PEN)		4.59	≥1	TBD
DMCHOCPEN		9.68	≥1	TBD

Note: Not shown in **Table 1** *is etoposide which has a low BB value of 0.05 and it is pumped out of the brain by the P-gP pump [14].*

Table 1.
Calculated and structure related activities for drugs with CNS activity [5].

4. Emerging technologies for crossing the brain blood barrier designing new agents

The medicinal chemists and molecular pathologists are constantly designing new agents for neuro-oncology. The goals are always the same—continue to develop new integrative drug designs vs. compliment specific receptors in the BBB or on vessels through which cancer cells penetrate the BBB.

5. Exosomes to deliver treatments across the blood-brain barrier

Matthew Wood et al. claim that exosomes can cross the blood-brain barrier and deliver siRNAs, antisense oligonucleotides, chemotherapeutic agents and proteins specifically to cells—normal and malignant in the brain [15]. Exosomes are cell-derived matrix-bound encapsulated vesicles that contain drugs [15]. They are naturally or synthetically generated, able to cross the blood-brain barrier and deliver poorly solubilized drugs into the CNS and directly to brain cancer, as well as other diseases. Again, they must be able to pass the BBB.

6. Nanoparticles

Nanoparticle drug delivery systems contain drugs bound to nanoparticles which are capable of traversing the blood-brain barrier [16]. Human serum albumin (HSA) is most widely used vehicle to design nanoparticles. The main benefits of HSA nanoparticles are that they are well tolerated with minimal side effects, as well as the albumin functional groups can be utilized for surface modification that allows for specific cell uptake. Nanoparticles have been shown to transverse the blood-brain

barrier carrying host drugs into the brain [16]. To enhance the effectiveness of nanoparticles to cross the blood-brain barrier, attempts have been made to coat the nanoparticles with polysorbate to make them more permeable [16]. Polysorbate 80 coated nanoparticles containing doxorubicin delivered up to 6 μg/g concentrations of the drug into the brain after intravenous injections of 5 mg/kg of the drug/nanoparticles [16]. No detectable drug was observed when given alone or with the uncoated nanoparticles. This technology continues to have promise in neuro-oncology.

7. Prodrugs

CNS prodrugs are derivatized forms of active drugs that are unable to cross the BBB. Designing active molecules that are derivatized with lipids, amino acids, esters, salts, etc., can improve the former molecules' ability to penetrate the BBB more efficiently [14]. In situ in the CNS the prodrugs are metabolized or degenerated after crossing the BBB, releasing the active form of the drug [14].

There are still major drawbacks to the use of prodrugs to treat tumors in the CNS. First, the prodrug may be able to pass through the BBB; however, it may be transported out of the CNS by the P-gP transport system without ever releasing the active drug. Second, the sheer size of these derivatized molecules makes it very difficult to pass through the BBB.

Nevertheless, this is a very promising area for new research endeavors [14].

8. Peptide masking

Similar to the prodrug concept, another method to improve drug CNS bioavailability is through derivatizing drugs with peptides and amino acids that have select transfer pathways through the BBB and into the brain [17].

One example is through the use of cholesterol [13]. Although the brain synthesizes its own cholesterol for support and metabolism, cholesteryl derivatized drugs behave like lipids and penetrate the BBB secondary to be lipophilic [13]. This type of masking works well and aids in traversing the blood-brain barrier. Also, a "target molecule" could be attached to the drug that helps it pass through the barrier and then once inside the brain released. If the drug is not transported out of the brain, then it is available for therapeutic use [13].

However, drawbacks to the above exist as well. Once the drug is in the brain there is a point where it needs to be degraded to prevent toxic changes in the brain tissue. Also the drug may not be transported out of the brain and could become toxic with increased concentration. There must always be a mechanism for the removal of the active form of the drug from the brain [13].

9. Receptor-mediated permeators

Drugs that increase the permeability of the BBB are described as receptor-mediated permeators (RMP) [18]. By decreasing the restrictiveness of the BBB, it is much easier for a molecule to pass through the barrier. RMPs increase the permeability of the blood-brain barrier temporarily by increasing the osmotic pressure in the blood which loosen the junctions between the endothelial cells and pericytes. By loosening the junctions, drugs can pass through the BBB and be available as therapy vs. cancer cells. These drugs must be administered in a very controlled environment because of the risk associated with their use [18].

First, a major concern is that the brain can be flooded with the drugs that are in the blood that are usually blocked by the BBB. Secondly, when the tight junctions loosen, the homeostasis of the brain can also be compromised, which can result in seizures and other dysfunctional events in the brain.

10. Microbubble-enhanced focused ultrasound

Microbubbles are small "bubbles" of mono-lipids that are able to pass through the BBB. One obstacle to this, however is that these microbubbles are large, which often prevents their diffusion through the BBB and into the brain. This can be counteracted by focused ultrasound. Ultrasound increases the permeability of the BBB by causing interference in the tight junctions in the BBB. In combination with ultrasound therapy, a very specific area of diffusion will develop, because microbubbles can only diffuse where the ultrasound has disrupted the barrier [19, 20].

The hypothesis and usefulness of this combination is the possibility of loading a microbubble with an active drug to diffuse through the barrier and target a specific area in the brain or spine. There are several important factors that make this a viable solution for drug delivery. The first is that the loaded microbubble must not be substantially greater than the unloaded bubble. This ensures that the diffusion will be similar and the ultrasound disruption will be sufficient to induce diffusion. A second factor is that the stability of the loaded micro-bubble must be stable. This means that the drug is fully retained in the bubble and there is no leakage.

Lastly, it must be determined how the drug is to be released from the microbubble in the CNS once it passes through the BBB. Studies have documented the effectiveness of employing microbubble technology to get drugs to specific sites in the brain in animal models and humans [19, 20].

The author hopes that the concepts discussed herein will be useful to stimulate research ideas that ultimately may lead to new treatments and approaches to the management of CNS and SNS tumors—primary and secondary.

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