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The Blood-Brain Barrier and Innovative Technology for Increasing Absorption of Medicine into Brain Tissue

Three Blood-Brain Barriers

The main blood-brain barrier is a system of zonula occludens that form an impermeable barrier between capillaries that provide blood to the brain and the cerebrospinal fluid in central nervous system itself. This physical barrier is composed of zonula occludens (protein and dimer bonds between endothelial cell layers) between endothelial cells in the central nervous system. The function of this protective layer is to prevent many common bacteria and viruses from entering the brain and causing infection. However, as a result of this protection, many antibiotics and other drugs with physically large crystal structures are also prevented from reaching the brain, making drug delivery difficult. Optimal configurations for passing the blood brain barrier are molecules that weight less than 180 atomic mass units and are significantly liposoluble (that is, their oil/water partition coefficient of 1.6). [3] Other important factors include less than ten hydrogen bonds in water, degree of ionization and plasma protein binding. “Consequently, passive diffusion of drugs across the blood-brain barrier is limited to small, lipophilic compounds, such as benzodiazepines and barbiturates.” [1] The blood brain barrier is important for drug design for this reason, but also because analysis of drugs designed for non-neurological targets can prevent drugs from being toxic to the brain.

How does the system of tightly closed capillaries prevent drugs from leaving the bloodstream and entering the brain? There are three main barrier systems that protect the brain

tissue. There are technically three distinct blood-brain barriers- the first between the bloodstream and the brain interstitial fluid, and the two barriers between the blood and spinal fliud- the choroid plexus epithelial barrier between the blood and cerebrospinal fluid, and the arachnoid epithelium between the bloodstream and subarachnoid cerebrospinal fluid. [3] The endothelial systems in the choroid plexus and the capillaries in the brain are not simply static 'walls' that prevent large or unusual molecules from entering the central nervous system, though they do perform that function, rather these are "dynamic tissues that express multiple transporters and drug-metabolizing enzymes."[1] Neurons and glia also serve in neurovascular units that work in unison to maintain molecular and pumping rate equilibria at the barrier. Astrocytes, also known as astroglia, are glial cells that support the function of the blood brain barrier. These astroglia maintain homeostasis at the barrier and regulate amino acids, neurotransmitters and water transport across the barrier. [6]

The second barrier, between the blood and the cerebrospinal fluid, is located within the choroid plexus, in the ventricles of the brain. Physically, endothelial cells also seal off capillaries from the surrounding brain tissue, but the most remarkable defense the choroid plexus has is the production of spinal fluid. The choroid plexus produces about 500 mL of cerebrospinal fluid daily, far in excess of the average brain's 135 to 150 mL capacity. According to Eyal, Hsiao and Unadkat, this yields a 'total turnover rate' of .38% per minute. This rapid flushing of the cerebrospinal fluid back into the blood stream produces a 'net diffusion gradient', which decreases the ability of drugs to remain in significant quantities within the brain.

Another defense that medication has to contend with is the difference in blood flow to various parts of the brain. Blood flow can vary up to four times between the gray and white matter, causing large variability in concentration and effectiveness of drugs inside the brain

tissue. Additionally, there is variation in blood flow between areas of the gray and white over time and space, which can be further manipulated by anesthetics, other drugs and conditions within the body. “Thus, a drug that affects regional cerebral blood flow may alter the regional distribution of itself, another drug, or related metabolites, that exhibit “flow limited” kinetics, such as desmethyl-loperamide (Liow et al., 2009).” Another complication related to blood flow is the incomplete protection offered by the blood brain barrier. The endothelial barrier is more permeable in some brain structures and is not present in others. For example, the circumventricular organs, which regulate some sensory and fluid secretion functions, are not protected by the blood brain barrier at all and are exposed to drugs and bacteria in the ‘regular’ bloodstream.

Coupled with this initial physical barrier comes the metabolic barrier. This defense consists of enzymes designed to break down bacteria, foreign molecules and endogenous proteins. If a molecule makes it past these enzymes, there are still three types of ‘efflux pumps’ that bind to toxic fat-soluble molecules that have managed to get through the capillary wall. These pumps remove waste products and toxic molecules from the cerebrospinal fluid and return them to the general bloodstream, along with helpful drugs.

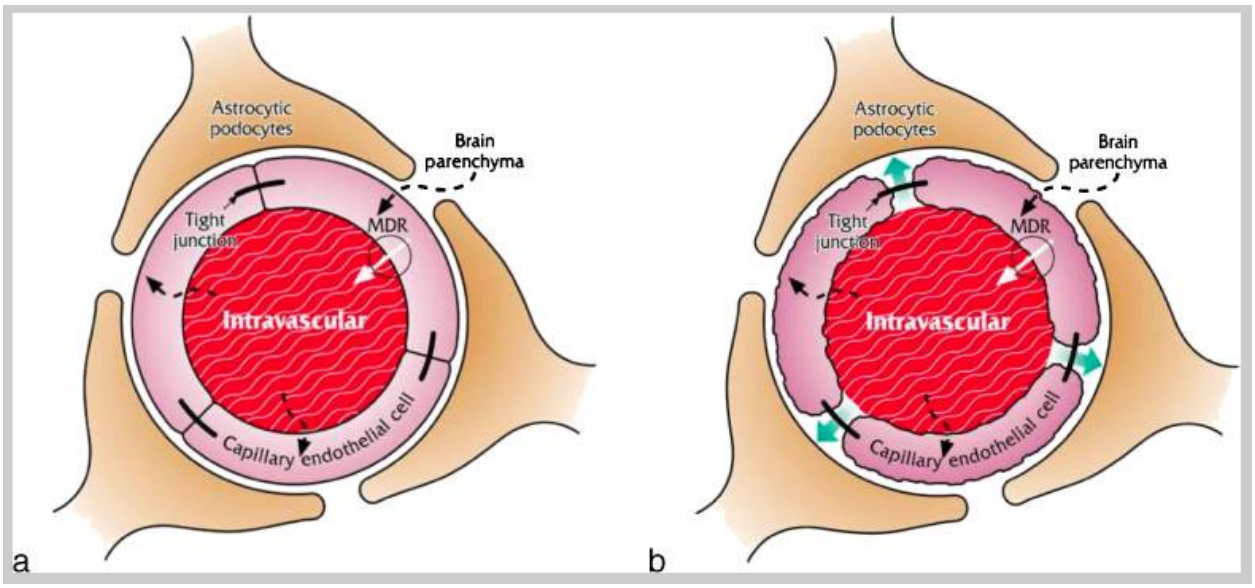
Besides these factors for removing undesirable compounds from the brain tissue, there are ports located on the luminal and abluminal membranes of endothelial and choroid plexus endothelial cells for amino acids and glucose to cross into the central nervous system, which present possibilities for modified drugs to cross the barrier. “For many drugs, the net transfer across these barriers is determined by interplay between several transport systems which can operate in the same direction or opposite directions.” [1] When designing brain medicines, multiple influx and efflux systems must be taken into account to determine absorption, as well as

variability in diffusion across the three different blood brain barriers. “Differences between the blood brain barrier and the blood cerebrospinal fluid barrier in expression and function of these transporters may contribute to the different pharmacokinetics of drugs in the interstitial fluid, compared to cerebrospinal fluid.” [1]

Pharmacokinetics and Drug-Drug Interactions

In addition to the three barriers, drug-drug and drug-protein interactions around transport sites also impact the ability of treatments to cross the blood brain barrier. There are a number of different pharmacokinetic drug interactions detailed in Eyal, Hsiao and Unadkat’s paper that can be used to a medicine’s advantage in increasing brain absorption.

One approach to increasing absorption is disrupting the ‘tight junctions’ in the physical barrier. Certain compounds will manipulate the junctions and open ports for medicine to pass through into the brain tissue by disrupting the barrier’s normal functions and by preventing the efflux transporters from working properly. One example of this is osmotic blood brain barrier disruption, first developed in 1972. Osmotic disruption injects hyperosmolar solutions into the carotid artery to pull water out of the brain via osmosis and thus open the zonula occludens that normally bind the blood brain barrier. “The authors proposed that hypertonic solutions increased BBB permeability by inducing the shrinkage of cerebrovascular endothelial cells, thus producing a disruption of inter-endothelial tight junctions.” [3] One study using methotrexate and osmotic barrier disruption increased absorption levels 10 to 100 times above the baseline. [1]



The image above demonstrates how the hypertonic solution causes the endothelial cells to shrink, opening pathways for drugs to reach the brain tissue.

Another passage for medicines to reach the brain is through p-glycoprotein inhibition. P-glycoprotein acts as a highly effective efflux pump in the blood brain barrier. The combined pumping and regulatory systems in the brain prevent 98% of small molecules and all large molecules (greater than 180 atomic mass unites) from reaching the brain tissue. [3] In studies of knockout mice without genes that coded for p-glycoprotein, there was an eight times increase in the brain uptake of the brain tumor drug paclitaxel.[1] However, there are varying rates of success when using p-glycoprotein inhibitors, possibly due to efflux and influx equilibria, or even the kinetics of how that particular drug affects other molecules in the barrier region.

Nanomedicine & Ultrasound Applications

[1] "One of the most extensively studied P-gp substrates is paclitaxel, a lipophilic anticancer drug that shows high potency against brain tumors in vitro, but is ineffective in vivo because it does not cross the BBB." (Miller et al., 2008).

Nanomedicine offers an interesting perspective on a solution to transporting molecules across the blood brain barrier. Paclitaxel's usefulness is greatly enhanced by nanomedicine. A research team coated the drug in polyactic co-glycolic acid nanoparticles that the barrier's receptors and ports can transport easily, and increased absorption of paclitaxel by 13 times compared to a dosage of paclitaxel without the nanoparticle coating.[2]

Silva's paper references the use of polybutylcyanoacrylate nanoparticles 'coated in polysorbate 80 [3-7]' to adsorb and transport many different types of drugs and molecules across the blood brain barrier. The polysorbate attracts and attaches apolipoproteins B and E, which is then transported via capillary endothelial cells to the brain tissue.

In addition to aiding in transport across the barrier, nanomedicine also offers breakthroughs in reducing side effects and build-up of drugs in the body, which can reduce dosage sizes as well. One study created a nanogel built of 'cross-linked PEG' and polyethylenimine that absorbed oligonucleotides with negative charges. "They demonstrated that intravenous injections resulted in a 15-fold accumulation of oligonucleotides in the brain after 1 hour, with a concurrent twofold decrease in accumulation in liver and spleen when compared with freely administered oligonucleotides (not encapsulated in nanogel particles)." [2][3]

There is a huge engineering advantage gained from using nanoparticles to enhance the absorption of medicine molecules. The first is that the nanoengineered parts of the combined molecule can be specially tailored to accomplish other tasks that would normally have to be included into the structure of the molecule itself. This saves on complexity and the need to

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[2] Nanoparticles of biodegradable polymers for clinical administration of Paclitaxel, by Feng SS, Mu L, Win KY, Huang G. (2004)

[3] Another very interesting use of nanoparticles unrelated to drug transport is in enhancing MRI. Solidified oil nanoparticles laced with iron oxide permeate the blood brain barrier very well, providing opportunities for more accurate magnetic imaging.

individually optimize each drug's molecules for compatibility while considering side affects, premature metabolization, blood-brain barrier crossing and cell targeting. Having the nanoparticles tackle these difficult tasks reduces drug complexity, and in some cases these particles can merely be attached to an existing drug while conferring the benefits of blood-brain barrier permeability and improved targeting.

A research group in Taiwan used focused ultrasound to interfere with the blood brain barrier in rats and permit the uptake of albumin and a blue dyeing agent. According to the article, "Sonications were applied at an ultrasound frequency of 1 MHz with a 5% duty cycle, and a repetition frequency of 1 Hz." [4] The group tested single treatments of ultrasound as well as repeated applications on the endothelial tissue of the rats with encouraging results. The more applications of ultrasound for longer durations of time led to the disruption of the blood-brain barrier for long durations of time, necessary for drugs to penetrate the tissue and accumulate. "At the same acoustic power, the extravasation caused by leakage of EB into the brain was found to be dependent on the applied sonication time." [4] The research group found that "there was a nearly twofold increase in EB extravasation in groups with a second sonication compared with the single sonication group." [4] However, ultrasound disruption works by damaging the barrier itself. While this tissue will recover, more research must be done into how permanent the damage is and if the brain tissue itself is harmed through this process.

Ultrasound offers the potential for drug absorption to be directly controlled by the location and duration of repeated sonic treatment. In addition, all of the parameters are controlled by the operating doctor or surgeon, which allows for incredibly precise disruption. "BBB disruption induced by FUS in the presence of an ultrasound contrast agent (UCA) is affected by

the ultrasound settings including the applied pressure amplitude, the frequency, the duty cycle, the number of cycles per pulse and the dose and bubble size of the UCA." [4]

It is fascinating to read about these new applications for advanced technology in treating some of the most recalcitrant and complicated brain diseases. Medicine's ability to treat patients effectively and safely will increase significantly as a result of the breakthroughs in these areas of opening the blood-brain barrier.

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