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Drug and Gene Delivery to the Brain: The Vascular Route

Minireview

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Brain drug development of either small molecule or large molecule (recombinant proteins, gene medicines) neurotherapeutics has been limited, owing to the restrictive transport properties of the brain microvasculature, which forms the blood-brain barrier (BBB) in vivo. Widespread drug delivery to the brain, while not feasible via craniotomy and intracerebral injection, is possible if the drug is delivered to brain via the transvascular route through the BBB. Novel brain drug delivery and drug targeting strategies can be developed from an understanding of the molecular and cellular biology of the brain microvascular and BBB transport processes.

Introduction

Brain drug delivery is the rate-limiting step in the translation of progress in the molecular neurosciences into clinically effective neurotherapeutics for patients with disorders of the central nervous system (CNS). Progress in brain drug delivery has lagged behind other areas in the molecular neurosciences, because of the difficulties posed by the blood-brain barrier (BBB). The brains of all vertebrates are perfused by a dense microvascular network, which is formed by the capillary endothelial cells within the brain (Bar, 1980).

The density of the microvasculature of the brain is illustrated with the India ink injection study in the adult rat brain shown in Figure 1A. The capillary network in the brain is so intricate that no neuron or glial cell is more than 20 μm from a neighboring capillary. Therefore, every neuron is virtually perfused by its own microvessel. Once a circulating neurotherapeutic crosses the brain microvascular wall, the drug or gene is immediately delivered to the "doorstep" of every neuron within the brain.

Brain Barrier Systems. In addition to the brain microvascular endothelial barrier, which forms the BBB, there are other barrier systems within the CNS, including the arachnoid epithelial membrane, which covers the surface of the brain, and the choroid plexus epithelium, which forms the blood-cerebrospinal fluid (CSF) barrier. In humans, there are approximately 400 miles of capillaries perfusing the brain, and the surface area of the brain microvascular endothelium is approximately 20 m^2 (Pardridge, 2001), which is 1000-fold greater than the surface area of either the blood-CSF barrier or the arachnoid membrane (Dohrmann, 1970). Therefore, the quantitatively important barrier system within the brain is the BBB at the capillary endothelium (Figure 1A). Despite the vast surface area of the human BBB, the thickness of the BBB is very thin, and the total intracellular volume of the brain capillary endothelium is only 5 ml in the entire human brain and 1 μl in the rat brain. The thickness of the brain capillary endothelial cell is about 200–300 nm. This very thin cellular barrier has some of the most restrictive permeability properties of any biological membrane (Oldendorf, 1971).

Multifunctional Basis of BBB to Drugs. Drug entry into brain from blood is restricted at the BBB through multiple mechanisms, including a physical endothelial barrier, an enzymatic BBB, and an efflux barrier. This multifunctionality of the BBB arises from the multicellularity of the brain microvasculature, which is formed by the triad of brain capillary endothelial cells, capillary pericytes, and perivascular astrocyte foot processes (Pardridge, 2001). The endothelium and pericyte share a common microvascular basement membrane, and 99% of the brain surface of the capillary basement membrane is invested by the end-feet of processes extending from astrocyte cell bodies originating within brain parenchyma.

Endothelial Tight Junctions. Capillaries perfusing peripheral organs have porous endothelial walls. Peripheral capillaries have open interendothelial junctional spaces and active pinocytosis, which form a paracellular route and a transcellular route, respectively, for the free diffusion of molecules from the blood to the organ interstitium. However, in the vertebrate brain, the capillary endothelial cells express epithelial-like high resistance tight junctions, which eliminate the paracellular pathway, and have minimal pinocytosis, which eliminates the nonspecific transcellular route of molecular transport from blood to brain (Brightman, 1977). The combination of the very high resistance endothelial tight junctions and the minimal endothelial pinocytosis forms a physical barrier to drug entry into brain from blood.

Enzymatic BBB. There is an "enzymatic BBB" to circulating drugs, in addition to the physical barrier formed by the endothelial tight junctions. The capillary endothelial cells, the capillary pericytes, and the astrocyte foot processes all express a variety of ecto-enzymes on the cellular plasma membranes, including aminopeptidases, carboxypeptidases, endopeptidases, cholinesterases, and others, which inactivate many drugs that may pass the endothelial barrier. For example, circulating adenosine enters brain from blood via the BBB concentrative nucleoside transporter type 2 (CNT2) but does not have pharmacological effects in the brain, owing to rapid inactivation at the BBB by adenosine metabolizing enzymes (Pardridge, 2001). Conversely, the enzymatic BBB may serve to activate pro-drugs. Circulating L-DOPA enters brain via the BBB large neutral amino acid transporter type 1 (LAT1) and is rapidly converted to dopamine, the pharmacologically active form of the drug, by microvascular aromatic amino acid decarboxylase (AAAD).

Active Efflux Barrier. Certain drugs may cross the endothelial barrier via free diffusion and undergo influx from the blood to the brain compartment. However, this influx can be immediately followed by active efflux from

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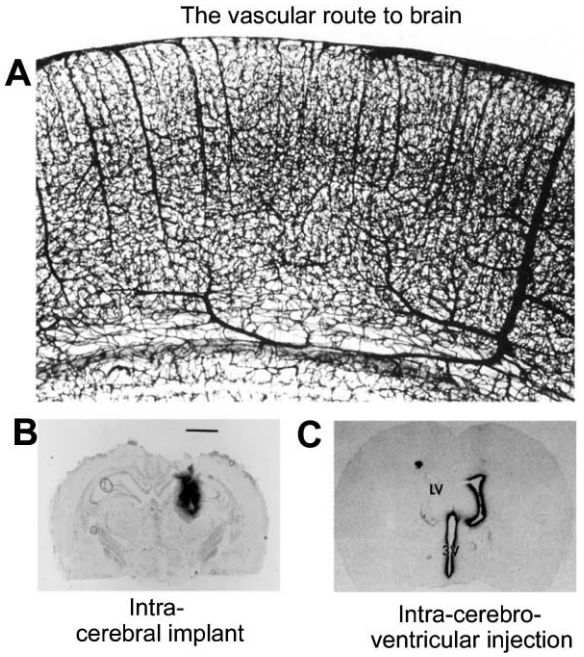

Figure 1. Transvascular and Transcranial Drug Delivery to the Brain

(A) India ink injection in adult rats shows the dense microvascular network within the brain (Bar, 1980). These brain capillaries form the BBB, and the delivery of drugs or genes across the BBB is the only pathway that enables widespread distribution of the drug to all cells within the brain.

(B) Film autoradiogram of rat brain 48 hr after the intracerebral (IC) implantation of a biodegradable polymer containing [^{125}I]-nerve growth factor (NGF) (Krewson et al., 1995). The distance bar is 2.5 mm, and the diameter of the polymer was 2 mm; therefore, there is minimal diffusion of the NGF into brain away from the IC implant.

(C) Film autoradiogram of rat brain 24 hr after the ICV injection of [^{125}I]-BDNF (Yan et al., 1994). The neurotrophin does not distribute into brain beyond the ipsilateral ependymal surface.

brain back to blood if the drug is a substrate for one of many different active efflux transporters (AET) expressed within the brain microvasculature. P-glycoprotein is the model AET at the BBB (Tsuji and Tamai, 1999), but there are many other efflux systems, such as organic anion transporting polypeptide type 2 (oatp2) (Asaba et al., 2000). One strategy to increase drug uptake into brain is the development of "co-drugs" (Pardridge, 2001). Co-drugs are inhibitors of BBB AET systems and are coadministered with the pharmacologically active drug that is an AET substrate. This is analogous to the administration of an AAAD-inhibitor with L-DOPA to prevent early degradation of the drug. In this case, the AAAD-inhibitor should be a drug that does not cross the BBB and selectively inhibits AAAD in the periphery.

Owing to these unique barrier properties of the microvascular endothelial barrier in the vertebrate brain, circulating molecules in the blood gain access to brain interstitial space via only one of two transport mechanisms:

- Lipid-mediated free diffusion of small molecules
- Catalyzed transport of small or large molecules

Free Diffusion of Small Molecules. Certain small molecules can traverse the BBB nonspecifically via lipid-mediated transport. A misconception with respect to small molecule transport across the BBB is that if a molecule is "small," then BBB transport is unrestricted. Only small molecules that are (1) lipid soluble and (2) have a molecular weight $<$500 Da threshold cross the BBB in pharmacologically significant amounts (Pardridge, 2001). Virtually all drugs presently in CNS clinical practice are small molecules that have these dual molecular characteristics. Similarly, if a small molecule is water soluble or has a molecular weight $>$500 Da, the drug may not cross biological barriers in pharmacologically significant amounts and have reduced absorption, as predicted by the "Rule of 5" (Lipinski et al., 1997). The adverse effect of molecular weight on membrane permeation is not observed if the molecular weight of the drug is $<$400 Daltons (Habgood et al., 2000). However, if the molecular weight of the drug causes the surface area of the drug to exceed 50–100 angstroms^2, then membrane permeation of the drug will not increase in proportion to the increase in lipid solubility of the drug (Fischer et al., 1998). The dependence of drug permeation through biological membranes on either molecular volume or molecular weight is not predicted by measurements of drug partitioning in solvents, because drug diffusion through solvents is not identical to drug diffusion across biological membranes (Lieb and Stein, 1986). Lipid mediation of small molecules through biological membranes requires molecular movement through channels within the lipid bilayer, and these channels have a finite size (references can be found in Pardridge, 2001). In summary, there are multiple impediments to small molecule transport across the BBB, and the following characteristics are associated with reduced BBB transport and reduced in vivo CNS pharmacologic activity:

- Molecular weight $>$500 Daltons
- Sum of hydrogen bond donor/acceptor groups $>$10
- Substrate for BBB enzyme system
- Substrate for BBB active efflux transporter
- Avid plasma protein binding of the drug

The above factors make it difficult to design a small molecule drug with effective CNS activity. Current small molecule neuropharmaceuticals only effectively treat a few CNS disorders, including pain, epilepsy, insomnia, and affective disorders (Ajay et al., 1999), and the majority of CNS disorders have thus far proven refractory to conventional small molecule therapy. A principal reason for this failure of conventional drug therapy of the brain is that $>$98% of all small molecule drug candidates do not cross the BBB, and no BBB drug targeting technology is used by the pharmaceutical industry (Pardridge, 2001).

Catalyzed Transport via CMT and RMT Systems. Small water-soluble nutrients and vitamins traverse the BBB rapidly via carrier-mediated transport (CMT). The CMT systems generally mediate the blood-to-brain transport of nutrients and include the GLUT1 glucose transporter, the LAT1 large neutral amino acid transporter, the MCT1 monocarboxylic acid transporter, the CNT2 nucleoside transporter, and many other small molecule transporters

(references can be found in Pardridge, 2001). The CMT systems are portals of entry of small molecule drugs that have a molecular structure similar to endogenous nutrients. L-DOPA, a neutral amino acid, gains access to the brain via CMT on the BBB large neutral amino acid transporter. In contrast, the conjugation of glucose to a peptide does not mediate transport via the BBB GLUT1 carrier (Witt et al., 2001), because the GLUT1 transporter does not recognize the peptide structure. In addition to the CMT systems, certain large molecule peptides or plasma proteins are selectively transported across the BBB via receptor-mediated transport (RMT) systems, including the insulin receptor, the transferrin receptor (TfR), or the leptin receptor. RMT of circulating peptides is comprised of three sequential steps: (1) receptor-mediated endocytosis of the circulating peptide at the luminal membrane of the capillary endothelium, (2) movement through the 200–300 nm of endothelial cytoplasm, and (3) exocytosis of the peptide into the brain interstitial fluid at the abluminal membrane of the capillary endothelium (Pardridge, 2001).

Brain Drug Delivery Strategies

Craniotomy-Based Drug Delivery to the Brain. Drugs or genes may be delivered to the brain via either intracerebroventricular (ICV) injection or intracerebral implantation (Shoichet and Winn, 2000). However, drug entry into brain parenchyma with either approach is limited by diffusion, and little drug diffuses into brain far from the depot site (Figures 1B and 1C). The most direct approach to drug or gene delivery to all parts of the brain is the vascular route (Figure 1A), and the transvascular brain drug delivery strategies include hyperosmolar BBB disruption, drug lipidization, protein cationization, and the chimeric peptide technology.

Hyperosmolar BBB Disruption. The intracarotid arterial infusion of hyperosmolar solutions causes a shrinking of the brain and the brain capillary endothelium, and this leads to a transient disruption of the BBB (Shoichet and Winn, 2000). BBB disruption has been used in humans for the delivery of chemotherapeutic agents to brain cancer (Dahlborg et al., 1998). The concern with BBB disruption is that this causes a generalized increase in the brain uptake of many plasma constituents. Blood proteins are toxic to brain cells (Nadal et al., 1995), and hyperosmolar BBB disruption causes chronic neuropathologic changes (Salahuddin et al., 1988).

Drug Lipidization. The BBB permeability-surface area (PS) product is an experimental measure of BBB permeability to a given drug. The BBB PS product for small molecules may be increased with lipidization via either (1) the reduction in hydrogen bonding of the drug through conjugation of lipid-soluble functional groups to water-soluble moieties on the drug, or (2) conjugation of the drug to a lipid carrier such as free fatty acid, adamantane, or dihyropyridine. A problem with drug lipidization is that the uptake of the lipidized drug by peripheral organs is also increased, and this causes a reduction in the plasma concentration of the drug. Therefore, the increased BBB transport caused by lipidization is offset by the increased uptake of the drug by peripheral tissues (Pardridge, 2001).

Protein Cationization. The cellular uptake of proteins may be increased by cationizing the protein, which triggers electrostatic interactions with anionic groups on the membrane, and this induces absorptive-mediated endocytosis into the cell (Pardridge, 2001). Proteins may be cationized via either (1) conjugation of amino groups such as hexamethylenediamine to surface carboxyl moieties, or (2) conjugation of cationic "import" peptides such as the arginine-rich tat peptide (Schwarze et al., 1999). Protein cationization has the same effect as drug lipidization. Both processes increase BBB permeability for the drug or protein but also cause a parallel increase in peripheral organ uptake and concomitant decrease in the plasma concentration of the drug or protein. Therefore, the brain uptake does not increase in proportion to the increase in BBB transport following protein cationization (Lee and Pardridge, 2001).

Chimeric Peptides. A chimeric peptide is formed when a small or large molecule drug that is normally not transported across the BBB is fused or conjugated to a BBB transport vector. The latter is comprised of an endogenous peptide, modified protein, or peptidomimetic monoclonal antibody (MAb) that undergoes RMT through the BBB on endogenous endothelial receptor systems (Pardridge, 2001). A peptidomimetic MAb transport vector binds an exofacial epitope on the BBB receptor. The MAb epitope is removed from the endogenous ligand binding site, and this binding enables the MAb to "piggyback" across the BBB on the endogenous RMT system, as demonstrated by electron microscopy of brain following the in vivo perfusion of anti-receptor MAb-gold conjugates. The MAb acts as a transport vector and can deliver to the brain any attached drug or gene. A panel of species-specific peptidomimetic MAbs has been developed to allow for transport of drugs or genes into the brain of either animal models or humans (references can be found in Pardridge, 2001).

Brain Drug Delivery of Large Molecule Drugs

Large molecule drugs are peptides, recombinant proteins, antisense agents, and gene medicines. It is widely believed that the BBB transport of large molecule drugs is not possible, and large molecule drug development programs are frequently terminated in favor of small molecule drug discovery. However, recombinant proteins, antisense drugs, and non-viral gene medicines can be delivered to the brain with brain drug targeting technology.

Recombinant Proteins. Neurotrophins could be used for a wide variety of brain diseases, but these recombinant proteins do not cross the BBB in pharmacologically significant amounts. Consequently, virtually all current neurotrophin CNS drug development programs are focused on the discovery of small molecule peptidomimetics. However, most small molecule peptidomimetics will not have molecular characteristics that pass the stringent criteria discussed above for effective BBB transport. Therefore, the small molecule peptidomimetic would benefit from reformulation with a BBB drug delivery strategy to be pharmacologically active in the brain. The development of a small molecule drug that crosses the BBB can be just as difficult as the development of a large molecule drug that is transported across the BBB. Therefore, one alternative is to reformulate the large molecule drug with a BBB drug delivery strategy. This has been done with the chimeric peptide technology, wherein a nontransportable peptide is conjugated to a BBB transport vector, which functions as a molecu-

lar Trojan horse and carries the peptide across the BBB. This approach has enabled the drug development of recombinant proteins, neuropeptides, and antisense drugs, which all cross the BBB and are pharmacologically active in the brain following intravenous administration. These chimeric peptides include (1) vasoactive intestinal peptide (VIP) for cerebral blood flow enhancement, (2) brain-derived neurotrophic factor for neuroprotection in either global or brain ischemia, (3) epidermal growth factor for the early detection of brain cancer, (4) Aβ analogs for the early detection of brain amyloid of Alzheimer's disease, and (5) peptide nucleic acid antisense agents for the in vivo imaging of brain gene expression (Pardridge, 2001). In all of these cases, the protein or antisense drug was not pharmacologically active in the absence of conjugation to the BBB Trojan horse, because the unmodified molecule did not cross the BBB in pharmacologically significant amounts.

The minimal transport of neuropeptides through the BBB is consistent with the high molecular weight and water solubility of these molecules. Some experimental results have led to the conclusion that peptides do cross the BBB, because radioactivity in brain is measured following the intravenous injection of neurotrophins labeled on tyrosine residues with ^{125}I. However, the neurotrophins are rapidly degraded by peripheral tissues and low molecular weight metabolites form in blood, which then enter brain to account for the cerebral uptake of radioactivity. When the peripheral metabolism of the peptide is inhibited, there is no measurable brain uptake of radioactivity following the intravenous injection of ^{125}I-neurotrophin (references can be found in Pardridge, 2001).

Gene Medicines. Widespread distribution of a therapeutic gene to brain, particularly the human brain, can only be achieved by delivery via the transvascular route (Figure 1A). It is possible to deliver non-viral plasmid DNA throughout the brain with gene targeting technology that uses pegylated immunoliposomes (PIL), which are able to access endogenous RMT systems within the BBB (references can be found in Zhang et al., 2002). In this approach, the supercoiled non-viral plasmid DNA is encapsulated in the *interior* of an 85 nm liposome, and this encapsulation within a nanocontainer protects the DNA from the ubiquitous endonucleases in the body in vivo. Uptake of the liposome by the reticulo-endothelial system (RES) is minimized by conjugating several thousand strands of 2000 Da polyethyleneglycol (PEG) to the surface of the liposome. The PIL is targeted across the BBB and across brain cell membranes by attaching receptor-specific MAbs to the tips of 1%–2% of the PEG strands. With this approach to brain gene delivery, intravenous antisense gene therapy led to a 100% increase in survival time in animals with experimental human brain cancer (Zhang et al., 2002).

Summary. The delivery of virtually any pharmaceutical to the brain via the vascular route is possible with the use of brain drug and gene targeting technology. The development of neurotherapeutics of the future can be accelerated by the merger of CNS drug discovery programs with parallel efforts in CNS drug targeting. Progress in CNS drug targeting is enabled by an understanding of the molecular and cellular biology of BBB transport processes.

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