From the liver to the brain across the blood–brain barrier

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The burden of brain diseases as established by a recent report of the World Health Organization represents 30% of the total burden of all diseases. This surprisingly high number is clearly related to the fact that the presently available CNS drugs treat only an extremely small percentage of brain diseases, leaving untreated major disorders, such as Alzheimer's disease, brain cancer, and stroke, or minor ones, such as autism, inherited mental retardation, and ataxia. There are also relatively few CNS drugs, although not for a lack of trying. In fact, ceaseless efforts have been made by the pharmaceutical industry to develop CNS drugs, but the number of failures has unfortunately paralleled the thousands of drugs that have been designed and tested. A major stumbling block has remained the fact that very few drugs have the ability to cross the blood–brain barrier (BBB) and reach their targets within the brain parenchyma (1). The BBB is created by the endothelial cells that provide the walls of the blood vessels perfusing the brain. However, in contrast to the peripheral endothelium, the brain endothelial cells lack capillary fenestrations, display low pinocytic activity, and form very tight junctions that are highly resistant to transendothelial ionic fluxes and strictly limit the entrance of endogenous and exogenous compounds into the CNS. How to successfully negotiate the barrier has required a deep understanding of its intimate properties and a great deal of ingenuity. The paper by Spencer and Verma (2) in this issue of PNAS is a good example of the latter with some extra creativity. To fully appreciate its novelty, one has to put this work in the context of what has been achieved so far in terms of the delivery of small drugs and therapeutic proteins to the brain.

CNS drugs nowadays consist mainly of small organic molecules, although the therapeutic potential of peptides or proteins for numerous brain pathologies is well recognized as well as their quasi-inaccessibility to the brain parenchyma. Access to the brain from blood circulation can take place either by diffusion or via specific transporters. The endothelial luminal membrane is in fact studded with specific transporters that gate the BBB and allow the selective entrance of saccharides, neutral amino acids, lipids, and vitamins as well as proteins, such as apolipoprotein E (ApoE), insulin, and transferrin.

So far, the vast majority of drugs targeting the brain are <400 Da and lipophilic, do not serve as a substrate of the P-glycoprotein (the product of the multidrug resistance protein), and cross the BBB by passive diffusion. However, drugs that do not display the latter permeation properties can be made to cross the BBB by several routes, although all at a price (Table 1). In the neurosurgery approach, the drug is directly injected intrathecally into the cerebrospinal fluid (3) or intracisternally into the ventricles (4). The drawback here is that the drug has a limited diffusion and has yet another barrier to cross: the ependyma that covers the ventricles. Drug-releasing polymeric implants (ethylene-covinyl acetate) introduced directly in the parenchyma do not encounter this barrier but also have a very restricted volume of action (5). To exploit the lipophilicity of the BBB, drugs have been encapsulated into liposomes and nanoparticles. However, this chemical approach as well as the drug cationization (6) cause an increased uptake in all organs and significantly decrease the drug's ability to reach the brain at effective therapeutic concentrations. To outwit the BBB, intracarotid arterial infusion of either hyperosmolar solutions or vasoactive drugs, such as bradykinin, along with drug administration have been used, but unfortunately the procedure leaves the BBB open for a short time only and allows the undesirable entry of toxic plasma proteins (7).

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Intranasal drug administration has been proposed to circumvent the BBB, yet the successes have been limited in terms of the drug concentrations reaching the brain (8). Moreover, the procedure has remained controversial because it is not clear whether the nasal route of drug administration is superior to the i.v. route.

Although crossing the BBB continues to be a very difficult exercise for numerous small organic molecules and prospective drugs, the problem is rather clear-cut for DNA or potential therapeutic proteins: They simply cannot cross unless one tricks, outwits, and fools the BBB. Thus, all of the successes encountered are testimony of imagination and creativity, all building on previous achievements and bringing small but very significant improvements. The major breakthrough occurred when it was recognized that the receptor-mediated transcytosis of proteins such as transferrin, insulin, ApoE is the key to the translocation of therapeutic proteins or DNA across the BBB. The basic mechanism coined by W. M. Pardridge, a pioneer in the field, as "molecular Trojan horses" (9) is as follows. The protein or DNA to be moved across the BBB is attached/conjugated to a motif that is recognized by a receptor present on the luminal side of the brain capillary endothelial cells. Once present in blood, the protein cargo binds the receptor and undergoes endocytosis. It moves through the endothelial cytoplasm, avoiding or exploiting the endosomal/lysosomal system, and exits on the brain side. This protein cargo system for delivery across the BBB has been used successfully for vasoactive intestinal peptide, BDNF, EGF, and pegylated immunoliposomes containing plasmid DNA encoding for β-galactosidase, among others (9–11). The technology is now ripe enough for start-up companies, such as ArmaGen Technologies and to-BBB, to start operating. Although proving the concept, the applicability of the various delivery protocols nevertheless has been limited by the lifetime of the protein in circulation, the need of repetitive injections, or the low yields of delivery to the brain.

The work of Spencer and Verma (2) provides a possible solution to the first two issues. The authors use a lentivirus (LV) vector system to deliver a protein (the secreted forms of glucocerebrosidase or GFP) to CNS neurons and astrocytes by fusing it to the low-density lipoprotein receptor (LDLR) binding domain of ApoB and adding a secretory leader sequence to allow its release. The LV vector was injected once i.p., and 2 weeks later

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Table 1. Methods used to deliver drugs across the BBB

Strategy	Method (representative refs.)	Major drawback
Neurosurgical approach	Injection within the CSF (3, 12)	Limited diffusion
	i.c.v. administration (13)	Limited diffusion
	Drug-releasing implants (5)	Local delivery
	Peritumoral infusion (14)	Invasive
Chemical approach	Liposome and nanoparticle encapsulation (15)	Enter all organs
	Cationization (6, 13)	Enter all organs
BBB opening	Intracarotid injection of hyperosmotic fluid (7)	Transient opening
BBB bypass	Intranasal delivery (8)	Limited size of <10 kDa, limited concentration
Cell therapy	Stem cell differentiation and engrafment in the CNS (16)	Low yield
Gene therapy	Receptor-mediated transcytosis of liposome-encapsulated RNAi and DNA (17)	Repeated injection
	Viral vectors (18)	Invasive, limited diffusion
Protein therapy	Protein overload (19)	Low yield
	Receptor-mediated transcytosis (9, 11)	Immunoreactivity

i.c.v., intracerebroventricular.

the authors were able to detect the protein within the CNS, demonstrating that it entered by transcytosis by binding to the LDLR. The beauty of the approach is that nonreplicating LV vector delivers genes to a peripheral organ (spleen or liver), which serves now as a source for the prolonged and continuous expression and secretion of a therapeutic protein that has the ability to cross the BBB and be delivered at the doorstep of LDLR-expressing brain cells. Using the LDLR to mediate the passage of a cerebrosidase through the BBB may possibly contribute to the remedy of lysosomal storage diseases that also involve the brain, such as type 2 and type 3 Gaucher's disease, for which there is currently no effective treatment. It is not that the approach of Spencer and Verma is without its own problems. A crucial one is the immunogenicity of the fusion protein, and another is related to the restricted brain distribution of the LDLR that results in only a minority of brain cells being targeted. The first problem will be difficult to tackle, whereas the second can possibly be solved by using other transcytosis-mediating receptors, such as the diphtheria toxin receptor (11). Although progress in the area is being made in small steps, these steps are highly significant because they slowly but surely bridge the great divide that separates us from a routine delivery of therapeutic proteins across the BBB that are effective enough to finally decrease the burden of brain diseases.

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