EDITORIAL

The Blood-Brain Barrier and Neurotherapeutics

INTRODUCTION

The blood-brain barrier (BBB) is a critical yet underdeveloped part of neurotherapeutics development. The BBB bottleneck is illustrated by these facts:

- More than 98% of all small-molecule drugs do not cross the BBB.
- Approximately 100% of large-molecule drugs or genes do not cross the BBB.
- No large pharmaceutical company in the world today has a BBB drug targeting program.

The logical consequence of this constellation is that it is very difficult to develop effective new neurotherapeutics that actually succeed in phase III clinical trials. This theme issue of *NeuroRx*® is devoted to the BBB, and to brain targeting of both small- and large-molecule drugs, including nonviral gene medicines. Investigators from Europe, Japan, and the U.S. have contributed to this issue

OVERVIEW OF BBB TRANSPORT

The first article provides an overview of the mechanisms by which molecules cross the BBB. A restricted class of small molecules, which have a molecular mass less than 400 Da and are lipid soluble, cross the BBB via free diffusion, and less than 2% of all small molecules fit this category. All other molecules either do not cross the BBB, or are transported across the BBB via catalyzed transport, owing to the specific interaction between the therapeutic and certain BBB transport systems. The BBB transporters are classified in one of three groups:

- Carrier-mediated transporters (CMT)
- Active efflux transporters (AET)
- Receptor-mediated transporters (RMT)

The second article by Ulrich Bickel provides an overview of the many methodologies available for the measurement of drug transport across the BBB, including pharmacokinetics. *In silico* models and *in vitro* BBB models provide information on the transport by free diffusion of small lipid-soluble drugs. However, these models have limitations with respect to drug transport via the CMT, AET, and RMT systems because many of the BBB transporters are either unknown or are downregulated in brain endothelial cell culture. A definitive examination of drug transport across the BBB requires the application of advanced *in vivo* physiological methods, which are reviewed in this issue.

SMALL-MOLECULE TRANSPORT

The BBB CMT and AET systems generally transport small molecules. The CMT systems include the Glut1 glucose carrier, the MCT1 monocarboxylic acid carrier, the LAT1 or CAT1 amino acid carriers, or the CNT2 purine nucleoside carrier. Generally, these systems are responsible for the transport of nutrients in the blood to brain direction. Drug transport via the CMT systems is reviewed by Akira Tsuji.

Active transport of drugs in the brain-to-blood direction is mediated by the AET systems. Active transport across the brain capillary endothelial interface requires drug transport across two membranes in series: the abluminal and luminal plasma membranes of the brain capillary endothelial cell, which forms the BBB *in vivo*. An energy-dependent transporter and an energy-independent cotransporter are differentially expressed on either the luminal or abluminal membranes. One class of energy-dependent transporters is derived from the ATP binding cassette (ABC) gene family, and the BBB ABC transporters are reviewed by Wolfgang Löscher and Heidrun Potschka. A class of energy-independent cotransporters are produced by the solute carrier (SLC) gene family, and the BBB SLC transporters are reviewed by Hiroyuki Kusuhara and Yuichi Sugiyama. The fundamental understanding of how the ABC and SLC transporters mediate active efflux transport at the BBB is not possible without definitive localization of the respective transporter at the BBB luminal or abluminal membrane. These two membranes are separated by a distance of only 200–300 nm, which makes it difficult to separate the luminal and abluminal membranes with confocal microscopy of brain sections. However, the localization of transporters on the two BBB membranes can be unambiguously identified with immunogold electron microscopy, which is reviewed by Eain Cornford and Shigeyo Hyman. Only a fraction of the BBB CMT or AET systems is known, and methods for the elucidation and

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characterization of new BBB transporters are reviewed by Tetsuya Terasaki and Sumio Ohtsuki.

LARGE-MOLECULE TRANSPORT

Certain endogenous large molecules, such as insulin, leptin, or transferrin, enter brain from blood via RMT across the BBB. In addition, receptor-specific peptidomimetic monoclonal antibodies (mAbs) may also bind specific BBB receptors to trigger RMT across the brain capillary endothelium. Endogenous peptides or peptidomimetic mAbs may act as molecular Trojan horses and ferry across the BBB any attached drug or gene. The delivery of peptides across the BBB via either CMT or RMT mechanisms is reviewed by Richard Egleton and Thomas Davis. Neurotrophins are potentially powerful neuroprotectives for the delayed intravenous administration in brain ischemia, should these molecules be made transportable across the BBB. Dafang Wu shows that if neurotrophins such as BDNF or FGF-2 are coupled to BBB molecular Trojan horses, such as a mAb to the transferrin receptor (TfR), then neuroprotection with intravenous administration of low doses is possible.

Drugs or genes may also be packaged in nanocontainers, such as liposomes or nanoparticles, which have a diameter of ~100 nm. To optimize the plasma pharmacokinetics of the nanocontainer, the surface should be modified such as with conjugation of several thousand strands of polymers such as polyethyleneglycol (PEG). The tips of PEGylated liposomes or nanoparticles can be conjugated with molecular Trojan horses to trigger RMT of the nanocontainer across the BBB. Drug delivery to brain with pegylated immunoliposomes (PILs) is reviewed by Anita Schnyder and Jörg Huwyler. Brain drug delivery with pegylated immunonanoparticles is reviewed by Jean-Christophe Olivier. Nonviral plasmid-derived gene medicines may be encapsulated in PILs. Using BBB drug targeting technology, it is now possible to achieve global expression in the brain of an exogenous gene after the intravenous injection of a nonviral formulation. The cover illustration of this journal shows the global expression in adult mouse brain of a β-galactosidase reporter gene driven by an opsin promoter. Reporter gene expression was examined at 2 days after an intravenous injection of the expression plasmid encapsulated in PILs, which were targeted across the BBB and the neuronal plasma membrane with a mAb to the TfR. This TfRmAb acts as a molecular Trojan horse to ferry the PIL across the BBB. This issue includes an article showing that it is possible to completely normalize striatal tyrosine hydroxylase (TH) gene expression in the 6-hydroxydopamine-lesioned rat brain after the intravenous injection of TfRmAb-targeted PILs carrying a TH expression plasmid. A new form of gene knockdown is RNA interference (RNAi). However, the limiting factor in the development of RNAi-based neurotherapeutics is delivery. BBB gene targeting technology enables intravenous RNA interference (RNAi) as reviewed by Ruben Boado.

FUTURE DISCOVERY OF NEW BBB TRANSPORTERS

Only a fraction of the BBB transporters is known to date, and research is needed to accelerate the discovery of novel CMT, AET, or RMT systems at the BBB. This is possible with the application of BBB genomics or BBB proteomics technologies, which are reviewed by Eric Shusta. It is not generally possible to identify BBB-specific genes in whole brain gene microarray. There is only 1 μl of brain capillary endothelial intracellular volume in an entire rat brain, and BBB-derived transcripts are about 10^{-3} parts of whole brain mRNA. Therefore, most BBB-specific gene products will be missed in a whole brain approach. BBB genomics starts with the isolation of capillaries from fresh animal or human brain, which enables the isolation of a pure population of BBB-derived mRNA. From there, BBB cDNA libraries may be constructed to enable the cloning of genes specifically expressed at the BBB.

In summary, fundamental research in the molecular and cellular biology of the brain microvascular endothelium has led to the development of new BBB drug and gene targeting technologies. These new technologies will create new opportunities for the future development of neurotherapeutics. If the CNS drug developer first produces a viable solution to the BBB problem, then the number of drug or gene candidates that can enter clinical trials is vastly amplified.

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Guest Editor