Pharmaceutical Research, Vol. 24, No. 9, September 2007 (© 2007)
DOI: 10.1007/s11095-007-9387-0

Editorial

Brain Drug Development and Brain Drug Targeting

Published online July 13, 2007

This issue of *Frontiers in the Pharmaceutical Sciences* highlights progress in the area of drug targeting to the brain. The goal of brain drug targeting is the re-formulation of pharmaceuticals to enable drug transport across the blood–brain barrier (BBB) via endogenous transport systems within the brain capillary endothelium.

Brain drug targeting science is derived from classical drug delivery. Drug delivery emanates from the materials sciences, and enables the re-formulation of drugs for controlled release. Drug targeting arises from the transport biology sciences, and aims to re-formulate drugs to enable membrane permeation via endogenous transport systems. Drug transport across the BBB involves movement through two membranes in series: the luminal membrane of the capillary endothelium and the abluminal membrane of the capillary endothelium, and these two membranes are separated by about 300 nm of endothelial cytoplasm.

BBB TRANSPORT BIOLOGY

There are three broad classes of transporters within the BBB:

- Carrier-mediated transporters (CMT) for small molecules
- Active efflux transporters (AET) for small molecules
- Receptor-mediated transporters (RMT) for large molecules

The CMT systems include the GLUT1 glucose transporter, the LAT1 large neutral amino acid transporter, the CAT1 cationic amino acid transporter, the MCT1 monocarboxylic acid transporter, and many other transporters that mediate either the influx of nutrients, hormones, or vitamins from blood into brain, or the bi-directional movement of these molecules between the blood and brain compartments. The CMT system may be expressed at both luminal and abluminal membranes of the BBB, or may be expressed only at the luminal membrane. In the latter situation, another CMT system must function at the abluminal membrane, so as to mediate the transport of the solute across both membranes.

The AET systems include P-glycoprotein, and other members of the ATP-binding cassette (ABC) gene family. The energy-dependent ABC transporters at the BBB work in concert with an energy-independent transporter, generally a member of the Solute Carrier (SLC) gene family, to mediate the active efflux of metabolites, and drugs, from brain to blood. While there is intensive focus on the role of P-glycoprotein in drug export from brain, (a) there are multiple other members of the ABC gene family that mediate drug efflux, apart from P-glycoprotein, and (b) the ABC transporter is expressed at one of the two endothelial membranes, while an SLC transporter is expressed at the opposite membrane. It is the coordinate action of energy dependent and energy independent transporters that mediates the net efflux of solute across both endothelial membranes.

The RMT systems include receptors such as the insulin receptor or transferrin receptor (TfR). Insulin is not made in the brain, but insulin is present in the brain. Brain insulin arises from the blood via transport across the BBB on the endothelial insulin receptor. Brain iron originates from transferrin (Tf) in blood, which is transported into brain on the BBB TfR. The BBB TfR mediates the bi-directional transport of holo-Tf, from blood to brain, and apo-Tf, from brain to blood. The BBB TfR is a bi-directional transcytosis system. An example of a "reverse transcytosis" system is the BBB Fc receptor (FcR), which mediates the asymmetric transcytosis of IgG molecules in the brain to blood direction, but not in the blood to brain direction.

BRAIN DRUG TARGETING

With the information on the biology of the BBB transporters, the brain drug developer may re-formulate small or large molecule drugs to cross the BBB on the endogenous transporters:

- The medicinal chemist may alter the structure of a lead molecule, not to increase lipid solubility, but to increase CMT affinity. For example, L-DOPA is a form of dopamine, and gabapentin is a form of gaba, and both drugs are effective neuropharmaceuticals because the drugs cross the BBB on LAT1. Just as the drug discoverer uses structure–activity relationships (SAR) to enhance drug affinity for a target receptor, the drug targeting scientist uses structure–transport relationships (STR) to enhance membrane permeation via a BBB CMT.
- Following the cloning and expression of a BBB AET, the drug developer might use high throughput screening (HTS) to isolate "co-drugs." A co-drug inhibits a BBB AET

system, and thereby increases brain permeation of a pharmacologically active molecule that has limited brain penetration, owing to its export from brain via the BBB AET system.

• The biotechnologist may discover receptor-specific ligands, or peptidomimetic monoclonal antibodies (MAb), that cross the BBB on an RMT system. Such molecules may act as a molecular Trojan horse, and ferry across the BBB a large molecule pharmaceutical, such as a recombinant protein, a MAb therapeutic, an antisense agent, a non-viral plasmid DNA therapeutic, or an RNA interference (RNAi) drug. The delivery of large molecule drugs to brain via the BBB RMT systems requires the merger of molecular biology, genetic engineering, biologics expression systems, and liposome or nanoparticle technology. In the case of advanced brain drug targeting formulations, such as Trojan horse liposomes, the materials sciences and the transport biology sciences are combined, and brain drug delivery and brain drug targeting are merged.

WILLIAM M. PARDRIDGE, M.D.

Dr. Pardridge is Professor of Medicine at the UCLA School of Medicine, Los Angeles, California. He graduated from Santa Monica High School in 1965, received a B.S. degree in Chemistry from UCLA in 1969, obtained an M.D. degree from Pennsylvania State University in 1974, completed internship and residency in Internal Medicine at Boston University Medical Center in 1976, completed a fellowship in Endocrinology and Metabolism at the UCLA Medical Center, was appointed Asst. Prof. of Medicine at UCLA in 1978, received tenure in 1981, and was promoted to Professor of Medicine at UCLA in 1985. He is a former NIH Research Career Development Award recipient, was elected to the American Society of Clinical Investigation in 1981, served on the NIH Endocrinology Study Section from 1983–1989, is an Alumni Fellow of the Pennsylvania State University, and is certified in both Internal Medicine and the Endocrinology and Metabolism Sub-Specialty by the American Board of Internal Medicine. Awards include the Joseph Erlanger Distinguished Lectureship of the American Physiological Society, the Meritorius Manuscript Award for most outstanding paper published in Pharmaceutical Research by the American Association for Pharmaceutical Research in 1996 and again in 2003, and the Tenth Annual Horace Magoun Lectureship of the UCLA Brain Research Institute. He is the author, editor, or inventor of five books, over 450 articles, and over 20 patents published in the blood–brain barrier field.

William M. Pardridge Interview

1. What do you think holds the key to your success as a pharmaceutical scientist?
   Response: The key was to learn physiology, and then molecular biology, before learning pharmaceutical science. I first learned the physiology of blood–brain barrier (BBB) transport of solutes and drugs. My initial training was in the use of an *in vivo* model—the Brain Uptake Index, or BUI methodology of Oldendorf. In a reductionist approach, I then learned techniques for the isolation of capillaries from brain, including human brain, since the capillaries formed the BBB, and housed the transporters I initially studied with the BUI method. Then, I learned the techniques of molecular biology, so that the BBB transporters expressed in the isolated brain capillaries could be cloned and evaluated at the molecular level. At this point, I was not a pharmaceutical scientist. But, early on, it occurred to me that the basic information on the biology of the BBB transporters could be used to re-formulate drugs to enable transport into brain via these transporters. I became a pharmaceutical scientist once I used the basic sciences to re-formulate drugs to enable penetration into the brain.
2. What do you consider to be your key research accomplishments?
   Response: I would identify key research accomplishments on the protein drug targeting side, and on the non-viral gene targeting side. In protein drug targeting, I would point to the conceptualization, genetic engineering, expression, and validation of a fusion protein therapeutic for Alzheimer's disease (AD), which was published in 2007 in *Bioconjugate Chemistry.* This genetically engineered fusion protein was designed to have three domains for three functionalities: (1) an engineered monoclonal antibody (MAb) against the human insulin receptor (HIR), which triggers the receptor-mediated transcytosis of the fusion protein across the human BBB, (2) a bivalent engineered single chain Fv (ScFv) antibody against the Aβ amyloid peptide of AD, to bind and disaggregate the amyloid plaque of AD behind the BBB, and (3) the CH2–CH3 region of a human IgG1 constant region, to cause the reverse transcytosis of the fusion antibody/amyloid complex from brain to blood via the BBB Fc receptor (FcR). I believe that an antibody treatment for AD must be able to mediate all three of these molecular events in order to reduce the amyloid burden of AD. On the gene targeting side, I believe my key research accomplishment is the "blue monkey brain," which was published in the 2003 *Molecular Therapy*. Sections of Rhesus monkey brain turned blue with β-galactosidase histochemistry. The brain was removed from adult Rhesus monkeys 48 h after a single intravenous (IV) injection of Trojan horse liposomes (THL), which encapsulated a non-viral plasmid DNA encoding for the β-galactosidase gene. The result meant that we had achieved the global expression of a transgene throughout the entire brain of an adult primate with a simple IV injection of a non-viral formulation. No other technology can do this, short of a pronuclear injection of the transgene in a monkey embryo and growth of the transgenic animal. The THL technology enabled pharmacologic effects with non-viral plasmid DNA therapeutics in experimental models of brain cancer, Parkinson's disease, lysosomal storage disorders, and intravenous RNA interference (RNAi).
3. What was the turning point in your career?
   Response: Meeting William H. Oldendorf.

4. Who are the individuals who most influenced your research career?
   Response: If I had not met Bill Oldendorf, I would not have worked on the BBB. Bill introduced me to this fascinating area of science in the summer of 1970 before I began medical school. He also gave me a technique, the Brain Uptake Index or BUI method, which he had just invented. The BUI method enabled the rapid acquisition of quantifiable research data on the transport of molecules across the BBB *in vivo*. With this robust method in hand, I was able to start my own independent research with little formal training.
5. Pharmaceutical scientists are faced with the dilemma of having to publish in biomedical or basic science journals. Does it mean cutting edge science will not likely be featured in the Pharmaceutical Research?
   Response: I would re-phrase the question by re-defining "cutting edge science." Basic science aims to acquire new information and applied science aims to put the new information to practical use. Applied science is identical to technology or translational science. Applied science or technology is still viewed as outside the realm of cutting edge basic research. Why would we apply the term, cutting edge, solely to basic science? The discovery of a new drug delivery or targeting technology is also cutting edge in that new science is created. Moreover, the extent to which the practice of molecular and cellular biology is required to implement a new drug targeting technology means there is now little distinction between the execution of the pure and applied sciences. In academia, we have, for decades, over-valued basic research and under-valued applied research. It is all cutting edge, and the sooner we realize that, and the sooner the applied or translational sciences are appropriately valuated, the sooner basic science will be translated into new medicines that actually work in people.
6. Where is the field of Drug Targeting to the Brain going, and how do the articles in the theme section fill the gap?
   Response: The field of drug targeting to the brain is going molecular, and the articles in this issue are based in molecular biology. Drugs are being targeted to the brain, across the BBB, by re-formulating the drug to access an endogenous BBB transporter. This cannot be done without basic science information on the molecular identity of the BBB endogenous transporters. The Ohtsuki and Terasaki article provides the blueprint for drug targeting via the CMT and AET transporters, and the Jones and Shusta article provides the blueprint for drug targeting via the RMT systems. The Boado chapter shows how the RMT basic science is translated into new RNAi and non-viral gene medicines. In all the chapters of this theme section, the emphasis is on the fundamental molecular biology of the BBB transporters, and how to use this information to create new approaches to solving the brain drug penetration problem.
7. What are the challenges for Drug Targeting to the Brain and how can be overcome?
   Response: The future challenge to the field of drug targeting to the brain is infrastructure. Infrastructure is people—people trained in the field. We presently are training only a handful of BBB scientists, much less scientists whose expertise is in brain drug targeting via endogenous transporters. No drug company in the world today has a BBB drug targeting program. Even if Big Pharma wanted to change that situation, there would be few to hire, because there is not a single academic neuroscience program in the USA that emphasizes BBB transporter biology and brain drug targeting. The few programs in the USA that do teach BBB transporter biology are all in Departments of Pharmaceutical Sciences. The chronic under-development of the BBB is difficult to understand considering the largeness of the human population with brain disorders, and the smallness of the population of drugs that cross the BBB.
8. What is the key to developing successful collaborative relationships?
   Response: Long-term collaborations have to be complementary, where each party brings their own expertise to bear on the problem, expertise which is complementary to the skills and attributes of the partner.
9. What is your philosophy of educating graduate students?
   Response: I am in a clinical department and have had only one graduate student in 30 years. However, I have trained over 70 post-doctoral fellows. My philosophy to education is to expose the trainee to as wide a variety of scientific problems and technological solutions as possible, and then hope they go off on their own and make their own innovations.
10. What are the challenges facing the pharmaceutical sciences?
    Response: The pharmaceutical sciences are a translational science, which means the pharmaceutical sciences bridge the gap between the basic sciences and the clinical sciences. We are very good at basic science—progress in the molecular sciences is breathtaking. We are also very good at clinical science—the double blind, placebo-controlled clinical trial standard established by the FDA means that only drugs that work are approved. Yet, the recent NIH-led emphasis on translational medicine attempts to respond to the difficulty we are encountering in jumping from the basic sciences to the clinical sciences. This transition is difficult, because we typically discover drugs that work in Petri dishes, but not in people. The drugs do not work in people, because the drugs encounter transport barriers *in vivo* that do not exist in a Petri dish. These transport barriers are circumvented with new technologies created by the pharmaceutical sciences. The drug delivery/drug targeting sciences are the paradigm of translational science. The challenge to the pharmaceutical sciences is to articulate the crucial role played by the pharmaceutical sciences in translational medicine.

11. What is the place for collaboration with industry in academia?
Response: The collaboration between industry and academia must necessarily be initiated by industry. First, industry needs to throw off its "not invented here" shackle, and seek out collaborations with academic scientists, and form programs that address critical problems not currently being advanced by existing in-house research. The delivery and targeting pharmaceutical sciences would be a good place to start, because the new medicines almost invariably have a delivery problem. The development of new technology that enables the successful targeting of a drug is a challenging proposition, and one that requires a multi-disciplinary team effort, which is enabled by industry-academic collaborations.

William M. Pardridge
Department of Medicine,
UCLA, Warren Hall 13-164,
900 Veteran Ave.,
Los Angeles, California 90024, USA
e-mail: wpardridge@mednet.UCLA.edu