William Pardridge discusses the lack of BBB research

Interviewed by Rebecca N. Lawrence

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William Pardridge is Professor of Medicine and has been at the UCLA School of Medicine since 1976. He was the Tenth Annual Horace Magoun Lecturer at UCLA, and is a member of the American Society for Clinical Investigation. He is the author or editor of five books and nearly 400 research articles published in the blood–brain barrier field.

Tell me about your current research and what you have found so far.

Our current research is devoted to drug and gene targeting across the blood–brain barrier (BBB) and can be divided into basically two platforms. First is brain targeting of therapeutics including large-molecule drugs such as recombinant proteins, non-viral gene medicines or antisense agents. Here, we use molecular 'Trojan horses' to re-formulate drugs so that these molecules can cross the BBB, and this work is fundamentally a drug discovery program. Second is transporter discovery, where we use a variety of expression cloning, brain vascular genomics, and BBB proteomics programs to clone novel BBB transporter targets. In the usual drug development process, drug discovery and drug targeting are two separate disciplines, but we believe that these two areas should be merged into the overall drug development program. Just as you would have a gene discovery platform for drug discovery, you need a gene discovery platform for drug targeting.

Protein drugs as neurotherapeutics

With respect to therapeutic drug discovery, we have a program developing recombinant neurotrophins as neuroprotective agents in stroke. There are over 30 neurotrophins on the brain that have the potential to be neuroprotective agents. Stroke is a devastating disease, and we spend US$40 billion each year in stroke rehabilitation, and so it is startling to think that there is no neuroprotective agent currently offered for stroke patients (they just receive supportive therapy). The neuroprotective agents that have been developed thus far have either been too toxic or they do not cross the BBB. Therefore, the key to neuroprotection in stroke is to build a BBB targeting platform. We have found that neurotrophins such as brain-derived neurotrophic factor (BDNF) are powerfully neuroprotective in stroke models following delayed intravenous administration, providing they are conjugated to BBB drug targeting systems.

It is startling to think that there is no neuroprotective agent currently offered for stroke patients.

Protein drugs as neurodiagnostics

We also have a protein neurodiagnostics program that involves using peptide radiopharmaceuticals for early detection of brain cancer and Alzheimer's disease (AD). Many brain cancers overexpress specific receptors such as the epidermal growth factor (EGF) receptor. The endogenous peptide ligand, EGF, for example, can be used as a peptide radiopharmaceutical for early detection of brain cancer. The problem is that the peptide itself does not cross the BBB in either the normal brain or in the brain tumour. However, the brain tumour BBB can be circumvented by reformulating the EGF with a BBB drug targeting technology. We are also developing a brain scan for early detection of the brain amyloid that is specific for AD. Deposition of amyloid in the brain starts up to 20 years before you develop symptoms of this disease. Hence, an amyloid brain scan for AD (similar to the current colonscopy program for early detection of colon cancer) could identify people (for example, over 50 years of age) who are at risk of ultimately developing AD and who could be prescribed amyloid inhibitors in advance. Delaying the onset of symptoms of AD by just five years would result in a saving to US healthcare costs of US$50 billion per year.

Gene therapy

We have recently developed an exciting program of non-invasive, non-viral gene targeting to the brain. Existing brain gene therapy approaches involve the intracerebral injection of a viral vector carrying the gene. This has two problems: first, the viral vector causes inflammation and demyelination in the brain; second, the intra-cerebral injection, or craniotomy-based brain drug delivery, is not only invasive and costly (US$15,000 per patient), but the gene is only delivered at the tip of the injection needle. Therefore, the treatment volume in the brain is about the size of a large grain of salt and, although this might be fairly extensive in a mouse brain, it is insignificant in the human brain. Hence, the only way to get widespread expression of the exogenous gene throughout the brain is to deliver the therapeutic gene through all of the 400 miles of brain capillaries that perfuse our brains, but this requires gene targeting technology.

We have shown that it is possible to achieve widespread expression of exogenous genes throughout the brain with a simple intravenous injection if you package the non-viral plasmid DNA within a special formulation that enables transport across the BBB. Moreover, we have shown that brain-specific expression of the exogenous gene is possible, if gene targeting technology is used in conjunction with brain-specific gene promoters, thus avoiding expression in peripheral tissues.

Antisense radiopharmaceuticals

With the availability of the human genome and disease-specific genomics, in a few years, people will be aware that they carry a certain disease-causing mutation. However, they would not know when this

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mutant gene was going to be expressed during their lifetime because, at the moment, the only way to determine this is to have a brain biopsy, which is not feasible. Gene expression in the brain *in vivo* could theoretically be imaged with sequence-specific antisense radiopharmaceuticals that hybridize to a particular gene sequence. However, because these molecules do not generally cross cell membranes and do not cross the BBB, antisense radiopharmaceuticals cannot be used *in vivo* without a BBB drug targeting technology. We have shown disease-specific gene expression in the brain can be imaged *in vivo* following the intravenous administration of targeted antisense radiopharmaceuticals in both an experimental brain cancer model and in a Huntington's disease (HD) model.

Transporter discovery platform

There are three different types of transporters at the BBB: carrier-mediated transporters (CMT), like the glucose or amino acid carrier that transport nutrients; active efflux transporters (AETs) that pump drugs out of the brain, like P-glycoprotein; and receptor-mediated transporters (RMT) that transport endogenous peptides like insulin, transferrin or leptin. Most of the BBB transporters that exist have yet to be discovered so we are using a variety of molecular biology approaches, such as expression cloning in *Xenopus oocytes*, BBB genomics, and BBB proteomics programs to uncover these novel transporters.

The BBB problem is a model for translational research.

What are the key hurdles to this research?

Within my own laboratory, we feel that we can deliver essentially any drug across the BBB in a non-invasive way. However, in general, the main current limiting factor in BBB research is the lack of infrastructure, that is, a critical mass of people trained in and working on the BBB. For a start, no drug company in the world today has a BBB program. Second, of all the academic neuroscience centres in the world, there are few, if any, academic training programs in the BBB field. Therefore, even if a large pharma company wanted to change this situation and commit funds for a free-standing BBB program within its company, there would be few to hire because the number of BBB trained scientists around the globe is just a handful.

This lack of BBB infrastructure is the result of long-term under-development of the field. For many years, both the academic neurosciences and the pharmaceutical industry have essentially ignored the BBB problem. I think the academic neurosciences have ignored the BBB because the BBB problem is a model for translational research, and the academic centres are generally not interested in translational research, but this area is crucial to the overall neuroscience mission. You have the progress in the molecular neurosciences in the laboratory and then you have patients in the clinic who have these serious neurological diseases. What we need is translational research that bridges the gap between the fundamental discoveries and enables clinical application. There are other cases of under-development in translational research, cancer being a particularly good example.

Why do you think the pharma industry has not really taken the lead in this?

The pharma industry is a chemistry-driven process devoted to small molecules and there is an erroneous belief in the industry that if a molecule is small, it will cross the BBB. In fact, more than 98% of small molecules do not cross the BBB because they are (a) water-miscible or (b) if they are lipid-miscible, they have a MW above the 500 Da threshold. Nearly all the drugs that come out of receptor-based HTS programs lack the two criteria necessary for BBB transport; that is, lipid-miscibility and a MW less than 500 Da.

The result of this approach is that there are only really a few brain diseases that are being widely treated today and these are the ones that are amenable to small-molecule drug therapy; namely, affective disorders, depressions and schizophrenia, epilepsy, and chronic pain. Progress has been slow in developing medicines for the major CNS patient groups: Alzheimer's disease, brain and spinal cord trauma, HD and other neurodegenerative disorders, brain cancer, stroke, AIDS infection in the brain, genetic diseases that lead to mental retardation and premature death, the ataxias, and so on. Parkinson's disease (PD) patients take L-DOPA (which crosses the BBB on a CMT system), but there is no neuroprotective agent for PD that prevents the inexorable neurodegeneration. Multiple sclerosis (MS) patients have cytokines that work on the peripheral immune system, but there is no drug that works in the brain to prevent the inevitable demyelination of MS. So, the vast majority of brain patients are not being adequately treated today, the reason being that they have diseases that do not really respond to small molecules. However, small molecules are the only drugs that are being developed by the pharma industry because this is the only kind of drug that it can develop: you cannot develop water-miscible small molecules or large-molecule drugs if you do not have a BBB drug targeting program. With such a program, however, you could greatly increase the number of drugs that could be developed, and you would not be limited to small-molecule drugs.

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Which are the key funding bodies that are providing academics with grants to support brain drug-targeting research?

The largest one is the NIH, but getting BBB research funded there has not been easy, because the BBB is just not on the neuroscience 'radar screen'. In the past, if you wrote a grant to the NIH concerning basic research on the BBB, it often would not get funded. A better approach was to write a brain ischaemia grant and say you are going to use BBB in order to execute the aims of that grant. However, the Neural Environment cluster of the NINDS has recently taken a leadership role in articulating the need for more BBB research. Technology-oriented agencies, such as the Department of Energy, have shown an interest in brain drug targeting technology of radiopharmaceuticals. Also, mission-oriented foundations, which seek out translational research, often fund BBB research.

Where do you think the extra funding should come from then?

Industry could do more to fund BBB research. Until the neuroscience leaders begin to articulate the importance of translational research, I do not think it is going to come from the academic side. If you go back to neuroscience research 30 years ago, the focus was on

mechanisms of learning. In the 1990s, the focus changed to mechanisms of disease, but until we start focusing on the mechanisms of treatment, we are not going to see progress in BBB research. We should be asking that all neuroscience grant applications include a section on mechanisms of treatment, and then the investigator would have to propose concrete ways of how their discoveries are actually going to be translated into new treatments that can be practically administered to millions of patients.

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By contrast, the industry has a large vested interest in this field because the number of patients with neurological disorders is huge compared with any other organ system. In the US alone, there are 80 million people with some disorder of the brain and I think we are only adequately treating a very small fraction of these patients. Hence, growth in the neuropharmaceutical industry in the future could far outstrip the growth in any other sector of the pharma industry if the BBB problem was solved. It is therefore a situation where the pharma industry has to take the leadership role. First, pharma companies could set up initial groups within their company that are devoted to focussing on the BBB. Second, the large pharma companies could form a consortium (like they did for the SNP Consortium) and have a www-based research grant application process that would focus on brain translational research, mechanisms of treatment and BBB issues, and this could provide a new source of funding for investigators. However, because there are so few people in the BBB field, we also need to do something that will bring in investigators from the molecular neuroscience area.

What do you think are the most innovative new therapeutic and diagnostic approaches for brain drug targeting at the moment?
Frankly, progress has been slow in this area. If you do an analysis of the Society for Neuroscience abstracts in the annual meeting in the US between the beginning and end of the 1990s (the decade of the brain), there is a doubling in the number of abstracts in BBB research. Essentially, 90% of this growth in the field was carried out in *in vitro* BBB models where cultured isolated endothelial cells are used to try to replicate the *in vivo* barrier properties. However, this is not a particularly accurate model as there is a tremendous down-regulation *in vitro* of tissue-specific gene expression in the brain capillary endothelium. What little new research there has been in the BBB has focused on these models and the new people who have come into the field have almost uniformly adopted this approach. Hence, the growth in BBB research in Europe and the US has been quite frankly static. The only real exception to this is the current work going on in Japan where multiple academic centres are developing exciting new BBB programs particularly focussing on the small-molecule transporters, CMTs and AETs.

Can you tell me a bit more about who are the key groups and what sort of work they are doing in Japan?
The groups that come to mind (and this is just a partial list) are Professors A. Tsuji at Kanazawa University, T. Terasaki at Tohoku University, and Y. Sugiyama at Tokyo University, who are all developing new methods and new approaches to study AETs and CMTs at the BBB. AETs (e.g. P-glycoprotein) are important because they pump drugs out of the brain and so if you develop a co-drug that inhibits the AETs and administer it in conjunction with the therapeutic drug, you can increase the brain uptake of that particular drug. One example of its potential would be with AZT, which does not get into the brain, enabling the brain to provide a sanctuary for the AIDS virus. The development of a co-drug would provide a treatment for the brain component of AIDS. This is an example of moving from a chemistry-based or 'pro-drug' approach to a biology-based or 'co-drug' approach to solving the BBB drug transport problem. The co-drug method emanates from an understanding of the basic molecular and cellular biology of the transporters at the BBB.

How have researchers been improving their knowledge of the basic molecular and cellular biology of the brain microvasculature?
There is increasing work on the molecular biology of the brain microvasculature, but a great deal more needs to be done. The application of molecular biological techniques to the brain microvasculature is possible if you develop gene libraries derived from animal or human BBB and clone the specific genes that are expressed in the BBB. The reason that I think a lot of people have not got involved in the molecular biology of the BBB is that the capillaries that make up the BBB occupy only a 1000th part of the brain. You can easily extract 20 μg of polyA$^+$ mRNA from whole brain, but to make a library of brain capillary-derived mRNA, you have to first isolate the brain microvessels, and throw away 99% of the brain. To get the same amount of polyA$^+$ mRNA from brain capillaries that you would from a fraction of 1 g of whole brain, you would have to start with 200 g of brain.

The pharma industry has to take the leadership role.

What kind of problems does the blood–tumour barrier (BTB) represent?
The BTB is formed by the capillaries perfusing the brain tumour. These tumour capillaries originate from normal brain, and in many instances, are similar to those of the non-tumour brain. Therefore, the BBB, or BTB, is a rate-limiting problem in developing new drugs or gene medicines for brain cancer. The barrier can become leaky in necrotic areas to small molecules but, in general, the BTB remains intact especially to compounds like EGF, which has a MW of 5000 Da. There are already several genomics programs in progress for brain cancer, and we know that many genes are selectively turned on in this condition. However, I think in parallel to this, there should be a cancer vascular genomics program that uses isolated capillaries from fresh brain tumour tissue (i.e. the BTB), rather than the usual procedure of immediately homogenizing the whole brain tumour tissue. We have done this for a couple of different types of human brain cancer and the work (which is so far unpublished) has led to many surprises and has demonstrated just how little we know about the vascular biology of brain cancer. There has been a large focus on angiogenesis in cancer, but this is only one aspect of many that are of biological significance to brain cancer vascular biology. If we knew much more

about the vascular biology of cancer, we would have several different treatment platforms that we could target to selectively shut off the vessels that perfuse the cancer.

We discussed earlier that most of the models for the BBB are* in vitro *models. How do you think they could be improved?
My lab started using *in vitro* BBB models over 15 years ago, but we could not find a way to make this model replicate the *in vivo* BBB. I think that we need to find the BBB-specific genes that are turned off in tissue culture. To do this, you could use a subtractive cloning procedure, in which you prepare cDNA from the freshly isolated brain capillaries, and then subtract the commonly expressed genes using cDNA obtained from cultured brain endothelial cells. Once we know what genes are turned off when brain endothelium is grown in cell culture, then we can begin the process of determining what factors must be added to the culture system so as to turn these BBB-specific genes back on. At that point, I think you will find that many of the BBB properties *in vivo* that had previously been lost in cell culture will now have been restored to the *in vitro* BBB model system. I have always thought that the problem of developing a functional *in vitro* BBB system was fundamentally one of molecular biology. However, until we have an *in vitro* BBB model that replicates the BBB *in vivo*, it is important to use *in vivo* model systems to evaluate drug transport at the BBB.

The 20th century was all about small molecules and a chemistry-driven platform… The 21st century will be all about a biology-driven pharmaceutics platform.

Where do you think brain drug targeting research will be in 5 or 10 years time?
We could be in exactly the same place in 5–10 years as we are now, if no changes are made in the way the pharma industry and academic neuroscience look at this important field. However, in 20 years, I think there will be a fundamental paradigm shift in the way we look at drug development in general.

What we have now is a situation where drug discovery is everything and drug delivery is an end-game issue that is more related to formulation of drugs that are already earmarked for drug development. I think that by 2020, drug discovery and gene discovery will be *passé* and the real innovation in drug development will be in drug and gene targeting. That will be because we will have developed drug targeting technologies and realized that we can then deliver any drug we want across the BBB including large-molecule drugs. The 20th century was all about small molecules and a chemistry-driven platform and that has proven to be less than adequate for coming up with new drugs for many brain disorders. By contrast, the 21st century will be all about a biology-driven pharmaceutics platform that focuses on drug targeting technology. However, I think it will take longer than five or 10 years for this to happen because of the infrastructure problem, and the need for more people to execute these changes.

What would you like to have achieved by the end of your career?
I would like to see what we are doing in the laboratory actually working in the clinic and I would like to have had a major impact on a major neurological disease. This is what keeps me going.

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