

# Delivery and Assessment of Potential Central Nervous System Therapeutics Across the Blood–Brain Barrier

a report by

Mohammad S Alavijeh,<sup>1</sup> Peter M Hoffmann,<sup>2</sup> Matthew D Roe<sup>2</sup> and Alan M Palmer<sup>1</sup>

1. Pharmidex; 2. Genzyme Pharmaceuticals

The human brain represents the pinnacle of biological evolution. With grooves and spirals that give it the appearance of a shelled walnut, it weighs on average 1.3kg and contains 100 billion nerve cells (or neurons), with 10 times as many glial support cells. These cells are organised in a highly complex three-dimensional (3-D) array of interconnecting fibres, with information flow mediated by flux of electrical charge along communicating neuronal fibres (axons) at a speed of up to 250 miles/hour. This flow of information is mediated by the movement of charged ions across the axonal membrane; communication between neurons is via release of neurotransmitter into the synaptic space between adjacent cells. These communication points are numerous and in total the human brain contains  $10^{14}$  such synapses. Any given neuron may have several thousand synaptic connections to many other neurons, forming a highly complex network – the number of possible connections is virtually without limit. The activity of the brain weaves meaningful patterns to control all of our senses, actions, emotions and thoughts.

Although constituting only 2% of the weight of the body, the brain utilises 20% of its blood supply. This is because of the huge demand for energy required to maintain electrochemical gradients across neuronal membranes. The blood is delivered through a uniquely complex network of blood vessels that extends more than 400 miles and spans a surface area of about 20m<sup>2</sup>. The mean distance between capillaries is 40µm,<sup>1</sup> which permits near-instantaneous solute equilibration throughout the brain interstitial space for small molecules. However, unlike nearly all other organs of the body where there is a free exchange between blood and interstitial fluid, the capillaries in the brain have evolved to constrain the movement of molecules and cells between blood and brain. This important characteristic provides a natural defence against toxic or infective agents circulating in the blood, and is conferred by cell adhesion molecules allowing endothelial cells to form tight junctions. This blood–brain barrier (BBB), while fulfilling a protective role, severely limits the movement of medicines and potential medicines into the brain. As a result, most molecules have to take a transcellular route into the brain, so the overwhelming majority of small molecules, proteins and peptides do not cross the BBB. A safe, reliable and consistent method of delivering compounds across the BBB and assessing their neuropharmacokinetics would therefore open new vistas for central nervous system (CNS) pharmacotherapy.

## Assessment of Brain Penetration

In order to develop CNS drug delivery technologies and therapies, it is essential to have reliable methods of assessing the ability of compounds to penetrate the BBB and permeate into the brain. These include *in vitro*, *ex vivo* and *in vivo* assessment.

## In Vitro Assessment

Numerous *in vitro* models of the BBB have been established, including primary bovine and human brain endothelial cells co-cultured with astrocytes, immortalised brain endothelial cell lines and models using cells not derived from endothelial cells – such as the Madine-Darby canine kidney (MDCK) cell lines.<sup>2</sup> The essential features of such models are that they reproduce key features of the intact BBB and, most importantly, that they predict brain penetration *in vivo*. A number of recent studies suggest that MDCK cells, which have tight junctions, provide the model with the best predictive validity. The utility of these cells for BBB research has been further enhanced by engineering them to overexpress P-glycoprotein (P-gp), which transports compounds out of the brain.

## Ex Vivo Assessment

### In Situ Brain Perfusion

This approach measures the rate of entry across brain endothelium *in situ* by measuring the concentration of administered compound in the brain following removal of blood from the cerebral vasculature.

### Brain/Plasma Ratio

This provides a simple ratio of drug concentration in brain and plasma. The ratio can then be used to calculate an apparent permeability coefficient. It provides a simple measure of partitioning, but does not take into account the presence of drug in the brain vasculature. Results are expressed as a brain/plasma ratio.

### Ex Vivo Assessment of Receptor Occupancy

This is used to establish the ability of a lead compound to bind to target receptor in the intact brain. It thus also provides a useful measure of the pharmacodynamics of a lead compound.

## In Vivo Assessment<sup>3</sup>

### Sampling Ventricular Cerebrospinal Fluid

Cerebrospinal fluid (CSF) concentrations provide a good measure of free drug concentrations in the brain and can be performed *post-mortem* or by repeated sampling of CSF from the cisterna magna *in vivo*.<sup>4</sup>

### Tissue Microdialysis

The key compartment for a CNS drug is the brain extracellular fluid, which can be assessed by tissue microdialysis. This permits measurement of the concentration of a compound in brain extracellular fluid over time, and so delivers a complete pharmacokinetic assessment. This, together with its anatomical precision, makes this a powerful tool for CNS drug discovery.<sup>5</sup>

## Imaging in Humans

Positron emission tomography (PET) permits receptor occupancy studies to be conducted in humans; however, it has been used more frequently to assess the pharmacodynamics of lead compounds than to assess neuropharmacokinetic profile directly.

## Prediction of Blood–Brain Barrier Penetration

There is great interest in establishing the key determinants of passive entry into the CNS, mainly using physicochemical properties. *In silico* prediction methods offer the prospect of screening libraries of actual or virtual compounds on the basis of BBB permeation. A number of such models are available. However, because of the paucity of consistent brain permeation data, effective modelling is difficult and the utility of the models questionable. What is needed is an algorithm that has been informed and improved on the basis of BBB permeation data obtained using both *in vitro* and *in vivo* models.<sup>6</sup>

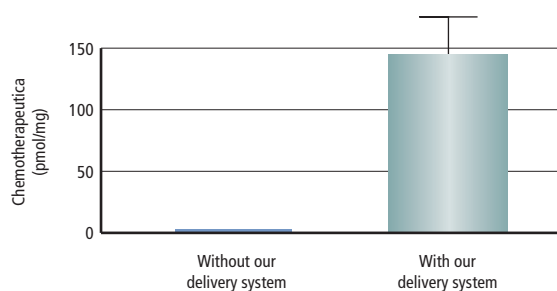
## Delivering Compounds Across the Blood–Brain Barrier

The brain endothelial cells are linked by various cell adhesion molecules that serve to make the junctions between such cells tight. This severely restricts the movement of hydrophilic solutes via the intercellular cleft (paracellular pathway), so most molecular traffic between blood and brain has to take a transcellular route; some compounds, such as the precursor to dopamine (L-DOPA), enter the brain by carrier-mediated transport. A safe, reliable and consistent method of delivering compounds across the BBB would therefore be highly desirable for the discovery and development of new therapeutics for disorders of the brain.

Traditional approaches to getting compounds into the brain are crude and include direct administration of therapeutic agents such as drugs or stem cells into the brain. This requires neurosurgery and is costly as well as invasive. An approach that does not require surgery is osmotic opening of the BBB by intracarotid infusion of hypertonic mannitol solution, which has been reported to increase the delivery of water-soluble drugs, peptides, antibodies and viral vectors to the brain.<sup>7</sup> Barrier opening via this method has been shown to last for six to eight hours,<sup>8</sup> but treatment-related toxicity was evident,<sup>9</sup> which has prevented the widespread use of this approach. The administration of the bradykinin B2 receptor agonist RMP-7 (also known as Cereport®) represents a more controlled method of opening the BBB, but results in only a very modest increase in drug transfer into the brain.<sup>10</sup>

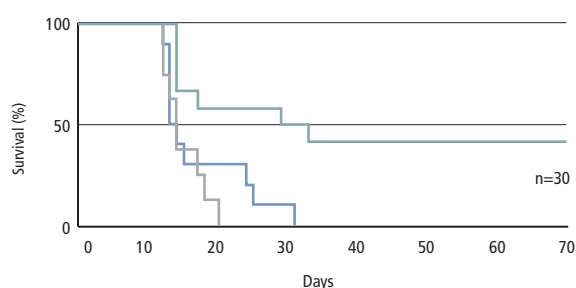
There is a need for a fresh approach to delivering compounds across the BBB that is both safe and effective. We have therefore developed a drug delivery system that transiently and reversibly opens the BBB to entry of compounds into the brain without inducing tissue injury. It is based on patented lipid-like structures and is offered as part of a package to comprehensively measure BBB penetration and kinetics using some of the assays described above. The lead delivery compound appears to be safe and well tolerated on the basis of pre-clinical testing and the system has

**Figure 1: Successful Delivery of a Chemotherapeutic Agent (Chemotherapeutica) into the Brain with Our Delivery System**



The figure shows the brain concentration of a systemically administered chemotherapeutic agent in the presence and absence of our delivery system.

**Figure 2: Chemotherapy in Animals with Experimental Glioma in the Presence and Absence of Our Delivery System**



The grey line represents the animals that were not treated. The animals that were treated with a standard concentration of a chemotherapeutic agent commonly used in the clinical environment are represented by the blue line. The green line represents the animals that were treated with a standard concentration of chemotherapeutica utilising our delivery system to enable a higher concentration of the chemotherapeutica to reach the tumour within the brain.

been shown to deliver both small molecules and large proteins effectively into the brain. It has been shown to increase substantially the brain penetration of a chemotherapeutic agent (see Figure 1) and markedly enhance its chemotherapeutic efficacy. Efficacy was transformed from a small extension of life to long-term survival in animals with experimental brain tumours (see Figure 2).

## Conclusion

The BBB fulfils a protective role for the brain, but severely limits the movement of potential medicines into the brain. A safe, reliable and consistent method of delivering compounds across the BBB and of assessing their kinetics will therefore open new vistas for CNS pharmacotherapy. In order to develop consequent CNS therapies, it is essential to have reliable methods of assessing the ability of compounds to penetrate the BBB and permeate into the brain. CerenseSM unlocks the potential of CNS therapeutic opportunities by providing a complete package that combines a novel technology that opens the blood–brain barrier – from Genzyme Pharmaceuticals – with the unique expertise of Pharmidex to assess brain penetration and neuropharmacokinetics. ■

- Duvernoy H, Delon S, Vannson JL, The vascularization of the human cerebella cortex, *Brain Res Bull*, 1983;11(4):419–80.
- Garberg P, Ball M, Borg N, et al., *In vitro* models for the blood brain barrier, *Toxicol In Vitro*, 2005;19(3):299–334.
- Alavijeh MS, Chisty M, Qaiser MZ, Palmer AM, Drug metabolism and pharmacokinetics, the blood brain barrier, and central nervous system drug discovery, *NeuroRx*, 2005;2(4):554–71.
- Walker MC, Tong X, Perry H, et al., Comparison of serum, cerebrospinal fluid and brain extra cellular fluid pharmacokinetics of aborigine, *Br J Pharmacol*, 2000;130:242–8.
- Alavijeh MS, Patsalos PN, A freely behaving animal model for the chronic and simultaneous study of pharmacokinetics and neuropharmacokinetics of drugs: an evaluation of carbamazepine, *Acta Neurologica Scandinavica*, 1990;82(Suppl. 133):S20.
- Clark DE, *In silico* prediction of blood brain barrier permeation, *Drug Discov Today*, 2003;8:927–33.
- Rapoport SI, Osmotic opening of the blood brain barrier: principles, mechanism, and therapeutic applications, *Cell Mol Neurobiol*, 2000;20:217–30.
- Siegal T, Ruinstein R, Bokstein F, et al., *In vivo* assessment of the window of barrier opening after osmotic blood brain barrier disruption in humans, *J Neurosurg*, 2000;92:599–605.
- Siegal T, Zylber-Katz E, Strategies for increasing drug delivery to the brain, *Clin Pharmacokinet*, 2002;41:171–86.
- Kroll RA, Neuwelt EA, Outwitting the blood–brain barrier for therapeutic purposes: osmotic opening and other means, *Neurosurgery*, 1998;42:1083–1100.