As a consequence of the growing ageing population, many neurodegenerative diseases – such as stroke, Alzheimer’s disease, and Parkinson’s disease – will become more prevalent. This, together with cancer, infections and a broad range of psychophysical and pain conditions are putting increasing pressure on pharmaceutical companies to produce more effective drugs for brain disorders. Although new neuropharmaceutical drugs are being developed, treatment is often complicated by the inability of these drugs to access their site of action because of the blood brain barrier (BBB). It is estimated that this barrier prevents more than 98% of systemically delivered potential therapeutic drugs from entering the brain. Many novel drugs are under development, but to exploit fully these new therapeutic approaches, there is a compelling need for drug developers to find improved ways of overcoming the blood brain barrier.

THE BLOOD BRAIN BARRIER

The fact that dye injected intravenously into animals stains all body tissues except the brain has been known for more than a century; this observation shows that the brain capillaries prevent molecules from entering the intact central nervous tissue. Thus, the concept of the blood brain barrier was established. The endothelial cells in the walls of blood vessels in the brain form a close-fitting monolayer connected by complex, and largely impassable, tight junctions. In addition, each brain capillary endothelial cell is composed of two lipid membranes: the luminal (facing the blood) and the abluminal (facing the brain).

The blood brain barrier is not entirely impenetrable, however. Like any other organ in the body, the brain requires nutrients and these are delivered via the bloodstream and must cross the BBB. There is a clear relationship between the lipid solubility of a drug and its CNS penetration; however, a significant number of molecules have a measured CNS penetration, which is not commensurate with that predicted on the basis of their lipid solubility. For example, several non-lipid molecules such as glucose and amino acids penetrate the brain far more readily than their lipid solubility would suggest. These molecules, together with others which are

Pep:Trans Peptide Vectors – A New Approach to Brain Delivery

Pep:trans technology overcomes many of the drawbacks associated with previous methods of crossing the blood brain barrier and offers a promising approach for the development of new drugs for the treatment of CNS diseases.

By Jamal Temsamani and Michel Kaczorek at Syntem

Jamal Temsamani, PhD, has been Director of Preclinical Research & Development at Syntem since March 1998; he is responsible for the preclinical programme and the development of Pep:trans technology. Between 1990 and 1998, he held several positions with US biotech company, Hybridon Inc, where he served as Senior Scientist, Associate Director and Scientific Director. Dr Temsamani received his PhD in Molecular Biology and Biochemistry at the University of Montpellier, France, and then completed a post-doctoral fellowship at the Worcester Foundation for Biomedical Research in the US. He has published more than 50 scientific articles and is co-inventor of numerous patents.

Michel Kaczorek, PhD, has 24 years’ experience in the biotechnology industry, and was a founder of Syntem in 1995. He received a PhD in Microbiology and a PhD in Virology at the University of Paris. In addition, he trained as a post-doctorate at Harvard Medical School and at the Institut Pasteur in Paris. Dr Kaczorek was in charge of research and development at Pasteur Vaccins in the area of recombinant vaccines; he was also one of the co-founders of Protéine Performance, SA, where he was the Scientific Director. He has published more than 40 scientific articles in international journals and is co-holder of 10 patents.
essential nutrients for the brain, have specialised carrier-mediated transport systems.

The accumulation of a therapeutic drug in the brain is a balance between its rate of influx and efflux. A variety of active efflux transporters may prevent therapeutic concentrations from being attained even if drug molecules are able to pass into the brain. A classic example is P-glycoprotein (P-gp) on the luminal surface of capillary endothelial cells; this restricts the passage of various molecules including anticancer agents (see Figure 1).

Researchers have investigated several strategies to deliver therapeutic molecules effectively to the brain in appropriate concentrations; these include bypassing the BBB, increasing the permeability of the BBB, modifying drugs so that they can make use of endogenous transport systems, and linking drug molecules to vectors or molecular transporters that can deliver them to the brain (1).

**STRATEGIES FOR DELIVERING DRUGS ACROSS THE BBB**

In order to avoid the brain barrier, invasive techniques such as craniotomy can be used to introduce drugs directly into the brain by intraventricular or intracerebral injection. This technique requires neurosurgery and carries the risk of infection and damage. It is used only in a few circumstances, such as the treatment of brain cancer.

Another way of getting neuropharmaceuticals into the brain is to ‘storm’ the BBB in order to temporarily and partially break down its walls. This can be achieved by using, for example, hyperosmolar disruption which causes tight junctions between endothelial cells to open and allows water-soluble compounds to enter the brain. Unfortunately, there are some causes for concern with this approach; while the BBB is disrupted, other blood molecules can enter the brain and may cause toxicity.

Since there is a clear relationship between the lipid solubility of a drug and its CNS penetration, designing a drug with optimal lipid solubility would be a desired solution. Unfortunately, this is often not possible. Simply increasing the lipid solubility of a drug molecule may have undesirable effects such as decreasing its solubility in aqueous fluids and its bioavailability.

If a small drug molecule can be modified to mimic nutrients such as amino acids or glucose, which are able to penetrate the BBB, then it may be possible for it to enter the brain using specialised carrier-mediated transport systems.

Another challenging situation is the development of large biomolecules, which generally cannot cross the BBB because of their size and polarity. This can be overcome by binding these large molecules to transporters that specifically bind to receptors on the BBB – such as monoclonal antibodies to transferrin receptors on the BBB – thereby enabling transport across the barrier. A possible drawback to this approach is that some vectors have receptors in organs other than the brain, and this may lead to unwanted drug delivery to these other organs.

**A NEW APPROACH: PEP:TRANS VECTORS**

An alternative approach, Pep:trans technology, overcomes many of the drawbacks of previous methods and involves the use of small linear synthetic peptides (SynB vectors) (1). These peptides, 10 to 15 amino acids long, are able to interact with the cell surface and to cross the plasma membrane without disrupting the membrane activity. Among the series of sequences available, some
are able to cross the BBB. Furthermore, the internalisation of these peptides into the brain does not appear to be dependent on a chiral receptor, since the D-enantiomer form penetrates as efficiently as the parent peptide (L-form). These peptides were the starting point for developing a new potent strategy for drug transport into complex biological membranes such as the BBB. Using linker molecules, drugs can be chemically conjugated to the peptide vectors and delivered through the BBB. If the linker were designed to be cleavable, then once the drug reached the brain it would be released into the brain parenchyma.

The ability to deliver neuropharmaceuticals to the brain represents a big challenge due to the restrictions imposed by the blood brain barrier. While researchers have devised many ingenious approaches that avoid, disrupt or exploit the BBB’s specialised transport mechanisms, many of these continue to have significant drawbacks. Rapid advances in neuroscience are leading to a proliferation of potent molecules, but if neuroscientists and clinicians are to benefit from these advances, the continuing refinement of new delivery methods will be essential to realising the potential of these molecular drugs.

Transport of Active Molecules
The efficacy of Pep:trans derived vectors (the SynB family) in enhancing the brain uptake of the anti-cancer agent doxorubicin was assessed using in situ cerebral perfusion in rats and mice (2). This ‘vectorisation’ of doxorubicin requires its conjugation to peptide vectors via a succinate linker, and was found to significantly enhance its brain uptake, without compromising BBB integrity. Following intravenous administration, the brain concentrations were higher for vectorised doxorubicin compared with free doxorubicin. Vectorised doxorubicin shows significantly lower levels in the heart, strongly suggesting that cardiotoxicity – the main side effect of doxorubicin – can be dramatically reduced using this strategy.

Interestingly, vectorisation of anticancer drugs such as doxorubicin and paclitaxel with SynB vectors led to bypass of the P-glycoprotein, which has been shown to be present in the luminal membrane of BBB endothelial cells and restricts entry into the brain of a broad number of therapeutic compounds, including cytotoxic drugs (2). This new property results in the ability of the vectorised anticancer drug to exercise its cytotoxicity even on resistant cancer cells.

A similar enhancement in brain uptake was obtained with another small molecule – the antibiotic, benzylpenicillin (B-Pc). Beta-lactam antibiotics are often used for treatment of CNS infections, but their poor penetration into the brain does not provide sufficient efficacy. The brain uptake of B-Pc coupled to SynB1 vector via a glycolamidic ester linker was measured using in situ brain perfusion. The brain uptake of coupled B-Pc showed an average eight-fold increase in comparison with free B-Pc. This increase was quite similar in the seven explored grey areas of the rat brain.

Pharmacological Applications
In a recent pharmacological application focused on pain management, we have coupled morphine-6-β-glucuronide (M6G) to SynB3, another Pep:trans vector. M6G is an active metabolite of morphine and was chosen because it has been reported to be more potent than morphine after central administration. As the affinity of both substances for the μ receptors has been reported to be similar, a possible explanation for this observation could involve a difference in the permeability of the BBB to M6G. In fact, several reports have indicated a significantly lower BBB permeability to M6G by systemic administration, in comparison with morphine.
Our study showed that SynB3 significantly enhances the brain uptake of M6G as measured by in situ brain perfusion in mice. Vectorisation of M6G with the SynB3 vector resulted in a significant enhancement in the analgesic effect of M6G using the tail-flick and hot-plate tests in mice. The ratio of the antinociceptive ED50 of M6G and morphine over vectorised M6G was approximately 3 and 4 respectively on a molar basis. This result indicates that vectorisation leads to the improvement of brain uptake and, as a consequence, to an enhancement of the antinociceptive activity of M6G.

Similar results were obtained with dalargin, a hexapeptide analogue of leu-enkephalin that has no systemic antinociceptive activity by systemic administration due to its poor brain uptake. Conjugation of dalargin to SynB vectors led to a considerable enhancement of analgesic activity immediately after intravenous injection.

**Mechanism of Transport of Pep:trans Vectors**

We have shown by in situ perfusion studies, a technique allowing a first-pass exposure, that the internalisation of Dox-SynB is a saturable process (3). The measured Km, which was in the range of 4 to 9 µM, compares well with the values observed for substrates reported to be taken up by adsorptive-mediated endocytosis. Furthermore, no difference in brain uptake was observed between doxorubicin linked to L-SynB or to D-SynB vectors, indicating that a stereospecific receptor is not required for its brain transport. In addition, we have reported that the passage of Pep:trans peptides can be inhibited in a competitive manner by polycationic molecules, such as poly(L)lysine or protamine, which act as endocytosis inhibitors. These observations suggest that the mechanism of transport across the BBB by SynB vectors acts via an energy dependent adsorptive-mediated endocytosis mechanism (3). It is known that at physiological pH values, the luminal surface of the brain endothelium presents an overall negative charge and thus creates an environment more selective for positively charged substances. The SynB peptides are positively charged, which likely plays a key role in the adsorptive-mediated endocytosis process. The electrostatic interactions of the peptide vector with the surface of endothelial cells may mediate surface-binding and subsequent internalisation of the peptide vectors into the brain capillaries.

**CONCLUSION**

The ability to deliver neuropharmaceuticals to the brain represents a big challenge due to the restrictions imposed by the blood brain barrier. While researchers have devised many ingenious approaches that avoid, disrupt or exploit the BBB’s specialised transport mechanisms, many of these continue to have significant drawbacks. Rapid advances in neuroscience are leading to a proliferation of potent molecules, but if neuroscientists and clinicians are to benefit from these advances, the continuing refinement of new delivery methods will be essential to realising the potential of these molecular drugs. The use of peptide vectors represents a novel and promising approach. Their small size, rapid uptake, ease of drug attachment and versatility in the range of molecules that they can deliver, provide an innovative approach for developing new drugs for the treatment of CNS diseases.

The authors can be contacted at jtemsamani@syntem.com

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