Enhanced Blood to Brain Drug Delivery

A pegylated liposomal drug delivery technology combined with the tripeptide glutathione as a targeting ligand provides a safe and versatile method to enhance drug delivery to the brain.

The central nervous system (CNS) is one of the most difficult organs to reach when it comes to the treatment of brain-related illnesses. This unmet medical market is very large as it affects 2 billion people worldwide - a number that is expected to grow with increasing life expectancy and an expanding global population (1). Although most drugs currently in development against neurological targets show high efficacy for their target, they often cannot reach the brain in sufficient amounts to exert an effect due to presence of the neuroprotective blood-brain barrier. This results in cessation of their development due to, for example, dose-limiting toxicity outside the CNS or off-target effects. This includes approximately 98 per cent of the small molecules and nearly all of the large biotechnology drugs, such as recombinant proteins or gene-based medicines (2).

GATEKEEPER OF THE CNS

To maintain homeostasis in the CNS, the blood-brain barrier regulates the entry of compounds into the brain; while it allows certain ions, neurotransmitters and nutrients to enter, it actively excludes, effluxes and metabolises potential neurotoxic compounds (including plasma proteins, cytokines, antibodies, drugs, bacteria and viruses). This sophisticated cellular barrier is formed by the interaction of at least four cell types (brain capillary endothelial cells, astrocytes, pericytes and neuronal cells), and is dynamically regulated to meet the intrinsic requirements of the CNS. Since the blood-brain barrier also keeps out most therapeutic compounds, treating CNS-related diseases is very difficult. Therefore, there is unprecedented demand for new methods to safely deliver these potential new drugs into the brain. Currently, there are only a few approaches for brain drug delivery and these tend to be limited in their application, highly invasive or disruptive to the neuroprotective blood-brain barrier (3-5).

A LIPOSOMAL BRAIN DELIVERY SYSTEM

At to-BBB, we have developed a drug delivery platform, G-Technology® , that comprises a pegylated liposomal drug delivery technology combined with the tripeptide glutathione as a targeting ligand at the tips of the polyethylene glycol (PEG) molecules. This provides a safe method to enhance drug delivery to the brain, as both components are already on the market; pegylated liposomes encapsulating chemotherapeutics, antifungal agents, vaccines and so on are in use for several indications. Furthermore, glutathione is used for instance as a supportive therapy in cancer (in high doses) and as a food supplement.

Glutathione is an endogenous tripeptide that possesses antioxidant-like properties and plays a central role in the detoxification of intracellular metabolites; it has specific and active uptake transporters expressed at the blood-brain barrier (6-8). Based on these properties and on previous unpublished validation results from Dr Maggie Lu at the Industrial Technology Research Institute (ITRI, Hsin-Chu,
Taiwan, Republic of China (RoC)) the ITRI was the first to file patents describing glutathione-mediated drug delivery... glutathione-mediated drug delivery

Table 1: Ten key development criteria for targeted blood-brain drug delivery

<table>
<thead>
<tr>
<th>Targeting the blood-brain barrier</th>
<th>Drug carriers</th>
<th>Drug development from lab to clinic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proven inherently safe receptor biology in humans</td>
<td>No modification of active ingredient</td>
<td>Low costs and straightforward manufacturing</td>
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<td>Safe and human applicable ligand</td>
<td>Able to carry various classes of molecules</td>
<td>Activity in all animal models</td>
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<td>Receptor specific binding</td>
<td></td>
<td>Strong IP protection</td>
</tr>
<tr>
<td>Applicable for acute and chronic indications</td>
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<td>Favourable pharmacokinetics</td>
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</tbody>
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In several proof-of-concept studies using rodent models for pain, brain tumours and viral encephalitis, to-BBB and ITRI have demonstrated that glutathione-PEG liposomes loaded with peptides and small molecules safely enhanced the delivery of these drugs to the brain, thereby decreasing pain sensitivity, inhibiting tumour growth and prolonging survival (10), and reducing the lethal effects of viral infections in the brain. Furthermore, a technological and mechanistic validation assay has shown that the free drug was delivered to the extracellular fluid of the brain; increasing the amount of glutathione on the outside of the liposomes resulted in a free drug concentration that was up to a five times higher when compared with non-targeted liposomes (11). Although the ultimate brain uptake and efficacy of any encapsulated compound will depend on the compound as well as the disease, to-BBB will be able to test and optimise the G-Technology for almost every specific situation.

Figure 1: The G-Technology consists of liposomes with glutathione-modified PEG on the outside. In this example, hydrophilic drugs are included in the aqueous core of the liposomes; however, lipophilic drugs can be included in the lipid bilayer.

STRENGTHENING THE PLATFORM

The versatility of the G-Technology comes from the liposomes – that is, vesicles comprising a lipid bilayer with an aqueous core in which both lipophilic and hydrophilic molecules can be entrapped (see Figure 1). Furthermore, the addition of PEG gives the liposomes stealth-like properties as it minimises scavenging of the liposomes by the body's defence system, thus enabling a long circulation time in the blood.

Over the past year, to-BBB has entered into several research collaborations to further investigate the possibilities for the G-Technology. Major interest from top-tier pharmaceutical and biotechnological companies is not only a reflection of the difficulty of transporting drugs across the blood-brain barrier, it also stresses the potential of the G-Technology. Research agreements have been made to investigate therapeutics ranging from small molecules to proteins. A typical research collaboration requires input from both sides. The in vivo testing is done at the pharmaceutical/biotechnology company, as they have the necessary equipment and scientists experienced in the selected pharmacology models. Scientists at to-BBB will concentrate on optimising the encapsulation and developing several batches that are then tested for stability and release of the compound, before preparation of the batches for in vivo testing. In most cases, two formulations with different release profiles will be made using a variety of lipid compositions. The transition temperature of the lipids influences the stability of the liposomes and thus the release profile of the encapsulated compound, as well as the plasma half-life of the lipidome. By tailor-making liposomes for each specific compound and pharmacology model, to-BBB, together with its partners, strives to optimise the G-Technology specifically for each situation.

Although versatile, the G-Technology is not a magic bullet delivering all drug compounds to the brain. to-BBB is, however, focusing on 10 key development criteria for safe and efficacious drug delivery to the brain with all of its research collaborations as well as with its lead compound. These criteria are related to targeting the blood-brain barrier, drug carriers and drug development from laboratory to clinic (see Table 1). Although the G-Technology is adhering to the criteria related to glutathione (as the targeting ligand) and liposomes (drug carriers), adherence to the last three criteria is also dependent on the compound that will be included (12). To bring a drug to the market, straightforward manufacturing and low (or justifiable) costs are favourable – if not essential. The encapsulation of recombinant proteins into the liposomes will be more expensive compared with the encapsulation of synthetic small molecules or peptides – but as long as these increased costs are justified and/or a means of reducing costs can be found in the production process (for example, recovery of non-
encapsulated material), it will be possible to develop these products.

2B3-101: CLINICAL LEAD

Our lead product at to-BBB, glutathione pegylated liposomal doxorubicin (2B3-101), is based on the already marketed pegylated liposomal doxorubicin (Doxil/Caelyx). Therefore, this product is adhering to all 10 of the key development criteria. The active ingredient, doxorubicin, is active in all animal models. Furthermore, the existing production process for Doxil/Caelyx can be easily adapted at minimal cost to include PEG modified with glutathione.

Studies in a mouse model of glioblastoma multiforme have shown that 2B3-101 reduces brain tumour growth more effectively than Doxil/Caelyx. In addition, 2B3-101 prolongs survival by up to 60 per cent when given at the maximum tolerated dose. Extensive GLP toxicity, safety and toxicokinetic evaluations have shown equal or less severe findings for 2B3-101 as compared with Doxil/Caelyx, possibly driven by the pharmacokinetic (PK) profile; the half-life of 2B3-101 in plasma was about 25 hours versus 28 hours for Doxil/Caelyx. For the development of 2B3-101, to-BBB obtained clinical scale batches of 2B3-101, produced according to cGMP standards by TTY Biopharm (Taipei, Taiwan, RoC).

FUTURE CHALLENGES

While to-BBB, together with its partners, has learned much about the possibilities of the G-Technology in the past year, many more challenges lie ahead. 2B3-101 as a lead product will be ready to enter clinical trials in 2011; however, to-BBB will continue to build the case by extending preclinical research. For the collaborations, further efforts will be made to optimise the G-Technology for active compounds to be able to meet the unmet need of patients with devastating brain diseases.

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References