When it comes to treatment of brain-related illnesses, the central nervous system (CNS) is one of the most difficult organs to reach. This unmet medical market is very large as it affects two billion people worldwide, a number which is expected to grow with increasing life expectancy and expanding global population.1 Although most drugs currently in development against neurological targets show high efficacy for the 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 the majority of the small molecules and nearly all of the large biotechnology drugs, such as recombinant proteins or gene-based medicine.2

THE BLOOD-BRAIN BARRIER: GATEKEEPER OF THE CNS

The blood-brain barrier dynamically regulates the entry of compounds into the brain to meet the intrinsic requirements of the CNS. Neurotransmitters, nutrients and certain ions are able to enter, while potential neurotoxic compounds (including plasma proteins, cytokines, antibodies, drugs, bacteria and viruses) are actively excluded, effluxed and metabolised. Since the blood-brain barrier also keeps out most therapeutic compounds, there is unprecedented demand for new methods to deliver these potential new drugs into the brain safely. Currently, there are only few approaches for brain drug delivery and these are limited in their application, highly invasive, or disruptive to the neuroprotective blood-brain barrier.3,4

THE G-TECHNOLOGY

The G-Technology is to-BBB’s core platform; it is a pegylated liposomal drug delivery technology with glutathione as targeting ligand at the tips of the 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, fungal...
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 optimise the encapsulation, and develop several batches that are tested for stability and the release of the compound before preparation of the batches used for in vivo testing. Mostly two formulations with different release profiles will be made, using different 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 liposome. 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. However, to-BBB is focusing on ten 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 (see Figure 2).

These criteria are related to targeting the blood-brain barrier, drug carriers, and drug development from laboratory to clinic. Although the G-Technology is adhering to the criteria related to glutathione (as targeting ligand) and the liposomes (drug carriers), adherence to the last three criteria is also dependent on the compound that will be included.

To bring a drug to the market, straightforward manufacturing and low (or justifiable) costs are favorable, if not essential. Inclusion of recombinant proteins into the liposomes will increase the costs more compared with the inclusion of synthetic small molecules or peptides, but as long as these are justified and/or means of reduction of costs can be found in the production process (e.g. high encapsulation efficiency or recovery of non-encapsulated material) it will be possible to develop these products.

<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>1. Proven inherently safe receptor biology in humans</td>
<td>6. No modification of active ingredient</td>
<td>8. Low costs &amp; straightforward manufacturing</td>
</tr>
<tr>
<td>2. Safe and human applicable ligand</td>
<td>7. Able to carry various classes of molecules</td>
<td>9. Activity in all animal models</td>
</tr>
<tr>
<td>3. Receptor specific binding</td>
<td></td>
<td>10. Strong IP protection</td>
</tr>
<tr>
<td>4. Applicable for acute and chronic indications</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Favorable pharmacokinetics</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 2: Ten key development criteria for targeted blood-to-brain drug delivery.

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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. Based on these properties and on previous unpublished validation results from Dr Maggie Lu (at the Industrial Technology Research Institute (ITRI) in Hsin-Chu, Taiwan, R.O.C.), ITRI was the first to file patents describing glutathione-mediated drug delivery to the brain. In 2008, to-BBB technologies BV obtained the exclusive worldwide rights to commercialise these patents for the targeted delivery of drugs to the brain.

In several proof-of-concept studies, to-BBB and ITRI have demonstrated that glutathione PEG liposomes loaded with peptides and small molecules safely enhanced the delivery of drugs to the brain, thereby exerting an effect in models for pain, brain tumors, viral encephalitis, and neuroinflammation. Furthermore, a technologic 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 up to five times higher compared with non-targeted liposomes. 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.

STRENGTHENING THE PLATFORM

The versatility of the G-Technology comes from the liposomes, i.e. vesicles existing of 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 blood.

In the past year, to-BBB has entered several research collaborations to investigate the possibilities of the G-Technology further. The large interest from top-tier pharmaceutical and biotechnological companies not only indicates the difficulty of transporting drugs across the blood-brain barrier, but it also stresses the potential of the G-Technology.

Research agreements have been made to investigate therapeutics ranging from small infections, vaccines, etc are in use for several indications. Furthermore, glutathione is used as supportive therapy in cancer (in high doses) and as a food supplement.
THE G-TECHNOLOGY IN PRACTICE: 2B3-101 AS PRIME EXAMPLE

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

The first clinical trial with 2B3-101 was initiated in June 2011. This trial is primarily designed to evaluate the safety of 2B3-101 in patients with solid tumors with brain metastases. However, specific efficacy data in patients with brain metastases from breast cancer will be obtained at the latest stage of the trial. Preclinical research with 2B3-101 has shown that 2B3-101 reduces brain tumor growth more efficacious than Doxil/Caelyx. Furthermore, 2B3-101 prolongs survival of mice with experimental brain tumors up to 60% when given at the maximum tolerated dose. Extensive GLP toxicity, safety and toxicokinetic evaluations have shown equal or less severe findings for 2B3-101 compared with Doxil/Caelyx, possibly driven by the pharmacokinetic (PK) profile – the half-life of 2B3-101 in plasma was about 30 hours versus 36 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 (Taiwan).

FUTURE CHALLENGES

While to-BBB, together with its partners, has gained much experience about the possibilities of the G-Technology in the past years, many more challenges lie ahead. 2B3-101 as a lead product has recently entered clinical trials. However, to-BBB will continue to build the case by extending preclinical research. Furthermore, to-BBB is continuing its efforts for the treatment of devastating brain diseases with its second lead, 2B3-201, glutathione pegylated liposomal methylprednisolone, for the treatment of neuro-inflammation. Initial preclinical studies have shown that 2B3-201 prolongs half-life and increases brain uptake of methylprednisolone compared with the free compound, thereby increasing its efficacy at a much lower dose.

Simultaneously, to-BBB will continue to work together with other pharmaceutical and biotechnical companies 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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ABOUT THE AUTHORS:

Willem van Weperen has more than 15 years’ experience as a pharma/biotech leader. Before becoming to-BBB’s CEO, he held several global commercial, clinical and general management positions mostly at Genzyme.

Pieter Gaillard is an entrepreneur and scientist who co-founded to-BBB and currently holds the position of CSO. With his extensive pharmacology and CNS experience he is globally recognised as an expert in the blood-brain barrier research field.

REFERENCES: