A focus on enzyme replacement therapies (ERT) for lysosomal storage diseases has led Shire Human Genetic Therapies, Inc (Shire HGT) to develop products for treating Fabry disease, Hunter syndrome, and type 1 Gaucher disease. These products are administered intravenously (IV) and are effective in treating the somatic symptoms of the disease.

Developing ERT for diseases involving the CNS is a challenge because IV administered enzyme does not adequately cross the blood-brain barrier (BBB) at a level needed for therapeutic effect. Moreover, formulations that are suitable for CNS administration are limited, posing a major challenge in generating stable products of adequate concentration.

By developing new methods and formulations to deliver enzymes to the CNS, Shire HGT is at the forefront of developing ERT for treating lysosomal storage diseases with CNS involvement. ERT for CNS symptoms in Hunter syndrome and Sanfilippo A syndrome are in Phase I clinical development, and efforts are ongoing in preclinical development for metachromatic leukodystrophy and globoid cell leukodystrophy.

**STRATEGIES FOR CNS DELIVERY OF THERAPEUTICS:**

The blood-brain barrier is a structural feature of endothelial cells which restricts the diffusion of microscopic objects (such as bacteria) and large or hydrophilic molecules (such as proteins) into the cerebrospinal fluid (CSF), while allowing the diffusion and active transport of selected small molecules. This barrier creates a challenge in delivering adequate levels of protein therapeutics to the brain when administered by IV injection. Several strategies have been designed to overcome this hurdle for CNS drug delivery:

- **Receptor-Mediated Transport:** Some large molecules essential for brain function are transported across the blood-brain barrier through active transport or transcytosis. These molecules or their mimetics can be used as vehicles for delivering peptides and other compounds to the brain. The transferrin uptake system has been well studied as a possible avenue into the brain following systemic administration. Covalent association of transferrin to a protein has resulted in enhanced delivery to the brain following intravenous administration.

- **Convection-Enhanced Delivery:** This modality involves the stereotactic placement of several catheters into brain parenchyma through cranial burr holes and the subsequent infusion of agents via a microinfusion pump. Convection-enhanced delivery uses a pressure gradient established at the tip of an infusion catheter to push a drug into the extracellular space. The intention is to distribute the drug more evenly, at higher concentrations, and over a larger area than when administered by diffusion alone. The placement and monitoring of these devices requires specialised technology which is limited to only a few centres across the globe. This factor, along with the invasive nature of the technique, has limited
The routes of CNS delivery are shown in Figure 1 and briefly described below:

- **Intraventricular (ICV) Delivery**: This route delivers drugs directly into the ventricle. ICV delivery can be facilitated via the Ommaya reservoir or other access port that is implanted or external. The catheter is most commonly inserted between the laminae of the lumbar vertebrae and the tip is threaded up the thecal space to the desired level (generally L3-L4). IT delivery of ERTs via lumbar puncture has been described.\(^2\)

- **Intracisternal (ICS) Delivery**: Direct delivery of 30 mg Enzyme into Neurons after an IT dose is connected to an implanted access port.

- **IT-Lumbar Delivery**: IT administration is the most common route for direct administration of drugs into the CSF.\(^3\) Procedurally, this is done by either lumbar puncture (slow bolus) or via port-catheter delivery systems (infusion or bolus). An implanted catheter is connected to a reservoir (for bolus) or an infusion pump, either implanted or external. The catheter is most commonly inserted between the laminae of the lumbar vertebrae and the tip is threaded up the thecal space to the desired level (generally L3-L4). IT delivery of ERTs via lumbar puncture has been described.\(^3\)

**INTRATHECAL DELIVERY CONSIDERATIONS**

Distribution of a therapeutic following IT administration is primarily dependent on CSF flow and diffusion into the brain tissue.\(^1\) The CSF is produced at 20 mL/hr in humans with a turnover of 3.7 times per day. CSF flow is initiated from its site of production (the choroid plexus) in all ventricles, enters cisterna magna via holes (foramina), circulates over the surface, and reabsorbs in arachnoid granulation. There is a bidirectional flow around the spinal chord which should facilitate diffusion of IT administered drugs towards the brain following lumbar puncture.

Protein delivery to the brain is typically diffusion limited. When nerve growth factor was administrated as a polymer implant into the brain interstitium, it diffused into brain tissues only 1-3 mm over several days.\(^12\) Simulation analyses indicated that this slow penetration is due to the protein’s slow diffusion rate.\(^13\)

Currently, CNS therapies requiring neural delivery of the drug are limited to small, hydrophobic molecules that enter cells by membrane diffusion or by modification of proteins to utilise active transport processes.\(^14\)

In contrast, we have observed considerable brain tissue distribution following IT delivery of our lysosomal enzyme therapeutics without any protein modification (Figure 2).

Such unique brain distribution may be due to axonal transport \(^{15}\) by way of the glycosylation structure that targets uptake into the target tissues and organelles. Mannose-6-phosphate (M6P) receptor-mediated uptake of M6P-containing glycoproteins targets our enzyme therapeutics to the cells and subsequently into the site of action in the lysosome. Neurons have been shown to contain M6P receptors.\(^16\)

**PROTEIN SOLUBILITY AND STABILITY CONSIDERATIONS**

General considerations for formulations for CNS delivery were summarised by Grouls.\(^17\)

IT-Lumbar delivery is limited by the delicate

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### Table: Composition of CSF and Elliott’s B Solution

<table>
<thead>
<tr>
<th>Solution</th>
<th>Na(^+) mEq/L</th>
<th>K(^+) mEq/L</th>
<th>Ca(^{2+}) mEq/L</th>
<th>Mg(^{2+}) mEq/L</th>
<th>HCO3(^-) mEq/L</th>
<th>Cl(^-) mEq/L</th>
<th>pH</th>
<th>Phosphorous mg/L</th>
<th>Glucose mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF</td>
<td>117-137</td>
<td>2.3</td>
<td>2.2</td>
<td>2.2</td>
<td>22.9</td>
<td>113-127</td>
<td>7.31</td>
<td>1.2-2.1</td>
<td>45-80</td>
</tr>
<tr>
<td>Elliott B</td>
<td>149</td>
<td>2.6</td>
<td>2.7</td>
<td>2.4</td>
<td>22.6</td>
<td>132</td>
<td>6.0-7.5</td>
<td>2.3</td>
<td>80</td>
</tr>
</tbody>
</table>

---

### Figure 3: Composition of CSF and Elliott’s B Solution

- CSF
- Elliott B

### Figure 4: List of Approved Intrathecal Formulations

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Method of IT Administration</th>
<th>Dose Volume</th>
<th>pH</th>
<th>Excipients</th>
</tr>
</thead>
<tbody>
<tr>
<td>baclofen</td>
<td>device, bolus and infusion</td>
<td>2 mL</td>
<td>5 to 7.0</td>
<td>NaCl, Water</td>
</tr>
<tr>
<td>bupivacaine</td>
<td>incremental doses per volume</td>
<td>2 to 20 mL</td>
<td>4.0 to 6.5</td>
<td>NaCl, Water</td>
</tr>
<tr>
<td>cytarabine</td>
<td>bolus injection</td>
<td>6 mL</td>
<td>7.4 to 7.7</td>
<td>NaCl, Water</td>
</tr>
<tr>
<td>Depocyt</td>
<td>bolus injection (1 to 5 minutes)</td>
<td>5 mL</td>
<td>5.5 to 8.5</td>
<td>NaCl, Water, Lipids</td>
</tr>
<tr>
<td>lohexol</td>
<td>bolus injection (1 to 2 minutes)</td>
<td>up to 17 mL</td>
<td>6.8 to 7.7</td>
<td>0.1 mg EDTA</td>
</tr>
<tr>
<td>morphine sulfate</td>
<td>bolus injection</td>
<td>1 to 2 mL</td>
<td>2.5 to 6.5</td>
<td>NaCl, Water</td>
</tr>
<tr>
<td>morphine sulfate</td>
<td>bolus injection</td>
<td>5 mL</td>
<td>5.0 to 8.0</td>
<td>NaCl, Water</td>
</tr>
<tr>
<td>penicillin G potassium buffered</td>
<td>bolus injection</td>
<td>6.0 to 8.5</td>
<td>6.8 mg NaCl, 65.6 mg KC1, buffered with Na citrate and citric acid</td>
<td></td>
</tr>
<tr>
<td>prialt</td>
<td>microinfusion device, long term</td>
<td>N/A</td>
<td>4.0 to 5.0</td>
<td>NaCl, L-methionine</td>
</tr>
<tr>
<td>sufentanil citrate</td>
<td>slow injection/infusion</td>
<td>0.2 to 1 mL</td>
<td>3.5 to 6.0</td>
<td>citric acid to adjust pH</td>
</tr>
</tbody>
</table>

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**Figure 4: List of Approved Intrathecal Formulations**

- baclofen
- bupivacaine
- cytarabine
- Depocyt
- lohexol
- morphine sulfate
- morphine sulfate
- penicillin G potassium buffered
- prialt
- sufentanil citrate

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balance of CSF composition and intracranial pressure. Thus, without removal of CSF, the dose volume is limited to ≤3 mL in humans (and ≤1 mL in the adult cynomolgus monkey).

Dose volume limitation necessitates high concentration protein formulation (>10 mg/mL) when doses are in the several tens of milligrams. Several factors can impact protein solubility to achieve the desired concentration, including ionic strength, amino acid sequence and co-solubilising agents.

Solution compositions used routinely for CNS administration are isotonic saline (unbuffered) or Elliott’s B solution (artificial CSF) with a composition listed in Figure 3. Isotonic solutions may not render adequate solubility for some proteins. Additionally, Elliott’s B solution contains a very low buffer concentration that may not provide adequate buffering capacity needed to stabilise protein formulations during long-term storage. The artificial CSF solution also contains various salts which are often not compatible with protein formulations. For example, calcium salts may mediate protein precipitation.

**IN VIVO TOLERABILITY**

As mentioned, saline and phosphate buffered saline are the most commonly used vehicles for formulating or diluting drugs for direct CNS delivery as well as for flushing the delivery system before and after dose administration. We have discovered that small differences in buffer concentration and pH have a very large impact on in vivo safety and tolerability of the administered solution. A preliminary study in adult cynomolgus monkeys was conducted to evaluate the toxicology and safety pharmacology of repeated IT-lumbar doses of our enzyme. Each animal was implanted with a port-catheter system to facilitate an every-other-week dosing regimen.

The device control animals received phosphate buffered saline at pH 7.2. The vehicle-control group was dosed with an aequous solution of 20 mM sodium phosphate, 130 mM NaCl, and 0.005% polysorbate 20 at pH 7.5. This formulation was evaluated as it rendered adequate solubility and stability to the protein.

Clinical signs were observed during and immediately after dosing; the incidence was comparable between the control groups (device control and/or vehicle-dosed group) and enzyme-dosed groups, with no evidence of a dose response. Consequently, the study was terminated after the second dose. A representative view of the histopathology is given in Figure 8.

These clinical observations, which occurred in all animals including the vehicle-dosed animals, prompted a series of toxicology studies
of vehicle formulations of varying phosphate buffer concentrations and pH, as well as dose volumes (Figure 9).

In this screening study, four animals per arm were dosed four times on days 1, 5, 14, 19. The clinical observations noted in the animals receiving the initial vehicle (20 mM sodium phosphate, 130 mM NaCl, 0.005% polysorbate 20, pH 7.5) were reproduced with formulations containing a sodium phosphate concentration of ≥ 10 mM and a pH above 7.0.

Tolerability was improved by lowering the dose volume from 1.5 mL to 1.0 mL. Formulations with lower phosphate concentration pH 5.5-7.0 were well tolerated. Of the vehicles which were well tolerated, a vehicle comprising 5 mM sodium phosphate, 145 mM NaCl, 0.005% polysorbate 20 at pH 7.0 was found to be suitable for solubility and stability of the product. There were no adverse clinical signs from four IT administrations of the enzyme in this vehicle (14 mg enzyme in 1.0 mL dose volume) over three weeks. This low-pH, low-phosphate vehicle provided suitable enzyme stability for clinical development. These studies defined the formulation design space suitable for IT dosing (Figure 10).

In summary, CNS delivery of protein therapeutics is an underdeveloped area of research that requires a balancing act of identifying compositions which render adequate solubility, in vivo tolerability of the pharmaceutical composition, and adequate long-term stability to be able to commercialise the product (Figure 11).

ACKNOWLEDGEMENTS

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