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Penetrating the impenetrable

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Nosing across the blood-brain barrier

The 10-year-old boy stares up at the ceiling from his bed, a crooked smile crossing his face.

His parents come and go, checking on him periodically to make sure he is OK. They know they won't disturb him; he doesn't really see them. And even when he does, there is little behind those eyes to suggest recognition.

The boy has Hurler Syndrome, the most severe form of mucopolysaccharidosis type I (MPS I). It is a condition that has effectively left him in a vegetative state, an infant in a preteen body—a life challenged by the lack of a single enzyme.

Enzyme replacement therapy (ERT) helps with some of the symptoms. It's likely that it has allowed him to live this long.

But the enzyme cannot reach his brain to stabilize his neurological decline. The enzyme can get close, passing through capillaries, but cannot cross the blood-brain barrier (BBB).

Facing our limits

Aside from a better understanding of why some central nervous system (CNS)-directed drugs work and others don't, says ICON's senior vice president of drug development services, Peter Schueler, there is a need to understand how we bring the drug to the brain itself.

"The BBB is a significant hurdle when it comes to bringing some, at least larger, molecules to the brain," he continues. "This is a highly protective physiological barrier that we use every day; we couldn't survive without it."

In other parts of the body, capillaries allow a relatively free exchange of substances between the blood and surrounding tissue. In the brain, this exchange is significantly curtailed by intra- and intercellular complexes and machinery (e.g., efflux pumps and tight junctions).

While ions and small compounds may pass between the cells in paracellular transport or through cells by passive diffusion, other molecules require active transport.

The importance of this interface is what takes President and CEO of Bioasis Mark Day by surprise when he sees how little importance the pharmaceutical industry gives the BBB, even though failure to do so can result in billions of dollars lost.

Rather than get a deeper understanding of the BBB, he says, "they go back to this old kind of cookbook approach to making drugs," putting too much onus on the results of in-vitro screens such as the PAMPA BBB assay.

Megan MacBride, director of commercial models at Taconic, shares Day's concerns, suggesting that in-vitro models have their place, but that they are ultimately too limited in scope.

"The ADMET folks have developed a huge number of in-vitro assays, and they are always starting in vitro," she explains. "Does this serve as a substrate for this transporter? Do I get transport in a Caco-2 model? They're looking at direct transport across in-vitro cells."

"When we're talking about the BBB, we're talking about transporters, receptors, tight junction proteins; you're not getting that whole system in vitro," MacBride continues.

It is this lack of understanding that Schueler believes might explain the doom of clinical studies from decades ago.

"There is good reason to believe that some of the clinical studies from 10 to 15 years ago did not really work in CNS because the drug just didn't reach the target," he says. "We were just too optimistic that the levels of drugs we reached in the body was representative of the amount of drug reaching the brain, and that is not always true."

"We cannot take a brain sample, like we do a blood sample, to indicate how much drug is reaching the liver or the lung," he continues, adding, "The blood sample itself is not telling us enough about the amount of drug in this cerebrospinal fluid."

For Schueler, this is the importance of imaging, where researchers can mark the drug with a tracer and monitor where and how it localizes.

"There are lots of ways we can indirectly visualize via imaging technology that the drug is doing something in the brain," he offers.

This, at least, would help explain whether a drug candidate failed because it didn't work or because it didn't reach its target.

Regardless of how you test the platform, however, the challenge remains to build molecules that can transverse the BBB. And in an ironic twist, the disease pathology itself might provide the first answer.

Leveraging the leaks

In some brain disorders—e.g., ischemic stroke, Parkinson's disease, multiple scelrosis (MS)—the BBB itself is compromised, making the brain more vulnerable to contamination from external substances. But this also affords researchers an opportunity.

"Otherwise, we probably wouldn't have great therapies available for MS as most of these are pretty large proteins," Schueler says. "Usually, they wouldn't get across the BBB, but in this inflamed brain of MS the BBB isn't functioning as usual, and so we have a chance to get these bigger molecules across."

Recently, Samira Azarin and colleagues at University of Minnesota modeled the childhood cerebral form of X-linked adrenoleukodystrophy (ccALD), directly differentiating induced pluripotent stem cells (iPSCs) into induced brain microvascular endothelial cells (iBMECs). They then tested the BBB properties of these cells—ccALD and healthy (WT)—with trans-endothelial electrical resistance (TEER), sodium fluorescein permeability and frayed junction analysis.

“In our study of the brain endothelium, we went beyond qualitative observations of tight junction proteins and quantified the integrity of the barrier formed by the WT- and ccALD-iBMECs using TEER,” the authors wrote. “By this metric, we found that the barrier integrity of the ccALD-iBMECs was decreased compared to WT-iBMECs.”

The researchers then examined the impact of amphiphilic block copolymers on their BBB model.

“Treatment of ccALD-iBMECs with either P188 or E182P16t at the end of the differentiation protocol yielded a slight but non-significant increase in TEER,” they noted. “However, efficacy of polymer treatment at a later stage might be improved upon optimization of pharmacodynamics and pharmacokinetic variables.

“Furthermore, the superior efficacy of treatment with E182P16t when added earlier in the iBMEC differentiation process compared to at the end of the differentiation process suggests that the treatment could be applied at an early stage of BBB development to inhibit the onset and progression of ccALD.”

Other groups, meanwhile, have tried to induce temporary BBB permeability either mechanically—using focused ultrasound or microbubbles—or chemically.

An example of the latter was described recently by Johns Hopkins University’s Sadhana Jackson and colleagues, who looked at the possibility of using regadenoson to transiently disrupt the BBB and thereby increase brain levels of the chemotherapy temozolomide.

“Preclinical models have demonstrated the effectiveness of A1 and/or A2A receptor agonism to increase BBB permeability,” the researchers wrote. “In additional studies that evaluated CNS barrier permeability with regadenoson, there was a 60-percent increase in temozolomide brain concentrations in non-tumor bearing rats, without changing the systemic pharmacology of temozolomide.”

In patients undergoing surgery for recurrent glioma, the researchers inserted microdialysis catheters to monitor brain interstitial temozolomide levels pre- and post-regadenoson administration.

Interestingly, the researchers were unable to reproduce in human patients the drug penetration seen in rodents.

“Regadenoson did not alter temozolomide plasma concentrations which could result in changes in temozolomide-related efficacy or toxicity,” they noted. “This difference in effect has raised further questions regarding BBB differences between mice and humans relating to expression and function of CNS adenosine A2A receptors.”

The researchers were quick to acknowledge that they offered patients the clinical dose of regadenoson used in cardiac stress testing.

“Thus, it is plausible that increased or decreased standard regadenoson dosing could optimally augment CNS temozolomide entry,” they concluded.

Understanding of the BBB permeability window of various disease pathologies has been slow, however, and leaving the BBB vulnerable through mechanical or chemical methods offers its own safety concerns, so researchers have looked for more targeted methods to transport drugs across the BBB.

Taking Troy

One approach that is finding increasing interest in the age of immunotherapy is the use of antibodies and other ligands against cell surface receptors that dot the BBB. This has become known as the Trojan horse approach, and it relies on receptor-mediated transcytosis to transport molecules from one side to the other.

Vect-Horus, for example, used phage display and chemical optimization to identify a series of cyclic peptides that specifically target the low-density lipoprotein receptor.

“Real-time two-photon microscopy experiments on mice demonstrated the ability of a lead peptide-vector to transport a non-permeable agent such as RhoRedX across the BBB and the blood–spinal cord barrier,” explained company co-founder Michel Khrestchatisky in a recent report on the 2017 CNS barrier congress.

“As a further proof of concept, following intravenous administration in mice, peptide-vectors efficiently transported to the brain molecules such as opiate peptides or neuropeptides, all known to poorly cross the BBB,” he continued. “In particular, a vector-neurotensin conjugate is under preclinical development for its potential to induce pharmacological hypothermia with neuroprotective effects in acute excitotoxic neurodegeneration.”

The plan seems simple enough, but according to ArmaGen CEO Mathias Schmidt, the road to success has been fraught with failure that stems from three basic challenges:

- What is the appropriate receptor to target for BBB transcytosis?
- What receptor-binding modality will have the optimal characteristics (e.g., affinity, stability)?
- How do you conjugate the therapeutic effector molecule and retain bioactivity?

Acknowledging that no single solution will suit all situations, Schmidt explains ArmaGen’s thinking by tackling the questions out of order.

“What kind of modality do you use for targeting the receptor?” he repeats. “What we have found out is if you use ligands, you have to come in relatively high doses. And if you over-activate certain receptors at the BBB, it’s going to have untoward side effects on the natural activity of those receptors.”

He then adds that peptides often don’t offer the optimal affinity for the receptors. Thus, ArmaGen has focused its efforts on antibodies.

As to conjugation, rather than rely on chemical or biochemical linkers, ArmaGen is largely focused on ERT, and therefore has the option to develop genetic fusion proteins.
“We have a lot of proprietary knowledge about the linker between the antibody and the therapeutic effector, and also where to fuse the therapeutic effector,” Schmidt explains.

Only when such a fusion is no longer biologically active, he adds, do they consider other conjugation methodologies.

What Trojan horse you ride, of course, depends on what receptor you target.

As Schmidt explains, much of the early work in this space was led by companies like Genentech and more recently Denali, which targeted the transferrin receptor. Despite the years of development, however, this choice has been fraught with safety concerns, not the least of which is reticulocytopenia.

For its part, based on the early academic work of company co-founder Bill Pardridge, ArmaGen settled on the insulin receptor (IR).

“Everybody knows that insulin is made in the pancreas, but insulin plays an important role in the brain,” says Schmidt. “The way it crosses the BBB is by binding to the insulin receptor, which then transcytoses insulin into the parenchymal space of the brain.”

As he explains, there was initially some concern that targeting the insulin receptor might interfere with glucose homeostasis.

“We give our antibody-enzyme fusion protein as an infusion in 5 percent dextrose, and that pretty much takes care of all concerns about changes in glucose control,” he says, recalling that the company has built its safety profile on upward of 1,000 independent patient infusions.

Although ArmaGen has a number of candidates in the neurodegenerative space, the primary focus of the company has been on lysosomal storage diseases such as MPS I.

“The lysosome is the trash can of our cells,” Schmidt says. “In those diseases, the trash can misses one particular enzyme so it cannot degrade certain substances.”

This leads to a build up of trash, which translates into both common and specific pathologies, depending on what enzyme is missing.

ERT was heralded as a sea change for patients, and to some extent it has been.

“The major limitation is that the enzyme doesn’t cross the BBB,” Schmidt continues.

In July, the company published the results of a clinical study of its lead AGT-181—an anti-IR/iduronidase fusion—in six adults (Ph 1) and 11 children (Ph 2) with MPS I.

Not only did treatment stabilize neurocognitive function and cortical grey matter volume in the children, but it also stabilized or improved somatic (non-CNS) manifestations.

According to Schmidt, “Our hypothesis was that our drug would somatically be non-inferior to the approved ERT Aldurazyme because we didn’t see any reason why we should be better or worse.”

As he explains, a mannose-6-phosphate (M6P) on the enzyme is effectively the zip code that binds to cell surface M6P receptor, which drags the enzyme into the lysosome.

“We thought we would have the same mechanism, and we were very surprised that we saw even a somatic differentiation,” Schmidt presses. “We saw a significant decrease in liver and spleen size, even in patients who had been treated with Aldurazyme for up to nine years.”

“And what is not published in the paper, we saw that the majority of the patients in the trial returned to a growth velocity that is comparable to age-equivalent healthy children,” he shares.

Schmidt wonders whether the company inadvertently discovered a dual-point entry into somatic cells: M6P and insulin receptors.

“We did experiments with serum from patients in Brazil,” he recounts. “We have seen that for patients who were poorly controlled on Aldurazyme, the serum of those patients neutralized the in-vitro uptake of Aldurazyme into Hurler fibroblasts, but our AGT-181 molecule could still enter via the insulin receptor into those Hurler fibroblasts in the presence of neutralizing antibodies.”

He also postulates that because insulin and M6P receptor expression does not overlap 100 percent, a dual-targeting mechanism could offer an advantage in targeting certain organs where the M6P receptor is not as efficacious as desired.

Like ArmaGen, Bioasis’ platform relies on receptor-mediated transcytosis, but where the former uses an antibody to target the insulin receptor, the latter uses melanotransferrin (MTf) to target the LRP-1 receptor.

As Day explains, MTf is a rather large protein of about 750 amino acids.

“At first blush, if you don’t know the mechanism, you’d say 'good luck getting that across the BBB,'” he recalls. “But it actually turns out to be a really potent delivery vector for antibodies, siRNAs, small molecules and ERTs.”

But the target LRP-1 is just as important in this equation for a couple of reasons, he continues.

First, the receptor is ubiquitous along the surface of the BBB, meaning that MTf conjugates have many access points to the brain, a factor that has been challenging to other Trojan horse approaches.

And second, the neuronal distribution of the LRP-1 receptor occurs throughout the brain and on a multitude of cell types, including radial glia, neuroblasts, microglia, oligodendrocyte progenitor cells, astrocytes and neurons.

Furthermore, as Texas Tech University collaborator Paul Lockman and colleagues suggested in a proof-of-concept study, the body only experiences MTf doing its normal function.

“As an autologous human protein, immune hypersensitivity or elimination via neutralizing antibodies are less likely to be an issue in clinical setting,” the study authors wrote. “Since MTf traverse the BBB as part of its normal function, MTf does not appear to pose toxicity-related issues for the delivery of MTf-drug conjugate into the brain.

“Subsequently, MTf poses less risk than cytokines TNF, which could disrupt the BBB by generating a focal inflammatory response.”

Lockman and colleagues examined the impact of xB3-conjugated Herceptin (trastuzumab) in a mouse model that bore brain metastases of human HER2+ breast cancer. Following treatment, the researchers performed quantitative autoradiography to look for correlations between brain foci and drug localization.

According to Day, tumor site drug levels peaked at about 140 ng/g, whereas the surrounding tissue only saw levels of about 25.1 ng/g, which they took as evidence of target engagement.

“What that tells us is that xB3 gets Herceptin across the BBB in significant quantities, and then, once its in the brain—because Herceptin is really good at seeking out HER2+ cells—that localizes the drug to the tissue,” he says.

Just as important as getting the drug to the target site is making sure the drug has a biological effect once there.

“In our studies, trastuzumab [alone] looks just like saline, which looks just like xB3 alone,” he recounts. “So, there’s no impact of the peptide alone, which is another important thing.”

The fusion, however, was quite effective. The researchers noted a 68-percent drop in tumor number and a 58-percent reduction in tumor volume.

“Based on those data, you’ve got really good target engagement and good biological impact that if you can recapitulate in a Phase 1 human study, you’d be the first company in the world to demonstrate that non-invasive delivery of a medicine like trastuzumab across the BBB to its target site,” Day enthuses.

Although the early work was performed with full-sized MTf, the company has more recently undertaken efforts to see if they could reduce the size of the transfer vector, and they managed to optimize the technology from a bulky 750 amino acids to a much more confined 12 amino acid peptide.

The optimized technology was validated in a recently published study led by collaborators at Medimmune, where researchers compared the abilities of full-sized MTf and MTfpep to transport an antibody across the BBB versus antibody alone.

“The Medimmune paper is important because it is a single-dose PK/PD study that ran for two weeks, and you’re getting about 4 to 5.5 percent of the injected dose into the brain,” Day explains.

“Following i.v. administration in mice, the MTf or MTfpep fusions showed a significant increased brain distribution compared to the unconjugated antibody, with 95 percent of all fluorescence localized in the brain parenchyma,” the study authors wrote.

“Molecules containing MTfpep have a significantly prolonged brain exposure when compared to either the MTf-containing molecule or the antibody alone,” they continued.

Just as important, Day says, was the biological response, echoing the earlier sentiments regarding the trastuzumab fusion.

The researchers undertook pharmacodynamic studies in a mouse model of neuropathic pain, looking for changes in mechanical hypersensitivity resulting from fusion to an IL-1 receptor

antagonist (IL-1RA). As expected, only fusions containing both IL-1RA and either MTf or MTfpep demonstrated reversal in mechanical hyperalgesia.

“What you’re seeing is a response up to two weeks,” Day recounts. “And it only goes away at two weeks because that’s when they ended the study.”

Perhaps more interestingly, the authors noted, reversal of hyperalgesia lasted significantly longer with the fusions than if IL-1RA was directly injected intrathecally.

Aside from these biologic activities, however, Day also foresees potential manufacturing benefits of shifting from a large protein to a small peptide, when cost of goods becomes more of an issue.

“I’d been at a company previously where we acquired an asset,” he says. “The manufacturing team there totally underestimated the cost of goods, and the company ended up with an asset that they couldn’t actually afford to manufacture.”

In May, Bioasis announced a collaboration with WuXi Biologics for the development and manufacturing of its xB3-001 lead targeting brain metastases of HER2+ breast cancer.

And in July, the company announced the results of a cortical brain-related activity study of xB3-001 that it conducted in collaboration with Charles River Labs using a freely moving in-vivo mouse microdialysis system.

Following a single injection, xB3-001 induced significant increases in brain cortical dopamine and serotonin activity within 60 to 90 minutes, in sharp contrast to trastuzumab alone, where the levels did not change. Similar patterns were seen with brain cortex norepinephrine.

“In addition to our recently published work with MedImmune, these new data further validated the utility of our xB3 platform technology to increase delivery of existing therapeutic compounds across the BBB,” said Bioasis vice president & head of external research Mei Mei Tian, in announcing the findings.

Trojan horses aren’t without challenges, however, including that receptors often differ between species, offers MacBride. The therapeutic molecule you develop for a human target may not recognize its mouse homologue.

“If your drug is specific for human and doesn’t recognize mouse, you need to do two things,” she says. “One is to develop a surrogate drug; basically, develop a human therapeutic and a surrogate. It’s parallel development and it’s a lot of work.”

The alternative is something Taconic has become quite good at and involves replacing that mouse target with its human version. Then you can use your human drug in your test species.

MacBride admits that she doesn’t have any specific BBB-related examples of this in action, but she says: “We are starting to see people ask can we use a humanized immune system now to look at neuroinflammation.”

“We have so many more tools in our tool box now,” she enthuses. “If you look at genetic editing, certainly, you can knockout almost anything much more easily and cheaply now.”

“Also, we really can do these large-scale humanizations, you can humanize eight or ten genes in a single mouse,” she presses. “You can start to influence human tissues and human organs.”

Again, she cannot offer examples where people try to humanize the meninges of the brain, but she’s open to the possibility.

With its G-technology, meanwhile, 2-BBB is giving the ligand approach a shot, conjugating glutathione (GSH) to its PEGylated liposome formulations.

“GSH was chosen as targeting moiety since it can be transported through the BBB in vivo via a sodium-dependent transporter,” explained 2-BBB founder Pieter Gaillard and colleagues in a recent paper. “Recently, we have investigated the uptake kinetics of the G-Technology and shown that both in vitro and in vivo, GSH PEGylated liposomes enhance delivery of a fluorescent marker in the brain.”

Using flow cytometry and fluorescence imaging, the researchers determined that liposomal uptake by in-vitro BBB model tissues was significantly influenced by the molar percentage of GSH-PEG chains. Similarly, microdialysis of mice treated with encapsulated ribivarin demonstrated that liposomes with little or no GSH could not deliver the drug to the brain parenchyma, whereas drug levels in the brain extracellular fluid jumped significantly and dose-dependently when GSH percentages reached a threshold.

“Further work is currently ongoing to determine in detail the exact membrane protein recognizing the GSH-PEG moiety and the subsequent fate of the liposomes in endothelial cells as well as delivery across the BBB,” the authors wrote. “This could potentially help us optimizing the affinity of the GSH-derived targeting agent of the G-Technology to its putative receptor and control the balance between targeting of and drug delivery across the BBB.”

“This issue was illustrated for transferrin-targeting antibodies that were unable to transcytose across the BBB when affinity for the transferrin receptor was too high,” they explained. “However, since increasing GSH-PEG density increases internalization in vitro and drug delivery in vivo, a potential issue of too high affinity and lack of brain delivery by the G-Technology is unlikely to be occurring.”

As always, though, bench success must translate.

Beyond the science

“On a very personal level, what really matters to me is that we’re working in rare diseases,” Schmidt says. “A paper is a paper, but it is the story behind the paper.”

A 10-year-old boy similar to the one described earlier was one of the 11 Brazilian children given AGT-181. His is the face that Schmidt sees when he describes the importance of ArmaGen’s work.

“When we interact with the parents of the children and the stories they tell us, how game-changing the outcome of the clinical study was for some of the parents and for some of the patients,” he shares.

Schmidt’s 10-year-old patient couldn’t use his hands, follow a moving finger, recognize his parents or sit for long periods.

Following treatment, the boy could stand, he could hold a spoon and feed himself. For his parents, as Schmidt relates, this was the world.

“This is what we should always be reminded of,” he reflects. “Anything that we’re doing here is not for us. It’s not for the vanity of science.”

“It’s about how do we create a better tomorrow for the patients whom we serve,” he vows. “And in rare diseases, they very easily tend to be forgotten.”

Nosing about

While several groups strive to slide things past the blood-brain barrier (BBB) or disrupt it with mechanical methods such as ultrasound, a separate group of researchers strive to completely circumvent the BBB by delivering drugs through an unusual portal: the nose.

“The nasal cavity has been employed not only as a portal for the local, but also for the systemic delivery of certain therapeutic agents (e.g., peptides, proteins, stem cells, etc.), due to its large surface area and high degree of vascularization,” explained Costas Kiparissides and colleagues at Aristotle University of Thessaloniki and the Centre for Research and Technology Hellas in a recent review.

Unlike conventional approaches, which either attempt to breech the BBB via systemic circulation or are delivered invasively via intracerebroventricular or intraparenchymal injections, drugs delivered through the nose have direct access to the olfactory and trigeminal nerve pathways.

“The olfactory nerve cells originate at the CNS and terminate at surface of olfactory epithelium in the olfactory region, which are located in the roof of the nasal cavity,” said Sapienza University of Rome’s Maria Carafa and colleagues in a separate publication. “Molecules are transported via the axon, using the paracellular or transcellular route, into olfactory cortex, and then cerebrum and cerebellum.”

“For trigeminal nerve route, it has been proven that drug molecules or nanoparticles diffuse into the maxillary and ophthalmic branches of the trigeminal nerve and enter the brainstem,” they added.

This non-systemic delivery offers the advantage of not only circumventing the BBB but also systemic gastrointestinal and hepatic elimination.

It is also associated with enhanced safety, ease of administration, and rapid onset of action, according to Kiparissides and colleagues.

To date, however, this method of delivery has largely been limited to extremely potent therapeutics for a handful of reasons.

“Direct nose-to-brain delivery of therapeutic entities is severely hampered by insufficient bioavailabilities, cytochrome P450-mediated degradation, short retention times, restrictions imposed by the geometry of the nasal cavity (e.g., small volume, limited surface area of the olfactory region, etc.), as well as lack of targeting specificity to the affected area of the brain,” Kiparissides and colleagues explained.

In a recent review of nose-to-brain delivery using in-situ gels, Blessing Atim Aderibigbe of University of Fort Hare suggested that some of these challenges are being overcome through the addition of bioadhesive compounds (e.g., chitosan) and absorption enhancers to drug formulations.

Research into these compounds has largely been preclinical, however, and any effort to translate findings from animals to human subjects is complicated by anatomical differences of the nasal passage.

“There is also the need to develop new excipients that can enhance the drug bioavailability,” Aderibigbe pressed. “Extensive toxicodynamic studies of excipients, nanoparticles and polymers used in the preparation of the gels are lacking.”

Even with these issues, a testament to the appeal of intranasal delivery comes when drugs that already manage to get by the BBB through systemic delivery are being reformulated.

In July, Therapeutic Solutions International releases a nano-formulation of pterostilbene, designed specifically for intranasal delivery of the antioxidant that may enhance chemotherapeutic impact in cancer and neurological conditions.

Although pterostilbene can pass the BBB naturally, the company anticipates that it can deliver higher concentrations intranasally.

“Our pharmacokinetic trial data showed a 55-percent increase in serum concentration of nanoparticle pterostilbene over a powdered and capsule form,” explained company president and CEO Timothy Dixon in the announcement. “We are therefore pleased to release a formulation of this product with double the potency and half-life over traditional powder formulation for intranasal use.”

Scientific advisory board member Santosh Kesar added that the generation of an intranasal formulation of pterostilbene facilitates its rapid evaluation in glioma and other brain cancer patients, where therapeutics options with good safety profiles are lacking.