ArmaGen's gatecrashers

By Tim Fulmer, Senior Writer

Over the past few years, **ArmaGen Technologies Inc.** and collaborators at the **University of California, Los Angeles**, have published a series of studies describing genetically engineered fusion proteins to shuttle therapeutics across the blood-brain barrier. In their newest paper in *Molecular Pharmaceutics*, the group presented *in vitro* data on AGT-185, a chemical nerve gas antidote created using the company's technology platform.¹

ArmaGen's approach takes a therapeutic that would not ordinarily cross the blood-brain barrier (BBB) and links it to a BBB-targeting mAb. The resulting fusion protein enters the CNS via a receptor-mediated molecular transport system in the vascular endothelial cells that make up the BBB (see **Figure 1**, "Breaching the blood-brain barrier").

The study in *Molecular Pharmaceutics* focused on paraoxonase 1 (PON1), a serum esterase that inactivates organophosphate toxicity caused by exposure to pesticides and nerve gas agents.

Naturally occurring PON1 cannot cross the BBB. In this case, the researchers linked it to a mAb targeting the human insulin receptor (INSR), which is expressed at the BBB. *In vitro*, this bifunctional fusion protein maintained PON1 activity following binding to the human INSR.

The next steps, said William Pardridge, corresponding author on the paper, include engineering a permanently transfected host cell line capable of producing large amounts of the fusion protein and testing it in animals. Pardridge is CSO of ArmaGen and professor of medicine at UCLA's David Geffen School of Medicine.

Figure 1. Breaching the blood-brain barrier. The relative impermeability of the blood-brain barrier (BBB), composed of vascular endothelial cells interspersed by tight junctions, ensures that only metabolically essential molecules enter the brain. However, this makes transport of therapeutics to diseased neural tissue especially challenging.

[a] A paper published in *Molecular Pharmaceutics* by **ArmaGen Technologies Inc.** and UCLA illustrates one strategy to overcome BBB resistance to drug delivery, dubbed a 'molecular Trojan horse'.¹ In this case, a BBB receptor-binding antibody or peptide conjugated to a therapeutic compound enters the brain via receptor-mediated endocytosis, a process by which molecules are absorbed into the cell. The researchers constructed a fusion protein consisting of the enzyme paraoxonase 1 (PON1), which ordinarily cannot cross the BBB, and linked it to an antibody that targets the insulin receptor on the BBB. PON1 inactivates organophosphate toxicity caused by exposure to pesticides and nerve gas agents.

Other strategies for crossing the BBB include: [b] Lipophilic small molecule therapeutics that passively diffuse across the BBB's plasma membrane.

[c] Small molecule inhibitors of BBB efflux transporters, which actively export foreign molecules from brain to blood, delivered in combination with the therapeutic.

[d] Soluble sugars delivered in combination with the therapeutic that disrupt tight junctions.

The researchers had previously shown that at least two other molecules linked to INSR-targeting antibodies were able to penetrate the BBB in rhesus monkeys following i.v. delivery. One study used the enzyme α -L-iduronidase (IDUA) to treat mucopolysaccharidosis type 1 (Hurler's syndrome), a lysosomal storage disorder that affects the CNS.² The other study used brain-derived neurotrophic factor (BDNF), which binds receptors on neurons, to trigger neuroprotective pathways that potentially can help treat stroke.³ Both of these compounds are in ongoing preclinical testing.

"Pardridge's Trojan horse strategy has shown promise in animal models of a number of different CNS disorders, and the approach should probably now be tested in patients," said Edward Neuwelt, professor of neurology and neurosurgery at the **Oregon Health & Science University** and the **Portland Veteran Affairs Medical Center**.

"An advantage of targeting a therapeutic to the BBB insulin receptor is that uptake occurs with the formation of intracellular vesicles, which can potentially accommodate a large drug cargo," said Eric Shusta, associate professor of chemical and biological engineering at the **University of Wisconsin-Madison**.

"An advantage of using an antibody to do that targeting is that it can be engineered to recognize an epitope on the receptor outside of the receptor's binding pocket. This should help ensure that the antibody doesn't compete with the receptor's natural ligand and interfere with the receptor's function," Shusta added.

Breaching the barrier

Although ArmaGen has opted for antibody-based fusion proteins to deliver payloads across the BBB, other groups are taking slightly different tacks. A case in point is **AngioChem Inc.**, which is taking a peptide-based approach that targets low-density lipoprotein-related protein 1 (α -2-macroglobulin receptor) (LRP1). LRP1 mediates transport across the BBB.

AngioChem's lead product is Ang1005, an i.v. LRP1-binding peptide conjugated to three molecules of paclitaxel. The therapeutic is in two Phase I/II trials to treat primary glioblastoma and metastatic brain tumors. In murine intracerebral tumor models, Ang1005 crossed the BBB and significantly increased survival compared with what was seen in untreated rodents ($p < 0.05$).^{4,5}

"We've chosen to rely on peptides rather than antibodies to help deliver therapies across the blood-brain barrier," said AngioChem CSO Reinhard Gabathuler. "Because our peptide sequences are derived from the endogenous ligands of a BBB receptor, we anticipate they will have better uptake and transport properties within the BBB compared with antibodies engineered to target a BBB receptor."

Like ArmaGen, AngioChem expects its technology will be able to deliver a variety of molecules across the BBB.

"In addition to small molecule chemotherapeutics like paclitaxel, we've already linked our BBB-targeting peptides to siRNA molecules and antibodies that could potentially treat brain cancer and neurodegenerative diseases," said Gabathuler.

Raptor Pharmaceutical Corp. also has a Trojan horse strategy in preclinical testing. The company's peptide-based NeuroTrans platform consists of variants of low-density lipoprotein receptor-related protein associated protein 1 (LRPAP1; RAP) conjugated to neurotrophic factors such as glial cell-derived neurotrophic factor (GDNF) and BDNF

for the treatment of neurodegenerative diseases. RAP, a protein that is known to assist in the folding and trafficking of LRP1 and LRP2 (megalin), has been shown to cross the BBB in mice following systemic delivery.⁶

Regardless of which BBB receptor is targeted, "there is always the likelihood that the receptor is also expressed on other cells in the body," noted Björn Bauer, assistant professor of pharmaceutical sciences at the **University of Minnesota College of Pharmacy**. "Thus, intravenous delivery of a BBB receptor-targeted Trojan horse construct could carry the risk of off-target toxicity."

Bauer also told *SciBX* that "the expression levels of the target receptor might vary considerably, putting the strategy at a disadvantage in patients whose BBB expresses low or negligible levels of the receptor."

Instead of employing receptors to gain access to the brain, Bauer's lab is targeting active efflux transporters within the BBB to enhance delivery of therapeutics into the brain. Active efflux transporters are proteins that export metabolites from the brain back to the blood. They are often responsible for excluding drugs and other foreign compounds from the brain.

Bauer said blocking efflux transporters could improve delivery of antiepileptic drugs across the BBB and help treat the 30–40% patients who fail to respond to medication.

In patients and in rodent models of epilepsy, upregulation of the efflux transporter P-glycoprotein in the BBB has been associated with epileptic brain seizures.⁷ Bauer and colleagues found that P-glycoprotein upregulation was lowered by administration of a cyclooxygenase (COX) inhibitor, suggesting that COX inhibition in the BBB may also increase brain uptake of some antiepileptic drugs.⁸

In Oregon, Neuwelt and colleagues are taking yet another approach in Phase I/II cancer trials, delivering chemotherapy in combination with a highly concentrated sugar solution intra-arterially to the patient. The sugar solution causes the BBB to reversibly shrink under osmotic stress, transiently opening the tight junctions between cells. This allows the chemotherapeutic to gain access to the brain. The combination therapy delivers considerably more therapeutic to the brain than chemotherapy alone.^{9,10}

In Wisconsin, Shusta and colleagues are pursuing genomics and proteomics strategies to increase the repertoire of potential BBB therapeutic targets.^{11,12}

"By using a combination of gene- and protein-expression profiling we expect to identify new blood-brain barrier receptors and transport systems that might be harnessed to deliver therapies to the brain," he said. "An ideal membrane receptor for therapeutic targeting would be selectively expressed in the BBB and expressed at high enough levels to ensure efficient and high-capacity delivery of drug to the brain."

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COMPANIES AND INSTITUTIONS MENTIONED

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