Breaching the retinal barrier

By Tim Fulmer, Senior Writer

Trinity College Dublin researchers have developed a way to use RNAi to reversibly enhance the permeability of the blood retina barrier.¹ The technique improved systemic delivery of two small molecules to the mouse retina, and the researchers are now adapting the approach for use in humans.

Much like the brain, the retina has evolved cellular barriers to exclude potentially harmful blood-borne agents while allowing passage of nutrients to ensure proper functioning. The retina has two such barriers: the inner blood retina barrier (BRB), which consists of retinal endothelial cells, and the outer BRB, which consists of a single layer of retinal pigment epithelial cells.

In both retinal barriers, protein complexes called tight junctions exist between individual cells to block passive diffusion of small molecules. Tight junctions consist of multiple types of proteins, including the claudins.

Previous work by the Trinity group showed that RNAi knockdown of claudin 5 (CLD5) in the mouse brain microvasculature led to a transient increase in diffusion of low-molecular weight compounds across the blood brain barrier (BBB).² Because of the similarities between the brain and retina barriers, the team hypothesized that the same approach could facilitate passage of systemically delivered therapies into the retina.

Injection of CLD5 small interfering RNA into the mouse tail vein led to lower levels of the protein in the retinal vasculature at 24 and 48 hours postinjection than levels in the retinal vasculature of control mice.

These reductions correlated with opening of the BRB. A small molecule MRI contrast agent was able to cross the BRB at 24 and 48 hours after injection of the CLD5 siRNA but not at 72 hours after injection.

In addition to transiently and reversibly opening the BRB to allow passage of small molecules from the blood into the retina, the siRNA did so without damaging the retina. DNA staining showed that the increased BRB permeability did not cause neuronal cell death in any layer of the retina.

Finally, the researchers looked at two different mouse models of disease to evaluate the therapeutic potential of the CLD5 siRNA approach.

In mice lacking an enzyme required to synthesize guanosine triphosphate (GTP), a key mediator of visual phototransduction, intraperitoneal delivery of GTP 48 hours after CLD5 siRNA injection significantly improved retinal function compared with that in control mice receiving nontargeted control siRNA ($p < 0.0001$).

In a mouse model of light-induced retinopathy, a small molecule antiapoptotic agent plus CLD5 siRNA significantly protected mice against photoreceptor cell death at 12 and 24 hours after light exposure compared with an antiapoptotic agent plus nontargeted siRNA ($p = 0.0011$ and $p = 0.0252$, respectively).

The findings were published in the *Proceedings of the National Academy of Sciences*.

Going viral

Matthew Campbell, corresponding author on the paper, told SciBX that the research team is now modifying the siRNA approach for use in patients. Campbell is a researcher in the Smurfit Institute of Genetics at Trinity College Dublin.

Rather than deliver naked siRNA—as was done in the mice—Campbell said the team is creating an adeno-associated viral (AAV) vector that expresses CLD5 short hairpin RNA and stably persists in retinal endothelial cells following a one-time injection into the eye. The viral vector should avoid the need for regular administration of RNAi, he said.

Once inside the retinal cells, the AAV construct could be turned on or off by delivering inducing agents. Those compounds would interact with the expression machinery of the

viral vector to increase or decrease CLD5 levels and thus increase or decrease the permeability of the BRB to systemic therapies, according to Campbell.

The authors wrote in the paper that the RNAi-based approach potentially could be used to deliver therapies to treat a variety of retinopathies for which there are no approved drugs, including dry age-related macular degeneration (AMD) and retinitis pigmentosa.

Anne Galy, project manager at Fovea Pharmaceuticals S.A., wants to see the siRNA approach tested in additional animal models of eye disease, “especially models of retinitis pigmentosa and dry AMD, where degeneration is slow and progressive. Based on the paper, it’s unclear that systemic siRNA delivery for periodically opening and closing the retinal blood barrier to systemic therapies will be safe and efficacious in a setting where chronic treatment is required,” she said.

Fovea, which is being acquired by sanofi-aventis Group, is developing compounds to treat back-of-the-eye diseases, two of which require intravitreal delivery. FOV2302, a plasma kallikrein-kinin inhibitor, is in Phase IIa testing to treat retinal vein occlusion (RVO)-induced acute macular edema. FOV2501, an intravitreal formulation of rod-derived cone viability factor (RdCVF), is in preclinical development to treat retinitis pigmentosa.

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—Anne Galy,
Fovea Pharmaceuticals S.A.

Campbell also thinks CLD5 siRNA could be used to improve delivery of approved ophthalmic drugs. For example, he said intraocular injections of Lucentis ranibizumab, a humanized mAb fragment against VEGF-A, “requires regular, often monthly injections for optimum efficacy, which can cost as much as \$2,000 per injection.”

Lucentis is marketed by **Roche**, Roche’s **Genentech Inc.** unit and **Novartis AG** to treat wet AMD. According to the drug’s label, serious adverse reactions have occurred in <0.1% of intravitreal injections, including endophthalmitis, retinal detachments and iatrogenic cataracts.

According to Campbell, an RNAi approach would require a single injection to get the AAV-RNAi into the retinal cells, after which the patient could take their medication less frequently and potentially in a systemic form.

However, Jayakrishna Ambati, vice chair of ophthalmology and visual sciences at the **University of Kentucky**, said an RNAi approach might not be necessary for a drug such as Lucentis. “Lucentis provides a good example of a therapeutic that is directly injected into the eye to treat a retinal disorder like wet AMD without any concern for enhancing the permeability of the retina blood barrier. In that case, intravitreal injection works, showing efficacy and safety with relatively minimal side effects,” he said.

Moreover, in at least some cases, it may be possible to chemically tweak small molecules that enter the retina from the blood without requiring that the permeability of the BRB be increased.

For example, **Acucela Inc.** and **Otsuka Pharmaceutical Co. Ltd.** are developing ACU-4429, a nonretinoid small molecule that inhibits an enzyme in retinal epithelial cells, to treat dry AMD. The orally delivered compound is in Phase I testing.

In mice, orally delivered analogs of ACU-4429 protected the retina from light-induced damage.³

Ambati also worried that siRNA molecules are known to bind and activate toll-like receptors on immune cells, which could trigger an unwanted immune response. But Campbell said that should not happen with the CLD5 shRNA expressed by the AAV vector because it is a few nucleotides longer than the naked siRNA used in the mouse experiments.

“That increase in size has been shown not to activate toll-like receptor 3 (TLR3) and trigger subsequent upregulation of proinflammatory cytokines in the retina,” Campbell said.

Campbell and Trinity colleagues Peter Humphries and Anna-Sophie Kiang have filed patent applications covering RNAi-mediated blood brain and blood retina barrier modulation to enhance delivery of therapeutic compounds that would otherwise be excluded from the brain and retina if delivered systemically.

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COMPANIES AND INSTITUTIONS MENTIONED

Acucela Inc., Bothell, Wash.

Fovea Pharmaceuticals S.A., Paris, France

Genentech Inc., South San Francisco, Calif.

Novartis AG (NYSE:NVS; SIX:NOVN), Basel, Switzerland

Otsuka Pharmaceutical Co. Ltd. (Tokyo:4768), Tokyo, Japan

Roche (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland

sanofi-aventis Group (Euronext:SAN; NYSE:SNY), Paris, France

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