TARGETING DRUGS TO DISEASED OCULAR CELLS

In this article, Frazer Coutinho, PhD Candidate, Colin Green, PhD, W&B Hadden Chair of Ophthalmology & Translational Vision Research, and Ilva Rupenthal, PhD, Senior Lecturer and Director of the Buchanan Ocular Therapeutics Unit, all of the University of Auckland's Department of Ophthalmology, discuss the targeted delivery of ocular therapeutics to diseased cells, using the cell-penetrating peptide, Xentry.

OVERCOMING THE BARRIERS TO EFFICIENT OCULAR DRUG DELIVERY

Ocular Barriers

The eye is a complex organ with multiple tissue layers that create anatomical and physiological barriers in order to protect it from the environment. These barriers include the cornea and sclera on the exterior while the inner limiting membrane (ILM) protects the retina on the interior. As a result, ocular drug delivery, especially to retinal cells, has long been a significant challenge. For example, drugs delivered orally or systemically have to be administered at very high concentrations in order to achieve a therapeutic effect at the target site. Even drugs delivered locally, in the form of eyedrops or intravitreal injections, face a number of barriers and elimination mechanisms necessitating the use of high drug concentrations to improve efficacy. The off-target effects resulting from such high drug concentrations can then create additional challenges, often resulting in secondary complications.

Improving Ocular Drug Delivery

Multiple approaches, including ultrasound, penetration enhancers and colloidal carriers such as nanoparticles and liposomes, have been developed in order to improve ocular drug delivery. While these systems may deliver the drug closer to its site of action, they are generally not targeted specifically to injured cells, with the cellular uptake of intracellularly acting molecules (siRNA, peptides, small molecules) also being limited. Cell-penetrating peptides (CPPs) can transport their cargo across the cell membrane in a biologically active and bioavailable form.[1] Well-established CPPs explored for ocular drug delivery include the transactivator of transcription (TAT) and penetratin, as well as newer CPPs, such as the peptide for ocular drug delivery (POD).[1] However, while most CPPs transport their cargo into cells, they are non-cell-specific, often delivering drugs into multiple cell types. This lack of specificity reduces the therapeutic dose in the target cells while also increasing the potential for off-target effects.

CPPS FOR TARGETED OCULAR DRUG DELIVERY

Xentry

Xentry is a short CPP (seven amino acids) which has been used to transport a range of cargo molecules into cells, including siRNA, oligonucleotides and antibodies.[2] Xentry is unique compared with most other CPPs, as it only enters Syndecan-4 expressing cells. Therefore, Xentry does not enter cells such as non-adherent monocytes and erythrocytes, which makes it ideal for systemic administration as it is not sequestered by the blood circulation. Furthermore, by specifically targeting Syndean-4 expressing cells and initiating rapid uptake, Xentry and its conjugated cargo can evade degradation by enzymes in the serum, allowing the overall administered dose to be reduced.

> "Xentry is unique compared with most other CPPs, as it only enters Syndecan-4 expressing cells. Therefore, Xentry does not enter cells such as non adherent monocytes and erythrocytes, which makes it ideal for systemic administration."

The Functions of Syndecan-4

Syndecans are a family of transmembrane heparan

Frazer P Coutinho
PhD candidate
T: +64 9 923 6471
E: f.coutinho@auckland.ac.nz

Prof Colin R Green
W&B Hadden Chair of Ophthalmology & Translational Vision Research
T: +64 9 923 6135
E: c.green@auckland.ac.nz

Dr Ilva D Rupenthal
Senior Lecturer and Director of BOTU
T: +64 9 923 6386
E: i.rupenthal@auckland.ac.nz

Buchanan Ocular Therapeutics Unit
Department of Ophthalmology
New Zealand National Eye Centre
University of Auckland,
Private Bag 92019
Auckland 1142
New Zealand

www.botu.nz

Copyright © 2019 Frederick Furness Publishing Ltd

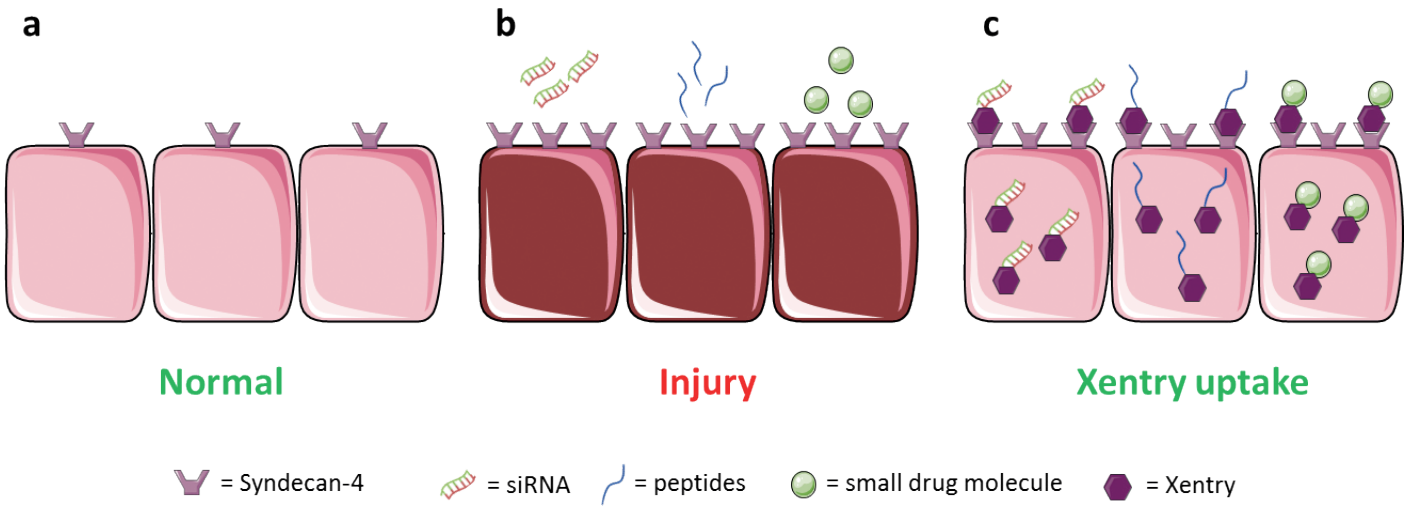

Figure 1: Xentry targets increased Syndecan-4 expression in injured cells. (a) Under normal conditions, cells express low levels of Syndecan-4. (b) During injury, Syndecan-4 levels are upregulated; however, untargeted therapeutics are unable to enter cells efficiently. (c) Conjugation of the therapeutic to Xentry enables specific targeting and increased uptake into Syndecan-4 overexpressing injured cells.

sulphate proteoglycans, with four syndecans having been identified in mammalian cells so far.[3] Low levels of Syndecan-4 are present in many different cell types under normal conditions, mediating numerous signalling pathways including proliferation, migration and endocytosis. Syndecan-4 binds multiple molecules such as fibronectin, integrin and paxillin for the formation of focal adhesions, as well as biochemical signalling by binding extracellular growth factors, including vascular endothelial growth factor (VEGF).[3] Most importantly, Syndecan-4 has been shown to be overexpressed in diseased cells.

Targeting Syndecan-4 with Xentry in Ocular Disease

In diseased ocular tissues, increased Syndecan-4 expression results in the binding of proteins such as VEGF in order to enhance interactions with the VEGF receptor, therefore stimulating blood vessel growth.[3] Consequently, there is potential to specifically target diseased cells that overexpress Syndecan-4 by using the CPP Xentry (Figure 1).

Recently, we have discovered that cultured retinal pigment epithelial cells and retinal microvascular endothelial cells increase cell-surface expression of Syndecan-4 under hypoxic, inflammatory and hyperglycaemic conditions. Human age-related macular degeneration (AMD) and diabetic retinopathy (DR) donor tissues also exhibited increased Syndecan-4 expression, primarily around large leaky blood vessels in the retina. Interestingly, the ILM of donor retinas, which contains astrocytic endfeet, also showed strong Syndecan-4 labelling. The ILM has long been a barrier to efficient drug delivery into the retina. Recently, astrocytic endfeet have been proposed as a way of delivering drugs across the ILM and further into the retina utilising astrocytic processes. Thus, higher Syndecan-4 expression on astrocytic endfeet in the ILM provides yet another opportunity for more efficient drug delivery into the retina when the therapeutic is injected intravitreally.

> "XG19 is a novel peptide therapeutic that specifically targets diseased cells in order to reduce inflammation and promote cell survival."

XG19 AS A NOVEL THERAPEUTIC FOR AMD

What is XG19?

XG19 is a novel peptide therapeutic that specifically targets diseased cells in order to reduce inflammation and promote cell survival. When used in ocular disease, XG19 promotes the survival of endothelial cells and repairs blood vessels, thus reducing vascular leak and inflammatory mediator concentrations in the environment. The repair of blood vessels also restores the normal blood supply to the tissues which helps to address the underlying ischaemia. Overall, XG19 restores and maintains blood-retinal barrier integrity by targeting both the hypoxic retinal pigment epithelium and the leaky blood vessels (Figure 2).

How Could XG19 Be Used Therapeutically?

Our group has studied biochemical changes and protein expression in a number of ocular diseases. Of the multitude of proteins elevated, Connexin43 (Cx43) is one that stands out. A number of studies have shown that blocking Cx43 hemichannels in inflammatory or hypoxic disease results in increased cell survival and tissue repair. This is particularly useful for vascular eye diseases, such as AMD and DR, where the blood supply is compromised and requires restoration.[4] Gap19 is an intracellularly acting Cx43 hemichannel blocker. However, the native peptide has low cell permeability, necessitating the administration of high doses in order to achieve a therapeutic effect.[5] To improve cell penetration and specifically target injured Syndecan-4 expressing retinal cells, Gap19 was conjugated to Xentry and was given the name XG19.

Our *in vitro* studies showed that XG19 uptake was greatly increased during hypoxic, inflammatory and hyperglycaemic conditions due to increased Syndecan-4 expression, confirming that XG19 could be primarily targeted to injured retinal cells. XG19 was able to specifically block uncontrolled Cx43 hemichannel opening in injured cells, inhibiting ATP release as well as increasing cell survival at concentrations as low as 5 µM, which is much lower than concentrations of native Gap19 (300 µM) used in similar assays.[5] This highlights that Xentry is able to improve the specificity and thus delivery of bioavailable Gap19 into injured cells.

Copyright © 2019 Frederick Furness Publishing Ltd

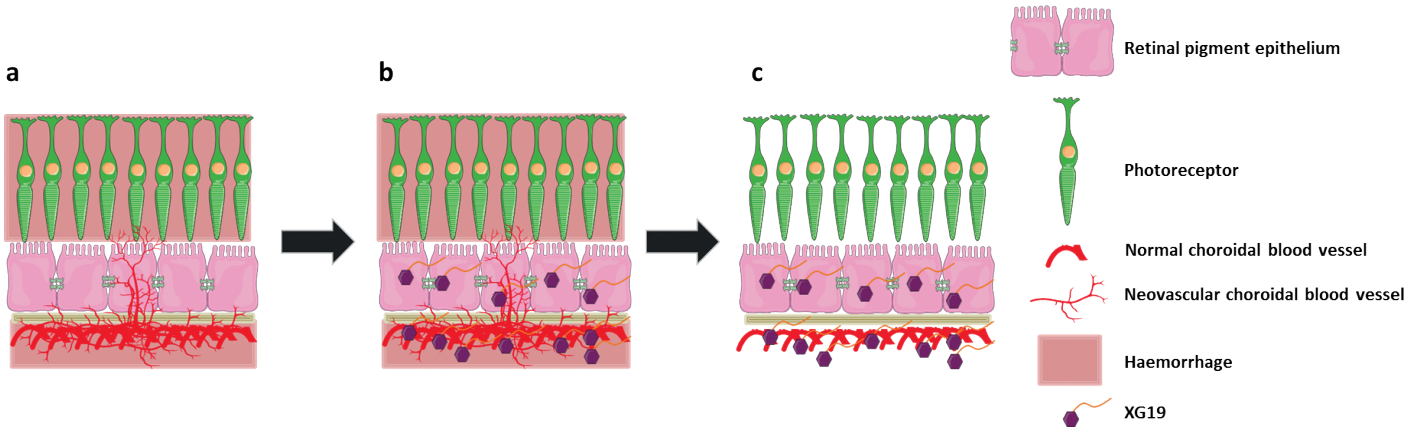

Figure 2: Therapeutic potential of XG19 in neovascular AMD. (a) Chronic unregulated blood vessel growth in the choroid (choroidal neovascularisation) results in poorly formed and leaky vessels. This disrupts the vascular supply leading to tissue ischaemia, hypoxia and inflammation. Retinal pigment epithelial cell death eventually disrupts the blood-retinal barrier and permits blood vessel growth into the sub-retinal space where further haemorrhage leads to vision loss. (b) Administration of Xentry-Gap19 (XG19) results in targeted delivery of the therapeutic peptide (Gap19) to hypoxic retinal pigment epithelial cells and choroidal blood vessels that overexpress Syndecan-4. (c) Efficient Cx43 hemichannel block reduces tissue inflammation and promotes blood vessel repair thus restoring the normal blood supply to the retina.

In an *in vivo* mouse model of choroidal neovascularisation (CNV), in which a laser is used to disrupt the blood-retinal barrier to mimic AMD pathologies, XG19 delivered via a single intraperitoneal injection was able to promote faster healing and reduce inflammation compared with control animals. Ellipsoid volumes of CNV lesions seven days post-laser treatment revealed that XG19 treated animals had significantly smaller lesion volumes, indicative of reduced blood vessel growth and inflammation. Immunohistochemistry of post-mortem tissues showed reduced Cx43, Syndecan-4 and glial fibrillary acidic protein (GFAP) expression levels in XG19 treated animals, indicative of reduced retinal inflammation.

Overall, XG19 can efficiently enter cells, especially during injury conditions, with the delivered cargo retaining its function. While given systemically during our initial studies, intravitreal injection and improved retinal transfer via Syndecan-4 expressing astrocytic endfeet is also possible.

FUTURE WORK

Our hope is to further develop XG19 as a therapeutic for vascular eye diseases as well as explore Xentry in combination with other intracellularly acting therapeutics to target diseased Syndecan-4 expressing cells of the eye, specifically.

ABOUT THE ORGANISATION

The Buchanan Ocular Therapeutics Unit (BOTU) aims to translate ocular therapeutic-related scientific research into the clinical setting, whether pharmaceutical, cell or technology based. The BOTU team is developing novel drugs and tailored controlled delivery systems with projects around dry eye, uveitis, glaucoma, diabetic retinopathy and age-related macular degeneration management.

REFERENCES

1. *Pescina S et al, "Cell penetrating peptides in ocular drug delivery: State of the art". J Control Release, 2018, Vol 284, pp 84–102.*
2. *Montrose K et al, "Xentry, a new class of cell-penetrating peptide uniquely equipped for delivery of drugs". Sci Rep, 2013, Vol 3, p 1661.*
3. *Elfenbein A, Simons M, "Syndecan-4 signaling at a glance". J Cell Sci, 2013, 126(Pt 17), pp 3799–3804.*
4. *Danesh-Meyer HV et al, "Connexin43 in retinal injury and disease". Prog Retin Eye Res, 2016, Vol 51, pp 41–68.*
5. *Abudara Vet al, "The connexin43 mimetic peptide Gap19 inhibits hemichannels without altering gap junctional communication in astrocytes". Front Cell Neurosci, 2014, Vol 8, p 306.*

ABOUT THE AUTHORS

Frazer Coutinho obtained his Bachelor's degree in Biomedical Science and Master's degree in Science majoring in Microbiology and Immunology from the University of Otago. He has been a PhD student within the Buchanan Ocular Therapeutics Unit, University of Auckland, since 2015, investigating the therapeutic potential of XG19 in ocular disease. He has a passion for innovation and is particularly interested in translational science, taking therapeutics from bench to bedside.

Colin Green holds the W&B Hadden Chair in Ophthalmology and Translational Vision Research in the Department of Ophthalmology at the University of Auckland. Professor Green's group focuses on cell reprogramming and connexin channel roles in disease, in particular chronic inflammatory diseases such as those affecting the retina. He has co-authored 185 manuscripts and book chapters and is a named inventor on over 255 patents in 29 patent families. He is a co-founder of CoDa Therapeutics (Auckland, NZ) and OcuNexus Therapeutics (US).

Ilva Rupenthal is a Senior Lecturer in the Department of Ophthalmology, University of Auckland, and the inaugural Director of the Buchanan Ocular Therapeutics Unit (BOTU), established in 2013. A pharmaceutical scientist by training, Dr Rupenthal's research focusses primarily on the development of novel ocular drug delivery systems. She is an author on over 60 peer-reviewed journal articles and has attracted more than NZ$5.6 million in research funding.

Copyright © 2019 Frederick Furness Publishing Ltd