OVERCOMING CHALLENGES WHEN DEVELOPING OLIGONUCLEOTIDES FOR OPHTHALMIC DRUGS

In this article, Shiyan Chen, PhD, and Binbin Tian, both Study Directors at WuXi AppTec, discuss the use of oligonucleotides for ophthalmic applications, including their potential uses, advantages, challenges and difficulties when it comes to pharmacokinetic and drug metabolism studies.

Oligonucleotides, or "oligos", are a class of small, single- or double-stranded synthetic nucleic acid polymers (approximately 20 mer) that can be used to modulate gene expression. They can target pre-mRNA, mRNA or non-coding RNA to induce degradation, modulate splicing events or interfere with protein translation.

Oligos have become a popular therapeutic because of their ability to target previously "undruggable" conditions, including cancers, metabolic disorders and genetic diseases. Specific retinal diseases, such as Leber's congenital amaurosis, X-linked retinitis pigmentosa and Usher syndrome, have also shown remarkable response to oligo therapy. Ocular oligos can also be used to treat glaucoma, age-related macular degeneration (AMD), dry eye syndrome, diabetic macular oedema, geographic atrophy and corneal disease. Several oligos have advanced to clinical trials for the treatment of these ocular diseases.

As of 2022, 10 antisense oligos (ASOs), five small-interference RNA (siRNA) and one aptamer have been approved for market use. Compared with the oligo drugs approved earlier by the US FDA, innovations in chemical modifications and delivery systems have greatly improved these drugs' stability and cellular uptake. However, the eye still remains a popular target for oligo drug development.

ADVANTAGES OF OLIGOS

While there are diverse groups of oligo therapeutics, they are all negatively charged, hydrophilic and in a similar range of molecular weights:

- ASOs: 4,000–10,000 Da
- siRNA: Approximately 14,000 Da
- Aptamers: 5,000–15,000 Da
- Micro-RNA (miRNA): 14,000–18,000 Da

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siRNA consists of small RNA duplexes comprising of "passenger" and "guide" strands, whereas ASOs are single-stranded fragments of nucleic acids (RNA or DNA) that identify targets without the aid of a "passenger" strand. ASOs use the RNase H1 degradation mechanism or splicing modulation mechanism, while siRNAs need the assistance of an RNA-induced silencing complex of proteins to regulate the mRNA degradation.

One major advantage of oligos is their potential to tackle targets that small molecules and protein-based therapeutics cannot. This is due to their unique mechanism of action – oligos primarily rely on Watson-Crick base pairing to the targeted mRNA molecules. This causes gene silencing, a steric block or altered splicing patterns. Aptamers function somewhat differently, folding into three-dimensional shapes, engulfing disease-causing mRNA and inhibiting protein interactions.

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Although small molecules often target membrane receptors and enzymes – and extracellular proteins or membrane receptors can be modulated with monoclonal antibodies – structural proteins or transcription factors are not often "druggable". However, because oligos act at the DNA or RNA level, they can alter gene expression without regard to the function of the proteins encoded by their target genes. This provides new opportunities to develop innovative ways of reaching therapeutic targets of interest. In principle, oligos can be rationally designed for virtually any genetic target, making them an exciting therapeutic option within precision medicine.

Around a third of blindness or severe visual impairment cases have a genetic basis, either as part of a multifactorial aetiology, such as AMD, diabetic retinopathy or glaucoma, or as the direct result of genetic mutations, such as inherited retinal diseases, inherited corneal dystrophies or optic neuropathies. Historically, some of these genetic eye disorders have been considered incurable. But the expanding knowledge of genotype-phenotype relationship and the growth in oligo therapies, means more oligos to treat them have reached clinical trials, giving hope to millions of suffering patients.

CHALLENGES OF OLIGOS

Scientists are justifiably excited about the potential of oligos to treat various diseases, however, their therapeutic application presents unique challenges. Specifically, the combination of the molecular weight of oligos and the eye's anatomical and physiological barriers results in inefficient delivery to intracellular targets. These barriers can affect drug entry into the eye across multiple routes of administration, including topical, systemic and injectable.

Because the eye is a closed, compartmentalised organ and is relatively small, it requires lower doses to achieve the desired drug concentration for local delivery of siRNA and ASO therapeutics. This can be achieved either by using topical eye drops or subconjunctival, intracameral or intravitreal injection. Lower doses could also minimise any potential interference with the complicated physiological environments encountered during oligo delivery.

Topical administration in the form of eye drops is preferable for the treatment of anterior segment diseases, as it is convenient and provides local delivery of drugs. However, topical delivery often demonstrates poor drug absorption and low bioavailability. Topical drug delivery to the posterior segment of the eye, such as for for glaucoma or uveitis, and to the anterior segment both demonstrate the same poor drug bioavailability.

Intraocular administration – injecting medicine into the eye – is an alternative method that provides greater bioavailability, drug concentration and cell targeting, but may come with a significant immune response. Failure to properly formulate and dose oligo therapies can result in severe inflammation, unsafe toxicity levels and vision loss.

Ultimately, choosing the most suitable drug delivery method depends on the target tissue. Different routes are more or less appropriate for ophthalmic administration – topical ocular and subconjunctival administration are best used to target the anterior segment, whereas intravitreal administration is better for reaching the posterior segment.

General strategies for improving oligo delivery include chemical or backbone modifications, the use of lipid nanoparticles and the direct covalent conjugation of various moieties that promote intracellular uptake, target the drug to specific cells or tissues, or that reduce clearance from systemic circulation. These include lipids, cholesterol that facilitates interactions with lipoprotein particles in the circulation; peptides, for improving cell targeting and/or cell penetration; aptamers; antibodies and sugars. For example, a 2´-O-hexadecyl (C16) conjugated to siRNA enables potent and durable silencing in the eye's aqueous humour and retinal pigmented epithelium after intravitreal administration.

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ADME PROPERTIES OF OLIGOS

Models used to study the absorption, distribution, metabolism and elimination (ADME) properties of oligos often include various *in vitro* and *in vivo* assays. The most common among these include:

In vitro models:

- Stability in serum or plasma
- Stability in liver S9 homogenates or other target tissue homogenates
- Plasma protein binding
- Metabolite identification in plasma or liver S9.

In vivo models:

- Pharmacokinetic (PK) profile
- Tissue distribution
- Urine, faeces and bile excretion
- Metabolite identification in biological matrices, such as tissue, plasma, urine, faeces, bile and cerebrospinal fluid.

Because oligos are metabolised by nucleases, they are not expected to be metabolised by cytochrome P450 (CYP) enzymes and thus have a low risk of drug-drug interaction (DDI). However, CYP enzyme induction and inhibition studies are still recommended to exclude the possibility of interference with the CYP enzyme. Similarly, studying the inhibition caused by oligos on transporters can provide additional information on DDI.

Diverse bioanalytical platforms are needed for oligo studies, including mass spectrometry, quantitative polymerase chain reaction techniques and fluorescence detectors. In addition to ADME properties, early exploration of the pharmacokinetic-pharmacodynamic (PKPD) correlation of an oligo will require analysing the target mRNA, target protein or downstream biomarkers.

The small size of the eyes and enhanced bioavailability from local delivery means that only small doses of oligos are required for effective treatment. It is worth noting

that the dose level of most ocular oligos is as low as a 100 µg per dose. The selection of appropriate bioanalytical methods with high specificity and sensitivity is critical to achieve accurate ocular PK data on oligos.

TIPS FOR CONDUCTING OCULAR OLIGO STUDIES

Preclinical *in vivo* models used to test small molecules depend on the species and its congruency with human biology. The anatomy of a rabbit's eye is similar to that of a human's, so they are some of the most commonly used animals for preclinical ocular studies. Various studies may require alternative species, but the most useful *in vivo* models for oligos are non-human primates.

Once the oligo drug is delivered to the eye, as long as the majority of the drug is retained in ocular tissues and the systemic exposure is negligible, scientists can focus more on the *in vivo* drug distribution and clearance within ocular tissues. Depending on the target tissues of the specific ocular disease, selecting and collecting dissected ocular tissues is critical for evaluating the tissue PK.

For example, fomivirsen was the first ASO approved for treating ocular disease. Following intravitreal administration, the drug was slowly cleared from the vitreous fluid through metabolism and distribution to other ocular tissues. It was also subsequently metabolised by nucleases in digestion, and fomivirsen concentration in the systemic circulation was found to be undetectable.

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In another scenario, if the oligo concentration in the systemic circulation is observable, scientists could consider conducting a quantitative whole-body tissue distribution study and an excretion study. Moreover, a plasma protein binding assay could help to understand potential DDIs with other highly protein-bound drugs.

A FINAL WORD ON OLIGOS

Ocular barriers present significant issues for delivering ophthalmic oligos. Establishing the PKPD correlation in preclinical models is critical for ensuring that the data transfers to clinical studies. The PK processes of ADME determine the lifespan of a drug in the body and the amount delivered to the site of action. Understanding these interrelated processes is critical for deciding a drug's dose and dosing frequency, thus influencing its efficacy and safety.

Ocular oligo PK studies place very high demands on the comprehensive capability of researchers and scientists. First, expert veterinarians and technicians must be able to perform different ocular administrations, such as intravitreal subretinal injections, and collect complicated ocular tissues. Next, oligos are notoriously unstable and, compared with other small molecules, require specialised handling and homogenisation conditions for tissue samples. Finally, oligos are diverse and impact biological matrices in different ways. Diverse bioanalytical approaches are often required to accomplish the full drug metabolism and pharmacokinetic characterisation of oligos and fulfil the requirement for high sensitivity.

There is very little official regulatory guidance for oligo drugs and, as noted prior, conducting PK studies on them can be challenging. A lab testing partner that has extensive experience with oligo drugs and comprehensive capabilities using various bioanalytical methods could greatly accelerate drug development in this area.

ABOUT THE COMPANY

As a global company with operations across Asia, Europe and North America, WuXi AppTec provides a broad portfolio of R&D and manufacturing services that enable the pharmaceutical and healthcare industry around the world to advance discoveries and deliver groundbreaking treatments to patients. Through its unique business model, WuXi AppTec's integrated, end-to-end services include chemistry drug CRDMO (Contract Research, Development and Manufacturing Organisation), biology discovery, preclinical testing, clinical research services and cell and gene therapies CTDMO (Contract Testing, Development and Manufacturing Organisation), helping customers improve the productivity of advancing healthcare products through cost-effective and efficient solutions. WuXi AppTec received an AA ESG rating from MSCI in 2022 and its open-access platform is enabling more than 5,900 customers from over 30 countries to improve the health of those in need – and to realise the vision that "every drug can be made and every disease can be treated".

ABOUT THE AUTHORS

Dr Shiyan Chen earned an PhD in Pharmaceutical Science and is a Study Director in the drug metabolism and pharmacokinetic service department at WuXi AppTec. She has extensive research experience in preclinical pharmacokinetics and gene regulation and is currently supporting the preclinical development of multiple oligonucleotide drugs.

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