The PK-Eye-the need of an ocular in vitro model during preclinical development

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# Glossary

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tr>
<td><strong>Age-related macular degeneration (AMD)</strong></td>
<td>A common eye disease affecting mostly elderly patients above 60 years and almost 10 million people worldwide. It causes damage to the macula (i.e. a small spot near the center of the retina). It results in the loss of vision.</td>
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<tr>
<td><strong>Angiogenesis</strong></td>
<td>The development of new blood vessels. It plays a vital role in growth and development including wound healing. However, when it goes out of control it can cause many medical conditions, including cancer and inflammation.</td>
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<td><strong>Anterior-hyaloid pathway</strong></td>
<td>The vast majority of this aqueous passes the anterior hyaloid membrane and flows into the front of the eye (anterior chamber) to then leave the eye via trabecular and uveoscleral pathways. Therapeutic proteins (or large molecular weight drugs) predominantly exit via this route.</td>
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<td><strong>Anti-drug antibodies (ADAs)</strong></td>
<td>An immunogenic response of a particular substance when tested in a living model. The ability of the body to induce a cell mediated immune response. For example, when testing a human therapeutic protein in an animal model, the formation of ADAs is possible in response to that protein and for the body to fight against it.</td>
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<td><strong>Antibodies</strong></td>
<td>Antibodies are immunoglobulins. They are Y-shaped proteins that are produced by the immune system to protect the body and to stop access to foreign substances.</td>
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<td><strong>Ciliary body</strong></td>
<td>Part of the eye that is responsible for the production of aqueous humour. The ciliary body includes the ciliary muscle, which controls the shape of the lens. Another function of the ciliary body is maintain the lens in place.</td>
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<td><strong>Dissolution models/testing</strong></td>
<td>They are routinely used to provide <em>in vitro</em> drug release data and are extensively used in pharmaceutical companies. They are used for both quality control (QC) and to assess batch-to-batch consistency of various dosage forms.</td>
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<td><strong>Half-life (t1/2)</strong></td>
<td>The time taken for a substance to fall to half its original value.</td>
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<td><strong>High molecular weight drugs</strong></td>
<td>These include molecules such as proteins and antibodies (150 kDa)</td>
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<td><strong>Intravitreal (IVT)</strong></td>
<td>Direct injection given to the eye to deliver drugs directly. As compared to topical administration, intravitreal injections give a higher therapeutic dose when targeted to the back of the eye.</td>
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<td><strong>Implant</strong></td>
<td>Usually made of polymers that are delivered to the body to allow a more sustained release of a drug i.e. to extend the duration of action of a drug to reduce the frequency of drug administration.</td>
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<tr>
<td><strong>In vitro</strong></td>
<td>A process performed outside a living organism, e.g. in a test tube, petri dish or an in vitro model.</td>
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**In vivo**  
A process performed in a living organism, e.g. in an animal model

**Low molecular weight drugs**  
These molecules usually refer to steroids, anti-virals and antibiotics, with a molecular weight below 1000 Da

**Pharmacokinetics**  
The movement of drugs within the body, which involves absorption, distribution, metabolism and excretion (ADME)

**Posterior segment**  
Refers to the back of the eye where the vitreous is present and where most blinding diseases occur

**Retina-choroid-sclera (RCS) pathway**  
Elimination of a drug towards the back of the eye. Most low molecular weight drugs (that are also lipophilic) will exit via this route. These drugs have a shorter $t_{1/2}$ as compared to the therapeutic proteins and macromolecules

**Vascular endothelial growth factor (VEGF)**  
VEGF is a signalling protein that helps restore the supply of oxygen to tissues when blood circulation is inadequate and is implicated in angiogenesis. Inhibiting VEGF has been clinically shown to slow the progression of medical conditions driven by angiogenesis

**Vitreous**  
A gel-like material present in the posterior segment of the eye, which is 98-99% water and is responsible for the shape of the eyeball. The viscosity of the vitreous can vary between a juvenile and an ageing vitreous, and between a healthy and unhealthy eye
Abstract
Much effort globally is focused on developing the next generation of therapeutic antibodies and other long acting therapeutics to treat ophthalmic diseases. Most treatments must be administered by intravitreal injection, so an important goal is to develop medicines with an extended residence time within the eye. The development of prolonged acting protein-based therapies is limited by the formation of anti-drug antibodies (ADAs) in animal models. We have developed and validated a two-compartment, aqueous outflow model called the PK-Eye to be used for ocular drug development.
Introduction

Posterior segment diseases accounts for over 70% of the blind registrations in Europe. Inflammatory, angiogenic and fibrotic processes cause tissue damage and vision loss. These processes are also major contributors to the failure of current treatments for these diseases. As the elderly population continues to grow, there is an urgent and unmet need to control the dose and optimise the duration of medicines in the posterior segment. Dose reproducibility is difficult to achieve with eye drops and most often the medicine is not able to adequately distribute into the posterior segment. Intravitreal injections are currently the only way clinically to ensure the largest therapeutic dose possible is administered into the vitreous body. Current treatment regimens however are onerous and not without risk when considered in the long-term management to control the progression of chronic blinding conditions. Treatment periods for a chronic condition such as age-related macular degeneration (AMD) can last for many years with patients receiving intravitreal injections monthly or every other month. Each time an injection is performed there is a small but real risk of bleeding, retinal detachment and infection, which may lead to complete vision loss (Jager et al. 2004). There remains the need to develop better medicines and final dosage forms with a longer duration of action that require less frequent administration.

Therapeutic proteins in ocular drug delivery

Increased knowledge of the molecular mechanisms of blinding diseases (de Oliveira Dias et al. 2011; Rodrigues et al. 2009) has driven the development of therapeutic proteins including antibody-based medicines. Intravitreal injections of therapeutic proteins and the use of steroid implants in the vitreous cavity are currently the best clinical methods to achieve prolonged exposure in the back of the eye. Vascular endothelial growth factor (VEGF) neutralising antibodies have considerably improved the treatment of exudative macular degeneration and diabetic retinopathy. Therapeutic biologics registered for ophthalmic use by intravitreal injection comprise a PEGylated-aptamer (Pegaptanib), antibody fragment (ranibizumab), and an Fc fusion (afibercept). The monoclonal antibody (mAb), bevacizumab is also widely used as an unlicensed medicine to treat AMD. Prior to introduction of the anti-VEGF antibody based medicines, it was difficult to treat these blinding diseases effectively (Ambati et al. 2003). A major challenge during both preclinical and clinical development is to determine the ocular clearance rates of new medicines. Even though the anti-VEGF antibody based
medicines can be administered once a month and in some cases every other month (Stewart et al. 2012), much research is focused on developing medicines which will require less frequent dosing regimens.

Another factor in the development of longer acting formulations is the cost of the drugs and overall treatment costs, which is a function of dosing frequency. Prior to the introduction of aflibercept, ranibizumab (licensed) and bevacizumab (unlicensed) were generally used to treat AMD. Each injection of ranibizumab (Lucentis®) can cost $2,000 and whereas bevacizumab (Avastin®) can cost only $50 (Raftery et al. 2007). The relatively low cost of bevacizumab drove its clinical use. Bevacizumab is presented in a vial containing 400 mg (25 mg/mL) for use to treat cancer patients and was fractionated into syringes in specialist pharmacies for intravitreal injection. The intravitreal dose of bevacizumab was simply a result of what amount of antibody was contained in 50 µL of the bevacizumab solution from the vial, rather than determined by a clinical trial. Considering that the formulation of bevacizumab was not developed for intravitreal injection and that there is no control of manufacture for the final dose presentation, the use of bevacizumab has been a remarkable example of how influential cost has become in clinical practice. Only recently has it been shown in a in head-to-head control trials in both the US and UK that a 1.25 mg dose of bevacizumab is clinically equally efficacious to the registered 0.5 mg dose of ranibizumab (Chakravarthy et al. 2012). New medicine that are being developed must take the cost of treatment into consideration to ensure wide patient access is possible.

**Issues with animal models during preclinical development**

The intrusive nature of the experiments to evaluate human ocular pharmacokinetics has limited the vast majority of studies to animal models (Rittenhouse & Pollack 2000). Frequent drug sampling from the vitreous of humans would be unethical due to the risks involved. Withdrawing a blood sample for a systemically administered drug is routine, whereas tapping the vitreous is not. So it is not generally possible to obtain human pharmacokinetics data during clinical trials.

During preclinical development, allometric modelling from *in vivo* models is not reliable due to (i) the multitude of anatomical differences between animal and human eyes (Laude et al. 2010) and (ii) the immunological response of animals caused by the administration of a humanised protein. The formation of ADAs in animals towards
humanised proteins results in accelerated clearance rates (Vugmeyster et al. 2012) making it impossible to evaluate prolonged dosage forms of new protein therapeutics (Brinks et al. 2011). The formation of ADAs is not predictive of the human immunological response, and may alter the biological activity and toxicity profile of preclinical candidates which can further delay development (Shankar et al. 2006).

**Use of *in vitro* models in ocular drug delivery**

Mass exchange within the eye is dominated by aqueous flow, which is secreted at 2.0–2.5 µL/min into the vitreous from the ciliary body. There are two main drug elimination pathways from the vitreous: (a) the aqueous outflow into the anterior chamber and (b) permeation through the retina via retinal-choroid-sclera (RCS) pathways. Therapeutic proteins are high molecular weight (MW) and charged molecules, and they clear predominantly by the anterior route. Proteins consequently have longer half-lives ($t_{1/2}$) i.e. days in the vitreous cavity than low MW drugs i.e. hours, many of which are lipophilic and permeable. Little has been reported to develop an *in vitro* model of the eye that accounts for the aqueous flow (Fogli et al. 2014; Repetto et al. 2005; Stocchino et al. 2007; Patel et al. 2015) to estimate clearance times for molecules that exit the eye predominantly via the anterior route through the front of the eye. Flow within the model is critical to allow drug clearance once it is injected into the posterior cavity and clearance mimics the anterior-hyaloid pathway.

**The PK-Eye and its use in drug delivery**

We have developed a low-cost *in vitro*, two-compartment model scaled to human dimensions with aqueous flow designed to mimic the total aqueous mass transfer through the anterior route. The model (called the PK-Eye (Awwad et al. 2015)) is specifically designed to mimic the intraocular aqueous outflow to estimate (1) the clearance of therapeutic proteins (Awwad et al. 2015), (2) the dissolution of drug suspensions (Awwad et al. 2015), and (3) the release profiles of implants from the vitreous cavity (Baskakova et al. 2016; Awwad et al. 2017). The PK-Eye (Awwad et al. 2015) was developed to estimate human vitreous clearance times and to predict drug stability.

One criticism of the model is it is “*in vitro*” and does not account for the clearance properties of living tissue (e.g. permeation, active transport) and eye movement. The PK-Eye model was designed for use during preclinical studies in an analogous way as a
multitude of *in vitro* models used for other modes of administration (e.g. oral, buccal, pulmonary, topical, etc). For example, the paddle dissolution apparatus (Barat et al. 2006) is routinely and widely used in the preclinical development of oral dosage forms to estimate the dissolution of preclinical candidates. Protocols are standardised and published in international pharmacopeia and simulated biological fluids are used to evaluate different features of dissolution (Marques et al. 2011; Farrugia 2002). While there are limitations in the paddle dissolution apparatus include the volume, geometry and mobility of the stirrer, this apparatus and many elated *in vitro* models for oral dosage forms are widely used formulation optimisation and in quality control (QC) efforts. The PK-Eye model can be used in an analogous way to other *in vitro* models to optimise long acting formulations that are designed (i) to prolong the dissolution or release of a low MW drug and (ii) to determine the clearance time of therapeutic proteins. The PK-Eye can be used in an iterative process to decrease preclinical development time lines and is expected to minimise the use of animal models with suboptimal preclinical candidates.

The PK-Eye is being used to evaluate new drug delivery strategies and devices that are being developed to prolong the vitreous $t_{1/2}$ and the duration of action of a protein therapeutic. Using the PK-Eye to estimate the pharmacokinetic profiles of long lasting dosage forms during preclinical research may accelerate development. The PK-Eye was designed to be simple and practical to use to allow for iterative processes to occur so that formulations can be optimised efficiently and while minimising the use of animal models for suboptimal preclinical candidates. The PK-Eye can be used with other *in vitro* or computational permeability models to develop *in vitro in vivo* correlations (IVIVCs) that include RCS clearance pathways (Awwad et al. 2017). Our studies show the PK-Eye model has many of the features needed to become a practical *in vitro* model with the capacity to contribute to research efforts focused on the development of new ophthalmic medicines.
Dr Sahar Awwad is a postdoctoral research scientist in UCL, School of Pharmacy and UCL, Institute of Ophthalmology (London, UK). She recently completed her PhD with two scholarships i.e. the UCL Overseas Research Scholarship (ORS) and NIHR Biomedical Research in Ophthalmology at Moorfields Hospital. She has worked on various projects that have resulted in the development of a new in vitro model of ocular pharmaceutics along with a new strategy for extending the duration of action of therapeutic proteins in the eye. Her projects are widely based on drug delivery, ocular pharmaceutics, pharmacokinetics and biodistribution properties, protein production and modification and extensive knowledge on protein characterisation. Dr Awwad is also a co-founder of Optceutics, a new company utilising the PK-Eye to develop new formulations.
References


