

# *In vitro* evaluation of the therapeutic tail of bevacizumab in the eye

Sahar Awwad<sup>1,2</sup>, Peng T. Khaw<sup>1</sup>, Steve Brocchini<sup>1,2</sup>

<sup>1</sup>National Institute for Health Research (NIHR) Biomedical Research Centre at Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology, London, United Kingdom (p.khaw@ucl.ac.uk)

<sup>2</sup>UCL School of Pharmacy, London, United Kingdom ([s.awwad@ucl.ac.uk](mailto:s.awwad@ucl.ac.uk))

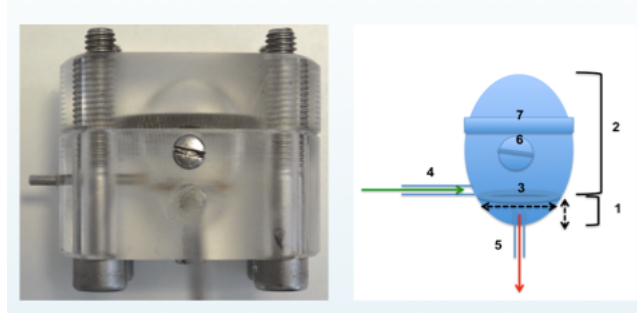
## INTRODUCTION

Prolonging therapeutic levels of a drug within the vitreous to treat blinding diseases is one of the most important goals in ophthalmic drug development. Intravitreal (IVT) injections of therapeutic proteins and the use of steroid implants in the vitreous are currently the best clinical methods to achieve prolonged exposure in the back of the eye. Therapeutic biologics registered for ophthalmic use by intravitreal (IVT) injection comprise a PEGylated-aptamer (Pegaptanib), antibody fragment (ranibizumab), and an Fc fusion (aflibercept). The monoclonal antibody, bevacizumab is also widely used as an unlicensed medicine to treat age related macular degeneration. It is anticipated that ophthalmic protein-based medicines, which tend to be potent and have a rapid onset of action, will continue to be developed as molecular mechanisms involved in blinding diseases become better understood

Mass exchange within the eye is dominated by aqueous which is secreted at 2.0–2.5  $\mu\text{L}/\text{min}$  into the vitreous from the ciliary body. The majority of aqueous passes the anterior hyaloid membrane and flows into the anterior chamber to exit the eye via trabecular and uveoscleral pathways. 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. As high molecular weight and charged molecules, therapeutic proteins clear predominantly through the anterior route. Proteins have been reported to have longer half-lives (i.e. days) in the vitreous than soluble RCS permeable molecules (e.g. lipophilic molecules), which generally clear in a matter of hours.

There is much research focused on developing new strategies to increase the vitreous residence times of macromolecular drugs to avoid the frequency of IVT injections. We have developed<sup>1</sup> and validated a two-compartment, aqueous outflow model to be used for ocular drug development called the PK-Eye. It has been shown to be particularly useful in evaluating protein PK and stability properties. Our model is used to aid the preclinical development of novel, long-acting therapies. As more stable protein therapeutics are developed, much higher concentration doses may be possible where exploitation of a therapeutic tail is difficult to evaluate in animal models because of the generation of anti-drug antibodies (ADAs). The less frequent dosing of aflibercept is primarily based on using a larger dose than what is used for ranibizumab.

In this study, we studied the different doses of bevacizumab (1.25, 2.5 and 5.0 mg) in the PK-Eye (Figure 1) with phosphate buffer saline (PBS, pH 7.4) and simulated vitreous (polymeric combination of hyaluronic acid and agar) to explore the protein's therapeutic tail.



**Figure 1:** The PK-Eye model comprises two plastic pieces that are held together with four screws with a fitted rubber washer (7) between the two pieces to seal the posterior cavity and to prevent leakage. A membrane (3) separates the anterior (1) and posterior (2) cavities, which have a volume of 200  $\mu\text{L}$  and 4.2 mL, respectively. The inlet port (4) allows flow immediately posterior to the membrane (3). The inlet port was connected with plastic tubing (1.5mm internal diameter) to a 16-channel Ismatec peristaltic pump to produce a flow rate of 2.0  $\mu\text{L}/\text{min}$  of PBS, pH 7.4 with 0.02% sodium azide. Aqueous outflow is collected from the anterior cavity at a single outlet port (5), which is elevated to about 3.0 cm to provide a small amount of back-pressure to maintain a full internal volume within the model. The inner diameter of the inlet and outlet ports was 1.0–1.5 mm.<sup>1</sup>

## METHOD

The PK-Eye has been designed to have both an anterior (0.2 mL) and a posterior (4.2 mL) cavity with a membrane in between the two cavities. There is an inlet port that allows phosphate buffered saline (PBS, pH 7.4) to flow at a fixed rate of 2.0  $\mu\text{L}/\text{min}$  at ambient temperature. The posterior cavity of the model was filled with either PBS, pH 7.4 or simulated vitreous, which comprised a combination of hyaluronic acid and agar. The combination of agar and hyaluronic acid has been found to have a dynamic viscosity of 0.6 Pa.s (average human vitreous viscosity is around 0.5 Pa.s). Bevacizumab (25 mg/mL) was injected via the injection port into the posterior cavity at different doses (1.25 mg in 50  $\mu\text{L}$ ; 2.5 mg in 100  $\mu\text{L}$  and 5.0 mg in 200  $\mu\text{L}$ ). Samples were obtained from the anterior outflow sampling port at various time intervals and were analysed by high performance liquid chromatography (HPLC) at 280 nm to determine their clearance kinetics. Experiments were

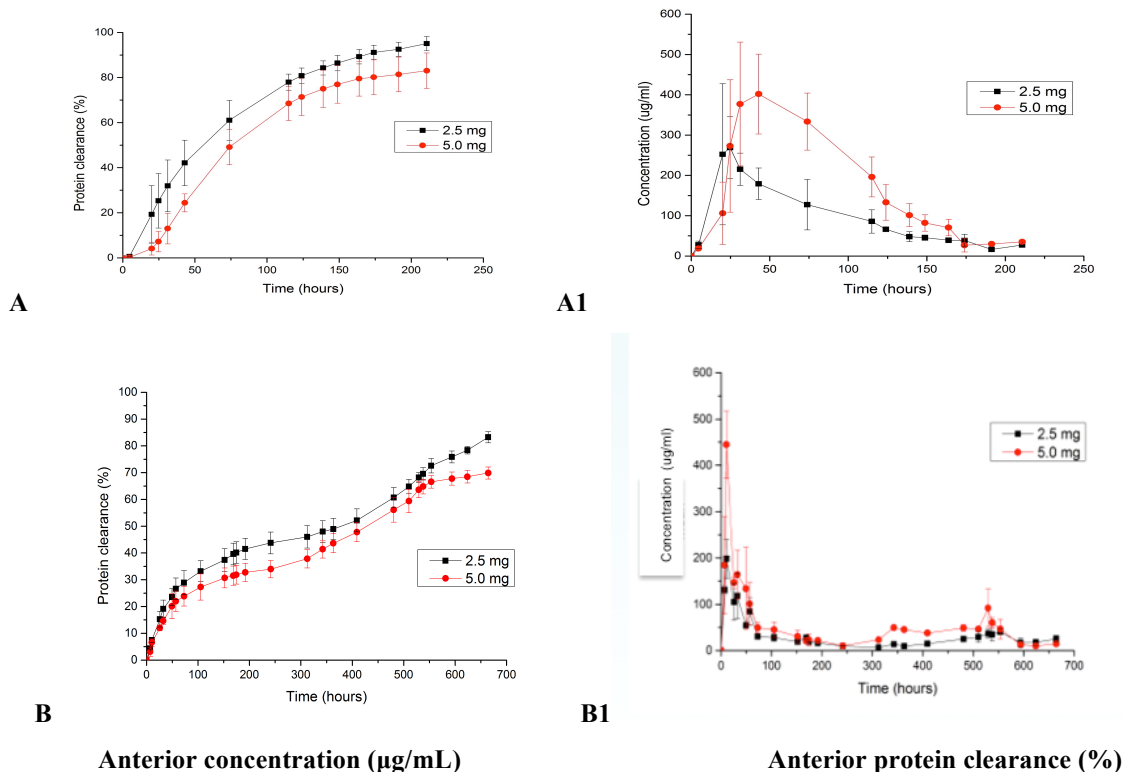
conducted in triplicate (n=3) and results were plotted in OriginPro with their means and standard deviations ( $\pm$  STD). The rate constant ( $k$ ) and half-life ( $t_{1/2}$ ) were calculated from the best fitting model with the data set.

## RESULTS

Clinically, the therapeutic dose of bevacizumab is usually 1.25 mg, in a 50  $\mu$ L injection. When this dose was tested in the PK-Eye containing PBS (to mimic vitrectomised eyes) and simulated vitreous, the half-lives ( $t_{1/2}$ ) for the residence times in the model were  $1.2 \pm 0.1$  days and  $10.1 \pm 0.7$  days respectively. These half-lives are comparable to what is observed in vitrectomised monkey eyes and human eyes.

## CONCLUSION

The model is currently being used to study the stability of proteins to develop strategies to maintain the presence of the protein in the posterior cavity for longer periods, e.g. in the form of implants and sustained release formulations. The PK-Eye can easily be used to evaluate protein function and stability. This is difficult to impossible to accomplish using animal models and is especially important for dosage forms that have longer clearance times that could extend over a 2–3-month period. 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 protein therapeutics.



**Figure 2:** Different doses of bevacizumab (2.5 mg and 5.0 mg) tested in the PK-Eye after injection into the posterior cavity of the PK-Eye containing PBS, pH 7.4 (A and A1) and simulated vitreous (B and B1). All data were obtained in triplicate (n=3) and were plotted as the mean and standard deviation ( $\pm$  STD).

(6.7–10.0 days)<sup>2-5</sup>. Larger doses (2.5 and 5.0 mg) of bevacizumab were then evaluated using PBS in the model, and the half-lives were  $2.3 \pm 0.8$  and  $3.4 \pm 0.7$  days respectively (Figure 2 (A and A1)). With simulated vitreous, the 2.5 and 5.0 mg doses displayed half-lives of approximately  $15.4 \pm 0.7$  and  $18.3 \pm 1.1$  days respectively (Figure 2, B and B1). Larger doses increased the clearance to a moderate extent, as would be expected with aflibercept (~115 kDa, 2.0 mg). Pegaptanib (~50 kDa, 0.3 mg) displayed a half-life in humans of about 7-8 days, which increased to  $10 \pm 4$  days using a 3.0 mg dose.

## REFERENCES

1. Awwad S, Lockwood A, Brocchini S, Khaw PT. The PK-Eye: A Novel *In Vitro* Ocular Flow Model for Use in Preclinical Drug Development. *J Pharm Sci.* 2015;104(10):3330-3342. doi:10.1002/jps.24480.
2. Krohne TU, Eter N, Holz FG, Meyer CH. Intraocular pharmacokinetics of bevacizumab after a single intravitreal injection in humans. *Am J Ophthalmol.* 2008;146(4):508-512. doi:10.1016/j.ajo.2008.05.036.
3. Meyer CH, Krohne TU, Holz FG. Intraocular pharmacokinetics after a single intravitreal injection of 1.5 mg versus 3.0 mg of bevacizumab in humans. *Retina.* 2011;31(9):1877-1884. doi:10.1097/IAE.0b013e318217373c.
4. Zhu Q, Ziemssen F, Henke-Fahle S, et al. Vitreous levels of bevacizumab and vascular endothelial growth factor-A in patients with choroidal neovascularization. *Ophthalmology.* 2008;115(10):1750-1755. 1755.e1. doi:10.1016/j.ophtha.2008.04.023.
5. Beer PM, Wong SJ, Hammad AM, Falk NS, O'Malley MR, Khan S. Vitreous levels of unbound bevacizumab and unbound vascular endothelial growth factor in two patients. *Retina.* 2006;26(8):871-876. doi:10.1097/01.iae.0000233327.68433.02.

