A SUSTAINED RELEASE PROTEIN FORMULATION FOR INTRAOCULAR USE

Sahar Awwad[1,2], Athmar Al-Shohani[1,2], Peng T. Khaw[2] and Steve Brocchini[1,2]
[1]UCL School of Pharmacy, London, United Kingdom. [2]National Institute for Health Research (NIHR) Biomedical Research Centre at Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology, London, United Kingdom.
E-mail: s.awwad@ucl.ac.uk

Introduction

An ageing population, together with a greater understanding of ocular disease, are driving the development of new generations of protein therapeutics for ocular use. Currently, antibodies to treat age related macular degeneration (AMD) are administered about every 4-6 weeks by intravitreal (IVT) injections. Although IVT injections are generally safe, they require a clinical procedure that can be uncomfortable for the patient. There is also an economic burden for treating increasingly large numbers of AMD patients, many of whom will be treated for decades.

Much effort is now focused on the need to develop ocular dosage forms that can be administered less frequently. We have recently developed an *in vitro* ocular outflow model (the PK-Eye) that can be used to estimate the human ocular half-life and stability of new protein therapeutics and new dosage forms[1] to be administered to the back of the eye. Our model is used to aid the pre-clinical development of novel, long-acting therapies. As more stable protein therapeutics are developed, doses with higher concentrations may be possible. This will allow the clinical exploitation of a therapeutic tail which is difficult to evaluate in animal models due to the generation of anti-drug antibodies (ADAs). To increase the half-life of bevacizumab, we now report the preparation of a N-Isopropylacrylamide (NIPAM) *in situ* forming gel in the presence of bevicizumab (Avastin), which is used in IVT injections. The half-life and binding activity of bevacizumab were determined in an effort to optimise the cross-link density of the NIPAM gel.

Results and Discussion

Bevacizumab (1.0 mL; 25.0 mg/mL, pharmaceutical preparation) was mixed with NIPAM (40.0 mg) and ammonium per sulfate (APS, 4.0 mg). Different amounts (~4, 8 and 12 µL) of poly(ethylene glycol) diacrylate (PEG-DA) crosslinker were added to the polymerisation mixture, along with *N*,*N*,*N*,*N*-tetramethylethylenediamine (TEMED), and the mixture was incubated for ~24 hours at 4°C. Both injection and gel formation of the bevacizumab loaded gels were separately evaluated in the PK-Eye (flow rate: 2.0 µL/min, fixed temperature at 37°C). Samples were analysed by high performance liquid chromatography (HPLC) at 280 nm to calculate the release kinetics. Binding properties of released bevacizumab were evaluated against its ligand (vascular endothelial growth factor, VEGF) using surface plasmon resonance (SPR).

For efficiency, PBS, rather than simulated vitreous was used in the PK-Eye to determine release kinetics. Bevacizumab gels (2.5 mg, 100 µL) with different amounts of PEGDA (4 uL, 8 uL and 12 uL) displayed a $t_{1/2}$ of approximately ~2.01 ± 0.01, 3.66 ± 1.21 and 2.55 ± 0.03 days respectively in a bimodal release profile. These studies showed continuous release of bevacizumab after a month. In contrast, gel-free doses of bevacizumab (2.5 and 5.0 mg) displayed a $t_{1/2}$ of 2.3 ± 0.8 and 3.4 ± 0.7 days respectively. Samples collected throughout the 4 week study were evaluated by SPR, and they all displayed binding affinity to VEGF. These binding studies indicated that bevacizumab maintained its binding properties in the gels and in the PK-Eye.

Conclusion

Different thermoresponsive NIPAM gels that were prepared in the presence of bevacizumab displayed a prolonged release profile compared to bevacizumab injection alone. The gel formulation was bimodal, with the release of bevacizumab being observed for a month. SPR results indicated that the bevacizumab released from the gels maintained its binding properites throughout the 4 week study. These studies indicate that it is possible to extend the duration of protein release using a thermoresponsive gel that is prepared in the presence of the protein.

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.