The Monthly Eye Drop: Preclinical testing of long-term, hydrogel/microsphere eye drops for glaucoma

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Statement of Purpose: Glaucoma is the second leading cause of blindness worldwide, expected to affect up to 3 million Americans by 2020. One of the main risk factors in glaucoma is an unsafe increase in intraocular pressure (IOP). IOP reduction in patients with glaucoma is typically accomplished through the administration of medicated eye drops several times daily, the difficult and frequent nature of which contributes to patient compliance rates estimated to be as low as 30%. Newer drug delivery methods for glaucoma aimed at improving patient compliance require clinician administration of invasive injections or implants. The purpose of this study was to develop and test a hydrogel/microparticle formulation that provides one month of therapeutic levels of glaucoma medication from a noninvasive, patient-administered drop. We hypothesize that this novel treatment method will address the issues of both compliance and poor bioavailability inherent to traditional eye drop medication while avoiding the need for more invasive techniques.

Methods: Poly(lactic-co-glycolic) acid (PLGA) microparticles containing BT were fabricated using a standard double emulsion procedure. Poly-(N-isopropylacrylamide) (pNIPAAm)-based hydrogels containing poly(ethylene glycol) (PEG) were prepared by polymerization in the presence of ammonium persulfate overnight and washing with PBS. The microparticles and gels were combined by mixing following polymerization of the gel. The properties of the microparticles and hydrogels were individually characterized as well as the properties of the combined gel/microparticle system. In vitro release of BT from the gel/microparticle system was quantified by incubating a known mass of microparticles in buffer and measuring the absorption using a spectrophotometer. For *in vivo* efficacy testing, a single drop was administered to the inferior fornix of New Zealand white rabbits. IOP was measured periodically using tonometry and histology was used to assess whether the gel/microparticle system resulted in any inflammation of the surrounding tissues.

Results: Microparticles were confirmed to have a diameter of 7.5±2.9 μm with a primarily poreless morphology. The pNIPAAm gel demonstrated a lower critical solution temperature (LCST) of approximately 34°C, which was unaffected by the addition of drug-loaded microparticles. Degradation of the gel at 37°C for over one month was negligible with or without microparticles. Drug release was similarly unaffected by the presence of the pNIPAAm-PEG hydrogel. *In vitro* cytotoxicity testing also demonstrated that the gel/microparticle system had no significant effect on conjunctival cell viability. Our *in vivo* study of the long term eye drop system demonstrated that the gel/microparticle drop could be easily administered in the same way as a traditional eye drop and form a stable, opaque gel. IOP reduction for a single administration of the hydrogel eye drops was comparable to twice-daily BT drops, with significantly lower systemic uptake. There was no evidence of irritation or inflammation due to the gel drops. *In vivo* pharmacokinetic and toxicity studies are ongoing.

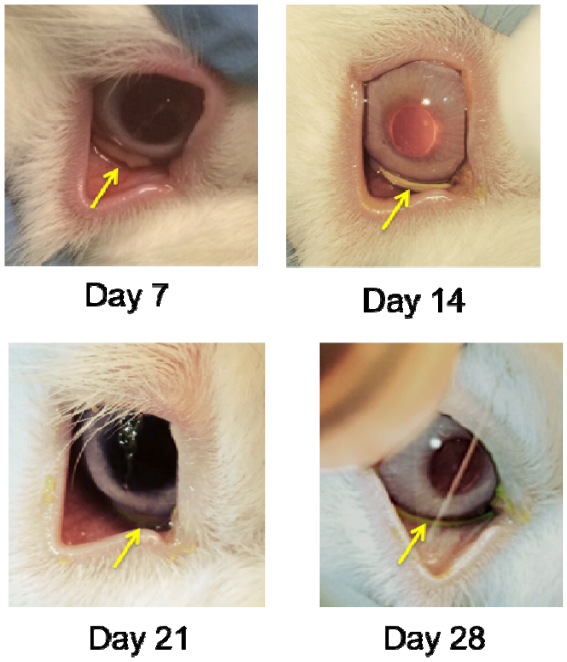

Figure 1. *In vivo* placement of drug loaded gel/microsphere eye drop over 28 days.

Conclusions: The BT-loaded microparticles presented in this study are capable of releasing a therapeutically relevant amount of a common glaucoma medication for four weeks. We have developed a cytocompatible, thermoresponsive hydrogel matrix for the microparticles that does not affect release. The non-degrading gel/microparticle system forms a stable, solid drop in the inferior fornix where it releases the encapsulated glaucoma drug BT. Overall IOP reduction was comparable to the clinical standard and achieved using 56 times fewer doses. This novel ocular drug delivery system represents a vast improvement in bioavailability and dosing frequency over current topical methods.