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OcuDrain-E™—A noninvasive technique for reduction of intraocular pressure

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ABSTRACT

OcuDrain-E™ is a noninvasive technique in which electrical pulses are applied across the cornea to enhance the rate of transcorneal water evaporation (TCWE). *In vitro* studies were carried out with rabbit cornea mounted on a Franz diffusion cell. Application of 30 pulses each of 1 millisecond (ms) duration at ≥40 V/cm^2 decreased the corneal resistivity ~80% indicating permeabilization of the cornea. The corneal resistivity was almost completely recovered within 6 h when the pulse voltage was <40 V/cm^2. The average TCWE at 40 V/cm^2 was significantly (~39-fold) higher than the control (*t*-test, $p < 0.0001$). Application of electrical pulses (40 V–30 pulses–1 ms–1 Hz) across the cornea resulted in significant decrease in the intraocular pressure (IOP) in rabbits. The electrical protocol was well tolerated by the rabbits. Microscopic studies revealed that the applied electrical protocol did not cause any edema or detachment of the epidermal layers. The results of current investigation suggest that OcuDrain-E™ could be developed as a potential technique for the treatment of glaucoma in patients who respond poorly to drugs.

1. Introduction

Glaucoma is a group of ophthalmic disorder associated with increase in the intraocular pressure (IOP). The IOP is determined by the balance between the aqueous humor production and outflow. The rate of drainage through schlemm's canal in human is ~2 μl/min. In case of partial or complete blockade of the trabecular and/or uveoscleral pathways, the IOP increases due to excessive accumulation of aqueous humor (Caprioli, 1985). Hyper IOP condition could lead to damage of the optic nerves and cause blindness. The goal of current glaucoma therapy is to control IOP by the use of drugs. Unfortunately, nearly 15–20% of the people respond poorly to drugs or suffer from severe systemic side effects and inevitably have to undergo surgery (Caprioli, 1985; Boswell et al., 1999). However, glaucoma surgeries are associated with some possible risks of severe post-surgical complications (Zarbin and Chu, 2005).

Cornea is a transparent tissue consisting of multiple layers of cells. However, corneal epithelium is the primary barrier that restricts the movement of water and hydrophilic substances across the cornea (Candia, 2004). Corneal epithelium consists of closely packed cells and the intercellular space filled with lipids. The gradient in water content across the cornea drives water always from the endothelial side to the epithelial surface of the cornea which evaporates after reaching the surface of the corneal epithelium. The normal transcorneal water evaporation rate (TCWE) in human is only ~5 μl/h and hence its contribution towards homoeostasis of IOP is negligible (Ortiz et al., 1998). However, when the cornea is rendered relatively more permeable, significant increase in TCWE could occur which in turn could potentially lead to considerable reduction in the IOP. This approach requires development of a technique that could bring about reversible permeabilization of the corneal epithelium and cause no damage to the internal tissues of the eye as well. Treatment with low and high voltage electrical pulses is known to render the biological barriers more permeable to water, ions and molecules (Vanbever et al., 1996; Oshima et al., 2002, 1998; Sakamoto et al., 1997; Zhang et al., 2004). OcuDrain-E™ is a technique of reversible permeabilization of the cornea by application of appropriate electrical protocol. This in turn is believed to enhance TCWE. A considerably high TCWE could potentially have significant impact on the IOP. The current study assessed the feasibility of the OcuDrain-E™ technique in reducing IOP by the way of reversible permeabilization of the cornea.

2. Materials and methods

2.1. Materials

Krebs ringer bicarbonate (KRB), hydroxypropylmethyl cellulose (HPMC), haematoxylin, eosin, glucose, fluorescein isothiocyanate labeled dextans (FITC) of different molecular weights (1 kDa,

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2.5 kDa, 4 kDa, 10 kDa and 35 kDa) and trinder reagent were purchased from Sigma chemicals (St. Louis, MO).

2.2. Ex vivo studies

2.2.1. Electrical resistivity

In vitro experiments were carried out using a two compartment diffusion cell fitted with Ag/AgCl electrodes in each compartment. Freshly excised rabbit cornea was sandwiched between the apical compartment and the basal compartments, with the help of a custom made corneal adapter. KRB buffer (300 μl in donor and 5 ml in the receiver) was placed in both the compartments and the electrical resistivity was measured after an equilibration time of ~30 min. The electrical resistivity across the cornea (R_C) was measured by placing a load resistor R_L (100 kΩ) in series with the cornea. The voltage drop across the whole circuit (V_O) and across the cornea (V_C) was measured using an electrical set up consisting of a wave form generator and a digital multimeter (Agilent Technologies, Santa Clara, CA). For measuring resistance, a low voltage of 100 mV was applied at 10 Hz and the cornea resistivity in kΩ was approximated from the formula:

$$R_C = \frac{V_C R_L}{V_O - V_C}$$

All the corneas used in this experiment had an electrical resistivity ≥2 kΩ cm^2. The voltage dependent drop in the transcorneal electrical resistivity following the application of 30 pulses of 1 millisecond (ms) duration, at 1 Hz was studied. Electrical pulses were applied using an ECM 830 Electro Square Porator (BTX, Holliston, MA) by connecting anode to apical and cathode to the basal compartment. The electrical resistivity of the cornea was noted immediately after cessation of electrical pulses. The post-pulse recovery was monitored by measuring the transcorneal electrical resistivity at different time points.

2.2.2. Transcorneal water evaporation (TCWE)

For TCWE studies, KRB buffer was placed only in the basal compartment and the apical compartment was kept empty. While applying electrical pulses, the donor electrode was directly placed on the corneal surface in the donor compartment and the electrode was withdrawn immediately after application of pulses. The whole set up was placed in a temperature (37 °C) and humidity (RH 55%) controlled chamber and the TCWE was measured using a VapoMeter (Delfin Technologies, Kuopio, Finland) by fixing the instrument directly on the apical compartment.

2.2.3. Permeability studies

The impact of application of electrical pulses (30 pulses of 1 ms duration at 40 V/cm^2) on the permeability of different molecular weight test permeants was investigated (Murthy et al., 2003). The test permeant solution prepared in KRB buffer (5 ml) was placed in the basal compartment and in the apical compartment 300 μl KRB buffer was placed. The electrical pulses were applied and the apical compartment buffer was sampled after 6 h. The amount of test permeant transported into the apical compartment buffer was measured. Glucose was quantitated by trinder reagent method (Sigma-Aldrich, St. Louis, MO) and FITC10K was quantitated by measuring the fluorescence intensity.

2.3. In vivo studies

In vivo studies were carried out in normotensive New Zealand rabbits (n = 5, baseline intraocular pressure [IOP] 23 ± 2 mmHg) (IACUC # 07-014). Rabbits were anesthetized by injecting ketamine and xylazine {(35 mg + 5 mg)/kg by i.p.}. In each animal, the left eye served as control and the right eye as "OcuDrain-E^{TM} test". An 8 mm diameter Ag/AgCl electrode with a concave curvature was filled with HPMC gel and used as corneal electrode. The other electrode was clamped up to ear on the same side. The electrodes were also placed on control eye similar to the test eye to mock the pulsing condition but no pulses were applied. Electrical pulses were applied (40 V/cm^2, 1 ms and 30 pulses) only on the test eye and the electrodes were removed immediately after treatment (or after 30 s in case of control). The adhering gel was washed off the corneal surface with deionized water and the corneal surface was dried by blowing ambient temperature air for 2 min. The IOP was measured subsequently at different time points.

2.4. Microscopic studies

The rabbits were anesthetized deeply and 30 electrical pulses of 1 ms duration at 40 V/cm^2 were applied on the right eye and left eye served as control similar to that described in Section 2.3. The cornea was excised before euthanizing the rabbit and the cornea were fixed in tissue-tek® embedding media (Electron Microscopy Sciences, Hatfield, PA) for sectioning. Transverse sections of 20 μm thick were prepared using a cryostat (Leica Cryostat CM 3050S) and the sections were stained with haematoxylin and eosin (Kim et al., 1999). The sections were examined using an optical microscope (Zeiss MI, with Axio Cam, Thornwood, NY). The sections were examined for loosening of the epidermis from underneath layers and occurrence of vacuoles in epidermal cells. The occurrence of necrotic cells is recognized by cell swelling and dark shrunken nuclei (Kim et al., 1999).

3. Results and discussion

The electrical resistivity of the cornea has been reported to be indicative of its permeability status. Therefore, the effect of application of electrical pulses at different voltages on the electrical resistivity of the cornea was investigated in this study (Ubels et al., 1994). Preliminary studies investigated the effect of different pulse lengths, frequencies and number of pulses on the change in transcorneal electrical resistivity and its recovery over time (data not shown). In this manuscript, a representative electrical protocol of 30 pulses each of 1 ms duration at 1 Hz is presented. The percentage drop in electrical resistivity $\{(R_0 - R_t)/R_0\} \times 100$, where R_t and R_0 are post-pulse and pre-pulse resistivity of the cornea, respectively) increased with the increase in applied pulse voltage. At pulse voltage ≥40 V/cm^2, the corneal resistivity dropped over ~80% indicating effective permeabilization of the cornea (Fig. 1).

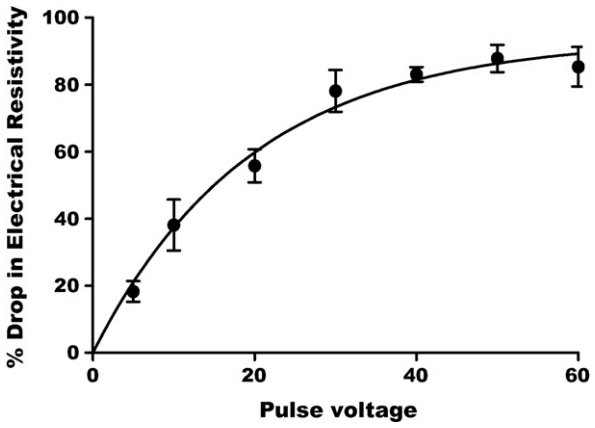

Fig. 1. The effect of application of 30 electrical pulses of 1 ms duration at different pulse voltages on the electrical resistivity of the cornea. (n = 6 ± S.E.M.).

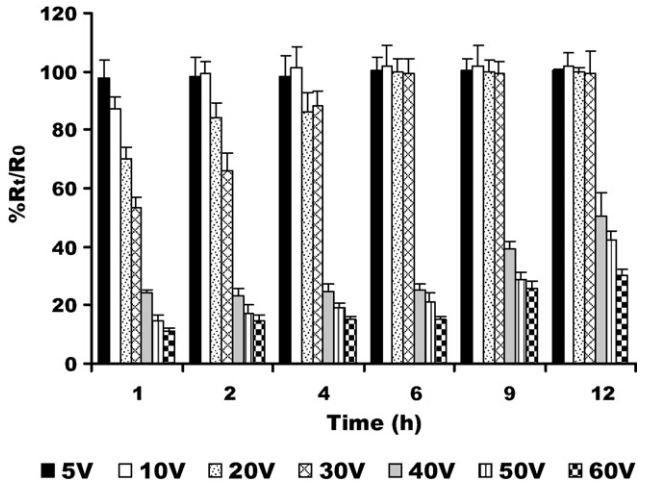

Fig. 2. The effect of application of 30 electrical pulses of 1 ms duration at different pulse voltages on the recovery of electrical resistivity of the cornea. ($n = 6 \pm$ S.E.M.).

The electrical treatment chosen should be effective and safe as well. Therefore, the most critical requirement of OcuDrain-E™ approach is to achieve prolonged permeabilized status yet reversibility of the corneal epithelial barrier. This means that the electrical protocol should be aggressive enough to effectively permeabilize the corneal epithelial membrane and the post-pulse high permeability status of the corneal epithelium should considerably prolong. However, the treatment should not cause any irreversible perturbation of the membrane. The post-treatment reversibility of the epithelial barrier was studied by monitoring the recovery of electrical resistivity of the cornea (Fig. 2). The relative resistivity of the cornea ($(R_t/R_0) \times 100$, where R_t is the resistivity at time t and R_0 is the pre-pulse resistivity of the cornea) was almost completely recovered within 6 h when the pulse voltage was <40 V/cm^2. At pulse voltage ≥40 V/cm^2, the recovery was significantly retarded and was ≤50% even after 12 h. However, the cornea recovered completely within 24–48 h.

At this stage it is not clear what structural changes were brought about by the applied electrical pulses in the cornea. The phenomenon of drop and recovery of electrical resistance due to application of electrical pulses observed in case of cornea was similar to that of transdermal electroporation (Pliquett, 1999). In case of skin, the applied high voltage electrical pulses are known to form transient aqueous pathways in the intercellular domains (Pliquett et al., 1996). The extent of drop in electrical resistivity of the skin and the duration for recovery depends on the applied electrical protocol. From the electrical resistivity data in the current study, it could be speculated that the electrical pulses create pathways in the cornea similar to that seen in case of skin. However, extensive structural studies need to be undertaken to visualize the existence of any such pathways in the corneal epithelium.

The TCWE studies assessed the basic hypothesis in OcuDrain-E™ approach. TCWE across the untreated cornea was 0.99 ± 0.06 mg/(h cm^2)$^{-1}$ (control). The TCWE across the electrically treated corneas was significantly higher than the control (t-test, $p < 0.0001$). At pulse voltage ≥40/cm^2, the TCWE remained consistently high throughout 12 h and the average steady state TCWE at 40 V/cm^2 was significantly (~39-fold) higher than the control (t-test, $p < 0.0001$) (Fig. 3). This data strongly supports our hypothesis that electropermeabilization of the cornea by treating with appropriate electrical protocol could bring about enhanced TCWE.

Aqueous humor composition includes several dissolved substances, of different molecular weight, along with the principle component, water. Increased permeability of the cornea is likely to lead to greater diffusion of dissolved substances across the membrane. Permeation studies of test permeants of different molecular weight were carried out across the cornea to assess the impact of electrical treatment on the molecular permeability. Application of electrical pulses at 40 V enhanced the flux significantly of glucose (Student t-test, $p < 0.0001$) and FITC dextrans up to 4 kDa (Student t-test, 1 kDa, $p < 0.0002$; 2.5 kDa, $p < 0.001$; 4 kDa, $p < 0.0001$) (Fig. 4). The FITC dextrans of molecular weight 10 kDa and 35 kDa did not permeate across the cornea (Fig. 4). From the data it is evident that the applied electrical protocol, not only enhanced loss of water, but also resulted in significant loss of dissolved substances as well. This eliminates the concern regarding the possibility of potential retention and accumulation of solutes in the ocular chambers due to drainage of water.

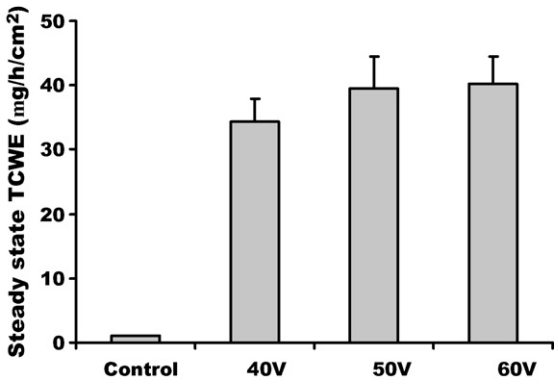

Fig. 3. The effect of application of 30 electrical pulses of 1 ms duration at different pulse voltages on the steady state TCWE of the cornea. ($n = 6 \pm$ S.E.M.).

Functionally, corneal epithelium is known to protect the eye from entry of exogenous materials like bacteria, virus and allergens. It may be argued that there is an increased potential risk of entry of microbes and harmful exogenous allergens across the cornea due to electrical permeabilization. Considering cut-off molecular size of electrical treated cornea (4 kDa), it is not likely that microbes and macromolecular allergens would be able to enter across the cornea. However, concern about potential risk of entry of smaller molecular size toxicants is still valid and need to be addressed during the development of OcuDrain-E™.

Rabbits are regarded as one of the appropriate models for evaluating the pharmacodynamic activity of several anti-glaucoma drugs

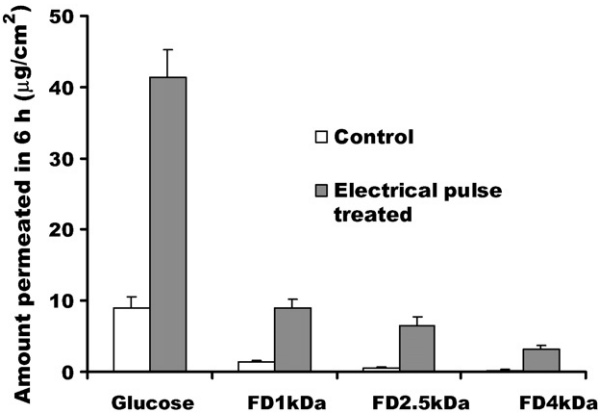

Fig. 4. The effect of application of 30 electrical pulses of 1 ms duration at different pulse voltages on the permeation of test permeant molecules of different molecular weights. ($n = 6 \pm$ S.E.M.).

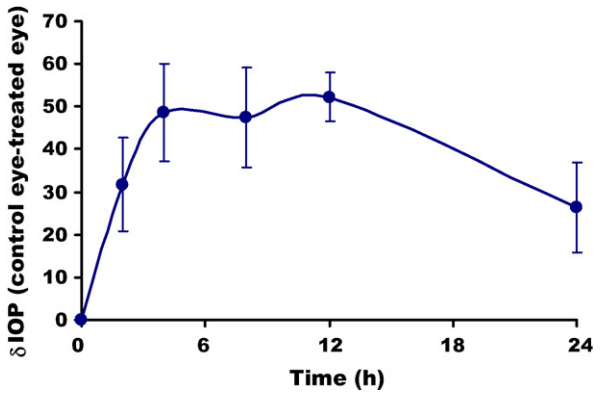

Fig. 5. The effect of application of electrical pulses at 40 V/cm^2 (1 ms, 30 pulses) at 1 Hz on the IOP (mmHg) in normotensive New Zealand rabbits. ($n = 5 \pm$ S.E.M.).

and dosage forms (Kalliopi et al., 2006; Hyung et al., 1994). Therefore, normotensive rabbits were chosen as animal model for the pharmacodynamic studies also in the current research. The applied electrical protocol was well tolerated by the animals. There was no visible evidence of any untoward effect due to pulsing up to 2 weeks after the study. It is noteworthy that the electroporation protocol applied in our study was comparable to that reported by others (Oshima et al., 2002, 1998; Sakamoto et al., 1997; Blair-Parks et al., 2002). Sakamoto et al. applied 8 pulses each of 100 ms at 5 V/cm, in rabbits for the delivery of bleomycin (Sakamoto et al., 1997). This electroporation protocol was well tolerated by rabbits and no local or systemic damage was caused. Oshima et al. assessed the efficacy of electroporation on gene transfer in the corneal cells of rats at pulse voltage in the range of 10–30 V (Oshima et al., 2002, 1998). They found that 20 V pulses of 50 ms duration were most effective for gene transfer. Blair-Parks applied 8 electrical pulses of 10 ms duration at 200 V/cm for delivery of genes and observed no trauma, corneal edema or inflammation in the mice. According to Blair-Parks, the corneal edema was observed only when the pulse voltage was above 200 V/ms (Blair-Parks et al., 2002).

The pharmacodynamic study was expected to answer the question whether an increased TCWE would lead to drop in IOP. In each animal, left eye served as control and the right eye was treated with electrical pulses at 40 V (30 pulses, each of 1 ms duration at 1 Hz). The difference in the IOP (δIOP = IOP control eye – IOP of test eye) between the control and the treated eyes was >30% after 2 h (Fig. 5). The difference remained consistently same up to 12 h. However, at 24 h, the δIOP decreased significantly likely due to the recovery of corneal epithelial barrier.

The corneal epithelium has greater electrical resistivity than any other layers of cornea. When electrical pulses are applied, the greatest electric fields are generated where the largest resistivity exist, thereby protects the already permeable viable parts of the cornea and deeper tissues. The results from microscopic studies revealed that the applied protocols did not result in corneal edema or loosening of the epithelium. No necrotic cells were found in test or control. So far there are no reports on ocular application of electrical pulses in humans. The low voltage and short pulse duration is expected to be tolerable in humans. However, systematic tolerability studies and histological studies need to be undertaken to demonstrate the safety of the electrical protocols in humans which will follow up in the next phase of development of OcuDrain-E^{TM} technique.

4. Conclusions

The present work provides proof of principle of OcuDrain-E^{TM}. Choosing an appropriate electrical protocol to achieve prolonged permeabilization of the cornea yet reversibility of the barrier is the most challenging task in this technique. Nevertheless, the study showed that the OcuDrain-E^{TM} could be developed as potential treatment technique of glaucoma condition that responds poorly to drugs. This noninvasive technique could most likely be combined with drugs and other methods for controlling IOP. Successful development of this technique is believed to replace the surgical treatment. Extensive studies using human corneal model need to be carried out to address the potential questions concerning the mechanism of permeabilization of the corneal epithelium, safety, tolerability and workability of the technique in humans.

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