Ultrasound-enhanced Delivery of Antibiotics and Anti-Inflammatory Drugs into the Eye

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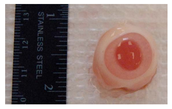
Introduction

Millions of people suffer from variety of ocular diseases which in some cases lead to vision impairment and eventually blindness. Topical administration of drugs, for treatment of ocular inflammations and infections, through cornea is the preferred method for drug delivery, but cornea's barriers makes delivery of a sufficient amount of therapeutic drugs a challenging task.

Ultrasound has been shown to enhance delivery of lytic agents into thrombi and anticancer drugs into cells. Further, application of ultrasound for delivery of drugs into skin has been one of extensively investigated research areas with promising results. Our research describes applying ultrasound for increasing corneal permeability for several ocular drugs while generating some changes in corneal structure. We have worked on developing procedures to determine effectiveness and safety of ultrasound application in enhancing ocular drug delivery.

Materials

Adult New Zealand white rabbit cornea: standard model for ocular drug delivery



Transducers

- Unfocused circular transducers with 15 mm active diameter at 400 KHz, 600 KHZ, 800 KHz, and 1 MHz frequencies.
- The d_{ff} calculated for these transducers are 1.5, 2.25, 3, and 3.75 cm respectively.

Drug Solutions

Sodium Fluorescein, 0.25%

- Used for diagnosis of corneal abrasions, corneal ulcers and herpetic corneal infections
- Hydrophilic
- Maximum absorption @ 490 nm

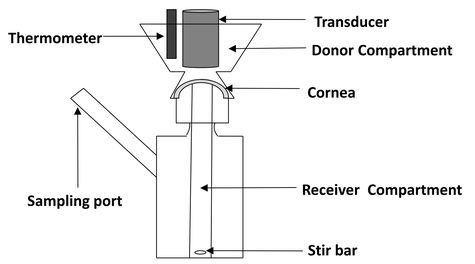
Tobramycin, 0.3%

- Ophthalmic antibiotic formulation for topical therapy of external infections
- Hydrophilic
- Maximum absorption @ 278 nm

Dexamethasone Sodium Phosphate, 0.1%

- Topical steroid solution used to suppress inflammatory response to different conditions
- Hydrophilic
- Maximum absorption @ 242 nm

Spherical Diffusion Cell



Eye cup used for *in vivo* study

Methods

In vitro Study

- Dissected cornea was placed over the spherical joint of diffusion cell, between donor and receiver compartments.

- Ultrasound was applied with intensity of 0.3, 0.5, 0.8, and 1 W/cm^2 at different frequencies of 400 KHz, 600 KHz, 800 KHz, and 1 MHz with application duration of 5 min.
- Temperature was measured while applying ultrasound.
- Sample from receiver compartment was collected after 60 min.
- The absorption of the sample was measured using spectrophotometer.

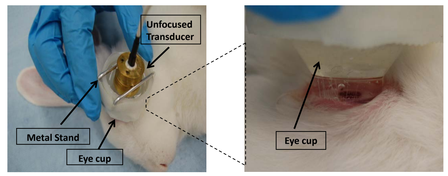
Corneal Permeability

$$p = \frac{V(C_{r,t} - \cancel{C_{r,0}^{0}})}{A(t - t_L)C_d} = \frac{VC_{r,t}}{A(t - t_L)C_d}$$

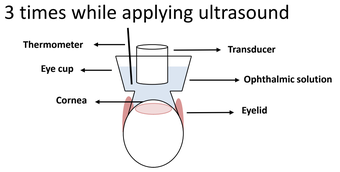
p= permeability, cm/sec
V= volume of the receiver compartment
$C_{r,t}$= drug concentration in receiver compartment
A= cross sectional area ~ 1 cm^2
t = 60 min
t_L= lag time, min
C_d= drug concentration in donor compartment

In vivo Study

- Animals were anesthetized before starting the experiment.
- The eye cup was placed on the eye filled with Dexamethasone.
- Transducer was placed on a metal stand and submerged inside solution.



- Ultrasound was applied with intensity of 0.8 W/cm^2 at different frequencies of 400 KHz and 600 KHz for 5 min.
- Temperature was measured 3 times while applying ultrasound (t = 0, 2.5, and 5 min).

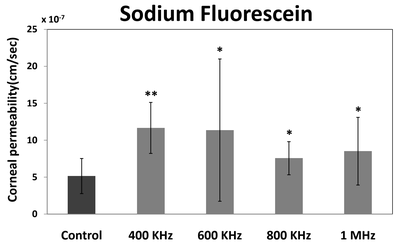


- Sample of aqueous humor was collected using a needle approximately 60 min after the ultrasound treatment and immediately after the animal was euthanized.

- Chromatography was used to investigate the amount of drug penetrated through cornea into aqueous humor.

Corneal Permeability Results

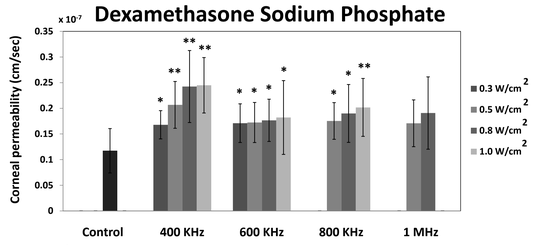
Sodium Fluorescein



* Indicates p-value < 0.05
** Indicates p-value < 0.001

In case of Sodium Fluorescein, ultrasound application for 5 minutes at 1.0 W/cm^2 produced permeability increase of 126% at 400 KHz (n=9), 121% at 600 KHz (n=13), 47% at 800 KHz (n=9), and 65% at 1 MHz (n=12) as compared to sham treated cases (n=9).

Dexamethasone Sodium Phosphate



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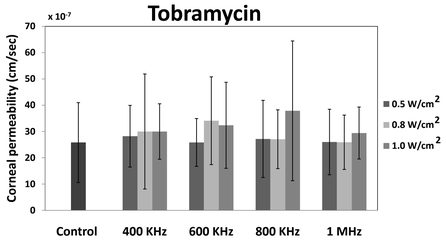
** Indicates p-value < 0.01

For Dexamethasone Sodium Phosphate, the changes in corneal permeability at 0.3 W/cm^2 were 43% for 400 KHz and 46% for 600 KHz (n=6), at 0.5 W/cm^2 was 76% for 400 KHz (n=6), 47% for 600 KHz (n=8), 50% for 800 KHz (n=6), and 46% for 1 MHz (n=5), at 0.8 W/cm^2 was107% for 400 KHz (n=6), 51% for 600 KHz (n=8), 62% for 800 KHz (n=6), and 63% for 1 MHz (n=6), and at 1.0 W/cm^2 was 109% for 400 KHz (n=6), 55% for 600 KHz (n=9), and 72% for 800 KHz (n=8).

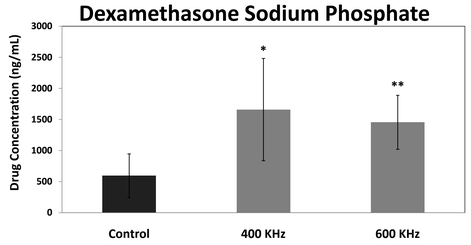
Tobramycin



For Tobramycin , no statistically significant changes were observed in the corneal permeability.

Result: *In vivo* study

Dexamethasone Sodium Phosphate



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Dexamethasone Sodium Phosphate concentration in aqueous humor samples as compared to sham treated samples (n=7) increased by:

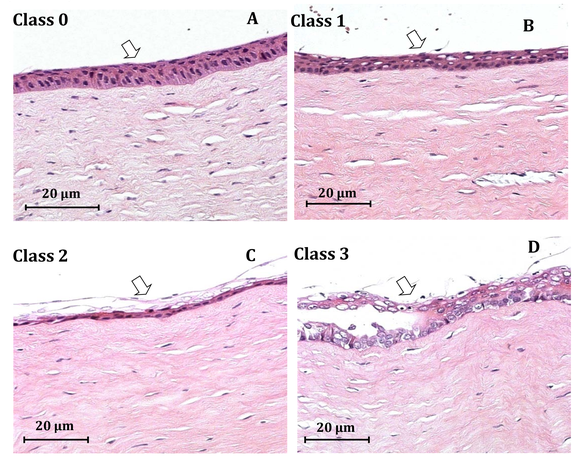
- 2.8 times using 400 KHz (n=5)
- 2.4 times using 600 KHz (n=6)

Histology

Cornea were dissected after both *in vitro* and *in vivo* study, kept in formalin and used for histology slides.

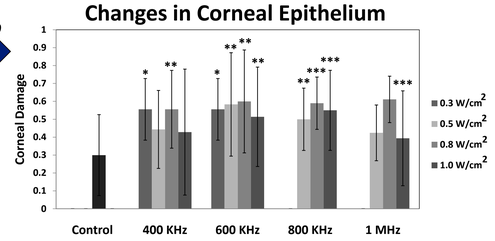
Damages observed in epithelium were categorized in four different groups based on the geometrical width of epithelial layer including all the five biological sub-layers:

- None of the layers are damaged or missing (class 0).
- Some cells are missing or first layer of epithelium is removed(class 1).
- Two layers are missing or damaged (class 2).
- All three layers are missing or epithelium is severely damaged (class 3).



Histological analysis showed structural changes which were limited to epithelial layers of cornea.

In vitro



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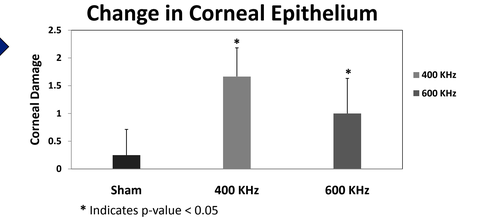
In vivo



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Conclusion

- Corneal permeability increase was highest at 400 kHz, both *in vitro* and *in vivo* study, and appeared to be higher at higher intensities.
- Ultrasound application provided enhancement of trans-corneal delivery of clinically relevant compounds.
- One of the main mechanism could be cavitation-induced production of pits on the surface of cornea which will be further investigate using Passive Cavitation Detection.
- Future studies involve establishing a stimulating model using PZFlex to investigate safety of this application and validating *in vitro* and *in vivo* results for temperature increase in cornea.

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