HISTOLOGICAL EVALUATION OF THE EFFECTS OF TRANSPORT ON BOVINE CORNEAS FOR OCULAR SAFETY ASSESSMENTS

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Abstract

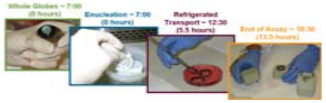
The bovine corneal opacity and permeability (BCOP) assay, originally developed by Gautheron (1992) and Sina (1995) has been used as an in vitro eye irritation screen for industrial hygiene, product development and safety testing. It has recently been validated as a screen for corrosive or severe irritation by ICCVAM and ECVAM. The assay measures changes in corneal opacity, and increases in permeability to fluorescein after chemical exposure. Since Curren and Evans (2000) proposed the use of histopathology to detect potential corneal injury, where the mode of chemical action might not induce opacity and permeability changes, histopathology has been used in BCOP studies for nearly a decade. Although the state of the negative control corneas at the end of the BCOP assay has been characterized histologically, no studies have been conducted to determine if there are artifactual changes in the cornea associated with the collection and storage of the enucleated eyes, or the BCOP methodology. Corneas were excised and fixed at various steps in the assay process, at the time of collection of freshly enucleated eyes, after refrigerated transport, and at the end of the BCOP assay. Corneas were fixed in 10% buffered formalin, embedded in paraffin, H&E stained and the corneas evaluated using light microscopy. Stromal thickness was measured primarily at the central cornea. No remarkable artifacts were observed in the corneal epithelium and endothelium as a result of the various conditions, and the corneal stroma appeared very similar histologically in all cases. The thickness of the corneas collected immediately after enucleation were approx. 600 to 650 µm; corneas collected after the refrigerated transport prior to the BCOP assay were approx. 675 to 775 µm, and typical negative control corneas at the end of the BCOP assay range from 680 to 800 µm. These results show that the corneas undergo minimal artifactual changes as a result of refrigerated transport and the BCOP assay procedures.

Introduction

- The BCOP assay has been used as an *in vitro* eye irritation screen for industrial hygiene, product development and safety testing.
- The BCOP assay measures changes in corneal opacity, and increases in permeability to fluorescein after chemical exposure.
- Histopathology has been used in BCOP studies for nearly a decade in order to
    1. determine depth and degree of injury which may be used to predict recovery
    2. help identify the pathologies associated with opacity and permeability increases
    3. detect potential corneal injury where the mode of chemical action might not induce opacity and permeability changes
- Although the state of the negative control corneas at the end of the BCOP assay has been characterized histologically, artifactual changes in the cornea associated with the collection and storage of the enucleated eye or the BCOP methodology have not been evaluated.

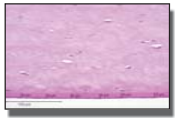
Materials and Methods

- Corneas were excised and fixed at various steps in the assay process
    1. At the time of enucleation (fixed with and without a histology cassette)
    2. After refrigerated transport
    3. At the end of the BCOP assay (untreated)
- As a control for corneal excision, whole globe eyes were fixed at the time of enucleation



- Corneas were fixed in 10% buffered formalin, embedded in paraffin, stained with Hematoxylin & Eosin
- Two cross-sections from each cornea were prepared and evaluated
- Corneal morphology was evaluated using light microscopy
- Stromal thickness was measured throughout the corneal cross-sections
- Thickness of Descemet's Membrane was measured throughout the corneal cross-sections
- Stromal disorganization immediately below the epithelium was examined

Stromal thickness measurements across the enucleated cornea

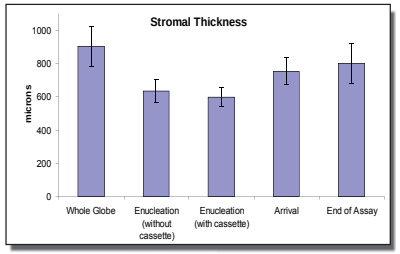


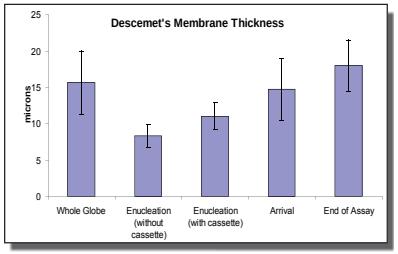
Descemet's Membrane thickness measurements across the enucleated cornea

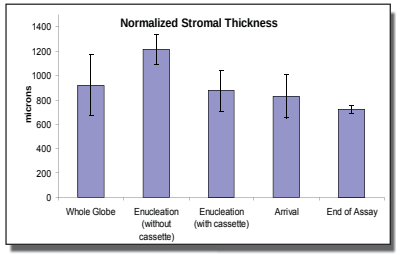
Results

- Thickness measurements indicate that the dissected cornea at the end of the BCOP assay is notably thicker than the dissected cornea at the abattoir.
- However, the cornea on the whole globe was notably thicker than any of the dissected corneas.
- The thickness of Descemet's Membrane was proportional with the increase in thickness of the corneas.
- Stromal thickness measurements normalized to the thickness of Descemet's Membrane showed no notable difference.

Condition	Number of Measurements (Number of Samples)	Average Stromal Thickness (Standard deviation)	Normalized Stromal Thickness (Standard deviation)
Whole Globe	279 (6)	903.8 µm (± 122.90 µm)	903.8 µm (± 122.90 µm)
Enucleation (no cassette)	147 (4)	636.3 µm (± 67.95 µm)	1211.9 µm (± 60.1 µm)
Enucleation (cassette)	113 (4)	598.2 µm (± 57.36 µm)	876.7 µm (± 84.2 µm)
Arrival (After Refrigerated Transport)	291 (8)	754.8 µm (± 80.32 µm)	829.8 µm (± 63.4 µm)
End of Assay	152 (4)	802.1 µm (± 120.51 µm)	721.2 µm (± 17.2 µm)

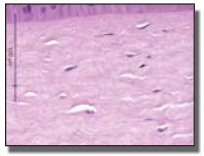




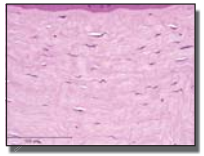


Stromal Organization

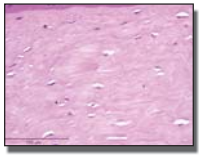
- Evaluation of the stroma immediately beneath the epithelium showed minimal differences in collagen organization or keratocyte morphology between the different groups evaluated.
- However, the interlamellar spacing, or clefting, appeared to be more prominent in the whole globe stroma, relative to the excised corneal stroma.
- The figures below show the stroma immediately beneath the epithelium



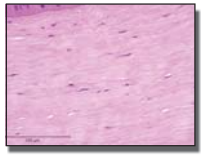
Whole Globe



Enucleation



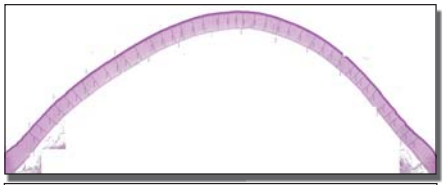
Arrival

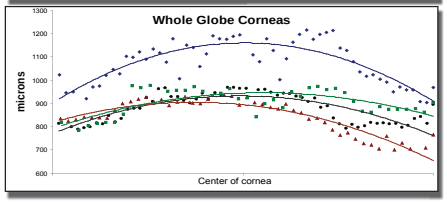


End of Assay

Discussion

- Examination of stromal thickness, including thickness of Descemet's Membrane, suggests corneas undergo minimal artifactual changes as a result of refrigerated transport and the BCOP assay procedures.
- Evaluation of the stromal layer immediately below the epithelium revealed consistent collagen organization and keratocyte morphology among all groups evaluated, which futher suggests that corneas undergo minimal artifactual changes.
- However, there is a large variability in the measurement of stromal thickness within each group, suggesting that stromal thickness alone is not a definite measure of stromal swelling and/or damage.
- Sources of differences in stromal thickness (or measured thickness)
    - Animal variability
    - Fixation and embedding technique
    - Microtome angle during sectioning
- Therefore, multiple stromal measurements for evaluating changes in stromal thickness and stromal swelling are needed.
- Stromal thickness was evaluated along the entire cornea
    - Parabolic curve fits were evaluated for the whole globe eyes in which the corneas were fixed in their natural state (r-squared values ranged from 0.46 to 0.85).
    - The curve fits suggest that the stromal thickness varies along the entire length of the cornea with the cornea being thickest in the center and thinner along the limbal edges.





Acknowledgements

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