Canine duplication of Descemet’s membrane:

A pathomorphological description of 66 eyes

C Kafarnik\textsuperscript{1}, S Carastro\textsuperscript{2}, CJ Murphy\textsuperscript{3}, RR Dubielzig\textsuperscript{1}

\textsuperscript{1} COPLOW, PBS Department, Vet. School, UW-Madison
\textsuperscript{2} Animal Eye Clinic, West Palm Beach, Florida
\textsuperscript{3} Department of Surgical Sciences, Vet. School, UW-Madison
Case: “eye hit with a bottle rocket“ two years ago

“Tommy”: 4.5 y, mc, Labrador retriever

History:
8/06 traumatic uveitis and cataract OD
4/08 Glaucoma, neg. ocular reflexes, pan-limbal corneal edema, Morgagnian cataract, hyper-reflective fundus

Enucleation

(www.ofallon.org)
Case: “eye hit with a bottle rocket” two years ago

200x, Alcian blue PAS

25x, H&E
Background

- Rel. common phenomenon in COPLOW
  Seen in dogs, cats, not in horses, exotics, birds


Objectives

1. Correlation to clinical history, signalment and ocular disease status?

2. Separation or reduplication of Descemet’s membrane?

3. Clinical relevance?
Material and Methods

- 66 canine globes (2000-2007)

- Signalment and clinical information

- Staining: H&E, Alcian blue PAS, 14/66: Masson Trichrome, Picrosirius red, Cytokeratin, Vimentin, α-Smooth muscle actin (SMA)

- Translectron microscopy (2 eyes, 1 normal control eye)

- Descemet’s membrane thickness (3 times per region, averaged), compared with control group (n=15)
Results

Signalment
- Mean age: 9.19 ± 2.91 y. (range: 3-14)
- No breed, laterality or gender predisposition

Time (median duration of clinical signs – enucleation)
- 17 month (Median), (range: 0.1 – 84)

Further anterior segment pathological features
- Chronic corneal disease = 80 %
- Otherwise normal cornea = 20 %
- Pre-iridal fibrovascular membrane
  /Peripheral anterior synechia = 53 %
- Retrocorneal membrane = 29 %
Results

Morphological features

- Anatomic location
  - multifocal = 33%
  - continuous = 10%
  - axial = 14%
  - peripheral = 43%

- Mean thickness of DM’s (μm)
  - Corneal layer (CL) = 14.6 ± 6.35
  - Ant. chamber layer (ACL) = 14.65 ± 11.05

100x, Alcian blue PAS
DM thickness in normal dogs

- 15 dogs mean 5.02 (range: 0.3 – 12 y.)
  - axial: 14.4 ± 6.15 µm
- 5 /15 mean age of 10 (range: 8-12 y.)
  - axial: 19.5 ± 5.95 µm

Doubling cases age: mean 9.19 (3 – 14 y.)
- 14.6 ± 6.35 µm CL
- 14.65 ± 11.05 µm ACL
  = approx. 30 µm
Results

Electron microscopic findings

CL

ACL
Results

- Matrix component in between CL/ACL
  - Collagen membrane (Masson +, Picrosirius red +)
  - Spindle cells = Vimentin +, α-SMA strongly +, CK - (14/14)

200x, Picrosirius red

600x, α-Smooth muscle actin
Results

Endothelium

- attenuated = 71 %
- normal = 14 %
- absent = 15 %
- CK 7/14 weakly +, 2/14 strongly +, 5/14 -

600x, Cytokeratin

600x, Cytokeratin
Results

- Descemetization = 8%
Results

**Further ocular features**

- Chronic glaucoma (2°) = 65 %
- Goniodysgenesis related glaucoma = 9%
- Acute glaucoma = 2%
- No glaucoma signs = 24%
- Intraocular surgery (aphakic, pseudophakic) = 36 %
- Lens luxation (1°, 2°) = 21 %
- Signs of blunt trauma = 15 %
- Retinal detachment = 18 %
Conclusion

1. Correlation to clinical history, signalment, ocular disease status?
   - Chronic glaucoma due to traumatizing conditions (blunt, intraocular surgery, lens luxation) were observed in eyes with doubling of DM
   - Middle age – older dog, chronic development

2. Separation or reduplication of Descemet's membrane?
   - CLS is the original DM, the ACL is a secondary reduplication of DM produced by activated corneal endothelial cells
   - Activation, migration (proliferation) of endothelial cells in association with direct trauma plays role in the pathogenesis
Conclusion

- Corneal opacity, reduce visual outcome post intraocular surgeries

3. Clinical relevance?

- Risk factor for developing post operative and post traumatic glaucoma
Acknowledgment

Thank you very much for your attention !!!!!

RR Dubielzig
Melanie Rawlings
Patricia Mundy
Kate Lieber
Discussion

1. Hypothesis:
   - Endothelium temporarily detached – reset – produce “stress collagen”
   - Contact inhibition lost, G1- phase cell cycle activated
     
     (Joyce et. al 2002, Inv Oph Visc Sci 43; 2152-2159)

   Canine endothelial cells can be activated, mitotic capacities higher than described

2. Clinical diagnosis/relevance:
   - Limited due to chronic corneal disease in most cases
   - Descemetization one reason of 2° glaucoma?
   - Human ophthalmology: reason for PK/DLK

3. Study limitation:
   - Retrospective, no complete and exact history available in all cases

4. Further characterization:
   - Serial section, Collagen IV, Laminin, Ki 67 (Proliferation markers)
   - In vivo confocal microscopy/ AC – OCT
Discussion

1. Role and origin of alpha – myofibroblasts:

Could arise from transformed stromal keratocytes or metaplastic endothelial cells
Could migrate in Inter-matrix-component and passing breaks in CL DM or through breaks in ACL

Leung et al. Molecular vision 2000:
Corneal endothelial cells of rabbits with RCFM have the capacity to produce basement membrane and non basement structures and in vitro model shows: under the influence of FGF-2 and protein factor released by PM Leukocytes, corneal endothelial cells transform in mesenchymal endothelial cells and produce Typ I collagen

Possible signaling from endothelial cells similar in wound healing mechanism and interaction known between epithelial cells and keratozytes (TGF beta, II)

We know, that the amount of mitochondria and rER in activated endothelial cells in Fuch’s dystrophy and retrocorneal membrane are much more higher – we can’t observe this due to the fixative destruction/deparaffine
basal laminae of corneal endothelium (major Typ 8 and 4 collagen, glycoprotein, proteoglycan)

- synthesis continuous throughout adult life
- human: 3-4 μm (birth), 5 μm (childhood), 10 - 12 μm (adult)

**fetal=banded zone** (anterior third, irregular banded pattern of type I collagen, tangential section show equilateral triangles interconnected wit electron dense nodes and internodes)

**postnatal=none banded zone** (posterior 2/3 of homogenous, fibrogranular material, modified hemidesmosomes attaches the endothelial cells)
Aging changes of mouse corneal endothelium and Descemet’s membrane
Albert S. Jun a,*, Shukti Chakravarti a,b, Henry F. Edelhauser c, Martha Kimos a
(Exp. Eye Research 2006)

Fig. 2. Transmission electron microscopy images of representative Descemet’s membrane (DM) from mice of indicated ages. Asterisks represent mean total DM thickness with P-values <0.05 compared with the preceding age group. Arrows indicate the transition from the posterior stroma to the anterior banded zone of DM. P indicates the posterior non-banded zone of DM. E indicates the endothelial cell layer. Original magnification × 25 000.
Literature

- Corneal “endothelial” cells = mesenchymal epithelial (correct), mesenchymal origin, vimentin +; neural crest origin (Cook et al. 1989) and neural specific enolase + in different species (Kwait et al. 1988)
- Adult human endothelial cell in G0/1-Phase (no mitotic activity)
- Rabbit: turn in G2 phase – DNS synthesis – mitosis (>1 year; mitotic index 50% less)
- Dog: migration, hypertrophy and cell mitosis (young dog)
Corneal endothelial healing (dogs) enlargement, sliding phenomenon, cell division, mitosis

Clinical experience: mitotic activity in dogs?

1993 Kay et al.: typical and atypical healing with retrocorneal membrane formation

Retrocorneal membrane (rabbit): fibrillin, fibroblast like cells with endothelial cell origin, typ I collagen

In vitro study in bovine: polymorpheous leucocytes produce CEMF (=corneal endothelium modulation factor) induce endothelial cells metaplasia in fibroblasts like cells (Kay et al. 1993, 1990)