Today’s Objectives

1) Discuss the “lacrimal functional unit” with emphasis on innervation, normal physiologic function, and a review of pathologic mechanisms in dry eye

2) Review mammalian eyelid anatomy

Today’s Objectives

3) Summary charts – helpful for boards

4) “The brain can only absorb what the #@$ can withstand”
   - Clinical cases – our passion
   - What does this lecturer “bring to the table”?
     - CALT and PDT
     - Encourage your creative juices

“Lacrimal Functional Unit (L.F.U.)”

- A complex functional unit which modulates the homeostasis of the ocular surface
  - Lacrimal gland
  - Tear film
  - Ocular surface epith.
  - Cornea, conjunctiva, meibomian glands
  - Eyelids
  - Interconnecting sensory and motor nerves
Control of Tear Secretion – New Concepts

- Traditional
  - Normal tears: the result of intrinsic lacrimal gland activity; neural participation in reflex tears only
- New concept: tears under constant neural regulation
  - On-going homeostatic regulation of the ocular surface
  - Suggests a relatively constant level of neural signals that precisely meter tear production; may mediate lipid & mucin secretion also

Control of Tear Secretion – New Concepts

- Control mechanism includes afferent nerves from the cornea & other ocular surface tissues → central nervous system relay nuclei → efferent nerves comprise the autonomic innervation to secretory tissues whose products contribute to the tear film

Lacrical Gland Innervation:
Afferent Pathway of Trigeminal Ganglion-Mediated Reflex

- Irritation of cornea/conjunctiva stimulates afferent nerves: Impulses are carried along lacrimal nerve → the ophthalmic division of the trigeminal nerve → sensory nuclei in trigeminal ganglion (TG)
- Lacrimal nerve - smallest branch of the ophthalmic n.
- Courses laterally within the orbital cavity above and along the upper border of the lateral rectus muscle
- Relevance?
Lacrimal Gland
Additional Sensory Innervation
- The afferent innervation of the lacrimal gland is also provided by the ipsilateral superior vagal ganglion (SVG) and superior glossopharyngeal ganglion (SGG)
- There may be SVG and SGG-mediated reflexes in addition to the TG-mediated reflex
- In humans, there is a connection between hypothalamus and lacrimal nucleus (emotional tears), and between olfactory system and lacrimal nucleus (“wasabe tears”)
Lacrimal Gland – Innervation

- Autonomic innervation – parasympathetic
- Postganglionic parasympathetic fibers innervate:
  - Acinar cells, duct cells, and blood vessels
  - Exert principal neural control of electrolyte, water, and protein secretion
  - Stimulatory effect mediated via acetylcholine and vasoactive intestinal peptide (VIP)
  - Increase in tear secretion through a G protein pathway and perhaps a calcium/calmodulin pathway

Parasympathetic innervation

- More than one parasympathetic ganglion is involved in the neural regulation of lacrimal gland secretion.
  - Ciliary ganglion (CG)
  - Otic ganglion (OG)

Some anatomical studies suggest that small neurons mediate the vasodilation of the lacrimal gland, while the large neurons mediate the lacrimal secretion.

Additional References


Text Books

- Milder B. The lacrimal system
- Snell RS, Lemp MA. Clinical Anatomy of the eye
- Kaufman PL, Alm A. Adler’s Physiology of the eye
- Krachmer JH, Mannis MJ, Holland EJ. Cornea
**“Lacrimal Functional Unit (L.F.U.)”**

- Normal tears essential:
  1. To prevent surface infection
  2. Provide a pure optical surface for light refraction
  3. Maintenance of surface “homeostatic” environment

- Describe the relationship between ocular surface and the lacrimal glands in normal tear secretion and during inflammation
- Composed of tear film, ocular surface epithelium, eyelids, interconnecting sensory and motor nerves

**Tear Film – Anatomy & Physiology**

- **Lipid**
  - Most superficial layer
  - Stabilizes & prevents evaporation of aqueous layer
  - Produced by the meibomian glands

- **Aqueous**
  - Intermediate layer
  - Provides corneal nutrition; removes waste products
  - Produced by orbital gland AND gland of the 3rd eyelid

- **Mucus**
  - Interface of tear film with hydrophobic cornea
  - Secretory IgA
  - Produced by conjunctival goblet cells

**Tear Film – Functional Anatomy**

- Traditionally, tear film has been described as having 3 layers with a total thickness of 7-10 µm (Adlers, older, 10th edition – note, typo in this edition, µm not mm)
- In the last 15-20 years, evidence has called this earlier estimate into question (newer techniques, mathematical models; hundreds of papers!)
  - Prydal et al, IOVS, 1992 estimated tear film thickness of 35-40 µm, composed mainly of a gel containing mucins
  - Danjo et al, Jpn J Ophthal, 1994 - 11 µm
  - Ewen King-Smith et al, IOVS, 2000 disputes Prydal’s and Danjo’s earlier work and states a value of 3 µm for the thickness of human precorneal tear film
- Another review: 6-20 µm, J Cataract Refract Surg 2007

**Continually Emerging Newer Techniques**

- **Measurement of Tear Film Thickness Using Ultrahigh-Resolution Optical Coherence Tomography**
  - René M. Werkebroek,1 Anesha A.2 Scnina Kaya,3 Angelika Unterhuber,4 Bernd Hofer,1 Jasmin Riedl,1 Michael Bronhuber,1 Martin Vietor,1 Doreen Schmidt,2 Tilman Schmoll,1 Gerhard Garföhl,4 Wolfgang Drägel,1 Rainer A. Leitgeb,1 Martin Grosschiß,2 and Georg Smitjersy,4
  - Center for Medical Physics and Biomedical Engineering, Medical University of Vienna, Vienna, Austria
  - Department of Clinical Pharmacology, Medical University of Vienna, Vienna, Austria
  - Institute of Applied Physics, Vienna University of Technology, Vienna, Austria

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*Table 2–5: SIGNS OF LESIONS IN FACIAL NERVE*

<table>
<thead>
<tr>
<th>Anesthetic Site</th>
<th>Facial Muscles</th>
<th>Tear</th>
<th>Deep Facial Muscle</th>
<th>Lacrimation</th>
<th>Venous Drainage</th>
<th>Hemorrhage</th>
<th>Syndrome</th>
<th>Brain Stem Signs</th>
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<td>Head</td>
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</tbody>
</table>

*See Figure 2–23.*

1. Level of consciousness, abscess, sensitivity to light, ptssus reactions, ptssue trauma, ptsses, ptsses to light.
2. Small animals.
Average central T.F. thickness: 4.78 +/- 0.88 µm

Dry eye disease (DED) is a common ophthalmic condition that is characterized by tear film instability and leads to ocular surface discomfort and visual disturbance. Advancements in the understanding and management of this condition have been limited by our ability to study the tear film secondary to its thin structure and dynamic nature. Here, we report a technique to simultaneously estimate the thickness of both the lipid and aqueous layers of the tear film in vivo using optical coherence tomography and maximum-likelihood estimation. After a blink, the lipid layer was rapidly thickened at an average rate of 10 µm/s over the first 2.5 s before stabilizing, whereas the aqueous layer continued thinning at an average rate of 0.29 µm/s of the 10 s blink cycle. Further development of this tear film imaging technique may allow for the elucidation of events that trigger tear film instability in DED. © 2016 Optical Society of America

Among different methods of measuring the tear film thickness in vivo [7–11], optical coherence tomography (OCT) was shown to be a promising approach due to its noninvasive nature and the recent advance of broadband light source techniques. A few methods based on spectral domain OCT were explored: indirect measurement of the tear film thickness with the application of a contact lens, and direct thickness measurements with an ultrahigh resolution OCT [12–14]. However, these OCT methods measure the total thickness of the tear film and cannot separate the lipid layer and the aqueous layer. We proposed an approach that is based on the combination of spectral domain OCT hardware and statistical decision theory [15–17] to simultaneously measure the thickness of both the lipid and aqueous layers of the tear film. The

Fig. 1. Tear film model (the mucin component is considered as a rough interface between the aqueous layer and the corneal epithelium).
Tear Film – Functional Anatomy

Adler’s Physiology of the Eye, 11th ed (2011) thickness of the precorneal tear film in humans: 3.4 ± 2.6 µm and composed of 4 layers (glycocalyx on corneal & conjunctival epithelia, mucous, aqueous, and lipid layers)

Whatever the true thickness of tear film, the structural rigidity of 3 discernible layers has changed with time

Tear layers are considered to be more of a continuum with the lipid layer most anterior to the aqueous and mucin components

Tear Film Content and Thickness

Analytical methods

- Tears – attractive for sampling (accessibility, rich in content, largely acellular)
- Volume of minimally stimulated tears (i.e. environmental stimulation) ~7 µL, collection of > 2 µL at any time point results in reflex tearing alters both volume and composition of tears
- Qualitative and quantitative techniques
  - 1 and 2-dimensional polyacrylamide gel electrophoresis (PAGE)
  - Isoelectric focusing (IEF)
  - Crossed immunoelectrophoresis
  - ELISA
  - Size-exclusion high-pressure liquid chromatography (HPLC)
  - Reversed phase and ion-exchange HPLC
  - Matrix-assisted laser absorption/ionization (MALDI) mass spectrometry
  - Surface-enhanced laser desorption-time of flight (SELDI-TOF) technology
  - OCT – latest and greatest versions (e.g. ultrahigh resolution OCT)
Tear Film Discrepancies in Vet. Med.?

- We are NOT immune to same issues/problems
- Non-invasive meibometry can be used in conscious dogs and has been described as a means to quantify meibomian gland secretions in this species, however results have been variable
- “Repeated measurement results obtained by two examiners, with the new device Meibometer MB550 linked to a computer, showed a wide range of values. The measuring precision of the new Meibometer MB550 is therefore questionable.”

Tear film osmolality and electrolyte composition in healthy horses

OBJECTIVE
To explore the tear film osmolality and electrolyte composition in healthy horses.

ANIMALS
15 healthy adult horses.

PROCEDURES
Each horse was manually restrained and an ophthalmic examination, which included slit-lamp biomicroscopy, indirect opthalmoscopy, and a Schirmer tear test, was performed. Tears samples were collected from both eyes with micropipettes taken 3 times at 5-minute intervals. The tear samples for each horse were pooled and the osmolality and electrolyte concentrations were measured. The mean (SD) was calculated for each variable to establish preliminary guidelines for tear film osmolality and electrolyte composition in healthy horses.

Tear Film – Functions
1) Protect cornea from desiccation and lubricate eyelids
2) Maintain refractive power of cornea by smoothing its surface for refraction of incoming rays
3) Protect against infections via specific and nonspecific antibacterial substances
4) Supply oxygen/nutrients to cornea and transport metabolic by-products from corneal surface
5) Avoid corneal dehydration due to hyperosmolarity
6) Remove foreign materials from the cornea and conjunctiva
7) Provide WBCs/other immune cells with access to cornea and conjunctiva

Tear Film – Lipid Layer
- Meibomian glands
  - Holocrine, modified sebaceous glands arranged linearly within the dense connective tissues (i.e., tarsal plate) of the eyelid margin
  - Secretions consist of wax monoesters, sterol esters, hydrocarbons, triglycerides, diglycerides, free sterols (i.e., cholesterol), free fatty acids, and polar lipids (including phospholipids) (Levin et al., 2011)

Tear Film – Lipid Layer
- Meibomian glands
  - Molecular weight of meibomian lipids (i.e., meibum) is higher, and the polarity is lower, than that of sebum, thus meibomian lipids are fluid at lid temperature
  - A recent model proposed that a combination of PTF proteins and lipids could interact and behave similarly to lung surfactant to provide a non-collapsible viscoelastic gel that would allow for proteins to remain in their lowest free energy states while in contact with lipids (Rantamaki et al., 2011, Butovich, 2011)
Tear Film – Meibomian Glands

- Highly developed in the dog, with 20 to 40 glands per eyelid typically being present
- Glands - located within the tarsal plate, in which they form linear aggregates of secretory acini that are usually visible through the semitransparent palpebral conjunctiva

Tear Film – Meibomian Glands

- These acini open into central ductules arranged at right angles to the eyelid margin, and they deliver lipid to the surface of the eyelid through small openings just external (i.e., anterior) to the mucocutaneous junction
- “Gray line” - an important surgical landmark in a variety of blepharoplasty procedures (photo courtesy Dr. Heinrich)

Tear Film – Lipid Layer

- Thickness varies throughout the day (maximum upon awakening) and composition may differ between individuals, age (children higher than adults)
- Compression of the eyelids during normal blinking contribute to release of meibomian secretions, but precise neural and hormonal mechanisms regulating secretion of meibomian lipid are not well understood

Tear Film – Lipid Layer

- Secretion influenced by several factors:
  - Mechanical (blinking reflex)
  - Nervous (as shown after trigeminal nerve sectioning)
  - Hormonal (stimulatory action of androgens, estrogens inhibitory)
  - Physical (feedback regulation according to surface tension – S.T. decreases when lipid spreads over surface)

Tear Film Break Up Time

Video courtesy Shelby L. Reinstein, DVM, MS, DACVO

Tear Film – Aqueous Layer

- Secreted by lacrimal glands of the orbit and nictitating membrane
- Aqueous tear component provides most of the avascular cornea’s metabolic needs by supplying glucose, electrolytes, oxygen, and water to superficial cornea
  - Lubricates the cornea, conjunctiva, and nictitating membrane
  - Removes metabolites such as carbon dioxide and lactic acid
  - Flushes away particulate debris and bacteria from the ocular surface
The aqueous portion of the PTF is 98.2% water and 1.8% solids (i.e., mostly proteins)

Consists of water, electrolytes, glucose, urea, surface-active polymers, glycoproteins, and tear proteins

Examples of primary tear proteins include globulins (i.e., secretory IgA, IgG, IgM), albumin, lysozyme, lactoferrin, lipocalin, epidermal growth factor, transforming growth factors, lactin, and interleukens

Antibodies, immunoglobulins, lysozyme, lactoferrin, transferrin, ceruloplasmin, and glycoproteins all contribute to the antibacterial properties of tears

Certain topical medications (e.g. EDTA) may reduce the gelatinase activity present in tears of normal dogs (Couture et al., 2006)

PTF contains protease inhibitors as well as proteinases - important in both ocular immunity and in the prevention of excessive degradation of normal healthy ocular tissues (de Souza et al., 2006)

Total proteolytic activity in tears has been found to be significantly increased after corneal wounding

Ulcerative keratitis in animals has been associated with initially high levels of tear film proteolytic activity which decrease as ulcers heal and protease levels in melting ulcers remain elevated leading to rapid progression of the ulcers (Ollivier et al., 2007)

The lacrimal glands of the orbit and the nictitating membrane are tubuloacinar and histologically similar

Ductules from these glands deliver aqueous tear secretions into the conjunctival fornices

In dogs 3-5 ductules from the orbital lacrimal gland open into the dorsolateral conjunctival fornix, whereas the nictitans gland delivers aqueous tears onto the corneal surface through multiple ducts opening between lymphoid follicles on the posterior central third eyelid

In humans, pH varies 7.14-7.82, osmotic pressure of 305 mOsm/kg, refractive index of 1.357

pH in our patients?

The relative contributions by each of the main lacrimal glands to reflex tear secretion have been investigated in the dog by surgical removal of either one or both glands and measurement of the resulting tear production (Helper, 1970, Helper, 1976, Saito et al., 2001, Helper et al., 1974)

Tear volume produced by each gland varied considerably among animals

The orbital lacrimal gland was the main source of aqueous tears in some dogs, whereas the nictitating membrane gland was the main source in others

When either gland was removed singly, a compensatory increase in tear production appeared to occur in the remaining gland

Removal of both glands resulted in near-total absence of secretions

Suggests that accessory conjunctival glands may not be present in the dog, or that they play an inconsequential role in aqueous secretions
Tear Film – Aqueous Layer

- The role of each gland (i.e., orbital or nictitans glands) in the production of basal secretions versus reflex tear secretions has not been determined.
- Destruction of lacrimal gland results in an estimated decrease of 23-46% and nictitans gland results in 12-26% decrease.

Chemical mediators of lacrimal gland secretion are cholinergic agonists, released from parasympathetic nerves, and norepinephrine, released from sympathetic nerves, located in both the cornea and conjunctiva (Dartt, 2004, Dartt, 2009, Tiffany, 2008).

These neurotransmitters activate signal transduction pathways affecting the myoepithelial, acinar, and duct cells, and blood vessels of the lacrimal gland leading to secretion.

Other stimuli of lacrimal gland secretion include various proteins (i.e. EGF growth factor, neuropeptide Y, substance P, calcitonin gene-related peptide) and hormones (Dartt, 2004, Davidson and Kuonen, 2004, Lemp, 2008).

Harderian Gland

- Specialized lacrimal gland found in amphibians, reptiles, birds, and mammals.
- 5 types recognized: serous, mucous, seromucoid, mixed, and lipid glands.
- Typically, located on nasal side of orbit and its single duct empties on to bulbar surface of nictitans.
- Contiguous with gland of 3rd eyelid except:
  - Rabbits – below and medial to lacrimal gland.
  - Pigs – separate from 3rd eyelid gland.
  - Rodents (rat, hamster, gerbil) – posterior to globe and produces porphyrin – imparts a reddish brown color to tears and will fluoresce under ultraviolet light.

Ultrasonography of the Harderian gland in the rabbit, guinea pig, and chinchilla

Katherine M. Hittmuller,* Alexander Tichy* and Barbara Noll*

*Department of Comparative Anatomy and Physiology, University of Veterinary Medicine, Vienna, Austria; and Vienna University Hospital, Department of Ophthalmology, University of Veterinary Medicine, Vienna, Austria

Abstract

Objective: To evaluate the Harderian gland in rabbits, guinea pigs, and chinchillas using ultrasonographically in horizontal and vertical planes. Normal Harderian gland size was then compared with size in 27 rabbits, 11 guinea pigs, and three chinchillas from the same breed.

Methods: Twenty-four Harderian glands in normal rabbits were 8.0 ± 0.07 mm in horizontal plane (86%), 3.5 ± 0.09 mm vertically in normal rabbits were 8.4 ± 0.07 mm in horizontal plane and 3.5 ± 0.09 mm vertically. In normal rabbits, the Harderian gland was 8.5 ± 0.07 mm horizontally and 3.5 ± 0.09 mm vertically. Harderian glands in rabbits were significantly larger in size of the cornea than in rabbits with conjunctivitis (P < 0.05). In rabbits, Harderian glands in rabbits with corneal epithelial ulceration were significantly larger in both horizontal and vertical plane compared with those of normal rabbits. Guineas pig and chinchilla with corneal ulceration had larger Harderian glands bilaterally in the normal plane.

Conclusions: Ultrasonography is a reliable diagnostic imaging technique to evaluate the Harderian gland in the rabbit, guinea pig, and chinchilla. Broader applicability of the technique in veterinary medicine may be evaluated in the future by comparing normal to diseased conditions of the ocular system.

Tear Film – Mucus Layer

- Deepest tear film layer.
- Adheres firmly to underlying epithelial cells.
- Thickness ranges from 0.8 µm over cornea to 1.4 µm over conjunctiva.
- Facilitates adherence of aqueous layer to surface of conjunctival and corneal epithelial cells.
Tear Film – Mucus Layer

- Mucin – composed of a heterogeneous group of hydrated O-linked oligosaccharides linked to protein
- Proteins synthesized in endoplasmic reticulum of goblet cells
- Saccharide branches added in Golgi apparatus
- Glycoproteins are condensed and stored in membrane-bound secretory granules at apical side of goblet cells
- Various compounds (secretagogues, serotonin, epinephrine, phenylephrine, dopamine) stimulate goblet cells to release mucin, as well as antigen, immune complexes, mechanical action, other factors
- Adler’s physiology of the eye (2011) – regulation of goblet cell and mucin production

Tear Film – Role in Ocular Immunity

- Rich in lysozyme, betalysin, lactoferrin, and antibody (low levels of lysozyme in cattle tears - Giensfriddo et al. AJVR, 2000)
- IgA & IgG secreted by lacrimal glands (rich in plasma cells and lymphocytes)
- Serum proteins
  - Derived from vascular compartment by filtration
  - Represent 1% of total tear proteins in the absence of infection
  - Albumin, haptoglobin, IgG, IgA, IgM, IgE, α2-macroglobulins, complement-derived proteins, transferrin, α1-antitrypsin, and β2-microglobulin
- Davidson HJ and Kuonen VI. The tear film and ocular mucins. Vet Ophtal 2004; 7 (2) 71-77

Clinical Significance
Keratoconjunctivitis Sicca

- “Abnormality in either the quantity or quality of any primary tear component may compromise tear function.” (C Moore, 1999)

- “Dry eye is a disorder of the tear film due to tear deficiency or excessive tear evaporation.” (National Eye Institute, NIH).

Immunopathogenesis of KCS in the dog

- Diagnosis and treatment of eKCS – familiar
- Mechanism by which inflammatory changes lead to reduced tear production?
  1. Lymphocyte-associated cytotoxicity
  2. Apoptosis of glandular epithelial cells
  3. Cytokine release from inflammatory cells
  4. Inflammatory cells/associated cytokines or autoantibodies may influence neurotransmitter function in lacrimal gland → inhibits neurologic stimulation of tear secretion

Immunopathogenesis of KCS in the dog

- One or more of the aforementioned proposed mechanisms may be involved in pathogenesis of disease
- Important murine models – significant body of literature on the immunologic aspects of dry eye & Sjögrens Syndrome
  - MRL/lpr mouse – defect in the Fas receptor
  - NOD mouse – CD4+ T cell infiltrate in submandibular, lacrimal, and pancreas glands

Want to learn about more about tear film
and practice your Italian?
Federica Maggio DVM, DACVO


Maggio F. Pizzirani S. Patologie del film lacrimale e delle superfici oculari nel cane e nel gatto. Parte II. Segni clinici, diagnosi e terapia. Veterinaria 2009 23:5;55-70
Dry Eye Therapy
(requested in last BSC reviews)

Precorneal Tear Film
- Lipid – Meibomian glands
- Aqueous – Lacrimal glands
- Mucin – Conjunctival goblet cells

Lacrimal Gland
Gland of 3rd Eyelid

Lacrimogenic agents
- For the treatment of dry eye, or keratoconjunctivitis sicca
- Lacrostimulants
  - cholinergic agonists
  - immunosuppressive agent (CsA)
- Lacrimomimetics ( tear substitutes)
  - aqueous replacement
  - mucinomimetics
  - lipid replacement

Lacrimostimulation
- Pilocarpine
  - Parasympathetic innervation to lacrimal glands
  - Topical solution is given orally
  - Systemic increase in parasympathetic activity
- Cyclosporine A
  - Topical

Cyclosporine A
- Inhibits T cell activation
- Decreases IL-2 release and IL-2 receptor expression
- Stimulates tear production
- Available as
  - 0.2% ointment (Optimmune® Schering Plough)
  - Compounded as a 1 or 2% solution at a licensed pharmacy
- Apply topically 2-3 times daily
- Systemic absorption may occur at higher dosing frequency and smaller dogs
- Non-inhibitory to corneal healing

CsA regulation of T lymphocytes
- CsA binds Cyclophilin (CpN)
  - Prevents Calcineurin (CaN) activity
- Nuclear Factor of Activated T cells (NF-AT-p)
  - Dephosphorylated by CaN
  - Translocated to nucleus
  - Promotes IL-2 transcription
- CsA decreases IL-2 production

CsA CsA
CpN
CaN
NF-AT
IL-2 promoter IL-2 Gene
Tacrolimus

- Tacrolimus belongs to a group of drugs called macrolide lactones or calcineurin inhibitors
- Similar immunosuppressant activity to cyclosporin
- Binds to a receptor within the cell called the FK binding protein
- This resulting drug-protein complex inhibits calcineurin (a calcium-dependent phosphatase) that in turn reduces the activity of T-lymphocytes in the immune system

Wedgewood 1-800-331-8272
Veterinary Pharmaceuticals 1-800-682-4664
Stokes Pharmacy 1-800-754-5222

Conjunctiva - overview

- Vascularized mucous membrane – the anterior surface of eyeball, posterior surface of eyelids, and ant. & post. surface of 3rd eyelid.
- Secretes mucus – required for tear film stability & corneal transparency
- Mucosal defense – immunocompetent cells
  - Initiate and mediate inflammatory reactions
  - Synthesize immunoglobulin
  - Morphologic characteristics (microvilli) and biochemical properties (enzyme activity) allow phagocytosis of foreign particles such as viruses

Conjunctiva - anatomy

- Palpebral conjunctiva
  - Mucocutaneous junction: zone behind the meibomian gland openings where stratified keratinized squamous epi of lid margin → stratified nonkeratinized squamous epi of conjunctiva
  - Tarsal conjunctiva
  - Orbital conjunctiva – from tarsal plate into fornix
  - Conjunctival Cul-de-sac, or Fornix
  - Bulbar conjunctiva
    - Scleral division: extends from fornix to limbus
      - Conj, sclera, and tenon’s capsule are firmly attached ~ 3mm from the limbus and conj is more difficult to mobilize in this area
      - Limbal division: ~ 3mm wide ring at junction of conj and corneal epithelia

Conjunctiva - histology

1) Epithelium
   - Between 2 and 8-10 layers thick, depending on location
   - Single layer of basal cells
   - Variable # of layers of intermediate cells
   - Superficial cells of variable shape
     - Flattening of superficial cells believed to be an adaptation to mechanical pressure
   - Melanocytes, located among basal cells
   - Immunocompetent cells (esp. Langerhans cells)
2) Basement Membrane Zone (BMZ)
   - Separates the epithelium from the conjunctival stroma or chorion
3) Chorion

Conjunctiva - histology

Chorion (conjunctival stroma)

1) Scleral division: extends from fornix to limbus
   - Conj, sclera, and tenon’s capsule are firmly attached ~ 3mm from the limbus and conj is more difficult to mobilize in this area
2) Limbal division: ~ 3mm wide ring at junction of conjunctival and corneal epithelia
3) Rich collagen framework
4) Abundant vessels and immunocompetent cells
   - Accounts for rapid and sometimes “violent” inflammatory reactions

Conjunctival Glands

1) Serous:
   1) Krause’s glands
      - Deep in the conj tissue of fornix (~ 40 in superior & 6-8 in inferior fornix in humans)
      - Histologically, similar to lacrimal glands
   2) Wolfring’s glands
      - 2-5 in upper lid (along the upper edge of tarsus) and fewer present along lower edge of inferior tarsus
2) Mucous:
   1) Henle’s glands or crypts
      - Epithelial invaginations within chorion and composed of goblet cells
      - Situated along upper edge of superior tarsus
   2) Mana’s glands
      - At limbus: reported in pigs, cattle, & dogs; absent in humans
   - Other: Goblet cells in the conjunctival epithelium
Goblet Cells
- Mucus production per eye per day: 2-3 mL (humans) = one thousandth of total tear production
- Mucins:
  - High molecular weight glycoproteins (2000-4000 kDa) with subunits of 0.5-2x10^6 Da, which form a gel when their concentration reaches 0.5-1%
- Peroxidases
  - Contribute to the anti-infectious defense of the ocular surface by the tear film
- Some goblet cells synthesize hyaluronic acid
  - Helps stabilize the tear film

Mucus – Functions:
- Anchor the aqueous layer of the tear film
- Tear film is organized into increasingly dense filaments as one approaches the cell layers
- Trap desquamated epithelial cells and acellular surface debris (microorganisms)
- Transported to medial canthus during blinking → evacuated
- Immunological barrier:
  - Immobilize more than 30% of the secretory IgA contained in tear film

Eyelid Functions
1) Screening and sensing – cilia and vibrissae
2) Mechanical wiping action
3) Secretions and spreading of glandular tissue
4) Screening of light to allow sleep

Glycocalyx:
- Glycoproteins and glycolipids that cover the microvilli and microplicae of corneal and conjunctival epithelium
- Extends ~ 300 nm from microvilli and microplicae
- Angular and branching and often extends laterally between microvilli
- Filaments branch distally and are associated with cell membrane
- Mucus layer of tear film attaches to the carbohydrate-rich glycocalyx
  - Protects epithelium by causing shear forces of blinking to break up mucus layer further away from cell surface
  - Mucus attachment to glycocalyx allows aqueous layer to spread evenly over corneal epithelium

Eyelid Functions
- Comparative approach: where are the exceptions?
  - Fish lack eyelids (constantly bathed in aqueous environment)
  - Land creatures need a way to “bring the ocean with them”
    - Amphibians: 1st creatures to have true eyelids and nictitans
      - Tadpoles have no eyelids → frogs have eyelids, lacrimal glands and a NL system
  - Specialized eyelids
    - Crocodilians – bony tarsus of upper lid
    - Chameleons – tight-fitting around globe and move with globe
    - Snakes, geckos, skinks – spectacle which has a vascular network
EyeLids - composition

Skin, collagen, muscle, glandular tissue, palpebral conjunctiva

<table>
<thead>
<tr>
<th>Eyelid</th>
<th>Skin</th>
<th>Musculofibrous layer</th>
<th>Palpebral Conjunctiva</th>
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Eyelid Skin

- Epidermis
  - Strata corneum & granular, spinous and basal layers
- Dermis
  - Dense, irregular C.T.
  - Most species, dermis devoid of fat
    - exception in some dogs: Shar Pei
- Hair follicles extend deep into dermis
- Palpebral margin
  - Skin changes keratinized, stratified squamous EPI → non-keratinized, stratified squamous EPI
- Eyelashes / CILIA located on eyelid leading edge
  - Normal turnover time: 3-5 months; regrow in 2 months

Cilia

<table>
<thead>
<tr>
<th>Species</th>
<th>Location on Eyelids</th>
</tr>
</thead>
<tbody>
<tr>
<td>People</td>
<td>Upper and lower</td>
</tr>
<tr>
<td>Dogs</td>
<td>Upper</td>
</tr>
<tr>
<td>Pigs</td>
<td>Upper</td>
</tr>
<tr>
<td>Horses</td>
<td>Upper and few on lower</td>
</tr>
<tr>
<td>Ruminants</td>
<td>Upper and lower</td>
</tr>
<tr>
<td>Cats</td>
<td>None per se</td>
</tr>
<tr>
<td>Birds</td>
<td>Some species (i.e. budgerigar) have filoplumes: rudimentary feathers without barbs</td>
</tr>
</tbody>
</table>

Eyelid Musculature

Eyelids - composition

1. Levator palpebrae superioris
2. Orbital septum & 2’ = tarsal plate
3. Orbicularis oculi
4. Puncta lacrimalis
5. Cilia w/ associated sebaceous glands
6. Tarsal or meibomian glands

Vetinary Anatomy, 1987, Dyce Sack & Wensing

Prominent orbito-palpebral sulcus (skin fold) of the superior eyelid (arrows) delineating the orbital portion (above the skin fold) and tarsal portion (below the skin fold) of the eyelid.

Textbook of Sm An Surg, 1993, Slatter
Eyelid Musculature – *Orbicularis oculi*
- Major eyelid muscle
- Arranged in concentric rings around the palpebral opening
- Fibers originate and terminate on the medial palpebral ligament
- Innervation: CN VII (Facial)
- Function: eyelid closure
- Specialized divisions:
  - Horner’s muscle:
    - Branch that runs under lacrimal sac and inserts on medial orbital wall
    - Negative pressure within lacrimal sac so as to pull tears into sac
  - Muscles of Riolan:
    - Travel along eyelid margin, surrounding the eyelash bulbs
    - May rotate eyelashes toward eye & propel glandular contents during blink

Musculature – *Levator palpebrae superioris* & Müllr’s muscle
- **Levator palpebrae superioris**
  - Originates deep within orbit, dorsal to optic canal between origins of dorsal rectus and dorsal oblique
  - Functions to elevate upper eyelid
  - Innervated by CN III (oculomotor)
- **Müllr’s muscle**
  - Portion of the levator palpebrae superioris that extends deeper into dermis
  - Composed of smooth muscle fibers
  - Innervated by sympathetic nervous system (carried by infratrochlear n, a branch of nasociliary n., branch of ophthalmic division of CN V)
  - Functions to widen/elevate palpebral fissure
  - Well described in cat, poorly described in horse, even less described in other species

Musculature – *Levator anguli oculi medialis* and *Frontalis*
- Both eyelid elevators
- Both muscles innervated by CN VII – palpebral branch
- LAOM - also known as the *corrugator supercilia*
  - Small muscle that arises caudodorsal to the medial commissure
  - Contraction raises the medial portion of the upper eyelid
  - In the horse, gives rise to a prominent lid notch

Musculature – *Retractor anguli oculi lateralis*
- Located parallel and superficial to lateral palpebral ligament
- Innervated by zygomatic branch of CN VII
- Functions to draw the lateral canthus posteriorly and laterally when eyelids close

Musculature – *Pars palpebralis of the m. sphincter colli profundus* (or the *Malaris muscle*)
- Consists of several delicate straps of muscle which originate near the ventral midline and course dorsally to insert on the lower eyelid
  - Ventral portion lies deep to the platysma
  - Dorsal portion is subcutaneous and close to eyelid skin
  - Innervated by buccal branches of CN VII
  - Functions to depress the lower eyelid
Eyelid Musculature

<table>
<thead>
<tr>
<th>MUSCLE</th>
<th>INNERVATION</th>
<th>FUNCTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orbicularis Oculi (Horner’s and Riolan)</td>
<td>CN VII (palpebral branch)</td>
<td>Eyelid closure</td>
</tr>
<tr>
<td>Levator palpebrae superioris</td>
<td>CN III</td>
<td>Maintain open pal. fissure</td>
</tr>
<tr>
<td>Müller’s muscle</td>
<td>Sympathetic N.S.</td>
<td>Maintain open pal. fissure</td>
</tr>
<tr>
<td>Frontalis (upper eyelid)</td>
<td>CN VII (palpebral branch)</td>
<td>Maintain open pal. fissure</td>
</tr>
<tr>
<td>Levator anguli oculi medialis</td>
<td>CN VII (auriculopalpebral)</td>
<td>Raises medial portion of superior eyelid</td>
</tr>
<tr>
<td>Retractor anguli oculi lateralis</td>
<td>CN VII (zygomatic branch of auriculopalpebral)</td>
<td>Contraction during eyelid closure pulls lateral canthus posteriorly &amp; laterally</td>
</tr>
<tr>
<td>Malaris (lower eyelid)</td>
<td>CN VII (dorsal buccal branch)</td>
<td>Maintain open pal. fissure</td>
</tr>
</tbody>
</table>

Eyelid Glands

- Glands of Zeis and Moll
  - Located in anterior lamella of eyelid
  - Associated with eyelash cilia
  - Secrete their contents around lash follicle shaft
- Zeis: Modified sebaceous glands
  - Surround base of hair follicles
- Moll: Eccrine, or modified sweat glands
  - Located just deep to the hair follicles
- Meibomian glands
  - Holocrine, sebaceous glands not associated with lash cilia
  - Produce the lipid layer of the tear film
  - Secretion may be partially under neural or hormonal control
  - Meibum contains waxy esters, sterols, triacylglycerols, cholestrols, polar lipids, free fatty acids
  - Lower melting temp than sebium, thus liquid on ocular surface

Eyelid Vasculature

- Well described in dog and horse
- In all species, variation exists between different animals – not all reports identical, but all similar

References:

Eyelid Movement

- Most domestic species: superior lid is most mobile
- Innervation to levator palpebrae superioris m follows Hering’s law:
  - Synergistic muscles receive simultaneous and equal innervation
  - Motor neurons for levator m. arise from a single unpaired central caudal nucleus of the oculomotor complex, and a single motor neuron may innervate the levator m. bilaterally
  - Hence, any supranuclear input into motor neuron influences BOTH levator muscles
- Clinical significance: When the levator on one side is weak, the lid on opposite side may be retracted in an unconscious attempt to elevate the ptotic lid

Birds, many reptiles: inferior lid raises to meet superior
Humans have ability to move eyebrows:
  - Elevation: frontalis muscle
  - Depression: orbicularis muscle in forced lid closure
  - Drawn together: corrugator supercili

WHERE DO YOU PLACE YOUR EQUINE LAVAGE TUBES??
Inferomedial Placement of a Single-Entry Subpalpebral Lavage Tube for Treatment of Equine Eye Disease

**Veterinary Ophthalmology**
Volume 3 (2-3), pp 153-156, September 2000
Giuliano EA, Maggs D, Moore CP, et al

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**Eyelid Movement - Blinking**

Types of eyelid closure:
1. Spontaneous blinking
   - Most common (15 / min humans)
   - Lateral → medial (part of lacrimal pump mechanism)

<table>
<thead>
<tr>
<th># blinks / min</th>
<th>% bilateral blinks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog: 3-5 b/min</td>
<td>85%</td>
</tr>
<tr>
<td>Cat: 1-5 b/min</td>
<td>70%</td>
</tr>
<tr>
<td>Horse: 5-25 b/min</td>
<td>30%</td>
</tr>
<tr>
<td>Pig: 10 b/min</td>
<td>90%</td>
</tr>
</tbody>
</table>

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**Third Eyelid**

Nictitating Membrane, Palpebral Tertia, Semilunar Fold of the Conjunctiva, Plica Semilunaris Conjunctivae

- **Topographical distribution:**
  - Originates in the anterior ventromedial orbit
  - Triangular in shape; covered with conjunctiva
  - “T-shaped” hyaline cartilage
  - Gland of the third eyelid
- **Function:**
  - Protects the globe
  - Secretion and distribution of tears
  - Aid in removal of particulate matter from the eye

---

**Nictitans (3rd Eyelid) – Anatomy & Physiology**

- **Movement - Passive**
  - Orbital tone
  - Orbital fat
  - Hydration status
  - Exception - CATS
    - Believed to have some smooth muscle and sympathetic innervation to their 3rd eyelid movement

---

**Nictitans (3rd Eyelid) – Anatomy & Physiology**

- **Gland of the 3rd Eyelid**
  - Encompasses base of cartilage
  - Seromucous secretions in dog (serous in horses) exit through ducts open in the posterior aspect of the TE between lymphoid follicles
  - Important contributor to basal tear production
Mucosa-Associated Lymphoid Tissue (MALT)
- A distinct network of diffuse aggregates of lymphoid tissue located in various mucosal surfaces
  - Gut (GALT)
  - Bronchus (BALT)
  - Conjunctiva (CALT)
  - Nasal mucosa (NALT)

Background Information
- Mammalian mucosae are routinely in contact with large numbers of different antigens
- Mucosa-Associated Lymphoid Tissue (MALT):
  - Afferent branch
  - Efferent branch
  - Effector functions may occur at some distance from original stimulus

Microfold (M) Cells
- Morphologic characteristics:
  - A less elaborate apical cell surface with small microvilli and microfolds
  - An invaginated basolateral membrane forming a cytoplasmic pocket containing lymphocytes, macrophages, & dendritic cells
  - A diminished distance between the apical and pocket membrane to enable more efficient transcytosis
  - Located in the Follicle Associated Epithelium (FAE) overlying organized immune cells

Significance of M-Cells
- Gut-Associated Lymphoid Tissue (GALT)
  - Exploited by infectious agents
  - Preferential binding and translocation of antigens across the mucosal barrier with subsequent delivery to underlying antigen presenting cells
  - Shigella, Salmonella, Yersinia, Campylobacter, Vibrio, E. coli, Polio, HIV (Neutra et al., 1996; Sansonetti & Phalipon, 1999)
SIGNIFICANCE of M-CELLS

- Intense research efforts aimed at the development of mucosal vaccines by targeting M-cells (reviewed by Killeen et al., 1999)
- New drug delivery strategies may be possible

OVERVIEW of Graduate Work

- Characterize the Follicle-Associated Epithelium (FAE) of Canine Conjunctiva-Associated Lymphoid Tissue (CALT)

Hypothesis:
- The FAE of CALT in healthy dogs contains microfold (M) cells morphologically and functionally analogous to those described in intestinal Mucosa-Associated Lymphoid Tissue (MALT)

Project design (3 phases):
- Morphologic examination using LM, TEM & SEM
- Immunohistochemistry
- Functional studies using Staphylococcus aureus organisms

RESULTS

- Morphologic studies:
  - Canine 3rd eyelid lymphoid follicles compared with surrounding conjunctival epithelium – light microscopy, transmission & scanning electron microscopy
- Healthy canine CALT contains cells structurally analogous to M-cells:
  - Diminished cytoplasmic thickness
  - Attenuated apical cell surface structures
  - Invaginated basolateral membranes forming cytoplasmic pockets with subtending lymphocytes and plasma cells

MORPHOLOGY
RESULTS

- Immunohistochemistry:
  - Lymphocytes have an organized distribution in MALT:
    - B-cells in germinal centers
    - T-cells comprise apical cap
  - Canine CALT lymphocytes (Nictitans from 5 dogs)
    - CD 79 staining for B-cells
      (monoclonal mouse anti-human B Cell, DAKO Corp., Carpinteria, CA)
    - CD 3 staining for T-cells
      (rabbit anti-human T-cell, DAKO Corp., Carpinteria, CA)
  - Findings consistent with other descriptions of organized MALT

IMMUNOHISTOCHEMISTRY

- CD 79 - B cells
- CD 3 - T cells

FUNCTIONAL STUDIES

- Hypothesis:
  Canine conjunctival M-cells exhibit selective binding and preferential uptake of antigens compared with surrounding epithelial cells

MATERIALS & METHODS

Functional Uptake Studies

- Nictitating membranes obtained from 10 healthy, 2 year old, M/C, tricolored research hounds.
- Health status based on:
  - Physical Examination
  - Blood work (CBC, Serum Chemistry Panel, Urinalysis)
  - Complete Ophthalmic Examination (Slit-lamp Biomicroscopy, Fluorescein Stain, and Schirmer Tear Test)

- 10 dogs treated with 30 µl of a heat-killed, 1 x 10^{10} Staphylococcus aureus (BioParticles, Molecular Probes, Eugene, OR) per 1 ml sterile eyewash (Dacirose, CIBA Vision Ophthalmics, Atlanta, GA)
  - 5 dogs: 1 gtt OU q 5 minutes for 30 minutes immediately prior to euthanasia
  - 5 dogs: 1 gtt OU q 5 minutes for 30 minutes 12 hours prior to euthanasia

- Primary fixation:
  - 2.5% glutaraldehyde-2% paraformaldehyde buffered solution for 12 hours

- Tissues subsectioned and processed using standard techniques for:
  - Scanning Electron Microscopy (SEM)
  - Transmission Electron Microscopy (TEM)
CONCLUSIONS

1) There are distinct differences in epithelial cell morphology overlying the canine conjunctival lymphoid follicles compared to the surrounding conjunctival epithelium.

2) The FAE overlying lymphoid follicles in canine CALT, as well as the distribution of T and B lymphocytes subtending this region, contain morphologic features analogous to MALT described in other regions.

CONCLUSIONS

3) Canine CALT contains M-cells that demonstrate selective binding and preferential uptake of heat-killed Staph. aureus organisms.

4) The transport of antigens across M-cells to the lymphoid cells in organized CALT appears to be time dependent.

CLINICAL RELEVANCE

1) Conjunctival M-cells capable of antigen sampling will link ocular immunology to a growing body of research concerned with targeted mucosal vaccines and potentially serve as a means of developing new strategies for drug delivery.

2) Recognition of conjunctival M-cells opens the door to new research aimed at determining if any ocular bacterial, parasitic, or viral pathogens exploit this entry site.
Future Directions

NATIONAL EYE INSTITUTE:
Project Title: M-cells in Mammalian Conjunctiva Grant Number – 1 R03
EV 13779-01


Characterization of membranous (M) cells in normal feline conjunctiva-associated lymphoid tissue (CALT)
EA Giuliano, K Finn
Veterinary Ophthalmology (2011) 14, Supplement 1, 60–66

PHOTODYNAMIC THERAPY FOR EQUINE PERIOCUCLAR TUMORS

Elizabeth A. Giuliano, DVM, MS
Diplomate ACVO

IEOC, 2013
OUR PROBLEM:
- Approximately 10% of equine neoplasms affect the eye or periocular structures
- Neoplastic adnexal disease represents the most common & often the most frustrating, of all equine eyelid diseases

EQUINE OPHTHALMIC NEOPLASIA
- Variability in clinical response → a myriad of different treatment options exist for any given equine neoplasm

<table>
<thead>
<tr>
<th>Type of Therapy</th>
<th>Description</th>
<th>Number of cases</th>
<th>% of non-recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgical excision</td>
<td>Excision</td>
<td>28</td>
<td>18%</td>
</tr>
<tr>
<td>Cryotherapy</td>
<td>Double or triple freeze – thaw to -25°C</td>
<td>23</td>
<td>9%</td>
</tr>
<tr>
<td>Hyperthermia</td>
<td>Tissue temperatures between 41°C and 45°C</td>
<td>2</td>
<td>9%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Radioisotope</th>
<th>Dose Range</th>
<th>Number of cases</th>
<th>% of non-recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>²²²Radon seeds</td>
<td>6000 cGy</td>
<td>19</td>
<td>92%</td>
</tr>
<tr>
<td>¹³¹Cesium seeds</td>
<td>7000 cGy</td>
<td>19</td>
<td>90%</td>
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<tr>
<td>¹⁹²Iodine seeds</td>
<td>60-100 cGy</td>
<td>115</td>
<td>87%, 100%</td>
</tr>
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<td>7000-9000 cGy</td>
<td>53</td>
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</tbody>
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Medical Treatment for Periocular Sarcoids in Horses

<table>
<thead>
<tr>
<th>Type of Therapy</th>
<th>Drug</th>
<th>Dose</th>
<th>Number of cases</th>
<th>% of non-recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topical Therapy</td>
<td>AW4-LUDES ointment</td>
<td>Once daily for 5 days</td>
<td>146</td>
<td>35%</td>
</tr>
<tr>
<td></td>
<td>5% 5-fluorouracil</td>
<td>Bid x 5 days, then qid for 5 days, then QOD for 5 application</td>
<td>9</td>
<td>67%</td>
</tr>
<tr>
<td>Immunotherapy</td>
<td>BCG</td>
<td>1 ml per cm² of tumor surface every 2-4 weeks</td>
<td>26</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>BCG</td>
<td>1 ml per cm² every 2-4 weeks</td>
<td>22</td>
<td>32%</td>
</tr>
</tbody>
</table>

Surgical Treatment for Periocular Sarcoids in Horses

<table>
<thead>
<tr>
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TREATMENT QUANDARIES…
- Definitive conclusions regarding biologic behavior or response to treatment from reviewing the veterinary literature difficult
  - Study design varies widely / limited to isolated case reports
  - Very few reports exist with >100 horses included and containing at least a year follow-up time after specific therapy for a particular tumor-type has been used
  - In stark contrast to studies routinely published in physician ophthalmology
TREATMENT QUANDARIES…

- Treatment efficacy: challenging to critically assess
- Some studies not designed with a control population for adequate comparison purposes & are often based on a subset of cases referred to specialty/teaching hospitals
- Referral cases are frequently those that are refractory to “conventional” treatment modalities
- May have already undergone treatment prior to being included in the study; therefore, the population of horses examined is skewed

**TREATMENT QUANDARIES…**

- Methods of data reporting (i.e. recurrence rate versus disease-free interval) also vary among publications
- Point: be aware of these limitations when reviewing the literature and in planning the most appropriate therapy for your patients

**LECTURE OBJECTIVES, PDT**

1) Overview of Photodynamic Therapy
2) My interest in this modality
   1) Summary of early work
   3) How I perform this procedure
   1) Review of treatment protocol and clinical cases
4) Response to frequently asked questions
   1) Laser type/source, cost, good vs. bad candidates for procedure, etc – discussion welcomed

**PHOTODYNAMIC THERAPY**

Historically, concept of combining light with chemical agent has ancient beginnings

- The success of PDT in early 1960s with subsequent PS approvals generated world-wide interest
  - 1900-1955: 112 publications
  - 1955-2013: > 16,000 publications; > 8000 papers in past 5 years

*Chem. Rev. 2010 (110) 2795-2838*

**PHOTODYNAMIC THERAPY (PDT)**

- Cancer therapy using photosensitizing agent and light in the presence of oxygen to produce a localized phototoxic effect
- 2-stage process
  - Delivery of photosensitizer (IV, PO, topical)
  - Light irradiation of target tissue

**“PHOTOCHEMISTRY”**

Mechanism of Action

- Photosensitizer
  - Ground state $\rightarrow$ Type I reaction: Energy transferred to other compounds
  - Superoxide, free radicals
  - Excited state $\rightarrow$ Type II reaction: Energy transferred to $^{1}O_2$
  - $O_2$ (singlet oxygen)

Cell Killing - $^{1}O_2$ and oxygen free radical production
1. Damage of cellular structures - plasma membrane, mitochondria, lysosomes
2. Inflammation
3. Vascular effects and tumor ischemia

Selective tissue destruction
- Preferential localization of photosensitizer in cancer cells
- Focussed light beam (lasers – major advancement in PDT)
- $^{1}O_2$ - short half-life (0.04 $\mu$s), diffusion distance (<0.1 $\mu$m)
**PHOTOPHYSICS & PHOTOCHEMISTRY**

- Light exposure takes a photosensitizer molecule from the ground singlet state ($S_0$) to an excited singlet state ($S_1$).
- Excited PS → very unstable
  - Emit its excess energy as fluorescence and/or heat
  - OR
  - Undergo “intersystem crossing” to form a more stable triplet state

- A Type I reaction can also occur:
  - PS reacts directly with organic substrates, acquiring a hydrogen atom or electron to form a radical
  - Superoxide anions, hydroxyl radicals, peroxides

**MECHANISMS OF PDT-MEDIATED CYTOTOXICITY**

- Apoptosis **
- Necrosis
- Autophagy-associated cell death

**PHOTOPHYSICS & PHOTOCHEMISTRY**

- Reactive oxygen species (ROS) (predominantly the generation of singlet oxygen or $^{1}\text{O}_2$) via Type II chemistry is mechanistically much simpler than via Type I
  - Most PSs are believed to predominantly exert their cytotoxic effects through Type II reactions

**APOPTOSIS**

- Characterized by chromatin condensation, DNA cleavage, cell shrinkage, membrane blebbing, with release of signals required for phagocytic cell activity (e.g. Damage Associated Molecular Patterns)
- At biochemical level: mitochondria outer membrane permeabilization (MOMP) after PDT → activation of caspases (highly conserved family of cysteine-dependent, aspartate-specific proteases)

NECROSIS
- Characterized by cytoplasm vacuolization & breakdown of plasma membrane → release of cellular contents and proinflammatory molecules
- Results from pathological insults or a bioenergetic catastrophe and ATP depletion
- At biochemical level: cytochrome c release and DNA oligonucleosomal fragmentation


AUTOPHAGY
- Characterized by formation of a double membrane structure called the autophagosome, which sequesters cytoplasmic organelles and traffics them to lysosomes
- Autophagosome-lysosome fusion results in degradation of cytoplasmic components by lysosomal hydrolases
- In adult organisms, functions as a self-digestion pathway promoting cell survival in an adverse environment & as a quality control mechanism by removing damaged organelles, toxic metabolites, or intracellular pathogens

PDT AND THE IMMUNE RESPONSE
- Inflammation elicited by PDT is a tumor antigen nonspecific process orchestrated by the innate immune system
- ROS within tumor cells after PDT leads to cell death (apoptosis & necrosis)
  - Tumor cell death potentiated by damage to microvasculature, restricting oxygen and nutrient supply
  - Cell death activates the complement cascade, secretion of proinflammatory cytokines, and rapid recruitment of neutrophils, macrophages, and dendritic cells

PDT AND THE IMMUNE RESPONSE
- Phagocytized tumor cells migrate to local lymph nodes
  - Differentiate into antigen-presenting cells
  - Tumor antigen presentation within lymph nodes is followed by clonal expansion of tumor-sensitized lymphocytes that home to the tumor and eliminate residual tumor cells


PHOTOSENSITIZERS
PHOTOSENSITIZERS (PSs)

- Most PSs used in cancer therapy based on a tetrapyrrole structure
- Similar to the protoporphyrin present in hemoglobin

![Tetrapyrrole Structures](image)

PSs – IDEAL PROPERTIES

- Single, pure compound (quality control analysis, low manufacturing costs, stable when stored)
- High absorption peak between 600-800 nm (red – deep red)
  - Absorption of photons with λ longer than 800 nm does not provide enough energy to excite oxygen to its singlet state
- No dark toxicity
- Relatively rapid clearance from normal tissues, minimizing phototoxic side effects


PSs – DEVELOPMENT

1st PS used in cancer therapy: HPD (hematoporphyrin derivative)

- A water-soluble mixture of porphyrins
- Later, a purified form, porfimer sodium (Photofrin®) developed
  - Still widely used
- Disadvantages: long-lasting skin photosensitivity, relatively low absorbance at 630 nm
- Major effort aimed at producing second generation PSs
  - Increased absorbance at longer λ, less toxicity

PSs – TUMOR-LOCALIZING PROPERTIES

”Enhanced permeability and retention effect”
Leaky tumor blood vessels due to neovascularization
Absence of lymphatic drainage
Some PSs preferentially bind to low-density lipoprotein (LDL)
LDL receptors up-regulated on tumor cells


LIGHT SOURCES IN PDT

LIGHT SOURCES in PDT

”Any light source, either laser or nonlaser, with suitable spectral characteristics and a high output at an absorption maximum of the photosensitizer can be used for PDT”

GOAL: match output $\lambda$ to spectral absorption peak of PS

CHOOSING A LIGHT SOURCE

No single light source is ideal for all PDT indications, even using the same PS
Choice based on:
- PS absorption
- Disease (location, lesion size, accessibility, and tissue characteristics
- Cost
- Size
Clinical efficacy of PDT is dependent on complex dosimetry:
- Total light dose, light exposure time, light delivery mode (single vs fractionated), etc

LIGHT SOURCES

First successful treatment of tumors in animals reported in 1975
Slow to gain acceptance
Lasers – major advancement
- Monochromatic
- Coherent
- Intense
- Fiberoptic cables

LASERS in PDT

ADV of Lasers:
- Monochromatic: provides maximum effectiveness if $\lambda$ corresponds with the peak absorption of PS
- Coherent, high irradiance: minimize therapeutic exposure time
- Can be readily coupled to fiberoptics, enabling light delivery to any organ (e.g. bladder, GI tract, lungs)

DIS of Lasers:
- Expensive
- Require special maintenance (automated dosimetry & calibration)
- When coupled with fiberoptics, may only be used on small skin lesions
NONCOHERENT LIGHT in PDT
* ADV:
  - Large illumination field compared to laser
  - Low cost, smaller size, and readily available
  - Polychromatic light sources allow the use of different photosensitizers with different absorption maxima
* DIS:
  - Recall: blue light penetrates least efficiently through tissue; red & infrared more deeply
  - 600-1200 nm = "Optical Window" of tissue
  - Do not exceed 800 nm (cannot generate $^{1}$O$_{2}$)

LIGHT EMITTING DIODES/DEVICES (LEDs) in PDT
* ADV:
  - Relatively narrow spectral bandwidths
  - High fluence rates
  - Large illumination field compared to laser
  - Low cost

LIGHT DOSIMETRY
* Definition: dose of light delivered from optical fiber
  - Fiber output and distance to surface
* Fluence: # photons to given area – J/cm²
  - Dose = amount of light delivered
* Power density: # photons per unit time – W/cm²
  - Dose rate = rate of light delivery
  - High – tissue hyperthermia at >125 mW/cm²
  - Low
  - Fewer microvascular effects
  - Slower O₂ depletion, more efficient tumor killing
  - Higher local control rate
* Treatment time = Fluence/Power density
  - Longer exposure times preferred in some cases

LIGHT DOSE
* Efficacy of PDT affected by power density and fluence
* **Energy**: Joules/cm²
  - Typical dose is 100 J/cm²
  - Typical treatment "spot" is 16 – 20 minutes
  - Some researchers advocate "pulsing" the laser energy so that the photosensitizer can return to ground state and then be "re-excited" to achieve better tumor necrosis

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**EXAMPLE**
* Area is $\pi r^2$, where $r = 3.1415927$
  - 3 cm spot $\rightarrow$ $1.5 cm$ radius $\rightarrow$ $r = 7.07 cm$
* Dose Rate = Power Density $\times$ Watt - Second $\rightarrow$ 100 mW/cm² $\times$ 100 sec $\times$ (Watt/sec)$^{-1}$ = 100 J/cm²

**Drug not commercially available**

**Pilot Study: Photochlor® (HPPH)**
* 2-[1-hexyloxy]-2-devinylpyropheophorbide-a
* Investigational cancer therapy at MU-VMTH
  - Drug not commercially available
REFERENCES

Peer Reviewed Publications


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Thank you!

*Enjoy Eye Camp, 2016*