OCULAR INFLAMMATION
AND PAIN

Dr. Flavio Mantelli, MD, PhD
The Ocular Surface

- Lacrimal glands
- Conjunctival epithelium with goblet cells
- Meibomian glands
- Corneal epithelium
- Tear Film
Ocular surface inflammation

• The ocular surface is a complex structure responsible for visual function and for protection of the eye against external insults.

• Comprising a variety of disorders on cornea, eyelid, conjunctiva, lacrimal apparatus and tear film, there are countless triggers of ocular surface inflammation.

• One of the main goals of ocular surface treatment is to control the inflammatory reaction in order to preserve corneal integrity and transparency.
Types of inflammation

• Allergic inflammation: cytokines, kemokines, histamine, etc…
• Inflammation in dry eye: mediators, hyperosmolar stress, etc…
• Ocular surface toxicity: medications, preservatives…

➡ Neurogenic inflammation: neuropeptides (SP, CGRP, NPY, VIP)
Allergic Inflammation

**Early phase response**
- \( \text{↑Ca}^{2+} \text{ and degranulation} \)
- Histamine, tryptase, chymase, 
  - \( \text{TGF}\beta 1, \text{NGF} \)

**Late phase response**
- \( \text{PKT down-stream (de novo synthesis)} \)
- Leukotrienes, tromboxanes, 
  - \( \text{TNF}\alpha, \text{TGF}\beta 1, \text{NGF} \)
  - \( \text{VEGF} \)

**Chronic Inflammation and Tissue remodeling**
- **ocular surface damage**

**Cellular Involvement**
- **Epithelium**
- **Endothelium**
- **EOS**
- **FBs vs. myoFBs**
- **Th2**
Mediators in tears are the main actors of dry eye-related inflammation.
Ocular surface Toxicity

Direct Reaction to Drug

Direct Reaction to Preservatives

Allergic Reaction to Drug

Allergic Reaction to Preservatives

“Dry eye like” Changes of Ocular Surface
Inflammation ➔ Pain
CORNEAL INNERVATION

• Corneal sensory innervation is the richest of the human body (100+ times more than than the tooth pulp)

• The cornea is an avascular tissue depending on sensory nerves not only for mechanical sensitivity but also for its trophic support

• Three types of sensory nerves: mechanic (20%), chemical (70%), cold fibers (10%).

Mechanical sensitivity testing is NOT sufficient!
Corneal sensitivity testing

• Mechanical stimulation of the corneal epithelium with a cotton thread:
  - Complete anhesthesia: no feel, no blink
  - Hypoesthesia: feeling without blinking
  - Normoesthesia: blink at every touch

• In the last 50 years corneal sensitivity testing was based only on mechanical stimuli.
  Improvement of cotton-thread test ➔ Cochet-Bonnet esthesiometer (0-6mm nylon thread)
Belmonte esthesiometer

- Nociceptive corneal sensation evoked by:
  - Mechanical stimulus (air)
  - Chemical stimulus (CO$_2$)
  - Thermal stimuli (hot / cold air)

- Possibility to investigate all the different corneal nerve fibers

- Possibility to understand roles of any kind of corneal hypo/hyperesthesia on the patogenesis of ocular surface disease, adjusted to peculiar characteristics of each patients
**STIMOLO MECCANICO**

- **ARIA**: 90 ml/min
- **STIMOLO MECCANICO ON/OFF**: REGISTRARE STÍMOLO MECCANICO
- **TEMPO STIMOLO**: 1.5 Sc.
- **TEMPERATURA D´USCITA**: 20º

**STIMOLO CHIMICO**

- **CO2**: 40 %
- **STIMOLO CHIMICO ON/OFF**: REGISTRARE STÍMOLO CHIMICO
- **TEMPERATURA D´USCITA**: 20º

**STIMOLO TERMICO FREDDO**

- **ARIA**: 90 ml/min
- **TEMPERATURA STIMOLO**: 20.2 ºC
- **STIMOLO TÉRMICO ON/OFF**: REGISTRARE STÍMOLO TERMICO
- **TEMPERATURA AMBIENTE**: 20º

**VALORI NORMALI ESTESIOMETRIA DELLA CORNEA**

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Neurogenic inflammation

- Inflammation that results from the release of substances from primary sensory nerve terminals.

- These neuromediators act on target cells and exert their biological activity on MC and immune cells to sustain inflammation (Richardson 2002).
The role of the nervous system in rhinitis

Seema Sarin, MD, Bradley Undem, PhD, Alvin Sanico, MD, and Alkis Togias, MD, Baltimore, Md
Not only allergens but any kind of noxious stimulus can induce neuropeptides’ release from corneal nerve endings.
Fig. 7. Neurochemistry of the corneal innervation and the pathways by which nerve transmitter substances reach the cornea.
The neuropeptides substance P (SP) and Calcitonine gene-related peptide (CGRP) are considered to be the major mediators of neurogenic inflammation and pain.

Bornes 2001, Groneberg 2004
SUBSTANCE P (SP)

Substance P induces pain, vasodilation, increase in vascular permeability, stimulation of mast cell, B-T lymphocytes chemoattractant for Eos (Lambiase et al 1998, 2013)

Substance P is produced by eosinophils, monocytes, macrophages, lymphocytes and dendritic cells (Lai 1998)
SUBSTANCE P (SP)

- Is present in the cornea in physiologically relevant concentrations
- It is a 11 amino acids peptide generally associated with intense, persistent, or chronic pain.

Substance P (SP) positive nerve fibers

Substance P and Pain

• Nociceptors in the damaged area initiate a sensation of pain.

• These receptors are stimulated after damage due to a release of chemicals to which they are sensitive. In the cornea, these receptors are primarily chemical sensors, but they also respond to mechanical and thermal stimulation.

• After stimulation, they send receptor potentials, which in turn trigger afferent action potentials.
Inflammation ➔ Pain

Diagram showing the relationship between inflammation and pain, with pathways involving PGE2 and other molecules such as COX-2, mPGES-1, EP1/4, and CGRP.
Increased Plasma Levels of Substance P in Vernal Keratoconjunctivitis

Alessandro Lambiase,*† Stefano Bonini,†‡
Alessandra Micera,* Paola Tirassa,*
Laura Magrini,§ Sergio Bonini,§
and Luigi Aloe*

0.001; Table 1). Moreover, NGF levels were dramatically increased in the plasma of VKC patients (11,037 ± 10,641 pg/ml; median, 130 pg/ml; P < 0.001) compared with levels in the plasma of control subjects (47.5 ± 8.5 pg/ml; median, 42.5 pg/ml).

FIGURE 1. Substance P (SP) plasma concentration in vernal keratoconjunctivitis (VKC) patients and control subjects. The horizontal bars represent the medians of the values. Readings in the two groups differ significantly (P < 0.001).

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Age (yr), Sex</th>
<th>Previous and Associated Atopic Diseases</th>
<th>Substance P (pg/ml)</th>
<th>NGF (pg/ml)</th>
<th>Total IgE (kU/l)</th>
<th>RAST Positive*</th>
<th>ECP (µg/l)</th>
<th>Circulating Eosinophils (× µl)</th>
<th>Biopsy</th>
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<td>—</td>
<td>956</td>
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<td>67.3</td>
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ECP = eosinophil cationic protein; NGF = nerve growth factor; ND = not done.
Altered Expression of Neurotransmitter Receptors and Neuromediators in Vernal Keratoconjunctivitis

Laura Motterle, MD; Yolanda Diebold, PhD; Amalia Enriquez de Salamanca, PhD; Victoria Saez, BS; Carmen Garcia-Vazquez, BS; Michael E. Stern, PhD; Margarita Calonge, MD; Andrea Leonardi, MD
Neuropeptides levels in tears of patients with hay fever

VIP

P=0.008

NPY

P=0.001

CGRP

P=0.003

SP

P=0.038

Healthy

Hay fever

N=12

N=12

N=5

N=8

N=8

N=8

N=8

N=11

N=10
Figure 2. In allergic patients SP, CGRP, and VIP but not NPY tear levels significantly increase after a positive CPT.

Table 1 Treatment with preservative-free cromolyn sodium 4%-chlorpheniramine maleate 0.2% eye drops inhibited the local release of substance P, calcitonine gene-related peptide, neuropeptide Y, and vasoactive intestinal peptide after conjunctival provocation test.

<table>
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<tr>
<th>Neuropeptides</th>
<th>Baseline (visit 1)</th>
<th>After treatment with cromolyn sodium (visit 2)</th>
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<tr>
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<td>Before CPT</td>
<td>After CPT</td>
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<tr>
<td>Substance P (ng/ml)</td>
<td>3.2 ± 2</td>
<td>5.1 ± 2.3</td>
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<td>CGRP (ng/ml)</td>
<td>3.9 ± 1.5</td>
<td>6.2 ± 2.4</td>
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<tr>
<td>NPY (ng/ml)</td>
<td>2.8 ± 0.4</td>
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<tr>
<td>VIP (ng/ml)</td>
<td>3.6 ± 0.6</td>
<td>5.2 ± 1.7</td>
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CGRP, calcitonine gene-related peptide; CPT, conjunctival provocation test; NPY, neuropeptide Y; NSS, non statistically significant; VIP, vasoactive intestinal peptide.
Percentage of patients showing an increase of Neuropeptides levels in tears after specific CPT

VIP
N=14

CGRP
N=13

NPY
N=8

SP
N=11
CGRP positive nerve fibers in the subbasal plexus
Tear CGRP and NPY levels in patients with hay fever and dry eye

CGRP

- Healthy: N=8
- Hay Fever: N=12
- Dry Eye: N=13

NPY

- Healthy: N=8
- Hay Fever: N=5
- Dry Eye: N=13
Tear CGRP and NPY levels in patients with dry eye

**CGRP**

- **Autoimmune** (N=3): [Bar Graph]
- **Dry Eye** (N=6): [Bar Graph]
- **OCP** (N=4): [Bar Graph]

**NPY**

- **Autoimmune** (N=3): [Bar Graph]
- **Dry Eye** (N=6): [Bar Graph]
- **OCP** (N=4): [Bar Graph]
Conclusions

- Sensory neuropeptides are the main actors of neurogenic pain during ocular surface inflammation.

- Possible strategy for pain management at the (inflammed) ocular surface?
  - Depletion of neuropeptides: topically applied capsaicin has been successfully used to reduce periocular post-herpetic pain induced by substance P released by damaged sensory nerve terminals.
  - However, capsaicin may reduce corneal sensitivity to reduce inflammation to reduce pain...
Wide spectrum of anti-inflammatory drugs available

- Steroids: for chronic use loteprednol preferable (better IOP control)

- Ciclosporin-a: FDA-approved, not yet approved by EMA

- Tacrolimus: already used for uveitis, in clinical trials for dry eye

- NSAIDs (better if preservative free to reduce toxicity in chronic use)
To avoid ocular surface toxicity ➔ systemic antibiotics available

Macrolides: from in vitro anti-inflammatory and immunomodulatory properties to clinical practice

Azithromycin decreases MMP-9 expression in the airways of lung transplant recipients

Azithromycin fails to reduce inflammation in cystic fibrosis airway epithelial cells