# PACK-CXL

Cross-Linking for infectious keratitis

Emilio Torres-Netto, MD

<table>
<thead>
<tr>
<th>Cornea Surgery</th>
<th>Cataract Surgery</th>
<th>Refractive Surgery</th>
<th>PhD Candidate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Federal University of São Paulo, Brazil</td>
<td>ISCMSP, São Paulo, Brazil</td>
<td>Rothschild Fonddation Paris, France</td>
<td>ELZA Institute Zurich, Switzerland</td>
</tr>
<tr>
<td></td>
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<td>Lab. for Ocular Cell Biology University of Zurich, Switzerland</td>
</tr>
</tbody>
</table>
1. Background

Reasons

PACK CXL

- Antibiotic Resistance
- Health costs
- Independent Experience
- Less Scar ??

emilioatorres@me.com
1. Background

Reasons

PACK-CXL

Antibiotic Resistance

Health costs

Independent Experience

Less Scar ??
1. Background

A global problem

Antibiotic Resistance
1. Background

Number deaths from infectious causes

- North America: 317,000
- Europe: 390,000
- Africa: 4,150,000
- Latin America: 392,000
- Asia: 4,730,000
- Oceania: 22,000

Mortality per 10,000 population:
- 5
- 6
- 7
- 8
- 9
- 10
- >
Over the last 30 years, no major new types of antibiotics have been developed.
1. Background

Reasons for PACK-CXL

- Antibiotic Resistance
- Health costs
- Independent Experience
- Less Scar
1. Background

Reasons

PACK-CXL

- Antibiotic Resistance
- Health costs
- Independent Experience
- Less Scar ??
1. Background

**Scenario 1**
Developing Country

Difficult access (doctor/lab)

Health costs
1. Background

Scenario 2
Developed Country

CL low-compliance patients & self medication

Health costs

10%
I. Background

Health costs

Benefits

Compliance-independent approach

Current

PACK-CXL

1. Background

Health costs

Benefits

Compliance-independent approach

Current

PACK-CXL
1. Background

**Reasons**

**PACK-CXL**

- Antibiotic Resistance
- Health costs
- Independent Experience
- Less Scar ??
1. Background
Diagnostic dilemma, therapeutic dilemma
Major geographic variations in etiology

1. Background

<table>
<thead>
<tr>
<th>Table 1. Central Corneal Ulcers in the Developing World: Geographical Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of study</td>
</tr>
<tr>
<td>No. of ulcers</td>
</tr>
<tr>
<td>Culture positive (%)</td>
</tr>
<tr>
<td>Organisms cultured</td>
</tr>
<tr>
<td>Bacteria (%)</td>
</tr>
<tr>
<td>Most frequent pathogens*</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
</tr>
<tr>
<td>Staphylococcus spp.</td>
</tr>
<tr>
<td>Pseudomonas species</td>
</tr>
<tr>
<td>Other bacteria (%)</td>
</tr>
<tr>
<td>Fungi (%)</td>
</tr>
</tbody>
</table>

*Each species as a percentage of the total number of bacteria cultured.

Whitcher et al., Int Ophthalmol Clin 2002
1. Background

- Antibiotic Resistance
- Health costs
- Independent Experience
- Less Scar ??

Reasons

PACK CXL
1. Background

Reasons

PACK, CXL

- Antibiotic Resistance
- Health costs
- Independent Experience
- Less Scar ??

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PACK-CXL effects

1. Background

UV-A + riboflavin = “disinfectant”

Goodrich et al, 2000, Vox Sang
1. Background

PACK-CXL effects

1. Oxidative stress

2. Intercalation with DNA = stops replication

3. Steric hindrance = Increased resistance to digestion

UV-A + riboflavin = “disinfectant”

Goodrich et al, 2000, Vox Sang
1. Background

PACK-CXL effects

Less Scar ??

Steric hindrance = Increased resistance to digestion

Torres Netto, personal data
1. Background

Reasons

PACK CXL

Antibiotic Resistance

Health costs

Independent Experience

Less Scar ??

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1. Background

2. First Results

Post-LASIK keratitis

Ten days after PACK-CXL

Iseli et al, 2008, Cornea
1. Background

2. First Results

Laboratory

Staph aureus growth inhibition by 97% in 30 minutes
(Dresden keratoconus protocol)

Figure 1. Percent of growth inhibition of organisms with exposure to riboflavin, ultraviolet (UV) light, or combined riboflavin and UV light.
1. Background

2. First Results

Pot and Hafezi, Vet Ophthalmol, 2013

Mortensen et al., Vet Ophthalmol, 2013
No antibiotics

Before PACK-CXL

Two weeks after PACK-CXL

Makdoumi et al., Curr Eye Res, 2011
1. Background

2. First Results

3. Optimize

PACK-CXL

Optimize

- Spectrum
- Stage of Disease
- Fluorescein
- Accelerate?
# Kill bacteria and fungi simultaneously

## BACTERIA
- Up to 98% *in vitro*
- With fluence currently used in clinical setting

## FUNGI
- 60-70% *in vitro*
- With high fluence currently used in clinical setting (7.2 J/cm²)

<table>
<thead>
<tr>
<th></th>
<th>MSSA</th>
<th>MRSA</th>
<th>P. aeruginosa</th>
<th>S. epidermidis</th>
<th>C. albicans</th>
<th>Fusarium</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.4 J/cm²</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>(✓)</td>
<td>(✓)</td>
</tr>
<tr>
<td>98%</td>
<td>99%</td>
<td>98%</td>
<td>97%</td>
<td>60-70%</td>
<td>60-70%</td>
<td></td>
</tr>
</tbody>
</table>

*Schrier et al., IOVS, 2008*  
*Martins et al., IOVS, 2008*  
*Richoz et al., JRS, 2014*  
*Richoz et al., unpublished data*
Current state: Acanthamoeba and H. simplex

**Acanthamoeba**
- Cornea: mixed results
- *SODIS: in vitro* 99.9% killing of trophozoites and cysts possible with fluence of 540 J/cm²

**Herpes Simplex Virus**
- Cornea: no effect
- *SODIS: in vitro* killing possible with fluence of 800 J/cm²

Heaselgrave et al., *Appl Environ Microbiol*, 2010
1. Background

2. First Results

3. Optimize

PACK-CXL
Optimize

Spectrum

Stage of Disease

Fluorescein

Accelerate?

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PACK-CXL
Optimize

1. Background

2. First Results

3. Optimize

- Spectrum
- Stage of Disease
- Fluorescein
- Accelerate?
1. Background

2. First Results

3. Optimize

Infiltrate / Early ulcer?

Advanced ulcer?

Price et al., JRS, 2012

Said et al., Ophthalmology, 2014
Prospective clinical study
40 eyes. All stages of ulcer, 0-12 mm
Bacterial, fungal, mixed
Overall: 15% required additional treatment

EARLY ulcers alone: did not require additional treatment
Photoactivated Riboflavin Treatment of Infectious Keratitis Using Collagen Cross-linking Technology

Marianne O. Price, PhD; Lawrence R. Tenkman, MD; Amilia Schriber, MD; Kelly M. Fairchild, BS; Stephen L. Trokel, MD; Francis W. Price, Jr, MD
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JRS, 2012
Collagen Cross-Linking with Photoactivated Riboflavin (PACK-CXL) for the Treatment of Advanced Infectious Keratitis with Corneal Melting

Dalia G. Said, MD, FRCS,1, * Mohamed S. Elfawy, MSc,1, * Zois Garboula, MD, PhD, 2
Elsh S. El-Zahargy, PhD, 1 Mansour A. Hassam, MD, 2 Mohamed Y. Alif, MD, 3 Ahmed A. Zaki, MD, 1
Hammam S. Duc, MD, PhD, 4 Forhad Hajiri, MD, PhD, 3

Ophthalmology, 2014

- Prospective randomised clinical study
- 40 patients with therapy-resistant **END-stage** ulcer
- Bacterial, fungal, mixed
- Arm 1: Medication only, 19 eyes
  Arm 2: Medication plus CXL, 21 eyes
- **Healing time comparable**
Collagen Cross-Linking with Photoactivated Riboflavin (PACK-CXL) for the Treatment of Advanced Infectious Keratitis with Corneal Melting

Dalia G. Said, MD, FRCS,1,11 Mohamed S. Elalfy, MSc,1,10 Zein Girgidas, MD, PhD,2
Ehab S. El-Zarkak, PhD,1 Manasser A. Hassam, MD,3 Mohamed Y. Afify, MD,3 Ahmed A. Zalz, MD,1
Hamed E. Dua, MD, PhD,1 Farhad Hajie, MD, PhD,5

Ophthalmology, 2014

1. Background

2. First Results

3. Optimize
Treat Early!!

1. Background

2. First Results

3. Optimize

Price et al., JRS, 2012

Said et al., Ophthalmology, 2014
1. Background

2. First Results

3. Optimize

PACK-CXL

Optimize

Spectrum

Stage of Disease

Accelerate?

Fluorescein

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PACK-CXL

Optimize

Spectrum

Stage of Disease

Accelerate?

Fluorescein
Major variations in outcome
Major variations in outcome

Richoz and Hafezi, JRS, 2013
Major variations in outcome

Richoz and Hafezi, JRS, 2013
Major variations in outcome
1. Background

2. First Results

3. Optimize

PACK-CXL
Optimize

Spectrum

Stage of Disease

Fluorescein

Accelerate?
1. Background

2. First Results

3. Optimize

- Spectrum
- Stage of Disease
- Fluorescein
- Accelerate?

PACK-CXL

Optimize

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Accelerate: corneal stiffness

For keratoconus? No

Hammer et al, IOVS, 2014
Accelerate: bactericidal effect

Richoz et al, JRS, 2014
Accelerated Corneal Cross-Linking With Photoactivated Chromophore for Moderate Therapy-Resistant Infectious Keratitis

Boris Knyazev, MD,‡ Yoni Krakauer, MD,‡ Yael Baumfalk, MD,‡ Tova Lifshitz, MD,‡ Sabine Kling, PhD,‡ and Farhad Hafezi, MD, PhD,§∥

1. Background

2. First Results

3. Optimize

180 seconds @ 30 mW/cm²

Adjuvant to antibiotics

Cornea, 2018
Different pathways for CXL and PACK-CXL

<table>
<thead>
<tr>
<th></th>
<th>Bunson Roscoe</th>
<th>O2 dependency</th>
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<tbody>
<tr>
<td>CXL</td>
<td>![Graph](IOVS 2013)</td>
<td>![Graph](TVST 2014)</td>
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<tr>
<td>PACK-CXL</td>
<td>![Graph](JRS 2014)</td>
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Different pathways for CXL and PACK-CXL

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<tr>
<th>Bunson Roscoe</th>
<th>O₂ dependency</th>
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<tbody>
<tr>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Yes</td>
<td>No?</td>
</tr>
</tbody>
</table>

1. Background
2. First Results
3. Optimize
Swiss PACK-CXL multicenter trial
Purpose

To analyze the time to corneal epithelization with PACK-CXL as a first-line treatment in early infectious corneal ulcers, and compare it to the current standard of care, antimicrobial therapy.
Methods

Prospective, interventional, multicenter, randomized and controlled phase-III clinical trial

Infiltrates and early superficial ulcers up to 4mm diameter
(suspected bacterial, fungal, or mixed origin)

Arm 1
PACK-CXL only

total energy
up to 7.2J/cm²

*Ricrolin+ (Sooft, Italy)

Arm 2
Medication only
(current standard of care treatment)

randomization
Average Size (mm)  | 2.4  | 1.8  
p=0.1113

Epithelization (days)  | 12.3  | 6.8  
p=0.1225

Medication  | PACK-CXL

1. Background
2. First Results
3. Optimize
4. Multicenter Trial

2.
2.
3.
3.

1.
4.

Multicenter Trial

Staphylococcus aureus
Positive cultures
No antibiotics

DAY 0

DAY 28

PACK (57.4J/cm²)

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**BACTERIA**
The most commonly identified Gram-positive cocci

**FUNGI**
Five eyes filamentous keratitis (2P/3M) *Aspergillus sp*

<table>
<thead>
<tr>
<th></th>
<th>Medication only</th>
<th>PACK-CXL only</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Number</td>
<td>13</td>
<td>10</td>
<td>23</td>
</tr>
<tr>
<td>Excluded</td>
<td></td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Evaluated</td>
<td>12</td>
<td>8</td>
<td>20</td>
</tr>
</tbody>
</table>

**MEDICATION 11\textsuperscript{st} DAY**
*Streptococcus pneumoniae* (PERFORATION)

**PACK DAY 0 (7.2J/cm\textsuperscript{2})**
4mm fil. fungal keratitis (Antifungals from 4\textsuperscript{th} day)
2. First Results

• PACK-CXL followed by antimicrobial therapy might be an alternative for infectious corneal infiltrates and early corneal ulcers;

• Even with a tendency for a longer healing (non-significant difference of 5.5 days), 80% eyes treated with PACK-CXL healed without the use of antimicrobial therapy;

• Need an experimental study to show how to achieve maximal bacterial killing rates.
Laboratory results

Antimicrobial efficacy for different bacterial strains as a function of UV fluence and irradiated volume

S. Kling, F. Hufschmied, E. Torres-Netto, R. Zbinden, H. Hafezi
Laboratory results

Bacterial killing rate
as a function of bacterial strain and UV fluence

Lethality dose (LD) curve

Bacterial killing rate and Lethality dose (LD) curve as a function of bacterial strain and UV fluence.
Final Conclusions

• Addresses important issues of the future:
  
  *antibiotic resistance, access to health care, costs and compliance issues*

• **Accelerated** to 3 minutes, still having efficiency

• **Highly efficient** in bacteria and fungi

• Further studies to establish optimal treatment settings
THANK YOU