Riboflavin, oxygen, and light – CXL protocols

Eberhard Spoerl
Department of Ophthalmology
Univ. Hospital Carl Gustav Carus, TU Dresden, Germany
Several riboflavin solutions

<table>
<thead>
<tr>
<th>solution</th>
<th>effect</th>
<th>before irradiation</th>
<th>irradiance</th>
<th>during irradiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1% riboflavin+ 20% dextran</td>
<td>thinning of cornea</td>
<td>every 5 min</td>
<td>3mW/cm²</td>
<td>every 5 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1% riboflavin+ methylcellulose</td>
<td>unchanged cornea</td>
<td>every 2 min</td>
<td>&gt;9mW/cm²</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyposomolar riboflavin 0.1%</td>
<td>swelling of cornea</td>
<td>every 2min</td>
<td>3mW/cm² &lt;9mW/cm²</td>
<td>every 5 min no</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transepithelial ribo 0.25%, BAC0.01%</td>
<td>epi-on</td>
<td>6/24 min two-stage</td>
<td>&gt;9 mW/cm²</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iontophoresis-Ribofalvin 0.1%, hypo</td>
<td>Ricrolin+</td>
<td>5min</td>
<td>&gt;9 mW/cm²</td>
<td>no</td>
</tr>
</tbody>
</table>
Adaptation of Riboflavin

- 0.1% riboflavin + methylcellulose
- Hypoosmolar riboflavin
  - no dextran
  - thinnest point < 400µm
- Transepithelial riboflavin
  - BAC 0.01%
  - no dextran
- Iontophoresis
  - dextran-free
  - Ricrolin+
- 0.11% riboflavin
  - Accelerated CXL
Riboflavin film

Riboflavin solutions of different viscosity form different thick films at the surface and show different break-up times.

<table>
<thead>
<tr>
<th>solution</th>
<th>Film thickness</th>
<th>Break-up time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextran 20%</td>
<td>70 µm</td>
<td>20 min</td>
</tr>
<tr>
<td>Methylcellulose 1.7%</td>
<td>100-300 µm</td>
<td>20 min</td>
</tr>
<tr>
<td>Methylcellulose 1%</td>
<td>60µm</td>
<td></td>
</tr>
<tr>
<td>hypo</td>
<td>40</td>
<td>2 min</td>
</tr>
</tbody>
</table>

- Thickness of riboflavin is related to the viscosity.
- Removal of film before irradiation.
- No dropping during the irradiation (A lot of UV would be absorbed in the riboflavin film if we would drop every 2-3 min.
- Also the oxygen penetration is not impaired.

Wollensak G, Significance of the riboflavin film in corneal collagen crosslinking. JCRS 2010;36:114-20
Riboflavin is unstable under illumination with light, it converts to lumiflavin and lumichrome.

Reduction of absorption coefficient by 3 - 4% in 5 min

Only a small fraction of the overall riboflavin molecules break down.


Bleaching and accelerated CXL

- During ACXL we do not drop riboflavin
- UV-intensity at the endothelial cells would be higher

\[
\frac{l_{accel}}{l_0} = \left(\frac{l_{stand}}{l_0}\right)^{0.8}
\]

\[
l_{accel} = 0.32 \text{ mW/cm}^2
\]

Toxic threshold \( l_t = 0.35 \text{ mW/cm}^2 \)

- Compensation of bleaching by increasing the riboflavin concentration to 0.11%
- Safety of endothelial cells is higher
Application of riboflavin

- During application of riboflavin the light intensity should be low (mesopic condition).
- Riboflavin should be stored in dark.

Absorption coefficient of fresh riboflavin and one day in the lab

100% 86%
Oxygen

Oxygen was also removed from the tissue.

Richoz O, The biomechanical effect of corneal collagen cross-linking (CXL) with riboflavin and UV-A is oxygen dependent. Trans Vis Sci Tech. 2013;2(7):6,
Oxygen

- Effective cross-linking requires the presence of oxygen.
- Oxygen partial pressure 155 mmHg

- A rigid semiscleral contact lens PMMA was sutured tightly over the cornea during CXL. The demarcation line was seen also in the reshaping group.
  (Beckman J. Initial results from mechanical compression of the cornea during crosslinking for keratoconus. Acta Ophthalmol 2014)

- A higher oxygen concentration (760 mmHg) in front of the cornea does not lead to higher stiffness (Spoerl, 2014)
Fractionated (pulsed) CXL

Krueger RR, First proposed efficacy study of high versus standard irradiance and fractionated riboflavin/UVA crosslinking with equivalent energy expose. Eye Contact Lens. 2014 Nov;40(6):353-7
Accelerated CXL

Biomechanical effect of CXL decreases significantly with higher irradiances?

Is this clinical relevant?

Wernli J. The effect of CXL shows a sudden decrease with very high intensity UV-light and short treatment time. IOVS 2013

Demarcation line

due to change in refractive index.

Standard CXL

3 mW/cm² 30 min

247±45 µm in ectasia

294±51 µm in keratoconus

(Yam J. Demarcation line depth after CXL for keratoconus and corneal ectasia. JRS 2012)
## Depth of demarcation line

<table>
<thead>
<tr>
<th>Method</th>
<th>Depth in µm</th>
<th>Irradiance</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard CXL</td>
<td>350.8±49.3</td>
<td>3 mW/cm²</td>
<td>30 min</td>
</tr>
<tr>
<td></td>
<td>294.2±51.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accelerated CXL</td>
<td>288.5±42.4</td>
<td>9 mW/cm²</td>
<td>10 min</td>
</tr>
<tr>
<td></td>
<td>160 (150-180)</td>
<td>30 mW/cm²</td>
<td>4 min</td>
</tr>
<tr>
<td>Pulsed CXL</td>
<td>200 (190-215)</td>
<td>30 mW/cm²</td>
<td>8 min</td>
</tr>
<tr>
<td>Hypoosmolar Riboflavin</td>
<td>262.9±45.6</td>
<td>3 mW/cm²</td>
<td>30 min</td>
</tr>
<tr>
<td>Transepithelial CXL (BAC)</td>
<td>150</td>
<td>3 mW/cm²</td>
<td>30 min</td>
</tr>
<tr>
<td>Iontophoresis CXL</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Demarcation line and irradiance

![Graph showing the relationship between depth of demarcation line in μm and irradiance in mW/cm². Key points include:
- S-CXL: black square
- Accelerated CXL: black square
- hypo Ribo ectasia: blue square
- CA-CXL: orange square
- Ionto-CXL: red square
- Pulsed CXL: green square
- TE-CXL(BAC): pink square.
- Irradiance values: 6.3 J/cm² and 5.4 J/cm².](image)
Demarcation line and swelling

![Graph showing the relationship between depth of demarcation line in μm and the ratio of swollen/deswollen thickness to original thickness.

- The graph indicates a decreasing trend as the ratio increases.
- The x-axis represents the ratio of swollen/deswollen thickness to original thickness.
- The y-axis represents the depth of the demarcation line in μm.

Key points:
- Swelling (hypoosmolar solution)
- Deswelling (dextran solution)
Summary

CXL is not a rigid procedure, it is under development and modifications are part of this development.

Carefull evaluation of efficacy and safety of this modifications by means of
- several methods and
- several study groups

should be done.
Thank you very much for your attention!