USE OF MITOMYCIN C in REFRACTIVE SURGERY

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Introduction

• Old PRK (photorefractive keratectomy):
  – SURGICAL TECHNIQUE:
    • Mechanical, chemical or laser deepithelialization
    • Laser ablation over Bowman and anterior stroma
    • No epithelial repositioning
  – ADVANTAGES:
    • Safe and stable (10-12 years)

Introduction

• Old PRK:
  – DISADVANTAGES:
    • Uncomfortable:
      – Slow visual recovery
      – Postoperative pain
    • Limited in the ttt of moderate to high myopia:
      – Haze and tendency to regression

Introduction

- **Haze:**
  - Decrease in corneal transparency
  - Histopathologic substrate:
    - Myofibroblasts: more light scatter
    - Extracellular matrix more dense and disorganized

Dawson DG et al. AJO 2005;139:168-78.
ADVANCED SURFACE ABLATION

- ADVANCED SURFACE ABLATION (ASA)
  - OBJECTIVE: to take advantage of PRK’s safety, but diminishing its disadvantages, (haze).
  - TECHNIQUES:
    - PRK
    - LASEK (laser subepithelial keratectomy)
    - Epi-LASIK (epipolis-LASIK)
  - All of them with the use of MMC
MMC: mechanism of action

- Activated by cytochrome p450 reductase
- Mechanism of action:
  - **Antiproliferative**: covalent union to DNA
  - **Cytotoxic**:
    - Upregulation of IL-8 and MCP-1
    - Fas-mediated apoptosis
    - Mitochondrial dysfunction
    - T-lymphocytes mediated cell lysis
    - Reactive oxygen radicals
    - Amplification of TNF


Introduction

• Corneal wound healing response:
  – Keratocyte apoptosis
  – Keratocyte activation and migration (bone marrow-derived cells)
  – Myofibroblast differentiation
  – New collagen and extracellular matrix, more dense and disorganized
  – Result in hypercellular fibrotic scar: with epithelial hyperplasia: regression

Barbosa et al. Exp Eye Res 2010
MMC: mechanism of action

- Effects over the ablated stroma:
  - First hours: increases keratocyte apoptosis (*cytotoxic effect*)
  - 24h: less keratocyte repopulation (*antimitotic effect*)
  - 4 weeks: lower density of keratocytes and myofibroblasts, less deposit of collagen and extracellular matrix: less haze and regression

First uses in refractive surgery

• Animal studies:
  – Less subepithelial collagen deposit, less scarring, lower keratocyte density

• Clinical studies:
  – Majmudar et al.: MMC improved transparency in corneas with haze
  – Carones et al.: MMC reduced the incidence and severity of haze after surface ablation for high myopia

Current uses in refractive surgery

- **Ttt of previous haze**: 0.02% x 2 min
- **Prophylactic use in primary ASA**:
  - Less haze, better visual and refractive results
  - ASA+MMC similar results to LASIK when treating high myopia
  - Undercorrection of aprox 10% of sphere

Teus MA, de Benito-Llopis L, Alio JL. Survey 2009;54:487-02

Prophylactic MMC

- **Prophylactic use in primary ASA:**
  - “Deep” myopic ablations:
    - >-6.00 D
    - Ablation depth > 50 or 75 or 100 µm
    - Ablation depth / corneal thickness ≥0.18
  - Hyperopic treatments:
    - >+2D: Better efficacy and predictability with MMC

Nagy ZZ et al. Surv Ophthalmol 1997;42:S64-76
Stojanovic A et al. JCRS 2001;27:404-10
Netto MV et al. JRS 2006;22:562-74
Mirza MA et al. JCRS 2004;30:709-14
Lin N et al. JRS 2004;20:797-802
Leccisotti A. JCRS 2009;35:682-7
Prophylactic MMC

• In ASA after previous corneal surgeries:
  – After RK, PK and DALK
  – To treat complications post-LASIK: striae, buttonholes, incomplete flaps
  – To treat refractive errors after previous uncomplicated LASIK?

Azar DT et al. JCRS 1998;24:303-11
Ribeiro JC et al. JRS 1995;11:165-9
La Tegola MG et al. JRS 2007;23:681-93
Nassaralla BA et al. JRS 2007;23:226-32
Solomon R et al. Eye Contact Lens 2004;30:156-8
Leccisotti Cornea 2008;27:417-20
Barreto JRS 2009;25:s131-5
Chalita MR et al. JRS 2004;20:176-81
Lane HA et al. JCRS 2003;29:390-2
Müller LT et al. JCRS 2005;31:291-6
Taneri S et al. JCRS 2005;31:2026-30
Alió JL et al. JCRS 2008;34:1727-35
Kuo IC et al. JCRS 2008;34:330-3
Prophylactic MMC

- **Dose:** MMC 0.002%:
  - More haze compared to 0.02% MMC:
    - Esp. in high myopia (>6 D) and deep ablation (>75 µm)
  - Some cases of mod-severe haze with 0.002% MMC

Camellin M. JRS 2004;20:S693-8
Netto MV et al. JRS 2006;22:562-74
Sadeghi HM et al. JRS 1998;14:534-40
Thornton I et al. AJO 2007;144:673-81
Thornton I et al. JRS 2008;24:S68-76
Prophylactic MMC

- **Exposure time:**
  - 0.02% for 12 sec-1 min
  - Virasch et al.: no dif in BSCVA and haze between 12 sec, 1 and 2 min.
  - Changes in exposure time have less impact on the absorption of MMC than changes in the concentration

References:

Teus MA, de Benito-Llopis L, Alio JL. Survey 2009;54:487-02
Camellin M. JRS 2004;20:S693-8
Netto MV et al. JRS 2006;22:562-74
Rajan MS et al. JCRS 2006;32:1741-7
Sadeghi HM et al. JRS 1998;14:534-40
Thornton I et al. AJO 2007;144:673-81
Thornton I et al. JRS 2008;24:S68-76
Argento C et al. JRS 2006;22:782-6
Lee DH et al. JCRS 2005;31:2293-8
Song JS et al. Cornea 2006;25:S20-3
Song JS et al. Cornea 2007;26:461-7
MMC in LASEK

- Currently, the criteria to apply MMC during LASEK are the same as in PRK

HOWEVER

- Ethanol obtained epith flap + replacement of the flap seem to lead to lower haze

- Synergy of MMC and ethanol: increased initial keratocyte apoptosis and lower keratocyte density 4 weeks postop

- Maybe less MMC needed in LASEK?

Chang SW, et al. JRS 2008;24:150-9
Shah S, et al. BJO 2001;85:393-6
Kim Ti et al. IOVS 2004;45:86-92
Netto MV et al. JRS 2008;24:626-32
Chen S et al. JRS 2011;17:1-12
Application of the MMC

• Several methods:
  – Round cellulose sponge 7-9 mm diameter
  – Ring of cellulose sponge
  – Brushstroke with a piece of cellulose sponge

Camellin M. JRS 2004;20:S693-8
Jain S et al. Cornea 2001;20:45-9
Khoury JM et al. JRS 2007;23:716-9
Maldonado MJ. Ophthalmology 2002;109:826-8
MMC safety

- No complications similar to those during pterygium and glaucoma surgeries: vascular endothelial injury
  - Sclera and conjunctiva: richly vascularized
  - Cornea: avascular: direct effect on:
    - epithelium
    - keratocytes
    - endothelium

Smith S et al. AJO 1994;118:332-7
Lee DH et al. JCRS 2005;31:2293-8
MMC - epithelium

- **Effect on epithelium:**
  - Delay in re-epithelialization?
  - Persistent epithelial problems?

- **Animal studies: variable results**
  - Dose-dependant delay in one study
  - No delay in another study

- **Human corneas in vitro:**
  - Delay in the latency until re-epith started
  - *No diff in epith migration rate*

Chang SW. *JCRS* 2004;30:1742-50
Rajan MS et al. *JCRS* 2006;32:1741-7
## MMC - epithelium

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment Details</th>
<th>Time (sec)</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argento et al.</td>
<td>30 MMC-treated 28 controls</td>
<td>75 sec</td>
<td>No difference in re-epithelialization</td>
</tr>
<tr>
<td>Camellin</td>
<td>86 MMC-treated 100 controls</td>
<td>Brushstroke</td>
<td></td>
</tr>
<tr>
<td>Carones et al.</td>
<td>30 MMC-treated 30 controls</td>
<td>30 sec</td>
<td></td>
</tr>
<tr>
<td>Gambato et al.</td>
<td>36 MMC-treated 36 controls</td>
<td>2 min</td>
<td></td>
</tr>
<tr>
<td>Leccisotti</td>
<td>52 MMC-treated 52 controls</td>
<td>45 sec</td>
<td></td>
</tr>
<tr>
<td>Vigo et al.</td>
<td>35 eyes</td>
<td>2 min</td>
<td>No epithelial toxicity reported</td>
</tr>
<tr>
<td>Lee et al.</td>
<td>1011 eyes</td>
<td>30 sec- 2 min</td>
<td>Delay in only 2 eyes (0.19%)</td>
</tr>
</tbody>
</table>

References:
- Argento C et al. JRS 2006;22:782-6
- Camellin M. JCRS 2004;20:S693-8
- Carones F et al. JCRS 2002;28:2088-95
- Kymionis GD et al. JRS 2006;22:511-3
- Leccisotti A. Cornea 2008;27:288-91
- Lee DH et al. JCRS 2005;31:2293-8
- Vigo L et al. JRS 2003;9:449-54
MMC - epithelium

- Pattern of hyperplasia altered by the MMC?
  - In human corneas in vitro:
    - epithelial layer thinner and less differentiated 1 month after a 2 min application
    - no diff with controls if applied 1 min
  - Clinical studies:
    - No clear differences in epith morphology
    - No diff in the increase of CCT
    - No diff in the epithelial thickness 5 years after the surgery

Dawson DG et al. AJO 2005;139:168-78
Rajan MS et al. JCRS 2006;32:1741-7
Chen WL et al. IOVS 2008;49:2416-23
Teus MA et al. EVER meeting, Portoroz, Slovenia, October 3-6th 2007
MMC-corneal nerves

- No difference in the number and characteristics of corneal nerves after MMC compared to no-MMC
- Higher nerve density in the MMC-treated eyes compared to the non-treated fellow eye

MMC - keratocytes

- **Effects on the stromal population:**
  - Anti-haze mechanism
  - Long-term keratocyte depletion in the stromal bed?

Teus MA, de Benito-Llopis L, Alio JL. Survey 2009;54:487-02
Netto MV et al. JRS 2006;22:562-74
Rajan MS et al. JCRS 2006;32:1741-7
MMC - keratocytes

- No agreement regarding the effect of surface ablation without MMC on keratocyte population:
  - No decrease
  - Depletion even 5 years postop

Dawson DG et al. AJO 2005;139:168-78
Rajan MS et al. JCRS 2005;31:1789-1801
Erie JC et al. AJO 2006;141:799-809
# MMC - keratocytes

<table>
<thead>
<tr>
<th>Study</th>
<th>Number of Eyes</th>
<th>Study Type</th>
<th>Duration</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kim et al.</td>
<td>18 eyes</td>
<td>Animal studies (rabbits)</td>
<td>3 months</td>
<td>Decreased</td>
</tr>
<tr>
<td>Netto et al.</td>
<td>182 eyes</td>
<td>Animal studies (rabbits)</td>
<td>1 month</td>
<td>Non-decreased (data not available)</td>
</tr>
<tr>
<td>Rajan et al.</td>
<td>16 eyes</td>
<td>Laboratory study (human corneas in vitro)</td>
<td>1 month</td>
<td>Non-decreased (data from Gambato et al. not available)</td>
</tr>
<tr>
<td>Xu et al.</td>
<td>20 eyes</td>
<td>Animal study (rabbits)</td>
<td>6 months</td>
<td>Non-decreased</td>
</tr>
<tr>
<td>Gambato et al.</td>
<td>36 eyes</td>
<td>Clinical studies</td>
<td>3 years</td>
<td>No difference compared to eyes without MMC</td>
</tr>
<tr>
<td>Midena et al.</td>
<td>56 eyes</td>
<td>Clinical studies</td>
<td>5 years</td>
<td>No difference compared to eyes without MMC</td>
</tr>
</tbody>
</table>

**References:**
- Netto MV et al. JRS 2006;22:562-74
- Rajan MS et al. JCRS 2006;32:1741-7
- Xu H et al. JRS 2001;17:342-9
- Midena E et al. JRS 2007;23:S1011-4
Material and methods

- Retrospective study of consecutive cases
- Myopic eyes treated with LASEK + intraop 0.02% MMC 30"
- Confocal keratocyte density (HRTII-Rostock Cornea Module)
  - 3 months, 15 months and 3 years postop
- Densities in the ant, mid (200µm above endoth) and post stroma (50µm above endoth) were compared with non-treated healthy eyes

De Benito-Llopis L et al. AJO 2010;150:642-9
De Benito-Llopis L et al. AJO 2011;in press
<table>
<thead>
<tr>
<th>Keratocyte density (Cells/mm³)</th>
<th>CONTROL n=32</th>
<th>LASEK+MMC n=32</th>
<th>LASEK+MMC n=27</th>
<th>P = 0.0001</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3 months</td>
<td>3 years</td>
<td></td>
</tr>
<tr>
<td>Stromal bed</td>
<td></td>
<td></td>
<td></td>
<td>Controls vs 3m / 3y</td>
</tr>
<tr>
<td>29,660.71 ± 5,904.80</td>
<td>16,993.75 ± 8,001.78</td>
<td>15,662.2 ± 2,839.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mid-stroma</td>
<td></td>
<td></td>
<td></td>
<td>Controls vs 3m 3m vs 3y</td>
</tr>
<tr>
<td>18,505.95 ± 1,996.06</td>
<td>30,783.33 ± 9,300.00</td>
<td>18,889.5 ± 3,474.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posterior stroma</td>
<td></td>
<td></td>
<td></td>
<td>Controls vs 3m 3m vs 3y</td>
</tr>
<tr>
<td>18,438.09 ± 2,139.68</td>
<td>30,268.75 ± 8,321.95</td>
<td>18,992.6 ± 3,402.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average (ant-mid-post)</td>
<td></td>
<td></td>
<td></td>
<td>Controls vs 3m/3y 3m vs 3y</td>
</tr>
<tr>
<td>22,201.58 ± 2,730.72</td>
<td>26,015.27 ± 7,193.52</td>
<td>17,848.16 ± 2,653.06</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Keratocytes

![Graph showing mean keratocyte density (cells/mm³) over different time periods (controls, 3 months, 15 months, 3 years) and stromal regions (Stromal bed, Mid stroma, Post stroma, Average).]
<table>
<thead>
<tr>
<th>Keratocyte density (Cells/mm³)</th>
<th>CONTROL n= 32</th>
<th>LASEK+MMC n=27 3 years</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 µm</td>
<td>21,668.19 ± 3,037.24</td>
<td>17,699.06 ± 2,530.57</td>
<td>0.0001</td>
</tr>
<tr>
<td>75 µm</td>
<td>21,155.47 ± 2,144.53</td>
<td>19,179.06 ± 2,653.87</td>
<td>0.003</td>
</tr>
<tr>
<td>Average (25-75 µm)</td>
<td>21,411.83 ± 1,917.69</td>
<td>18,439.06 ± 2,278.01</td>
<td>0.0001</td>
</tr>
<tr>
<td>Total average</td>
<td>19,451.96 ± 1,533.21</td>
<td>18,738.03 ± 2,555.77</td>
<td>0.2</td>
</tr>
</tbody>
</table>
Keratocytes - anterior stroma
Discussion

- Increase 3 months postop in mid and post stroma density previously described after LASIK

- Possible explanations:
  - Mitotic activity of the keratocytes of the underlying corneal layers
  - Repopulation by bone marrow-derived cells

Perez-Gomez et al. Optom Vis Sci 2003;80:690-7
Barbosa et al. Exp Eye Res 2010
MMC - keratocytes

- Surface ablation with intraop MMC does not seem to lead to permanent keratocyte depletion, but to a redistribution of the cell density in different corneal layers:
  1. temporary decrease in the anterior stromal cells, compensated with an increase in deeper layers,
  2. normalisation of stromal cell density throughout the cornea, despite lower density in the stromal bed

De Benito-Llopis L et al. AJO 2010;150:642-9
De Benito-Llopis L et al. AJO 2011;in press
MMC - endothelium

• Direct application of MMC on the endothelium:
  – No changes with 0.01%
  – Rapid toxicity with 0.02%

• MMC in the AC after MMC 0.02% 2min over the cornea: <0.002%, suggesting no toxicity

• But even such small concentrations caused cross-linking and double-strand breaks of corneal endothelial DNA in goats

Torres RM, et al. JCRS 2006;32:67-71
<table>
<thead>
<tr>
<th>Study</th>
<th>Eyes</th>
<th>Follow-up (months)</th>
<th>Endothelial cell density</th>
<th>Endothelial cell morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>De Benito-Llopis, Teus et al.</td>
<td>48</td>
<td>3</td>
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<tr>
<td>Diakonis et al.</td>
<td>15</td>
<td>12</td>
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<td>Non-decreased</td>
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<td>Gambbato et al.</td>
<td>28</td>
<td>5 years</td>
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<tr>
<td>Goldsberry et al.</td>
<td>16</td>
<td>12-24</td>
<td></td>
<td>Non-decreased</td>
</tr>
<tr>
<td>Leccisotti</td>
<td>52</td>
<td>12</td>
<td></td>
<td>Unchanged</td>
</tr>
<tr>
<td>Lee et al.</td>
<td>359</td>
<td>3</td>
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<td>Unchanged</td>
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<td>Nassaralla et al.</td>
<td>22</td>
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<td>Unchanged</td>
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<tr>
<td>Wallau et al.</td>
<td>44</td>
<td>6</td>
<td></td>
<td>Unchanged</td>
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<tr>
<td>Zhao et al.</td>
<td>174</td>
<td>6</td>
<td></td>
<td>Unchanged</td>
</tr>
<tr>
<td>Morales et al.</td>
<td>9</td>
<td>3</td>
<td></td>
<td>Decreased</td>
</tr>
<tr>
<td>Nassiri et al.</td>
<td>76</td>
<td>6</td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>
MMC - ciliary body

• MMC applied over the corneal stroma (rabbits):
  – No diff in IOP
  – No morphological changes in the ciliary body

Kymionis GD, et al. JRS 2008;24:633-8
Safety: conclusions

- **MMC-EPI THELIUM**: using MMC does not seem to cause a clinically significant delay in corneal reepithelialization or in final epithelial thickness.

- **MMC-KERATOCYTES**: the ability of the cells to repopulate the cornea seems to be maintained after the use of MMC.

- **MMC-ENDOTHELIUM**: a single intraoperative application of MMC 0.02% after laser surface ablation does not seem to cause a substantial change in corneal endothelial cell density. Effect on the long-term viability of corneal endothelium needs investigation.
Other methods to avoid haze

• Cooling of the surface at the end of the surgery: might help to control pain, but no evidence for haze
• Ciclosporine A: no effect.
• Corticosteroids: only effect in the early postop corneal haze, but no long-term effect
• Ascorbate (vit. C) p.o.: might decrease UV-activation of keratocytes. 1gr/d 1month postop.
• Smooth ablation profiles.

Nien CJ et al. JCRS 2011;37:937-44
Stojanovic A et al. JRS 2003;338-43
Vinciguerra P et al. JRS 1998;14:S204-6