Rho-kinase inhibitors and corneal transplantation

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Rho-associated coiled-coil protein kinase
(ROCK 1 & ROCK 2)

What’s the fuss about Rho-kinase in glaucoma and corneal disease?

A new approach to glaucoma and corneal diseases?

Rho Kinases in Health and Disease: From Basic Science to Translational Research

Garance Lalande, MD, PhD, Saleh Al-Abbad, MD, New York City

"Rho-associated kinases ROCK1 and ROCK2 are key regulators of actin cytoskeleton dynamics downstream of Rho GTPases that participate in the control of important physiologic functions, including cell contraction, migration, proliferation, adhesion, and inflammation."

The difficult bit – the ROCK pathways

Figure 2. ROCK targets. Rho proteins can be activated by guanine nucleotide exchange factors (GEFs), which are themselves activated by receptor tyrosine kinases (RTKs), G protein coupled receptors (GPCRs), cytokines and integrins.

Rho-GTP subsequently activates ROCK1 and ROCK2 that have a broad range of substrates and are responsible for diverse cellular responses.

ROCK1 and ROCK2 Cellular Targets

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Therapeutic potential of ROCK inhibitors

- Potential therapeutic applicability in wide variety of conditions including:
  - asthma, cancer, erectile dysfunction, glaucoma, insulin resistance, kidney failure, neuronal degeneration, and osteoporosis.
- More than 170 ROCK inhibitors have been developed
  - Two ROCK inhibitors approved for clinical use in Japan (Fasudil and Ripasudil) and one in China (Fasudil)
  - 1995: Fasudil was approved for the treatment of cerebral vasospasm,
  - 2014: Ripasudil was approved for the treatment of glaucoma

Actions of ROCK inhibitors in Glaucoma

Advantage of ROCK inhibitors is that IOP reduction is achieved by four or five different mechanisms:

- Increased outflow via trabecular meshwork
- Increased outflow via the uveo-scleral pathway
- Decreased aqueous production
- Reduced episcleral venous pressure
- Possible neuroprotective role

Major limitation is high incidence of ocular hyperemia which can affect the patients’ persistence and adherence.

ROCK inhibitors in glaucoma practice: effectiveness

ROCK inhibitors typically produce IOP reduction comparable to Prostaglandins

ROCK inhibitors also work successfully in combination with PGA & Beta-blockers:
IOP reduction 9-12mm Hg in a combination of Rho-kinase Inhibitor and prostaglandin analogue

Most common side effect of Rx is ocular hyperemia:
Incidence of up to 65% in clinical trials
Once-daily dosing at night minimizes this to 11%

Glaucma drugs and the corneal endothelium

The human corneal endothelium

- Innermost layer of the cornea, separating stroma from aqueous humor
- A non-replication hexagonal monolayer of flat cells of ~5 μm thickness on Descemet’s membrane
- Highly metabolic essential for corneal clarity
- Core roles in corneal homeostasis
  - passage of nutrients and metabolites
  - control of stromal hydration

Corneal endothelial cells and ageing

Graph 1. \[ \text{Graph showing data on corneal endothelial cell density decreasing with age in emmetropic eyes} \]

Corneal endothelial cell density decreases with age in emmetropic eyes

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Treating advanced corneal endothelial disease
Currently only definitive treatment is transplantation

Five most common Indications for keratoplasty 1991-2015 (N=5043)


NZNEB: Indications for keratoplasty the rise & rise of regraft 1991-2019

Indications for repeat keratoplasty

Repeat keratoplasty Indication N=279 Percent
Endothelial decompensation without history of rejection 105 37.6%
Endothelial decompensation with history of rejection 88 31.5%
Recurrent ectasia or high astigmatism in keratoplasty 44 15.8%
Acute infection 11 3.9%
Acute trauma 10 3.6%
Corneal scar 10 3.6%
Primary graft failure 62 2.2%
Other 51 1.8%

Most common keratoplasty techniques 1991-2019

DMEK vs DSAEK for corneal endothelial failure (Cochrane Systematic Review 2018)

- DMEK may be associated with more early surgical complications
- Graft dislocation in 1-2% in DSAEK and x5 more with DMEK
- DMEK may result in better vision compared with DSAEK
  - (low-certainty evidence). This difference is equivalent to 1-2 lines.
- Endothelial density after surgery found inconsistent result
- Almost everyone in reported studies had good graft survival

Emerging superiority of DMEK?

<table>
<thead>
<tr>
<th>AAO review</th>
<th>DSEK</th>
<th>DMEK</th>
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</thead>
<tbody>
<tr>
<td>BCVA ≥ 20/25</td>
<td>9% - 32%</td>
<td>50% - 55%</td>
</tr>
<tr>
<td>Immune rejection</td>
<td>10%</td>
<td>1.9%</td>
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<tr>
<td>Visual recovery</td>
<td>Slower</td>
<td>Faster</td>
</tr>
<tr>
<td>Primary failure</td>
<td>5%</td>
<td>1.9%</td>
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<tr>
<td>EC loss @ 6 months</td>
<td>37%</td>
<td>33%</td>
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<tr>
<td>Secondary failure</td>
<td>4%</td>
<td>2.2%</td>
</tr>
<tr>
<td>Re-bubble</td>
<td>14%</td>
<td>28.8%</td>
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ROCK Inhibitor therapy of damaged corneal endothelium

ROCK inhibitor Y-27632 eye drops promoted recovery of cell density in a corneal-endothelial partially cryo-damaged primate model.

(A) Slit-lamp microscopy Y-27632–treated and – non-treated recovered transparency at 1/12.

(B) Control group, specular microscopy shows enlarged corneal endothelium & 1500 cells/mm²

Y-27632–treated normal cells 3000 cells/mm²

(C) Specular microscopy analysis revealed CEC density significantly higher in Y-27632–treated group than controls throughout the 4-week (*P < 0.01).

Clinical trial of ROCK inhibitor Y-27632 eye drops for treating human central corneal oedema and diffuse corneal oedema (N=7) 2013
The future of Rho-kinase in treating corneal endothelial disease

Roles to be confirmed by further study

- Slowing progression of Fuchs endothelial dystrophy
- Preventing endothelial damage intra/post-operatively
- Augmenting DSAEK or DMEK endothelial grafts
- Augmenting Descemet’s stripping surgery (DWEK)
- As an additive to cellular endothelial transplant

Future modifications and alternatives to established endothelial keratoplasty

1. Use of Rho-Kinase (ROCK) inhibitors
2. Small diameter Descemet’s stripping (DWEK)
3. 1 donor → split cornea, hemi/quarter DMEK
4. Descemet’s membrane endothelial transfer
5. Tissue engineered grafts
6. Endothelial Cell therapy
7. Stem cell therapy


Cultured endothelial cells

- Cultured human ECs, 1 donor can treat many patients
  - Minimally invasive
  - Poor cell attachment when injected
  - Removed by aqueous humour drainage
- Clinical trial (n=11) of bullous keratopathy + ECs cultured in Y-27632 medium
  - ECs mechanically scraped off
  - Attachment after cell injection
  - Corneal transparency ↑, Corneal thickness and Visual acuity @24 weeks

THE TAKE HOME POINTS

- Rho-kinase is ubiquitous throughout the body
- Rho-kinase activation may have role in Cardiovascular & Central Nervous System disease and glaucoma
- Over 20 years >200 Rho-kinase inhibitor molecules developed but only two drugs currently licenced
- Developing role in glaucoma and corneal disease
- Further clinical studies awaited

Rho Rho Rho Rho the corneal revolution?

Thank you