Rho-kinase inhibitors and corneal transplantation

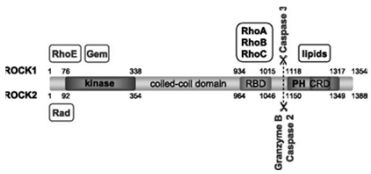
Professor Charles NJ McGhee
ONZM FRSNZ PhD DSc FRCS FRCOphth FRANZCO

Professor and Chair of Ophthalmology,
Director, New Zealand National Eye Centre
Faculty of Medical & Health Sciences
University of Auckland

Consultant, Auckland District Health Board
Consultant, Eye Institute, Remuera, Auckland

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

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



nz national eye centre

ASPET | PHARMACOLOGICAL REVIEWS

search
Advanced Search

Home | Articles | Information | About Us | Editorial Board | Alerts

Review Article | Review Article

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

Gervaise Loirand
Rhian M. Touyz, ASSOCIATE EDITOR

Pharmacological Reviews October 2015, 67 (4) 1074-1095; DOI: https://doi.org/10.1124/pr.115.010595

"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."

REVIEW ophthalmology

A new approach to glaucoma and **corneal dis**eases?

Lama Al-Aswad, MD, New York City

PUBLISHED 20 MARCH 2009

Rho-kinase Approach

These drugs may help

Rho-associated kinase inhibitors: a novel glaucoma therapy.

Rho-associated kinase inhibitor eye drop treatment as a possible medical treatment for Fuchs corneal dystrophy.

REVIEW OPHTHALMOLOGY — HOME | CE | TOPICS | EVENTS

Published February 15, 2016

ROCKin' a New Treatment Option

Rho-kinase inhibition may soon help glaucoma and some cornea patients. Here's what it does.

By Andrew Dixon, OD, and Andrew S. Gurwood, OD

As primary eye care practitioners working with a rapidly aging population, our role in glaucoma management is expanding. Unfortunately for patients and practitioners, the pathogenesis of glaucoma is not yet fully understood. A multitude of causal mechanisms have been proposed, including: low cerebrospinal fluid pressure leading to laminar cribrosa non-support, impaired

The difficult bit – the ROCK pathways

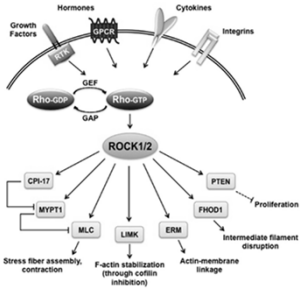
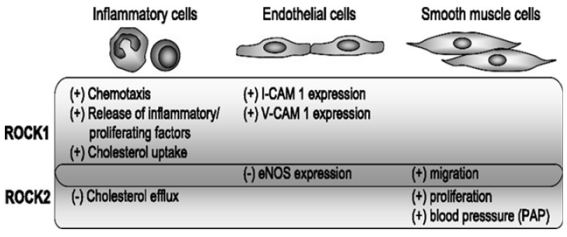


Figure 2. ROCK targets. Rho proteins can be activated by guanine nucleotide exchange factors (GEFs), which are themselves activated by receptor tyrosine kinases (RTKs), G protein coupled receptors (GPCRs), cytokines and integrins.

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

CIP-17, kinase C-potentiated phosphatase inhibitor of 17 kDa; ERM, ezrin-radixin-moesin; FHOD1, formin homology 2 domain-containing 1; GAP, GTPase activating protein; LIMK, LIM-kinase; MLC, myosin II regulatory light chain; MYPT1, myosin phosphatase target subunit 1; PTEN, phosphatase and tensin homolog deleted on chromosome 10

ROCK 1 and ROCK 2 Cellular Targets



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

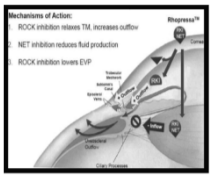
nz national eye centre

Rho Kinase (ROCK) Inhibitors and Their Therapeutic Potential.
Feng Y, LoGrasso PV, Defert O, Li R J Med Chem. **2016*** Mar 24;59(6):2269-300.

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.

nz national eye centre

ROCK inhibitors in glaucoma practice: effectivity

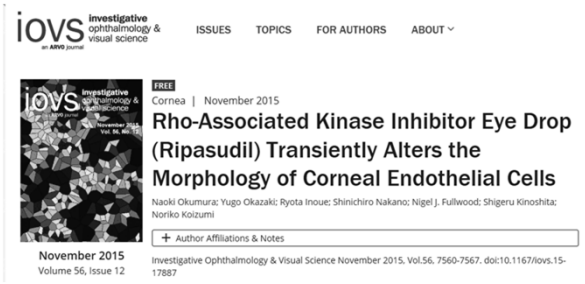
ROCK inhibitors typically produce IOP reduction comparable to Prostaglandins

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%

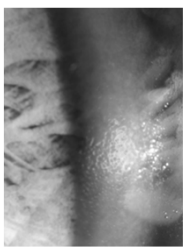
nz national eye centre

Glaucoma drugs and the corneal endothelium



The human corneal endothelium

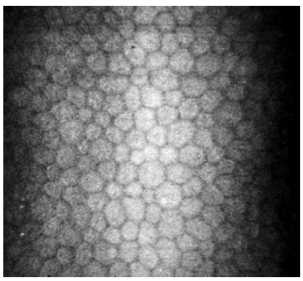
- Innermost layer of the cornea, separating stroma from aqueous humor
- A non-replication hexagonal monolayer of flat cells of ~5 µm thickness on Descemet's membrane
- Highly metabolic essential for corneal clarity
- **Core roles in corneal homeostasis**
  - passage of nutrients and metabolites
  - control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

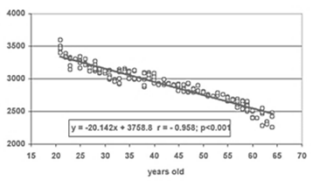


nz national eye centre

Corneal endothelial cells and ageing

nz national eye centre





Graph 1. Individual values of the emmetropic corneal endothelial cell density according to age.

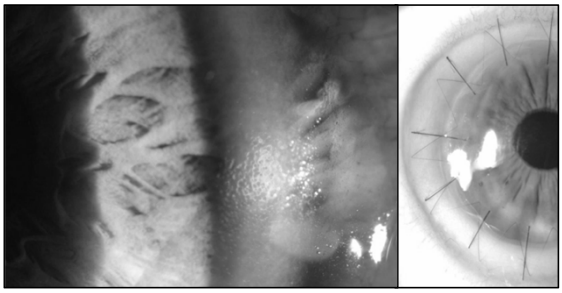
Corneal endothelial cell density decreases with age in emmetropic eyes

J.A. Sanchis-Gimeno[1], A. Lleó-Pérez[2], L. Alonso[2], M.S. Rahhal[2] and F. Martínez Soriano[1]
[1]Department of Anatomy and Human Embryology, Faculty of Medicine, University of Valencia and [2]Rahhal Ophthalmology Clinic, Valencia, Spain

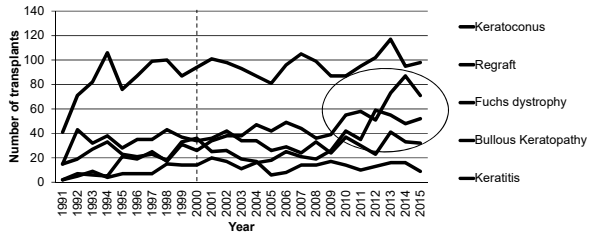
Histol Histopathol (2005) 20: 423-427

Treating advanced corneal endothelial disease

Currently only definitive treatment is transplantation

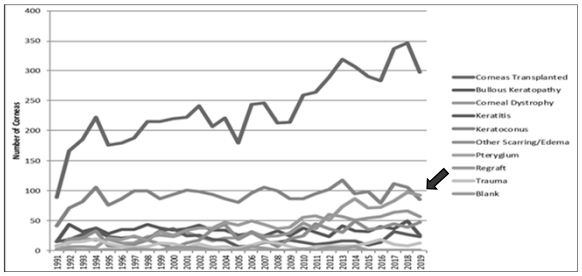


Five most common Indications for keratoplasty 1991-2015 (N=5043)



New Zealand trends in corneal transplantation over the 25 years 1991-2015. Kim BZ, Meyer JJ, Brookes NH, Moffatt SL, Twohill HC, Pendergrast DG, Sherwin T, McGhee CNJ. Br J Ophthalmol. **2017** Jun;101(6):834-838.

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

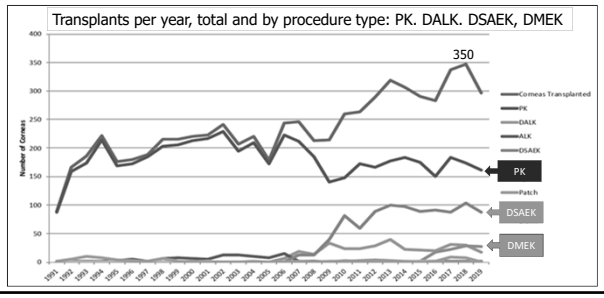


Indications for repeat keratoplasty

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	6	2.2%
Other	5	1.8%

Repeat corneal transplantation in Auckland, New Zealand: indications, visual outcomes and risk factors for repeat keratoplasty failure. Lu LM, Boyle AB, Niederer RL, Brookes NH, McGhee CNJ, Patel DV. Clin Exp Ophthalmol **2019** Nov; 47(8): 987-994

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

Stuart AJ, Romano V, Virgili G, Shortt AJ. Descemet's membrane endothelial keratoplasty (DMEK) versus Descemet's stripping automated endothelial keratoplasty (DSAEK) for corneal endothelial failure. Cochrane Database Syst Rev.**2018** Jun 25;6:CD012097.

Emerging superiority of DMEK?

AAO review	DSEK	DMEK
BCVA ≥ 20/25	6% - 31%	50% - 55%
Immune rejection	10%	1.9%
Visual recovery	Slower	Faster
Primary failure	5%	1.9%
EC loss @ 6 months	37%	33%
Secondary failure	4%	2.2%
Re-bubble	14%	28.8%

Descemet Membrane Endothelial Keratoplasty: Safety and Outcomes. Deng, Sophie X. et al., Ophthalmology, 125(2), 295 – 310.

The rapid evolution of ROCK-inhibitor therapy for corneal endothelial dysfunction

www.nature.com/scientificreports

SCIENTIFIC REPORTS

OPEN

Rho kinase inhibitor enables cell-based therapy for corneal endothelial dysfunction

Received: 06 January 2016
Accepted: 27 April 2016
Published: 18 May 2016

Naoki Okumura[1], Yuji Sakamoto[2], Keita Fujii[2], Junji Kitano[2], Shinichiro Nakano[1], Yuki Tsujimoto[2], Shin-ichiro Nakamura[1], Morio Ueno[3], Michio Hayiya[2], Junji Hamuro[1], Akifumi Matsuyama[1], Shingo Suzuki[4], Takashi Shiina[4], Shigeru Kinoshita[4,5] & Noriko Koizumi[1]

The corneal endothelium maintains corneal transparency; consequently, its dysfunction causes severe vision loss. Tissue engineering-based therapy, as an alternative to conventional donor corneal transplantation, is anticipated to provide a less invasive and more effective therapeutic modality. We conducted a preclinical study for cell-based therapy in a primate model and demonstrated regeneration of the corneal endothelium following injection of cultured monkey corneal endothelial cells (MCECs) or human CECs (HCECs), in combination with a Rho-kinase (ROCK) inhibitor, Y-27632, into the anterior chamber. We also evaluated the safety and efficacy of Good Manufacturing Practice (GMP)-grade HCECs, similar to those planned for use as transplant material for human patients in a clinical trial, and we showed that the corneal endothelium was regenerated without adverse effect. We also showed that CEC engraftment is impaired by limited substrate adhesion, which is due to actomyosin contraction induced by dissociation-induced activation of ROCK/MLC signaling. Inclusion of a ROCK inhibitor improves efficiency of engraftment of CECs and enables cell-based therapy for treating corneal endothelial dysfunction as a clinically relevant therapy.

Rapid Progress in RHO-Kinase inhibitors

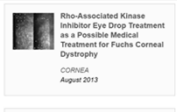
The New Therapeutic Concept of Using a Rho Kinase Inhibitor for the Treatment of Corneal Endothelial Dysfunction

Okumura, Naoki MD, PhD; Koizumi, Noriko MD, PhD; Ueno, Morio MD, PhD; Sakamoto, Yuji; Takahashi, Hiroaki BSc; Hamuro, Junji PhD; Kinoshita, Shigeru MD, PhD

Cornea: October 2011 - Volume 30 - Issue - p S54-S59
doi: 10.1097/ICO.0b013e3182281ee1
Article

2011 → 2019
concept to clinical trials


The New Therapeutic Concept of Using a Rho Kinase Inhibitor for the Treatment of Corneal Endothelial Dysfunction
CORNEA
October 2011


Rho-Associated Kinase Inhibitor Eye Drop Treatment as a Possible Medical Treatment for Fuchs Corneal Dystrophy
CORNEA
August 2013


Topical Rho-Associated Kinase Inhibitor, Y27632, Accelerates Corneal Endothelial Regeneration in a Canine Cryoinjury Model
CORNEA
March 2019

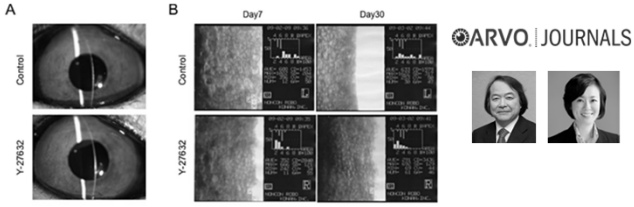

Cell-Based Approach for Treatment of Corneal Endothelial Dysfunction
CORNEA
November 2014

Use of Topical Rho Kinase Inhibitors in the Treatment of Fuchs Dystrophy After Descemet Stripping Only

CORNEA
May 2019

ROCK Inhibitor therapy of damaged corneal endothelium

The ROCK Inhibitor Eye Drop Accelerates Corneal Endothelium Wound Healing Invest. Ophthalmol. Vis. Sci.. 2013;54(4):2493-2502.

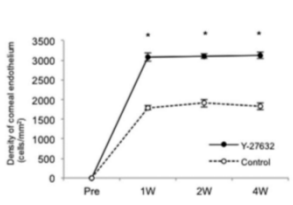


ARVO JOURNALS

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/mm^2
Y-27632–treated normal cells 3000 cells/mm^2

ROCK Inhibitor therapy of damaged corneal endothelium

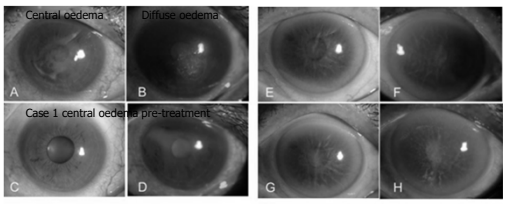
The ROCK Inhibitor Eye Drop Accelerates Corneal Endothelium Wound Healing Invest. Ophthalmol. Vis. Sci.. 2013;54(4):2493-2502.



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

ARVO JOURNALS ROCK Inhibitor Rx damaged endothelium

Clinical trial of ROCK inhibitor Y-27632 eye drops for treating **human** central corneal oedema and diffuse corneal oedema (N=7) 2013



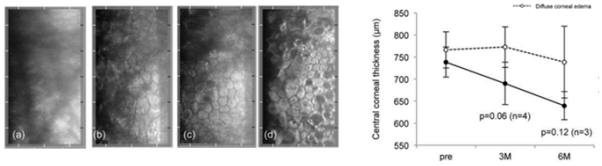
Central oedema
6/12 post treatment the central corneal edema is significantly reduced and visual acuity improved logMAR 0.70 to –0.18

Diffuse oedema
6/12 post treatment of persisting diffuse corneal oedema with no improvement in visual acuity or oedema

The ROCK Inhibitor Eye Drop Accelerates Corneal Endothelium Wound Healing Invest. Ophthalmol. Vis. Sci.. 2013;54(4):2493-2502.

ARVO JOURNALS ROCK Inhibitor Rx damaged endothelium

Clinical trial of ROCK inhibitor Y-27632 eye drops for treating human central corneal oedema and diffuse corneal oedema (2013)



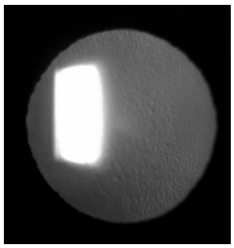
Case 1 specular microscopy before (A, B) and 6 months after treatment (C, D). Pre-treatment no clear image of central cornea due to edema in contrast with guttae observed para-central area. Six months post-treatment, specular microscopic images obtained from both of center (C) and peripheral cornea (D).

From: The ROCK Inhibitor Eye Drop Accelerates Corneal Endothelium Wound Healing Invest. Ophthalmol. Vis. Sci.. 2013;54(4):2493-2502.

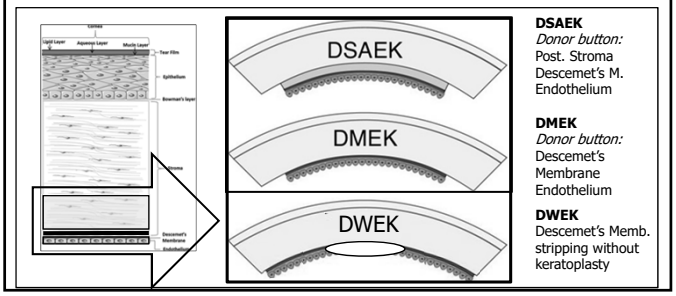
The future of Rho-kinase in treating corneal endothelial disease

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



Trends in Descemet's Endothelial Keratoplasty: DSAEK, DMEK or DWEK



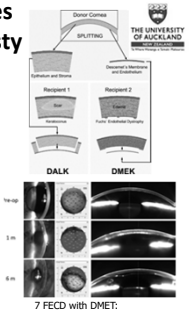
DSAEK
Donor button:
Post. Stroma
Descemet's M.
Endothelium

DMEK
Donor button:
Descemet's
Membrane
Endothelium

DWEK
Descemet's Memb.
stripping without
keratoplasty

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



7 FECD with DMET: progressive corneal clearance

Dirisamer M, Yeh RY, van Dijk K, et al. Recipient endothelium may relate to corneal clearance in Descemet membrane endothelial transfer. Am J Ophthalmol 2012; 154:290–296.

Cultured endothelial cells

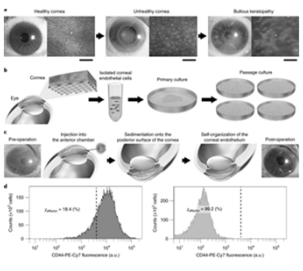
nature biomedical engineering

Article | Published: 22 July 2019

A physical biomarker of the quality of cultured corneal endothelial cells and of the long-term prognosis of corneal restoration in patients

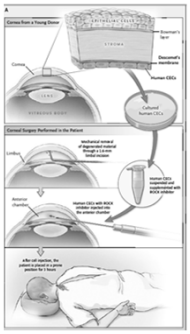
Akihisa Yamamoto, Hiroshi Tanaka, Munetoyo Toda, Chie Sotozono, Junji Hamuro, Shigeru Kinoshita, Morio Ueno & Motomu Tanaka

Nature Biomedical Engineering (2019) | Download Citation



The Future? Endothelial Cell therapy

- 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



Kinoshita S. et. al., (2018). Injection of Cultured Cells with a ROCK Inhibitor for Bullous Keratopathy. New Engl J Med 2018; 378:995-1003

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

nz national eye centre

Rho Rho Rho Rho the corneal revolution?

nz national eye centre