Mechano- and Glucocorticoid-Sensitive TREK-1 Channels Regulate Conventional Outflow and Intraocular Pressure

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Purpose. The purpose of this study was twofold: to determine the molecular link between corticosteroid exposure and mechanosensation and to establish the role of mechanosensitive TWIK-related potassium channel-1 (TREK-1) in the regulation of aqueous humor outflow and corticosteroid-induced ocular hypertension (OHT).

Methods. Real-time PCR was used to determine the corticosteroid dexamethasone (DEX) dependence of expression of tandem-pore potassium (K_{2P}), transient receptor potential vanilloid (TRPV), Piezo channel, extracellular matrix (ECM), and fibrotic marker genes in mouse trabecular meshwork (mTM) cells. Immunohistochemistry was employed to assess TREK-1 localization, iPerfusion to determine the TREK-1 dependence of conventional outflow, and tonometry to track intraocular pressure (IOP) in mouse eyes. Telemetry additionally tested TREK-1 dependence of OHT in rat. Steroid-induced transcriptional suppression of mTM *Kcnk2* was validated by whole-cell recording in primary human trabecular meshwork (TM) cells.

Results. The tandem pore K^+ channel transcriptome in mTM cells was dominated by *Trek-1* (*Kcnk2*) mRNA; with residual levels of *Traak* and *Thik-2* transcripts; and low levels of *Trek-2*, *Twik3*, and *Task1* expression. DEX upregulated *Fsp1* and suppressed *Kcnk2* expression without affecting *Trpv4*, *Piezo1*, or *Trpc1* mRNA content. The TREK-1 agonist ML-402 doubled outflow facility in mouse eyes and reduced IOP in the mouse model of DEX-induced OHT and in rat eyes with spontaneously elevated IOP. Chronic DEX exposure depolarized human primary TM (pTM) cells and reduced the amplitude of the ML-402-evoked current.

Conclusions. Ocular overexposure to corticosteroids may compromise IOP homeostasis by impairing TM expression and function of TREK-1. Pharmacological activation of TREK-1 facilitates conventional outflow and could help lower IOP in eyes with steroid-induced ocular hypertension.

Keywords: intraocular pressure, steroid glaucoma, mechanosensation, TREK-1, outflow facility, electrophysiology

Glucocorticoids are widely used in the clinic to combat inflammation associated with autoimmune disorders, eye conditions (Graves' ophthalmopathy, uveitis), allergies, asthma, heart failure, skin conditions, and surgery. Persistent treatment increases the risk for osteoporosis, weight gain, high blood pressure, and cognitive defects[1] and may cause permanent vision loss due to chronic elevation of intraocular pressure (IOP).[2,3] Clinicians thus often face the dilemma of whether to battle ocular inflammation or preserve vision imperiled by elevated IOP. An estimated one-third of the population is moderately steroid responsive (rise of IOP 6–15 mm Hg from baseline with steroid treatment), and 4% to 6% of the population is highly steroid responsive (IOP increase of >15 mm Hg from baseline or IOP over 31 mm Hg after steroid exposure).[1–3] Steroid sensitivity is augmented in patients with primary open-angle glaucoma (POAG), 90% of whom display elevated blood cortisol levels and glucocorticoid-induced ocular hypertension (OHT).[2,3]

Steroids elevate IOP by augmenting the resistance of the conventional outflow pathway to the flow of aqueous humor.[4,5] The pathway is comprised of episcleral vessels, the inner wall endothelium of the canal of Schlemm, and trabecular meshwork (TM) that is populated by cells

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in three distinct extracellular matrix (ECM) layers. TM cells are highly mechanosensitive[6–8] and contractile[9,10] and may exhibit properties of a pressure-activated biomechanical pump.[11,12] Non-glaucomatous rabbit, dog, rodent, nonhuman primate, and human TMs respond to glucocorticoids with changes in nuclear size, cell–cell adhesion, cell cycle, metabolism, inflammatory and TGF-β2 signaling, and expression of cytoskeletal and ECM proteins[5,13–21] that may together underlie the observed decrease in outflow facility in vitro and in vivo.[5,21] We do not know, however, which aspects of steroid-associated signaling cause OHT and which represent its epiphenomenon. It would therefore be useful to determine whether and how steroids affect IOP sensing and homeostasis and, conversely, whether manipulation of TM mechanosignaling impacts steroid-induced OHT.

The TWIK-related potassium channel-1 (TREK-1) channel, a member of the tandem-pore potassium (K_{2P}) channel family encoded by the *Kcnk2* gene, is a bona fide mechanochannel that is sensitive to stretch, temperature, pH, and C22:6 ω-3/C18:2 ω-6 polyunsaturated fatty acids[22–24] and is activated by physiological acidification.[25] Studies in heart, vasculature, gut, and bladder preparations have associated TREK-1–mediated K^+ efflux with protection from mechanical stress and inflammation[26–28] and our previous research in human primary TM (pTM) cells has uncovered a central role for TREK-1 channels as the principal mediators of the K^+ leak conductance in these cells. We showed TREK-1 to be activated by modest (<15 mm Hg) pressure steps and pH transients,[6,23,25] with the selective activator ML-402 promoting permeability of pTM cell monolayers.[23] Whether TREK-1 activity contributes to the regulation of aqueous humor outflow and IOP, however, remains unknown.

In the present study, we identified TREK-1 as a major positive regulator of conventional outflow facility and showed that its activation lowers IOP in the glucocorticoid-induced mouse OHT model and a rat model of spontaneously elevated IOP. Chronic exposure to the steroid dexamethasone (DEX) profoundly suppressed TREK-1 transcription in mouse TM cells. We tested the translatability of this phenomenon to cells isolated from human donors and identified potent, DEX-induced suppression of resting and evoked TREK-1 activity. These findings support a model whereby the protective function of TREK-1 activity in mammalian IOP homeostasis may be compromised in glaucoma.

Materials and Methods

Animals and Ethical Approval

This study investigated TREK-1 signaling in rodents acquired from three separate sources, with experiments performed in compliance with institutional Institutional Animal Care and Use Committees and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Studies in Utah utilized 1.5- to 4-month-old C57BL/6J mice purchased from The Jackson Laboratory (Bar Harbor, ME, USA). The animals were housed at the Moran Eye Center vivarium in individually ventilated enclosures at 21°C with a 12-hour light/dark cycle and ad libitum food and water; they were used for tonometry and derivation of mouse trabecular meshwork (mTM) cell lines. iPerfusion experiments in London utilized C57BL/6J mice obtained from Charles River UK Ltd. (Margate, UK), and telemetry studies employed rats purchased from Envigo (Indianapolis, IN, USA). There is no evidence that C57BL/6 mice from Charles River and The Jackson Laboratory showed any differences with regard to cellular mechanosensing, outflow, and IOP regulation.

Reagents

The TREK-1 agonist was synthesized as described by Lolicato et al.[29] and stored at −40°C. The 20- to 40-mM solutions were obtained by diluting aliquots of the stock solution before each experiment. Salts were purchased from Sigma-Aldrich (St. Louis, MO, USA) and VWR International (Radnor, PA).

mTM/pTM Purification and Culture

The pTM cells were isolated from human donor eyes (Lions Eye Bank at the Moran Eye Center)[10,30,31] according to consensus recommendations[32] and used in concordance with the tenets of the Declaration of Helsinki and the U.S. Department of Health and Human Services Belmont Report. The trabecular meshwork was dissected out of the anterior eye and plated on 10-mg/mL collagen I–coated tissue flasks, with cells migrating from the tissue over 2 to 4 weeks. Trypsinized cells were replated on collagen I–coated flasks in Trabecular Meshwork Cell Medium (TMCM) medium (ScienCell Research Laboratories, Carlsbad, CA, USA) supplemented with growth serum (TCGS; ScienCell Research Laboratories), 2% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 mg/mL streptomycin. The experiments were conducted with P1 to P4 cells, which were validated as described.[8,31,33]

Isolation and culturing of mTM cells followed a previously published protocol.[34,35] Briefly, retina, choroid, vitreous, lens, and cornea were removed; limbal rings were dissected, rinsed in PBS containing 1% acetic acid (AA; Thermo Fisher Scientific, Waltham, MA, USA), and digested in 1% AA, 4-mg/mL collagenase A (MilliporeSigma, Burlington, MA, USA), and 4 mg/mL bovine serum albumin (BSA) in PBS for 2 hours at 37°C. Dissociated cells were transferred to flasks at 37°C and incubated for 24 hours. Then, 0.001% magnetic polystyrene smooth-surfaced beads (2.0–2.9 µm; Spherotech, Lake Forest, IL, USA) were added, and cells were detached with Gibco TrypLE Express (Thermo Fisher Scientific). Phagocytosing cells were magnetoprecipitated, resuspended in 6 mL of media, and seeded into Gibco Dulbecco's Modified Eagle Medium (DMEM) media (1 g/L glucose), supplemented with 10% FBS (Genesee Scientific, Morrisville, NC, USA) and 1% AA (Thermo Fisher Scientific) at 37°C and 5% CO_2. The cells were used between passages 2 and 4. TM identity was validated by the expression of *Myoc*, *Chi3L*, *Acta2*, and *Timp3* marker genes and DEX-induced upregulation of *Myoc*.[34]

Gene Expression Analysis

mTM cells were used for analyses of mRNA expression. Total RNA was isolated with the Arcturus RNeasy Plus Micro Kit (Qiagen, Hilden, Germany) and 500 ng of total RNA used for reverse transcription. First-strand cDNA synthesis and PCR amplification of cDNA were performed using a qScript Ultra SuperMix cDNA synthesis kit (Quantabio, Beverly, MA, USA). SYBR Green–based real-time PCR was performed using Apex qPCR Master Mix (Genesee Scientific). The results were obtained in triplicate, with at least

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Table. Primer Sequences

Gene	Forward Primer	Reverse Primer	Product Size (bp)	NCBI Ref. No.
Task1	CCATCGGCTGCACCTCTACCTCT	GGGCCCCAGCTGCTTGTTCAC	196	NM_175130.4
Trek-1	AGTCCAGTCCCTGCATCTAGC	ATCACGTAAGCCCAAGCCTC	162	NM_001159850.1
Traak	GAGTCCTCAGACACGCAGAG	CATCAGCAAGAGCGAAAGCC	191	NM_008431.2
Task5	GCGAGCGTCTAAACACACTG	CCGAAGCCTATGGTGGTGAG	223	NM_001030292.1
Twik1	TTACCTGCTCCTCGGACTCA	TCCTCATAGGGTGGGGACTG	240	NM_008430.2
Tresk2	ACGGCTGTATCTTGTCTCCC	TTTTGGCAACGGCTGGAATC	197	NM_207261.3
Thik-1	TTGGCCTCAGGCAAGAGTAG	CACGTTCCAGGACAGAAGCA	227	NM_001164427.1
Twik3	CGATCGGCCTAGGAGACTTG	TAACAACATGGCCAGGAGCC	119	NM_010609.3
Trpm4	TCTTCGAGCACTTCCGTGTC	AGCAAGGCAGAGTGGCTAAG	283	NM_175130.4
Acta2	GCCAAGGGTGTCCCCAAAAA	ACTAGGAGTAGAGGCCAGGTC	244	NM_007392.3
Piezo1	TGGTGGCCATCCTTACAC	GGTACAGCCACTTGATGAGG	195	NM_001357349.1
Fn1	CCCTCCATCTTTGAGTGGTCC	AACCCTGAAGCAGAACAGGG	163	NM_010233.2
Trpv4	TCCTGAGGCCGAGAAGTACA	TCCCCCTCAAACAGATTGGC	166	NM_022017.3
Fsp1	GGACAGCAACAGGGACAATG	ACTGGCAAACTACACCCCAA	160	NM_011311.3
Mmp9	TCTACTGGGCGTTAGGGACA	AGGAGTCTGGGGTCTGGTTT	101	NM_013599.5
Trpc1	AGCCTCAGACATTCCAGGTTT	AACATTTTGCACTGACGGGC	188	NM_011643.4
Gapdh	GGTTGTCTCCTGCGACTTCA	TAGGGCCTCTCTTGCTCAGT	220	NM_001289726.2

NCBI, National Center for Biotechnology Information.

three experiments. The comparative C_T method ($\Delta\Delta C_T$) was used to measure relative gene expression. The fold enrichment was calculated as: $2^{[\Delta CT\ (sample) - \Delta CT\ (calibrator)]}$ after normalization. *Gapdh* levels were utilized to normalize fluorescence signals. Primer sequences are shown in the Table.

Immunohistochemistry

Mice were euthanized, and eyes were fixed in 4% PFA for 1 hour at room temperature (RT).[36,37] The anterior eye tissue was cryoprotected in 15% sucrose or 30% sucrose for 45 minutes at RT or overnight at 4°C, respectively, and the eyes were embedded in Tissue-Tek OCT (Sakura Finetek USA, Torrance, CA, USA). Then, 12-µm slices were cut with a cryostat, mounted on Superfrost Plus slides (Thermo Fisher Scientific), dried, and kept at −80°C. For staining, cryosections were rinsed in PBS (3 × 5 minutes) and blocked in 5% FBS and 0.3% Triton-X in 1× PBS for 30 minutes (sections) at RT. Primary antibodies—mouse α-smooth muscle actin (αSMA; 1:500; Abcam, Cambridge, UK) or rabbit TREK-1 (1:200; Santa Cruz Biotechnology, Santa Cruz, CA, USA) were prepared in 2% BSA and 0.2% Triton-X in 1× PBS. Cryosections were incubated overnight at 4°C, rinsed three times for ∼5 minutes in 1× PBS, incubated with secondary antibodies for 1 hour at RT, and washed three times for 5 minutes in 1× PBS. Secondary antibodies were Invitrogen Goat anti-Mouse Alexa Fluor 594 (1:1000; Thermo Fisher Scientific), Goat anti-Rabbit Alexa Fluor 488 or 594 (1:1000; Thermo Fisher Scientific), and Goat anti-Guinea Pig Cy3 (1:1000; Jackson ImmunoResearch, West Grove, PA, USA). Cryosections were counterstained (Fluoromount-G with 4′,6-diamidino-2-phenylindole [DAPI]; Southern Biotech, Birmingham, AL, USA) and coverslipped. Immunofluorescence was acquired with a confocal microscope (FV1200; 1024 × 1024 pixels with a 20× water superobjective; Olympus, Tokyo, Japan).

Electrophysiology

Whole-cell patch current in human pTM cells was recorded[23,31,38] at RT (22°–24°C) with borosilicate pipettes (5–8 MΩ; World Precision Instruments, Sarasota, FL, USA) pulled on a P-2000 puller (Sutter Instrument, Novato CA, USA). Pipettes were fabricated from borosilicate glass capillaries (outer diameter, 1.5 mm; inner diameter, 0.84 mm; World Precision Instruments). The external solution contained 126-mM NaCl, 2.5-mM KCl, 1.5-mM $MgCl_2$, 1.5-mM $CaCl_2$, 1.25-mM NaH_2PO_4, 26-mM $NaHCO_3$, and 10-mM D-glucose (pH 7.4, adjusted with NaOH). The pipette solution contained 135-mM potassium D-gluconate, 10-mM KCl, 1-mM $MgCl_2$, 10-mM HEPES, 0.5-mM EGTA, 0.01-mM $CaCl_2$, 0.3-mM guanosine 5′-diphosphate sodium salt (Na-GTP; pH 7.3, adjusted with potassium hydroxide), and 1-mM adenosine 5′-triphosphate magnesium salt (Mg-ATP). Acquisition was controlled via a MultiClamp 700B amplifier, Axon Digidata 1550 board, and pCLAMP Clampex 10.7 acquisition software (all Molecular Devices, San Jose, CA, USA). Cells were held at the resting potential (typically between −30 mV and −40 mV). Currents were sampled at 10 kHz, filtered at 5 kHz with an 8-pole Bessel filter, and analyzed with the pCLAMP Clampfit Advanced Analysis Module (version 10.7; Molecular Devices) and OriginPro 8 (OriginLab, Northampton, MA, USA).

iPerfusion Flow Measurement

Outflow facility was measured in enucleated eyes from C57BL/6J mice ($n = 6$ mice) using iPerfusion.[31,39,40] Briefly, enucleated eyes were affixed to a support platform and submerged in a PBS bath at 35°C. The anterior chamber was cannulated using a glass micropipette pulled to a 100-µm diameter beveled tip. The perfusion fluid was Dulbecco's PBS containing divalent cations and sterile (0.25 µm) 5.5-mM glucose. The ipsilateral eye was perfused with ML-402 (30 µM), and the contralateral eye perfused with vehicle. IOP was set to 9 mm Hg for 1 hour to pressurize and acclimatize the eye to the perfusion environment and to allow sufficient time for the TREK-1 agonist to reach the anterior chamber. Flow was measured over eight increasing pressure steps from 6.5 mm Hg to 17 mm Hg. Steady state for each step was evaluated when the ratio of the flow rate to pressure changed by less than 0.1 nL/min/mm Hg per minute over a 5-minute window. The

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stable pressure (P) and flow rate (Q) were calculated over the last 4 minutes of each step. A power–law relationship of the form

$$Q = C_r \left(\frac{P}{P_r} \right)^{\beta} P \quad (1)$$

was fit to the Q–P data. The reference outflow facility, C_r, represents the value of outflow facility at a reference pressure (P_r) of 8 mm Hg, and β characterizes the non-linearity of the Q–P relationship. The relative difference in C_r between treated and untreated contralateral eyes was calculated as the ratio of C_r in the treated eye relative to that in the contralateral control eye minus unity. Facility values and relative changes in facility are reported in terms of geometric means and 95% confidence intervals (CIs).

Tonometry and IOP Elevation

DEX-Induced OHT. Mice were habituated to handling, with topical 0.1% DEX sodium phosphate ophthalmic eyedrops (Bausch & Lomb, Bridgewater, NJ, USA) administered to awake animals three times a day (10–11 AM, 2–3 PM, 6–7 PM) for ~12 weeks; age-matched cohorts were treated with sterile PBS drops. The animals were gently restrained for ~30 seconds after drop application to allow for glucocorticoid permeation. IOP in awake mice was measured with a rebound tonometer (TonoLab; iCare, Vantaa, Finland) between noon and 2 PM, with each IOP data point representing an average of 10 to 20 tonometer readings. No sex differences were noted, and data from male and female animals were pooled.

IOP Telemetry. Data were gathered from male and female animals with no noted gender differences. Rats were anesthetized, a fluid-filled silicone microcannula was implanted in the anterior chamber of one eye, and the cannula was connected to a wireless pressure sensor via a plastic coupler secured to the skull.[41,42] When the animal woke, IOP was transmitted at 0.25 Hz around-the-clock to a laptop for display and storage. At sporadic times, a drop of drug (ML-402; 40 µM) or vehicle (PBS saline) was instilled on the implanted eye.

Statistical Analysis

Group sizes were determined based on preliminary experiments, and data were analyzed by Prism 10.0 (GraphPad, Boston, MA, USA). Two-way repeated-measures ANOVAs were conducted for longitudinal IOP data in ipsi-/contralateral eyes treated with vehicle PBS and ML-402. Paired t-tests were utilized to compare two groups with pre-injection and post-injection. The details are included in the figure legends for each panel.

Results

Histology: TREK-1 Channel Expression in the Anterior Eye of the Mouse

Previous studies combined mouse tonometry with investigations of TREK-1 and K_{2P} channel gene expression and TREK-1 localization in cultured human cells.[23,25,43] To gain insight into channel expression and function in the same species, we investigated K_{2P} transcripts, TREK-1 immunolocalization, and the role of TREK-1 in outflow regulation and IOP

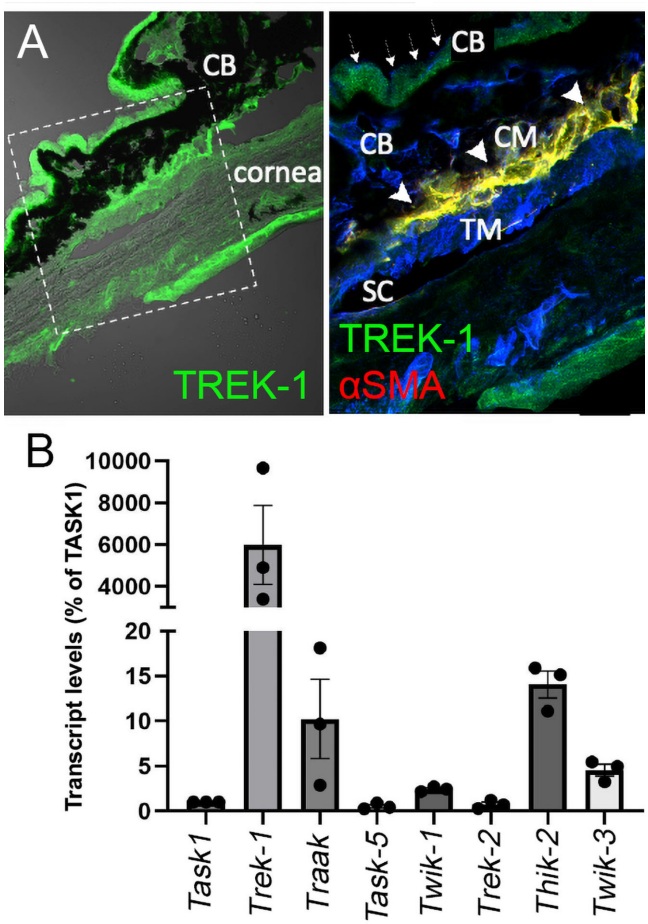


Figure 1. TREK-1 expression in the anterior eye of C57BL/6 mice. (**A**) *Left panel*: TREK-1-ir in the anterior eye. The TM, corneal epithelium and the CB are labeled. *Right panel*: Higher magnitude view. TREK-1-ir (*green*) colocalized with αSMA (*red*) and collagen IV (Col4)-ir (*blue*) (*arrowheads*); TREK-1 is also expressed in corneal epithelium and the CB (*arrows*). (**B**) Quantitative PCR of mTM cells. Relative K_{2P} gene expression is dominated by the *Trek-1* gene. *Error bars* ± SEM.

signaling in cells and tissue derived from the mouse. Anterior segment sections from wild-type C57BL/6 mice showed TREK-1 immunoreactivity (TREK1-ir) in nonpigmented cells of the ciliary body (CB) (Fig. 1A, arrows), corneal epithelium and the TM (Fig. 1A, arrowheads). TM localization was confirmed through colocalization with the marker αSMA. To gain insight into relative expression in mouse versus human preparations, we evaluated the relative expression of the *Kcnk2* gene that encodes TREK-1 and cognate K_{2P} family isoforms. mTM cells showed robust expression of *Myoc* (myocilin), *Mgp* (matrix Gla protein), *Chil3l1* (chitinase-3-like protein 1), and *Mmp2* (matrix metalloproteinase 2).[34] The K_{2P} transcriptome was notable for the prevalence of *Trek-1* expression, modest levels of *Traak* (TWIK-related arachidonic acid-stimulated K^+ channel member 4 [*Kcnk4*]) and *Thik-2* (tandem-pore-domain halothane-inhibited K^+ channel member 12 [*Kcnk12*]), and low to undetectable expression of *Trek-2* (*Kcnk10*, a mechanosensitive paralog of *Trek-1*), *Twik3* (TWIK-related acid-sensitive K^+ channel member 9 [*Kcnk9*]), *Task1* (TWIK-related acid-sensitive K^+ channel member 3 [*Kcnk3*]) and *Task5* (TWIK-related spinal cord K^+ channel member 15 [*Kcnk15*]) genes. K_{2P} channel

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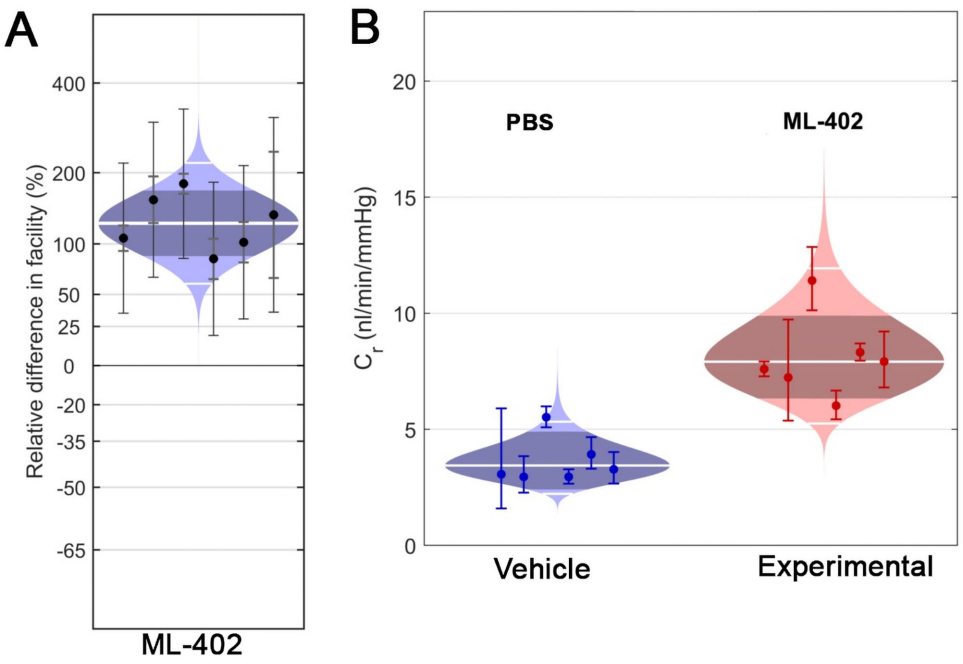


Figure 2. TREK-1 agonism increases outflow facility in enucleated mouse eyes perfused with ML-402 (30 µM) versus vehicle (Dulbecco phosphate-buffered saline [DPBS] + 5.5-mM glucose). (**A**) Cello plot showing the relative difference in reference outflow facility (klC_r) between contralateral eyes perfused with 30-µM ML-402 versus vehicle, showing an average increase in Cr of 125% (95% CI, 86–172; $n = 6$). (**B**) Control facility plotted against experimental cohort. The central white lines represent the geometric means, outer white lines represent 95% data boundaries, and central dark regions indicate the 95% CIs.

expression pattern in mTMs is thus similar yet distinct from human pTM cells.[23]

Pharmacological TREK-1 Activation Doubles the Ex Vivo Outflow Facility

TREK-1 channels contribute to the pressure response and subserve the resting K^+ leak conductance in pTM cells,[23] but their role in outflow modulation and IOP homeostasis remains unknown. We utilized iPerfusion, a cannulation-based fluidic system that quantifies the facility via differential pressure measurements,[39,40] to test the effect of ML-402, a selective TREK channel activator,[29] on conventional outflow. Mouse eyes were pressurized at 8 mm Hg and subjected to incremental pressure steps with and without the agonist (30 µM). ML-402 approximately doubled the flow rate through the outflow pathway at each level of applied pressure, with mean C_r for ML-402-treated eyes of 7.9 nL/min/mm Hg (95% CI, 6.3–9.9) versus 3.4 nL/min/mm Hg (95% CI, 2.4–4.9) for vehicle-treated eyes (Fig. 2), corresponding to an average increase of 125% (95% CI, 86–172) relative to contralateral vehicle-perfused eyes ($P = 0.0006$, $n = 6$) (Fig. 2A). Hence, TREK-1 activity functions as a positive regulator of conventional outflow.

TREK-1 Activation Lowers IOP in Glucocorticoid-Induced Ocular Hypertensive Eyes

We employed tonometry to test whether TREK-1 activation modulates IOP in hypertensive eyes that experienced chronic exposure to glucocorticosteroids. As shown previously,[16] daily DEX (0.1%) administration induced gradual increase in IOP that stabilized at 19.4 ± 0.3 mm Hg at ~5 weeks. A single eye drop of ML-402 (500 µM) was sufficient to reduce IOP for 4 to 6 hours ($P < 0.0001$, $n = 11$) (Fig. 3), suggesting that induced TREK-1 activity could protect eyes challenged by steroid overexposure.

TREK-1 Activation Lowers IOP in Rat Eyes Recorded With a High-Resolution Telemetry Sensor

Mechanical stimuli activate TREK-1 within milliseconds,[22,24] suggesting that the agonist could influence IOP at temporal dynamics beyond the time resolution of perfusion-based systems. Mouse eyes are too small for implantation with wireless IOP telemetry systems. We thus utilized the Brown–Norway rat model[41,42] for high-resolution tracking of the effect of ML-402 (40 µM) on eyes with elevated IOP, and also gained information about the role of the channel in another species. Proof-of-principle experiments ($n = 3$) showed that, similar to mice (Fig. 3), corneal administration of the TREK-1 agonist ML-402 lowers IOP ($P < 0.05$) (Fig. 4).

Glucocorticoid Treatment Downregulates *Kcnk2* But Not *Trpv4* or *Piezo1* Expression

Given the facility-promoting effect of TREK-1 activation (Fig. 2), the simplest explanation for ML-402–induced lowering of IOP in DEX-treated eyes (Fig. 3) is that the glucocorticoid modulates *Kcnk2* expression itself. Consistent with previous reports,[44] we found that mTM cells respond to DEX with upregulation of fibroblast-specific protein 1 (*Fsp1*), a TGF-β2–sensitive RhoA-activated myofibroblast marker gene. Expression of *Trpm4*, *Acta2*, and *Mmp9* genes was

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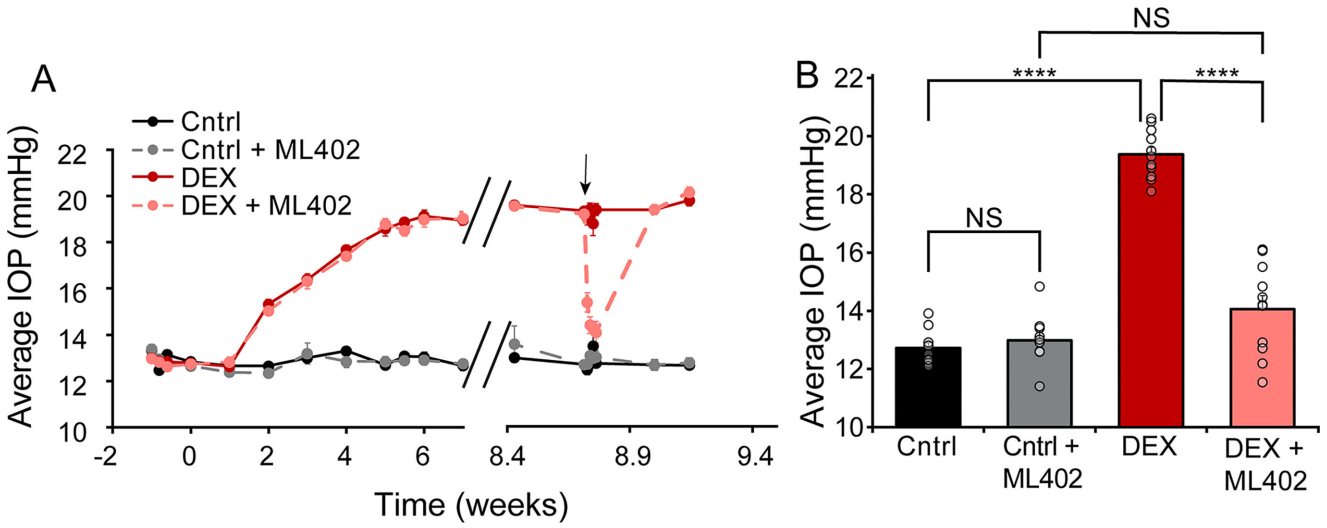


Figure 3. TREK-1 activation lowers IOP in mouse eyes exposed to chronic DEX treatment. (**A**) Time course of IOP elevations in eyes topically treated with DEX (three times daily) and vehicle (PBS) in the presence and absence of ML-402. ML-402 ($n = 11$; *pink trace*) but not PBS ($n = 11$; *red trace*) lowered IOP in DEX-treated eyes. Neither PBS ($n = 10$) nor ML-402 ($n = 10$) affected normotension (*gray* and *black traces*). (**B**) Data summary for the experiments shown in **A** 8 hours after ML-402 treatment. Repeated-measures one-way ANOVA with Dunnett's post hoc comparisons are presented as average mean ± SEM. ****$P < 0.001$.

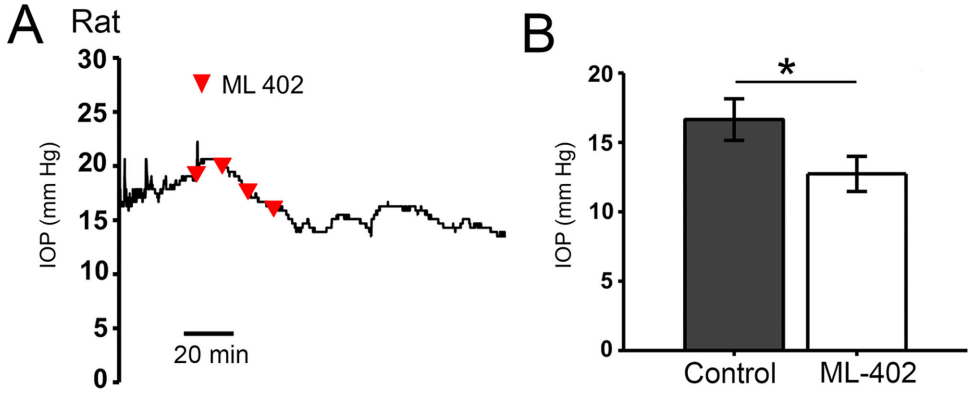


Figure 4. Rat eye telemetry. Isoflurane-anesthetized rats. (**A**) Representative recording of IOP lowering induced by ML-402. *Red arrows* denote additions of ML-402-containing eye drops (**B**) Averaged data for rat eyes before administration of first eye drop (*black bar*) and 20 min after after last administration of ML-402 ($n = 3$). Paired-sample *t*-test results are shown as mean ± SEM. *$P < 0.05$.

suppressed (Fig. 6), possibly due to fibrotic dedifferentiation. We also observed a remarkable ~80% downregulation of *Kcnk2* expression ($P < 0.5$, $n = 4$) in contrast to *Piezo1*, *Trpv4*, or *Trpc1* genes, which appeared to be unaffected by the steroid.

DEX-Induced Suppression of Constitutive TREK-1 Activity Is Mitigated by Pharmacological Agonism

Data shown in Figures 1 to 5 show that mTM expresses TREK-1, which regulates conventional outflow and IOP in mouse eyes and is downregulated by chronic exposure to DEX. Given the prominent TREK-1 expression in mouse and human TM, we investigated whether DEX-induced modulation of TREK-1 signaling is relevant in the primate context by testing the role of chronic exposure on the membrane potential of cells and TREK-1–mediated current in primary human TM cells. Physiologically, the cells would be predicted to respond to suppression of the dominant resting K^+ conductance with change in the resting potential (V_{rest}). Whole-cell recordings from TM cells isolated from donors without history of glaucoma indeed revealed significant depolarization that was associated with a reduction in the amplitude of the evoked TREK-1 current. Perfusion with ML-402 (40 µM) hyperpolarized the membrane potential in current-clamped control cells (ranging between −30 and −40 mV) (Fig. 6A) by approximately 15 mV ($n = 8$). Chronic exposure to DEX (500 nM for 7 days) depolarized the cells to approximately −16 mV and significantly attenuated the amplitude of ML-402–evoked hyperpolarizations ($P < 0.05$, $n = 6$). Glucocorticoids thus suppress the steady-state leak K^+ conductance and reduce the size of the reserve pool of activatable TREK-1 channels.

Discussion

This study identified the TREK-1 channel as an important component of the molecular–physiological bridge that links

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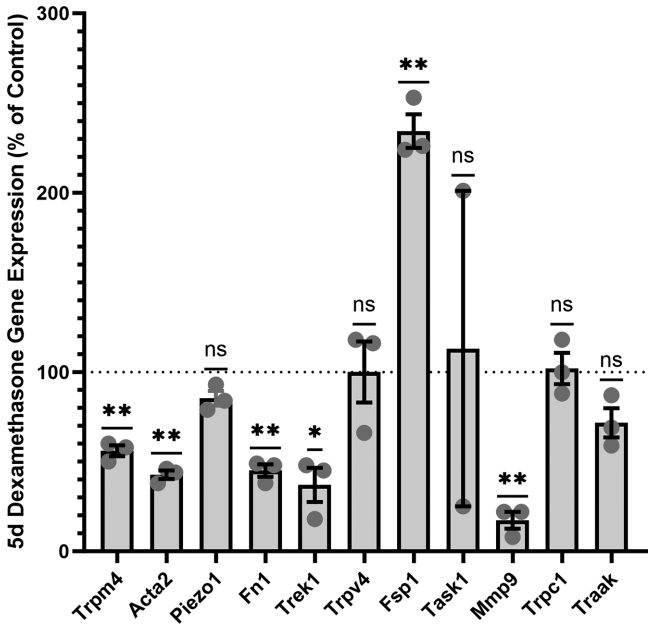


Figure 5. DEX modulates expression of K_{2P} and myofibroblast marker genes. mTM, 10-day exposure to DEX (100-nM), semiquantitative qPCR. Shown are the approximate fold changes in relative abundance of transcripts that encode fibrosis- and mechanosensation-related proteins ($n = 4$). DEX downregulated *Trek-1* expression without impacting the expression of *Trpc1*, *Task1*, *Piezo1*, and *Trpv4* genes. One-way ANOVA results shown as mean ± SEM. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

overexposure to corticosteroids to impaired mechanosensation, increased conventional outflow resistance, and OHT. Glucocorticoid therapy is commonly used to treat many ocular and systemic conditions, yet it entails many serious health risks that include disruption of the hypothalamic–pituitary–adrenal axis, impaired cardiovascular function, and blindness: Approximately 40% of people without glaucoma develop steroid-induced OHT, with glaucoma risk rising to ~90% in POAG patients and ~80% in patients with Cushing's syndrome.[1–3] Increased glucocorticoid levels during aging are likely to additionally predispose individuals toward IOP dysregulation.[45]

Four sets of findings laid out by our study bring insight into this process. First, TREK-1 channel activation facilitated conventional outflow all pressures, thereby contributing to IOP stability in healthy eyes. Second, TREK-1 activity was protective, with channel activation lowering acute and chronic OHT. Third, DEX depolarized TM cells and elevated IOP in part by downregulating *Trek-1* gene expression and reducing resting and evoked TREK-1 conductance, whereas *Piezo1* or *Trpv4* expression appeared unaffected by the steroid. Fourth, glucocorticoid-induced OHT could be reversed pharmacologically with eye drops containing selective TREK-1 agonists. The consistency of transcriptomic and functional evidence about TREK-1 signaling in rodent (mouse and rat) and human preparations validates the usefulness of rodent models for the investigation of molecular–functional remodeling mechanisms that lead to the etiology of and treatment for steroid glaucoma.

Mice and humans show many similarities in the anatomy and physiology of the outflow pathway[32,46] and the conserved expression of phenotype-defining *Myoc*, *Pitx2*, *Acta2*, transient receptor potential vanilloid (*Trpv*), *Chi3l1*, *Timp3*, and *Mmp9* genes, as well as similar responsiveness to glucocorticoids.[34,47–49] Historically, IOP measurements in mice have been complemented by in vitro experiments in cultured human TM cells, whereas few studies have correlated in vivo tonometry and ex vivo analyses of outflow facility in mice with electrophysiological and molecular experiments in cells from the same species. We report that the K_{2P} transcriptome in mouse TM cells is similar to yet distinct from the human counterpart. Both preparations showed prominent expression of *Trek-1* and low levels of the cognate *Trek-2* mRNA.[23] mTM, but not pTM, showed modest expression of *Traak* (i.e., another mechanosensitive K_{2P} channel)[26] and *Thik-2* (which encodes a "nonfunctional" K_{2P} subunit)[50] and vanishingly low expression of *Task1*. These observations suggest that mechanosensitive K^+ efflux in mTM is likely to be predominantly carried by TREK-1, with residual involvement of homomeric TRAAK channels and/or TREK-1–TRAAK heteromers. We also report translatability of genes that encode mechanosensitive channels, with the TM of both species exhibiting prominent expression of KCNK2/*Kcnk2*, TRPV4/*Trpv4*, PIEZO1/*Piezo1*, and TRPC1/*Trpc1* but not PIEZO2/*Piezo2* genes[10,34] (Fig. 6). Nonetheless, these observations advocate for in vivo mouse experiments to be complemented by in vitro studies that employ mTM rather than pTM cells.

DEX-induced transcriptional suppression of *Mmp9* (which encodes a type IV collagenase necessary for maintenance of normotension)[51] and upregulation of *Fsp1* (myofibroblast marker gene linked to RhoA-dependent cytoskeletal remodeling)[44] mirror the suppression of ECM turnover and increased stiffness observed in human cells.[17,48,52–55] Chronic exposure to prednisolone acetate, for example, upregulates polymerization of actin in pTM cells together with suppressed expression of MMP2 and MMP3 isoforms implicated in the degradation of TM collagen IV and fibronectin.[21]

This study underscores a central role for the mechanosensitive TREK-1 channel in modulation of resting potential, conventional outflow, and IOP. Our observation that steroid-induced OHT was temporarily reversed by ML-402, which hyperpolarized TM cells, doubled the outflow facility, and lowered IOP suggests the potential for deploying pharmacological stratagems to counter steroid-induced transcriptional suppression of KCNK2. It is important to note that the downregulation of *Kcnk2* expression due to DEX and IOP lowering by ML-402 cannot be interpreted as indicating that impaired TREK-1 signaling causes glucocorticoid-induced OHT, as similar facilitatory effects have been reported for other compounds that stimulate TM K^+ efflux. The outflow facility increases and IOP lowering observed in mouse, rat, pig, and human preparations exposed to agonists of K_{ATP} (Kir 6.1) and BK_{Ca} (KCa1/.1) channels suggest that the stabilizing function of TREK-1 activity reflects its facilitation of K^+ efflux, which in turn might drive aquaporin-mediated redistribution of water and paracellular flow of aqueous humor through the expanded extracellular volume fraction.[56,57] Studies in gastrointestinal tract, bladder, uterus, lung, and cardiovascular cells similarly suggest that TREK-1–mediated hyperpolarization reduces actomyosin contractility by lowering $[Ca^{2+}]_i$ and reducing RhoA activation.[26,27] We propose that DEX suppresses trabecular outflow through fibrotic remodeling that approximately doubles tissue stiffness[5,16,58,59] and by suppressing the protective, outflow-promoting role of TREK-1 activity. In future experiments, it will be important to explore the relationship between

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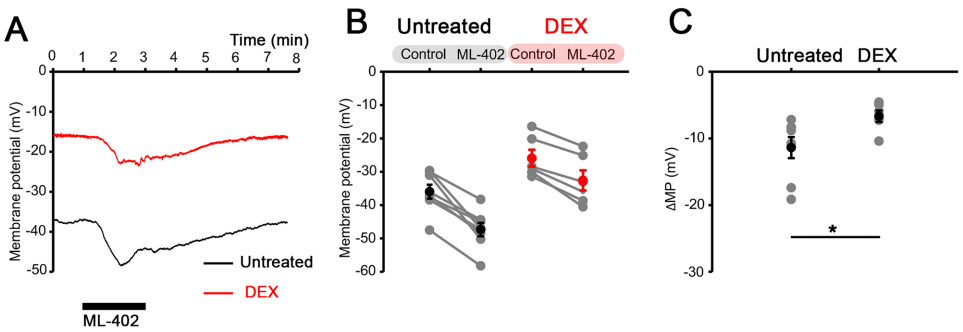


Figure 6. DEX depolarizes pTM cells and reduces the amplitude of the ML-402-evoked current in human cells. Shown are whole-cell recordings, current-clamp mode. (**A**) Representative recordings of resting membrane potential (V_{rest}) and current evoked by ML-402 (40 µM) from control and DEX-treated (500 nM, 7 days) cells. Steroid treatment depolarized the cells and attenuated the amplitude of the ML-402-evoked current. (**B**) Summary of ML-402–evoked hyperpolarizations in control ($n = 8$) and DEX-treated ($n = 6$) cells. (**C**) Average membrane potential in control and DEX-treated pTM cells. Black markers indicate mean ± SEM, paired-sample *t*-test. *$P < 0.05$.

TREK-1 signaling and TM biomechanics by combining physiological assessments of TREK-1 dependence of contractility, calcium signaling, and dynamics of integrin-based focal adhesions with atomic force spectroscopy, nanoindentation, shear rheology, and/or optical coherence elastography. Another viable approach would be to employ electric cell–substrate impedance sensing (ECIS) to determine the effect of transcriptional suppression of *Kcnk2* (Fig. 5) on impedance of TM monolayers as proxies of paracellular fluid flow.[21] Monolayers treated with TREK-1 activators ML-402 and ML67-33 exhibit dose-dependent increases in permeability,[23] which could reflect changes in ionic permeation, paracellular spacing, contractility, and/or ECM adhesion.

TREK-1 expression in the ciliary body, Schlemm's canal, and cornea (Fig. 1) suggests that these tissues are mechanosensitive and might be susceptible to corticosteroid modulation. Given that iPerfusion obviates pressure-independent contributions from inflow (ciliary body), choroid (uveovortical outflow), and suprachoroidal (uveoscleral outflow) pathways,[39] the doubling of outflow in the presence of ML-402 reflects the TREK-1 dependence of flow the trabecular meshwork and/oir the canal of Schlemm. A potential contribution of the ciliary body to IOP lowering in agonist-treated DEX-exposed eyes cannot, however, be excluded. Animal models with conditional loss of TREK-1 function are necessary to dissect the contribution from specific inflow- and outflow-regulating cell types and tissues.

TREK-1 may help maintain the homeostatic IOP setpoint[60] by serving as a brake for pressure- and TRPV4-dependent fibronectin release and Ca^{2+}- and RhoA-dependent actomyosin contractility that underlie IOP elevations.[8,10,25,34,36,61] Together with Piezo1, TRPV4, and TRPM4 channels, TREK-1 contributes to an integrated mechanosensory network that underlies homeostatic IOP regulation but may be compromised by aging, mechanical stress, glucocorticoids, TGF-β2, ECM remodeling, and circadian factors, which, we propose, reset the setpoint by altering mechanochannel expression, trafficking, and activation/inactivation states. The setpoint, therefore, can be shifted through TGF-β2–dependent modulation of TRPV4 and Piezo1 expression,[10] DEX-induced reduction in KCNK2 expression (Fig. 5), and circadian modulation of TRPV4 activation.[34] TRPV4 and TREK-1 are attractive candidates for setting a mechanodynamic steady state, as both are stretch-sensitive thermochannels with comparable temperature activation profiles; sensitivity to arachidonic acid, phosphatidyl inositol biphosphate, cholesterol, and protons; and shared participation in cell–ECM interactions.[25,27,30,43,61–63] *Trek-1*$^{-/-}$ mice accordingly have shown increased[64,65] and *Trpv4*$^{-/-}$ mice decreased sensitivity to mechanical stressors (allodynia and hyperalgesia).[66] We hypothesize that dysregulated IOP setpoint in hypertensive eyes might be restored through TRPV4 inhibition[10,34] or TREK-1 activation (Figs. 2–4).

Conclusions

In summary, these findings may expand the toolbox of clinicians faced with the dilemma of whether to battle ocular inflammation or preserve vision by bringing insight into the molecular mechanism of corticosteroid-impaired pressure transduction and offering the possibility of treatments that transcend current ciliary body/ciliary muscle–centered approaches. In addition, they underscore the need to consider IOP regulation as reflecting parallel activity of multiple mechanosensing channels that respond differently to pressure steps and glaucoma risk factors. Examples include TGF-β2, which elevates IOP by promoting TM expression of TRPV4 but not TREK-1,[10] whereas glucocorticoids elevate IOP by suppressing TREK-1 but not TRPV4 or Piezo1 transcription (Fig. 5). Steroid-responsive patients are generally treated with aqueous humor suppressants (inhibitors of carbonic anhydrase and/or adrenergic modulators) and prostaglandin analogs; however, they do not appear to adequately control IOP.[67] Alternative approaches might include TRPV4 inhibitors to suppress stretch-induced RhoA activity[10,34,61] or TREK-1 activators to counter transcriptional suppression of the facility. Given that ML-402 increases permeability of TM monolayers,[23] promotes conventional outflow (Fig. 2), and reverses DEX-induced IOP elevations (Fig. 3), TREK-1 modulation appears to be a potential alternative paradigm that could be utilized to treat refractory cases of corticosteroid-induced OHT.

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