Supplementary Methods

Mouse strains used

Strains used were: C57BL/6J (Stock Number 000664, B6); B6.129P2-$Nos3^{<tm1Unc>}$/J (Stock Number 002684, B6.$Nos3^{-/-}$ or $Nos3^{-/-}$) and B6.Cg-$Cav1^{<tm1Mls>}$/J (Stock Number 007083, B6.$Cav1^{-/-}$ or $Cav1^{-/-}$). B6.$Prox1$-GFP mice have been described previously [1].

Drugs

Insolution^{TM} Y-27632 (Cat. No. 688001, EMD Millipore) used at 200 µM. L-N^G-Nitroarginine methyl ester (L-NAME, Cat. No. 80210, Cayman Chemical) was used at 100 µM. The drugs were diluted from stocks in either BSS PLUS irrigating solution (Alcon) or D-PBS (Sigma).

Dissection of the mouse eye for mounting on the device

An enucleated eye was oriented so that the superior limbal region runs across the field of view. An incision (2.5 mm, about one fourth of the circumference) was created using micro scissors on the lower half of the eye (midway between the optic nerve and the limbus, Figure S1-S2). This permitted flaps to be cut into the lower half of the eye in an orchestrated manner (Figure S1-S2). Flaps were created so that the eye can sit comfortably on the perfusion device, without causing any undue stress in the limbal tissue. After cutting the flaps, the lens and retina were removed. Initially the iris was not dissected out.

However, the iris tended to intermittently occlude the drainage structures/inlet pore at the top of the frustum and reduced the success rate of experimentation. Hence, we removed the iris by carefully grasping with forceps as long a length as possible along a radial axis of the undulating iris and gently pulling it without disturbing the iris root. When experiments were successful in eyes with the iris intact, C was similar to that obtained in eyes after iris removal.

Technical aspects of the DTX pressure transducer

This transducer has a range of -30 mmHg to 300 mmHg. The maximum error due to the total effects of nonlinearity, hysteresis and sensitivity variations is no more than 2% of the reading or ±1 mmHg, whichever is greater, over the operating range. This pressure transducer has been previously validated for our experiments using a water manometer and it accurately reads pressures set within the mouse eye using a manometer (R^2=0.98) [2].

Measurement of length of drainage tissue available for perfusion in mounted eyes

We measured the length of the Schlemm's canal (SC) spanning the frustum and free from the O-ring. This length reflects the length of conventional outflow structures exposed to flow. To measure this length of the SC, we made pinpricks at the two ends of the limbal region (spanning the frustum) abutting the inner surface of the O-ring. Tissue was fixed in 4% PFA in PBS and imaged (Schlemm's canal was visualized by GFP fluorescence or by endomucin antibody

staining) [1]. Using Imaris, this length of the SC was traced using the polygon tool and measured to be 3.7±0.18 mm (n=10). We also measured the length of the SC around the entire limbus in 4% PFA fixed anterior segment wholemounts to be 8.27±0.4 mm (n=10). Thus, we determined that the length of SC exposed to flow in our perfusion system was ~45% of the entire length of SC in whole eye.

Facility calculation

Facility (nl/min/mmHg) was measured by obtaining the slope of the linear region of the relationship between average flow-rate and pressure as described before [3]. This was based on the Goldman equation [4] : $F = (IOP-EVP) \times C + F_u$, where F = total outflow rate, IOP = is intraocular pressure, EVP = Episcleral venous pressure, C is the conventional outflow facility and F_u is pressure independent flow. In our case, since EVP = 0 the equation can be rewritten as $F = C \times P + F_u$, where P is the pressure we set. This equation is of the form $y = mx + b$ where m hence C is the slope of the line and b is the y-intercept F_u. A flow-rate vs. pressure graph was created in Matlab from the measured average flow rate during the record phase. The Matlab script then computed the slope of the linear part of the graph (between 5-25 mmHg in Protocol A or between 4-24 mmHg in Protocol B). To avoid adding error/uncertainties, resulting from the correction factor of 2.2 we statistically compared the raw data from different treatment or genotype groups prior to correction.

Resistance and Compliance measurements

To measure resistance, we determined if any pressure changes occurred over the atmospheric pressure upon utilizing a flow rate of 660 μl/min (far larger than the flow observed by an eye) and leaving the inlet port open (no eye secured to system). System compliance was determined by measuring the pressure changes caused by injecting fluid using the pump after sealing the inlet port. We then plotted the volume against the pressure change. We used a linear fit on the plot to obtain the slope of the plot namely the compliance.

Washout measurement

To measure washout rates, i.e. facility change with time, eyes were perfused at 15 mmHg for 2 hours. Prior to these measurements, we allowed the system to stabilize for 30 min. We then calculated the facility (change in total flow volume /pressure) at 10 min intervals and compared each interval to determine if there was an increase over the 2 hours period (represented as a % change).

Immunofluorescence, tracer perfusion and tissue sections

To determine the effect of perfusion on the drainage structures, eyes from B6.*Prox1-GFP* mice were used. Control eyes were mounted on the device and inflated at low pressure, but not subjected to the pressure protocols. Eyes were also perfused with 3-kDa lysine fixable Dextran Texas Red (Thermo Fisher Scientific). After perfusion, eyes were placed into 4% paraformaldehyde (PFA) in D-PBS overnight and then placed into 0.4% PFA prior to processing.

Immunofluorescence was performed on wholemount eyes using anti-VE-cadherin rat monoclonal antibody (BV13, eBioscience) as described before [1]. Eyes perfused with dextran were stained with rabbit polyclonal ZO1 (Thermo Fisher Scientific). Eyes were mounted on slides and subjected to confocal microscopy (Leica TCSSP8).

Eyes perfused using Protocol A and control eyes were fixed in Smith–Rudt fixative and plastic sections were cut and toluidine blue stained as described previously [5]. The sections were imaged on a Zeiss AxioObserver- Z1 (20X and 40X objectives).

Reference

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