Intraocular Pressure Homeostasis: Maintaining Balance in a High-Pressure Environment

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Abstract

Although glaucoma is a relatively common blinding disease, most people do not develop glaucoma. A robust intraocular pressure (IOP) homeostatic mechanism keeps ocular pressures within relatively narrow acceptable bounds throughout most peoples' lives. The trabecular meshwork and/or Schlemm's canal inner wall cells respond to sustained IOP elevation and adjust the aqueous humor outflow resistance to restore IOP to acceptable levels. It appears that the cells sense IOP elevations as mechanical stretch or distortion of the actual outflow resistance and respond by initiating a complex extracellular matrix (ECM) turnover process that takes several days to complete. Although considerable information pertinent to this process is available, many aspects of the IOP homeostatic process remain to be elucidated. Components and mechanisms beyond ECM turnover could also be relevant to IOP homeostasis, but will not be addressed in detail here. Known aspects of the IOP homeostasis process as well as possible ways that it might function and impact glaucoma are discussed.

Glaucoma

GLAUCOMA IS AN OPTIC neuropathy characterized by a distinctive pattern of permanent visual field loss.^{1,2} Optic disk cupping is also a diagnostic parameter. Elevated intraocular pressure (IOP) is the primary risk factor for glaucomatous optic nerve damage and reducing pressure remains the only treatable component of disease progression.^{2,3} Although glaucoma is a relatively common blinding disease affecting over 67 million persons worldwide,^{3–5} it is noteworthy that only 2%–8% of people actually develop this disease within their lifetime and most only at advanced ages. The implication of this observation is that some very efficacious mechanism exists to maintain IOP within acceptable ranges throughout the life of most people.⁶

Intraocular Pressure

IOP is maintained primarily by changes in the aqueous humor outflow resistance, which is thought to reside predominantly within the cribriform or juxtacanalicular (JCT) region of the trabecular meshwork (TM) and the inner wall of Schlemm's canal (SC).^{6–10} Aqueous humor inflow rates are relatively stable and are not pressure dependent, until very high pressures are achieved.^{11,12} Although outflow through the alternative or uveoscleral pathway is clearly important, most of the outflow in humans is through the conventional TM/SC route.^{2,7,8,12,13}

IOP Homeostasis

For our purposes, in this study, we will define *IOP homeostasis* as *corrective adjustments of the aqueous humor outflow resistance, which occur in direct response to sustained pressure changes and which maintain IOP within acceptable physiological ranges*.

We hypothesize that the flow resistance within the conventional outflow pathway is continually being adjusted with time frames measured in many hours and that sustained pressure changes serve as a guide for the direction and extent of homeostatic resistance modifications. Since the outflow resistance is thought to be comprised primarily of extracellular matrix (ECM)^{6,7,9,10,14,15} and since ongoing ECM turnover is required to maintain the outflow resistance,^{6,16,17} this hypothesis has some basis in our understanding of how the system works.

Caveats and Disclaimers

It should be noted that the studies, observations, and ideas discussed in this study are based primarily on evidence from perfused human anterior segment organ culture, perfused *ex vivo* human and mouse eyes, or cell culture. These are well-accepted models and the flow rate and pressure relationships obtained with them reflect the measured *in vivo* values and behavior quite closely. However, these models are just models. Important differences relating to IOP

homeostasis between these models and *in vivo* humans may well exist. Although we are unaware of any direct and controlled *in vivo* studies of IOP homeostasis, as defined above, in either an animal model or in humans, our exclusive focus in this study on these *ex vivo* models should temper reader interpretation to reflect this context. Our focus herein is also primarily on the role of ECM turnover in this processes. Processes other than ECM turnover may be involved in IOP homeostasis, but they will not be discussed in detail here.

Experimental Evidence for IOP Homeostasis

Although the existence of an effective IOP homeostatic mechanism seems obvious, it is surprising how little literature is available that addresses IOP homeostasis directly. Some years ago, we conducted studies to evaluate the potential of perfused human anterior segment organ cultures to show IOP homeostatic behavior.¹⁸ After we had perfused anterior segments for several days at standard physiological flow rates (2.5 $\mu\text{L}/\text{min}$), we doubled the flow rate (5 $\mu\text{L}/\text{min}$) and measured pressures for several days. The pressure approximately doubled immediately after flow rates were doubled, that is, outflow facility ($C = \text{flow rate}/\text{pressure}$; in this system) did not change significantly. However, over the next several days at the sustained $2\times$ flow rate, the measured pressure slowly declined, returning to approximately the initial level measured before doubling the flow rate (Fig. 1, a schematic representation of typical results). The outflow system sensed that the pressure was elevated and had adjusted the outflow resistance over several days to return the pressure to the pre-elevation levels, although the flow rate was still at $2\times$. This appears to be the first actual controlled experimental study demonstrating the IOP homeostatic effect.¹⁸

Since that time, we and others have conducted studies showing that anterior segments in perfusion organ culture can sense pressure elevation and respond by adjusting the outflow resistance.^{6,17,19,20} We get similar adjustments for the outflow resistance when using a constant flow rate or constant pressure perfusion protocols in human or porcine

anterior segment organ culture. Of course, in constant pressure perfusion, the resistance adjustment does not actually reduce the pressure, which is held constant, so the system just keeps trying to do so by further reducing the resistance. Since the difference between IOP and the episcleral venous pressures in humans is around 6–9 mm Hg and typical human outflow rates are around 2.5–3 $\mu\text{L}/\text{min}$, we normally perfuse for $1\times$ at rates or levels in this range and use $2\times$ values for homeostatic challenge.^{18,20} Figure 2 shows a compilation of outflow facility data from different human IOP homeostasis experiments conducted in our laboratories over the last 15 years. Sustained adjustments to the outflow resistance following $2\times$ pressure increases are relatively slow, taking many hours to several days for a strong homeostatic adjustment to be apparent.

Short-Term Moderate Pressure Responses

Normally, doubling the pressure has no significant immediate effect on outflow facility, that is, going from $1\times$ to $2\times$ perfusion pressure, the flow rate approximately doubles immediately and does not change further for many hours (see Figs. 1 and 2). There are many examples of this in the literature. For example, in one study, using perfused human anterior segment organ culture, increases from 10 to 25 mm Hg showed no change in facility over a 1–3-h period.²¹ Perfusion of mouse eyes *ex vivo*, where stepped pressure readings are used to strip out conventional (pressure sensitive) from alternative (pressure insensitive) outflow, showed no changes in conventional outflow facility after or during flow readings at moderate pressures, that is, 8–35 mm Hg.²² In these relatively short-term studies (20-min perfusions at each pressure level with a total study of only a few hours), no IOP homeostatic changes were observed and the return to low (15 mm Hg) pressure value was approximately the same as the initial value at that pressure.^{22–24} In one study, similar facilities were obtained in the same mouse when anesthetized compared with when euthanized.²⁴ In addition, short-term mechanical stretching lasting less than 6 h does not trigger any homeostatic response, as judged by biochemical readouts.¹⁸

FIG. 1. Schematic of typical intraocular pressure (IOP) homeostatic response. Constant flow perfusion of human anterior segment organ cultures for 48 h at $1\times$ flow rate followed by sustained perfusion at $2\times$ flow rate during which time, the outflow system adjusts the outflow resistance to restore normal pressure.

Higher Pressures Can Decrease Facility Acutely

It has long been known that large pressure increases, that is, much greater than $2\times$, or starting at much higher than

FIG. 2. IOP homeostatic response to $2\times$ pressure. Human anterior segment organ cultures subjected to constant pressure perfusion at $1\times$ pressure for 48 h followed by perfusion at $2\times$ pressure. Normalized outflow facility is plotted for 23 separate experiments. Mean facility before homeostatic corrections ($1\times$) was 0.34 $\mu\text{L}/\text{min}/\text{mm Hg}$ and significance (***) was assessed by one-way ANOVA with the Dunnett's Multiple Comparison Test.

our $1 \times$ initial pressure, can produce facility decreases that are large and fairly immediate.^{25–29} This appears to be primarily due to large changes in the physical conformation of the outflow pathway, where the JCT/SC distends far out into and obstructs the SC.^{26–28,30} Images in some of these studies show dramatic partial or total collapse of the SC, which nearly or actually occludes the canal further obstructing lateral circumferential flow and may completely block fluid egress into collector channels.^{25–27,29} At higher pressures, particularly when sustained as in glaucoma, the JCT/SC forms actual herniations out into the collector channels.²⁶ In these high-pressure studies, where flow was measured, facility often decreases immediately and significantly at higher perfusion pressures and the effective filtration length (a measure of regions of the JCT, with flow compared with total JCT regions, where flow could occur) is reduced.²⁶ Although these studies provide valuable insight into aqueous outflow and regulation, their duration of only a few hours limits direct application to understanding the normal IOP homeostatic response, which takes hours.

Sensing Resistance or Pressure Misbalance as Mechanical Stretch

The probable site of the outflow resistance is within the deepest portion of the JCT and the SC inner wall basement membrane.^{6–9,31} These thin layers (probably, no more than 3–7 μm thick) of highest flow resistance would thus be the foci of the forces created by IOP. We hypothesized that these IOP forces would produce mechanical stretching or distortion of the ECM and attached cells of the JCT and SC inner wall when pressures increased.^{6,18} Presumably, the pressure would be expressed as force focused primarily upon the outflow resistance itself, which would distend modestly out into the SC creating a stretching or distorting effect within the resistance.⁶ This is quite compatible with studies showing that modest pressure elevations tend to push the JCT/SC out into the SC, exactly as this hypothesis would predict.^{26,27,32} For normal pressure differentials, that is, 5–15 mm Hg would likely be the focus of day-to-day IOP homeostatic adjustments, the stretching or distortion would be relatively small, perhaps in the range of 10%–20%.

How Could the IOP Homeostatic Process Work?

If the outflow resistance itself is somewhat dynamic and is continuously being adjusted at a moderate pace, for example, over many hours to days, then the resistance could occasionally drift to levels that were too high or too low. Other perturbations, such as genetic or environmental influences, would exacerbate this drift over time. When the IOP changes were large enough to need attention, TM cells may sense this as mechanical stretch/distortion of the outflow resistance. Since much of the resistance is putatively provided by ECM, this ECM itself would be stretched or distorted by the pressure change. This would be sensed by JCT and/or SC cells, likely through specific integrins and other cell surface ECM receptors such as CD44, VCAM-1, syndecans, and NG2/CSPG4. This would be transmitted within JCT and possible SC inner wall cells by activation of specific signal transduction pathways and by changes in cytoskeletal tensions and organization. The signal transduction pathways would trigger key gene expression and

activity level changes, including specific ECM turnover enzymes like the matrix metalloproteinases (MMPs). ECM turnover would be increased, including increased degradation and replacement of ECM components by increased biosynthesis or endocytic recycling. Presumably, the replacement ECM would be slightly different in composition, organization, or amount, thus modifying the outflow resistance. IOP would thus be adjusted due to the modified outflow resistance. The exact changes in the resistance would likely depend somewhat on the extent and specific type of sensing, a nuance that has not currently been addressed.

Further inserted into this conceptualization is the complexity recently identified in TM cellular behavior depending on the type and rigidity of the substratum.^{33–35} In addition, a direct cellular contribution, possibly beyond the ECM turnover involvement, appears to affect the outflow resistance in some manner that is currently only partially understood.^{10,36–42} A large number of cellular cytoskeletal tension/relaxation studies showing acute and more sustained effects on outflow facility have been reported. Numerous studies where cell-related properties, for example, nitric oxide and eNOS, RhoA GTPase, are associated with direct or indirect cellular effects on outflow, suggest additional complexity.^{23,37,43–47} These effects have been discussed elsewhere and are not central to this article, but do suggest intriguing areas for investigation.

Evidence for ECM Turnover in IOP Homeostasis

We had shown that ongoing ECM turnover, initiated by MMPs in the TM, was absolutely necessary to maintain outflow facility.¹⁶ Perfusing or stimulating levels of MMPs increased outflow and specific inhibition of endogenous MMPs decreased outflow.^{16,48} These processes took days. When we subjected TM cells to mechanical stretching, we found that MMP14 and MMP2 were increased, while their primary inhibitor, tissue inhibitor of metalloproteinases 2 (TIMP2), was decreased.¹⁸ Mechanical stretch also increased ADAMTS4 (a disintegrin and metalloproteinase with thrombospondin type I motifs).⁴⁸ We hypothesized that JCT cells in the TM sensed ECM stretch/distortion when IOP varied, probably through ECM-integrin interactions, but possibly assisted by other ECM-cell receptors in the TM/SC.^{6,17,18} We showed that acute stretching, for times less than 6 h, did not trigger the IOP homeostatic response, at least in terms of triggering ECM turnover enzymes.¹⁸ The time course for MMP activity changes to affect outflow are very compatible with the IOP homeostasis time observed with pressure elevation.^{16–18,20} Mechanical stretching or perfusion pressure increases trigger numerous changes in ECM protein expression levels at times compatible with ECM remodeling.^{17,28,49–51} Thus, all of our data are compatible with the concept that sustained JCT stretching is the signal to initiate the IOP homeostatic response and begin adjusting the outflow resistance.^{6,17}

Evidence for Modified ECM Production in IOP Homeostasis

We conducted microarray gene expression studies after 12, 24, and 48 h of mechanical stretching of TM cells.⁴⁹ Others conducted similar analyses after subjecting perfused

anterior segments to elevated pressure for 2–4 days.²⁸ In this study, the control pressure was approximately 15 mm Hg and elevated pressure was 30–45 mm Hg, both much larger than our normal 1× and 2× pressures. More recently, as part of an analysis of differential gene expression relating to segmental outflow patterns, we have also analyzed additional ECM gene expression patterns using 8.8 mm Hg for 1× and 17.6 mm Hg for 2× perfusion pressure. These several gene expression studies identified several hundred genes that exhibited increased or decreased mRNA levels. Table 1 shows a few of the most interesting that were observed in several studies.^{48,49,52}

In addition to changes in gene expression, we found a number of ECM genes that show differences in mRNA splice usage with pressure or stretch.^{49,52} Some of these are also shown in Table 1. The splice variants included some

typical patterns seen often in other systems as well as some unique splice forms not previously reported.⁵² Some of these variants are in molecules that have been implicated in the outflow resistance or as being associated with molecules that are.^{6,17,52} Since these alternative splice forms include or lose exons that have specific binding domains for other ECM components, the splice forms would be expected to integrate into the ECM differently. However, most of the molecular details are yet to be worked out.

Cyclic Mechanical Stretch

In addition to sustained static mechanical stretching of cells, several studies have been conducted utilizing cyclic mechanical stretch.^{51,53–57} Since an ocular pulse of 2–3 mm Hg could perturb the TM, there is some logic to evaluating cyclic mechanical stretching. Studies showing a TM displacement due to an ocular pulse have been conducted recently.^{58,59} The temporal responsiveness of the stretch or pressure stimulus that was discussed above, seems to preclude a direct cyclic ocular pulse-related contribution to IOP homeostasis. Cyclic stretch and IOP have been assessed, but these tend to reduce facility rather than increase it.^{57,60} However, a pressure or stretch increase that occurred on a cyclic background, could conceptually produce different responses than would be observed with an equivalent sustained noncyclic stretch.⁶¹ The group of genes with expression profiles changed by TM cell cyclic stretching (15% stretching at 1 cycle per second for 6 h), shows some similarity, but also comprises some unique genes compared with those found with sustained stretching.^{49,53} In addition, signal transduction pathways triggered by cyclic stretching are partially overlapping with those involved in static stretching.^{50,62}

Signal Transduction and Cellular Responses to Stretching/Pressure

When we analyzed the signaling pathways responsible for MMP14 and MMP2 increases following mechanical stretching of TM cells, we found a translational, but not a transcription-mediated process.⁶³ Putative integrin triggering was processed downstream through integrins-linked kinase (ILK), protein kinase B (PKB), phosphoinositide-dependent kinase (PDK1), phosphoinositol-3-kinase (PI3K), and mammalian target of rapamycin (mTOR). Signaling then progressed to p70/p85 S6 kinase and eIF-4E in the translational machinery.⁶³ This set of signaling pathways may impact some other IOP homeostatic changes that occur, but it seems probable that other pathways are also involved in some of the other changes that happen concurrently.

Other investigators have identified stretch-triggered actin cytoskeletal changes,⁶⁴ and other signaling gene changes.^{21,55,56,65,66} Superimposed on the direct effects of mechanical stretching on TM cells, a number of signaling molecules are released or upregulated by mechanical stretch, such as CTGF, IL-6, TGFβ, and adenosine.^{49,55,56,66} Many of these genes have dramatic effects on the TM and on outflow.^{15,67–70} The same can be said for the cytoskeletal changes. Many cytoskeletal manipulations have dramatic effects on both outflow facility and TM cell behavior.^{36,37,39,44} Although most of the cytoskeletal research has not been directly applicable to the IOP homeostatic pressure

TABLE 1. STRETCH/PRESSURE RESPONSE OF EXTRACELLULAR MATRIX GENES FROM MICROARRAY, QUANTITATIVE RT-PCR, QUANTITATIVE PCR ARRAY, OR PROTEOMICS ANALYSES

<i>Gene name</i>	<i>Up or down^a</i>	<i>Splicing in response to stretch</i>
Versican	– ^{49,52}	Versican ⁵²
Tenascin C	++ ^{49,52}	Tenascin C ⁵²
Fibronectin	+ ^{49,52}	Fibronectin ^{49,52}
CD44, hyaluronan receptor	+ ^{49,52}	CD44 ⁵²
Collagen 12	+ ⁵²	Collagen 12 ⁵²
Collagen 14	+++ ⁴⁹	
Collagen 1α2	++ ⁴⁹	
Collagen 5α1	+ ⁴⁹	
NELL2	+++ ⁴⁹	
ADAMTS4, a disintegrin and metalloproteinase with thrombospondin motifs 4	++ ⁴⁸	
Matrix Gla	++ ⁴⁹	
VCAM 1, vascular cell adhesion molecule 1	+ ⁴⁹	
MMP-2	+ ⁴⁹	
MMP-15	+ ⁴⁹	
MMP-16	++ ⁴⁹	
Periostin	– ⁴⁹	
Syndecan2	+ ⁴⁹	
LAMC1, laminin, gamma 1	+ ⁴⁹	
SPARC, secreted protein, acidic, cys-rich (osteonectin)	++ ⁴⁹	
CSPG4, chondroitin sulfate proteoglycan NG2	+ ⁴⁹	
Fibromodulin	+ ⁴⁹	
Biglycan	+ ⁴⁹	
CTGF, connective tissue growth factor	+ ⁴⁹	
Mimecan	– ⁴⁹	
LTBP2, latent TGFβ-binding protein 2	+ ⁴⁹	
Endoglin	+++ ⁴⁹	

^aApproximate fold changes in response to stretch where “–” <0.75, “+” ≥1.5, “++” ≥2.0, “+++” ≥3.0.

RT-PCR, reverse transcriptase-polymerase chain reaction; MMP, matrix metalloproteinase.

responses, several studies have shown clear effects that may relate to the IOP homeostatic mechanism.^{40,44–47}

Focal ECM Turnover

Initially, it was not fully appreciated that the IOP homeostatic ECM turnover events needed to be highly constrained and very focal.¹⁶ Studies colocalizing MMP2 and MMP14 facilitated the identification of specialized regions of ECM degradation and attachment that we named PILS for podosome- and invadopodia-like structures.⁷¹ Upon extended analysis, these regions turn out to be sites of ECM degradation, endocytosis, and recycling. These very focal regions facilitate controlled ECM turnover producing restrained ECM processing zones within the flow channels. The focal and highly controlled nature of PILS allows managed adjustments in the outflow resistance without disorganization of the whole structure.^{6,17,71} Recently, a glaucoma-associated SNIP was identified between caveolins 1 and 2.^{72,73} Additional studies⁷⁴ suggest that these proteins may have direct involvement in PILS function and outflow, although these studies are still incomplete (Aga, et al., article in preparation).

Segmental Outflow and IOP Homeostasis

Although it has long been known that outflow is uneven or segmental around the circumference of the eye,^{26,27,75–78} the impact of this observation on IOP homeostasis has only recently become fully appreciated.^{79–81} As with other aspects of aqueous outflow, segmental flow has dramatic implications on the resistance adjustments that occur during IOP homeostasis. Questions about the site of resistance adjustments, that is, does it occur in high or in low flow regions? impact analysis, where the complete circumference of the eye is assessed. Aside from a number of studies in process or reported only in abstract form, little literature is available to clarify aspects of this important issue. The molecular differences between high and low flow regions, particularly as changed during the IOP homeostatic resistance adjustment, are critical to understanding the behavior of the system.

IOP Homeostasis and Glaucoma

Although there appears to be many causal triggers for glaucoma, including different genetic^{82,83} and possibly environmental contributing factors, the loss of IOP homeostatic capability is clearly a hallmark of much of glaucoma. The inability to maintain the outflow resistance within acceptable ranges to avoid sustained IOP elevation triggers a significant portion of glaucomatous optic nerve damage, which is why IOP elevation remains a leading risk factor for glaucoma. Conceptually, the loss of effective IOP homeostasis in much of glaucoma could be due to (1) inadequate IOP sensing; (2) inability to mount an effective adjustment of the outflow resistance; or (3) perhaps, a disrupted or disorganized resistance and restoration system that is beyond repair.

TM cellularity is often diminished,^{84–86} probably as a consequence of the various genetic and environmental insults and damages that trigger glaucoma. Some specific glaucoma triggers or initiating factors may directly affect specific key pathways that are necessary to maintain IOP

homeostasis. Alternatively, the glaucoma triggers and their consequences may diminish general capability of TM JCT/SC cells to function; thus, the effect on the IOP homeostasis process could be more of a collateral effect than a direct one.⁶⁵

ECM changes with incompletely characterized causes, such as the increased stiffness detected in glaucomatous eyes,³⁴ may also result in ECM that is more difficult to remodel.^{87,88} This stiffness could also modulate TM cell sensing, responsiveness, or direct behavior.^{89–95} An array of other related ECM effects are also likely involved, but IOP homeostatic relationships are not well defined for any of these.^{15,67,68,96,97}

Conclusion

The IOP homeostatic process, which appears to be a key mechanism explaining why most people do not develop glaucoma, remains only partially understood. Extension of our understanding of this process should provide strong guidance for enhanced therapeutic approaches to glaucoma, which may be categorized as a disease where IOP homeostatic capability has been compromised or lost.

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